

19th World Congress of Soil Science

Symposium 3.5.1

Heavy metal contaminated soils

Soil Solutions for a Changing World,

Brisbane, Australia

1 – 6 August 2010

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Adsorption and leaching of aromatic arsenicals in Japanese agricultural soils

Yuji Maejima^A, Hirotatsu Murano^A, Takashi Iwafune^A, Tomohito Arao^A and Koji Baba^A

^ANational Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, Japan, Email ymaeji@niaes.affrc.go.jp

Abstract

We investigated the adsorption and mobility of aromatic arsenicals (AAs) in two types of Japanese agricultural soils through the batch and column leaching experiments. AAs were composed of both phenyl or methyl group and arsenate. The amounts of adsorbed AAs onto both Fluvisols, sandy loam, and Andosol, light clay, increased in the order of phenylarsonic acid (PAA) > methylphenylarsinic acid (MPAA) > diphenylarsinic acid (DPAA) > methylphenylarsine oxide (MDPAO) = dimethylphenylarsine oxide (DMPAO). The adsorbed amounts of all AAs onto Andosols, which had relatively high amorphous minerals and organic carbon, were higher than those of Fluvisols. The adsorbability of AAs on soils increased with the increasing a portion of arsenate of AAs. The mobility in Fluvisol was in the orders of DMPAO (30cm) > MDPAO(25cm) > MPAA(20cm) > DPAA(15cm) = PAA(15cm). The mobility in Andosol was in the orders of DMPAO (15cm)=MDPAO(15cm) > DPAA(10cm) > MPAA(5cm) = PAA(5cm). The mobility of MDPAO and DMPAO in soils was higher than that of PAA and MPAA, and all AAs's mobility in Andosols was lower than that in Fluvisols. Consequently, the mobility of AAs in soils decreased with increasing the adsorbability of AAs on soils.

Key Words

Chemisorption, Freundlich equation, hydrophobic bonding, ligand exchange, variable-charge minerals.

Introduction

Diphenylarsinic acid (DPAA) and methylphenylarsinic acid (MPAA) were detected in paddy rice grown in Kamisu-town, Ibaraki Prefecture, Japan in 2004. It is necessary to evaluate the risk to the human of these aromatic arsenicals (AAs) through the soil, water, and farm product. Recently, Arao *et al.* (2009) investigated the uptake of AAs from agricultural soils by rice with compared to the results from AAs-added solution culture experiment, and they reported that MPAA and DPAA were methylated not in the rice plant but in the soil under flooded conditions. However, there has been little research on the behavior of AAs in the agricultural soils and farm products. The objectives of this study were to understand the basis of the adsorption characteristics of AAs in soils and to evaluate the mobility of AAs in agricultural fields.

Materials and methods

Chemicals

Analytical grade AAs as shown by Figure.1 were used in this study. Standard stock solution of AAs (100 mg/L) was prepared in 0.02 mol/L sodium hydroxide or 10% ethanol, and diluted with ultra-pure water.

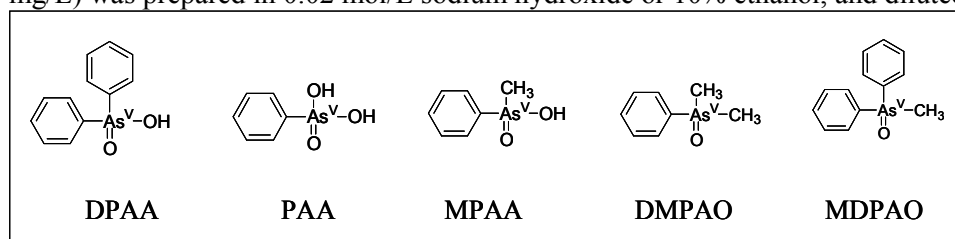


Figure 1. Chemical structure of AAs in this study. DPAA: diphenylarsinic acid; PAA: phenylarsonic acid; MPAA: methylphenylarsinic acid; DMPAO: dimethylphenylarsine oxide; MDPAO : methylphenylarsine oxide

Soil samples

Two types of non-contaminated soils were collected from the surface (top15 cm) in fields in Japan (Kamisu and Tsukuba, Ibaraki Pref.). Soil samples were air-dried, and were then passed through a 2 mm sieve. Soil pH, particle size distribution, cation exchange capacity (CEC), carbon and nitrogen contents, acid oxalate extractable Fe, Al, and Si contents, 1M HCl soluble and total arsenic (As), and phosphate adsorption coefficients were measured.

Adsorption experiment

Adsorption experiments were carried out in a 12-mL glass tube at 25 ± 2 °C according to the method of OECD Guideline for the Testing of Chemicals 106 (OECD 2000). Two grams of soil was put into the glass tube, and 10 mL of AAs solution, such as DPAA, PAA, MPAA, MDPAO, and DMPAO, was added to each soil sample at the soil to solution ratio of 1:5. The final concentration of arsenic (As) in each solution was set to 0, 100, 300, 1000, 3000, and 10000 µg/L including 0.01 mol/L CaCl₂. Each glass tube was shaken for 24 hours at 25 ± 2 °C. After centrifuged at 3000 rpm for 20 min, the supernatant was filtered with a 0.2 µm membrane filter. The solution was diluted with ultra-pure water, and As in the solution was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES).

Column leaching experiment

DPAA, PAA, MPAA, MDPAO, and DMPAO, were added to the soil according to the method of OECD Guideline for the Testing of Chemicals 312 (OECD 2004). Soil was filled to become 30cm of soil layer thickness with seven fractional columns (5cm in diameter, 5cm in height, and no active alumina). Soil column was filled in 0.01 mol/L CaCl₂ solution during a day. After then, the AAs solution was added to surface water (each AAs was 1 mg As, and total As was 5mg), and 0.01 mol/L CaCl₂ solution flowed at the rate of 0.136 mL/min for 48 hours (corresponded to precipitation 200mm/2day). The percolating water during the experiment was collected using the fraction collector every hour, and As concentration in the percolating water was measured by ICP-OES. The soil column after leaching experiment was divided into 6 sections of soil. AAs were extracted from these soils according to the methods of Baba *et al.* (2008).

Results and discussion

Physico-chemical properties of soils

Physico-chemical properties of soils (Kamisu and Tsukuba) are summarized in Table 1 and Table 2. Tsukuba soil had relatively high organic matter (74.3 g/kg) and clay content (38.5 %), and cation exchange capacity (CEC) (37.9 cmol_c/kg) in comparison to Kamisu soil (Table 1). Tsukuba soil had also high oxalate-extractable Fe, Al, and Si contents, while low oxalate-extractable Fe, Al, and Si contents characterized Kamisu soil (Table 2). Therefore, high amorphous Fe, Al and Si contents of Tsukuba soil will increase the soil surface area, which will potentially reduce the bioavailability of AAs. According to Soil taxonomy (Soil Survey Staff 1998) and WRB (FAO, ISRIC, and ISSS 1998), Kamisu soil was classified as Typic Udifluvents or Eutric Fluvisols, and Tsukuba soil was Typic Fluvudands or Haplic Andosols, respectively.

Table 1. Physico-chemical properties of soil samples.*

| Sample | Hor. Depth (cm) | Particle size distribution | | | Texture | pH | | CEC (cmol _c kg ⁻¹) | Total | | | |
|---------|-----------------|----------------------------|----------|----------|---------|------------------|-----|---|-------------------------|-------------------------|----------|------|
| | | Sand (%) | Silt (%) | Clay (%) | | H ₂ O | KCl | | C (g kg ⁻¹) | N (g kg ⁻¹) | CN ratio | |
| Kamisu | Ap | 0-15 | 78.9 | 9.1 | 12.0 | SL** | 5.8 | 5.2 | 14.0 | 9.8 | 0.8 | 12.1 |
| Tsukuba | Ap | 0-15 | 26.4 | 35.1 | 38.5 | LiC | 5.8 | 5.0 | 37.9 | 74.3 | 4.8 | 15.5 |

*, oven-dry basis, **, SL: sandy loam, LiC: light clay

Table 2. Physico-chemical properties of soil samples (continued).*

| Sample | Oxalate extractable | | | | As | | Phosphate abs. coeff.*** (g P ₂ O ₅ kg ⁻¹) | Soil classification | |
|---------|---------------------|------|------|----------|--------|-------|--|---------------------|--------------------|
| | Fe | Al | Si | Al+1/2Fe | 1M HCl | Total | | WRB (1998) | USDA system (1998) |
| Kamisu | 7.0 | 1.8 | 1.5 | 5.3 | 0.55 | 6.2 | 7.1 | Eutric Fluvisols | Typic Udifluvents |
| Tsukuba | 19.0 | 43.7 | 17.0 | 53.2 | 1.05 | 12.1 | 21.7 | Haplic Andosols | Typic Fluvudands |

***, phosphate absorption coefficient

Adsorption experiment

The amounts of adsorbed AAs onto both Fluvisol (Kamisu) and Andosol (Tsukuba) increased in the order of PAA>MPAA>DPAA>MDPAO=DMPAO (Figure 2a, b). Moreover, all of AAs were strongly adsorbed onto Andosols compared with Fluvisols. Adsorption isotherms can be fitted using Freundlich equation. PAA, MPAA, and DPAA, which have hydroxyl group in those structural formulas, were seemed to be mainly adsorbed onto the surface hydroxyl groups at layer silicate edges, on oxyhydroxides of iron and aluminium, and on noncrystalline aluminosilicates. These AAs were chemisorbed by variable-charge minerals to make

inner-sphere complexes by ligand exchange reaction as well as phosphate sorption (Obihara and Russell 1972; Nanzyo and Watanabe 1981). In addition, a part of the structures in these AAs might be adsorbed onto soil organic matter with both phenyl and methyl group by the hydrophobic bonding. On the other hand, both DMPAO and MDPAO, which have no hydroxyl group, would be mainly adsorbed onto soil organic matter by the hydrophobic bonding only. Judging from these results, an adsorption of AAs, which have hydroxyl group, by ligand exchange reaction, might be stronger than that of AAs, which have no hydroxyl group, by the hydrophobic bonding.

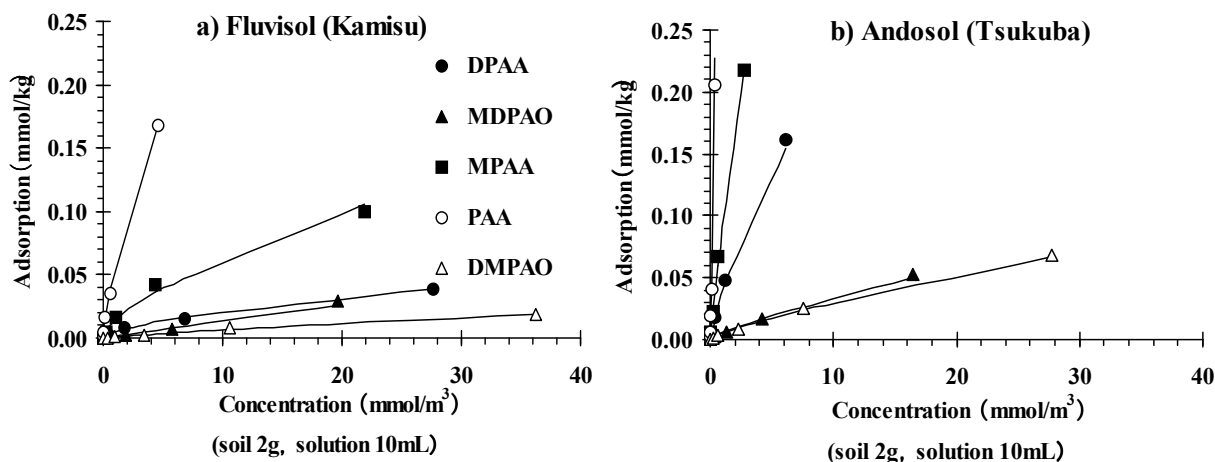


Figure 2. Adsorption isotherms of aromatic arsenicals (AAs) on Japanese agricultural soils. a) Fluvisol (Kamisu) and b) Andosol (Tsukuba).

Column leaching experiment

Figure 3 shows AAs distribution of soil column two days after leaching experiments. It was clear that the mobility of AAs in Andosol was lower than that in Fluvisol (Figure 3a, b). The mobility in Fluvisol (0.5% or more of the total AAs was detected) was in the orders of DMPAO(30cm) > MDPAO(25cm) > MPAA(20cm) > DPAA(15cm) = PAA(15cm). The mobility in Andosol was in the orders of DMPAO(15cm) = MDPAO(15cm) > DPAA(10cm) > MPAA(5cm) = PAA(5cm) (Table 3). Any AAs could not be detected in the percolating water during two days (data not shown). Both PAA and MPAA were retained at the surface of both soils because PAA and MPAA were seemed to be tightly adsorbed onto variable-charge minerals in the soil by ligand exchange reaction as well as the results of adsorption experiment. On the other hand, both DMPAO and MDPAO can move down relatively because these chemicals would be less adsorbed than PAA and MPAA. Therefore, it was confirmed that these results of adsorption experiment were corresponded to those of soil column leaching experiment.

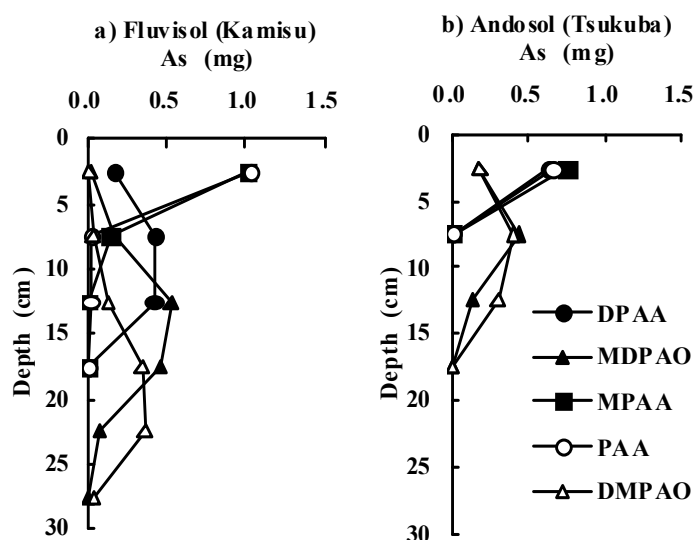


Figure 3. Aromatic arsenicals (AAs) distribution of soil column two days after leaching test. a) Fluvisol (Kamisu) and b) Andosol (Tsukuba).

Table 3. Aromatic arsenicals (AAs) distribution of soil column two days after leaching test on Fluvisol (Kamisu) and Andosol (Tsukuba).

| Depth (cm) | Fluvisol (Kamisu) | | | | | Andosol (Tsukuba) | | | | |
|------------|-------------------|-------|------|------|-------|-------------------|-------|------|------|-------|
| | Distribution (%) | | | | | Distribution (%) | | | | |
| | DPAA | MDPAO | PAA | MPAA | DMPAO | DPAA | MDPAO | PAA | MPAA | DMPAO |
| 0-5 | 16.4 | 1.8 | 96.1 | 86.1 | 1.2 | 97.8 | 23.8 | 100 | 100 | 20.9 |
| 5-10 | 41.8 | 13.6 | 2.4 | 12.3 | 3.9 | 2.2 | 58.6 | n.d. | n.d. | 45.1 |
| 10-15 | 41.5 | 41.6 | 1.6 | 0.7 | 14.2 | n.d. | 17.5 | n.d. | n.d. | 34.0 |
| 15-20 | 0.4 | 36.4 | n.d. | 0.9 | 37.4 | n.d. | n.d. | n.d. | n.d. | n.d. |
| 20-25 | n.d. | 6.6 | n.d. | n.d. | 39.3 | n.d. | n.d. | n.d. | n.d. | n.d. |
| 25-30 | n.d. | n.d. | n.d. | n.d. | 4.0 | n.d. | n.d. | n.d. | n.d. | n.d. |

*n.d.:not detected

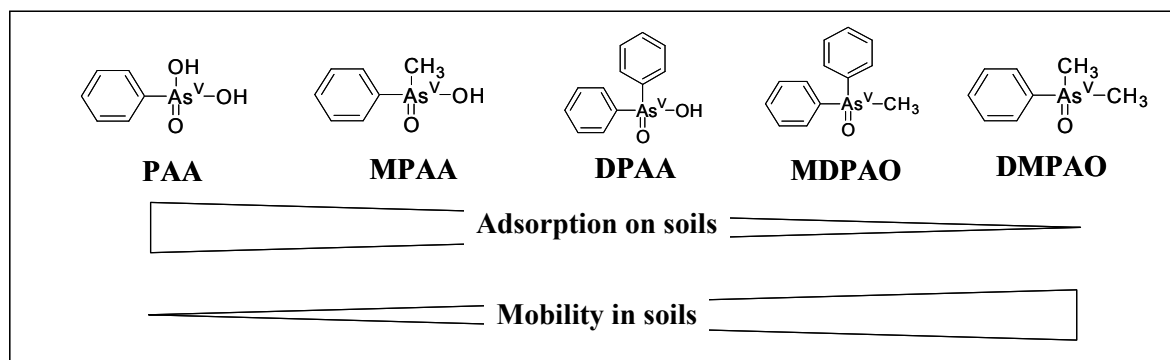


Figure 4. Relationship between adsorption and mobility of aromatic arsenicals (AAs) on soils.

Conclusion

The adsorption characteristics and mobility of AAs were summarized by Figure 4. The adsorption mechanism of PAA, MPAA and DPAA onto soils might be different from that of MDPAO and DMPAO due to the difference in these AAs's structural formulas. However, all AAs were strongly adsorbed onto Andosols compared with Fluvisols. The mobility of MDPAO and DMPAO in acid soils with low clay and oxide content like Fluvisols was higher than that of PAA and MPAA. Judging from the viewpoint of the long-term fate, it is possible that both MDPAO and DMPAO in Fluvisols move down to ground water.

References

- Arao T, Maejima Y, Baba K (2009) Uptake of aromatic arsenicals from soil contaminated with diphenylarsinic acid by rice. *Environ. Sci. Technol.* **43**, 1097-1101.
- Baba K, Arao T, Maejima Y, Watanabe E, Eun H, Ishizaka M (2008) Arsenic Speciation in Rice and Soil Containing Related Compounds of Chemical Warfare Agents. *Anal. Chem.* **80**, 5768-5775.
- FAO, ISRIC, ISSS (1998) 'World reference base for soil resources'. world soil resources reports 84, (FAO: Rome)
- Nanzyo M and Watanabe Y (1981) Material balance associated with phosphate sorption by amorphous clays, silica-alumina gel and synthetic goethite, *Soil Sci. Plant Nutr.* **27**, 329-337
- Obihara CH, Russell EW (1972) Specific adsorption of silicate and phosphate by soils. *J. Soil Sci.* **23**, 105-117.
- OECD (2004) OECD Guideline for the Testing of Chemicals 312, Leaching in Soil Columns.
- OECD (2000) OECD Guideline for the Testing of Chemicals 106, Adsorption-desorption using a batch equilibrium method.
- Soil Survey Staff (1998) 'Keys to soil taxonomy' (8th Ed.). (USDA, Natural Resources Conservation Service: Washington)

Application effect of magnesium oxide material on cadmium uptake by wheat (*Triticum aestivum*)

Masanori Okazaki^A, Naomi Aoyama^A, Genki Kobori^A, Takashi Motobayashi^B, Kouji Matsukawa^B and Masaharu Murakami^C

^AGraduate school of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, 2-24-16, Nakacho, Koganei, Tokyo 184-8588 Japan, Email masaok24@st.tuat.ac.jp

^BField Science Center for Education and Research, Tokyo University of Agriculture and Technology, 3-7-1, Honmachi, Fuchu, Tokyo 183-0027 Japan.

^CNational Institute of Agro-Environmental Sciences, 3-1-3, Kannondai, Tsukuba, Ibaraki, 305-8604 Japan.

Abstract

The effect of magnesium oxide material on cadmium uptake by wheat (Ayahikari and Norin No. 61) was investigated in the upland field (Fluvaquept) rotated from paddy rice field of Tokyo, Central Japan. Soil pH was relatively high due to long history of paddy rice cultivation. Magnesium oxide material (also containing antigorite + vermiculite) was used for neutralization to achieve the suppression of cadmium uptake by wheat, associated with improvement of the imbalance of calcium and magnesium with calcium carbonate for a long time. However, the application of magnesium oxide material at the level of 3000 kg/ha, which maintained soil at pH less than 6.5, did not significantly suppress cadmium uptake by two different wheat cultivars. It is suggested more suitable methods are required to suppress cadmium uptake by wheat than the application of magnesium oxide material in the case of this upland field rotated from paddy rice field which showed neutral soil pH.

Key Words

Antigorite, cadmium, vermiculite, wheat grain.

Introduction

Cadmium (Cd) is one of the highly toxic elements for human beings (Kobayashi 1978). Excessive Cd intake has caused serious disease of renal and bone. The Codex Committee on Food Additives and Contaminants has announced allowable limits for Cd concentration in polished rice, wheat grain and vegetables (Codex Committee 2005). Meanwhile, there is no allowable limit on wheat grain in Japan. The large-scale survey of domestic agricultural products revealed that the Cd concentration of 3 % of wheat grain exceeded intake of 0.2 mg/kg that is the international allowable limit proposed by the Codex Committee (Ministry of Agriculture, Forestry, and Fisheries of Japan 2002). With respect to genetic characteristics of wheat for Cd uptake, genotypic differences in cadmium uptake and distribution in wheat cultivated in pot and low Cd concentration field have been reported (Grant *et al.* 2008). Ministry of Agriculture, Forestry, and Fisheries of Japan intend to recommend low Cd uptake wheat cultivars and encouraged to cultivate them in relatively high Cd concentration field. The wheat cultivar of low Cd uptake accumulated much higher Cd in their roots than those of the cultivar with high Cd uptake. In this study the amount of Cd uptake and accumulation in two different wheat cultivars and application effect of magnesium oxide material to improve the imbalance of calcium and magnesium on Cd uptake by wheat were investigated.

Methods

Magnesium oxide material

The magnesium oxide material (powder) consists of magnesium oxide, antigorite and vermiculite (Figure 1) (Okazaki *et al.* 2008). It was able to adsorb cadmium as cadmium hydroxide on its surface in aqueous solution, which provided the sharp X-ray diffraction pattern. The element composition of the magnesium oxide material is as follows, Na₂O: 0.207 wt %, MgO: 61.41, Al₂O₃: 0.894, SiO₂: 21.11, P₂O₅: 0.913, SO₃: 0.0318, K₂O: 0.0162, CaO: 3.81, TiO₂: 0.0483, V₂O₅: 0.005, Cr₂O₃: 0.163, MnO: 0.107, Fe₂O₃: 4.42, Others: 6.62, Total: 99.76. The magnesium oxide material was pH 10.7 and its surface area was 165.0 m²/g. Magnesium oxide material was used for neutralization to achieve the suppression of cadmium uptake by wheat, associated with improvement of the imbalance of calcium and magnesium due to applying calcium carbonate for a long time.

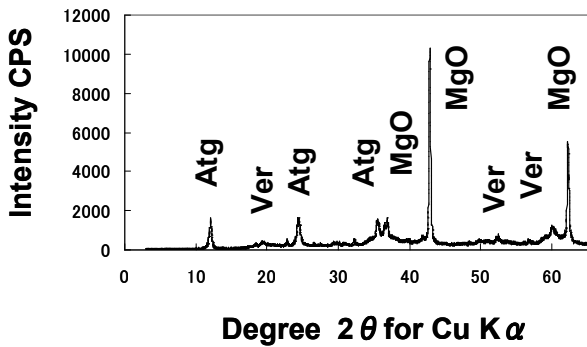


Figure 1. X-ray diffraction pattern of magnesium rich material
Atg: antigorite, Ver: vermiculite, MgO: magnesium oxide.

Soil neutralization by magnesium oxide material

Air-dried soil samples (Fluvaquept) were mixed with 0 to 6 wt % of magnesium oxide material. Twenty five mL of distilled water was added to the mixture of 5 g air-dried soil and magnesium oxide material. The suspension was lightly shaken, left for 24 hours and aerated for 2 min to let CO₂ gas out. The pH was determined by a pH meter equipped with a glass electrode.

Cultivation of wheat

The cultivation experiment of wheat was performed at Honmachi Farm of Tokyo University of Agriculture and Technology, shown in Figure 2, on an alluvial plain derived from Tama River sediment. The application of magnesium oxide material at the rate of 3000 kg/ha according to the previous experimental result (Figure 3) was carried out to neutralize soil acidity in order to suppress Cd uptake by wheat, comparing to control plot. Two different wheat cultivars, Ayahikari and Norin No. 61, were selected from a lot of cultivars in Japan.

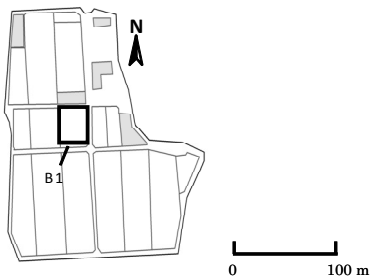


Figure 2. Hommachi Farm, Tokyo University of Agriculture and Technology
B1: 40 m x 27 m, Soil pH: 6.1 ± 0.1

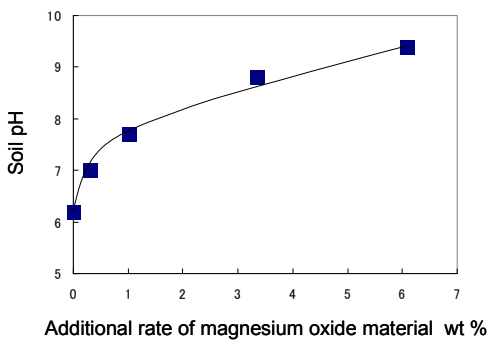


Figure 3. Neutralization of soil with magnesium oxide material

Norin No. 61 was common in Japan and recognized as low Cd uptake cultivar and recommended by Ministry of Agriculture, Forestry, and Fisheries of Japan (2007). Akihikari was newly developed for Japanese wheat noodle. Wheat was seeded in November of 2008 and harvested in July of 2009.

Cd in soil

Soil samples (n=36) at the point of wheat cultivation field were collected from just below harvested wheat. After air-drying soil samples were passed through a 2 mm nylon sieve. Twenty five mL of 0.1 mol/L HCl solution was added to 5 g soil samples. The mixture was shaken by a mechanical end-over-end shaker (Daiki, DIK 2102) at 25 °C. The extract was filtered with a No. 5 C filter paper (Advantec) and Cd concentration in the filtrate was determined by a flame atomic absorption spectrophotometer (Hitachi Z-5010).

Cd in wheat

Wheat grain samples were collected as whole body, including root. After that, wheat grain samples were washed by tap water and then deionized water, dried at 70 °C for 36 hours in a ventilated oven and ground with a mixer mill (MM301, Restch) for subsequent Cd analysis. The ground samples were digested using extra-pure water, H₂O₂ and HNO₃ (1:1:8 mL) in teflon containers in a microwave apparatus. The solution was filtered with a 0.45 µm Membrane filter (Millipore). The Cd concentration in the digests was determined using Inductively Coupled Plasma Mass Spectrometry (Thermo Fisher, X series).

Statistical analysis

All statistical analyses were performed using JMP 8 (SAS Institute Inc., Cary, NC, USA).

Results

The physicochemical properties of soils in Hommachi Farm of Tokyo University of Agriculture and Technology were described by Sakagami *et al.* (1978). The mean value of 0.1 mol/L HCl extractable Cd concentration in soil was 0.99 ± 0.07 mg/kg dry weight (DW), which is almost 3 times of median total Cd concentration (HNO₃ + H₂SO₄ digestion) in soils of Japan. This area including Hommachi Farm was polluted by Cd-polluted irrigation water through Fuchu Irrigation Canal for paddy rice cultivation. Kikuchi *et al.* (2009) reported that wheat has greater uptake and accumulation of Cd than rice. Although the extraction of Cd in soil with 0.025 mol/L HCl solution was suitable for prediction of Cd concentration in wheat grain (Ibaraki 2003), 0.1 mol/L HCl solution was used in this study to determine Cd concentration in soil, because of easy comparison with much data accumulated in Japan. Wheat can accumulate Cd in grain at low level of 0.1 mol/L HCl extractable cadmium (0.3 mg/kg DW). This upland field still revealed the past Cd pollution through irrigation systems for paddy rice, indicating that the region around the inlet of irrigation gave higher Cd concentration than those in the central and outlet regions.

The mean value, standard deviation (SD) and coefficient of variance (CV) of Cd concentration in grain of different wheat cultivars cultivated in the experiment site is shown in Table 1. The mean value of Cd concentration in Akihikari of the control plot was 0.236 ± 0.0592 mg/kg fresh weight (FW),

Table 1. Cadmium concentration in wheat grain.

| | | Cd mg/kg FW | SD | CV (%) |
|--------------|---------|-------------|--------|--------|
| | | Mean | | |
| Ayahikari | Control | 0.236 | 0.0592 | 25.1 |
| | MgO | 0.237 | 0.0060 | 2.55 |
| Norin No. 61 | Control | 0.146 | 0.0524 | 35.8 |
| | MgO | 0.153 | 0.0508 | 33.2 |

MgO: 3000 kg/ha application of magnesium oxide material

which provided a higher value than the allowable limit (0.2 mg/kg FW) of the Codex Committee. The variations of Cd concentration in wheat grain coincided with the result of Kusa *et al.* (2005). The application of magnesium oxide material at the rate of 3000 kg/ha did not suppress Cd uptake by Ayahikari. In case of Norin No. 61, the mean value of Cd concentration in wheat grain was 0.146 ± 0.0524 mg/kg FW, is a lower value than for Akihikari. The effect of magnesium oxide material on Cd uptake by Norin No. 61 was minimal, due to the relatively higher soil pH.

Conclusion

There were significant differences of Cd uptake between two different wheat cultivars; Ayahikari and Norin No. 61. The application of magnesium oxide material at the rate of 3000 kg/ha in the upland field rotated from paddy rice field did not affect Cd uptake by wheat. It is suggested that more suitable methods to suppress cadmium uptake by wheat than the application of magnesium oxide material should be used for upland fields rotated from paddy rice fields which have neutral soil pH.

References

- Bowen, HJM (1979) 'Environmental Chemistry of the Elements'. (Academic Press: London)
- Codex Alimentarius Commission (2005) Report of the 37th session of the Codex Committee on Food Additives and Contaminants. Codex Alimentarius Commission, Alinorm 05/28/12, 25
- Grant CA, Clarke JM, Duguid S, Chaney RL (2008) Selection and breeding of plant cultivars to minimize cadmium accumulation. *Sci. Total Envir.* **390**, 301-310.
- Ibaraki T (2003): Evaluation of bio-available cadmium for wheat. Promising Agricultural Practices Technologies for Reducing Heavy Contamination in Relevant Staple crops With a Special Emphasis on Cadmium Contamination 23, pp. 98-99 (NIAES: Tsukuba)
- Kikuchi T, Okazaki M, Motobayashi T (2009) Suppressive effect of magnesium oxide materials on cadmium accumulation in winter wheat grain cultivated in a cadmium-contaminated paddy field under annual rice-wheat rotation cultivation. *J. Hazardous Materials* **168**, 89-93
- Kobayashi J (1978) Pollution by cadmium and the Itai-itai disease in Japan. In 'Toxicity of Heavy Metals in the Environment, Part 1' (Ed FW Oehme) pp. 199-260. (Marcel Dekker Inc.: New York and Basel)
- Kusa K, Hatta K, Hara Y, Tsuchiya K (2005) Variation difference in concentration and location of cadmium in wheat and barley grain. Rep. of Kyushu Agr. 67, p. 46 (in Japanese)
- Ministry of Agriculture, Forestry and Fisheries of Japan (2002) Investigation of Cd concentration of staple crops: the out line of the results. December 2, Press release, Tokyo (in Japanese)
- Okazaki M, Kimura SD, Kikuchi T, Hattori T, Abe T (2008) Suppressive effects of magnesium oxides on cadmium uptake and accumulation into rice grain, I. Characteristics of magnesium oxides materials for cadmium adsorption. *J. Hazardous Materials* **154**, 287-293
- Sakagami K, Shimoda H, Watanabe N (1978) Soils of University Farm of Tokyo University of Agriculture and Technology (No. 2), Physicochemical properties of soils and nitrogen status of Hommachi Farm (paddy rice field). Rep. University Farm of Tokyo University of Agriculture and Technology No. 8, pp. 23-35 (in Japanese)

Arsenic levels, chemistry and bioavailability in Hawaii soils

Nguyen V. Hue

Tropical Plant and Soil Sciences, University of Hawaii, Honolulu, HI, USA, Email nvhue@hawaii.edu

Abstract

Past use of arsenical pesticides has resulted in elevated levels of arsenic (As) in some Hawaii soils. Total As concentrations of 50 -100 mg/kg are not uncommon, and can exceed 900 mg/kg in some former sugarcane lands. Given the high content of amorphous aluminosilicates and iron oxides in many Hawaii Andisols, a high proportion (25 – 30%) of soil As seemed to associate with either these mineral phases or with organic matter. Fractions that are water soluble or exchangeable, thus potentially bioavailable, contributed less than 1% of the total As. In contrast, soils having more crystalline minerals (e.g. Oxisols) often contain less As and also sorb less. Phosphate fertilization increases As bioaccessibility, whereas Fe(OH)₃ addition decreases it. Brake fern (*Pteris vittata* L.) could be used to remove some soil As. Concentration of As in fern fronds varied from approximately 40 mg/kg when grown on a low-As Oxisol to 800 mg/kg when grown on a high-As Andisol. The bioaccumulation ratio (plant As/soil As) apparently was rather constant at ~2:1 for this fern.

Key Words

Bioaccessible As, sorption, sequential extraction, phytoremediation.

Introduction

Arsenic (As) is a serious global environmental toxicant (Ravenscroft *et al.* 2009). Chronic exposure to As is known to cause adverse health effects in human, notably skin hyperpigmentation, lesions and hardening (keratosis), and to a lesser extent, cancer and neurological disorders (Ravenscroft *et al.* 2009). In Hawaii, the chemical control of weeds in the sugar industry began in 1913 when experiments with sodium arsenite (NaAsO₂) were undertaken on the island of Hawaii. From its first use until about 1945, NaAsO₂ dissolved in water was the primary herbicide used on sugarcane fields throughout the State. Although background levels of As in Hawaii soils are typically less than 20 mg/kg, significant elevated As levels have been identified at a number of locations. In fact, levels of 50 – 100 mg/kg are not uncommon throughout Hawaii. On former sugarcane lands, a broad region has been identified with total soil As as high as 900 mg/kg (Ortiz-Escobar *et al.* 2006). In soils, As is found in -3, 0, +3, and +5 oxidation states. Its prevalent forms are the inorganic species: arsenate (As[+5]) and arsenite (As [+3]). Arsenic may occur in methylated forms but these organic species are much less bio-toxic and rare in soils and surface waters (Smith *et al.* 1998). Soil As is distributed among different soil components, specifically metal oxides (Al, Fe, Mn oxides) and short-range ordered aluminosilicates (allophane, ferrihydrite, imogolite) (Violante *et al.* 2008). It is the form of chemical associations of As with various soil solid phases, rather than its total concentration, that affects its mobility, bioavailability and toxicity (Goh and Lim 2005). Sequential extraction is often used to provide operationally defined phase associations of As species. Such fractionations help identify some of the main binding sites and assess the potential for remobilization and bioavailability of As in polluted soils (Martin *et al.* 2007). Many plants do not absorb much As (approximately 1 – 10 mg/kg in leaves), but a few do (Schat *et al.* 2000). The Chinese brake fern (*Pteris vittata* L.) can accumulate between 1,440 and 7,500 mg As/kg in its fronds from some As-contaminated sandy soils of Florida (Ma *et al.* 2001). Arsenic may be taken up as arsenate by the phosphate transport system and subsequently interfering with P-related metabolic processes (Meharg *et al.* 1994; Miteva *et al.* 2005; Ravenscroft *et al.* 2009). Many former sugarcane lands, and some with high As, are being used for commercial agriculture, for residential housing and family gardening, for school and play ground building. Thus, good understanding of As reactions in Hawaii soils is urgently needed so As contaminated soils can be properly managed and remediated, if necessary. That was the objective of this study.

Methods

Survey of As levels in selected Hawaii soils

Using descriptions of soil map units published by the National Resources Conservation Services of America and geographic coordinations, we sampled about two dozen soil samples (series) at 1-10 cm from the surface throughout the Hawaiian Islands. Detailed sampling was performed on an Andisol and an Oxisol.

Chemical analysis of arsenic

Total As: a 1.00 g soil sample was shaken in 50 mL of (30% concentrated HNO₃ and 70% concentrated HClO₄) for 24 hours. Arsenic in the filtrate was measured with an inductively coupled plasma spectrometer (ICP). Bioaccessible As: a 1.00 g sample was shaken in 100 mL of HCl adjusted to pH 1.5 for 1 hour at 37 °C. Fractional As: four different extractants were used sequentially to extract soil As. (1) a 2.00 g sample was first shaken with 20 mL, 0.1 M CaCl₂ for 24 hours, then centrifuged, to obtain exchangeable fraction; (2) The solid was sonicated in 20 mL, 0.10 M sodium pyrophosphate then shaken for 24 hours, and centrifuged (organic matter-associated As); (3) step (2) was repeated, but with 20 mL, 0.30 M NH₄-oxalate, and shaken in the dark (amorphous Al and Fe oxides-associated As); (4) step (2) was repeated, but with 20 mL of 8 M HNO₃ (recalcitrant As).

Arsenic sorption/desorption and treatments with phosphate or Fe(OH)₃

Sorption isotherms of As were constructed by equilibrating 2.00 g soil in 20 mL of 10 mM CaCl₂ containing various concentrations of As (+5) as Na₂HAsO₄. The samples were shaken continuously for 7 days. Sorbed As was calculated as the difference between the initially added As and As remained in solution. Desorption was carried out by continuously extracting 2.00 g soil with 20 mL of 10 mM CaCl₂ for 10 times, equilibration time between each extraction was 1 hour. Effects of phosphate or Fe(OH)₃ on bioaccessible As were studied by mixing a high-As soil with different rates of Ca(H₂PO₄)₂ or amorphous Fe(OH)₃, and subjected the treated samples to two wetting (4 days)-drying (3 days) cycles before subsamples were taken for As analysis with ICP.

Arsenic uptake by brake fern (Pteris vittata L.) (phytoremediation)

A greenhouse experiment with 4 treatments: [control, 250 mg P/kg, 0.5% Fe as Fe(OH)₃, 0.5% compost] was established on a high-As Andisol (300 mg/kg total As) and a low-As Oxisol (18 mg/kg). The experiment had a completely randomized design with three replications in pots of 2 kg soils. One brake fern seedling (approximately 7.5 cm tall) was transplanted to each pot and grown for 6 months. Recently fully mature leaf samples were collected and analyzed for As every month starting on the 2nd month after transplanting.

Results

Total As concentration in some soils, sediments, and stream waters of Hawaii

Our limited data (Table 1) suggest the followings: (1) As levels in Hawaii soils are quite variable, ranging from 15 to 950 mg/kg. (2) Observed elevated As levels in some stream waters are likely related to As-impacted soils. (3) Andisols seem to contain more total As than the other soil orders, perhaps because of high As sorption capacity of amorphous Al and Fe oxides that are predominant in the Andisols, and where sugarcane used to be grown and arsenical herbicides applied.

Table 1. Total As levels in soils, sediments, and stream waters in Hawaii.

| Total As mg/kg | Soil Order | | | | | Sediment (3) | Water mg/L (4) |
|-------------------|-----------------------|-------------------|-----------------|---------------|-----------------|-----------------|----------------------|
| | Andisol (7 series) | Inceptisol (2) | Mollisol (1) | Oxisol (6) | Ultisol (11) | | |
| Range | 25-950 | 15-67 | 55 | 15-133 | 37-84 | 12-45 | 0.080-0.005 |
| Mean | 163 | 41 | 55 | 72 | 60 | 29 | 0.029 |

Arsenic fractionation and chemical reactions in Hawaii soils

Table 2 presents different As fractions in two mineralogically different soils of Hawaii. The Olaa Andisol has 10.0% organic carbon (OC), and is X-ray amorphous, whereas the Lihue Oxisol has 1.5% OC and over 50% kaolinite and crystalline Fe oxides (mainly hematite). These contrasting properties in part explain for the high total As (448 mg/kg) in the Andisol vs. 15.6 mg/kg in the Oxisol. The distribution patterns of As fractions in the two soils may also have serious environmental and health implications: The Andisol has only 0.5% total As as exchangeable, implying most of its As is not easily released to the solution nor become available to living organisms. On the other hand, nearly 25% of its As is associated with soil organic matter (SOM), suggesting that some As may be gradually released upon the oxidation of SOM. Another 30% of total As was associated with amorphous iron oxides (as extracted by ammonium oxalate) that may be subjected to redox conditions of the soil (Table 2).

Table 2. Arsenic fractions in an Andisol and an Oxisol of Hawaii.

| As fraction, mg/kg | Andisol (Olaa Series) | Oxisol (Lehue Series) |
|---|-----------------------|-----------------------|
| CaCl ₂ (exchangeable) | 2.4 (0.54%) | 2.77 (17.72%) |
| Na-Pyrophosphate (organic matter) | 110.4 (24.62%) | 1.34 (8.57%) |
| NH ₄ -Oxalate (amorphous Fe/Al oxides) | 132.7 (29.59%) | 2.18 (13.95%) |
| HNO ₃ (recalcitrant) | 202.9 (45.25%) | 9.34 (59.75%) |
| Total | 448.4 (100%) | 15.63 (100%) |

Desorption and sorption of As in Hawaii soils

Consistent with the different As fractions, As was desorbed (released) faster in the Oxisol (maximum desorption at cycle 3 or 4) than in the Andisol (maximum at cycle 5) (Figure 1). In general, it took at least 10 cycles to desorb soil As to levels below our detection (< 0.005 mg/L in solution or 0.05 mg/kg in soil). In contrast, the two soils can still sorb considerable amounts of As (Figure 1). For example, to maintain the 0.20 mg As/L in solution, it requires an addition of 220 mg As/kg to the Andisol and 150 mg/kg to the Oxisol (The action level of As in stream water in Hawaii is set at 0.19 mg/L). The sorption is much stronger (steeper slope) for the Andisol when As in the equilibrium solution is below 0.10 mg/L (Figure 1).

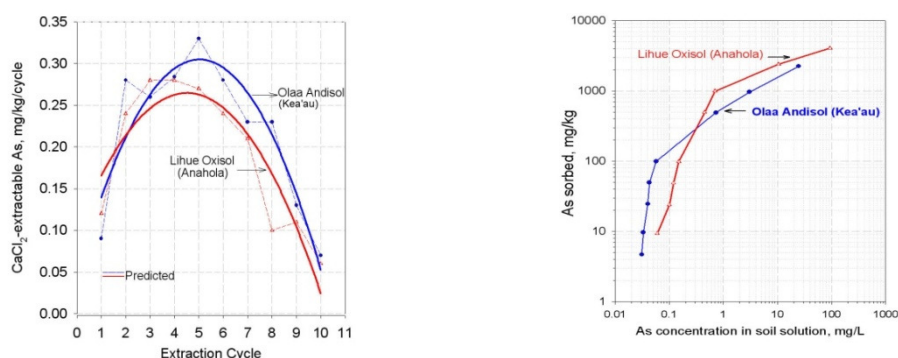


Figure 1. Desorption (left) and sorption of As in an Andisol and an Oxisol of Hawaii.

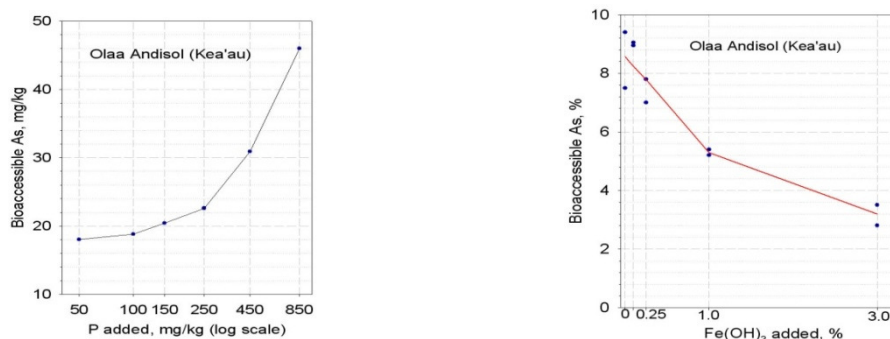


Figure 2. Effects of P fertilizer (left) and amorphous Fe(OH)₃ additions on HCl-extractable (bioaccessible) As in a high-As Andisol of Hawaii.

Effects of soil amendments on As bioaccessibility

Adding P fertilizer to the high-As Andisol releases significant amounts of As (Figure 2). For example, bioaccessible As increased from 18 to 31 mg/kg when 450 mg P/kg as Ca(H₂PO₄)₂ was added. On the other hand, adding colloidal Fe(OH)₃ decreases bioaccessible As markedly. For example, 1% Fe(OH)₃ reduced bioaccessible As from 9% of total As to 5% (Figure 2).

Phytoremediation of soil As with brake fern (*Pteris vittata* L.)

Brake ferns grown on the high As Andisol had between 500 and 1000 mg As/kg in their leaves. The above-ground biomass produced was between 30 and 70 g/pot dry weight for a 3-month growth; and the compost treatment provided the best growth whereas the Fe(OH)₃ treatment the least (Figure 3). However, As concentration in ferns grown on the low-As Oxisol was only 30-50 mg/kg, suggesting As uptake depends on soil As, or perhaps the ratio of As bioaccumulation (As concentration in leaf/As concentration in soil) is rather constant (at approximately 2.5:1 of plant As:soil As) for this fern.



Figure 3. Chinese brake fern (*Pteris vittata* L.) grown on an Andisol of Hawaii treated with $\text{Fe}(\text{OH})_3$, P fertilizer or compost (3 weeks after transplanting).

Conclusion

Chronic exposure to As often manifests as hyperpigmentation and hyperkeratosis, particularly in hands and feet. Skin and other types of cancer may follow. Past use of arsenical pesticides has resulted in elevated levels of As in many areas of Hawaii. High proportions of total soil As, however, are associated with iron oxides, organic matter, or are recalcitrant, thus not bioavailable in the short term. On the other hand, phosphate fertilization or compost application could release more As into the soil solution; and Chinese brake fern (*Pteris vittata* L.) could be used to extract soil As for remediation purpose.

References

- Goh KH, Lim TT (2005) Arsenic fractionation in a fine soil fraction and influence of various anions on its mobility in the subsurface environment. *Applied geochemistry* **2**, 229-239.
- Ma LQ, Komar KM, Tu C, Zhang WH, Cai Y, Kennelley E D (2001) A fern that hyperaccumulates arsenic: a hardly, versatile, fast-growing plant helps to remove arsenic from contaminated soils. *Nature* **409**, 579.
- Martin M, Violante A, and Barberis E (2007) Fate of arsenite and arsenate in flooded and not flooded soils of southwest Bangladesh irrigated with arsenic contaminated water. *Journal of Environmental Science and Health part A* **42**, 1775-1783.
- Meharg AA, Naylor J, Macnair MR (1994) Phosphorus nutrient of arsenate tolerant and non-tolerant phenotypes of velvetgrass. *Journal of Environmental Analysis* **23**, 234-238.
- Miteva E, Hristova D, Nenova V, Manava S (2005) Arsenic as a factor affecting virus infection in tomato plants: changes in plant growth, peroxidase activity and chloroplast pigments. *Scientia Horticulturae* **105**, 343-358.
- Ortiz-Escobar ME, Hue NV, Cutler WG (2006) Recent developments on arsenic: contamination and remediation. *Recent Research and Development in Bioenergy* **4**, 1-32 (Kerala: India).
- Ravenscroft P, Brammer H, Richards K (2009) Arsenic pollution: a global synthesis. (John Wiley & Sons, Inc. Massachusetts).
- Schat H, Llugany M, Bernhard R (2000) Metal-specific patterns of tolerance, uptake, and transport of heavy metals in hyperaccumulating and nonhyperaccumulating metallophytes. In 'Phytoremediation of contaminated soil and water' (Eds. N Terry, G Banuelos), pp. 171-188. (Lewis Publ.: Boca Raton, FL).
- Smith E, Naidu R, Alston AM (1998) Arsenic in the soil environment: a review. *Advances in agronomy* **64**, 149-195.
- Violante A, Gaudio SD, Pigma M, Pucci M, Amalfitano C (2008) Sorption and desorption of arsenic by soil minerals and soils in the presence of nutrients and organics. In: 'Soil mineral-microbe-organic interactions' (Eds X Huang, PM Huang, A Violante), pp. 39-69. (Springer-Verlag: Berlin).

Assessment of lead contamination in urban soils in an area of southern Italy

Ilaria Guagliardi^A, Gabriele Buttafuoco^B, Domenico Cicchella^A, Rosanna De Rosa^C

^ADipartimento di Studi Geologici ed Ambientali, Università degli Studi del Sannio, Benevento Italy, Email ilguagli@unisannio.it, cidom@unisannio.it

^BCNR - Institute for Agricultural and Forest Systems in the Mediterranean (ISAFOM), Rende (CS), Italy, Email gabriele.buttafuoco@cnr.it

^CDipartimento di Scienze della Terra, Università della Calabria, Ponte Bucci, 31-C, 87036 Rende (CS), Italy, Email derosa@unical.it.

Abstract

Lead concentration of urban soils in Cosenza-Rende territory (southern Italy) was investigated to assess and map contaminated areas. Samples were collected at 149 residual and non-residual topsoils (0.20 m) and were analysed for lead (Pb) using X-Ray Fluorescence Spectrometry method. Geostatistical methods were used to study spatial structure and risk assessment of lead concentration in urban soils. Results showed areas in which lead concentration values were higher than the Italian regulatory values regarding soil pollution with lead. These polluted areas were quite large and it is likely that they could create a significant health risk for human beings and vegetation in the near future. The results demonstrated that the proposed approach can be used to study urban soils contamination to produce geochemical maps, identify hot-spot areas and assess the probability of exceeding given regulatory values for soil lead concentration.

Key Words

Urban soils, lead in soils, soil pollution, geostatistics, stochastic simulation

Introduction

The influence of environmental geochemistry on human health is extremely important above all if some heavy metals, such as lead (Pb), appear to cause ill health through either deficiency or toxicity or both (Oliver 1997). Soil in urban environment has a direct influence on public health not related directly with production of food (Madrid *et al.* 2002). Urban soil acts as a sink for heavy metals and many other pollutants (Biasoli *et al.* 2005; Lee *et al.* 2005). Sources of heavy metals and other pollutants are related to human activities, such as vehicular emissions, industrial discharges and urban development (Wong, 2006). Among these sources, a vehicular emission is commonly known to be significant and increasing source of soil pollution in urban environment (Rossini and Fernández 2007). Many urban soils are contaminated with high concentrations of lead and exposure to it disrupts the development of the nervous system, causing delays in growth and learning disabilities. Geostatistical methods provide us a valuable tool to study spatial structure of lead concentration and mapping its spatial distribution. Stochastic simulation is a development of geostatistics and estimates the conditional cumulative distribution functions at each location. Statistical information deriving from stochastic simulation allows us to estimate the probability that each pixel exceeds a threshold value and to produce the probability map of high lead concentrations in urban soils. The main objective of this study was to explore the spatial structure and risk assessment of lead concentration in urban soils in Cosenza-Rende territory (southern Italy).

Methods

The study area (92 km²), characterized by high vehicular traffic load, was located in the Cosenza-Rende territory (southern Italy) (Figure 1). Soil samples (Figure 1) were collected from residual and non-residual topsoil (0.20 m) and from flower-beds in the urban areas at 149 georeferenced locations. The total number of samples was dictated by financial constraints. Fine earth fraction (< 2 mm) of soil samples was analyzed for lead by X-Ray Fluorescence spectrometry (XRF). Geostatistical methods (Matheron 1971) were used to explore the spatial structure and risk assessment (Deutsch and Journel, 1998) of lead in urban soils. Soil lead data were used to yield 500 alternative equi-probable images of the unknown soil lead concentrations using conditional sequential Gaussian simulation (SGS) (Deutsch and Journel 1998). Stochastic sequential Gaussian simulation estimates the conditional cumulative distribution functions (CDFs) at each location, using neighbouring sample data and neighbouring estimates obtained previously. Statistical information was extracted from the set of the simulated images: 1) averaging the values for each pixel and producing the map of the “expected” soil lead concentration at any node of the interpolation grid (E-type or Expected-value estimate) and 2) counting the number of times that each pixel exceeded the threshold value and converting the sum to a proportion in order to produce the probability map of exceeding the threshold.

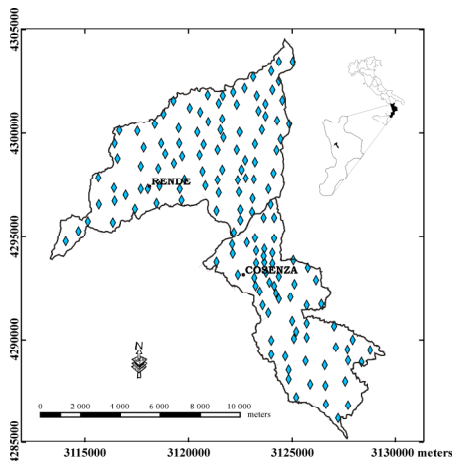


Figure 1. Study area and sample data location.

Results

Lead concentration data were characterized by a positively skewed distribution (Figure 2) with values 3 times higher than the local background.

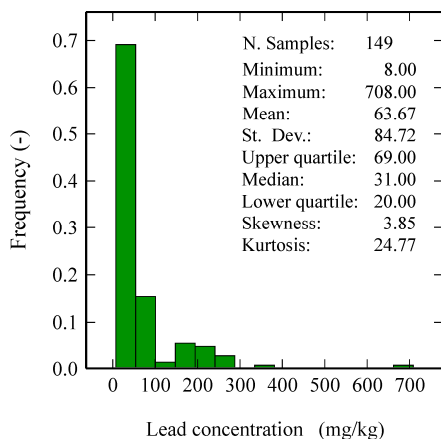


Figure 2. Histogram and basic statistics of lead concentration.

Conditional sequential Gaussian simulation is based on a multiGaussian model and it required a prior Gaussian transformation of the initial lead concentration data into a Gaussian-shaped variable with zero mean and unit variance. Such a procedure is known as Gaussian anamorphosis (Wackernagel, 2003) and the lead concentration values were transformed by using an expansion in terms broken to the first 30 Hermite polynomials. An isotropic variogram model was fitted to experimental semivariance values (Figure 3) because no anisotropy was observed in the variogram map (not shown). The model fitted (Figure 3) to the experimental variogram has three basic structures: (1) a nugget effect of 0.2079; (2) an exponential model with a practical range of 2000 m and a sill of 0.5453; (3) a spherical model with a range of 8000 m and a sill of 0.1790.

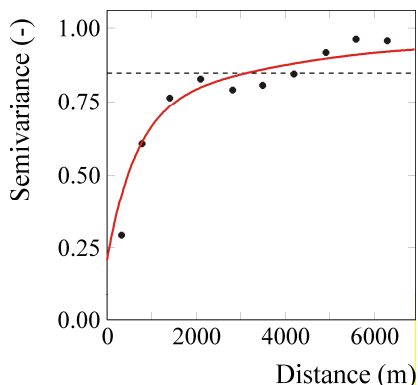


Figure 3. Variogram of lead concentration transformed to Gaussian values. The points are the experimental variances and the solid line is the fitted model.

Map of mean simulated lead concentration (Figure 4a) and probability map (Figure 4b) show a clear evidence of a strict correlation between location of emission sources, i.e. roads, (Figure 5) and the highest values of lead concentration.

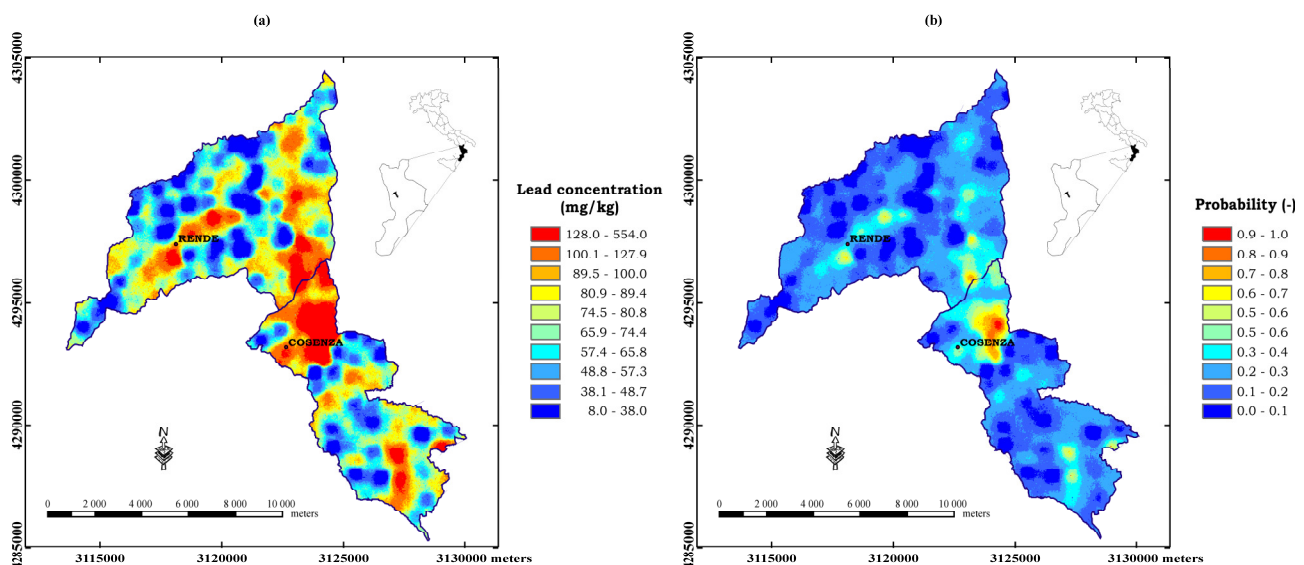


Figure 4. Maps of the mean simulated lead concentration (a) and of the probability of exceeding the threshold of 100 mg/kg

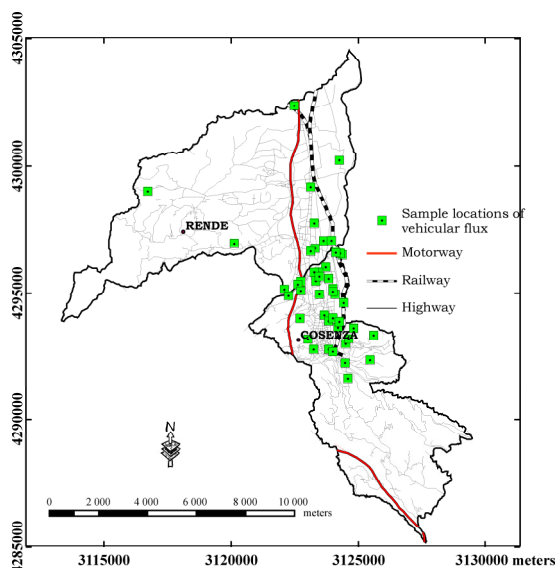


Figure 5. Road network.

The polluted areas were quite large and this is likely to create a significant health risk for human beings and vegetation in the near future.

Conclusion

The study showed that the proposed approach can be useful in contaminated urban soils from lead to produce geochemical maps, identify hot-spot areas and assess the probability of exceeding given regulatory values for soil lead concentration.

References

- Biasioli M, Barberis R, Ajmone-Marsan F (2006) The influence of a large city on some soil properties and metals content. *Science of the Total Environment* **356**, 154-164.
- Deutsch CV, Journel AG (1998) 'GSLIB: Geostatistical Software Library and User's Guide' (Oxford University Press: New York).
- Lee CSL, Li XD, Shi WZ, Cheung SC, Thornton I (2005) Metal contamination in urban, suburban and country park soils of Hong Kong: A study based on GIS and multivariate statistics. *Science of the Total Environment* **335**, 1-12.

- Environment* **356**, 45-61.
- Li X, Poon C, Liu PS (2001) Heavy metal contamination of urban soils and street dusts in Hong Kong. *Applied Geochemistry* **20**, 611-625.
- Madrid L, Díaz-Barrientos E, Madrid F (2002) Distribution of heavy metal contents of urban soils in parks of Seville. *Chemosphere* **49**, 1301-1308.
- Oliver MA (1997) Soil and human health. *European Journal of Soil Science* **48**, 573-592.
- Rossini Oliva S, Fernández Espinosa AJ (2007) Monitoring of heavy metals in topsoils, atmospheric particles and plant leaves to identify possible contamination sources. *Microchemical Journal* **86**, 131-139.
- Wackernagel H (2003) 'Multivariate Geostatistics: an introduction with Applications' (Springer-Verlag: Berlin).
- Wong CSC, Li XD, Thornton I (2006) Urban environmental geochemistry of trace metals. *Environmental Pollution* **142**, 1-16.

Background of some heavy metals on the Croatian Carst

Boris Vrbek^A, Ivan Pilas^B and Mirjana Vrbek^C

^ACroatian Forest Research Institute, Trnjanska 35, 10 000 Zagreb, Croatia, Email: borisv@sumins.hr

^BCroatian Forest Research Institute, Cvjetno naselje 41, 10 450 Jastrebarsko, Croatia, Email: ivanp@sumins.hr

^CCroatian Natural History Museum, Demetrova 1, 10 000 Zagreb, Croatia, Email: Mirjana.Vrbek@hpm.hr

Abstract

The aim of the research is to prove the influence of air pollution with heavy metals on the Carst area of Croatia. The following heavy metals were monitored: lead (Pb) copper (Cu), zinc (Zn) and cadmium (Cd). At 55 localities in the Croatian carst the organic layer (0-3 cm) and mineral soil of cambisols (5-25 cm) were collected and analyzed. On average 1 kg of soil was collected from each locality for analysis. On the mountain Velebit 4 samples from a deep pit-cave were also collected (Lukina jama-Trojama pit) as a reference point. During sampling maximum caution is required in order to avoid errors when taking 'natural' and pure sediment samples in "Lukina jama" pit. Research was undertaken in the carst forest ecosystem area in the Mediterranean region of Croatia. From the results obtained, it can be concluded that there is a higher level of lead, and especially cadmium, in the upper layers of the soil (0-3 cm), while in the mineral layer (5-25 cm) this level is somewhat lower. The amount of cadmium is close to the boundary value of 2,0 mg/kg. The mean value for lead in the upper layer is 43,1 mg Pb/kg while for the mineral area of the soil it is 29,8 mg Pb/kg, for cadmium the mean value in the upper layer is 1,7 mg Cd/kg while for the mineral layer it is 1,8 mg Cd/kg. The mean values for copper are 9,9 mg Cu/kg for the upper layer and 10,1 mg Cu/kg for the mineral layer of the soil; while for zinc they are 19,0 mg Zn/kg for the upper layer and 7,6 mg Zn/kg for the mineral layer of the soil. Copper and zinc can be found in low concentrations in the carst area of the Mediterranean region of Croatia. Samples from deep pit Lukina jama-Trojama (-1343m) have low concentration of heavy metals compared with the soil on the carst area.

Key Words

Heavy metals, Croatian carst, cambisol, "Lukina jama-Trojama" pit.

Introduction

The forest ecosystem in central Europe has been exposed to pollution for decades, some forest stands have even been exposed for more than a century. Gases such as SO₂ and NO_x not only pollute the leaves but also acidify the soil. At the same time, acid in the soil mobilizes heavy metals that have accumulated through atmospheric accumulation. Less mobile heavy metals such as lead and to a certain extent copper accumulate through a long period of time in the upper layers of the soil. Depending on the distance from the pollution's source heavy metals pollution can be measured in lower or higher concentrations. The way that any heavy metal moves within an ecosystem depends on the biogeochemical cycle. There are a number of ways of circulation between the atmosphere, hydrosphere, geosphere and biosphere. The transmission of heavy metals can be observed through atmospheric flows in the form of gases as well as sedimentation of dry and wet deposits in the forest ecosystem and soil.

Materials and methods

A soil pit was dug at each location in order to define the soil type. During the procedure only cambisols was taken (the brown soil on the limestone or calcocambisol). The external morphological profile of the soil was described and samples were taken at two depths, each weighing one kilogram, in plastic bags. They were then taken for physical and chemical analysis in the laboratory. The basic ecological characteristics of each locality were also described (altitude, exposure, incline, depth, etc.). For comparison samples were collected in four different parts of Lukina jama-Trojama pit, from bottom (-1392m) to Camp II (-928 m). The heavy metal content (lead, copper, zinc and cadmium) per mg/kg was analyzed. Heavy metals were determined by an atomic absorption spectrophotometer AAS Perkin Elmer 3001 S, using the extracting method with a 2N HCl (Brune-Ellinghaus 1981). This procedure separates the total amount of lead and cadmium, 75% of the copper and 30% of the zinc from the soil. Other soil analysis was undertaken using routine methods that are prescribed for analysing soil samples. Analysis was carried out in the laboratory of the Forestry Research Institute Jastrebarsko. The following was determined: The reaction of the soil upon applying glass electrodes in H₂O and M-KCl, the amount of carbonate, the amount of humus, particle size distribution and texture classification of the soil samples.

Results and discussion

Upon analysing the soil samples for the amount of heavy metals it was established that in certain areas of the carst there were higher amounts of lead in the upper layers of the soil. The largest amounts of lead were found near major roads, but also at high altitudes. According to data from literature, the values are varying for unpolluted soils. Nrigau (1978) states mean values of approximately 17 mg Pb/kg, Ure & Berrow (1982) states 29 mg Pb/kg. According to Reaves *et al* (1984) the humus layer has a mean value of 30 mg Pb/kg, while the mineral area of the soil has 13 mg Pb/kg. Zinc values are also variable (depending on the parent material). The natural content of lead is 10-35 mg/kg of soil, zinc 70-90 mg/kg of soil while copper is 20-30 mg/kg soil (Smith 1990). However, according to Kabata-Pendias *et al* (1992) they are between 10 and 300 mg Zn/kg soil with a mean value of approximately 50 mg Zn/kg. For copper and cadmium, according to Alloway (1995) mean values for carst soils (lime) are around 40 mg Cu/kg, while for cadmium they are 0,53 mg Cd/kg. In sediment samples taken in cave systems and deep carst pits that are not under the influence of dry and wet deposits, it was determined that values in the mineral area for lead were 9-13 mg Pb/kg, copper 10-22 mg Cu/kg, zinc 22-29 mg Zn/kg and cadmium 0,10-0,80 mg Cd/kg. Research was undertaken in the carst forest ecosystem area in the Mediterranean region of Croatia. Cambisol was monitored while other soil types such as calci-molic leptosol, rendzic leptosol and chromic-cambisol were omitted. Cambisol covers 10.53% of the total forest soils that are present in Croatia. Analysis results taken at 55 localities of cambisol and 4 samples from Lukina jama-Trojama pit. In Table 1 and Table 2 the mean values of heavy metals as well as the minimum and maximum values are reported. One can conclude from the results obtained that a higher level of lead and especially cadmium are present in the upper layers of soil, at depths of 0-3 cm, while in the mineral area at depths of 5-25 cm there is a slightly higher level of lead while cadmium is still close to boundary value of 2,0 mg/kg.

Table 1. Some chemical and physical properties of soil samples and heavy metal content

| Sample Deep (in cm) | Cambisol samples | pH in | | CaCO ₃ % | Humus g/kg | Clay % | Heavy metals (mg/kg) | | | |
|---------------------------|---------------------|------------------|-------|------------------------|---------------|-----------|----------------------|------|------|-----|
| | | H ₂ O | M-KCl | | | | Pb | Cu | Zn | Cd |
| 0-3 humus layer | Min. | 5.2 | 3.9 | 0.0 | 49.7 | 20.0 | 23 | 4 | 4 | 0.4 |
| | Max. | 7.7 | 7.4 | 32.99 | 639.5 | 43.3 | 86 | 36 | 78 | 4.0 |
| | n | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 30 |
| | Mean | 6.9 | 6.2 | 2.60 | 181.1 | 32.7 | 43.1 | 9.9 | 19.0 | 1.7 |
| 5-25 mineral layer | Min. | 5.7 | 4.6 | 0.0 | 31.3 | 26.9 | 15 | 5 | 4 | 0.4 |
| | Max. | 8.0 | 7.5 | 35.54 | 126.5 | 70.1 | 56 | 16 | 17 | 3.7 |
| | n | 53 | 53 | 53 | 53 | 53 | 53 | 53 | 53 | 40 |
| | Mean | 7.4 | 6.9 | 3.59 | 73.3 | 39.9 | 29.8 | 10.1 | 7.6 | 1.8 |

Table 2. Some chemical and physical properties of pit samples and heavy metal content

| Sample Deep (in cm) | Pit samples | pH in | | CaCO ₃ % | Humus g/kg | Clay % | Heavy metals (mg/kg) | | | |
|----------------------------|----------------|------------------|-------|------------------------|---------------|-----------|----------------------|------|------|-----|
| | | H ₂ O | M-KCl | | | | Pb | Cu | Zn | Cd |
| 5-25 | Min. | 8.3 | 7.1 | 22.3 | 5.9 | 7.9 | 9 | 10 | 22 | 0.1 |
| Lukina jama- Trojama | Max. | 9.0 | 7.9 | 49.9 | 18.3 | 48.9 | 16 | 22 | 31 | 0.8 |
| | n | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | Mean | 8.7 | 7.6 | 31.9 | 18.1 | 21.1 | 12.3 | 16.5 | 26.8 | 0.6 |

Conclusion

According to the content of heavy metals in samples taken from 55 localities, it can be concluded that there is a higher content of lead, and especially cadmium, in the Mediterranean area of Croatia with a parent material of lime. The mean value for lead for the upper layer is 43,1 mg Pb/kg, for the mineral area of the soil 29,8 mg Pb/kg, and while for cadmium the mean value in the upper layer is 1,7 mg Cd/kg in the mineral layer Cd concentrations was 1,8 mg Cd/kg on average. According to data from literature these are boundary values for the content of cadmium in soils. In the light of this analysis, the soil is likely to be contaminated with cadmium. Mean values for copper are 9,9 mg Cu/kg in the upper layer and 10,1 mg Cu/kg in the mineral layer, while for zinc they are from 19,0 mg Zn/kg for the upper layer and 7,6 mg Zn/kg for the mineral layer of the soil. Copper and zinc are present in low concentrations in the carst area of the Mediterranean region of Croatia. The results show the various values of lead, copper, zinc and cadmium content, depending on the structure and position of samples in the carst area. According to the content of heavy metals in the samples collected from Lukina jama-Trojama pit on the "Veletit" National Park, it can be concluded that the sediments in the speleological objects have natural content of heavy metals in

comparison to the soil samples taken from the humus horizon and mineral part of soil in the carst area under the limestone parent material.

References:

Alloway BJ (1995) Heavy Metals in Soils. Blackie Academic and Professional, London. pp.123-305.

Brune HR Ellinghaus (1981) Schwermetallgehalte in hessischen Böden. *Landw. Forschung. Kongressband Trier*. **38**, 338-349.

Kabata-Pendias A, Pendias H (1992) Soil and Plants, 2nd Edition Lewis Publ. Inc. Boca Raton, Florida, USA.

Reaves GA, Berrow ML (1984) *Geoderma* **32**, 1-8.

Smith WH (1990) 'Air pollution and forest'. Second edition. (Springer Verlag: New York).

Nrigau JO (1978) Biogeochemistry of Lead. Elsevier Biomedical Press Amsterdam. pp.18-88.

Ure AM, Berrow ML (1982) *Environmental Chemistry* **2**, 94-204.

Cadmium adsorption behavior of some soils from northern of Iran

Maryam Esfandbod^A, Akbar Forghani^B, Ebrahim Adhami^C and Mehran Rezaei Rashti^D

^A Soil Science Department of, Guilan University, Rasht, Iran, Email maryam.esfandbod@gmail.com

^B Faculty of Soil Science, Guilan University, Rasht, Iran, Email

^C Soil Science Department, College of Agriculture, Yasouj University, Yasouj, Iran, Email eadhami@mail.yu.ac.ir

^D Department of Soil Science, Guilan University, Rasht, Iran, Email mehran_325@yahoo.com

Abstract

Cadmium adsorption behavior (Cd) of soils is an important process which exerts a major influence on its uptake by plant roots. Cadmium (Cd) sorption characteristics of 20 surface soils (0-30 cm) with a wide range of soil physicochemical properties, was investigated. Two grams of each sample were equilibrated for 24h at laboratory temperature ($25 \pm 2^\circ\text{C}$) with 40ml, 0.01M CaCl_2 containing 5 to 500 mg Cd/L. Langmuir, Freundlich and Temkin adsorption isotherms were used to evaluate the trend of Cd adsorption. The data of Cd adsorption was well fitted to Langmuir and Freundlich adsorption isotherms. However, the fit to Langmuir adsorption model was closer than that of Freundlich. Multiple stepwise regression analysis indicated that, the adsorption capacity of the soils for Cd increased with an increase in pH, CEC and calcium carbonate equivalent (CCE).

Key Words

Adsorption, Cadmium, Freundlich, Isotherm, Langmuir, Temkin.

Introduction

Cadmium (Cd) contamination imposes an adverse effect on environmental quality and constitutes a serious threat not only to plants and animals but also to human lives (Martin-Garin *et al.* 2002). Cadmium adsorption and desorption by soils is one of the factors that control its bioavailability and transfer in the environment (Singh and Nayyar 1993). Langmuir (Ramachandran and Souza 1999; Singh and Nayyar 1993), Freundlich (Ramachandran and Souza 1999), and Temkin (Maftoun *et al.* 2004) equations have been commonly used to assess the pattern of heavy metals adsorption by soils. The objectives of the present study were to (1) evaluate the pattern of Cd adsorption of some soils from Guilan province, and (2) to determine the effect of soil properties on Cd adsorption.

Methods

Twenty surface soil samples (0-30 cm) varying widely in physicochemical properties (Table 1) from Guilan province in northern part of Iran, were used in the present study. 2g subsample of each soil was placed in centrifuge tubes and 40ml of 0.01M CaCl_2 containing different concentrations of Cd (ranging from 5 to 500 mg Cd/L) was added and shaken for 1h at room temperature and allowed to equilibrate at $25 \pm 2^\circ\text{C}$ for 24h. The suspensions were then shaken for additional 30 min. Samples were centrifuged for 10 min and the supernatant was analysed for Cd by atomic absorption spectrophotometry. The amount of Cd adsorbed was calculated as the difference between initial and final Cd concentrations. The adsorption data were fitted to Freundlich, Langmuir, and Temkin adsorption isotherms. The stepwise multiple regression procedure was used to study the relationship between the Cd sorption coefficients and the physicochemical properties of the soils.

Table 1. Range of physicochemical characteristics of the soils.

| | Clay (%) | pH | OM (%) | CCE (%) | CEC (cmol _c /kg) |
|------|----------|------|--------|---------|-----------------------------|
| Min. | 8.00 | 4.21 | 1.53 | 1.59 | 14.18 |
| Max. | 52.00 | 7.61 | 9.77 | 16.51 | 58.46 |
| Ave. | 31.92 | 6.58 | 4.99 | 7.37 | 37.20 |

Equations

$$\text{Freundlich} \quad \log X = \log k_F + n_F \log C \quad (1)$$

$$\text{Langmuir} \quad C / X = [1 / (K_L b_L)] + (1 / b_L) C \quad (2)$$

In Langmuir equation, (X / C) is termed the distribution coefficient (K_d) (Bolt and Bruggenwert 1976):

$$X / C = K b = K_d \quad (3)$$

$$\text{Temkin} \quad X = k_{T1} + k_{T2} \ln C \quad (4)$$

Where X is the amount of Cd sorbed per unit weight of soil (mg/kg soil), C is Cd concentration in equilibrium solution (mg/L), b_L is Cd adsorption maxima (mg/kg soil), K_L is a coefficient related to Cd bonding energy (L/mg), K_d is Cd distribution coefficient (L/kg soil), k_F is Cd adsorption capacity (mg/kg soil), n_F is rate of Cd adsorption, k_{T1} and k_{T2} are Cd adsorption coefficients.

Results

Langmuir and Freundlich isotherms were well fitted to the adsorption data (Table 2).

Table 2. Freundlich and Langmuir adsorption isotherm parameters and coefficients of determination (r^2).

| | Freundlich Eq. | | | Langmuir Eq. | | | |
|------|-------------------|--------|-------|-------------------|-------|-------|--------|
| | r^2 | k_F | n_F | r^2 | K_L | b_L | K_d |
| Min. | 0.82 ^a | 29.7 | 0.22 | 0.86 ^a | 0.01 | 1105 | 17.10 |
| Max. | 0.98 ^a | 1072.9 | 0.72 | 1.00 ^a | 0.18 | 5000 | 702.10 |
| Ave. | 0.92 ^a | 504.5 | 0.41 | 0.97 ^a | 0.07 | 3506 | 236.60 |

^a Significant at $p \leq 0.001$

k_F value ranged from 29.7 to 1072. The K_F value indicates the quantity of adsorption when the equilibrium concentration is 1 mg/L (Kariman and Cox, 1978). Multiple linear regression analysis between K_F and soil properties (Eq. (5) and Eq.(6)) indicated that 68 percent of K_F variation could be explained by cation exchange capacity (CEC) and pH. As it can be clearly seen from these equations, adsorbed Cd increased linearly with increase in pH and CEC. However these properties showed significant negative correlation with n_F coefficient, similar result has been reported by Ramachandran and Souza (1999).

$$k_F = -1658.47 + 328.97 \text{ pH} \quad r^2 = 0.60 \quad p \leq 0.001 \quad (5)$$

$$k_F = -2163.45 + 342.58 \text{ pH} + 11.17 \text{ CEC} \quad r^2 = 0.68 \quad p \leq 0.001 \quad (6)$$

Clay and OM surfaces are the main sorbent of Cd; however, the nonsignificant correlation found between k_F with clay and OM might be due to the fact that the adsorption of Cd by tested soils could be more affected by the kind of clay and OM than the amount of clay and OM.

K_L coefficient was correlated with some soil properties. Multiple stepwise regression analysis showed that the simple regression equation could adequately describe such relations:

$$K_L = 0.001 + 0.009 \text{ CCE} \quad r^2 = 0.63 \quad p \leq 0.001 \quad (7)$$

$$K_L = -0.21 + 0.04 \text{ pH} \quad r^2 = 0.56 \quad p \leq 0.001 \quad (8)$$

$$K_L = -0.123 + 0.006 \text{ CCE} + 0.022 \text{ pH} \quad r^2 = 0.71 \quad p \leq 0.001 \quad (9)$$

These findings are similar to those reported by Maftoun *et al.* (2004) and Ramachandran and Souza (1999).

In the present study, based on soil pH, and initial Cd concentration, the Cavallaro and McBride solubility diagram (Cavallaro and McBride 1978) indicates the possibility of solid phase Octavite (CdCO_3) precipitation in some soils. The calculated Langmuir b_L , ranged from 1105 to 5000 (Table 2). The relatively high b_L values in the present study revealed that the soil removed a large amount of Cd from solution.

Stepwise regression analysis between b_L and soil properties showed the following relationships:

$$b_L = 1133.05 + 63.80 \text{ CEC} \quad r^2 = 0.48 \quad p \leq 0.001 \quad (10)$$

$$b_L = -2482.02 + 68.79 \text{ CEC} + 521.62 \text{ pH} \quad r^2 = 0.75 \quad p \leq 0.001 \quad (11)$$

which, indicates that CEC and pH are the main soil properties controlling maximum Cd adsorption in the studied soils.

The distribution coefficient, K_d shows the ratio of the Cd concentration in the soil to the concentration of Cd^{+2} in the solution phase. K_d values varied greatly between soils, ranging from 17.10 to 702.10 (L/kg soil) and showed a highly significant correlation with CCE ($r^2 = 0.63$, $p \leq 0.001$) and pH ($r^2 = 0.52$, $p \leq 0.001$). These results clearly demonstrate that pH and, to a greater extent CCE, contribute to the variation of K_d in the soils studied (Eq. (12)). A portion of the removal of Cd from the solution phase may have been a result of precipitation of CdCO_3 . Similar results have been reported by Yin *et al.* (2002) and Sauve *et al.* (2000).

$$K_d = -435.31 + 23.03 \text{ CCE} + 76.38 \text{ pH} \quad r^2 = 0.70 \quad p \leq 0.001 \quad (12)$$

Conclusion

From results reported herein, it is concluded that the adsorption of Cd increased with increasing solution Cd concentration and the soils varied in Cd sorption capacity. The results showed that CEC and pH were the most important soil properties affecting Cd adsorption in these soils. Alkaline soils favoured high adsorption of Cd and possibly precipitation as CdCO₃, which may lead to less bioavailability of Cd; whereas, acid soils adsorbed less Cd, and more Cd was present in solution, which may result in high availability. Although, the Cd adsorption data showed a highly significant fit to Freundlich and Langmuir isotherms, Comparison of coefficients of determination of Langmuir and Freundlich equation demonstrated that Langmuir adsorption isotherm has a better fit than Freundlich.

References

- Bolt GH, Bruggenwert MGM (1976) Soil chemistry. In 'Basic elements-Part A. (Elsevier Scientific Publishing Co: Amsterdam. the Netherlands)
- Cavallaro N, McBride MB (1978) Copper and cadmium adsorption characteristics of selected acid and calcareous soils. *Soil Sci. Soc. Am. J.* **42**, 550-556.
- Karimian N, Cox FR (1978) Adsorption and extractability of molybdenum in relation to some chemical properties of soil. *Soil Sci. Soc. Am. J.* **42**, 757-761.
- Maftoun M, Rassooli F, Ali-Nejad Z, Karimian N (2004) Cadmium sorption behavior in some highly calcareous soils of Iran. *Commun. Soil Sci. Plant Anal* **35**, 1271-1282.
- Martin-Garin A, Gaudet JP, Charlet L, Vitart X (2002) A dynamic study of the sorption and the transport processes of cadmium in calcareous sandy soils. *Waste Manage* **22**, 201-207.
- Ramachandran V, Souza TJD (1999) Adsorption of cadmium by Indian soils. *Water Air Soil Pollut.* **111**, 225-234.
- Sauve S, Hendershot W, Allen HE (2000) Solid-solution partitioning of metals in contaminated soils: Dependence on pH, total metal burden and organic matter. *Environ. Sci. Technol* **34**, 1125-1131.
- Singh SP, Nayyar VK (1993) Adsorption of cadmium on alkaline and calcareous soils. *J. Indian Soc. Soil Sci* **41**, 270-273.
- Sukreeyapongse O, Holm PE, Strobel BW, Panichsakpatana S, Magid J, Hansen HCB (2002) pH-dependent release of cadmium, copper and lead from natural and sludge-amended soils. *J. Environ. Qual* **31**, 1901-1909.
- Yin Y, Impellitteri CA, You SJ, Allen HE (2002) The importance of organic matter distribution and extract soil: Solution ratio on the desorption of heavy metals from soils. *Sci. Total Environ* **287**, 107-119.

Can be reference values of heavy metals useful as soil quality standards? Contributions from assays in representative Mediterranean agricultural soils

Luis Recatalá^{A,C}, Juan Sánchez^A, Carmen Dolores Arbelo^B and Daniel Sacristán^A

^ADepartamento de Planificación territorial, Centro de Investigaciones sobre Desertificación-CIDE (CSIC-Universitat de València-Generalitat Valenciana), Camí de la Marjal s/n, 46470 Albal (Valencia) España.

^BDepartamento de Edafología y Geología, Facultad de Biología, Universidad de La Laguna, 38206 La Laguna (Tenerife), Islas Canarias, España.

^CCorresponding author. Email luis.recatala@uv.es

Abstract

This paper presents the results of the research project “Assays of reference concentrations of heavy metals (Cd, Cu, Pb and Zn) in representative agricultural soils of the Mediterranean region under controlled horticultural crops”. This project aimed to determine the maximum concentration for Cd, Cu, Pb and Zn related to the reference values for these metals in representative soils of horticultural areas of the Mediterranean region, which do not exceed the maximum concentration established by the legislation for these metals in crops for human consumption. This concentration has been determined for each soil after several assays using reference concentrations of these metals in controlled cultures of lettuce, which represents an important component of the Mediterranean diet and furthermore it is a metal accumulator crop. Reference concentrations are multiples of the reference value established for each metal in horticultural areas of the Valencian Region, a representative area of the European Mediterranean Region. Representative soils were selected each having different properties relevant to regulating the behaviour of heavy metals in soil. The results obtained suggest that the usefulness of criteria based on soil quality standards to define contaminated soils in Mediterranean soils contaminated by heavy metals can vary depending on soil properties.

Key Words

Trace elements, toxicity, root test, biomass test.

Introduction

Heavy metals are toxic elements for the human health when specific concentrations for each element are exceeded. Particularly, cadmium (Cd) and lead (Pb) are non-essential elements for life and highly dangerous, since they cause serious dysfunctions in organisms, including humans. On the other hand, copper (Cu) and zinc (Zn) are essential elements for organisms but can be dangerous if high concentrations in agricultural soils are reached. These elements have generally an anthropic origin, due to the excessive use of agrochemical products (e.g. Cu and Pb), to the wastewater irrigation with an urban-industrial origin (e.g. Cd, and Pb) and to the atmospheric depositions from several emissions (e.g. Cd and Pb). These processes are affecting soils under vegetable crops in the Mediterranean Region (Peris *et al.* 2007), and therefore further research on these metals is needed in this region, where only few studies on heavy metal in soils at a regional level have been carried out (Micó *et al.* 2006). The intake of contaminated food is the main way for introducing these elements to the food web. In this context, the current legislation establishes the maximum concentration of heavy metals allowed in different types of food (including vegetable crops). Specifically, the Regulation EC n°466/2001 of the European Commission fixes the maximum content of Cd and Pb in some crops for human consumption (DOCE 2001). Cu and Zn are not included in this Regulation. However, other international legislations, like the Australian regulation, establish the maximum concentration of Cu and Zn in some vegetable crops (e.g. McLaughlin *et al.* 2000) and, therefore, it can be expected their incorporation in the European regulations in the near future.

It is widely recognized that heavy metal levels in crops are influenced by a number of factors such as soil properties and the type of crop. Leaf crops have a high accumulation capacity of heavy metals in their leaves according to different authors (e.g. Ramos *et al.* 2002). The determination of the maximum concentrations of Cd, Cu, Pb and Zn in different representative Mediterranean agricultural soils, that does not cause the maximum limit allowed in lettuces (*Lactuca sativa L.*) established by the current international legislation, would be of great interest since leaf crops represent an important percentage of the crops cultivated and consumed in the Mediterranean region. Exceeding this concentration in soils would lead to the declaration of such soils as contaminated.

The Spanish Royal Decree 9/2005 (BOE 2005) establishes criteria to declare contaminated soils such as toxicity tests, risk analysis and the comparison of contaminant concentrations in soils with reference values. According to this Royal Decree, a soil will be considered “contaminated” when heavy metal concentrations in soils exceed 100-times the reference values established for them. In this context, this paper presents the framework, methodology and results of the research project “Assays of reference concentrations of heavy metals (Cd, Cu, Pb and Zn) in representative agricultural soils of the Mediterranean region under controlled horticultural crops”. This project aimed to determine the maximum concentrations of Cd, Cu, Pb and Zn related to the reference value for each metal in agricultural soils representative of the Mediterranean Region that do not cause the overcoming of the maximum limit for lettuces established by the international legislation. The assay of different doses has enabled to determine these maximum concentrations. The different doses were established taking into account the reference levels for Cd, Cu, Pb and Zn in agricultural soils under vegetable crops of the Valencia Region, a representative area of the European Mediterranean Region, situated in the east of Spain.

Material and methods

Soil samples were collected from the surface horizons (25 cm depth topsoil, approximately) of four agricultural soils (Rojales, Sollana, Nules and Peñíscola) representative of the Mediterranean region (Recatalá *et al.* 2002, Recatalá *et al.* 2004). All the samples were transported to the laboratory where they were air dried at ambient temperature and sieved to pass a 2-mm mesh sieve. Selected soils properties relevant to control the mobility and bioavailability of heavy metals (Adriano 2001) were analyzed according to the official laboratory methods of the Spanish Ministry of Agriculture, Fisheries and Food (MAPA 1994). Soil pH was measured in a 1:2.5 soil:water suspension and electrical conductivity was determined in the saturation paste extract. Organic matter content was determined by the Walkley-Black method and the total carbonate content by the Bernard calcimeter. Particle-size distribution was determined by the Robinson Pipette method to determine the sand, silt and clay percentages. Cation exchange capacity was determined by saturation with sodium acetate solution (pH=8.2), displacement of the absorbed sodium with ammonium acetate (pH=7.0) and determination of displaced sodium by flame atomic absorption spectrometry (FAAS).

Biomass assays based on OECD test 208 (OECD 2006) were performed to assess the toxicity of the different heavy metals considered on the biomass production of lettuce crops (*Lactuca sativa* L.). Heavy metals were added to soils by spraying appropriate volumes of a solution (e.g. CdCl₂, CuCl₂, Pb(NO₃)₂, ZnSO₄) diluted with deionised water. This procedure ensured an even distribution of the metal solution in the soil while maintaining its pore structure. The soils were contaminated to obtain a range of six concentrations, the control and five doses that were multiple concentrations of reference values established for horticultural soils in the Valencian Mediterranean region (Sánchez *et al.* 2004). For each metal, the doses were established after considering the results of a rangefinder test. For instance, in the case of Cu, the five doses were concentrations representing 1, 10, 25, 50 and 100-times, respectively, the reference value (65.9 mg Cu kg⁻¹) for this metal. After contamination, each sample was completely mixed in a plastic bag by hand. Control samples were similarly treated using deionised water only. All treatments were equilibrated for seven days before using in the mentioned assays. The crops for each biomass assay were dried in an oven (60 °C) and sieved to 0.5 mm for analysis of heavy metal contents. Heavy metal contents in crops were determined using the USEPA 3052 method for organic matrices (USEPA 1996). This method was selected because it provided satisfactory results in relation to recovery and precision data (Micó 2005; Peris 2005).

Results and discussion

Soil properties of the four soils used in the assays are shown in Table 1. Soil pH was between 7.8 and 8.5, organic matter between 1.6 and 9.7%, clay content between 20 and 41% and carbonate content between 36 and 52%. The results of the assays show that soil properties greatly influenced metal toxicity in the crop. For instance, the effective concentration of added Cd causing 50% inhibition (EC₅₀) on biomass production increased with the calcium carbonate content of soils, being 839 mg/kg for Rojales, 169 mg/kg for Sollana, 285 mg/kg for Nules and 658 mg/kg for Peñíscola (Recatalá *et al.* 2009a). This means that Cd toxicity in the crop was higher in the soils having less carbonate content because of calcium carbonate has a high affinity for Cd and retains it by adsorption and/or precipitation (which predominates at higher Cd concentrations) reactions (e.g. Papadopoulus and Rowell 1986) Other soil properties (high salinity and a coarse texture) facilitate the Cd availability and mobility in soil. In saline soils, the formation of ion chloride complexes can facilitate the transfer of Cd from soil to plant (e.g. Khoshgoftar *et al.* 2004). Soils having a coarse texture have a low metal sorption capacity (e.g. Hooda and Alloway 1998).

Table 1. Selected properties of the soils used in the assays.

| Soil | pH | EC _{se} dS/m | OM % | CaCO ₃ % | CEC cmol(+)/kg | Sand % | Silt % | Clay % | Texture |
|-----------|-----|--------------------------|---------|------------------------|-------------------|-----------|-----------|-----------|-----------------|
| Rojales | 7.8 | 17.9 | 1.6 | 50 | 10.1 | 31 | 44 | 25 | Loam |
| Sollana | 8.5 | 1.5 | 3.5 | 36 | 19.6 | 28 | 37 | 35 | Clay loam |
| Nules | 8.2 | 2.2 | 9.7 | 37 | 31.0 | 16 | 43 | 41 | Silty clay |
| Peñíscola | 8.2 | 1.1 | 2.1 | 52 | 9.9 | 56 | 24 | 20 | Sandy clay loam |

EC_{se}, electrical conductivity; OM, organic matter content; CEC, cation exchange capacity

In addition, it should be highlighted that for all soils the EC₅₀ was greater than 100-times the reference value for Cd (0.74 mg/kg). However, for all the soils, from the second dose, which represents 10-times the reference value for Cd, the crop had Cd contents (Table 2) that exceeded the maximum levels established by the Commission Regulation no. 466/2001 (DOCE 2001). Although Rojales and Peñíscola are the most carbonated soils the lettuces grown in these soils accumulated more Cd than those ones grown in Sollana and Nules due to the effect of salinity and a coarse texture, respectively. Similar results were obtained for Cu (Recatalá *et al.* 2009b), Pb and Zn (Recatalá 2010). However, for these metals the EC₅₀ increased with the organic content of soils as soil organic matter has a high affinity for these metals. Lettuces grown in Rojales and Peñíscola also accumulated more Cu, Pb and Zn than those grown in Sollana and Nules because of salinity and texture. As for Cd, for all soils the Pb contents in the crop exceeded the maximum level from the dose equivalent to 10-times the reference value for Pb. For Rojales, Sollana and Peñíscola, the Cu contents in the crop exceeded the maximum level from the dose equivalent to 25-times the reference value for Cu whereas for Nules from the dose equivalent to 50-times. In the case of Zn, for all soils the Zn contents in the crop exceeded the maximum level after the dose equivalent to 100-times the reference value for Zn.

Table 2. Cd content in lettuce crops for the different doses added to soils.

| Doses Cd (mg/kg) added to soils | Cd content in crop (mg/kg) | | | |
|---------------------------------|----------------------------|---------|-------|-----------|
| | Rojales | Sollana | Nules | Peñíscola |
| 1 (control) | <0.5 | 0.5 | <0.5 | 1 |
| 2 (7.4 mg/kg) | 47 | 26 | 14 | 32 |
| 3 (74 mg/kg) | 77 | 66 | 66 | 65 |
| 4 (740 mg/kg) | 206 | 147 | 158 | 715 |
| 5 (1480 mg/kg) | 390 | 342 | 318 | -- |
| 6 (2220 mg/kg) | -- | 500 | 517 | -- |

-- = no biomass produced.

It can be, therefore, concluded that the criteria established by the Spanish Royal Decree 9/2005 (BOE 2005) to declare a contaminated soil when the concentration of a metal is higher than 100-times the reference value is not valid for the metals studied neither from the point of view of the growth of the crop nor from the point of view of the toxicity due to the accumulation of the metal in the crop that can affect human health through the food web. Giving that reference values are also proposed in other parts of the European Mediterranean region (e.g. Pérez *et al.* 2002) the results achieved in this study can be relevant to proposed criteria to declare contaminated soils in this region.

Acknowledgements

The authors thank the Spanish Ministry of Science and Innovation for funding the research project CGL-2006-07250/BTE.

References

- Adriano DC (2001) 'Trace Elements in Terrestrial Environments. Biochemistry, Bioavailability and Risks of Metals'. (Springer-Verlag: New York).
- BOE (2005) 'Real Decreto 9/2005, de 14 de enero, por el que se establece la Relación de Actividades Potencialmente Contaminantes del Suelo y los Criterios y Estándares para la Declaración de Suelos Contaminados'. (Boletín Oficial del Estado BOE nº 15 de 18 de enero de 2005: Madrid).
- DOCE (2001) 'Reglamento CE nº466/2001 de la Comisión de 8 de marzo de 2001 por el que se fija el contenido máximo de determinados contaminantes en productos alimenticios'. (Diario Oficial de las Comunidades Europeas: Bruselas).
- Hooda PS, Alloway BJ (1998) Cadmium and lead sorption behaviour of selected English and Indian soils. *Geoderma* **84**, 121-134.

- Khoshgofar AH, Shariatmadari H, Karimian N, Kalbasi M, Van der Zee, SEATM, Parker DR (2004) Salinity and zinc application effects on phytoavailability of cadmium and zinc. *Soil Science Society of American Journal* **68**, 1885-1889.
- MAPA (1994). 'Métodos oficiales de análisis de suelos y aguas para riegos. Tomo III'. (Servicio de Publicaciones del Ministerio de Agricultura, Pesca y Alimentación: Madrid).
- McLaughlin MJ, Hamon RE, McLaren RG, Speir TW, Rogers SL (2000) Review: A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. *Australian Journal of Agricultural Research* **38**, 1037-1086.
- Micó C (2005) 'Estudio de metales pesados en suelos con cultivos hortícolas de la provincia de Alicante'. PhD thesis, Universitat de València. (Servei de Publicacions de la Universitat de València: Valencia).
- Micó C, Recatalá L, Peris M, Sánchez J (2006) Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis. *Chemosphere* **65**, 863-872
- OECD (2006). 'OECD Guidelines for the Testing of Chemicals. Section 2: Effects on Biotic Systems Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test'. (Organisation for Economic Cooperation and Development: Paris),
- Papadopoulos P, Rowell DL (1986) The reactions of cadmium with calcium carbonate surfaces. *European Journal of Soil Science* **39**, 23-36.
- Pérez, C.; Martínez, M.J.; Vidal, J.; Navarro, C. (2002). Proposed reference values for heavy metals in Calcaric Fluvisols of the Huerta de Murcia (SE Spain). In 'Proceedings of the International Symposium on Sustainable Use and Management of Soils in Arid and Semiarid Regions' (Eds A Faz, R Ortiz, AR Mermut) pp. 495-496. Cartagena, Murcia, Spain.
- Peris M (2005) 'Estudio de metales pesados en suelos bajo cultivos hortícolas de la provincia de Castellón'. PhD thesis, Universitat de València. (Servei de Publicacions de la Universitat de València: Valencia).
- Peris M, Micó C, Recatalá L, Sánchez R, Sánchez J (2007) Heavy metal contents in horticultural crops of a representative area of the European Mediterranean region. *Science of the Total Environment* **378**, 42-48.
- Ramos I, Esteban E, Lucena J J, Garate A (2002) Cadmium uptake and subcellular distribution in plants of *Lactuca sp.* Cd-Mn interaction. *Plant Science* **162**, 761-767.
- Recatalá L (2010) Ensayo de concentraciones de referencia para los metales pesados (Cd, Cu, Pb y Zn) en suelos agrícolas mediterráneos representativos bajo cultivos hortícolas controlados (CORESMED). Informe Final. CIDE-Centro de Investigaciones sobre Desertificación (CSIC, Universitat de València, Generalitat Valenciana). Valencia, Spain .
- Recatalá L, Micó C, Peris M, Sáez E, Sánchez J (2002) Analysis of Heavy Metals in Mediterranean Soils (Alicante, Spain) for Soil Quality Policy Making. In 'Proceedings of the International Symposium on Sustainable Use and Management of Soils in Arid and Semiarid Regions' (Eds A Faz, R Ortiz, AR Mermut) pp. 318-320. Cartagena, Murcia, Spain.
- Recatalá L, Peris M, Micó C, Sánchez R, Sánchez J (2004) Analysis of Heavy Metals in Agricultural Soils (Castellón, Spain). In 'Proceedings of the Fourth International Conference on Land Degradation' (Eds A Faz, R Ortiz, G García), pp. 325-326. Cartagena, Murcia, Spain.
- Recatalá L, Arbelo CD, Sánchez J (2009a) Transferencia de cadmio de suelo a planta en ensayos controlados de concentraciones de referencia en suelos agrícolas Mediterráneos representativos. In 'Actas del IV Simposio Nacional sobre Control de la degradación de los Suelos y Cambio Global'. (Eds J Sánchez Díaz, S Asins Velis) pp.127-129. Valencia, Spain.
- Recatalá L, Arbelo CD, Sánchez J (2009b) Determinación de cobre en planta en ensayos controlados de concentraciones de referencia en suelos agrícolas Mediterráneos representativos. In 'Actas del IV Simposio Nacional sobre Control de la degradación de los suelos y cambio global'. (Eds J Sánchez Díaz, S Asins Velis) pp.189-191. Valencia, Spain.
- Sánchez J, Recatalá L, Micó C, Peris M (2004) Contenido de metales pesados en suelos agrícolas y su influencia en cultivos hortícolas. Informe Final. CIDE-Centro de Investigaciones sobre Desertificación (CSIC, Universitat de València, Generalitat Valenciana). Valencia, Spain.
- USEPA (1996) Method 3052. Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices. Revision 0. U.S. Environmental Protection Agency (USEPA), Washington D.C.

Characteristics of soil heavy metal contents in the agricultural areas near closed mine in Korea

Byong-Gu Ko^A, Seong-Jin Park^A, Gu-Bok Jung^B, Min-Kyeong Kim^A, Gun-Yeob Kim^A, Suk-Young Hong^A and Deog-Bae Lee^A

^A National Academy of Agricultural Science (NAAS), RDA, Suwon 441-707, Rep. of Korea, Emails bgko@korea.kr, archha98@korea.kr, kimmk72@korea.kr, gykim@rda.go.kr, syhong67@korea.kr, leedb419@korea.kr

^B Extension Service Bureau, RDA, Suwon 441-707, Rep. of Korea, Email gbjung@korea.kr

Abstract

There is an increasing concern over heavy metal contamination of soil in agricultural areas. This study was carried out for monitoring the status and long term trend of heavy metal contents in agricultural fields near closed metal mines on a regular basis. Six hundred soil samples were collected in 58 paddy fields near mine sites nationwide in 2000, 2004 and 2008, respectively. Six heavy metals including cadmium (Cd), copper (Cu), lead (Pb), arsenic (As), zinc (Zn) and nickel (Ni) were analysed and the data were used for further statistical data analysis such as correlation analysis. Average concentrations of cadmium, copper, and lead in the soil samples were 0.4, 13.3 and 14.0 mg/kg, respectively. It indicated that the concentration of the heavy metals in the soil were higher in the paddy fields located at closed mine areas than that in the conventional paddy fields. There were 120 sampling points which exceeded the threshold level (TL) and 57 points which showed the corrective action level (CL) according to the Soil Environment Conservation Act enacted by Ministry of Environment (ME) out of 600 sampling points in 2000. The heavy metal concentrations of cadmium, copper, lead, and arsenic have gradually decreased intervals of every four years since the monitoring started in 2000. The highest correlations ($R^2=0.79^{**}$) exist between zinc and cadmium concentrations and the regression model estimating zinc from cadmium exhibited high R^2 value of 0.69^{**}.

Key Words

Paddy fields, soil heavy metals, mine, monitoring, data analysis.

Introduction

Research on soil heavy metals, which are known harmful and residual substances affecting food safety have been becoming popular in recent years. Some mine tailings have been left without proper management in closed metal mines and have become the source of heavy metal contamination in adjacent agricultural soils and crops (Jung *et al.* 2005). Exchangeable cadmium in the soil correlates to cadmium content increments in polished rice (Kim *et al.* 2008). We have monitored soil heavy metals and chemical properties in agricultural fields as well as in the vulnerable agricultural paddy fields such as the wastewater flowing area, closed metal mines area, an industrial complex area, and around of highway on a regular basis since 1999 as a nationwide agri-environmental project carried by Rural Development Administration (RDA), Korea to grasp the spatial and temporal changes over the country. In this study, we analysed soil heavy metal data collected in 2000, 2004, and 2008 from vulnerable agricultural paddy fields which were near closed metal mines to understand the characteristic heavy metal contents of vulnerable paddy fields in terms of concentration levels and correlations.

Methods

Soil sampling

In the agricultural paddy fields near closed metal mines, 600 soil samples of a nationwide paddy field surveying of 58 sites were collected at fixed sites in 2000, 2004 and 2008.

Chemical analysis

The heavy metal contents were analysed by using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer) after 0.1N-HCl extraction, and arsenic was analysed by the above same method after 1N-HCl extraction (Guide book of ME 2002). Zinc and nickel were extracted using a microwave oven (Mars-x, CEM Co. Ltd., USA) according to EPA 3051 method (US Environmental Protection Agency 1996).

Statistical Analysis

The data were analysed for correlation between heavy metal concentrations in the soil samples using the statistical software, SPSS 12.0 (SPSS Inc.; Chicago).

Results

The distribution of the 58 sites for nationwide monitoring of soil heavy metals in agricultural fields located at near closed metal mines every four years since 2000 are shown in Figure 1. The 600 sampling points and the chemical data were established as a spatial database for spatial and temporal analysis.

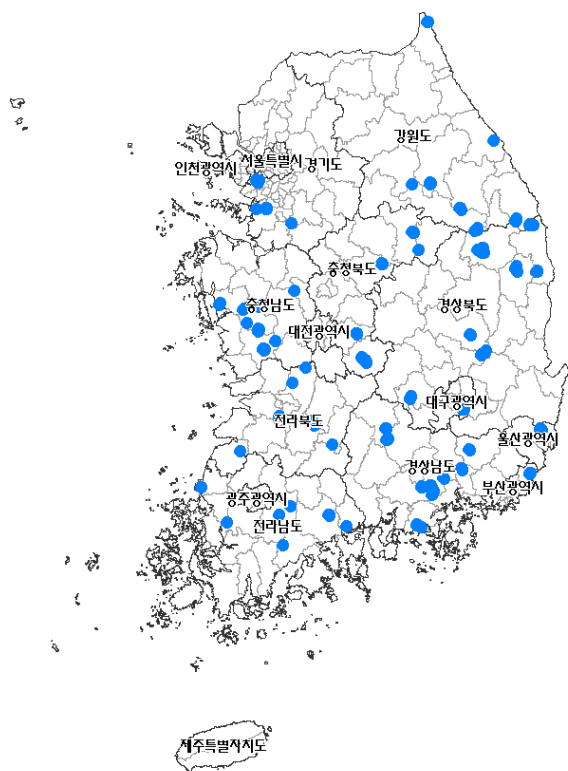


Figure 1. Nationwide monitoring sites and sampling points for soil heavy metals in agricultural fields located at near closed metal mines.

Mean value and the range of soil heavy metal concentrations in paddy field samples collected in 2000, 2004, and 2008 near closed mines as well as the threshold level (TL) and the corrective action level (CL) are shown in Table 1. Some of heavy metal concentrations from soil samples which were collected in the paddy fields near the closed mine are above TL and CL for 2000, 2004, and 2008 (Table 1). Mean value of cadmium, copper, and lead in the soil samples were 0.4, 13.3 and 14.0 mg/kg, respectively. Concentrations of the heavy metals in the soil samples were remarkably higher than those in conventional farmland investigated by our research group in 2007(Kim *et al.* 2007).

Table 1. Mean value and the range of soil heavy metal concentrations in paddy fields near closed mines. (unit : mg/kg)

| Year | Cd | Cu | Pb | As | Zn | Ni |
|--|-------------------|---------------------|--------------------|------------------|----------------------|---------------------|
| 2000 | 0.6 (0.01~8.6) | 17.9 (0.1~305.8) | 22.6 (tr~557.5) | 3.7 (tr~62.0) | - | - |
| 2004 | 0.46 (tr~5.6) | 13.9 (tr~292.0) | 14.8 (tr~402.9) | 1.9 (tr~43.4) | - | - |
| 2008 | 0.4 (tr~7.7) | 13.3 (tr~284.0) | 14.0 (tr~329.7) | 1.9 (tr~29.5) | 132.4 (17.4~1548) | 22.0 (18.4~1157) |
| Mean of conventional paddy fields ^x | 0.1 | 3.3 | 4.8 | 0.02 | - | - |
| Threshold level (TL) | 1.5 | 50 | 100 | 6 | 300 | 40 |
| Corrective action level(CL) | 4 | 125 | 300 | 15 | 700 | 100 |

x : Average concentrations of heavy metal in paddy fields in Korea (Kim *et al.* 2007)

The number of points which exceeded TL and CL are shown in Table 2. Analyses of Ni and Zn were only conducted in 2008. There were 120 sampling points which exceeded TL and 57 points which showed CL according to the Soil Environment Conservation Act enacted by Ministry of Environment (ME) out of 600 sampling points in 2000 (Table 2). The concentrations of cadmium, copper, lead, and arsenic have gradually decreased for an interval of every four year since monitoring started in 2000. Our institute, Rural Development Administration(RDA), has suggested soil remediation for the sites around closed mines which

showed the TL and CL to Ministry for Food, Agriculture, Forestry and Fisheries (MFAFF) of Korea. MFAFF conducted soil remediation for those sites.

Table 2. The number of exceeding points on the threshold level (TL) and the corrective action level (CL).

| Year | Cd | Cu | Pb | As | Zn | Ni | Total |
|------|-----------------------------------|---------|--------|---------|--------|--------|-----------------------|
| 2000 | 54 ^x (7 ^y) | 38 (13) | 25 (3) | 61 (40) | - | - | 120 (57) ^z |
| 2004 | 31 (1) | 26 (10) | 13 (1) | 26 (10) | - | - | 74 (20) |
| 2008 | 17 (2) | 19 (11) | 11 (1) | 20 (14) | 26 (9) | 33 (3) | 100 (39) |

x, y: Number of exceeding points on TL and CL, respectively. z : Some of soil samples were observed more than two metals exceeded on TL and CL per sample.

Table 3 is the result of correlation analysis among heavy metals collected in paddy fields near closed mines. The highest correlation was observed between zinc and cadmium concentrations ($R^2=0.788^{**}$), the next highest correlation was found between zinc and arsenic ($R^2=0.409^{**}$). Figure 2 shows a linear relationship between zinc and cadmium concentrations collected in paddy fields near closed mine in 2008. The regression model for estimating zinc content from cadmium content has a R^2 value of 0.69^{**}.

Table 3. Correlation analysis among heavy metal concentrations for soils collected in paddy fields near closed mine.

| Heavy metal | Cd | Cu | Pb | Zn | As | Ni |
|-------------|---------------------|---------------------|-------|---------------------|--------------------|-------|
| Cd | 1.000 | | | | | |
| Cu | 0.286 ^{**} | 1.000 | | | | |
| Pb | 0.143 [*] | 0.266 ^{**} | 1.000 | | | |
| Zn | 0.788 ^{**} | NS | NS | 1.000 | | |
| As | 0.358 ^{**} | NS | NS | 0.409 ^{**} | 1.000 | |
| Ni | NS | NS | NS | NS | 0.150 [*] | 1.000 |

^{*}, ^{**} Significant at the 0.05, 0.01 probability level

NS : Not Significant

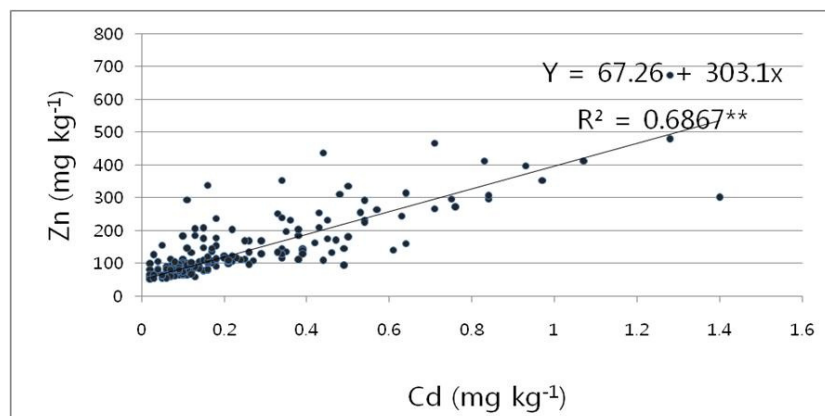


Figure 2. A linear relationship between zinc and cadmium concentrations for soils collected in paddy field near closed mines in 2008.

Conclusion

The result shows that the heavy metal concentrations were higher for the closed mine areas than for conventional paddy field, and some of samples exceed the TL and CL according to the Soil Environment Conservation Act. The highest correlation ($R^2=0.79^{**}$) was between zinc and cadmium concentrations and the regression model estimating zinc from cadmium exhibited the high R^2 value of 0.69^{**}. During the experimental period, soil remediation of the farmlands around closed mine areas has been conducted by the Korea Ministry for Food, Agriculture, Forestry and Fisheries (MFAFF). Critical decreasing of the heavy metal concentration was due to the remediation as shown in the above results. However, several reports indicate that farmlands around closed mines are still affected by the heavy metals exceeding the TL and CL based on the prescription of the Soil Environment Conservation Act in Korea. Continuous monitoring related to heavy metal flows in the soil-water-plant system are done by our research group for maintaining of food safety.

References

- Kim WI (2008) Long-term monitoring of heavy metal contents in paddy soils. *J. Kor. Soil Sci. Fert.* **41**, 190-198.
- Kim KT, Kang MA (2008) Mathematical Approach for Environmental Impact Analysis of Soils from Abandoned Mines. *J. Eng. Geo.* **18**, 297-302.
- Kim MS (2007) 'Survey on the change of chemical properties of Agricultural Fields in Korea'.
- KOlfhoff IM, Elving PJ (1961) Treatise on Analytical Chemistry Part 2. 3, *Interscience* **171**,
- Ministry of Environment (2003) 'Soil Environmental Conservation Act'.
- US EPA (1996) Microwave assisted acid dissolution of sediments, sludges, soils and oils (Method 3051A).

Characterization of deposited flue-dust slurry from a former Pb-smelter site and release of inorganic contaminants

Thilo Rennert^A, Stephan Kaufhold^B, Matthias Händel^A and Kai U. Totsche^A

^AInstitut für Geowissenschaften, Friedrich-Schiller-Universität Jena, Burgweg 11, 07749 Jena, Germany, Email thilo.rennert@uni-jena.de

^BBundesanstalt für Geowissenschaften und Rohstoffe, Stilleweg 2, 30655 Hannover, Germany

Abstract

As a by-product of Cu smelting, a flue dust originally enriched in metal sulphides evolved. The dust was deposited as slurry in unsealed heaps and is characterized by very high contents of toxic metals such as Pb and Zn. We studied a Technosol profile in the Mansfeld region, Germany, developed from deposited flue-dust slurry by means of chemical, mineralogical, and microscopic characterization (SEM-EDX, XRD, FTIR, DTA-MS), by column experiments on contaminant release under water-saturated conditions, and by geochemical modelling. The flue-dust slurry contained large amounts of ash particles, and quartz, Fe oxides, silicates, sulphates and sulphides (in varying states of weathering) were identified. In both open-flow and closed-flow column experiments, high metal concentrations were determined (e.g., $c_{\max}(\text{Zn}) = 87 \text{ mg/l}$). The concentration patterns in both experimental column approaches indicated that the transport scale approximated the reaction scale and that contaminant release was near equilibrium. Geochemical modelling indicated the dissolution of sulphates and precipitation of secondary carbonates that prevented the complete dissolution of sulphates, which would have led to even higher aqueous concentrations. The combination of intensive material characterization, adequate column experiments and modelling allowed for a quantitative and qualitative estimation of contaminant release.

Key Words

soil contamination, column experiment, modelling, equilibrium, rate limitation

Introduction

Until 1990, for more than 800 years, a metalliferous Permian marine black shale was mined and smelted in the Mansfeld region south-east of the Harz Mountains, central Germany. The shale is a bituminous rock mainly consisting of quartz, calcite, dolomite, feldspars and further aluminosilicates, but also of sulphide ore minerals such as chalcopyrite (CuFeS_2), bornite (Cu_5FeS_4), galena (PbS), sphalerite and wurtzite (ZnS) (Knitzschke 1966). During smelting the shale for Cu production, fine particles evolved as flue dust. The purified flue dust was used as feed stock for a Pb smelter after partial smouldering. After closure of the Pb smelter in 1978, the flue-dust slurry was deposited in unsealed heaps. Deposited flue-dust slurry is characterized by high contents of potentially toxic metals such as Cd (0.4 g/kg), Cu (12 g/kg), Pb (140 g/kg), Zn (180 g/kg) as well as S and C species (S_{tot} 160 g/kg; C_{tot} 110 g/kg) (Weiss *et al.* 1997). Heavy metals in the flue-dust slurry were originally present as sulphides, but they were oxidized when exposed to the atmosphere to the respective sulphates (e.g., anglesite, PbSO_4). Flue-dust slurry heaps have been identified as the main contaminant source in local sediments and in lakes (Schubert *et al.* 2003, 2008). Oxidation of sulphide minerals in the uppermost layers of a flue-dust slurry deposit proceeded quickly leading to the increased aqueous concentrations of Cd, Cu, Pb as well as Zn, and mobilization of these metals was explained by higher solubility of metal sulphates compared to the respective sulphides (Schubert *et al.* 2003). Therefore, the aim of this study was i) to intensively characterize a Technosol developed from flue-dust slurry by means of chemical, mineralogical, and microscopic methods, ii) to estimate the contaminant release from the substrates by using column experiments, iii) to identify the phases that control the aqueous contaminant concentrations, and iv) to integrate the characterization results and the release studies with geochemical modelling.

Methods

Characterization

We took three samples from a Technosol profile on the former Pb-smelter site in Hettstedt (Mansfeld region, Germany) in an open pit during a remediation campaign. The samples #1 (from 0-50 cm depth) and #2 (from 51-90 cm depth) originated from deposited flue-dust slurry, whereas #3 (depth > 91 cm) was taken from the underlying sediment (quaternary glacio-fluviatile and fluviatile sand). The samples were intensively

characterized by means of chemical composition (total metal contents by digestion and X-ray fluorescence (XRF), C, S, pH), X-ray diffractometry (XRD), Fourier-transform infrared (FTIR) spectroscopy, differential thermal analysis-mass spectrometry (DTA-MS), and scanning electron microscopy (SEM) with energy-dispersive X-ray (EDX) detection.

Release experiments and modelling

We carried out column experiments at water-saturated flow ($q = 0.01$ cm/min with de-ionized water as eluent) with columns made of polymethylmethacrylate (length 10 cm, inner diameter 4 cm). The eluates were collected with a fraction collector at an interval of 0.17 to 2.2 pore volumes exchanged. The flow was interrupted for 4 times ranging from 4 h to 21.5 d. The transport regime was characterized by fitting Cl^- breakthrough data from tracer experiments to the advection-dispersion equation. Additionally, we carried out closed-flow column experiments in duplicate with #1 and #2. Here, the solution leaving the column after the passage of the sample was pumped back into the water-storage vessel so that a closed loop was established. The solution in the vessel was sampled after 3, 7, and 10 d.

We used Visual MINTEQ 2.51 to evaluate mineral phases that possibly control the aqueous contaminant concentrations. Prior to the computational runs, we defined systems that considered the aqueous concentrations of Ca, Cd, Cu, Pb, Zn, and SO_4^{2-} after the first flow interruption in the open-flow experiments or at the end of the closed-flow experiments. Furthermore, we defined sulphates of the metals mentioned before (gypsum, CdSO_4 , CuSO_4 , anglesite, ZnSO_4) as well as CaCO_3 as finite phases.

Results

Characterization

Due to their different origins, the samples from the flue-dust slurry largely differed from the underlying sediment. The flue-dust slurry samples were brownish black (10 YR 2/2 (#1) and 10 YR 2/1 (#2)), whereas #3 was light yellow (10 YR 7/4). The flue-dust slurry samples revealed very high contents of As, Cd, Cu, Pb, and Zn (Table 1). In contrast, the underlying layer was less contaminated as the percentage of contaminants analyzed in #3 related to the upper most #1 was low (As, 1.2%; Cd, 6%; Cu, 0.4%; Pb, 0.6%; Zn 2.5%). According to SEM-EDX analyses, in both #1 and #2 quartz, feldspars, (Cu, Pb, Zn) sulphides (in varying states of weathering), sulphates, Fe oxides (e.g., hematite) and Fe silicates were identified. The samples also contained ash particles, which were enriched in Ca, S, and O as well as in Pb, Cu, Fe, Zn, and Co, and remnants of wood and plants. Gypsum crystals were identified in #1, whereas we assume gypsum present in #2 as particles $< 1 \mu\text{m}$ or accumulated at the rims of ash particles. The FTIR spectra of both flue-dust slurry samples were characterized by bands mainly attributed to quartz, gypsum and aliphatic species. The X-ray diffractograms of these samples revealed quartz as the dominant mineral, and gypsum, anglesite (PbSO_4) and possibly smithsonite (ZnCO_3) as further minerals. Our XRD results largely differed from those previously reported on flue-dust slurry from a deposit in this region (Weiss *et al.* 1997). They noticed that the most prominent minerals were sulphides (wurtzite, sphalerite, galena) that summed up to 43.9%, but also anglesite resulting from sulphide oxidation was detected (6.3%). As shown by DTA-MS analyses, the content of sulphide S in our flue-dust samples was in the range 0.1 to 1 g kg^{-1} confirming that the vast majority of sulphides initially present have been oxidized to sulphates.

Table 1. Chemical characterization of a Technosol developed from flue-dust slurry on a Pb-smelter site.

| | #1 | | #2 | | #3 ^b |
|------------------|------------------|-------------------|------|------|-----------------|
| | (g/kg) | | | | (mg/kg) |
| SiO ₂ | 430 ^a | nd ^{b,c} | 420 | nd | nd |
| Fe | 76.9 | 77.1 | 55.9 | 63.8 | 21,590 |
| As | 1.1 | 1.3 | 1.2 | 1.3 | 16 |
| Ca | 28.6 | 25.5 | 21.4 | 24.7 | 34,150 |
| Cd | nd | 0.1 | nd | 0.1 | 6 |
| Cu | 8.1 | 8.2 | 6.2 | 6.5 | 31 |
| Pb | 21.3 | 18.3 | 22.9 | 19.4 | 116 |
| Zn | 24.3 | 24.0 | 26.2 | 27.1 | 613 |
| organic C | 149 | | 165 | | 3,600 |
| inorganic C | 8 | | 16 | | 10,200 |
| total S | 21.3 | | 20.3 | | 1,400 |

^a determined by XRF in the first data column for #1 and #2; ^b determined by sample digestion in the second data column for #1 and #2, and generally for #3; ^c not determined

Column experiments

The transport regime was dominated by advection, and we did not identify preferential water flow. In the open-flow experiments, the aqueous concentrations of ions under study (except for Cu) resembled similar patterns as exemplified in Figure 1 for #1. The patterns were very similar for #2 in both experimental approaches (data not shown). Concentrations of toxic metals such as Pb and Zn were on a very high level. The flow interruptions had only minor impact on the concentrations indicating that the transport scale was near the reaction scale of the process inducing the release. In the case of Ca and SO_4^{2-} , the molar concentration ratio varied between 0.98 and 1.13 indicating (near-) equilibrium dissolution of gypsum. In contrast to Cd, Pb, and Zn, the concentrations of Cu distinctly increased after the flow interruption indicating rate-limited Cu release, whereas that of the other metals was near equilibrium. The rapid decrease of concentrations in the course of the experiments was caused by the continuous removal of dissolved ions and replacement with de-ionized water.

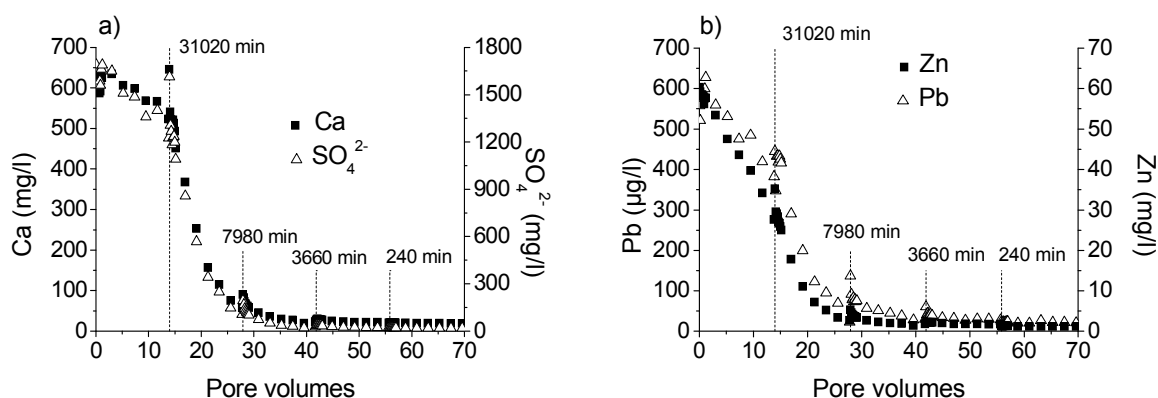


Figure 1. Concentrations of selected substances in the eluates of the open-flow experiment with a Technosol (sample #1): a) Ca, SO_4^{2-} ; b) Pb, Zn. Vertical lines indicate the point in time and the duration of a flow interruption.

The closed-flow experiments (Figure 2) confirmed the results of the open-flow experiments both quantitatively and qualitatively. The increasing concentrations (except for Cu) that converged to a constant level again indicated that equilibrium between the liquid and the solid phase had established which could be explained by the dissolution of mineral phases. However, the concentration patterns for Cu indicated slow retention of Cu initially mobilized.

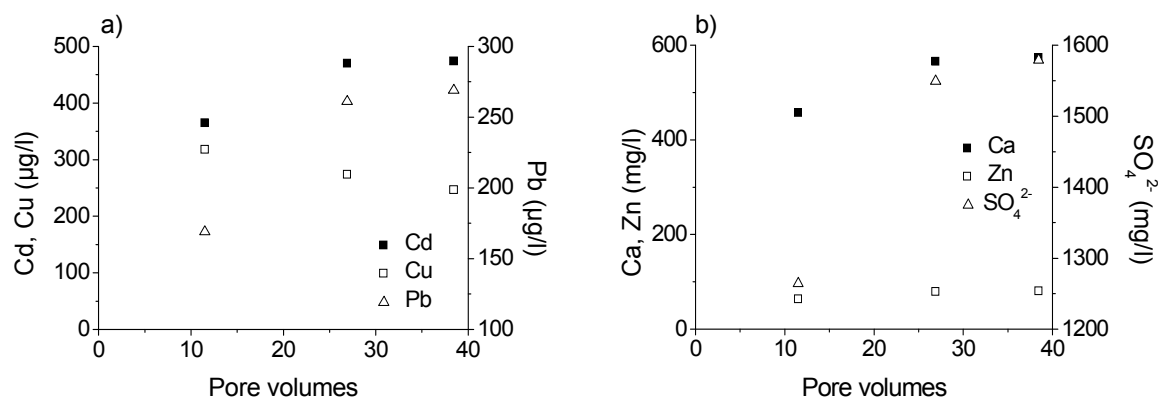


Figure 2. Concentrations of selected substances in the closed-flow experiments with a Technosol (sample #1): a) Cd, Cu, Pb; b) Ca, SO_4^{2-} , Zn.

The proposed dissolution of mineral phases was checked by model calculations. When estimating aqueous concentrations under the assumption of dissolution of sulphates, these modelled concentrations were very similar to those measured except for Cu (Table 2). The measured pHs ranged from 6.7 to 7.5, and the modelled pHs based on the mass balance were only 0.4 to 0.6 lower than the measured ones. In all calculations, the dissolution of gypsum was at equilibrium, and the saturation indices (SI) of the sulphates of Cd, Cu, Pb and Zn, which we defined as finite phases, were < 0 so that these minerals dissolved.

Table 2. Measured (mea.) and modelled (mod.) concentrations of selected elements and sulphate. Measured concentrations refer to those after the first flow interruption in the open-flow experiments and to those at the end of the closed-flow experiments.

| Experiment | Ca | | Cd | | Cu | | Pb | | SO ₄ ²⁻ | | Zn | |
|--------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------------------------------|------|------|------|
| | mea. | mod. | mea. | mod. | mea. | mod. | mea. | mod. | mea. | mod. | mea. | mod. |
| #1 OF ^a | 646.0 | 791.8 | 0.360 | 0.652 | 0.160 | 0.502 | 0.440 | 0.258 | 1614 | 1537 | 35.2 | 22.8 |
| #1 CF ^b | 563.5 | 747.9 | 0.470 | 0.619 | 0.241 | 0.431 | 0.443 | 0.238 | 1579 | 1563 | 79.3 | 21.4 |
| #2 OF | 441.0 | 797.4 | 0.246 | 0.661 | 0.182 | 0.422 | 0.390 | 0.241 | 1487 | 1603 | 25.1 | 23.3 |
| #2 CF | 562.5 | 763.9 | 0.484 | 0.637 | 0.149 | 0.398 | 0.311 | 0.232 | 1586 | 1647 | 82.7 | 22.3 |

^a open-flow experiment; ^b closed-flow experiment

However, the dissolution of sulphates was not complete, because carbonates precipitated as indicated by their SI > 0. According to the calculations, these carbonates include cerussite (PbCO₃), otavite (CdCO₃), amorphous ZnCO₃, and smithsonite, the latter of which we assume present in the samples as deduced from the XRD results. Thus, the modelling strongly indicated an important inhibitory effect of carbonates on the release of toxic metals from deposited flue-dust slurry. For verification, we carried out the same modelling without considering CaCO₃. Then, the pH would drop to < 4.5 and the metal concentrations were largely overestimated in all calculations (e.g., Pb by a factor of 10, Zn by a factor > 400). Therefore, our approach resulting in the formation of carbonates following the dissolution of sulphates was capable of reproducing measured concentrations in column experiments adequately. Similar to our modelling results, initially Zn-Cu-Al hydroxides precipitated at a discharge point of another heap of flue-dust slurry and Cu slag in the Mansfeld region that subsequently transformed into a Zn-hydroxide carbonate (Schubert *et al.* 2005).

Conclusion

Deposited flue-dust slurry poses an environmental threat, because initially present sulphides are oxidized to more soluble sulphates as a result of weathering under atmospheric conditions. Therefore, water that passes soils contaminated with such substrates is loaded with dissolved toxic metals. However, complete dissolution of metal sulphates is inhibited by precipitation of secondary carbonates as derived from model calculations that coincide with aqueous concentrations in column experiments.

References

- Knitzschke G (1966) Zur Erzmineralisation, Petrographie, Hauptmetall- und Spurenelementführung des Kupferschiefers im SE-Harzvorland. Freiburger Forschungshefte C 207. (VEB Deutscher Verlag für Grundstoffindustrie: Leipzig).
- Schubert M, Morgenstern P, Wennrich R, Freyer K, Weiss H (2003) The weathering behavior of heavy metals in ore processing residues (Mansfeld Region, Germany). *Mine Water and the Environment* **22**, 2-6.
- Schubert M, Wennrich R, Weiß H, Schreck P, Zeller T, Otto HH, Wolfram H (2005) Formation of heavy metal bearing phases at a spring affected by the weathering of ore processing residues. *European Journal of Mineralogy* **17**, 119-128.
- Schubert M, Osenbrück K, Knöller K (2008) Using stable and radioactive isotopes for the investigation of contaminant metal mobilization in a metal mining district. *Applied Geochemistry* **23**, 2945-2954.
- Weiss H, Morency M, Freyer K, Bourne J, Fontaine D, Ghaleb B, Mineau R, Möder M, Morgenstern P, Popp P, Preda M, Treutler, H-C, Wennrich R (1997) Physical and chemical characterization of a complexly contaminated scrubber dust - a by-product of copper smelting in Sachsen-Anhalt, Germany. *The Science of the Total Environment* **203**, 65-78.

Chemical remediation of cadmium-contaminated paddy soils by washing with ferric chloride: Cd extraction mechanism and on-site verification

Tomoyuki Makino^A, Takashi Kamiya^B, Naoki Sekiya^C, Yuji Maejima^A, Ikuko Akahane^A and Hiroyuki Takano^B

^A Soil Environment Division, National Institute for Agro-Environmental Sciences, Tsukuba, Japan, Email t_makino@affrc.go.jp

^B Research & Development Center, Taiheiyo Cement Corp., Sakura, Chiba, Japan, Email Takashi_Kamiya@taiheiyo-cement.co.jp

^C Nagano Agricultural Research Center, Suzaka, Nagano, Japan, Email n.sekiya@alps.pref.nagano.jp

Abstract

Codex Alimentarius Commission (Codex) has adopted the maximum permissible concentration of cadmium (Cd) in polished rice and other relevant crops, which requires an alleviation of the Cd contamination in rice grain. Various chemicals were tested for their Cd extraction efficiency by using three paddy soils, selecting ferric chloride (FeCl₃) as a promising chemical for on-site soil washing. The comparison of FeCl₃ extraction ability to that of various iron, manganese, and zinc salts revealed the primary extraction mechanism of FeCl₃ to be proton release coupled with hydroxide generation (hydrolysis). This indicates that proton release from FeCl₃ is controlled by the chemical equilibrium of hydroxide formation, and minimizes the negative effect on soil properties and environment, which are different from hydrochloric acid (HCl). Washing with FeCl₃ led to the formation of Cd–chloride complexes, enhancing Cd extraction from the soils. We also developed in situ three-step washing method for Cd-contaminated paddy fields with FeCl₃. The method comprised 1) chemically washing the field soil with a FeCl₃ solution; 2) washing the treated soil with water to eliminate residual Cd and FeCl₃; and 3) on-site treatment of wastewater using a portable wastewater treatment system. Concentrations of Cd in the treated water were below Japan's environmental quality standard (0.01 mg/L). The on-site soil washing confirmed the effectiveness of FeCl₃ for decreasing Cd in soil and rice grains without negative effect on rice yield.

Key Words

Heavy metal, cadmium, remediation, soil washing, rice.

Introduction

Japanese agricultural soils, in particular, paddy soils in some regions, have been heavily polluted with cadmium (Cd), owing to fast industrialization during the 1960s. The Japanese government enacted the Agricultural Land Soil Pollution Prevention Law in 1970 which demarcated Cd-contaminated paddy fields which produced rice grains containing more than 1 mg/kg. Since the Law was in effect, the polluted paddy soils have been remedied mainly by unpolluted soil dressing. However, Codex has adopted the maximum permissible concentration of Cd in polished rice (0.4 mg/kg), which requires an alleviation of the Cd contamination in rice grain. In addition, the soil dressing has become increasingly difficult to implement because of its high cost and difficulty in obtaining unpolluted soil. Thus, it is a matter of urgency to develop promising technologies to remediate the Cd polluted paddy soils. On-site soil washing could be one of the promising technologies, which is suitable for paddy fields which usually have an impervious layer that keeps the wash solution in the surface layer. For application of the soil-wash method to paddy fields, we have set up four points to guide the development of potential on-site remedial technologies for Cd-contaminated paddy soils; (1) selection of chemicals that have low environmental impact but high efficiency, (2) development of an on-site washing and wastewater-treatment system, (3) ensuring favorable post-washing soil fertility and plant growth and (4) maintenance of the washing effect (Makino *et al.* 2007).

As washing chemicals, strong metal chelating agents, neutral salts and strong acids have been used (Davis, 2000). Especially, ethylenediaminetetraacetic acid (EDTA) could efficiently remove Cd from contaminated soils (Abumaizar and Smith 1999). EDTA, however, has the disadvantage of remaining in the environment for quite some time due to its low biodegradability (Tandy *et al.* 2004). Because EDTA has a high environmental burden, some researchers have used biodegradable chelating agents (Tandy *et al.* 2004). Though biodegradable chelating agents are favorable washing chemicals from the viewpoint of environmental impact, the costs of these chemicals are relatively high. Cost-effective and environmentally friendly chemicals are needed for soil washing. This paper aimed the selection of the promising extraction agents and developed the on-site soil washing for Cd-contaminated paddy fields.

Methods

Selection of washing chemicals

Three paddy soils were used for a Cd extraction test: Nagano soil (Fluvaquents), Toyama soil (Epiaquepts), and Hyogo soil (Fluvaquents). 10 g each of the three paddy soils polluted with Cd were shaken for 1hr with 15 ml of solutions containing 20 or 100 mmol/L chemicals such as acids, chelating materials, neutral salts, iron salts, manganese salts and zinc salts to extract Cd from soils. The Cd in the extracts was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES).

Extraction with metal salts

Same extraction procedure, as mentioned above, was conducted using the three soils and 100 mmol/L of a variety of acids and metal salts, such as HCl, HNO₃, H₂SO₄, FeCl₃, MnCl₂, ZnCl₂, Fe(NO₃)₃, Mn(NO₃)₂, Zn(NO₃)₂, Fe₂(SO₄)₃, MnSO₄, and ZnSO₄. Various ions extracted using FeCl₃, Fe(NO₃)₃, and Fe₂(SO₄)₃ were determined by the following analytical methods with duplicate: ICP-OES for Na, K, Ca, and Mg, and distillation with MgO for NH₄⁺. An ion chromatograph (DX-320, Dionex Corp., USA) was used to measure anions (Cl⁻, NO₃⁻, PO₄³⁻, SO₄²⁻). Dissolved organic carbon (DOC) was analyzed using a total organic carbon analyzer (TOC-5000, Shimadzu Corp., Japan). Visual MINTEQ software was used to analyze the ionic, DOC, and pH data sets to estimate Cd speciation in the extracts (Gustafsson 2004).

On-site soil washing

An on-site testing plot (ca. 100 m²) was prepared in a paddy field in Nagano Prefecture. The soil-washing procedure consisted of three steps: (1) chemical washing with FeCl₃ solution, (2) following water washing to eliminate the remaining chemicals, and (3) on-site treatment of the wastewater by a portable purification system with a chelating material. Soil samples were taken from the washed and unwashed plots. 0.1 mol/L HCl was used to extract soil Cd and the amounts of Cd extracted were determined by ICP-OES. Soil pH, electrical conductivity, total-C, total-N, available P, available N, and exchangeable cations were determined before and after soil washing. Rice plant was transplanted into the paddy field. Rice yields were measured after harvest. Part of the rice straw and some of the brown rice were ground, and digested with concentrated HNO₃ and then HClO₄. The Cd concentrations in the solutions were determined by ICP-OES.

Table 1. Soil properties (Makino *et al.* 2006).

| Sampling site | Horizon | Depth cm | pH(H ₂ O) | pH(KCl) | ^a TC g/kg | ^b TN g/kg | ^c TCd mg/kg | Clay % | Silt % | Sand % | *Clay minerals | **Soil type |
|---------------|---------|-------------|----------------------|---------|-------------------------|-------------------------|---------------------------|-----------|-----------|-----------|-------------------|-------------|
| Nagano | Ap | 0-12 | 5.77 | 4.6 | 26.4 | 2.3 | 0.71 | 16.3 | 29.9 | 53.9 | Kl, Ch, Mi | Fluvaquents |
| Hyogo | Ap | 0-11 | 6.77 | 5.87 | 21.4 | 1.8 | 4.65 | 16.6 | 21.2 | 62.2 | Kl, Ch, Mi | Fluvaquents |
| Toyama | Ap | 0-10 | 4.96 | 3.87 | 15.6 | 1.4 | 1.21 | 15.3 | 17.6 | 67.1 | Kl, Ch-Sm, Ch, Sm | Epiaquepts |

Kl:kaolin minerals, Sm:smectite, Ch:chlorite, Mi:mica, Ch-Sm:chlorite-smectite intergrade.

**Classified by Soil Taxonomy (Soil Survey Staff 1998). ^aTC, ^bTN and ^cTCd indicate total carbon, total nitrogen and total Cd, respectively.

Results

The extraction efficiency of the neutral salt was relatively low compared with that of the strong acids. FeCl₃ extracted more than 90% of the total Cd extractable by the strong acids and EDTA (Figure 1)(Makino *et al.* 2006). Based on the results obtained, FeCl₃ was selected as an extracting agent in terms of its extraction efficiency and environmental friendliness. The Cd extraction capacity was compared with other metal salts to elucidate the mechanism of Cd extraction by FeCl₃. The proportion of total soil Cd extracted by the washing chemicals (i.e., the Cd extraction efficiency) increased in the following order: Mn salts ≤ Zn salts << ferric Fe salts in all the three soils, with efficiencies ranging from 4-41%, 8-44%, and 24-66%, respectively (Figure 2) (Makino *et al.* 2008). The amount of Cd extracted was negatively correlated with the extraction pH, suggesting that extraction pH plays an important role in determining the Cd extraction efficiency. When metal salts are added to soils, the dissociated metal cations that may form hydroxide precipitates with releasing protons according to the following equations (Hydrolysis):



$$K^om = \frac{[M(OH)_n][H^+]^n}{[M^{n+}][H_2O]^n} \quad (3)$$

where *MmAn* denotes a metal salt, M a metal cation (Fe, Zn, or Mn) and A an anion (Cl⁻, NO₃⁻, or SO₄²⁻). *m* and *n* represent the charge numbers of the anion and cation respectively. *K^om* denotes the equilibrium

constants (expressed in terms of activities) for metal M^{n+} in Eq. (2), corresponding to 2.88×10^{-4} , 3.31×10^{-13} , and 6.46×10^{-16} for Fe^{3+} , Zn^{2+} , and Mn^{2+} , respectively (Lindsay, 1979).

The precipitation of the metal hydroxide (hydrolysis of the metal ion) generates protons at a rate that depends on K^0m , and these protons may decrease the extraction pH (Eqs. 1-3). Figure 3 illustrates the theoretical relationships between pH and activity of metal ions in the metal hydrolysis reactions at the equilibrium with soil iron (calculated using Eq. 3 and the K^0m values). The pH of ferric hydroxide is around 2 (Figure 3), which is much lower than the original soil-pH (H_2O) of the three soils. Thus, the Fe-hydrolysis is associated with a high decrease in soil pH compared to other two metals. This indicates that a driving force of the Cd extraction by $FeCl_3$ is proton release, which results in a sharp decrease in soil pH. Heavy metal solubilization was greatly enhanced by acidification, and at pH 1.3, reached more than 80% of the total Cd content of the soil (Dube and Galvez-Cloutier, 2005). Our results and these previous reports endorse the effectiveness of iron salts as washing chemicals to remove soil Cd. Determination of the chemical speciation of Cd using MINTEQA software indicated that Cd-chloride complexes were formed, and this would enhance Cd extraction from the soils (data not shown).

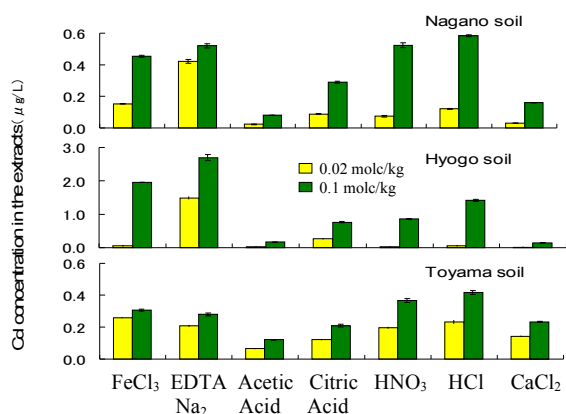


Figure 1. Efficiency of Cd extraction with various chemicals from the three soils.

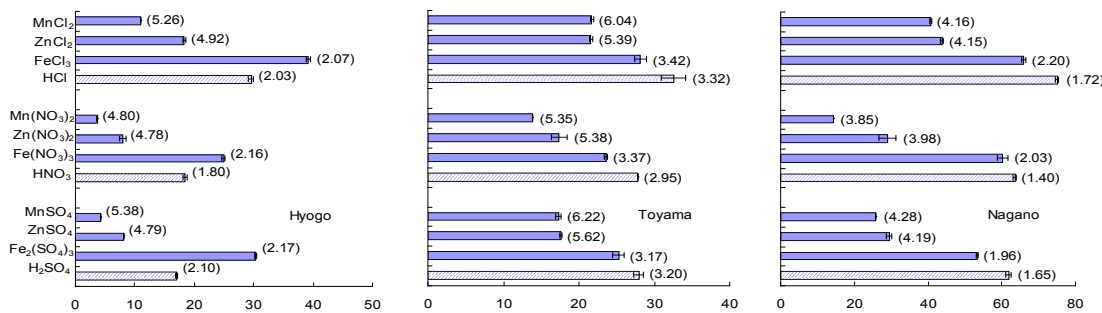


Figure 2. Comparison of cadmium extraction efficiency from the three soils by metal salts (gray bars) and strong acids (shaded bars). The extraction pH is shown in the parenthesis.

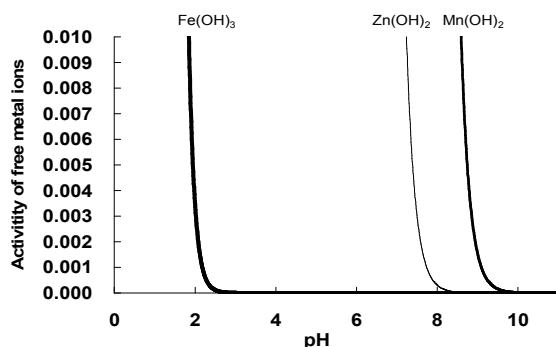


Figure 3. Diagram of pH and metal activity to precipitate metal hydroxides

During soil washing, the Cd concentration in the wastewater treated by the portable purification system was far below Japan's environmental quality standard (0.01 mg/L), proving that this technology was effective and promising for *in situ* treatment of wastewater. The Cl concentration was less than 500 mg/L after three times' washing by water. This concentration is the threshold value for healthy rice crops.

The Cd content extracted with 0.1M HCl in the washed soils was 55% of that in unwashed soils. The washing markedly decreased the Cd concentration (Figure 4). The pH(H₂O) and pH(KCl) of the soil were significantly decreased by on-site washing treatment. The soil EC increased with the treatment; however, it did not reach the critical level at which rice growth starts to retard. Exchangeable cations were decreased by soil washing. The Mg and K deficit was corrected by application of fertilizers to the washed soil. Total carbon and total nitrogen content were scarcely changed by washing.

Although changes in some relevant soil properties were observed, the changes can be easily corrected and does not affect relevant soil fertility. Soil washing considerably decreased the Cd content in the rice straw and brown rice from 0.91 and 0.31 mg/kg, respectively in the unwashed soil, to 0.18 and 0.053 mg/kg in the washed soil (Figure 5).

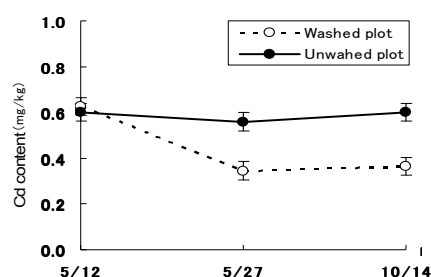


Figure 4. Changes of Cd contents in soils with washing treatment extracted with 0.1M HCl.

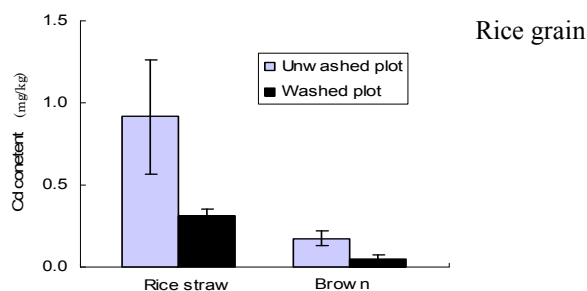


Figure 5. Effects of the washing treatment on the Cd contents in rice grain.

Conclusion

We selected ferric iron chloride for soil washing and revealed primary extraction mechanism of FeCl₃ is proton release coupled with hydroxide generation. We also developed an on-site soil washing technology for Cd-contaminated paddy fields. The washing had no negative affect on rice growth, and reduced the average Cd concentration in soil and rice grains.

References

- Abumaizar RJ, Smith EH (1999) Heavy metal contaminants removal by soil washing. *Journal of Hazardous Materials* **70**, 71-86.
- Davis AP (2000) Chemical and engineering aspects of heavy metal-contaminated soils. *Revista Internacional Contaminacion Ambiental* **16**, 169-174.
- Dube JS, Galvez-Cloutier R (2005) Applications of data on the mobility of heavy metals in contaminated soil to the definition of site-specific remediation criteria. *Journal of Environmental Engineering and Science* **4**, 399-411.
- Gustafsson JP (2004) 'Visual MINTEQ, version 2.30'. Department of Land and Water Resources <http://www.lwr.kth.se/English/OurSoftware/vminteq/index.htm>, 6th December, 2004
- Makino T, Kamiya T, Takano H, Itou T, Sekiya N, Sasaki K, Maejima Y, Sugahara K (2007) Remediation of cadmium-contaminated paddy soils by washing with calcium chloride -Verification of on-site washing. *Environmental Pollution* **147**, 112-119.
- Makino T, Sugahara K, Sakurai Y, Takano H, Kamiya T, Sas+-66-6aki K, Itou T, Sekiya N (2006) Remediation of cadmium contamination in paddy soils by washing with chemicals: Selection of washing chemicals. *Environmental Pollution* **144**, 2-10.
- Makino T, Takano H, Kamiya T, Itou T, Sekiya N, Inahara M, Sakurai Y (2008) Restoration of cadmium-contaminated paddy soils by washing with ferric chloride: Cd extraction mechanism and bench-scale verifeication, *Chemosphere* **70**, 1035-1043.
- Soil Survey Staff (1998) 'Keys to Soil Taxonomy 8th'. Natural Resources Conservation Services,(USDA:Washington D.C.)
- Tandy S, Bossart K, Mueller R, Ritschel J, Hauser L, Schulin R, Nowack B (2004) Extraction of heavy metals from soils using biodegradable chelating agents. *Environmental Science and Technology* **38**, 937-944.

Chemically induced phytoextraction of caesium -137

Meena Sadhasivam^A, Senthilvalavan Pitchamuthu^B and Vadivel Ayyavu^A

^ARadioisotope Laboratory, Dept.of Soil Science and Agrl. Chemistry, Tamil Nadu Agrl. University, Coimbatore-641 003, India, Email meenus_69@yahoo.com

^BFaculty of Agriculture, Annamalai University, Annamalainagar, Chidambaram-608 002, India, Email senvalavan_m2002@yahoo.co.in

Abstract

Radiocaesium (¹³⁷Cs) is one of the important radionuclides from the point of environmental contamination. Phytoextraction, a green technology to remediate the contaminated soils uses plants to accumulate contaminants in the above ground tissue. Monovalent cations such as potassium (K⁺) or ammonium (NH₄⁺) may enhance the phytoextraction of ¹³⁷Cs. Potential for chemically induced phytoextraction of a Typic Haplustalf contaminated with radiocaesium was investigated in a pot culture experiment. Bioaccumulation of ¹³⁷Cs was studied in shoots of four plants viz., amaranthus (*Amaranthus viridis* L.), maize (*Zea mays* L.), cowpea (*Phaseolus vulgaris* L.) and sunflower (*Helianthus annuus* L.) using ammonium chloride as a chemical extractant. The ability for ¹³⁷Cs extraction from a *Typic Haplustalf* contaminated with ¹³⁷Cs at 20 Bq/g was in the order of *A. viridis* L. > *Z. mays* L. > *P.vulgaris* L. > *H. annuus* L. Bioaccumulation ratio (BAR) of more than one was recorded for *A. viridis* L. at flowering stage. BAR was increased statistically by the addition of ammonium chloride in all crops. *A.viridis* with ammonium chloride as a chemical extractant could be a viable phytoextractant for a ¹³⁷ Cs contaminated soil.

Key Words

Caesium-137, phytoextraction, bioaccumulation ratio, *Amaranthus viridis*.

Introduction

Contamination of soil with radionuclides is a worldwide problem and of the various radionuclide contaminations, contamination of soil with ¹³⁷Cs deserves special attention because of its high fission yields, long half-life (t_{1/2} = 30.2 years) and influence on human health (Dushenkov 2003). Current remediation practices are costly and include excavation, shipping and burial of contaminated soil at licensed radioactive waste disposal facilities. These often have adverse effect on biological activity, soil structure and fertility and disturb the ecosystem (Negri and Hibchman 2000). One possible low cost and environment friendly technique is phytoextraction, a process that uses plants to accumulate contaminants in the aboveground tissue. The efficiency of phytoextraction of ¹³⁷Cs is ultimately a product of simple equation – biomass x element concentration in biomass. Therefore, the important aspect to consider is the choice of plants, which on one hand should not be susceptible to the contaminant but on the other hand should extract the contaminant from the soil to the highest possible extent. BAR of more than one were reported in shoots of grasses (Smolders and Shaw 1995; Lasat *et al.* 1997) grown in hydroponics culture. However, accumulation of caesium from soil into shoots is usually limited and frequently BAR of less than one has been reported for plants grown in radiocaesium contaminated soil (Nisbet and Shaw 1994). A major factor limiting radiocaesium uptake in to roots is its strong retention in clay particles (Cremers *et al.* 1988). Although earlier reports suggest that fixed radiocaesium can be desorbed to some extent by monovalent cations NH₄⁺ and to a lesser extent by K⁺ (Field *et al.* 1993), the extent of this release was shown to be highly dependent on soil properties (Kirk and Staunton 1989). With this background, the investigation was carried out to study the i) bioaccumulation of ¹³⁷ Cs by selected plant species during different physiological stages and ii) the effect of ammonium chloride application on ¹³⁷ Cs extraction from soil in to shoots. The resulting information will contribute to the selection of suitable plant species and the effect of chemical extractants on phytoextraction of ¹³⁷ Cs in a Typic Haplustalf.

Materials and methods

The soil selected for the study was classified as a Typic Haplustalf with sandy loam texture. The soil has 0.53 % organic carbon, pH (1:2.5 soil: water extract) of 8.45 and an electrical conductivity of 0.26 dS/m. The ammonium acetate extractable potassium content was 1139 kg/ha (Table 1). The experimental soil was filled in ceramic pots which consists of 10 kg of soil and was contaminated with ¹³⁷Cs @ 20 Bq/ g soil [Caesium - 137 was obtained as carrier free caesium nitrate in dilute nitric acid medium from the Board of Radiation and Isotope Technology (BRIT), Mumbai]. Following ¹³⁷Cs application, seeds of the test plants, maize (*Zea mays* L.),

sunflower (*Helianthus annuus* L.), cowpea (*Phaseolus vulgaris* L.) and amaranthus (*Amaranthus viridis* L.) were sown. Common cultural practices were followed in raising the crop. To induce chemical extraction, ammonium chloride, identified as the best extractant in the laboratory experiment, was applied at 0.5 M (0.262 g in 10 mL) one week prior to harvest. One more set of pots were maintained without ammonium chloride application. The design of the pot experiment was a two-factorial replicated in three completely randomized blocks.

During flowering stage plant samples were harvested. Fresh weight of shoots was recorded and expressed in g/pot. The weighed samples were dried in an oven at 70° C to a constant weight. From the dry weight recorded the total dry matter yield (g/ pot) was calculated. After processing, the radioactivity of the samples was determined in a Gamma Ray Spectrometer (Type GRS 101P of PLA) by differential counting by keeping the single channel analyzer at optimal settings. Using the radioassay data obtained, the content of ¹³⁷Cs radionuclide in the plant (Bq/ g), uptake of radionuclide by the plant (Bq/ pot) and Bioaccumulation ratio of ¹³⁷Cs were worked out.

Results

Biomass production

Caesium -137 phytoextracted from the soil also depends on the shoot biomass. The crops studied registered considerable difference in the biomass yield at flowering stage (Table 1). The greatest amount of biomass was produced by *Z.mays* followed by *A.viridis*. Irrespective of the crops, ammonium chloride addition has a significant positive effect on shoot biomass production.

Caesium -137 content and uptake

Regardless of ammonium chloride application, shoot ¹³⁷Cs content was highest in *A.viridis* and was significantly superior to other crops (Table 1). Addition of ammonium chloride significantly increased the ¹³⁷Cs content in shoots of all the plants studied. In *A.viridis* ammonium chloride application increased the ¹³⁷Cs accumulation in shoots by 16%. *Amaranthus viridis* L. recorded invariably higher ¹³⁷Cs uptake because of high ¹³⁷Cs activity even though biomass production was less than maize. The ¹³⁷Cs uptake values recorded in *A.viridis* L was 983.33 Bq/ pot with addition of ammonium chloride and 570.85 Bq /pot without ammonium chloride addition (Table 1).

Table 1. Shoot biomass yield, ¹³⁷Cs content, ¹³⁷Cs uptake and bioaccumulation ratio in crops as influenced by ammonium chloride addition

| Parameters | Extractant | Crop | | | |
|------------------------|---------------------|--------|-----------|--------|------------|
| | | Maize | Sunflower | Cowpea | Amaranthus |
| Biomass yield (g/pot) | + ammonium chloride | 111.82 | 32.50 | 24.30 | 40.35 |
| | - ammonium chloride | 106.10 | 30.70 | 22.76 | 35.88 |
| | | SEd | | CD | |
| | Crop (c) | 2.30 | | 4.60 | |
| | Extractant (e) | 1.63 | | 3.25 | |
| | c x e | 2.30 | | 4.60 | |
| Cs-137 content (Bq/g) | + ammonium chloride | 1.94 | 1.69 | 1.69 | 24.37 |
| | - ammonium chloride | 1.63 | 1.48 | 1.64 | 20.46 |
| | | SEd | | CD | |
| | Crop (c) | 0.81 | | 1.62 | |
| | Extractant (e) | 0.58 | | 1.15 | |
| | c x e | 0.36 | | 0.71 | |
| Cs-137 uptake (Bq/pot) | + ammonium chloride | 217.49 | 54.86 | 41.07 | 983.33 |
| | - ammonium chloride | 173.37 | 45.56 | 37.21 | 570.85 |
| | | SEd | | CD | |
| | Crop (c) | 34.11 | | 58.15 | |
| | Extractant (e) | 24.12 | | 48.19 | |
| | c x e | 48.25 | | 96.38 | |
| BAR | + ammonium chloride | 0.10 | 0.08 | 0.09 | 1.42 |
| | - ammonium chloride | 0.08 | 0.07 | 0.08 | 0.80 |
| | | SEd | | CD | |
| | Crop (c) | 0.010 | | 0.020 | |
| | Extractant (e) | 0.007 | | 0.014 | |
| | c x e | 0.015 | | 0.030 | |

Bioaccumulation Ratio (BAR) of Caesium -137

The ^{137}Cs BAR was significantly higher for *A. viridis* L. (1.42) than for *Z. mays* L. (0.10), *P. vulgaris* L. (0.09) and *H. annuus* L. (0.09) (Table 1). Though ammonium chloride application increased BAR in all the crops in *A. viridis* it was highly significant with an increase of 77.5 % over control (no ammonium chloride).

Discussion

Careful species selection that increase the bioavailability of ^{137}Cs could greatly enhance the prospects for the use of plants to remediate ^{137}Cs contaminated soil. Broadly and Willey (1997) found maximum 30 fold differences in ^{134}Cs concentration between a total of 30 plant taxa grown on one soil. In this investigation also significant variation was observed among the plant species studied. Greatest ^{137}Cs accumulation occurred in shoots of *A. viridis* L. which recorded 14 fold increases in ^{137}Cs concentration over *H. annuus* L. and *P. vulgaris* L. Application of ammonium salts to contaminated soil might have decreased the ^{137}Cs sorption capacity of the soil and thereby increased the ^{137}Cs bioavailability, and subsequently its accumulation in plants. When ammonium salts are added to the soil, they dissociate in the soil solution to positively charged ions and negatively charged anions. The cationic components can exchange ^{137}Cs from sorption sites in soil. This is in line with the findings of Lasat *et al.* 1997 and Blaylock and Huang 2000.

Uptake of a particular radionuclide depends on plant species and physiology of the plant. In this study *A. viridis* L. is an effective accumulator of ^{137}Cs which is characterized by high degree of uptake of ^{137}Cs . Since crops differ with regard to their physiological and metabolic characteristics they vary in their ability with regard to uptake, accumulation and translocation of ^{137}Cs . Different BAR can result from differences in rooting pattern, rooting depth, absorption characteristics of the root surface, root turn over, root growth rate etc., *A. viridis* L. recorded the highest BAR of 1.42 with addition of ammonium chloride. Lasat *et al.* (1998) also recorded significantly higher BAR for *A. retroflexes* grown in a coarse loamy soil. The effect of NH_4^+ on ^{137}Cs bioaccumulation was caused by increased radiocaesium bioavailability in the soil following NH_4^+ application. Results obtained from this study suggest that phytoextraction of ^{137}Cs contaminated soil is feasible and *A. viridis* L. seems to be one of the promising crops. Selection of a chemical extractant such as ammonium chloride (0.5 M) that can increase the bioavailability of ^{137}Cs will help in efficient phytoextraction of ^{137}Cs contaminated soil.

References

- Blaylock MJ, Huang, JW (2000) Phytoextraction of metals. In 'Phytoremediation of toxic metals'. (Eds J Raskin, BD Ensley) pp. 71-88 (Wiley: New York).
- Broadley MR, Willey NJ (1997) Differences in root uptake of radiocaesium by 30 plant taxa. *Environmental Pollution* **97**, 11–15.
- Cremers A, Elsen A, De Preter P, Maes A (1988) Quantitative analysis of radiocaesium retention in soils. *Nature* **335**, 247-249.
- Dushenkov S (2003) Trends in phytoremediation of radionuclides. *Plant and Soil* **249**, 167–175.
- Field JG, Belden RD, Serne RJ, Mattigod SV, Freeman HD, Scheck RW, Goller ED (1993) 100 area Hanford soil washing treatability tests. 'Meeting the Challenge' In 'Environmental Remediation Conference, October 24–28, Augusta, Georgia. 1, 377–381'. (US Dept of Energy, Office of Environmental Restoration: Washington, DC).
- Kirk GJ, Staunton S (1989) On predicting the fate of radioactive caesium in soil beneath grassland. *Journal of Soil Science* **40**, 71–84.
- Lasat MM, Fuhrmann ME, Kochian C (1998) Phytoremediation of a radiocaesium – contaminated soil. Evaluation of Caesium -137 bioaccumulation in the shoots of three plant species. *Journal of Environmental Quality* **27**, 165-169.
- Lasat MM, Norvell WA, Kochian C (1997) Potential for phytoextraction of ^{137}Cs from a contaminated soil. *Plant and Soil* **195**, 99-106.
- Negri MC, Hibchman RR (2000) The use of plants for the treatment of radionuclides. In 'Phytoremediation of toxic metals'. (Eds I Raskin, BD Ensley) pp. 107-132. (Wiley: New York).
- Nisbet AF, Shaw S (1994) Summary of five year lysimeter study on the time dependant transfer of ^{137}Cs , ^{90}Sr , $^{239,240}\text{Pu}$ and ^{241}Am to crops from three contrasting soil types. 1. Transfer to the edible portion. *Journal of Environmental Radioactivity* **23**, 1-17.
- Smolders E, Shaw G (1995) Changes in radiocaesium uptake and distribution in wheat during plant development: a solution culture study. *Plant and Soil* **176**, 1-6.

Concentrations of arsenic and other metals in agricultural soils of Bangladesh

Mohammad Mahmudur Rahman^{A,B} and Ravi Naidu^{A,B}

^ACentre for Environmental Risk Assessment and Remediation (CERAR), University of South Australia, Mawson Lakes campus, South Australia 5095, Australia, Email mohammad.rahman@unisa.edu.au

^BCooperative Research Centre of Contamination Assessment and Remediation of the Environment (CRC-CARE), P.O. Box 486, Salisbury South, South Australia 5106, Australia, Email ravi.naidu@crccare.com

Abstract

To ascertain the magnitude of arsenic (As) and other metals contamination in soils of agricultural lands, a preliminary study has been carried out from an As affected area of Bangladesh. The agricultural irrigation system of Bangladesh is completely reliant on groundwater. Large amounts of As and other heavy metals are added to agricultural soils due to the irrigation. A total of 20 agricultural land soils were analyzed by using inductively coupled plasma mass spectrometry (ICP-MS) for As and other metals following microwave assisted digestion. National Institute of Standard and Technology (NIST) standard reference material (SRM) 2711 (Montana soil) was analyzed to validate the results of As and other metals. Results from these studies show slight elevation of As in surface soils relative to background concentrations with no effect of irrigation on other metals. Further studies with a larger sample size are needed for detailed assessment of the effect of ground water irrigation on heavy metal content of agricultural soils.

Key Words

Groundwater, irrigation, microwave digestion, ICP-MS.

Introduction

Naturally elevated levels of As have been detected in the groundwater of Bangladesh. Groundwater is the primary supply of potable water for Bangladeshi inhabitants. In addition, As contaminated groundwater is generally used for agricultural irrigation. So, it is likely that huge quantity of As is pumping out with groundwater in As-affected regions and falling on agricultural irrigated lands. Therefore, it is probable that As is entering in human food chain. Accumulation of As in food crops pose potential health threats to humans. A number of studies have already been reported on As concentrations of Bangladeshi soils. Ahsan *et al.* (2009) recently reported that the range of As in floodplain soils from Faridpur district of Bangladesh was 18-65 mg/kg whereas the range of As in Dhamrai soils was 3.1-8.9 mg/kg. Another study showed that As levels of rice field soils were much higher compared to those in the non-contaminated areas (Saha and Ali, 2007). Ullah (1998) reported that irrigation with groundwater increased the levels of As in soil up to 83 mg/kg. The As content in soils ranged from 1.27–56.68, 3.18–54.77, 1.27–50.95, 1.27–39.48 and 3.18–35.66 mg/kg in Chapainawabganj Sadar, Kustia Sadar, Bera, Ishurdi and Sarishabari thanas of Bangladesh, respectively (Alam and Sattar 2000). This study reports a preliminary assessment of As and other metals content in agricultural soils of Bangladesh to determine the degree of contamination.

Materials and method

The soil samples were collected from the agricultural lands of Bangladesh during 2004. A microwave digestion system (Model: MARS 5, CEM) was used for the digestion of soil samples. The USEPA method 3051 was followed for sample digestion. ICP-MS (Agilent Technologies, Tokyo, Japan, model 7500c) was used for the determination of As and other metals. National Institute of Standard and Technology (NIST) standard reference material (SRM) 2711 (Montana soil) was used to validate the results of As and other metals. The values of soil pH and EC were given elsewhere (Rahman *et al.* 2009).

Results and discussion

Concentrations of As and other metals in agricultural soils

The analytical results of As and other metals in Montana soil showed that experimental values are very close to the certified values (Table 1). Concentrations of As and other metals in soil from each individual agricultural land are summarized in Table 2. The mean, median and range of As and other metal concentrations are presented in Table 3. The mean and median As concentrations of soils were 6.9 µg/g and 6.8 µg/g, with a range of 2.3-11.9 µg/g. Arsenic levels in paddy soils varied between 3.1 and 42.5 µg/g in a study reported from Bangladesh (Meharg and Rahman 2003). Alam and Sattar (2000) found that As levels in soils (depth 0-15 cm) ranged from not detectable level to 31.8 µg/g. They found that As content in soils was

Table 1. Concentration of elements in NIST standard reference material 2711 (Montana Soil) by ICPMS after microwave digestion.

| Element | n | Certified /non-certified values | Observed values | Percentage of recovery |
|------------------------|---|---------------------------------|-------------------|------------------------|
| As ($\mu\text{g/g}$) | 4 | 105 \pm 8 | 107 \pm 7 | 102 |
| Mg (%) | 4 | 1.05 \pm 0.03 | 0.95 \pm 0.06 | 85.3 |
| Al (%) | 4 | 6.53 \pm 0.09 | 5.05 \pm 0.15 | 77.4 |
| P (%) | 4 | 0.086 \pm 0.007 | 0.080 \pm 0.007 | 91.3 |
| Fe (%) | 4 | 2.89 \pm 0.06 | 2.51 \pm 0.04 | 87.0 |
| Cr ($\mu\text{g/g}$) | 4 | 47 | 45.3 \pm 4.2 | 96.5 |
| Mn ($\mu\text{g/g}$) | 4 | 638 \pm 28 | 529 \pm 34 | 82.9 |
| Ni ($\mu\text{g/g}$) | 4 | 20.6 \pm 1.1 | 18.2 \pm 0.3 | 88.2 |
| Cu ($\mu\text{g/g}$) | 4 | 114 \pm 2 | 113 \pm 34 | 99.5 |
| Zn ($\mu\text{g/g}$) | 4 | 350.4 \pm 4.8 | 331.4 \pm 15.7 | 94.6 |
| Cd ($\mu\text{g/g}$) | 4 | 41.7 \pm 0.25 | 43.5 \pm 0.49 | 104 |
| Pb ($\mu\text{g/g}$) | 4 | 1162 \pm 31 | 1153 \pm 34 | 99.2 |

Table 2. Concentration ($\mu\text{g/g}$) of arsenic and other heavy metals in soils collected from the agricultural lands (n =20) individually, Bangladesh.

| SMPL ID | As) | Mg | Al | P | Fe | Cr | Mn | Ni | Cu | Zn | Cd | Pb |
|-------------------------------|------|------|-------|-----|-------|------|-----|------|------|-------|------|------|
| (----- $\mu\text{g/g}$ -----) | | | | | | | | | | | | |
| B 2 | 4.7 | 2912 | 16238 | 293 | 16192 | 29.3 | 203 | 18.6 | 9.9 | 72.7 | 0.07 | 7.7 |
| B 3 | 6.6 | 3427 | 18546 | 440 | 17965 | 33.6 | 255 | 19.4 | 10.3 | 155.4 | 0.07 | 7.9 |
| B 7 | 9.4 | 5099 | 21055 | 356 | 23098 | 40.8 | 279 | 28.1 | 13.8 | 65.0 | 0.08 | 9.5 |
| B 8 | 10.6 | 5174 | 26421 | 405 | 25910 | 48.1 | 285 | 33.3 | 16.5 | 63.7 | 0.11 | 11.5 |
| B 9 | 5.3 | 4709 | 25508 | 293 | 23318 | 46.3 | 267 | 29.9 | 14.5 | 56.8 | 0.07 | 10.7 |
| B 10 | 5.9 | 4685 | 28560 | 282 | 23718 | 49.1 | 267 | 32.8 | 13.6 | 100.9 | 0.07 | 10.9 |
| B 53 | 3.9 | 3102 | 18945 | 201 | 17574 | 32.8 | 245 | 20.4 | 11.0 | 142.5 | 0.06 | 8.3 |
| B 54 | 3.9 | 2611 | 16973 | 162 | 15109 | 30.0 | 222 | 16.6 | 8.8 | 32.8 | 0.06 | 7.5 |
| B 56 | 7.1 | 3100 | 16565 | 624 | 17934 | 30.6 | 268 | 17.4 | 9.8 | 78.3 | 0.08 | 7.8 |
| B 60 | 6.2 | 3316 | 23248 | 195 | 23194 | 38.5 | 430 | 22.7 | 10.3 | 35.9 | 0.04 | 8.0 |
| B 61 | 2.3 | 582 | 11171 | 175 | 6763 | 21.3 | 90 | 10.8 | 6.1 | 19.3 | 0.03 | 6.2 |
| B 73 | 7.6 | 3691 | 24375 | 488 | 19610 | 41.3 | 327 | 21.3 | 11.2 | 293.5 | 0.07 | 8.1 |
| B 78 | 9.3 | 5627 | 29573 | 291 | 28719 | 52.1 | 345 | 35.0 | 14.3 | 51.4 | 0.08 | 10.7 |
| B 81 | 9.5 | 3852 | 23283 | 248 | 19819 | 39.7 | 233 | 23.5 | 12.1 | 142.8 | 0.06 | 8.2 |
| B 83 | 11.9 | 5216 | 27418 | 373 | 24372 | 47.5 | 296 | 31.4 | 15.6 | 62.7 | 0.08 | 9.9 |
| B 89 | 5.8 | 4007 | 15947 | 447 | 18035 | 32.5 | 305 | 19.5 | 11.2 | 157.7 | 0.06 | 6.1 |
| B 92 | 8.3 | 4897 | 18646 | 505 | 22643 | 37.9 | 310 | 27.5 | 17.8 | 297.5 | 0.13 | 11.0 |
| B 97 | 7.7 | 3536 | 20579 | 390 | 21648 | 36.5 | 360 | 23.0 | 9.8 | 44.2 | 0.06 | 7.9 |
| B 101 | 4.9 | 4097 | 14655 | 507 | 22833 | 31.3 | 324 | 21.5 | 9.9 | 44.4 | 0.04 | 6.2 |
| B 103 | 7.7 | 3179 | 15932 | 388 | 18991 | 30.0 | 321 | 18.5 | 8.6 | 137.7 | 0.05 | 6.5 |

Table 3. Parametric presentation of As and other metals ($\mu\text{g/g}$) in 20 soils collected from agricultural lands of Bangladesh.

| Parameter | As | Mg | Al | P | Fe | Cr | Mn | Ni | Cu | Zn | Cd | Pb |
|-------------------------------|------|------|-------|-----|-------|------|-----|------|------|-------|------|------|
| (----- $\mu\text{g/g}$ -----) | | | | | | | | | | | | |
| Mean | 6.9 | 3841 | 20682 | 353 | 20372 | 37.5 | 282 | 23.6 | 11.8 | 102.8 | 0.07 | 8.5 |
| Median | 6.8 | 3771 | 19762 | 365 | 20733 | 37.2 | 282 | 22.1 | 11.1 | 68.8 | 0.07 | 8.1 |
| Min | 2.3 | 582 | 11172 | 162 | 6763 | 21.3 | 90 | 10.8 | 6.1 | 19.3 | 0.03 | 6.1 |
| Max | 11.9 | 5627 | 29573 | 624 | 28719 | 52.1 | 430 | 35.0 | 17.8 | 297.5 | 0.13 | 11.5 |
| SD | 2.5 | 1174 | 5149 | 126 | 4716 | 8.1 | 69 | 6.49 | 2.9 | 78.9 | 0.02 | 1.7 |

positively correlated with As content in irrigation water. Roychowdhury *et al.* (2002) reported that the mean As concentration of agricultural lands of West Bengal, India was 10.7 mg/kg, with a range of 3.3-31.6 mg/kg. In this study, As content in examined soils was lower compared to the studies reported from Bangladesh and West Bengal. The agricultural soils have been exposed to groundwater due to irrigation, rainwater and, on occasion, floodwater from rivers or ponds following heavy rain during the wet season (Roychowdhury *et al.* 2002). Arsenic levels in soils may thus vary throughout the year. May be this is one of the probable reasons that As levels in this study were lower compared to the other studies.

The mean concentrations of Cr, Ni and Pb in the examined soils were almost similar compared to the study conducted by Roychowdhury *et al.* (2002). The mean concentrations of Mg, Fe and Zn were much higher and Cd, Cu and Mn were lower in the examined soils compared to the study of Roychowdhury *et al.* (2002). Most of the metals in this study were much lower in the examined soils compared to the study conducted from Faridpur district by Ahsan *et al.* (2009). Figure 1 presents the bar diagram of mean concentrations of As and other metals compared with the data reported from As-affected areas of Bangladesh and West Bengal.

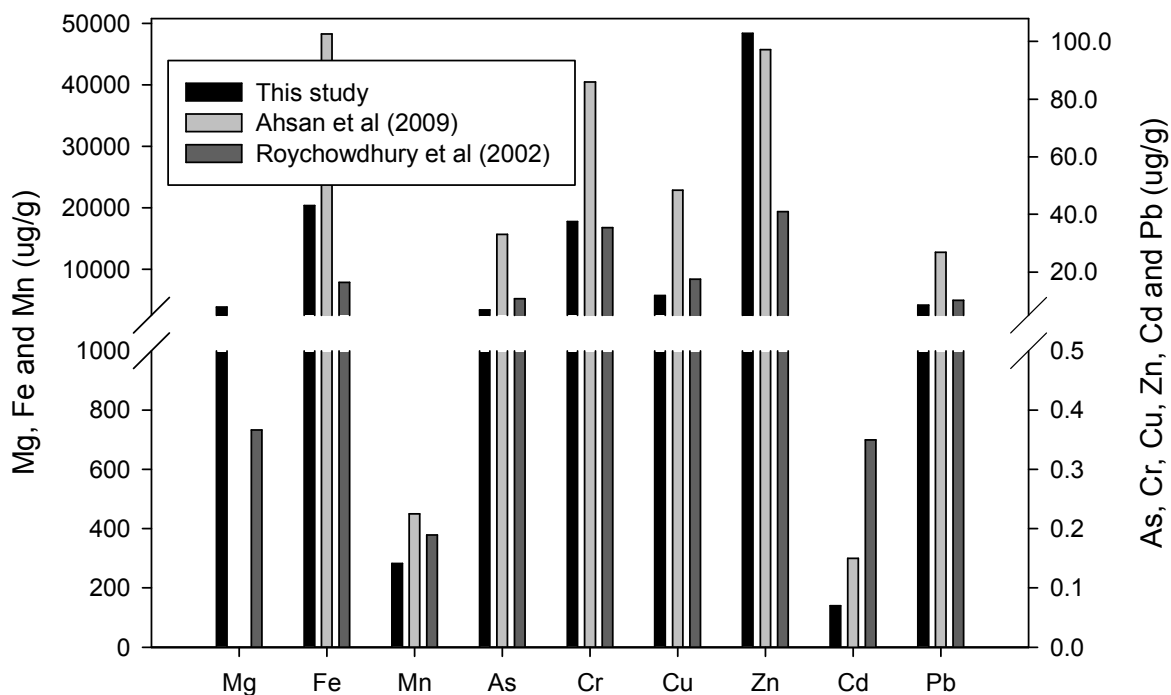


Figure 1. Comparative mean concentrations of As and other metals in agricultural land soils.

Relation between As and other metals in soils

The regression analyses were carried out between As and other metals in soil samples. The study showed positive significant correlation between As and Fe ($r^2 = 0.479$, $p < 0.05$), As and Mg ($r^2 = 0.539$, $p < 0.05$), As and Cr ($r^2 = 0.451$, $p < 0.05$), As and Ni ($r^2 = 0.449$, $p < 0.05$) and As and Cu ($r^2 = 0.457$, $p < 0.05$). Ahsan *et al.* (2009) also found a positive although very weak correlation between As and Fe in both Faridpur and Dhamrai soils. Roychowdhury *et al.* (2002) found a strongly significant correlations between As and other metals such as Cu, Pb, Ni, Mn, Zn, Mg, Cr, Cd in agricultural soils of As affected area of West Bengal, but did not get any fruitful correlation between As and Fe.

Speciation of As in soils

Ion Chromatography (IC) coupled with ICP-MS was used for the determination of As species [arsenite (AsIII), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenate (AsV)] in soils. The soils were extracted using 1M ortho-phosphoric acid. As(V) was detected in all 20 soil samples (mean: 2.3 µg/g and range: 0.25-8.16 µg/g) whereas As(III) was present in 9 soils (mean: 0.38 µg/g and range: 0.21-1.12 µg/g). None of the examined soils contained DMA and MMA. The detail As extraction method and analytical procedures have been discussed elsewhere (Rahman *et al.* 2009).

Conclusion

The study revealed that mean As level in soils in our study was lower compared to the other studies conducted from Bangladesh and West Bengal. Heavy withdrawal of groundwater may be the reason to discharge As and other metals and deposits on agricultural soil. In addition, crops grown in these areas are also contaminated by As and other metals. A detailed study is required to know the contamination status of food crops such as rice and vegetables. Further studies are needed to know the As load in agricultural soils in all contaminated districts of Bangladesh. More investigation is required to determine the accumulation of As and other metals in food crops from contaminated soils.

References

- Ahsan DA, DeIValls TA, Blasco J (2009) Distribution of arsenic and trace metals in the floodplain agricultural soil of Bangladesh. *Bulletin of Environmental Contamination and Toxicology* **82**, 11-15.
- Alam MD, Sattar MA (2000) Assessment of arsenic contamination in soils and waters in some areas of Bangladesh. *Water Science and Technology* **42**, 185-192.
- Meharg AA, Rahman MM (2003) Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption. *Environmental Science and Technology* **37**, 229-234.
- Rahman MM, Chen Z, Naidu R (2009) Extraction of arsenic species in soils using microwave-assisted extraction detected by ion chromatography coupled to inductively coupled plasma mass spectrometry. *Environmental Geochemistry and Health* **31**, 93-102.
- Roychowdhury T, Uchino T, Tokunaga H, Ando M (2002) Arsenic and other heavy metals in soils from an arsenic affected area of West Bengal, India. *Chemosphere* **49**, 605-618.
- Saha GC, Ali MA (2007) Dynamics of arsenic in agricultural soils irrigated with arsenic contaminated groundwater in Bangladesh. *Science of the Total Environment* **379**, 180-189.
- Ullah SM (1998) International conference on arsenic pollution of ground water in Bangladesh: causes, effects and remedies, Dhaka Community Hospital, Dhaka, Bangladesh, p 133.

Copper dynamics and impact on microbial communities in a vineyard soil. Influence of the soil organic status

Aline Navel^A, Jean M.F. Martins^A, David P.H. Lejon^B, Isabelle Lamy^C, Lionel Ranjard^B, Jean Lévêque^B and Lorenzo Spadini^{A,D}

^ACNRS; Université Grenoble I, UMR5564 LTHE, 1025 Rue de la piscine, BP53, 38041 Grenoble Cedex 9, France, Email jean.martins@hmg.inpg.fr

^BINRA-Université Bourgogne, UMR MSE, CMSE, 17 rue Sully, BP-86510, F-21065 Dijon, France, Email Lionel.Ranjard@dijon.inra.fr

^CINRA Versailles Grignon, UR 251, PESSAC, RD 10, F-78026 Versailles Cedex, Email lamy@versailles.inra.fr

^DCNRS; Université Grenoble I. UMR5559 LGIT, Grenoble, France, Email lorenzo.spadini@ujf-grenoble.fr

Abstract

In a coupled microcosm and lysimeter study, the effect of the soil organic status (SOS) on copper dynamics and impact was investigated in a vineyard soil that had been amended with varying types of organic matter during a previous long-term field experiment. Soil microcosms and lysimeters were contaminated at 240 mg Cu/kg and incubated for 12 months. Copper distribution and dynamics were assessed in the solid matrix by size fractionation and sequential extraction procedures and in the soil solution by measuring total and free exchangeable copper concentrations. Copper bioavailability was also measured with a whole-cell biosensor. Variations in microbial communities were assessed by means of bacteria enumeration and characterization of genetic structure using ARISA (Automated-Ribosomal-Intergenic-Spacer-Analysis). Results showed that copper distribution, speciation and bioavailability are strongly different between organically amended and non-amended soils and largely driven by the SOS. Unexpectedly, in solution, bioavailable copper correlated with total-copper, suggesting that non-free copper remains bioavailable to microorganisms. Similarly the observed differential copper impact on micro-organisms suggested that organic matter controlled copper toxicity through the control of the structure of the microbial communities in the different soils. In the two soils, microorganisms were dominantly distributed in the <20 μ m fraction, where native and spiked copper also accumulate. This soil smallest size fraction is also the one that more releases free and bioavailable copper, explaining thus the important amount of copper-resistant bacteria inhabiting this fraction. A clear relation between copper speciation, bioavailability, distribution and impact was established in the present study and will permit better predicting the fate and impact of trace elements in complex heterogeneous soils.

Key Word

Copper speciation, soil, organic matter, impact, bioavailability, micro-organism, biodiversity.

Introduction

Since many decades, copper is widely applied on soil through various land use practices (biosolids application, mining, pesticides use, etc.) leading to large increases of total Cu content, i.e., up to 1000 mg /kg of soil. At such high concentrations, copper has detrimental and irreversible effects on soil biocenosis and impacts the population structure of soil microbial communities. The impact of copper depends on its speciation, which controls its bioavailability. Factors such as pH, redox potential, mineralogical, and soil organic matter contents and types are all known to affect copper speciation (Tom-Peterson *et al.* 1999, Sauvé *et al.* 2008, Dumestre *et al.* 1999). Among the soil constituents, soil organic matter (SOM) is one of the more reactive towards copper, as demonstrated by several studies with soil organic matter or with organic ligands (Dumestre *et al.* 1999). Organic ligands interact with trace elements essentially through complexation reactions (Guiné *et al.* 2006). Consequently, the correlation between total and bioavailable copper content was shown to be poor in complex soil systems (Kunito *et al.* 1999). In order to better understand the reasons of this poor correlation, we studied the fate of copper in a soil variably amended with organic matter under different physical chemical conditions and at different scales.

Material and methods

The studied soil is a vineyard soil from Macon (France) variably amended with organic matter for over twenty years. After collection and sieving at 4 mm, the control and OM amended soils were incubated in lysimeters at 20°C in the dark, and supplemented with water or copper solution at 240 mg Cu./kg dry soil. At given times the different soils were submitted to granulometric fractionation (Jocteur-Monrozier *et al.* 1991) in order to obtain 4 size fractions (>250 μ m, 250-63 μ m, 63-20 μ m, and <20 μ m). Copper distribution and

dynamic were assessed i) in the solid matrix after size fractionation and sequential extraction procedures and ii) in the soil solution by measuring total and free exchangeable copper concentrations (Lejon *et al.* 2008). Copper bioavailability was also measured with a whole-cell biosensor (Lejon *et al.* 2008). Variations in microbial communities were assessed by means of biomass-C measurements and characterization of genetic structure using the ARISA fingerprinting technique (Lejon *et al.* 2007).

Results and discussion

Mineral and organic distribution in the size fractions of the amended and non amended soils

The mass distribution of the different size fractions confirmed the soil as a loamy clay (Figure 1a). No significant differences between the two soils were observed indicating that the organic amendment did not change the size fraction distribution, i.e. soil structure. The size fractions below 63 μm were dominant and represented up to 80 % of the total soil mass. Clay particles account for more than 50%.

The carbon concentration in the OM amended soil is slightly higher than in the non amended control soil (Figure 1b). This was observed also on the distribution of the organic matter in the different fractions of the two soils (Figure 1b). The differences were observed mostly in the coarser fractions where large fragments of raw organic matter coming from the compost amendment are observed. The micro-aggregated fraction (63-20 μm) is OM-poor and does not vary significantly between soils. Similarly the finest size fraction (<20 μm) that contains the more evolved OM was only slightly enriched by the compost amendment.

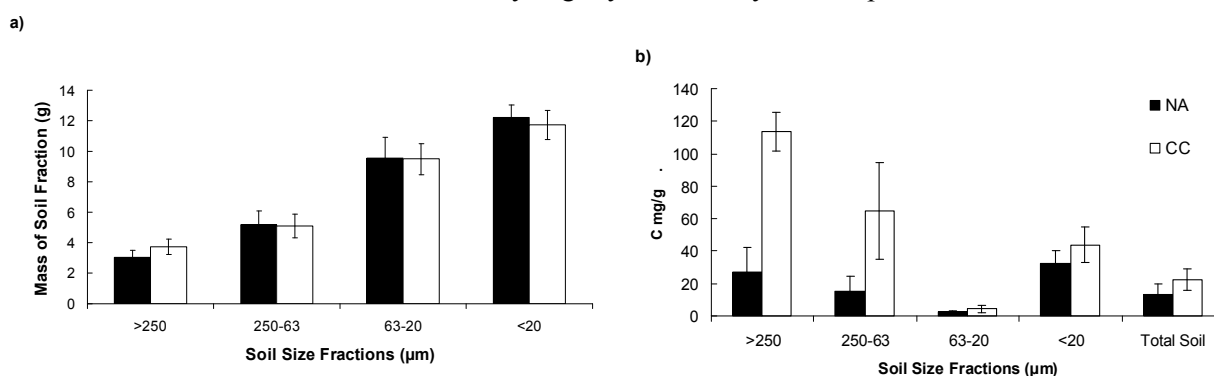


Figure 1. Distribution of the mass (a) and the total organic carbon (b) of the Non-Amended (NA) and compost amended soil size fractions. Error bars correspond to the standard deviations calculated with triplicates.

Distribution of copper in the size fractions

The distribution of copper in the fractions of both soils is presented in Figure 2. The results show that the distribution of native copper (low amount compared to the spiked copper) was shown to be very similar than that of the spiked copper. These two types of copper accumulated preferentially in the finest fraction (<20 μm) of the two soils. The solid speciation measurements (sequential extractions) revealed furthermore that copper is firstly bound to OM and then redistributed over time among OM and reactive iron and manganese oxides (Lejon *et al.* 2008), especially in the conifer compost amended soil. Copper speciation measured in solution after exchange with Ca^{2+} showed a fast decrease of free copper concentration during the two first days and then a stabilisation, whatever the organic matter status (Lejon *et al.* 2008). Figure 3 shows that copper released from the soil size fractions (exchanged with Ca^{2+}) is dominated by the smallest fraction (<20 μm), that contributes to about 50% of copper in solution. The observed total copper exchange is in agreement with the solid copper distribution measured in the soil size fractions. However the release of free copper varies with the size of the fraction, clearly indicating a different copper reactivity with size fractions constituents of both soils. Especially, copper present in the coarse fractions (>63 μm) appears strongly bound with the poorly-degraded organic matter that is thus less available (free) than copper weakly bound with the constituents of the smallest fractions (<20 μm) (Figure 3). This is also in agreement with the observed enrichment in copper-resistant bacteria in the smallest soil fractions presented below.

Copper impact

The results on the impact of Cu on the distribution of total and Cu-resistant cultivable bacteria in both soils are presented in Figure 4. These results showed that about 99% of bacteria are located in the fine fractions (<20 μm) of the two soils with a slight enrichment of bacteria (total and Cu-resistant) over time. Bacteria distribution is well correlated with the distribution of the readily degradable OM in the soil (<20 μm). Cu-resistant bacteria represented about 0.1% of the total heterotrophic bacteria in all soil fractions and are the more abundant in the <20 μm soil fraction that also accumulates dominantly native and spiked Cu (Figure 2).

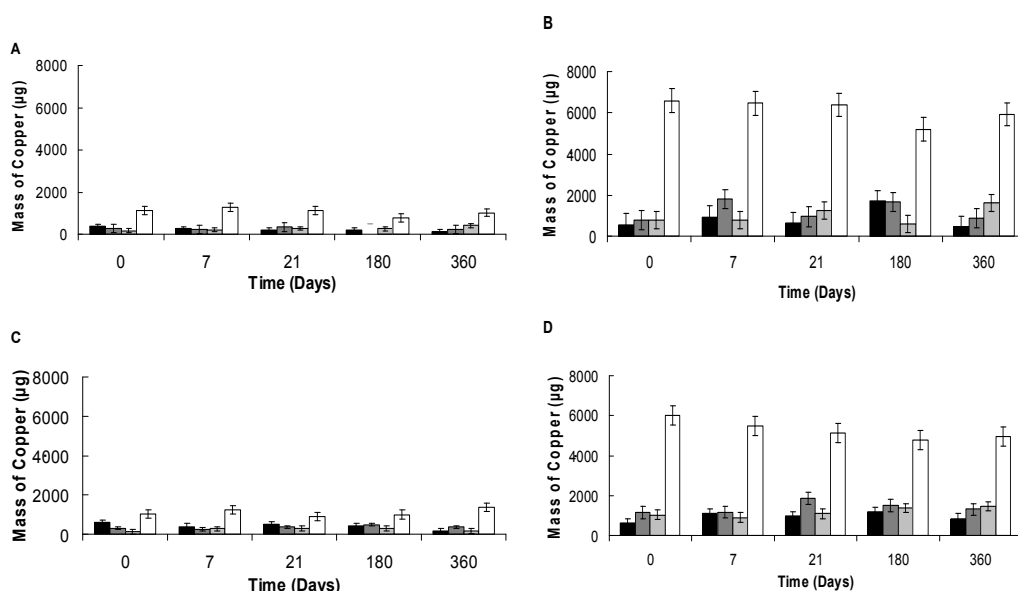


Figure 2. Temporal evolution of the mass of copper in soil fractions (>250µm : black; 250-63µm : dark grey; 63-20 µm : light grey; <20µm : white). **A:** Non amended and uncontaminated control soil. **B:** Non amended control soil contaminated at 240 mg/kg of copper. **C:** Uncontaminated conifer compost amended soil. **D:** Conifer compost amended soil contaminated at 240 mg/kg of copper.

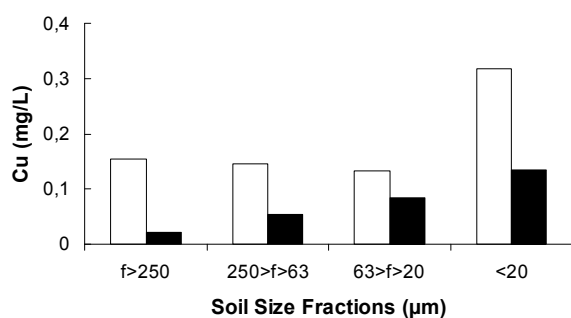


Figure 3. Total (white bars) and free (dark bars) copper concentrations exchanged with Ca²⁺ from the fractions of the compost-amended soil.

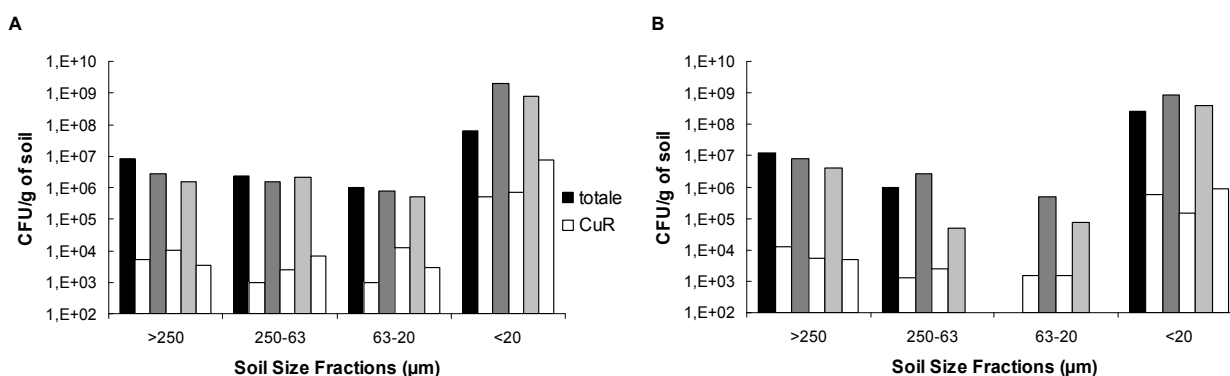


Figure 4. Temporal evolution (T0j : black, T7j : dark grey and T21j : light grey) of the distribution of total heterotrophic (black) and copper resistant (white) bacteria in fractions of the non-amended control soil (A) and the conifer compost amended soil (B).

The results about the toxicity of copper on soil microbial communities (Figure 5) revealed an impact of copper in both soils: the ARISA profiles were always significantly different between the control (water) and the copper (Cu) contaminated soils in the unfractionated soils and in the four size fractions. However, when considering the temporal evolution of the ARISA profiles, the smallest fraction appears clearly as the more impacted by copper spiking (Fig. 5a and b, right), especially in the non amended soil, indicating that the

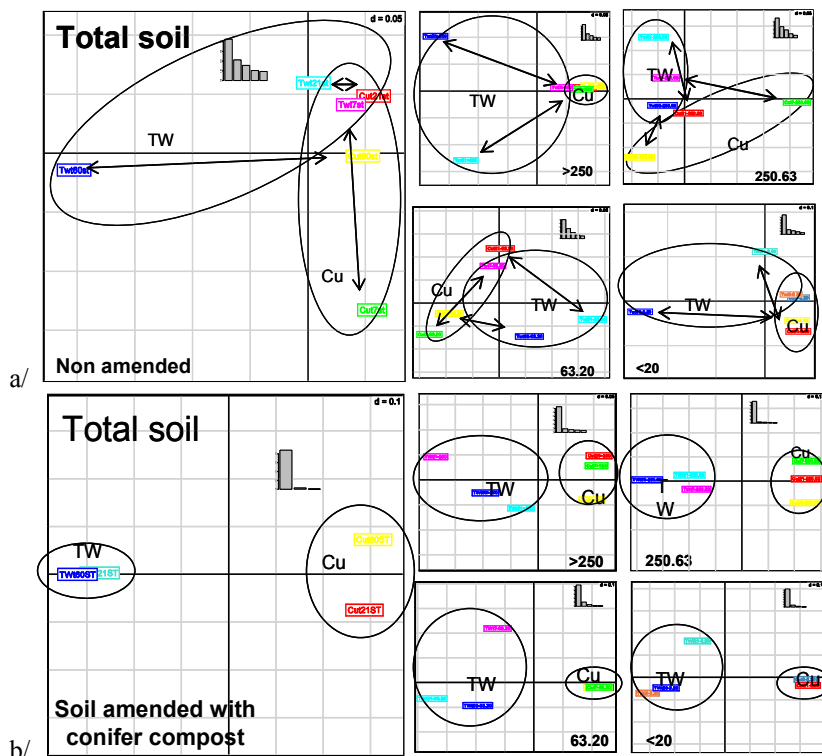


Figure 5. Principal component (PC1 × PC2) factorial maps generated from B-ARISA profiles obtained from the total (left) and size fractions (right) of the non-amended (a) and compost amended (b) soils (at t0d, t7d, t21, and t60d) for the water (W) and copper (Cu) treatments.

compost amendment protected microorganisms from copper toxicity to an extent that still needs to be established. The observed variations consisted mostly of an enrichment of *Actinobacteria*, especially in the non amended soil where copper is the more bioavailable (Figure 2 and 3). These results confirm that the structure and the sensitivity to metal contamination of the microbial community is controlled by the soil organic status (Lejon *et al.* 2008).

Conclusion

In the present study, we showed that the organic status of natural soils controls largely the solid and liquid speciation of copper as well as its availability to microorganisms. The SOS was shown to influence strongly the microbial community structure and its sensitivity to a Cu contamination. Cu was shown to be dominantly distributed in the smallest size fractions of both control and amended soils. This distribution was well correlated with organic matter and microorganisms distributions in these two soils. The preferential impacts of copper observed in the non amended soil and in the smallest size fractions demonstrated that copper toxicity and impact is controlled by the reactivity of the soil fractions (amount of reactive organic matter). This reactivity controls especially the release and the liquid speciation of Cu and thus bacteria-metal contact.

References

- Dumestre A, Sauvé S, McBride M, Baveye, P, Berthelin J (1999) Copper speciation and microbial activity in long-term contaminated soils. *Arch. Environ. Contam. Toxicol.* **36**, 124–131.
- Guiné V, Spadini L, Muris M, Sarret G, Delolme C, Gaudet JP, Martins JMF (2006) Zn Sorption to cell wall components of three gram- bacteria. *Environ Sc. Technol.* **40**, 1806-1813
- Jocteur-Monrozier L, Ladd JN, Fitzpatrick RW, Foster RC, Raupach M, (1991) Component and microbial biomass content of size fractions in soils of contrasting aggregation. *Geodermatology.* **50**, 37-62
- Kunito T, Saeki K, Oyaizu H, Matsumoto S (1999) Influences of copper forms on the toxicity to microorganisms in soils. *Ecotoxicol. Environ. Saf.* **44**, 174–181.
- Lejon DPH, Martins JMF, Lévêque J, Spadini L, Pascualt N, Landry D, Chaussod R, Ranjard L (2008) Cu dynamics and impact on microbial communities in vineyard soils. *Environ. Sci. Technol.* **42**, 2819-2825.
- Sauvé S, McBride MB, Norvell, WA, Hendershot WH (1997) Copper solubility and speciation of in situ contaminated soils: Effects of copper level, pH and organic matter. *Water Air Soil Pollut.* **100**, 133–149.
- Tom-Petersen A, Leser T. D, Marsh, T.L, Nybroe O (2003) Effects of copper amendment on the bacterial community in agricultural soil analyzed by T-RFLP. *FEMS Microbiol. Ecol.* **46**, 53–62.

Correlating accumulated cadmium and soil characteristics in wheat farm grain

A. R. Jafarnejadi^{A,C}, M. Homaei^A, Gh. Sayyad^B, M. Bybordi^A

^ASoil Science Department, Tarbiat Modares University, Tehran, Iran.

^BSoil Science Department, College of Agriculture, Shahid Chamran University, Ahvaz, Iran.

^CCorresponding author: A.R. Jafarnejadi, Email arjafarnejady@gmail.com

Abstract

Cadmium (Cd) accumulation in edible crops is undesirable due to its hazardous influences on human health. The objective of this study was to evaluate grain Cd content and its relation with soil properties in 4000 km² wheat farms. A number of 255 soil (0-20 cm) and grain samples were taken in an irregular weighted sampling scheme, using a GPS apparatus. Cadmium concentrations in grain samples and some soil properties were measured. The results indicated that grain Cd was distributed in an approximately normal distribution. Skewness, kurtosis and range of grain Cd were 1.2, 1.7 and 0.15-1.75 mg/kg, respectively. The grain Cd concentrations in 95 percent of the samples exceeded the threshold of 0.27 mg/kg grain. There was a significant ($p < 0.01$) correlation between grain Cd and organic carbon ($r = 0.66^{**}$), CEC ($r = 0.77^{**}$) and DTPA-extractable Cd ($p < 0.05$) ($r = 0.57^*$) of the soils. However, total Cd, soil pH and lime content (TNV) did not have significant ($p < 0.05$) correlations with grain Cd in the whole study area. The grain and soil analysis revealed some pollution mainly at the east and west of the study area. Organic carbon and CEC were the effective factors controlling soil Cd availability for plants.

Key Words

Cadmium, contaminated soils, soil characteristics, wheat farms

Introduction

Cadmium (Cd) is a heavy metal, toxic to humans, animals and plants. About 75 percent of the Cd in food chain originates from cereals and vegetables (Wangstrand *et al.* 2006). Wheat (*Triticum aestivum*), especially durum wheat, can accumulate Cd in its tissues more than the other currently grown cereals. The European Community limit value for Cd in wheat grain is 0.2 mg/kg. Soil is the main Cd source for plant uptake. Cadmium phytoavailability in soils is related to soil properties such as concentration and form of metal, pH, organic matter, clay content, cation exchange capacity (CEC), soluble Cl, sulfur (S) and sodium (Na) (Wu *et al.* 2002; Sayyad *et al.* 2009). Cadmium is transported from soil to plant roots by convection, diffusion and interception (Ingwersen 2005). Some previous studies have evaluated the relationships between different soil properties and Cd uptake in cereals under field conditions. Cd accumulation in wheat grain was significantly affected by soil chemical characteristics and cultivar. Wu *et al.* (2002) showed that the variations of grain Cd and most soil properties ($n = 124$) in a durum wheat field were strongly spatially dependent. Adams *et al.* (2004) reported that wheat grain Cd concentrations ($n = 162$) could be predicted reasonably well from soil total Cd and pH using a model with 53 percent of the variance being accounted for. In this study, a large scale study was conducted to assess the correlations among grain Cd concentration and soil properties, as well as to determine the effects of soil properties on grain wheat Cd concentration.

Methods

The study area was about 4000 km² based on the distribution of wheat fields in Khuzestan province, Southwest Iran. The fields were located from an east direction at 47°40' to 50°33' longitude and to a north direction at 29°57' to 30°00' latitude. This area included irrigated and dry land farms. Most dry land farms are located in the east of the province, while others are mainly under irrigation. Grain winter wheat and soil samples were collected from different regions using a weighing method, i.e., the more wheat grown in a farm area, the more grain and soil samples were taken. The required grain and soil samples (0-20 cm) were collected in mid May, 2008, from all 255 wheat farms. At this time, the wheat was near maturity. At each farm, a 1-m² quadrat was randomly placed in the field for sampling. A combined 0-20-cm topsoil sample (approximately 1 kg in weight and comprised of 10 cores) was obtained by manual coring within the quadrat area. A combined crop sample was collected at the same time by hand-cutting the crops near the ground level from all the quadrat areas.

Sub-samples of 0.2 g wheat grains were digested in HNO₃ and H₂O₂ and distilled water. The solution was analyzed for grain Cd concentration by graphite furnace atomic absorption spectrometry. All soil samples were then air-dried and passed through a 2-mm sieve. Total Cd concentration was determined using concentrated HCl and HNO₃, and the samples were analyzed by an atomic absorption spectrophotometer. Available cadmium was extracted using diethylenetriaminepentaacetic acid (DTPA) and analyzed by the graphite atomic absorption spectrophotometer. Electrical conductivity and pH of soil samples were measured in a saturated extract. Clay content was determined using the hydrometric method. Organic carbon was determined by wet oxidation and lime content was determined as the total neutralizing value (TNV) by titration with NaOH. Correlation analyses between total and DTPA-extractable Cd and soil parameters were done using the SPSS14 software.

Results and discussion

Summary of descriptive statistics for Cd grain and topsoil properties are presented in Table 1. The results indicated that grain Cd was distributed in an approximately normal distribution. The calculated skewness, kurtosis and range of grain Cd data were 1.2, 1.7 and 0.15-1.75 mg/kg, respectively. Soil Cd_t and Cd_{DTPA} varied about 3-fold and 11-fold between their minimum and maximum, respectively. The mean concentration of 1.47 mg/kg for Cd_t and 0.084 mg/kg for Cd_{DTPA} are very close to the values for agricultural soils in central Iran reported by Amini *et al.* (2005).

Table 1 The calculated statistics for grain Cd and some soil properties in the study area (n=255).

| Variable ^(A) | Cd _g mg/kg grain | Cd _{DTPA} mg/kg soil | Cd _t | pH | CEC cmol _c /kg | OC % | ECe dS/m | TNV % |
|-------------------------|--------------------------------|----------------------------------|-----------------|------|------------------------------|---------|-------------|----------|
| Mean | 0.70 | 0.084 | 1.47 | 7.40 | 13.75 | 0.77 | 4.08 | 46.20 |
| SD | 0.31 | 0.06 | 0.26 | 0.26 | 4.00 | 0.30 | 4.18 | 9.14 |
| CV% | 44.00 | 71.00 | 18.00 | 3.00 | 29.00 | 39.00 | 102.00 | 0.20 |
| Maximum | 1.75 | 0.35 | 2.19 | 8.40 | 28.30 | 1.71 | 24.30 | 75.00 |
| Skewness | 1.20 | 1.30 | 0.03 | 0.04 | 0.60 | 0.61 | 2.00 | -0.22 |
| Kurtosis | 1.70 | 2.40 | 0.24 | 0.25 | 0.46 | 0.33 | 4.70 | 0.85 |

^(A) Cd_g: concentration of Cd in grain; Cd_{DTPA}: DTPA-extractable Cd; Cd_t: total Cd; CEC: cation exchange capacity; OC: organic carbon; EC_e: electrical conductivity in saturated paste extract; TNV: total neutralizing value respectively

The Pearson correlations between grain Cd and soil parameters were calculated. A significant correlation ($p < 0.01$) was obtained between grain Cd and organic carbon ($r = 0.66^{**}$). Also, the correlation between grain Cd and both CEC ($r = 0.77^{**}$) and DTPA-extractable Cd ($r = 0.57^*$) was significant ($p < 0.05$). However, grain Cd was not significantly correlated with other soil properties such as EC_e, pH, TNV and Cd_t.

The correlation between grain Cd and CEC was the highest among soil properties. No significant ($p < 0.05$) correlation was obtained between grain Cd and total Cd in all locations. This clearly indicates that the availability of Cd was controlled by other soil factors than total Cd concentration. It is common conception nowadays that the total concentrations of metals in soils are not a good indicator of phytoavailability, or a good tool for potential risk assessment, due to different and complex distribution patterns of metals among various chemical species or solid phases. Grain Cd in different regions was compared, using a one-way ANOVA method. Various soil and management systems in the regions caused significant differences ($p < 0.05$) in grain Cd concentrations.

Grain Cd concentrations in 95 percent of the samples exceeded a concentration of 0.27 mg/kg. The greatest grain Cd concentrations were measured in the east regions with a range of 1.15 to 1.2 mg/kg. The lowest range was 0.32 to 0.63 mg/kg and it was distributed in different regions. Greater grain Cd contents were measured in regions with low Cd_t, which confirms the results of some previous studies that showed no relationships between Cd_t and grain Cd.

One reason for having such high Cd content in the grains at the east region can be attributed to the wheat cultivar. The durum wheat cultivar can accumulate more Cd in its grains than bread wheat. Another possible reason can be related to the dominant crop rotation system. In the east part the rotation is rice-wheat, while for the center and south it is wheat-wheat.

Conclusion

Agricultural activities have significantly ($p < 0.05$) increased Cd concentration in the topsoils and grains of the wheat farms in the study area. Also, in 95 percent of the grain samples, Cd concentration exceeded the 0.27

mg/kg limit. The organic carbon content, CEC, and DTPA-extractable Cd concentrations were significantly ($p < 0.05$) correlated with the grain Cd concentrations. This indicated that the Cd availability for plants could be quantified by soil organic carbon and CEC. The results showed that some polluted samples were measured mainly in the east and west of the study area. Agricultural mismanagement due to overusing P-fertilizers seems to be the main reason for the increase in Cd concentration in the topsoils and grains of wheat grown in wheat farms in the study.

References

- Adams ML, Zhao FJ, McGrath SP, Nicholson FA, Chambers BJ (2004). Predicting cadmium concentrations in wheat and barley grain using soil properties. *Journal Environmental Quality* **33**, 532-541.
- Amini, M, khademi H, Afyuni M, Abbaspour KC (2005). Variability of available cadmium in relation to soil properties and land use in an arid region in central Iran. *Water Air and Soil pollution Journal* **162**, 205-218.
- Ingwersen J, Streck T (2005). A regional-scale study on the crop uptake of cadmium from sandy soils; measurement and modeling. *Journal Environmental Quality* **34**, 1026-1035.
- Sayyad G, Mousavi SF, Abbaspour KC, Hajabbasi MA, Richards B and Schulin R (2009). Effects of cadmium, copper, lead, and zinc contamination on metal accumulation by safflower and wheat. *Soil & Sediment Contamination* **18**, 216-228.
- Wangstrand H, Eriksson J, Oborn I (2006). Cadmium concentration in winter wheat as affected by nitrogen fertilization. *European Journal of Agronomy*. *In press*.
- Wu J, Norvell WA, Hopkins DG, Welch RM (2002). Spatial variability of grain cadmium and soil characteristics in a durum wheat field. *Soil Science Society American Journal* **66**, 268-275.

Development of a rational sampling method for evaluation of Cd concentration in wheat (*Triticum aestivum*) grain and soil

Naomi Aoyama^A, Genki Kobori^B, Masanori Okazaki^B, Takashi Motobayashi^C and Masaharu Murakami^D

^ATokyo University of Agriculture and Technology, 3-5-8, Saiwai-cho, Fuchu, Tokyo 183-8509, Japan, 50006153003@st.tuat.ac.jp

^BGraduate School of Bio-Applications and System Engineering, Tokyo University of Agriculture and Technology, 2-24-16, Nakacho, Koganei, Tokyo 184-8588, Japan

^CField Science Center for Education and Research, Tokyo University of Agriculture and Technology, 3-7-1, Hommachi, Fuchu, Tokyo 183-0027, Japan

^DNational Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki 305-8604 Japan

Abstract

The rational method of sampling for the evaluation of Cd concentrations in wheat (*Triticum aestivum*) (Norin No. 61 and Ayahikari cultivar) grain and soil was investigated. Wheat grain and soil samples were collected from Hommachi Farm and Saiwaicho Farm, Tokyo University of Agriculture and Technology (TUAT). Soil of Hommachi is Gray Lowland Soils (Haplaquept) and soil of Saiwaicho is Cumulic Andosols (Melanudand). Hommachi Farm was polluted by cadmium in 1970's and contained higher cadmium concentration than Saiwaicho Farm. The intersections of the 5 meter mesh of the field were used for sampling sites. Sampling sites of 5, 9 and 32 or 120 for wheat grain and soil were prepared. The concentration of Cadmium was determined by ICP-MS. There was no significant difference of the mean values by the sampling numbers and sampling methods of wheat grain and soil, although increasing number of sampling sites decrease in the 95% confidence interval. The variation of the Cd concentration in Hommachi Farm was larger than that in Saiwaicho Farm. The error (percent) (sampling error / mean value x 100) at the probability level of 95 % decreased with increasing the number of wheat grain and soil sampling. For wheat grain, more than 30 sampling at Hommachi Farm and more than 100 sampling at Saiwaicho Farm provided the acceptable error value of less than 10 %. Meanwhile, for soil sample, 5 sites sampling at Hommachi Farm and 9 sites sampling at Saiwaicho Farm gave the acceptable error value of less than 10 %. It can be recommended that more than 30 wheat panicles at the place with high risk of cadmium pollution are collected from whole field and soil sampling is done at up to 5 sites.

Key Words

Cadmium, wheat, sampling method.

Introduction

Cadmium (Cd) is one of the most toxic and carcinogenic elements. Long term exposure to Cd even at low concentration affects human body, as it is accumulated in the kidney. Cadmium intake source for human is mainly from foods. Humans eat crops and vegetables which take up and accumulate Cd from soil. It is important to control Cd concentration in cereals. The Codex Committee (Codex, 2005) adopted 0.2 mg kg⁻¹ FW (Fresh Weight) of cadmium concentration in wheat grain in 2005, based on 7 µg per kg weight per week of Provisional Tolerable Weekly Intake (PTWI) after long discussion in Joint FAO/WHO Expert Committee on Food Additives (JECFA). Wheat, a major cereal for many areas, can absorb more Cd than rice when cultivated in the same field (Kikuchi *et al.* 2007). There were several reports on prediction of Cd concentration in wheat by total/extractable soil Cd concentration and pH using a regression analysis (Kuboi *et al.* 1986; Kuboi *et al.* 1987; Eriksson 1990; Singh *et al.* 1995; Robert *et al.* 1998; Gray *et al.* 2001; Ibaraki 2003; Mench and Baize 2003; Adams *et al.* 2004; Kusa *et al.* 2005; Spiegel *et al.* 2006; Matsumoto *et al.* 2007; Römkens *et al.* 2009). The objectives of this study are to show the relationship between Cd concentration in wheat grain and soil at two different fields of Hommachi Farm (Haplaquept) and Saiwaicho Farm (Melanudand) which hold different Cd levels and to propose the rational sampling method for evaluation of Cd concentration in wheat grain and soil.

Methods

Site description

This study was conducted at two Farms, Field Science Center for Education and Research of TUAT. Hommachi Farm is located on an alluvial lowland plain of Tama river in Fuchu City, Tokyo (35°39'N, 139°25'E) and consists of 13 rice paddy plots with a total area of 2.4 ha. It was reported in 1970's that the irrigation water to this farm derived from the Fuchu Irrigation Canal had been polluted by Cd from industrial

effluents (Fuchu City 1978). Since then, the ground water from a 150-m depth has been used for irrigation (Saito and Shimoda 1984; Okazaki and Saito 1989). Saiwaicho Farm, also is located in Fuchu City, has soil derived from volcanic ash.

Cultivation practices of winter wheat

In 2009, 32 sites of intersections 5m × 5m at Hommachi Farm and 120 sites at Saiwaicho Farm were used for sampling (Figure 1). The fertilizer rate of N: P₂O₅: K₂O = 40: 40: 40 kg/ha and 14: 14: 14 kg/ha were applied at Hommachi Farm and Saiwaicho Farm on the 18th of November, 2008. The application rate of fertilizer was 50% of the standard fertilization amount in Tokyo. *Triticum aestivum* L. cv. Norin No.61 and *Triticum aestivum* L. cv. Ayahikari were sown at Hommachi on the 1st of December and Saiwaicho Farm on the 2nd of October, 2008.

Sampling and analysis

The soil samples were taken from 120, 32, 9 and 5 sites in the field by an auger. After air-drying soil samples were passed through a 2mm nylon sieve. Five gram of soil sample was treated with 25 mL of 0.1 mol L⁻¹ hydrochloric acid solution and the mixture was shaken by a mechanical end-over-end shaker (Daiki, DIK 2102) at 25°C. The extract was filtered with a Whatman No.5 C filter paper and cadmium concentration in the filtrate was determined by an atomic absorption spectrophotometer (Hitachi Z-5010) with a flame at 228.8 nm. The grain samples of wheat obtained from the selected sampling at 120, 32, 9 and 5 sites were washed with tap water and deionized water, dried at 70 °C for 48 hours. These were ground with a ball mill (MM301, Retsch, Haan, Germany) for subsequent Cd analysis. The ground samples were digested using concentrated HNO₃, H₂O₂ and H₂O by a microwave apparatus, and the Cd concentration in the digests was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS/Thermo X-SERIES II, Japan). Statistical analysis by Student's t-test using JMP 8 program (SAS Institute Inc., Cary, NC, USA)

Results

Suggestion of sampling methods

The yields of wheat were 3.0 t ha⁻¹ in Hommachi Farm (Norin No.61) and 4.2 t ha⁻¹ in Saiwaicho Farm (Ayahikari). The moisture content of wheat grain was 13.5 % and 12.5 %, respectively.

The mean, median, standard deviation, coefficient of variation, standard error and variation of 95% confidence interval of Cd concentrations in wheat grain and soil at two Farms are shown in Table 1 and 2. The increasing number of sampling plots of wheat grain and soil showed no difference in mean value of soil Cd concentration. To increase the confidence level (decrease in standard error), the increase in number of sampling was necessary (Table 1 and 2).

Relationship between Cd concentration in wheat grain and extractable Cd concentrations in soil

The relationship between Cd concentration in grain of two cultivars and extractable Cd concentration in soil is shown in Figure 2. Cd concentration in wheat grain increased with increasing Cd concentration in soil, even though cultivars and soils were different. This coincided with the results that there were positive correlations between Cd concentration in wheat grain and soil (0.025 mol/L HCl as an extraction solution) (Ibaraki, 2003). Yoshida and Sugito (2007) proposed that 0.01 mol/L HCl extraction gave a good variable to predict Cd concentration in wheat grain. While Eriksson (1990) reported that 2 mol/L HNO₃ extractable Cd concentration in soil should be recommended to predict Cd concentration in edible part using multiple regression analysis.

Sampling number and reliability of data

The relationship between the number of sampling and sampling error (D / mean value × 100) was calculated, based on Table 1 and 2 according to Yanai *et al.* (2008). The variance (s^2) had been given by standard deviation (s). The equation using confidence interval (L), mean (M), number of sampling (N) and probability of 95 % (t_{α}) (N-1 degree of freedom) was introduced from t-test for estimation of interval of mean value.

$$L = M \pm t_{\alpha} s / N^{0.5} \quad (1)$$

The precision for estimating error (D) of sampling number was calculated according to the following equation

$$D = t_{\alpha} s / N^{0.5} \quad (2)$$

The relationship between the sampling number and the error from equation (1) and (2) is shown in Figure 3, indicating that increasing sampling number made the error small. For wheat grain sampling number should be required to be more than 30 at Hommachi Farm and more than 100 at Saiwaicho Farm. In case of the soil,

the error at the probability level of 95% was 7.98 % for sampling number of 5, 3.92 % for sampling number of 9 and 2.55 % for sampling number of 32. It suggested that less than 10% of the error was often found in the mean value of Cd concentration in soil when more than 9 sampling of soil was carried out. Considering that the error in the number of sampling was within 5 %, soil sampling was acceptable to be less than 5 in Hommachi Farm and 9 sites in Saiwaicho Farm.

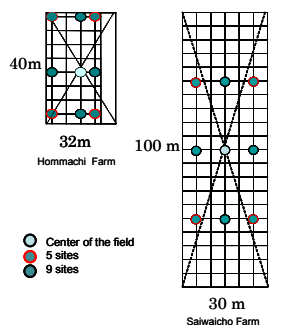


Figure 1. The arrangement of the sites in the experiment field (Hommachi Farm and Saiwaicho Farm).

Table 1. Cd concentration in wheat grain and its variation at Hommachi and Saiwaicho Farm.

| Number of sites | Hommachi Farm | | | Saiwaicho Farm | | |
|-------------------------|---------------|-------------|-------------|----------------|---------------|---------------|
| | 32 | 9 | 5 | 120 | 9 | 5 |
| Mean | 0.18 | 0.18 | 0.20 | 0.011 | 0.013 | 0.012 |
| Median | 0.18 | 0.18 | 0.18 | 0.010 | 0.010 | 0.010 |
| SD. | 0.037 | 0.064 | 0.074 | 0.0048 | 0.0075 | 0.0065 |
| CV (%) | 20.3 | 36.0 | 37.4 | 43.6 | 57.7 | 54.2 |
| Standard Error | 0.0064 | 0.021 | 0.033 | 0.00044 | 0.0025 | 0.0029 |
| 95% Confidence Interval | 0.18±0.0131 | 0.18±0.0480 | 0.20±0.0850 | 0.011±0.000867 | 0.013±0.00566 | 0.012±0.00748 |

Table 2. Cd concentration in soil and its variation at Hommachi and Saiwaicho Farm.

| Number of sites | Hommachi Farm | | | Saiwaicho Farm | | |
|-------------------------|---------------|------------|------------|----------------|-------------|------------|
| | 32 | 9 | 5 | 120 | 9 | 5 |
| Mean | 0.99 | 0.98 | 0.98 | 0.10 | 0.10 | 0.11 |
| Median | 0.99 | 0.99 | 0.99 | 0.098 | 0.098 | 0.098 |
| SD. | 0.07 | 0.05 | 0.063 | 0.014 | 0.012 | 0.013 |
| CV (%) | 7.00 | 5.10 | 6.40 | 14.4 | 11.8 | 12.8 |
| Standard Error | 0.0124 | 0.0167 | 0.0282 | 0.0013 | 0.0040 | 0.0058 |
| 95% Confidence Interval | 0.99±0.025 | 0.98±0.039 | 0.98±0.078 | 0.10±0.0025 | 0.10±0.0095 | 0.11±0.016 |

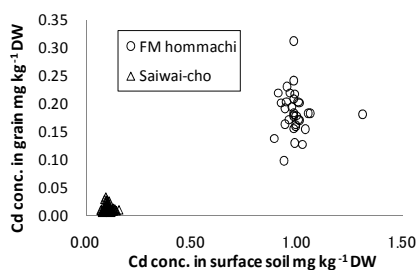


Figure 2. The relationship between Cd concentration in wheat grain and soil.

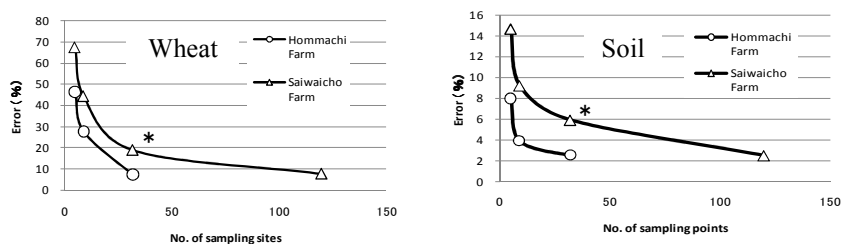


Figure 3. The relationship between the estimation error and number of sampling sites in soil and wheat *32 sites were randomly selected in Saiwaicho Farm.

Conclusion

The error at probability level of 95 % remarkably decreased with increasing number of sampling. For wheat grain the panicle sampling of more than 30 at the field with high risk of Cd pollution is performed from the whole field by walking along the row. Five sampling sites for soil in the field are prepared to evaluate high risk of Cd pollution.

Reference

- Adams ML, Zhao FJ, McGrath SP, Nicholson FA, Chambers BJ (2004) Predicting cadmium concentrations in wheat and barley grain using soil properties. *Journal of Environmental Quality* **33**, 532-541.
- Codex (2005) Report of the 37th session of the Codex Committee on Food Additives and Contaminants. Codex Alimentarius Commission 25, Alinorm.
- Eriksson JE (1990) A field study on factors influencing Cd levels in soils and in grain of oats and winter wheat. *Water, Air, and Soil Pollution* **53**, 69-81.
- Fuchu City (1978) Environmental Pollution in Fuchu City, Musashi-Fuchu Sosho No.6, p.75-80, Fuchu City.
- Gray CW, McLaren RG, Roberts AHC, Condron LM (1999) Cadmium phytoavailability in some New Zealand soils. *Australian Journal of Soil Research* **37**, 461-477.
- Gray CW, McLaren RG, Roberts AHC (2001) Cadmium concentrations in some New Zealand wheat grain. *New Zealand J. Crop and Horticultural Science* **29**, 125-136.
- Ibaraki T (2003) Evaluation of bio-available cadmium for wheat. *Promising Agricultural Practices Technologies for Reducing Heavy Contamination in Relevant Staple crops With a Special Emphasis on Cadmium Contamination* **23**, 98-99.
- Kikuchi T, Okazaki M, Toyota K, Motobayashi T, Kato M (2007) The input–output balance of cadmium in a paddy field of Tokyo. *Chemosphere* **68**, 920-927.
- Kikuchi T, Okazaki M, Motobayashi T (2009) Suppressive effect of magnesium oxide materials on cadmium accumulation in winter wheat grain cultivated in a cadmium-contaminated paddy field under annual rice–wheat rotational cultivation. *Journal of Hazardous Materials* **168**, 89-93.
- Kuboi T, Noguchi A, Yazaki J (1986) Family-dependant cadmium accumulation characteristics in higher plants. *Plant and Soil* **92**, 405-415.
- Kuboi T, Noguchi A, Yazaki J (1987) Relationship between tolerance and accumulation characteristics of cadmium in higher plants. *Plant and Soil* **104**, 275-280.
- Kusa K, Hatta K, Hara Y, Tsuchiya K (2005) Varietal difference in concentration and location of cadmium in wheat and barley grain. Report of Kyushu Agriculture **67**, 46.
- Matsumoto T, Kara H, Higasa Y (2007) Evaluation of pollution risk of cadmium in crops by chemical characteristics of arable soils as an indicator. Report Sophisticated Project of Ministry of Agriculture, Forestry and Fisheries, pp. 3. (MAFF:Tsukuba).
- Mench M, Baize D (2003) Trace element distribution in relevant arable soils and staple crops in France. *Promising Agricultural Practices Technologies for Reducing Heavy Contamination in Relevant Staple crops With a Special Emphasis on Cadmium Contamination* **23**, 8-12.
- Garrett RG, MacLaurin AI, Gawalko EJ, Tkachuk R, Hall GEM (1998) A prediction model for estimating the cadmium content of durum wheat from soil chemistry. *Journal of Geochemical Exploration* **64**, 101-110.
- Römken PFAM, Guo HY, Chu CL, Liu TS, Chiang CF, Koopmans GF (2009) Prediction of Cadmium uptake by brown rice and derivation of soil–plant transfer models to improve soil protection guidelines. *Environmental Pollution* **157**, 2435-2444.
- Singh BR, Narwal RP, Jeng AS, Almas A (1995) Crop uptake and extractability of cadmium in soils naturally high in metals at different pH levels. *Commun. Soil Science and Plant Analysis* **26**, 213-2142.
- Spiegel H, Dersch G, Oberforster M, Mechtier K, Baumgarten A (2006) Cadmium in Austrian wheat and potatoes - an inventory of sites and varieties. Abstract of 18th World Congress of Soil Science, pp. 168-17, July 9-15, 2006, Philadelphia, USA
- Yanai J, Matsubara T, Lee CK, Moritsuka N, Shinjo H, Kosaki T (2008) Establishment of Rational Sampling Science for the Paddy Field-Relationship between Sampling frequency and Data Reliability-, Japanese Society of Soil Science and Plant Nutrition **79**, 61-67 (in Japanese).
- Yoshida K, Sugito T (2007) Determination method of cadmium highly correlated to crop uptake. Report Sophisticated Project of Ministry of Agriculture, Forestry and Fisheries, pp. 3. (MAFF:Tsukuba).

Differences of cadmium uptake and accumulation among soybean (*Glycine max*) cultivars

Genki Kobori^A, Masanori Okazaki^A, Takashi Motobayashi^B and Kouji Matsukawa^B

^AGraduate school of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, 2-24-16, Nakacho, Koganei, Tokyo 184-8588, Japan, Email 50009401308@st.tuat.ac.jp

^BField Science Center for Education and Research, Tokyo University of Agriculture and Technology, 3-7-1, Honmachi, Fuchu, Tokyo 183-0027, Japan.

Abstract

Soybean (*Glycine max*) was cultivated in the field with high Cd concentration of FM Honmachi, Tokyo University of Agriculture and Technology, to investigate the differences in the amount of Cd uptake and accumulation among soybean cultivars. Three cultivars of soybean; Enrei, Tsurunoko and Tsukui were used in this study. The order of Cd concentration in green beans (pea in a pod, usually served after boiling as it is) and soybean seeds was Enrei < Tsurunoko < Tsukui with the mean value of 0.21, 0.27 and 0.42 mg/kg DW for green beans and 0.27, 0.34 and 0.51 mg/kg DW for soybean seeds. The relationship between Cd concentration in soil and soybean seeds was different among cultivars; Enrei had a negative trend, but Tsukui had a positive trend. The Cd uptake rate by plant was also different among cultivars. Tsukui had a faster Cd uptake rate than the others. Before the green bean stage, Tsukui showed the fastest Cd uptake, of 0.10 Cd µg/d/plant. After green bean stage, Tsurunoko was the fastest with 0.85 Cd µg/d/plant.

Key Words

Heavy metal, variety, upland, bean.

Introduction

Cadmium (Cd) is a highly toxic element for human beings because of its extremely long biological half-life. Excessive Cd intake causes serious disease of renal and bone tissue in a human body. Therefore, the Codex Committee on Food Additives and Contaminants has announced an upper allowable limit for Cd concentration in staple crops, such as polished rice, wheat and vegetables (Codex Committee 2005). However, Codex Committee decided not to determine the allowable limit of Cd concentration in soybean, because soybean is not a main food product in the world (Codex Committee 2004). Meanwhile in Japan, soybean products are traditional major food like *nattou*, *tofu*, *soysource* and *miso*. The results of a large-scale survey of domestic agricultural products revealed that the Cd concentration of 16.7 % of soybean seeds exceeded intake of 0.2 mg/kg that is the international allowable limit proposed by the Codex Committee until 2004, and is much higher proportion than that of other upland crops (Ministry of Agriculture, Forestry, and Fisheries of Japan 2002). With respect to genetic characteristic for Cd uptake, Arao *et al.* (2002) reported genotypic differences in cadmium uptake and distribution in soybeans cultivated in pot and under low Cd concentrations in the field. Ministry of Agriculture, Forestry, and Fisheries of Japan decided to promote low Cd uptake soybean cultivars and encouraged farmers to cultivate them in high Cd concentration fields (2007). Sugiyama *et al.* (2007) found the cultivars with low Cd uptake accumulated much higher Cd in their roots than those of the cultivar with high Cd uptake. Using tracer Cd, Kawasaki and Oda (2004) and Kawasaki *et al.* (2005) reported that Cd transported to seeds was absorbed before full seed stage and Cd absorbed at the beginning of growing stage was accumulated in leaf. Oda *et al.* (2004) showed the growing stage where Cd concentration in seeds become the highest was from full pod to full seed stage. Decreasing soil Cd concentration reduced Cd concentration in soybean seeds (Maejima *et al.* 2007). In this study, the amount of Cd uptake and accumulation in green beans and soybean seeds among different cultivars and differences of Cd uptake rate among cultivars were investigated.

Methods

Cultivation of soybean

The cultivation experiment on soybeans was conducted at Honmachi Farm shown in Figure1 (A3N, A3W and B1), Field Science Center for Education and Research of Tokyo University of Agriculture and Technology on an alluvial lowland derived from Tama River sediment (Figure 1). The mean values of soil Cd concentration at A3N, A3W and B1 field (Gray Lowland Soils is comparable to an Aquic Fluvents (Soil Survey Staff 1990) were 1.05, 1.31 and 1.00 mg/kg, respectively. Three different soybean cultivars, Enrei,

Tsurunoko and Tsukui, were selected from many varieties in Japan. Enrei was regarded as a low Cd uptake cultivar and recommended by Ministry of Agriculture, Forestry, and Fisheries of Japan, 2007. Soybean was seeded in May-July of 2008 and harvested in October-November of 2008.

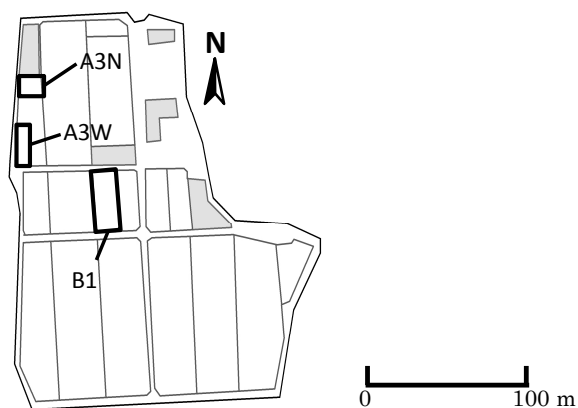


Figure 1. The Honmachi Farm, Field Science Center for Education and Research of Tokyo University of Agriculture and Technology.

Soil analysis

Soil samples ($n=36$) at the point of soybean cultivation field were collected from just below the harvested soybean. After air-drying, soil samples were passed through a 2 mm nylon sieve. Twenty five mL of 0.1 mol/L HCl solution was added to 5 g soil samples. The mixture was shaken by a mechanical end-over-end shaker (Daiki, DIK 2102) at 25 °C. The extract was filtered with a No. 5 C filter paper (Advantec) and Cd concentration in the filtrate was determined by a flame atomic absorption spectrophotometer (Hitachi Z-5010).

Plant analysis

Green beans and soybean seeds were collected as whole body including root, which was removed from the stem. Soybean seeds were washed with tap water and then deionized water, dried at 70 °C for 36 hours in a ventilated oven and ground with a mixer mill (MM301, Restch) for subsequent Cd analysis. The ground samples were digested using extra-pure water, H₂O₂ and HNO₃ (1:1:8 mL) in teflon containers in a microwave apparatus. The solution was filtered with a 0.45 µm Membrane filter (Millipore). The Cd concentration in the digests was determined using Inductively Coupled Plasma Mass Spectrometry (Thermo Fisher, X series II).

Statistical analysis

All statistical analyses were performed using JMP 8 (SAS Institute Inc., Cary, NC, USA). Tukey's HSD test was used to determine the differences of Cd concentration in seeds among cultivars.

Results

The mean value of Cd concentration in green beans and soybean seeds among cultivars are shown in Figure 2. The mean value of Cd concentration in green bean seeds, harvested after about 87~107 days from seeding, was 0.21, 0.27 and 0.42 mg/kg DW for Enrei, Tsurunoko and Tsukui, respectively. The mean value of Cd concentration in soybean seeds, harvested after about 140 days from seeding, was 0.27, 0.34 and 0.51 mg/kg DW for Enrei, Tsurunoko and Tsukui, respectively. There was a significant difference of Cd concentration in green beans and soybean seeds among cultivars ($p<0.05$). Enrei had the lowest Cd concentration for soybean seeds and Tsukui gave the highest value. Tsurunoko and Tsukui showed significant difference in Cd concentration between green bean and soybean seeds ($p<0.05$).

The relationships between Cd concentration in soil and soybean seeds are shown in Figure 3. This result revealed that the relationship was different among cultivars. When soil Cd concentration increased, Cd concentration in Enrei seeds, which provided low Cd concentration in seeds, slightly decreased but Cd concentration in Tsukui seeds, which exhibited high Cd concentration in seeds, increased. However, these trends are not highly significant.

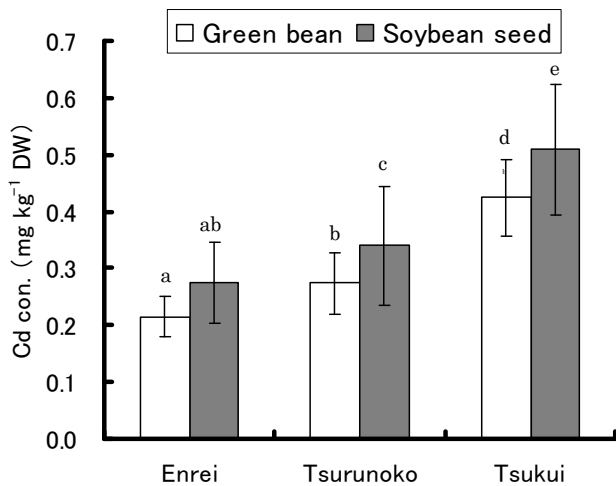


Figure 2. Cd concentration in green bean and soybean seed of three cultivars. Error bar shows standard deviation. Means followed by the same letter are not significantly different at the 5% level (Tukey's HSD).

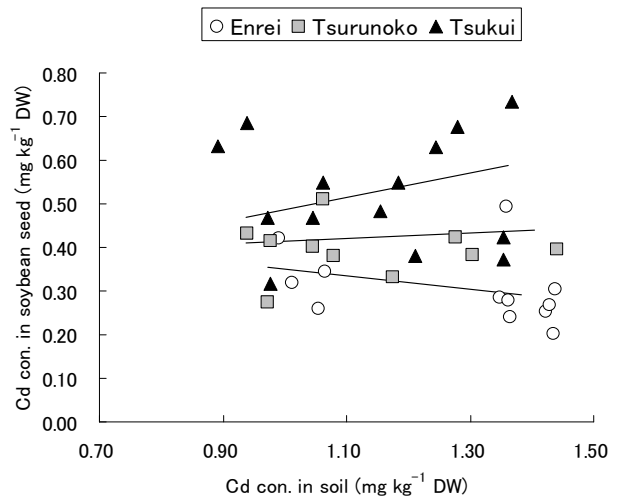


Figure 3. The relationship between Cd concentration in soil and soybean seeds for each cultivar.

Figure 4 shows Cd uptake rate to soybean seed during cultivation period. This result showed Cd translocation and accumulation in seed from root. From this result, rapid Cd accumulation in seeds occurred when green bean changed to soybean, and the Cd uptake rate was different among cultivars. While, Oda *et al.* (2004) reported that the growing stage, where Cd concentration in seeds cultivated hydroponically the highest, was from full pod stage to full seed stage (peas in pods become bigger). This discrepancy depends on the cultivation methods and period of soybean. Before they had green beans, Tsukui had the fastest Cd uptake rate. After they had green beans, Tsurunoko had the fastest Cd uptake rate. This might be caused by the number of pods (Tsurunoko had the most pods among them: date not shown). In order to survey Cd in soybean seeds translocated from other organs or soil directly, Cd concentration in other organs should be analysed.

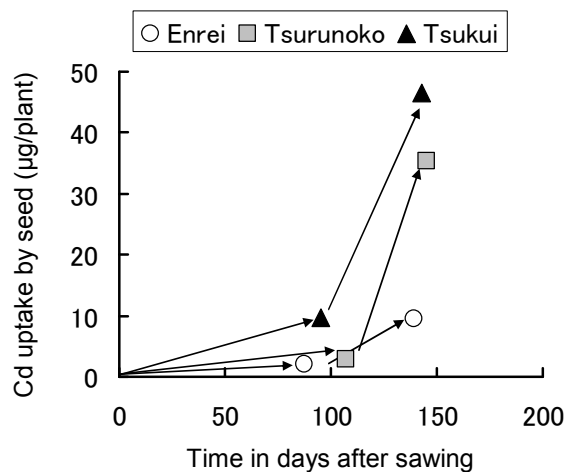


Figure 4. Cd uptake by seeds during the cultivation period.

Conclusion

There were significant differences of Cd uptake among soybean cultivars cultivated in the same upland fields. The relationship between soil Cd concentration and soybean Cd concentration was different between cultivars. Cd uptake by soybean seeds happened extensively when green beans changed to soybeans.

References

- Arao T, Ae N, Sugiyama M, Takahash M (2003) Genotypic differences in cadmium uptake and distribution in soybeans. *Plant and Soil* **251**, 247-253.
- Codex Alimentarius Commission (2004) 'Report of the 36th session of the Codex Committee on Food

- Additives and Contaminants'. (Codex Alimentarius Commission: Alinorm).
- Codex Alimentarius Commission (2005) 'Report of the 37th session of the Codex Committee on Food Additives and Contaminants'. (Codex Alimentarius Commission: Alinorm).
- Kawasaki A, Oda H, Yamada M (2004) Application of ^{113}Cd as a tracer in evaluation of cadmium uptake by soybeans under pot conditions. *Japanese Journal of Soil Science and Plant Nutrition* **75**, 667-672.
- Kawasaki A, Oda H (2005) Application of ^{113}Cd as a tracer in evaluation of cadmium uptake by soybeans under field conditions. *Japanese Journal of Soil Science and Plant Nutrition* **76**, 261-267.
- Maejima Y, Makino T, Takano H, Kamiya T (2007) Remediation of cadmium-contaminated paddy soils by washing with chemicals: Effect of soil washing on cadmium uptake by soybean. *Chemosphere* **67**, 748-754.
- Ministry of Agriculture, Forestry, and Fisheries of Japan (2002) 'Investigation of Cd concentration of staple crops: the outline of the results'. (Tokyo).
- Ministry of Agriculture, Forestry, and Fisheries of Japan (2007) 'The manual to confirm the technology for suppression of Cd uptake in soybean'.
- Oda H, Senda S, Kawasaki A (2004) Uptake and transport of Cd supplied at different growth stages in hydroponically cultured soybean plants. *Biomedical Research on Trace Elements* **15**, 289-291.
- Soil Survey Staff (1990) 'Keys to Soil Taxonomy'. (USDA-Natural Resources Conservation Service: Washington, DC).
- Sugiyama M, Ae N, Arai T (2007) Role of roots in differences in seed cadmium concentration among soybean cultivars-proof by grafting experiment. *Plant and Soil* **295**, 1-11.

Different patterns of organic acid exudation in metallophyte and agricultural plants at increasing copper levels

Sebastián Meier^A, Marysol Alvear^A, Fernando Borie^A, Paula Aguilera^A and Pablo Cornejo^{A,B}

^AScientific and Technological Bioresource Nucleus, BIOREN-UFRO, Universidad de La Frontera, P.O. Box 54-D, Temuco, Chile.

^BCorresponding author. Email pcornejo@ufro.cl.

Abstract

Copper (Cu) accumulation in shoot and root tissues and low molecular weight organic acids (LMWOA) root exudation have been studied in two metallophytes from Central Chile (*Oenothera affinis* and *Imperata condensata*) and two agricultural plants (*Lupinus albus* and *Helianthus annuus*) growing under hydroponics conditions at increasing Cu levels (0, 0.125, 0.250, 0.5, 1 and 2 mg Cu/L). The plants were grown in mineral solution for four weeks and after that in Cu-added solution for ten days, harvested and analyzed after this time. All the plant species showed high Cu accumulation, highlighting *O. affinis* with 116 and 2657 mg Cu/kg in shoots and roots, respectively. Strong differences in the LMWOA exudated by the different plant species were determined. *O. affinis* exudated high amounts of succinic acid, while *I. condensata* exuded citric and oxalic acids. Acid root exudations from agricultural plants were mainly composed by citric acid. This result suggests that LMWOA root exudates by metallophytes play an important role against high Cu levels in polluted soils, being an important factor for plant selection in the design and implementation of phytoremediation programs.

Key Words

Cu tolerance, metallophytes, organic acid exudation, phytoremediation.

Introduction

The main sources of metals in the environment are anthropogenic, such as agriculture, domestic and industrial wastes and mining, being the latter the more important (Ginocchio *et al.* 2004). Under this environmental stress, some plants have developed diverse mechanisms for tolerating high metal levels such as exudation of low molecular weight organic acids (LMWOA) (Tao *et al.* 2004). These exudates play an important function mobilizing low mobile/soluble nutrients (e.g., P, Fe, Zn), increasing microbial activity and complexing metals thus affecting the metal bioavailability. In this sense, the extracellular metal complexation could be an important adaptative mechanism for plant establishment in nutrient deficient soils or in those polluted with high metal levels (Shen *et al.* 2002).

On the other hand, it has been found that LMWOA are involved in the metal transport and storage in plants (Nigam *et al.* 2001). Under high Cu levels the exudation of LMWOA by agricultural plants has been widely studied, and several reports have shown that roots might exude diverse LMWOA, including citric, oxalic and succinic acid, which could play a important role in alleviating Cu toxicity (Quartacci *et al.* 2009). However, the exudation of LMWOA by plants tolerant to high Cu levels has not been extensively studied. In this way, there are no studies which contrast the Cu tolerance, Cu accumulation and exudation of LMWOA between Cu metallophyte and agricultural plants. Based on the aforementioned we hypothesized that LMWOA exudation may be a crucial Cu tolerance mechanism developed by metallophytes naturally growing in Cu polluted areas.

Therefore, the aim of this work was to determine the exudation pattern of LMWOA in mineral solutions, and Cu concentration of two metallophyte species and to contrast their behavior with two agricultural plants growing in the same conditions, for analyzing its possible use in phytoremediation.

Methods

Biological material and culture conditions

Commercial seeds of the agricultural plant species *Helianthus annuus* and *Lupinus albus* cv. Rumbo-B, and seeds of *Oenothera affinis* obtained *in situ* in Cu polluted soils were sown. Additionally, stolons of *Imperata condensata* were collected from the polluted area to produce plantlets. The collect area was a Mediterranean ecosystem strongly affected by the deposit of metal-rich particles, located approximately at 1.5 km southeast from the Ventanas smelter (CODELCO), in the Puchuncaví valley, Central Chile (32°46' 30" S 71° 28' 17"

W). All seedlings were grown in perlite/sand/vermiculite substrate (1:1:1, v:v:v), supplemented with sterile ddH₂O and maintained in a growth chamber until roots reached > 2 cm-length. Then, the plants were transferred to 1-L polyethylene containers and were continuously aerated by air pumping. The nutrient solution used was 1.3 mM MgSO₄, 2.0 mM Ca(NO₃)₂, 2.0 mM KNO₃, 2.0 mM K₂HPO₄, and (in μ M) 0.2 μ M CuSO₄, 1.0 μ M ZnSO₄, 2.0 μ M MnCl₂, 20 μ M H₃BO₃, 0.1 μ M (NH₄)₆Mo₇O₂₄, and 200 μ M FeEDTA. The containers were placed into a plant growth chamber room under controlled conditions. After 4 weeks growing in this culture conditions the nutrient solution was replaced by the same solution described above, but supplied with 0, 0.125, 0.5, 1.0 and 2.0 mg Cu/L as CuSO₄. This test solution was replaced every two days to keep constant concentrations of nutrients and Cu. The plants grew in these conditions for 10 days, and after the root exudates were collected using the methodology proposed by Rosas *et al.* (2007) with minor modifications.

Measurements

Plants with intact roots were rinsed thoroughly with 50 mL of deionized water ($\leq 1 \mu$ S/cm) and then were immersed in deionized water under constant aeration for 1 h. The solution was filtered (0.22 μ m) and freeze-dried. In order to quantify the concentration of LMWOA the residue was resuspended in 300-500 μ L of deionized-sterilised water for HPLC injection. The separation was conducted on a 250 \times 4 mm reverse phase column (LiChrospher 100 RP-18 5 mm particle size; Merck, Darmstadt, Germany). Sample solutions (20 μ L) were injected onto the column and 200 mM orthophosphoric acid (pH 2.1) was used for isocratic elution, with a flow rate of 1 mL/min and UV detection at 210 nm. Identification of organic acids was performed by comparison of retention times and absorption spectra with standards for each organic acid. After to obtain the root exudates, the plants were cut separating shoot and roots, washed with distilled water, oven-dried at 60 °C for 48 h and weighed. The tissue samples obtained were crushed and converted into ash in a furnace and digested using a H₂O/HCl/HNO₃ mixture (8/1/1 v/v/v). The Cu concentrations were determined by Atomic Absorption Spectroscopy (Perkin-Elmer 3110).

Results

Copper accumulation

All the plant species studied showed Cu concentrations above normal (superior to 20 μ g Cu/g DW, Adriano 2001), presenting values between 14 and 40 μ g Cu/g DW in shoots, with the exception of *O. affinis* which presented a mean accumulation of 116 μ g Cu/g DW at the highest Cu concentration (Figure 1, A). However, the highest differences in Cu concentration were registered in the root, where all species studied showed a sharp increase directly related with the increase in Cu concentration in the solution (Figure 1, B), reaching means of 330-660 μ g Cu/g DW in the roots of all species at the highest Cu level, with the exception of *O. affinis* which at the same conditions sowed up to 2660 μ g Cu/g (4 times higher than concentration in shoots and roots of the other species evaluated) (Figure 1).

Root exudates

Four different LMWOA were detected: succinic acid, oxalic acid, citric acid and small amounts of fumaric acid, which were strictly dependent of the plant species analyzed and the amount of Cu added to the solution (Figure 2). Succinic acid was principally exudated by the metallophyte *O. affinis*, which exuded high amounts at increasing Cu concentration, reaching values of 1048,56 μ mol/h DW at the higher Cu level (Figure 2C). On the other hand, *Imperata condensata* exhibited LMWOA exudation rate was very variable under different Cu conditions. At low Cu concentrations (between control treatment and 0.125 mg Cu/L) *I. condensata* showed a low exudation of succinic acid (Figure 2C); however, at increasing Cu concentrations this metallophyte produced large amounts of citric and oxalic acid, reaching values of 164,05 and 1,61 μ mol/h.g DW respectively at the highest Cu concentrations (Figure 2 A, B). Root exudates of *L. albus* and *H. annuus* contained exclusively citric acid (Figure 1A), which was exuded in high amounts, reaching on an average of 46,01 and 51,44 μ mol/h.g DW; however, the concentration of citric acid remained relatively constant with increasing copper concentrations in both plants.

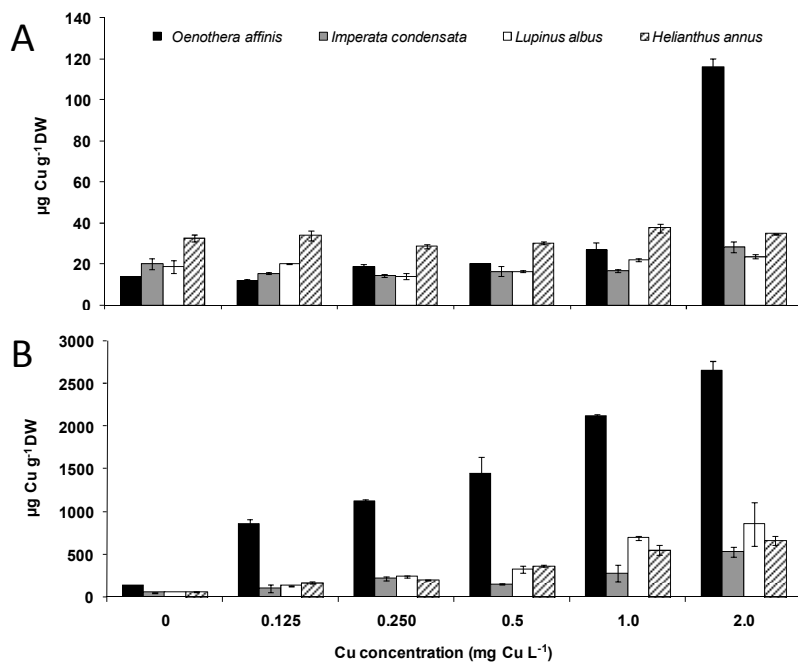


Figure 1. Copper (Cu) concentration in (A) shoots and (B) roots of four plants species in response to increasing Cu levels (0; 0.12; 0.250, 0.5; 1.0 and 2.0 mg Cu/L). Bars denote means \pm S.E (n = 6). DW= Dry weight.

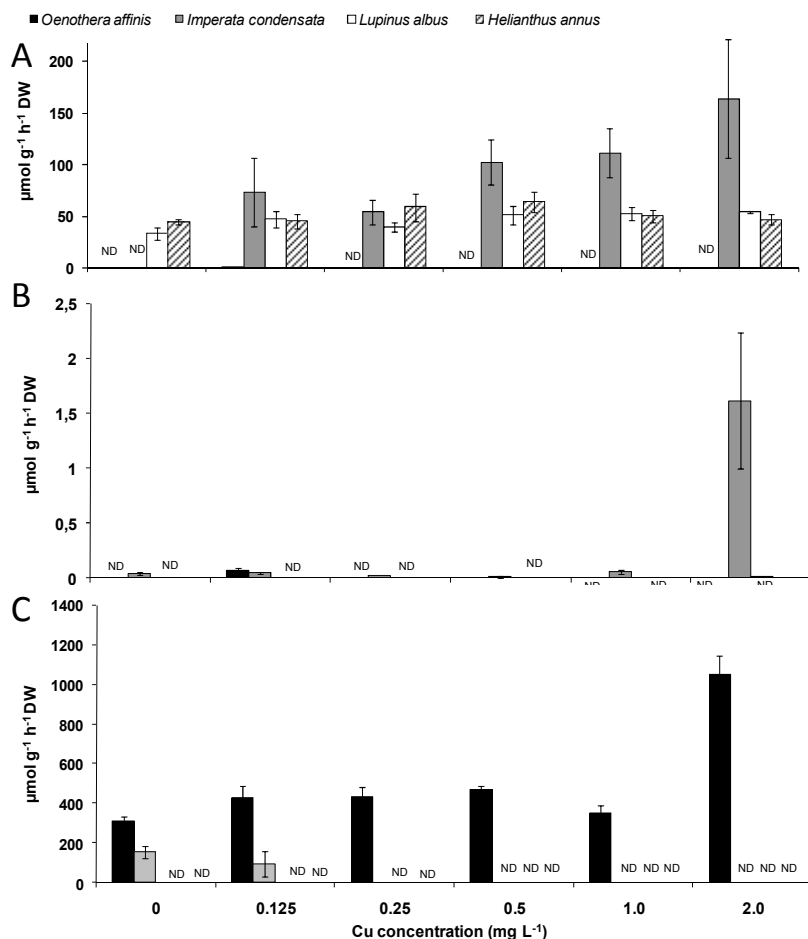


Figure 2. Exudation of low molecular weight organic acids in four plant species in response to increasing copper levels (0; 0.12; 0.250, 0.5; 1.0 and 2.0 mg Cu/kg). A) Citric acid, B) oxalic acid, C) succinic acid. Bars denote means \pm S.E (n = 6). DW= Dry weight, ND= Not detected.

Conclusion

These results suggest that the different metallophytes and agricultural plants present differences with respect to its Cu tolerance. If metal tolerance is considered to be manifested as a series of physiological traits (including root elongation, metal uptake and accumulation; Baker and Walker 1990), the most tolerant species would be the metallophyte *O. affinis*, due to its high Cu accumulation capacity. Similarly, the differential exudations of LMWOA by plants, and the large amounts exuded by the metallophytes suggest that this is an important defense mechanism developed to tolerate high Cu concentrations. Further studies aimed to investigate the metal binding properties of each compounds released by roots and the possible involvement of other exudates (phenols) are necessary in order to assess their role in Cu tolerance and accumulating capacity of the metallophytes and agricultural plants as technological tool to be used in potential phytoremediation programs in Cu polluted soils.

References

- Adriano D (2001) Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risk of Metals. Springer-Verlag, New York. 857p.
- Baker AJM, Walker PL (1990) Ecophysiology of metal uptake by tolerant plants. In 'Heavy Metal Tolerance in Plants: Evolutionary Aspects'. (Ed AJ Shaw) pp. 155-177. (CRC Press, Boca Raton, FL)
- Ginocchio R, Carvallo G, Toro I, Bustamante E, Silva Y, Sepúlveda N (2004) Micro-spatial variation of soil metal pollution and plant recruitment near a copper smelter in Central Chile. *Environmental Pollution* **127**, 343-352.
- Nigam R, Srivastava S, Prakash S, Srivastava M (2001) Cadmium mobilization and plant availability: the impact of organic acids commonly exuded from roots. *Plant and Soil* **230**, 107-113
- Quartacci M, Irtelli B, Gonnelli C, Gabbriellini R, Navari-Izzo F (2009) Naturally-assisted metal phytoextraction by *Brassica carinata*: Role of root exudates. *Environmental Pollution* **157**, 2697-2703.
- Rosas A, Rengel Z, Mora ML (2007) Manganese supply and pH influence growth, carboxylate exudation and peroxidase activity on ryegrass and white clover. *Journal of Plant Nutrition* **30**, 253-270.
- Shen H, Yan X, Zhao M, Zheng S, Wang X (2002) Exudation of organic acids in common bean as related to mobilization of aluminum and iron bound phosphates. *Environmental and Experimental Botany* **48**, 1-9.
- Tao S, Liu WX, Chen YJ, Xu FL, Dawson RW, Li BG, Cao J, Wang XJ, Hu JY, Fang JY (2004) Evaluation of factors influencing root-induced changes of copper fractionation in rhizosphere of a calcareous soil. *Environmental Pollution* **129**, 5-12.

Distribution and availability of metal contaminants in shooting range soils around Australia

Peter Sanderson^{A,B,D}, Nanthi Bolan^{A,B}, Mark Bowman^C and Ravi Naidu^{A,B}

^ACentre for Environmental Risk Assessment and Remediation and CRC CARE, University of South Australia, Mawson Lakes, SA.

^BCRC CARE, University of South Australia, Mawson Lakes, SA.

^CDirectorate of Environmental Impact Management, Department of Defence, Canberra ACT 2600.

^DCorresponding Author Email: peter.sanderson@postgrads.unisa.edu.au

Abstract

The Department of Defence operates shooting ranges across Australia to train soldiers in marksmanship and maintain their capabilities. It has been recognised that this practice can lead to significant contamination of the environment. Shooting ranges are characterised by high loading of bullets, which releases lead (Pb), antimony (Sb), and arsenic (As), copper (Cu), nickel (Ni) and zinc (Zn) and into the soil. Spent ammunition is subject to weathering in the soil; therefore intact bullets represent a significant source for Pb release. Lead concentrations exceeding 10,000 mg Pb/kg soil are commonly reported at shooting ranges around the world. The concentration of Pb including intact bullets may be upward of 500,000 mg/kg.

Soil samples collected from the surface and subsurface of Defence shooting ranges around Australia have been analysed to characterise the distribution and bioavailability of metal contaminants. The highest concentration of soil Pb found in sieved surface samples was up to 81,000 mg/kg in stop butt. The range floors contained as little as 22 mg/kg. Concentrations in the subsurface were up to 21,000 mg/kg. Comprehensive characterisation and analysis of shooting range soils will serve as a basis for the management and remediation of contamination.

Key Words

Shooting range, lead, antimony, bioavailability, risk assessment.

Introduction

To defend Australian national interests Defence requires shooting ranges to train and maintain the capabilities of soldiers in marksmanship. It has been recognised that this practice can result in significant contamination of the local environment. Therefore, to continue the necessary operation of these ranges and ongoing use of the land requires a response to the issue of contamination. Lead poses a serious threat to human and ecosystem health, due to potential exposure and uptake of Pb, which remains near the surface of the soil (Ryan *et al.* 2004). It is toxic to humans having deleterious effects on organ systems, particularly the central nervous system, the kidneys and the blood (Tong *et al.* 2000). It is readily absorbed by and accumulated by plant roots, though translocation to shoots is relatively poor. Pb contamination has been reported to decrease microbial biomass and respiration and the biomass of enchytraeid worms (Rantalainen *et al.* 2006). Secondary contamination of fauna may also occur by consumption of contaminated plant material, soil organisms and other contaminated prey.

Contamination of Shooting Range soils

Lead contamination at shooting ranges has been under increased scrutiny in recent years, due to concern over the high intensity of Pb loading, which may become mobile through the weathering of spent bullets. In many countries Pb-based ammunition is a significant source of Pb pollution. Annual deposition by hunting and shooting is around 500 tons in Switzerland (Scheinost *et al.* 2006), 800 tons annually in Denmark (Jorgensen and Willems 1987) and up to 60,000 tons per year in the United States (Craig *et al.* 1999).

Spent ammunition is subject to weathering when it comes into contact with the soil or water. Metals may be oxidized to a number of secondary forms such as oxides and carbonates, which are soluble and serve as a source that can release labile metals into the soil. Though much of the research on shooting range contamination has focused solely on Pb, the other components of bullets Sb (a hardening agent), As (an impurity in the Pb), Cu (casing of the bullet), Ni and Zn (alloys with copper) in addition to Pb have been identified as contaminants of concern at shooting ranges (Peddicord and LaKind 2000). Although these metals are only present in small amounts in Pb-based bullets they can also accumulate at elevated levels in the soils (Table 1).

Studies on the distribution of contaminants in shooting ranges have focused on the characterization of the contamination and the effect of weathering processes, primarily with respect to Pb (Cao *et al.* 2003, Hardison *et al.* 2004, Duggan and Dhawan 2007). More recently studies have examined the distribution and weathering of Sb (Johnson *et al.* 2005, Scheinost *et al.* 2006). Hydrocerussite [(Pb(CO₃)₂(OH)₂] is the predominant weathering product of Pb in many of the ranges examined (Cao *et al.* 2003 and Duggan and Dhawan 2007). Other common minerals include cerussite [(PbCO₃)], massicot [(PbO)], and litharge [(PbO)] (Cao *et al.* 2003; Dermatas *et al.* 2006). In the presence of high P, Pb solubility may be controlled by the less soluble Pb phosphate (Cao *et al.* 2003).

Table 1. The highest levels of metals reported in Shooting Range Studies.

| Pb | Sb | As | Cu | Ni | Zn | |
|--------|-------|------|------|------|------|----------------------------------|
| 80935 | 4022 | | 552 | 37.5 | 79.8 | Knechtenhofer <i>et al.</i> 2003 |
| 515800 | 17500 | | 4450 | 770 | | Johnson <i>et al.</i> 2005 |
| 397840 | 845 | 1057 | 318 | | | Dermatas <i>et al.</i> 2006 |
| 167760 | 437 | | 817 | | | Spuller <i>et al.</i> 2007 |
| 156000 | 8230 | | 6200 | 1490 | | Robinson <i>et al.</i> 2008 |

Risk based management and Remediation

The need to manage the environment in a sustainable way in order to carry out operations now and into the future has been recognised by the Department of Defence, which operates shooting ranges at army bases around the country to train soldiers in marksmanship. This study determined the distribution and bioavailability of contaminants at Defence shooting ranges across Australia as it relates to differing soil types and soil properties to aid the implementation of best management practices at these sites. Remediation by in situ chemical stabilisation of metal contaminants was to be examined at these sites.

Methods

Soil samples were collected from several defence shooting range sites around Australia. Samples were taken at a depth of 0-10 cm, along a transect from the firing line (1.5, 31.5, 61.5 and 91.5m). A soil profile sample was taken just before the backstop berm to 100 cm depth using a soil core. Samples were also collected from the top, middle and bottom of the berm at the 25 cm depth and combined into a composite sample. A final sample was collected from 5 m behind the backstop berm. Samples were weighed and dried for 24 hours, and re-weighed to determine moisture content. Soils were classified using the Australian Soil Classification key. The soil physical and chemical properties (pH, EC Eh (redox potential) CEC, OC, DOC, Total P, metal content) were determined by standard methods. Soil pH and EC were measured in a 5:1 deionised (DI) water suspension and a 5 mmol/L CaCl₂ suspension, using as Orion pH/EC meter. CEC was determined by the USEPA 9080 ammonium method. The Walkley Black (1934) procedure was used to measure organic carbon and DOC was measured using the Zhou (2001) method and a total organic carbon analyser. The metal content was examined by microwave assisted acid digestion using the USEPA SW-846 Method 3051A. The digests were analysed by ICPMS according to SW-846 Standard Method 6010. Sequential Extraction (SE) by Tessier (1979) was used to determine contaminant distribution among the soil fractions. XRD was used to examine contaminant mineralogy.

Results

The distribution of metal contaminants at shooting ranges is predominantly associated with spent bullets in the impact berm. Soil properties play a large role in determining distribution of metal contaminants in shooting range soils in Australia. Concentrations of Pb may be as low as 22 mg/kg on the range floor and up to 81,000 mg/kg in the impact berm. Significant contamination of subsurface soil may also be found in more acidic soils, with concentrations of up to 21,000 mg/kg reported. Concentrations of TCLP leachable Pb were found to be as high as 300 mg/l. The highest reported concentrations of the other metallic elements were Sb 600, As 50, Cu 350, Ni 260, and Zn 100 mg/kg, which were associated with the highest densities of bullets.

Conclusions

This study indicates that concentrations of Pb were elevated in surface soils at defence shooting ranges, particularly at the impact berm. The speciation and distribution of metal contaminants in range soils reflect the soil properties which influence the weathering of spent bullets.

References

- Cao X, Ma LQ, Chen M, Hardison DW, Harris WG (2003) Lead transformation and distribution in the soils of shooting ranges in Florida, USA. *The Science of The Total Environment* **307**, 179-189.
- Craig JR, Rimstidt JD, Bonnaffon CA, Collins TK, Scanlon PF (1999) Surface Water Transport of Lead at a Shooting Range. *Bulletin of Environmental Contamination and Toxicology* **63**, 312-319.
- Duggan J, Dhawan A (2007) Speciation and Vertical Distribution of Lead and Lead Shot in Soil at a Recreational Firing Range. *Soil & Sediment Contamination* **16**, 351-369.
- Dermatas D, Cao X, Tsaneva V, Shen G, Grubb D (2006) Fate and behavior of metal(loid) contaminants in an organic matter-rich shooting range soil: Implications for remediation. *Water, Air, & Soil Pollution: Focus* **6**, 143-155.
- Hardison DW, Ma LQ, Luongo T, Harris WG (2004) Lead contamination in shooting range soils from abrasion of lead bullets and subsequent weathering. *Science of The Total Environment* **328**, 175-183.
- Johnson CA, Moench H, Wersin P, Kugler P, Wenger C (2005) Solubility of Antimony and Other Elements in Samples Taken from Shooting Ranges. *J Environ Qual* **34**, 248-254.
- Jorgensen SS, Willems. M (1987) The fate of lead in soils: The transformation of lead pellets in shooting range soils. *Ambio* **16**, 11-15.
- Knechtenhofer LA, Xifra IO, Scheinost AC, Fluhler H, Kretzschmar R (2003) Fate of heavy metals in a strongly acidic shooting-range soil: Small-scale metal distribution and its relation to preferential water flow. *Journal of Plant Nutrition and Soil Science* **166**, 84-92.
- Peddicord RK, LaKind JS (2000) Ecological and human health risks at an outdoor firing range. *Environmental Toxicology and Chemistry* **19**, 2602-2613.
- Rantalainen M-L, Torkkeli M, Strömmer R, Setälä H (2006) Lead contamination of an old shooting range affecting the local ecosystem -- A case study with a holistic approach. *Science of The Total Environment* **369**, 99-108.
- Robinson BH, Bischofberger S, Stoll A, Schroer D, Furrer G, Roulier S, Gruenwald A, Attinger W, Schulin R (2008) Plant uptake of trace elements on a Swiss military shooting range: Uptake pathways and land management implications. *Environmental Pollution* **153**, 668-676.
- Ryan JA, Scheckel KG, *et al.* (2004) Peer Reviewed: Reducing Children's Risk from Lead in Soil. *Environmental Science & Technology* **38**, 18A-24A.
- Scheinost AC, Rossberg A, Vantelon D, Xifra I, Kretzschmar R, Leuz A-K, Funke H, Johnson CA (2006) Quantitative antimony speciation in shooting-range soils by EXAFS spectroscopy. *Geochimica et Cosmochimica Acta* **70**, 3299-3312.
- Spuller C, Weigand H, Marb C (2007) Trace metal stabilisation in a shooting range soil: Mobility and phytotoxicity. *Journal of Hazardous Materials Stabilisation/Solidification Treatment and Remediation: Advances in S/S for Waste and Contaminated Land* **141**, 378-387.
- Tessier A, Campbell PGC, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry* **51**, 844-851.
- Tong S, Von Schirnding YE, Prapamontol T (2000) Environmental lead exposure: A public health problem of global dimensions. *Bulletin of the World Health Organization* **78**, 1068-1077.
- Walkley A, black IA (1934) An Examination of the Degtjareff Method for Determining Soil Organic Matter, and A Proposed Modification of the Chromic Acid Titration Method. *Soil Science* **37**, 29-38.
- Zhou LX, Wong JWC (2001) Effect of dissolved organic matter from sludge and sludge compost on soil copper sorption. *Journal of Environmental Quality* **30**, 878-883.

Distribution of arsenic species in solutions of as-contaminated flooding soils and the toxicity of arsenic to rice plants

Dar-Yuan Lee^A, Tzu-Huei Lin^A and Yi-Fang Wu^A

^ADepartment of Agricultural Chemistry, National Taiwan University, Taipei 106, Taiwan, R.O.C., Email dylee@ntu.edu.tw

Abstract

The mobility and toxicity of As species in soils is different. Therefore, understanding As species distribution is very important for evaluating the mobility and phytotoxicity of As in As-contaminated soils. In this study, the distribution of As species in solutions of a geogenic As-contaminated soil and three As(V)-spiked soils after flooding was determined and the toxicity of As to rice plants was investigated. Results showed that before flooding incubation, the major As species in solutions of tested soils was As(V). However, after flooding incubation, As(III) concentrations in solutions of tested soils increased with incubation time, as As(V) was reduced into As(III) under the flooding condition. Among the relationships between As species concentration and rice seedling growth, the soil NaH₂PO₄-extractable total As and height and dry weight of rice seedlings has the best fits for the dose-response mode and thus it is proposed for use as an index for the assessment of phytoavailability and phytotoxicity of As in As-contaminated paddy soils.

Key Words

As-contaminated paddy soils, As phytotoxicity, As speciation, HPLC-ICP-MS.

Introduction

Arsenic enters into soil and aquatic ecosystems from anthropogenic, geogenic, and biogenic sources. Long-term exposure to As has caused health problems. In some cases, As-contaminated water is used for irrigating rice crops, resulting in an accumulation of As in paddy soils and elevated uptake of As by rice plants (Xu *et al.* 2008). In addition, recent studies have shown that human As intake from consumption of rice can be substantial. Since the toxicity and mobility of As species is different, to have suitable methods to evaluate As speciation and the availability of As in paddy soils is very important for assessing the phytotoxicity of As-contaminated soils. In this study, the distribution and transformation of arsenic species in soil solutions of As-contaminated soils under flooded conditions was determined using HPLC-ICP-MS. The relationship between the As speciation in soils and toxicity of As to rice plants was also investigated.

Materials and Methods

Studied Soils

Four As-contaminated Guandu soils, containing different levels of As, 16 (Gd1), 83 (Gd4), 192 (Gd2), and 528 (Gd3) mg/kg, were used. In addition, three uncontaminated soils having various pH, Pinchen (Pc), Taikang (Tk) and Chenchung (Cf), spiked with six levels of As(V), 0, 30, 60, 120, 240, 480 mg/kg and underwent three wetting-drying cycles were also used in this study.

Preservation of flooding soil solutions

100 g Gd4 soil and 100 mL distilled water were put in a 250 mL centrifuge tube. After shaking for 30 minutes and incubating at 25°C for 6 days, soil solutions were used for testing the solution preservation method. Soil solutions were immediately added with or without phosphoric acid (10 mM) and the concentration of As species was determined using HPLC-ICP-MS one day later to investigate if the distribution of As species could be preserved by adding phosphoric acid.

Soil flooding incubation and soil solution analysis

Put 20 g studied soil and 20 mL distilled water in a 50 mL centrifuge tube (three replicates). After shaking for 30 minutes and incubating at 25°C for 0, 3, 6, 12, 24, and 42 days respectively, soil solutions were collected. One part of the solutions was preserved in 0.01 M H₃PO₄ immediately and used for determining As species, As(III), DMA, MMA and As(V), by using HPLC-ICP-MS. One part was preserved immediately in 5% HNO₃ and used for determining Fe, Mn, Si and P by using ICP-OES. Finally, the pH of soil solutions was also measured. All of the analyses were completed in one day.

Soil NaH₂PO₄-extractable As (Huang et al. 2006)

Put 2 g studied soil and 30 mL 0.5 M NaH₂PO₄ in a 50 mL centrifuge tube. After shaking for 2 hrs, soil solutions were collected and preserved with H₃PO₄ immediately. Then arsenic species [As(III), MMA, DMA, and As(V)] were determined by HPLC-ICP-MS.

Arsenic phytoavailability (phytotoxicity) to rice seedlings

Put 200 g studied soil and 200 mL distilled water in a 300 mL beaker (three replicates). After stirring for 30 minutes and incubating for 12 days, five rice seedlings (*Oryza sativa L.*) having four appeared leaves were transplanted to each beaker and grown for 30 days in a glass room. Then, the plant height, dry weight, and As uptake by shoot and root of rice seedlings were measured.

Results and Discussion

Preservation of flooded soil solutions

The change of color and thus the composition of the soil solutions were observed after soil solutions were taken from flooding soils. The total As concentration in the flooded soil solution without preserving with phosphoric acid was lower than that preserving with phosphoric acid (Table 1). It suggests that oxidation of Fe(II) to Fe(III)-oxide may occur after soil solutions taken from flooded soils, and Fe-oxide may adsorb As species, resulting in the change of total As concentration and distribution of As species in soil solutions. However, if soil solution was preserved with phosphoric acid immediately after it was taken from flooded soil, the As concentration and speciation would not vary (Table 2).

Table 1. Preservation of flooded soil solution with or without phosphoric acid (10 mM).

| | As(III) | DMA | MMA | As(V) | Total As |
|--|------------------|-------------|-------------|-------------|------------|
| | (-----µg/L-----) | | | | |
| Without H ₃ PO ₄ | 35.7 ± 2.7 | 1.28 ± 0.24 | ND | 2.84 ± 0.03 | 39.8 ± 2.9 |
| With H ₃ PO ₄ | 145 ± 9 | 1.46 ± 0.05 | 0.50 ± 0.02 | 11.6 ± 0.7 | 159 ± 9 |

Table 2. The recovery of As species spiked in flooded soil solution preserved with phosphoric acid (10 mM).

| | As(III) | DMA | MMA | As(V) | Total As |
|-----------------|------------------|-------------|-------------|------------|------------|
| | (-----µg/L-----) | | | | |
| Sample | 145 ± 9 | 1.46 ± 0.05 | 0.50 ± 0.02 | 11.6 ± 0.7 | 159 ± 9 |
| Standard spiked | 49.9 ± 1.4 | 49.1 ± 1.2 | 55.2 ± 2.3 | 49.9 ± 1.7 | 204 ± 7 |
| Spiked sample | 192 ± 1 | 50.8 ± 0.4 | 56.5 ± 0.6 | 63.9 ± 4.1 | 363 ± 3 |
| Recovery % | 92.5 ± 15.6 | 98.8 ± 0.6 | 101 ± 1 | 105 ± 7 | 99.3 ± 5.6 |

The total As concentration in soil solutions before flooded incubation

As shown in Figure 1, the concentration of total As in soil solutions increasing with the amounts of As(V) spiked for As(V)-spiked soils was found. In addition, the total As concentration in soil solution of Pc acid soil was lower than those of other two alkaline soils, Tk and Cf, at the same level of As(V) spiked. For As-contaminated Gd soils, the total As concentration in soil solutions was low although the Gd soils had high content of total As in soils.

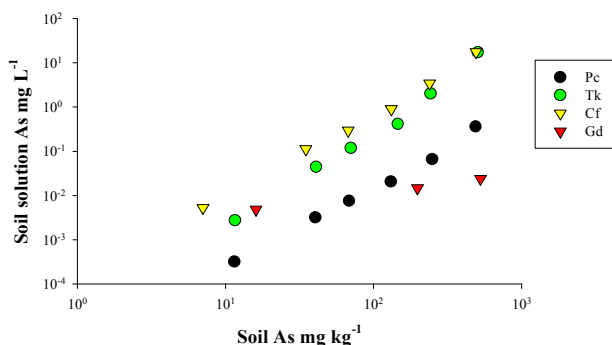


Figure 1. The relationships between the As content in studied soils and the total As concentration in soil solution before flooded incubation

Arsenic species in soil solutions before flooded incubation

The major As species in As-contaminated Gd soil was As(III). It may result from that the soils were used for paddy rice production for many years and they were under waterlogged and anaerobic conditions for a long

period of time. For the As(V)-spiked soils, the major As species in soil solutions of Tk and Cf alkaline soils was As(V) before flooded incubation. However, the percentages of As(V) in Pc acidic soil solutions were lower than those of Tk and Cf soils. It may be due to that in low pH the spiked As(V) was more easily adsorbed relative to As(III) that already existed in soils before As(V) spiking.

As concentration and speciation in soil solutions after 42 days flooded incubation

The total As concentrations in soil solutions of Gd soils increased greatly with flooding time. The main reason was that after flooding, soil Eh decreased and thus favoring the reduction of Fe/Mn-oxide into Fe⁺²/Mn⁺². Thus As adsorbed on Fe/Mn-oxide was released into soil solutions and thus the total As concentration increased. In addition, the As(V) was also favorably reduced into As(III) and thus the As(III) concentration increased after 42 days flooding. For As(V)-spiked soils, As(III) concentrations in soil solutions also increased with flooded incubation time. It suggests that As(V) was reduced to As(III) under the flooding condition.

Arsenic phytoavailability (phytotoxicity) to rice seedlings grown on soils

The relationships between the concentrations of total As, As(V) and As(III) in soil solutions (before flooding and after 42 days flooding) and rice seedling growth (plant height and dry weight) all were fitted well with the dose-response model (significant at $P = 0.01$). Among the dose-response relationships, the soil NaH₂PO₄-extractable total As with the heights and dry weights of rice seedlings has the best fit for the model and the effective toxicity concentration of decreasing 20% of rice plant heights (EC₂₀) was 78.7 mg As/kg (Figure 2). Therefore, soil NaH₂PO₄-extractable total As could be used as an index for the assessment of phytoavailability and phytotoxicity of arsenic in paddy soils. The results of the total As uptake in shoots and roots of rice seedlings were shown in Figure 3. It shows that As uptake ratio (root: shoot) is high which can be explained by the formation of iron plaque. The iron plaque was found on the roots of the rice seedlings grown in the tested soils, especially in the As-contaminated Gd soil. The iron plaque has high affinity for adsorbing As. That is why As uptake ratio (root: shoot) of As-contaminated Gd soil was higher than those of other studied soils. Therefore, the iron plaque may act as a barrier for uptake of As by rice plants.

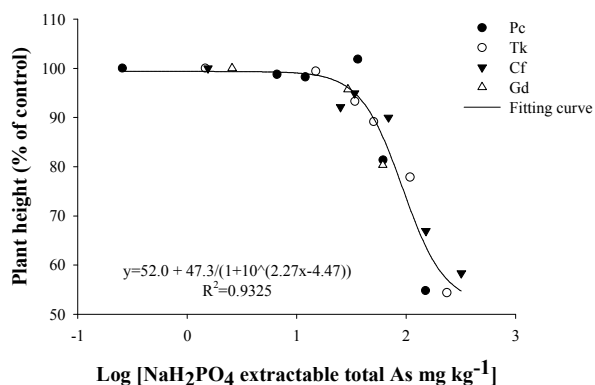


Figure 2. The dose-response relationship between the amounts of NaH₂PO₄-extractable total As and the plant heights of rice seedlings.

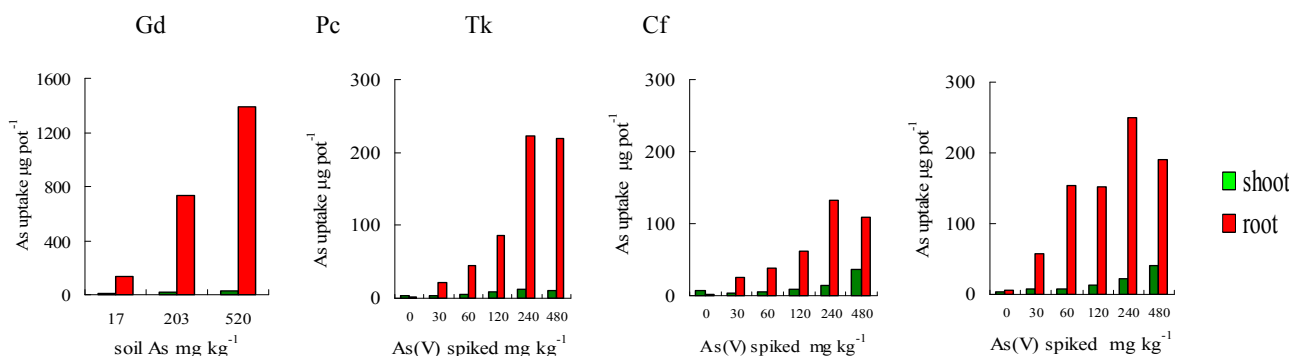


Figure 3. Total As uptake in shoots and roots of rice seedlings.

Conclusion

Phosphoric acid could be used as a preservation agent for preventing from the change of As species distribution in flooding soil solutions after the soil solutions are taken from soils for As species analysis. Before flooded incubation, the major As species in solutions of tested soils was As(V). However, after flooded incubation, As(III) concentrations in solutions of tested soils increased with incubation time, resulting from that As(V) was reduced into As(III) under the flooded condition. Among the relationships between As species concentration and rice seedling growth, the soil NaH_2PO_4 -extractable total As with the heights and dry weights of rice seedlings has the best fit for the dose-response mode and thus is proposed for using as an index for the assessment of phytoavailability and phytotoxicity of As in As-contaminated paddy soils. Iron plaque was found on the roots of rice seedlings and it may act as a barrier for uptake of As by rice plants.

References

- Huang RQ, Gao SH, Wang WL, Staunton S, Wang G (2006) Soil arsenic availability and the transfer of soil arsenic to crops in suburban areas in Fujian Province, southeast China. *Science of the Total Environment* **368**, 531-541.
- Xu XY, McGrath SP, Meharg AA, Zhao FJ (2008) Growing rice aerobically markedly decreases arsenic accumulation. *Environmental Science and Technology* **42**, 5574-5579.

Distribution pattern of heavy metals in soil fertilized with industrial sludge

Rozita Ahmad^A, Wan Rasidah Abdul Kadir^A and Rosazlin Abdullah^B

^AForest Plantation Programme, Biotechnology Forestry Division, Forest Research Institute of Malaysia (FRIM), 52109, Kepong, Malaysia, Email rozita@frim.gov.my, rashidah@frim.gov.my

^BDepartment of Land Management, Faculty of Agriculture, Universiti Putra Malaysia (UPM), 43400, Serdang, Selangor, Malaysia, Email rosazlin@yahoo.com

Abstract

Bio-solids waste from pulp and paper mill industries have been linked to containing various metals as contaminants from the chemicals added during the pulping process. Metal pollution of soils is of great environmental concern especially when using paper mill sludge (pms) for land application. The aim of this study is to investigate the distribution of heavy metals in soil after being amended with composted and raw PMS as organic fertilizer for 14 months. A field plot on a *Tectona grandis* or Teak plantation was established at FRIM Research Station in Mata Ayer, Perlis by the application of different rates of composted and raw paper mill sludge (PMS). Inorganic fertilizer and control plot were set up to compare the effectiveness of the PMS as sludge fertilizer. Soil samples were collected at two depths of 0-15cm and 15-30cm from the surface, with three distances, 0.5m, 1.0m and 1.5m from the Teak trees. The distribution of Cadmium (Cd) in soil increased with depth for every distance. However there were no clear trends observed for Arsenic (As) and Lead (Pb) for different layers and distances. The concentration levels of As and Pb in soils were found to be higher than Cd for all the treatments, but the values were within the permissible range of the Maximum Permitted Concentration (MPC) for land application.

Key Words

Soil, heavy metals, sludge, composted paper mill sludge, teak.

Introduction

Pulp and paper manufacturing mill in Malaysia generates substantial amount of bio-solids from different stages of paper making. These waste residues are categorized under hazardous toxic materials by the Department of Environment, Malaysia (DOE). The current practices of sludge disposal such as land spreading, landfilling and incineration are becoming unfavourable due to environment and economic considerations (Krigstin 2005). Safe management and environmental friendly approaches must be developed to utilize these waste materials. PMS can provide beneficial attributes to soil ecosystem and crop yield due to its physical properties and good plant nutrient content. Through composting, paper mill waste can also be biodegraded into organic soils. The purpose of this study is to determine leaching of heavy metals in soil after 14 months application with composted and raw PMS.

Materials and Methods

Raw and Composted Paper Mill Sludge

Raw paper mill sludge (RPMS) was collected from the biological treatment pond from paper mill in Selangor, Malaysia. The material obtained was wet with approximately 75% moisture content, sticky, compact, caking in nature and have strong unpleasant smell. Dried shredded oil palm fibers were added as enhancement material to provide and improve aeration of the sludge. Pile composting method was applied with ratio of 1:1 of sludge and oil palm fiber mixture and evenly turned for the production of composted paper mill sludge (CPMS). This final product has soil like structure with organic soil colour, friable with no unpleasant smell. Table 1 provides the properties of raw and composted PMS.

Field Plot

The study plot was established at FRIM Research Station in Mata Ayer, Perlis, Malaysia. The soil in site has pH of 6.62 with 0.12% N and 1.00 % C content (Rosazlin *et.al.* 2008). A total of 480 *Tectona grandis* seedlings were planted at 3m by 4m distance. Eight treatments were carried out and described in Table 2.

Soil Sampling and Chemical Analysis

Soil samples were taken using soil auger at two depths 0-15cm and 15-30cm from the surface, with three distances, 0.5m, 1.0m and 1.5m from Teak plants. This is to represent the application area, soil underneath

and surrounding of the treated site. Heavy metal elements in the soil were extracted using microwave digestion procedure followed the EPA 3051A method and concentration analysed on Varian 725 ICP-OES spectrometer. Duncan New Multiple Range Test (DMRT) was carried out to analyse the significant difference of each metals between the treatments. A one way ANOVA statistical analysis was also applied to test the significant difference in concentration of the elements between the depth and distance.

Table 1. Properties of composted and raw paper mill sludge.

| Parameter | Composted PMS | Raw PMS | Maximum Permitted permission (MPC) |
|-------------|---------------|---------|------------------------------------|
| pH | 4.74 | 7.84 | |
| Organic C % | 10.02 | 13.9 | |
| Total N % | 1.48 | 4.84 | |
| C/N ratio | 6.77 | 2.90 | |
| P % | 0.56 | 0.78 | |
| K % | 0.80 | 0.42 | |
| Ca % | 0.93 | 0.53 | |
| Mg % | 0.25 | 0.15 | |
| Cu ppm | 88 | 70 | 1,000 – 1,750 |
| Zn ppm | 188 | 153 | 2,500 – 4,000 |
| Mn ppm | 91 | 111 | |
| Pb ppm | 114 | 155 | 750 – 1,200 |
| Cd ppm | 0.21 | 0.29 | 20 – 40 |
| Fe ppm | 0.30 | 0.11 | |
| Al ppm | 0.88 | 0.90 | |
| As ppm | 8.07 | 15.54 | |

(Source: Wan Rashidah *et.al.*,2004)

Table 2. Application treatment carried out.

| Treatment | Application per plant | Treatment | Application per plant | Treatment | Application per plant |
|-----------|------------------------------------|-----------|-----------------------|-----------|-----------------------|
| T1 | Control | T3 | 20 kg N/ha CPMS | T6 | 40 kg N/ha RPMS |
| T2 | 50 kg N/ha Inorganic Fertilizer | T4 | 40 kg N/ha CPMS | T7 | 80 kg N/ha RPMS |
| | | T5 | 60 kg N/ha CPMS | T8 | 120 kg N/ha RPMS |

Result and Discussion

Distribution patterns of heavy metals between treatments

Concentration of As, Pb and Cd in soil at different depths and distances after 14 months of applying composted and raw PMS are presented in Tables 3, 4 and 5 respectively. Applications with inorganic fertilizer contribute the highest concentration of As compared to other treatments except at 1.0 m distance. The highest As detected at 1 m distance is obtained from application of 60 kg N/ha and 40 kg N/ha of composted PMS at 0-15 cm and 15-30 cm depths respectively. Further away from the sampling point which was at 1.5 m distance, high content of As was observed from treatment with 20kg N/ha of composted PMS at 15-30 cm soil layer compared to control and other sludge application. However, at the same distance, there is no significant difference between treatments for As at 0-15 cm soil layer. The results show that high amount of As leached out into the soil are obtained from composted PMS treatment. This could be attributed to the inorganic fertilizer added into the composted PMS treatment.

Lead values are found to be higher in both soil layers at 0.5m distance for all the sludge treatments compared to control except for application with 80 kg N/ha raw PMS. Further away from the sampling site in 15-30 cm soil layer at 1.0 m distance, higher Pb content is observed for all application with composted and raw PMS compared to control and inorganic fertilizer treatment. No significant difference was found between treatments at soil depth of 0-15 cm for 1.0 m and 1.5 m distance. At 1.5 m distance, highest Pb content was detected from application with 40 kg N/ha of composted PMS at 15-30 cm soil horizon. The results indicated that there is a release of Pb from all the sludge application in soil depth of 15-30 cm soil at 1.0 m distance after 14 months treatment but the concentration are within the accepted MPC range. Addition of organic admendments containing a proportion of organic matter can reduced the mobility of heavy metals through formation of insoluble organic matter and metal complexes. On the other hand, these metal complexes can become soluble releasing the metals into the soil at high pH values (Killbride 2004). In this investigation, there was no significant difference of Cd in soil between any of the treatments when the results were compared to control which has no sludge and fertilizer treatment. This suggested that application of PMS did not lead to an increase in Cd content in soil.

Table 3. As content in soil taken at different depths and distances after 14 months application with composted and raw PMS.

| Treatment | 0.5 m distance | | 1 m distance | | 1.5 m distance | |
|--------------------------|----------------|------------|--------------|------------|----------------|-----------|
| | 0-15 cm | 15-30 cm | 0-15 cm | 15-30 cm | 0-15 cm | 15-30 cm |
| T1- control | 7.216 b | 10.217 bc | 8.074 ab | 10.624 abc | 8.183 a | 9.811 b |
| T2- inorganic fertilizer | 13.078 a | 14.079 a | 11.312 a | 12.549 ab | 11.675 a | 15.234 a |
| T3 – composted PMS | 6.756 b | 9.493 bc | 7.269 b | 10.989 abc | 9.151 a | 11.454 ab |
| T4 – composted PMS | 8.507 ab | 7.175 c | 5.759 b | 13.586 a | 7.243 a | 7.179 b |
| T5- composted PMS | 4.265 b | 10.725 abc | 11.397 a | 9.832 abc | 9.660 a | 9.947 b |
| T6 – raw PMS | 8.677 ab | 10.910 ab | 8.341 ab | 9.851 abc | 7.636 a | 10.045 b |
| T7 – raw PMS | 6.540 b | 8.874 bc | 6.313 b | 6.591 c | 9.616 a | 8.375 b |
| T8 – raw PMS | 6.433 b | 8.115 bc | 7.594 ab | 7.990 bc | 7.932 a | 7.723 b |

Table 4. Pb content in soil taken at different depths and distances after 14 months application with composted and raw PMS.

| Treatment | 0.5 m distance | | 1 m distance | | 1.5 m distance | |
|--------------------------|----------------|-----------|--------------|-----------|----------------|-----------|
| | 0-15 cm | 15-30 cm | 0-15 cm | 15-30 cm | 0-15 cm | 15-30 cm |
| T1- control | 6.339 ab | 7.354 b | 8.955 a | 8.094 b | 9.805 a | 12.209 ab |
| T2- inorganic fertilizer | 6.218 ab | 13.631 a | 6.449 a | 7.733 b | 2.565 a | 6.239 c |
| T3 – composted PMS | 9.431 a | 9.245 ab | 8.880 a | 13.385 a | 9.741 a | 10.520 ab |
| T4 – composted PMS | 8.789 a | 9.709 ab | 9.102 a | 9.222 ab | 8.661 a | 12.814 a |
| T5- composted PMS | 10.349 a | 9.796 ab | 10.426 a | 11.600 ab | 8.542 a | 11.773 ab |
| T6 – raw PMS | 9.381 a | 10.324 ab | 10.174 a | 9.593 ab | 7.203 a | 7.958 bc |
| T7 – raw PMS | 3.894 b | 7.302 b | 6.845 a | 9.671 ab | 7.939 a | 8.159 bc |
| T8 – raw PMS | 8.192 a | 10.723 ab | 7.727 a | 8.196 ab | 10.507 a | 8.301 bc |

Table 5. Cd content in soil taken at different depths and distances after 14 months application with composted and raw PMS.

| Treatment | 0.5 m distance | | 1 m distance | | 1.5 m distance | |
|--------------------------|----------------|----------|--------------|----------|----------------|----------|
| | 0-15 cm | 15-30 cm | 0-15 cm | 15-30 cm | 0-15 cm | 15-30 cm |
| T1- control | 3.628 a | 5.499 a | 4.083 a | 5.815 a | 4.495 a | 5.300 a |
| T2- inorganic fertilizer | 4.804 a | 5.707 a | 3.673 a | 4.604 a | 3.471 a | 4.741 a |
| T3 – composted PMS | 3.991 a | 4.398 a | 3.716 a | 4.432 a | 3.364 a | 4.398 a |
| T4 – composted PMS | 3.929 a | 5.533 a | 4.127 a | 5.929 a | 4.207 a | 5.716 a |
| T5- composted PMS | 4.542 a | 5.651 a | 5.235 a | 5.923 a | 4.499 a | 5.563 a |
| T6 – raw PMS | 4.297 a | 6.093 a | 4.328 a | 5.310 a | 3.988 a | 5.449 a |
| T7 – raw PMS | 4.748 a | 5.804 a | 4.706 a | 5.705 a | 4.737 a | 4.934 a |
| T8 – raw PMS | 4.036 a | 5.277 a | 3.983 a | 4.756 a | 4.096 a | 4.161 a |

Letter with the same alphabet list in the column for the different treatments are not significant different at $p < 0.05$, according to the Duncan New Multiple Range Test (DMRT)

Distribution trends of heavy metals between soil depth and distance

Analysis of Variance tabulated in Table 6 indicated significant difference for Cd in soil between the two soil layers and distances. Concentration of Cd is observed to be higher in 15-30 cm soil layer compare to the upper soil horizon for all treatments applied. The distribution of Cd in soil increased with increasing depth for every distance. But Cd content did not show any clear trends with increasing distances.

No significant difference is observed for As and Pb concentration between the two soil layers and distances. These two elements did not exhibit any obvious pattern on the distribution in different depths and its surroundings. However the concentration levels of As and Pb in soils were found to be higher than Cd for all the treatments but the values did not exceed the Maximum Permitted Concentration (MPC) for land application.

Table 6. Statistical variation (Anova) for As, Cd and Pb between the two soil layers and distances.

| Metal | F | P-value | F crit | P-value < 0.05 indicate significant difference |
|-------|-------|----------|--------|--|
| As | 1.96 | 0.105 | 2.44 | Not significant |
| Pb | 1.43 | 0.235 | 2.44 | Not significant |
| Cd | 10.89 | 9.21E-07 | 2.44 | Highly significant |

Conclusion

The data obtained in this study demonstrate that application of PMS did not lead to an increase in heavy metals concentration in soil. There were no clear pattern to relate the leaching of metals to the lower soil layer and its surrounding.

Acknowledgement

The authors would like to thank FRIM and MOA for the financial support and permission in presenting this paper. The help from all staff of Soil Chemistry Laboratory, FRIM and students from Universiti Malaysia Terengganu are greatly appreciated. Special thanks to FRIM and MOSTI for the financial support through Science Fund Grant in running this project.

Reference

- Commission of the European Communities (EU) (1986) Council directive (86/278/Eec) on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. *Official Journal L* **181**, 0006 – 0012.
- Killbride C (2004) 'Application of Sewage Sludges and Composts. Best Practice Guidance for Land Regeneration'. Note 6.(The Land and Urban Greening Research Group: Forest Research, Surrey).
- Krigstin S, Sain M (2005) Characterization and potential utilization of recycled paper mill sludge. In 'Natural Fiber and Bio-Composite Group, Faculty of Forestry Earth Sciences, Ontario. PAPTAC 91st Annual Meeting'.
- Method 3051A (2007) Microwave assisted acid digestion of sediments, sludges, soils and oils. SW-846. In 'Test Methods for Evaluating Solid Waste, Physical/Chemical Methods'. Revision 1.
- Rosazlin A, Wan Rasidah K, Che Fauziah I, Wan Asma I, Rozita A (2008) Heavy metas in soil applied with composted and raw paper mill sludge. In 'Proceeding 14th International Conference on heavy metals in the environment. Taipei, Taiwan'.
- Wan Rasidah K, Mahmudin S, Wan Asma I, Rosazlin A (2004) Properties of sludge from paper mill processing recycle paper. In 'Proceedings of the 7th Pacific Rim Bio-Based Composites Symposium, Nanjing, China' Vol.II. (Eds Zhou X *et.al.*) pp.53-58.

Dual metal solution mapping study of competitive adsorption between cadmium and zinc in soil

Hui Ming^{A,B}, Ravi Naidu^{A,B}, Dane T. Lamb^{A,B} and Mallavarapu Megharaj^{A,B}

^ACentre for Environmental Risk Assessment and Remediation, University of South Australia, Mawson Lakes Campus, Mawson Lakes, SA 5095, Australia.

^BCRC CARE - Cooperative Research Centre for Contamination Assessment and Remediation of the Environment, Mawson Lakes, SA 5095, Australia.

Abstract

Coherent common heavy metal Cd(II) and Zn(II) adsorption between two virgin Australian soils with different pH are studied using solution mapping in the metal concentration range up to 2×10^{-4} mol. Zn exhibited a greater affinity in single metal soil sorption system with alkaline soil PB than Cd but only marginal better in distribution behaviour in acidic soil AT. While in the competitive dual metal sorption system, the sorption preference for both metals in alkaline soil is Zn slightly over Cd, but Cd slightly over Zn in acidic soil. Furthermore, in dual metal sorption system, variations in sorption behaviour strongly in acidic soil but weakly in alkaline soil were observed suggesting competition of two metals on surfaces being dependent upon the nature of the soil.

Key Words

Adsorption, competition, soil, solution mapping, cadmium, zinc.

Introduction

Cadmium (Cd) and zinc (Zn) are heavy metals with similar chemical characteristics and mineralogical origin. Much of the physiological effect of cadmium has been attributed to chemical similarity to zinc (Hamon *et al.* 1998). Soil contamination by both metals resulted from adsorption may impact on the plant growth process and may later enter into the food chain thus posing a great health risk to humans (Alloway 1990). Cd is more mobile in the sorption process than Zn and tends to form stable ionic species in soil solution at a wide range of soil pH. Due to heterogeneity of the real surface, sorption affinity varies across the surface. The high affinity sorption sites will be occupied at low concentration, whereas at higher concentrations sites of lower affinity are also occupied. The partition coefficient of metal in soil is not constant, but depends on the conditions in the solid and the solution (Sauve *et al.* 2000). Furthermore, metal adsorption can vary significantly between the competitive systems (multiple metals present in a soil) and monometal adsorption systems (the corresponding individual metal in the same soil) (Christensen 1984).

In this work, metal a solution mapping method was used to examine adsorption behaviour of a Cd and Zn in either none competitive sorption, i.e., mono metal system or competitive dual metal system on two Australian soils with different acidity. The variation in distribution behaviour of Cd and Zn in mono and dual metal adsorption systems can be attributed to the competition between both metals in soil systems..

Methods

Materials

Two virgin soils, one acidic loamy soil and one alkaline sandy soil, were collected from different location in Australia. Both soils were sampled vertically between 0-20 cm from the A horizon. The collected samples were air dried, sieved to 2 mm fraction and stored in plastic containers in an ambient temperature before used for this study. Prior to the adsorption study, characterization of both soils was conducted (Table 1) to gain general knowledge on both soils and to ensure minimum interference from the soil substrates for adsorption results.

Sorption study

Batch studies of heavy metal sorption on both soils were performed according to widely used procedures (Ravi *et al.* 1994). 1.000 gram of less than 2 mm soil was weighed into a 50ml PE centrifuge tube followed by adding 30 ml 0.01 M $\text{Ca}(\text{NO}_3)_2$ as background electrolyte containing various concentrations of individual metal ion from 0 to 2×10^{-4} M. Samples were then equilibrated using a spinning shaker for 24 h at 25 °C. At the end of the shaking period, the suspensions were centrifuged at 3000 rpm for 30 min and filtered through

Table 1. General characteristics of the soil.

| Sample name | Unit | PB | AT |
|-----------------|----------------------------|----------|----------|
| Soil type | | Alfisols | Ultisols |
| pH | in H ₂ O | 7.95 | 3.80 |
| | in 0.01M CaCl ₂ | 8.44 | 5.34 |
| EC | uS/cm | 520 | 26 |
| CEC | cmol/kg | 11 | 9.47 |
| Surface Area | m ² /g | 9.97 | 0.91 |
| Organic Content | % | 1.32 | 0.56 |
| Clay content | Clay % | 8.46 | 19 |
| | Silt % | 0.53 | 39 |
| | Sand % | 91 | 42 |
| Soil Texture | | Sandy | Loamy |

a 0.22 mm Millipore filter before metal analysis. For both mono metal and mixed dual metal sorption experiments, the batch study for a particular concentration of metal was conducted simultaneously on all soils, while also using a blank sample (no soil), so that the results would be comparable. The solution before and after sorption was analysed by an Agilent 7500C inductively coupled plasma-mass spectrometer (ICP-MS) (Agilent, USA).

Calculation

For monometal adsorption study, sorption at equilibrium was fitted using the Freundlich adsorption isotherm expressed as follows:

$$C_{\text{sorb}} = k(C_{\text{soln}})^n$$

Where, $C_{\text{Pb}_{\text{sorb}}}$ is the sorbed Pb concentration in soil and $C_{\text{Pb}_{\text{soln}}}$ is the Pb concentration in solution phase. k is the slope constant and also estimated to be the partition coefficient K_d (K_d ; also known as the sorption distribution coefficient). n is a constant for a particular sorption system. For competitive dual metal adsorption, 3D graphs were used to display sorption behaviour as Freundlich adsorption isotherm is no longer suitable to describe the sorption behaviour of a competitive metal sorption.

Results

Adsorption isotherms of Cd and Zn in single metal adsorption systems are listed in Figure 1. Both metals sorption behaviour in either acidic AT or in alkaline PB are generally well fitted with Freundlich adsorption equation with correlation coefficients are all close to 1 except Zn in acidic soil AT. In the acidic soil AT, the of both metals uptake by soil showed a great mobility in solution phases with only small difference in which Zn was just slightly higher bondable to soil ($K_d=9.640$ L/kg) than Cd did ($K_d=1.211$ L/kg). In contrast, Cd and Zn uptake in alkaline soil PB showed significant changes in sorption behaviour to those in acidic soil as an increase in metal concentration in solution resulted in decreasing slope of the sorption curve. The mobility of both Cd and Zn in alkaline soil PB was reduced dramatically. The difference in partition coefficient of Cd and Zn, i.e., the distribution ratio between solid and soil solution, in alkaline soil is 174 and 180 times greater than for corresponding metals uptake by acidic soil. The difference for Zn and Cd uptake in acidic soil was relatively small, but it became much larger for both metals in the alkaline soil PB which showed about 15 times more Zn adsorbed in PB than for Cd.

The variation in metal concentration in soil phases among mixed dual metal adsorption in acidic soil and alkaline soil are displayed as 3D graphs in Figure 2. In addition, a mesh surface provides information regarding the trend of variation in individual metal concentration in the system. The distribution behaviour of both metals in solid phase in alkaline soil displayed a similar behaviour. If the total Zn concentration in soil remains constant, increase total Cd concentration in soil will result in increases in amount of Zn or Cd sorbed in soil. The similar trend was also observed when total Cd concentration remains constant with increasing total Zn concentration. At constant high concentration of total Cd; however, uptake of Cd soil was deviated slightly from a linear increase with a slight drop in uptake but increased as total Zn concentration increased further. In fact, a similar variation in Zn uptake in soil at high total Zn concentration was also observed at a much smaller extent. This kind of variation beyond normal adsorption behaviour (single metal adsorption) implies interference from other factors to reduce the sorbed metal species in the soil phase. Much greater variation in the soil phase for uptaked both metal were exhibited. When the total Zn concentration in the

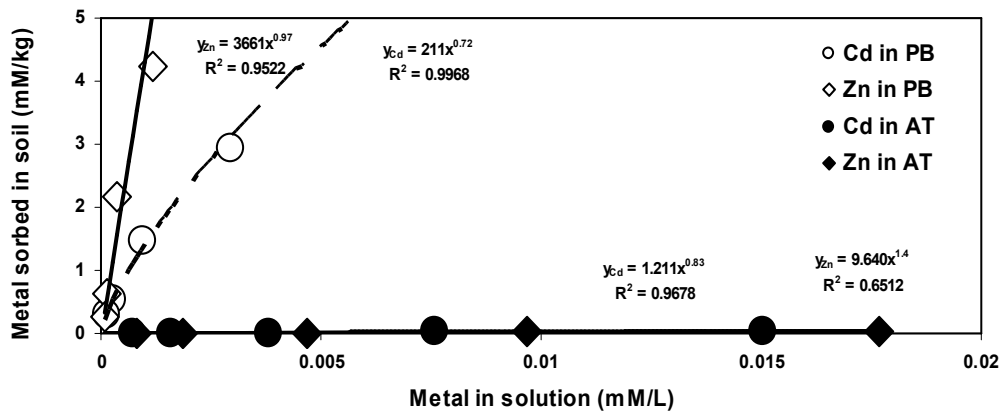
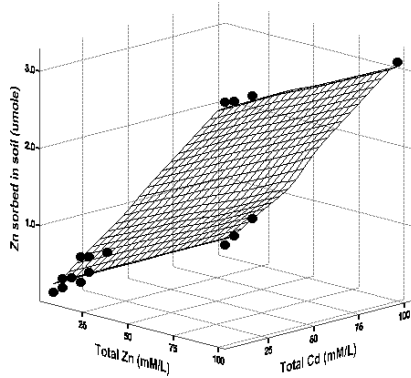
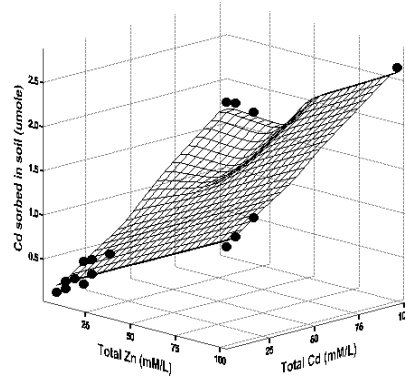


Figure 1. Variation in adsorption behaviour of single metal Cd and Zn sorbed on acidic soil AT and alkaline soil PB.

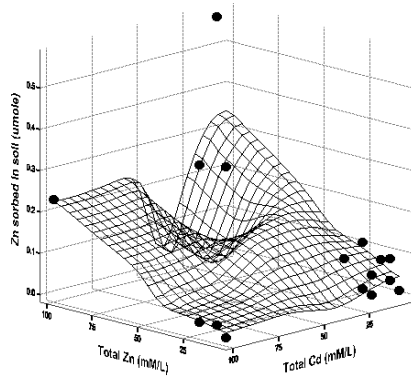
system remains constant with increase in total Cd concentration, the sorbed Zn will decrease slowly at low total Zn concentration but drop dramatically in uptake of Zn when the total Zn concentration becomes high (10^{-4} M total Zn). Under the similar condition for both Cd and Zn, the Cd sorption will generally increase. In contrast, when total Cd concentration remain constant with an increase in total Zn concentration, the adsorbed Zn in soil will increase nearly linearly at low total Zn concentration until total Cd concentration become very high (10^{-4} M Zn) with a big jump in adsorbed Zn. Adsorbed Cd in soil under the same conditions did not change a lot until the total Cd concentration was very high (10^{-4} M) so that Cd uptake changed from very high to dropping sharply and increasing again at high total Zn concentration (Figure 3D). As no such a distribution behaviour occurred for the single Cd sorption system or at low total Cd concentration, it is most likely due to present of large amount of Zn in soil preventing the normal adsorption process, i.e. metal competition in the sorption system.



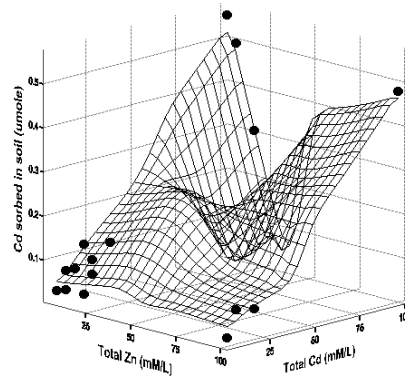
Zn sorbed in alkaline soil



Cd sorbed in alkaline soil



Zn sorbed in acid soil



Cd sorbed in acid soil

Figure 2. Cd-Zn competition for acidic soil and alkaline soil

Conclusions

The adsorption behaviour of Cd(II) and Zn(II) onto two virgin Australian soils with different pH was studied. Zn exhibited a greater affinity with alkaline soil PB ($K_d=3661$ kg/L) than Cd ($K_d=211.1$ kg/L) but much lower reactivity in acidic soil AT with only a marginal difference in distribution behaviour between Zn ($K_d=9.641$ kg/L) and Cd ($K_d=1.211$ kg/L). For the competitive dual metal sorption system, the sorption preference for both metals in alkaline soil is Zn slightly over Cd, but Cd slightly over Zn in acidic soil. Furthermore, for the dual metal sorption system, variations in sorption behaviour were strong in acidic soil but weakly in alkaline soil suggesting competition of two metals was dependent upon the nature of the soil.

References

- Hamon RE, Mclaughlin MJ, Naidu R, Correll R (1998) Long-term changes in cadmium bioavailability. *Environ. Sci. Technol.* **32**, 3699-3703.
- Alloway BJ (1990) 'Heavy Metals in Soils'. (New York: JohnWiley and Sons).
- Sauve S, Hendershot W, Allen HE (2000) Solid-solution partitioning of metals in contaminated soils: dependence on pH, total burden and organic matter. *Environ. Sci. Technol.* **34**, 1125-1131.
- Christensen T (1984) Cadmium soil sorption at low concentration: II Reversibility, effect of changes in solute composition and effect of soil aging. *Water Air Soil Pollut.* **21**, 115-125.
- Naidu R, Bolan NS, Kookana RS, Tiller KG (1994) Ionic-strength and pH effects on the sorption of cadmium and the surface-charge of soils. *Euro. Soil* **45**, 419-429.

Effect of calcined eggshell on fractional distribution and plant uptake of Cd, Pb and Zn in contaminated soils near mine

Rog-Young Kim^A, Jwa-Kyung Sung^A, Seok-Cheol Kim^A, Byoung-Choon Jang^A, and Yong-Sik Ok^B

^ANational Academy of Agricultural Science, RDA, Suwon, Republic of Korea, rogkim@korea.kr

^BDepartment of Biological Environment, Kangwon National University, Chuncheon, Republic of Korea, soilok@kangwon.ac.kr

Abstract

The fractional distribution of Cd, Pb and Zn in a soil contaminated by mine tailings and their uptake to lettuce (*Lactuca sativa* var. *longifolia*) were investigated after application of varying amounts of calcined eggshell (0, 1, 3, and 5 %). The experiments were conducted under greenhouse conditions during a 30-d period. A sequential extraction method was used to determine the binding forms of these metals in soils. The application of calcined eggshell decreased particularly the mobile fraction of these metals: from 43.3 to 0.2 % for Cd, from 4.8 to 1.4 % for Pb and from 5.4 to 0.4 % for Zn. Instead, the easily exchangeable fraction of Cd and the organically bound fraction of Pb and Zn were increased. This alteration of binding forms resulted in a diminished uptake of Cd, Pb and Zn by lettuce. The ratios of Cd, Pb and Zn uptake from soils to roots (total contents) decreased from 0.25 to < 0.01 for Cd, from 0.01 to < 0.001 for Pb and from 0.27 to 0.16 for Zn. The results suggested that the calcined eggshell can be used as an effective immobilization agent for the remediation of Cd, Pb and Zn contaminated soils.

Key Words

Heavy metals, contamination, stabilisation, transfer factor, waste material.

Introduction

Agricultural soils surrounding mining sites in South Korea are often contaminated with multiple metals such as Cd, Pb and Zn. It poses potential risks to plants, groundwater, and eventually human health. The contents of heavy metals in plants grown on those soils often exceeded the safe limits of Codex standards (1995; 0.2 and 0.3 mg/kg for Cd and Pb, respectively). For the remediation of those contaminated soils, the reuse of waste materials such as calcined eggshell can represent an environmentally-friendly and cost-effective alternative. Therefore, the objective of this study was to evaluate the effectiveness of calcined eggshell on the immobilization of Cd, Pb and Zn in soils using the sequential extraction method, because binding forms of heavy metals determine the plant availability and the potential for environmental contamination.

Materials and Methods

Greenhouse experiments

Soils were collected from a contaminated agricultural field (0 - 30 cm) near the closed mine Seosung, located in Seosan, the western South Korea. The soil samples showed a pH(H₂O; 1: 5) of 5.9, a OC content of 14.9 g/kg and a CEC of 11.4 cmol_e/kg. Total contents of Cd, Pb and Zn were 8.04, 1590 and 645 mg/kg (aqua regia digestion). The soil texture was clay loam. Four levels of calcined eggshell (ES 0, 1, 3, 5 %), which was prepared by calcination of raw eggshell at 900 °C (CaCO₃ → CaO + CO₂ (g); Ok unpublished), were thoroughly mixed with soil samples. The mixed soil samples were placed in each pot (500-600 g a pot) in the greenhouse, brought to field capacity and incubated for three days. Each treatment was replicated twice. Seedlings of lettuce (4 weeks after germination) were transplanted into each pot and the plants were grown for 30 days in the greenhouse at 25 ± 2 °C. At harvest, leaves, roots and the soils were separated.

Soil and plant analyses

The leaves and roots dried at 85 °C were finely ground and portions (ca. 0.5 g) were digested with HNO₃ + H₂SO₄ + HClO₄ (10: 1: 4; 200 °C). Soil samples were air-dried and subjected to sequential extraction according to Zeien and Bruemmer (1989): (I) mobile fraction (NH₄NO₃), (II) easily mobilizable fraction (NH₄OAc), (III) in Mn-oxides occluded fraction (NH₂OH-HCl + NH₄OAc), (IV) organically bound fraction (NH₄-EDTA), (V) in poorly crystalline Fe-oxides occluded fraction (NH₄ oxalate buffer), (VI) in well crystalline Fe-oxides occluded fraction (ascorbic acid in 0.2 M NH₄ oxalate) and (VII) residual fraction (conc. HNO₃/conc. HClO₄). The extraction was carried out in three replicates. All metals in extracts were determined using a GBC Integra XL Dual ICP-OES.

Results

Fractional distribution of Cd, Pb and Zn in soils

Figure 1 showed that the dominant fraction of Cd was the mobile fraction (43.3 %) in soils without application of calcined eggshell (ES 0 %), whereas Pb was found mainly in the organically bound fraction (31.7 %) and Zn in the residual fraction (59.5 %). Increasing the application of calcined eggshell from 0 to 5 % decreased particularly the mobile fraction of all metals: from 43.3 to 0.2 % for Cd, from 4.8 to 1.4 % for Pb and from 5.4 to 0.4 % for Zn. Instead, the easily exchangeable fraction of Cd (up to 63.8 %) and the organically bound fraction of Pb (up to 52.5 %) and Zn (up to 6.4 %) were increased.

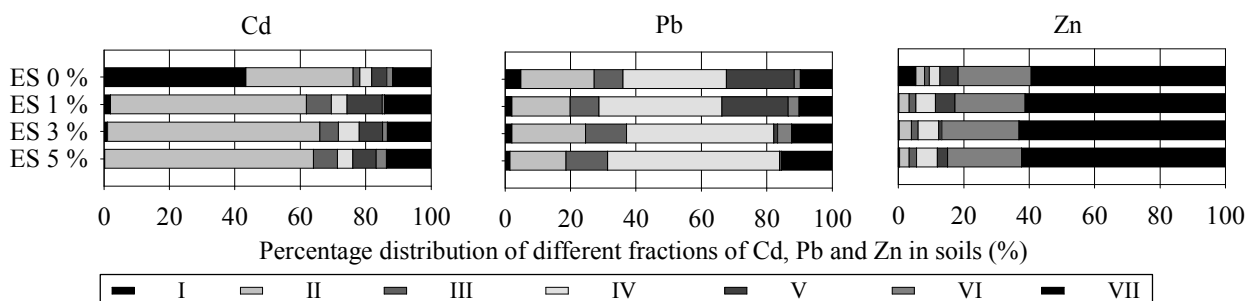


Figure 1. Percentage distribution of Cd, Pb and Zn fractions in contaminated soils with and without application of calcined eggshell (ES: calcined eggshell; I: mobile, II: easily mobilizable, III: in Mn-oxides occluded, IV: organically bound, V: in poorly crystalline Fe-oxides occluded, VI: in well crystalline Fe-oxides occluded, VII: residual fraction)

Plant uptake

Figure 2 presents the total contents of Cd, Pb and Zn in leaves and roots of lettuce. The Cd, Pb and Zn contents without application of calcined eggshell (ES 0 %) were 4.19, 0.78 and 133 mg/kg in leaves and 2.08, 12.9 and 180 mg/kg in roots, respectively. Increasing the amount of calcined eggshell from 0 to 5 % lowered the contents of all metals both in leaves and roots. The ratios of Cd, Pb and Zn uptake from soils to roots decreased from 0.25 to < 0.01 for Cd, from 0.01 to < 0.001 for Pb and from 0.27 to 0.16 for Zn. The translocation factors from roots to leaves revealed that Cd (2.0) was mainly accumulated in leaves, whereas Pb (< 0.1) and Zn (0.7) were mainly accumulated in roots.

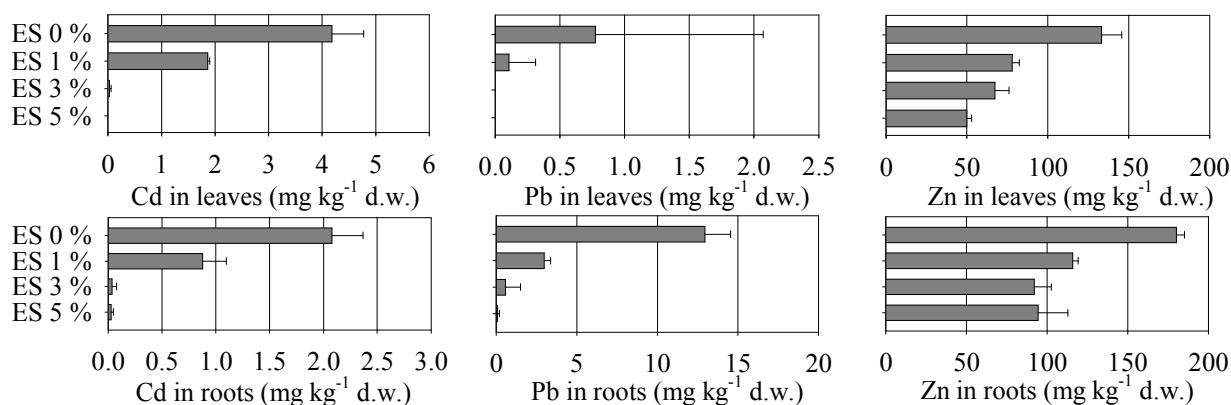


Figure 2. Cd, Pb and Zn uptake in leaves and roots of lettuce from contaminated soils with and without application of calcined eggshell (ES: calcined eggshell)

Conclusion

The application of calcined eggshell altered the mobile fraction of Cd, Pb and Zn to less mobile or immobile fraction in soils by increasing the soil pH. This resulted in a diminished uptake of Cd, Pb and Zn to lettuce. The calcined eggshell can be considered as an effective immobilization agent for remediation of Cd, Pb and Zn contaminated soils.

References

- Codex Alimentarius Commission (1995) General Standard for Contaminants and Toxins in Food and Feed CODEX STAN 193, amended 2009, pp. 32-33.
- Zeien H, Bruemmer GW (1989) Chemische Extraktion zur Bestimmung von Schwermetallbindungsformen in Böden. *Mitt. Dt. Bodenkundl. Ges.* **59**, 505-510 (in German).

Effect of flue gas desulfurization waste on corn plants

S. Ebanks^A, S. Paramasivam^A, A. Alva^A and K. Sajwan^A

^ADepartment of Natural Sciences, Savannah State University, Savannah, GA 31404, USA.

^BVegetables and Crops Research Laboratory, USDA-ARS, Prosser, WA 99350, USA.

Abstract

The results of this research indicated that any increase beyond 1% FGD waste to soils an amendment would not be ideal for corn plants. However, other crops should also be tested for the phytotoxic effects of FGD wastes.

Introduction

Flue gas desulfurization (FGD) waste results from the addition of limestone and/or dolomite to the coal either before (in the case of fluidized bed combustion) or after (in the case of flue gas desulfurization) combustion. These wastes are typically a combination of ash and various Ca, Mg, and S compounds (Sajwan *et al.* 1999). Similar to fly ash these wastes also contain several trace elements (Sajwan *et al.* 1999). Like fly ash, FGD sludge contains high concentrations of soluble salts and high pH (Sajwan *et al.* 1993). As with fly ash, the principal concerns with FGD disposal include groundwater contamination from leachate, and elevated concentrations of trace elements in plants and soils in disposal area (Santhanam *et al.* 1979). It has been reported that that FGD waste could be used as a source of B and Se for soils deficient in these elements (Santhanam *et al.* 1979). This study investigated the effect FGD waste on biomass production and selected elemental uptake by corn plants.

Materials and methods

This study was conducted under greenhouse conditions. The FGD was collected from a Coal Fired Power plant in Cope, South Carolina. The FGD was weathered by flushing it through water periodically for over a month. The Ogeechee loamy sand soil (pH of 5.5) used in this study. The FGD was added at 0 (T1), 1% (T2), 2% (T3), 4% (T4), 6% (T5), 8% (T6), and 10 % (T7) by weight to 6 Kg soil in pots and blended thoroughly. The experiment was arranged in complete randomized block design with three replications. Pots were incubated for two weeks at field capacity moisture content. After two weeks of soil incubation corn seeds (*Zea mays L.*, var. Pioneer 3165) were sown in each pot. All the pots were maintained at field capacity moisture content throughout the six week growth period. Plant visual symptoms were also monitored for signs of any stress, growth abundance or lack of growth as a result of FGD applications. At the end of the six week growth period above ground plant parts were harvested, oven dried, weighed and analyzed for elemental composition.

Results and discussion

Dry matter yield of six-week-old maize started to decrease with the second dose of FGD (2%) and the lowest yield was obtained in treatment with the highest dose of FGD (10%). Increasing level of FGD caused a steady decline in dry matter yield, with the highest treatment (10%) approximately half the biomass of the control plants. No visual metal toxicity symptoms in plants were observed. Our preliminary assessment has shown that up to 1% FGD could be used as effective soil amendment. The chemical analysis of plant tissue indicated that As, B, and Se concentrations significantly increased as the FGD rate was increased from 1% to 10%. The As, B and Se concentrations increased respectively from 1% to 10% FGD applications. The Mo and Cr levels in plant tissue were below the detection limit.

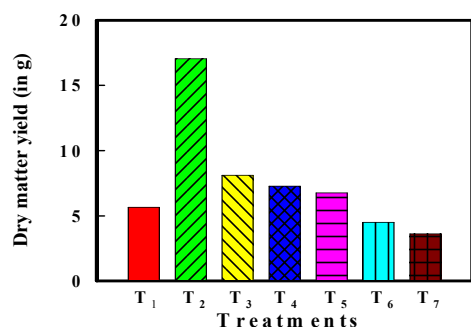


Figure 1. Dry matter yield under various FGD treatments.

Table 1. As, B, Se, Mo, and Cr in plant tissues under various FGD treatments.

| Treatments | As | B | Se | Mo | Cr |
|--------------------------|-------------------|--------|-------|----|----|
| | (-----mg/kg-----) | | | | |
| T ₁ (Control) | 0.30 | 11.49 | 0.3 | <1 | <1 |
| T ₂ (1%) | 1.95 | 38.25 | 1.0 | <1 | <1 |
| T ₃ (2%) | 2.06 | 52.73 | 3.13 | <1 | <1 |
| T ₄ (4%) | 2.10 | 70.48 | 5.48 | <1 | <1 |
| T ₅ (6%) | 2.20 | 85.82 | 6.56 | <1 | <1 |
| T ₆ (8%) | 2.68 | 182.47 | 12.81 | <1 | <1 |
| T ₇ (10%) | 2.73 | 189.60 | 14.34 | <1 | <1 |

Conclusion

The results of this research indicated that any increase beyond 1% FGD waste to soils an amendment would not be ideal for corn plants. However, other crops should also be tested for the phytotoxic effects of FGD wastes.

Acknowledgement

This research was funded by the Department of Energy under contract DE-FG09-02SR2248 at Savannah State University. Authors also express their sincere appreciation to PLSAMP for funding student intern research program.

References

- Sajwan KS, Alva AK, Keefer RF (1993) 'Chemistry of Trace Elements in Fly Ash'. (Kluwer Academic/Plenum Publishers: New York, NY).
- Sajwan KS, Keefer RF, Alva AK (1999) 'Biogeochemistry of Trace Elements in Coal and Coal Combustion Residues'. (Kluwer Academic/Plenum Publishers: New York, NY).
- Santhanam CJ, Lunt RR, Johnson SL, Cooper CB, Thayer PS, Jones JW (1979) Health and environment impacts of increased generation of coal ash and FGD sludges. *Environ. Health Perspect.* **33**, 131-157.

Effect of organic amendments on heavy metals uptake by potato plants

Violina Angelova^A, Radka Ivanova^B, Galina Pevicharova^C and Krasimir Ivanov^A

^ADepartment of Chemistry, Agricultural University, Plovdiv, Bulgaria, Email vileriz@yahoo.com

^BDepartment of Plant science, Agricultural University, Plovdiv, Bulgaria, Email radkay@yahoo.com

^CMaritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria, Email gpevicharova@abv.bg

Abstract

Comparative research on the impact of organic soil additives (peat, compost and vermicompost) on the quantity of mobile forms of Pb, Zn, Cd and Cu and uptake of these elements by potato (*Solanum tuberosum* L.) plants was carried out. The application of soil amendments favours plant growth and development. Development and fruit yield demonstrated a stimulating effect with all amendments and this effect was best expressed after 10% compost addition. Organic amendments led to an increase of starch yield, absolute dry substance and quantity and to a decrease of reducing sugars in potatoes. Peat, compost and vermicompost application led to effective immobilization of Pb, Cu, Zn and Cd phytoaccessible forms in soil. A correlation was found between the quantity of the mobile forms and the uptake of Pb, Zn, Cu and Cd by the potato. Organic amendments led to decreased heavy metal content in potato peel and tubers, and this decrease was best expressed with 10% compost and 10% vermicompost (separately). Organic amendments were especially effective for reduction of cadmium content in potato tubers.

Key Words

Phytoremediation, heavy metals, organic amendments, potato.

Introduction

Phytoremediation can be defined as the combined use of plants, soil amendments and agronomic practices to remove pollutants from the environment or to decrease their toxicity (Salt *et al.* 1998). This technique has many advantages compared with other remediation procedures – low economic costs and the possibility of being applied to soils, causing a minimum environmental impact. Addition of organic matter amendments, such as compost, fertilizers and wastes, is a common practice for immobilization of heavy metals and soil amelioration of contaminated soils (Clemente *et al.* 2005). The effect of organic matter amendments on heavy metal bioavailability depends on the nature of the organic matter, their microbial degradability, salt content and effects on soil pH and redox potential, as well as on the particular soil type and metals concerned (Walker *et al.* 2003, 2004). The present study has the following three objectives: (i) to broaden and increase the knowledge of the effect of organic additives on the quantity of mobile forms of Pb, Zn, Cd and Cu (ii) to compare the effect of the selected additives on accumulation of heavy metals by the potato and (iii) to estimate the effect of the introduction of additives on the phytoremediation of contaminated with heavy metals soils.

Methods

The soil used in this experiment was sampled from the vicinity of the area contaminated by a copper smelter near Pirdop, Bulgaria. It is characterized by acid reaction (pH 5.5), loamy texture and a moderate content of organic matter (2.0%). The total content of Cu, Zn, Pb and Cd is high (340 mg/kg Cu, 110 mg/kg Zn, 54 mg/kg Pb and 0.4 mg/kg Cd, respectively) and exceeds the maximum permissible concentrations (60 mg/kg Cu, 90 mg/kg Zn, 50 mg/kg Pb) except for Cd (1.0 mg/kg Cd). The pot experiment was conducted on soil with organic amendments (peat, compost and vermicompost at 5.0%, 7.5% and 10.0% addition rates (calculated on soil dry weight basis). Soils were passed through a 1-cm sieve. Amendments were added and thoroughly mixed by hand. The pots were filled with 9 kg soil. All treatments were performed in triplicate. Three control pots were also set up without amendment. Pots were watered and stored in a greenhouse, where they were left to settle a minimum of 6 weeks at room temperature before planting the potato. The potato plants were grown in a climate chamber for 56 days with regular watering and random rotation of the position of the pots. After 56 days, all plants were harvested. The plants were cleaned and washed under running water to remove stuck soil. Following drying, they were divided, with the help of a scalpel, into their constituent parts - root, potato (peel and tuber), leaves and stems, and analyzed separately. The contents of the heavy metals in the plant material were determined.

Total content of heavy metals in soils was determined in accordance with ISO 11466. The mobile heavy metals contents in soils were determined by 1 M NH_4NO_3 . The plant samples were treated by the method of dry ashing. To determine the heavy metal content in the samples, inductively coupled emission spectrometer (Jobin Yvon Horiba "ULTIMA 2", France) was used.

Results

Effect of soil amendments on the mobile forms of Pb, Zn, Cu and Cd

In many plants there is direct relation between the content of microelements in the soil solution and their uptake by the plants. This relation is most evident with cadmium and less evident with zinc and lead (Kabata Pendias, 2001). The soil amendments used for phytostabilization may have a significant effect on the mobile forms of Pb, Zn, Cu and Cd as a result of sedimentation, absorption and change in the degree of oxidation. The quantity of mobile forms of Pb, Zn, Cu and Cd depended on the soil amendments and the treatment (type and rate). The results presented in Figure 1 showed that the impact of soil amendments on mobile forms of Pb, Zn, Cu and Cd was explicitly expressed and led to their effective immobilization.

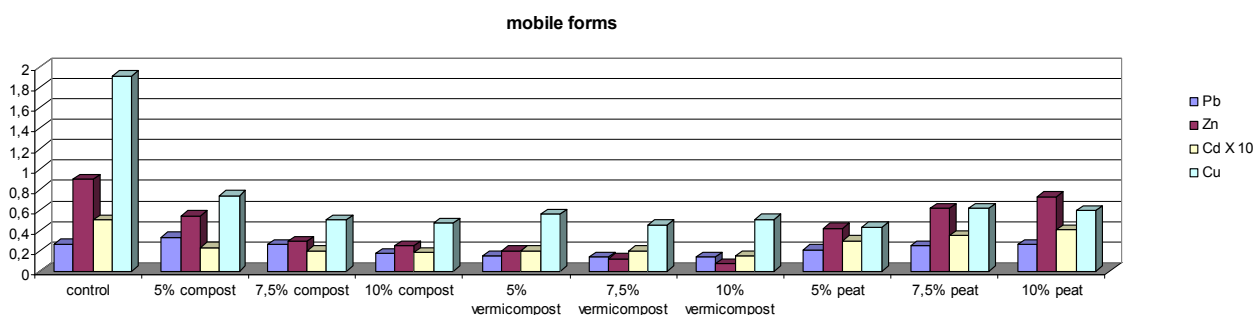


Figure 1. Effect of the soil amendments on the quantity of the mobile forms of Pb, Zn, Cd and Cu.

Effect of organic amendments on the Pb, Zn, Cu and Cd accumulation in potato plants

Impact of soil amendments on plant biological and physiological reactions

The application of soil amendments favours plant growth and development. Development and fruit yielding were stimulated with all amendments and this effect was best expressed after 10% compost addition. Organic amendments led to an increase of starch yield, absolute dry substance and quantity and to a decrease of reducing sugars in potatoes in comparison to those in the control samples.

Accumulation of Pb, Zn, Cu and Cd by potato plants

The results for the influence of the organic additives on the accumulation and distribution of Pb, Zn, Cu and Cd in the potatoes are presented in Figure 2. The contamination of the potatoes was due mainly to the presence in the soil of heavy metals, which entered the plants through their root system, as well as by diffusion through the peel. The movement and the accumulation of the heavy metals in the vegetative organs of the potato plants differed considerably. Their quantity in the stems and leaves of the plants of potato was considerably higher compared to the root system. Probably via the conductive system the heavy metals moved to above-ground parts and predominantly accumulated there. The peel played the role of a selective filter for the heavy metals preventing them from moving into the tuber. The content of Pb in the tuber in plants without amendments reached to 0.38 mg/kg, Zn – 3.7 mg/kg, Cu - 2.7 mg/kg and Cd – 0.04 mg/kg. Pb, Zn and Cu content in the potato tuber were below the proposed guideline values for vegetable plants (0.5 mg/kg Pb, 10 mg/kg Cu and 10 mg/kg Zn). In the tuber of potato, Cd accumulated in quantities considerably above the proposed guideline value for vegetable plants (0.03 mg/kg).

Organic additives impact

According to the literature the content of organic substance in soil has a significant impact on absorption and translocation of heavy metals in soil and their uptake by plants. Cu, Zn, Pb and Cd are adsorbed on organic matter, which generate stable forms and lead to their accumulation in organic horizons of soil and peat (Kabata Pendias, 2001). The results obtained by us showed that Pb, Zn, Cu and Cd uptake by potato depended on the soil amendments and treatment (type and rate). Compost, vermicompost and peat addition led to increased Pb and Cu content in potato roots, stems and leaves, and this increase was better expressed with 10% compost, 10% vermicompost or 5% peat. Organic amendments led to decreased Pb and Cu content in the potato peel and tubers, and this decrease was best expressed with 10% compost or 10% vermicompost.

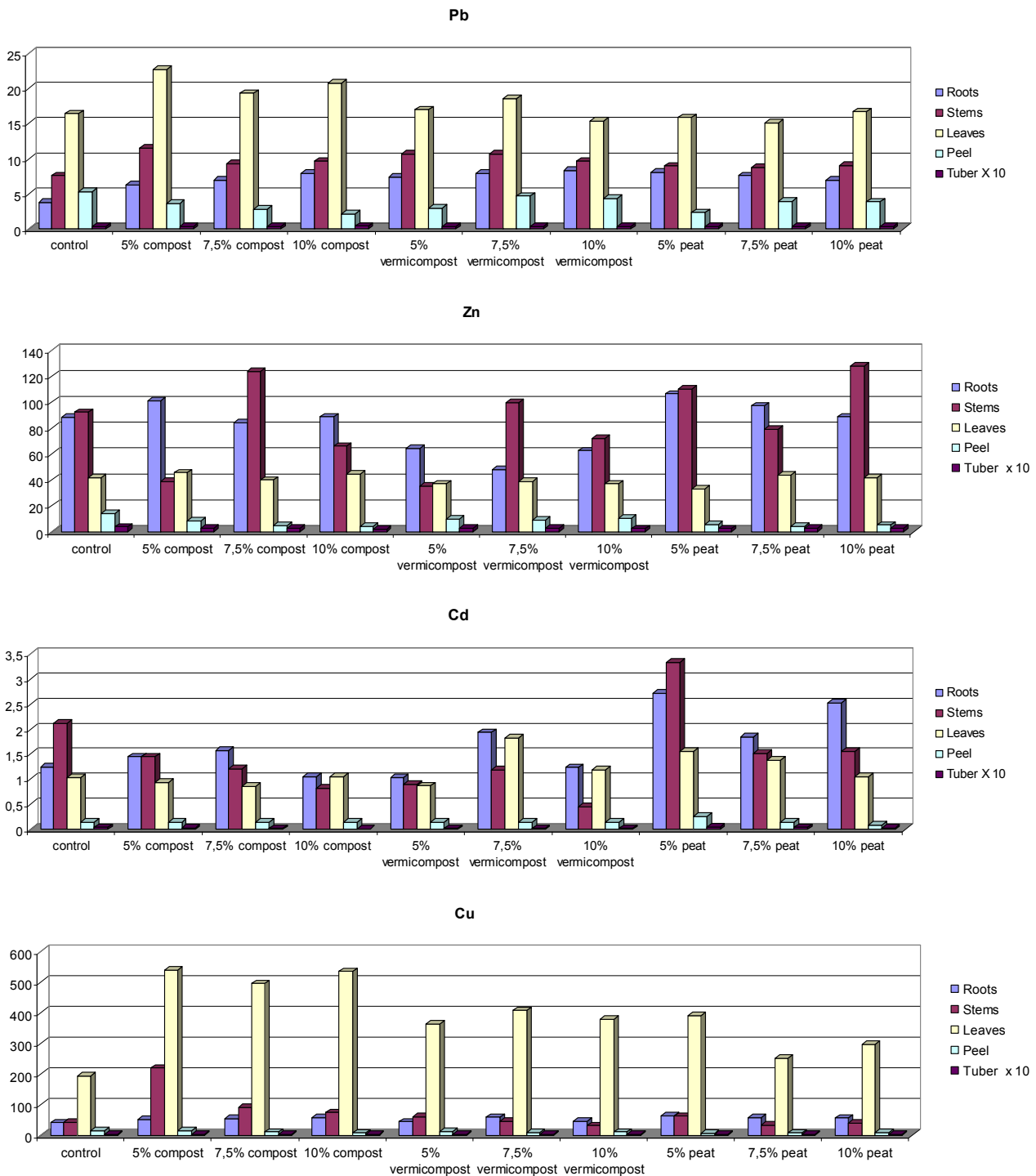


Figure 2. Effect of the organic amendments on the quantity of Pb, Zn, Cd and Cu (mg/kg) in potato plants.

Impact of organic amendments on Pb accumulation in potato peel depended significantly on their quantity. Increase of compost quantity led to a decrease of the Pb content in potato tubers: the concentration of Pb with compost was 0.34 mg/kg. When the soil was treated with vermicompost, Pb content remained practically unchanged (0.38 mg/kg). When the soil was treated with peat, Pb concentration decreased from 0.36 to 0.33 mg/kg. Cu showed similar tendency. Increase in the quantity of compost and vermicompost led to a decrease of Cu content in tubers and concentrations were 1.6 and 1.7 mg/kg, respectively. Increase in the quantity of peat led to an increase in Cu from 1.7 to 2.0 mg/kg. Changes in Cd and Zn content in potato organs were rather complex. Zn content in roots and stems increased in the plants treated with compost and peat amendments and decreased with vermicompost amendment. Zn in leaves showed a similar, but less obvious tendency. Organic amendments led to decreased Zn content in the potato peel and tubers, and this decrease was best expressed with 10% compost, 10% vermicompost and 5% peat.

Cadmium content in roots increased with all amendments used in the experiments. Cadmium content tended to decrease in stems and leaves when treated with the amendments. The only exception was the case where 5% peat amendment was applied, in which case Cd content in stems and leaves increased by 58% and 33%, respectively. Cadmium content in the peel remained practically unchanged in plants treated with compost and vermicompost. Organic amendment addition was especially effective for the reduction of Cd content in potato tubers. Increase of compost, vermicompost and peat doses led to a decrease of Cd content in potato tubers by up to 0.011 mg/kg (10% compost), 0.016 mg/kg (10% vermicompost) and 0.029 mg/kg (10% peat) and these concentrations were below the allowable concentration. The results agreed with those obtained by Chlopecka and Adriano (1997) who found that amendments were more efficient when Cd concentrations in soil were low, probably because at higher Cd concentrations in the soil the amount of amendment added was not sufficient to immobilize all available Cd.

Conclusion

1. The application of soil amendments favours plant growth and development. Development and fruit yielding were stimulated effect with all amendments and this effect was best expressed after 10% compost addition. Organic amendments led to an increase of starch yield, absolute dry substance and quantity and to a decrease of reducing sugars in potatoes.
2. Organic amendment application led to an effective immobilization of Pb, Cu, Zn and Cd phytoaccessible forms in soil. A correlation was found between the quantity of the mobile forms and the uptake of Pb, Zn, Cu and Cd by the potato.
3. Organic amendments led to decreased heavy metal content in the potato peel and tubers, and this decrease was best expressed with 10% compost or 10% vermicompost. Organic amendments were especially effective for reduction of the Cd content in potato tubers.
4. The organic amendments are of great interest for the purpose of phytostabilization. Evaluation of their potential, however, requires further study of the effect of organic amendments on a wider range of agricultural crops.

Acknowledgement

This work is supported by Bulgarian Ministry of Education, Project DO-02-87/08 and NSFB project GAMA DO 02-70 11/12/2008.

References

- Chlopecka A, Adriano DC (1997) Influence of zeolite, apatite and Fe-oxide on Cd and Pb uptake by crops. *The Science of the Total Environment* **207**, 195-206.
- Clemente R, Waljker DJ, Bernal MP (2005) Uptake of heavy metals and As by Brassica Juncea grown in a contamination soil in Arnalcollar (Spain): The effect of soil amendments. *Environmental Pollution* **136**, 46-58.
- Kabata-Pendias A (2001) *Trace Elements in Soils and Plants*, 3rd ed. CRC Press LLC, Boca Raton.
- Salt DE, Smith RD, Raskin I (1998) Phytoremediation. *Annual Rev. Plant Physiol. Plant Mol. Biol.* **49**, 643-668.
- Walker DJ, Clemente R, Roig A, Bernal MP (2003) The effect of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. *Environ Pollution* **22**, 303-312.
- Walker DJ, Clemente R, Bernal MP (2004) Contrasting effects of manure and compost on soil pH, heavy metal availability and growth of *Chenopodium album* L. in a soil contaminated by pyritic mine waste. *Chemosphere* **57**, 215-224.

Effect of phosphate-induced immobilization of lead on its mobility and bioavailability

Jinhee Park^{A,B}, Nanthi Bolan^{A,B}, Megharaj Mallavarapu^{A,B} and Ravi Naidu^{A,B}

^ACentre for Environmental Risk Assessment and Remediation, University of South Australia, Mawson Lakes, SA, Australia, Email parjy014@students.unisa.edu.au

^BCooperative Research Centre for Contamination Assessment and Remediation of the Environment, Adelaide, SA, Australia.

Abstract

Lead (Pb) can be immobilized using insoluble phosphate compounds in the presence of phosphate solubilizing bacteria (PSB) which facilitate phosphorus (P) solubilization from insoluble P sources. The mobility and bioavailability of Pb as affected by P-induced immobilization was examined using leaching and plant growth experiments. Phosphate solubilizing bacteria promoted the solubilization of rock phosphate, thereby leading to the immobilization of Pb without causing any significant leaching of P in soils. Phosphate solubilizing bacteria reduced Pb in pore water, and rock phosphate and soluble P amendments significantly reduced Pb accumulation by sunflower.

Key Words

Lead, phosphate solubilizing bacteria, immobilization, phosphorus, rock phosphate.

Introduction

Lead is one of the major metal contaminants attracting particular attention because of its widespread use in mining, industry and agricultural activity and distribution in the earth's crust (Wong 1985). It is generally recognized that the mobility and bioavailability of Pb in soil are more important than total Pb concentration (Tongtavee 2005). Therefore, remediation options for Pb include amelioration of soils to minimize Pb bioavailability. Phosphorus compounds are used to immobilize heavy metal contaminated environments by phosphate-heavy metal precipitation (Cotter-Howells 1996). Insoluble P compounds can be solubilized by phosphate solubilizing bacteria (PSB) by means of phosphatase enzyme activity and organic acid production (Chen 2006). Therefore, PSB can facilitate Pb-P precipitation and increase Pb immobilization with the amendment of insoluble P compounds. The objective of this research was to examine the immobilization of Pb in soils using soluble and insoluble P compounds in the presence of PSB, thereby reducing Pb mobility and bioavailability.

Methods

Incubation and leaching of Pb in spiked soil

A Pb spiked soil (2000 mg Pb/kg) was treated with phosphate sources and PSB as indicated in Table 1. The levels of P addition were 200, 800 and 1600 mg P/kg for rock phosphate and 200 and 800 mg P/kg for soluble P as KH_2PO_4 . After 2 weeks of incubation, NH_4NO_3 extractable Pb was analyzed by Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES, Agilent).

Table 1. Soil treatment.

| Symbol | Treatment (Moisture 60 % of Water holding capacity) |
|--------|---|
| S | Pb contaminated soil |
| SB | Pb contaminated soil + PSB bacterial solution (ca. 5.5×10^8 CFU/mL) |
| SRP | Pb contaminated soil + rock phosphate |
| SRPB | Pb contaminated soil + rock phosphate + PSB bacterial solution (ca. 5.5×10^8 CFU/mL) |
| SP | Pb contaminated soil + KH_2PO_4 |

A column experiment with the Pb spiked soil was conducted to examine P and Pb leaching potential in P amended soil. The Pb spiked soil samples (45 g) pre-amended with various treatments (Table 1) were placed in a 60 mL syringe, leached with 1 mM CaCl_2 solution and analyzed for P and Pb concentration using Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS, Agilent).

Pot experiment

The Pb spiked soil samples (400 g, <2 mm) were mixed with rock phosphate (Table 1) and placed into 600 mL opaque plastic pots. Rhizon samplers (one per pot) were horizontally placed at 2 cm from the bottom of

the pot. Sunflower seeds (5 seeds per pot) were sown directly into the soils and the number of seedlings was thinned to 2 per pot one week after germination. The pots were watered daily and plants were harvested 6 weeks after germination. After harvesting plants, shoots and roots were separated, washed with Milli Q water, oven dried and ground. The ground plant material (0.1000 – 0.5000 g) was digested using a temperature controlled digestion block (AI Scientific Block Digestion System AIM 500). The digested plant extracts (along with blanks and spiked samples) were analyzed for metals by ICP-OES.

Results

Leaching of P and Pb

The addition of phosphate compounds immobilized Pb as shown by a decrease in NH_4NO_3 extractable Pb in soil. Phosphate solubilizing bacterial inoculation decreased NH_4NO_3 extractable Pb concentration significantly at all levels of rock phosphate amendment, and the decrease in Pb concentration increased with increasing levels of P addition. In the case of soluble P addition, the decrease in Pb concentration was not significant at 200 mg/kg of level, but Pb concentration was decreased by 57 % at 800 mg/kg of soluble P level (Figure 1) compared to the control (0 mg/kg P concentration). Rock phosphate alone treatment immobilized 0.48 %, 7.8 % and 18.8 % of Pb at 200, 400, 800 and 1600 mg P/kg, respectively, after 2 weeks of incubation compared to the control (0 mg/kg P concentration). Phosphate solubilizing bacteria in the presence of rock phosphate treatment immobilized 6.98 %, 25.6 % and 32.0 % of Pb in soil at 200, 400, 800 and 1600 mg P/kg, respectively compared to the control (0 mg/kg P concentration).

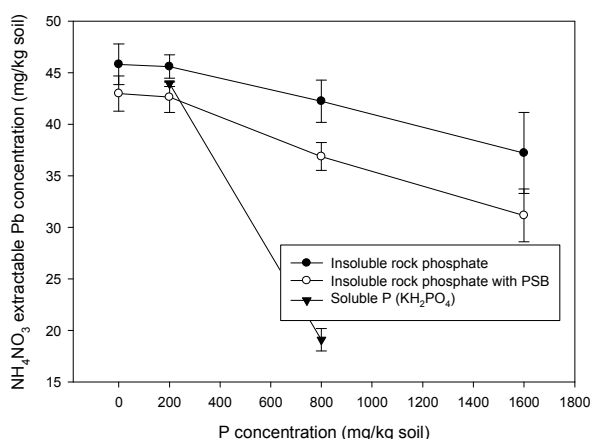


Figure 1. Ammonium nitrate extractable Pb concentration in Pb spiked soil treated with various levels of soluble P and rock phosphate and in the presence of PSB for rock phosphate.

The total leached Pb concentration was the highest in soluble P treated Pb spiked soil and the lowest in rock phosphate treatment with PSB (Figure 2 (a)). Phosphate solubilizing bacterial inoculation with rock phosphate reduced Pb leaching by 18 % compared to the control. The total leached P concentration in Pb spiked soil was 0.04% and 8.9 % from the rock phosphate and soluble P treated soils, respectively (Figure 2 (b)). Higher levels of P application increase the risk of eutrophication of ground water because increased P application increased the leaching of P from the treated soil columns (Basta and McGowen 2004).

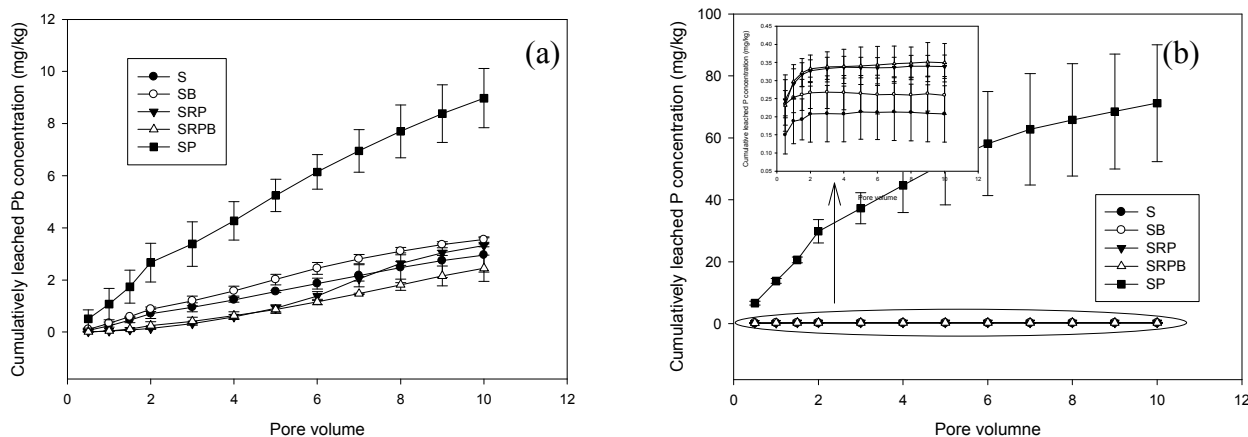


Figure 2. Cumulative concentration of Pb (a) and P (b) in the leachate.

Pot experiment

Lead concentration in pore water in soluble P amended soil was higher than other treatments. The high Pb concentration in pore water from soluble P amended soil may be attributed to the mobilization of dissolved organic carbon (DOC). There was a significant correlation between DOC and Pb concentrations in pore water indicating that DOC formed complexes with Pb, thereby increasing Pb mobility (Levonmaki *et al.* 2006). Phosphate solubilizing bacterial inoculation with rock phosphate reduced Pb concentration in soil solution up to 40 days of plant growth (Figure 3). This effect can be explained by P solubilization by PSB from rock phosphate and subsequent immobilization of Pb through Pb-P precipitation. Phosphorus amendments significantly reduced shoot and root Pb concentration (Figure 4). Rock phosphate, rock phosphate with PSB and KH_2PO_4 decreased Pb concentration of sunflower shoot by 57 %, 56 % and 76 %, respectively, compared to the control.

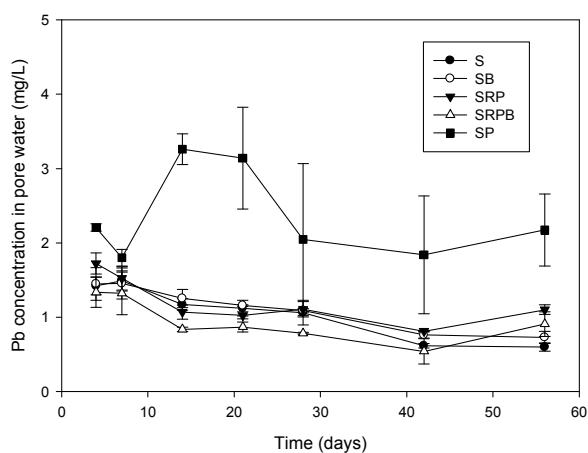


Figure 3. Lead concentration in pore water with sunflower growth.

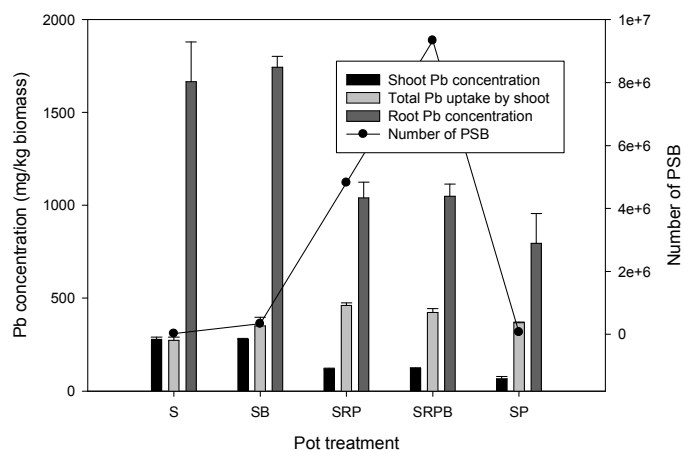


Figure 4. Lead accumulation by sunflower shoot and root.

Conclusion

Insoluble P sources in the presence of PSB showed the potential to immobilize Pb without any detrimental effects on P and Pb leaching. Phosphate solubilizing bacteria enhanced the immobilization of Pb in soil, thereby reducing the mobility and bioavailability of Pb in soil.

References

- Basta NT, McGowen SL (2004) Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil. *Environmental Pollution* **127**, 73-82.
- Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecology* **34**, 33-41.
- Cotter-Howells J, Caporn S (1996) Remediation of contaminated land by formation of heavy metal phosphates. *Applied Geochemistry* **11**, 335-342.
- Levonmaki M, Hartikainen H, Kairesalo T (2006) Effect of organic amendment and plant roots on the solubility and mobilization of lead in soils at a shooting range. *Journal of Environmental Quality* **35**, 1026-1031.
- Tongtavee N, Shiowatana J, McLaren RG, Gray CW (2005) Assessment of lead availability in contaminated soil using isotope dilution techniques. *Science of the Total Environment* **348**, 244-256.
- Wong MH (2003) Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere* **50**, 775-780.

Effects of arsenic and its interaction with phosphorus on yield and arsenic accumulation in rice

M. R. Islam^{A,B} and M. Jahiruddin^A

^ADepartment of Soil Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

^BIRRI, Bangladesh, Email M.R.Islam@irri.org

Abstract

A study was made on the effect of arsenic (As) and As × P interaction in Boro (dry season) rice. Arsenic was added to soil (having 2.6 mg/kg initial As) @ 0, 15 and 30 mg As/kg from Na₂HAsO₄·7H₂O and phosphorus @ 0, 20, 40, 60 and 80 mg P/kg from KH₂PO₄. Each pot had received 100 mg/kg N, 40 mg/kg K and 25 mg/kg S from urea, muriate of potash (MoP) and gypsum, respectively. The grain yield of rice was reduced by 20.6 % for 15 mg/kg As treatment and 63.8 % due to 30 mg/kg As. Such reductions for straw yield were 21.0 and 65.2 % with these two As treatments, respectively. Arsenic concentration in grain and straw significantly increased due to arsenic application. The adverse effect of arsenic on rice was further enhanced by P addition. This reaction has an implication to P fertilizer management in rice.

Key Words

Arsenic, phosphorus, rice.

Introduction

Arsenic (As) contamination of groundwater is a severe problem in Bangladesh and this has affected at least 25 million people (Ravenscroft *et al.* 2005). Next to drinking water, rice could be a potential source of As exposure of the people living in the As affected areas of Bangladesh (Hossain *et al.* 2008, Panaullah *et al.* 2009; William *et al.* 2009). Roberts *et al.* (2007) estimated that over 1000 tons of As might be transferred to arable land each year from As contaminated groundwater irrigation, creating a potential risk for future agricultural sustainability and food security of the country. Arsenic may enter into human body directly through drinking water and indirectly through foods, chiefly rice for Bangladeshi people. Rice covers about 75% of the total cropped areas in this country. Many areas have high groundwater and soil arsenic contents which are likely to be taken up by plants through roots and transported to the aerial portion. Islam *et al.* (2005) reported 12.3 mg/kg mean As over 456 soil samples across the country. Phosphorus fertilization is common in rice cultivation which might interact with arsenic uptake since phosphate is an analogue of arsenate and compete for the same sorption sites (Qafoku *et al.* 1999). Considering the above points in view, the present study was conducted to evaluate the effect of arsenic and its interaction with P on yield loss and arsenic accumulation in rice. The experiment was conducted with varying doses of arsenic addition to soil having low arsenic content in order to understand the situation in high arsenic soil.

Materials and methods

A pot-culture experiment was set up during Boro season (January-May) of 2006 with Bangladesh Agricultural University (BAU) farm soil having low arsenic content (2.6 mg/kg) in a net-house of the Department of Soil Science of BAU, Mymensingh. Texturally the soil was silt loam with 6.7 pH, 2.28% organic carbon, 0.28% total N, 9.8 mg/kg available P, 14 mg/kg available S, 0.7 mg/kg available Zn and 0.11 mol/kg exchangeable K. The size of each pot was 43 cm in diameter and 40 cm in height. There were 15 treatments consisting of three doses of As (0, 15 and 30 mg/kg) and five doses of P (0, 20, 40, 60 and 80 mg/kg), their sources being Na₂HAsO₄·7H₂O and KH₂PO₄, respectively. The experiment was conducted in a two factorial completely randomized design with three replications. The seedlings (cv. BRRI dhan29) were transplanted in the pots on 26 January 2006. Every pot had received 100 mg/kg N from urea, 40 mg/kg K from muriate of potash (MoP) and 25 mg/kg S from gypsum. Nitrogen was added in three splits, the first split during final land preparation and the remaining splits at 35 days and 60 days after transplanting. Intercultural operations such as weeding and irrigation were done whenever required. The crop was harvested at maturity and the data on yield and yield parameters were recorded. Plants of all three hills from each pot were measured and averaged to record the yield contributing characters. The grain and straw As content was determined after digesting the samples with HNO₃ and H₂O₂ at 120°C followed by flow injection hydride generation atomic absorption spectrophotometer (UNICAM model No. 969) with hydride generator assembly using matrix-matched standards. Nitrogen content of grain and straw was determined after digesting the samples with H₂O₂ and H₂SO₄ in presence of catalyst mixture. Phosphorus was determined

from HNO₃ and HClO₄ (3:1 ratio) digest following phosphomolybdate blue colour method. All the plants data were statistically analyzed following F-test and the difference between treatment means was adjudged by Duncan's Multiple Range Test.

Results

Yield

Both grain and straw yields were significantly reduced due to arsenic toxicity. The grain yields due to three arsenic treatments (0, 15 and 30 mg As/kg) were 74.17, 58.86 and 26.84 g/pot and the corresponding straw yields were 86.00, 67.94 and 29.94 g/pot, all were significantly different from one another (Table 1). Compared to the control pot, the yield reductions were 20.6 and 63.8 % in grain, and 21.0 and 65.2 % in straw, for the As₁₅ and As₃₀ treatments, respectively. It appeared that the toxic effect of added arsenic on grain and straw yields were deteriorated due to added P showing a negative interaction (Fig. 1). For example, the grain yields due to As₃₀ treatment with different doses of P viz. P₀, P₂₀, P₄₀, P₆₀, & P₈₀ were 35.55, 23.22, 19.96, 29.20 and 26.28 g per pot, respectively. The highest yield in both cases was observed with P₈₀As₀ treatment and the lowest yields with P₈₀As₃₀.

Yield contributing characters

Addition of arsenic at 30 mg/kg rate produced a highly significant toxic effect on the effective tillers/pot, filled grains panicle⁻¹ and 1000-grain weight (Table 1). However, the toxic effect due to 15 mg/kg arsenic treatment was not significant. The number of effective tillers/pot for 0, 15 and 30 mg/kg arsenic treatments were 33.60, 31.33 and 23.40, respectively. The corresponding values for the number of filled grains panicle⁻¹ were 96.72, 92.14 and 49.57. The 1000-grain weights for the arsenic treatments followed the order of 24.53, 24.94 and 21.11 g. There was no significant interaction of As and P on these three characters.

Table 1. Effects of arsenic and phosphorus on the yield and yield components of rice (cv. BRR1 dhan29).

| Treatment (As or P) | Effective tillers/pot | Filled grains/panicle | 1000-grain weight (g) | Grain yield (g/pot) | Straw yield (g/pot) |
|---------------------|-----------------------|-----------------------|-----------------------|---------------------|---------------------|
| As (mg/kg) | | | | | |
| 0 | 33.60a | 96.72a | 24.53a | 74.17a | 86.00a |
| 15 | 31.33b | 92.14a | 24.94a | 58.86 b | 67.94b |
| 30 | 23.40c | 49.57b | 21.11b | 23.81 c | 29.94c |
| S.E. (±) | 0.89 | 4.55 | 0.42 | 2.98 | 1.98 |
| P (mg/kg) | | | | | |
| 0 | 29.67 | 82.15 | 22.58 | 58.95 | 64.91 |
| 20 | 29.44 | 77.38 | 22.75 | 54.09 | 66.62 |
| 40 | 32.00 | 71.97 | 23.52 | 48.37 | 57.51 |
| 60 | 27.89 | 79.43 | 24.49 | 50.92 | 58.51 |
| 80 | 28.22 | 86.41 | 24.28 | 49.06 | 58.92 |
| S.E. (±) | NS | NS | NS | NS | NS |

In a column, the figures having same letter do not differ significantly at 5% level of probability

Arsenic and nutrient content

Arsenic concentrations of both grain and straw had increased as the dose of arsenic increased, the range being 0.214 – 0.507 mg/kg As for grain and 2.55 – 8.27 mg/kg As for straw (Table 2). The treatment with 15 mg/kg As had a grain-As concentration of 0.376 mg/kg and straw-As of 6.01 mg/kg. The As content also varied with the P doses with a result of 0.339 – 0.442 mg/kg in grain and 4.84 -7.47 mg/kg in straw. The As × P interaction was significant showing the maximum As concentration (0.75 mg/kg in grain and 10.17 mg/kg in straw) for P₈₀As₃₀ treatment in both grain and straw (Figure 1, straw data not shown).

Similar to arsenic, the N content in grain and straw increased with increasing addition of arsenic. The grain-N varied from 1.264 -1.520% and the straw-N from 0.572 - 0.839% (Table 2). There was a significant As × P interaction for grain-N content while the straw N-content remained unaffected.

Phosphorus content of rice grain also increased with arsenic doses but the reverse was true for straw-P content (Table 2). Thus, the highest grain-P concentration (0.41%) was recorded by the 30 mg/kg As treatment while for the straw-P (0.107%) the highest record was obtained with the As control. The As × P interaction was found significant with the maximum grain-P concentration (0.437%) in P₈₀As₃₀ and the maximum straw-P content (0.151%) in P₂₀As₀ treatments

Table 2. Effects of arsenic and phosphorus on the As, N and P concentrations in rice (cv. BRRI dhan29).

| Treatment (As or P) | N conc. (%) | | P conc. (%) | | As conc. (mg/kg) | |
|------------------------|-------------|---------|-------------|--------|------------------|-------|
| | Grain | Straw | Grain | Straw | Grain | Straw |
| As (mg/kg) | | | | | | |
| 0 | 1.264c | 0.572b | 0.301c | 0.107a | 0.214c | 2.55c |
| 15 | 1.441b | 0.800a | 0.345b | 0.076b | 0.376b | 6.01b |
| 30 | 1.520a | 0.839a | 0.410a | 0.085b | 0.507a | 8.27a |
| S.E. (\pm) | 0.012 | 0.027 | 0.009 | 0.005 | 0.012 | 0.20 |
| P (mg/kg) | | | | | | |
| 0 | 1.291c | 0.796a | 0.291c | 0.065c | 0.433ab | 5.03b |
| 20 | 1.284c | 0.588b | 0.353b | 0.071c | 0.352c | 5.27b |
| 40 | 1.524b | 0.773a | 0.339bc | 0.094b | 0.442a | 4.84b |
| 60 | 1.298c | 0.844a | 0.367ab | 0.118a | 0.339c | 5.46b |
| 80 | 1.644a | 0.684ab | 0.410a | 0.099b | 0.369abc | 7.47a |
| S.E. (\pm) | 0.016 | 0.035 | 0.011 | 0.006 | 0.016 | 0.26 |

In a column, the figures having same letter do not differ significantly at 5% level of probability

The study reveals that the grain yield was drastically reduced due to arsenic addition and it was associated with reduction in tillers and grain set. The toxic effect of arsenic was further deteriorated due to P supplement to soil (Fig. 1). This result can be interpreted in that added phosphate displaced sorbed arsenate from exchange sites, and therefore, increased the concentration of arsenic in the rhizosphere soil solution for subsequent uptake by rice roots.

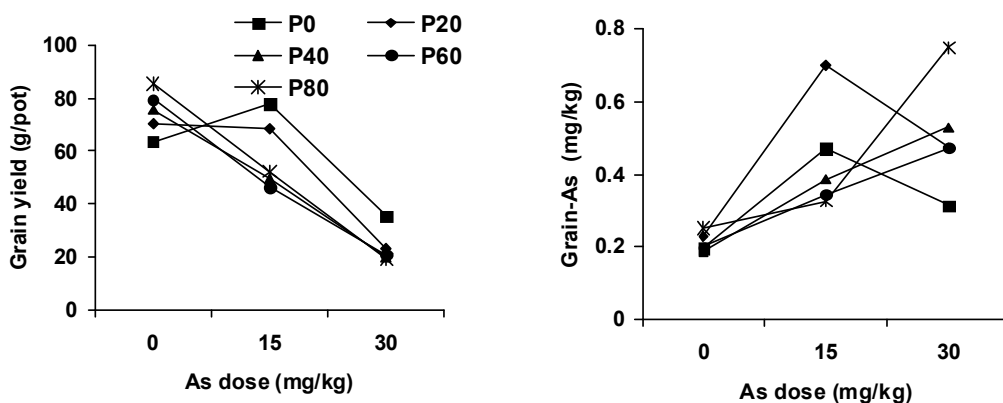


Figure 1. Interaction effect of arsenic and phosphorus on the yield and arsenic concentrations of rice.

Conclusion

The addition of arsenic markedly increased the arsenic concentration of grain and straw, with a concomitant reduction in grain and straw yields of rice. The situation was further deteriorated due to P application to soil, probably due to displacement of arsenic from adsorption sites in the bulk soil, with an increased concentration of arsenic in the rhizosphere soil solution for subsequent uptake by rice roots. Rice straw can be a potential exposure of arsenic to cattle.

References

- Hossain MB, Jahiruddin M, Panaullah GM, Loeppert RH, Islam MR, Duxbury JM (2008) Spatial variability of arsenic concentration in soils and plants, and its relationship with iron, manganese and phosphorus. *Environmental Pollution* **156**, 739-744.
- Islam M, Jahiruddin M, Rahman GKMM, Miah MAM, Farid ATM, Panaullah GM, Loeppert RH, Duxbury JM, Meisner CA (2005) Arsenic in paddy soils in Bangladesh: levels, distribution and contribution of irrigation and sediments. In 'Behaviour of Arsenic in Aquifers, Soil and Plants: Implications for Management'. Dhaka.
- Ravenscroft P, Burgess WL, Ahmed KM, Burren M, Perrin J (2005) Arsenic in groundwater of the Bengal Basin, Bangladesh: Distribution, field relations, and hydrogeological setting. *Hydrogeol. J.* **13**, 727-751.
- Roberts LC, Hug SJ, Dittmar J, Voegelin A, Saha GC, Ali MA, Badruzzaman ABM, Kretzschmar R (2007) Spatial distribution and temporal variability of arsenic in irrigated rice fields in Bangladesh. I. Irrigation

- Water. *Environmental Science and Technology* **41**, 5960-5966.
- Qafoku NP, Kukier U, Sumner ME, Miller WP, Radcliffe DE (1999) Arsenate displacement from fly ash amended soils. *Water Air and Soil Pollution* **114**, 185-198.
- William PN, Islam S, Islam MR, Jahiruddin M, Adomako E, Solaiman ARM, Rahman GKMM, Lu Y, Deacon C, Zhu YG, Meharg AA (2009) Arsenic limits trace mineral nutrition (selenium, zinc, and nickel) in Bangladesh rice grain. *Environmental Science and Technology* DOI: 10.1021/es901825t.
- Panaullah GM, Alam T, Hossain MB, Loeppert RH, Lauren JG, Meisner CA, Ahmed ZU, Duxbury JM (2009) Arsenic toxicity to rice (*Oryza sativa* L.) in Bangladesh. *Plant and Soil* **317**, 31-39.

Effects of chemical amendment on heavy metal leachability and bioavailability of an acidic mine water-contaminated soil and growth of a vegetable

Chuxia Lin^{A,B}, Wenzhou Lu^B, Yong Liu^B, Yingqun Ma^B, Fengxian Wan^B and Aisheng Chen^B

^AAustralian Centre for Sustainable Catchments, University of Southern Queensland, Toowoomba QLD, Australia, Email Chuxia.Lin@usq.edu.au

^BCentre for Ecological and Environmental Technologies, South China Agricultural University, Guangzhou, China.

Abstract

A pot experiment was conducted to examine the effects of acid neutralization treatment of a mine water-polluted soil on the leachability and bioavailability of the heavy metals in the soil, as well as the growth of a vegetable using the treated soils as growth media. The results show that the leachability of the soil-borne heavy metals can be effectively reduced after application of lime and red mud (pH 11.58, acid-neutralizing capacity of about 10 mole/kg). The amount of heavy metals extracted by the plant differed significantly among the different treatments despite that the soluble forms of the metals were consistently low or non-detectable for various treatments. This indicates that non-soluble heavy metal pools were more important sources for heavy metal uptake by the plant. The bioavailability of the heavy metals was not dependent on soil pH, at least for Cu, Zn and Cd. Soil pH might have certain effects on the uptake of Pb by the plant. The growth performance of the vegetable was significantly affected by the amount of red mud added to the soil, which does not appear to be related to soil pH conditions and heavy metal toxicity.

Key Words

Chemical amendment, heavy metal, metal mobility, soil contamination, acid mine drainage.

Introduction

Agricultural soils contaminated by acidic mine water tend to have elevated concentration of heavy metals, not only the total amount but also the soluble fraction. This could have significantly adverse impacts on the quality of crop and groundwater due to mobility of these metals from the soil to the crop plants and near-surface aquifers (Lin *et al.* 2005; Chen *et al.* 2007). Chemical amendments have been widely practised to remediate heavy metal-contaminated soils based on the general belief that they can immobilize the heavy metals and therefore reduce their export from the soil system (Lombi *et al.* 2002). In this study, a pot experiment was conducted to examine the growth performance of a vegetable and the chemical behaviours of a few heavy metals in a mine water-contaminated soil under acid-neutralizing treatments. The objective was to assess the effects of the acid neutralization on the leachability and bioavailability of the heavy metals in the soil.

Materials and methods

Experimental soil and crop plant

The soil used in this experiment was collected from a floodplain downstream of the Dabaoshan Mine in the South China. The agricultural land had been irrigated with acidic mine water for two decades. The soil was abandoned due to severely acidic conditions when it was collected. The topsoil layer (0-30 cm) was sampled, air-dried and crushed to pass a 3 mm sieve before it was used for pot experiment. Some basic characteristics of the experimental soil are given in Table 1. The red mud used in the experiment had a pH of 11.58 and an acid-neutralizing capacity of about 10 mole/kg (Liu *et al.* 2007). *Brassica chinensis* L, a common vegetable species, was used as a test plant in the experiment.

Pot experiments

One control and 6 treatments (in triplicate) were set for the pot experiment. For all the 6 treatments, equal amount of 5 g hydrated lime (analytical grade) was used for each pot. From Treatment 1 (T1) to Treatment 6 (T6), 0, 1, 3, 5, 10, 20 g fresh red mud was used accordingly. One kilogram of the original soil was completely mixed with the additives and then put in a plastic pot (2 kg capacity). The control contained original soil without addition of hydrated lime and red mud. Prior to vegetable growth experiment, the soils were irrigated to keep the soil moisture content at field capacity level. This was done by placing each pot on a plastic tray containing a layer of water (at the very beginning, the dry soil needed to be wet by watering from top). After soil incubation for 20 days, the vegetable growth experiment commenced on July 20, 2005

and harvested on September 30, 2005. The pots were placed randomly in a green house with free ventilation. In each pot, three seedlings with two leaves were initially planted and only two healthier plants were remained in each pot by removing the weakest plant from the pot about two weeks after transplanting. In addition, a dose of compound fertilizer containing 0.2 g N, 0.2 g P and 0.2 g K was added to each pot twice, one at 25th day and another at 50th day following transplanting. At the first day of the growth experiment (i.e. after 20 days of soil incubation) and the harvest day (i.e. on September 30, 2005), soil samples were taken from each pot for chemical analysis.

Table 1. Some basic characteristics of the experimental soil.

| Soil chemical parameter | Value |
|--|-------|
| Organic matter content (%) | 26.67 |
| Total N (mg/kg) | 860 |
| Total P (mg/kg) | 1640 |
| Total K (mg/kg) | 2110 |
| Available N (mg/kg) | 75.6 |
| Available P (mg/kg) | 50.57 |
| Available K (mg/kg) | 42.86 |
| pH | 3.49 |
| EC (dS m ⁻¹) | 0.970 |
| Water-extractable acidity (mmol/kg) | 1.5 |
| NH ₄ Cl-extractable acidity (mmol/kg) | 16.1 |
| Total actual acidity (mmol/kg) | 19.4 |

Chemical analysis

The plant materials were washed using deionized water, separated into root and the above-ground portion and then oven-dried at 70 °C to constant weight for hours to determine the dry biomass of the plant materials. The dried plant tissue was milled. Two grams of each milled plant sample were ignited (at 550°C) in a Muffle furnace for 6 hours and then digested with 2 mol/L HCl (Long *et al.* 2002). Various heavy metals contained in the plant materials were determined by atomic absorption spectrometry. Soil samples were oven-dried at 60°C for 1 day and then ground to pass a 2 mm sieve. 1:5 (soil:water) extracts was prepared. pH of the water extract was measured by a calibrated pH meter; water-extractable acidity was determined by titrating an aliquot of extract with a standardized NaOH solution to pH 5.5; concentrations of various heavy metals in the extract was determined by atomic absorption spectrometry;

Results

Changes in pH over time for the control and various treatments

Soil pH measured for the samples collected on the first day and last day of the pot experiment is given in Table 2. For the control, the mean pH of the soil was 3.49 and 3.89 for the first day and the 73rd day of the growth experiment, respectively. The mean soil pH of all the treatments was significantly higher than that of the control for all the sampling occasions. On the 1st day of the growth experiment, the soil pH increased from about 7.9 for T1 to 8.87 for T6.

Table 2. Variation of soil pH for various treatments during the period of experiment.

| Treatment | First day | Last day |
|-----------|-----------|-----------|
| Control | 3.49±0.01 | 3.89±0.02 |
| T1 | 7.94±0.04 | 6.53±0.04 |
| T2 | 8.45±0.11 | 7.60±0.12 |
| T3 | 8.44±0.12 | 7.77±0.12 |
| T4 | 8.46±0.05 | 7.87±0.05 |
| T5 | 8.58±0.04 | 8.12±0.04 |
| T6 | 8.87±0.04 | 8.35±0.04 |

Heavy metal concentration in the soils of the control and various treatments

After acid-neutralization treatments, water-extractable Cu, Pb and Cd become non-detectable except for Cu in T3 and T4. Water-extractable Zn was detected but markedly reduced, as compared to that in the control (Table 3).

Table 3. Concentration (mg/kg) of the water-extractable heavy metals for various treatments on the last day of experiment.

| Treatment | Cu | Pb | Zn | Cd |
|-----------|-----------|-----------|-----------|-----------|
| Control | 0.89±0.06 | 0.14±0.02 | 9.30±0.12 | 0.03±0.00 |
| T1 | nd | nd | 0.29±0.10 | nd |
| T2 | nd | nd | 0.32±0.01 | nd |
| T3 | 0.27±0.06 | nd | 0.15±0.05 | nd |
| T4 | 0.31±0.09 | nd | 0.13±0.02 | nd |
| T5 | nd | nd | 0.14±0.05 | nd |
| T6 | nd | nd | 0.42±0.12 | nd |

nd: not detectable

Biomass of the crop plants for the control and various treatments at harvest

The untreated soil did not support growth of the test plant. T2 had the best growth performance with a dry biomass of 21.2 mg/kg, followed by T3. There was no statistically significant difference in the dry biomass among T1, T4, T5 and T6 (Table 4).

Table 4. Dry biomass (mg/kg) of the crop plants for the control and various treatments at harvest.

| Treatment | Above-ground portion | Underground portion |
|-----------|----------------------|---------------------|
| Control | 0.00d | 0.00d |
| T1 | 4.38±1.08bc | 0.02±0.01cd |
| T2 | 21.2±7.48a | 0.04±0.01cd |
| T3 | 13.16±9.41ab | 0.15±0.12bcd |
| T4 | 7.32±1.84bc | 0.26±0.09ab |
| T5 | 4.03±1.12bc | 0.2±0.06abc |
| T6 | 5.29±0.66bc | 0.32±0.046a |

Means with the same letters in the same column do not differ significantly at $P > 0.05$

Heavy metal concentration in the soils of the control and various treatments

The degree of heavy metal accumulation in the plant tissue in relation to the amount of added red mud varied among different metals. Pb showed a trend to decrease with increasing amount of added red mud but other metals exhibited no clear relationship between the amount of added red mud and the metal concentration in the tissue (Table 5).

Table 5. Heavy metal concentration (mg/kg) of plant tissue for the control and various treatments at harvest.

| Treatment | Cu | Pb | Zn | Cd |
|-----------|--------------|-------------|---------------|---------------|
| T1 | 34.91±2.69bc | 4.26±1.05a | 58.96±1.09bc | 3.25±1.08abc |
| T2 | 31.92±0.69c | 3.56±0.35a | 65.06±2.37abc | 0.90±0.24d |
| T3 | 40.33±1.3abc | 3.08±0.10ab | 72.19±6.66ab | 0.97±0.52cd |
| T4 | 33.95±3.26bc | 3.42±0.51ab | 62.58±1.62abc | 1.28±0.35cd |
| T5 | 44.9±5.58abc | 3.36±0.34ab | 66.27±5.18abc | 2.80±0.21abcd |
| T6 | 32.33±13.24c | 2.84±0.71ab | 46.31±9.78bc | 1.43±0.49bcd |

Means with the same letters in the same column do not differ significantly at $P > 0.05$

Discussion

The results obtained from this experiment suggests that the leachability of the heavy metals in the soil, as indicated by elimination or significant reduction in water-extractable forms of the metals, can be effectively reduced after amendment of the soil with the acid-neutralizing agents. This is attributable to the maintenance of a high soil pH during the period of the experiment.

The much better growth performance of the vegetable in T2 and T3, compared to those of other treatments, either higher or lower dose of added red mud, suggests that addition of appropriate amount of acid-neutralizing agents is extremely important in terms of achieving remediation goals. It is unknown that T1 had such poor growth performance despite that its soil pH ranged from 6.53 to 7.94, which is even more optimal for the growth of *Brassica chinensis* L. (Huxley, 1992), compared to the soil pH in T2 and T3.

The significant difference in heavy metal uptake by the crop plant among the different treatments is not related to the status of soluble heavy metal pools since the concentration of the heavy metals in the soils was consistently very low or non-detectable for all the treatments. This suggests that the certain non-soluble

heavy metal pools played a significant role in supplying heavy metals for the plant uptake. Further work is required to investigate the mechanisms responsible for this. Under the current experimental conditions, the bioavailability of the heavy metals was not dependent on soil pH, at least for Cu, Zn and Cd. Soil pH might have certain impacts on the uptake of Pb by the plant but this requires further work to confirm it.

Conclusion

The leachability of the soil-borne heavy metals can be effectively reduced after application of lime and red mud. The amount of heavy metals extracted by the plant differed significantly among the different treatments despite that the soluble forms of the metals were consistently low or non-detectable for various treatments. It is evident that that non-soluble heavy metal pools were more important sources for heavy metal uptake by the plant. The bioavailability of the heavy metals was not dependent on soil pH, at least for Cu, Zn and Cd. Soil pH might have certain effects on the uptake of Pb by the plant. The growth performance of the vegetable was significantly affected by the amount of red mud added to the soil, which does not appear to be related to soil pH conditions and heavy metal toxicity.

References

- Chen A, Lin C, Lu W, Wu Y, Ma Y, Li J, Zhu L (2007) Well water contaminated by acidic mine water from the Dabaoshan Mine, South China: Chemistry and toxicity. *Chemosphere* **70**, 248-255
- Huxley A (1992) 'The New RHS Dictionary of Gardening.' (MacMillan Press: New York).
- Lin C, Lu W, Wu Y (2005) Agricultural soils irrigated with acidic mine water: acidity, heavy metals, and crop contamination *Australian Journal of Soil Research* **43**, 819–826.
- Liu Y, Lin C, Wu Y (2007) Characterization of red mud derived from a combined Bayer Process and bauxite calcination method. *Journal of Hazardous Materials* **146**, 255-261.
- Lombi E, Zhao F, Zhang G, Sun B, Fitz W, Zhang H, McGrath SP (2002) In situ fixation of metals in soils using bauxite residue: Chemical assessment. *Environ. Pollut.* **118**, 435-443.
- Long X., Yang X., Ye Z., (2002) Study of the difference of uptake and accumulation of zinc in four species of *Sedum linn.* *Acta Bot. Sin.* **44**, 152-157.

Effects of compost application on remediation and the growth of maize planted on lead contaminated soil

S. A. Adejumo^A, A. O. Togun^A, J. A. Adediran^B and M. B. Ogundiran^C

^ADepartment of Crop Protection and Environmental Biology, University of Ibadan, Ibadan. Nigeria. Email; nikade_05@yahoo.com

^BInstitute of Agricultural Research and training, Moor Plantation, Ibadan. Nigeria.

^CDepartment of Chemistry, University of Ibadan, Ibadan. Nigeria.

Abstract

A field experiment was conducted in 2008 and 2009 to test for the effects of Mexican Sunflower (SW) and Cassava waste (CW) composts as well as inorganic fertilizer on the growth of maize planted on lead contaminated soil. Different application rates of 0t/ha, 20t/ha and 40t/ha were used for the two types of compost while inorganic fertilizer and 0t/ha were used as checks. The experiment was laid out in Randomized Complete Block Design with four replicates. It was observed that compost application increased significantly the vegetative and yield parameters of maize and performed better than inorganic fertilizer ($P < 0.05$). Higher dose (40t/ha) of MSW compost gave the highest plant height, dry matter yield, leaf area and grain yield. More importantly, the experiment shows that there was a reduction in the final concentration of lead in soils of all the compost treated plots by 50-70%. Though, application of compost did not restrict the uptake of lead by the maize crop the concentrations were lower than those of control and inorganic fertilizer plants. Higher concentrations were also detected in the root than other plant parts. This method of remediation could be recommended for restoration of lead contaminated sites.

Key Words

Soil, compost, heavy metals, contamination, maize.

Introduction

Lack of and reduction in cultivable and productive land has been attributed to soil contamination from high rate of industrialization and urbanization (UNEP 2000). UNEP (1992) calculated that 2 billion hectares of land that was once biologically productive has been irreversibly degraded in the past 100 years due to contamination and inaccessibility. Land contamination/degradation is a threat to sustainable agricultural development and food security in developing countries. Among all the degraded lands, those contaminated with heavy metals are largely irreversible and where reversibility is attempted, it is at high cost (Oldema, 1994). It has therefore become imperative that the environment and its resources should be managed judiciously to enhance sustainable national and socio-economic development. Efforts have been made by different researchers to remediate contaminated soils using different methods. However, it is very important that any method used should be environment-friendly and cost-effective. This explains the present promotion of compost-bioremediation for land reclamation (USEPA 1997). However, there is dearth of information on the use of compost on contaminated land in Nigeria. This research work was designed to test for the efficacy and optimum application rates of composts and inorganic fertilizer on the performance of maize grown directly on the field contaminated with battery wastes.

Methods

Description of experimental site;

In this study, the dumpsite of the defunct Lead - Acid Battery Manufacturing Company in Ibadan, Nigeria was used (Figure 1). Initial soil analysis for environmentally available form of heavy metals such as Pb, Cu, Zn, Cd, and Cr in mg/kg using model 210A of the Buck Scientific Atomic Absorption Spectrophotometer series with Air-Acetylene gas mixture as oxidant under different wavelengths after hot digestion with 2M HNO₃ (Ogundiran, 2007) was carried out before application of compost.

Experimental procedure

The treatments used include CONTROL (without compost or inorganic fertilizer), SWR2 (Mexican Sunflower compost at 20t/ha), SWR5 (Mexican Sunflower compost at 40t/ha), CWR2 (Cassava waste at 20t/ha), CWR5 (Cassava waste at 40t/ha) and F1 (Inorganic fertilizer - NPK at 100KgN/ha) using experimental design of Randomized Complete Block (RCBD) and replicated four times. Data were collected on the vegetative parameters and grain yield of maize, Post-harvesting soil and plant tissue Pb

concentrations. Analysis of variance and Duncan's multiple range test of Statistical Analysis System package (1998) was used to analyze the data.

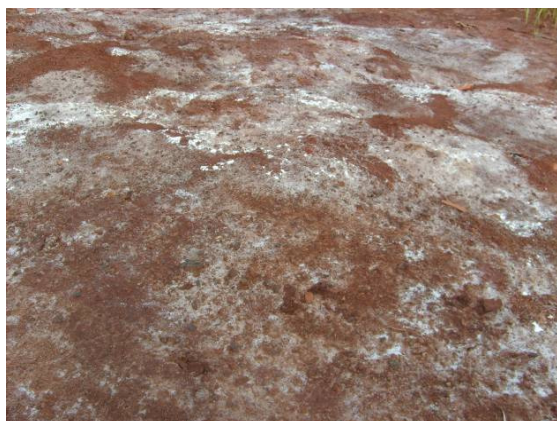


Figure 1. Experimental site before demarcation.

Results

The level of lead and Cd were extremely high in this soil while those of Zn, Cu, and Cr are relatively high (Table 1) when compared with the levels of these metals in uncontaminated soil (Kabata-Pendias and Pendias, 2001; Ogundiran and Osibanjo, 2009). Based on the mean values of the growth and yield of maize, application of compost produced significantly taller plants, higher number of leaf and leaf area over control and inorganic fertilizer (Figure 2) due to the ability of compost to supply the soil with required nutrients (Sadovnikova, 2002). Among the two types of compost applied at the rate of 40t/ha, mexican sunflower compost gave the highest maize yield (Figure 3). Application of sunflower compost at the rate of 40t/ha reduced the level of Pb in the soil by 72% followed by that of cassava waste compost, also applied at the same rate (67%) (Figure 4) due to reduction in the solubility and mobility of trace metals in the soil with appreciable content of organic matter (Williams *et al.* 1980; Renevan *et al.* 2007). The total level of Pb in the maize plant tissues from all the compost treated plots were significantly lower than those treated with inorganic fertilizer and control ($P < 0.05$) (Table 2).

Table 1. Pre-cropping soil physico-chemical analysis.

| Properties | Pb | Cu | Zn | P | Cr | Cd | pH | OC | N | Ca | Mg | K |
|------------|-------------------|-----|-----|-----|----|----|-----|------|-----|-------------------|------|------|
| | (-----mg/kg-----) | | | | | | | (%) | (%) | (----cmol/kg----) | | |
| Soil | 138,000 | 612 | 990 | 138 | 8 | 34 | 4.2 | 1.03 | 0.1 | 4.3 | 1.48 | 0.03 |

Conclusion;

It could be concluded that application of compost improved soil fertility and increased the plant resistance to heavy metal toxicity thereby enhancing plant growth and dry matter yield of maize in heavy metal polluted soil.

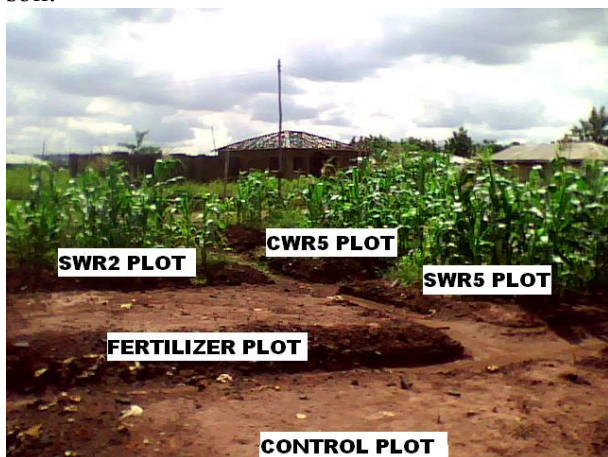


Figure 2. Experimental site at twelve weeks after planting showing the plants in control plot and the plots treated with inorganic fertilizer, Mexican sunflower compost at 20t/ha (SWR2), Mexican sunflower compost at 40t/ha (SWR5), Cassava waste compost at 20t/ha (CWR2) and Cassava waste compost at 40t/ha (CWR5).

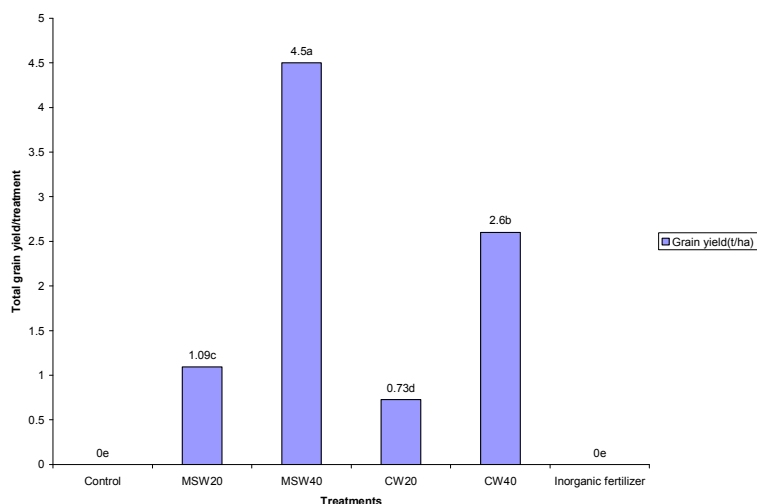


Figure 3. Effects of compost source and rates on total grain yield by maize
 Bars carrying the same letter are not significantly different from each other at 5% level of significance
 F1= Inorganic fertilizer, MSW20= Mexican Sunflower at 20t/ha, MSW40= Mexican Sunflower at 40t/ha, CW20= Cassava waste at 20t/ha, CW40= Cassava waste at 40t/ha.

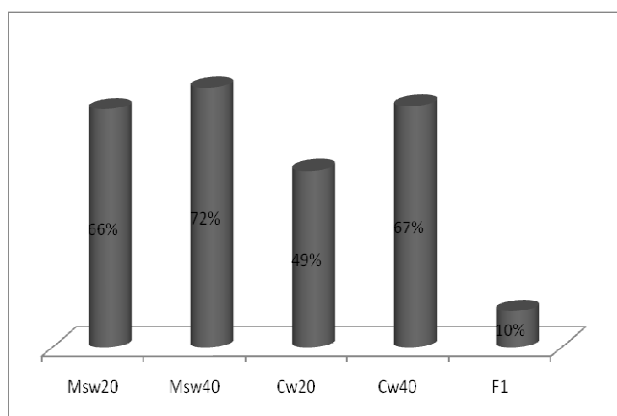


Figure 4. Effects of compost and inorganic fertilizer on percentage reduction in Pb concentration of post harvesting soil; F1= Inorganic fertilizer, MSW20= Mexican Sunflower at 20t/ha, MSW40= Mexican Sunflower at 40t/ha, CW20 = Cassava waste at 20t/ha, CW40 = Cassava waste at 40t/ha.

Table 2. Effects of different rates of sunflower, cassava waste compost and inorganic fertilizer on the Pb(%) concentration in maize plant parts at harvesting on the field.

| Treatments | Seed (%) | Cob (%) | Sheath (%) | Leaf (%) | Pb (%) | Stem (%) | Root (%) | Total (%) |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|-----------|
| Control | 0.02 ^b | 0.01 ^a | 0.02 ^b | 0.05 ^b | 0.03 ^c | 1.34 ^a | 1.46 ^a | |
| MSW ₂₀ | 0.02 ^b | 0.01 ^a | 0.01 ^c | 0.02 ^c | 0.02 ^d | 1.12 ^b | 1.25 ^b | |
| MSW ₄₀ | 0.02 ^b | 0.01 ^a | 0.02 ^b | 0.05 ^b | 0.03 ^c | 1.14 ^{ab} | 1.26 ^b | |
| CW ₂₀ | 0.03 ^a | 0.00 ^b | 0.03 ^a | 0.02 ^c | 0.04 ^b | 1.14 ^{ab} | 1.27 ^b | |
| CW ₄₀ | 0.03 ^a | 0.01 ^a | 0.03 ^a | 0.02 ^c | 0.12 ^a | 0.87 ^c | 1.07 ^d | |
| F1 | 0.02 ^b | 0.01 ^a | 0.02 ^b | 0.06 ^a | 0.03 ^c | 1.35 ^a | 1.47 ^a | |

Means followed by the same letter in a column for the treatments and weeks are not significantly different from each other at P<0.05.

MSW20= Mexican sunflower compost at 20t/ha, MSW40= Mexican sunflower compost at 20t/ha, CW20= Cassava waste compost at 20t/ha, CW40= Cassava waste compost at 20t/ha, F1=Inorganic fertilizer.

References

- Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants 3rd edn. CRC, New York.
- Ogundiran MB, Osibanjo O (2009) Mobility and speciation of heavy metals in soils impacted by hazardous wastes. *Journal of Chemical Speciation and Bioavailability* **21**, 59-69.
- Oldema LR (1994) The global extent of soil degradation. In 'Soil Resilience and Sustainable Land Use' (Eds. Greenland DJ, Szabolcs T). Wallingford, Commonwealth Agricultural Bureau International <http://www>.

Isric.nl/GLASOD.htm.

Sadovnikova LK (2002) The remediation experience of oil and heavy metal polluted soils.

UNEP (1992) Land degradation, In, Global Environment Outlook 3, (2002), pp. 63-64.

United Nations Environment Program (UNEP) (2000) The urban environment: facts and figures. Industry and Environment. Global Environment Outlook (GEO). Latin American and the Caribbean Environment Outlook. Mexico City, United Nations Environment Programme. Regional Office for Latin America and the Caribbean. **23**, 61-88.

USEPA (1997) Innovative uses of compost Bioremediation and pollution prevention.

Williams DE, Vlamis J, Pukite AH, Corey JE (1980) Trace element accumulation movement and distribution in the soil profile from massive application of sewage sludge. *Soil Science* **129**, 119-132.

Effects of water management on Cd and As content in rice grain

Tomohito Arao^A, Akira Kawasaki^A, Koji Baba^A, Shinsuke Mori^A and Shingo Matsumoto^B

^ANational Institute for Agro-Environmental Sciences, Kannondai 3-1-3, Tsukuba, Ibaraki, 305-8604, Japan, Email arao@affrc.go.jp

^BShimane University, 2059 Kamihonjomachi, Matsue, Shimane, 690-1102, Japan, Email smatsu@life.shimane-u.ac.jp

Abstract

Rice consumption is a major source of cadmium and arsenic for the population of Asia. We investigated the effects of water management in rice paddy on levels of cadmium and arsenic in Japanese rice grains. Flooding increased arsenic concentrations in rice grains, whereas aerobic treatment increased the concentration of cadmium. Flooding for three weeks before and after heading was most effective in reducing grain cadmium concentrations, but this treatment increased the arsenic concentration considerably, whereas aerobic treatment during the same period was effective in reducing arsenic concentrations but increased the cadmium concentration markedly. Flooding treatment after heading was found to be more effective than flooding treatment before heading in reducing rice grain cadmium without a concomitant increase in total arsenic levels.

Key Words

Codex Alimentarius Commission, JECFA, pot experiments, PTWI, redox potential, soil solution.

Introduction

Cadmium (Cd) is toxic to humans at concentrations lower than those at which it is toxic to plants, because its effects on humans are cumulative (Singh and McLaughlin 1999). Soil pollution by Cd has been of public concern since the 1970s, when it was discovered that daily ingestion of rice (*Oryza sativa* L.) containing high levels of Cd is the main cause of itai-itai disease (Kobayashi 1978). A health-based guidance value for Cd of 7 µg/kg bodyweight per week [the provisional tolerable weekly intake (PTWI)] has been established by the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization and the World Health Organization. Recently, the European Food Safety Authority established a tolerable weekly intake for Cd of 2.5 µg/kg body weight. The weekly intake of Cd from foods in Japan in 2001 was estimated to be 4.1 µg/kg body weight, and about half the Cd intake from foods was from rice. A re-evaluation of Cd is scheduled for the 2010 meeting of the JECFA. A maximum concentration of 0.4 mg/kg for Cd in white rice grain has been adopted by the Codex Alimentarius Commission. Rice is a staple crop in Asia, and is the principal source of dietary intake of Cd in the Japanese population; therefore, minimizing the intake of Cd from rice is an important health issue.

Arsenic (As) is a carcinogen and the intake of inorganic As in rice is a significant risk factor for cancer in populations for whom rice is a staple foodstuff (Mondal and Polya 2008). In some cases, human As intake from the consumption of rice exceeds that from drinking water (Williams *et al.* 2006). For inorganic As, a PTWI of 15 µg/kg body weight has been established by the JECFA. The Ministry of Agriculture, Forestry, and Fisheries of Japan analyzed the As contents of staple crops in Japan and found that As concentrations in brown rice ranged from 0.04 to 0.33 mg/kg, with an average value of 0.16 mg/kg (n = 199); the average values for wheat, soybean, and spinach were 0.008, 0.005, and 0.010 mg/kg, respectively. Rice is therefore a major source of dietary intake of inorganic As in the Japanese population. Contamination by As occurs to a greater extent in paddy rice than in other upland crops because anaerobic conditions in paddy soil lead to arsenic mobilization and thus enhanced bioavailability to rice (Takahashi *et al.* 2004).

Flooding of paddy fields is effective in reducing grain levels of Cd; however, anaerobic conditions in paddy soil lead to arsenic mobilization and, therefore, As uptake by rice could increase (Koyama, 1975, Kyuma, 2004). The main objective of the present study was to investigate the simultaneous effects on Cd and As levels in rice grains induced by water management of paddy soil before and after emergence of the rice ears.

Methods

Pot experiments, with three or six (treatment 1) replications each, were performed in 2008 in a greenhouse at ambient temperatures (7–36 °C) under sunlight. Wagner pots (1/5000 a, Fujiwara Scientific Co., Tokyo, Japan) were filled with 3 kg of two kinds of soil collected from the plow layer of paddy fields. Soil A

contained 1.6% total C, 0.15% total N, 0.56 mg/kg total Cd, and 25 mg/kg total As, and it had a pH of 5.6. Soil B contained 3.4% total C, 0.32% total N, 0.66 mg/kg total Cd, and 48 mg/kg total As, and it had a pH of 5.5. Eh was measured at a depth of 10 cm. A soil-water sampler (DIK8393, Daiki Rika Kogyo Co., Saitama, Japan) was buried in the middle of the soil of each pot for collecting soil solution. The soil solution was sampled 33, 54, 70, 83, and 90 days after transplanting and diluted with 10% HNO₃ at a ratio of 9:1 immediately after collection and filtered through a sterilized 0.45- μ m filter. Seedlings of rice (*O. sativa* L. cv. Koshihikari) were germinated on perlite and transplanted into the soil samples on 14 May 2008. A compound fertilizer containing 0.2 g of N, 0.04 g of P, and 0.08 g of K was supplied to each pot by basal application. Ammonium sulfate containing 0.2 g of N was also supplied to each pot by top dressing 60 days after transplantation of the rice seedlings.

Seven water-management treatments were examined in the experiment: treatment 1 involved flooding throughout the entire growth period; treatment 2, flooding from transplanting to three weeks after heading; treatment 3, flooding from transplanting to heading; treatment 4, flooding from transplanting to three weeks before heading and from heading to three weeks after heading; treatment 5: flooding from transplanting to three weeks before heading; treatment 6, flooding from transplanting for two weeks and then from three weeks before heading to three weeks after heading; and treatment 7, flooding from transplanting for two weeks. The heading days occurred between August 1st and August 6th. Water management was changed at the beginning of the last heading day of each treatment of the pot experiments. At the time of heading in treatment 1, the stems of the plants grown in three pots were cut 2 cm above the soil surface and the xylem sap that exuded from the cut surface was collected by trapping in a 1.5-mL plastic vial containing a small piece of cotton for 2 h after cutting the shoots. After the seeds had matured, the plants were cut off at the stem above the point at which they were immersed in water.

Results

Yields of rice grain and straw were significantly different for the various water treatments, and were highest for the continuous flooding treatment 1 and lowest for the aerobic treatment 7. In both the soils, rice from the continuous flooding treatment 1 had the lowest Cd concentration and the highest As concentration in grain (Table 1). Rice from treatment 5 had the highest Cd concentration in grain in soil A, and rice from treatments 5 and 7 had higher Cd concentrations in grain than rice from other treatments in soil B; rice grains from treatments 5 and 7 also had the lowest As concentration in both. The As concentrations in grain were significantly different between treatment 1 and treatment 2 in both soils. In both soils, rice grain from treatment 6, where flooded conditions existed between 3 weeks before heading and 3 weeks after heading, had a higher concentration of As and a lower concentration of Cd than rice grain from treatments 3, 4, 5, and 7. Rice grain from treatment 4 had a 59–62% lower Cd concentration than that from treatment 3 in both soils; however, the As concentrations in the rice grains were not significantly different between treatments 3 and 4 in both soils.

In soils A and B, rice straw from the continuous flooding treatments 1 and 2 had a higher As concentration and a lower Cd concentration than rice straw from other treatments (Table 1). Rice straw from treatment 4 had a significantly lower Cd concentration than rice straw from treatment 3 in both soils. Rice straw from treatment 4 also had a significantly lower As concentration than rice straw from treatment 3 in soil A. Rice straw from the aerobic treatment 7 had the lowest As concentration in both soils. Flooding treatment

Table 1. Effects of water management on As speciation and Cd concentration in grain and straw.

| Water management | grain | | | | straw | | | |
|------------------|---------------------|-----------------|----------------|-----------------|---------------------|----------------|---------------|----------------|
| | Soil A | | Soil B | | Soil A | | Soil B | |
| | As | Cd | As | Cd | As | Cd | As | Cd |
| | mg kg ⁻¹ | | | | mg kg ⁻¹ | | | |
| 1 | 0.95 ± 0.044 a | 0.005 ± 0.001 a | 1.7 ± 0.118 a | 0.010 ± 0.003 a | 27.3 ± 0.66 b | 0.02 ± 0.004 a | 26.2 ± 0.85 a | 0.03 ± 0.004 a |
| 2 | 0.92 ± 0.029 a | 0.016 ± 0.002 a | 1.7 ± 0.077 a | 0.046 ± 0.011 b | 29.5 ± 0.29 a | 0.14 ± 0.014 a | 26.7 ± 0.27 a | 0.22 ± 0.054 a |
| 3 | 0.30 ± 0.020 c | 0.36 ± 0.003 d | 0.59 ± 0.014 c | 0.27 ± 0.012 d | 15.9 ± 0.59 d | 1.4 ± 0.043 d | 17.0 ± 0.76 c | 1.3 ± 0.093 c |
| 4 | 0.36 ± 0.007 c | 0.21 ± 0.018 b | 0.60 ± 0.037 c | 0.16 ± 0.011 c | 11.7 ± 0.70 e | 1.1 ± 0.10 c | 18.1 ± 0.57 c | 0.85 ± 0.097 b |
| 5 | 0.11 ± 0.026 d | 0.41 ± 0.034 e | 0.17 ± 0.030 d | 0.34 ± 0.020 e | 1.8 ± 0.09 f | 2.0 ± 0.14 e | 5.0 ± 0.18 d | 1.3 ± 0.051 c |
| 6 | 0.55 ± 0.006 b | 0.066 ± 0.006 a | 1.26 ± 0.044 b | 0.063 ± 0.006 b | 18.4 ± 0.84 c | 0.66 ± 0.12 b | 23.2 ± 0.64 b | 0.57 ± 0.013 b |
| 7 | 0.10 ± 0.014 d | 0.28 ± 0.021 c | 0.14 ± 0.027 d | 0.38 ± 0.011 e | 1.1 ± 0.53 f | 1.4 ± 0.10 d | 0.9 ± 0.22 e | 2.4 ± 0.24 d |

The same letters are not significant at the 5% level.

increased the concentration of As in rice grain and straw, whereas aerobic treatment increased the concentration of Cd in rice grain and straw (Table 1). This occurs because flooding decreases the redox potential of the soil and increases the As concentration in the soil solution, whereas aerobic treatment increases the redox potential of the soil and increases the Cd concentration in the soil solution (Figure 1). For reducing the Cd concentration in grain, flooding three weeks before and after heading (treatments 1, 2, and 6) was most effective; however, the As concentration in grain increased considerably as a result. On the other hand, for reducing the As concentration in grain, aerobic treatment for three weeks before and after heading (treatments 5 and 7) was most effective, but the concentration of Cd in the grain increased considerably as a result.

Flooding for three weeks after heading was more effective in reducing Cd concentrations in grain than was flooding for three weeks before heading (Table 1); the effects on the As concentration in grain were similar for flooding three weeks after heading and for flooding for three weeks before heading. The value of Eh

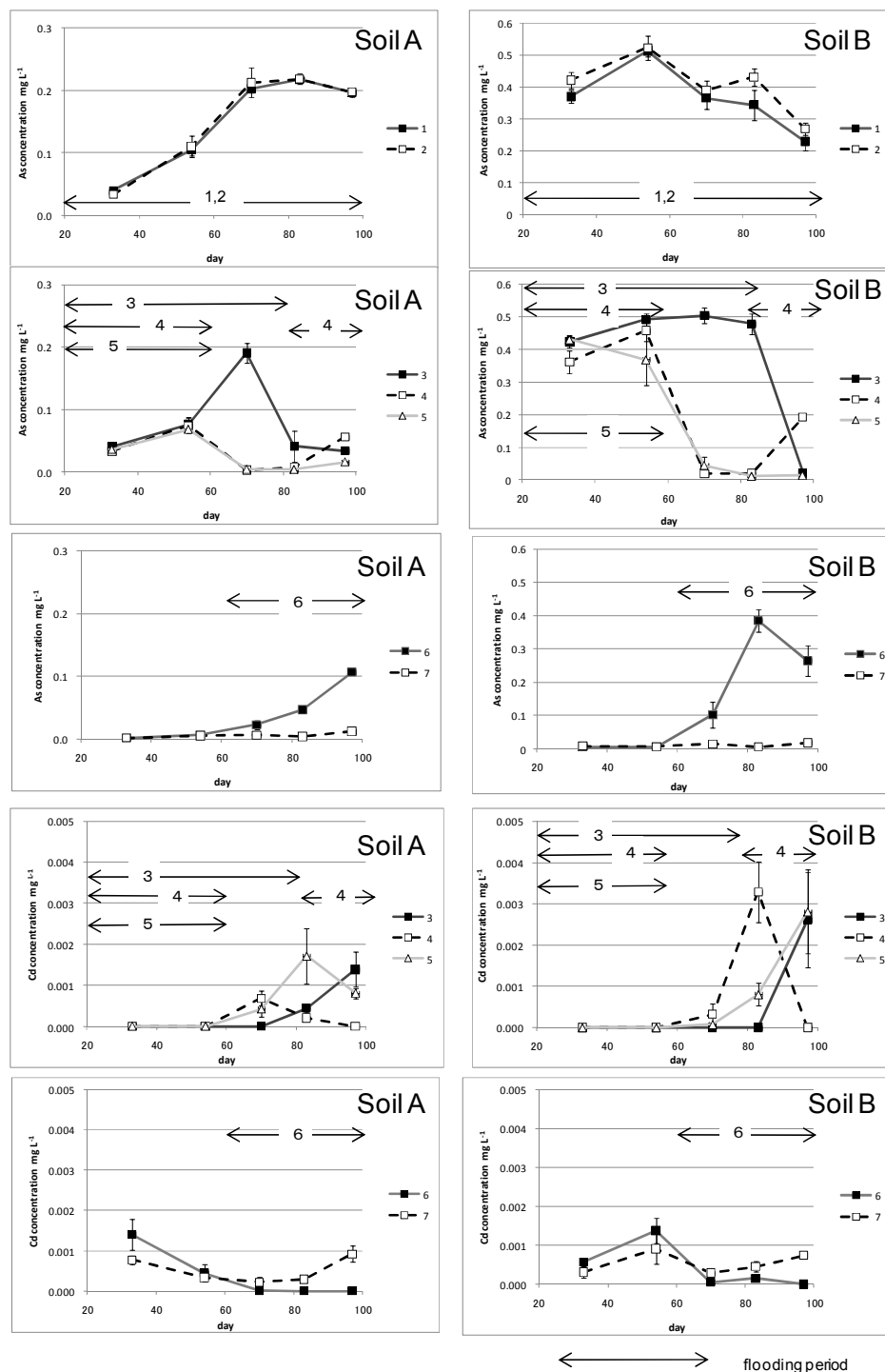


Figure 1. Effects of water management on As and Cd concentrations in soil solution.

increased to above 0 mV immediately after aerobic treatment in treatment 3, and the Cd concentrations in the soil solutions of both soils on day 97 in treatment 4 were below detectable limits (Figure 1). However, the value of Eh gradually decreased and fell below -200 mV about 100 days after flooding in treatment 4. Therefore, the uptake of Cd by rice from the soil solution should increase immediately after the aerobic treatment of treatment 3, and the uptake of As by rice from the soil solution should not increase immediately after the flooding treatment of treatment 4. The effect of flooding treatment after heading on the accumulation of Cd by rice grain should be greater than that on accumulation of As.

Levels of inorganic As in grain were much higher in treatment 4 than in treatment 5 or treatment 3. Therefore, flooding after heading should produce a greater increase in inorganic As levels than flooding before heading. Because As accumulation in grain should be greater after heading than before heading, flooding after heading should lead to arsenic mobilization in soil and thus grain levels of inorganic As should increase. Another possibility is that the relocation of As from the leaves increases. Grain As concentrations in treatments 5 and 7 were almost the same (Table 1), so flooding until 52 days after transplanting did not affect the grain As concentrations when rice was grown in aerobic conditions after day 52. The grain As concentrations in treatment 1 were 1.2-fold higher than those in treatment 2 in both soils, whereas grain Cd concentrations in treatment 2 were 2.3- and 4.7-fold higher than in treatment 1 in soil A and soil B, respectively (Table 1). Therefore, water management just before harvesting time should have a greater impact on the Cd concentration in grain than on the As concentration.

The same water-management regime could cause different changes in the redox potentials for various types of soils because of differences in the properties of the soils, such as aggregate development. It may therefore be difficult to maintain low Cd and As concentrations in grain simultaneously by means of water management alone. Silicon fertilization decreases As and Cd concentrations in rice grain (Inahara *et al.* 2007; Li *et al.* 2009), so it will be necessary to screen for more materials that could reduce As and/or Cd concentrations in rice grain. Some tropical japonica cultivars with low levels of As in their grains have the potential to be used in breeding (Norton *et al.* 2009).

Conclusion

In conclusion, our study showed that water management before and after the heading time is important in managing the Cd and As concentrations simultaneously in rice grain.

References

- Inahara M, Ogawa Y, Azuma H (2007) Effect of alkalinity application on cadmium uptake by lowland rice under different water managements. *Jpn. J. Soil Sci. Plant Nutr.* **78**, 253–260 (in Japanese with English abstract)
- Kobayashi J (1978) Pollution by cadmium and the itai-itai disease in Japan. In 'Toxicity of Heavy Metals in the Environment' (Eds FW Oehme) pp 199–260. (Marcel Dekker: New York).
- Koyama T (1975) Arsenic in soil–plant system. *Jpn. J. Soil Sci. Manure* **46**, 491–502. (in Japanese)
- Kyuma K (2004) Soils polluted with hazardous elements. In 'Paddy Soil Science' pp 241–254. (Kyoto Univ. Press: Kyoto, Japan).
- Li RY, Stroud JL, Ma JF, McGrath SP, Zhao FJ (2009) Mitigation of arsenic accumulation in rice with water management and silicon fertilization. *Environ. Sci. Technol.* **43**, 3778–3783.
- Mondal D, Polya DA (2008) Rice is a major exposure route for arsenic in Chakdaha block, Nadia district, West Bengal, India: A probabilistic risk assessment. *Appl. Geochem.* **23**, 2987–2998.
- Norton GJ, Islam MR, Deacon CM, Zhao FJ, Stroud JL, McGrath SP, Islam S, Jahiruddin M, Feldmann J, Price AH, Meharg A (2009) Identification of low inorganic and total grain arsenic rice cultivars from Bangladesh. *Environ. Sci. Technol.* **43**, 6070–6075.
- Singh BR, McLaughlin MJ (1999) Cadmium in soils and plants: summary and research perspective. In 'Cadmium in Soils and Plants' (Eds McLaughlin MJ, Singh BR) pp. 257–267. (Kluwer Academic Publishing: Dordrecht).
- Takahashi Y, Minamikawa R, Hattori KH, Kurishima K, Kihou N, Yuita K (2004) Arsenic behavior in paddy fields during the cycle of flooded and non-flooded periods. *Environ. Sci. Technol.* **38**, 1038–1044.
- Williams PN, Islam MR, Adomako EE, Raab A, Hossain SA, Zhu YG, Feldmann J, Meharg AA (2006) Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. *Environ. Sci. Technol.* **40**, 4903–4908.

EXAFS analysis of Pb speciation in bullet-contaminated range soils

Jeffrey Lewis^{A, B}, Jan Sjöström^A, Ulf Skyllberg^B and Lars Hägglund^A

^ATotalförsvarets forskningsinstitut FOI-CBRN, Umeå, Sweden.

^BSveriges Lantbruksuniversitet (SLU) Skogsekologi, Umeå, Sweden.

Abstract

This study was a lab-based investigation of range soils contaminated by 500 high velocity 7.62 mm jacketed Swedish military rounds. The 0.06 mm fraction of soil was analyzed using EXAFS to determine the speciation of Pb in the soil both before and after a simulated springtime snow melt. Contrary to previously published material, in both cases PbO and not hydrocerrusite was found to be the dominant lead species, which was attributed to the low organic content and pH of the soil when compared to previous studies.

Key Words

EXAFS, Pb speciation, shooting range.

Introduction

Civilian and military shooting ranges concentrate spent bullets in relatively small areas. The United States (U.S.) Environmental Protection Agency (EPA) estimates that 7.26×10^7 kg of Pb shot and bullets enter the U.S. environment each year at approximately 9000 non-military shooting ranges. In Denmark, annual deposition rates of bullets and Pb shot have been estimated at 800 tons (Joergensen and Willems 1987). One serious difficulty with in-situ research approaches when dealing with ranges is that a great deal of uncertainty exists concerning what ammunition has been fired during the lifetime of the range. Such records are usually difficult to come by and, when available, are usually not detailed enough to indicate accurately what shooting activities have taken place on a given firing point. These uncertainties make it difficult to establish a quantitative link between shooting activity and the eventual fate and distribution of the resulting metals. For example, it is plausible that unjacketed, subsonic, low caliber ammunition will produce different heavy metal loads in the berm sand than jacketed, high velocity, large caliber munitions. Differentiating the influence of the two munitions on an existing range without accurate records is nearly impossible.

Another factor which will influence the fate of metals in sand is the local climate (Dermatas *et al.* 2004). In northern latitudes, up to 70% of the annual groundwater recharge occurs in the three or four week period when the snow pack melts in springtime. At this time of year, evapotranspiration losses are minimal due to the lack of photosynthesis and the low temperatures, so most of the infiltrate eventually reaches the water table and ultimately surface water bodies. This snowmelt has been shown to have a lower pH than the average annual precipitation in northern Sweden (Laudon *et al.* 1999), and low pH has been cited as one factor which could increase the dissolution (Dermatas *et al.* 2004) or colloidal mobility of metals (Klitzke *et al.* 2008). The objective of this study was to use EXAFS (extended X-ray absorption fine structure) spectroscopy to identify the Pb minerals which exist in a bullet-contaminated soil before and after exposure to a simulated snowmelt.

Materials and methods

Bullet impacted sand

To produce the source term for the experiment, 500 rounds of 7.62 mm jacketed military ammunition were fired into an uncontaminated range sand obtained from an existing military range in Umeå, northern Sweden.

Lysimeter construction

Impact berm samples were used to construct two column-type lysimeters 0.4 m in diameter and 0.4 m deep. A 4 cm thick layer of bullet-impacted sand was then placed on the surface of the lysimeters. Based on historical water table fluctuation data, precipitation data and temperature data for the springtime snowmelt in northern Sweden, the infiltration rate for each lysimeter was calculated to be 0.48 liters/day or 1.00 mL/min for 8 hours daily. The chemical composition of the snowmelt was determined using rainfall chemistry from Svartbergets Research Station (Swedish University of Agricultural Sciences) in Vindeln (Lat./Long. 67°11' N; 19°38' E). The pH of the infiltrate was adjusted to a value of 4.8, which is an historical average value of the springtime snowmelt runoff in the area of interest (Laudon *et al.* 1999). The lysimeters were placed in an

environmental chamber and the ambient temperature was held at a constant value of 4° C, again to simulate environmental conditions during the period of snowmelt as closely as possible.

EXAFS analyses

Only the finest fraction of sand (< 0.06 mm) was investigated by EXAFS analysis. This fraction of bullet-impacted sand was analyzed both before and after the infiltration protocol. In addition, a 1 cm layer of initially uncontaminated sand taken from a depth 1 cm below the bottom of the bullet impacted sand was analyzed to determine the chemical speciation of Pb reaching this depth as a consequence of mobilization during infiltration. EXAFS data were collected in fluorescent mode using a Lytle detector filled with Kr, at the Pb L_{III}-edge at the super conducting multi-pole wiggler beamline i811 at MAX-Lab, Lund University, Sweden. The machine conditions were 1.5 GeV energy and 100–200 mA electron current. Spectra were recorded at room temperature at ambient atmospheric pressure using a double crystal monochromator (Si [111]). The energy was calibrated by a Pb foil, and the first maximum of the first derivative of the Pb L_{III}-edge set at 13.035 keV. Samples were positioned at 45° to the incident beam and a Ga filter was placed between the sample and the detector. The monochromator was detuned 15–25% to remove higher order harmonics. EXAFS data were collected at an exposure time of 2–4 s for each data point with 5 eV steps from 200 eV before the edge and 1 eV steps from the edge and up to 800 eV after the edge. Each EXAFS spectrum represents the average of 2–3 scans, each scan taking about 60 min to collect. For reduction and analysis of EXAFS data, the program WinXAS97 (Ressler, 1998) was used. From each averaged spectrum a polynomial pre-edge function was subtracted and the data were normalized. Above the absorption edge a cubic spline fit was used to remove the background. Data were k₃-weighted, normalized and the edge-energy was set as the first maximum of the first-derivative. Transmission EXAFS data were obtained for a selection of model compounds diluted in boron nitride; hydrocercite [Pb₃(CO₃)₂(OH)₂]; cerrucite (PbCO₃); litharge (PbO, tetragonal); and Pb bound to soil organic matter (SOM). These model compounds were used to reproduce the experimental curves of contaminated sand by a least-square, linear combination fitting (LCF) procedure. The fitting interval was performed over the k-range 1.0 – 8.7 Å⁻¹. The model compound for Pb bound to SOM was prepared by adding an aqueous solution of Pb(II) nitrate to an organic, fen peat soil (54% organic C content by mass), resulting in a final concentration of 4,000 µg Pb(II) g⁻¹. pH was adjusted to 5.0 by NaOH. Iron k-edge X-ray absorption near-edge spectroscopy (XANES) analyses revealed no detectable goethite in the organic soil (unpublished data).

Results

The EXAFS analyses and model fitting are shown in Figure 1. A simple visual comparison of EXAFS spectra reveals a similarity between the contaminated sand (before and after leaching) and the model compound litharge, indicating PbO(s) to be the most dominant Pb species in the first 30 cm. Also, the layer of soil 1 cm below the contaminated sand indicated that PbO(s) was present as the predominant Pb species, even if data were not fitted (because of a lower signal-to-noise ratio). The PbO(s) model compound showed a distinctive secondary peak in the normalized EXAFS spectrum at 3 Å⁻¹, which was shared by all experimental sands. In contrast, cerrucite had a trough at 3 Å⁻¹ and hydrocercite had a distinctive double peak. The best model for the experimental spectra of the contaminated sand was a linear combination of 25% Pb bound to organic matter and 75% PbO, and 30% Pb bound to organic matter and 70% PbO in the leached, contaminated sand. Given that the error sum of squares was decreased by 48% by adding organically bound Pb to the model only including PbO, the contribution from organic Pb complexes were highly significant.

Discussion

Based on our EXAFS analysis, a relatively small quantity of organic matter in the <0.06 mm fraction of the contaminated sand (0.65 mass-% organic C) would be enough to account for the complexation of 25% of total Pb. Given that natural organic matter on average contains one carboxyl group per six organic carbon atoms (Stevenson, 1994), a soil content of 0.65% organic C corresponds to a concentration of 45 mmol kg⁻¹ carboxyls, which is more than enough to complex 25% of the total concentration of 110 mmol Pb kg⁻¹ found in the contaminated soil. In a similar EXAFS study, Hashimoto and co-workers (2009) found that 38% of total Pb (at a concentration of 18,000 mg kg⁻¹ or 87 mmol Pb kg⁻¹) was organically complexed in a shooting range soil containing 8.6% organic matter.

Most of the literature concerning field observations of Pb species on active firing ranges reports that hydrocercite (Pb₃(CO₃)₂(OH)₂) and cerrucite (PbCO₃) are the dominant solid species of Pb to be found (Johnson *et al.* 2005). In other studies, PbO(s) has been determined to be a minor component of the

weathering products of bullets (Dermatas *et al.* 2004; Hardison *et al.* 2004). However, Hashimoto *et al.* (2009) recently determined that between 22% and 46% of the total lead in an amended shooting range soil was in the form of PbO.

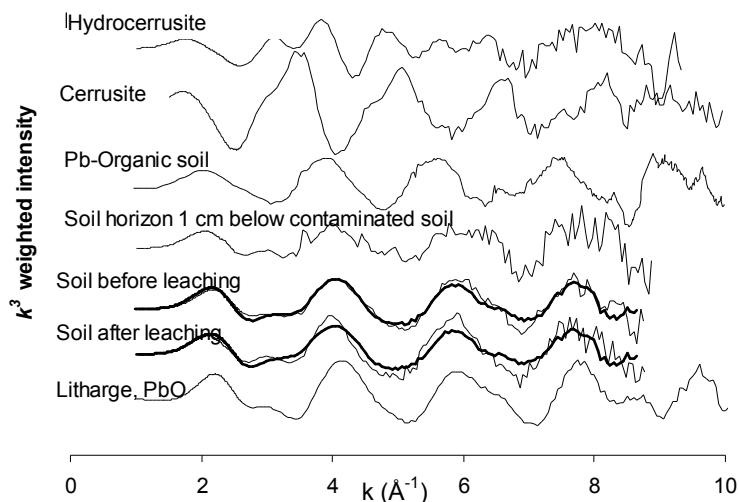


Figure 1. EXAFS spectra of model compounds and experimental samples

The formation of cerrusite and hydrocerrusite are predicated on the availability of the carbonate ion (Basta *et al.* 1993). Microbial activity in sands with significant amounts of organic matter may raise pore concentrations of CO₂ and it has been shown that an elevated partial pressure of CO₂ relative to the atmosphere favours the formation of Pb carbonates (Lindsay, 1979). The sand and gravel mixture used on Swedish ranges is quite low in organic material. Together with the low pH of 4.8 (shifting the equilibrium to carbonic acid and degassing of CO₂), this suggests a lack of available carbonate anions, which will favour the formation of PbO(s) over Pb carbonates.

Lead oxide exists in two forms; orthorhombic β -PbO (massicot) and tetragonal α -PbO (litharge). In most previous spectroscopic studies (Hashimoto *et al.* 2009; Scheckel, 2004), only massicot has been investigated, while Ma *et al.* (2007) only investigated litharge. Vantelon *et al.* (2005) considered both massicot and litharge but concluded that the signal of PbO was too weak to allow a non-ambiguous assignment to either litharge or massicot. In the current study, we only used litharge as model compound for PbO. However, given the similarity of EXAFS spectra for litharge and massicot, we cannot exclude possible contribution from massicot in our samples. Therefore, we have chosen to refer only to PbO throughout this article rather than to specify either litharge or massicot.

Based on their experimental observations, Ma *et al.* (2007) have proposed a three step model for the weathering of Pb in sand in which the first step is the oxidation of metallic Pb to PbO. Vantelon *et al.* (2005) have shown that in the weathered crust of bullets, PbO is the mineral which is found closest to the metal surface, indicating that it is the first product of weathering. Our results are in agreement with these observations and suggest that PbO is in fact the first product of weathering when a bullet is exposed to the environment, and that the kinetics of carbonate formation are significantly slower. The study reported by Hardison *et al.* (2004) is especially interesting in the context of our results because the sand used was the type used in children's play areas, which implies an organic content that is presumably at least as low as that in the current experiment. One possible explanation for these differences is that the kinetics of reaction between the various Pb minerals is different at 4°C than it is at 20°C. The effect of temperature on Pb speciation in sands has not been discussed in the literature.

Conclusions

The majority of the Pb in the sands was found to be in the form of PbO both before and after infiltration. This was explained by the low organic content and pH of the soil compared to previous studies which prevented the formation of carbonates, the rapid kinetics of reaction of PbO as compared to the Pb carbonates, and the higher ambient redox potential used in this experiment.

References

- Basta NT, Pantone DJ, Tabatabai MA (1993) Path analysis of heavy metal adsorption. *Soil Agron. J.* **85**, 1054-1057.
- Dermatas D, Dadachov M, Dutko P, Menounou M, Arienti P, Shen G (2004) Weathering of lead in Fort Irwin firing range soils. *Global Nest* **6**(2), 167-175
- Hardison DW, Ma LQ, Luongo T, Harris WG (2004) Lead contamination in shooting range soils from abrasion of lead bullets and subsequent weathering. *Sci. of the Tot. Environ.* **328**, 175-183
- Hashimoto Y, Takaoka M, Oshita K, Tanida H (2009) Incomplete transformations of Pb to pyromorphite by phosphate-induced immobilization investigated by X-ray absorption fine structure (XAFS) spectroscopy. *Chemosphere* **76** (5).
- Johnson CA, Moench H, Wersin P, Kugler P, Wenger C (2005) Solubility of antimony and other elements in samples taken from shooting ranges. *J. Environ. Qual.* **24**, 248-254.
- Jorgensen SS, Willems J (1987) The fate of lead in soils: The transformation of lead pellets in shooting range soils. *Ambio.* **16**, 11-15.
- Klitzke SF, Lang, Kaupenjohann M (2008) Increasing pH releases colloidal lead in a highly contaminated forest soil. *Euro. J. of Soil. Sci.* **59**(2), 265-273
- Laudon H, Köhler S, Bishop, KH (1999) Natural acidity or anthropogenic acidification in the spring flood of northern Sweden. *Sci. Tot. Environ.* **234**, 63-73
- Lindsay WL (1979) 'Chemical equilibria in sands' (John Wiley Publishers: New York).
- Ma LQ, Hardison DW, Harris WG, Cao X, Zhou Q (2007) Effects of soil property and soil amendment on weathering of abraded metallic Pb in shooting ranges. *Water Air Soil Pollut.* **178**, 297-307
- Scheckel KG, Ryan JA, (2004) Spectroscopic speciation and quantification of lead in phosphate-amended soils. *J. Environ. Qual.* **33**, 1288-1295.
- Stevenson FJ (1994) 'Humus chemistry' (John Wiley and sons: Hoboken, NJ).
- Vantelon D, Lanzirrotti A, Scheinost AC, Kretzschmar R (2005) Spacial distribution and speciation of lead around corroded bullets in a shooting range soil studied by micro X-ray fluorescence and absorption spectroscopy. *Environ. Sci. Technol.* **39**, 4808-4815.

Geochemistry and contents of trace metals in the soils and sediments of Daliao River System watershed, China

Mengchang He, Xitao Liu and Chunye Lin

State Key Joint Laboratory of Environmental Simulation and Pollution Control, School of Environment, Beijing Normal University, Beijing 100875, China. Email c.lin@bnu.edu.cn

Abstract

The Daliao River System (DRS) watershed has the long-term been affected by intensive industrial, urban and agricultural activities. The objectives of this study were to compare contents of trace metals and geochemical relationships between trace metals and Mn in the watershed soils and estuarine sediments of the DRS. Thirty five surface sediment samples were collected from the DRS estuary and analysed for trace metals. Trace metal contents in the various soils of the DRS watershed were collected from the earlier survey in the 1980s. Results indicate that As, Cr, Cu, Hg, Ni, Pb and Zn contents and their geochemical baselines in the estuarine sediments were similar to those in the watershed soils. However, Cd content was much higher in the estuarine sediments than in the watershed soils, while Co and V contents in the estuarine sediments were much lower. Higher Cd content in the estuarine sediments might be caused by contamination; while lower Co and V contents might be caused by the sedimentary environment, resulting in release of Co and V in the sediments. Our study documented that the geochemical baselines of most trace metals for the watershed soils were generally similar to those for the estuarine sediments of the DRS.

Key Words

Trace metals, soils, sediments, estuary, watershed.

Introduction

The products of rock weathering constitute most of the inorganic minerals in soils and sediments. In addition, aquatic sediments generally originate from erosion of soils. Therefore, elemental composition and geochemistry in soils are generally similar to those in sediments in the same watershed (Bowen, 1979). However, internal and external factors, such as contamination, precipitation, dissolution, postdepositional effects of diagenesis, etc., might change elemental composition and geochemistry in sediments (Forstner and Wittmann 1983). Thus, a knowledge of elemental geochemical relationship from soils to sediments is of considerable importance in understanding the elemental geochemistry and contamination within watersheds.

The Daliao River System (DRS) consists of the Daliao river, Hun river, and Taizi river, with 94 km, 415 km, and 413 km in length, and draining 1390 km², 11500 km², and 13900 km² of land area, respectively (Figure 1). The DRS is the most important water course in Liaoning Province of China. These rivers drain large industrial, urban and agricultural areas. Average annual outputs of Cu, Pb and Zn were 70.3×10³, 40.3×10³ and 265.9×10³ t, respectively, in the province during the period of 1990 to 2006. In addition, the output of pig iron reached 37.6×10⁶ t in 2006. Big industrial cities in the watershed of the DRS include Fushun, Shenyang, Benxi, Anshan, Liaoyang, Panjin, and Yingkou, with populations of 2.24, 6.98, 1.56, 3.47, 1.82, 1.25, 2.30 million, respectively, in 2005. Hence, there is interest in investigating composition and geochemistry of the trace metals in the soils and sediments of the DRS watershed. The objectives of this study were to compare contents of trace metals and geochemical relationships between trace metals and mineral matrix element Mn in the watershed soils and estuarine sediments of the DRS.

Methods

Data collection

Elemental contents in the soils of DRS watershed as well as Liaoning province were investigated in 1980s, for thousands of soil samples. We collected the data on contents of trace metals (As, Cd, Co, Cr, Cu, Hg, Ni, Pb, V, and Zn) and mineral matrix element Mn in various types of soil obtained by this investigation.

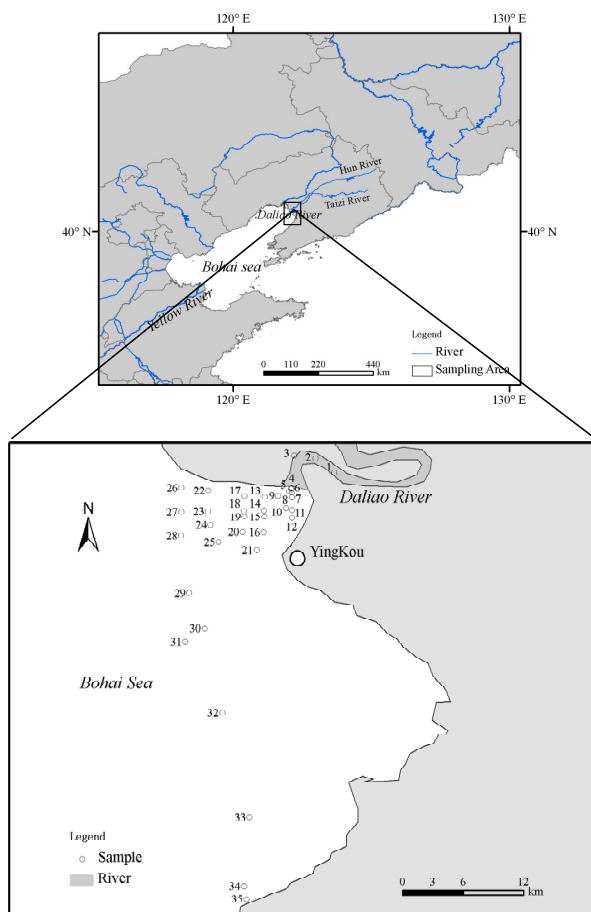


Figure 1. Schematic graph of DRS watershed and sediment sampling sites.

10.02%, 6.01%, 5.10%, 6.74%, 3.81%, 2.27%, 6.21%, 1.65%, 2.75%, 1.99% and 1.52% for As, Cd, Co, Cr, Cu, Hg, Ni, Pb, V, Zn, Mn, Al, Fe and Ca, respectively.

Results

Comparison between elemental contents in the estuarine sediments and watershed soils of the DRS

The contents of Mn, Al, Fe and Ca in the estuarine sediments of the DRS were 264.7-1073.0 mg/kg, 3.77-7.97%, 0.67-4.19% and 0.75-2.02%, respectively, with means of 623.9 mg/kg, 6.72%, 2.61% and 1.20%. Compared with world average contents of 770 mg/kg, 7.2%, 4.1% and 6.6% of Mn, Al, Fe and Ca, respectively (Bowen, 1979), the surface sediments of the estuary contained slight lower Mn, Al and Fe and much lower Ca. The average contents of As, Cd, Co, Cr, Cu, Hg, Ni, Pb, V and Zn in the estuarine sediments of the DRS were 10.68, 0.39, 10.60, 65.20, 21.06, 0.06, 23.47, 26.19, 62.37, 76.62 mg/kg, respectively; while in the watershed soils of the DRS they were 8.90, 0.12, 15.36, 52.21, 22.54, 0.04, 27.03, 22.05, 82.40 and 67.93 mg/kg, respectively (Figure 2).

As, Cr, Cu, Hg, Ni, Pb and Zn contents in the estuarine sediments were similar to those in the watershed soils. This may indicate that these trace metals in the estuarine sediments mainly originated from soil erosion of watershed as natural source. On the other hand, Cd content was much higher in the estuarine sediments than in the watershed soil, while Co and V contents were much lower in the estuarine sediments than in the

Sediment sampling and analysis

Surface sediment samples were collected at 35 sites within the estuarine coastal area in 2007, using cable operated sediment samplers (Van Veen grab sampler, Eijkelkamp) (Figure 1). All sediment samples were freeze-dried, slightly crushed, passed through 2 mm sieve, and stored in glass bottles.

Portions of the sediment samples were digested with HNO₃-HF-HClO₄ and the filtrated extracts were measured for Co, Cr, Cu, Hg, Ni, V, Zn, Mn, Al, Fe and Ca by ICP-OES (IRIS Intrepid II, Thermo Electron) and for Cd and Pb by ICP-MS (X Series II, Thermo Electron) (Lin *et al.* 2008). In addition, sediment samples were digested with aqua regia, 1% KMnO₄ solution, and 1% oxalic acid, with the supernatant was measured by atomic fluorescence spectroscopy (XGY-1011A, Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences) for As and Hg concentrations. The accuracy of the method was assessed by conducting analyses of three reference materials (GSS1, GSS2, GSS9) from Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences. Average relative errors (triplicate) of As, Cd, Co, Cr, Cu, Hg, Ni, Pb, V, Zn, Mn, Al, Fe and Ca for the three reference materials were -1.7~1.0%, -5.2~12.5%, -1.8~1.9%, -6.0~1.7%, 4.5~7.67%, -1.3~4.2%, -2.0~7.8%, -0.9~5.0%, -3.3~2.8%, -4.7~7.1%, -1.9~-0.1%, -1.6~3.1%, -0.1~2.7% and 1.2~2.0%, respectively. The precision was calculated as the variation coefficients (CV%) of two replicates from 14 sediment samples, and average CV% was 4.51%, 8.92%, 4.03%,

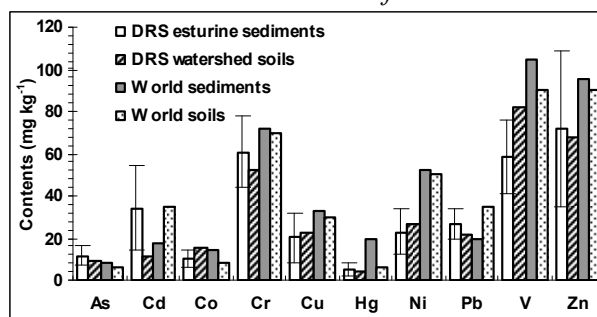
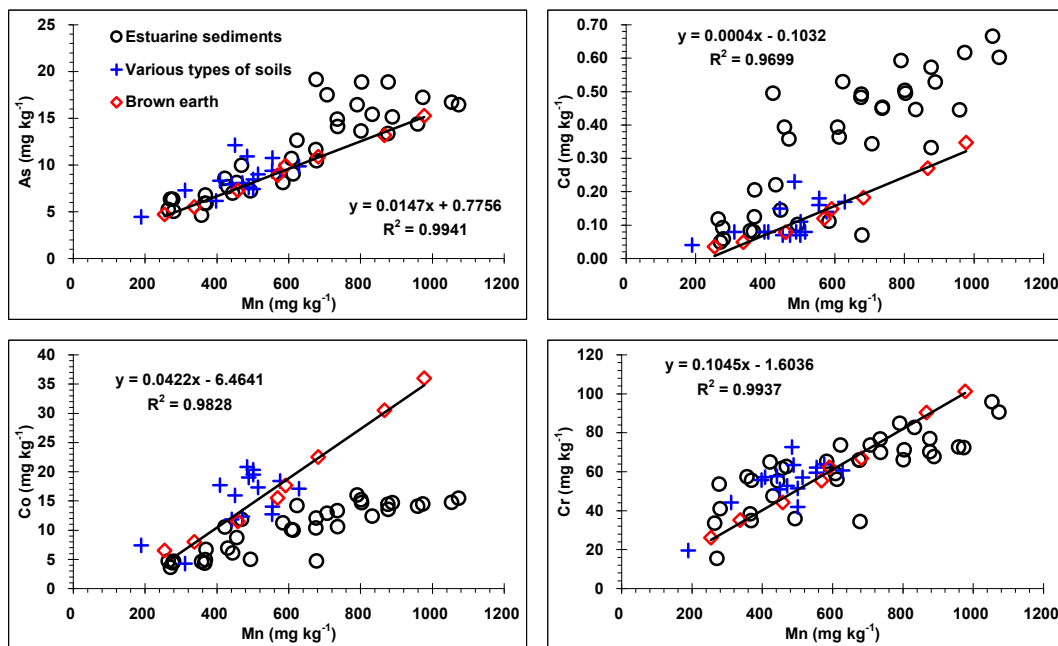


Figure 2. Trace metal contents in the watershed soils and sediments of the DRS and world sediments and soils (Bowen, 1979).

watershed soils. The higher Cd content in the estuarine sediments indicated its anthropogenic sources such as industrial and municipal effluent. The lower Co and V contents in the estuarine sediments may show their dissolution, following sedimentation of suspended particles. In addition, whereas Cd and Pb contents in the watershed soils of the DRS were much lower than those in the world soils, Cd and Pb contents in the estuarine sediments of the DRS were higher than those in the world sediments (Figure 2), indicating relatively higher enrichment of Cd and Pb in the estuarine sediments. This might be related to the Pb mining and smelting in the watershed. Cu, Ni and Zn contents in the both soils and estuarine sediments of the DRS were much lower than those in the world soils and sediments.

Comparison between elemental geochemical relationships in the estuarine sediments and watershed soils of the DRS

Trace metal contents in the soils and estuarine sediments were plotted as function of the content of mineral matrix element Mn (Figure 3). Trace metal contents were positively correlated with Mn content in the both watershed soils and estuarine sediments of the DRS. In non-contaminated soils and sediments, trace element contents are generally correlated significantly with the contents of co-occurring elements or mineral matrix elements and the regression lines (equations) are defined as the geochemical baselines of trace metals for soils and sediments (Windom, 1989). The cross symbol in Figure 3 represents average contents of elements in the various types of the watershed soils, including brown earths, weakly brown earths, aquic brown earths, dark-brown earths, meadow soils, cinnamon soils, leached cinnamon soils, calcic cinnamon soils, meadow soils, calcic meadow soils etc. The square symbol in Figure 3 represents elemental content average and percentiles at 5%, 10%, 25%, 50%, 75%, 90%, and 95% of 267 soil samples of brown earths covering the most area of the watershed. For As, Cr, Cu, Hg, Ni, Pb and Zn, their geochemical relationships with Mn in the watershed soils were similar to those in the estuarine sediments. However, Cd content was relatively higher and Co and V contents were relatively lower in the estuarine sediments. Generally, changes of geochemical conditions from soils to sediments and contamination can change geochemical relationships of trace metals with co-occurring elements or mineral matrix elements. Relatively higher Cd content in the estuarine sediments might be caused by contamination; while relatively lower Co and V contents might be caused by the geochemical conditions of the estuarine sediments, resulting in release of Co and V in the sediments. The regression lines (equations) were fitted with datum points of square symbol (Figure 3), indicating trace metals contents were significantly correlated to the mineral matrix Mn in the brown earths covering the most area of the watershed. For As, Cr, Cu, Ni, Pb, and Zn, the regression lines generally fitted well datum points for the estuarine sediments, indicating similar geochemical relationships between trace elements and Mn in the watershed soils and estuarine sediments. Therefore, these regression lines might be geochemical baselines of the trace metals in the watershed soils and estuarine sediments to identify their sources and contamination.



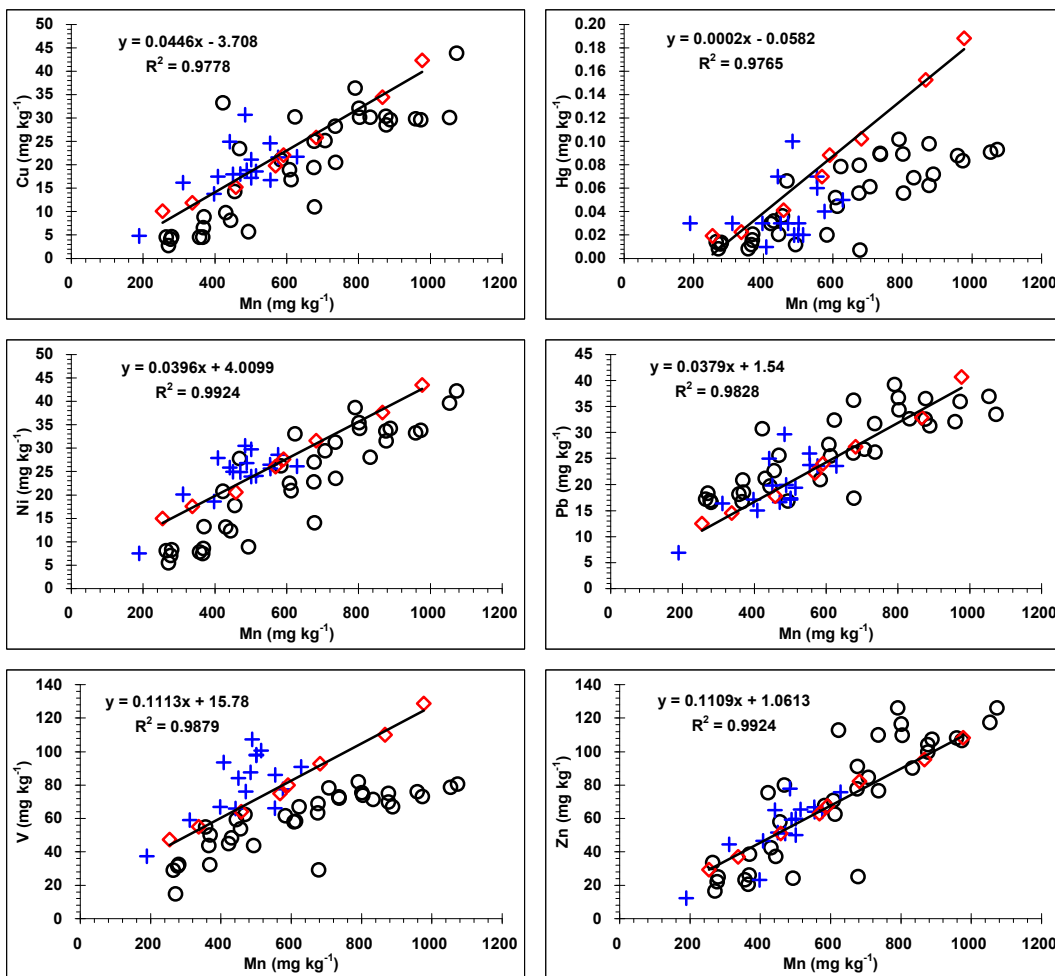


Figure 3. Geochemical relationship between trace metal contents and mineral matrix element Mn content in the watershed soils and estuarine sediments of the DRS. The detailed explanation are given in text.

Conclusion

As, Cr, Cu, Hg, Ni, Pb and Zn contents in the estuarine sediments were similar to those in the watershed soils, indicating that these trace metals mainly originated from soil erosion of watershed as natural source. On the other hand, anthropogenic input of Cd into the DRS led to the higher content of Cd in the estuarine sediments, but dissolution of V and Co, caused by the estuarine sedimentary condition, resulted in the lower content of V and Co in the estuarine sediments. The geochemical relationships of As, Cr, Cu, Hg, Ni, Pb and Zn with Mn in the watershed soils were similar to those in the estuarine sediments, documenting the geochemical baselines of these trace metals were similar in the watershed soils and estuarine sediments of the DRS.

Acknowledgements

This study was supported by the National Basic Key Research Program of China (2004CB418502) and the National Natural Science Foundation of China (40971058, 40873077).

References

- Bowen HJM (1979) 'Environmental Chemistry of the Elements.' (Academic Press: London)
- Försterner U, Wittmann GTW (1981) 'Metal Pollution in the Aquatic Environment, 2nd edn.' (Springer Verlag: Berlin)
- Lin C, He MC, Zhou YX, Guo W, Yang ZF (2008) Distribution and contamination assessment of heavy metals in sediment of the Second Songhua River, China. *Environ. Monit. Assess.* **137**, 329-342.
- Windom HL, Schropp SJ, Calder FD, Ryan JD, Smith RG, Burney LC, Lewis FG, Rawlinsont CH (1989) Natural trace metal concentrations in estuarine and coastal marine sediments of the Southeastern United States. *Environ. Sci. Technol.* **23**, 314-320.

Heavy metal contents and chemical speciations in sewage-irrigated soils from the eastern suburb of Beijing, China

Ye Zhao, Zhifan Chen, Qiang Li, Xitao Liu

School of Environment, Beijing Normal University, Beijing 100875, China, Email zhaoye@bnu.edu.cn

Abstract

Accumulation of heavy metals in agricultural sewage-irrigated soils has caused increasing concern. This study analyzed the total concentrations and chemical speciations of heavy metals including Cd, Cr, Cu, Zn and Ni in sewage-irrigated soils in the eastern suburb of Beijing, China. The results showed that there was remarkable buildup of Cd, Cr, Zn and Cu in sewage-irrigated topsoils compared to reference topsoils. Besides, the total Cd and a part of Ni were beyond Chinese agricultural soil environmental quality criteria. In sewage-irrigated soils, Cd, Cr, Ni, Zn and Cu were dominated by residual fractions and few were present in exchangeable and carbonate fractions as a result of higher soil pH. The mobility and bioavailability of the five metals declined in the following order: Zn, Cu, Ni, Cd and Cr. However, other than the four metals, Cd was more mobile and bioavailable in the sewage-irrigated topsoils than in the reference topsoils. This indicated higher Cd contents in sewage-irrigated soils may constitute a potential risk to food security and human health.

Key Words

Heavy metals, chemical speciation, sewage irrigation, agricultural soil, bioavailability.

Introduction

The rapid development of urbanization and industrialization, together with the shortage of availability of fresh water to be used for irrigation led to the rising use of sewage for agricultural land irrigation. While sewage provides water and valuable plant nutrients, it leads to the potential accumulation of heavy metals (HMs) in agricultural soils (Abdel-Sabour 2003; Zhang *et al.* 2008). When the contents of HMs exceed the permitted threshold, they will impact the normal growth of crops or even might enter food chain to threat human and animal health (Akoumianakis *et al.* 2009; Fu *et al.* 2009; Salvatore *et al.* 2009). In the study of S. Khan *et al.* (2008), it was suggested that there was a substantial buildup of HMs in plants grown in wastewater-irrigated soils of Beijing, in which the HMs concentrations were significantly higher than those in the reference soil, and exceeded the permissible limits set by SEPA in China and WHO.

The toxicity and the mobility of heavy metals in soils depend not only on the total concentration, but also on their specific chemical form and soil properties like pH, organic matter content etc. (Lu *et al.* 2003). The chemical fractionation of heavy metals must be taken into account in pollution studies since total contents in soils provide, in most cases, limited information on the mobility and bioavailability of heavy metals (Kartal *et al.* 2006) and can be misleading when assessing environmental effects due to a potential overestimation of exposure risk (Cuong and Obbard 2006). The objective of this study was to determine the total concentrations and geochemical speciation of Cd, Cr, Cu, Zn and Ni in Calcaric Cambisol under sewage irrigation in the eastern suburb of Beijing, China.

Materials and methods

Description of study area

The sewage-irrigated area studied is located in the connection region between the Tongzhou District of Beijing City and the Xianghe County of Hebei Province, where several rivers form a fan-shaped alluvial plain, characterized by continental monsoon climate. The predominant soil type in the area is Calcaric Cambisol. And the agricultural land has been mainly irrigated using sewage from the Beiyun River which runs through the urban area to rural agriculture land, and from the Fenggangjian River, or the Gaobeidian wastewater treatment plant. The Beiyun River and the Fenggangjian River are the main domestic and production effluent recipients and also are the main sources for irrigation in the southeast area of Beijing.

Collection of soil samples

Sewage-irrigated and reference soils were collected in the summer of 2007. Sewage-irrigated soil samples were collected in the area irrigated with sewage from the Beiyun River and the Fenggangjian River, including 20 samples of topsoil (0-20 cm) and 10 samples of subsoil (20-40 cm). Reference soils were

collected in non-sewage-irrigated areas from the northeast area of the Chaobai River, including 6 topsoil samples. All soil samples were collected from farmlands far from the highways as shown in Fig. 1.

Analytical methods

Immediately after collection, all samples were air-dried at room temperature and ground in an agate mortar to allow passage through a 2-mm nylon sieve. A subsample of each soil sample was further ground to allow passage through a 0.149-mm nylon sieve. The sequential extraction scheme proposed by Tessier *et al.* was adopted to partition HMs into five fractions: exchangeable (exch.), bound to carbonates (carb.), bound to Fe-Mn oxides (Fe/Mn ox.), bound to organic matter (orga.) and residual (resi.) (Tessier *et al.* 1979). The defining and specific approach were performed according to our published literature (Chen *et al.* 2009). The total elemental contents of soil samples and residual fractions were digested 0.1000 g of dried soil samples with HNO₃-HF-HClO₄ mixture followed by instrumental analysis. The elemental concentrations of all solutions were determined using HR-ICP-MS (Element 2, Finnigan Co., USA). The soil pH was measured in a 1:2.5 (w/v) mixture of soil and water using a glass electrode, the CaCO₃ content was measured using the gas-volumetric method and the organic carbon content of the soil was determined by the K₂Cr₂O₇-H₂SO₄ digestion method (Liu *et al.* 2005).

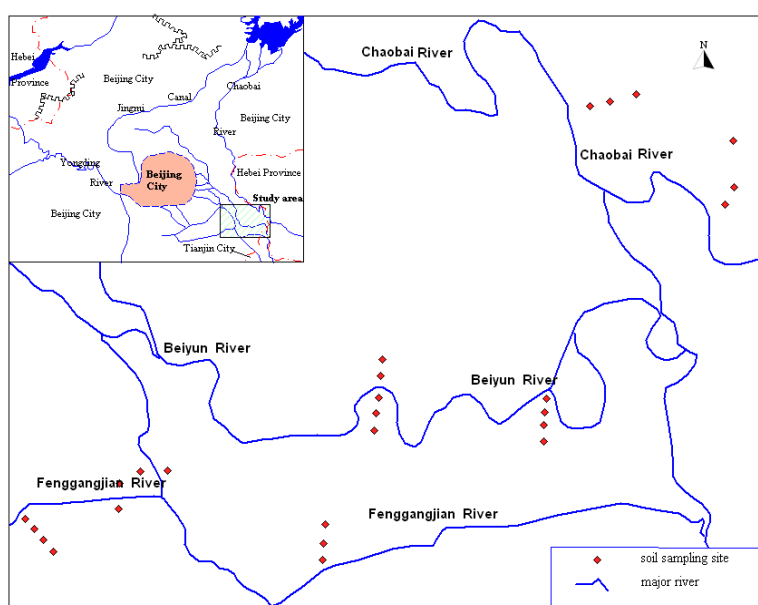


Figure 1 Soil sampling sites for heavy metal investigation in the east suburb of Beijing

Results

Total concentrations of heavy metals in the soils were shown in Table 1. The Cd, Cr, Ni, Zn and Cu fractions expressed as percentages of the sum of individual chemical forms in ST, SS and RT are presented in Table 2, respectively.

Table 1 Total content of heavy metals in the soils (unit: mg/kg)

| | | | Cd | Cr | Ni | Zn | Cu |
|--|-----------------|-------|-----------|------------|-----------|-----------|-----------|
| sewage-irrigated soils | 0-20cm n=20 | Mean, | 0.81±0.16 | 78.3±20.2 | 31.8±17.4 | 71.5±14.0 | 25.5±4.3 |
| | | SD | | | | | |
| | | Range | 0.62-1.19 | 55.7-124.4 | 14.7-85.1 | 52.1-91.1 | 17.2-32.8 |
| reference soils | 20-40cm n=10 | Mean, | 0.45±0.34 | 58.7±13.7 | 23.3±5.5 | 66.0±11.7 | 21.8±4.6 |
| | | SD | | | | | |
| | | Range | 0.27-0.65 | 41.7-73.2 | 15.7-28.8 | 54.3-81.9 | 15.1-25.8 |
| reference soils | 0-20cm n=6 | Mean, | 0.36±0.25 | 53.8±8.1 | 18.7±3.0 | 51.9±4.4 | 16.0±3.7 |
| | | SD | | | | | |
| | | Range | 0.12-0.68 | 47.4-65.7 | 14.9-22.0 | 45.4-53.6 | 12.2-21.1 |
| Criteria of agricultural soil quality ^a | | | 0.60 | 250 | 60 | 300 | 100 |
| Background of soils in Beijing ^b | | | 0.12 | 29.8 | 26.8 | 57.5 | 18.7 |

^a National Standard of PR China (1995) (GB 15618-1995).

^b Chen T B et al (2004).

Table 2 Chemical fractionation of heavy metals in the study area (unit: %)

| | | | Exch. | Carb. | Fe/Mn ox. | Orga. | Resi. |
|----|----|----------|-------------|-----------|-------------|-------------|--------------|
| Cd | ST | Mean, SD | 6.98±10.16 | n.d. | 23.85±12.18 | 7.37±11.46 | 61.77±13.36 |
| | | Range | n.d.-32.97 | | n.d.-40.47 | n.d.-45.92 | 0.34-0.69 |
| | SS | Mean, SD | 13.38±12.05 | n.d. | n.d. | 4.60±2.41 | 82.01±12.72 |
| | | Range | n.d.-24.37 | | | 1.95-7.80 | 67.83-95.72 |
| | RT | Mean, SD | 20.24±14.93 | n.d. | 4.30±8.61 | n.d. | 75.46±20.59 |
| | | Range | n.d.-31.37 | | n.d.-17.22 | | 51.49-100.00 |
| Cr | ST | Mean, SD | 0.04±0.07 | 0.08±0.16 | 2.30±0.88 | 13.46±5.10 | 84.12±5.41 |
| | | Range | n.d.-0.17 | n.d.-0.55 | 1.30-3.84 | 6.26-27.17 | 70.33-92.42 |
| | SS | Mean, SD | 0.04±0.02 | 0.55±0.22 | 2.00±0.44 | 22.72±5.57 | 74.71±6.16 |
| | | Range | 0.01-0.06 | 0.29-0.79 | 1.56-2.58 | 17.65-28.94 | 67.69-80.04 |
| | RT | Mean, SD | 0.08±0.10 | 0.36±0.26 | 2.11±0.83 | 15.47±4.98 | 81.99±4.83 |
| | | Range | n.d.-0.20 | n.d.-0.58 | 1.47-3.33 | 9.96-20.47 | 77.10-86.71 |
| Ni | ST | Mean, SD | 0.43±0.57 | 0.19±0.48 | 10.75±4.41 | 33.87±12.56 | 54.75±13.58 |
| | | Range | n.d.-1.92 | n.d.-1.77 | 4.10-19.22 | 8.88-62.36 | 29.86-86.78 |
| | SS | Mean, SD | 0.70±0.84 | 2.21±0.52 | 6.26±1.25 | 55.37±11.31 | 35.47±10.81 |
| | | Range | 0.12-1.93 | 1.65-2.86 | 4.84-7.39 | 44.21-67.53 | 25.45-47.98 |
| | RT | Mean, SD | 0.38±0.42 | 1.31±1.07 | 6.47±4.94 | 38.80±7.35 | 53.05±6.48 |
| | | Range | 0.12-1.00 | n.d.-2.44 | 3.44-13.85 | 32.11-49.17 | 43.89-57.98 |
| Zn | ST | Mean, SD | 1.76±3.67 | 1.55±1.67 | 14.92±6.78 | 32.55±5.75 | 49.23±8.73 |
| | | Range | n.d.-10.52 | 0.24-5.45 | 5.25-29.64 | 23.77-45.60 | 31.12-67.23 |
| | SS | Mean, SD | 9.93±2.05 | 1.95±1.21 | 7.19±1.07 | 45.20±6.27 | 35.73±9.54 |
| | | Range | 7.96-12.61 | 1.01-3.70 | 6.16-8.63 | 37.05-50.82 | 26.49-46.20 |
| | RT | Mean, SD | 3.57±3.34 | 2.86±1.17 | 12.25±5.49 | 37.89±6.31 | 43.43±4.16 |
| | | Range | n.d.-7.84 | 2.00-4.54 | 7.94-20.29 | 29.80-44.78 | 39.18-48.29 |
| Cu | ST | Mean, SD | 0.37±0.27 | 0.12±0.26 | 17.50±9.98 | 30.25±6.57 | 51.76±11.19 |
| | | Range | 0.17-1.24 | n.d.-0.83 | 6.91-40.55 | 18.00-43.19 | 27.78-69.36 |
| | SS | Mean, SD | 0.41±0.30 | 1.31±0.48 | 20.73±3.65 | 39.32±5.44 | 38.24±9.10 |
| | | Range | 0.22-0.86 | 0.85-1.79 | 17.23-25.13 | 34.66-45.23 | 30.12-46.24 |
| | RT | Mean, SD | 0.52±0.20 | 0.19±0.20 | 11.64±3.31 | 37.32±8.06 | 50.33±8.22 |
| | | Range | 0.27-0.72 | n.d.-0.38 | 8.24-15.09 | 25.51-43.64 | 40.43-60.44 |

n.d. means not detected.

Discussion and conclusion

In ST, an obvious accumulation of Cd, Cr, Zn and Cu was found compared with reference soils, and the concentrations of the HMs except Cd and Ni were below the Chinese agricultural soil environmental quality criteria. In sewage-irrigated soils, Cd, Cr, Ni, Zn and Cu were predominately present in the residual fraction. The order of Cu, Zn and Ni in sewage-irrigated topsoils in each fraction was residual > organic > Fe-Mn oxide > exchangeable > carbonate, for Cd in sewage-irrigated soils, the order was residual > Fe-Mn oxide > organic > exchangeable > carbonate, and for Cr in sewage-irrigated soils, the order was residual > organic > Fe-Mn oxide > carbonate > exchangeable. However, for Cu and Ni in sewage-irrigated subsoils, the order was organic > residual > Fe-Mn oxide > carbonate > exchangeable, for Zn the order was organic > residual > exchangeable > Fe-Mn oxide > carbonate, and for Cd the order was residual > exchangeable > organic > Fe-Mn oxide and carbonate. The contents of the exchangeable and carbonate fractions were very low, which may be related to higher soil pH. It was observed that the contents of residual speciation and the Fe-Mn oxygenation state in ST were higher than those in RT for all heavy metals except Cd, while the contents of the organic state and other states were relatively lower than those in RT. Likewise, the contents of the residual and Fe-Mn oxide fractions in ST were higher than those in SS for all the metals with the exception of Cu and Cd, while the contents of organic, carbonate and other states were relatively lower than those in SS. The relatively high percentage of heavy metal carbonated bound fraction in subsoils may be related to adsorption and consolidation of CaCO₃ in subsoils. The mobility and bioavailability of the five metals declined in the following order: Zn, Cu, Ni, Cd and Cr. Cd being different from other four heavy metals was more mobile and bioavailable in the sewage-irrigated topsoils than in the reference topsoils. Therefore, higher Cd contents in sewage-irrigated soils will have potential risk for human health.

References

- Abdel-Sabour MF (2003) Impact of wastewater reuse on cobalt status in Egyptian environment. *Journal of Environmental Sciences-China* **15**, 388-395.
- Akoumianakis KA, Passam HC, Barouchas PE, Moustakas NK (2009) Effect of cadmium on yield and cadmium concentration in the edible tissues of endive (*Cichorium endivia* L.) and rocket (*Eruca sativa* Mill.). *Journal of Food, Agriculture & Environment* **6**, 206-209.
- Chen TB, Zheng YM, Chen H, Zheng GD (2004) Background concentrations of soil heavy metals in Beijing. *Environmental Science* **25**, 117-122.
- Chen Z, Zhao Y, Li Q, Qiao J, Tian Q, Liu X (2009) Heavy metal contents and chemical speciations in sewage-irrigated soils from the eastern suburb of Beijing, China. *Journal of Food, Agriculture & Environment* **7**, 690-695.
- China P (1995) Soil Environmental Quality (GB 15618-1995). (Ed NSoP China). (Standards Press of China: Beijing).
- Cuong DT, Obbard JP (2006) Metal speciation in coastal marine sediments from Singapore using a modified BCR-sequential extraction procedure. *Applied Geochemistry* **21**, 1335-1346.
- Fu Q, Hu H, Li J, Huang L, Yang H, Lv Y (2009) Effects of soil polluted by cadmium and lead on production and quality of pepper (*Capsicum annuum* L.) and radish (*Raphanus sativus* L.). *Journal of Food, Agriculture & Environment* **7**, 698-702.
- Kartal S, Aydin Z, Tokalioglu S (2006) Fractionation of metals in street sediment samples by using the BCR sequential extraction procedure and multivariate statistical elucidation of the data. *Journal of Hazardous Materials* **132**, 80-89.
- Khan S, Cao Q, Zheng YM, Huang YZ, Zhu YG (2008) Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution* **152**, 686-692.
- Liu WH, Zhao JZ, Ouyang ZY, Soderlund L, Liu GH (2005) Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. *Environment International* **31**, 805-812.
- Lu Y, Gong ZT, Zhang GL, Burghardt W (2003) Concentrations and chemical speciations of Cu, Zn, Pb and Cr of urban soils in Nanjing, China. *Geoderma* **115**, 101-111.
- Salvatore MD, Carratù G, Carafa AM (2009) Assessment of heavy metals transfer from a moderately polluted soil into the edible parts of vegetables. *Journal of Food, Agriculture & Environment* **7**, 683- 688.
- Tessier A, Campbell PGC, Blsson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.* **51**, 844-851.
- Zhang YL, Dai JL, Wang RQ, Zhang J (2008) Effects of long-term sewage irrigation on agricultural soil microbial structural and functional characterizations in Shandong, China. *European Journal of Soil Biology* **44**, 84-91.

Heavy metal contamination of soils from organic paddy fields in Thailand

Nanthana Chinoim^A and Nusara Sinbuathong^A

^AKasetsart University Research and Development Institute, Kasetsart University, Bangkok, Thailand, Email rdinnc@ku.ac.th

Abstract

The objective of this study was to investigate the heavy metal concentrations (Cd, Cr, Pb, Cu, Ni, Zn) in paddy fields used for production of organic rice in Thailand. The researchers evaluated soil samples from four sites located north and west of Bangkok. From visual assessment and historical study, the locations did not appear to have been affected by industrial waste. Soil samples were collected from the 0–15 cm depth, air dried, and ground to pass a 2 mm sieve. Heavy metals were extracted from soil samples by acid mixture and then determined by atomic absorption spectrophotometry. Heavy metal concentrations as measured across the soil samples ranged from 0 – 0.0727 mg Cd/kg, 0 – 1.92 mg Cr/kg, 0.186 – 1.39 mg Pb/kg, 0.372 – 2.57 mg Ni/kg, 0.698 – 2.90 mg Cu/kg, and 0.987 – 14.4 mg Zn/kg. All of the heavy metal concentrations measured in these soils were much lower than the critical levels of the heavy metals.

Key Words

Heavy metals, contamination, paddy field, critical heavy metal values.

Introduction

The consumption of organic agricultural products is increasing worldwide. To take advantage of this growing market, Thailand, as a world leading rice exporter, has encouraged its farmers to produce rice using organic methods. In addition, the Thai government's policy in organic rice export is also stated.

To cultivate organic rice, Thai farmers have commonly applied cow or pig manure as a low cost fertilizer. However, accumulation of heavy metals from the manures has become a concern of consumers, farmers and researchers. Accumulation of the heavy metals in humans, particularly cadmium (Cd), chromium (Cr), lead (Pb), copper (Cu), nickel (Ni), and zinc (Zn), can have toxic effects. For example, cadmium, one of the most common and most widely distributed of environmental metal poisons, when consumed in rice causes proximal tubule damage, anaemia and a severe loss of bone minerals resulting in fractures (Reilly 2002). In addition, cases of *itai-itai* disease were identified among people living in cadmium-polluted areas (Yoshioka 1964; Waalkes 2000). An intake of 1–2 g/d of the hexavalent form of chromium causes kidney and liver necrosis (Kaufman *et al.* 1970). Lead, widely distributed in the environment, affects the central nervous system. Zinc salts at intake levels greater than 250 mg/d in adults causes intestinal irritation, nausea, vomiting and abdominal pain (Faila 1999). Although, emissions of heavy metals have declined in most developed countries, exposure still continues in developing countries, especially among the poorer populations where the toxic effects have not been widely known. Since rice is important economically to Thailand and is a major component of the daily diet of its citizens, this research has focused on the contamination of the heavy metals of soils from organic rice production fields.

Methods

Soil samples

Soil samples were collected from four paddy fields used in organic rice production. There were no indications of industrial pollution, from visual assessment and historical study. Two sites, with cow manure applied, were located approximately 100 km north, and two sites, with pig manure applied, were located approximately 120 km west of Bangkok, Thailand (Figure 1). At each site, ten randomly distributed samples were taken from the 0–15 cm depth (Tandon 1995; Soil and Plant Analysis Council Inc, 2000). The soil samples were air dried, ground to pass a 2 mm sieve and kept in plastic bags prior to analysis.

Analysis methods

Heavy metal ions in the soil samples were extracted by acid mixture (1N HCl/1N H₂SO₄) according to Perkins (1970). Concentrations of Cd, Cr, Pb, Cu, Ni, and Zn in the extractants were then determined by atomic absorption spectrophotometry (GBC AVANTA).



Figure 1. Sampling sites located in the north and west of Bangkok.

Results

The concentration of heavy metals in forty soil samples from the organic paddy fields varied widely. The ranges of concentration are shown in Table 1.

Table 1. Heavy metal concentrations of Cd, Cr, Pb, Ni, Cu, and Zn in 40 soil samples from Thailand rice paddy fields.

| Heavy metal | Concentration (mg/kg) | Mean | Standard deviation |
|-------------|-----------------------|-------|--------------------|
| Cd | 0.000 – 0.0727 | 0.034 | 0.03 |
| Cr | 0.00 – 1.92 | 0.385 | 0.52 |
| Pb | 0.186 – 1.39 | 0.925 | 0.49 |
| Ni | 0.372 – 2.57 | 1.38 | 0.85 |
| Cu | 0.698 – 2.90 | 1.82 | 0.67 |
| Zn | 0.987 – 14.4 | 5.41 | 4.45 |

A survey of heavy metals in soils from the central, east, northeast, north and south of Thailand by Pongsakul and Attajarusit (1999) showed wide ranges but generally higher concentrations of Cd, Cr, Pb, Ni, Cu, and Zn compared to our study (Table 2). All of the soils in our study had heavy metal concentrations much lower than the critical levels for each element (Fergusson 1990; Table 3).

Table 2 Heavy metal concentrations in 40 soil samples compared with Pongsakul and Attajarusit (1999)*.

| Heavy metal | Concentration (mg/kg) | Concentration (mg/kg)* |
|-------------|-----------------------|------------------------|
| Cd | 0.000 – 0.0727 | 0.001–0.294 |
| Cr | 0.00 – 1.92 | 0.1–114 |
| Pb | 0.186 – 1.39 | 0.2–295 |
| Ni | 0.372 – 2.57 | 0.5–550 |
| Cu | 0.698 – 2.90 | 0.2–350 |
| Zn | 0.987 – 14.4 | 0.1–138 |

Table 3. Heavy metal concentration in soils from four sites compared with critical levels of heavy metals as determined by Fergusson (1990).

| Heavy metal | Concentration (mg/kg) | | | | Critical heavy metal values (mg/kg) |
|-------------|----------------------------|----------------------------|----------------------------|----------------------------|-------------------------------------|
| | Site 1 ^a | Site 2 ^a | Site 3 ^b | Site 4 ^b | |
| Cd | 0.0033 – 0.0267 (0.011) | 0.0033 – 0.0727 (0.054) | 0.0066 – 0.0298 (0.021) | 0.0000 – 0.0670 (0.049) | 3 |
| Cr | 0.396 – 1.92 (1.17) | 0.036 – 0.245 (0.166) | 0.0000 | 0.190 – 0.223 (0.202) | 50–200 |
| Pb | 0.303 – 0.186 (0.234) | 1.21 – 1.88 (1.38) | 1.28 – 1.39 (1.36) | 0.661 – 0.800 (0.725) | 100 |
| Ni | 2.28 – 2.57 (2.41) | 1.45 – 2.47 (1.97) | 0.372 – 0.871 (0.522) | 0.598 – 0.670 (0.633) | 50–125 |
| Cu | 2.30 – 2.68 (2.49) | 1.81 – 2.90 (2.31) | 1.27 – 1.74 (1.57) | 0.698 – 1.10 (0.892) | 50 |
| Zn | 11.3 – 14.4 (12.9) | 1.26 – 4.02 (3.04) | 3.49 – 4.65 (4.21) | 0.987 – 1.77 (1.54) | 300 |

^a cow manure application

^b pig manure application

() mean

Conclusion

This study surveyed the contamination levels of Cd, Cr, Pb, Ni, Cu, and Zn in organic rice paddies at four sites north and west of Bangkok, Thailand. All of the heavy metal concentrations measured were very low and much lower than critical levels. So the researchers recommended that Thai farmers can apply cow or pig manure as an organic fertilizer to their paddy fields.

References

- Faila ML (1999) Considerations for determining optimal nutrition for copper, zinc, manganese and molybdenum. *Proceedings of the Nutrition Society* **58**,497–505.
- Fergusson JE (1990) *The Heavy Elements: Chemistry Environmental Impact and Health Effects*. Pergamon Press, England.
- Kaufman DB, Nicola W, McIntosh R (1970) Acute potassium dichromate poisoning. *American Journal of the Diseases of Childhood* **119**, 374–376.
- Perkins HF (1970) A rapid method of evaluating the zinc status of coastal plain. *Community of Soil Science and Plant Analysis* **1**, 35–42.
- Pongsakul P, Attajarusit S (1999) Assessment of heavy metal contaminations in soils. *Thai Journal of Soils and Fertilizers* **21**, 71–82
- Reilly C (2002) *Metal contamination of food*. 3rd Edn. Blackwell Science, Oxford, UK.
- Soil and Plant Analysis Council, Inc. (2000) *Soil Analysis : Handbook of Reference Methods*. CRC Press. London, UK.
- Tandon HLS (1995) *Methods of Analysis of Soils, Plants, Waters and Fertilisers*. Fertiliser Development and Consultation Organisation. New Delhi, India.
- Waalkes MP (2000) Cadmium carcinogenesis in review. *Journal of Inorganic. Biochemistry* **79**, 241–244.
- Yoshioka K (1964) Epidemiological study on the relationship between *itai-itai* disease and mining nuisance. *Yamaguchi Medical Journal* **13**, 146–170.

Heavy metal concentration in crops and soils collected from intensively cultivated areas of Sri Lanka

H .M. P. Lakmalie Premarathna^{A,B}, S. P Indraratne^A and Ganga Hettiarachchi^{A,C}

^ADepartment of Soil science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.

^BSoil and Land Systems, School of Earth and Environmental Sciences, University of Adelaide, Waite Campus, 5064, SA, Australia, Email herath.premarathna@adelaide.edu.au, srimathii@pdn.ac.lk

^CDepartment of Agronomy, 2004 Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506, USA, Email ganga@ksu.edu

Abstract

Intensification of agriculture could have resulted in accumulation of heavy metals due to excess use of agrochemicals and amendments. Surface horizons of 40 agricultural soils from low country and up country wet zone of Sri Lanka under vegetable crops were sampled to determine concentrations of Cd, Cu, Ni, Pb and Zn. Some selected soil chemical properties were determined to understand the relationships between the metal concentrations in soils and other soil properties. Crops which were grown in the same fields where the soils were collected also analyzed for the heavy metals. Elevated levels of Cd, Cu, Ni, Pb and Zn than the uncultivated soils were observed in both up country and low country wet zone of Sri Lanka. Measured mean values of Cd in few soils exceeded the maximum allowable limits imposed by the European standard; however, Cd concentrations in the vegetable crops were still below the standard levels. The Cd to Zn ratio in studied soils indicated safer limits for Cd in more than 95% of the soils studied. Metals in soils correlated significantly with number of years cultivated. These results provided the initial evidence of elevated heavy metal concentrations in Sri Lankan leafy vegetables and soils emphasizing the importance of extensive investigations.

Key Words

Heavy metals, vegetable crops, intensive cultivation, agrochemicals.

Introduction

Heavy metals are important environmental pollutants threatening the health of human population and natural ecosystems. Heavy metals can affect for the quality of agricultural soils, including phytotoxicity and transfer of heavy metals to the human diet from crop uptake (Nicholson *et al.* 2003). Fertilizers and organic amendments contain many impurities leading to contaminate soils subsequently transferring to the humans through food chains. In Sri Lanka use of fertilizers and organic manures in more than recommended doses is a common practice in intensively cultivated vegetable growing soils. Though the agricultural soils can be a long-term sink for heavy metals, data on heavy metal concentrations in soils and crops are not available for Sri Lankan soils. Analysis of heavy metals concentrations in soils and identifying the sources are essential to implement control measures to reduce heavy metal inputs to soils through agrochemicals. The objectives of the study were to determine concentrations of Cd, Cu, Ni, Pb and Zn in soils and vegetable crops in locations where the intensive agriculture has been performing more than 10 years, and to establish relationships between heavy metal concentrations and soil properties.

Materials and methods

Sample collection

Soil (0-20 cm) samples were collected from wet zone low country (22 samples) and wet zone up country (18 samples) of Sri Lanka where annual rainfall was common (1700-3300 mm) for both places and annual average temperature was 32 – 35 °C and 13 – 18 °C respectively for up country and low country. In these selected places leafy vegetables and vegetable crops were grown intensively for long period of time.

Soil digestion

Soil pH, available N, exchangeable potassium, available phosphorous, cation exchange capacity and soil organic matter were determined as the basic soil properties. The total concentration of cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) in soils were determined after digesting soil with 4M HNO₃ acid (1:10 ratio) at 80 °C water bath for four hours. Exchangeable Cd, Cu, Ni, Pb and Zn were determined using DTPA extraction at 1:2 soil to solution ratio.

Plant and fertilizer digestion

Plant samples of leafy vegetables at the harvesting stage were collected along with the soil samples from the same field. Metals in the oven dried (55 °C) plant tissues were determined after digestion with conc. HNO₃ acid. Total heavy metals in fertilizer samples were determined by digestion with conc. HCl acid of the powdered fertilizer sample. After ash the ground manure sample it was dissolved in conc. HCl and measured the heavy metals (Hettiarachchi and Pierzynski 2004).

Sample analysis

Metals of Cu, Ni, Pb and Zn extracted from soils, plants, fertilizers and manures were determined using an atomic absorption spectrophotometer (GBC Avanta Ver 1.33) and Cd was determined by atomic absorption spectrophotometer coupled with graphite furnace (GBC Avanta Ver 1.33). Correlation matrix was used to identify the relationship between heavy metal concentrations and soil properties.

Results and discussion

The mean values of the heavy metal concentrations (mg/kg) in soils were in the following order; Cd (1.16±0.69) < Ni (21±25) < Cu (51±34) < Pb (60±58) < Zn (227±106). Among the soil properties tested, pH, CEC and available P showed positive significant correlations with some metals. The mean values of the heavy metals in the plants were recorded as Cd 0.59±0.44; Cu 11±6; Ni 13±9; Pb 8±3 and Zn 40±20. The differences of soil and plant heavy metal contents in the studied two regions were not significantly different. Some elevated levels of Cd, Cu, Ni, Pb and Zn were observed in soils under long-term intensive vegetable cultivation as compared to uncultivated soils collected from the same areas. Only two fields out of the 40 exceeded the maximum acceptable upper limit (3 mg/kg) imposed by the European community set standards in 1986 (McGrath *et al.* 1994). Among the soils studied only three soils indicated Cd:Zn ratio exceeding 0.015 in this study. The soils contain usual geochemical ratio of Cd:Zn (0.005 to 0.015) indicates that the human or wildlife species are not at risk of chronic Cd consumption (Chaney *et al.* 1999).

Measured mean values of Cd in leafy vegetables, vegetables and root/tuber crop samples changed from 0.17 to 2.05 mg/kg dry weight, where 87.5% of the values were < 1.00 mg/kg. Positive significant correlation between the total and exchangeable Cd ($r^2=0.68$; $p=0.001$), Cu ($r^2=0.91$; $p<0.05$), Ni ($r^2=0.05$; $p<0.001$), Pb ($r^2=0.30$; $p<0.1$) and Zn ($r^2=0.51$; $p<0.05$) were observed for the studied soils. (Figure 1)

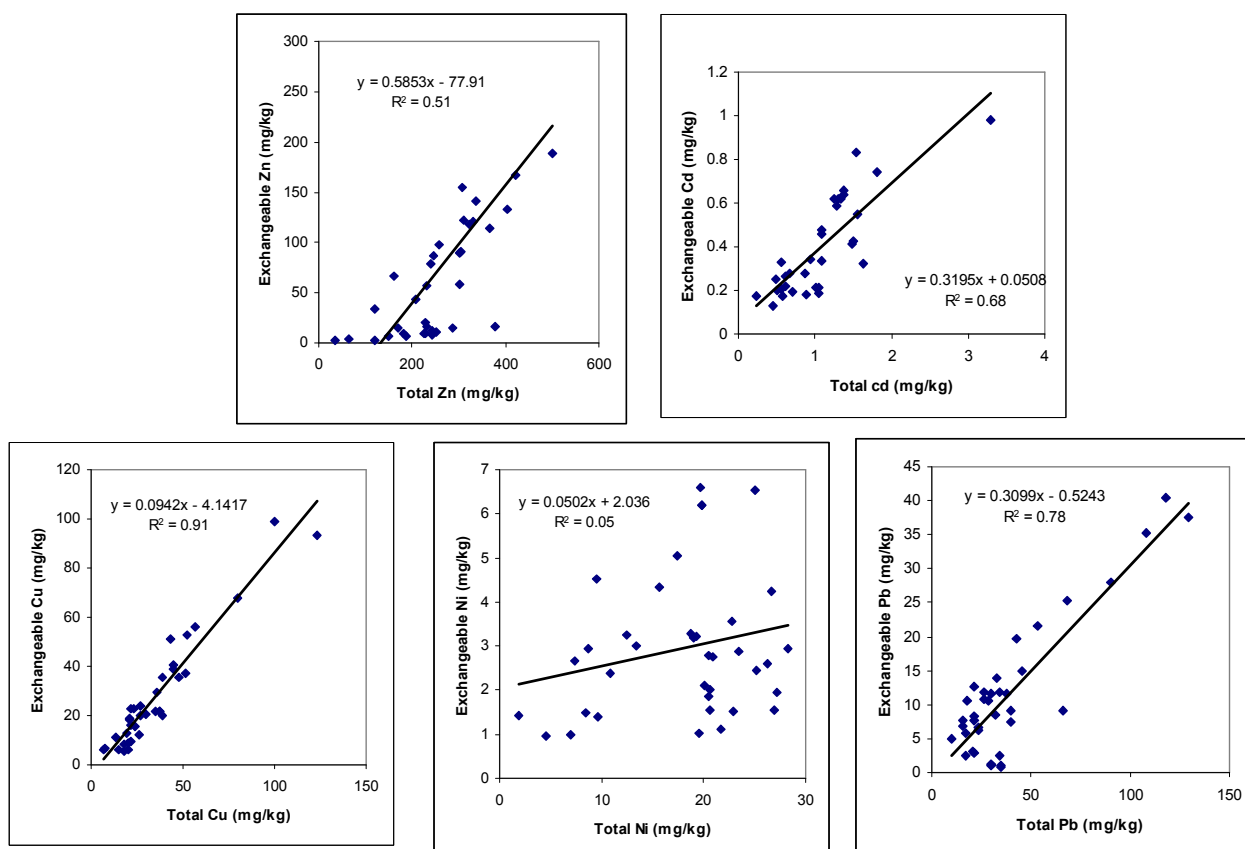


Figure 1. Regression relation of soil total and exchangeable Zn, Cd, Cu and Ni, concentrations in tested soils.

Among organic and inorganic manures/fertilizers analyzed, triple super phosphate (TSP) had the highest Cd concentration (23.5mg/kg). Even though the concentrations of heavy metal in those manure and fertilizers (except for Cd concentration in TSP) did not exceed the maximum permissible levels of heavy metals recommended by the Sri Lankan Standard Institute, they can be accumulated in agricultural soils with time. In Sri Lanka application of phosphate fertilizers, pesticides, fungicides and organic manures 2-3 times higher than the agrochemical doses recommended by the Department of Agriculture, has been a common practice among the farmers for many years. Therefore, long-term over application of agrochemicals may be responsible for elevated Cd in observed in some fields.

Conclusion

These results provide initial evidence that, Sri Lankan leafy vegetables and soils have elevated heavy metal concentrations, and emphasize the importance of extensive investigations on the extent of heavy metal contamination in Sri Lankan soils and vegetables, and their sources as well as possible control measures to reduce the associated risk due to food chain transfer of toxic heavy metals. Regulations should be introduced on agrochemical applications to protect soil resources from further contamination.

References

- Chaney RL, Ryan JA, Li YM, Brown SL (1999) Soil Cd as a threat to human health. In 'Cadmium in soils and plants'. (Eds MJ McLaughlin, Singh BR) pp. 219-256. (Kluwer Academic Publ.: Dordrecht, the Netherlands).
- McGrath SP, Chang AC, Page AL, Witter E (1994) Land applications of sewage sludge: Scientific perspectives of heavy metal loading limits in Europe and the United States. *Environ. Rev.* **2**, 108–118
- Nicholson FA, Smith SR, Alloway BJ, Carlton-Smith C, Chambers BJ (2003) An inventory of heavy metals inputs to agricultural soils in England and Wales. *Sci. Total Environ.* **311**, 205–219.
- Hettiarachchi GM, Pierzynski GM (2004) Soil lead bioavailability and in situ remediation of lead-contaminated soils. *Environmental Progress* **23**, 78-93.

Highlighting processes involved in mobility and bioavailability of zinc within contaminated substrates: Multiple approaches

Eléonore Couder^A, Nadine Mattielli^B, David Houben^A, Charlotte Cambier^A and Bruno Delvaux^A

^AUnité des Sciences du Sol, Earth and Life Institute, Université catholique de Louvain, Belgium,
Email eleonore.couder@uclouvain.be

^BIsotopes: pétrologie et environnement, DSTE, Université Libre de Bruxelles, Belgium, Email nmattiel@ulb.ac.be

Abstract

Zinc is an essential element for plants but excessive uptake can lead to (phyto) toxicity and contamination of the foodweb. Mobility and bioavailability depend on zinc speciation in solid and liquid phases. Considering the importance of solid phase, three contaminated substrates were studied: two soils and a slag heap. The relative amounts of exchangeable zinc were estimated by $MgCl_2$ extraction. We investigated chemical composition of solution collected *in situ* using zero-tension lysimeters. Furthermore, in order to complete the description of the substrate-plant system, zinc concentrations were measured in plants growing in the field. Zinc mobilization was determined by substrate-solution equilibrium and thus exchange processes. Importance of exchange processes was confirmed by the positive correlation between proportion of zinc leached and proportion of exchangeable zinc. Finally, zinc in solution and exchangeable zinc were positively correlated with plant uptake and transfer coefficient. As a general conclusion, exchange processes played a crucial role in mobility and bioavailability.

Key Words

Exchangeable zinc, substrate-solution equilibrium, divalent competition, pH, predictive equation, chemical assessment.

Introduction

Due to the past metallurgical industry in Belgium, zinc is one of the country's main inorganic pollutants. Potential contamination of foodweb or hydrological system by heavy metals motivated researchers to study their mobility and bioavailability (e.g. Marseille *et al.* 2000; Sterckeman *et al.* 2000; Chaignon and Hinsinger, 2003; Impellitteri *et al.* 2002; Ettler *et al.* 2007; Schwab *et al.* 2007). It is well-known that solid phase and soil parameters such as pH, redox conditions and ligands or competitive cations concentrations impact on mobility and availability of heavy metals. Therefore, in addition to total concentrations, solid and liquid phases have to be characterized to predict the mobility or the bioavailability of zinc and, consequently, potential risks.

Numerous methods have been developed to describe zinc bound to solid phase. Chemical methods such as single or sequential extractions give information about fractionation of zinc between bearing-phases. The latter can be identified by X-ray diffraction, electronic microscopy with microprobe and other physical methods. Previous studies have shown that exchangeable zinc was the most important labile form and its determination was useful to predict zinc mobility and bioavailability (e.g. Ettler *et al.* 2007; Menzies *et al.* 2007; Burgos *et al.* 2008). In the field, zero-tension lysimeters are the easiest and cheapest device to collect gravity water.

The aim of this study is to highlight processes controlling zinc mobility in order to identify the essential parameters to predict its mobility and availability. Considering the importance of solid phase, we choose three contrasted substrates – two soils contaminated by aerial fallout and a slag heap – presenting different characteristics.

Materials and methods

Substrates characterization

Two soils contaminated by aerial fallout (Prayon, Belgium) – a calcareous soil (CaS) and a shale-derived soil (ShS) – and a slag heap (SH) (Angleur, Belgium) were chosen (Table 1). Depending on the cover type, three stations were identified on the slag heap: bare (BSH), under *Armeria maritima* (ASH) and under the grass *Agrostis tenuis* (GSH). In slag heap, organic-horizons were identified thanks to visual and textural characteristics and organic matter contents. Sampling of organic horizon (designate as O or 0 in soils and

slag heap respectively) of all substrates and organo-mineral horizon Ah1 (A) of soils only was performed in November and December 2005. Organo-mineral horizon samples (A) were air-dried and organic horizon samples (O and 0) were stored at 4°C after removing roots without drying. Fine earth fraction was obtained by dry sieving (< 2 mm).

Table 1. Substrates characterization.

| Substrates | Horizons | Depth cm | C _{org} ¹ % | pH-H ₂ O ² | Exchangeable bases (exch) ³ | | | | | CEC (pH 7) ³ cmol _c /kg | Zn _{tot} ⁴ mg/kg |
|------------|----------|-------------|------------------------------------|----------------------------------|--|------|------|------|-------|--|---|
| | | | | | Ca | K | Mg | Na | Zn | | |
| | | | | | ----- cmol _c /kg ----- | | | | | | |
| Ca-S | O | 0-7 | 11.05 | 6.40 | 4.65 | 0.32 | 0.25 | 0.18 | 24.32 | 22.65 | 74800 |
| | A | 7-17 | 2.46 | 7.05 | 11.30 | 0.50 | 0.39 | 0.03 | 6.16 | 18.21 | 9700 |
| Sh-S | O | 0-6 | 21.65 | 4.78 | 16.77 | 0.45 | 1.38 | 0.12 | 3.37 | 37.96 | 7400 |
| | A | 6-12 | 2.66 | 5.35 | 3.20 | 0.24 | 0.27 | 0.03 | 1.12 | 13.41 | 2200 |
| BSh | 0 | 0-7 | 4.05 | 7.34 | 5.72 | 0.17 | 0.22 | 0.01 | 6.76 | 11.43 | 54800 |
| ASH | 0 | 0-7 | 14.91 | 6.72 | 9.51 | 1.81 | 1.36 | 0.11 | 48.92 | 44.72 | 52900 |
| GSH | 0 | 0-7 | 8.53 | 6.55 | 6.30 | 0.20 | 0.35 | 0.08 | 20.67 | 29.61 | 65600 |

¹ Organic carbon content (C_{org}) was determined using the Walkley and Black method.

² pH was measured in solid:water suspension (1:5).

³ Exchangeable cations (Ca, K, Mg, Na and Zn) concentrations and cation exchangeable capacity pH 7 (CEC) were determined according to the Metson method (NH₄AcO).

⁴ Zinc concentration was measured by ICP-AES (Thermo Jarrell Ash Iris Advantage) after calcination at 450°C and tri-acid digestion (HNO_{3conc}, HF_{conc} and HClO_{4conc}).

Exchangeable zinc

1 g of substrate (fraction < 2 mm) was agitated with 8 ml of MgCl₂ 1N for one hour. Extractions were replicated two or three times on each sample. Zinc concentration was measured by ICP-AES after centrifugation (10 000 rpm) and filtration (0.45 µm).

Collect and analysis of substrate solutions

In situ gravity solutions were collected using zero-tension lysimeters (adapted from Titeux 2005) buried in triplicates under organic horizon of each substrate and under organo-mineral horizon of soils only. After filtration (0.45 µm), we determined cations (ICP-AES), anions (HPLC-Dionex) and dissolved organic carbon (DOC) (Dohrman DC-180) concentrations. Four collecting campaigns were performed under 0 horizon of slag heap and 8 under O and A horizons of soils.

Shoot sampling and analysis

Shoots of plants growing on the different substrates were randomly sampled and zinc concentrations were measured by ICP-AES after tri-acid digestion (3ml HNO_{3conc}, 5ml HF_{conc} and 5ml HClO_{4conc}).

Results and discussion

Zinc in solution

In soils, after passing through A horizon, release of zinc (Zn_{liq}), calcium (Ca_{liq}) and DOC (Table 2) decreases because substrates and solutions equilibrated. Adsorption processes occurred in A horizons while in O horizons zinc and calcium were desorbed, processes involving weakly bound elements, probably exchangeable zinc.

According to Ettler *et al.* (2007), mobile zinc could be evaluated by exchangeable zinc extraction with CaCl₂. Percentage of leached zinc (Table 2), defined as the ratio between quantity of leached zinc and quantity of zinc in horizons above lysimeter, is well-correlated with percentage of zinc extracted with MgCl₂ under agitation (Zn_{MgCl2}) ($r^2=0.64$, $n=7$) and with pH of substrates ($r^2=0.78$, $n=7$). These results confirmed both the importance of exchangeable zinc, and thus exchange processes in mobilization of zinc but highlighted also a pH effect. The highest mobility of zinc in ShS could be explained by its larger proportion of exchangeable zinc and its lower pH.

Moreover, Zn_{liq} is positively correlated with Ca_{liq} ($r^2=0.77$, $n=124$) and with DOC ($r^2=0.60$, $n=124$), as well as Ca_{liq} with DOC ($r^2=0.84$, $n=124$). These results indicated that zinc and calcium had the same behaviour during substrate-solution equilibration and complexation with DOC. So, competition between divalent cations for adsorption, desorption and complexation with DOC played a key role in zinc mobilization.

Table 2. Exchangeable zinc (Zn_{MgCl_2}) and characterization of substrate solutions ($\pm SD$).

| | | Zn_{MgCl_2} | Characterization of solutions | | | | |
|-----|--------------|------------------|-------------------------------|-------------------|-------------------|-----------------|---------------------|
| | | % | Zn_{liq} $\mu mol_c/kg$ | Ca_{liq} | DOC mg/kg | pH_{liq} | $Zn_{liq}\%$ % |
| CaS | O ($n=24$) | 3.93 ± 0.14 | 268.4 ± 101.3 | 312.8 ± 103.7 | 12.89 ± 4.64 | 6.97 ± 0.33 | 0.0117 ± 0.0044 |
| | A ($n=24$) | 10.23 ± 0.18 | 42.8 ± 18.8 | 170.1 ± 75.2 | 3.30 ± 2.71 | 7.13 ± 0.40 | 0.0050 ± 0.0063 |
| ShS | O ($n=16$) | 18.65 ± 0.22 | 413.3 ± 251.7 | 978.6 ± 492.4 | 51.70 ± 18.59 | 5.85 ± 0.30 | 0.1855 ± 0.1111 |
| | A ($n=24$) | 10.31 ± 0.10 | 54.3 ± 24.2 | 146.1 ± 62.4 | 9.27 ± 2.53 | 5.71 ± 0.38 | 0.0567 ± 0.0359 |
| BSh | 0 ($n=12$) | 1.69 ± 0.03 | 17.2 ± 7.1 | 15.4 ± 4.5 | 0.74 ± 0.45 | 6.89 ± 0.48 | 0.0010 ± 0.0004 |
| ASH | 0 ($n=12$) | 11.16 ± 0.09 | 1.5 ± 0.7 | 8.7 ± 4.7 | 0.48 ± 0.32 | 7.11 ± 0.37 | 0.0001 ± 0.0001 |
| GSH | 0 ($n=12$) | 4.98 ± 0.11 | 92.7 ± 48.4 | 67.6 ± 34.7 | 1.65 ± 1.13 | 7.06 ± 0.16 | 0.0046 ± 0.0024 |

Zinc in plants

Zinc concentrations in shoots (Zn_{sh} in Table 3) do not follow the same trend that total zinc concentration in substrates. On one hand, uptake mechanisms of plant species could explain this difference. On the other hand exchangeable zinc should be a better indicator than total concentration since it is easily mobilized and thus immediately available for plant uptake. Indeed, our results show that zinc concentration in shoots is positively correlated with the quantity of zinc extracted from O/O horizon sample with $MgCl_2$ under agitation [mg/kg](data not shown) ($r^2=0.97$, $n=4$) and Zn_{liq} ($r^2=0.72$, $n=4$). This corroborates the study of Burgos *et al.* (2008).

Table 3. Zinc in shoots ($n=3$).

| Substrates | Species | Zn_{sh} mg/kg _{DM} | Transfer % |
|------------|--------------------------|----------------------------------|-----------------|
| CaS | <i>Viola calaminaria</i> | 1390 ± 228 | 1.85 ± 0.30 |
| ShS | <i>Agrostis tenuis</i> | 450 ± 68 | 6.08 ± 0.11 |
| ASH | <i>Armeria maritima</i> | 4760 ± 59 | 8.99 ± 0.92 |
| GSH | <i>Agrostis tenuis</i> | 1600 ± 68 | 2.44 ± 0.10 |

Prediction of zinc in substrate solutions

In the view of the importance of exchangeable zinc and zinc competition with calcium, a multiple regression was developed to predict zinc leached ($\mu mol_c.kg^{-1}$) from parameters generally involved in exchange processes: Zn_{tot} (mg/kg), Ca_{exch} (cmol_c/kg), pH and Corg (%) ($r^2=0.83$, $n=7$, $p=0.05$ (Figure 2)). This last parameter was integrated instead of CEC because, in the chosen substrates, it is the main component of CEC.

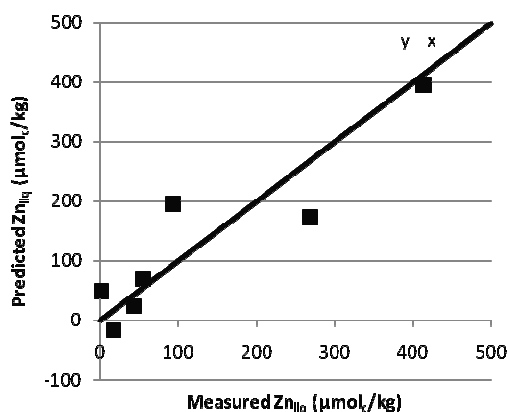


Figure 2. Prediction of zinc leached using a multiple regression involving values of Zn_{tot} (mg/kg), Ca_{exch} (cmol_c/kg), pH and Corg (%).

Conclusions

Determination of exchangeable zinc proportions by $MgCl_2$ extraction allows a good assessment of zinc mobility and phytoavailability. Moreover, values of parameters usually and easily measured for soil samples (pH, organic carbon content, exchangeable calcium and total concentration in zinc) could be used to predict leached zinc and thus zinc uptake since uptake is correlated with zinc in solution. Predicting regression should be improved by comparison with other substrates but it highlighted, once again, the crucial importance of exchange processes in mobilization of zinc.

References

- Burgos P, Perez-De-Mora A, Madejon P, Cabrera F, Madejon E (2008) Trace Elements in Wild Grasses: a Phytoavailability Study on a Remediated Field. *Environmental Geochemistry and Health* **30**, 109-114.
- Chaignon V, Hinsinger P (2003) A Biotest for Evaluating Copper Bioavailability to Plants in a Contaminated Soil. *Journal of Environmental Quality* **32**, 824-833.
- Ettler V, Mihaljevic M, Sebek O, Grygar T (2007) Assessment of Single Extractions for the Determination of Mobile Forms of Metals in Highly Polluted Soils and Sediments - Analytical and Thermodynamic Approaches. *Analytica Chimica Acta* **602**, 131-140.
- Impellitteri CA, Lu YF, Saxe JK, Allen HE, Peijnenburg WJGM (2002) Correlation of the Partitioning of Dissolved Organic Matter Fractions With the Desorption of Cd, Cu, Ni, Pb and Zn From 18 Dutch Soils. *Environment International* **28**, 401-410.
- Marseille F, Tiffreau C, Laboudigue A, Lecomte P (2000) Impact of Vegetation on the Mobility and Bioavailability of Trace Elements in a Dredged Sediment Deposit: a Greenhouse Study. *Agronomie* **20**, 547-556.
- Menzies NW, Donn MJ, Kopittke PM (2007) Evaluation of Extractants for Estimation of the Phytoavailable Trace Metals in Soils. *Environmental Pollution* **145**, 121-130.
- Schwab P, Zhu D, Banks MK (2007) Heavy Metal Leaching From Mine Tailings as Affected by Organic Amendments. *Bioresource Technology* **98**, 2935-2941.
- Sterckeman T, Douay F, Proix N, Fourrier H (2000) Vertical Distribution of Cd, Pb and Zn in Soils Near Smelters in the North of France. *Environmental Pollution* **107**, 377-389.
- Titeux H (2005) Transferts de carbone dissous et de métaux dans des sols forestiers acides : influence du fonctionnement de la litière et des réserves minérales du sol (Thesis: Université catholique de Louvain, Belgium).

Immobilization of cadmium and lead in biosolids as affected by lime, red mud, fly ash and bentonite addition

Thammared Chuasavathi^{A,B}, Nanthi S. Bolan^B and Ravi Naidu^{A,B}

^ACRC CARE, University of South Australia, Mawson Lakes, SA, Australia, Email Thammared.Chuasavathi@postgrads.unisa.edu

^BCentre for Environmental Risk Assessment and Remediation, University of South Australia, Mawson Lakes, SA, Australia, Email Nanthi.Bolan@unisa.edu.au

Abstract

Biosolid samples from the Bolivar Wastewater Treatment Plant (South Australia) were blended with lime (1%, 3% and 5%; w/w basis), red mud, fly ash and bentonite (5%, 10% and 20%; w/w basis) and incubated under aerobic condition at room temperature for seven months to examine the effects of various additives on: (i) the redistribution of metals in the biosolids and (ii) the mobilization of metals and their subsequent release to pore water. Both the NH_4NO_3 extractable concentration of Cd (Cd_{NN}) and Pb (Pb_{NN}) from biosolids and the concentration of these metals in pore water was lower in all amendments than biosolid alone. The effect of amendments on the immobilization of biosolid-derived Cd and Pb varied with both the nature and level of amendments addition. The immobilization of Cd and Pb in biosolids and pore water increased with increasing level of amendment addition and red mud was most efficient in the immobilization of Cd and Pb. For example, red mud at the highest rate of application decreased Cd_{NN} and Pb_{NN} from 1.73 and 11.018 $\mu\text{g}/\text{kg}$ (biosolid alone) to 0.24 and 1.12 $\mu\text{g}/\text{kg}$, respectively. It decreased the pore water concentration of these metals from 8.88 and 4.82 $\mu\text{g}/\text{L}$ (biosolid alone) to 1.34 and 1.68 $\mu\text{g}/\text{L}$, respectively.

Key Words

Biosolids, heavy metal, lime, red mud, fly ash, bentonite.

Introduction

Millions of tons of biosolids are generated worldwide every year. As an option of disposal, application of biosolids to land has been proved to be quite effective, especially in reviving degraded soils and also as a cost effective soil amendment for growing agricultural crops. Biosolids possess an abundant supply of nutrients and organic matter that improve soil chemical, physical and biological properties. However, biosolids can cause some environmental risk because they are usually a potential source of heavy metals when applied to soil. Thus, there is a need to develop suitable treatment methods for biosolids to overcome this problem. One effective approach might be co-composting of biosolids with other waste resources, such as lime, red mud, fly ash and bentonite, which can immobilize heavy metals, thereby reducing their mobility and bioavailability. The overall objective is to produce co-additive products by mixing biosolids with other waste by-products that are effective in the immobilization of heavy metals and to compare the effectiveness of various co-compost blends on the immobilization of metals in biosolids.

Methods

The immobilization of Cd and Pb in biosolid amended with lime, red mud, coal fly ash and bentonite (Table 1) was investigated in laboratory incubation experiments. Biosolid samples from the Bolivar Wastewater Treatment Plant in South Australia were used for this study. Two incubation experiments were conducted; one to examine the effect of various additives on the redistribution of metals in the biosolids and the other to examine the effect of these additives on the mobilization of metals and their subsequent release to pore water. The experimental treatments were comprised of control (only biosolid without any additive), addition of lime (1%, 3% and 5%; w/w basis), red mud, fly ash and bentonite (5%, 10% and 20%; w/w basis). All treatments were duplicated and incubated under aerobic condition at room temperature for seven months. Pore water samples were collected at 0, 1, 2, 3, 5 and 7 months of incubation using a rhizon extraction technique. The concentration of heavy metals in the pore water samples was measured to examine the solubility and mobility of metals. Similarly, biosolid subsamples were taken at various intervals to monitor the redistribution of metals and these samples were analyzed for pH (1:5 biosolid: water), dissolved organic carbon (DOC) and 1M NH_4NO_3 extractable Cd and Pb.

Table 1. Some chemical characteristics of biosolids and other by-products.

| Material | pH | EC ($\mu\text{s}/\text{cm}$) | OM (%) | N (%) | P (-----mg/kg-----) | K (-----mg/kg-----) | Cd (-----mg/kg-----) | Cu (-----mg/kg-----) | Pb (-----mg/kg-----) |
|-----------|------|-----------------------------------|-----------|----------|------------------------|------------------------|-------------------------|-------------------------|-------------------------|
| Biosolids | 6.64 | 8680 | 43.4 | 0.85 | 800 | 2285 | 1.23 | 359.6 | 51.6 |
| Lime | 8.73 | 420 | - | - | - | - | 0.03 | 4.21 | 2.34 |
| Red mud | 9.1 | 13750 | - | - | - | - | 0.14 | 10.1 | 44.0 |
| Fly ash | 9.86 | 1360 | 0.01 | 0.02 | 272 | 530 | 0.24 | 74.9 | 46.1 |
| Bentonite | 7.2 | 897 | - | - | - | - | 0.18 | 6.9 | 46.3 |

Results

The NH_4NO_3 extractable concentration of Cd (Cd_{NN}), which represents bioavailable Cd was lower in all amendments than biosolid alone. The effect of amendments on the immobilization of Cd present in biosolids varied with both the nature and level of amendments addition. The Cd_{NN} concentration decreased with increasing level of amendment addition, indicating that immobilization of Cd increased with application rate. For example, at the highest rate of application, red mud, lime, fly ash and bentonite in biosolid sub-samples decreased the Cd_{NN} concentration from 1.73 $\mu\text{g}/\text{kg}$ (biosolid alone) to 0.24, 0.65, 0.67 and 0.59 $\mu\text{g}/\text{kg}$, respectively (Figure 1), thereby achieving 83.4, 38.1, 53.5, and 59.1% immobilization of bioavailable Cd (Cd_{NN}). In biosolid pore water, at the highest rate of application, red mud, lime, fly ash and bentonite decreased the Cd level from 8.88 $\mu\text{g}/\text{L}$ (biosolid alone) to 1.344, 2.152, 3.065 and 2.036 $\mu\text{g}/\text{L}$, respectively (Figure 2), thereby achieving 81.8, 70.9, 58.6 and 72.5% immobilization of bioavailable Cd.

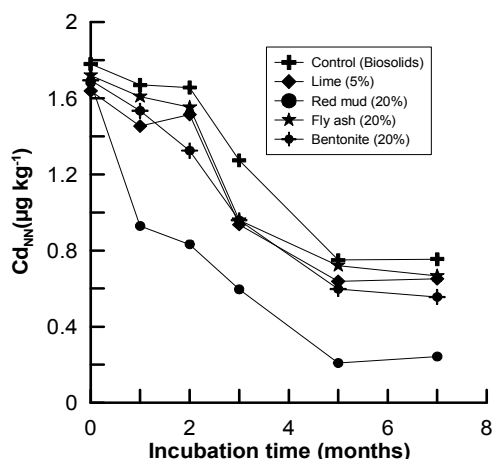


Figure 1. The effect of lime, red mud, flies ash and bentonite on the NH_4NO_3 extractable Cd.

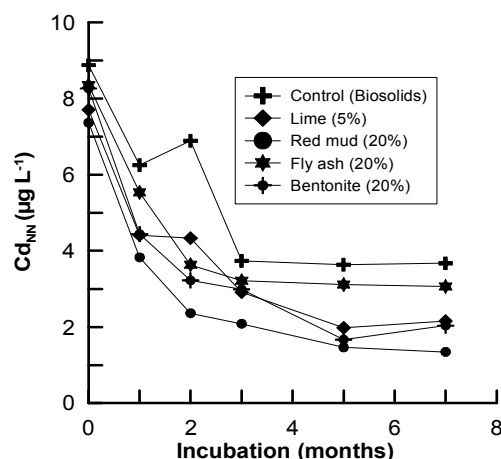


Figure 2. The effect of lime, red mud, fly ash and bentonite on Cd in biosolids in biosolid pore water.

The effect of amendments on biosolid-derived Pb varied with both the nature and level of amendments addition. The immobilization of Pb increased with increasing application rates for all amendments. For example, at the highest rate of application, red mud, lime, fly ash and bentonite in biosolid sub-samples decreased the Pb level from 11.018 $\mu\text{g}/\text{kg}$ (biosolid alone) to 1.118, 1.232, 1.743 and 1.334 $\mu\text{g}/\text{kg}$, respectively (Figure 3), thereby achieving 87.8, 86.6, 81.0 and 85.47% immobilization of bioavailable Pb (Pb_{NN}). In biosolid pore water, at the highest rate of application, red mud, lime, fly ash and bentonite decreased the Pb level from 4.822 $\mu\text{g}/\text{kg}$ (biosolid alone) to 1.804, 2.667, 2.787 and 2.684 $\mu\text{g}/\text{kg}$, respectively (Figure 4), thereby achieving 55.1, 33.6, 30.6, and 33.2% immobilization of bioavailable Pb.

Application of these amendments increased pH of biosolids both in solid phase and pore water, but it decreased the concentration of soluble Cd and Pb in biosolid and pore water. The DOC concentration in the biosolids also decreased with the amendment addition in the solid phase and pore water, except for lime and fly ash. In a separate DOC sorption experiment, it was noticed that red mud sorbed the highest amount of DOC followed by bentonite (Figure 5). The decrease in bioavailable Cd and Pb in biosolids and pore water resulting from co-composting with amendments may be attributed to the increases in pH and decrease in DOC concentration (Figure 6). The pH has a significant controlling effect on the chemical behavior of metals and heavy metal cations are more mobile under acid conditions (Bolan *et al.* 2003a and Merrington *et al.* 2002). The reduction in DOC in biosolids after application of all amendments may be a contributing factor to the availability and mobility of biosolid-borne metals. DOC can mobilize metals by forming strong complexes with metals in biosolid pore water solutions, whereas solid organic matter can immobilize metals (Bolan *et al.* 2003b; Bradl 2004 and Basta *et al.* 2005).

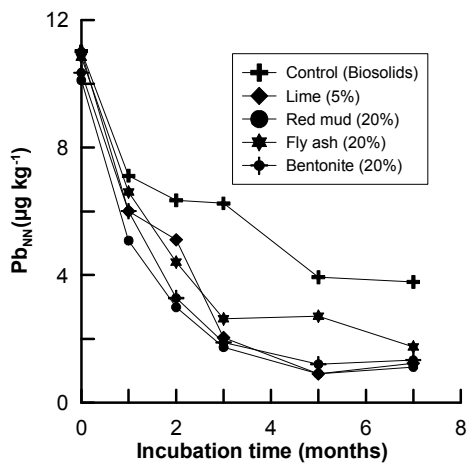


Figure 3. The effect of lime, red mud, flies ash and bentonite on the NH_4NO_3 extractable Pb.

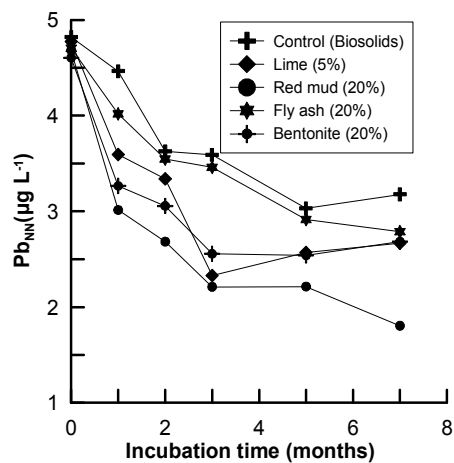


Figure 4. The effect of lime, red mud, fly ash and bentonite on Pb in biosolid pore water in biosolids.

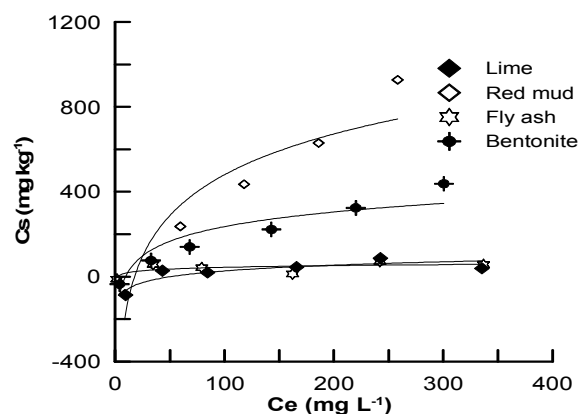


Figure 5. DOC sorption curves for lime, red mud, fly ash and bentonite.

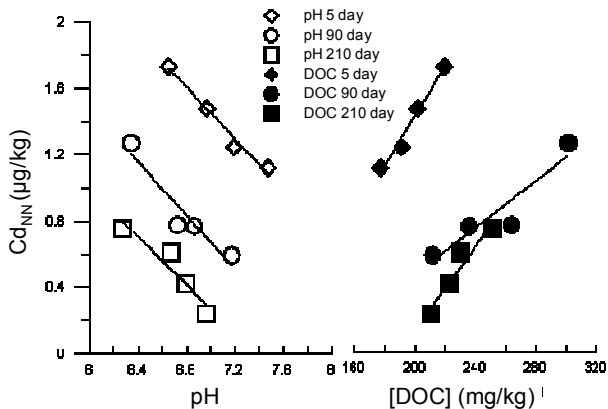


Figure 6. Relationships between pH and NH_4NO_3 extractable Cd (A) and DOC and NH_4NO_3 extractable Cd (B) in biosolid amended with various levels of red mud.

Conclusions

It was found that red mud was more effective than lime, coal fly ash and bentonite in reducing the availability of Cd and Pb. This may be attributed to the high pH and surface reactivity of red mud, thereby resulting in the immobilization of metals. The DOC concentration in biosolids decreased with increasing red mud application, thereby decreasing the DOC-induced mobilization of metals.

References

- Basta NT, Ryan JA, Chaney RL (2005) Trace element chemistry in residual-treated soil: key concepts and metal bioavailability. *Environmental Quality* **34**, 49-63
- Bradl HB (2004) Adsorption of heavy metal ions on soils and soils constituents. *Journal of Colloid and Interface Science* **277**, 1-18
- Merington G, Oliver I, Smernik RJ, McLaughlin MJ (2002) The influence of sewage sludge properties on sludge-borne metal availability. *Advances in Environmental Research* **8**, 21-36
- Bolan NS, Adriano DC, Curtin D (2003a) Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. *Advances in Agronomy* **78**, 216-272.
- Bolan NS, Adriano DC, Mani S, Khan AR (2003b). Adsorption, complexation and phytoavailability of copper as influenced by organic manure. *Environmental Toxicology and Chemistry* **22**, 450-456.

Impact of long-term application of phosphate fertilizer on cadmium accumulation in crops

Cynthia Grant^A, Don Flaten^B, Mario Tenuta^B, Xiaopeng Gao^B, Sukdev Malhi^C and Eugene Gowalko^D

^AAgriculture and Agri-Food Canada, Brandon, MB, Canada, Email Cynthia.grant@agr.gc.ca

^BDepartment of Soil Science, University of Manitoba, Winnipeg, MB, Canada, Email flatendn@ms.umanitoba.ca

^CAgriculture and Agri-Food Canada, Melfort, SK Canada, Email malhis@agr.gc.ca

^DCanadian Grain Commission, Winnipeg, MB, Canada, Email Eugene.gowalko@cgcc.ca

Abstract

Field studies were conducted since 2002 at five locations across the Canadian prairies to study the long-term effects of P fertilizer application rate and Cd content on seed Cd concentration of durum wheat (*Triticum turgidum* L.) and flax (*Linum usitatissimum* L.). Cadmium concentration was higher in durum wheat than flax and varied with location. Cadmium concentration in the seed of both crops increased with application of P fertilizer even when the fertilizer contained only trace concentrations of Cd, indicating that P fertilization directly influenced Cd concentration of crops apart from the effect of Cd addition. Seed Cd concentration was higher when the fertilizer contained greater Cd concentrations, particularly when rate of fertilizer application was also high. Cadmium concentration in crops was directly proportional to the total amount of Cd applied over time, but the effect of fertilizer application varied with soil characteristics. Highest availability of Cd added in P fertilizer was on light-textured or acidic soil, while availability of applied Cd was lower on heavier-textured or higher pH soils. Therefore, soil characteristics that affect phytoavailability must be taken into account when assessing the risk of transfer of Cd into the food chain from P fertilization.

Key Words

Cadmium, heavy metal, linseed.

Introduction

Cadmium (Cd) is a trace element that can accumulate to high levels in specific crops. Cadmium has been linked to negative health effects, so it is desirable to reduce the Cd concentrations of crops entering the human diet. Cadmium concentration in crops is influenced by a wide range of factors, including crop genetics, soil characteristics such as texture, pH and salinity, weather, crop sequence, crop management practices and soil Cd concentration (Grant *et al.* 1999). Cadmium is present at varying concentrations as a contaminant in phosphate (P) fertilizers (Grant *et al.* 2002; Grant and Sheppard 2008; Taylor 1997) and repeated applications of Cd in P fertilizers may increase Cd content of soils, potentially increasing Cd content of crops. However, the long-term availability of the Cd applied in phosphate fertilizer will vary with soil characteristics. This study was conducted to determine the long-term influence of Cd applied in monoammonium phosphate (MAP) fertilizer on Cd concentration of durum wheat and flax, across a range of soils and environments.

Methods

Field studies were conducted at five sites across the Canadian prairies from 2002 to 2009, to determine the influence of repeated applications of monoammonium phosphate fertilizer on accumulation of Cd in crops. At each location, three rates of P fertilizers (0, 20, 40 and 80 kg P ha⁻¹) from three different sources varying in Cd concentration (0.38 mg Cd/kg, 71 mg Cd/kg, and 211 mg Cd/kg) were applied annually and sites were seeded following a durum wheat-flax-durum wheat-flax crop sequence. Treatments were applied to the same plots each year so that the cumulative effects of P applications could be assessed over time. Treatments were arranged at each location in a randomized complete block design with four replications. Crops were harvested at maturity using a small plot combine and seed yield and concentrations of Cd and nutrient elements were determined for flax in 2007 and durum wheat in 2008. Seed samples were ground in a coffee grinder and digested with a mixture of HNO₃-HClO₄ (9:1). Digests were analysed for Zn on an ARL (Sunland, CA, USA) ICP unit and for Cd on a Varian (Palo Alto, CA, USA) 300/400 atomic absorption spectrophotometer at a wavelength of 228.8 nm using a graphite furnace with deuterium correction (detection limit 0.01 ng Cd /mL). Reliability of the analysis was assessed by including standard reference materials from NIST (i.e., durum wheat flour #8436, peach leaves #1547, tomato leaves #1573 and wheat

flour #1567a), and internal standards (with concentration values confirmed from ‘round-robin’ analyses with 7-10 co-operating laboratories) in each of the plant digests. Statistical analysis was conducted using Proc Mixed of SAS (Littell *et al.* 1996), with differences among treatments being considered significant at the $p < 0.05$ level.

Results and discussion

Seed Cd concentration was measured in flax grown in 2007 (data not presented) and durum wheat grown in 2008 (Figure 1). At a given site, flax seed was higher in Cd concentration than was durum wheat grain. Grain Cd in the unfertilized crop varied as much as three-fold from location to location, indicating that soil and environmental characteristics have a dominating effect on Cd concentration of crops (Figure 1). Highest Cd concentrations in the unfertilized control in both the flax and the durum wheat occurred on the Carman site, a light-textured, acidic soil. Previous studies had shown that this soil had a high solution Cd concentration, indicating high Cd phytoavailability (Lambert *et al.* 2007).

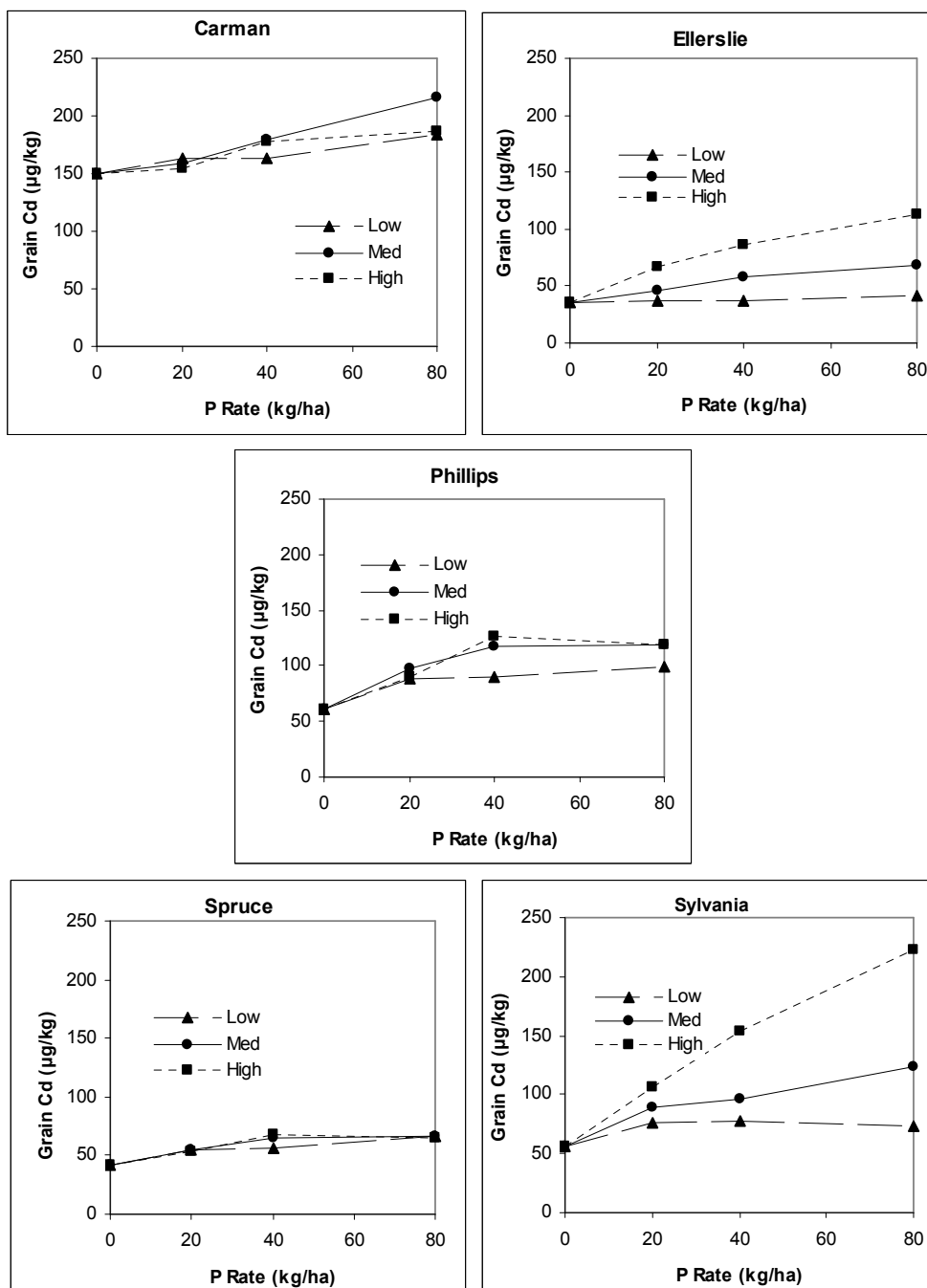


Figure 1. Effect of 8 years of application of varying rates of MAP fertilizer with low, medium and high Cd concentration on concentration of Cd in durum wheat grain at five sites in Western Canada (2008).

Phosphorus application increased grain Cd content at all sites in both flax and durum wheat, even when Cd concentrations in the P fertilizer were low, indicating an effect of fertilization on Cd availability for crop uptake apart from the direct addition of Cd. However, grain Cd increased to a greater extent with MAP application on most of the sites when the fertilizer contained a higher concentration of Cd. When the data were averaged over sites, Cd concentration in durum grain showed a quadratic relation to the total amount of Cd added over the seven years of the study with an R^2 of 0.89.

While the pattern of crop response to fertilizer application was similar from location to location, the magnitude of response differed substantially. The largest effect of fertilizer application on flax was at the Ellerslie site where seed Cd increased from 103 $\mu\text{g Cd/kg}$ in the control to 441 $\mu\text{g Cd/kg}$ in the high Cd high P treatment. Similarly at the Sylvania site seed Cd increased from 127 $\mu\text{g Cd/kg}$ in the control to 365 $\mu\text{g Cd/kg}$ with the high rate of high-Cd MAP. Durum wheat grain Cd at the Sylvania site increased by more than four-fold from 55 $\mu\text{g Cd/kg}$ in the control to 223 $\mu\text{g Cd/kg}$ with the high rate of high-Cd MAP. Cadmium concentration in durum wheat at the Ellerslie site also increased substantially from 35 $\mu\text{g Cd/kg}$ in the control to 113 $\mu\text{g Cd/kg}$ with the high rate of high-Cd MAP. The Sylvania site was a sandy loam soil, which may have led to the high availability of added Cd on this site. The Ellerslie site was a silt clay loam with pH near 6.2 and the Carman site was a loam with a pH near 5.2. Cadmium concentration in flax and durum wheat at the Carmen site, a coarse-textured soil with low pH, also increased substantially with application of high Cd MAP. Grain Cd response to fertilizer application was much smaller at the Phillips and Spruce sites, which were clay to clay-loam sites with pH of 7.0 or higher.

Conclusions

Cadmium concentration in durum wheat increased with application of MAP fertilizer, even when the fertilizer did not contain significant amounts of Cd. Therefore MAP fertilizer can increase Cd concentration of crops through mechanisms other than the direct addition of Cd. However, the impact of MAP fertilizer on Cd concentration of crops increased with Cd concentration in the fertilizer, particularly at high fertilizer rates and with repeated application. Soil characteristics such as texture or pH appear to be important in influencing the long-term phytoavailability of the added Cd. Therefore, soil characteristics that affect phytoavailability must be taken into account when assessing the risk of transfer of Cd into the food chain from P fertilization.

Acknowledgements

Funding for this project was provided by Westco Fertilizers, Ltd., Agriculture and Agri-Food Canada's Matching Investment Initiative program and the Metals in the Human Environment Research Network, supported by NSERC. Technical assistance from Dick Purveen, Mike Svistovski, Josh Price, and Darwin Leach was greatly appreciated.

References

- Grant CA, Bailey LD, Harapiak JT, Flore NA (2002) Effect of phosphate source, rate and cadmium content and use of *Penicillium bilaii* on phosphorus, zinc and cadmium concentration in durum wheat grain. *Journal of the Science of Food and Agriculture* **82**, 301-308.
- Grant CA, Bailey LD, McLaughlin MJ, Singh BR (1999) Management Factors which Influence Cadmium Concentration in Crops. In 'Cadmium in Soils and Plants'. (Eds MJ McLaughlin, BR Singh) pp. 151-198. (Kluwer Academic Publishers: Dordrecht).
- Grant CA, Sheppard SC (2008) Fertilizer impacts on cadmium availability in agricultural soils and crops. *Human and Ecological Risk Assessment* **14**, 210-228.
- Lambert R, Grant C, Sauve S (2007) Cadmium and zinc in soil solution extracts following the application of phosphate fertilizers. *Science of the Total Environment* **378**, 293-305.
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) 'SAS System for Mixed Models.' (SAS Institute, Inc.: Cary, North Carolina).
- Taylor MD (1997) Accumulation of cadmium derived from fertilisers in New Zealand soils. *Science of the Total Environment* **208**, 123-126.

Impact of Mn-ore mining on heavy metal accumulation in the soils and vegetables nearby in Hunan Province, China

Xiao-Hong Wu^A, Xue-Feng Hu^{A,B}, Guo Zhang^A, Xu Cao^A, Qi Jiang^A, Shan Li^A, and Yang Li^A

^ADepartment of Environmental Science and Engineering, School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, China.

^BCorresponding author. Email xfhu@shu.edu.cn

Abstract

A stream near a Mn mine is heavily polluted by the discharge of untreated mining wastewater in Hunan province, China, which poses a threat to the agricultural land. In this study, heavy metal concentrations of the stream water and sediments and the soils and vegetables along the stream were determined. The results showed that the stream water and sediments were severely contaminated by heavy metals. The mean values of Cd, Cu, Zn, Pb, Co, Ni, Mn and Cr in the stream water are 0.0009, 0.0004, 0.315, 0.0132, 0.0049, 0.0355, 0.9795 and 0.0212 mg/L, respectively, and those in the soils irrigated by the water are 0.70, 72.7, 1343.2, 60.9, 34.8, 184.8, 21507.7 and 793.3 mg/kg, respectively, which are significantly higher than those in the soils far away from the stream. The concentrations of Cu, Ni, Mn and Cr in the soils irrigated by the stream water are 6.2, 9.7, 40.9 and 17.3 times the local background values. It was also found that the edible parts of vegetables growing on the soils with stream water irrigation contain higher concentrations of heavy metals than those in the soils irrigated by other water. Cd, Zn, Ni and Cr contents in the vegetables exceed the recommended values of the Chinese hygienic standards.

Key Words

Mn mine, heavy metals, soil, vegetables, Hunan province.

Introduction

Hunan province has abundant mineral resources and is a famous location for nonferrous metals in China. However, a large amount of acid mine drainage released from mining activities as well as many poisonous filtrates containing heavy metals from the tailing dump pose a severe threat to water sources and nearby farmlands (Conesa 2007; Lin 2005; Hussein 2005; Granero 2002). Heavy metal concentrations in the soils and vegetables beside a stream polluted by exploiting a Mn mine were determined in this study, aiming to evaluate the hazard risk of mining activities on the local agriculture.

Methods

A stream near a Mn mine in Taojiang county, Hunan province, China, was seriously polluted, with the water being black. There is a large area of agriculture land along the polluted stream (Figure 1). Three water and sediment samples from the different points of the stream were collected; four soil and vegetable samples in the area irrigated by the stream water, and two soil and vegetable samples far away from the stream and irrigated by other water were collected. In addition, two tailing and three soil samples from undisturbed natural soil profiles were also collected as controls.

The samples of sediments and soils were digested with the mixed acids ($\text{HNO}_3 + \text{HF} + \text{HClO}_4$); those of water and vegetables with HNO_3 and HClO_4 . Cu, Zn, Pb, Ni, Co, Mn and Cr in the solution were determined with the Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES), and Cd with the Graphite Furnace Atomic Absorption Spectrometry (AAS).

Results

Heavy metal accumulation in soil

Heavy metal concentrations of the stream water and sediments and the soils were determined (Table 1). The stream sediments are heavily contaminated with Mn, Zn, Ni and Cr, whose mean values are 77.2, 5.0, 9.5 and 8.2 times the local undisturbed natural soil, respectively. Heavy metal concentrations in the sediments were in the order of $\text{Mn} > \text{Zn} > \text{Cr} > \text{Ni} > \text{Cu} > \text{Pb} > \text{Co} > \text{Cd}$. The concentrations of Cu, Zn, Pb, Co, Ni and Cr in the sediments decrease significantly from the upper to the lower reaches of the stream, with the distance from the Mn mine becoming far, which suggests the mining is the main reason for the stream pollution. Cd, Cu, Zn, Pb, Co, Ni, Mn and Cr are heavily accumulated in the tailing from the Mn mining, which are 3.5, 28.8, 9.8, 14.9, 5.4, 46.4, 147.5 and 5.0 times those in the local natural soil, respectively. Moreover, a significantly

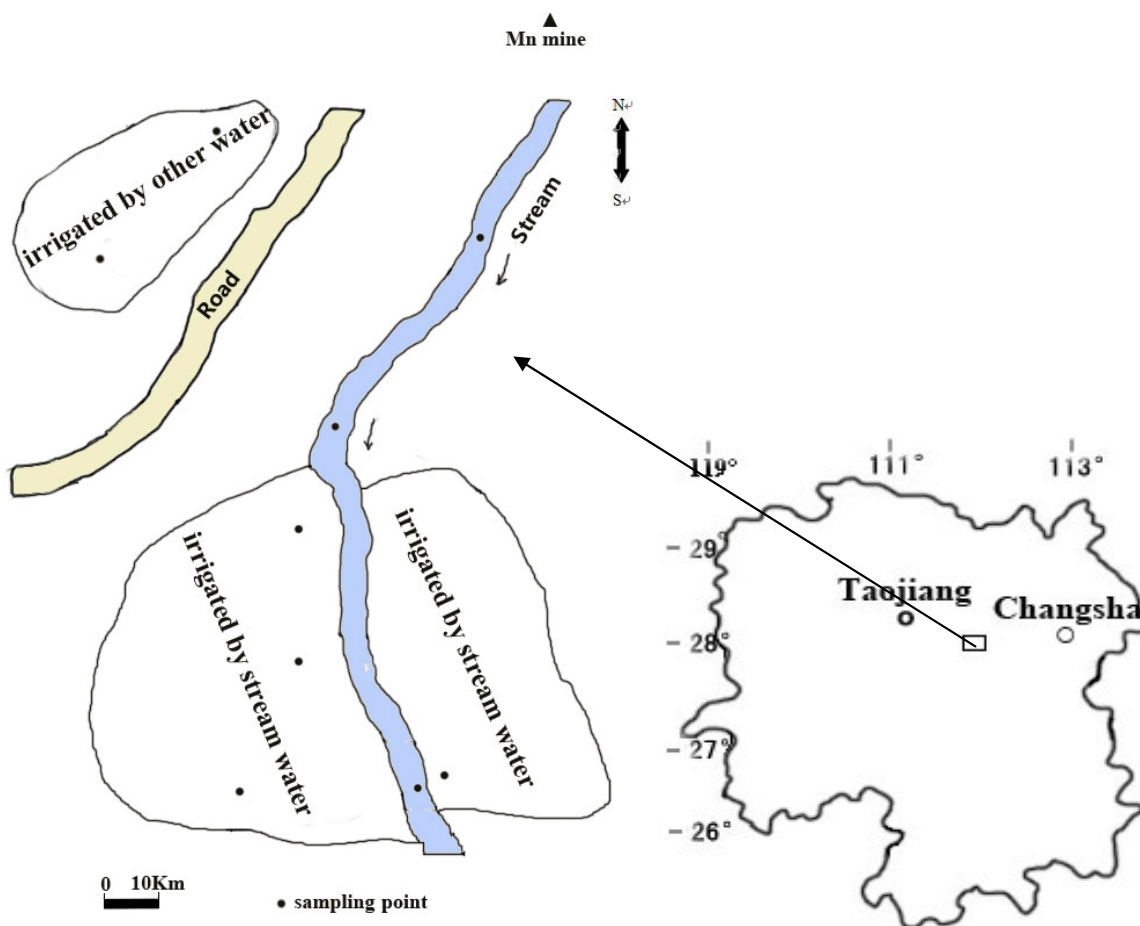


Figure 1. Sketch map showing the sampling points in study area (left) and the study area in Hunan province, China (right).

positive correlation was found between the contents of Cu, Zn, Pb, Co, Ni, Cr, Cd and Mn in the stream sediments and those in the tailings ($r=0.989$, $p<0.01$), which suggests the same source provenance of heavy metals in the stream sediments and the tailings, further proving that the stream was polluted by the mining.

The mean concentrations of Cd, Cu, Zn, Pb, Co, Ni, Mn and Cr in the soils irrigated by the stream water are 0.70, 72.7, 1343.2, 60.9, 34.8, 184.8, 21507.7 and 793.3 mg/kg, respectively, which are 3.5, 6.2, 4.2, 4.1, 2.2, 9.7, 40.9 and 17.3 times those in the local natural soils; while the mean concentrations of Cd, Cu, Zn, Pb, Co, Ni, Mn and Cr in the soils far away from the stream are 0.3, 22.2, 445.1, 17.3, 16.5, 31.6, 4299.8 and 247.3 mg/kg, respectively, which are 1.5, 1.9, 1.4, 1.2, 1.1, 1.7, 8.2 and 5.4 times those in the natural soil. Apparently, the long-term irrigation with the stream water has severely contaminated the soils nearby.

Heavy metals accumulation in vegetables

Heavy metal concentrations in the edible parts of the vegetables irrigated with the stream water are significantly higher than those irrigated with other water (Table 2). Cd, Zn, Ni and Cr in the vegetables irrigated with the stream water significantly exceed the recommended values. For example, Cd, Zn, Ni and Cr in the edible part of *Allium sativum L.* from the area with the stream irrigation are 22.4, 7.5, 3.6 and 2.8 times higher than the recommended values of the Chinese vegetable hygienic standards, respectively. The edible part of *Lactuca sativa L.* from the area irrigated with the stream water has the highest concentrations of Cd, Cu, Zn and Cr, which are 24.8, 2.7, 16.2 and 10.6 times the recommended values of the Chinese vegetable hygienic standards, respectively. The concentration of Ni is 17.36 mg/kg in *Pisum Sativum Linn* and 5.1 mg/kg in *Bulbus Allii Chinensis* in the area irrigated by the stream water, which exceed the recommended value by 27.9 and 7.5 times, respectively. Cd and Mn concentrations in *Allium sativum L.* in the area irrigated with the stream water are 166.1 and 4.7 times those in the area irrigated with other water. Cd and Zn concentrations in *Lactuca sativa L.* in the area with the stream irrigation are 14.4 and 4.5 times those in the area irrigated with other water. This suggests that the irrigation of the stream water has caused the significant accumulation of heavy metals in the vegetables nearby the stream, which poses severe health risks on the local residents.

Table 1. Concentrations of heavy metals in the stream water, sediments and the soils in the study area, in Hunan province, China

| Sampling points | Heavy metals (mg/kg; water, mg/L) | | | | | | | | |
|--|-----------------------------------|------------------|------------------|------------------|-----------------|-----------------|-----------------|------------------|-------|
| | Cd | Cu | Zn | Pb | Co | Ni | Mn | Cr | |
| Soils irrigated by stream water | 0.70 | 72.7 | 1343.2 | 60.9 | 34.8 | 184.8 | 21507.7 | 793.3 | |
| Soils irrigated by other water | 0.30 | 22.2 | 445.1 | 17.3 | 16.5 | 31.6 | 4298.8 | 247.3 | |
| Stream water | 0.0009 | 0.0004 | 0.315 | 0.0132 | 0.0049 | 0.0355 | 0.9795 | 0.0212 | |
| Other water | ND ^A | ND ^A | 0.101 | ND ^A | ND ^A | ND ^A | 0.186 | ND ^A | |
| Stream sediments | Upper stream | 1.3 | 78.9 | 1840.3 | 90.3 | 32.6 | 233.6 | 32056.3 | 440.1 |
| | Middle stream | 0.60 | 62.3 | 1607.5 | 42.5 | 21.4 | 184.6 | 46907.1 | 352.1 |
| | Lower stream | 0.90 | 32.9 | 1289.1 | 29.4 | 14.2 | 120.2 | 42811.7 | 328.2 |
| | Mean | 0.90 | 58.0 | 1579.0 | 54.1 | 22.7 | 179.5 | 40591.7 | 373.5 |
| Tailing | 0.90 | 349 | 3447.8 | 234.3 | 101.3 | 899.9 | 78087.3 | 271.7 | |
| Local non-disturbed natural soil | 0.20 | 11.7 | 318.0 | 14.7 | 15.7 | 19.0 | 525.7 | 45.6 | |
| Soil background value in Hunan province ^B | 0.10 | 26.0 | 94.0 | 27.0 | 14.0 | 32.0 | 459.0 | 68.0 | |
| Recommended values | 0.60 ^C | 100 ^C | 300 ^C | 350 ^C | 20 ^D | 60 ^C | NA ^E | 150 ^C | |

^A ND means No Detected; ^B China environment monitoring station (1990); ^C Soil Environmental Quality Standard in China (GB 15618, 1995); ^D Tentative Netherlands Soil Quality Criteria (VROM, 1983); ^E NA means no recommended value

Table 2. Heavy metal concentrations in the edible parts of the vegetables growing in study area irrigated with the stream water and other water

| | Vegetable species | Heavy metals (mg/kg) | | | | | |
|---|-------------------------------|----------------------|-------|--------|-------|-----------------|------|
| | | Cd | Cu | Zn | Ni | Mn | Cr |
| Irrigated by stream water | <i>Allium sativum L.</i> | 1.17 | 14.47 | 170.48 | 2.73 | 111.95 | 1.88 |
| | <i>Pisum Sativum Linn</i> | 0.008 | 15.57 | 133.89 | 17.36 | 53.79 | 0.47 |
| | <i>Bulbus Allii Chinensis</i> | 0.008 | 15.46 | 47.87 | 5.10 | 43.56 | 2.31 |
| | <i>Lactuca sativa L.</i> | 1.24 | 26.51 | 324.54 | 5.13 | 483.10 | 5.32 |
| | | | | | | | |
| Irrigated by other water | <i>Allium sativum L.</i> | 0.007 | 14.24 | 62.74 | 0.74 | 19.58 | 1.14 |
| | <i>Lactuca sativa L.</i> | 0.086 | 14.75 | 72.25 | 1.73 | 161.69 | 1.81 |
| Chinese vegetable hygienic standard (Yang 1998) | | <0.05 | <10 | <20 | <0.6 | NA ^A | <0.5 |

^A NA means no recommended value

Conclusion

The exploitation of a Mn mine in Hunan province, China, has made a stream nearby severely polluted, with mean concentrations of Mn, Zn, Ni and Cr in the stream sediment of 40592, 1579, 180 and 373.5 mg/kg, respectively, 77.2, 5.0, 9.5 and 8.2 times those in the local undisturbed natural soil. The significantly positive correlations between heavy metal contents in the stream sediments and those in the tailings prove that the stream pollution is mainly caused by the Mn mining. Cu, Ni, Mn and Cr concentrations of the soils irrigated with the stream water are significantly higher than those irrigated with other water, which are 6.2, 9.7, 40.9 and 17.3 times these in the local natural soil, respectively. Apparently, the irrigation with the stream water has caused the accumulation of heavy metals in the soils nearby. Cd and Mn concentrations in the edible parts of *Allium sativum L.* in the area irrigated with the stream water are 166.1 and 4.7 times those in the area irrigated with other water. Cd and Zn concentrations of *Lactuca sativa L.* in the area irrigated with the stream water are 14.4 and 4.5 times those in the area irrigated with other water. This suggests that the stream water irrigation has caused the heavy metal contamination of vegetables nearby, which poses severe health risks on the local residents.

Acknowledgements

This work was supported by the National Science & Technology Pillar Program of China during the Eleventh Five-year Plan Period (No. 2006BAD20B07) and the National Natural Science Foundation of China (40771093).

References

- China Environment Monitoring Station (1990) 'China Soil Element Background Values.' (Chinese Environment Science Publishing House: Beijing)
- Conesa HM, Robinson BH, Schulin R (2007) Growth of *Lygeum spartum* in acid mine tailings: Response of plants developed from seedlings, rhizomes and at field conditions. *Environmental Pollution* **145**, 700-707.
- GB15618 (1995) 'Soil Environmental Quality Standard of People's Republic of China.'
- Granero S, Domingo JL (2002) Levels of metals in soils of Alcaade Henares, Spain: Human health risks. *Environment International* **28**, 159-164.
- Hussein H, Farag S, Kandil K (2005) Tolerance and uptake of heavy metals by *Pseudomonads*. *Process of Biochemistry* **40**, 955-961.
- Lin C, Wu Y, Lu W (2005) Water chemistry and ecotoxicity of an acid mine drainage-affected stream in subtropical China during a major flood event. *Journal of Hazardous Materials* **142**, 199-207.
- VROM (1983) 'Leidrand Bodem sanering - Guidelines for Soil Clean Up.' (The Hague: Netherland)
- Yang HF, Li MY, Shen W (1998) 'Food Hygienic Physicochemical Test Standard Manual.' (China Standard Publishing House: Beijing)

Influence of inorganic and organic amendments for mine soils reclamation on spontaneous vegetation colonization and metal plant bioaccumulation

Dora M. Carmona^A, Ángel Faz,^A Raúl Zornoza^A, Asuman Büyükkiliç^A, Sebla Kabas^A, Jose A. Acosta^{A,B} and Silvia Martínez-Martínez^A.

^ASustainable Use, Management, and Reclamation of Soil and Water Research Group. Department of Agrarian Science and Technology. Technical University of Cartagena. Paseo Alfonso XIII, 52, 30203 Cartagena. Murcia. Spain. E-mail: angel.fazcano@upct.es

^BInstitute for Biodiversity and Ecosystem Dynamics. University of Amsterdam. Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands.

Abstract

The effectiveness of two organic amendments (pig manure and sewage sludge) and marble mud for the in situ remediation of contaminated soils by heavy metals owing to former mining activities in SE Spain was assessed. Concretely, we evaluated the spontaneous colonization of plant species and increments in richness and vegetation cover in the amended plots, as well as the bioaccumulation of heavy metals in plants, to avoid the risk of mobility in the food chain. Results showed that although the untreated plots remained without vegetation, natural plant species spontaneously colonised the amended plots. Increases in vegetation cover and richness were related with the application dose, with pig manure plots having the highest values. Metal concentrations in shoots of plant species were similar except for *Zygophyllum fabago* which had highest levels of Cd, Cu and Zn. The calculation of the bioaccumulation factor (BF) showed that Cd, Cu and Zn bioaccumulation was carried out in most species, while for Pb no bioaccumulation was observed. Moreover, *Piptatherum hordeum* only showed bioaccumulation for Cu, although it showed lower BF factors for all the other studied metals.

Key Words

Metal pollution; in situ remediation; phytostabilization; sewage sludge; pig slurry; marble waste.

Introduction

The environmental impacts of the long-history of mining activities in southeast Spain include large areas of soils characterized by strong acidification processes, high salinity and accumulation of metals. These mining activities have generated high amounts of sterile materials for many years; the wastes being accumulated in pyramidal structures called tailing ponds. Mine sites contain materials of high Fe-oxyhydroxides, sulphates, and potentially leachable elevated contents of heavy metals (mainly Cd, Pb, Cu and Zn) due to extreme acidic conditions. As a consequence, these mine soils have scarce or null vegetation due to very poor properties, including extremely low soil organic matter. These metal-contaminated soils contribute to human and animal metal exposure, through food chain transfer or inhalation of wind blown dust (Pierzynski 1997). In response to a growing concern for human health and environmental quality, many technologies have been developed to treat and remediate metal-contaminated soils. One of the remediation options gaining considerable interest over the last decade is the in situ immobilization of metals using metals immobilizing agents (Vangronsveld and Cunnungham 1998). Thus, the transformation of metals into harmless species or their removal in a suitable recycled mineral form such as carbonates using marble wastes or lime (Geebelen *et al.* 2003) is a promising solution for the remediation of a mining area, decreasing the concentration of available metal pools in the amended soil. In addition, incorporation of organic amendments into contaminated mine soils has been proposed as feasible, inexpensive and environmentally sound disposal practice, as generally such wastes can improve soil physical and chemical properties and contain nutrients beneficial to initialize plant colonization (Barker, 1997). The increment in vegetation cover reduces or even prevents the dispersion of the contamination through wind and water erosion, and improves the aesthetic value of formerly bare areas (Vangronsveld and Cunnungham 1998). Besides, vegetation itself may contribute to metal immobilization processes through biological activities in the production of organic matter, (Bouwman and Vangronsveld 2004), an emerging technology called phytostabilization. Thus, the goal of this work is to assess the establishment of spontaneous vegetation cover after the remediation of contaminated mine soils with different inorganic and organic amendments, and to evaluate the bioaccumulation of heavy metals in plants, to avoid the risk of mobility in the food chain.

Methods

Study site and experimental design

The study was conducted in the province of Murcia (SE Spain), in the Cartagena-La Unión Mining District, where extensive mining activity has been carried out for more than 2500 years, till the nineties. The climate of the area is semiarid Mediterranean type with mean annual temperature of 18°C and mean annual rainfall of 275 mm. Two tailing ponds generated by mining activities were selected: El Lirio (L) and Brunita (B), representative of the rest of existent ponds in Cartagena-La Unión Mining District.

The field trial was established in 2004. Plots (2 m x 2 m) were randomised and replicated 3 times. Two different organic amendments were used to reclaim the soils, pig manure (P) and sewage sludge (S). In addition, 3 different doses per amendment were applied. Thus, the treatments were: Untreated contaminated soil (Control: C), soil treated with pig manure at dose 1 (P1), dose 2 (P2) and dose 3 (P3); and soil treated with sewage sludge at dose 1 (S1), dose 2 (S2) and dose 3 (S3). For pig manure, dose 1, 2 and 3 were 2.5, 5 and 10 kg per plot, respectively. For sewage sludge, doses were 1.99, 3.98 and 7.97 kg per plot, respectively. Doses were established by thresholds imposed by legislation regarding the addition of N to soil (Council Directive 91/676/EEC, 1991) With the purpose of increasing soil pH to immobilise metals and create better conditions for microbial and plant development, marble mud was applied in all plots except for control, adding 22 kg of this inorganic waste per plot.

Soil and plant samplings and analytical methods

The soil sampling was carried out in May 2009 (5 years after application of amendments). One sample (0-15 cm depth) was collected for each plot, taken to the lab, air-dried for 7 days, passed through a 2-mm sieve and stored at room temperature prior to laboratory analyses. The physico-chemical characterization of the plots is shown in Table 1. At the same time, the identification of all plants that spontaneously colonized the plots was carried out (richness), as well as the percentage of vegetation cover. In addition, the shoot of one of the two most dominant species in P3 (the treatment with highest vegetation cover and richness) was collected in each of the three replicated plots for analyses of metal concentration. These species were *Piptatherum hordeum* and *Zygophyllum fabago* in El Lirio, *Dactylis glomerata* and *Brassica fruticulosa* in Brunita.

To determine the concentration of bioavailable metals in soils DTPA was used in the ratio of 1:2 as a soil-extractant (Lindsay and Norwell, 1978). For plant metals concentration, dry plant material was ground and combusted at 450°C for 24 h. After that, the digested material was suspended in HNO₃ and filtered. Measurements of metals were carried out using atomic absorption spectrophotometer (AAAnalyst 800, Perkin Elmer). The bioaccumulation factor (BF) was also calculated as $[\text{metal}]_{\text{shoot}}/[\text{bioavailable metal}]_{\text{soil}}$.

Table 1. Main physico-chemical properties and bioavailable metals of control and amended plots.

| Treatment | pH | EC (dS/m) | SOC (g/kg) | Nt (g/kg) | Texture | Cd (mg/kg) | Cu (mg/kg) | Pb (mg/kg) | Zn (mg/kg) |
|-----------------|-----|--------------|---------------|--------------|------------|---------------|---------------|---------------|---------------|
| El Lirio | | | | | | | | | |
| Control | 7.1 | 2.5 | 2.13 | 0.05 | Sandy loam | 5.63 | 1.94 | 177 | 495 |
| LP1 | 7.6 | 2.4 | 2.96 | 0.12 | Sandy loam | 4.21 | 2.45 | 320 | 418 |
| LP2 | 7.5 | 2.5 | 2.92 | 0.15 | Sandy loam | 3.29 | 2.21 | 333 | 352 |
| LP3 | 7.3 | 2.4 | 2.14 | 0.06 | Sandy loam | 2.92 | 0.84 | 309 | 446 |
| LS1 | 7.5 | 2.4 | 2.66 | 0.05 | Sandy loam | 4.02 | 1.72 | 223 | 428 |
| LS2 | 7.6 | 2.3 | 2.29 | 0.06 | Sandy loam | 3.86 | 1.68 | 295 | 433 |
| LS3 | 7.4 | 2.5 | 2.33 | 0.06 | Sandy loam | 3.70 | 1.38 | 288 | 406 |
| Brunita | | | | | | | | | |
| Control | 2.8 | 2.7 | 0.91 | 0.06 | Sandy loam | 0.02 | 0.40 | 2.13 | 5.74 |
| BP1 | 6.8 | 2.5 | 1.34 | 0.15 | Sandy loam | 0.13 | 0.85 | 4.72 | 12.39 |
| BP2 | 6.4 | 2.5 | 1.92 | 0.16 | Sandy loam | 0.32 | 0.91 | 4.66 | 19.65 |
| BP3 | 5.0 | 2.5 | 1.84 | 0.09 | Sandy loam | 0.16 | 0.55 | 6.67 | 10.02 |
| BS1 | 6.7 | 2.5 | 1.52 | 0.10 | Sandy loam | 0.15 | 0.26 | 4.03 | 10.67 |
| BS2 | 6.7 | 2.5 | 1.60 | 0.11 | Sandy loam | 0.23 | 0.28 | 5.26 | 13.04 |
| BS3 | 6.7 | 2.5 | 1.81 | 0.12 | Sandy loam | 0.22 | 0.40 | 5.34 | 11.14 |

EC: Electrical conductivity, SOC: soil organic carbon, Nt: total nitrogen..

Results and discussion

The untreated plots for both sites remained without vegetation, while natural plant species spontaneously colonised the amended plots. The vegetation cover increased with the dose as a general trend, although plots

amended with pig manure showed highest vegetation cover (Table 2). Richness also increased with the application dose of amendments, with highest values in plots amended with pig manure. In fact, vegetation cover and richness were significantly positively correlated in both zones, El Lirio ($r=0.78$; $P<0.001$) and Brunita ($r=0.67$; $P<0.01$). These results are promising in an area like Murcia province where more than 10% of pig production in Spain is located. Annually, Murcia province generates an estimated 8 millions m³ of waste residues from the pork industry (CAAMA, 2003), which is continuously increase with high demands for pork, and consequently creating disposal problem for many pig producers. SOC presents similar values in all plots in each different zone (Table 1), which indicates a mineralization or leaching of the organic amendments, since treated plots had initially significantly higher values of SOC (Zanuzzi, 2007). Nonetheless, this initial incorporation of organic matter has triggered the establishment of vegetation, which remains after 5 years of amendments application. The maintenance of this vegetation cover is essential for true landscape reclamation, reactivating nutrient cycles and microbial activity (Bouwman and Vangronsveld, 2004).

Table 2. Natural colonization of plant species on the plots.

| Treatment | Vegetation cover (%) | Richness | Plant species |
|-----------------|----------------------|----------|--|
| El Lirio | | | |
| Control | 0 | 0 | - |
| LP1 | 43 | 4 | <i>Zigophyllum fabago</i> , <i>Piptatherum hordeum</i> , <i>Dittrichia viscosa</i> , <i>Phragmites australis</i> |
| LP2 | 45 | 4 | <i>Zigophyllum fabago</i> , <i>Piptatherum hordeum</i> , <i>Helichrysum decumbens</i> , <i>Sonchus tenerrimus</i> |
| LP3 | 60 | 5 | <i>Zigophyllum fabago</i> , <i>Piptatherum hordeum</i> , <i>Helichrysum decumbens</i> , <i>Dittrichia viscosa</i> , <i>Phragmites australis</i> |
| LS1 | 13 | 2 | <i>Zigophyllum fabago</i> , <i>Piptatherum hordeum</i> , |
| LS2 | 27 | 3 | <i>Zigophyllum fabago</i> , <i>Piptatherum hordeum</i> , <i>Helichrysum decumbens</i> , |
| LS3 | 32 | 4 | <i>Zigophyllum fabago</i> , <i>Piptatherum hordeum</i> , <i>Helichrysum decumbens</i> , <i>Sonchus tenerrimus</i> |
| Brunita | | | |
| Control | 0 | 0 | - |
| BP1 | 23 | 5 | <i>Dactylis glomerata</i> , <i>Brassica fruticulosa</i> , <i>Piptatherum miliaceum</i> , <i>Bromus rubens</i> , <i>Helichrysum decumbens</i> |
| BP2 | 30 | 8 | <i>Bromus rubens</i> , <i>Brassica fruticulosa</i> , <i>Helichrysum decumbens</i> , <i>Sonchus tenerrimus</i> , <i>Phagnalon saxalite</i> , <i>Dactylis glomerata</i> , <i>Zigophyllum fabago</i> , <i>Spergularia bocconeii</i> |
| BP3 | 47 | 7 | <i>Dactylis glomerata</i> , <i>Bromus rubens</i> , <i>Helichrysum decumbens</i> , <i>Dittrichia viscosa</i> , <i>Phagnalon saxalite</i> , <i>Phalaris canariensis</i> , <i>Sonchus tenerrimus</i> |
| BS1 | 19 | 5 | <i>Dactylis glomerata</i> , <i>Brassica fruticulosa</i> , <i>Bromus rubens</i> , <i>Phagnalon saxalite</i> , <i>Phalaris canariensis</i> |
| BS2 | 25 | 6 | <i>Brassica fruticulosa</i> , <i>Phalaris canariensis</i> , <i>Bromus rubens</i> , <i>Sedum sediforme</i> , <i>Dactylis glomerata</i> , <i>Piptatherum miliaceum</i> |
| BS3 | 26 | 7 | <i>Dactylis glomerata</i> , <i>Brassica fruticulosa</i> , <i>Sonchus tenerrimus</i> , <i>Bromus rubens</i> , <i>Helichrysum decumbens</i> , <i>Phalaris canariensis</i> , <i>Spergularia bocconeii</i> |

The contents of the different metals in shoots were similar in the most dominant plant species in the P3 plots, except for *Zigophyllum fabago*, which had significantly higher values of Cd, Cu and Zn (Table 3).

Table 3. Metal concentrations in shoots for the most dominant plant species in P3 plots. Values are mean \pm Standard Deviation (n=3).

| Metal concentration | Plant species | | | | F value ^a |
|---------------------|---------------------------|----------------------------|-----------------------------|---------------------------|----------------------|
| | <i>Dactylis glomerata</i> | <i>Piptatherum hordeum</i> | <i>Brassica fruticulosa</i> | <i>Zigophyllum fabago</i> | |
| Cd (mg/kg) | 4.2 \pm 0.0 a | 4.4 \pm 0.1 a | 4.2 \pm 0.1 a | 13.2 \pm 2.0 b | 61.9*** |
| Cu (mg/kg) | 3.4 \pm 0.4 a | 3.8 \pm 0.4 a | 4.4 \pm 0.7 a | 12.0 \pm 0.9 b | 116.2*** |
| Pb (mg/kg) | 23.7 \pm 6.2 ab | 13.1 \pm 6.6 a | 49.9 \pm 18.5 b | 21.6 \pm 4.8 a | 6.7* |
| Zn (mg/kg) | 21.8 \pm 5.7 a | 90.9 \pm 27.0 b | 49.0 \pm 8.4 ab | 288.2 \pm 30.2 c | 100.0*** |
| Fe (mg/kg) | 171 \pm 53 | 325 \pm 116 | 225 \pm 119 | 180 \pm 38 | 1.8 ns |
| Mn (mg/kg) | 46.1 \pm 10.0 a | 28.0 \pm 2.0 b | 31.0 \pm 6.9 ab | 30.4 \pm 4.5 ab | 4.8* |

^a Significant at: * $P<0.05$, ** $P<0.01$, *** $P<0.001$ after one-way ANOVA; ns: not significant ($P>0.05$). Different letters indicate significant differences ($P<0.05$) among means in each location after Tukey's honestly significant difference.

The BF showed that Cd, Cu and Zn bioaccumulation occurred in most species, (with values >1), while for Pb no accumulation was observed. Moreover, *P. hordeum* only showed bioaccumulation for Cu, being the plant species with lower BF factors for the rest of metals. In this sense, plant species studied here (except for *P. hordeum*), translocated to the shoots high quantities of heavy metals, being *B. fruticulosa* and *D. glomerata* the species with highest BF for Cd, especially toxic for the food chain. Thus, these species may be more suitable for phytoextraction technique, rather than phytostabilization, since the most suitable species are those that show mechanisms for protecting themselves against uptake of metals and restricting their transport within the plant (Lefèvre *et al.* 2005). However, most species used in this study are not eaten by herbivores (Zanuzzi, 2007), acting like a sink for metals and preventing it from becoming available to other organisms.

Table 4. Bioaccumulation factors (BF) of each metal in the most dominant plant species in P3 plots. Values are mean ± Standard Deviation (n=3).

| BF | Plant species | | | | F value ^a |
|----|---------------------------|----------------------------|-----------------------------|---------------------------|----------------------|
| | <i>Dactylis glomerata</i> | <i>Piptatherum hordeum</i> | <i>Brassica fruticulosa</i> | <i>Zygophyllum fabago</i> | |
| Cd | 18.7 ± 7.8 a | 1.0 ± 0.1 b | 18.8 ± 6.7 a | 3.1 ± 0.9 b | 4.9* |
| Cu | 6.2 ± 0.8 a | 5.9 ± 3.7 a | 8.0 ± 0.9 ab | 12.7 ± 1.5 b | 6.8 * |
| Pb | 0.4 ± 0.1 a | 0.1 ± 0.0 b | 0.7 ± 0.2 c | 0.1 ± 0.0 b | 23.4 *** |
| Zn | 3.2 ± 1.1 ab | 0.3 ± 0.1 a | 5.0 ± 1.5 b | 1.0 ± 0.3 a | 10.1 ** |

^aSignificant at: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ after one-way ANOVA; ns: not significant ($P > 0.05$). Different letters indicate significant differences ($P < 0.05$) among means in each location after Tukey's honestly significant difference.

Conclusion

The application of pig manure and sewage sludge together with marble wastes has proved to be effective to initialize natural spontaneous vegetation colonization, richness and vegetation cover being highest in pig manure plots, also increasing with the dose of application. *Zygophyllum fabago* accumulated moderated quantities of metals, not observed in the other plant species. However, BF was high for all plants except for *P. hordeum*. Thus, even though most species used in this study are refused by herbivores, a better through selection of the most suitable species to continue with phytostabilization progress and mine soils remediation in SE Spain should be developed in the immediate future, focusing on reduction of erosion, tolerance to metals and salinity, nitrogen fixation and low accumulations of metals (Zanuzzi, 2007), so that risks in the food chain are minimised.

References

- Bouwman LA, Vangronsveld J (2004) Rehabilitation of the nematode fauna in a phytoestabilized heavily zinc-contaminated, sandy soil. *Journal of Soils and Sediments* **4**, 17-23.
- CAAMA (2003) Consejería de Agricultura, Agua y Medio Ambiente de la Comunidad Autónoma de la Región de Murcia. Registro de explotaciones Porcinas de la Región de Murcia.
- Council Directive 91/676/EEC. Protection of waters against pollution caused by nitrates from agricultural sources. *Official Journal L* 375, pp. 1-8, 31-12-1991
- Geebelen W, Adriano DC, van der Lelie D, Mench M, Carleer R, Clijsters H, Vangronsveld J (2003) Selected bioavailability assays to test the effect of amendment-induced immobilization of lead in soils. *Plant and Soil* **249**, 217-228.
- Lefèvre I, Correal E, Lutts S (2005a) Cadmium tolerance and accumulation in the noxious weed *Zygophyllum fabago*. *Canadian Journal of Botany* **83**, 1655-1662.
- Lindsay W, Norvell W (1978) Development of a DTPA soil test for Zn, Fe, Mn, and Cu. *Soil Science Society of America Journal* **42**, 421-428.
- Pierzinski GM (1997). Strategies for remediating trace-element contaminated sites. In 'Remediation of soils contaminated with metals'. (Eds IK Iskandar, DC Adriano), pp.67-88. (Science Reviews, Northwood, UK).
- Vangronsveld J, Cunningham S (1998). Introduction to concepts. In 'In situ inactivation and phytoremediation of metal contaminated soils'. (Eds. J Vangronsveld, S Cunningham), pp. 1-15. (Springer-Verlag, Berlin and RG Landes Company Georgetown).
- Zanuzzi A (2007) Reducción de impactos ambientales asociados a suelos contaminados por metales pesados mediante el uso de residuos antropogénicos. PhD Dissertation. Departamento de Ciencia y Tecnología Agraria. Universidad Politécnica de Cartagena.

Influence of percolation pattern on growth and yields of rice plants and uptake of cadmium with soil dressing models

Paul Shyamal Kumar^A, Sasaki Choichi^B, Nobuhiko Matuyama^C, Yoshimura Takeshi^D and Kaori Noda^E

^AThe United Graduate school of Agricultural Sciences, Iwate University, Morioka 020-8550, Japan, Email skumarpaul79@yahoo.com

^BFaculty of Agriculture and Life Science, University of Hirosaki, Hirosaki 036-8560, Aomori, Japan, Email- chsasaki@cc.hirosaki-u.ac.jp

^C Faculty of Science and Technology, University of Hirosaki, 3 Bunkyo-cho, Hirosaki-shi, Aomori, 036-8561, Japan.

Abstract

A greenhouse experiment was conducted to evaluate the effect of open system percolation and close system percolation on accumulation of Cadmium in various parts of the rice plant using cadmium polluted paddy field model with soil dressing. Low groundwater level in an open system percolation model caused oxidation of soil, on the other hand, the closed system percolation model with a high ground water level resulted in grayish reduced soil. The concentration of cadmium in polluted soil was 3.39 mg/kg. Cadmium accumulation in all parts of rice plants (roots, stems, leaves and grains) was higher for open system percolation in the paddy field model than for the closed system percolation. Cadmium uptake by rice plants was enhanced in the open system percolation model due to the interactions of oxidation and reduction. Finally it can be concluded that the low redox potential (Eh) value in a closed system percolation model was effective in reducing cadmium accumulation in rice plant.

Key Words

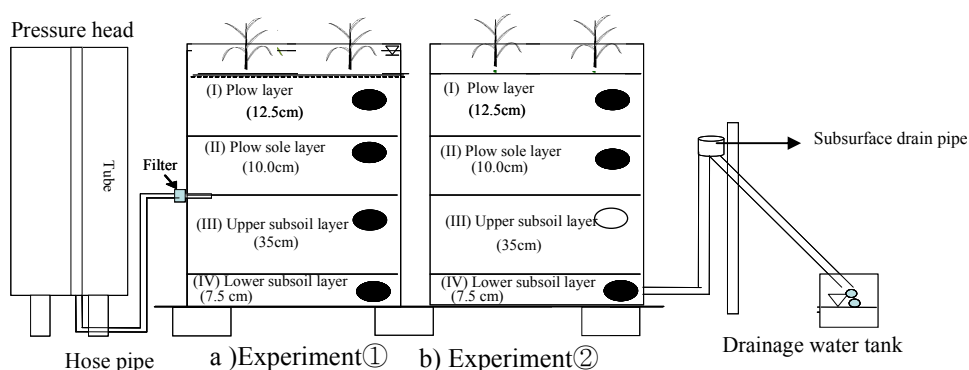
Percolation pattern, cadmium concentration of rice plant, growth and yields, soil dressing.

Introduction

Heavy metal pollution in soil has an adverse effect on plant growth. Cadmium is one of heavy metals for soil pollution. WHO set the cadmium concentration for edible rice grain <0.001 mg/ kg. Eating of high cadmium content rice may cause Itai-Itai disease, which was documented in Toyama prefecture of Japan. Many technologies have been studied for reduction of cadmium in rice grain. Water management in paddy fields may be used as a method to reduce cadmium uptake in rice plants as the Ministry of Agriculture, Forestry and Fisheries and National Institute for Agro-Environmental Sciences, Japan (2002) recommended the flooding water of paddy before and after the heading, and soil improvement using fertilizers for minimizing cadmium uptake in rice plants. The percolation pattern is related to hydraulic conductivities of the soil layers and hydraulic conditions such as groundwater level (Adachi *et al.* 1992), which may acts as controllers of soil redox potentials, consequently the uptake of cadmium in the rice plants. In paddy field, water is needed for saturating soil, surface ponding, percolation seepage (bund percolation) and evapo-transpiration (Adachi *et al.* 1992). Sasaki *et al.* (2009) studied a polluted paddy field model but did not include soil dressing treatment, therefore the purpose of this study is to clarify the influence of percolation pattern on growth and yields of rice plants and uptake of cadmium with soil dressing polluted paddy fields models.

Experimental design and method

The experiment was conducted in a green house of Hirosaki University, Japan. Using stratified paddy field model consisting of 50cm × 30cm × 70cm in size steel box filled with four layers of soil materials. Plow layer (I layer; 0-12.5cm), plow sole (II layer; 12.5-22.5cm), upper subsoil (III layer; 22.5-57.5cm), and lower subsoil (IV layer; 57.5-65.0cm) were constructed with Andosol (I layer), cadmium polluted soil (II layer), gravel (III layer) and gravel with sand (IV layer). Stratified paddy field models were subjected to two kinds of percolation pattern as an open system percolation and a closed system percolation. These two percolation systems were imposed in paddy field models by controlling water level at 52.5cm and 7.5cm, respectively using a subsurface drainage pipe. The ground water level in an open and a closed percolation pattern was 52.5 and 7.5 cm, respectively. Platinum electrodes were placed in each soil layer to measure the value of oxidation reduction potential in soil.



●: Close system percolation① ○:Open system percolation②

Figure 1. Experimental design of stratified paddy field models

Table 1. Physical and chemical properties of sample soil.

| Measurement items | Density (g/cm ³) | Soil texture | Total iron * | Cd * | T-N (%) | T-P (%) | Organic matter content (%) |
|-------------------|---------------------------------|--------------|-----------------|---------|------------|------------|-------------------------------|
| Andosol | 2.592 | SCL | 5,683 | 0.19 | 0.44 | 0.60 | 10.0 |
| Polluted soil | 2.453 | L | 2,820 | 3.39 | 0.40 | 0.15 | 9.4 |
| Gravel | 2.680 | - | 600 | 0.13 | 0.00 | 0.35 | 0.00 |

Soil texture is based on the International Soil Society classification; explain SCL and L stands for: L = Loamy; SCL = Silt Clay Loam *represents mg/kg dry soil.

After construction of paddy field model, 15 lines of rice plants (Cultivar: Tsugaru-Roman) were transplanted in each paddy field model with 10 cm planting distance. Fertilizers were applied as recommended dose for Tsugaru-Roman cultivar. Oxidation-reduction potential (ORP) is referred to as redox potential (Eh) was measured electrometrically in each soil layer using a ORP meter (Central Science Co., Ltd., Model UC-203). Analysis of Cd in grains, roots, stems and leaves of rice plant by atomic absorption spectrometry analysis (Hitachi High –technologies. Co., Model Z2000). Growth of rice plants was investigated by measurement of plant height, number of stem, dry matter weight, per plant grain yield and filled grain ratio.

Results and discussion

In an open system percolation model, the pressure head distribution was under positive pressure in the plow layer but changed to negative pressure in the plowsole layer and upper subsoil layer. However in a closed system percolation system, the pressure head distribution had positive pressure in all layers. Positive and negative pressure was controlled by ground water level. We postulated that Cd in rice plants for the negative hydraulic pressure model was higher than that with positive hydraulic pressure model. According to Sukthai P. *et al.* (2005) Cd uptake was higher by rice plants with positive hydraulic pressure than those with negative hydraulic pressure.

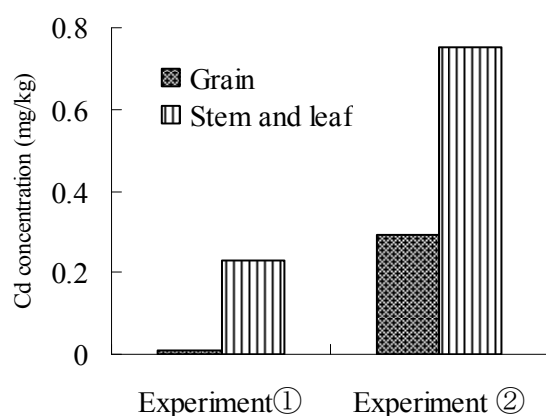
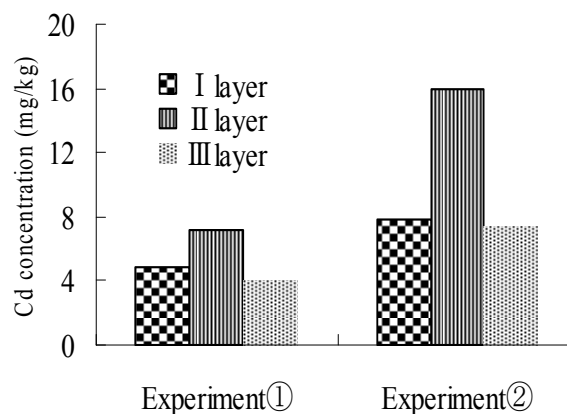
The ORP value in plow layer and plowsole layer was lower than 300 mV in both the open percolation and closed percolation models. However, the ORP value in subsoil was more than 300 mV in the open percolation system, whereas it was lower than 300 mV in the closed percolation system. The ORP value of more than 300 mV is indicative of oxidising conditions and lower than 300 mV indicates reductive conditions. In principle, soil layers with oxidising conditions promote the accumulation of cadmium in plant.

Rice plants in the closed system percolation with paddy field model showed better vegetative growth and grain yield than those in the open system percolation. In experiment ①, plant height, number of stems and dry matter weight of rice plants were higher than for experiment ② but no difference was found for heading time. The weight of grains and filled grain ratio were also higher in experiment ① than experiment ② (Table 2). High cadmium uptake in rice plant might have an adverse effect on growth and yield of the rice plant as Zhou *et al.* (2003) found that rice plant height and grain yield were decreased by about 4 to 5 cm and 20 to 30 %, respectively due to the effect of Cd.

Table 2. Growth and yield of rice plant in different percolation system.

| Treatment | Plant height(cm) | Stem no. | Leaf no. | Dry matter weight (g/stump) | Weight of grains (g/stump) | Filled grain ratio (%) |
|--------------|------------------|-----------|-----------|-----------------------------|----------------------------|------------------------|
| Experiment ① | 103.8±4.14 | 12.7±1.95 | 14.80±0.4 | 814 | 40.6 | 89.0 |
| Experiment ② | 102.6±2.97 | 12.0±2.10 | 15.0±0.0 | 731 | 36.5 | 86.8 |

Cadmium concentrations in rice plants followed: root > stem > grain in both paddy field models. Percolation pattern also influences uptake of Cd by rice plants. In experiment ② Cd concentrations were higher in all parts of rice plants than for experiment ① (Figure 2 and 3). Cadmium concentration in stem and leaf and grain were 0.23 and 0.01 mg/kg in experiment ① whereas in experiment ② values were 0.75 and 0.29 mg/kg, respectively. With an open system percolation, atmospheric air entered through air flow path at the subsoil layer. In the presence of oxygen, cadmium forms cadmium sulphate (CdSO₄), which is more available for uptake by rice plants but in close system percolation, air could not enter into soil profile in absence of air flow path. Moreover, high groundwater level in the closed system percolation model favored the reduced condition of soil layers. Cadmium concentrations of roots in each layer were relatively higher for experiment ② than experiment ① (Figure 3).

**Figure 2. Cadmium concentration in brown rice grain and stem plus leaf****Figure 3. Cadmium concentration in roots**

Conclusion

Percolation pattern is one of the important factors together with soil pH, temperature, anaerobic bacteria, heavy metal concentration, gravel size and soil fertility for the growth and development of rice plants. Percolation pattern controls the oxidation- reduction status of soil, consequently the uptake of cadmium by rice plants. A closed system percolation pattern can be considered a tool to reduce cadmium uptake by rice plants, growing in cadmium polluted paddy fields.

References

- Adachi K, Sasaki C (1992) Percolation and Seepage. *Advanced Paddy Field Engineering*. (Eds M Mizutani, S Hasegawa, K Koga, A Goto and VVN Murty) pp .71-88. (JSIDRE publishing: Shinzan-sha Sci. & Tech, Tokyo, Japan)
- Forestry and Fisheries and National Institute for Agro-Environmental Sciences (2002) Ministry of Japan. www.maff.go.jp/e/annual_report/pdf/t_2002.pdf.
- Sasaki C, Matuyama N, Kubota M, Noda K, Kato K (2009) Influence of percolation pattern on the Cadmium concentration, the growth and yields of rice plants in Cadmium polluted paddy fields. *Trans of JSIDRE*. **261**, 65-72.
- Sukthai P, Sasaki C, Matuyama N, Tonouchi A, Noda K (2005) Influence of percolation pattern on removal of soluble elements in downward water and cadmium transfer using a stratified paddy field model. *J. Jpn. Soc. Soil Phys.* **101**, 17-26.
- Zhou Q, Wang X, Liang R, Wu Y (2003) Effects of Cadmium and Mixed Heavy Metals on Rice Growth in Liaoning, *China Soil and Sediment Contamination* **12**, 851-864.

Interaction of mercury contaminated soils with iron oxides and potential remediation of river/runoff/storm water by an aquatic plant

Fengxiang X.Han, Yi Su, David L. Monts and Yunju Xia

Institute for Clean Energy Technology (ICET) Mississippi State University, Starkville, MS, USA.

Abstract

Historically as part of its national security mission, the U.S. Department of Energy's Y-12 National Security Facility in Oak Ridge, TN, USA acquired a significant fraction of the world's supply of elemental mercury. During the 1950's and 1960's, a large amount of elemental mercury escaped confinement and is still present in the buildings and grounds of the Y-12 Facility and in the Y-12 Watershed. Because of the adverse effects of elemental mercury and mercury compounds upon human health, the Oak Ridge Site is engaged in an on-going effort to monitor and remediate the area. The main thrust of the Oak Ridge mercury remediation effort is currently scheduled for implementation in FY09. In order to more cost effectively implement those extensive remediation efforts, it is necessary now to obtain an improved understanding of the role that mercury and mercury compounds play in the Oak Ridge ecosystem.

Most recently, concentrations of both total mercury and methylmercury in fish and water of lower East Fork Poplar Creek (LEFPC) of Oak Ridge increased although the majority of mercury in the site is mercury sulfide. This drives the US DOE and the Oak Ridge Site to study the long-term bioavailability of mercury and speciation at the site. The stability and bioavailability of mercury sulfide as affected by various biogeochemical conditions, such as presence of iron oxides have been studied. We examined the kinetic rate of dissolution of cinnabar from Oak Ridge soils and possible mechanisms and pathways in triggering the most recent increases of mercury solubility, bioavailability and mobility in Oak Ridge site. The effects of pH and chlorine on oxidative dissolution of cinnabar from cinnabar-contaminated Oak Ridge soils is discussed.

On the other hand, aquatic plants might be good candidate for phytoremediate contaminated waste water and phytofiltration of collective storm water and surface runoff and river. Our greenhouse studies on uptake of Hg by water lettuce (*Pistia stratiotes*) show that water lettuce is effectively removing Hg from water solution and Hg was mostly stored in roots. One day of growing could remove 93-98% of Hg from water solutions. However, Hg causes acute toxicity to water lettuce as indicated by decreases in fresh biomass and moisture contents.

Key Words

Mercury, Oak Ridge, iron oxides, mercury sulfide, water lettuce.

Introduction

The Y-12 National Security Facility site encompasses 3.24×10^6 m² near the city of Oak Ridge, Tennessee, USA. Y-12 is a manufacturing and developmental engineering facility that formerly produced components for various nuclear weapons systems. Mercury contamination is ubiquitous in the Y-12 watershed and has been identified as a key contaminant in soil, sediment, surface water, groundwater, buildings, drains, and sumps (Han *et al.* 2006). The source of the mercury is from elemental mercury used during the 1950s and early 1960s for the manufacture of nuclear weapons. Mercury was a key element used to capture enriched lithium by separating the lithium isotopes. The total mercury release to the environment has been estimated to range from about 75 to 150 metric tons. Most of the contamination is accumulated in the upper 3 m of floodplain soils and the sediments of a 24-km length of East Fork Poplar Creek (EFPC), which has its headwaters at Y-12. The concentrations of mercury in the Upper EFPC watershed (soil) range from 0.01 to 7700 mg/kg. Some sediment cores contain 460 mg/kg mercury at depths of 80-84 cm. Mercury has been detected at higher than background levels in sediments of the Clinch River and the Tennessee River near Chattanooga, some 190 km downstream of Oak Ridge.

A series of remediation efforts have been employed in the Oak Ridge watersheds. The U.S. Department of Energy has removed soil at several locations along the creek where mercury concentrations were particularly high (1). The predominant form of mercury in the floodplain soils of the region is mercuric sulfide (cinnabar and meta-cinnabar forms) (1). This indicates that after long-term transformation and redistribution, mercury is finally transferred into the insoluble sulfide form with decreasing mobility, volatility and phyto- and bio-

availability. Mercury sulfide, in general, has been regarded to be the stable mercury form in soils and sediments since its solubility is limited. However, the concentration of total mercury in fish and water of the lower East Fork Poplar Creek (EFPC) of Oak Ridge has recently increased. It was reported that dissolved organic carbon increased dissolution of mercury from HgS (1) and thus increased mercury bioavailability in soils. Other inorganic (chloride and sulfate) and organic ligands (salicylic acid, acetic acid, EDTA, or cysteine) were not found to enhance the dissolution of mercury from the mineral cinnabar. Due to interaction of cinnabar and various soil organic and mineral in soil environment, solubility and extractability of mercury sulfide mineral in soil has been found to be enhanced compare to pure cinnabar mineral (1).

Phytoremediation has been accepted and utilized widely because of cost-effectiveness, permanent removal and protection of nature. Our laboratory has investigated uptake and accumulation of Hg by Indian mustard (*Brassica juncea*), Beard grass (*Polypogon monspeliensis*), Chinese brake fern (*Pteris vittata*) and Boston fern (*Nephrolepis exaltata*) as well as its oxidative phytotoxicity (Chen *et al.* 2009; Han *et al.* 2004). All these plants are upland plants. No detailed studies have been done on application of aquatic plants to phytofilter Hg from river, lake and storm waters. The objectives of this study were to 1) investigate effects of chloride and pH on solubility of soil mercury sulfide in contaminated Oak Ridge soil reacted with iron oxides and 2) uptake of Hg by water Lettuce (*Pistia stratiotes*) from water.

Materials and methods

Soil sampling and laboratory experiments

The soil at the study site is Armuchee soil (clayey, mixed, thermic Ochreptic Hapludults). This is a moderately deep soil with a clayey subsoil. Armuchee soils are formed in residuum of shale. The total Hg concentration of this soil was 0.20 ± 0.15 mg/kg, indicating that the soils has not been contaminated. One gram of iron oxides (magnetite, Fe₃O₄, 325 mesh or hematite Fe₂O₃, 325 mesh) were used to react with either 0.1 g pure mercury sulfide cinnabar mineral or 5 g of contaminated Oak Ridge soil. Soil contained 2000 mg/kg Hg as HgS. For the experiment with various pHs, 35 ml of 0.01 M NaNO₃ at pH 4, 6 and 8 was added to the mixture and the mixture was shaken for 24 hours and then centrifuged. Supernatant was filtered with 0.45 um filter for analyses. For the experiment with various Cl concentrations, 35 ml of 0.01, 0.1 and 1 M NaCl was added to the mixture.

Greenhouse study with water lettuce (pistia stratiotes)

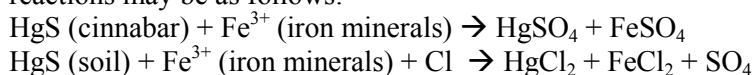
Water lettuce (*Pistia stratiotes*) plants with a similar size (4-5 full leaves) were collected from a clean water pond for the experiment. Hg was not found in the pond water. The plants were transferred into 1000 mL of modified Hoagland's solution containing HgCl₂ and grew for 7 days. The pH of the nutritant solution is 5.5. HgCl₂ with different Hg concentrations (1, 5, 10, and 20 mg/l) was used. After seven days, plant samples were collected and roots were washed by 1% (v/v) HCl and distilled water to remove the mercury adhering to the surface of the roots. Plants were dried for 48h at 70°C, and then weighed for mercury content analysis.

Results and discussion

Effects of Cl and pH on reaction of HgS and iron oxides in pure mineral systems and in HgS-contaminated oak ridge soil

We observed the increase in SO₄ concentrations in pure HgS cinnabar-iron oxide (both magnetite and hematite) systems. The SO₄, Fe and Hg concentrations in (NaCl) solution increased with increasing in Cl concentrations (Figure 1). This clearly indicates that Cl promoted oxidation-induced dissolution of Hg and S from HgS cinnabar mineral surface via surface complexing. The possible mechanisms of dissolution of HgS were suggested to include surface complexation of mercury and oxidation of surface sulfur species by trivalent iron atoms from iron oxide. This mechanism was proposed to explain the increase in dissolution of Hg by the organic matter. The complexing of Cl with iron and dissolved Hg may promote the oxidation-induced dissolution reaction. This resulted in a significant decrease in pH from 4.9 to 4.1.

The similar increase in SO₄ was observed in HgS contaminated Oak Ridge soil reacted with iron oxide (magnetite). Significant pH decrease was also found in the soil system with increasing Cl. The possible reactions may be as follows:



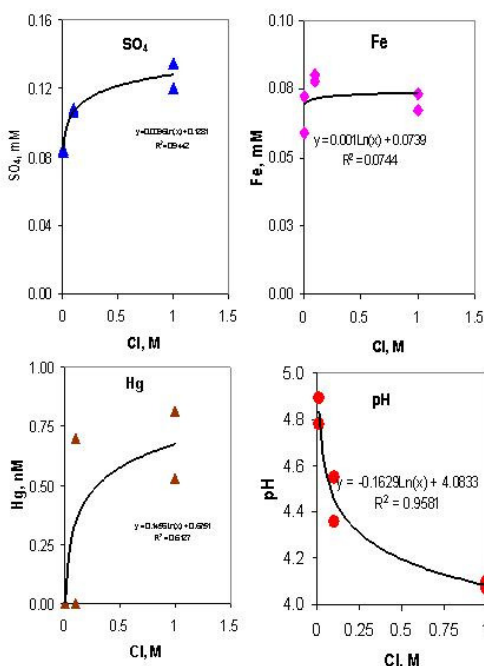


Figure 1. Changes in SO_4 , Hg and Fe concentrations in NaCl extracts and pH in the HgS cinnabar- Fe_3O_4 mineral systems with increasing Cl concentration (NaCl).

The effects of initial pH on concentration of SO_4 released from Oak Ridge soils were observed, but not significant. A slight increase in SO_4 release from contaminated soils reacted with magnetite was found with increase in initial pH of the suspension.

Mercury sulfide is stable in soils under normal conditions. The solubility product constant of HgS is $10^{(-52)-(-54)}$ (Han *et al.* 2008). Thus, many common chemical extractants are not able to solubilize and extract mercury from pure HgS. Strong acids, such as 4M HNO_3 and 12 M HNO_3 , only extracted <0.033% and <0.086% of Hg from commercial chemical HgS, respectively (3). The extractability of HgS from HgS-contaminated Oak Ridge soil significantly increased. However, neutral salts, such as NH_4OAc , and other weak extractants (e.g., $\text{NH}_2\text{OH}\cdot\text{HCl}$, pH 2) did not extract any detectable amounts of Hg from pure chemical or from HgS-contaminated soils (3). EDTA also effectively released mercury from cinnabar-contaminated Oak Ridge soils (3).

Phytofiltration of Hg from water with water lettuce

Water lettuce effectively removed Hg from Hg solution in the range of 0-20 mg/L. Kinetics of Hg concentrations showed that even first day of reaction effectively removed a majority of Hg (Figure 2)

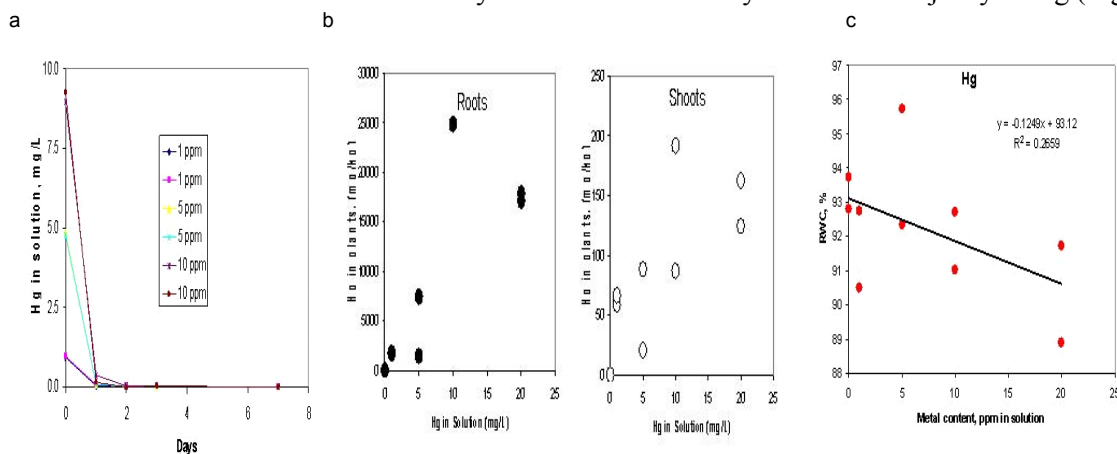


Figure 2. a. Kinetics of mercury concentrations in water with days of growing of plants; b. Uptake of mercury by shoots and roots of water lettuce; c. Relationships between relative moisture contents of shoots and mercury concentrations in water.

After the end of 7 days of cultivation, no any mercury residual was found in solution (Figure 2). Of course, some of Hg may be evaporated into the air, but the majority of Hg was cumulated in roots of plants. The maximum Hg accumulation in roots of water lettuce could be up to 2.5%. Mercury concentrations in both roots and shoots increased with Hg in solution (Figure 2). However, Hg phytotoxicity was also found in water lettuce over the experiment. Both fresh weight and relative moisture contents decreased with increase in Hg concentrations in solution (Figure 2). Relative moisture (water) contents (RWC) of shoots have been suggested to be correlated to phytotoxicity of Hg and other heavy metals in plants (2). Significant decrease in RWC due to exposure to Hg was also observed in ferns and Indian mustard (4).

Our preliminary experiments showed increased oxidation-induced dissolution of Hg from both pure HgS cinnabar mineral surface and HgS contaminated Oak Ridge soil via Cl complexing. Increases in Cl concentrations resulted in increases in Hg, Fe, and SO₄ concentrations, but decreased pH in the pure and soil systems. However, the detailed mechanisms and kinetics require further study. On the other hand, water lettuce proves a very promising candidate for phytofiltering Hg from contaminated lake/river/story water from the site.

References

- Han FX, Su Y, Monts DL, Waggoner CA, Plodinec MJ (2006) Binding, Distribution, and Plant Uptake of Mercury in a Soil from Oak Ridge, Tennessee, USA. *Science of Total Environment* **368**, 753-768.
- Chen J, Shiyab S, Han FX, Monts DL, Waggoner CA, Yang ZM, Su Y (2009) Bioaccumulation and Physiological Effects of Mercury in *Pteris vittata* and *Nephrolepis exaltata*. *Ecotoxicology* **18**, 110-121
- Han FX, Shiyab S, Chen J, Su Y, Monts DL, Waggoner CA, Matta FB (2008) Extractability and bioavailability of mercury from a mercury sulfide contaminated soil from Oak Ridge, Tennessee, USA. *Water, Air and Soil Pollution* **194**, 67-75.
- Han FX, Sridhar BBM, Monts DL, Su Y (2004) Phytoavailability and Toxicity of Trivalent and Hexavalent Chromium to *Brassica juncea* L. *Czern. New Phytologist* **162**, 489-499.

Interactive effects of salinity and lime on the phytoextraction of cadmium by *Salix* species in biosolids

Hannah G Dannatt^A, Alison Monsant^A and Caixian Tang^B

^AGolder Associates Pty Ltd, Building 7, Botanicca Corporate Park, Richmond, Vic 3121, Australia. HDannatt@golder.com.au

^BDepartment of Agriculture, La Trobe University, Bundoora, Vic 3086, Australia. C.Tang@latrobe.edu.au

Abstract

A glasshouse experiment was set to examine the effect of lime amendments and increasing salinity on the phytoextraction of Cd by three *Salix* species grown in biosolids. Liming greatly enhanced the Cd phytoextraction by *Salix* crop by up to 12 times, via increasing shoot biomass, Cd uptake and translocation. Increases in salinity (NaCl) further enhanced Cd uptake, however salt stress dramatically reduced plant growth. In biosolids of low salinity *S. reichardtii* had the highest rate of Cd extraction, whereas in moderately saline biosolids *S. viminalis* had the highest biomass and Cd accumulation. This study revealed that lime addition to saline biosolids is an effective method of increasing Cd phytoextraction and reducing salt stress, thus allowing irrigation of phytoextraction crops with saline water to be a feasible option.

Key Words

Phytoremediation, salty water irrigation, species variation, willow.

Introduction

Fresh water is limited in many areas of Australia and much of the water available for the irrigation of phytoremediation crops is burdened with dissolved salts. Hence, to phytoextract metals from many areas, and from substrates that are naturally saline, such as biosolids, plants must not only accumulate high concentrations of metals but also be able to tolerate increasingly saline conditions. Currently, the potential of short rotation *Salix* stands for the phytoextraction of Cd is being investigated at Werribee Treatment Plant (Laidlaw *et al.* 2007). It is suggested that saline lagoon water is used for the irrigation of *Salix* plantations. However, the effects of introducing additional salts to the already moderately saline biosolids (EC 2.6 dS/m) on: (i) Cd mobility; (ii) plant growth and (iii) the overall phytoextraction of Cd are unknown. As biosolids at the Western Treatment Plant are acidic (pH =4.6, 1:5 H₂O) liming may heighten the growth of *Salix* crops and thus enhance phytoremediation practices. The combined effects of salinity and lime on the phytoremediation of Cd from biosolids requires further research and hold potential for enhanced phytoextraction efficiency. The aim of this study was to determine the effects of increasing concentrations of salts (NaCl) and lime on Cd bioavailability and phytoextraction by three *Salix* species (*Salix viminalis*, *Salix reichardtii* and *Salix purpurea*).

Materials and methods

A glasshouse experiment was set up which consisted of two levels of lime (0 and 8 g CaCO₃/kg dry biosolids) applications, three *Salix* species and five salinity levels with three replicates. The salt application rates were 0, 1.5, 4, 8 and 11 g NaCl/kg biosolids. The three species of *Salix* used were: 1) *Salix purpurea*; 2) *Salix reichardtii*, which is a hybrid taxon of *Salix caprea* × *Salix cinera* and has been found to be most successful at extracting Cd from biosolids (Laidlaw *et al.* 2007); and 3) *Salix viminalis*, which is well known for its high biomass production, but is less tolerant to heavy metals than other *Salix* species (Punshon and Dickinson 1997). Stem cuttings (10 cm length) of *Salix* species were collected from 3-year plantations. Prior to planting, the cuttings were chilled (4°C) for three weeks to stimulate growth, as winter approached. All cuttings were dipped in rooting hormone and grown in a heated sandbed with a mist watering system in a glasshouse (25°C). After 30 days, uniform cuttings were transferred to lined 3-L pots containing 1.8 kg of biosolids. Lime treatments were added in powder form to the biosolids, well mixed and then incubated for 14 days prior to planting. Basal nutrients were added at the time of planting (5 g Grocote fertilizer/kg biosolid). After allowing the cuttings to stabilize in the biosolids for 16 days, salt treatments were added in 4 parts over 14 days. Pots were watered with tap water to field capacity every second day.

Plant shoots and roots were harvested 70 days after transplanting into the biosolids. Roots were rinsed four times in distilled water and then soaked in ice cold 5 mM PbNO₃ (1h) to desorb metals adhering to the root apoplasts (Assunção *et al.* 2001). The dry weights of shoots and roots were recorded before grinding the

samples. Cadmium concentrations in plant tissues were determined by atomic absorption spectroscopy after plant material (0.1 g) was digested in a 1:4 mixture of nitric: perchloric acids using Tecator DS40 Digestion systems. The translocation percentage was used to indicate the ability of *Salix* plants to transport Cd from the roots to the shoots and was calculated by dividing the total metal content in shoots by the total metal content in roots (Ghnaya *et al.* 2007).

Results

Plant growth

The growth rate of all *Salix* species was low. By Day 10 after salt addition, new growth of all plants grown with high salt levels (8 to 11 g NaCl/kg) showed signs of chlorosis, particularly *S. viminalis*. By Day 21 after salt addition, the leaves of many of the plants, particularly those grown in non-limed biosolids, began to senesce. Liming increased the shoot and root dry mass of *Salix* species by up to 4 fold (Figure 1). Conversely, increasing salinity significantly decreased plant growth, most markedly when salt additions exceeded 1.5 g NaCl/kg. Shoot and root dry weights differed significantly between *Salix* species, in varying salt and lime environments. *Salix purpurea* had the greatest shoot biomass in biosolids with low salt additions (compared other *Salix* species), whilst *S. viminalis* had the greatest biomass in the most saline biosolids.

The pH and EC of bulk and rhizosphere biosolids

Salt treatments increased the bulk EC from 1.45 to 3.58 dS/m in the non-limed biosolids and from 1.55-4.72 dS/m in the limed biosolids. The EC was highly variable between replicates, particularly in the rhizosphere, and did not significantly differ between species. The addition of lime to the biosolids increased the pH from 4.5 to 6.2 in the bulk and rhizosphere biosolids and did not significantly differ between *Salix* species. Salt treatments did not significantly influence the bulk soil or rhizosphere pH.

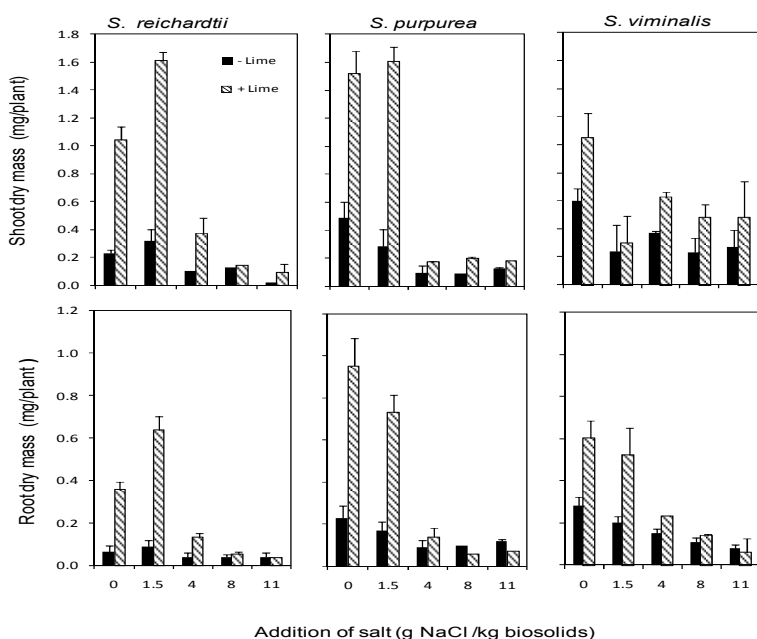


Figure 1. The dry mass of the roots and shoots of each *Salix* species after 70 days growth in biosolids with various salt treatments with (+ lime, 8 g/kg) and without lime (-lime, 0 g/kg). The bars indicate the standard error of means of 3 replicates.

Cadmium concentration and content in *Salix* shoots and roots

Liming significantly elevated Cd concentrations in the shoots of all *Salix* species (Figure 2). In particular, liming increased Cd uptake in *S. reichardtii* by 12 times at 1.5 g NaCl/kg. However, liming did not consistently increase Cd concentrations in the plant roots. The highest concentrations of Cd in shoots were found in *Salix* grown on limed biosolids with 4 g/kg salt treatments. However, total Cd uptake in the plant shoots was greatest for biosolids with 1.5 g/kg salt addition, due to greater plant growth. Overall Cd extraction in biosolids with low salt additions was revealed with the following decreasing order: *S. reichardtii* > *S. purpurea* > *S. viminalis*. However, *S. viminalis* had the greatest total Cd uptake in the moderate saline environments.

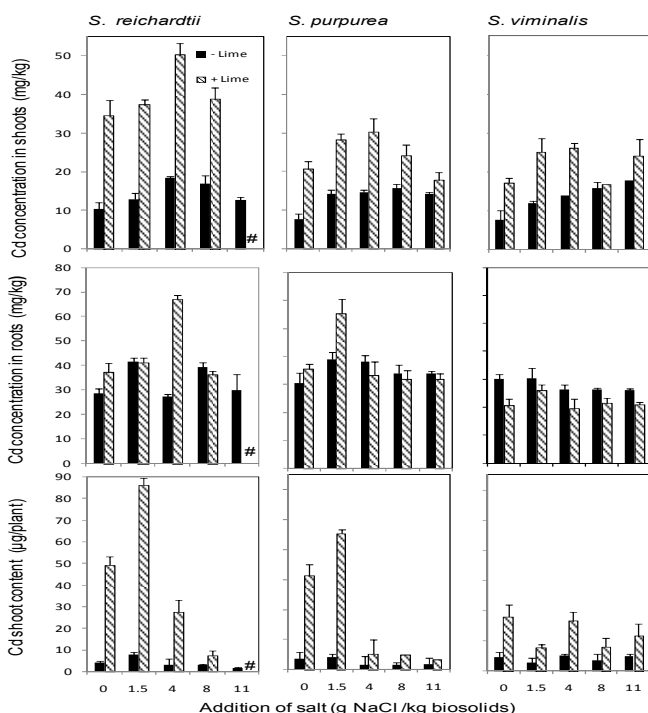


Figure 2. Concentration of Cd in the shoots and roots and total Cd content in the shoots of each *Salix* species after 70 days growth in biosolids with various salt treatments with (+ lime 8 g/kg) and without lime (-lime 0 g/kg). The bars indicate the standard error of means of 3 replicates. Missing data are indicated by “#”.

Cadmium translocation

Salix grown on limed biosolids had significantly greater translocation of Cd from the roots to the shoots, as shown in Figure 3. The translocation rates of Cd ranged from 29 to 135%. *Salix viminalis* had significantly greater translocation of Cd compared to the other *Salix* species, particularly in limed biosolids with high salt levels. Both salt and lime additions were shown to increase Cd translocation.

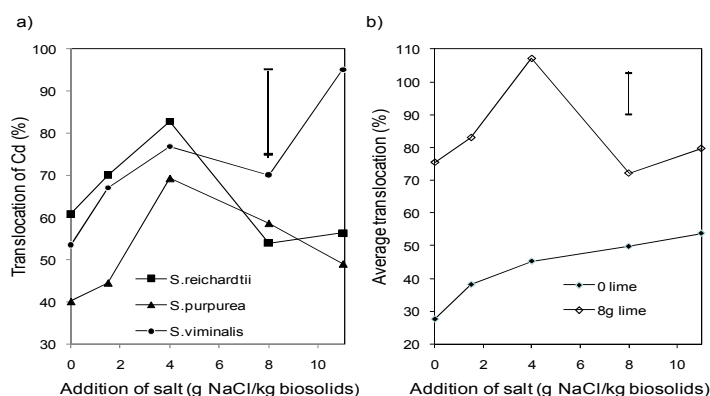


Figure 3. a) Average translocation of Cd in *S. reichardtii*, *S. purpurea* and *S. viminalis* with increasing salt additions. b) Average translocation of Cd from the roots to the shoots of *Salix* plants when grown with lime (lime 8 g lime) and without lime (0 lime), with increasing salt additions. The bars represent the L.S.D of the means.

Discussion

Salix species varied in their capacity to tolerate increasing salinity and accumulate Cd. *Salix viminalis* had the greatest shoot biomass at high salt levels. Correspondingly, *S. viminalis* had the greatest Cd accumulation in the most saline biosolids. *Salix reichardtii* had the highest concentrations of Cd when grown in biosolids with low salt additions. *Salix viminalis* showed greater selectivity for K accumulation than other *Salix* species and consequently had the lowest Na/K concentration ratios in its shoots (Dannatt, unpublished), indicating its tolerance to salinity (Schachtman and Liu 1999).

Liming significantly reduced salt stress in all *Salix* species. Lime reduced Na concentrations in plant shoots by more than 50 % (unpublished). This was most likely due to increased Ca availability in the root zone of limed plants. In addition, lime may have alleviated salt stress indirectly by amelioration of biosolid acidity

and increased nutrient availability. The alleviation of simultaneous environmental stresses, such as acidity, metal toxicity and salinity by lime remain largely unknown.

Liming enhanced Cd uptake in *Salix* but did not significantly decrease the DTPA-extractable Cd in the biosolids (Dannatt unpublished). These results are contradictory to the results of most studies, which suggest that liming decreases Cd bioavailability and thus plant uptake (Singh *et al.* 1995). There are several mechanisms that may have caused the observed increases in Cd concentrations found in limed *Salix*: (i) liming ameliorated biosolid acidity and hence increased shoot biomass and root function; (ii) liming reduced competition of other metal elements for plant uptake; (iii) addition of Ca increased Cd bioavailability and/or (iv) liming increased translocation of Cd due to increased transpiration and improved root function.

Increasing salinity increased Cd accumulation in *Salix* shoots. It is likely that increases in salinity elevated Cd translocation due to the formation of chloro-complexes with Cd, resulting in heightened Cd solubility, increased uptake of Cd in the form of chloro-complexes and/or increased transport of Cd within the root apoplasts, in the presence of Cl (Helal *et al.* 1999). In addition, lime increased plant vitality and transpiration, augmenting Cd translocation. Further research on the influence of Cl on Cd translocation is required, and holds potential for increasing the efficiency of Cd phytoremediation methods.

Conclusion

Liming has great potential at increasing the efficiency of Cd phytoextraction by *Salix* crops. Amendment of biosolids with lime, at a rate of 8 g/kg, increased *Salix* growth by 4 fold and increased total Cd content by up to 12 times. Liming also increased Cd translocation from plant roots to shoots, probably due to increased transpiration. The use of saline water to irrigate phytoextraction crops can further increase Cd phytoextraction as a result of increased Cd bioavailability and transport within plant tissues. However, *Salix* species have limited tolerance to salinity. Further research is required to elucidate the influence of lime on *Salix* growth in field conditions and to determine if phytoextraction of other contaminants such as Zn and Cu is also enhanced in these environments.

Acknowledgement

We thank Dr Scott Laidlaw and Prof Alan Baker for helpful discussion and providing *Salix* cuttings.

References

- Assunção AGL, da Costa Martins C, de Folter S, Vooijs R, Schat H, Aarts MGM (2001) Elevated expression of metal transporter genes in three accessions of the metal hyperaccumulator *Thlaspi caerulescens*. *Plant, Cell and Environment* **24**, 217-226.
- Dannatt H (2008) 'Interactive effects of salinity and lime on microbial activity and the phytoextraction of cadmium by *Salix* species in biosolids' Honours Thesis (Latrobe University: Melbourne).
- Ghnaya T, Slama I, Messedi D, Grignon C, Ghorbel M, Abdelly C (2007) Cd-induced growth reduction in the halophyte *Sesuvium portulacastrum* is significantly improved by NaCl. *Journal of Plant Research* **120**, 309-316.
- Helal HM, Upenov A, Issa GJ (1999) Growth and uptake of Cd and Zn by *Leucaena leucocephala* in reclaimed soils as affected by NaCl salinity. *Journal of Plant Nutrition and Soil Science* **162**, 589-592.
- Laidlaw WS, Baker AJM, Gregory D, Huynh TTT, Popa K, Lamonte C (2007) Phytoremediation of biosolids and soils contaminated with heavy metals at the Western Treatment Plant, Werribee. Progress Report 3 (Research and Technology Division, Melbourne Water cooperation).
- Punshon T, Dickinson NM (1997) Mobilization of heavy metals using short rotation coppice. *Aspects of Applied Biology* **49**, 285-292.
- Schachtman D, Liu W (1999) Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants. *Trends in Plant Science Reviews* **4**, 281-287.
- Singh BR, Narwal RP, Jeng AS, Almas A (1995) Crop uptake and extractability of cadmium in soils naturally high in metals at different pH levels. *Communications in Soil Science and Plant Analysis* **26**, 2123-2142.

Kinetics of cadmium desorption from some soils of Iran

Maryam Esfandbod^A, Ebrahim Adhami^B, Mehran Rezaei Rashti^C and Malahat Esfandbod^D

^ADepartment of Soil Science, University of Guilan, Rasht, Iran, Email maryam.esfandbod@gmail.com

^BFaculty of Soil Science, University of Yasuj, Yasuj, Iran, Email eadhami@mail.yu.ac.ir

^CDepartment of Soil Science, University of Guilan, Rasht, Iran, Email mehran_325@yahoo.com

^DPublichealth School, Medical university of Shahid Beheshti, Tehran, Iran, Email malahatesfandbod@yahoo.com

Abstract

Cadmium (Cd) is highly toxic and therefore represents a potential threat to the environment and human health. To improve predictions of the toxicity and threat from Cd contaminated soil, knowledge of time-dependent desorption behavior of these soils is crucial. Kinetics of Cd desorption from 15 Cd-spiked surface soil samples (0-30 cm) from the northern part of Iran with a wide range of physical and chemical properties, were studied. Treatments consisted of two levels of Cd (15 and 30 mg/kg as CdCl₂) with two replications. Samples were incubated under 20% w/w moisture content and 25±°C. After 3 and 6 weeks, Cd desorption pattern was investigated by DTPA (Diethylene-Triamine-Pentaacetic-Acid) extraction with shaking times from 30 to 960 min. Results showed that two-constant-rate, parabolic-diffusion and simple Elovich equations were the best fitted equations among five kinetic models used. The coefficients of kinetic equations had significant correlation with each other. Stepwise multiple regression equations indicated that pH and CEC (cation exchange capacity) were the most important soil characteristics for predicting the rate constants of the kinetic models.

Key Words

Cadmium, desorption-kinetic, two-constant, parabolic-diffusion, simple Elovich.

Introduction

Of the heavy metals often found contaminating soil and ground water, cadmium (Cd) is both readily available and highly toxic. Cadmium pollution of the environment has also increased in recent years due to an increase in its use (Alloway 1990). Cadmium (Cd) bioavailability, and hence, potential toxicity depends on its concentration in the soil solution and on the ability of soils to release it from the solid phase to replenish those removed from solution by plants (Backes *et al.* 1995). Although desorption experiments are simple extensions of adsorption experiments, there are relatively few publications have examined Cd desorption, and even fewer that have measured Cd desorption kinetics (Tran *et al.* 2002). Therefore, the objective of the present study were (1) to evaluate different kinetic models in describing Cd desorption from some selected soils with a wide range of soil physicochemical properties and (2) to investigate factors influencing desorption of Cd.

Methods

Fifteen surface soil samples (0-30 cm), with a wide range of soil physicochemical properties, were collected from Guilan province in northern part of Iran (Table 1). A laboratory experiment was designed with two levels of Cd (15 or 30 mg/kg as CdCl₂) with two replications. Cadmium was added to 200 g of each soil sample in polyethelene pots. The pots were kept at 25±2°C, and soil moisture was maintained about 20 % w/w. After three and six weeks, 10 g of each soil sample was extracted with 20ml of DTPA extractant (Lindsay and Norvell 1978) for periods of 30, 60, 120, 240, 480 and 960 min at room temperature. Desorbed Cd concentration was determined by atomic absorption spectrophotometry. Kinetic equations including, Zero Order (Dang *et al.* 1994), First Order (Kue and Lotse 1973), Parabolic Diffusion (Khater and Zaghoul 2002), Two Constant Rate (Dang *et al.* 1994) and Simple Elovich (Polyzopoulos *et al.* 1986) were used in soil desorption studies. A relatively high coefficient of determination (R²) and low standard error (SE) were used as criteria for the best fit. The relationships between desorption coefficients and soil properties were investigated by correlation and regression analysis using Excel and SPSS software.

Table 1. Mean and range of physicochemical characteristics of the soils.

| | Clay (%) | pH 1:2.5 (soil:CaCl ₂ 0.01M) | OM (%) | CCE (%) | Fe _o (mg/kg) | CEC (cmol _c /kg) |
|------|----------|---|--------|---------|-------------------------|-----------------------------|
| Min. | 8.00 | 4.21 | 1.53 | 1.59 | 582 | 14.18 |
| Max. | 52.00 | 7.61 | 9.77 | 16.51 | 9348 | 51.94 |
| Ave. | 32.00 | 6.65 | 4.91 | 7.78 | 4833 | 35.00 |

Equations

Desorption data were fitted to Zero order, First order, Parabolic diffusion, Two constant rate and Simple Elovich models.

$$\text{Zero Order} \quad q_t = q_0 - k_0 t \quad (1)$$

$$\text{First Order} \quad \ln q_t = \ln q_0 - k_1 t \quad (2)$$

$$\text{Parabolic Diffusion} \quad q_t = q_0 + k_p t^{0.5} \quad (3)$$

$$\text{Two Constant Rate} \quad q_t = a t^b \quad (4)$$

$$\text{Simple Elovich} \quad q_t = 1 / \beta_s \ln \alpha_s \beta_s + 1 / \beta_s \ln t \quad (5)$$

Where q_t and q_0 are the amounts of Cd desorbed by DTPA (mg/kg) after t (s) period of extraction and at $t = 0$, respectively, k_0 is Zero Order rate constant (mg Cd/kg/s), k_1 is First Order rate constant (s^{-1}), k_p is diffusion rate constant (mg Cd/kg)^{-0.5}, a is initial Cd desorption rate constant (mg Cd/kg/s)^b, b is Cd desorption rate coefficient (mg Cd/kg)⁻¹, α_s is initial Cd desorption rate (mg Cd/kg/s), β_s is Cd desorption constant (mgCd/kg)⁻¹.

Results

Results of the statistical analysis showed that Parabolic Diffusion ($R^2_{\text{mean}}=0.81$), Two Constant Rate ($R^2_{\text{mean}}=0.88$), and simple Elovich ($R^2_{\text{mean}}=0.93$) predicted the pattern of the Cd desorption from treated soils better than the other kinetic equations (Table 2). The range of these kinetic model coefficients are shown in Table 2.

Table 2. Mean and range of values of the rate constants for the kinetic models that best described Cd desorption from soils

| | Parabolic diffusion | Two constant rate | | Simple Elovich | |
|------|---|--|-----------------|---|-------------------------|
| | ($k_p \times 10^3$) (mg Cd/kg ^{s-0.5}) | a (mg Cd/kg/s) ^b /(mg Cd/kg) | b (mg Cd/kg) | $\alpha_s \times 10^3$ (mg Cd/kg/s)/(mg Cd/kg) | β_s (mg Cd/kg) |
| Min. | 10.85 | 0.053 | 0.066 | 1.33 | 0.25 |
| Max. | 64.92 | 8.505 | 0.413 | 1157.40 | 1.48 |
| Ave. | 31.10 | 2.187 | 0.225 | 226.90 | 0.67 |

A plot of the rate of Cd desorption vs. $t^{0.5}$ (linear form of parabolic diffusion equation) showed that the initial desorption of Cd from these soils was a rapid process (Figure 1). Most of the desorbable Cd desorbed within 2h, although limited Cd desorption was still ongoing after 16h. For instance, the pattern of Cd desorption based on linear form of Parabolic Diffusion equation and the average rates of Cd desorption of the soils after 3 and 6 weeks with two levels of Cd (15 or 30 mg Cd/kg soil), showed a discontinuity in slope at 2h in most soils (Figure 1). The findings of Dang *et al.* (1994) on the studies of kinetics of zinc desorption, revealed that the most desorption of zinc has occurred within 4h. The trend proposed that two different mechanisms are controlling the rate of Cd desorption. Film diffusion was minimized by continuous shaking during the experiment (Sparks *et al.* 1980), and therefore it is likely that at least two particle diffusion mechanisms were involved (Sivasubramaniam and Talibudeen 1972). Probably in the first 2h Cd desorption is occurred from macroaggregates or outer surfaces microaggregates. The low desorbability of Cd may be due to entrapment of Cd in the imperfections/cracks in the structure of soil particles or due to specific adsorption on high energy sites on soil. If the former is true, the desorbability of Cd should decrease with increased time of Cd reaction with soil. This is consistent with the Cd diffusion model of Brummer *et al.* (1988); initially, Cd complexes with DTPA, desorbs from external surfaces of adsorbents, and diffuses into soil solution then, Cd from internal binding sites diffuses to external surfaces, or complexes with DTPA and diffuses into soil solution.

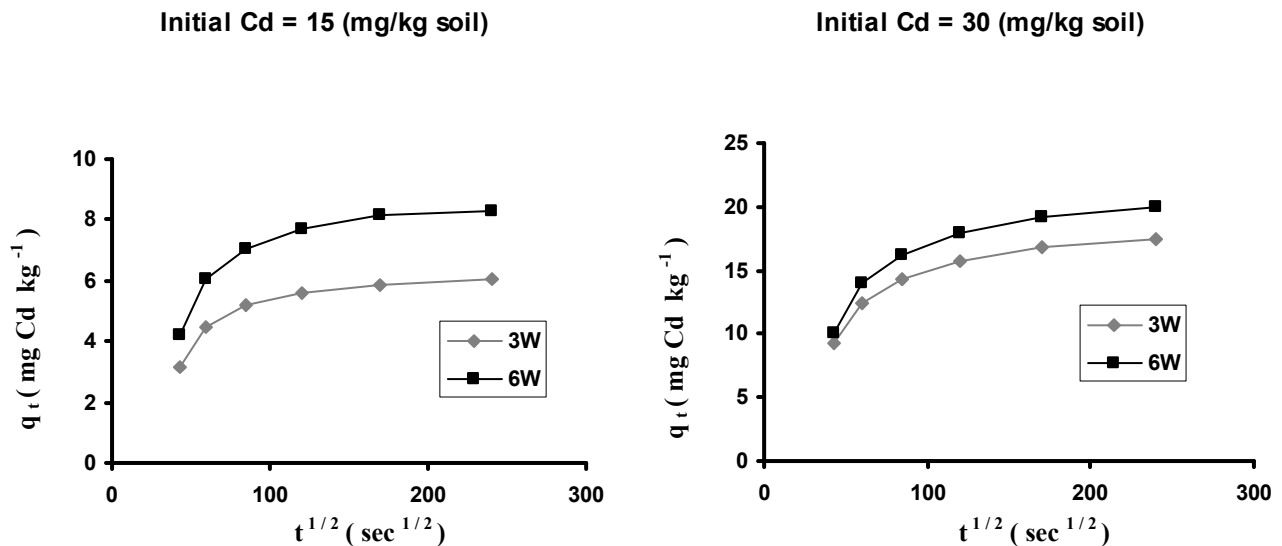


Figure 1. Kinetics of initial Cd desorption (15 or 30 mg Cd/kg oil) by DTPA from the average rates of Cd desorption of the soils after 3 and 6 weeks as described by linear form of Parabolic Diffusion equation.

The constant rates of these different kinetic models had significant correlation with each other (Table 3).

Table 3. Correlation coefficient (r) between constant rates of kinetic models

| Constant rates | Constant rates | | | |
|-----------------------------|-------------------------------|--------------------------------|--------------------------|--------------------------|
| | β_s (Simple Elovich) | α_s (Simple Elovich) | b (Two constant rate) | a (Two constant rate) |
| k_p (Paraboli Diffusion) | -0.834** | NS | NS | NS |
| a (Tow constant rate) | NS | 0.780** | -0.608* | |
| b (Tow constant rate) | NS | -0.556* | | |
| α_s (Simple Elovich) | NS | | | |

* and ** significant at $p \leq 0.05$, and $p \leq 0.01$, respectively.

NS : not significant

The negative sign of a correlation coefficient between β_s , value of the Simple Elovich equation that relates to the mount of Cd desorption, and k_p , value of the Parabolic Diffusion equation that relates to the mount of Cd diffusion, indicated that the rate of Cd diffusion increased as the value of β_s decreased, which confirm the findings of pavlatou and polyzopoulos (Pavlatou and Polyzopoulos 1988). There had a significant negative correlation between a. and b, coefficient of Two Constant Rate equation. Dang *et al.* (1994) proposed that an increase in the value of a, and a decrease in the value of b in Two Constant Rate equation probably indicates an increase in the rate of desorption from soils.

Conclusion

This study showed that Cd desorption was characterized by a rapid initial desorption, which lasted for about 2h, followed by a slower desorption, which was still proceeding after 960 min (16h). The trend well describes the relatively high ability of the DTPA soil test in predicting Cd availability in these soils. The Cd desorption by DTPA from these soils was adequately described by the Parabolic Diffusion, the Two Constant Rate, and the Simple Elovich. Moreover, the constant rates of these kinetic models have shown significant correlation with each other.

References

- Alloway BJ (1990) Cadmium, In 'Heavy metals in soils'. (Eds BJ Alloway) p. 100-24. (Haltsland Publishing: New York)
- Backes CA, McLaren RG, Rate AW, Swift RS (1995) Kinetics of cadmium and cobalt desorption from iron and manganese oxides. *Soil Sci. Soc. Am. J.* **59**, 778-785.

- Bruemmer GW, Gerth J, Tiller KG (1988) Reaction kinetics of the adsorption and desorption of nickel, zinc and cadmium by goethite. I: Adsorption and diffusion of metals. *J. Soil Sci.* **39**, 37-52.
- Dang YP, Dalal DG, Edwards DG, Tiller KG (1994) Kinetics of zinc desorption from vertisols. *Soil Sci. Soc. Am. J.* **58**, 1392-1399.
- Khater AH, Zaghoul AM (2002) Copper and zinc desorption kinetics from soil: Effect of pH. In ' the 17th World Conference on Soil Science, Thailand ', (Symposium No. 47, Paper No. August 2001, 1-9).
- Kue S, Lotse EG (1973) Kinetics of phosphate adsorption and desorption by hematite and gibbsite. *Soil Science* **116**, 400-406.
- Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal* **48**, 769-772
- Pavalatou A, Polyzopoulos NA (1988) The role of diffusion in the kinetics of phosphate desorption: The relevance of the Elovich equation. *J. Soil Sci.* **39**, 425-436.
- Polyzopoulos NA, Keramidas VZ, Pavlatou A (1986) On the limitation of the simplified Elovich equation in describing the kinetics of phosphate sorption and release from soils. *Journal of Soil Science* **37**, 81-87.
- Sivasubramaniam S, Talibudeen O (1972) Potassium-aluminium exchange in acid soils. I: Kinetics. *J. Soil Sci.* **23**, 163-176.
- Sparks DL, Zelazny LW, Martens DC (1980) Kinetics of potassium exchange in a Paleudult from the Coastal Plain of Virginia. *Soil Sci. Soc. Am. J.* **44**, 37-40.
- Tran YT, Barry DA, Bajracharya K (2002) Cadmium desorption in sand. *Environmental International* **28**, 493-502.

Leaching and phytoavailability of zinc and cadmium in a contaminated soil treated with zero-valent iron

David Houben and Philippe Sonnet

Earth and Life Institute – Environmental Sciences, Université catholique de Louvain (UCL), Belgium,
Email David.Houben@uclouvain.be

Abstract

Immobilization of heavy metals by stabilization amendments is a promising method to restore contaminated soils. In our study, we investigated the efficiency of zero-valent iron (Fe^0) added to the soil in the form of iron grit to reduce leaching and uptake of Zn and Cd by *Lupinus albus* L. Results of column leaching experiments show that metal leaching decreased proportionally to the rate of amendment application (1%, 2% or 5%: w/w) and that the reduction reached up to 98% and 83% for Zn and Cd respectively. An increase in pH and in the number of sorption sites which bind ionic free metals and organometal complexes are possible mechanisms for this attenuation. Moreover, a rhizobox experiment has demonstrated that phytoavailability of Zn and Cd was reduced by 63% and 45% respectively when soil was treated with 5% (w/w) iron grit, and that *L. albus* did not develop strategies to reduce Zn and Cd uptake in untreated soil. We conclude that covering contaminated soil with *L. albus* could be used in association with immobilization techniques for soil remediation. However, it is crucial that the amendment and the soil be thoroughly homogenized in order to ensure the maximum reduction of metal uptake.

Key Words

Heavy metals, metal immobilization, stabilization amendments, mobility; *Lupinus albus*, iron grit.

Introduction

In Belgium, the non-ferrous metallurgical industry has caused severe contamination of soils by heavy metals. Since the beginning of the Industrial Revolution, metal refining plants using pyrometallurgical processes were responsible for significant atmospheric emissions of metals such as Zn and Cd, which have affected soils in widespread areas. Due to the large size of the contaminated areas, classical remediation techniques (e.g. excavation) are not appropriate because of the prohibitive cost. It is thus crucial to find cheaper alternatives. Among these technologies, *in situ* stabilization, having low environmental impact and thus better accepted by the public, is one of the most promising (Guo *et al.* 2006). This technique consists in reducing the risks of groundwater contamination, plant uptake, and exposure of other living organisms by immobilizing metals (Boisson *et al.* 1999). This objective can be reached by the application of contaminant immobilizing additives to the soil (Kumpiene *et al.* 2008). Due to its oxidation by corrosion (Dries *et al.* 2005) which slightly modifies the pH and offers new surfaces for sorption of both cations and anions (Cornell and Schwertmann 2003), zero-valent iron (Fe^0) could be an effective stabilizing amendment. Compared with other Fe containing compounds, Fe^0 also offers the advantage of being available in large quantities (Kumpiene *et al.* 2007) as industrial by-products.

In addition to the immobilization of heavy metals, covering soil with suitable plants has proven helpful in preventing the dispersion of the contaminant through erosion (Ruttens *et al.* 2006). *Lupinus albus* L. (white lupin), a nitrogen-fixing (i.e. fertility-improving) plant, adaptable to poor acid soils and tolerant to nitrate and lime excess, high salinity and high heavy metal contents in soils, appears to be an excellent candidate combined with heavy metal immobilization by amendments (*cfr* authors cited by Castaldi *et al.* 2005).

This study aims at assessing the efficiency of Fe^0 in reducing mobility and phytoavailability of Zn and Cd in soil contaminated by heavy atmospheric metal fallouts. Emphasis will be put on the physico-chemical processes responsible for the immobilization of heavy metals and on the feasibility of using white lupin in order to cover metal stabilized soils.

Materials and methods

Soil sampling and soil treatment

Organic horizon of a shale soil (pH=4.8; CEC=38.0 cml./kg; organic carbon=38.5%) contaminated by atmospheric metal fallouts was collected in Prayon (Belgium). Zn and Cd total contents were 7400 mg/kg

and 150 mg/kg respectively. The sample was air-dried, passed through a 2 mm nylon sieve and then stored at 4°C before analysis. The Fe⁰ source, referred to here as iron grit, was in the form of iron filings produced by machining tools in a machine shop. Sub-samples of soil were treated with iron grit as follows (%w/w): untreated polluted soil (UNT); iron grit 1% (IG1); iron grit 2% (IG2); iron grit 5% (IG5). Mixtures were prepared by 2 hours of thorough agitation just before use.

Leaching column design

Leaching column experiments were carried out to investigate the effect of the zero-valent iron amendment in soil on the mobility of Zn and Cd. Each PVC column (8 cm diameter, 10 cm height) was filled with 50.0 g of UNT, IG1, IG2 or IG5. The complete design is shown in Figure 1a. Control experiments, consisting of columns containing only sand, were also conducted. All the experiments were carried out in triplicate. Deionized water input, based on the average annual rainfall recorded for Prayon (731 mm), was 10 ml four times a week during 12 weeks. The water input began one week after equilibrating the substrates at 80% of the water holding capacity (WHC). Sampling was performed every 14 days, which was the time required to reach a sufficient volume to allow for pH analysis, conductivity, anions, cations and DOC. Aqueous speciation using the Stockholm Humic Model (SHM) was performed using Visual MINTEQ (version 2.61).

Rhizobox experiments

Rhizobox growth chambers were based on the design of Whiting *et al.* (2000) and constructed from 10 x 10 x 2 cm square Petri dishes. A large opening at the top makes it possible for the plant stem to emerge and facilitates the watering while a 1.5 cm sand layer at the bottom prevents water stagnation in the soil (Figure 1b1). When filling the Petri dishes with soil, a cardboard strip was temporarily inserted into the dish to divide the box into two equal left and right compartments. Homogeneous treatments were obtained by filling both compartments with the same substrate (UNT or IG5) while heterogeneous treatments were obtained by filling one side with UNT and the other side with IG5 (Figure 1b2). The cardboard strip was then removed, the lid was fixed over the base with silicon and each rhizobox was wrapped in a black plastic sheet to avoid exposing the roots to light. Finally, the rhizoboxes were placed at an angle of 50° on an inclined support with the lid on the underside to ensure root growth close to the lid. The substrates in each rhizobox were equilibrated during one week by maintaining the WHC at 80%. A 7-day-old seedling of *Lupinus albus* was then transplanted in each rhizobox taking care that the roots were well positioned on the central line where the cardboard strip had been previously placed. All experiments were conducted in triplicate. The plant growth was carried out in a phytotron (16 h at 20°C with light; 8 h at 15°C without light) during 28 days and the plants were watered regularly. The shoots were then harvested, dried (60°C) and their Zn and Cd contents were determined by ICP-AES after *aqua regia* digestion.

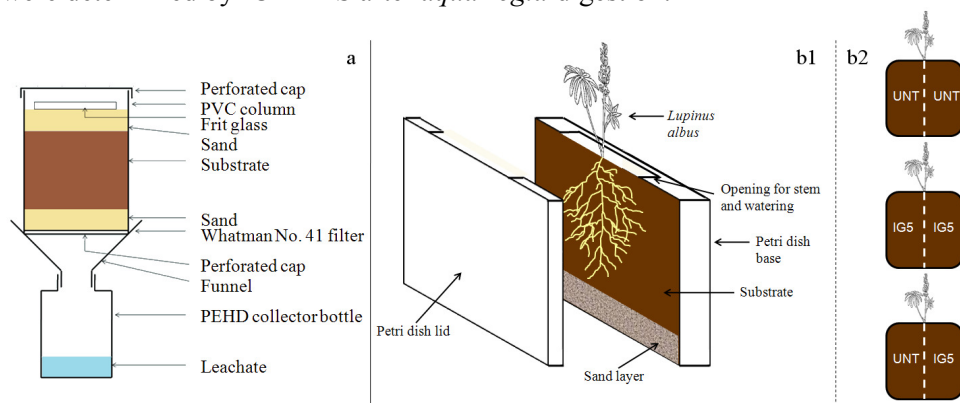


Figure 1. Design of the leaching column (a) and rhizobox (b1) and experimental designs (b2).

Results and discussion

Effects of zero-valent iron amendment on Zn and Cd leaching

Figure 2 shows a clear decrease of Zn and Cd leaching in iron grit amended soils. At the end of the experiment, the total amount of leached Zn (mg) was reduced by up to 73%, 87% and 98% in the leachate from IG1, IG2 and IG5 respectively while the total of leached Cd (µg) was reduced by up to 48%, 66% and 83% in the leachate from IG1, IG2 and IG5 respectively. Zn and Cd leaching attenuation by zero-valent iron amendment is thus very effective and is slightly more efficient for Zn. In addition, this decrease in leaching is a function of the proportion of iron grit in soils and occurs immediately, as is it observed even in the first leachate samples. This can be explained by the higher number of sorption sites in the amended soil and the fast sorption of metals to oxide surface (equilibrium time ranging from 24 to 72 h; Smith 1996).

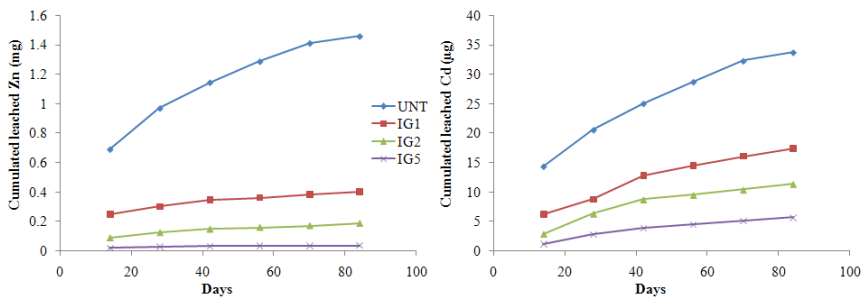


Figure 2. Cumulated quantity of Zn (mg) and Cd (µg) in column leachates.

Moreover, Figure 3 shows a strong correlation between Zn and Cd concentrations and pH ($R^2=0.82$ and $R^2=0.75$ respectively). As an oxidation phenomenon, iron grit increases the pH. As a consequence, metals are immobilized because of (i) the higher number of negative sites for cation sorption and (ii) the higher proportion of hydrolysed metals species which are preferentially adsorbed compared to the free ionic metals (Cappuyns and Swennen 2008). As shown by Kumpiene *et al.* (2007), pH is thus one of the most significant factors affecting the mobility of heavy metals in Fe^0 -stabilized soil.

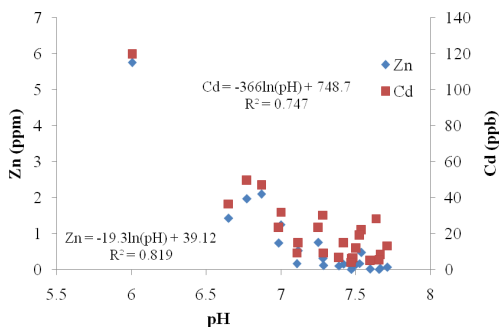


Figure 3. Correlations between Zn or Cd concentrations (ppm and ppb respectively) and pH in leachates.

In addition to increased ionic free metal sorption, the quantity of organometal complexes in soil solution is considerably diminished by the addition of iron grit as shown by the average Zn and Cd speciations (Figure 4). Consequently, as described by Davis and Bhatnagar (1995) and Jones and Brassington (1998), in iron enriched systems, metal immobilization can also be caused by the adsorption of organometal complexes on iron oxides. Since organometal complexes are known to enhance Zn and Cd transport (Schwab *et al.* 2008), their adsorption also contributes in reducing the leaching of these metals.

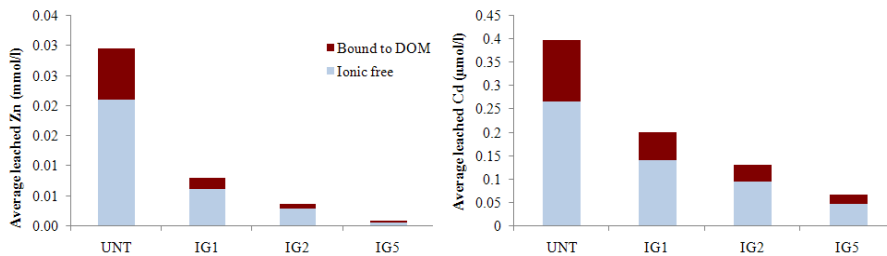


Figure 4. Speciation of average Zn and Cd concentrations ($mmol\ l^{-1}$ and $\mu mol\ l^{-1}$ respectively) in leachates.

Effects of zero-valent iron amendment on Zn and Cd phytoavailability

Table 1 shows (i) a decrease of 33% and 63% in the Zn uptake by *Lupinus albus* in UNT/IG5 and IG5/IG5 respectively and (ii) a decrease of 26% and 45% in the Cd uptake in UNT/IG5 and IG5/IG5 respectively. Similar to what was observed in the column experiments, the reduction of Zn and Cd transfers to plant by amendment of Fe^0 is very effective and proves to be the highest for Zn. Moreover, the Zn and Cd contents in plants grown using the UNT/IG5 treatment indicate that the white lupin has not only taken up Zn and Cd from the amended compartment but also from the untreated side. This suggests that, within the time span of the experiment (28 days), *Lupinus albus* did not develop strategies to avoid metal uptake from the untreated soil. This absence of adaptation is probably due to a tolerance to heavy metals. This indicates that *Lupinus albus*, because of its tolerance to high Zn and Cd concentrations, could be used in combination with heavy metal immobilization techniques. However, the amendment and the soil must be thoroughly homogenized in order to obtain the highest reduction of metal uptake.

Table 1. Zn and Cd contents (mg/kg) in *Lupinus albus* grown in treated and untreated soils.

| Rhizobox | Zn content (mg/kg) | Cd content (mg/kg) |
|----------|-----------------------|-----------------------|
| UNT/UNT | 1223.4 ± 92.8 | 14.8 ± 1.7 |
| UNT/IG5 | 818.9 ± 139.5 | 10.9 ± 1.5 |
| IG5/IG5 | 460.4 ± 93.8 | 8.1 ± 1.5 |

Conclusion

As a by-product of industrial processes, zero-valent iron in the form of iron grit was found to be a promising additive to immobilize heavy metals in soils. This study has shown that the amendment of iron grit in soil was very effective in reducing Zn and Cd leaching (up to 98% and 83% respectively). Likely mechanisms are, among others, a pH increase and the sorption of ionic free metals and organometal complexes. This amendment also reduces the uptake of Zn and Cd (up to 63% and 45% respectively) by *Lupinus albus* plants. However, the iron grit and soil must be perfectly homogenized in order to obtain the most efficient uptake reduction. In order to elucidate with more precision the possible mechanisms responsible for causing the decrease in Zn and Cd transfer, we have planned to analyse treated soil and iron grit with physical techniques (e.g. SEM-EDS, EPMA and X-ray diffraction) in the upcoming months.

References

- Boisson J, Ruttens A, Mench M, Vangronsveld J (1999) Evaluation of hydroxyapatite as a metal immobilizing soil additive for the remediation of polluted soils. Part 1. Influence of hydroxyapatite on metal exchangeability in soil, plant growth and plant metal accumulation. *Environmental Pollution* **104**, 225-233.
- Cappuyns V, Swennen R (2008) The use of leaching tests to study the potential mobilization of heavy metals from soils and sediments: A comparison. *Water Air And Soil Pollution* **191**, 95-111.
- Castaldi P, Santona L, Melis P (2005) Heavy metal immobilization by chemical amendments in a polluted soil and influence on white lupin growth. *Chemosphere* **60**, 365-371.
- Cornell RM, Schwertmann U (2003). The Iron Oxides: Structure, Properties, Reactions, Occurrences and Uses. 2nd Edition. (Wiley-VCH; Weinheim).
- Davis AP, Bhatnagar V (1995) Adsorption of cadmium and humic acid onto hematite. *Chemosphere* **30**, 243-256
- Dries J, Bastiaens L, Springael D, Kuypers S, Agathos SN, Diels L (2005) Effect of humic acids on heavy metal removal by zero-valent iron in batch and continuous flow column systems. *Water Research* **39**, 3531-3540.
- Guo GL, Zhou QX, Ma LQ (2006) Availability and assessment of fixing additives for the in situ remediation of heavy metal contaminated soils: A review. *Environmental Monitoring and Assessment* **116**, 513-528.
- Jones DL, Brassington DS (1998) Sorption of organic acids in acid soils and its implication in the rhizosphere. *European Journal of Soil science* **49**, 447-455.
- Kumpiene J, Montesinos I, Lagerkvist A, Maurice C (2007) Evaluation of the critical factors controlling stability of chromium, copper, arsenic and zinc in iron-treated soil. *Chemosphere* **67**, 410-417.
- Kumpiene J, Lagerkvist A, Maurice C (2008) Stabilization of As, Cr, Cu, Pb and Zn in soil using amendments - A review. *Waste Management* **28**, 215-225.
- Ruttens A, Mench M, Colpaert J, Boisson J, Carleer R, Vangronsveld J (2006) Phytostabilization of a metal contaminated sandy soil. I: Influence of compost and/or inorganic metal immobilizing soil amendments on phytotoxicity and plant availability of metals. *Environmental Pollution* **144**, 524 – 532.
- Schwab AP, Zhu DS, Banks MK (2008) Influence of organic acids on the transport of heavy metals in soil. *Chemosphere* **72**, 986-994.
- Smith E (1996) Uptake of heavy metals in batch systems by a recycled iron-bearing material. *Water Research* **30**, 2424-2434
- Whiting S, Leake J, McGrath S, Baker A (2000) Positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens* *New Phytologist* **145**, 199-210

Leaching of heavy metals (Cu, Mn, Zn, Ni, Pb and As) after six months application of raw and composted recycled paper mill sludge

A. Rosazlin^A, C. I. Fauziah^A, W. Wan Rasidah^B and A. B. Rosenani^A

^ADepartment of Land Management, Faculty of Agriculture, Universiti Putra Malaysia (UPM), 43400, Serdang, Selangor, Malaysia, Email rosazlin@yahoo.com, fauziah@agri.upm.edu.my, rosenani@agri.upm.edu.my

^BForest Biotechnology Division, Forest Research Institute of Malaysia (FRIM), 52109, Kepong, Selangor, Malaysia, Email rashidah@frim.gov.my

Abstract

Sludge from paper mill with recycled paper feedstock (RPMS) is a waste from secondary process of paper making, generated at final stage of paper production after biological treatment. Land application of RPMS could provide substantial benefit to plant growth and contributes to increase in organic matter that may result in chemical and physical changes in soil. Nevertheless, the potential for groundwater contamination from land application of RPMS is an important concern. Therefore, the objective of this study is to evaluate the cumulative amount of heavy metals (Cu, Mn, Zn, Ni, Pb and As) leached at 15, 30, 60 and 120 cm depths after six months application of raw and composted RPMS. The cumulative amount of Cu, Mn, Zn, Ni, Pb and As leached after six months application of raw and composted RPMS ranged from 7.9 to 72 µg, 8.6 to 212 µg, 17.9 to 462 µg, 1.2 to 18.4 µg, 0.4 to 3.5 µg and 0.05 to 2.4 µg, respectively. After 6 months of application of raw and composted RPMS, the trend of heavy metals leached seemed to increase with increasing depth (15, 30, 60 and 120 cm) for every treatment. The general trend was that only very small fractions (below 1%) of Cu, Mn, Zn, Ni, Pb and As leached relative to the total amount added with the raw and composted RPMS after six months of experiment. The percentage and cumulative amounts of heavy metals leached were very low; possibly maybe the sludge was not fully mineralized within the duration of this experiment.

Key Words

Leaching, heavy metals, recycled paper mill sludge, cumulative.

Introduction

Utilization of organic by-products as soil amendments in agricultural production exemplifies a strategy for converting wastes to resources. At present, Malaysia generates about 1,000,000 metric tons annually of recycled paper mill sludge and the quantity that needs to be disposed of is accumulating every year. Some of the wastes from the paper manufacturing mill are categorized under hazardous toxic waste by the Department of Environment, Malaysia, due to which, paper mill sludge is not well utilized in Malaysia. Paper manufacturing mills opted for land fill disposal, which might not be viable in the long run as land cost is becoming expensive. Recycled paper mill sludge is an active organic material and has potential benefits to supply nutrients for crop growth. The trial showed encouraging plant growth and demonstrated the effect of sludge in improving soil fertility and nutrient properties, as well as soil physical properties (Bellamy *et al.* 1995; Phillips *et al.* 1997; Ritter *et al.* 1992). However, if excessive pollutants are introduced by application of low quality sludge, the practice may have an adverse effect on soil and groundwater quality, and lead to contamination of the food chain. Direct utilization of raw RPMS for plant growth needs close monitoring. In this study, RPMS were turned into compost for converting these chemically complex materials into useful soil amendments. The objective of this study was to evaluate the cumulative amount of heavy metals (Cu, Mn, Zn, Ni, Pb and As) leached at 15, 30, 60 and 120 cm depths after six months application of raw and composted RPMS.

Methods

Experimental design

The field study was located at University Agriculture Park, Puchong, Selangor, Malaysia. Twelve plots (10m × 6 m) were established and each plot consists of 9 plants of *Khaya senegalensis* seedlings planted at 4m × 3m distance with 3 treatments and 4 replications arranged in a completely randomized block design (CRBD). *Khaya senegalensis* is a forest tree identified under the 9th Malaysian Plan as one of the potential timber species for large scale forest plantation. Composted and raw RPMS were air dried and passed through a 4.7 mm sieve and applied around the tree within 50 cm radius. Treatments established were control, 0.7 t/ha raw RPMS and 1.3 t/ha composted RPMS.

Soil, raw and composted recycled paper mill sludge

Raw RPMS from the biological treatment pond was collected from the United Paper Mill in Selangor, Malaysia. Raw RPMS mixed with EFB fibres at 1:1 ratio (v/v) was used in this study as composted RPMS was found suitable for land application (Rosazlin *et al.* 2009). pH and mean concentrations of heavy metals in the soil, composted and raw RPMS used in this experiments are shown in Table 1.

Table 1. pH and mean concentrations of heavy metals in the soil, composted and raw recycled paper mill sludge used in the experiments (on a dry weight basis).

| | pH | Cu (mg/kg) | Mn (mg/kg) | Zn (mg/kg) | Ni (mg/kg) | Pb (mg/kg) | As (mg/kg) |
|----------------|------|---------------|---------------|---------------|---------------|---------------|---------------|
| Soil | 5.06 | 7.3 | 23 | 30 | 5.7 | 7.9 | 35 |
| Composted RPMS | 7.84 | 72 | 342 | 183 | 22 | 89 | 150 |
| Raw RPMS | 7.18 | 88 | 325 | 251 | 26 | 177 | 186 |

Sampling

Leachate solutions were sampled at 15, 30, 60 and 120 cm depth, with 1905 slim tube water sampler (Soil Moisture Equipment Corp., SA). Slim tube water samplers were located at 50 cm radius from the plant. Water samples were collected every week to avoid overflow of water in the tube. On dry season, water samples were combined for following weeks to get enough water samples for analysis. Finally, a total of 11 water samples were collected between July 2009 and January 2010 with total rainfall of 1609mm. Leachate were sampled by application of a vacuum to the sampler at a level of 70 kPa, and then transported to the laboratory and stored in a refrigerator at ≤ 4 °C.

Laboratory Analysis

Water samples were filtered and concentrations of Cu, Mn and Zn in the leachate samples were determined by atomic absorption spectrophotometer (5100 PerkinElmer, USA). Given the low concentration of Ni, Pb and As in the leachate, these elements were measured out using atomic absorption spectrophotometer with graphite chamber (4100 Graphite Perkin Elmer Zeeman).

Results

The cumulative amount of Cu, Mn, Zn, Ni, Pb and As leached after six months application of raw and composted RPMS ranged from 7.9 to 72 μg , 8.6 to 212 μg , 17.9 to 462 μg , 1.2 to 18.4 μg , 0.4 to 3.5 μg and 0.05 to 2.4 μg , respectively (Figure 1). The amounts were very low, maybe the applied sludge was not fully mineralized within duration of this experiment. Generally, quantities of metal leached followed the order of $\text{Zn} > \text{Mn} > \text{Cu} > \text{Ni} > \text{Pb} > \text{As}$.

After 6 months of experiments, the composted RPMS gave the highest cumulative leached amount at 120 cm depth compared raw RPMS. Treatment with raw RPMS shows significantly higher leaching compared to composted RPMS and control in cumulative leached amount of Mn and As except 60 and 30 cm depth, respectively. Application of raw RPMS was significantly different at 60 and 120 cm depth but not significant different at 15 and 30cm in leached Zn. Meanwhile, the cumulative amount of leached Zn is highest compared to other metals. These observations support assertions that there is a soluble or mobile fraction of heavy metals in soil (Sloan *et al.* 1997). The cumulative amount of Ni and Pb after six months application of raw and composted RPMS was significantly higher than control for every depth except at 60 cm depth, despite their differing chemical properties (Table 1). This suggests that soil physical processes may be more important than chemical processes in determining the distribution and mobility of these metals.

After 6 months of application the raw and composted RPMS, trend of heavy metals leaching seemed to increase with increasing depths for every treatments. This observation is supported by Chaney and Ryan (1993) who reported that the specific metal adsorption capacity added to soil by sludge plays a significant role in controlling phytoavailability of metals. Overall, treatment with raw RPMS showed significantly higher heavy metal in the leachate than composted RPMS indicative of more rapid leaching compared composted RPMS and control even the amount of application is half than composted RPMS.

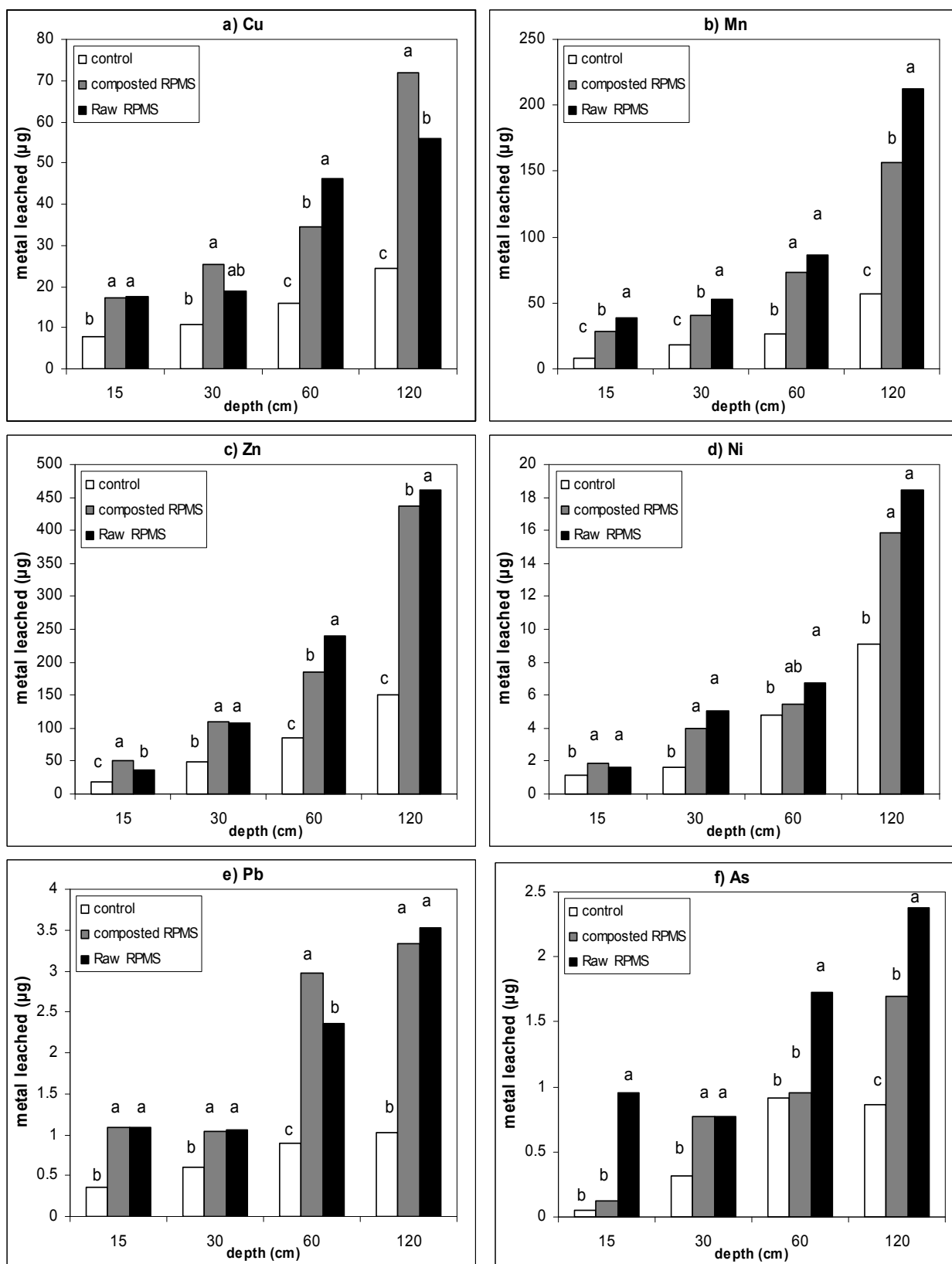


Figure 1. Cumulative amount of a) Cu, b) Mn, c) Zn, d) Ni, e) Pb and f) As leached at different depths after 6 months application of raw and composted recycled paper mill sludge. Similar letter above bar within treatment in each depth indicate the bar are not significantly different at $p < 0.05$, according to the Tukey.

Table 2 shows the percentage of total amounts of leached Cu, Mn, Zn, Ni, Pb and As relative to the corresponding total amount added with the raw and composted recycled paper mill sludge after six months of experiment. There was no significant difference for Cu, Ni, Pb and As in the percentage leached between raw and composted RPMS treatments. Generally, only very small fractions (below 1%) of the total amounts of Cu, Mn, Zn, Ni, Pb and As were leached after six months for the raw and composted RPMS treatments. Six months duration of experiment is not enough to show the trend of percentage loss as water through soil profile. According to Egiarte *et al.* 2008, after 2.5 years study, application of 17 Mg/ha sludge only gave 1.5% and 0.7% of Zn and Pb leached at 50 cm depth.

Table 2. Percentage of total amounts of Cu, Mn, Zn, Ni, Pb and As leached relative to the corresponding total amount added with the raw and composted recycled paper mill sludge after six months of experiment.

| | Cu | Mn | Zn | Ni | Pb | As |
|----------------|---------|---------|---------|---------|---------|----------|
| Composted RPMS | 0.08% a | 0.04% b | 0.20% b | 0.05% a | 0.02 %a | 0.001% a |
| Raw RPMS | 0.16% a | 0.12% a | 0.32% a | 0.10% a | 0.02% a | 0.003% a |

Letter with the same alphabet list in the column are not significant different at $p < 0.05$, according to the T-Test

Conclusion

Application of raw and composted RPMS for six months showed an increase in cumulative amount of leached Cu, Mn, Zn, Ni, Pb and As with increasing depth (15, 30, 60 and 120 cm) for every treatments. Generally, the amount of metal leached followed the order of $Zn > Mn > Cu > Ni > Pb > As$. Only very small fractions (below 1%) of the total amounts of Cu, Mn, Zn, Ni, Pb and As were leached after six months for the raw and composted RPMS treatments.

References

- Bellamy KL, Chong C, Cline RA (1995) Paper sludge utilization in agriculture and container nursery culture. *Journal of Environmental Quality* **24**, 1074-1082.
- Chaney RL, Ryan JA (1993) Heavy metals and toxic organic pollutants in MSW-compost. In 'Science and engineering of composting: Design, environmental, microbiological and utilization aspects. Renaissance Publication (Ed Keener HM). pp. 451-506 (Renaissance Publication: Ohio).
- Egiarte G, Pinto M, Ruiz-Romera E, Camps Arbestain M (2008) Monitoring heavy metal concentrations in leachates from forest soil subjected to repeated applications of sewage sludge. *Environmental Pollution* **156**, 840-848.
- Phillips R, Kirkpatrick K, Scotford IM, White RP, Burton RGO (1997) The use of paper mill sludges on agricultural land. *Bioresource Technology* **60**, 73-80.
- Ritter WF, McDermott JG, Chirside AEM (1992). Use of paper mill residuals as soil amendments. *Communication Soil Science ad Plant Analysis* **23**, 469-488.
- Rosazlin A, Fauziah CI, Wan Rasidah K, Rosenani AB (2009) Characteristics of compost from recycled paper mill sludge mixed with oil palm empty fruit bunches. In 'Proceedings of the Agriculture Congress' (Ed Dzolkifli O), pp. 286-288 (UPM Publication; Malaysia).
- Sloan JJ, Dowdy RH, Dolan MS, Linden DR (1997) Long-term effects of biosolids applications on heavy metal bioavailability in agricultural soils. *Journal of Environmental Quality* **26**, 966-974.

Levels of trace elements in soils on the Apodi-Mossoró River margin in Brazil

Monalisa Gurgel De Medeiros^A, Francisco Nildo Da Silva^B, Glécia Mesquita Freire^C, Humbelina Silva Siqueira Lopes^A and Fábio Henrique Nascimento^A

^AUniversidade Federal Rural do Semi-árido – UFERSA. Br 110 – Km 47/ Presidente Costa e Silva, CEP: 59625-900, Mossoró – RN, Email monalisa_gurgel@hotmail.com, humbelinas@hormail.com, fa_bill05@hotmail.com

^BDepartment of Environmental Sciences, Universidade Federal Rural do Semi-árido – UFERSA. Br 110 – Km 47/ Presidente Costa e Silva, CEP: 59625-900, Mossoró – RN, Email fnildos@yahoo.com

^CUniversidade Federal Rural do Semi-árido - UFERSA. Br 110 – Km 47/ Presidente Costa e Silva, CEP: 59625-900, Mossoró – RN, Email glecia_freyre@yahoo.com.br

Abstract

The study examined the levels of trace elements in soil on the margin of the river Apodi / Mossoró, which has great importance for the state, being the largest river in Rio Grande do Norte. Samples of the soil layer 0-20 cm were taken at 24 points on the river Apodi-Mossoro, distributed along its length from the begin (Luiz Gomes-RN) to the end on the Atlantic Ocean (Areia Branca-RN). Samples were collected in July and October 2008. Zn, Mn and Pb were determined for Luiz Gomes, Rafael Fernandes, Pau dos Ferros, Apodi, Governador Dix-Sept Rosado, Mossoró and Areia Branca in the state of Rio Grande do Norte. The worst values were in Mossoró city high concentrations of Pb and Mn of 106 and 41,095 mg/kg, respectively, while cities that were less affected are those located closer to the beginning of the river, like Pau dos Ferros.

Key Words

Trace elements, monitoring, soil pollution.

Introduction

The rivers have been used like deposit of variety products, from human activity. The presence of potentially toxic elements is responsible for adverse effects on the environment, with repercussions on the economy and public health. The introduction of metals in aquatic systems occurs naturally through geochemical processes in the weathering and the contribution to human activity is a reflection of its widespread use by industry. (Campos 2005; Yabe and Oliveira 1998). According to KICuen and Watkins, cited by Schwanz (2008), some metals are considered essential to the development of plants or animals, however, the tolerance limits for these trace elements, in general, are very low. Among them we highlight the cobalt (Co), manganese (Mn) and nickel (Ni). However, all the elements in high concentrations may cause damage to the body. Apodi River has a great importance to the state, being the largest river in Rio Grande do Norte, with approximately 210 km long. According to Mitchell and Spencer (2008), it is a resource of great economic importance, enabling the use of fisheries, the use of its water for agriculture and livestock, and the source of income and livelihood of many rivers. According Petta *et al.* (2007), the most dominant economic activities in the region, such as salt production, irrigated horticulture, extraction of oil and gas, fishing and shrimp grow so significantly dependent on water bodies. However, since the spring, the river shows clear signs of environmental problems such as pollution from the release of sewage, reduction of near vegetation and sedimentation. The framework of environmental degradation is further aggravated because of the degree of contamination of water in the urban area of Rio Grande do Norte. According to Oliveira and Queiroz (2008) is this breakage that the problem is more serious, since it is launched, directly, the liquid waste from industrial activity, the provision of services and even organic household waste, generating high levels of chemical pollution, evidenced by the existence heavy metals. Thus, the monitoring of soil, over time, is of fundamental importance to verification of changes in the levels of chemical elements, mainly heavy metals. The contamination of rivers, usually associated with land on margins of rivers, which has contributed greatly to the increase of contaminants in river water and decreased agricultural productivity of soils. This study aimed to verification the accumulation and distribution of the trace elements Cu, Zn, Mn, Ni, Pb and Co in soils along the River Apodi / Mossoró.

Methods

There were two samples of the soil layer 0-20 cm in 24 points on the river Apodi-Mossoró, distributed along the begin (Luiz Gomes-RN) to the end (Areia Branca-RN). Samples were collected in July and October, both in 2008. The samples were air dried, then pass through harrowing and mesh wire 2mm. To perform the digestion, we used the method USEPA 3050B, the U.S. Environmental Protection Agency, as described by

Campos *et al.* (2005). After digestion took place in the reading atomic absorption of quantity of zinc, manganese, nickel, lead, cobalt, calcium, magnesium. The potassium was determined by flame photometer, and boron in the spectrophotometer for each sample collected.

Results

In agreement with the obtained results, after analysis in laboratory, it was observed that were present in the soil the following elements: Cu, Zn, Pb, Co and Ni, as values presented in the Table 1. Among the certain points for the evaluation, Luiz Gomes, Rafael Fernandes, Pau dos Ferros, Apodi, Governador Dix-Sept Rosado, Mossoró and Areia Branca in the state of Rio Grande do Norte, the most affected city was Mossoró, because obtained larger presence of chemical elements and larger concentration of Cu, Zn, Co and Ni with 108.48; 173.83; 17.60 and 9.61 mg/kg respectively, while the less affected cities were the located ones closer to the begin of the river, like Pau dos Ferros city, presenting low values for the elements compared to other places.

Table 1. Points, collected sites and averages of values trace elements studying (mg/kg). Nd = Not detected.

| Points | Identification Local collection and coordinated | Trace Elements | | | | |
|--------|--|----------------|--------|-------|-------|------|
| | | Cu | Zn | Pb | Co | Ni |
| 1 | Luiz Gomes-RN (6°22'8.00"S e 38°27'22.60"O) | 0.87 | nd | 26.77 | 7.33 | 5.79 |
| 2 | Luiz Gomes-RN (6°22'11.00"S e 38°26'6.00"O) | 4.02 | 8.88 | 37.98 | 13.39 | 3.08 |
| 3 | Rafael Fernandes-RN (6°14'50.00"S e 38°14'54.00"O) | nd | nd | 24.91 | 6.93 | nd |
| 4 | Pau dos Ferros-RN (6°8'50.00"S e 38°11'35.00"O) | nd | nd | 35.49 | 8.51 | nd |
| 5 | Pau dos Ferros-RN (6°6'28.00"S e 38°12'13.00"O) | nd | nd | 27.4 | 7.19 | nd |
| 6 | Apodi-RN (5°45'46.86"S e 37°48'4.76"O) | nd | nd | 28.64 | 7.46 | nd |
| 7 | Apodi-RN (5°45'46.86"S e 37°48'4.76"O) | nd | nd | 24.28 | 9.83 | 1.60 |
| 8 | Governador Dix-Sept Rosado-RN (5°28'9.65"S e 37°31'29.99"O) | nd | nd | 26.15 | 8.64 | 0.86 |
| 9 | Mossoró (5°13'9.13"S e 37°21'46.25"O) | nd | nd | 26.77 | 7.85 | 0.25 |
| 10 | Mossoró (5°13'9.13"S e 37°21'46.25"O) | nd | 15.56 | 27.4 | 13.39 | 6.40 |
| 11 | Mossoró-RN (5°12'22.64"S e 37°21'1.84"O) | nd | 173.83 | 36.11 | 17.60 | 9.61 |
| 12 | Mossoró-RN (5°12'22.64"S e 37°21'1.84"O) | nd | 0.93 | 23.66 | 9.44 | 2.71 |
| 13 | Mossoró-RN (5°12'16.61"S e 37°20'22.66"O) | nd | 1.18 | 31.13 | 8.64 | 1.23 |
| 14 | Mossoró-RN (5°12'11.72"S e 37°20'14.76"O) | nd | 5.25 | 27.39 | 10.09 | 4.92 |
| 15 | Mossoró-RN (5°11'43.32"S e 37°20'26.59"O) | nd | 11.81 | 25.53 | 12.20 | 7.63 |
| 16 | Mossoró-RN (5°11'43.32"S e 37°20'26.59"O) | nd | 12.72 | 21.79 | 11.80 | 4.43 |
| 17 | Mossoró-RN (5°11'43.32"S e 37°20'26.59"O) | nd | 1.63 | 20.55 | 9.96 | 1.97 |
| 18 | Mossoró-RN (5°11'52.55"S e 37°19'36.54"O) | nd | nd | 25.53 | 6.8 | nd |
| 19 | Mossoró-RN (5°11'45.60"S e 37°19'43.48"O) | nd | nd | 13.07 | 5.74 | nd |
| 20 | Mossoró-RN (5°10'36.81"S e 37°19'46.99"O) | nd | nd | 16.81 | 12.33 | 4.68 |
| 21 | Mossoró-RN (5°9'21.11"S e 37°17'8.99"O) | nd | 41.48 | 22.42 | 16.02 | 7.39 |
| 22 | Mossoró-RN (5°7'35.07"S e 37°16'5.37"O) | nd | nd | 1.24 | 3.50 | nd |
| 23 | Mossoró-RN (5°7'35.07"S e 37°16'5.37"O) | nd | nd | 2.49 | 2.32 | nd |
| 24 | Areia Branca-RN (4°57'15.17"S e 37°8'11.81"O) | 180.48 | 132.02 | 35.49 | 8.38 | 2.46 |

The Company of Technology of Environmental Sanitation, Cetesb (2005) it determines prevention values (VP) and intervention values (VI) for metals in soils. VP is concentration that can cause harmful alterations to the quality of the soil, VI is the concentration above which risks potential, direct or indirect exist to the human health. In agreement with the company, VP in soils is of: Cu 60 mg/kg; Zn 300 mg/kg; Pb 72 mg/kg; Co 25 mg/kg; Ni 30 mg/kg, while the value of VI established for agricultural soils for the metals is: Cu 200 mg/kg; Zn 450 mg/kg; Pb 180 mg/kg; Co 35 mg/kg; Ni 70 mg/kg. The results for zinc presented values varying from 0 to 173.83 mg/kg, in other words, not presenting superior values to the established. The variation along the points can be observed in the Figure 1. Studies accomplished by Zuliani (2006), Duarte and Pasqual (2000) and Ramalho *et al.* (2000) observed values of Zn varying from 2.9 to 191 mg/kg; 0.5 to 5.0 mg/kg; 21.07 to 109 mg/kg respectively, similar to the results obtained this work. The concentration of cobalt and nickel were found along the river with grand intensity, like can be observed on Figure 2 with highest value in Mossoró city. This result can be happen because the largest pollution associated with the biggest population liven on this city, about 250.000 habitats.

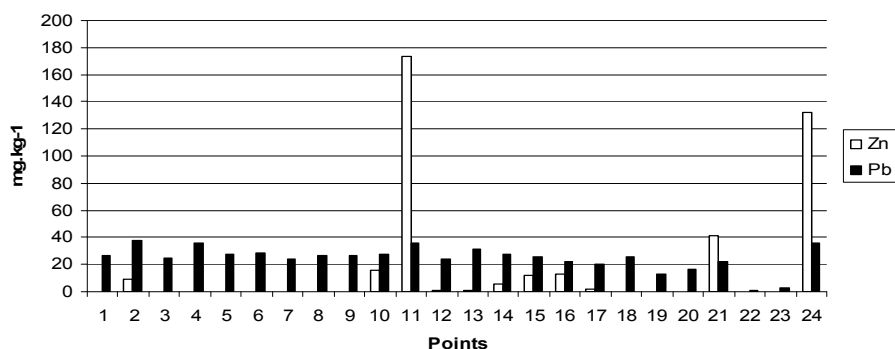


Figure 1. Variation of the concentration of zinc (Zn) and lead (Pb) in mg/kg along the collected points.

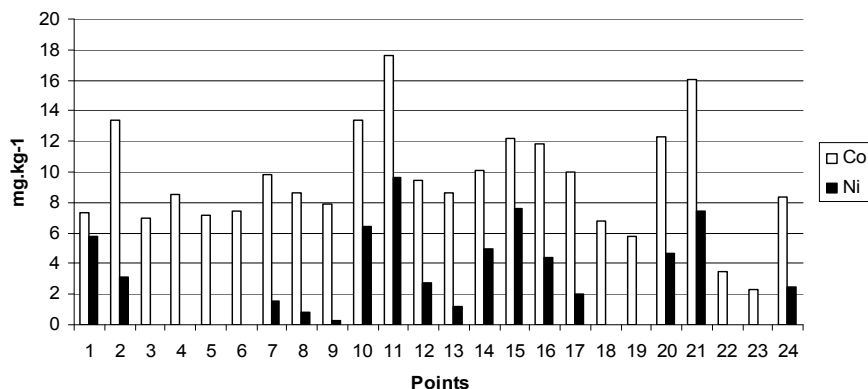


Figure 2. Concentration of cobalt (Co) and nickel (Ni) in mg/kg along the collected points.

The lead, cobalt and nickel were also inferior to the value established by CETESB (2005), being found along the points values among 1.24 to 37.98 mg/kg for Pb, 2.32 to 17.60 mg/kg for Co and 0 to 9.61 mg/kg of Ni. Duarte and Pasqual (2000), evaluating soil sample near highways of intense traffic in the state of São Paulo, verified values of Zn varying from 0.5 to 5.0 mg/kg, besides Pb varying from 1.3 to 8.9 mg/kg. Ramalho *et al.* (2000), evaluating the contamination of Caetés River by agrochemistry use, observed values in soils of Zn varying from 21.07 to 109.0 mg/kg and Pb varying from 18.34 to 30.73 mg/kg. In the present work, it is possible to observe in the river Apodi/Mossoró, higher values of the elements in some collected points when compared to the other mentioned authors. The presence of those metals in great amounts, as it happened in Mossoró city, it can take several consequences, like unavailability of drinking water for consumption, or soil for planting and of the use of fishing by the people live near the river.

Conclusion

The analysis made clear show the importance of studying the presence of trace elements in soil, with the objective to removal or treatment this lands, divide nearest soil river is present in an area of great economic importance for the region, this pollution can cause permanent damage to population.

References

- Campos ML *et al.* (2005) Determinação de cádmio, cobre, cromo, níquel, chumbo e zinco em fosfatos de rocha. *Pesquisa Agropecuária Brasileira*, Brasília **40**, 361-367
- Yabe MJS, Oliveira E (1998) Metais pesados em águas superficiais como estratégia de caracterização de bacias hidrográficas. *Quím. Nova* **21**, 551-556.
- Schwanz M, Ferreira JJ, Fröhlich P, Zuanazzi JAS, Henriques AT (2008) Análise de metais pesados em amostras de *Peumus boldus* Mol. (Monimiaceae). *Rev. bras. farmacogn.* **18**, 98-101.
- Morais PRF, Souza LD (2008) 'Efeito da sazonalidade e das ações antrópicas no teor de sólidos e na turbidez das águas da Bacia do Rio Apodi Mossoró-RN'. <http://www.abq.org.br/cbq/2008/trabalhos/5/5-265-3026.htm>
- Petta RA, Gomes RC, Erasmi S, Campos TFC, Nascimento PSR (2007) 'Análise da Bacia Hidrográfica do Rio Apodi-Mossoró no contexto de alterações ambientais e socioeconômicas ligadas a exploração de petróleo'. http://www.portalabpg.org.br/PDPetro/4/resumos/4PDPETRO_6_2_0289-2.pdf
- Oliveira MA, Queiroz RAC (2008) 'A poluição do Rio Mossoró (RN) e a Ação Intervencionista do

Ministério Público'. <http://www.anppas.org.br/encontro4/cd/ARQUIVOS/GT6-518-1004-20080517230550.pdf>

CETESB (2005) Valores orientadores para solo e água subterrânea no Estado de São Paulo.

<http://www.agsolve.com.br/pdf/artigos/novatabelacetesb2005.pdf>

Zuliani DQ (2006) Elementos-traço em águas, sedimentos e solos da bacia do rio das Mortes, Minas Gerais. 168f. Tese (Doutorado em Solos e Nutrição de Plantas) – Universidade Federal de Lavras, Lavras-MG.

Duarte RPS, Pasqual A (2000) Avaliação de cádmio (Ca), chumbo (Pb), níquel (Ni) e zinco (Zn) em solos, plantas e cabelos humanos. *Energia na agricultura* **15**.

Ramalho JFGP, Sobrinho N, Velloso ACX (2000) Contaminação da microbacia de Caetés com metais pesados pelo uso de agroquímicos. *Pesq. Agroppec.bras.* **35**, 1289-1303.

Long term effect of irrigation with the treated sewage effluent on some soil properties for date palms in Al Hassa, Saudi Arabia

A. M. Al-Omran^A

^ADepartment of Soil Science, Faculty of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia, Email rasoul@ksu.edu.sa

Abstract

There is a gradual decline in availability of fresh water to be used for irrigation in Saudi Arabia. As a consequence, the use of sewage and other industrial effluents for irrigating agricultural lands is on the rise particularly in developing areas. On the other hand, there is increasing concern regarding statutory and advisory food standards for trace metals throughout the world. Hence, a case study was undertaken to assess the long-term effect of sewage irrigation on some soil properties and heavy metals concentrations in the soils under the date palm in Al- Hassa; Saudi Arabia. To achieve this goal, thirty two surface soil samples were collected from the study area. Half were collected from an area irrigated for 13 years with treated sewage effluent. The rest of soil samples were collected from an area irrigated with well water in addition to reference samples from uncultivated areas. Sewage effluent, ground water, soil and plant samples were collected and analyzed mainly for metal contents. The results emphasized the role of sewage effluent irrigation on increasing heavy metal content as well as organic matter content in the soils when compared with the respective values obtained under well water irrigation. Furthermore, the soil salinity ranged from 2.5 to 3.69 dS/m with an average of 2.8 dS/m due to irrigation with well water. Soil salinity ranged from 3.58 to 20.7 dS/m with an average of 7.9 dS/m after irrigation for a long period with the treated sewage effluent. Sewage effluent contained much higher amounts of Zn, Cu, Fe, Mn, Co, Cr, Pb and Ni compared to ground well water. Generally soils receiving sewage irrigation for more than 13 years exhibited significant increases in Zn, Fe, Ni and Pb.

Key Words

Long-term sewage irrigation, heavy metals, soils, well water, date palm, sandy soil.

Introduction

In arid and semiarid regions such as Saudi Arabia, water demand has exceeded the reliable supply of surface water and renewable ground water due to rapid growth in municipal and industrial use. The agricultural sector is the major consumer of water in Saudi Arabia, using two-thirds of available resources. The growing competition for scarce water resources, coupled with laws limiting ground water pumping, has led to utilization of low quality water in irrigated agriculture in Saudi Arabia. However, applying effluent to arable lands also involves certain environmental and agricultural risks. Effluent differs from fresh water with higher contents of electrolytes, dissolved organic matter, suspended solids, and biochemical and chemical oxygen demand (BOD, COD). These varied constituents in effluent can affect soil physical and hydraulic properties. Although the concentration of heavy metals in sewage effluents are low, long-term use of these waste waters on agricultural lands often results in the build-up of elevated levels of these metals in soils (Rattan *et al.* 2002; Madyiwa *et al.* 2002; Yadar *et al.* 2002; Angin *et al.* 2004). On the other hand, crops raised on the metal-contaminated soils may accumulate metals in sufficient quantities to cause clinical problems both to animals and human beings consuming these metal rich plants (Miller, 1986). The objectives of this study were: (i) to study the chemical composition of sewage effluent and ground well water; (ii) to assess the effects of long-term irrigation with sewage effluent on metal contents in soils which are cultivated with date palm at Al Hassa area, Saudi Arabia.

Materials and methods

Study area and collection of samples

The study area is located between (N 24 ° 21' 325") and (E 46 ° 55' 906") where sewage effluent originating from Al- Hassa Sewage Treatment Plant have been used for irrigation purposes since 1995. Sewage effluent was collected periodically from the study area. Similarly, groundwater samples were also collected from wells used for irrigation in the study area. Thirty two surface soil samples (0–20 cm) were collected from sewage and adjacent well water-irrigated plots. Soil samples were dried, ground and passed through 2 mm sieve and kept for physical and chemical analysis.

Chemical analysis of sewage effluents, well water, soil and plant samples

An aliquot of 500 mL of sewage effluents and well water samples with 15 mL of HNO₃ was evaporated to near dryness on a hot plate. Then contents were digested with 15 mL of HNO₃ and 20 mL HClO₄ (70%) (Brar *et al.* 2000). The residue was taken up in 15 mL of 6N HCl and made to volume (50 mL) and contents were filtered. The filtrate was analyzed for P, K, S, Zn, Cu, Fe, Mn, Ni, Pb and Cd using inductively coupled plasma-atomic emission spectrophotometer (ICP-AES(Perkin-Elmer, Model 4300 DV)). Sewage effluent samples were analyzed for a pH and electrical conductivity using pH meter and salt-bridge, respectively. Carbonate and bicarbonates were estimated by titrating an aliquot of effluent samples with H₂SO₄. Soil pH was measured in suspension (soil paste) according to Datta *et al.* (1997). Organic carbon contents in soil were determined by the wet digestion method (Walkley and Black 1934; Nelson and Sommers 1982.). Further, 30 soil samples were selected and analyzed for texture by the hydrometer method (Day 1965; Gee and Bauder 1994). For heavy metal analysis, the soil samples were digested with HF; H₂SO₄ and HClO₄ according to Hossner (1997). The concentrations of metals were determined using an inductively coupled plasma-atomic emission spectrophotometer (ICP-AES). Plant samples were dried at 60 °C in hot air oven, ground and digested in an acid mixture of (HNO₃:HClO₄:9:4) (Jackson 1973). Metal contents in the plant digests were determined using an inductively coupled plasma-atomic emission spectrophotometer (ICP-AES). Due care was taken to avoid metal contamination in the process of sampling, drying, grinding, extracting and analysis. All equipment and containers were soaked in 10% NHO₃ for 24 h then rinsed thoroughly in de- ionized water before use. Also, quality control was assured by performing duplicate analyses on all samples and by using reagent blanks and standard reference soil (Till 1) which yielded As, Ni, Fe, Cu, Mn, Cd, Cr, Pb and Zn contents close to the certified values.

Results and discussion

The sewage effluents were found to contain much higher amount of Zn, Cu, Fe, Mn Co; Cr; Pb and Ni compared to the ground well water. Generally soils receiving sewage irrigation for more than 13 years exhibited significant increase in Zn, Fe, Ni and Pb. On the other hand sewage effluents were alkaline in reaction with pH values ranging from 7.5 to 8.5 (data not shown). The tolerance limit of pH for irrigation water ranged from 6.0 to 9.0 (Patel *et al.* 2004). Thus, pH of the effluent samples is within the permissible limit. Electrical conductivity of sewage effluents exceeded 1 dS/m (2.1–2.2 dS/m) indicating that these effluents were saline in nature. The carbonate and bicarbonate contents in effluent samples varied from traces to 1.1 and 1.5 to 3.9 meq L⁻¹, respectively. Carbonate concentrations in all the samples were much lower as compared to bicarbonate concentrations. The sand; silt and clay contents of soils receiving sewage effluent ranged from (70.3- 86.3), (5-14 %) and (7.7 – 15.7%), in the respectively. Soil pH varied from 7.6 to 8.1 and 7.9 to 8.7 in sewage and well water-irrigated soils, respectively. The organic matter content varied from 0.8 to 2.4% in sewage-irrigated soils, the corresponding values for well water-irrigated soils were 1.0–1.09 %. Soil salinity ranged from 2.5 to 3.69 dS/m with an average of 2.8 dS/m due to irrigation with well water. Soil salinity ranged from 3.58 to 20.7 dS/m with an average of 7.9 dS/m due to irrigation for a long period with the treated sewage effluent. The results emphasize the role of sewage effluent irrigation in increasing heavy metals contents in the soils when compared with values well water irrigated soils.

References

- Angin I, Yaganoglu AV, Turan M (2004) Effect of long term water waste irrigation on soil properties. *Journal of substantial Agriculture* **26**, 31 – 42.
- Brar MS, Mahli SS, Singh AP, Arora CL Gill KS (2000) Sewer water irrigation effects on some potentially toxic trace elements in soil and potato plants in northwestern India. *Can. J. Soil Sci.* **80**, 465–471.
- Datta SP, Subba A, Rao Ganeshamurthy AN (1997). Effect of electrolytes coupled with variable stirring on soil pH. *J. Indian Soc. Soil Sci.* **45**, 185–187.
- Day PR (1965). Fractionation and particle size analysis. In: ‘Methods of soil analysis. Part 1. Physical and mineralogical methods’ (Ed CA Black) pp. 545–567. (ASA/SSSA: Madison, WI).
- Gee GW Bauder JW (1994). Particle-size Analysis. In: ‘Methods of soil ANALYSIS. Part 1. 3rd edition Physical and mineralogical methods’ (Ed A Klute) pp. 377-382. (ASA/SSSA: Madison, WI).
- Hossner LH (1996). Dissolution for total elemental analysis. In ‘Methods of soil analysis. Part 3. 3rd edition Chemical Methods’. (Eds Sparks *et al.*) pp. 46-64. (ASA/SSSA: Madison, WI).
- Jackson ML (1973) ‘Soil Chemical Analysis’. (Prentice-hall of India Pvt. Ltd.: New Delhi).
- Madyiwa SM Chimbari J, Nyamangara Bangira C (2002) Cumulative effects on sewage sludge and effluent mixture application on soil properties of a sandy soil under a mixture of star and kikuyu grasses in Zimbabwe. *Physics and Chemistry of the Earth.* **27**, 747-753.

- Nelson DW, Sommers LE (1982) Total carbon, organic carbon and organic matter. In Methods of soil analysis. Part 2. 2 edition. (Eds AL Page *et al.*) pp. 539-579. (ASA/SSSA: Madison, WI).
- Patel KP, Pandya RR, Maliwal GL, Patel KC, Ramani VP George V (2004) Heavy metal content of different effluents and their relative availability in soils irrigated with effluent waters around major industrial cities of Gujarat, *J. Indian Soc. Soil Sci.* **52**, 89–94.
- Rattan RK, Datta SP, Chhankar PK, Suribabu K, Singh AK (2004) Long term impact of irrigation with sewage effluents on heavy metal contents in soils, crops and groundwater- a case study. *Agriculture, Ecosystems and Environment.* **109**, 310-322.
- Tiller KG (1986). Essential and toxic heavy metals in soils and their ecological relevance, Trans. XIII Congr. *Intern. Soc. Soil Sci.* **1**, 29–44.
- Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **37**, 29–38.
- Yadar RK, Goyal R, Sharma RK, Dubey SK, Minchas RS (2002). Post irrigation impact of domestic sewage effluent on composition of soils, crops and ground water- A case study. *Journal of Environmental international* **28**, 481-486.

Long-term effects of mine soil reclamation using different amendments on microbial and biochemical properties in Southeast Spain

Raúl Zornoza, Ángel Faz, Dora M. Carmona, Asuman Büyükkiliç, Sebla Kabas, Silvia Martínez-Martínez^A and Jose A. Acosta^{A,B}

^ASustainable Use, Management, and Reclamation of Soil and Water Research Group. Department of Agrarian Science and Technology. Technical University of Cartagena. Paseo Alfonso XIII, 52, 30203 Cartagena. Murcia. Spain. E-mail: angel.fazcano@upct.es

^BInstitute for Biodiversity and Ecosystem Dynamics. University of Amsterdam. Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands.

Abstract

Microbial biomass carbon, soil respiration and enzyme activities were measured five years after reclamation of a polluted soil affected by former mining activities in SE Spain. This abandoned mine site contains materials of high Fe-oxyhydroxides, sulphates, and elevated contents of potentially leachable heavy metals due to extreme acidic conditions. Thus, soils have scarce or null vegetation due to very poor properties including low soil organic matter. A field trial was established in 2004 in which different experimental plots were designed, using marble wastes, pig manure and sewage sludge as amendments to recover soil properties. Results show that all biochemical properties were higher in treated soils than in control, despite soil organic matter being similar amongst the treatments. As general pattern, biochemical properties showed higher values after application of pig manure than after application of sewage sludge. The different doses used did not have a great effect, being only significant for β -glucosidase, phosphodiesterase and arylsulfatase. This study confirms the high sensitivity of biochemical properties in the evaluation of soil quality and reactivation of nutrients cycles.

Key Words

Metal pollution, in situ remediation, pig slurry, sewage sludge, microbial biomass, hydrolase activities

Introduction

The environmental impacts of the long-time mining activities in southeast Spain include large areas of soils being affected by strong acidification processes, high salinity and accumulation of metals. These mining activities have generated high amounts of sterile materials for many years; the wastes are accumulated in pyramidal structures called tailing ponds. Mine sites contain materials of high Fe-oxyhydroxides, sulphates, and potentially leachable elevated contents of heavy metals (mainly Cd, Pb, Cu and Zn) due to extreme acidic conditions. As a consequence, these mine soils have scarce or null vegetation due to very poor properties, including extremely low soil organic matter. Since the polishing of metals from a mining area is a difficult task, the transformation of metals into harmless species or their removal in a suitable recycled mineral form such as carbonates using marble wastes or lime (Geebelen *et al.* 2003) is a possible solution for the remediation of a mining area. In addition, incorporation of organic amendments into contaminated mine soils has been proposed as feasible, inexpensive and environmentally sound disposal practice, as generally such wastes can improve soil physical and chemical properties and contain nutrients beneficial to microorganisms and plants (Barker 1997), favouring the reactivation of biogeochemical cycles and the natural establishment of vegetation. Although there is a general consensus that efficiency of soil remediation also depends on the presence and activity of microorganisms, the long-term ecological consequences of inorganic and organic amendments for these features have received little attention (Mench *et al.* 2006). The goal of this work was the evaluation of the long-term effectiveness of the remediation of contaminated mine soils by means of different inorganic and organic amendments using soil microbial and biochemical properties as indicators.

Methods

Study site and experimental design

The study was conducted in the province of Murcia (SE Spain), in the Cartagena-La Unión Mining District, where great mining activity has been carried out for more than 2500 years, the activity being stopped only in the nineties. The climate of the area is semiarid Mediterranean with mean annual temperature of 18°C and mean annual rainfall of 275 mm. One tailing pond generated by mining activities was selected (El Lirio), representative of the rest of existing ponds in Cartagena-La Unión Mining District.

The field trial was established in 2004. Plots (2 m x 2 m) were randomised and replicated 3 times. Two different organic amendments were used to reclaim the soils, pig manure (P) and sewage sludge (S). In addition, 3 different doses per amendment were applied. Thus, the treatments were: Untreated contaminated soil (Control: C), soil treated with pig manure at dose 1 (P1), dose 2 (P2) and dose 3 (P3); and soil treated with sewage sludge at dose 1 (S1), dose 2 (S2) and dose 3 (S3). For pig manure, dose 1, 2 and 3 were 2.5, 5 and 10 kg per plot, respectively. For sewage sludge, doses were 1.99, 3.98 and 7.97 kg per plot, respectively. Doses were established by thresholds imposed by legislation regarding the addition of N to soil (Council Directive 91/676/EEC, 1991). With the purpose of increasing soil pH to immobilise metals and create better conditions for microbial and plant development, marble mud was applied in all plots except for control, at the rate of 22 kg per plot.

Soil sampling and analytical methods

The soil sampling was carried out in May 2009 (5 years after application of amendments). One sample (0-15 cm depth) was collected for each plot, taken to the lab, air-dried for 7 days, passed through a 2-mm sieve and stored at room temperature prior to laboratory analyses. The physico-chemical characterization of the plots is shown in Table 1.

Microbial biomass carbon (MBC) was determined using the fumigation-extraction procedure (Vance *et al.* 1987); basal soil respiration (BSR) determined according to Anderson (1982); β -glucosidase activity according to Tabatabai (1982); arylesterase activity according to Zornoza *et al.* (2009); acid and alkaline phosphatase according to Tabatabai and Bremner (1969); phosphodiesterase following the method of Browman and Tabatabai (1978); and arylsulphatase measured by the method of Tabatabai and Bremner (1970).

Table 1. Main physico-chemical properties of control and amended plots from El Lirio pond.

| Treatment | pH | EC (dS/m) | SOC (g/kg) | Nt (g/kg) | WSC (mg/kg) | Cd (mg/kg) | Cu (mg/kg) | Pb (mg/kg) | Zn (mg/kg) |
|-----------|-----|--------------|---------------|--------------|----------------|---------------|---------------|---------------|---------------|
| Control | 7.1 | 2.5 | 2.13 | 0.05 | 33 | 5.6 | 1.9 | 177 | 495 |
| P1 | 7.6 | 2.4 | 2.96 | 0.12 | 25 | 4.2 | 2.5 | 320 | 418 |
| P2 | 7.5 | 2.5 | 2.92 | 0.15 | 29 | 3.3 | 2.2 | 333 | 352 |
| P3 | 7.3 | 2.4 | 2.14 | 0.06 | 29 | 2.9 | 0.8 | 309 | 446 |
| S1 | 7.5 | 2.4 | 2.66 | 0.05 | 26 | 4.0 | 1.7 | 223 | 428 |
| S2 | 7.6 | 2.3 | 2.29 | 0.06 | 33 | 3.9 | 1.7 | 295 | 433 |
| S3 | 7.4 | 2.5 | 2.33 | 0.06 | 33 | 3.7 | 1.4 | 288 | 406 |

EC: Electrical conductivity, SOC: soil organic carbon, Nt: total nitrogen, WSC: water soluble carbon. Cd, Cu, Pb and Zn have been extracted with DTPA (Bioavailable metals).

Results and discussion

Results of the different biochemical properties determined in the plots 5 years after the application of the amendments are shown in Figure 1. According to the general trends, all biochemical properties were higher in treated soils than in control, despite the fact that soil organic carbon, total nitrogen or water soluble carbon were similar amongst the treatments after 5 years of the application (Table 1). The highest increases with respect to control were for MBC in P plots, (100%), β -glucosidase in P plots (250%), phosphodiesterase in P plots (210%) and arylsulphatase in P3 (4000%). This confirms the high sensitivity of biochemical properties to evaluate soil quality (Nannipieri *et al.* 1990), as undetected shifts occurred with other chemical properties. In addition, also as general pattern, biochemical properties showed higher values after application of pig manure than after application of sewage sludge. These results are promising in an area like Murcia province where more than 10% of pig production in Spain is located. Annually, Murcia province generates an estimated 8 millions m³ of waste residues from the pork industry (CAAMA, 2003). This generation of large volume of pig slurry continuously increases with high demands for pork, and consequently creates disposal problem for many pig producers. However, doses did not have a great effect, being only significant for β -glucosidase, phosphodiesterase and arylsulphatase. The fact that SOC remains similar in all plots indicate a mineralization or leaching of the organic amendments, since treated plots had initially significantly higher values of SOC (Zanuzzi, 2007). Nonetheless, this initial incorporation of organic matter has triggered the activation of microbial populations which has increased their activity, favouring the recovery of soils and the establishment of vegetation, since spontaneous colonization of vegetation has taken place in treated plots (data not shown). However, the values of microbial biomass, respiration and enzyme activities are still low comparing with non contaminated soils from other zones from SE Spain with the same climatic conditions

(Zornoza *et al.* 2006; 2007; Bastida *et al.* 2008). This can be explained by the still extreme edaphic conditions, like the already moderate levels of heavy metals, low organic matter and high salinity.

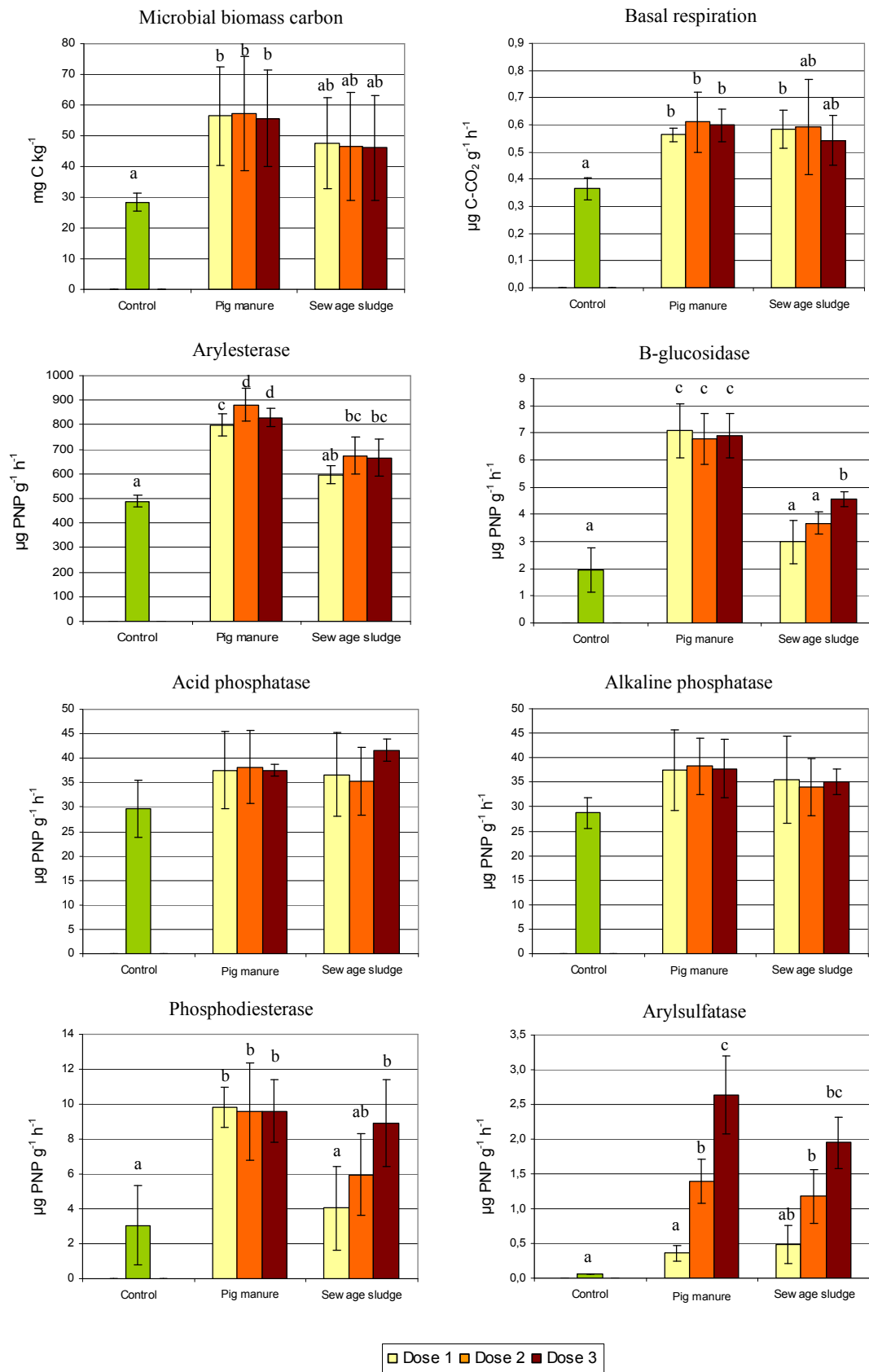


Figure 1. Microbial biomass carbon, soil respiration and enzyme activities of the control soil and remediated plots with different organic amendments at three different doses. Different letters indicate mean values significantly different after Tukey's honestly significant difference at $P < 0.05$.

Conclusion

The application of pig manure and sewage sludge together with marble wastes has proved to be effective to activate microbial populations and activity even after 5 years of application, pig manure being the organic amendment with the best effects on microbial biomass and activity. However, the values of the biochemical properties are still low even for a semiarid environment. Thus, future research is needed to monitor the evolution of soil properties to guarantee long-term rehabilitation of polluted mine soils in SE Spain, and assess if phytostabilization or more applications of organic amendments could help to facilitate reclamation.

References

- Anderson JPE (1982) Soil respiration. In 'Methods of soil analysis, part 2, 2nd edition'. (Eds. Page AL, Miller RH, and Keeney DR), pp. 837-871. (American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, USA).
- Barker AV (1997) Composition and uses of compost. In 'Agricultural uses of by-products and wastes'. (Eds JE Rechling, HC Mackimmon), pp. 140-162. (ACS Symposium Series No. 668, Vol 10. American Chemical Society, Washington DC).
- Bastida F, Barberá GG, García C, Hernández T (2008) Influence of orientation, vegetation and season on soil microbial and biochemical characteristics under semiarid conditions. *Soil Biology & Biochemistry* **38**, 3463-3473.
- Browman MG, Tabatabai MA (1978) Phosphodiesterase activity of soils. *Soil Science Society of America Journal* **42**, 284-290.
- CAAMA (2003) Consejería de Agricultura, Agua y Medio Ambiente de la Comunidad Autónoma de la Región de Murcia. Registro de explotaciones Porcinas de la Región de Murcia.
- Council Directive 91/676/EEC. Protection of waters against pollution caused by nitrates from agricultural sources. Official Journal L 375, pp. 1-8, 31-12-1991
- Geebelen W, Adriano DC, van der Lelie D, Mench M, Carleer R, Clijsters H, Vangronsveld J (2003) Selected bioavailability assays to test the effect of amendment-induced immobilization of lead in soils. *Plant and Soil* **249**, 217-228.
- Mench M, Renella G, Gelsomino A, Landi L, Nannipieri P (2006) Biochemical parameters and bacterial species richness in soils contaminated by sludge-borne metals and remediated with inorganic soil amendments. *Environmental Pollution* **144**, 24-31. Methods of Soil Analysis, Part 2, 2nd ed.
- Nannipieri P, Greco S, Ceccanti B (1990) Ecological significance of the biological activity in soil. In: 'Soil Biochemistry. Volume 6' (Eds JM Bollag, G. Stotzky). pp. 293-355. (Marcel Dekker Inc., New York).
- Tabatabai MA (1982) Soil enzymes. In: 'Methods of soil analysis, part 2, 2nd edition'. (Eds. Page AL, Miller RH, Keeney DR), pp. 501-538. (American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin, USA).
- Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate in assay of soil phosphatase activity. *Soil Biology & Biochemistry* **1**, 301-307.
- Tabatabai MA, Bremner JM (1970). Arylsulphatase activity of soils. *Soil Science Society of America Journal* **34**, 225-229.
- Vance ED, Brookes PC, Jenkinson DS (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry* **19**, 703-707.
- Zanuzzi A (2007) Reducción de impactos ambientales asociados a suelos contaminados por metales pesados mediante el uso de residuos antropogénicos. PhD Dissertation. Departamento de Ciencia y Tecnología Agraria. Universidad Politécnica de Cartagena.
- Zornoza R, Guerrero C, Mataix-Solera J, Arcenegui V, García-Orenes F, Mataix-Beneyto J (2006) Assessing air-drying and rewetting pretreatment effect on some soil enzyme activities under semiarid Mediterranean conditions. *Soil Biology and Biochemistry* **38**, 2125-2134.
- Zornoza R, Guerrero C, Mataix-Solera J, Arcenegui V, García-Orenes F, Mataix-Beneyto J (2007) Assessing the effects of air-drying and rewetting pre-treatment on soil microbial biomass, basal respiration, metabolic quotient and soluble carbon under Mediterranean conditions. *European Journal of Soil Biology* **43**, 120-129.
- Zornoza R, Landi L, Nannipieri P, Renella G (2009) A protocol for the assay of arylesterase activity in soil. *Soil Biology & Biochemistry* **41**, 659-662.

Magnitude and kinetics of metal rhizotoxicity in cowpea

Peter M. Kopittke^A, F. Pax C. Blamey^A, J. Bernhard Wehr^A and Neal W. Menzies^A

^AThe University of Queensland, School of Land, Crop and Food Sciences, St Lucia, QLD, Australia, and Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC-CARE), Email p.kopittke@uq.edu.au

Abstract

Many difficulties exist in establishing the concentrations of metals in solution that are toxic to the growth of plant roots. To limit these difficulties, short-term solution culture experiments were conducted using the same technique on 3-d-old cowpea (*Vigna unguiculata* (L.) Walp.) cv. Caloona seedlings. These were grown for 48 h in solutions with ca. 1000 μM Ca and 5 μM B plus one of 24 metals, concentrations of which were determined after filtering (0.22 μm). The decrease in root elongation rate (RER) varied markedly among the metals tested, with a 50 % reduction in RER (i.e. EC50) evident at 0.02 μM Ag to 132 mM K. The rapidity with which RER was reduced varied also, as did the symptoms of rhizotoxicity. A range of metals caused rupturing of the rhizodermis and outer cortex in the roots elongation and transition zones within 2 – 24 h of exposure. These metals were all highly rhizotoxic, but not all highly rhizotoxic metals caused ruptures. We conclude that rhizotoxicity results from disruption of a range of underlying biochemical mechanisms. There were some common effects, but no metal could be considered an analogue for another in all respects.

Key Words

Cell wall, plasmalemma, root, rymptoms, toxicity.

Introduction

In the early 16th Century, Paracelsus stated: “All things are poison and nothing is without poison, only the dose permits something not to be poisonous”. This applies equally to metals of no known benefit to biota (e.g. Hg, Pb) and known for centuries to be toxic, as well as to those that are essential for life (e.g. Cu, Mn).

Kopittke *et al.* (Submitted) reviewed 34 y of published information on the toxicity of eight metals, and included only those data that met strict criteria which allowed comparison with other studies. There was decreasing trace metal toxicity in the order of Pb \approx Hg > Cu \approx Cd > As \approx Co \approx Ni \approx Zn > Mn. Even with strict criteria for inclusion, there was an order of magnitude difference in the concentration of each metal reported to be toxic. For example, the 25 – 75 percentile phytotoxic range for Cd was 0.99 to 10 μM .

It is difficult to compare results on metal phytotoxicity across studies because of differences in (i) experimental conditions and (ii) sensitivity of plant genotypes to one or more metals. With regard to experimental conditions, increased concentrations of other ions reduce the toxicity of a metal in solution. This results from three mechanisms: (i) reduced electronegativity of the plasma membrane (PM), (ii) restoration of Ca²⁺ activity at the PM, and (iii) other unknown mechanisms (Kinraide 2004). An example of this last-named mechanism is the alleviation of Zn²⁺ toxicity by low concentrations (\leq 5 μM) of Mg (Pedler *et al.* 2004). The range in sensitivity among genotypes to elevated Mn in solution, for example, was demonstrated by Edwards and Asher (1982). This study showed that across 13 crop and pasture species, the external Mn concentration needed to reduce plant dry mass by 10 % varied from 1.4 μM in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) to 65 μM in sunflower (*Helianthus annuus* L.). To limit problems arising from differences in technique or genotypic sensitivity, this paper reports results using the same solution culture procedure on the rhizotoxic effects of 24 metals, both essential and non-essential, in cowpea seedlings.

Methods

Solution culture experiments were conducted to determine root growth of 3-d-old cowpea cv. Caloona seedlings over a 48 h period when exposed to elevated concentrations of a range in metals (Kopittke *et al.* 2008). Initially, seedlings were grown for 16 h in continuously-aerated solutions nominally containing 1000 μM Ca (as CaCl₂ or Ca(NO₃)₂) and 5 μM B (as H₃BO₃) at ca. pH 5. After this acclimatisation period, the seedlings (seven per 650 mL of solution) were transferred to solutions with the same concentrations of Ca and B plus one of 10 nominal concentrations of 24 metals. Solutions were sampled at the beginning and end of the experimental period (i.e. 48 h); mean data are presented. In some cases there was considerable loss of

metal from solution, making it difficult to determine the actual concentration of metal detrimental to root growth. In these instances, mean measured values at the beginning and end of the experiment was used. The 10 mL samples were filtered to 0.22 μm (Millipore, Millex-GS), acidified with 20 μL concentrated HCl or HNO₃. The concentrations of metals were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES), inductively coupled plasma mass spectroscopy (ICP-MS), or by flow injection mercury system atomic absorption spectroscopy (FIMS-AAS, for Hg). Solution pH was measured at the beginning and end of the experimental period, but was not adjusted in any instance (other than for Al, where pH was reduced to 4.5 in all treatments using 0.1 M HCl).

Seedlings were grown for 48 h and digital images captured at the time of transfer (0 h) and 4, 8, 12, 24, 36, and 48 h thereafter. The length of each root in each treatment was measured using ImageTool 3.0. These data were used to calculate the root elongation rate (RER) of each root for each time period. All roots were harvested after 48 h growth, and stored in 10 % ethanol in deionised water prior to examination using light microscopy. In some instances, scanning electron microscopy (SEM) was used to produce high resolution images of the symptoms evident close to the root tip. There were two replicates of each treatment, with data being presented as either the mean \pm standard error (SE) or as non-linear relationships estimated by GenStat 7.2 (GenStat 2003). Metal ions were classified according to The Chemogenesis Web Book available at http://www.meta-synthesis.com/webbook/43_hsab/HSAB.html.

Results

The EC₅₀ for cowpea RER ranged from 0.02 μM Ag to 130 mM K using the same technique to assess metal rhizotoxicity (Table 1). It is noteworthy also that some hard, soft, and borderline metals were highly rhizotoxic, and that this classification provided no clear distinction among metals in the magnitude of their rhizotoxic effects. However, the least rhizotoxic metals were all hard metals – but not necessarily those essential for plant growth.

Table 1. Rhizotoxicity of 24 metal ions in cowpea in decreasing order of toxicity, with their classification as hard (H), soft (S) or borderline (B) cations and whether (✓) or not (✗) they cause ruptures in the root's elongation and transition zones.

| Metal Ion | Hard, soft, or borderline | Ruptures | EC ₅₀ (μM) |
|-----------|---------------------------|----------|------------------------------------|
| Ag(I) | S | ✓ | 0.02 |
| Tl(I) | S | ✗ | 0.44 |
| Cu(II) | B | ✓ | 0.5 |
| Hg(II) | S | ✓ | 1.0 |
| In(III) | H | ✓ | 1.5 |
| Ni(II) | B | ✗ | 1.5 |
| Cd(II) | S | ✗ | 2.0 |
| La(III) | H | ✓ | 2.0 |
| Sc(III) | H | ✓ | 2.0 |
| Gd(III) | H | ✓ | 3.0 |
| Cs(I) | H | ✗ | 3.1 |
| Pb(II) | B | ✗ | 3.5 |
| Co(II) | H | ✗ | 4.0 |
| Ru(III) | H | ✓ | 10 |
| Zn(II) | B | ✗ | 28 |
| Al(III) | H | ✓ | 30 |
| Ga(III) | H | ✓ | 30 |
| Ba(II) | H | ✗ | 3200 |
| Sr(II) | H | ✗ | 3400 |
| Li(I) | H | ✗ | 12000 |
| Mg(II) | H | ✗ | 25000 |
| Ca(II) | H | ✗ | 48000 |
| Na(I) | H | ✗ | 69000 |
| K(I) | H | ✗ | 130000 |

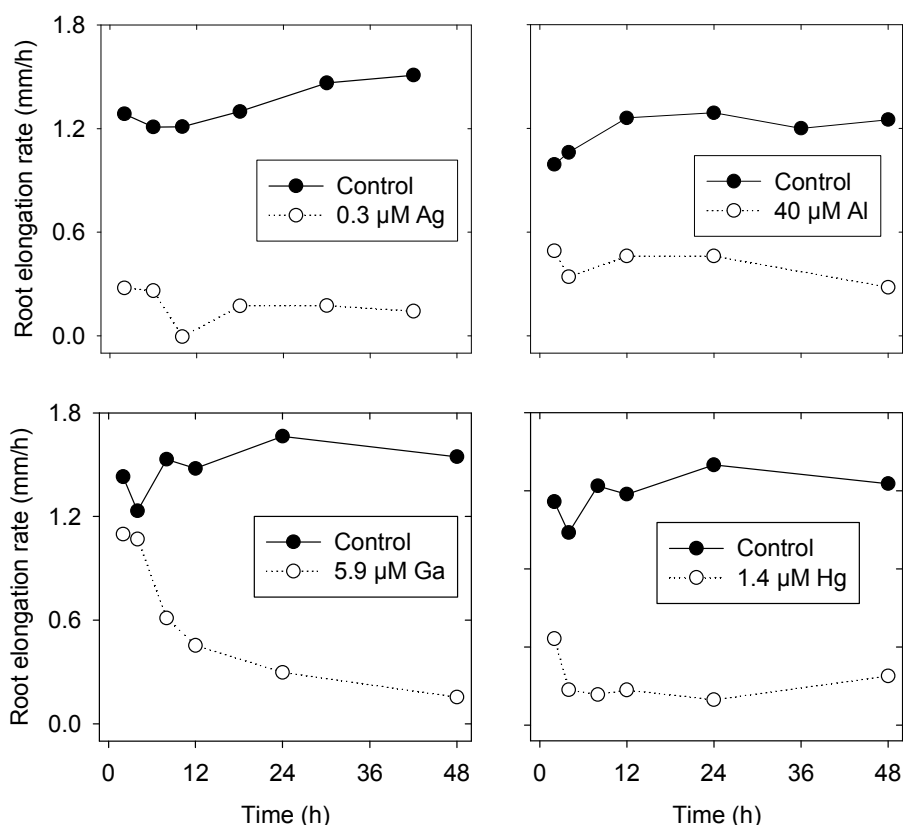


Figure 1. Kinetics of reduced root elongation rate (RER) in cowpea seedlings exposed to Ag, Al, Ga, and Hg sufficient to reduce root elongation by ca. 85 %.

Besides a reduction in RER, 10 metals caused ruptures to the rhizodermis and outer cortex (Table 1). This occurred with many, but not all, of the highly rhizotoxic metals. None of the least rhizotoxic metals did so, however, and these were all hard metals. Classification of metals as hard, soft, and borderline did not discriminate among the metals that rupture cowpea roots.

There were marked differences in the rapidity with which the metals reduced root growth. It was evident that Ag, Al, and Hg reduced root growth within 4 h of exposure (Figure 1). In contrast, there was a continually detrimental effect of Ga on root growth for up to 48 h.

Some metals caused ruptures to the rhizodermis and outer cortex of the root's transition and elongation zones (Figure 2). These ruptures were evident over an extended range of 30 – 600 μM Al, but this was not the case with most metals (e.g. 0.85 to 1.8 μM Cu or 2.0 to 5.5 μM La) (Kopittke *et al.* 2008). This study also showed that rupturing occurred within 4 h of exposure to the EC50 Al concentration, but only after 24 h on exposure to Cu and La also at EC50. It appeared that no ruptures developed where metal concentrations caused complete cessation of root elongation. This suggests that ruptures result from a decrease in wall loosening of the outer cells in the root's elongation zone while cells of the stele and inner cortex continue to grow.

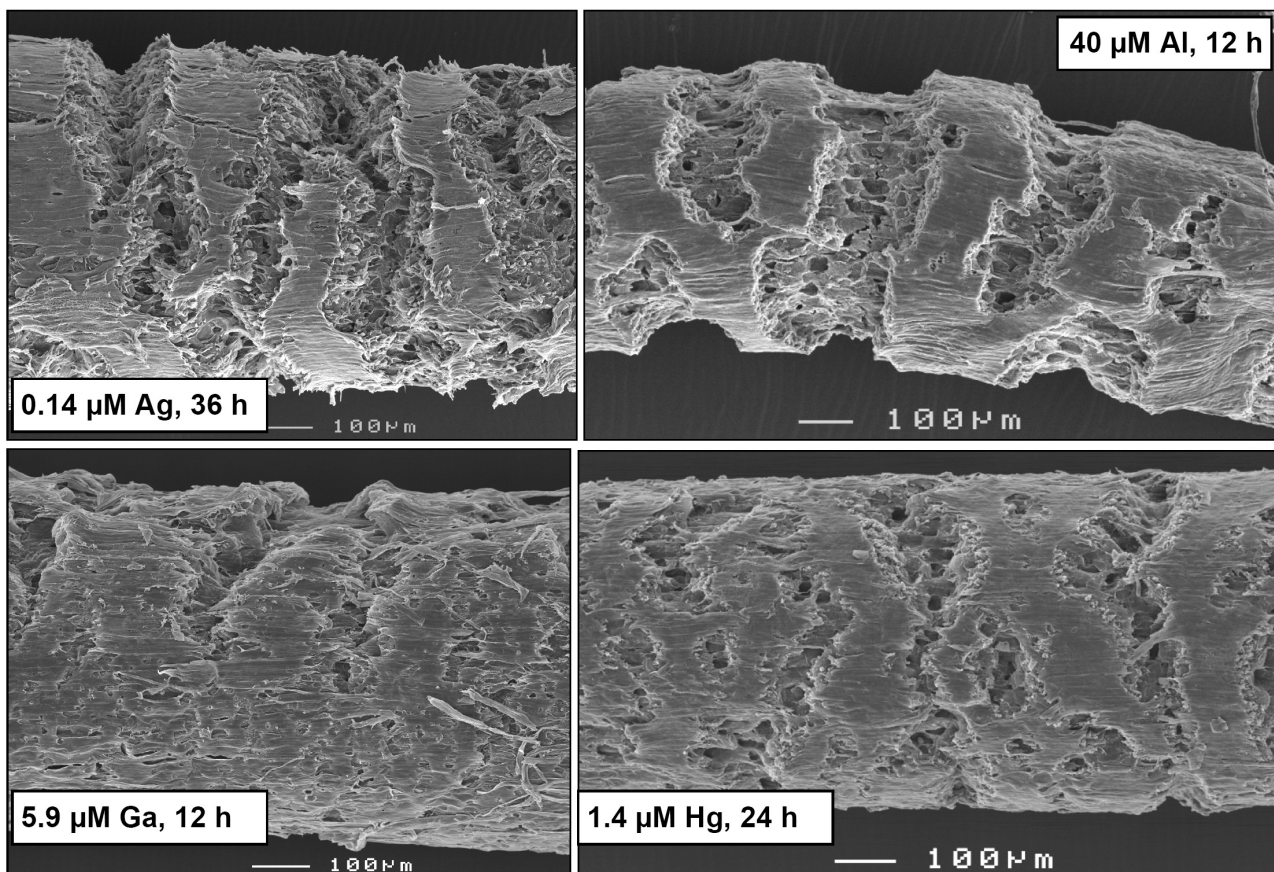


Figure 2. Ruptures developed in the rhizodermis and outer cortex of the elongation and transition zones when exposed to rhizotoxic concentrations of some metals (Table 1) as evident in scanning electron micrographs of roots exposed to Ag, Al, Ga, and Hg.

Conclusions

A range of over six orders of magnitude in EC₅₀ rhizotoxicity was found, from 0.02 μM Ag to 132 mM K, among the 24 metals tested using the same technique. It was evident that elevated concentrations of the metals caused a range in effects, with many (but not all) highly rhizotoxic metals causing ruptures to the epidermis and outer cortex. We conclude that the metals' rhizotoxic effects result from the disruption of many underlying biochemical mechanisms, with some commonality among some metals. However, no metal could be considered an analogue for another in all respects.

References

- Edwards DG, Asher CJ (1982) Tolerance of crop and pasture species to manganese toxicity. In 'Proceedings of the Ninth International Plant Nutrition Colloquium'. Warwick University, England. (Ed A Scaife) pp. 145-150. (Commonwealth Agricultural Bureaux).
- GenStat (2003) 'GenStat for Windows. Release 7.2. Seventh Edition.' (VSN International Ltd: Oxford).
- Kinraide TB (2004) Possible influence of cell walls upon ion concentrations at plasma membrane surfaces. Toward a comprehensive view of cell-surface electrical effects upon ion uptake, intoxication, and amelioration. *Plant Physiology* **136**, 3804-3813.
- Kopittke PM, Blamey FPC, Menzies NW (2008) Toxicities of soluble Al, Cu, and La include ruptures to rhizodermal and root cortical cells of cowpea. *Plant and Soil* **303**, 217-227.
- Pedler JF, Kinraide TB, Parker DR (2004) Zinc rhizotoxicity in wheat and radish is alleviated by micromolar levels of magnesium and potassium in solution culture. *Plant and Soil* **259**, 191-199.

Mercury soil pollution on Spanish islands: Methods to assess Hg input

Jose Antonio Rodríguez-Martín^A, Nikolaos Nanos^B, Gregoria Carbonel-Martín^C and Jose Manuel Grau-Corbí^A

^ACIFOR-INIA. Ctra. de la Coruña km, 7.5. 28040 Madrid, Spain, Email rmartin@inia.es

^BUniversidad Politécnica de Madrid. ETSI Montes. 28040 Madrid. Spain.

^CDpto. Medio Ambiente. INIA. Ctra. de la Coruña km, 7.5. 28040 Madrid, Spain.

Abstract

The purpose of this study was to quantitatively characterise and to provide a basic understanding of mercury concentrations in soils in order to distinguish the “natural” mercury contribution from that of human-induced pollution in two Spanish archipelagos. The Canary Islands are an archipelago formed by 7 islands of volcanic origin. They are located in the Atlantic Ocean to the southwest of Spain, near the Tropic of Cancer and the western Moroccan coastline. The Balearics (limestones) are formed by 4 islands located to the east of the Iberian Peninsula and to the extreme west of the Mediterranean Sea. This study uses geostatistical methods to assess mercury concentration in topsoil, as opposed to its content in the original matter. The level of mercury was higher in topsoil than rocky fragments, specifically in the Balearics where the mean mercury content was 61 µg/kg in topsoil and 11 µg/kg in rocky fragments. Maps of the spatial distribution indicate various areas with high top/rock mercury content that are linked to emissions from the nearby thermal power plant (Es Murterar). A significant portion of the increased mercury content in the Majorca island topsoil probably originates from atmospheric deposition.

Key Words

Mercury, soil pollution, heavy metals, geostatistics, spatial variability.

Introduction

Mercury is not abundant in nature, although its presence in soils poses an important risk (Mark and Ralph 2001). The naturally occurring concentration of mercury in arable soil depends primarily on the geological parent material. However, it has become widespread as a result of many industrial practices that often cause enrichment. Human activities, including combustion of fossil fuels, waste incineration, among others, have significantly increased the emission of Hg into the atmosphere. Mercury pollution is a major worldwide environmental problem with serious immediate and long-term implications for human health. In general, mercury accumulations in soils are associated with atmospheric deposition (Engle *et al.* 2005). The anthropogenic emission of Hg is about 60–80% of global Hg emissions. Mercury is an extremely volatile metal that can remain present in the atmosphere for between 0.5 and 2 years before being deposited in soil (Navarro *et al.* 1993), and may then be transported over long distances. Thermal power plants are an important source of mercury emissions. Coal-burning power plants are the largest single source of mercury pollution, and the only major source that governments do not regulate (Coequyt and Willes 1999). The “Mercury Falling” study found that an estimated 49 tons of mercury are emitted directly into the air by hundreds of coal-burning power plants in the U.S. each year (Coequyt and Willes 1999).

The characterisation of spatial variability is essential to achieve a better understanding of the complex relationships among soil properties, environmental factors and soil pollution. Geostatistical techniques, such as kriging, incorporate the spatial characteristics of current data into the statistical estimation, which classical statistical approaches ignore (Korre 1999; Lin 2002). One example of geostatistics applications to soil science is the estimation and mapping of some heavy metals by the existing spatial dependence between observations (Goovaerts 1997).

Materials and methods

Soil samples

The sampling scheme was based on an 8x8 km grid. Soil samples were collected in 2007. Each sample was defined as a composite made up of 21 sub-samples collected with the Eijkelkamp soil sampling kit from the upper 25 cm of soil in a cross pattern. Further details can be found in Rodríguez Martín *et al.* (2006).

Analytical methods

A standard soil analysis was carried out and soil texture was determined for each sample. Total Hg (THg) analyses were performed using a direct Hg analyzer (DMA80, an atomic absorption spectrophotometer, Milestone, Wesleyan University, Middletown, CT, USA). The results of this detection system were previously validated for solid and liquid matrices (EPA 7473). A calcareous loam soil, BCR-141 R, obtained from the European Commission Community Bureau of Reference, was used as a certified reference material to check the accuracy of the method. All the materials used for the Hg analysis in this study were acid-washed with 10% HNO₃ and carefully rinsed with ultrapure water (Milli-Q system, Bedford, MA). The limits of quantification (LOQ) and detection (LOD) were 0.6 µg/kg and 0.24 µg/kg, respectively.

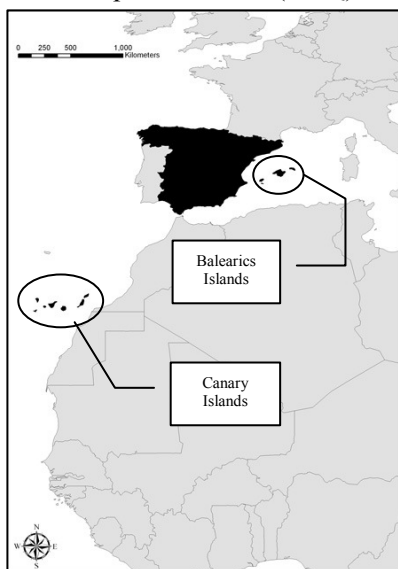


Figure 1. Location of two Spanish archipelagos. Study area.

Geostatistical analysis

A semivariogram was developed to establish the degree of spatial continuity of mercury among the data points and to establish the range of spatial dependence. The variogram γ is calculated using the relative locations of the samples (Lin, 2002) defined as (1):

$$\gamma(h) = \frac{1}{2n} \sum_{i=1}^{i=n} [Z(u_i) - Z(u_{i+h})]^2 \quad (1)$$

where $Z(u_i)$ is the value of Z at location u_i and $Z(u_{i+h})$ is the value of Z at a location separated from u_i by distance h .

The spherical model was used to fit the experimental semivariogram, and soil and rocky mercury contents were mapped by ordinary kriging (OK). There are many different models and kriging algorithms, most of which are reviewed in Goovaerts (1999) with references to soil applications. Textbooks (Gressie 1991; Goovaerts 1997; Kanevski and Maignan 2004) offer further detailed geostatistical methods.

Results and discussion

The statistics of the mercury contents are summarised in Table 1. The mercury topsoil concentration in this study fall between 12 and 350 µg/kg (mean 61 µg/kg) in the Balearics, and between 3 and 159 µg/kg (mean 33 µg/kg) in the Canary islands. The normal range in soils is 10–500 µg/kg (Alloway 1995). Using 4090 samples, Wu *et al.* (1991) established Hg levels of 100 µg/kg for natural or pristine areas, and of 200 µg/kg for agricultural and pastoral areas. In general, 300 µg/kg is the threshold value at which toxicity symptoms may occur. This critical value is exceeded only in two samples from the Balearics, and only seven samples are higher than 200 µg/kg.

Table 1. Statistical summary of Hg concentrations (in µg/kg).

| | | No. samples | Mean | Median | Stan. Dev. | 1 st Qu. | 3 rd Qu. | P 90 | P 95 | P 99 |
|---------|-----------|-------------|------|--------|------------|---------------------|---------------------|-------|-------|-------|
| Topsoil | Balearics | 125 | 61.1 | 39.09 | 63.51 | 28.29 | 59.33 | 116.9 | 225.2 | 327.9 |
| | Canaries | 193 | 33.2 | 20.39 | 32.94 | 12.13 | 42.78 | 82.72 | 95.56 | 158.9 |
| Rock | Balearics | 129 | 11.1 | 5.65 | 14.43 | 3.74 | 12.06 | 22.56 | 43.93 | 82.6 |
| | Canaries | 188 | 9.12 | 6.58 | 8.03 | 4.35 | 11.63 | 16.12 | 20.74 | 46.94 |

Stan. Dev: Standard deviation. 1st Qu, 3rd Qu: first and third quartile. P90, P95 and P99 percentiles = P 90, P 95 and P 99, respectively.

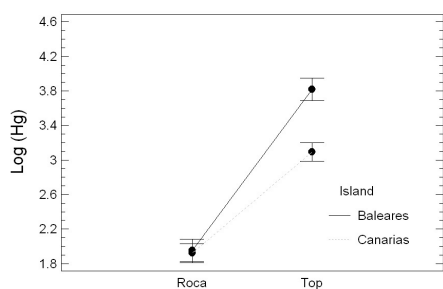
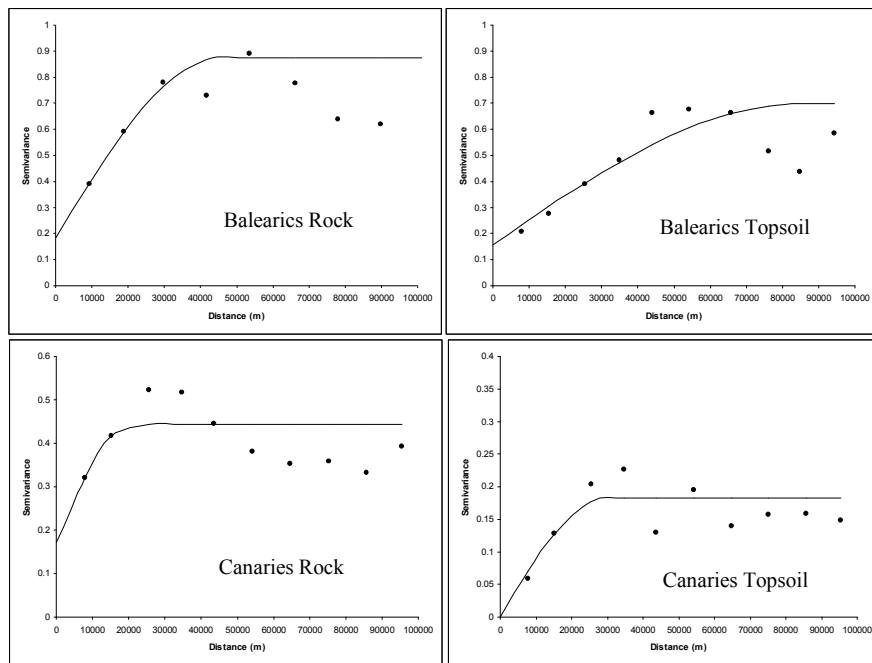


Figure 2. Top and rock mercury content in two

are considered to be strong and stable (Liu *et al.* 2003). Furthermore, mobility and retention are strongly affected by soil pH and carbonates.

The mercury in rock (original matter) is similar in both the Canaries (mean 9 µg/kg) and the Balearics (mean 11 µg/kg) (Figure 2), although it has different lithologies. The concentration ranges in rocky fragments do not present high values. In general, mercury contents tend to be higher in soils than in rock. Mercury contents usually tend to be higher in soils with high contents of clay and/or organic matter (Rodríguez Martín *et al.* 2006) due to the capacity of clay mineral to absorb cations. Organic carbon increases the binding capacity of soil for metals (Boluda 1988; Chen *et al.* 1999); this effect is due to the cation exchange capacity of organic material (Di Giulio and Ryan 1987). Complexes between Hg and organic matter

The spherical model was used to fit the experimental semivariograms (Figure 3). Spatial variation in the mercury content of rocky fragments can be attributed to inherent geographical properties and to geochemical processes that correspond to both mineralogical structures and a bedrock influence.



Variations in relation to the Hg concentration in topsoil can be largely attributed to major human perturbations such as industrialisation, agricultural practices, urban development, and a long list of many industrial practices.

In this study, ordinary kriging was used to map the mercury contents in rock and soil samples. The top/rock mercury content ratio (Figure 4) indicates three areas of high concentration levels.

Figure 3. Semivariogram for the topsoil and rock mercury values.

Soil contamination may be considered when the metal concentration in soil is eight times higher than the lithogenic content (the real geochemical baseline). These levels were higher in the Majorca island with areas whose lithogenic content is sixteen times higher. High map values were also located near the Es Murterar thermal power plant (Figure 5). This power plant used coal as fuel. Coal-burning power plants are an important source of mercury emissions (Coeqyt and Willes 1999).

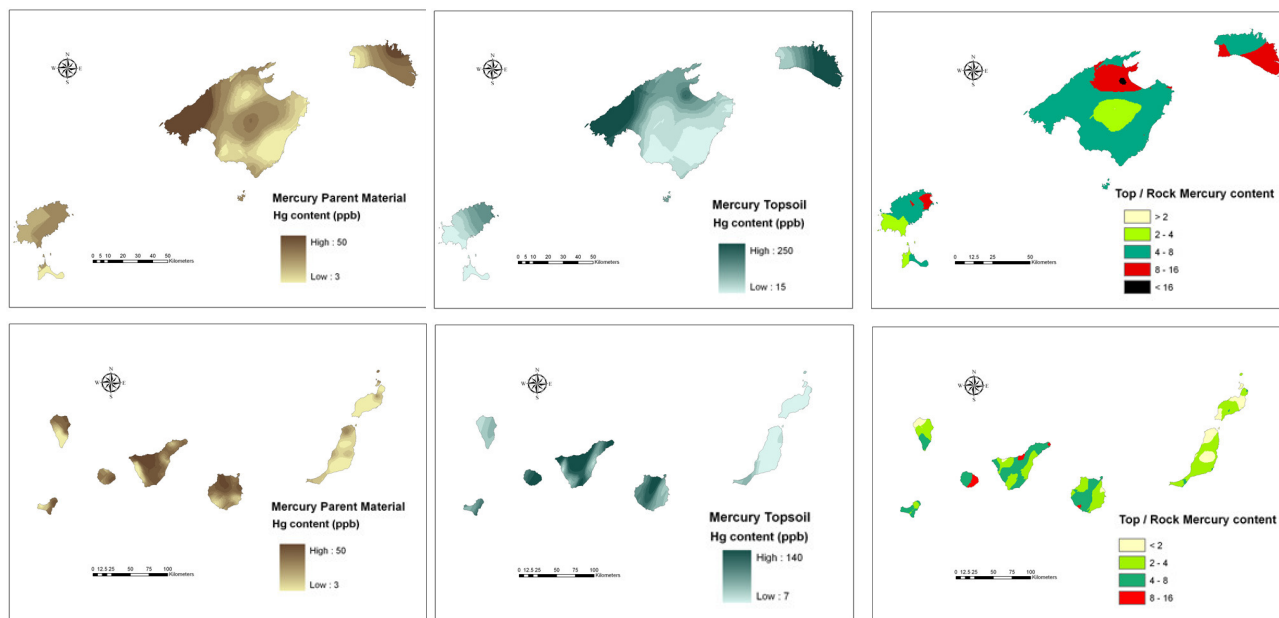


Figure 4. Kriging maps of the mercury content in top soil and parent material. Map showing

Acknowledgments:

This study was supported by MARM (Spanish Ministries of the Environment and Agriculture). We are also grateful to Ministerio de Ciencia e Innovacion, project: CGL2009-14686-C02-02 and to CAM project: P2009/AMB-1648 CARESOIL

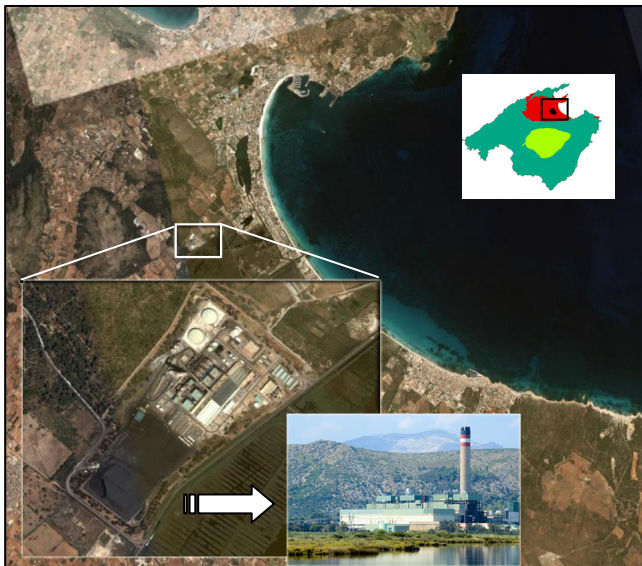


Figure 5. Es Murterar thermal power plant (Majorca).

Conclusion

A geostatistical analysis has been essential to understand the mercury pollution and spatial relationships.

The top/rock mercury content ratio was higher in Majorca (Balearics). A significant portion of the increased mercury content in topsoils of the Balearics originated from the Es Murterar thermal power plant. The specific amount of mercury cannot be determined on the basis of the data collected in this research, but this power plant is an important source of mercury pollution.

However, the soil of this Mediterranean region is principally calcareous, with an alkaline pH and a low organic matter, thereby helping to minimise the effect of mercury content.

References

- Alloway BJ (1995) *Heavy Metals in Soils*. Chapman & Hall, Glasgow, UK.
- Boluda R (1988) Relaciones estadísticas de los valores de metales pesados (Cd, Co, Cu, Cr, Ni, Pb y Zn) con el pH, contenido en materia orgánica, carbonatos totales y arcilla de los suelos de la comarca La Plana de Requena-Utiel (Valencia). *Anales de Edafología y Agrobiología* **47**, 1503-1524.
- Chen M, Ma LQ, Harris WG (1999) Baseline concentrations of 15 trace elements in Florida surface soils. *J Environ. Qual.* **28**, 1173-1181.
- Coequyt J, Willes R (1999) *Mercury Falling: An analysis of mercury pollution from coal-burning power plants*. Eds EWG. Washington USA.
- Di Giulio RT, Ryan EA (1987) Mercury in soil, sediments, and clams from a North Carolina peatland. *Water, Air and Soil Pollution* **33**, 205-219.
- Engle MA, Gustin MS, Lindberg AW, Ariya PA (2005) The influence of ozone on atmospheric emissions of gaseous elemental mercury and relative gaseous mercury from substrates. *Atmospheric Environment* **39**, 7506-7517.
- Goovaerts P (1999) Geostatistical in soil science: state-of-the-art and perspectives. *Geoderma* **89**, 1-45.
- Goovaerts P (1997) *Geostatistics for natural resources evaluation*. Applied geostatistics series. Oxford University Press, New York.
- Gressie NAC (1991) *Statistics for Spatial Data*. John Wiley, New York, USA.
- Kanevski M, Maignan M (2004) *Analysis and modelling of spatial environmental data*. Marcel Dekker EPFL press. Basel, Switzerland.
- Korre A (1999) Statistical and spatial assessment of soil heavy metal contamination in areas of poorly recorded, complex sources of pollution. *Stoch. Environ. Res. Assess.* **13**, 288-316.
- Lin YP (2002) Multivariate geostatistical methods to identify and map spatial variations of soil heavy metals. *Environmental Geology* **42**, 1-10.
- Liu R, Wang Q, Lu X, Fang F, Wang Y (2003) Distribution and speciation of mercury in the peat bog of Xiaoxing'an Mountain, northeastern China. *Environmental Pollution* **124**, 39-46.
- Mark OB, Ralph RT (2001) Bioaccessibility of mercury in soil. *Soil and Sediment Contamination* **10**, 301-316.
- Navarro M, López H, Sánchez M, López M (1993) The effect of industrial pollution on mercury levels in water, soil, and sludge in the coastal area of Motril, Southeast Spain. *Archives of Environmental Contamination and Toxicology* **24**, 11-15.
- Rodríguez Martín JA, López Arias M, Grau Corbí JM (2006) Heavy metal contents in agricultural topsoils in the Ebro basin (Spain). Application of multivariate geostatistical methods to study spatial variations. *Environmental Pollution* **144**, 1001-1012.
- Wu Y, Zhou Q, Adriano DC (1991) Interim environmental guidelines for cadmium and mercury in soils of China. *Water, Air and Soil Pollution* **57-58**, 733-743.

Monitoring soil properties and heavy metals concentrations in reclaimed mine soils from SE Spain by application of different amendments

Raúl Zornoza, Dora M. Carmona, Rosa M. Rosales, Ángel Faz, Asuman Büyükkiliç, Sebla Kabas and Andrea Zanuzzi

Sustainable Use, Management, and Reclamation of Soil and Water Research Group. Department of Agrarian Science and Technology. Technical University of Cartagena. Paseo Alfonso XIII, 52, 30203 Cartagena. Murcia. Spain. E-mail: angel.fazcano@upct.es

Abstract

Mine soils stabilization is an in situ remediation method that uses inexpensive amendments to reduce heavy metals availability and increase soil organic matter, nutrients and water retention in polluted soils. We tested the long-term effects of several amendments (pig manure, sewage sludge and marble mud) on the immobilization of heavy metals and improvement in soil physico-chemical properties in abandoned tailing ponds from SE Spain affected by former mining activities. Results showed that an initial increase in pH close to neutrality occurred and remained practically stable with time. However, soil organic matter decreased owing to mineralization and leaching. Total contents of heavy metals remained stable for a long time, but bioavailable metals decreased in the amended plots. However, increments in Pb and Zn in El Lirio, and in Cd and Zn in Brunita in the amended plots after 5 years of monitoring were observed. The main reasons could be slight decreases in pH, water and wind erosion of surface particles that provokes shifts in bioavailable metals, decrease in soil organic matter, which initially immobilized metals, and release of root exudates by colonizer plants in amended plots, which tend to mobilize nutrients and as a consequence metals.

Key Words

Metal pollution, in situ remediation, pig slurry, swage sludge, marble waste, long-term effects.

Introduction

In SE Spain, there are many mining tailing ponds due to intensive mining activities. Although mining activity was abandoned decades ago, those ponds with high amount of heavy metals still remain in the area. As a consequence, these mine soils have scarce or null vegetation due to very poor properties, including extremely low soil organic matter. Since a long time those mine residues has been transported downstream during periods of high flow, erosion is evident in these areas, causing migration of pollutants into surface and ground water. Thus, efforts are needed for long-term reclamation of these contaminated areas to stabilise soil metals, create a structured soil and avoid health risks in the ecosystems. The transformation of metals into harmless species or their removal in a suitable recycled mineral form such as carbonates using marble wastes or lime (Geebelen *et al.* 2003) is a possible solution for the remediation of a mining area. In addition, incorporation of organic amendments into contaminated mine soils has been proposed as feasible, inexpensive and environmentally sound disposal practice leading to an improvement of soil characteristics needed for microorganisms and plants development (Barker 1997). Thus, the objective of this study is to monitor the evolution of some physico-chemical properties and availability of heavy metals during five years to assess the capacity of several amendments for long-term immobilization of heavy metals and recover soil properties to guarantee true landscape rehabilitation.

Methods

The study was conducted in the province of Murcia (SE Spain), in the Cartagena-La Unión Mining District. The climate of the area is semiarid Mediterranean with mean annual temperature of 18°C and mean annual rainfall of 275 mm. Two tailing ponds generated by mining activities were selected: El Lirio (L) and Brunita (B), representative of the rest of existent ponds in Cartagena-La Unión Mining District. The field trial was established in 2004. Plots (2 m x 2 m) were randomised replicated 3 times. Two different organic amendments were used to reclaim the soils, pig manure (P) and sewage sludge (S). In addition, 3 different doses per amendment were applied. Thus, the treatments were: Untreated contaminated soil (C), soil treated with pig manure at dose 1 (P1), dose 2 (P2) and dose 3 (P3); and soil treated with sewage sludge at dose 1 (S1), dose 2 (S2) and dose 3 (S3). For pig manure, doses were 2.5, 5 and 10 kg per plot, respectively. For sewage sludge, doses were 1.99, 3.98 and 7.97 kg per plot, respectively. With the purpose of increasing soil pH to immobilise metals marble mud was applied in all plots except for control, at the rate of 22 kg/ plot.

The soil samplings were carried out previous to the application of amendments (time 0), and at 6 months, 1, 2 and 5 years after application of amendments. One sample (0-15 cm depth) was collected for each plot, taken to the lab, air-dried, passed through a 2-mm sieve and stored at room temperature. Soil pH and electrical conductivity (EC) were measured in deionised water (1:2.5 and 1:5 w/v, respectively). Soil organic carbon (SOC) and total nitrogen (N_t) were determined according to Duchaufour (1970). For the quantification of the total metals an acid digestion was used and bioavailable metals were extracted with DTPA. Metals were measured using atomic absorption spectrophotometer (AAAnalyst 800, Perkin Elmer).

Results and discussion

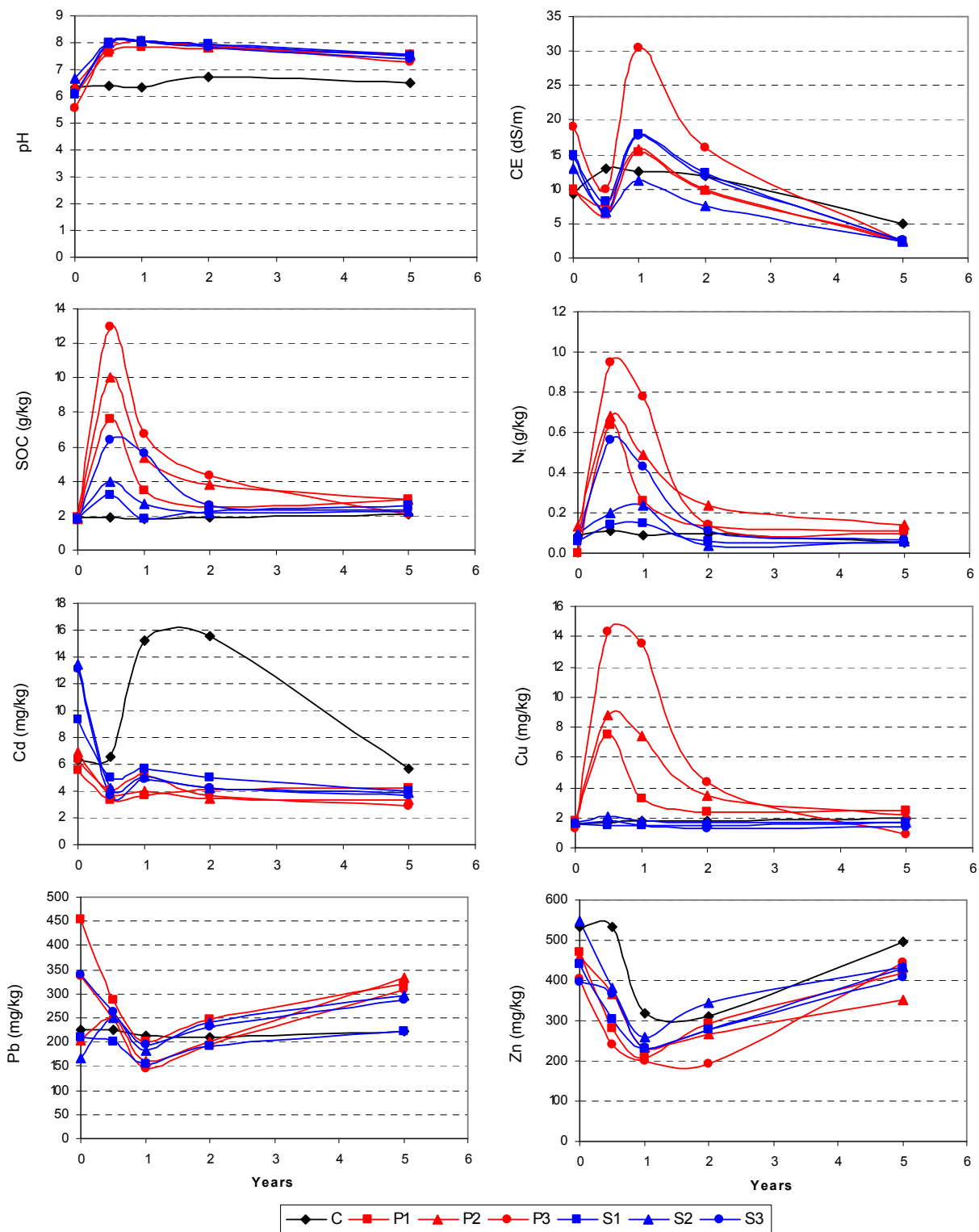


Figure 1. Evolution of pH, electrical conductivity (EC), soil organic carbon (SOC), total nitrogen (N_t) and bioavailable metals in El Lirio plots (see the text for the meaning of plots abbreviations).

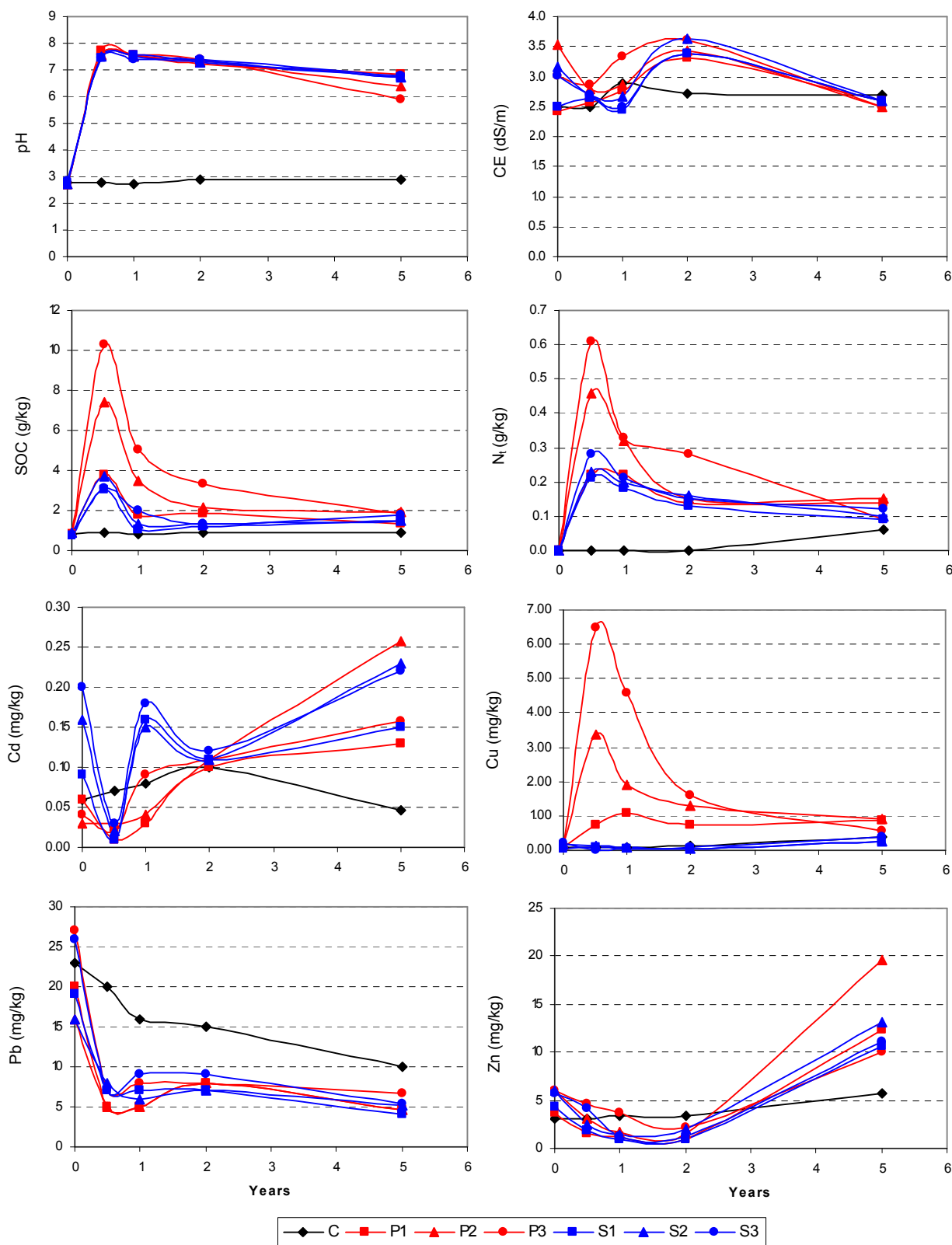


Figure 2. Evolution of pH, electrical conductivity (EC), soil organic carbon (SOC), total nitrogen (Nt) and bioavailable metals in Brunita plots (see the text for the meaning of plots abbreviations).

The evolution of soil properties and available metals for 5 years are represented in Figures 1 (El Lirio) and Figure 2 (Brunita). After the application of amendments, we observed an increase in pH in both ponds, being higher in Brunita, since the initial pH value in this pond was extremely low (<3). The pH remained practically stable with time, without differences among treatments, although it tended to decrease after 5 years, mainly due to decreases in carbonates content (data not shown). With regards to EC, we observed an increment after one year of amendments application, owing to the high quantity of salts provided by the

organic amendments and the solution of carbonates. After two years, there is a decreasing trend in EC, owing to leaching of soluble ions. SOC and N_t initially increased with the application of amendments, mainly in P plots. This increase was in general terms related with the dose of application. However, the values of these two properties decreased owing to leaching and mineralization, shifting down after 5 years of applications to values slightly higher to control.

Regarding total metals, Cd, Cu, Zn and Pb were above European legislation thresholds, and did not change with time (data not shown). Bioavailable metals decreased as general pattern in the amended plots in both ponds. However, we detected slight increments in Pb and Zn in El Lirio, and in Cd and Zn in Brunita in amended plots after 5 years of monitoring. The possible explanation for this behavior is difficult owing to the different factors implied in the mobility of heavy metals and their interactions with soil properties. The detected slight decreases in pH and solubilization of carbonates may have likely contributed to increments in bioavailability of some metals. Moreover, the fact that we have also detected increments in the control plot in some metals could be indicating changes in bioavailability of these metals owing to water and wind erosion of surface particles that migrate to other zones, thereby exposing subsurface soils. In addition, the parent material is rich in Cd, Pb, Cu and Zn sulfides. Thus, oxidation processes of these sulfides may have also released some metals to the soil. On the other hand, decreases in soil organic matter could have had an important effect, since the application of organic amendments initially immobilized metals by complexation (Zanuzzi 2007). Bioavailable Pb and Zn in El Lirio were positively correlated with plant richness and vegetation cover, whilst Cd and Zn were also positively correlated with plant richness in Brunita. This could indicate that the natural establishment of vegetation could be influencing the availability of these metals. In fact, plant roots are known to exude organic compounds capable of complexing metals, which can increment the metals availability in the rhizosphere, and this process differs among different plant species (Jones 1998; Almeida *et al.* 2006). Plants release some labile compounds to soil to promote the availability and uptake of nutrients, provoking also the availability and uptake of heavy metals (Séguin *et al.* 2005).

Conclusion

The application of pig manure and sewage sludge together with marble wastes has proved to be effective for long-term decrease in the bioavailability of most toxic heavy metals present in two tailing ponds from SE Spain, besides maintaining pH close to neutrality. Despite the initial decrease in SOC and N_t, mineralization and leaching have led to levels of organic matter only slightly higher in comparison to control plots. Since increments in some bioavailable metals have been monitored, future studies are needed to determine the causes, or mitigate this trend by new applications of organic amendments.

Acknowledgements

This work has been funded by the Project Nr.: CP-IP 213968-2 IRIS, funded by the European Union FP7.

References

- Almeida CMR, Mucha AP, Vasconcelos MTSD (2006) Comparison of the role of the sea club-rush *Scirpus maritimus* and the sea rush *Juncus maritimus* in terms of concentration, speciation and bioaccumulation of metals in estuarine sediment. *Environmental Pollution* **142**, 151-159.
- Barker AV (1997) Composition and uses of compost. In 'Agricultural uses of by-products and wastes'. (Eds JE Rechling, HC Mackimom) pp. 140-162. (ACS Symposium Series No. 668, Vol 10. American Chemical Society, Washington DC).
- Duchaufour P (1970) Précis de Pedologie. Masson and Cie, Paris.
- Geebelen W, Adriano DC, van der Lelie D, Mench M, Carleer R, Clijsters H, Vangronsveld J (2003) Selected bioavailability assays to test the effect of amendment-induced immobilization of lead in soils. *Plant and Soil* **249**, 217-228.
- Jones DL (1998) Organic acids in the rhizosphere – a critical review. *Plant and Soil* **205**, 25-44.
- Séguin V, Gagnon C, Courchesne F (2004) Changes in water extractable metals, pH and organic carbon sequestrations at the soil-root interface of forested soils. *Plant and Soil* **260**, 1-17.
- Zanuzzi A (2007) Reducción de impactos ambientales asociados a suelos contaminados por metales pesados mediante el uso de residuos antropogénicos. PhD Dissertation, Departamento de Ciencia y Tecnología Agraria. Universidad Politécnica de Cartagena.

Phytoremediation of arsenic contaminated soil and water

Mahimairaja Santiago^A and Nanthi S. Bolan^B

^ADepartment of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore, India, Email rajasmahimai@yahoo.co.in

^BCentre for Environmental Risk Assessment and Remediation, University of South Australia, Mawson Lake, SA

Abstract

Arsenic-contamination of soil and water is widespread which poses a serious threat to plants, animals and humans. There has been growing interest in developing remediation of As-contaminated ecosystem. Studies were conducted to examine the uptake of Arsenic (III) and Arsenic (V) by mustard plants from water and soil. The results have shown that the As content and uptake by mustard plants were significantly affected due to varied levels of As (As III and As V) and P in soil and water. Increase in As concentration markedly increased the As uptake by crop and the effect was more pronounced on As (V) than As (III). Addition of P was found to inhibit the uptake and accumulation of As in plants. In general, roots accumulated large amount of As than shoots and flowers. Phosphate addition was found to decrease the bioavailability of As, particularly, As (III). The two soils (Egmont and Manawatu) significantly differed in influencing the bioavailability of As. However, at high rate (500 mg/kg) of P application was found to increase the bioavailability and thus As uptake by plants. The results provided evidence for P-induced As mobilization in contaminated soil at high rate of P addition. Introduction of earthworms to contaminated soil also increased the bioavailability of As.

Key Words

Arsenic, Phytoremediation, biotransformation, bioavailability, phosphate.

Introduction

Arsenic (As) is a toxic metalloid which enters terrestrial and aquatic ecosystems through both natural (geological) processes and anthropogenic (industrial and agricultural) activities. Arsenic-contamination of soil and water is widespread which poses a serious threat to plants, animals and humans. Arsenic is a unique carcinogen. Therefore, there is a growing interest in developing regulatory guidelines and remediation technologies for mitigating As-contaminated ecosystems. A range of technologies, including bioremediation, has been applied with varying levels of success either to remove As from the contaminated medium or to reduce its bio-toxicity. Plants are increasingly being used to enhance the removal of As. This technology is known as Phytoremediation and it attracts intensive research and commercial interests. The effectiveness of this technology is, however, variable and depends on several factors associated with soil and plant characteristics and the chemistry of As in the environment. Several soil amendments like lime, phosphate (P), compost etc., are used to enhance the effectiveness of phytoremediation. In the current study we examined the potential of mustard crop in hyper accumulating the As from soil and water and the impact of P on As uptake by crop.

Methods

Hydroponic experiment

A hydroponics experiment was conducted to examine the P-induced As uptake by Indian mustard crop (*Brassica juncea*). The experimental set up consists of plastic containers (15 × 15 × 15 cm) which were placed in long PVC trays where the water was continuously circulated to prevent excessive evaporation of the nutrient solution. Provision was made for aerating the nutrient solution in the container by inserting a rubber scrubber and continuous aeration was done. Seeds of mustard plants were germinated separately in small pots filled with vermiculite as inert media. After ten days, seedlings of even size were carefully selected and transferred along with the vermiculite pots to plastic containers containing 1.75 litres of nutrient solutions. After a week of acclimatization of the plants in the hydroponics culture, the nutrient solution was replaced with fresh nutrient solution containing varied levels of As either As (III) or As(V), in combination with different concentration of P. There were three replicates of each treatment and the containers were placed randomly within a long plastic tray. After eight weeks the individual plants were harvested by carefully removing from the vermiculite-pots. The shoots, roots and flowers were separated, oven-dried at 60°C for 72 hours and analysed for As by using a Graphite Furnace Atomic Absorption Spectrometry (GFAAS).

Pot Experiment-I

A glass house pot experiment was conducted using two soils viz., Egmont (a high P fixing soil) and Manawatu, (silt loam, low P fixing soil) differ in chemical characteristics. Some important characteristics of soils are given in Table 1.

Table 1. Some important characteristics of Egmont and Manawatu soils

| Characteristics | Egmont | Manawatu |
|---------------------|-----------|---------------|
| pH | 5.21 | 6.01 |
| Organic C (g/kg) | 78.5 | 29.1 |
| P retention (%) | 83 | 33 |
| CEC (cmol/kg) | 26.2 | 7.6 |
| Dominant clay | Allophane | Mica / illite |
| Total As (mg/kg) | 13.6 | 6.7 |
| Acid oxalate Al (%) | 3.54 | 0.09 |
| Acid oxalate Fe (%) | 1.63 | 0.31 |

Bulk soil samples were air-dried and crushed to pass through 5-mm screen to ensure homogeneity of soil before use in the glasshouse pot study. A sub-sample of 1000 g of soil was weighed into heavy grade plastic bags. The soils were mixed with graded levels of As (0, 50, 100 and 200 mg/kg) as arsenite [As(III)] or arsenate [As(V)] and thoroughly mixed. The moisture content was brought to approximately 75 per cent of field capacity and the As-spiked soils were incubated at 25°C for 60 days. At the end of 60 days phosphate solutions at a rate equivalent to 0, 100, 250, 500 mg P/kg as KH_2PO_4 were added to soil and the incubation was continued for further 60 days. At the end of 60 days the soils were transferred into 9-inch plastic pots (1725 cm³). The soil in each pot was slightly compacted to a bulk density of approximately 1 g/cm³ and the moisture content was brought to field capacity with distilled water. Approximately 10 to 15 seeds of Indian mustard (*Brassica juncea*) were sprinkled on soil surface and gently covered with soil. After germination the plants were thinned and only three plants per pot were allowed to grow. After eight weeks the individual plants were harvested and analysed for As. The dynamics and bio-availability of As in soils were examined by determining the amount of readily labile As extracted by 0.05 M $(\text{NH}_4)_2\text{SO}_4$. The speciation of As in soils spiked with As(III) and As(V) was determined for selected treatments as per the fractionation procedure outlined by Wenzel *et al.* (2001).

Pot Experiment-II

The As-contaminated soil samples were collected from an abandoned sheep dip site in Hamilton, New Zealand. About 1500 g of air-dried soil was placed in heavy grade polyethylene bags. Phosphate solutions as KH_2PO_4 were added to soil at a rate equivalent to 0, 100, 250 and 500 mg P/kg and the moisture content was adjusted to field capacity with distilled water. The soil and P solutions were thoroughly mixed in polyethylene bags and incubated at 25°C for 60 days. At the end of 60 days the soil was transferred into 9-inch plastic pots (1725 cm³). The soil in each pot was slightly compacted to a bulk density of approximately 1 g/cm³ and the moisture content was brought to field capacity with distilled water. Approximately 10 to 15 seeds of Indian mustard (*Brassica juncea*) were sprinkled on soil surface and gently covered with soil. Pots were randomised on the bench and their positions were changed weekly to minimize variations in microenvironment. After germination the plants were thinned and only three plants per pot were allowed to grow. Initially for two weeks 100 ml of nutrient solution was added to all pots. At alternate days the plants were watered and grown for eight weeks. After eight weeks the individual plants were harvested and As content was determined.

In another set of treatment, approximately 1500 g of soil was placed in heavy grade polyethylene bags and added with different rates of P (0, 100, 250 and 500 mg P/kg). The soil and P solutions were thoroughly mixed in polyethylene bags and incubated at 25°C for 60 days. There were four treatments replicated four times. At weekly intervals the soil was mixed and moisture content was adjusted for any weight loss. At the end of 60 days the soil was transferred into 9-inch plastic pots (1725 cm³). The pots were kept as such without growing any plants. At weekly intervals the soil was mixed and moisture content was adjusted for any weight loss. The effect of earthworms on the bioavailability of As in the contaminated soil was examined by incubating the contaminated soil (500 g) with 10 medium sized earthworms, which were collected from the contaminated soil. This treatment was replicated three times. This incubation was conducted for 180 days. The soil samples were analysed for various fractions of As. The plant samples were dried and As was determined using a Graphite Furnace Atomic Absorption Spectrometry (GFAAS). The

dynamics and bio-availability of As in soils were examined by determining the amount of readily labile As extracted by 0.05 M (NH₄)₂SO₄.

Results

Arsenic uptake from water

The presence of P in nutrient solution significantly decreased both As(III) and As(V) uptake by crop. The crop removal of As from solution ranged from 252 to 876 mg/kg. Significant difference was observed due to different levels of As and P. In general, crop removal of As(V) was relatively higher than As(III). Addition of P markedly reduced the plant uptake of As (Table 2).

Table 2. Effect of As and P on As uptake by mustard plants

| Treatments | Arsenic (mg/kg) | | |
|--------------------------|-----------------|---------------------|----------------|
| | P0 | P (0.005 mmol) | P (0.025 mmol) |
| 1. No As(III) | 0.0 | 0.0 | 0.0 |
| 2. As (III) (0.005 mmol) | 270.2 | 416.5 | 291.2 |
| 3. As(III) (0.025 mmol) | 623.7 | 467.0 | 513.1 |
| 4. No As(V) | 0.0 | 0.0 | 0.0 |
| 5. As (V) (0.005 mmol) | 252.0 | 254.0 | 456.0 |
| 6. As(V) (0.025 mmol) | 876.0 | 472.7 | 603.0 |
| LSD (0.05) | As:78.8, | P:39.7, As × P : NS | |

The relative distribution of As in plants shows that Brassica sp. accumulated As mainly in the roots followed by shoots and flower. On a dry weight basis, the roots contained the highest mean As concentration being several fold higher than As in shoots and flowers. Only a small amount of As was found accumulated in flowers. Upon uptake mustard plants accumulated about 69 to 75% of As in roots. The As concentration in roots, shoots and flowers were highly influenced by both As and P levels in solution. Both As and P addition markedly reduced the As accumulation in roots. Arsenic concentrations in shoots of plants have been reported to depend mainly on the root system activity (Carbonell-Barrachina *et al.* 1997).

Arsenic uptake by mustard and relative distribution in different parts

The uptake of As by mustard was significantly influenced by varied levels of As both in the presence and absence of P. Increase in As(III) markedly increased the As uptake. The highest value was observed at an application rate of 200 mg/kg. Increase in P levels up to 250 mg/kg notably increased the As uptake and at 500 mg/kg the As uptake was found decreased. This trend was observed with both As(III) and As(V) application. Arsenic uptake was relatively higher in Manawatu soil than Egmont soil. The As uptake varied between 29.5 and 209 mg/kg for As(III), whereas it was from 7.42 and 283.4 mg/kg for As(V). Such variation could be due to differential chemical and mineralogical properties of these two soils. While P application at 100 mg/kg remarkably increased the As uptake, further increase in P levels significantly reduced the As uptake. The highest uptake was due to the combined application of 200 mg As/kg and 100 mg P/kg.

Relative distribution of As in different parts

The relative distribution of As in different parts of mustard is depicted in Figure 1. The results showed that the mustard plants accumulated As primarily in the roots. Only relatively low quantities of As were found translocated to the shoot and flowers. On a dry weight basis, the roots contained the highest mean As concentration being several fold higher than As in shoots and flowers. Only a small amount of As was found accumulated in flowers with the application of As(V). Upon uptake brassica accumulated about >70% of As in roots. The As concentration in roots, shoots and flowers were highly influenced by both the As and P levels in solution. Both As and P addition had markedly reduced the As accumulation in roots and shoots.

Dynamic, bioavailability and speciation of As in soil

Phosphate treatment was found to decrease the bioavailability of As in soil, particularly when added as As(III). Consistently up to 4 months, P application remarkably increased the (NH₄)₂SO₄-As during incubation. This could be due to P induced mobility of As in soil. However, the soils collected after the harvest of crops have shown marked reduction in the (NH₄)₂SO₄-As due to P applications. A comparison of the data on (NH₄)₂SO₄-As in Egmont and Manawatu soils after the harvest of crops showed that the soils differed markedly with regard to P effect. In Egmont soil, increase in P levels appeared to decreased the bioavailability of As(III) and As(V), whereas in Manawatu soil, increase in P levels increased the bioavailability of both As(III) and As(V).

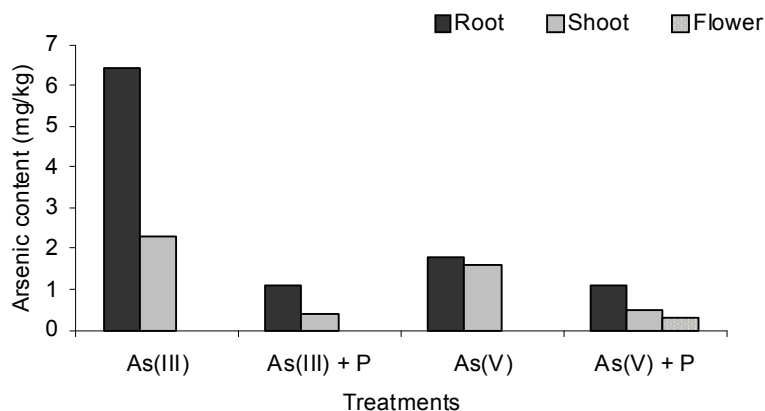


Figure 1. Relative distribution of As in plant parts

The data on the bioavailability of As in the contaminated soil as measured by the 0.05 M (NH₄)₂SO₄ extractable As showed that the addition of P remarkably increased the bioavailability of As in the contaminated soil. Initially, the concentrations of (NH₄)₂SO₄-As in soil was only 171 µg/kg which was markedly increased due to P application. This trend was also observed in contaminated soil incubated with P but without any plants. Further, P application remarkably decreased the specifically-sorbed As [as extracted in 0.05 M (NH₄)H₂PO₄] in soil. This provides clear evidence for the P induced mobility of As in soil. It is interesting to observe that the bioavailability of As in the contaminated soil was tremendously increased due to the introduction of earthworms. Earthworms also appeared to have enhanced the adsorption of As in soil as the data have shown high values for sorbed-As [as extracted in 0.05 M (NH₄)H₂PO₄]. Large amounts of As (25 mg/kg) was also found accumulated in the earthworm tissues.

Acknowledgments

We thank the Massey University, Palmerston North, New Zealand for awarding a Postdoctoral fellowship to undertake the research and Tamil Nadu Agricultural University, Coimbatore, India for granting study leave.

Reference

- Carbonell-Barrachina AA, Burlo F, Burgos-Hernandez A, Lopez E, Mataix J (1997) The influence of arsenite concentration on arsenic accumulation in tomato and bean plants. *Scientia Hort* **71**, 167-176.
- Wenzel WW, Kirchbaumer N, Prohaska T, Stingeder G, Lombi E, Adriano DC (2001) Arsenic fractionation in soils using an improved sequential extraction procedure. *Analytica Chimica Acta* **436**, 309-323.

Potential use of *MuSI* transgenic tobacco for phytoremediation of the soils contaminated with Cd

Kye-Hoon Kim^A and Young-Nam Kim^A

^ADept. of Environmental Horticulture, The University of Seoul, Dongdaemun-Gu, Seoul, Korea, Email johnkim@uos.ac.kr

Abstract

This study was carried out to identify the potential for phytoremediation of soils contaminated with Cd using *MuSI* (line 6) transgenic tobacco. The experiment was composed of two parts: i) Effect of cadmium treatment on germination rate; and ii) Effect of cadmium treatment on growth with hydroponic system. *MuSI* tobacco seeds germinated and grew better than wild-type tobacco seeds. The tolerance of *MuSI* transgenic tobacco to Cd stress was better than that of wild-type tobacco at all Cd treatment levels. Especially, wild-type tobacco showed chlorosis and withering with 200 μ M Cd treatment, but *MuSI* tobacco gradually recovered from Cd damage. Results of this study showed potential for phytoremediation of soils contaminated with Cd using *MuSI* transgenic tobacco. They also indicated potential for biofuel production from the area where soils are contaminated with Cd using *MuSI* transgenic tobacco.

Key Words

MuSI gene, cadmium, phytoremediation, chlorosis.

Introduction

Soil contamination by human being goes back as far as the Bronze Age (2500 B.C.) (Kabata-Pendias and Mukherjee 2007). Industrial activities such as mining and smelting of metalliferous ores, brick and pipe manufacture as well as power generation and agricultural practices significantly contributed to soil contamination. Remediation of the contaminated soil and water is very popular not only scientifically but also for business aspects. Phytoremediation is a promising soil remediation technique among many remediation techniques including chemical, physical, biological and thermal ones. One of the disadvantages of the phytoremediation is that the amount of the biomass of most of the hyperaccumulators is not enough. If a plant has greater biomass as well as deep root system it would be perfect for phytoremediation. Since it is not easy to find such a plant, scientists try to prepare such plant with the help of genetic engineering. The *MuSI* is known to a multiple stress related gene with several lines extracted from sweet-potato (*Ipomoea batatas* L. cv. Yulmi). The previous study using RT-PCR showed that the expression of *MuSI* gene in tobacco plant induced the tolerance to cadmium stress. There the objective of this study was to identify potential of *MuSI* (line 6) transgenic tobacco for phytoremediation of the soils contaminated with Cd.

Materials and methods

Materials

MuSI (line 6) transgenic tobacco and wild-type tobacco were used in this study. The previous study using RT-PCR showed that the expression of *MuSI* gene in tobacco plant induced the tolerance to cadmium stress (Yang, 2009). The total genomic DNA of *MuSI* gene was extracted from sweet-potato (*Ipomoea batatas* L. cv. Yulmi) using the modified CTAB method (Kim and Hamada 2005).

Methods

MS medium with 0, 50, 100 and 200 μ M Cd was used to compare germination rate between *MuSI* (line 6) transgenic tobacco seeds and wild-type tobacco (control) seeds. At each Cd concentration, fifteen seeds of each tobacco were placed in petri dish. Germination rate was determined after two weeks.

To find out effect of Cd stress on the growth of tobacco plants, *MuSI* (line 6) transgenic tobacco and wild-type tobacco seedlings were transplanted 2 weeks after germination and were cultivated in a hydroponic system with 0, 50, 100 and 200 μ M Cd treatment for 3 weeks (Kawashima *et al.* 2004). Three weeks later tobacco were harvested and fresh weight, dry weight, shoot length and relative damage content (RDC) of the leaves were measured. RDC is defined as number of damaged leaves relative to number of total leaves. After measuring fresh weight, dry weight, length of shoot and Cd damage of leaves, both *MuSI* (line 6) transgenic tobacco and wild-type tobacco (control) were separated into leaf, shoot and root, and then dried at 65 $^{\circ}$ C for 3 days. The concentration of Cd in each part of the plant was determined by AAS (Cresser and Parsons 1979) after digestion with mixed acid (conc. H₂SO₄:60% HClO₄= 1:10, v/v).

Results and discussion

Germination rate

MuS1 (line 6) tobacco seeds showed better germination rate and growth than wild-type tobacco seeds. Especially, 100% of *MuS1* (line 6) tobacco seeds germinated with 100 μM Cd treatment at 14 days (Figure 1). There was visual difference of growth between *MuS1* (line 6) transgenic tobacco and wild-type tobacco. The cotyledon of *MuS1* (line 6) transgenic tobacco seedling was greener and bigger than that of the wild-type tobacco. The elongation of the roots of *MuS1* (line 6) transgenic tobacco the seedlings was better than that of wild-type tobacco.

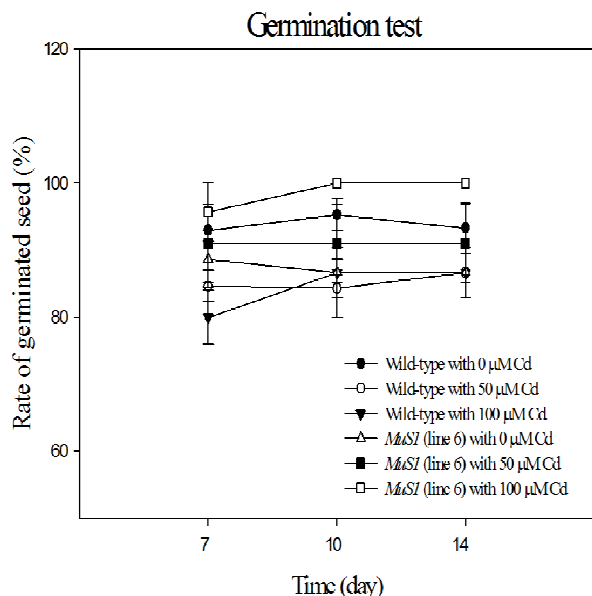


Figure 1. Comparison of the germination rate between *MuS1* (line 6) tobacco and wild-type tobacco with 0, 50, 100 μM Cd treatment for 2 weeks.

Growth experiment

Figure 2 shows fresh weight and dry weight of wild-type tobacco and *MuS1* transgenic tobacco with different Cd treatment. The growth of both plants decreased with increasing Cd concentration. The fresh and dry weight of *MuS1* transgenic tobacco was more than that of wild-type tobacco with 0-100 μM Cd treatment except for 200 μM Cd. Starting from the second day of the experiment, all of tobaccos with Cd treatment showed damage of leaves to Cd stress. Damaged leaves gradually got chlorosis and withered from undermost. With 200 μM Cd treatment, the leaf damage of the wild-type tobacco became worse showing chlorosis and withering, whereas *MuS1* (line 6) transgenic tobacco gradually recovered from Cd damage from the tenth day of the experiment. *MuS1* transgenic tobacco expressed tolerance to cadmium stress at all Cd concentration, showing withered leaves at only some of the lower parts.

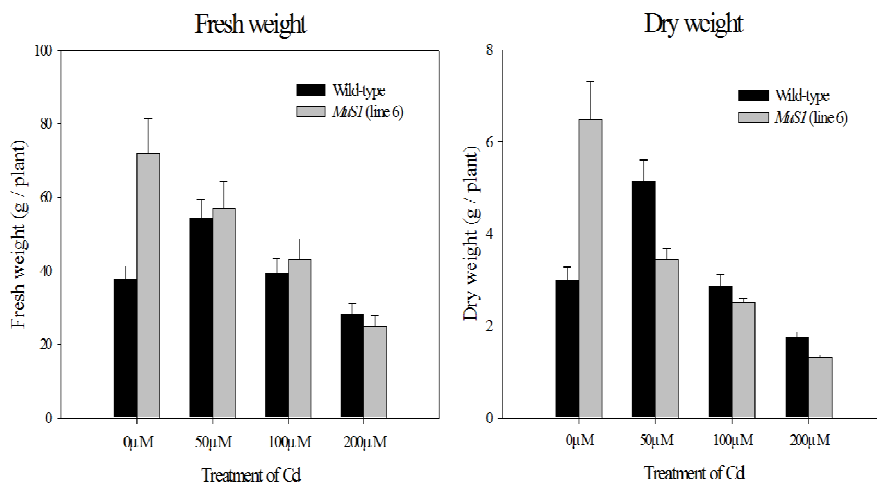


Figure 2. Comparison of the fresh and dry weight between *MuS1* (line 6) transgenic and wild-type tobacco treated with 0, 50, 100, 200 μM Cd.

Figure 3 (a) shows RDC at harvest. RDC difference between wild-type tobacco and *MuSI* transgenic tobacco increased with increasing Cd concentration, indicating Cd tolerance of *MuSI* transgenic tobacco. Figure 3 (b) shows effect of 200 μM Cd treatment on growth of wild-type tobacco and *MuSI* transgenic tobacco.

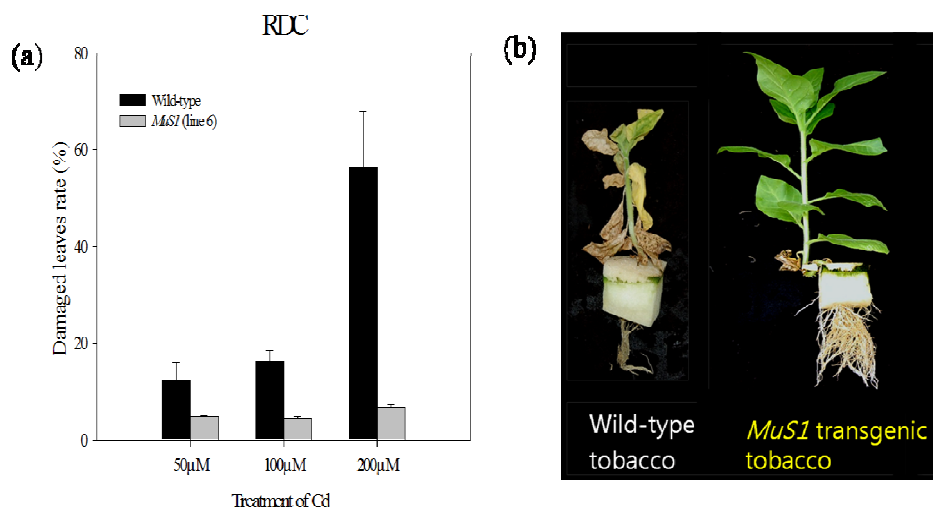


Figure 3. Comparison of the growth of *MuSI* transgenic tobacco and wild-type tobacco with Cd treatment: (a) Relative damage contents (RDC) of leaves of both tobacco with 50, 100, 200 μM Cd; (b) Symptoms of both tobacco to Cd stress with 200 μM Cd at harvest.

Concentration and accumulation of Cd

Expression of the Cd resistant *MuSI* gene resulted in less Cd uptake by *MuSI* (line 6) transgenic tobacco than by wild-type tobacco with 100 and 200 μM Cd treatment. Wild-type tobacco accumulated more Cd (2.28 mg per plant) with 200 μM Cd treatment than *MuSI* (line 6) transgenic tobacco (Table 1). Rate of Cd translocation from root to leaves was 81.8 % for wild-type tobacco, while 37.1 % for *MuSI* transgenic tobacco. *MuSI* gene may reduce the translocation of Cd from roots to shoot, leading to an overall decrease of Cd in leaves, which was similar to the results by Maiti *et al.* (1989). According to these results, the mechanism of the recovery of the *MuSI* (line 6) transgenic tobacco plant is not by high level of Cd uptake and accumulation in the plant but by revealing resistance to Cd through inducing less Cd uptake and/or more Cd immobilization around roots, resulting in less translocation to shoot.

Table 1. Cd concentration and accumulated amount for *MuSI* (line 6) transgenic tobacco and wild-type tobacco in root, shoot and leaf with 50, 100, 200 μM Cd treatment at harvested.

| Treatment of Cd | Root | | Shoot | | Leaf | | Accumulated amount | |
|-------------------|---------------------|-------------|-----------|-------------|-----------|-------------|--------------------|-------------|
| | Wild-type | <i>MuSI</i> | Wild-type | <i>MuSI</i> | Wild-type | <i>MuSI</i> | Wild-type | <i>MuSI</i> |
| | (----- mg/kg -----) | | | | | | (----- mg -----) | |
| 50 μM | 1165.8 | 1206.9 | 105.4 | 109.5 | 662.8 | 595.8 | 3.24 | 2.11 |
| 100 μM | 3645.4 | 2366.7 | 160.2 | 104.2 | 1264.6 | 1121.8 | 4.08 | 2.88 |
| 200 μM | 7796.8 | 5262.4 | 1355.6 | 622.2 | 1746.8 | 865.3 | 4.65 | 2.37 |

Conclusion

This study showed the potential for phytoremediation of the soils contaminated with Cd using *MuSI* (line 6) transgenic tobacco. They also indicated potential for biofuel production from the area where soils are contaminated with Cd using *MuSI* (line 6) transgenic tobacco in the future.

References

- Cresser MS, Parsons JW (1979) Sulfuric-perchloric acid digestion of plant material for the determination of nitrogen, phosphorus, potassium, calcium and magnesium. *Analytica Chimica Acta* **109**, 431-436.
- Kabata-Pendias A, Mukherjee AB (2007) 'Trace Elements from Soil to Human'. (Springer).
- Kawashima CG, Noji M, Nakamura M, Ogra Y, Suzuki KT, Saito K (2004) Heavy metal tolerance of transgenic tobacco plants over-expressing cysteine synthase. *Biotechnology Letters* **26**, 153-157.
- Kim SH, Hamada T (2005) Rapid and reliable method of extracting DNA and RNA from sweet potato (*Ipomoea batatas* (L.) Lam. *Biotechnology Letters* **27**, 1841-1845.
- Maiti IB, Wagner GJ, Yeagan R, Hunt AG (1989) Inheritance and Expression of the Mouse Metallothionein

Gene in Tobacco. *Plant Physiology* **91**, 1020-1024.

Yang YS (2009) Characterization and Expression Pattern of *MuSI*, a Novel Stress Related Gene from Sweet potato. MS. thesis, The University of Seoul, Seoul, Korea.

Prediction of cadmium removal from highly contaminated soils by phytoextraction with different switchgrass cultivars

Kai-Wei Juang^A and Dar-Yuan Lee^B

^ADepartment of Agronomy, National Chiayi University, Chiayi City, Taiwan, Email kwjuang@mail.ncyu.edu.tw

^BDepartment of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan, Email dylee@ntu.edu.tw

Abstract

The energy crop, switchgrass (*Panicum virginatum* L.), has been studied for phytoextraction of heavy metals in soil because of its high biomass production and moderate tolerance to heavy metals. The potential of switchgrass to remove heavy metals from contaminated soils is promising. The performance of phytoextraction of heavy metal by switchgrass plant from highly contaminated soils has never been discussed. In this study, the maximum removal of Cd from soil during harvest of aboveground tissues of switchgrass was predicted with the relationship between plant Cd concentration and dry weight of switchgrass. Two eco-type cultivars, Alamo and Blackwell, were used for illustration. And, two soil series (Lukang and Pinchen) with spiked Cd (0, 20, 60, and 120 mg Cd/kg soil) were used in pot experiments. The aboveground tissues of switchgrass were harvested after a growth period of three months, and then dry weight and plant Cd concentration were measured. The results showed that the two eco-types of switchgrass, Alamo and Blackwell, were showed different phytotoxic responses to Cd. The phytotoxicity thresholds of 63% reduction in plant Cd concentration (PT63) for Alamo and Blackwell were respectively 46.51 and 98.04 mg/kg. This suggested that Blackwell certainly had higher Cd tolerance than Alamo did. Nevertheless, the maximum phytoextraction of Cd by Alamo and Blackwell, respectively, 141.3 and 47.1 µg/pot were happening on plant Cd concentrations of PT63. Alamo thus had higher efficiency to remove Cd from soil than Blackwell did. Because the maximum phytoextraction of Cd by Alamo was much higher than that by Blackwell, Alamo would be the priority cultivar to be used for Cd removal from heavily contaminated soils.

Key Words

Heavy metal, phytoavailability, phytotoxicity, phytoremediation, energy crop.

Introduction

Among heavy metals, cadmium (Cd) is of special concern due to its relatively high mobility in soils and potential toxicity to biota at low concentrations. Cadmium is usually released into the environment via various industrial activities. Smelters, heating systems, metal-working industries and cement factories are the main contributors to Cd pollution. Increasing concentrations of Cd have been observed in agricultural soils due to long term application of phosphorous fertilizers and sewage sludge (Stephens and Calder 2005). Since Cd tends to adsorb to the topsoil, phytoextraction has been proposed as a low-cost technique to reclaim Cd contamination of soils (Cosio *et al.* 2006).

For efficient remediation, the phytoextraction process needs to take into account good biomass yields and heavy metal hyperaccumulation simultaneously (McGrath and Zhao 2003). Maxted *et al.* (2007) used the product of plant Cd and biomass yield weight to measure phytoextractable Cd by *Thalpi caerulescens* grown under various pH and organic carbon concentration of lowly contaminated soils. While high plant Cd accumulation results in decrease of biomass yield under highly contaminated soils, the phytotoxic symptoms will violate measurement of phytoextractable Cd. So far, there have been few studies to show phytoextraction of heavy metal in highly contaminated soils.

Switchgrass (*Panicum virgatum* L.), which is a perennial grass of importance as a forage and hay crop in soil conservation and as a potential source for biofuels, is a promising plant for phytoextraction of heavy metals (Entry and Watrud 1998; Shahandeh and Hossner 2000). Reed *et al.* (1999) reported that switchgrass was tolerant of Cd while grown in sand culture. Accumulation of Cd in switchgrass was dependent upon soil pH, Cd concentration and plant cultivars (Reed *et al.* 2002). Because switchgrass could yield high biomass (Sladden *et al.* 1991) and tolerate moderate levels of Cd in soils to accumulate Cd in aboveground tissues (Reed *et al.* 1999; Reed *et al.* 2002), the potential of switchgrass for Cd phytoextraction in contaminated soil is thus promising.

Currently, the performance of Cd phytoextraction by switchgrass plant under high contamination has never been discussed. In order to assess the potential of switchgrass used for phytoremediation of Cd contaminated soils, the maximum removal of Cd from soil during harvest of aboveground tissues should be figured out. The objective of the present study was to predict the phytoextraction of Cd by switchgrass in highly contaminated soils with a specified phytotoxicity model.

Methods

Soil characterization and preparation of Cd-spiked soils

Two soils, Pinchen and Lukang, were sampled from the agricultural lands in Taiwan. They were, respectively, classified into Typic Kandiodox and Aquic Dystrudept. Soil samples were air-dried and passed through a 2 mm sieve to be prepared for soil analysis and a pot experiment. The selected soil properties are listed in Table 1. The texture classes, which would be of concern for soil water regime management, are sandy loam for Pinchen and sandy clayey loam for Lukang, respectively. The pH value of Pinchen soil (pH = 4.6) is much lower than that of Lukang soil (pH = 7.4). Compared with Lukang soil, Pinchen soil also showed a lower CEC, OC and total Kjeldahl nitrogen. Air-dried soil samples were amended with four levels of Cd: 0, 20, 60 and 120 mg/kg, in the form of CdCl₂. Cd-spiked soils underwent three wetting-drying cycles at room temperature (25°C). Each wetting-drying cycle was about 4 weeks. Each Cd-spiked soil sample after three wetting-drying cycles was then ground and passed through a 2 mm sieve to thoroughly mix for the pot experiment with switchgrass.

Pot experiments and plant growth and Cd measurements

Two switchgrass cultivars, Alamo and Blackwell, were used as the model plants in pot experiments. Alamo is a lowland eco-type which has high yields in a given environment (Cassida *et al.* 2005). Blackwell is an upland eco-type which usually shows a small response to drought stress (Stroup *et al.* 2003). Five seedlings of Alamo and Blackwell germinated for three weeks were transplanted into a pot (16.5 cm diameter × 14 cm height) with 1 kg Cd-spiked soil. The pot plants were grown in the greenhouse under natural light and the plant growth period was for four months. At harvest, aboveground tissues of plant were then collected by cutting the shoots at the pot soil surface. The harvested tissues were oven-dried at 65°C for 72 h to measure the dry weight. The Cd concentration of each dried plant sample was then determined with an AAS (PerkinElmer AAnalyst 200) following a H₂SO₄ / H₂O₂ digestion procedure.

Prediction of Cd phytoextraction by switchgrass with a phytotoxicity model

An exponential decay model $f(x)$ was used to present plant dry weight, which decreased with increase of plant Cd concentration (x), to assess Cd toxicity of switchgrass:

$$f(x) = a \exp(-bx), \quad [1]$$

where the parameters a and b respectively indicate the maximum dry weight and the reduction rate of dry weight during increase of plant Cd concentration. Then, phytoextraction of Cd (w) could be expressed as the product of x and $f(x)$:

$$w = ax \exp(-bx). \quad [2]$$

Theoretically, there is a maximum value (w_{max}) if x is equal to $1/b$:

$$w_{max} = \frac{a}{b} \exp(-1). \quad [3]$$

Moreover, there is reduction of dry weight of about 63% when x is equal to $1/b$. The value of $1/b$ thus was identified as the phytotoxicity threshold of 63% reduction (PT63) and used to assess the plant tolerance to Cd.

Results

The effects of Cd-spiked soils on plant Cd concentration and dry weight are shown in Figure 1. Plant Cd concentration increased with increase of Cd level in the soil. However, dry weight was significantly decreased by increasing the levels of Cd in the soil. This suggested that Cd phytoavailability was dependent on Cd-contaminated levels and that phytotoxic symptoms of Cd in switchgrass plant were associated with an increase of Cd phytoavailability. In Figure 2, the effects of soil properties on plant Cd concentration and dry weight of switchgrass are shown. Switchgrass plants grown in Pinchen soil had a higher plant Cd concentration than those grown in Lukang soil (Figure 2(a)). Compared with the Lukang soil, Cd phytoavailability in the Pinchen soil was relatively high. This was because the values of pH, CEC and OC of the Pinchen soil were much lower than those of the Lukang soil. The low pH would raise the labile Cd of soil and the low CEC and OC would allow less sorption of Cd. More serious damage due to Cd phytotoxicity thus occurred when switchgrass plants were grown on the Pinchen soil (Figure 2(b)).

In Figure 3, relationships between plant Cd concentration and dry weight were expressed to show the Cd phytotoxicity of switchgrass. The observations for the Pinchen soil were clustered and obviously lower than those for the Lukang soil. This indicated that the phytotoxicity of Cd was more pronounced in the Pinchen soil than in the Lukang soil. Phytotoxicity models as Eq. [1] for Alamo and Blackwell cultivars were obtained by using best-fit regression. For the Alamo cultivar, $a = 8.45 \text{ g/pot}$ and $b = 0.022$, and for the Blackwell cultivar, $a = 1.28 \text{ g/pot}$ and $b = 0.010$. The maximum dry weight of Alamo was obviously higher than that of Blackwell; however, the reduction rate of dry weight of Blackwell was much lower than that of Alamo. By contrast, Alamo did not only have higher biomass production in aboveground tissues but also showed more dramatically Cd toxicity symptoms. Moreover, the PT63 values in plant Cd concentration for Alamo and Blackwell were, respectively, 46.51 and 98.04 mg/kg. This revealed that Cd tolerance of Blackwell was higher than that of Alamo.

Moreover, plant Cd concentrations of PT63 could indicate a desirable Cd phytoavailability to meet the maximum Cd phytoextraction (w_{max}), as shown Eq. [3]. The w_{max} values for Alamo and Blackwell at PT63 were, respectively, 141.3 and 47.1 $\mu\text{g/pot}$. This suggested that Alamo had a higher efficiency for Cd removal from soil than Blackwell did. For Cd accumulation by plants during phytoextraction, Blackwell might be suitable for a soil with highly available Cd and Alamo would be suitable in a soil with relatively low Cd availability.

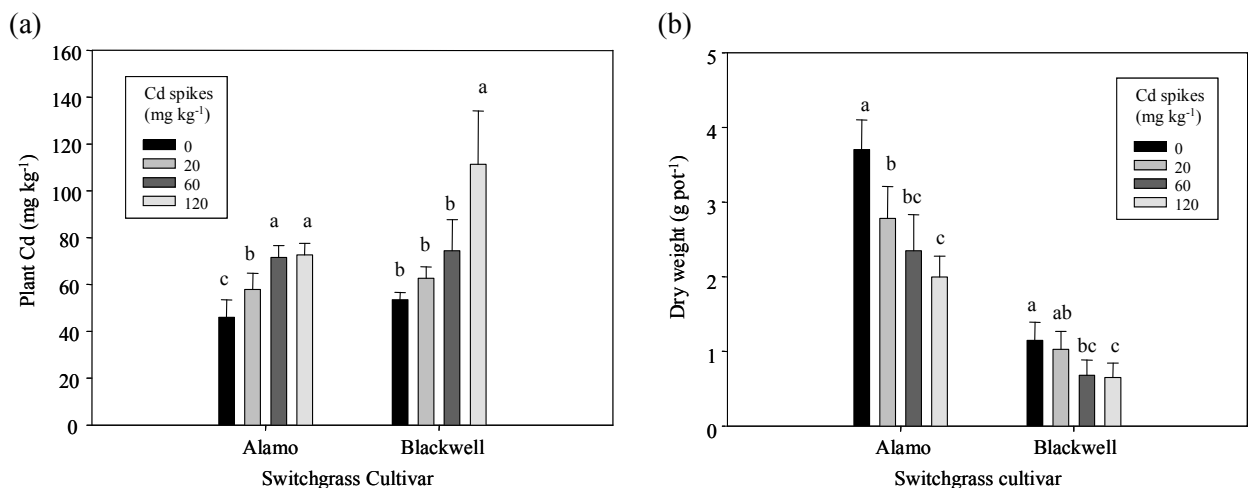


Figure 1. Effects of the four levels of Cd (0, 20, 60, and 120 mg/kg) in soils on (a) plant Cd concentration and (b) dry weight of switchgrass (Alamo or Blackwell) grown in the soils. Error bar is standard error of the mean for a given Cd level. Significantly different effects on switchgrass at the 0.05 level according to the LSD test are labeled with different letters.

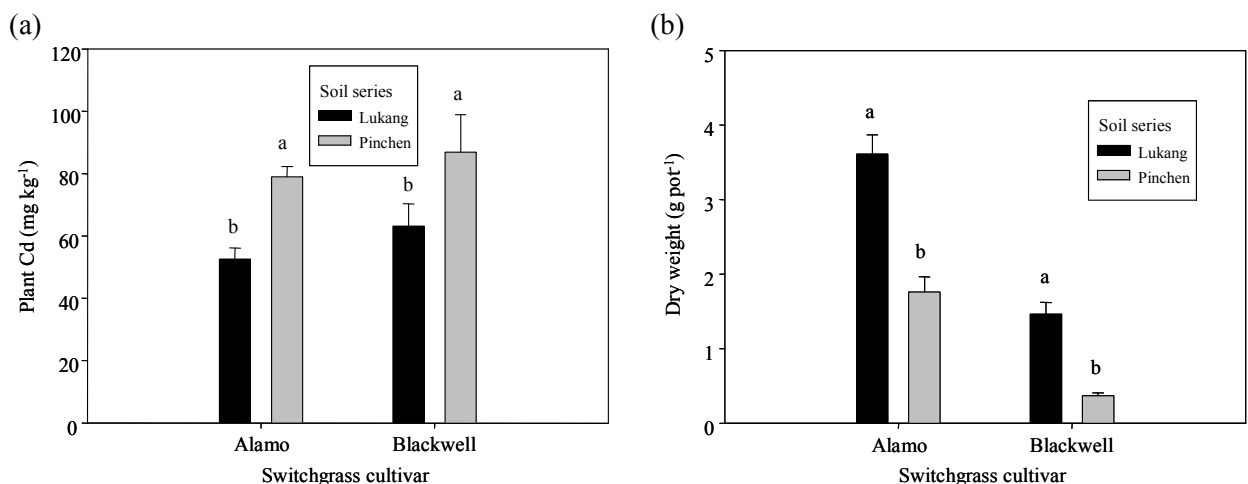


Figure 2. Effects of the two soil series (Lukang and Pinchen) on (a) plant Cd concentration and (b) dry weight of switchgrass (Alamo or Blackwell) grown in Cd-spiked soil. Error bar is standard error of the mean for a given soil series. Significantly different effects on switchgrass at the 0.05 level according to the LSD test are labeled with different letters.

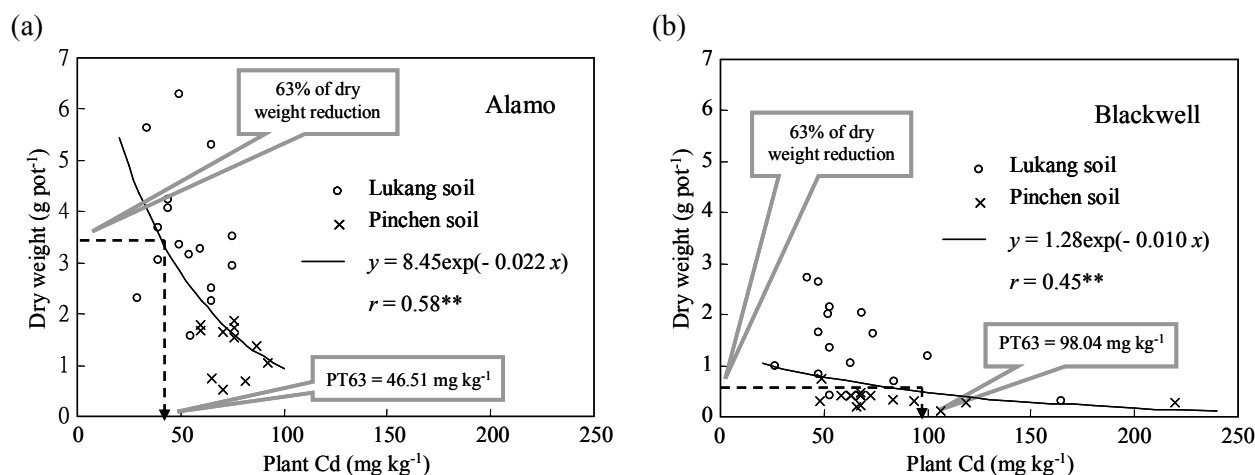


Figure 3. Relationships between plant Cd concentration and dry weight for the switchgrass cultivars, (a) Alamo and (b) Blackwell, grown in Cd-spiked soils. Plant Cd concentrations, with phytotoxicity thresholds of 63% dry weight reductions (PT63), for Alamo and Blackwell, respectively, are denoted. Best-fit regression models are represented by solid lines and with correlation coefficients r significant at $P < 0.01$ ().**

Conclusion

The two eco-types of switchgrass, Alamo and Blackwell, showed different phytotoxic responses to Cd. The PT63 values for Alamo and Blackwell were, respectively, 46.51 and 98.04 mg/kg. This revealed that Blackwell had higher tolerance to Cd than Alamo did. Nevertheless, the maximum phytoextraction of Cd by Alamo (141.3 $\mu\text{g}/\text{pot}$) was much higher than that by Blackwell (47.1 $\mu\text{g}/\text{pot}$). Alamo had higher efficiency for Cd removal from soil than Blackwell did. Therefore, Alamo switchgrass is suggested to be the priority plant for phytoextraction of Cd in heavily contaminated soils.

References

- Cassida KA, Muir JP, Hussey MA, Reed JC, Venuto BC, Ocumpaugh WR (2005) Biomass yield and stand characteristics of switchgrass in south central U.S. environments. *Crop Science* **45**, 673-681.
- Cosio C, Vollenweider P, Keller C (2006) Localization and effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.): I. Macrolocalization and phytotoxic effects of cadmium. *Environmental and Experimental Botany* **58**, 64-74.
- Entry JA, Watrud LS (1998) Potential remediation of ^{137}Cs and ^{90}Sr contaminated soil by accumulation in Alamo switchgrass. *Water, Air, and Soil Pollution* **104**, 339-352.
- Maxted AP, Black CR, West HM, Crout NMJ, McGrath SP, Young SD (2007) Phytoextraction of cadmium and zinc from arable soils amended with sewage sludge using *Thlaspi caerulescens*: Development of a predictive model. *Environmental Pollution* **150**, 363-372.
- McGrath SP, Zhao FJ (2003) Phytoextraction of metals and metalloids from contaminated soils. *Current Opinion in Biotechnology* **14**, 277-282.
- Reed RL, Sanderson MA, Allen VG, Matches AG (1999) Growth and cadmium accumulation in selected switchgrass cultivars. *Communications in Soil Science and Plant Analysis* **30**, 2655-2667.
- Reed RL, Sanderson MA, Allen VG, Zartman RE (2002) Cadmium application and pH effects on growth and cadmium accumulation in switchgrass. *Communications in Soil Science and Plant Analysis* **33**, 1187-1203.
- Shahandeh H, Hossner LR (2000) Plant screening for chromium phytoremediation. *International Journal of Phytoremediation* **2**, 31-51.
- Sladden SE, Bransby DI, Aiken GE (1991) Biomass yield, composition and production costs for eight switchgrass varieties in Alabama. *Biomass and Bioenergy* **1**, 119-122.
- Stephens WE, Calder A (2005) Source and health implications of high toxic metal concentrations in illicit tobacco products. *Environmental Science and Technology* **38**, 937-944.
- Stroup AJ, Sanderson MA, Muir JP, McFarland MJ, Reed RL (2003) Comparison of growth and performance in upland and lowland switchgrass types to water and nitrogen stress. *Bioresource Technology* **86**, 65-72.

Proteases activities of Andosols after the addition of clay and humic substances in the presence of cadmium

Fereshteh Shahriari^A and Teruo Higashi^A

^AGraduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Ten-nou-dai, Tsukuba, Ibaraki, 305-8572, Japan, Email frshahriari@yahoo.com, Email labsoils@sakura.cc.tsukuba.ac.jp

Abstract

The addition of clay and humic substances to soils may control the negative effect of Cd through both ion adsorption and the stabilization of soil enzymes. In this regard we investigated the response of proteases activities in soils to Cd after the addition of allophanic clay or aromatic humic substances, separated from the soil samples used. The experiment was done at three levels of clay, humic substances and Cd (0, +5%, and +10% of original content of clay, 0, +5%, and +10% of original soil organic matter content as carbon, and 0, 10, and 50 mg Cd kg⁻¹ soil). Two surface soil samples were obtained from Andosols under a forest and a cultivated field in an upland area of Japan's Kanto district. We determined proteases activities and Exchangeable Cd in both soil samples after 0, 2, 10 and 40 days of incubation. Cd addition decreased the proteases activities in both soil samples, while the addition of allophanic clay or aromatic humic substances significantly enhanced proteases activities even in the presence of Cd. Thus the addition of clay or humic substances did not have a significant positive effect on the inhibitory effect of Cd. These results indicate that both allophanic clay and aromatic humic substances separated from Andosols stabilize proteases of Andosols, but have little capacity to adsorb Cd²⁺.

Key Words

Protease activity, cadmium, allophane, humic substances, soil improvement, Andosol.

Introduction

Heavy metals like Cd have long-term toxic effects on soil biological functions, including soil microbial communities and enzyme activities (Majer *et al.* 2002). Since Cd is not biodegradable, it is considered as one of the most serious groups of environmental contaminants (Bailey *et al.* 1999). Therefore the reclamation of contaminated soils with Cd is only possible by using the techniques that extract or stabilize the contaminant (Pérez-de-Mora *et al.* 2006). Our objectives of this study were to examine the response of the proteases activities of Andosols to the addition of clay or humic substances in the presence of Cd during a short-term incubation experiment, and also to find the factors affecting proteases activities. The hypothesis was that addition of clay or humic substances would reduce Cd solubility and increase soil proteases activities.

Methods

Study sites and soil samples

Two surface soil samples were collected from a forest and a cultivated field at the Agricultural and Forestry Research Center, University of Tsukuba, located in an upland area of Kanto district, Japan. The forest was a natural mixed wood stand of warm-temperate trees (mainly *Quercus serrata* and *Pinus densiflora*), and the field has been continuously cultivated with corn for more than 10 years. The two sites are 200 m apart. Both soils are classified as Umbric Andosols (FAO 2006). Soil samples were sieved through a 2-mm mesh and kept moist in plastic containers for one week at room temperature (20-25°C) before use. Clay fractions were separated from soil samples by the sedimentation method. Extraction and preparation of humic substances were carried out according to the combination of IHSS method (International Humic Substances Society, <http://www.ihss.gatech.edu/>, 1996) and Hiradate *et al.* (2007).

Characterization of clay and humic substances

Oriented clay fraction samples were subjected to X-ray diffraction (XRD), after the DCB treatment, using a X-ray diffractometer (RAD-X; Rigaku Intl. Co., Tokyo, Japan) with Cu-K α radiation ($\lambda=1.54$ Å). XRD analyses of the samples were conducted after the separate treatments by air drying (Mg-saturated; K-saturated); ethylene glycol (Mg-saturated); and heating to 350°C and 550°C (both K-saturated). Infrared spectroscopic analysis of Mg-saturated clays was also carried out by a FT-IR spectrometer (FT-720; Horiba, Ltd., Tokyo, Japan) over the range 400-4000 cm⁻¹. Chemical analyses of Fe (Fe_d) and Al (Al_d) extracted by DCB and Si (Si_o), Fe (Fe_o) and Al (Al_o) extracted by acid ammonium oxalate (0.3 mol/L, pH 3) were done by

ICP-AES (ICAP-757, Nippon Jarrell-Ash, Tokyo, Japan). Weight loss (%) arising from the treatment by ammonium oxalate was also calculated. Elemental analysis (C, H and N) of humic substances was carried out in triplicate using Flash EA 1112 NCS Analyzer (Thermo Finnigan, Italy). Ratios of C/N and atomic ratio of H/C and O/C were calculated. Infrared spectroscopic analysis of humic substances was also carried out by a FT-IR spectrometer (FT-720; Horiba, Ltd., Tokyo, Japan) in the range of 4000-400 cm^{-1} . Solid-state CP/MAS ^{13}C NMR spectra of the humic substances were recorded with a FT-NMR system (Alpha 300, JEOL, Tokyo).

Addition of clay fractions or humic substances to the soil samples

In the present study two levels of clay fraction and or humic substances (separated from the soil samples used) were added to the soils, designated as low and high concentrations (LC and HC, respectively). Experiments were also performed with the soil samples containing the original concentration of clay and soil organic matter (OC: no addition of clay fractions or humic substances). These two amendments were added as their water suspensions in the LC and HC systems, giving 5% and 10% higher content than OC, respectively. In case of humic substances the content was calculated as carbon. Then doses of 0-, 1- and 5-fold of the critical Cd level, 10 mg Cd kg^{-1} soil (Chang and Broadbent 1981; Effron *et al.* 2004), were added to the soil samples using the solutions of $\text{Cd}(\text{NO}_3)_2$ of different concentrations, designated as Cd0, Cd10, and Cd50, respectively. Incubation periods were 0, 2, 10 and 40 days, designated as d0, d2, d10 and d40, respectively.

Proteases activities

Protease activity was measured after 0, 2, 10 and 40 days of incubation based on the method of Ladd and Butler (1972) with slight modifications by using colorimetric determination of leucine released by *N*-benzyloxycarbonyl L-phenylalanine L-leucine as substrate, followed by the incubation for 1 h at 40°C in dark. The absorbance at 570 nm was measured by a spectrophotometer (U-3210, Hitachi Co., Tokyo, Japan). Proteases activities were expressed as ng leucine g^{-1} dried soil sec^{-1} .

Exchangeable Cd

Incubated soil samples were subjected to the analysis of exchangeable Cd, based on the method of Sadamoto *et al.* (1994) in each incubation day. The extraction of Cd was done with $\text{Ca}(\text{NO}_3)_2$ (0.05 mol/L) with a soil/solution ratio of 1/10 after the end to end shaking (200 rpm) for 24 h at 30 °C. The supernatant, obtained after centrifuging at $1700 \times g$ for 10 min, was filtered through a 0.2 μm filter (Millipore Co., Bedford, MA, USA), and Cd was measured by ICP-AES (Optima 5300 DV, Tokyo, Japan). Exchangeable Cd was expressed as mg Cd kg^{-1} soil.

Results

Characterization of clay and humic substances

X-ray diffraction patterns of oriented clay fractions from the forest and the cultivated field soil samples showed that the crystalline layer silicates are composed of kaolinite, illite and chlorite in addition to the primary minerals of quartz and feldspars. The atomic ratios of Al/Si extracted by acid ammonium oxalate were 2.02 and 1.95 for the forest and the cultivated field soil sample, respectively. Moreover considering the total weight loss (%) on acid oxalate treatment (56% and 61%, respectively), the two separated clay fractions are largely composed of allophane (probably including imogolite) and ferrihydrite in addition to the layer silicates mentioned above.

Humic substances from the forest soil sample had higher C, H and N contents than those from the cultivated field soil sample. The H/C ratio of humic substances from both samples was low, probably due to high O content in both samples. The higher O content and O/C ratio in the cultivated field soil than the forest soil may indicate higher amounts of more oxidized organic materials in the cultivated field sample. ^{13}C NMR spectra showed that a small difference was observed in the alkyl region (0-60 ppm) between the forest and the cultivated field soil samples. In the cultivated field soil sample relative percentage of the alkyl group was less than the forest soil sample. Humic substances from the cultivated field soil sample included higher content of O-alkyl C (60-110 ppm). Percentage of aromatic C (110-160 ppm) in the forest soil sample was slightly higher than the cultivated field soil sample, while carbonyl-C (160-190 ppm) of the cultivated field soil was slightly higher than the forest soil. The ratio of $\text{C}_{\text{alip}}/\text{C}_{\text{arom}}$ also indicated higher amount of aromatic C in the forest soil samples. Moreover hydrophobicity was less for the cultivated field soil sample. Thus the NMR spectra of both humic substances showed aromatic nature in general.

Proteases activities and exchangeable Cd after the addition of clay in the presence of Cd

As shown in Figure 1 the addition of separated clay to both the forest and the cultivated field soil samples significantly increased proteases activities ($p < 0.01$) compared with the control (OC) on each incubation time. Proteases activities were almost in the order of HC > LC > OC. Addition of Cd significantly inhibited proteases activities during incubation periods ($p < 0.01$) in most of the two soil samples. The effect of time on proteases activities also was significant ($p < 0.01$), fluctuated over the incubation periods. This may relate to the changes in the microbial community during the incubation time (Pérez-de-Mora *et al.* 2006).

Exchangeable Cd increased in proportion to the total added Cd in both soil samples. However there were little changes in the exchangeable Cd in term of the amount of added clay or incubation time. Exchangeable Cd in the forest soil samples was higher than the cultivated field soil samples. This can be attributed to the difference in pH between these soils, since soil pH has the greatest impact on the availability and mobility of metal cations. Exchangeable Cd showed no clear change in LC and HC systems in the forest soil samples, and seemed to fluctuate among the different incubation times. In the cultivated field soil samples the addition of clay led to a significant decrease of exchangeable Cd at all doses of Cd, but again it was fluctuated during incubation times.

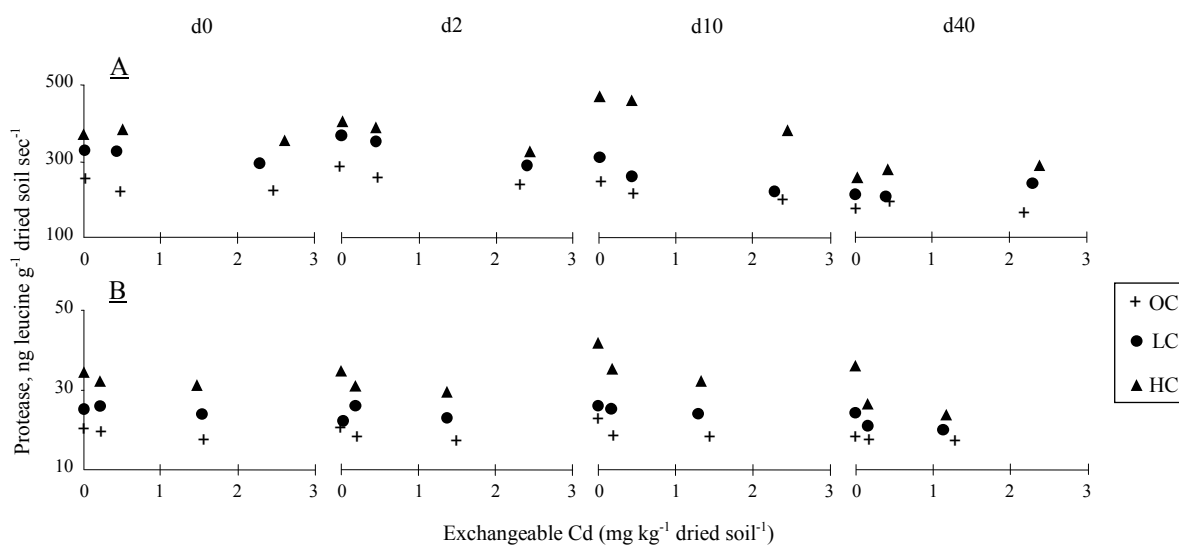


Figure 1. Correlation between proteases activities and exchangeable Cd in (A) forest and (B) cultivated field soil samples after the addition of clay fractions and Cd.

Proteases activities and exchangeable Cd after the addition of humic substances in the presence of Cd

The response of proteases activities after the addition of aromatic humic substances was analogous with that of allophanic clay addition. As shown in Figure 2 the addition of different concentrations of humic substances significantly increased proteases activities ($p < 0.01$) compared with control (OC) in both soil samples on each incubation days, except 40 days of incubation (d40) of the forest soil samples. The increase in the activity was observed in three levels of Cd addition. However these results have shown that the activity enhancement did not occur in proportion to the increase in the levels of humic substances. In the present study the enhancement of enzyme activity is more evident for forest soil samples than the cultivated field sample. This may be attributed to the difference in the contents of functional groups of humic substances, as described in the characterization of humic substances used in the present study.

Levels of exchangeable Cd became higher in proportion to the total Cd content of the soils after the addition of Cd in both the forest and the cultivated field soil samples. But the response of exchangeable Cd in the forest and the cultivated field soil was different after the addition of humic substances. In the forest soil samples it didn't show clear change after the addition of different contents of humic substances and the exchangeable Cd decreased significantly in LC, but it was fluctuated in HC during different incubation days. While, in the cultivated field soil samples, it significantly increased in both LC and HC ($p < 0.01$) at all levels of Cd doses.

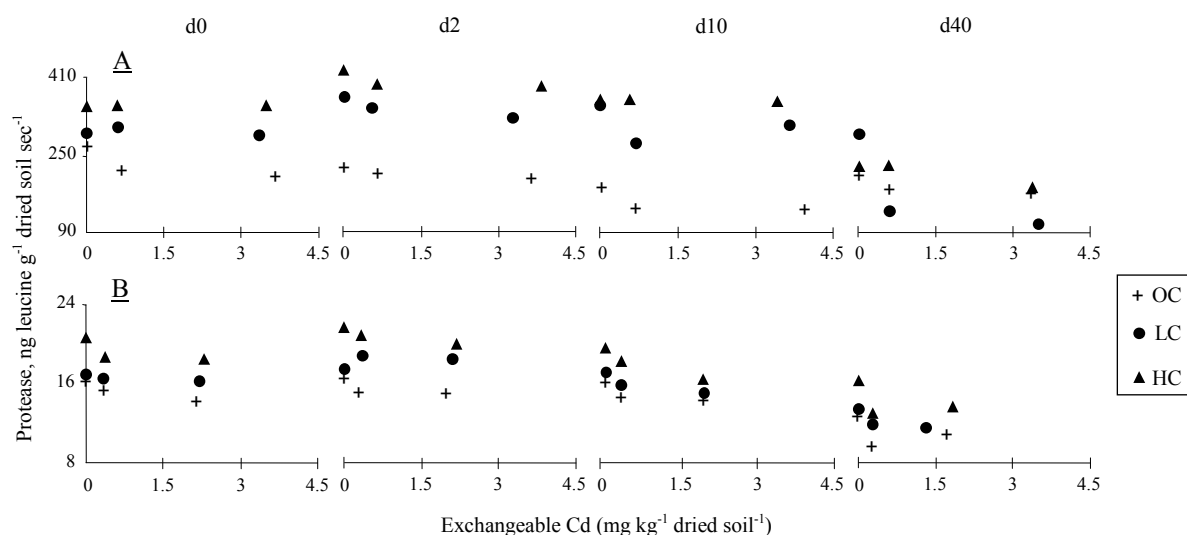


Figure 2. Correlation between proteases activities and exchangeable Cd in (A) forest and (B) cultivated field soil samples after the addition of humic substances and Cd.

Conclusion

Soluble and exchangeable fractions of heavy metals are the most important pools regarding the toxicity and bioavailability of heavy metals. One of the main reasons for the application of both clay minerals and humic substances was to change bioavailability of Cd in soils. Although the addition of humic substances seems to be an important factor affecting proteases activities in the presence of Cd, the addition of both amendments showed the stabilization of proteases, but had little contribution on the inhibitory effect of Cd.

References

- Bailey SE, Olin TJ, Bricka RM, Adrian DD (1999) A review of potentially low-cost sorbents for heavy metals. *Water Research* **33**, 2469-2479.
- Chang FH, Broadbent FE (1981) Influence of Trace-Metals on Carbon-Dioxide Evolution from a Yolo Soil. *Soil Science* **132**, 416-421.
- Effron D, de la Horra AM, Defrieri RL, Fontanive V, Palma RM (2004) Effect of cadmium, copper, and lead on different enzyme activities in a native forest soil. *Communications in Soil Science and Plant Analysis* **35**, 1309-1321.
- FAO (2006) World reference base for soil resources. World Soil Resource Reports, p. 70.
- Hiradate S, Yonezawa T, Takesako H (2007) Fine fractionation and purification of the fulvic acid fraction using adsorption and precipitation procedures. *Soil Science and Plant Nutrition* **53**, 413-419.
- Ladd JN, Butler JHA (1972) Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biology & Biochemistry* **4**, 19-30.
- Majer BJ, Tschierko D, Paschke A, Wennrich R, Kundi M, Kandeler E, Knasmüller S (2002) Effects of heavy metal contamination of soils on micronucleus induction in *Tradescantia* and on microbial enzyme activities: a comparative investigation. *Mutation Research-Genetic Toxicology and Environmental Mutagenesis* **515**, 111-124.
- Pérez-de-Mora A, Burgos P, Madejon E, Cabrera F, Jaekel P, Schlöter M (2006) Microbial community structure and function in a soil contaminated by heavy metals: effects of plant growth and different amendments. *Soil Biology & Biochemistry* **38**, 327-341.
- Sadamoto H, Iimura K, Honna T, Yamamoto S (1994) Examination of fractionation of heavy metals in soils. *Japanese Journal of Soil Science and Plant Nutrition* **65**, 645-653 (in Japanese).

Relation between soil characteristics and heavy metal content in Virginia tobacco

Penka Zapryanova^A, Krasimir Ivanov^B, Violina Angelova^B and Lilko Dospatliev^B

^AInstitute of Tobacco and Tobacco Products, Plovdiv, Bulgaria, Email p_alexieva@abv.bg

^BDepartment of Chemistry, Agricultural University, Plovdiv, Bulgaria, Email kivanov1@abv.bg

Abstract

The study was conducted on alluvial-meadow soils (Fluvisols), cinnamonic forest soils (Chromic Luvisols) and smolnitzi (Vertisols) with Virginia tobacco. pH, humus, total content and mobile forms of Pb, Cd, Cu and Zn in soils, as well as concentration of the elements in roots and aboveground biomass of tobacco were specified. Statistically significant dependencies between soil reaction and Cd content in leaves, stems and blossoms of Virginia tobacco were established. pH increase led to reduction of Pb, Cu and Zn concentration in plant organs but these relations were not statistically proven. The humus content influenced Pb and Cd accumulation in leaves of the three harvesting zones and of Cu in stems and blossoms. Statistically significant dependencies with varying degree of correlation were established between total Pb, Cd, Cu, Zn content, their mobile forms in soil and element concentration in plant organs (mainly in leaves) of Virginia tobacco. The obtained regression dependencies in the soil-tobacco system may be used for solving various scientific as well as scientific and practical tasks for prognostication and prevention of tobacco heavy metal contamination.

Key Words

Soil, Heavy Metals, Tobacco (Virginia)

Introduction

The heavy metal content in tobacco leaves was variable and depended on the conditions, under which tobacco was grown and mostly on the composition and properties of soils. One of the major factors influencing the element concentration in tobacco leaves was soil reaction (Adamu *et al.* 1989; Gondola and Kadar 1995). Golia *et al.* 2003 found a significant negative correlation between pH of soil and heavy metal content in oriental tobacco, while in the case of Virginia variety group, such correlation was determined only with cadmium, copper and manganese. The same authors have not found any relation between the total content of metals in soil and in leaves of oriental tobacco, Virginia and Burley. Another soil characteristic influencing the heavy metal concentration in tobacco was the humus content (Adamu *et al.* 1989). The same authors have found statistically important relations between the mobile forms of zinc, manganese, nickel and cadmium extracted from the soil by means of different extragents and their concentration in tobacco leaves. Such dependency has not been determined for lead. The purpose of this study was to provide information on the relation between pH, humus, total content and mobile forms of lead, cadmium, copper and zinc in soil, as well as the concentration of these elements in roots and aboveground biomass of Virginia tobacco.

Methods

The study was conducted in the period 2005-2007 on alluvial-meadow soils (Fluvisols), cinnamonic forest soils (Chromic Luvisols) and smolnitzi (Vertisols) with Virginia tobacco (37 soil samples). The samples were taken from depth of 0-30 cm. The following soil characteristics were determined: pH in water (1:1), humus, total content of Pb, Cd, Cu and Zn through decomposition by HF, HClO₄ and HNO₃ acids. A solution of 0.005M DTPA + 0.1M TEA, pH 7.3 was used for extraction of element mobile forms from soils. The preparation of plant samples (roots, stems, leaves of the three harvesting zones and blossoms) was made by means of dry ashing and dissolution in 3 M HCl. An atomic-absorptional spectrometer "Spektra AA 220" of the Australian company Varian was used for determination of heavy metal content at the following operating wavelengths: Cd – 228.8 nm, Pb - 217.0 nm, Cu - 324.8 nm, Zn – 213.9 nm. Certified Reference Material of soil (Light Meadow Cinnamonic Soil PS-2, SOOMET № 0002 - 1999 BG, SOD №311^a-98) and of Virginia tobacco leaves (a Polish reference material CTA-VTL-2) was also analyzed for the verification of the accuracy of trace element determination in soil and in tobacco. SPSS program for Windows was used for statistical data processing. A correlation-regression analysis was conducted between pH, humus content, total and mobile forms of Pb, Cd, Cu and Zn in soil, as well as the concentration of the elements in roots and aboveground biomass of Virginia tobacco.

Results

Soils

Table 1 presents pH values for the tested soils. As evident from the table, soil reaction was within the range from 5.25 to 7.73. The arithmetic mean was 6.65 – a value which stood close to the value considered optimal by Stephenson *et al.* 1987 for flue-cured tobacco. The humus content was within the range from 1.07 to 2.45 (low to medium), as most of the soils had low humus content and they were suitable for the Virginia variety group.

Table 1 Soil properties, content of Heavy metals in soil (n = 37)

| Statistical index | pH | Humus % | Pb, mg/kg | | Cd, mg/kg d | | Cu, mg/kg | | Zn, mg/kg | |
|-------------------|-------|---------|-----------|--------------|-------------|--------------|-----------|--------------|-----------|--------------|
| | | | total | Mobile forms | total | Mobile forms | total | Mobile forms | total | Mobile forms |
| mean | 6.65 | 1.82 | 19.78 | 3.74 | 0.78 | 0.24 | 62.92 | 4.19 | 95.51 | 2.83 |
| Minimum | 5.25 | 1.07 | 4.00 | 0.50 | 0.40 | 0.05 | 15.00 | 0.46 | 34.6 | 0.31 |
| Maximum | 7.73 | 2.45 | 116.00 | 31.60 | 3.00 | 1.44 | 399.20 | 24.28 | 280.00 | 25.26 |
| CV, % | 11.34 | 22.70 | 129.64 | 191.98 | 68.53 | 124.90 | 113.92 | 128.17 | 52.77 | 198.20 |

The total lead content in the soils varied broadly – from 4 to 116 mg/kg. According to the requirements of the Bulgarian standards lead content was above the allowable concentration in only two types of soil. These types of soil could be found close to an industrial source of contamination, which was the reason for the higher lead content. The mobile lead content also varied broadly – from 0.5 to 31.6 mg/kg. The highest values were measured in soils near the source of contamination, where the total content of the element was the highest.

The total cadmium content in soils varied from 0.4 to 3 mg/kg. All measured values were below the allowable concentration with the respective pH. Mobile cadmium in soils varied broadly from 0.05 to 1.44 mg/kg, as highest concentrations were measured in soils near an industrial source of contamination.

The total Cu content was within the range from 15 to 399.2 mg/kg. It exceeded the allowable concentration with the respective pH with one soil only. Generally speaking, the element content was higher than the state-average one, which was 30 mg/kg. The arithmetic mean reached 62.92 mg/kg. Mobile Cu content also varied in a wide range. According to MAFF classification (Mitsios *et al.* 2005) some soils which were subject of the present study, were characterized as having very large reserves of mobile copper.

With many soils the total zinc content was close to the state-average. It was higher with some soils, but it was below the allowable concentration. The mobile Zn content varied broadly and most soils had small or medium reserves.

Tobacco

Table 2 presents the values of lead concentration in plant organs of tobacco grown in all studied regions. If you trace the average element content in the separate parts of tobacco plants you will see that it is lowest in stems, followed by blossoms, leaves and roots. The highest Pb values were measured in leaves of tobacco grown on soils having high lead content in regions located near the source of contamination. Very high Cd values in leaves (13.4–15.4 mg/kg) were measured in these regions. The data of Jancheva *et al.* 2007 showed that up to 21.8 mg/kg of cadmium were accumulated in leaves of oriental tobacco grown near an industrial source of contamination. Apparently, tobacco is a crop, accumulating large cadmium quantities in leaves and acting as a hyper accumulator of this element. In regions located away from a source of contamination cadmium concentration in tobacco was lower than the concentrations considered critical to plants: 5 -30 mg/kg (Kabata Pendias and Pendias 1989). If you trace the Cd content in the separate parts of tobacco plants you will see that it was highest in leaves, followed by roots, blossoms and stems.

For the studied tobacco the average copper content in leaves of the lower, medium and upper harvesting zone was within the range from 21 to 30 mg/kg. It was higher than the content, cited in some scientific sources (Adamu 1988; Campbell 2000) – from 5 to 10 mg/kg, but it complied with the results obtained by other authors (Radojicic *et al.* 2003; Pelivanoska 2007).

The zinc content was highest in leaves followed by blossoms, roots and stems. It varied from 8.5 to 138.6 mg/kg in leaves as the highest values were measured in soils having higher total and mobile Zn content. Increase of the average and minimum values from lower to upper harvesting zones was observed.

Table 2. Content of heavy metals in roots and aboveground biomass of Virginia tobacco (n = 37)

| Heavy Metals | Statistical index | Roots | Stems | Lower leaves | Middle leaves | Upper leaves | Blossoms |
|--------------|-------------------|--------|-------|--------------|---------------|--------------|----------|
| Pb | Mean, mg/kg | 14.85 | 5.27 | 11.86 | 13.34 | 11.60 | 8.30 |
| | Minimum, mg/kg | 0.80 | 1.00 | 0.75 | 0.10 | 0.10 | 1.00 |
| | Maximum, mg/kg | 88.00 | 9.00 | 105.00 | 117.00 | 85.00 | 34.00 |
| | CV, % | 150.98 | 46.83 | 163.37 | 200.11 | 127.53 | 71.52 |
| Cd | Mean, mg/kg | 1.74 | 0.53 | 3.81 | 2.77 | 3.09 | 1.16 |
| | Minimum, mg/kg | 0.30 | 0.10 | 0.60 | 0.40 | 0.10 | 0.10 |
| | Maximum, mg/kg | 10.50 | 2.30 | 13.40 | 11.90 | 15.40 | 5.20 |
| | CV, % | 119.54 | 97.74 | 91.80 | 105.21 | 118.90 | 95.07 |
| Cu | Mean, mg/kg | 26.05 | 15.33 | 21.37 | 26.71 | 30.49 | 28.76 |
| | Minimum, mg/kg | 12.60 | 9.40 | 3.60 | 9.20 | 7.20 | 17.50 |
| | Maximum, mg/kg | 52.20 | 29.20 | 52.40 | 53.80 | 58.90 | 45.50 |
| | CV, % | 33.90 | 33.59 | 54.14 | 46.20 | 54.51 | 27.29 |
| Zn | Mean, mg/kg | 44.85 | 16.42 | 43.37 | 51.96 | 62.86 | 55.61 |
| | Minimum, mg/kg | 25.60 | 8.50 | 9.24 | 17.67 | 19.20 | 40 |
| | Maximum, mg/kg | 72.40 | 25.20 | 112.00 | 138.60 | 124.00 | 80.40 |
| | CV, % | 29.65 | 23.08 | 68.82 | 64.40 | 54.22 | 14.58 |

Table 3. Correlation among soil parameters and concentration of Heavy Metals in the Virginia tobacco (n = 37)

| Heavy Metals | Soil parameters | Roots | Stems | Lower leaves | Middle leaves | Upper leaves | Blossoms |
|--------------|-----------------|---------|---------|--------------|---------------|--------------|----------|
| Pb | pH | ns | ns | ns | ns | ns | ns |
| | Humus | ns | ns | 0.707** | 0.628** | 0.678** | ns |
| | Total content | 0.407* | ns | 0.898** | 0.870** | 0.844** | 0.792** |
| | Mobile forms | 0.602** | ns | 0.902** | 0.972** | 0.864** | 0.865** |
| Cd | pH | ns | -0.411* | -0.370* | -0.351* | -0.555** | -0.496** |
| | Humus | ns | ns | 0.512** | 0.725** | 0.668** | 0.655** |
| | Total content | ns | ns | 0.359* | 0.431** | 0.350* | ns |
| | Mobile forms | 0.337* | ns | 0.460** | 0.462** | 0.361* | ns |
| Cu | pH | ns | ns | ns | ns | ns | ns |
| | Humus | ns | 0.332* | ns | ns | ns | 0.431** |
| | Total content | ns | ns | 0.628** | 0.639** | 0.414* | ns |
| | Mobile forms | ns | ns | 0.507** | 0.611** | 0.681** | 0.691** |
| Zn | pH | ns | ns | ns | ns | ns | ns |
| | Humus | ns | ns | ns | ns | ns | ns |
| | Total content | ns | ns | 0.620** | 0.719** | 0.719** | ns |
| | Mobile forms | ns | ns | 0.739** | 0.650** | 0.650** | ns |

ns - no significant correlation;

*correlation is significant at the 0.05 level;

**correlation is significant at the 0.01 level

The results of the conducted correlation and regression analysis showed that statistically significant dependencies were determined between pH of soils and cadmium content in all organs of tobacco plants (Table 3). The obtained results complied with the data, indicated in other scientific sources, which referred mainly to the relation between pH and element content in leaves (Gondola and Kadar 1995; Golia *et al.* 2003). pH increase led to reduction of Pb, Cu and Zn concentration in plant organs but these relations were not statistically proven.

The humus content influenced mainly Pb and Cd accumulation in leaves of the three harvesting zones and of Cu in stems and blossoms. The third order polynomial model (cubic model) adequately reflected the relation between humus and content of lead and cadmium at a critical level of significance of 0.01. There were no statistically significant dependencies determined between humus and zinc concentration in tobacco plants. The Pb content in leaves and blossoms increased linearly with the increase of the total element content in soils. The observed level of significance (α_s) had a value smaller than the critical level of significance of

0.01; the correlation coefficients were high and statistically significant. The determination coefficients showed that approximately 70-80% of the values of lead concentration in leaves and 60% of the values of lead concentration in blossoms depended on the total element content in soils. The increase of the total cadmium, copper and zinc content in soils led to increase of their concentration in the leaves of the lower, medium and upper harvesting zone. The increase of mobile lead and copper in soils resulted in the increase of the element content in leaves of the three zones and in blossoms. Statistically significant relations were determined between mobile cadmium and element content in roots and leaves of the three zones. The third order polynomial model adequately reflected the dependence of zinc content in leaves on mobile zinc content in soils.

Conclusions

1. Statistically significant dependencies were established between soil reaction and Cd content in leaves, stems and blossoms of Virginia tobacco. pH increase led to reduction of Pb, Cu and Zn concentration in plant organs but these relations were not statistically proven.
2. The humus content influenced Pb and Cd accumulation in leaves of the three harvesting zones and of Cu in stems and blossoms. There were no statistically significant dependencies determined between humus and Zn concentration in tobacco plants.
3. Statistically significant dependencies with varying degree of correlation were established between total Pb, Cd, Cu, Zn content, their mobile forms in soil and element concentration in plant organs (mainly in leaves) of Virginia tobacco.
4. The obtained regression dependencies in the soil-tobacco system may be used for solving various scientific as well as scientific and practical tasks for prognostication and prevention of tobacco heavy metal contamination.

Acknowledgement

This work is supported by Bulgarian Ministry of Education, Project DO-02-87/08 and NSFB project GAMA DO 02-70 11/12/2008

References

- Adamu CA (1988) The role of soil extractants and soil characteristics in assessing the availability of heavy metal in Maryland soils for the growth of tobacco. *Ph.D. dissertation, University of Maryland College Park*.
- Adamu CA, Mulchi CL, Bell PF (1989) Relationships between soil pH, clay, organic matter and CEC [cation exchange capacity] and heavy metal concentrations in soils and tobacco. *Tob. Sci.* **33**, 96-100.
- Campbell C, (2000) Reference Sufficiency Ranges Field Crops, Tobacco, Flue-cured, www.ncagr.com/agronomi/saesd/fluecure.htm
- Golia EE, Mitsios IK, Tsadilas CD (2003) Concentration of Heavy Metals in Burley, Virginia and Oriental Tobacco leaves in the Thessaly region of Central Greece. *Communication in Soil Science and Plant Analysis* **36**, 200 – 230.
- Gondola I, Kadar I (1995) Heavy metal content of flue-cured tobacco in different growing regions of Hungary. *Acta Agronomica Hungarica* **43**, 243–251.
- Jancheva D., Boginova R, Stanislavova L (2007) To the dynamics of tobacco area heavy metal contamination in the region of the Lead and Zinc Complex in Kardzhali. International conference „Pedology as a basis for sustained agriculture and environment protection”, 13-17 May 2007, Sofia, 640-643.
- Kabata Pendias A, Pendias H (1989) Trace Elements in Soils and Plants, 2nd ed; CRC Press: Boca Raton, FL, 424.
- Mitsios IK, Golia EE, Tsadilas CD (2005) Heavy metal concentrations in soils and irrigation waters in Thessaly region, central Greece. *Communications in Soil Science and Plant Analysis* **36**, 487-501.
- Pelivanoska V (2007) The Heavy Metals Content in Tobacco Grown in the Region of Radovis. *Tutun/Tobacco* **57**, 5-6, 103-112.
- Radojičić V, Cvetković O, Dukić M (2003) Uticaj agroekoloških uslova gajenja na sadržaj mineralnih materija u duvanu tipa Virdžinija. *Tutun/Tobacco* **53**, 3-4, 96-104.
- Stephenson MG, Parker MB, Gaines TP, Scinos AS (1987) Manganese and soil pH affects on yield and quality of flue-cured tobacco. *Tob. Sci.* **31**, 109-113.

Relationship between magnetic susceptibility and heavy metal content of soil

Jae Gon Kim^A, Jung Shik Park^B, Chul Min Chon^C and Youn Soo Lee^D

^ADepartment of Geologic Hazard, Korea Institute of Geoscience and Mineral Resources, Daejeon, Korea, Email jgkim@kigam.re.kr

^BDepartment of Geologic Hazard, Korea Institute of Geoscience and Mineral Resources, Daejeon, Korea, Email remoniejs@hanmail.net

^CDepartment of Groundwater and Soil, Korea Institute of Geoscience and Mineral Resources, Daejeon, Korea, Email femini@kigam.re.kr

^DDepartment of Geological Mapping, Korea Institute of Geoscience and Mineral Resources, Daejeon, Korea, Email leeys@kigam.re.kr

Abstract

The magnetic susceptibility and the contamination level of soil have been showed a strong correlation since magnetic particle and contaminant are genetically related. The magnetic susceptibility, mineralogical composition and heavy metal content of 30 surface soil samples collected from uncontaminated forest, road side in industrial area and abandoned mine area were determined to examine their correlation. The soil samples from industrial and mine areas had a higher magnetic susceptibility and higher contents of heavy metal, magnetite and hematite comparing with the soil samples from uncontaminated forest. There was a strong correlation between the magnetic susceptibility and the content of Fe, Mn, Cr, Ni, Pb and Zn of the soils. They also showed a strong correlation with the content of Fe-oxide and Mn-oxide. The contents of As and Cu revealed a weak correlation with the magnetic susceptibility. However, they showed a strong correlation with the Fe oxide content. The correlation data indicate that the magnetic susceptibility measurement can be used as a screening tool of heavy metal level of soil.

Key Words

Magnetic susceptibility, magnetite, hematite, heavy metal.

Introduction

Magnetic susceptibility of a soil has been utilized in a variety of soil science researches such as soil genesis and morphology. Recently, the magnetic susceptibility has been adapted as a tool for the mapping of pollutant distribution (Wang and Qin 2005). The magnetic measurement is considered as a rapid and cheap screening tool for the determination of the spatial distribution of contamination level. The use of magnetic measurement as a proxy for chemical method is possible because pollutant and magnetic mineral are genetically related (Hanesch and Scholger 2002). Hematite and magnetite are common minerals and occur as primary and secondary minerals in soil and solid waste and provide a major sink for pollutants such as heavy metals in soils. They have been known as the major minerals contributing to the magnetic susceptibility of a soil. In addition to the presence of those minerals, the content of Fe, Mn, Cr, Co and Ni also affects the magnetic susceptibility of a soil. The magnetic susceptibility, the heavy metal content and the iron and manganese oxide content of 30 surface soil samples collected from forest, road side in industrial area and abandoned mine area were tested. We report the correlation between the magnetic susceptibility and heavy metal content of the soils.

Methods

Soil sampling and characterization

Thirty surface soil samples were collected from uncontaminated forest, road side in industrial area, and abandoned mine area. The collected soil samples were air-dried and gently ground with a rubber stopper to pass a 2 mm sieve. The samples with less than 2 mm in diameter were stored in a polyethylene bottle for further chemical and mineralogical analysis and magnetic measurement. The mineralogical composition of the samples was determined with an X-ray diffractometer. The metal contents were determined with the aqua regia extraction method. The Fe- and Mn-oxide contents were determined with the dithionite-citrate-bicarbonate method.

Measurement of magnetic susceptibility

The air-dried sample was filled in an acrylic cube for the magnetic susceptibility measurement. The measurement was conducted with a magnetic susceptibility meter (MS2, Bartington Instruments Ltd., UK) at low and high fields.

Results

The soils collected from abandoned mine and industrial areas had a higher metal content and a higher magnetic susceptibility comparing with the soils collected from forest. There was little difference between magnetic susceptibilities measured at high field and low fields except one soil with high clay content. The soil containing a significant amount of magnetite and hematite had a relatively higher magnetic susceptibility ($1586 - 31963^{-10}$ SI) than the others ($76 - 1500^{-10}$ SI). The magnetic susceptibility showed a strong correlation with the contents of aqua regia extractable Fe, Mn, Cd, Cr, Pb, Ni, and Zn (Table 1). The content of aqua regia extractable As and Cu had a weak correlation with the magnetic susceptibility. The heavy metal content of the soil showed a strong correlation with the Fe-oxide content and/or Mn-oxide content. It implies that a significant portion of heavy metals in the soils occurs with Fe-oxide and/or Mn-oxide as adsorbed or coprecipitate form. The magnetic susceptibility data and the heavy metal contents of the soils from collected industrial area and abandoned mine area indicate that the magnetic measurement can be used as an indicator or a screening tool of metal contamination level of soil.

Table 1. Correlation coefficient (R^2) between the magnetic susceptibility measured at low field and the aqua regia extractable metal contents.

| Metal | Fe | Mn | As | Cd | Cr | Cu | Pb | Ni | Zn |
|-----------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Correlation coefficient (R^2) | 0.608 | 0.615 | 0.089 | 0.579 | 0.778 | 0.258 | 0.759 | 0.855 | 0.625 |

Conclusion

The combined data of magnetic and chemical measurements of the soils indicate that the magnetic susceptibility measurement can be a useful tool for the screening of contamination level.

References

- Hanesch M, Schloger R (2002) Mapping of heavy metal loadings in soils by means of magnetic susceptibility measurements. *Environ. Geology* **42**, 857-870.
- Wang ZS, Qin Y (2005) Correlation between magnetic susceptibility and heavy metals in urban topsoil: a case study from the city of Xuzhou, China. *Environ, Geology* **49**, 10-18.

Remediation of chromium contaminated soils: Potential for phyto and bioremediation

Mahimairaja Santiago and Shenbagavalli Santhamani

Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore, India, Email rajasmahimai@yahoo.co.in

Abstract

Long term disposal of tannery wastes has resulted in extensive contamination of agricultural land and water sources in many parts of India. Chromium (Cr), a toxic heavy metal, is a major contaminant in tannery wastes and its accumulation in soil and water is an environmental issue of increasing public concern in India, particularly in Tamil Nadu. Remediation of Cr-contaminated soils is a challenging task which may not only help in sustaining agriculture, but also in minimizing adverse environmental impacts. We examined the potential of phytoremediation and bioremediation techniques suitable for Cr-contaminated soils. Non-edible flower crops were found suitable in remediation as they grow well under Cr-rich effluent irrigation. Amongst the plants examined, *Jasminum* species showed a high degree of tolerance towards soil Cr. While a mustard crop did not establish, a sunflower crop established very well and exhibited higher tolerance towards soil Cr. However, the accumulation of Cr in seeds is likely to diminish its potential for phytoremediation. Application of biological wastes namely, coir pith and poultry manure, to Cr-contaminated soil was found effective in reducing the bioavailable fractions of Cr mainly by forming organic complexes and demonstrated their potential in bioremediating the Cr-contaminated soil.

Key Words

Heavy metals, Cr-hyper accumulators, tanneries, bioavailability, poultry manure, coir pith.

Introduction

The tanning industry is one of the major sources of pollution in Tamil Nadu, India as it disposes large quantities of effluents and sludge rich in chromium (Cr) and salts. The indiscriminate disposal of such wastes into rivers and onto land has resulted in extensive degradation of productive agricultural land and water sources. There are several contaminated sites in Vellore, Erode and Dindigul districts in Tamil Nadu, where, more than 60 per cent of Indian tanneries are located. Assessment of Cr in contaminated soils in Vellore district showed that the soils around tannery industries are severely contaminated with Cr and in most places exceeded the maximum threshold limit prescribed in different countries. The Cr concentration in ground waters was also much higher than the normal average background value reported in different parts of India (Mahimairaja *et al.* 2000).

Phytoremediation is an emerging technology which involves the use of metal-accumulating plants or metal-tolerant plants for remediating metal contaminated soils. Some plants have inherent capacity to absorb and hyperaccumulate heavy metals in their tissues and such capacity can be harnessed to remove toxic heavy metals from contaminated soils. Bioremediation of contaminated soils is a widely accepted technology in which native or introduced microorganisms and/or biological wastes such as compost, animal manures and plant residues are used to detoxify or transform toxic Cr to less toxic forms. Though it has several limitations, this technology holds continuing interest because of its cost-effectiveness. We examined the potential of certain crops (flower, and oilseed crops) and biological wastes (coir pith and poultry manure), in remediating the Cr-contaminated soil.

Methods

Field experiments

A field experiment was conducted on a sandy loam soil (*Typic ustropept*) to examine the growth of plants and accumulation of Cr in plants under Cr-rich effluent irrigation. The soil was slightly saline (pH 8.2; EC 0.23 dS/m) with a cation exchange capacity of 13.4 cmol (p+)/kg. The flower crops *viz.*, *Jasminum sambac* (Gundumalli), *Jasminum grandiflorum* (Jathimalli), *Polianthus tuberosa* (Tuberose) and *Nerium oleander* (Nerium) were evaluated for their phytoremediation potential. The growth and marketable flower yield of plants and the accumulation of Cr in plant parts were examined. In another field experiment, the growth and Cr accumulation in two field crops *viz.*, sunflower and mustard grown on soils amended with different levels of Cr-laden tannery sludge at rates equivalent to 2500, 5000 and 7500 mg Cr per kg soil were examined. The

growth of crops and the Cr accumulation in different parts were examined. The plant samples (flowers, leaves and roots) were collected after 10 months, dried, ground and digested with aqua-regia at 110°C for 2 hrs. After filtration, the Cr was determined using an Atomic Absorption Spectrophotometer (Varian SpectrAA-200) using air-acetylene flame (USEPA 1979).

Laboratory experiment

The potential of some biological wastes viz., coir pith (a waste product from coconut industry) and poultry manure in remediation of Cr-contaminated soil was assessed by examining their effect on the transformation of Cr in sludge amended soils in a closed incubation experiment. Chrome sludge (29560 mg Cr/kg) was mixed with either 100 g clay loam (*Typic haplustert*) or silt clay loam (*Typic ustropept*) at a rate equivalent to 3000 mg Cr/kg soil. The coir pith and poultry manure were added at a rate equivalent to 12.5 t/ha and 5 t/ha, respectively. After thorough mixing of soil, sludge and coir pith / poultry manure, the soil-mixture was incubated in 150 ml plastic cups at field capacity moisture (0.58 g/g). The moisture content was maintained throughout the incubation. The transformation of Cr was examined by determining the different fractions of Cr in soil following a sequential fractionation procedure (Noble and Hughes 1991).

Results

Chromium accumulation in flower crops

The results of field experiment showed that considerable amount of Cr was accumulated in flower crops due to irrigation with tannery effluent. The Cr content in plants varied from 0.74 to 4.83 mg/kg in flowers, 1.69 to 7.85 mg/kg in leaves and 2.83 to 14.02 mg/kg in roots. Invariably, in all crops, roots accumulated higher levels of Cr than leaves and flowers. The irrigation with undiluted effluent resulted in the highest accumulation of Cr (14.0 mg/kg) in roots of *P. tuberosa* followed by *J. grandiflorum* (7.89 mg/kg) and *J. sambac* (7.82 /kg) and *N. oleander* (7.32 mg/kg). Leaves of Jathimalli appeared to have accumulated significantly higher Cr than leaves of other crops. A concentration of Cr up to 14.02 mg/kg did not exhibit any toxicity symptoms in flower crops. Large amounts of Cr were found accumulated in different parts of crops. The translocation of Cr from root to flower was found to be low in flower crops. This suggests that Cr was relatively less mobile, due mostly to its being in chromic (Cr⁶⁺) form. The crops namely *J. sambac*, *J. grandiflorum* and *P. tuberosa* showed high degree of tolerance towards Cr rich-effluent irrigation, whereas, *N. oleander* was relatively sensitive to Cr-effluent.

According to Reeves *et al.* (1996) the concentration of Cr in the dry matter of the aboveground tissues as threshold to define Cr-hyper accumulator is 1000 mg/kg (0.1%). Though Cr accumulation in tested plants was far below the threshold concentration it may be noted that these plants survive at a high level of Cr contamination in soil. Therefore, such Cr-tolerant crops may be suitable for phytoremediation of Cr-contaminated soil. Evidently in the study, none of the flower crops showed any toxicity symptoms at high Cr concentration. This suggests that even at this high concentration of Cr the flower crops could grow well and therefore could safely be used for remediating the Cr-contaminated soils. In Tamil Nadu these flowers are used for worship and more by ladies as decorative in their hairstyle.

Chromium accumulation in sunflower

In the field experiment conducted with Cr-sludge, the mustard crop did not establish due to high concentration of soil Cr. However, sunflower established well and showed a high degree of tolerance towards soil Cr. The amount of Cr taken up by sunflower increased with increasing levels of Cr-sludge-Cr (Table 1). The accumulation of Cr appeared to be distributed in all the plant parts, but with more concentration in the roots. Roots accumulated the highest amount of Cr (190 mg/kg) at the highest dose of Cr-sludge application, followed by leaves (up to 9.18 mg/kg) and stem (4.23 mg/kg). Substantial amount of Cr (5.10 mg/kg) was detected in the seeds, particularly at the highest dose of sludge-Cr. Though the mustard crop was reported to accumulate large amounts of Cr our study demonstrated that this crop may not be suitable for remediating Cr-contaminated soil. The sunflower crop established very well and tolerated a high concentration of soil Cr. However, accumulation of Cr in seeds raises concern that Cr may enter into food chain and therefore it diminishes its potential for remediation.

Table 1. Accumulation of chromium in different parts of sunflower grown on soil amended with tannery sludge.

| Treatments | Cr content (mg/kg) | | | | Soil Cr * (mg/kg) |
|--------------------------------|--------------------|------|--------|-------|----------------------|
| | Roots | Stem | Leaves | Seeds | |
| 1. Control | 9.84 | 0.60 | 5.10 | bdl | 110 |
| 2. Sludge @ 2500 mg Cr/kg soil | 50.03 | 1.01 | 7.86 | 0.41 | 2576 |
| 3. Sludge @ 5000 mg Cr/kg soil | 158.90 | 2.09 | 8.79 | 0.66 | 5464 |
| 4. Sludge @ 7500 mg Cr/kg soil | 190.49 | 4.23 | 9.18 | 5.10 | 6843 |
| LSD (0.05) | 34.5 | 0.92 | 0.68 | 0.29 | - |

bdl = below detectable limit, * at harvest

Effect of biological wastes on chromium transformation in soil

The result of laboratory study showed that irrespective of soils, both coir pith and poultry manure markedly reduced the concentration of soluble plus exchangeable-Cr which represent toxic forms of Cr in soils (Figure 1). While 61 (clay loam) and 75 (silt clay loam) per cent reduction in the concentration of bioavailable fractions (soluble plus exchangeable) of Cr was observed with the application of coir pith, a reduction of 62.3 (clay loam) and 68 (silt clay loam) per cent was observed due to poultry manure addition.

The application of coir pith or poultry manure appeared to have reduced bioavailability of Cr in soil. This may be due to either formation of organo-chromic complexes (immobilization) or reduction of toxic, soluble Cr (VI) to non-toxic, less soluble Cr (III) in soil. The biological wastes represent a significant reservoir of electron donors for the reduction of Cr (VI) to Cr (III) (James and Bartlett 1983). During the decomposition of organic matter, compounds such as citric acid or gallic acid are formed which have the potential for chelating Cr (III) or reducing Cr (VI), and thereby reducing the toxicity of Cr (James and Bartlett 1983). Humic substances present in the biological wastes play a major role in reduction of Cr (VI). In general, it appeared that the addition of an easily degradable substrate with a low C:N ratio (poultry manure) stimulated more Cr (VI) reducers compared to high C:N ratio substrate (coir pith).

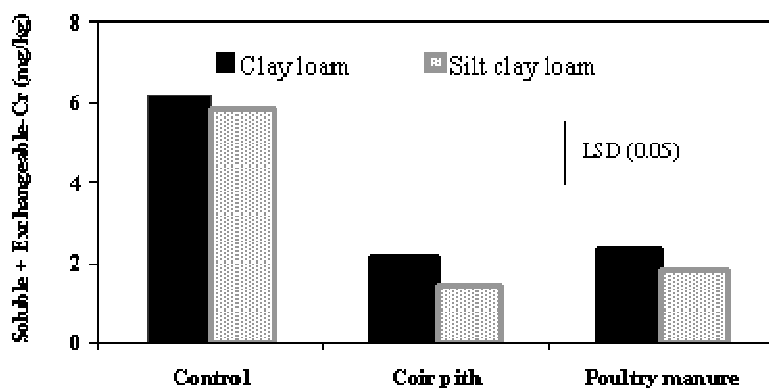


Figure 1. Effect of biological wastes on soluble and exchangeable fractions of Cr in soils.

Conclusion

The non-edible crops, like flower crops, were found suitable for phytoremediation of the Cr-contaminated soils as the entry of Cr into the food chain is largely avoided. Bioremediation, using biological wastes offers another avenue for the remediation. Addition of biological wastes and compost is known to immobilize Cr through complexation and thereby reducing the bioavailable fractions of Cr. Application of farm yard manure (FYM) or compost or biological wastes is a common practice and traditionally followed in Indian agriculture. This technology can also be exploited for the remediation of Cr-contaminated soils. Integrated approach, involving suitable chemical and biological amendments, plants and microbial strains should be required for successful remediation of Cr-contaminated soils.

Acknowledgments

We thank the Australian Centre for International Agricultural Research (ACIAR) for funding part of this research project and CSIRO Land and Water, Adelaide, for collaboration.

References

- James BR, Bartlett RJ (1983) Behaviour of chromium in soils. VII. Adsorption and reduction of hexavalent forms. *Journal of Environmental Quality* **12**, 177-181.
- Mahimairaja S, Sakthivel S, Divakaran J, Naidu R, Ramasamy K (2000) Extent and severity of contamination around tanning industries in Vellore district. In 'Towards Better Management of Soils Contaminated with Tannery Wastes. ACIAR Publication No **88**, 75-82.
- Noble AD, Hughes JC (1991) Sequential fractionation of chromium and nickel from serpentinite derived soils from the Eastern Transvaal. *Communications in Soil Science and Plant Analysis* **22**, 1963-1972.
- Reeves RD, Baker AJM, Borhidi A, Berzain R (1996) Nickel-accumulating plants from the ancient serpentine soils of Cuba. *New Phytology* **133**, 217-224.
- USEPA (1979) Method 218.1. Atomic Absorption, direct aspiration. In 'Methods for Chemical Analysis of Water and Waste Water. Report No. EPA-600/4-79-00. March 1979. United States Environmental Protection Agency Office of Drinking Water, Washington DC

Response of *Pinus radiata* and soil microbial activity to increasing copper and zinc contamination in a soil treated with metal-amended biosolids

Paramsothy Jeyakumar^A, Paripurnanda Loganathan^A, Christopher W.N. Anderson^A, Sivalingam Sivakumaran^B and Ronald G. McLaren^C

^ASoil and Earth Sciences, Institute of Natural Resources, Massey University, Palmerston North, New Zealand,

Email j.jeyakumar@massey.ac.nz

^BSustainable Production–Soil Water Environment, Plant & Food Research, Palmerston North, New Zealand.

^CSoil and Physical Sciences Dept, Agriculture and Life Sciences Faculty, Lincoln University, Lincoln, New Zealand.

Abstract

The effects of elevated concentrations of copper (Cu) and zinc (Zn) in a soil treated with biosolids previously spiked with these metals on *Pinus radiata* were investigated in a 312 day glasshouse pot trial. The total soil metal concentrations in the treatments were 16, 48, 146 and 232 mg Cu/kg and 36, 141, 430 and 668 mg Zn/kg. Increased total soil Cu concentration increased the soil solution Cu concentration (0.03 to 0.54 mg/L) but had no effect on leaf and root dry matter contents. Increased total soil Zn concentration also increased the soil solution Zn concentration (0.9 to 362 mg/L). Decreased leaf and root dry matter were recorded above the second level of Zn. Neither Cu nor Zn had any effect on the mycorrhizal colony of *P. radiata*. A lower percentage of Cu in the soil exchangeable fraction (5–12%) and lower Cu²⁺ concentration in soil solution (0.001–0.06 µM) relative to Zn (soil exchangeable fraction, 12–66%; soil solution Zn²⁺ concentration, 4.5–4419 µM) indicated lower bioavailability of Cu. Soil dehydrogenase activity decreased with every successive level of Cu and Zn applied. It was reduced by 50% at the total solution–phase Cu and Zn concentrations of 0.3 and 38 mg/L, respectively, and solid–phase exchangeable Cu and Zn concentrations of 8 and 185 mg/kg, respectively.

Key Words

Radiata pine, forest, speciation, soil metal fractions, EC₅₀, rhizosphere.

Introduction

Forestry has a very important place in the economy of New Zealand and contributes NZ\$ 3.2 billion to the export economy annually. Production forests comprise 7% of New Zealand's total land area (1.8 million ha) of which radiata pine contributes 89.2% of the national plantation forestry estate (NZFOA 2008/2009). Radiata pine forests have in place intensive management regimes and grow relatively quickly. Biosolids application to forestry land is permitted in New Zealand, and this represents a major nutrient and organic carbon input for radiata pine plantations. However, heavy metals accumulating in soil through biosolids application may present an environmental risk. Contaminants could potentially enter the food chain as a result of possible future land–use change. The environmental effects of biosolids must be evaluated. Part of this assessment includes quantification of the long-term capacity of forests to receive biosolids without significant degradation. The concentration of bioavailable rather than total metal is an accurate index to assess the biotoxicity of heavy metals. In order to develop such an index, data are required on the effect of variable concentrations of different metal species on soil microbiological activity and plant growth. This paper presents the results of a study that was conducted to determine the bioavailability of Cu and Zn in soils amended with biosolids containing different Cu and Zn concentrations, and the effect of these metals on the growth and level of metal uptake by radiata pine.

Method

A glasshouse trial was conducted using one–year old radiata pine (*Pinus radiata*) in pots containing soil (13 kg) that was amended with biosolids previously spiked with 3 levels of Cu or Zn. The control treatment was soil amended with biosolids not spiked with metal (Jeyakumar *et al.* 2008 and 2009). Total metal concentrations (mg/kg soil) in the treatments were for Cu 16 (control), 48 (Cu1), 146 (Cu2), 232 (Cu3); for Zn 36 (control), 141 (Zn1), 430 (Zn2), 668 (Zn3). These metal concentration ranges cover the soil metal concentration limits (100 and 300 mg/kg for Cu and Zn, respectively) recommended by the NZWWA (2003). The 7 treatments were replicated three times and arranged in a Randomized Complete Block Design (RCBD). One–year old radiata clones (R96004) were transplanted, one per pot, and regularly watered to maintain soil moisture at 80% 'pot field capacity'. The plants were harvested 312 days after planting. The

following parameters were measured at harvest: metal fractions, soil–solution total metal concentration, pH, and dehydrogenase activity in rhizosphere and bulk soils, as well as radiata pine foliar and root dry matter (DM) yields, mycorrhizae hyphal density, and plant metal concentration.

Results

Dry matter yield and metal availability to the plants

Increased Cu levels had no significant effect on both leaf and root DM yield. Increasing the soil Zn concentration decreased leaf DM yields beyond Zn1 treatment (Table 1). In roots a much greater DM yield reduction was observed between the Zn1 and Zn2 treatment than was observed for leaves.

The leaf Cu concentration at all levels of Cu was higher than the critical Cu concentration of 2.1–2.3 mg/kg, generally considered the limit for Cu deficiency in juvenile radiata pine plants (Boardman *et al.* 1997). Plant leaves at the Zn2 treatment showed severe yellowing and withering. The Zn3 treatment plants died during the experiment. The concentration of Zn in leaves and roots for all Zn treatments was significantly increased with every successive level of Zn applied. The leaf Zn concentration above the Zn1 treatments was much higher than the phytotoxic level of 200 mg Zn/kg DM defined by Boardman *et al.* (1997). The bioconcentration factor (BCF) (Table 1) was much lower for Cu (0.03–0.26) than for Zn (0.79–1.5) probably due to lower plant availability of Cu in soils. The leaf:root concentration ratio was also lower for Cu than for Zn (Table 1) suggesting that the translocation of Cu from root to leaf was lower than that for Zn.

Table 1. Effect of Cu and Zn on radiata leaf metal concentration and dry matter yield.

| Treatment | Soil solution Conc. (mg/L) | Dry matter (g) | | Metal conc. (mg/kg) | | Metal conc. Ratio (leaf:root) | BCF |
|-----------|-------------------------------|-------------------|--------|------------------------|--------|----------------------------------|------|
| | | Leaf | Root | Leaf | Root | | |
| Cu0 | 0.03 | 77.6 a | 34.8 a | 4.2 d | 10 d | 0.42 | 0.26 |
| Cu1 | 0.27 | 67.5 a | 32.7 a | 5.3 c | 65 c | 0.08 | 0.11 |
| Cu2 | 0.37 | 65.6 a | 35.6 a | 6.3 b | 166 b | 0.04 | 0.04 |
| Cu3 | 0.54 | 65 a | 39.6 a | 7.3 a | 267 a | 0.03 | 0.03 |
| Zn0 | 0.9 | 72.3 a | 34.8 a | 39 d | 45 d | 0.85 | 1.08 |
| Zn1 | 4.4 | 68.1 a | 33.7 a | 205 c | 550 c | 0.37 | 1.45 |
| Zn2 | 89 | 33.5 b | 3.1 b | 341 b | 2464 b | 0.14 | 0.79 |
| Zn3 | 362 | 12.6 c | 1.5 b | 1000 a | 5033 a | 0.2 | 1.5 |

Values in columns followed by different letters are significantly different ($p \leq 0.05$)

Metal concentration in soil solution and solid phase

The total Cu concentrations (mg/L) in rhizosphere soil solution were 0.03, 0.27, 0.37 and 0.54 for the control, Cu1, Cu2 and Cu3 treatments, respectively. For Zn, the concentrations were 0.9, 4.4, 89 and 362 for the control, Zn1, Zn2 and Zn3 treatments, respectively. When the soil solution Cu species in the rhizosphere soils were investigated using the Windermere Humic Aqueous Model (WHAM) (Centre for Ecology and Hydrology 2002), Cu^{2+} concentrations (μM) in soil solution were 0.001, 0.008, 0.04 and 0.06 for the control, Cu1, Cu2 and Cu3 treatments, respectively. These Cu^{2+} concentrations are lower than the critical Cu^{2+} concentration of 0.22 μM , below which rhode grass growth was reported to be reduced (Sheldon and Menzies 2005). Though the Cu^{2+} concentrations are very low, the growth of radiata did not increase with an increase in the Cu^{2+} concentration probably because mycorrhizal association in the roots was assisting the plants to take up adequate Cu from the soil even at the lowest Cu^{2+} concentration. The low Cu^{2+} concentration is attributed to about 99% of Cu in soil solution being complexed to dissolved organic matter. Zn^{2+} concentrations (μM) for the corresponding Zn treatments were 4.5, 41, 983 and 4419, respectively; and they contributed 61–80% of soil solution Zn. Copper, at all rates of addition, and Zn at the low rates of addition, was mainly associated with the oxide bound soil fraction (21–54% for Cu and 64% for Zn1). However, Zn at high rates of addition (Zn2 and Zn3) was mainly found in the bioavailable soluble & exchangeable soil fraction (58–66%) (Figure 1).

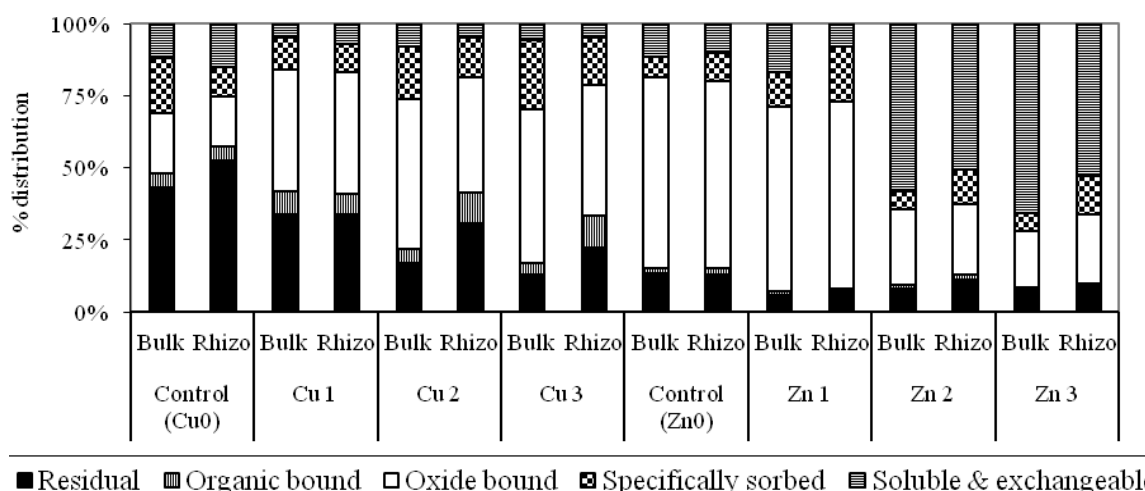


Figure 1. Percentage distribution of Cu and Zn fractions in rhizosphere and bulk soils amended with biosolids under poplar

Mycorrhiza and dehydrogenase activity

Both Cu and Zn at all levels of addition had no significant effect on mycorrhizal colonization of the roots. Although Cu was not found to be toxic to plants or mycorrhiza, it was found to decrease total microbial activity at all levels of Cu addition, as observed from the decrease in soil dehydrogenase activity with increasing Cu (Figure 2). As for Cu, increasing the rate of Zn also decreased soil dehydrogenase activity. The concentration of metal in the soluble+exchangeable solid phase and in the total solution phase that corresponded to a 50% reduction in dehydrogenase activity (EC_{50}) was determined. The EC_{50} values for total solution phase Cu and Zn were 0.3 and 38 mg/L, respectively, and for solid-phase exchangeable Cu and Zn were 8 and 185 mg/kg, respectively.

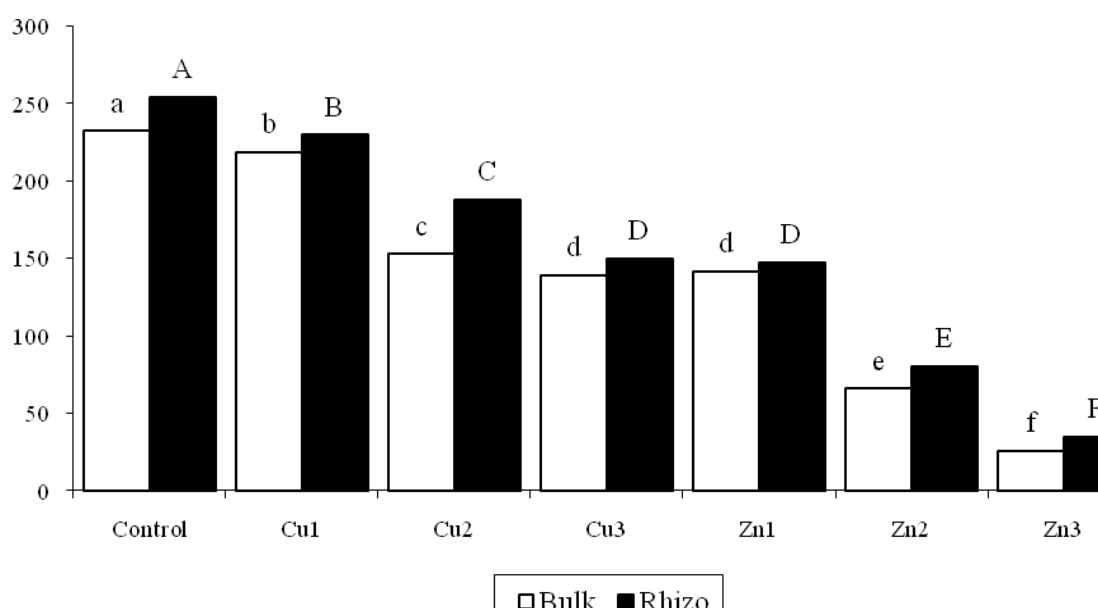


Figure 2. Dehydrogenase activity (TPF) in bulk and rhizosphere soils at different levels of Cu and Zn. Bars with different letters are significantly different ($p \leq 0.05$). Simple letters indicate the variance among the bulk soils and capital letters for the rhizosphere soils.

Conclusion

Biosolid-derived Cu in soils at rates of up to the highest level of 232 mg/kg tested in this study (total soil solution Cu concentration of 0.54 mg/L and Cu^{2+} concentration of 0.06 μM) had no effect on radiata leaf and root dry matter yield and root population of mycorrhiza but reduced total soil microbial activity as measured by dehydrogenase activity even at the lowest total soil Cu concentration of 48 mg/kg tested (total soil solution Cu concentration of 0.27 mg/L and Cu^{2+} concentration of 0.008 μM). Biosolid-derived Zn in soils at rates of 430 mg/kg (total solution Zn concentration of 89 mg/L) and above reduced radiata leaf and root dry

matter yield but had no effect at 141 mg/kg total soil Zn concentration. As for Cu, Zn at all rates of addition reduced dehydrogenase activity but had no effect on mycorrhiza. Therefore, applications of biosolids–derived Cu at rates of up to 232 mg/kg and Zn up to 141 mg/kg are not likely to pose any phytotoxic risk to radiata pine but can present a risk to microorganisms at much lower concentrations. Considering these results the current recommendation of soil Cu and Zn limits (100 mg/kg for Cu and 300 mg/kg for Zn) are high with respect to soil microbial activity but low for Cu with respect to radiata pine growth. In terms of bioavailable metal concentrations affecting microbial activity the Cu concentration limit is much lower than that of Zn. Cu is more toxic to microorganisms than Zn per unit total soil metal concentration.

Acknowledgement

We thank Mark Ryan, Arborgen, New Zealand for providing radiata pine clones R96004 and Stéphanie Caille for measuring mycorrhizae colonized roots infection.

References

- Boardman R, Cromer RN, Lambert MJ, Webb MJ (1997) Forest plantations. In 'Plant analysis: An interpretation manual' 2nd edn (Eds DJ Reuter, JB Robinson) pp. 505–566. (CSIRO Publishing: Melbourne)
- Centre for Ecology and Hydrology (2002) 'Windermere humic aqueous model (WHAM): Equilibrium chemical speciation for natural waters.' (Natural Environmental Research Council: UK)
- Jeyakumar P, Loganathan P, Sivakumaran S, Anderson CWN, McLaren RG (2009) Comparative tolerance of poplar and microorganisms to copper and zinc toxicity in a biosolids–amended soil. In 'Proceedings of the 10th International Conference on the Biochemistry of Trace Elements, Chihuahua, Mexico'. <http://icobte2009.cimav.edu.mx/proceedings.php>
- Jeyakumar P, Loganathan P, Sivakumaran S, Anderson CWN, McLaren RG (2008) Copper and zinc spiking of biosolids: effect of incubation period on metal fractionation and speciation and microbial activity. *Environmental Chemistry* **5**, 347–354
- NZWWA (2003) 'Guidelines for the safe application of biosolids to land in New Zealand'. (New Zealand Water and Wastes Association: Wellington, NZ)
- NZFOA (2008/2009) 'New Zealand Forest Industry Facts and Figures 2008/2009'. (New Zealand Forest Owners Association: Wellington, NZ)
- Sheldon A, Menzies NW (2005) The effect of copper toxicity on the growth and morphology of Rhodes grass (*Chloris gayana* Knuth.) in resin buffered solution culture. *Plant and Soil* **278**, 341–349

Rhizosphere bacterial community PCR-DGGE profiles and metal speciation in shooting range soils

Yohey Hashimoto^A, Kiyoshi Tajima^B, Hiroko Yamada^C and Takeshi Sato^D

^ADepartment of Bioresources, Mie University, 1577 Kurima-machiya, Mie, 514-8507 Japan, Email yhashim@bio.mie-u.ac.jp

^BDepartment of Bioproduction and Environment Engineering, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya, Tokyo, 156-8502 Japan.

^CContig-i Co. Ltd., 631-7 Masaki, Gifu, 502-0857 Japan.

^DDepartment of Civil Engineering, Gifu University, 1-1 Yanagido, Gifu, 501-1193 Japan.

Abstract

Chemical immobilization in conjunction with vegetation coverage is a practical remediation technology for metal contaminated soils. This paper evaluated metal solubility and bacterial community structure in a highly contaminated shooting range soil treated with a phosphorus amendment (AP) and plant growth. The low dose of amendment addition decreased the SPLP-Pb concentration to 2.1 mg/L. However, the high dose treatment increased Pb solubility more than that of Control soil. The soils treated with the amendment along with plant growth (Plant + AP) had a lower SPLP-Pb concentration than the soils treated solely with the amendment. The soils with the amendment addition had a decreased number of bands in their DGGE profile, and the detrimental effect of amendment appeared more significant for the high dosage than in low dosage. According to the DNA sequence identification, the band corresponding to *Bacillus sp.*, and *Rhizobium sp.* that dominated in the Control and Plant soils was not observed in the soil with the high dose of amendment.

Key Words

Microbial activity, heavy metals, lead, antimony, phytoremediation.

Introduction

In situ chemical immobilization is a practical remediation technology for metal-contaminated soils because of its capability to reduce cost and environmental impacts. We have suggested a new immobilization technology (chemically-enhanced phytostabilization) that can be applied along with a vegetation cover as a form of phytostabilization (Hashimoto *et al.* 2009). For development and evaluation of chemically-enhanced phytostabilization technology for shooting ranges, it is necessary to investigate how the plant rhizosphere affects speciation and solubility of preexisting heavy metals in the soil. In addition to mineralogical and chemical perspectives of heavy metals, the technology should be assessed with respect to remediation of soil biological functions in the rhizosphere soil. This paper evaluated metal solubility and bacterial community structure in a highly contaminated shooting range soil treated with a phosphorus amendment and plant growth.

Methods

Experiment setup

Ryegrass (*Lolium perenne* L.) was grown with a modified a split-root system described elsewhere (Perez *et al.* 2007). The split-root system was designed to isolate rhizosphere and non-rhizosphere soils. Two PVC tubes with a 14 cm internal diameter and 10 cm length formed lower and upper sections, both of which contained contaminated soils collected from a shooting range in Central Japan. These sections were connected by a central compartment with 32 PVC tubes each with 10 mm internal diameter and 10 cm in length. The tubes of central compartment were filled with a 0.45 mm sieved soil and treated with 2% and 4%_(w/w) of hydroxyapatite (AP_{low} and AP_{high}). The tubes without amendment but allowing root entry were also prepared (Plant). The soil tubes where roots were blocked from entry using a 10 µm nylon filter served as non-rhizosphere soils (Control).

Heavy metal analysis in soils

Synthetic Precipitation Leaching Procedure (SPLP, EPA Method 1312) was used to determine extractable Pb and Sb in the soil. Twenty (20) mL SPLP solution (H₂SO₄/HNO₃ = 60/40% wt.) was added to 1.0 g soil, and the mixture was equilibrated for 24 hours on a shaker. The supernatant passed through a paper filter was analyzed by ICP-AES. Equilibrium modeling was performed by a thermodynamic code, Visual MINTEQ ver. 2.61 (KTH, Stockholm, Sweden) to compute the saturation indices (SI) of Pb precipitates that may control Pb solubility in liquid phases. The SI represents the degree of saturation with respect to a specific Pb

solid phase is defined as $SI = \log IAP - \log K_{sp}$ where IAP is the ion activity product and K_{sp} is the solubility product constant. If $-1 < SI < 0$, the solution is saturated with respect to the solid; if $SI < -1$, the solution is undersaturated with respect to the solid; and if $SI > 0$, the solution is supersaturated with respect to the solid. The extraction procedure of soil solution and following elemental analysis were the modifications of previous study (Hashimoto *et al.* 2008).

Soil DGGE analysis

Nucleic acid was extracted directly from duplicate 0.5-1.0g composite samples. PCR amplification of the 16S rDNA fragments prior to DGGE was performed as described by Muyzer *et al.* (1993). DGGE was performed by using a D-Code 16/16-cm gel system with a 1.5-mm gel width (Bio-Rad, Hercules, Calif.) maintained at a constant temperature of 60°C in 7 liters of $1 \times$ TAE buffer. The central 1-mm 2 portions of strong DGGE bands were excised with a razor blade and soaked in 50 μ l of purified water overnight. Purified DNA was sequenced with an ABI-Prism model 373 automatic sequencer. Sequence identification was performed by use of the BLASTN facility of the National Center for Biotechnology Information and the Sequence Match facility of the Ribosomal Database Project.

Results

Soil characterization

The Pb-contaminated soil had a sandy clay loam texture based on the USDA soil textural classification system. The soil had a relatively high proportion of organic matter (8.6%) since the shooting range is located in a forested area. The soil had a pH value of 5.9. Total concentration of Pb and Sb in the soil was 29600 and 50 mg/kg, respectively. An XRD analysis revealed that cerussite ($PbCO_3$) was a predominant Pb mineral in the soil.

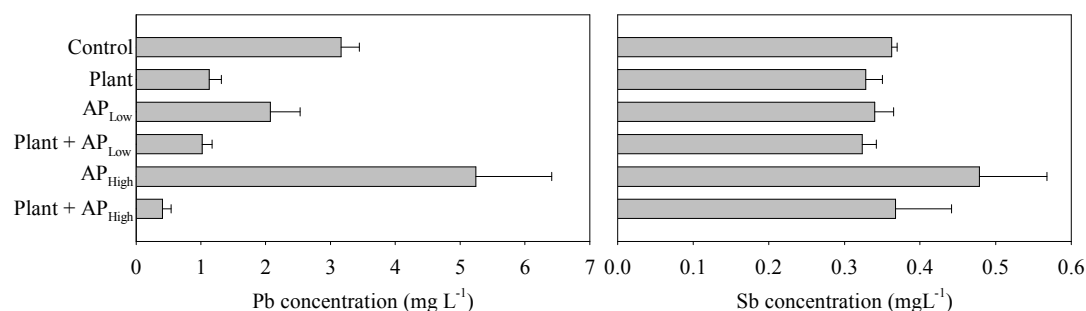


Figure 1. Concentrations of SPLP extracted Pb and Sb in the soils treated with and without amendment and plant

Pb and Sb solubility

The SPLP-Pb concentration for the Control soil was 3.2 mg/L (Figure 1). The low dose amendment decreased the SPLP-Pb concentration to 2.1 mg/L. As expected, the decreased Pb solubility resulted in transformation of pre-existing cerussite into pyromorphite. However, the high dose amendment increased Pb solubility more than that of Control soil. The soils treated with the amendment along with plant growth (Plant + AP) had a lower SPLP-Pb concentration than the soils treated solely with the amendment (AP_{low} and AP_{high}). The rhizosphere processes including proton and organic acid secretions may enhance the dissolution of soil Pb and amendment P and induce their reformation as pyromorphite precipitates. Solubility of Sb was not attenuated by the amendment rather appeared to be increased by the high dose amendment (AP_{high}). The dissolution of Sb was attributable to anion exchange processes between soil Sb and amendment P. Our result suggests that a high dose of apatite application increased both Pb and Sb solubility.

Aqueous Pb speciation

The SI values of solubility controlling phases were calculated to examine the Pb solubility in elutes among the treatments (Table 1). Over 98% of total dissolved Pb in all treatments was present as organically complexed forms. Chloropyromorphite [$Pb_5(PO_4)_3Cl$] had SI values of over -1.0 in the Control, Plant, AP_{low} and AP_{high} treatments, indicating a possible saturation in solution. The SI value of the combination treatments with amendment and plant growth indicated that chloropyromorphite appeared to be undersaturated in solution. Increased DOC concentration observed in these solutions accelerated the Pb-organic complex which may block the formation of chloropyromorphite.

Table 1. Saturation index values of minerals in the soils treated with plant and different levels of amendments (AP).

| Minerals | Control | Plant | AP _{low} | Plant+AP _{low} | AP _{high} | Plant+AP _{high} |
|---|---------|-------|-------------------|-------------------------|--------------------|--------------------------|
| CaCO ₃ | -1.6 | -2.0 | -0.9 | -1.1 | -0.9 | -0.5 |
| Ca ₅ (PO ₄) ₃ OH | -4.7 | -6.9 | -0.4 | -3.3 | 0.0 | -0.8 |
| PbCO ₃ | -1.8 | -2.1 | -2.1 | -2.1 | -2.0 | -2.7 |
| Pb ₃ (CO ₃) ₂ (OH) ₂ | -5.6 | -6.4 | -6.5 | -6.6 | -6.3 | -8.4 |
| Pb ₅ (PO ₄) ₃ OH | -10.9 | -12.1 | -11.6 | -13.6 | -10.5 | -17.1 |
| Pb ₅ (PO ₄) ₃ Cl | 0.0 | -0.9 | -1.0 | -2.8 | 0.0 | -6.5 |
| PbO | -7.3 | -7.6 | -7.6 | -7.6 | -7.5 | -8.2 |
| Pb(OH) ₂ | -2.6 | -2.8 | -2.9 | -2.9 | -2.8 | -3.5 |

Soil DGGE analysis

Apparent differences in the bacterial community among the treatments were observed in the DGGE profiles (Figure 2). Plant growth in the contaminated soil appeared to enrich bacterial populations since the general band profile exhibited in the Plant soil was brighter than the Control soil. The soils with the amendment addition decreased the number of bands in their DGGE profile, and the detrimental effect appeared more significantly in AP_{high} than in AP_{low} soils. However, the number of bands lost in the solo application of amendment was recovered in the soil treated with amendment along with plant growth. According to the DNA sequence identification, the band corresponding to *Bacillus sp.*, and *Rhizobium sp.* dominated in the Control and Plant soils was not observed in the soil with the high dose of amendment.

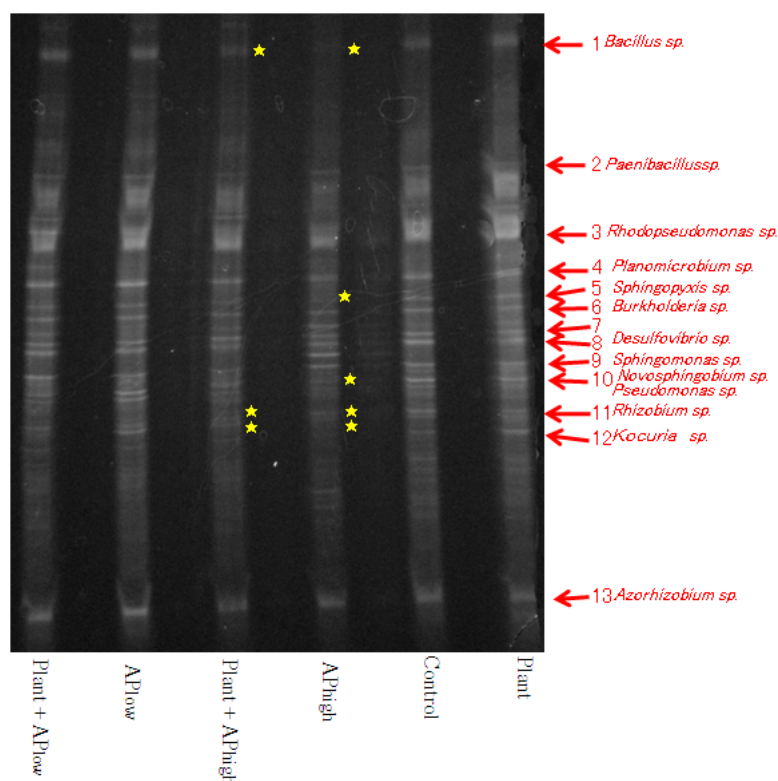


Figure 2. DGGE profiles and DNA sequence identification from the soils treated with and without amendment and plant.

Discussion and summary

Solubility of soil Pb was attenuated by the amendment application. When the amendment was added, Pb immobilization was more enhanced in the rhizosphere than the non-rhizosphere soils. Previous studies identified the formation of phosphate-bound Pb precipitates at the root surface (Cotter-Howells *et al.* 1999). Original Pb species in the soil may be transformed via rhizosphere processes that have a catalysing role for chloropyromorphite formations, although the exact mechanism remains unclear. A significant decrease of

number of band in the DGGE profile was found in the AP_{high} soils where solubility of Pb and Sb increased by the high dose of amendment. The detrimental impact of amendment on soil bacterial community may be attributed to the increased metal toxicity and salinity levels which were induced by the high application dosage. Plant growth in combination with the amendment even at high dosage attenuated Pb solubility and generally conserved bacterial community found in the Control soil. Our study suggests that phytostabilization assisted with the amendment enhance Pb immobilization and maintain bacterial community even in a highly contaminated shooting range soil.

References

- Cotter-Howells JD, Champness PE, Charnock JM (1999) Mineralogy of Pb-P grains in the roots of *Agrostis capillaris* L. by ATEM and EXAFS. *Mineralogical Magazine* **63**, 777-789.
- Hashimoto Y, Matsufuru H, Sato T (2008) Attenuation of lead leachability in shooting range soils using poultry waste amendments in combination with indigenous plant species. *Chemosphere* **73**, 643-649.
- Hashimoto Y, Matsufuru H, Takaoka M, Tanida H, Sato T (2009) Impacts of chemical amendment and plant growth on Pb speciation and enzyme activities in a shooting range soil: an X-ray absorption fine structure (XAFS) investigation. *Journal of Environmental Quality* **38**, 1420-1428.
- Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied Environmental Microbiology* **59**, 695-700.
- Perez MJ, Smyth, TJ, Israel, DW (2007) Comparative effects of two forage species on rhizosphere acidification and solubilization of phosphate rocks of different reactivity. *Journal of Plant Nutrition* **30**, 1421-1439.

Role of PGP arsenic-resistant bacteria in As mobilization and translocation in *Helianthus annuus* L.

Lucia Cavalca, Anna Corsini, Sachin Bachate and Vincenza Andreoni

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Email lucia.cavalca@unimi.it, anna.corsini@yahoo.it, sachin.bachate@unimi.it, vincenza.andreoni@unimi.it

Abstract

This study investigated the effect of arsenic-resistant *Alcaligenes* sp. strain DhalL with potential plant growth promoting characteristics on growth and arsenic uptake by sunflower (*Helianthus annuus* L.). Pot experiments were prepared using an agricultural As-contaminated soil sown with seeds of sunflower uninoculated and inoculated with the strain. A Real Time PCR method, based on the quantification of *ACR3(2)* gene carried by DhalL, was set up in order to monitor presence and colonisation of the soil by the strain. The arsenic content was significantly higher in inoculated than in the uninoculated aboveground part of the plants (1.63 mg kg d.w.⁻¹ vs. 0.77), highlighting the effect of the strain on arsenic uptake by sunflower. *ACR3(2)* gene copy number was one hundred times higher in inoculated than in uninoculated pots, especially in the rhizospheric soil, indicating that colonisation occurred. The results suggest that the presence of arsenic resistant strain such as *Alcaligenes* sp. DhalL in the rhizosphere of sunflower could influence As mobilization and uptake by plant.

Key Words

Arsenic, *ACR3*, qPCR, PGPR, sunflower.

Introduction

Arsenic (As) concentrations range from 1 to 40 mg As/kg in uncontaminated soil (Fitz and Wenzel, 2002). However, natural- and anthropogenic processes can elevate soil As levels. Arsenic mainly occurs in two inorganic forms, viz., arsenite (AsIII) and arsenate (AsV). Constituents of minerals, pH, redox potential, organic matter, interactions with Fe and Mn oxides, chemical speciation as well as biological activity affect the bioavailability and mobility of As in soil, enhancing As concentration in soil solution and causing toxic effect for plants (Adriano 2001; Islam *et al.* 2000; Bauer and Blodau 2006; Mukhopadhyay *et al.* 2002). To counteract the toxicity of As, microorganisms have evolved several mechanisms, that can be divided into different categories, consisting of detoxification reactions through the *ars* operon genes (Silver and Phung 1996), and of energy-gaining reactions through dissimilatory AsV reduction by a periplasmic AsV-reductase and of AsIII oxidation by a AsIII-oxidase (Oremland and Stolz 2005). In plants arsenate, acting as an analogue of phosphate, is transported across the plasma membrane via phosphate co-transport systems while arsenite enters aspecifically through aquaglyceroporins. Some plants respond to biological- and environmental-stresses by synthesising “stress” ethylene from the precursor 1-amino-cyclopropane-1-carboxylic acid (ACC) (Morgan and Drew 1997). Plant growth-promoting rhizobacteria (PGPR) that produce the enzyme ACC deaminase cleave ACC and lower the level of ethylene, facilitating the formation of longer roots in plants growing in the presence of heavy metals (Grichko *et al.* 2000). Synergistic use of plants and metal-resistant PGPR is a promising approach for remediation of metal contaminated soils. Plants sequester the metals in their shoots, which can then be harvested. Different crop plants, among which sunflower (*Helianthus annuus* L.), can be used for phyto-extraction purpose because they can uptake relatively high concentrations of metals and can have high yield biomass (Marchiol *et al.* 2007). The objective of this study was to evaluate the effect of *Alcaligenes* sp. strain DhalL, an As-resistant strain with PGP characteristics, on the growth of *H. annuus* and on As translocation in the plant.

Methods

Experimental design

Pot experiments were set up using 250 g of soil sown with sunflower seeds (3 seeds in each pot). Five pots were sown with seeds treated according to Dell'Amico *et al.* (2008) with cell suspension (10⁸ CFU/mL) of *Alcaligenes* sp. DhalL previously isolated from an As-contaminated soil (Bachate *et al.* 2009). Five additional pots represented uninoculated controls. Pots were placed in growth chamber with a 16/18 h photoperiod, 25°/18° C temperature, UR 80/60%. The soil under study was physico-chemically characterized according to the MIPAF Official Methods (2000). As fractions were determined by sequential extraction method (Wenzel *et al.* 2001).

Microbiological analysis

The number of total heterotrophic (THB) and As-resistant bacteria (ARB) in soil was determined by conventional plating techniques on 1/10 strength Tryptic Soy Agar (TSA/10) in the absence or in the presence of AsV (15 mmol/L) or AsIII (3 mmol/L), respectively. At 0, 27 and 50 days of incubation, rhizosphere soil samples were collected and divided into two fractions: the “bulk”, obtained by manually shaking the roots, and the “rhizospheric” soil, obtained by washing the roots with sterile distilled water. For each fraction the number of THB and ARB was determined. Strains were isolated from As plates and their As resistance level was determined by growth in Tris Mineral Medium supplemented with gluconate (0.6%, w/v) containing increasing amounts of AsV or AsIII. Strains were identified by 16S rRNA gene sequence analysis.

Molecular analysis and quantitative Real Time PCR

Genes *ArsC*, *ArsB*, *ACR3(1)* and *ACR3(2)* were amplified in the strains as reported in Bachate *et al.* (2009). At the different incubation times, DNA was extracted from bulk and rhizosphere soil fractions (0.5g) by using UltraClean Soil DNA Isolation Kit (MOBIO, USA). Soil DNA was used in Real-Time quantitative PCR (q-PCR) experiments to quantify *ACR3(2)* gene, in order to monitor the presence of *Alcaligenes* sp. Dhall. Primer pair acr475F/611R (targeting a 137 bp fragment internal to *ACR3(2)* gene sequence of *Alcaligenes* sp. Dhall) and P1369F/1492R (targeting a 123 bp region of 16S rRNA gene) were designed. The reactions were set up with 1X SsoFast EvaGreen Supermix (BIORAD, USA). For generation of calibration curves of *ACR3(2)* and 16S rRNA genes, serial dilutions of total DNA extracted from *Alcaligenes* sp. Dhall were prepared. Relative quantification of *ACR3(2)* gene, normalized to 16S rRNA as reference gene (Livack Method, $\Delta\Delta Ct$) was performed in all the soil fractions.

Analytical methods

At 27 and 50 day of incubation, sunflower shoots dry biomass (105 °C) was measured and the As content was determined by ICP-MS (Inductively Coupled Plasma-Mass Spectrometry, Varian Inc.). Analytical data were compared by applying t-Student test using SPSS version 17.0 (Inc., Chicago).

Results

The soil used in this study was a loamy agricultural soil from Scarlino (Italy). The soil was moderately alkaline (pH 8.1), with a medium content of organic matter (11.5 g/kg) and a low content of available P (7.4 mg/kg). Total As content was 214 mg/kg: 181 mg/kg were associated with well crystallised Fe hydroxides, 25 mg/kg were specifically sorbed and 0.25 mg/kg were present as labile fraction. The soil contained THB in the order of 10^6 CFU g soil/dw, and AsV- and AsIII-resistant bacteria were 42% and 10% of the total heterotrophs, respectively. As evidence of the presence of As-resistant bacteria, several strains possessing *ArsB*, *ArsC*, *ACR3(1)* and *ACR3(2)* genes were isolated and characterized (Table 1). *Alcaligenes* sp. strain Dhall is an As-resistant strain (300 mM AsV and 70 mM AsIII) able to convert completely 2 mM AsV into AsIII within 72 hours (Figure 1).

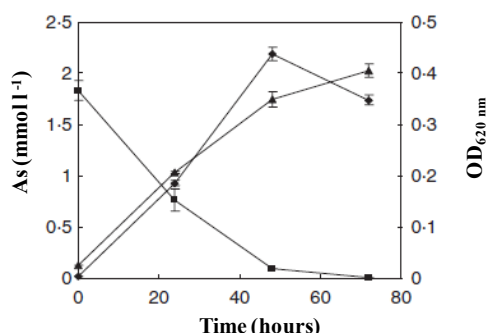


Figure 1. Bacterial growth (OD_{620nm}) (◆) and corresponding reduction of AsV (■) to AsIII (▲) by *Alcaligenes* sp. Dhall. The OD values reported are 1:10 dilution of original sample. Data are represented as means ± standard error, n = 4.

Alcaligenes sp. Dhall exerted an effect on As uptake by plant, as demonstrated by the higher As content in the inoculated than the uninoculated plants (Table 2). Although the strain possessed an ACC-deaminase activity (0.33 $\mu\text{mol/h}/\mu\text{g}$), no significant difference on plant biomass was recorded, indicating that the strain did not promote sunflower growth (Table 2).

During the experiment, no significant difference in THB and ARB of the bulk fraction was recorded between inoculated and uninoculated pots (Table 2). On the contrary, in the rhizospheric fraction of inoculated pots THB and ARB were higher, suggesting that the strain colonized sunflower roots.

Although microorganisms with *ACR3(2)* gene were already present in the soil, q-PCR of *ACR3(2)* gene of *Alcaligenes* sp. DhalL was able to detect differences in inoculated and uninoculated soil DNA of bulk and rhizospheric fractions. Calibration curves (Figure 2) showed good correlation between DNA concentration (ranging from 0.026 to 6.5 ng/ μ L) of DhalL and fluorescence signal at each amplification cycle of *ACR3(2)* and of 16S rRNA genes. The melting curves for the genes showed only one peak, indicating that no primer-dimers formed during the amplification steps. Data elaborated with the relative quantification Livak method ($\Delta\Delta C_t$), showed that at 27 days of incubation *ACR3(2)* gene was more abundant in the inoculated soil than in the uninoculated soil and it was higher in the rhizospheric than in the bulk fraction (Table 3). At 50 days, q-PCR data showed that strain DhalL multiplied, particularly in the rhizospheric fraction, indicating a preferential colonization of sunflower rhizosphere.

Table 1. Characterization of bacterial strains isolated from the soil: arsenic resistance and presence of *ars* genes.

| Isolates | As resistance [§] mmol/L | | Gene fragments | | | |
|--|--------------------------------------|------------|----------------|-------------|----------------|----------------|
| | AsIII | AsV | <i>ArsC</i> | <i>ArsB</i> | <i>ACR3(1)</i> | <i>ACR3(2)</i> |
| As3-1b <i>Ancylobacter dichloromethanicum</i> 98% EU589386 | 3 | 100 | + | + | - | - |
| As3-1 <i>Brevundimonas intermedia</i> 99% FJ609705 | 25 | 100 | - | - | + | - |
| As3-2b <i>Achromobacter xylosoxidans</i> 99% AF439314 | 3 | 100 | - | + | - | - |
| As3-3 <i>Bordetella</i> sp. 99% FJ598334 | 10 | 100 | - | + | - | + |
| As5-16 <i>Brevundimonas bacteroides</i> 97% AJ227782 | 5 | 200 | + | - | + | - |
| As3-5a <i>Ensifer adhaerens</i> 100% FJ609715 | 3 | 10 | - | - | - | + |
| As3-5 <i>Microbacterium</i> sp. 98% FJ595885 | 25 | 100 | - | - | - | - |
| As3-8 <i>Ochrobactrum tritici</i> 100% EU870448 | 25 | 100 | - | - | - | + |
| As3-9a <i>Ensifer adhaerens</i> | 3 | 100 | - | - | - | + |
| As5-11a <i>Microbacterium</i> sp. 99% FJ595885 | 5 | 200 | - | - | - | - |
| As5-12 <i>Georgenia ferrireducens</i> 99% EU095256 | 2 | 200 | - | - | - | - |
| As3-10a <i>Achromobacter xylosoxidans</i> | 3 | 100 | - | + | - | + |
| As3-10 <i>Bacillus</i> sp. 99% EU124558 | 50 | 100 | + | + | - | - |
| As3-11 <i>Pseudomonas veronii</i> 99% FM162562 | 25 | 100 | - | - | - | - |
| As5-3 <i>Sinorhizobium</i> sp. 99% AY505132 | 10 | 200 | - | - | - | + |
| As5-4a <i>Rhodococcus erythropolis</i> 99% U81990 | 2 | 200 | + | - | - | - |
| As5-4b <i>Bosea thiooxidans</i> 99% AJ250798 | 10 | 200 | - | - | - | - |
| As3-15b <i>Ochrobactrum tritici</i> | 25 | 50 | - | - | - | + |
| As3-21 <i>Bacillus</i> sp. 99% EU124558 | 25 | 100 | + | + | - | - |
| As3-22 <i>Ochrobactrum tritici</i> 100% EU301689 | 25 | 50 | - | - | - | + |
| As3-25 <i>Ochrobactrum tritici</i> 100% EU301689 | 25 | 100 | - | - | - | + |
| As5-8 <i>Sinorhizobium</i> sp. | 10 | 200 | - | - | + | + |
| As5-9 <i>Sinorhizobium</i> sp. | 5 | 200 | - | - | - | + |
| As5-10 <i>Achromobacter</i> sp. 99% EU073119 | 5 | 200 | - | + | - | + |
| As3-30 <i>Bacillus</i> sp. 100% EU612335 | 50 | 100 | + | + | - | - |
| As3-33 <i>Brevundimonas intermedia</i> 99% FJ609705 | 25 | 50 | - | - | + | - |
| As3-35 <i>Microbacterium</i> sp. 99% FJ595885 | 25 | 50 | - | - | - | - |
| As3-37 <i>Brevundimonas intermedia</i> 99% FJ609705 | 25 | 100 | - | - | + | - |
| As3-38 <i>Achromobacter</i> sp. 100% EU073119 | 25 | 10 | + | + | - | - |

[§], the resistance to As(V) and As(III) was evaluated by an increase in OD₆₂₀ nm (from twofold to fivefold) of the inoculum

Table 2. Total As content in shoots (mg/kg d.w.), aboveground plant biomass (g d.w.), counts of THB^A and ARB^B (CFUg⁻¹ d.w. soil).

| Plants | Total As content | | Plant biomass | | THB | | | | ARB | | | |
|--------------|------------------|---------|---------------|---------|----------------------------|----------------------------|----------------------------|----------------------------|-----------|----------------------------|-----------|----------------------------|
| | | | | | 27 days | | 50 days | | 27 days | | 50 days | |
| | 27 days | 50 days | 27 days | 50 days | Bulk soil | Rhizospheric soil | Bulk soil | Rhizospheric soil | Bulk soil | Rhizospheric soil | Bulk soil | Rhizospheric soil |
| Inoculated | 1.18* | 1.96* | 0.48 | 2.25 | 5.60±0.94 x10 ⁶ | 6.29±0.95 x10 ⁵ | 2.90±0.47 x10 ⁶ | 1.11±0.07 x10 ⁷ | - | 6.32±2.34 x10 ⁵ | - | 1.56±0.17 x10 ⁶ |
| Uninoculated | 0.77 | 1.76 | 0.46 | 1.84 | 5.25±0.77 x10 ⁶ | 4.50±0.45 x10 ⁶ | 3.13±0.77 x10 ⁶ | 1.13±0.18 x10 ⁷ | - | 5.08±0.93 x10 ⁵ | - | 1.25±0.23 x10 ⁶ |

*, statistically different from the uninoculated (t Student, p=0.1)

^A, total heterotrophic bacteria; ^B, As-resistant bacteria

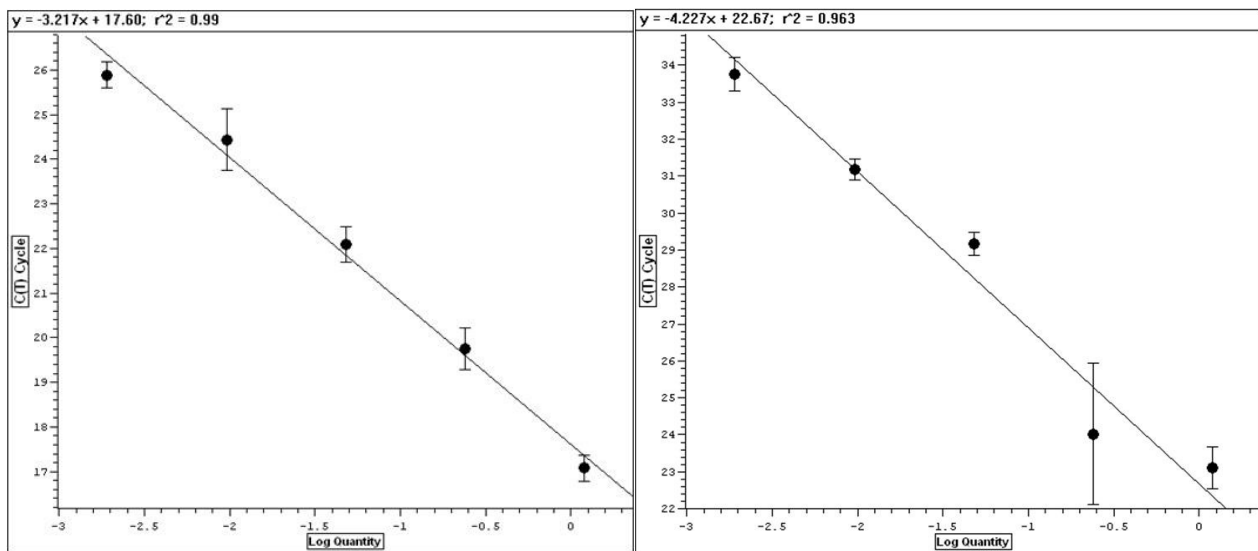


Figure 2. Calibration curves for 16S rRNA (A) and *ACR3(2)* (B) genes. C(t) values are plotted against Log of total DNA initial quantity (ng).

Table 3. Relative quantification of *ACR3(2)* in inoculated vs uninoculated pots, normalised to 16S rRNA calculated by Livak method, $\Delta\Delta C_t$ (average ΔC_t of inoculated pots – average ΔC_t of uninoculated pots).

| | T27 | | T50 | |
|---------------------|-----------|-------------------|-----------|-------------------|
| | Bulk soil | Rhizospheric soil | Bulk soil | Rhizospheric soil |
| $\Delta\Delta C(t)$ | 70 | 690 | 148 | 400 |

Conclusion

The results evidence that the potential PGPR *Alcaligenes* sp. Dhall colonized the sunflower rhizosphere and promoted the As uptake by plants. These data confirm that in soil As mobilization and uptake by plants would be influenced by the presence of As-resistant bacteria possessing an *Ars* operon (Meagher and Heaton 2005). As also reported by other authors (Marchiol *et al.* 2007), sunflower could be a candidate for phyto-extraction purposes and in a synergistic use with metal-resistant PGPR could represent a promising approach for remediation of metal contaminated soils.

References

- Adriano DC (2001) 'Trace elements in terrestrial environments: biogeochemistry, bioavailability and risks of metals.' (2nd ed.) (Springer, New York)
- Bachate SP, Cavalca L, Andreoni V (2009) Arsenic-resistant bacteria isolated from agricultural soils of Bangladesh and characterization of arsenate-reducing strains. *Journal of Applied Microbiology* **107**, 145-156.
- Bauer M, Blodau C (2006) Mobilization of arsenic by dissolved organic matter from iron oxides, soils, and sediments. *Science of the Total Environment* **354**, 179-190.
- Dell'Amico E, Cavalca L, Andreoni V (2008) Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria. *Soil Biology & Biochemistry* **40**, 74-84.
- Fitz WJ, Wenzel WW (2002) Arsenic transformations in the soil-rhizosphere-plant system: fundamentals and potential application to phytoremediation. *Journal of Biotechnology* **99**, 259-278.
- Grichko VP, Filby B, Glick BR (2000) Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb and Zn. *Journal of Biotechnology* **81**, 45-53.
- Islam FS, Gault AG, Boothman C, Polya DA, Charnock JM, Chatterjee D, Lloyd JR (2004) Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. *Nature* **430**, 68-71.
- Marchiol L, Fellet G, Perosa D, Zerbi G (2007) In situ plant uptake and removal of heavy metals in a soil polluted by pyrite cinders during the crop cycle. *Plant Physiology & Biochemistry* **45**, 379-387.
- Meagher RB, Heaton ACP (2005) Strategies for the engineered phytoremediation of toxic element pollution: mercury and arsenic. *Journal of Industrial Microbiology Biotechnology* **32**, 502-513.
- MIPAF Official Methods (2000) Franco Angeli, Milano-Italy.
- Morgan PW, Drew CD (1997) Ethylene and plant responses to stress. *Plant Physiology* **100**, 620-630.
- Mukhopadhyay R, Rosen BP, Phung LT, Silver S (2002) Microbial arsenic: from geocycles to genes and enzymes. *FEMS Microbiology Reviews* **26**, 311-325.
- Oremland RS, Stolz JF (2005) Arsenic, microbes and contaminated aquifers. *Trends in Microbiology* **13**, 45-49.
- Silver S, Phung T (1996) Bacterial heavy metal resistance: new surprises. *Annual Review of Microbiology* **50**, 753-789.
- Wenzel WW, Kirchbaumer N, Prohaska T, Stingeder G, Lombi E, Adriano DC (2001) Arsenic fractionation in soils using an improved sequential extraction procedure. *Analytica Chimica Acta* **436**, 309-323.

Soil amendment to remediate copper contaminated soils

Zhenli He^A, Jinghua Fan^A, Xiaoe Yang^{A,B} and Peter J. Stoffella^B

^AUniversity of Florida, IFAS, Indian River Research and Education Center, 2199 S Rock Rd, Fort Pierce, FL 34945, USA, Email zhe@ufl.edu, jfan@ufl.edu; pjs@ufl.edu, xyang@zju.edu.cn.

^BMOE Key Laboratory of Environment Remediation and Ecological Health, Zhejiang University, Hangzhou 310129, China.

Abstract

Copper contamination to agricultural soils has been accelerated due to its wide and repeated use in agriculture and horticulture as fertilizers or fungicides to protect vines, citrus trees, and other fruit crops against fungus diseases. Laboratory incubation and field survey were conducted to understand the mechanisms of Cu inactivation by Ca-water plant residuals (Ca-WTRs containing mainly CaCO₃ and minor CaO) and to estimate the optimal rate of Ca-WTRs for remediation of Cu-contaminated soils. The results indicate that Ca-WTRs can effectively raise soil pH and convert water soluble and exchangeable Cu to more stable oxides-bound and residual Cu fractions in the soils. A pH value of 6.5 was found to divide the high (pH<6.5) and low (pH>6.5) availability of Cu in the soils. Based on this criterion and the response curve of pH to Ca-WTRs application rate, the optimal rates of Ca-WTRs can be estimated for different Cu-contaminated soils.

Key Words

Acidic soils, chemical remediation, heavy metal contamination, Cu transformation.

Introduction

Copper contamination to agricultural soils has been accelerated due to its wide and repeated use in agriculture and horticulture as fertilizers or fungicides to protect vines, citrus trees, and other fruit crops against fungus diseases (He *et al.* 2005). In Florida, due to a long history of citrus production, Cu has accumulated in the soils (Alva 1993). A large proportion of soils under citrus production contains total Cu content above 85 mg/kg, the critical level for ecosystem health (Schuler and Hoang 2008). Soil contamination with Cu causes soil degradation, Cu phytotoxicity, and increased transport of Cu to surface and ground waters. Total Cu as high as 300 mg/kg was measured in the sediment from St. Lucie River, South Florida, which is 20-60 times greater than background levels (5–15 mg/kg) (Ma *et al.* 1997; Zhang *et al.* 2003). Because of the persistent nature of heavy metals, remediation of Cu-contaminated soils has become a great challenge. Physical approaches such as scavenging or burial of the contaminated surface soil or ‘washing out’ of Cu from the contaminated field with or without electrical dialysis are effective but often too expensive for a large scale remediation. Phytoremediation is cost-effective, but requires a longer time to accomplish desired results as compared to other approaches. Therefore, chemical approaches are frequently adopted for the remediation of agricultural soils. Soil amendment is one of the best management practices in Florida for reducing nutrient losses from agricultural production systems on sandy soils. Amendment of Ca-water plant residues (Ca-WTRs, pH 9.1, containing mainly CaCO₃ and minor CaO) to acidic sandy soils can effectively reduce Cu loading in surface runoff water based on our field observation, but the mechanisms of the effects are not clear. In this study laboratory incubation experiments were conducted to understand the mechanisms of Cu inactivation by Ca-WTRs and to estimate the optimal rate of Ca-WTRs for remediation of Cu-contaminated soils.

Materials and methods

Eighteen representative soils were collected at the 0-15 cm depth from commercial citrus groves in the Indian River area, South Florida, of which two typical soils were selected for incubation study (Table 1). The soil samples were air-dried, ground, and passed through a 2-mm sieve prior to the incubation study and physical and chemical analyses. The Ca-WTR was collected from the Fort Pierce Utility Authority facility with the following properties: pH 9.05, electrical conductivity (EC) 659 μ S/cm, and total recoverable Cu 0.4 mg/kg. pH and EC of soil and Ca-WTRs were measured using a pH/ion/conductivity meter (DIM 200, Denver Instrument, Denver, CO). Total organic carbon (C) was determined using a C/N analyzer (Vario Max, Elemental Analysensystem GmbH, Hanau, Germany). Total recoverable Cu in soil was determined following EPA method 3050B and soil extractable Cu by the Mehlich-3 method (Mehlich 1984). Copper

concentration in the digested solution or Mehlich-3 extracts was determined using inductively coupled plasma optical emission spectrometry (ICP-OES, Ultima, J. Y. Horiba, Edison, NJ, USA) following USEPA method 200.7.

Table 1. Relevant properties of the soils

| Soils | Soil classification | Cropping history* | Org. C (g/kg) | pH (H ₂ O) | Recoverable Cu (mg/kg) | M-3 Extr. Cu (mg/kg) |
|----------|---|-------------------|---------------|-----------------------|------------------------|----------------------|
| Alfisol | Fine loamy siliceous hyperthermic Typic Glossaqualfs | Red GF/1988 | 3.29 | 5.83 | 63.2 | 39.9 |
| Spodosol | Sandy siliceous hyperthermic Alfic Haplaquods | Red GF/1979 | 7.24 | 4.18 | 114 | 72.3 |

*GF=grapefruit

Incubation study

Soils (each 1-kg oven dried basis) were amended with Ca-WTRs at the rates of 0, 5, 10, and 20 g/kg for the Alfisol and 0, 5, 50, and 100 g/kg for the Spodosol. There were two levels of Cu for each soil: with or without being enriched with 400 g/kg Cu in the form of Cu(NO₃)₂. The amended soils were then incubated at room temperature for 70 d and soil moisture was maintained at 70% of water holding capacity (WHC) during the incubation. At the end of incubation, subsamples of the soils were collected for the determination of pH, Mehlich-3 extractable Cu, and Cu fractionation into water soluble and exchangeable, carbonate-bound, organic-bound, oxide-bound, and residual fraction following the procedure modified from that of Amacher (1996).

Results and discussion

Soil pH and extractable Cu

Amendment of Ca-WTRs effectively raised soil pH (Figure 1). At the rate of 5 g/kg soil pH was increased from 5.4-5.8 to 6.4-7.0 for the Alfisol and from 3.9-4.2 to 6.2 for the Spodosol. Copper loading at 400 mg/kg decreased soil pH by 0.45 and 0.26 units, respectively for the Alfisol and Spodosol, indicating that Cu contamination may result in secondary acidification. As a consequence, more Ca-WTRs were required to raise pH to the same level for Cu polluted soils. Extractable Cu decreased with increasing pH (Figure 2). Amendment of Ca-WTRs at the rate of 5 g/kg reduced M3-extractable Cu by 24-36% in the Alfisol and 22-47% in the Spodosol. Based on a linear model of the M-3 extractable Cu vs. soil pH, increasing soil pH by one unit decreased M-3 extractable Cu by 12-90 mg/kg, depending on soil type and Cu level in the soil. At pH 6.5, M3 extractable Cu was dropped by 28-30% in the Alfisol and by 42-44% in the Spodosol. These results indicate that Ca-WTRs amendment can effectively convert labile Cu to stable Cu.

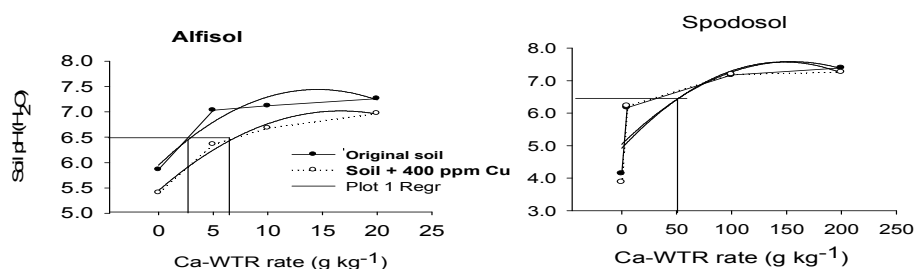


Figure 1. Response of soil pH to application rates of Ca-Water plant residuals in an Alfisol and a Spodosol with and without Cu enrichment at 40 mg/kg.

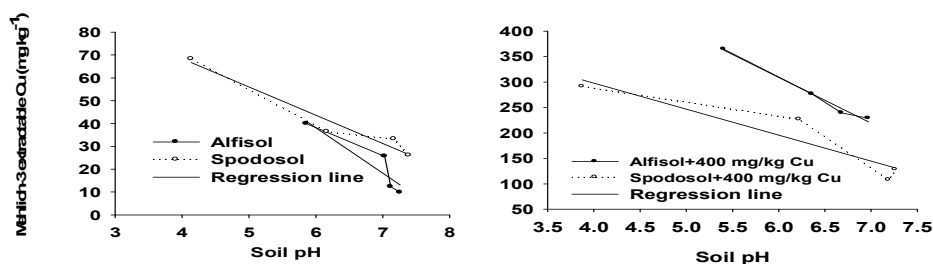


Figure 2. Relationship between Mehlich 3-extractable Cu and soil pH: Alfisol, $y = 157-19.8x$, $r = 0.85$; Spodosol, $y = 118-12.3x$, $r = 0.96$; Alfisol + 400 mg/kg Cu, $y = 854-90.3x$, $r = 0.99$; Spodosol + 400 mg/kg Cu, $y = 501-51x$, $r = 0.99$.

Soil Cu fractionation

The amendment of Ca-WTRs drastically reduced water soluble and exchangeable Cu but increased oxide-bound and residual Cu fractions (Figure 3). Carbonate-bound slightly increased at a low-medium rate of Ca-WTRs but decreased at a high rate whereas organic-bound Cu was slightly reduced. At the application rate of 5 g/kg, Ca-WTRs reduced water soluble and exchangeable Cu²⁺ by 63% in the original soils, and by 92% in the Cu-enriched soils regardless of soil type. Apparently Cu from the labile pool was largely adsorbed by some newly formed Fe and Al oxides/hydroxides due to increased pH, and converted into more stable forms. The carbonate-bound Cu was also at high Ca-WTR rates (>reduced by Ca-WTRs), which is unexpected as we thought that this Cu fraction would be increased due to the large input of CaCO₃/CaO from Ca-WTRs. This result probably indicates that the binding of Cu is stronger to oxides than carbonates.

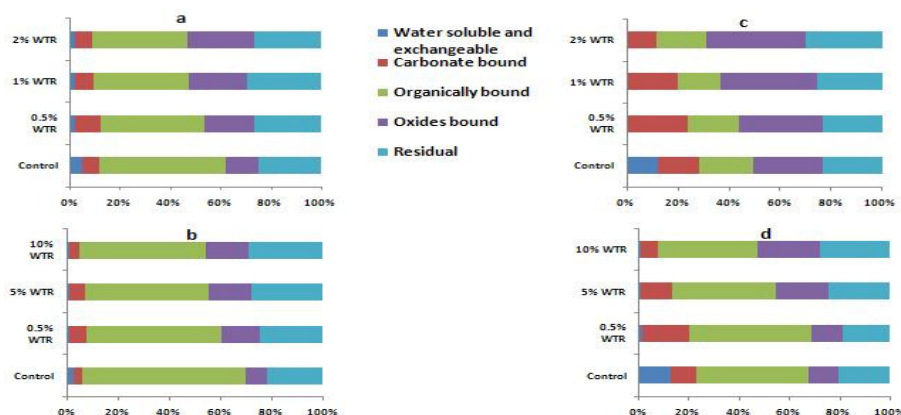


Figure 3. Cu Fractionation in soils amended with different Ca-WTRs rates: (a) Alfisol; (b) Spodosol; (c) Alfisol + 400mg Cu/kg soil; (d) Spodosol + 400mg Cu/kg soil.

Optimal rate of Ca-WTRs

A great challenge to chemical remediation of metal contaminated soil is to determine adequate rate of amendment, i.e. how much is the optimal. The results from our field survey indicate that the relationship between readily available Cu (as determined by CaCl₂ extraction) and total recoverable Cu is affected by soil pH, and a pH value of 6.5 divides the high (pH<6.5) and low (pH>6.5) availability status of Cu in the soils as reflected in the slope of available-total Cu relationship curve (Figure 4). This finding agrees with field observation that phytotoxicity symptoms often disappear in Cu contaminated soils at pH ≥ 6.5. Based on this criterion and the response curve of pH to Ca-WTRs application rate, it was calculated that the optimal rates of Ca-WTRs are 3, 6.5, and 50 g/kg, respectively for the Alfisol, Cu-enriched Alfisol, and Spodosol with or without Cu enrichment.

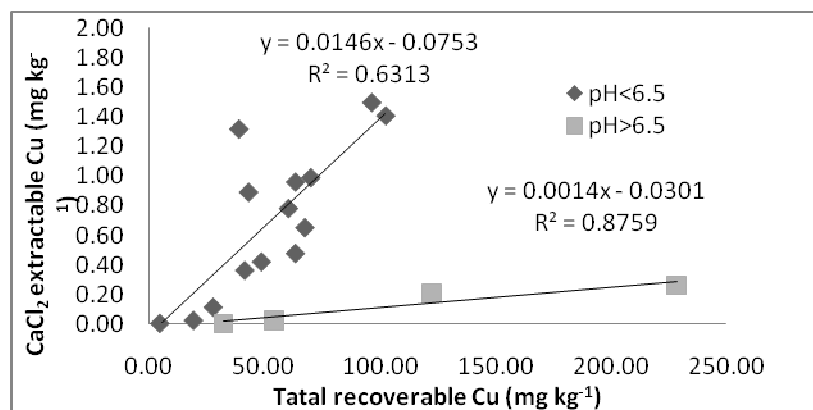


Figure 4. Relationship between CaCl₂ extractable Cu and total recoverable Cu as affected by soil pH.

Conclusion

Amendment of Ca-WTRs can effectively raise soil pH and convert labile Cu into more stable oxides-bound and residual Cu fractions. Ca-WTRs, which can be obtained from a drinking water treatment facility at no cost and contain no detectable contaminants, thus have great application potential for remediation of Cu contaminated soils, particularly those acidic sandy soils under citrus production.

Acknowledgment

This study was in part supported by a grant from South Florida Water Management District (contract#4600001773), University of Florida and MOE Key Laboratory of Environmental Remediation and Ecological Health, Zhejiang University, Hangzhou, China.

References

- Alva AK (1993) Copper contamination of sandy soils and effects on young Hamlin orange trees. *Bulletin of Environmental Contamination and Toxicology* **51**:857-864.
- Amacher MC (1996) Nickel, cadmium, and lead. In 'Methods of soil analysis, Part 3: Chemical methods'. (Ed DL Sparks.) pp. 739-768. (SSSA Book Series No 5, ASA-CSA-SSSA Inc. Madison, WI).
- He ZL, Yang XE, Stoffella PJ (2005) Trace elements in agroecosystems and impacts on the environment. *Journal of Trace Elements in Medicine and Biology* **19**, 125–140.
- He ZL, Zhang MK, Stoffella PJ, Yang XE (2006) Vertical distribution and water solubility of phosphorus and heavy metals in sediments of the St. Lucie Estuary, South Florida, USA. *Environmental Geology* **50**, 250-260.
- Ma LQ, Tan F, Willie GH (1997) Concentrations and distributions of eleven metals in Florida soils. *Journal of Environmental Quality* **26**, 769-775.
- Mehlich A (1984) Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. *Communication in Soil Science and Plant Nutrition* **15**, 1409-1416.
- Schuler LJ, Hoang TC (2008) Aquatic risk assessment of copper in freshwater and saltwater ecosystem of south Florida. *Ecotoxicology* **17**, 642-659.
- Zhang MK, He ZL, Calvert DV, Stoffella PJ, Yang XE (2003) Surface runoff losses of copper and zinc in sandy soils. *Journal of Environmental Quality* **32**, 909–915.

Soil pH and free Fe/Al oxides control As availability and fractionation in representative Taiwan soils contaminated by As

Shaw-Wei Su and Zueng-Sang Chen

Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan, Email soilchen@ntu.edu.tw

Abstract

A large area of agricultural soils mostly growing rice in Guandu Plain, Taipei was contaminated by arsenic (As). Rice produced in the As-contaminated area was safe for consumers even total As in soils reached as high as 535 mg/kg. Results of As fractionation in soils in this area indicated that As availability in soils were very low and supposed that this may be explained by high contents of amorphous materials in soils. To further prove this hypothesis and find out the major soil properties affecting As availability in representative Taiwan soils contaminated by As, this study apply As solution in 9 different soil samples with various characteristics and investigate the dynamics of As fractionation in soils under two cultivation systems, paddy fields or upland. We found that As transferred to non-labile fractions with time and the relative portions of As fractions were quite stable after 90 days of incubation in both cultivation systems. The ageing effect is more apparent in paddy fields condition compared with dry land condition. This study concluded that soil pH and free Fe/Al oxides are the major soil properties affecting As availability and fractionation in the representative Taiwan soils contaminated by As.

Key Words

Arsenic, contamination, fractionation, amorphous materials, cultivation systems, Guandu Plain.

Introduction

Arsenic is a contaminant of public concern since it is highly toxic and carcinogenic. It may be accumulated in plants and eventually be transferred to humans through the food chain (Rahman *et al.* 2008). According to a survey conducted in 2006, more than 60 hectares of soils located in Guandu Plain, Taipei, were contaminated by arsenic (≥ 60 mg/kg). The maximum As concentration in topsoil (0-15 cm) reached 535 mg/kg in this area. The contamination source of arsenic in this area may come from the soil parent material, andesite, and the hot spring water of Thermal Valley located around 5 km northeast away from Guandu Plain. The hot spring water flowed out and mixed with the stream water which was used as irrigation water for the As-contaminated area of the Guandu Plain.

The food safety of rice produced in this area was investigated (Su *et al.* 2008). It was found that the arsenic concentrations in rice grain were all below 0.5 mg/kg DW, no matter how high the arsenic concentration in soil was. No relationship or trend was found between total arsenic concentration in soil and in brown rice. It was concluded that the rice cultivated in the Guandu Plain is safe for consumers. Another study that conducted arsenic fractionation in 13 soil samples collected from Guandu Plain showed that the non-specifically-bound As in soils, which is related to bioavailability, is extremely low in this area (<1% of total As) and the amorphous hydrous Fe and Al oxide-bound As was the major fraction in soils (>50% of total As). A hypothesis was proposed that the amorphous materials in soils may play a central role in limiting the availability of arsenic in the soils (Su and Chen, 2008). To prove the hypothesis and find out the major soil properties affecting As availability in representative Taiwan soils, this study apply As solution in soil samples collected from 9 representative soil series in Taiwan and investigate the dynamics of As fractionation in soils with time under two cultivation systems, paddy fields condition or dry land condition.

Methods

Soil samples collection

According to the reports of previous soil survey in Taiwan, around 20 kilograms of 9 soil samples were collected from agricultural topsoil (0-20 cm) belong to the following soil series representative in Taiwan: Chengchung (Cf) and Annei (An), weathered from sandstone and shale; Erhlin (Eh) and Liuchieh (Lc), weathered from clay slate; Shanhua (Sk) and Linfengying (Lh), weathered from Taiwan clay; Pinchen (Pc) and Chentsoliao (CCe), weathered from red earth; Kuantu (TKt), weathered from andesite (Table 1). The collected soil samples were air-dried, ground, 2 mm sieved, and stored in plastic bottles for further laboratory analysis.

Soil sample analysis

The soil pH (soil:water = 1:1), organic carbon (Walkley-Black method), particle-size analysis (hydrometer method), amorphous Fe, Al, and Mn (acid ammonium oxalate in darkness method), free Fe, Al, and Mn (citrate-bicarbonate-dithionite method), available P (Bray-1 method), and total As (digested by HNO₃/H₂O₂) were analyzed.

Soil spiked by As solution

The solution of Na₂HAsO₄•7H₂O was added in one kilogram of each soil samples to reach As load of 60 mg/kg, the soil regulation standard in Taiwan, and incubated for 300 days in two cultivation systems. The cultivation system of paddy fields was simulated by immersing soil with water for 90 days and then maintained soil water content at 70% of water holding capacity (WHC) of soil samples for 60 days. Repeat the same cycle again for the whole period of 300 days incubation. The cultivation system of upland was simulated by maintaining soil water content at 70% of WHC of soil samples for 300 days. Spiked soil samples were stored on indoor bench.

Soil sequential extraction procedures

Arsenic fractionation in soil samples on the 15th, 90th, 150th, and 300th days of incubation were conducted by sequential extraction procedures (SEPs) proposed by Wenzel *et al.* (2001). Five As fractions were operationally defined by authors as: (1) non-specifically-bound As (extracted by (NH₄)₂SO₄); (2) specifically-bound As (extracted by (NH₄)₂HPO₄); (3) amorphous hydrous Fe and Al oxide-bound As (extracted by NH₄-oxalate buffer); (4) crystalline hydrous Fe and Al oxide-bound As (extracted by NH₄-oxalate buffer + ascorbic acid); and (5) the residual As (digested by HNO₃/H₂O₂).

Results

The characteristics of 9 collected soil samples varied widely and total As concentrations in soils were all below 20 mg/kg (Table 1). After As solution application, total As concentrations in soil samples reached the expected value and ranged from 61.1 mg/kg to 76.3 mg/kg. The accuracy of SEPs was tested by comparing the sum of five As fractions in soil samples on the 15th day of incubation against the total As concentrations independently analyzed in a single acid digest. The recovery ranged from 93% to 137%.

Table 1. Characteristics of 9 soil samples.

| Soil sample | pH | Organic C (%) | Total As (mg/kg) | | Bray-1 P (mg/kg) | Sand (g/kg) | Silt (g/kg) | Clay (g/kg) |
|-------------------|------|---------------|------------------|--------|------------------|-------------|-------------|-------------|
| | | | Original | Spiked | | | | |
| Chengchung (Cf) | 6.84 | 0.817 | 6.60 | 62.9 | 38.4 | 281 | 566 | 153 |
| Annei (An) | 6.72 | 1.22 | 12.8 | 72.3 | 12.6 | 104 | 546 | 350 |
| Erhlin (Eh) | 6.39 | 1.23 | 9.82 | 65.7 | 23.8 | 245 | 483 | 272 |
| Liuchieh (Lc) | 6.23 | 1.38 | 10.9 | 62.0 | 11.0 | 238 | 507 | 255 |
| Shanhua (Sk) | 7.09 | 1.33 | 8.58 | 76.3 | 69.9 | 291 | 408 | 301 |
| Linfengying (Lh) | 6.23 | 3.28 | 11.8 | 61.1 | 66.4 | 72 | 434 | 494 |
| Pinchen (Pc) | 4.67 | 0.915 | 9.54 | 66.9 | 3.98 | 80 | 384 | 536 |
| Chentsoliao (CCe) | 4.86 | 0.809 | 8.91 | 72.8 | 66.9 | 226 | 334 | 440 |
| Kuantu (TKt) | 5.35 | 1.92 | 19.1 | 73.3 | 51.9 | 283 | 316 | 401 |

Table 1. (continued)

| Soil sample | Free Fe (g/kg) | Amor. Fe (g/kg) | Free Al (g/kg) | Amor. Al (g/kg) | Free Mn (g/kg) | Amor. Mn (g/kg) | Parent materials |
|-------------------|----------------|-----------------|----------------|-----------------|----------------|-----------------|-------------------|
| Chengchung (Cf) | 5.06 | 2.95 | 0.421 | 0.471 | 0.043 | 0.036 | sandstone & shale |
| Annei (An) | 11.7 | 5.80 | 0.896 | 0.832 | 0.384 | 0.400 | sandstone & shale |
| Erhlin (Eh) | 9.61 | 4.69 | 0.462 | 0.432 | 0.103 | 0.092 | clay slate |
| Liuchieh (Lc) | 12.6 | 3.78 | 0.600 | 0.497 | 0.186 | 0.194 | clay slate |
| Shanhua (Sk) | 11.5 | 4.02 | 0.915 | 0.582 | 0.209 | 0.210 | Taiwan clay |
| Linfengying (Lh) | 16.5 | 3.61 | 1.54 | 0.844 | 0.239 | 0.251 | Taiwan clay |
| Pinchen (Pc) | 26.4 | 1.91 | 5.86 | 2.17 | 0.047 | 0.019 | red earth |
| Chentsoliao (CCe) | 22.8 | 1.77 | 2.51 | 1.16 | 0.248 | 0.263 | red earth |
| Kuantu (TKt) | 19.8 | 7.49 | 1.57 | 1.80 | 0.144 | 0.130 | andesite |

Amor.: Amorphous.

Arsenic in all soil samples transferred from labile fractions (fraction (1) and (2)) to non-labile fractions (fraction (3) to (5)) with time and this ageing effect was more apparent in paddy fields condition compared with dry land condition (Fig. 1). The relative portions of As fractions were quite stable and did not change very much after 90 days of incubation in both cultivation systems. The specifically-bound As and the amorphous hydrous Fe and Al oxide-bound As were the major fractions in most soil samples, however, for Pc, CCe, and TKt soil samples, more As existed as non-labile fractions.

All the 9 soil samples can be divided into 2 groups according to relative portions of labile As fractions. Group 1 includes the first 6 soil samples collected from soil series of Cf, An, Eh, Lc, Sk, and Lh. The other 3 soil samples collected from soil series of Pc, CCe, and TKt were categorized as Group 2. The relative portions of labile As fractions in Group 2 was around 60% of those in Group 1 after 90 days incubation in both cultivation systems. This indicated that soils belong to Group 2 can effectively reduce environmental risks of As contamination in soils. Compared with Group 1, soil samples in Group 2 contained high amounts of free Fe, free Al, and amorphous Al and the soil pH were all lower than 5.5. This can be interpreted by the facts that Fe/Al oxides in soils had high sorption capacity of As and net surface charge of soil colloids tend to be positive at lower pH, which facilitates the sorption of As by soil colloids because As existed as oxyanions in soils. Compared with the other two soil samples in Group 2, the soil sample of TKt collected from Guandu Plain had apparently higher amounts of amorphous Fe. This supported the hypothesis that the amorphous materials in soils of Guandu Plain play an important role in limiting the availability of As in the soils. From this study we further found that low pH and high amounts of free Fe/Al in soils of Guandu Plain also inhibited As availability in highly As-contaminated soils.

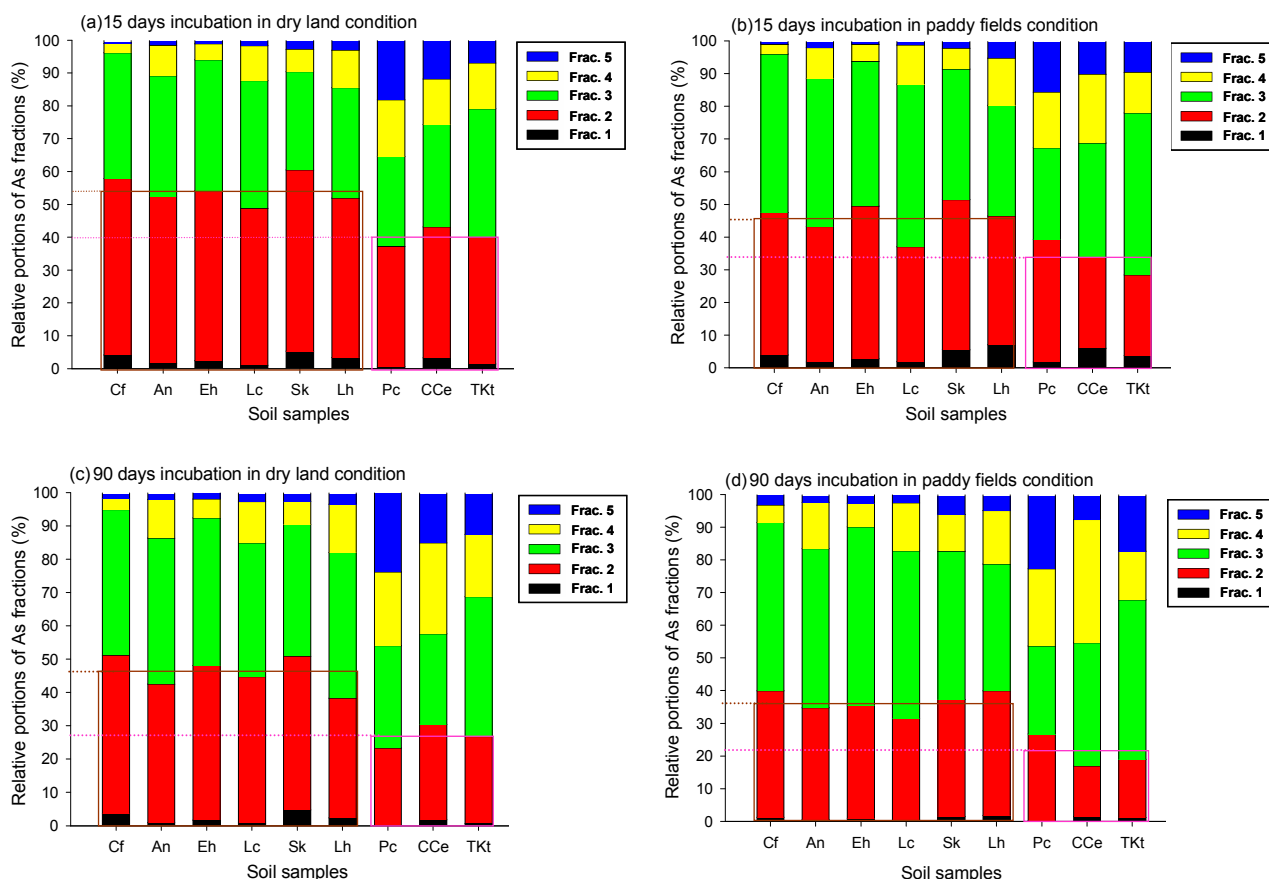


Figure 1. The distributions of As fractions in 9 soil samples after spiking on 15th and 90th day of incubation in dry land and paddy fields condition. The first 6 soil samples (Cf, An, Eh, Lc, Sk, and Lh) are categorized as Group 1 and the latter 3 soil samples (Pc, CCe, and TKt) are categorized as Group 2 based on the relative portions of labile As fractions (fraction 1 and fraction 2).

The Pearson correlation coefficients between the measured soil properties and relative portions of As fractions in soils on the 90th day of incubation showed that the distribution of As fractions in soils was mainly related to soil pH and free Fe/Al (Table 2). This study indicated that the impacts of As-contaminated soils on crop production and agro-ecosystems should not be assessed only by total As in soils because soils

with lower pH and higher contents of free Fe/Al oxides can effectively reduce the availability of As in soils. From the results of this study, we supposed that acidic soils (pH < 5.5) containing free Fe + free Al higher than 20 g/kg can be categorized as Group 2. Soils belong to Group 2 had higher capacity to endure As contamination and reduce environmental risks.

Table 2. Pearson's correlation coefficients between soil properties and As fractions in two cultivation systems.

| Parameters | pH | O.C. | Total As | Bray-1 P | Free Fe | Amor. Fe | Free Al | Amor. Al | Sand | Silt | Clay |
|------------|---------|-------|----------|----------|---------|----------|---------|----------|-------|--------|---------|
| D.-F. 1 | 0.65 | 0.01 | -0.53 | 0.63 | -0.57 | -0.18 | -0.46 | -0.59 | 0.46 | 0.19 | -0.47 |
| D.-F. 2 | 0.92** | -0.09 | -0.45 | -0.05 | -0.95** | 0.09 | -0.81** | -0.94** | 0.37 | 0.79* | -0.85** |
| D.-F. 3 | 0.77* | 0.43 | 0.25 | -0.08 | -0.76* | 0.65 | -0.73* | -0.54 | 0.12 | 0.61 | -0.53 |
| D.-F. 4 | -0.91** | 0.01 | 0.30 | 0.11 | 0.94** | -0.25 | 0.70* | 0.75* | -0.34 | -0.75* | 0.80** |
| D.-F. 5 | -0.93** | -0.23 | 0.14 | -0.11 | 0.92** | -0.33 | 0.93** | 0.92** | -0.29 | -0.71* | 0.72* |
| P.-F. 1 | 0.21 | 0.54 | -0.01 | 0.95** | -0.13 | 0.01 | -0.36 | -0.28 | 0.21 | -0.27 | 0.05 |
| P.-F. 2 | 0.83** | 0.22 | -0.44 | -0.14 | -0.74* | -0.04 | -0.44 | -0.66* | -0.15 | 0.79* | -0.46 |
| P.-F. 3 | 0.68* | -0.05 | 0.13 | -0.06 | -0.83** | 0.58 | -0.92** | -0.71* | 0.62 | 0.54 | -0.84** |
| P.-F. 4 | -0.78* | -0.14 | 0.01 | 0.20 | 0.81** | -0.47 | 0.61 | 0.52 | -0.29 | -0.64 | 0.68* |
| P.-F. 5 | -0.77* | -0.06 | 0.36 | -0.11 | 0.80** | -0.04 | 0.84** | 0.96** | -0.20 | -0.69* | 0.65 |

D.-F.: Dry land-Fraction; P.-F.: Paddy fields-Fraction; O.C.: Organic Carbon; Amor.: Amorphous; *: significant at $\alpha = 0.05$; **: significant at $\alpha = 0.01$.

Conclusion

After spiking soil samples with As solution to the level of soil regulation standard enacted in Taiwan, the dynamics of As fractions in soils from 9 common soil series under simulated paddy fields or dry land conditions showed that As transferred to non-labile fractions with time and this trend is more apparent in paddy fields condition. Soil pH and free Fe/Al oxides are the major soil properties affecting As availability and fractionation in the representative Taiwan soils contaminated by As. Soils weathered from red soil and andesite are acidic (pH < 5.5) and containing high amounts of free Fe/Al (> 20 g/kg) can effectively reduce As availability if contaminated by As. Further studies are required to quantify the influence of soil pH and free Fe/Al oxides on environmental risks caused by As-contaminated soils.

References

- Rahman MA, Hasegawa H, Rahman MM, Miah MAM, Tasmin A. (2008) Arsenic accumulation in rice (*Oryza sativa* L.): Human exposure through food chain. *Ecotoxicology and Environmental Safety* **69**, 317-324.
- Su SW, Lin SC, Huang WD, Chang TK, Chen ZS. (2008) The food safety of rice cultivated in arsenic contaminated soils in Guandu Plain of northern Taiwan. In 'Programme and Abstracts for the Joint Conference of the Australia and New Zealand Societies of Soil Science in conjunction with the International Year of Planet Earth'. (Ed. V Neall) pp. 98.
- Su SW, Chen ZS. (2008) Impacts of arsenic contaminated soils on agroecosystems in Guandu plain, Taipei: Assessment by As fractionation. In 'Proceedings of 14th International conference on heavy metals in the environment'. (Ed. ZS Chen *et al.*) pp. 93-96.
- Wenzel WW, Kirchbaumer N, Prohaska T, Stingeder G, Lombi E, Adriano DC. (2001) Arsenic fractionation in soils using an improved sequential extraction procedure. *Analytica Chimica Acta* **436**, 309-323.

Sorption and bioavailability of copper as affected by recycled water sources

Anitha Kunhikrishnan^{A,B}, Nanthi Bolan^B and Ravi Naidu^{A,B}

^ACRC CARE-CRC for Contamination Assessment and Remediation of the Environment.

^BCERAR-Centre for Environmental Risk Assessment and Remediation, University of South Australia, Mawson Lakes, South Australia 5095, Email Anitha.Kunhikrishnan@postgrads.unisa.edu.au

Abstract

Copper (Cu) is applied in vineyards as a fungicide, and these soils are irrigated with recycled water sources that include raw, treated sewage effluent, winery wastewater and farm effluents. These are likely to affect the transformation and mobility of Cu in soils. Batch sorption studies and various microbial characteristics were used to investigate the effect of recycled water sources on Cu in 3 contrasting soils from South Australia varying in pH (4.88-7.83), texture and organic matter (2.5-7.2%). The soils were treated with copper nitrate solutions ranging in concentration from 0 – 400 mg Cu/L, and the adsorption of Cu was measured. The results indicated that adsorption was affected by both the soil type and the origin of recycled water. The adsorption of Cu was less in recycled water sources than in deionised water and decreased with increasing concentration of DOC in the recycled water. An incubation study was also performed to examine the influence of recycled water sources on the bioavailability of Cu as measured by microbial respiration. Respiration decreased significantly with increasing Cu concentration. Respiration, microbial biomass C and metabolic quotient were higher in the presence of recycled water than deionized water, but it varied among the effluents and soils.

Key Words

CO₂ production, qCO₂, substrate induced respiration (SIR), Freundlich isotherm, distribution coefficient.

Introduction

In a number of countries including Australia, the decrease in groundwater levels has been compounded by prolonged periods of drought or seasons of low rainfall. One possible approach by which the pressure on fresh water resources can be mitigated is by the recycled water technology. Recycled water can act both as a source and sink for heavy metals (Martin and Bullock 1994; Singh *et al.* 2005). Recycled water contains a range of heavy metals, and the concentration of these metals depends on the level of treatment; when recycled water is used for irrigation it increases the heavy metal content of soils. However various organic and inorganic components in recycled water immobilize metals through adsorption, complexation and precipitation, thereby affecting their bioavailability (Antoniadis and Alloway 2002). Recycled water sources, such as winery and piggery effluents in particular, contain carbon (dissolved and particulate), which is likely to interfere with metal dynamics in soil. Copper (Cu) enters the agricultural ecosystems through applications of Cu-containing fertilizers, manures (e.g. pig slurry), fungicides and liquid or solid wastes from mining and manufacturing industries (Saria *et al.* 2006).

There is a need to understand the factors such as soil pH, cation exchange capacity, organic matter content, and soil texture affecting Cu concentrations in soils, and its speciation in soils, so that, benefits and potential hazards can be identified. Copper availability to biota (as a nutrient or toxin) and its mobility are the most important factors to be considered when assessing its effect on the soil environment. Sorption measurement is a useful technique to study the retention of metals in soil (Morera *et al.* 2001), and it is a significant process in terms of controlling the distribution of Cu in soils. Sorption of Cu is pH dependent and is attributed to organic matter and iron/manganese oxides (Alloway 1995). Soil microbial biomass has been shown to decrease with the addition of heavy metals (Chaney and Ryan 1993). Microbial biomass size and composition are affected by the changes in the soil management and pollution and hence can be used as an early warning of such changes. Soil microorganisms which are typically associated with the organic fractions of the soil are expected to influence the mobilization-immobilization equilibrium of metals by changing the chemical composition of their immediate microenvironment (Beveridge 1988). The activity of soil microorganisms is measured either by the conventional plate-count technique or by soil respiration and microbial biomass (Jenkinson 1988). The aim of the work reported in this paper is to examine the effect of recycled water on soil physicochemical properties, sorption and microbial respiration process in relation to the interaction of Cu.

Methods

Five recycled water sources [treated sewage water (TS), storm water (SW), farm dairy effluent (FDE), winery effluent (WE) and piggery effluent (PE)] and 3 soil samples varying in texture and organic matter content were used in this study. Soil samples were collected from the surface layer (0-10 cm) of Adelaide Hills (AH; pH_{water} 4.88), Flaxley (FL; pH_{water} 6.07) and Gawler (GL; pH_{water} 7.83) in South Australia.

Sorption of copper in soil

The effect of recycled water on the sorption of Cu was examined using batch experiments. The sorption experiment was carried out by taking one g of soil in Cu solutions of different concentrations in the presence of recycled water. The solution concentrations were 0, 50, 100, 150, 200 and 400 mg/L Cu, prepared from copper nitrate. The samples were then placed in an end-over-end shaker for 24 hours, centrifuged at 2500 rpm for 30 minutes, filtered and analysed for pH and EC. The concentrations of total Cu in the supernatant solution were measured by Inductively Coupled Plasma with Optical Emission Spectroscopy (ICP-OES).

Microbial respiration

An incubation experiment was conducted for 45 days using 3 different soils to examine the effect of recycled water sources on various microbial characteristics as affected by Cu. Basal respiration, substrate induced respiration and microbial biomass carbon were measured in soils spiked with Cu (0-1000 mg/kg) in the presence of three recycled water sources (farm dairy, piggery and winery effluent) differing in DOC and compared with deionized water. Substrate induced (respiratory response on the supply of glucose) and basal respiration rates were determined by trapping evolved CO_2 from soil samples under incubation at 1, 14, 30 and 45 days interval using 0.05 M NaOH. Determination of the amount of evolved CO_2 was done by back-titration of unreacted NaOH with 0.03M HCl. The metabolically active bacterial and fungal biomass was measured by the inhibition of substrate-induced respiration by streptomycin sulphate and actidione, respectively (Wardle and Parkinson 1990). Microbial biomass was calculated using the chloroform-fumigation extraction procedure (Vance *et al.* 1987). 10 g soil were weighed into glass beakers and placed in a desiccator containing about 35 ml ethanol-free CHCl_3 in a small beaker. After 24h of fumigation the beaker of CHCl_3 was removed and the residual CHCl_3 vapor in the soil was removed by repeated evacuations. The fumigated and non-fumigated samples were extracted using 0.5M K_2SO_4 at a solid: solution ratio of 1:4 in an end-over-end shaker. The extract was filtered and analyzed using the TOC analyzer. Microbial biomass C was calculated as E_C/k_{EC} , where E_C = (organic C extracted from fumigated soils - organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Joergensen 1995). Soil microbial basal respiration (R_{mic}) and soil microbial biomass (C_{mic}) ratio were used to calculate the metabolic quotient ($q\text{CO}_2$), which is the amount of CO_2 -C produced per unit of microbial biomass carbon (Anderson and Domsch 1978).

Results

Sorption

The difference between the total Cu added and the total concentration remaining in the equilibrium solution gives the adsorbed Cu. The relationship between the Cu concentrations in the equilibrium solution, and the amounts of Cu adsorbed in the solid phase yield an adsorption isotherm. Gawler soil had the highest sorption capacity when compared to other soils (Figure 1A). For all three soils adsorption was less in piggery and winery effluents than in other effluents. The sorption data were fitted to the two most common equilibrium isotherms Langmuir and Freundlich. The Cu sorption isotherms fitted better to the Freundlich equation, $S = kC^n$ (1)

where, S is the amount of Cu sorbed (mg/kg), C is the equilibrium Cu concentration (mg/L), k is the equilibrium partition/distribution coefficient, and n is the sorption intensity. The distribution coefficient, k, is positively related to the Cu sorption capacity of soils and provides an index of a metal's potential mobility. Copper sorption, as indicated by k values, was higher in DI water than in the effluents and decreased with increasing concentration of dissolved organic carbon (DOC) in the recycled water (Figure 1B). The sorption of Cu was affected by the origin of recycled water sources, and the effect of effluents on sorption varied between the soils.

Microbial Respiration

Substrate induced respiration was higher than basal respiration (Figure 2A), indicating that basal respiration is limited by carbon substrate. Respiration rates decreased significantly with increasing Cu concentration during the 45 d incubation period and varied between the recycled water sources, which may be attributed to the difference in DOC (Figure 2B). Microbial biomass carbon, bacterial: fungal ratio and metabolic quotient

also decreased with increasing Cu concentration and varied between the effluents and soils (Figure 2C and 2D). These indices were higher in soils in the presence of recycled water than in the deionized water, indicating that recycled water sources decreased the inhibitory effect of metals on microbial activity as measured by respiration and microbial biomass carbon.

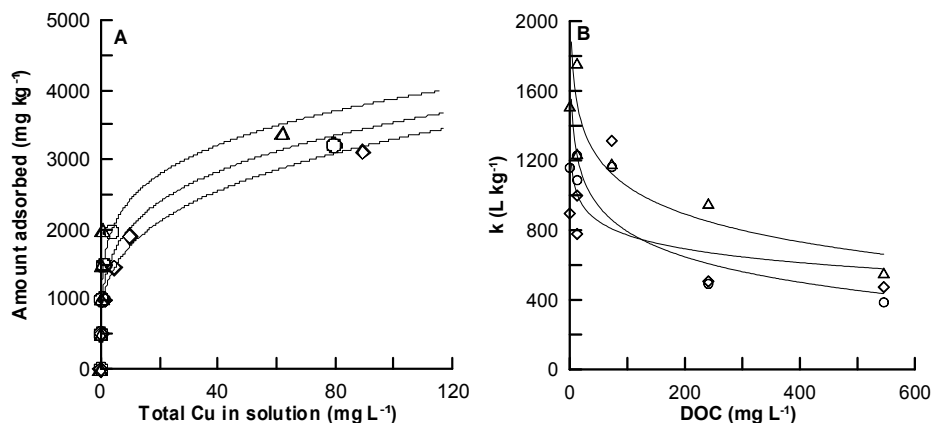


Figure 1. Comparison of Cu sorption between various soil types for DI water (A) and Relationship between DOC in various effluents and k values for Cu sorption (B) (○ Adelaide Hills □ Flaxley △ Gawler).

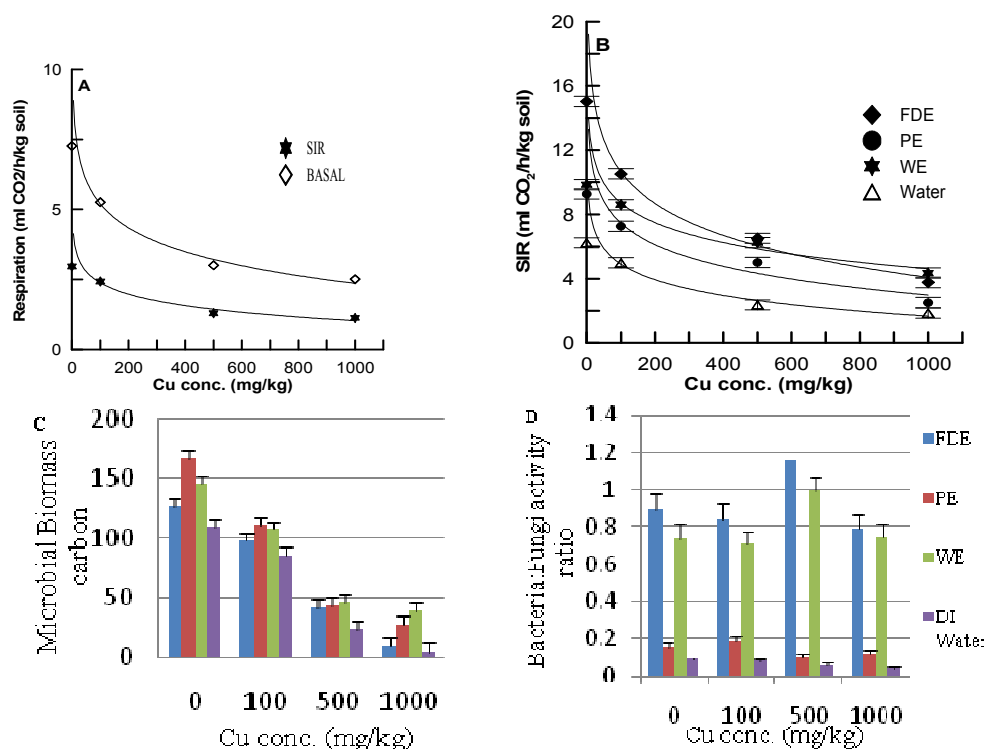


Figure 2. SIR and Basal respiration in Gawler soil in the presence of WE at 30 days interval (A), SIR in Gawler soil in the presence of 3 effluents and water at 30 days interval (B), Difference in microbial biomass carbon (C) and bacterial: fungal ratio among the effluents and water (D).

Conclusions

The results indicated that, DOC in these recycled water sources reduced Cu sorption in soils by forming Cu-DOC complexes. Recycled water resources modify the sorption behaviour of metals in soils, which is attributed to the presence of DOC and other ions such as Al, Fe and Ca in the recycled water. Copper significantly reduced microbial biomass carbon and respiration, indicating that Cu has negative effects on soil microorganisms. Soil disturbance and stress cause a decrease in microbial efficiency and enhance the qCO_2 , because the microbial populations need to spend more energy on maintenance limiting the incorporation of added substrate into the cell components. This effect could be explained by the fact that metal sensitive species may be replaced by other more tolerant groups which respire at a higher rate. Thus when farms, e.g. vineyards and vegetable farms, use recycled water for irrigation, such as winery and piggery effluents that contain high DOC, soluble Cu complexes may affect leaching, plant and microbial uptake.

References

- Alloway BJ (1995) 'Heavy metals in soils'. 2nd edition. (Blackie Academic and Professional: Glasgow).
- Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology & Biochemistry* **10**, 215–221.
- Antoniadis V, Alloway BJ (2002). The role of dissolved organic carbon in the mobility of Cd, Ni and Zn in sewage sludge-amended soils. *Environmental Pollution* **117**, 515–521.
- Beveridge TJ (1988) The bacterial surface: general considerations towards design and function. *Canadian Journal of Microbiology* **34**, 363–372.
- Chaney RL, Ryan JA (1993) Heavy metals and toxic organic pollutants in MSW-composts: Research results on phytoavailability, bioavailability, fate, etc. In 'Science and Engineering of Composting: Design, Environmental, Microbiological and Utilization Aspects'. (Ed Keener) pp.451-506. (Renaissance Publ.: Worthington, OH).
- Jenkinson DS (1988) Determination of microbial biomass nitrogen and carbon in soil. In 'Advances in Nitrogen Cycling in Agricultural Ecosystems'. (Ed JT Wilson) pp. 368–386. (Commonwealth Agricultural Bureau International: Wallingford).
- Joergensen RG (1995) The fumigation extraction method to estimate soil microbial biomass: Extraction with 0.01 M CaCl₂. *Agribiological Research* **48**, 319–324.
- Martin MH, Bullock RJ (1994) The Impact and Fate of Heavy Metals in an Oak Woodland Ecosystem. In 'Toxic Metals in Soil-Plants Systems'. (Ed M Ross) pp. 327–363. (John Wiley & Sons: New York).
- Morera MT, Echeverria JC, Mazkaran C, Garrido JJ (2001) Isotherms and sequential extraction procedures for evaluating sorption and distribution of heavy metals in soils. *Environmental Pollution* **113**, 135–144.
- Saria L, Shimaoka T, Miyawaki K (2006). Leaching of heavy metals in acid mine drainage. *Waste Management and Research* **24**, 134-140.
- Singh KP, Malik A, Mohan D, Sinha S, Singh VK (2005) Chemometric data analysis of pollutants in wastewater – A case study. *Analytica Chimica Acta* **532**, 15–25.
- Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry* **19**, 703–707.
- Wardle DA, Parkinson D (1990) Response of the soil microbial biomass to glucose, and selective inhibitors, across a soil moisture gradient. *Soil Biology & Biochemistry* **22**, 825–834.

Sorption and mobility of chromium species in a range of soil types

Girish Choppala^{A,B}, Nanthi Bolan^{A,B}, Megharaj Mallavarapu^{A,B} and Zuliang Chen^{A,B}

^A CRC-Contamination Assessment and Remediation of Environment (CRC CARE), Mawson Lakes, SA-5095.

^B CERAR, University of South Australia, Mawson Lakes, SA-5095; Girish.Choppala@postgrads.unisa.edu.au

Abstract

The adsorption of chromium species in soils has received much attention because of its toxicity to environment as adsorption controls the bioavailability. Batch studies were performed to evaluate the effect of soil properties on the adsorption of Cr(III) and Cr(VI) in 12 different Australian soils, which varied in their origin, mineralogy, CEC and organic content. Soil properties, including pH, CEC, OM, clay, water extractable SO_4^{2-} and PO_4^{3-} were investigated on the adsorption of Cr(III) & Cr(VI). The adsorption data were fitted to Freundlich equation and the adsorption parameters, K_f and n were calculated. In all soils, the amount of adsorption, as measured by K_f value was higher for Cr(III) than Cr(VI). The amount of Cr(III) adsorbed increased with an increase in pH and CEC of soils. There was no significant effect of soil properties on the adsorption of Cr(VI) and its adsorption was high in soils with high iron content. The mobility of Cr species, as estimated by the retardation factor was higher for Cr(VI) than for Cr(III).

Key Words

Redox reactions, heavy metal, retardation, bioavailability, bulk density, remediation.

Introduction

In many countries, including Australia, tannery and timber treatment industries contribute a major source of effluents containing heavy metals including chromium (Cr). The oxidation state of Cr in contaminated sites is an important indicator of toxicity. Hexavalent chromium (hex chrome) is highly toxic, carcinogenic, mutagenic and teratogenic. Chromium is used as Cr(VI) in timber treatment and as Cr(III) in tannery industries (Barnhart 1997). The processes that control the environmental chemistry and fate of Cr include adsorption, redox transformations and precipitation reactions (Adriano 2001).

While Cr(III) is strongly retained onto soil particles, Cr(VI) species such as chromate (CrO_4^{2-}), bichromate (HCrO_4^-) and dichromate ($\text{Cr}_2\text{O}_7^{2-}$) are weakly sorbed to soils under alkaline to slightly acidic conditions, leading to their movement to sub surface environments (James and Bartlett 1983; Carey *et al.* 1996). The maximum threshold level of Cr(VI) in water is 0.05 mg/L (World Health Organization 1996). Chromium(VI) is more stable form in equilibrium with atmospheric oxygen, but in the presence of soil organic matter it is reduced to Cr(III) (Cary *et al.* 1977; Grove *et al.* 1980; Bolan *et al.* 2003; Banks *et al.* 2006; Jiang *et al.* 2008).

Oxidation-reduction (redox) reactions are largely controlled by the adsorption of Cr species in soils, the supply of redox compounds and microbial activity. The detoxification of Cr(VI) through reduction to Cr(III) and its subsequent adsorption has been considered as an effective method for remediation management of Cr contaminated soils. The study of adsorption of Cr(VI) and Cr(III) is important in soils to understand the bioavailability of these two species. Despite the persistence of Cr contamination in soils, little data exist in the literature (Avudainayagam *et al.* 2001; Bolan and Thiagarajan. 2001) about the interaction of soil properties with Cr(III) and Cr(VI) in a range of Australian soils. The objectives of this research are to quantify the relative adsorption of Cr(III) and Cr(VI) and to examine the effect of soil properties on adsorption and mobility of these species in soils.

Materials and methods

Soils

Twelve different soils from 0-10 cm depth of representative profiles of soils in South Australia, Queensland and New South Wales were collected from uncontaminated land. The soil samples were analysed for pH, clay content, cation exchange capacity (CEC) (Gillman and Sumpter 1986), organic matter (Walkley and Black 1934) and water extractable SO_4^{2-} and PO_4^{3-} with 1:10 ratio of soil and solution by Ion chromatography (Dionex, ICS 2000-RFIC, Japan). The pH of the soils ranged from 3.01 to 7.80 and the organic matter ranged from 0.37 to 7.9 %.

Batch sorption

A batch technique was used to perform all adsorption studies. Two gram of soil samples were taken in duplicates in 50 mL centrifuge tube, mixed with 20 mL of Cr solutions [$\text{Cr}(\text{NO}_3)_3$ for Cr(III) and $\text{K}_2\text{Cr}_2\text{O}_7$ for Cr(VI)] at different concentrations (100, 200, 300, 400, 500 mg/L) and shaken on an end-over-end shaker at room temperature (22°C). After 16 hours of shaking, the soil suspensions were centrifuged, filtered through $0.45\ \mu\text{m}$ filter and analysed for Cr by Inductively Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES, Perkin Elmer, Optima 5300V, Japan). The amount of Cr(III) and Cr(VI) sorbed by soils was calculated from (Eq 1):

$$C_s = (C_o - C_e) V / W \quad (1)$$

Where C_s is the amount sorbed (mg/kg), C_o is the initial concentration (mg/L) and C_e is the equilibrium concentration (mg/L), V is the solution volume (L) and W is the weight of the air-dried soil sample (kg).

Results and discussion

Sorption isotherm

The sorption isotherms indicate the difference in sorption between Cr(III) and Cr(VI). The Freundlich adsorption isotherm (Eq 2) was used to model Cr sorption in 12 different soils:

$$S = K_f C^n \quad (2)$$

where S is amount sorbed (mg/kg), C is equilibrium concentration (mg/L) and K_f & n are linear and exponential coefficients.

The sorption of Cr, as measured by K_f values was higher for Cr(III) than for Cr(VI). While Cr(III) adsorption increased with increasing pH (Figure 1a), the effect of pH on the Cr(VI) adsorption was not consistent (Figure 1b).

The adsorption of Cr(III) increased with an increase in pH, which is attributed to cation exchange reactions of the hydrolysed species (Figure 2a) (Grove *et al.* 1980). Chromium (III) adsorption is generally influenced by pH and cation exchange capacity. When pH increases, negative surface charge of the soils increases, so there was an increased adsorption of Cr(III). (Griffin *et al.* 1977; Adriano 2001). Similarly clay and organic matter carry negative functional groups on their surface, thereby increasing the adsorption of Cr(III) in soils.

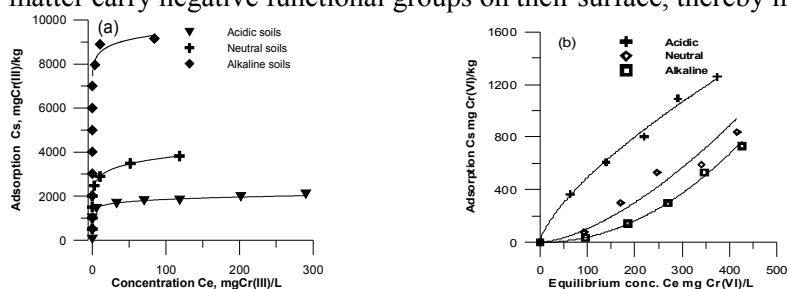


Figure 1. Adsorption of Cr(III) (Figure 1a), and Cr(VI) (Figure 1b) by 3 different soils.

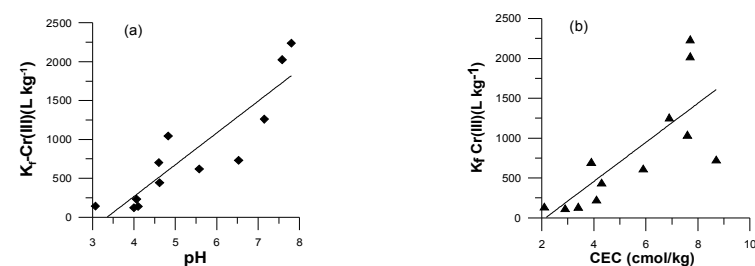


Figure 2. Effect of pH (Figure 2a) and CEC (Figure 2b) on the adsorption of Cr(III).

In all soils, Cr(VI) adsorption was much lower than that of Cr(III). There was no significant effect of pH and OM on the adsorption of Cr(VI) and adsorption was higher in soils containing high Fe and Al content (Griffin *et al.* 1977; Rai *et al.* 1989; Zachara *et al.* 1989), which may be attributed to high positive charge.

Multiple regression (Eq 3) analysis indicated that 88% (adjusted R^2 value) of the variation in adsorption of Cr(III) between the soils was attributed to the difference in pH, CEC, OM and clay content.

$$K_d = -1287.6 (\pm 275.2) + 410.1 (\pm 59.7) \text{pH} + -9.9 (\pm 6.605) \text{CEC} + 64.8 (\pm 28.75) \text{OM} + -5.273 (\pm$$

59.7) clay (3)
 Where k_d is dependent variable (adsorption of Cr(III) (L/kg) and independent variables are pH, CEC (cmol/kg), OM % and clay %).

Mobility of Cr

The mobility of Cr species was also estimated in terms of retardation factor (R) according to the following equation (Eq 3) (Kookana *et al.* 1990)

$$R_f = 1 + [(D_b/\theta_s)^n K_f C^{n-1}] \quad (4)$$

Where R_f is retardation factor which is an index of mobility of Cr species, D_b is bulk density (kg m^{-3}), θ_s is volumetric water content ($\text{m}^3 \text{m}^{-3}$). Parameters, K_f and n are Freundlich sorption constants, and C is equilibrium solution concentration. The higher the R_f value, the greater is the retardation of Cr(III) and Cr(VI) in soils. Values of R are lower for Cr(VI) than for Cr(III) (Figure 3) indicating that Cr(VI) is relatively more mobile and bioavailable.

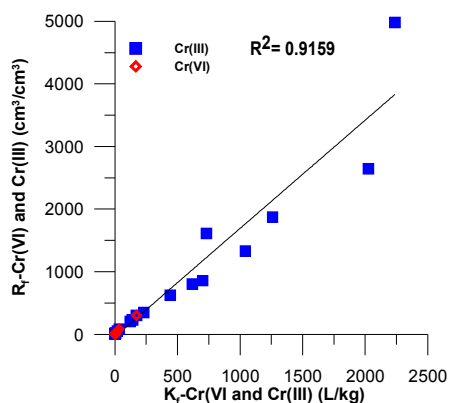


Figure 3. Mobility of Cr(VI) and Cr(III) in 12 soils

Conclusions

The results showed that Cr(III) adsorption was more than that of Cr(VI) in all 12 Australian soils and Cr(III) adsorption was greatly influenced by pH, CEC, OM and Clay content. However there was no significant effect of soil properties on the adsorption of Cr(VI). The retardation factor was less for Cr(VI) than for Cr(III) indicating greater mobility and bioavailability of Cr(VI) species in soils.

References

- Adriano DC (2001) Trace elements in terrestrial environments: Biogeochemistry, bioavailability, and risks of metals. 2nd ed. (Springer, New York).
- Avudainayagam S, Naidu R, Kookana R, Alston AM, McClure S, Smith L (2001) Effects of electrolyte composition on chromium desorption in soils contaminated by tannery waste. *Australian Journal of Soil Research* **39**, 1077-1090.
- Banks M, Schwab A, Henderson C (2006) Leaching and reduction of chromium in soil as affected by soil organic content and plants. *Chemosphere* **62**, 255-264.
- Barnhart J (1997) Chromium chemistry and implications for environmental fate and toxicity. *Soil and Sediment Contamination* **6**, 561-568.
- Bolan NS, Adriano, DC, Natesan R, Koo BJ (2003) Effects of organic amendments on the reduction and phytoavailability of chromate in mineral soil. *Journal of Environmental Quality* **32**, 120-128.
- Bolan NS, Thiagarajan S (2001) Retention and plant availability of chromium in soils as affected by lime and organic matter amendments. *Australian Journal of Soil Research* **39**, 1091-1104.
- Carey P, McLaren R, Cameron K, Sedcole J (1996) Leaching of copper, chromium, and arsenic through some free-draining New Zealand soils. *Australian Journal of Soil Research* **34**, 583-597.
- Cary EE, Allaway WH, Olson OE (1977) Control of chromium concentrations in food plants. 2. Chemistry of chromium in soils and its availability to plants. *Journal of Agricultural and Food Chemistry* **25**, 305-309.
- Gillman GP, Sumpter EA (1986) Modification to the compulsive exchange method for measuring exchange characteristics of soils. *Australian Journal of Soil Research* **24**, 61-66.
- Griffin R, Au AK, Frost R (1977) Effect of pH on adsorption of chromium from landfill-leachate by clay

- minerals. *Journal of Environmental Science and Health, Part A* **12**, 431-449.
- Grove JH, Ellis BG (1980) Extractable chromium as related to soil pH and applied chromium. *Soil Science Society of America Journal* **44**, 238-242.
- James BR, Bartlett RJ (1983) Behavior of chromium in soils: VII, Adsorption and reduction of hexavalent form. *Journal of Environmental Quality* **12**, 177-181.
- Jiang J, Xu R, Wang Y, Zhao A (2008) The mechanism of chromate sorption by three variable charge soils. *Chemosphere* **71**, 1469-1475.
- Ko BG, Vogeler I, Bolan NS, Clothier B, Green S, Kennedy J (2007) Mobility of copper, chromium and arsenic from treated timber into grapevines. *Science of the Total Environment* **388**, 35-42.
- Kookana R, Gerritse R, Aylmore L (1990) Effect of organic co-solvent on adsorption and desorption of linuron and simazine in soil. *Australian Journal of Soil Research* **28**, 717-725.
- Nriagu JO, Pacyna JM (1988) Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature* **33**, 134-139.
- Rai D, Eary LE, Zachara JM (1989) Environmental chemistry of chromium. *The Science of the Total Environment* **86**, 15-23.
- US EPA (2000) In situ treatment of soil and ground water contaminated with chromium- technical resource guide, *United States Environmental Protection Agency*. (Cincinnati, Ohio).
- World Health Organisation (1996) Health criteria and other supporting information, **2** WHO, Geneva, **2**. WHO. Geneva.
- Walkley A, Black IA (1934) An examination of Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* **37**, 29-38.
- Zachara JM, Ainsworth CC, Cowan CE, Resch CT (1989) Adsorption of chromate by subsurface soil horizons. *Soil Science Society of America Journal* **53**, 418-428.

Spatial variability of cadmium concentration in wheat farm soils

A. R. Jafarnejadi^{A,C}, M. Homaei^A, Gh. Sayyad^B and M. Bybordi^A

^ASoil Science Department, Tarbiat Modares University, Tehran, Iran;

^BSoil Science Department, College of Agriculture, Shahid Chamran University, Ahvaz, Iran.

^CCorresponding author. Email arjafarnejady@gmail.com

Abstract

Heavy metals are naturally present in the environment, but increasing levels of these elements are becoming a serious problem worldwide. The objective of this study was to assess the spatial variability and availability of cadmium (Cd) in the topsoils of 4000 km² wheat farms. Consequently, 255 soil samples were collected from the study area. The DTPA-extractable and total Cd concentrations, pH, EC, organic carbon and clay contents of soil samples were measured. The total Cd concentrations in 95 percent of samples exceeded 0.8 mg/kg value. However DTPA-Cd concentrations in 25 percent of the samples were more than 0.1 mg/kg. The correlation between DTPA-Cd and both organic carbon ($r=0.63^{**}$) and soil pH ($r=0.58^{**}$) was significant ($p<0.01$). Also, a significant ($p<0.05$) correlation was obtained between DTPA-Cd concentration and clay content ($r=0.55^*$). A significant ($p<0.05$) correlation was obtained between total Cd and organic carbon ($r=-0.48^*$). The correlation analysis indicated that soil organic carbon alone explains 63 percent of DTPA-Cd variation in the study area. All the obtained semivariograms showed a high degree of spatial dependency. The semivariograms of total Cd and root square of DTPA-Cd were fitted well with a spherical model. Risk probability maps were made, using ordinary Kriging for both variables.

Key Words

Cadmium, soil pollution, soil properties, spatial variability, wheat farms.

Introduction

Rising levels of heavy metals are becoming a serious problem worldwide (Norvell *et al.* 2000). Cadmium is recognized as being one of the most mobile trace metals circulating in the environment and can readily enter the human food chain from agricultural and industrial sources and causes some problems for human health. Plants are the main pathways for cadmium entry into the food chain (Ingwersen and Streck 2005). The uptake of cadmium by plants and ingestion by humans depend on the bioavailability of Cd in the soil. Therefore, the knowledge on spatial variability and availability of Cd in agricultural soils are essential to develop effective management recommendations (Norvell *et al.* 2000). Predictions of polluted areas are often based on geostatistical methods, which calculate unbiased estimates of heavy metals at unsampled locations. Geostatistical methods have been widely used to document the spatial variability of soil properties. Wu *et al.* (2002) reported that the variation of grain cadmium and soil characteristics in a durum wheat field were spatially dependent with range distances that varied from about 30 to 55 m. A spherical model was fitted successfully to the variograms for the studied characteristics. In other study, Reis *et al.* (2005) predicted soil arsenic concentrations at unsampled locations of a mine in Portugal using a spherical model. In arid regions of Iran, farmers extensively use fertilizers to obtain more products. In addition, an abundance soil and water resources and suitable temperatures in Khuzestan province provide excellent conditions for crop production during the whole year. Therefore, high loads of heavy metals are imposed by fertilizer application into intensively farmed and overly irrigated soils, which can cause problems for food chain health. Up to now, no comprehensive survey of soil Cd contamination has been carried out in the region. In this study, a large scale investigation was conducted to assess the spatial variability and availability of Cd in wheat-farm soils of Khuzestan province. Since wheat is the main crop and food in Iran, the wheat farms were chosen for the study.

Methods

The designated soil samples (0-20 cm) were collected from 255 wheat farms in a study area of 4000 km² in Khuzestan province (47°40'E to 50°33'E and 29°57'N to 30°00'N), Iran, before plant harvesting in mid May, 2008. Soil samples were collected from different cities using a weighing method, i.e., the more wheat in a farm area, the more soil samples. The coordinates were recorded with a GPS apparatus. All soil samples were then air-dried and passed through a 2-mm sieve. Total Cd concentration were extracted, using concentrated HCl and HNO₃ and were analyzed by an atomic absorption spectrophotometer. Available cadmium was extracted by diethylenetriaminepentaacetic acid (DTPA) and analyzed by a graphite atomic

absorption spectrophotometer. Electrical conductivity and pH of soil samples were measured in a saturated extract. Clay content was determined using the hydrometric method. Organic carbon was determined by wet oxidation. Spatial variability was studied with semivariograms, which were created and evaluated according to standard geostatistical methods. Before creating semivariograms, a square root (sqrt) transformation was applied to the available cadmium data, which normalized the distribution. The best model for semivariograms was selected comparing statistical parameters, correlation coefficient (r^2), mean absolute error (MAE) and mean bias error (MBE), and was used for kriging of cadmium (total and available). The GS⁺ (v3.1 for windows, Gamma Design Software, Plainville, MI) package was used for geostatistical analyses. Correlation and regression analyses between total and available Cd and soil parameters were done using the SPSS14 software.

Results and discussion

The descriptive statistics for topsoil Cd concentration (total and DTPA-extractable (available)) is presented in Table 1.

Table 1. Summary statistics of soil cadmium concentrations (mg/kg).

| Variable [†] | Minimum | Maximum | Std | CV | Skewness | Kurtosis |
|------------------------|---------|---------|------|------|----------|----------|
| Cd _t | 0.75 | 2.19 | 0.26 | 17.5 | 0.029 | 0.235 |
| sqrtCd _{DTPA} | 0.002 | 0.54 | 0.11 | 41 | -0.15 | 0.22 |

[†]Cd_t: Total Cd concentrations, sqrtCd_{DTPA}: Square root of DTPA-extractable Cd

Total cadmium was distributed in an approximately normal distribution. Although DTPA-extractable Cd concentrations had a highly skewed distribution, the square root transformation caused a normal distribution for the available data. Based on the coefficient of variation, the DTPA-extractable Cd was nearly twice as variable as the total concentration of Cd on the regional scale. This indicates that the availability of Cd is being affected by different soil properties in this region. Total cadmium concentrations in 95 percent of the samples exceeded the suggested Swiss threshold of 0.8 mg/kg, while available cadmium concentrations in 25 percent of the samples were more than 0.1 mg/kg. Analysis of the correlation coefficients showed that there was no significant ($p < 0.05$) correlation between total Cd concentration and soil properties (clay, EC and pH) in this study except for organic carbon ($r = -0.47^*$). However, the correlation between DTPA-extractable Cd and both organic carbon ($r = 0.63^{**}$) and soil pH ($r = 0.58^{**}$) was significant ($p < 0.01$). Also, a significant ($p < 0.05$) correlation was obtained between DTPA-extractable Cd concentration and clay content ($r = 0.55^*$). All the obtained semivariograms indicate a high degree of spatial dependency (Figure 1).

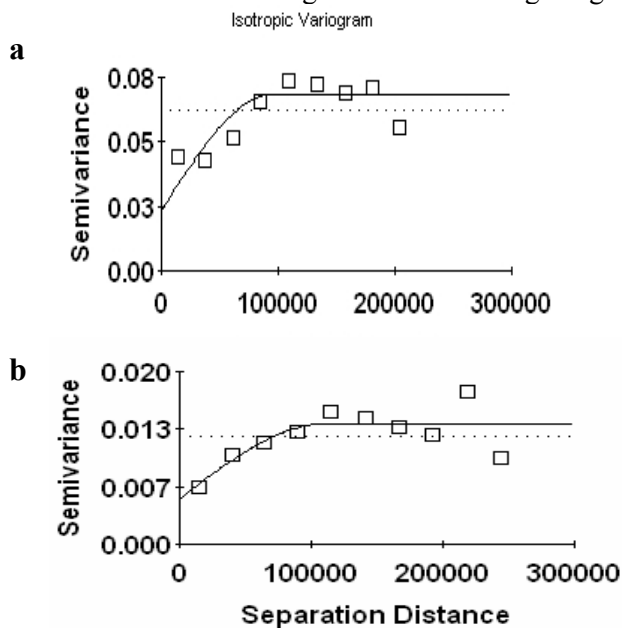


Figure 1. Semivariograms for the total (a) and available (b) soil Cd concentration.

The semivariograms of total Cd and root square of DTPA-extractable Cd were fitted well with a spherical model. This model had the largest r^2 and the least MAE and MBE values compared to other models. The nugget, sill and range values for the spherical model of total Cd were 0.025, 0.073 and 95000 m, respectively. The values for the spherical model of available Cd were 0.0053, 0.014 and 112000 m for nugget, sill and range, respectively. Regarding normal distribution of total and transformed DTPA-extractable Cd data, ordinary kriging was selected. The produced kriging map of DTPA-extractable Cd revealed two polluted zones in the study area. The first hot spot is located at the east of the province where dry land farming is the common practice, and this needs to be studied in more detail. The other hot spot is located at the south of the province where the farms were affected mostly by intensive irrigated agriculture and also 8 years of the Iraq-Iran war. As a consequence of these two reasons, a high amount of metals was added to agricultural soil.

Conclusion

Agricultural activities have significantly increased the Cd concentration in the topsoils of wheat farms in the study area. Both total and available Cd concentration exceeded the permissible threshold value in the study area (95% and 25% of the samples, respectively). Only organic carbon content significantly affected the total Cd concentrations, while clay content, organic carbon and soil pH showed significant contributions to Cd availability. The results also indicated that the risk of Cd availability for plants could not be quantified only by its total or available concentration. The other factors (in this study clay content, organic carbon and soil pH) should also be considered to assess the state of soil contamination by Cd. Future investigations should focus on the effect of clay mineralogy and the compounds of organic carbon on soil Cd availability.

References

- Ingwersen J, Streck T (2005) A regional-scale study on the crop uptake of cadmium from sandy soils; measurement and modeling. *Journal Environmental Quality* **34**, 1026-1035.
- Norvell WA, Wu JD, Hopkins G and Welch RM (2000) Association of cadmium in durum wheat grain with soil chloride and chelate-extractable soil cadmium. *Soil Science Society American Journal* **64**, 2162-2168.
- Reis AP, Sousa AJ, Ferreira D, Silva E, Cardoso Fonseca E (2005) Application of geostatistical methods to arsenic data from soil samples of the Cova dos Mouros mine (Vila Verde-Portugal). *Environmental Geochemistry and Health* **27**, 259-270.
- Wu J, Norvell WA, Hopkins DG, Welch RM (2002) Spatial variability of grain cadmium and soil characteristics in a durum wheat field. *Soil Science Society American Journal* **66**, 268-275.

The impact of phlogopite-rich mine tailings on the speciation of water-soluble Pb in contaminated boreal shooting range soil

Salla Venäläinen

Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Finland, Email salla.venalainen@helsinki.fi

Abstract

Changes in the water-solubility and the speciation of bullet-derived lead (Pb) in a contaminated shooting range soil were investigated in a pot experiment. Samples from the uppermost organic soil were incubated for 21 months with different particle-size fractions of untreated and artificially weathered (i.e. acid-treated) tailings from apatite ore beneficiation. Soil samples were subjected to water extraction. To estimate the proportion of the less toxic, non-cationic and organic Pb compounds in the total water-soluble Pb, the extracts were passed through cation exchange columns and analysed for Pb and dissolved organic carbon (DOC) before and after the cation exchange. Addition of untreated tailings to the organic soil decreased the water soluble Pb and increased the relative abundance of water-soluble and non-cationic Pb. On the contrary, incubation with the unsieved or the small particle-size fraction of the acid-treated tailings increased the water-solubility of Pb as well as the relative abundance of the most toxic Pb^{2+} and PbOH^+ species. The results indicated that the untreated and the large-sized acid-treated tailings might be suitable to the remediation of Pb-contaminated soils, whereas the unsieved and the small-sized acid-treated tailings may enhance leaching of Pb and, thus, cannot be considered as amendments.

Introduction

Lead (Pb) is toxic to living organisms and, if dissolved to the soil water, may end up in food chain and harm the ecosystem and human health. Due to its excellent ballistic properties, Pb is a commonly used element in shotgun pellets. Thus, shooting range soils are often subjected to Pb contamination.

The speciation and the mobility of Pb are strongly dictated by soil organic matter (SOM), soil pH and the nature of the mineral material present. In Finland, shooting ranges are typically located in boreal forest areas, where the topsoil is peaty and rich in humic substances, primarily humic and fulvic acids (HA and FA). In contact with such compounds, Pb is likely to form chelates and other organic complexes (Stevenson 1982). These compounds are more mobile and less toxic than the free Pb cation (Pb^{2+}) or the Pb hydroxycomplex (PbOH^+). The formation of organic Pb complexes is promoted by elevated soil pH, which contributes to the dissociation of H^+ from carboxyl and other organic functional groups and to the formation of negatively charged surfaces. Furthermore, high pH increases the stability of organometallic complexes but, on the other hand, increases the solubility of HAs (Klucakova & Pekar 2008) and, thus, the mobility of organically bound Pb. On the contrary, acid conditions promote the dissolution of Pb from pellets and increase the abundance of free Pb^{2+} .

If leached to the mineral soil horizons, Pb may be retained by hydrated Al and Fe oxides or amorphous silica emerging from the edges of weathered silicate minerals (Aualiitia and Pickering 1987; Elzinga and Sparks 2002). Such retention is specific in nature and favoured by low pH. Pb is also known to form various compounds with apatite and, in calcareous soils, with carbonate minerals. In certain conditions, the reaction between apatite and Pb may result in the formation of sparingly soluble pyromorphites [$\text{Pb}_{10}(\text{PO}_4)_6(\text{OH}, \text{F}, \text{Cl})_2$] (Cao *et al.* 2004). It can also be adsorbed through cation exchange with apatitic Ca^{2+} (Chen *et al.* 1997) or be specifically sorbed by functional surface groups of apatite (Mavropoulos *et al.* 2002). The free Pb^{2+} may also be adsorbed by calcite surfaces (Fulghum *et al.* 1988) through a specific mechanism (Rouff *et al.* 2002). Precipitation of Pb as carbonates also occurs (Taylor and Lopata 1984; Al-Degs *et al.* 2006).

Apatite ore mining at the Siilinjärvi carbonatite complex (described in detail by Puustinen 1971) produces mine tailings that mainly consist of a trioctahedral silicate mineral, phlogopite [$\text{KMg}_3(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH})_2$], accompanied by carbonate minerals, mostly calcite (CaCO_3), and fluorapatite [$\text{Ca}_5(\text{PO}_4)_3\text{F}$]. The chemical composition of the tailings in relation to Pb immobilisation may enable the material to act as an *in situ* remediation media on contaminated shooting ranges.

The aim of the study was to investigate the tailings-induced changes in the water-solubility, i.e. potential bioavailability, and in the speciation of water-soluble Pb (Pb_{ws}) in contaminated shooting range soil. For this purpose, an incubation experiment was carried out by amending contaminated soil with mine tailings of various particle sizes differing in the mineralogical composition (Hartikainen and Hartikainen 2008). A portion of the material was artificially weathered with strong acid, since acid-treatment has been shown to increase the abundance of reactive Al and Fe oxyhydroxides in the tailings (Hartikainen and Hartikainen 2008). The beneficial utilisation of the mine tailings aims to the reduction of bioavailability of Pb and, thus, protection of ecosystem and the groundwater, as well as to the decrease in the amount of waste produced by the mining industry.

Materials and methods

Soil samples

The soil samples originated from the organic horizon of Hälvälä shooting range (61°00.644' N, 025°28.406' E) located in Hollolla, southern Finland (area described in detail by Hartikainen and Kerko (2009)). The soil in the area is sandy with a moss layer on the top. In the laboratory, the shotgun pellets were picked out of the soil by hand and the pellet-free material was homogenized.

Tailings materials

The tailings provided by Kemira GrowHow Oyj originated in Siilinjärvi apatite ore, eastern Finland (63°6.893' N, 27°43.994' E). To investigate the impact of artificial weathering on the Pb immobilizing effect of the tailings, the material was treated with strong sulphuric acid. A portion of both untreated (UT) and acid-treated (AT) tailings was passed through a 2-mm sieve. Unsieved material was also included in the experiment.

Design of the incubation experiment

For the incubation experiment, 50-g samples of the homogenized peaty soil were amended with each of the tailings materials in four replicates. Four soil samples without tailings treatments served as controls. All the experimental units were moistened with 25 mL of mQ-H₂O (30% of water holding capacity) and incubated at 20°C for 21 months. During the incubation, the evaporated water was replaced by watering the samples according to weight loss.

Pb speciation

Following the incubation, water-extractable Pb was extracted at a soil:solution ratio of 1:200 (dw:V, 2h shaking, 250 rpm). A portion of the suspensions were filtered through a coarse (12-25- μ m) filter paper (Pb_{tot}) and the rest of the solutions through a 0.2- μ m membrane filter (Pb_{ws}). The membrane filtrates were subjected to cation exchange (CE) by passing them through solid phase extraction (SPE) tubes that were preconditioned with 0.4 M KH₂PO₄ (pH 5). The solutions were analysed for Pb with ICP-OES before and after the CE. The abundance of cationic Pb in various treatments was calculated as the difference between soluble Pb before and after the CE. To differentiate between the inorganic cationic Pb species (Pb^{2+} and $PbOH^+$) from the less toxic, organic cationic Pb species the solutions were also analysed for dissolved organic carbon (DOC) before and after the CE. The proportion of organic cationic Pb species in each solution was estimated as the difference between DOC before and after the CE. Finally, to determine the total Pb (Pb_{tot}) in each sample, the paper filtrates were subjected to microwave digestion and analysed for Pb with ICP-OES.

Results and discussion

Figure 1 depicts the Pb_{tot} and the Pb_{ws} (mg/l) and the solution pH before the CE and Figure 2 Pb_{ws} after the CE (note the scales). All size fractions of the UT tailings as well as the large particles of the AT tailings elevated the solution pH, whereas the unsieved and the small particle-size fraction of the AT tailings had no effect on pH. All particle-size fractions of the UT tailings decreased Pb_{ws} , especially the unsieved material. The large particle-size fraction of the AT tailings had no effect on Pb_{ws} , while both the unsieved and the small particle-size fraction of this material increased this Pb pool notably. In the soil amended with the unsieved and the small particle-size fraction AT tailings, Pb_{ws} was equal to the Pb_{tot} , i.e. no Pb was bound by the large-molecular humic substances or by particulate organic matter. This was probably due to the low pH of the tailings that resulted in the disintegration of Pb from its compounds.

Passing the solutions through the CE columns decreased the Pb_{ws} substantially in all treatments (Figure 2). The proportion of non-cationic Pb to the total soluble Pb was substantially higher for the soil amended with the UT tailings (9–12%) than for the soil amended with the AT tailings (1–4%). In the control soil, the ratio was 5%. This suggests that the UT tailings increased the abundance of the less toxic, non-cationic Pb in the soil water at the expense of the more toxic, cationic Pb species. The phenomenon can be explained by the liming effect of the UT tailings that resulted in increased stability of organic Pb complexes. The proportion of non-cationic DOC to the total DOC ranged between 81–97%. The high ratios indicate that the CE did not affect the DOC notably and that nearly all of the Pb retained by the CE column was in inorganic form.

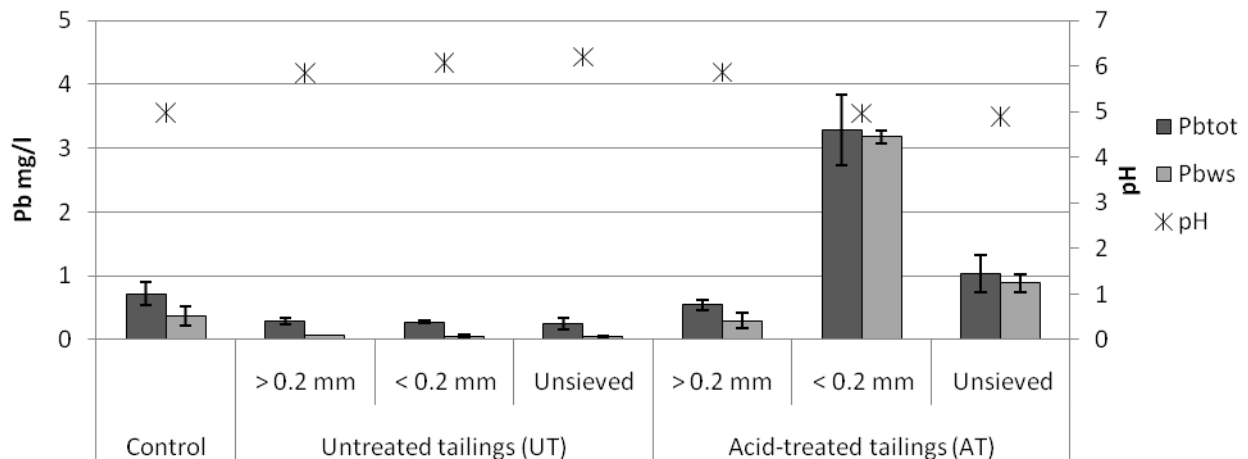


Figure 1. Total Pb in the samples (Pb_{tot}), soluble Pb (Pb_{ws}) and pH in the water extract before cation exchange. The error bars indicate the standard deviations of Pb.

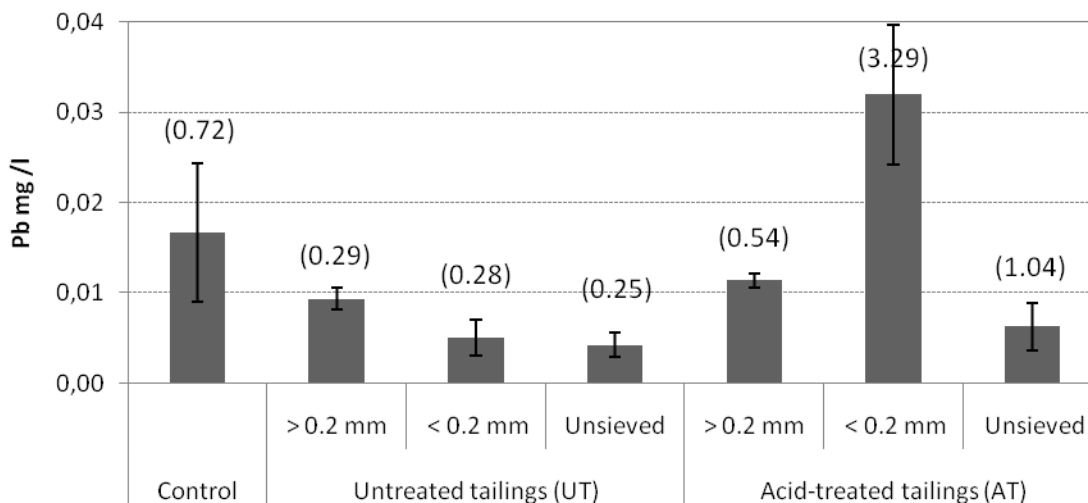


Figure 2. Non-cationic Pb in the water solution. The values in the brackets indicate Pb_{tot} (mg/l) and the error bars indicate the standard deviations of Pb.

Conclusion

The results showed that amending Pb-contaminated, organic shooting range soil with untreated tailings from apatite ore beneficiation may reduce the water-solubility of Pb and increase the abundance of organic Pb complexes at the expense of the more toxic, inorganic cationic Pb species. The untreated tailings elevate soil pH thus resulting in increased stability of organic Pb-complexes. Since high pH increases the solubility of organic matter, the untreated tailings may induce increased leaching of Pb complexes deeper into the mineral soil horizons. However, it is likely that, in mineral soil, Pb is strongly retained by the hydrated Al and Fe oxides and other inorganic soil components present. Acid-treated tailings substantially increase the water-solubility of Pb and disintegrate Pb from its organic compounds increasing the abundance of the most toxic Pb^{2+} and $PbOH^+$ species. Thus, it cannot be considered to be utilized as a soil amendment.

References

- Al-Degs YS, El-Barghouthi MI, Issa AA, Khraisheh MA, Walker GM (2006) Sorption of Zn(II), Pb(II), and Co(II) using natural sorbents: Equilibrium and kinetic studies. *Water Research* **40**, 2645–2658.
- Aualiita TU, Pickering WF (1987) The specific sorption of trace amounts of Cu, Pb and Cd by inorganic aperticulates. *Water Air and Soil Pollution* **35**, 171–185.
- Cao X, Ma LQ, Rhue DR, Appel CS (2004) Mechanisms of lead, copper, and zinc retention by phosphate rock. *Environmental Pollution* **131**, 435–444.
- Chen X-B, Wright JV, Conca JL, Peurrung LM (1997) Effects of pH on heavy metal sorption on mineral apatite. *Environmental Science and Technology* **31**, 624–631.
- Elzinga EJ, Sparks DL (2002) X-ray absorption spectroscopy study of the effects of pH and ionic strength on Pb(II) sorption to amorphous silica. *Environmental Science and Technology* **36**, 4352–4357.
- Fulghum JE, Bryan SR, Linton RW, Bauer CF, Griffis DP (1988) Discrimination between adsorption and coprecipitation in aquatic particle standards by surface analysis techniques: lead distributions in calcium carbonates. *Environmental Science and Technology* **22**, 463–467.
- Hartikainen H, Kerko E (2009) Lead in various chemical pools in soil depth profiles on two shooting ranges of different age. *Boreal Environmental Research* **14 (Suppl. A)**, 61–69.
- Hartikainen SH, Hartikainen HH (2008) Phosphorus retention by phlogopite-rich mine tailings. *Applied Geochemistry* **23**, 2716–2723.
- Klucakova M, Pekar M (2008) Behaviour of partially soluble humic acids in aqueous suspension. *Colloids and Surfaces A-Physicochemical and Engineering Aspects* **318**, 106–110.
- Mavropoulos E, Rossi AM, Costa AM, Perez CAC, Moreira JC (2002) Studies on the mechanism of lead immobilization by hydroxyapatite. *Environmental Science and Technology* **36**, 1625–1629.
- Puustinen K (1971) Tetraferriphlogopite from the Siilinjärvi carbonatite complex, Finland. *Bulletin of the Geological Society of Finland* **45**, 35–42.
- Rouff AA, Reeder RJ, Fisher NS (2002) Pb (II) sorption with calcite: A radiotracer study. *Aquatic Geochemistry* **8**, 203–228.
- Stevenson FJ (1982) *Humus Chemistry*. John Wiley & Sons, Inc., Canada.
- Taylor P, Lopata VJ (1984) Stability and solubility relationships between some solids in the system lead oxide-carbon dioxide-water. *Canadian Journal of Chemistry* **62**, 395–402.

The mobility of Pb and Sb originating from small arms ammunition in a coarse grained unsaturated surface sand

Jeffrey Lewis^{A,B}, Jan Sjöström^A, Ulf Skyllberg^B and Lars Hägglund^A

^ATotalförsvarets forskningsinstitut FOI-CBRN, Umeå, Sweden

^BSveriges Lantbruksuniversitet (SLU) Skogsekologi, Umeå, Sweden

Abstract

Uncontaminated soil obtained from two shooting ranges in Sweden was used to construct two bench scale lysimeters. These lysimeters were then used to quantify the transport of Pb and Sb leaching from bullet-contaminated sand placed on the surface of both lysimeters. The lysimeters were subjected to infiltration which reproduced the springtime snowmelt in northern Sweden, and the effluent was analyzed for dissolved Pb and Sb. The Pb concentrations in the effluent stayed at or near background levels for the duration of the experiment while the Sb concentrations showed a classic breakthrough curve.

Key Words

Shooting range, leaching, lysimeters.

Introduction

Several studies have been published which examine Pb concentrations and mineralization in shooting range sands but fewer papers exist which examine Sb (Cao *et al.* 2003; Dermatas *et al.* 2004; Knechtenhofer *et al.* 2003; Ma *et al.* 2007). The transport of Pb and other metals from shooting range soils has been experimentally examined in some detail (Johnson *et al.* 2005; Jorgensen and Willems, 1987). The mobility of Pb has been shown to be affected by the redox potential (Eh), pH, cation exchange capacity (CEC) and available anions – including carbonate, phosphate and sulfate (Basta *et al.* 1993). Factors which have been observed as influencing Pb chemistry in firing range sand include pH (Cao *et al.* 2003; Dermatas *et al.* 2004; Ma *et al.* 2007; Lin *et al.* 1995), mineralogy (Dermatas *et al.* 2004), natural organic matter and water content (Ma *et al.* 2007). There is very little experimental information in the literature about the effects of such basic attributes as temperature and Eh on Pb chemistry and conflicting reports exist concerning the relative solubility of Pb minerals (Lindsay, 1979, Knechthofer *et al.* 2003). Furthermore, there are no studies concerning the kinetics of reaction of various Pb minerals existing on firing ranges. The complexity of the chemistry and the significant gaps in the current state of knowledge necessitates a site-specific approach to deal with the potential fate and transport of metals on various firing ranges (Dermatas *et al.* 2004; Cao *et al.* 2003). Little information is available in the literature concerning the transport and environmental fate of any of the non-Pb heavy metal alloying agents in metal bullets, particularly Sb (Johnson *et al.* 2005, Scheinost *et al.* 2006). While little is known about the speciation and geochemical fate of Sb in sands and sediments (Krupka and Serne 2002), is that the toxicity of Sb is thought to be similar to that of arsenic (Scheinost *et al.* 2006) and standardized tests have shown Sb to be toxic to invertebrates (Kuperman *et al.* 2005). Therefore, the evidence indicates that if Sb is mobilized from a shooting range, it could pose a significant environmental concern.

The purpose of this experiment was to quantify the transport of Pb and Sb through the standardized soil used in the berms of Swedish military ranges. All military range berms in Sweden are constructed using a standardized mixture of sand and pea gravel, making experimental observations at one site cautiously relevant to other sites. Unfortunately, such a mixture of sand and gravel is also highly permeable, allowing the rapid movement of water through its pores. Furthermore, it is very low in both clay and natural organic matter (NOM), which are the soil components which typically contribute the most to the retardation of dissolved contaminants (Boulding and Ginn 2004).

Materials and methods

Bullet impacted sand

Uncontaminated range sand was obtained from an existing military range in Umeå, northern Sweden, where a new impact berm had been installed. The sampling was performed on the side of the berm facing away from the firing point to minimize the potential of pre-existing heavy metal contamination. X-Ray fluorescence (XRF) analysis confirmed that the sand did not have prior heavy metal contamination. A total

of 500 rounds of 7.62 mm jacketed military ammunition were then fired into the soil in a controlled fashion on an indoor range.

Lysimeter construction

Uncontaminated range sand was used in the construction of two lysimeters measuring 40 cm in diameter and 40 cm in depth. Sand samples were obtained from two Swedish military shooting ranges, from the back side of the berm facing away from the firing points. X-Ray fluorescence (XRF) analysis confirmed that the sand did not have prior heavy metal contamination. The sand columns were built up gradually by compacting 2 cm thick layers of sand sequentially to ensure maximal density. Following the compaction of each layer, the sand surface was scarified with a nylon comb. This was done both to ensure complete hydraulic connectivity with the subsequent sand layer and to minimize the potential for horizontal preferential flow. A 4 cm thick layer of bullet-impacted sand was then placed on the surface of each of the two lysimeters.

A 40 cm diameter 1-bar ceramic pressure plate cell (Soilmoisture Ceramic-B0.5M2) was installed at the base of each lysimeter. This pressure plate has an effective pore size of 6.0 μm . The negative head was created with a hanging column of effluent in the outflow hose from the pressure plate. The negative head determined the degree of saturation of the sand column. Time domain reflectometer (TDR) probes were installed at depths of 5 cm, 20 cm and 35 cm below the surface of the lysimeter to ensure that the sand moisture was maintained within an acceptable range. The TDR probes automatically read sand moisture content and sent the information to a datalogger every 30 seconds. The head was adjusted by trial and error to produce a sand moisture content of $0.20 + 0.05$ (mass ratio) throughout the lysimeter, which is consistent with surface conditions of the sand at the shooting range. Saturated conditions occurred at a moisture content of 0.37.

Infiltration protocol

The infiltration rate for a sand during the springtime snowmelt in northern Sweden was determined by integrating historical water table fluctuation data, precipitation data and temperature data from SGU, Geological Survey of Sweden; SMHI, the Swedish meteorological society and from BALTEX, the Baltic Sea Experiment (unpublished data). A 15 year period from 1985 – 2000 was used to calculate the average time for complete snowmelt (31 days) and the average total infiltration caused by the snowmelt (115 mm). Historical hourly temperature data was not available, so it was assumed that melting and therefore infiltration occurred between 10 am and 6 pm. Given the surface area of the lysimeters (0.126 m²), the infiltration rate for each lysimeter was calculated to be 0.48 liters day⁻¹ or 1.00 ml min⁻¹ for 8 hours daily.

Infiltrate chemistry and ambient temperature

The chemical composition of the snowmelt was determined using rainfall chemistry from Svartbergets Research Station (Swedish University of Agricultural Sciences) in Vindeln (Lat./Long. 67°11' N; 19°38' E). Aqueous concentrations are shown in Table 1. The pH of the infiltrate was adjusted to a value of 4.8, which is a historical average value of the springtime snowmelt runoff in the area of interest (Laudon *et al.* 1999). The lysimeters were placed in an environmental chamber and the ambient temperature was held at a constant value of 4° C, again to simulate environmental conditions during the period of snowmelt as closely as possible.

Results

The leachate from the lysimeters was chemically analyzed for Pb and Sb for the duration of the experiment. The pH of the leachate remained stable throughout the period of analysis at 4.8 ± 0.1 and while the redox potential was not monitored, ambient conditions and temperature were not changed for the duration of the experiment. The water samples were not filtered, but the use of the ceramic pressure plate removed all particles greater than the pore size of 6.0 μm . The data are shown in Figure 1. Lead concentrations generally remained stable or decreased slightly over time throughout the experiment in both lysimeters. The concentration remained under 2 $\mu\text{g/L}$, which is lower than the Swedish Environmental Protection Agency (EPA) limit of 10 $\mu\text{g/L}$ set for drinking water (Naturvårdsverket 1999, Annex 4). Thus, the origin of the Pb observed in the leachate is likely to be background and not originate from the bullet fragments. This implies that the Pb from the bullet impacted sand is relatively immobile, even in sands which have no clay fraction and little organic matter to retard the contaminants in the lysimeter effluent.

In contrast, the Sb in the effluent from both lysimeters showed a classic breakthrough curve. At the peak concentration, the Sb in the effluent was over two orders of magnitude higher than the Pb concentrations,

despite Sb being a minor alloying agent in the bullets. Figure 1 shows that peak concentrations of Sb in the lysimeter containing the sand from Umeå reached 124 µg/L and this occurred after 29 days of infiltration. This effluent would be considered “very seriously” contaminated according to the Swedish EPA guidelines for groundwater (Naturvårdsverket 1999, Annex 4). The transport of Sb in the lysimeter containing the sand from the first range is retarded by approximately 10 – 15 days relative to that of the second range and peak concentrations of Sb were not achieved before the snowmelt infiltration protocol ended. The area under the right hand curves of figure 1 provides the total mass of Sb which was mobilized. 1.7 mg and 0.6 mg of Sb were captured in the effluent of the two lysimeters. Given the standardized berm sand used by the Swedish military, the results should be applicable to military ranges throughout Sweden assuming a similar bullet distribution and infiltration pattern.

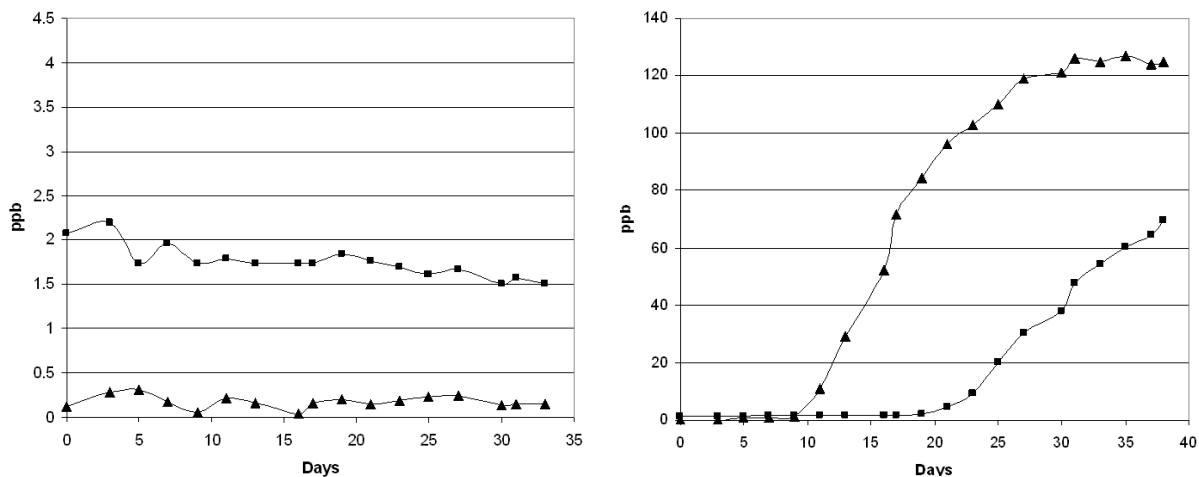


Figure 1. Pb (left) and Sb (right) concentrations in lysimeter effluent. Triangle and square symbols show results from the two different lysimeters

Discussion

As was pointed out in the introduction, there are significant knowledge gaps in the state of the art concerning the environmental impacts of Sb. Our observations indicate that Sb is highly mobile both in speed of transport and mass of transport in the vadose zone of Swedish berm sands. This contrasts with the findings of Knechtenhofer *et al.* (2003) who showed that within 40 cm sand depth Sb concentrations on a shooting range approached background concentrations. However, the sands investigated by Knechtenhofer *et al.* (2003) had a very low pH of 3.6 and contained 15% organic carbon, making comparisons with our results difficult. There is some evidence in the literature that complexation with organic ligands may stabilize Sb compounds (Brookins, 1972).

The difference in the rate of Sb transport between the two lysimeters may reasonably be attributed to slightly different concentrations of either Fe (hydro)xides or clays, either of which have been shown to have a considerable impact on the sorption of antimony (Tighe *et al.* 2005). However, our experimental observations are in general consistent with the observations of Johnson *et al.* (2005) who noted that the release of Sb from shooting range sands was significant and considerably higher than the release of Pb. Further work will have to be performed to determine the redox processes that control the oxidation of Sb in surface sands.

Conclusions

Transport of Pb in the Swedish range sands was found to be negligible, with no clear solute breakthrough curve and with aqueous concentrations remaining stable at below 2 ppb. However, Sb was found to be far more mobile, with solute breakthrough occurring between 5 and 14 days and concentrations rising to over 125 ppb within one month.

References

- Basta NT, Pantone DJ, Tabatai MA (1993) Path analysis of heavy metal adsorption. *Soil Agron. J.* **85**,1054-1057.
- Boulding JR, Ginn JS (2004) 'Practical handbook of soil, vadose zone, and ground water contamination.' 2nd Ed. (Lewis Publishers)
- Brookins DG (1972) Stability of stibnite, metastibnite and some probably dissolved antimony species at 278.1°K and 1 atm. *Econ. Geol.* **67**, 369-372.
- Cao RX, Ma LQ, Chen M, Hardison DW, Harris WG (2003) Weathering of lead bullets and their environmental effects at outdoor shooting ranges. *J. Environ. Qual.* **32**, 526-534
- Dermatas D, Dadachov M, Dutko P, Menounou N, Arienti P, Shen G (2004) Weathering of lead in Fort Irwin firing range soils. *Global Nest* **6(2)**, 167-175.
- Johnson CA, Moench H, Wersin P, Kugler P, Wenger C (2005) Solubility of antimony and other elements in samples taken from shooting ranges. *J Environ Qual.* **24**, 248-254
- Jorgensen SS, Willems M (1987) The fate of lead in soils: the transformation of lead pellets in shooting range soils. *Ambio* **16**, 11-15.
- Knechtenhofer LA, Xifra IO, Scheinost AC, Fluhler H, Kretzschmar R (2003) Fate of heavy metals in a strongly acidic shooting range soil: small scale metal distribution and its relation to preferential water flow. *J. Plant Nutr. Sand Sci.* **166**, 84-92.
- Krupka KM, Serne RJ (2002) Geochemical Factors Affecting the Behavior of Antimony, Cobalt, Europium, Technetium, and Uranium in Vadose Sediments. *Pacific Northwest National Laboratory, Richland, Washington. PNNL-14126*
- Kuperman RG, Checkai RT, Simini M, Phillips CT, Speicher JA, Barclift D (2005) Toxicity benchmarks for antimony, barium and beryllium determined using reproduction endpoints for *Folsomia candida*, *Eisenia fetida* and *Enchytraeus crypticus*. *Env. Toxicol. and Chem.* 754-762.
- Laudon HS, Köhler S, Bishop KH (1999) Natural acidity or anthropogenic acidification in the spring flood of northern Sweden. *Sci. Tot. Environ.* **234**, 63-73.
- Lindsay WL (1979) 'Chemical equilibria in sands' (John Wiley Publishers, New York)
- Ma.LQ, Hardison DW, Harris WG, Cao X, Zhou Q. (2007) Effects of soil property and soil amendment on weathering of abraded metallic Pb in shooting ranges. *Water Air Soil Pollut.* **178**, 297-307.
- Naturvårdsverket (1999) 'Metodik för inventering av förorenade områden.'
- Scheinost AC, Rossberg A, Vantelon D, Xifra I, Kretzschmar R, Leuz AK, Funke H, Johnson A (2006) Quantitative antimony speciation in shooting-range sands by EXAFS spectroscopy. *Geo. Cosmo. Acta*, **70**,3299-3312.
- Tighe M, Lockwood P, Wilson S (2005) Adsorption of antimony (V) by floodplain soils, amorphous iron(III) hydroxide and humic acid. *J. Environ. Monit.* 1177 – 1185.

The Tri-State Mining Region USA: Twenty years of trace element research

G.M. Pierzynski^A, L. R. Baker^A, G.M. Hettiarachchi^A, K. G. Scheckel^B, V. Gudichuttu^A and R. Pannu^A

^ADepartment of Agronomy, Kansas State University, Manhattan, Kansas, USA, Email gmp@ksu.edu, lbaker@blinc.com, ganga@ksu.edu, vindhya@ksu.edu, rpannu@ksu.edu.

^BUnited States Environmental Protection Agency, Cincinnati, Ohio, USA, scheckel.kirk@epa.gov,

Abstract

Lead and Zn were mined extensively in the Tri-State Mining Region for over 100 years leaving a multitude of environmental issues. Large areas are void of vegetative cover, communities have been impacted by smelter emissions, and vast quantities of mine wastes and soils enriched with Pb, Zn, and Cd remain. Phytostabilization, soil amendments, and sub-aqueous disposal of mine wastes have been proposed as remediation approaches and the purpose of this paper is to present a synopsis of key research projects in support of such activities. Long-term viability of vegetative cover for phytostabilization is a significant concern and soil microbial ecology likely plays a role in the success or failure of this approach. Our results indicate that very high levels of compost (>200 Mg/ha) are needed to sustain soil microbial enzyme activities over time, which will facilitate long-term viability of vegetation. Phosphorus additions to soils induce the formation of Pb phosphate minerals, reducing bioavailability, and the source of P and time influence the reaction products. The conversion of metals to sulfide forms is slow under reducing conditions in the absence of added C. When in sulfides form, metal solubility and mobility should be greatly reduced.

Key Words

Zinc, cadmium, lead, mining, nonferrous, heavy metals.

Introduction

The Tri-State Mining Region is comprised of portions of the states of Kansas, Missouri, and Oklahoma in the central United States. Lead and Zn were mined extensively from the mid 1800's until the early 1950's, and smelted until the early 1970's. A variety of environmental issues remain including Pb-contaminated soils in a number of communities, highly contaminated abandoned smelter sites, large quantities of mine wastes, and thousands of hectares of land with little or no vegetation. A lack of vegetative cover can be attributed to poor soil chemical, physical, and biological properties that adversely impact the establishment or maintenance of vegetation. One particularly challenging aspect of phytostabilization is long-term viability of the vegetation. Soil amendments allow rapid growth of vegetation shortly after application, often in areas completely void of vegetation previously, but the vegetation slowly declines and can be completely gone within three to five years. Some investigators have proposed using very high application rates of organic amendments (>200 Mg/ha) to help establish a viable soil ecosystem. The use of P to reduce Pb bioavailability in contaminated soils has been proposed as an alternative to soil excavation and replacement. Early work has demonstrated the treatments are effective in reducing Pb uptake by rats with relative bioavailability (as compared to Pb acetate) reduced from 0.34 in unamended soil to 0.24 in the same soil amended with rock phosphate. What isn't known is the nature of the mineralogical changes in Pb induced by various P sources and how they may change over time. Subaqueous disposal of mine wastes involves collecting mine wastes present in large piles and placing it into mine voids. This removes the waste from the surface and fills numerous mine voids, facilitating remediation. Shallow groundwater is already negatively impacted by metals. It is assumed that metals in the mine wastes will be converted to insoluble sulfide forms in the anaerobic environment and, therefore, that subaqueous disposal will not increase existing groundwater concerns. The low C content of the mine wastes and aquifer may inhibit microbial activity and the conversion of metals to sulfide forms.

Materials and methods

Phytostabilization

Two field studies were established in areas highly contaminated in Pb and Zn. Of particular interest here are the contaminated control (C), low compost (LC, 45 Mg/ha), and high compost (HC, 269 Mg/ha) treatments. Soil samples were taken approximately one week after application to establish a Time 0 and switch grass (*Panicum virgatum*) was sown into each plot. Soil samples were again taken after 157, 371, 553, 729, and 1157 d. Soil enzyme activities (β -glucosidase, alkaline, and arylsulfatase) were assayed within 2 weeks after

sampling on field-moist samples and are reported on a dry-weight basis. Changes to soil microbial community were assessed at 729 DAT by measuring the soil phospholipid fatty acid (PLFA) content. Immediately after sampling/sieving, approximately 20 g of moist material was frozen at -20 °C and lyophilized. The total lipids were extracted from the lyophilized material, the total lipid extract was separated into PLFA and waste lipids using silicic acid chromatography, the fatty acids were cleaved from the glycerol backbone by KOH saponification, and the harvested fatty acids were methylated to form fatty acid methyl esters. The nomenclature used to designate the identified fatty acids is the total number of carbon atoms and number of double bonds, followed by the position of the double bond from the methyl end of the molecule.

Laboratory incubations of P-amended Pb-contaminated mine spoils

The material used in this study was collected from an abandoned Pb/Zn smelter. The material was brought to 25% gravimetric water content and placed into plastic containers. Phosphorus treatments (phosphate rock (PR) or phosphoric acid (PA)) were applied in a line across the container. Treatments were covered with additional moist contaminated soil, covered, incubated for 4 or 52 weeks, and air dried before impregnation two part (epoxy/hardener) resin. The hardened resin was then cut using a petrographic trim saw to expose the P treatments and surrounding waste material. At the Advanced Photon Source (APS), μ -XRF maps (typically 6000- by 3000- μ m) were collected for Pb near the point of P application. Pb points of interest (POI) were selected from each map to perform Pb μ -XANES (13,035 eV) to detect changes in Pb speciation as affected by P source, time, and distance from the point of application.

Subaqueous metal transformations of Pb- contaminated mine-spoils

Two contrasting metal-rich mine-spoil materials were used for this study. Oxygen-free feed solution was used to submerge these materials. Organic C (OC as sodium lactate) was added tri weekly to support growth of microorganisms. Samples with and without added OC were incubated for 1 and 2; 11 and 10 months in an anaerobic glove box chamber in a sequential manner. Solution samples were analyzed periodically for pH, Eh, and soluble constituents. Characterization and speciation of solids samples were performed using SEM-EDX analysis, μ -XRF maps and μ -XAS.

Results and discussion

Alkaline phosphatase activity is presented as a representative enzyme. Phosphatase activities were significantly increased by the HC treatment at all sample times (Figure 1) and remain relatively constant with time. PLFA analysis indicated that gram (+), gram (-), fungi, and total microbial biomass were significantly increased by compost additions at 729 d, but actinomycetes were unchanged relative to the control.

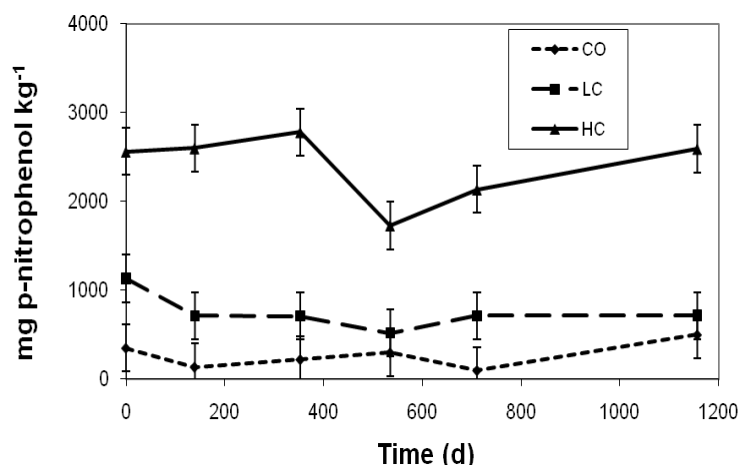


Figure 1. Alkaline phosphatase activity with time.

The Pb μ -XANES spectra for the untreated control samples indicated the presence of cerussite [PbCO₃], galena [PbS], anglesite [PbSO₄], magnetoplumbite [PbFe₆Mn₆O₁₉], and plumboferrite [Pb₂Mn_{0.2}Mg_{0.1}Fe_{10.6}O_{18.4}] as the dominant Pb mineral species, while no phosphate minerals were detected. Plumbogummite [PbAl₃(PO₄)₂(OH)₅·H₂O] was present at 4 wks for all P sources and at 52 weeks, plumbogummite and pyromorphite [Pb₅(PO₄)₃Cl] were found. Micro-XRD analysis was able to confirm results obtained with linear combination fitting.

In reaction vessels with no added OC, E_h remained stable around +250 mV (moderately reduced) from 42 to 98 d. Then within a week time (between 98 to 105 d) E_h dropped down to ~115 mV indicating a major shift in terminal electron acceptors. In contrast, in vessels with OC added E_h dropped down to ~+50 mV after 28 d, remained below 0 mV between 59 to 70 d, and then increased to +50 mV again by 112 d of submergence. The pH in these systems appeared to be unaffected by OC addition and stabilized around 7.6 after 42 d of submergence indicating that the level of OC in these systems are critical in determining redox changes. Recent SEM-EDXA analyses revealed more C in metal-rich particles examined (appeared more like precipitates in SE and BSE images) from systems in which OC was added (Figure 2). In contrast, S concentrations in metal rich particles were either non-detectable or very low. We suspect that high carbonate in these geological materials and microbial respiration might have caused increased levels of bicarbonate concentrations and the formation of metal carbonate solid solutions instead of sulfide solid solutions. This hypothesis will be verified using μ -XRF maps and μ -XAS at the APS in December 2009. Soluble Fe^{2+} concentrations in samples collected from systems with OC added were significantly higher ($23 \mu\text{mol/L}^1$) than the no-OC added samples ($\sim 4 \mu\text{mol/L}^1$) at 110 d of submergence. In contrast, soluble Mn, Pb and Zn concentrations in samples collected from systems with OC added were significantly lower than the no-OC added samples indicating major differences in transformation products of these two (with and without OC) systems.

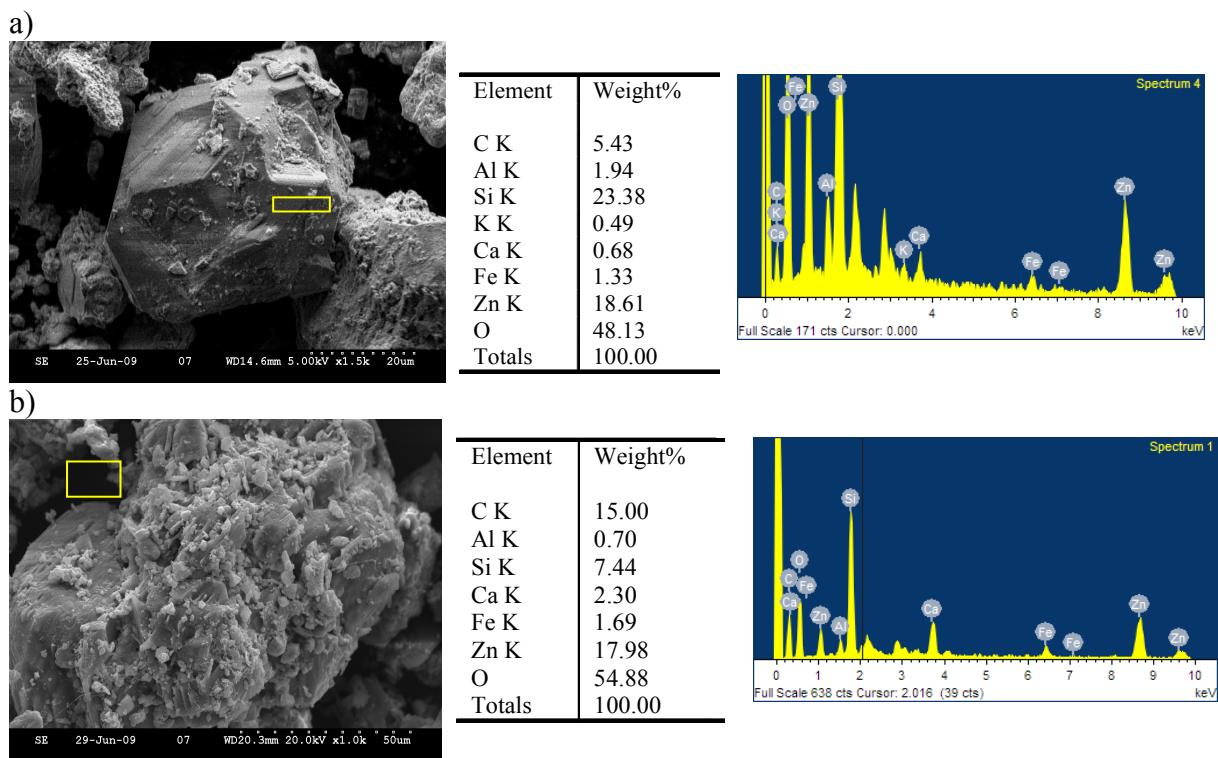


Figure 2. SEM image of original mine-spoil with a) no OC added and b) OC added, submerged for 110 days, and EDXA spectra of particle, located on the larger particle or separately, shown on the SEM image.

The use of calcium peroxide (CaO₂) as a process applied to arsenic contaminated soil around an abandoned tungsten mine, southern China

Liu Chuan-Ping, Wu Chang-An and Li Fang-Bai

Guangdong Open Lab of Environmental Science & Technology, Guangdong Institute of Eco-Environmental and Soil Sciences, Guangzhou 510650, China

Abstract

To reduce As bio-available and uptake in celery, calcium peroxide (CaO₂) was applied as a stabilizer. In the batch experiments, with the increase of CaO₂ concentration addition, the pH of soil was increased significantly. With the application of CaO₂ at low concentration, the bio-available As was decreased significantly, while application of high concentration of CaO₂ increased As mobility significantly. In the field experiments, CaO₂ application could alleviate As toxicity to celery. The biomass of celery shoots increased significantly while the As concentration in the shoots of celery decreased significantly with CaO₂ application. The optimal dose of CaO₂ application was 750 kg/ha, at this dosage, the celery had the highest biomass and lowest As concentration.

Key Words

Arsenic, calcium peroxide, stabilization, celery.

Introduction

Lianhuashan tungsten mine, one of largest tungsten mines in southern China, had a typical polymetallic sulfide ore, with the main components being wolframite, scheelite, arsenopyrite, pyrite, magnetite, chalcopyrite, quartz, sericite, chlorite and feldspar. Arsenic (As) was found to be the most important contaminant in the tailing and soil samples of the area (Liu *et al.* 2009). As is known to be a very toxic element and a carcinogen to humans (Duker *et al.* 2005; Williams *et al.* 2005). As (III) and As (V) are the most widespread forms in nature and As (III) was both more mobile and toxic (Boyle and Jonasson 1973; Pansar-Kallio and Manninen 1997). Stabilization is one of the most effective methods to reduce the mobility of heavy metals (Yukselen and Alpaslan 2001). Various combinations such as lime, type F fly ash, silica fumes, iron (II) or (III), silicates and blast furnace slag have been used in the treatment of soils contaminated with As (Akhter *et al.* 1997; Leist *et al.* 2000). Although the leaching of As is significantly reduced by lime application, such a soil treatment is rather disruptive and considered only when the material is disposed of at a landfill. In this research, calcium peroxide (CaO₂) was selected as a stabilizer to reduce As uptake and accumulation in celery.

Methods

In the batch experiments, the soil was collected from a paddy field contaminated with arsenic around Lianhuashan tungsten mine. Air dried soil was passed through a 2 mm diameter sieve, and then calcium peroxide (CaO₂) was added at the concentrations of 0, 10, 20, 50, 100 mmol/kg. After undergoing three cycles of saturation with deionized water and air-drying for 10 d, the pH of these soils was measured by 0.01 M CaCl₂ at a 1:5 ratio (w/v) using a pH meter and the bio-available As concentrations in these soils were measured by extraction with 0.1 M Na₂HPO₄ and 0.1 M NaH₂PO₄. The fields near Lianhuashan tungsten mine were selected for the field experiments. Arsenic concentration in the soil was 935.3 mg/kg and the pH of the soil was 5.71. To reduce As uptake, CaO₂ @ 375, 750 and 1500 kg/ha was applied 15 d before planting, and the soil without CaO₂ application was set as the control. After harvest, the biomass and As concentration were determined.

Results

As shown in Table 1, the pH of control soil was 5.59. With the increasing concentration of CaO₂ added in the soils, the pH increased significantly. At a concentration of 50 mmol/kg CaO₂, the pH of soil was neutral. Total As concentration in the soil was 837.2 mg/kg, but the bio-available As was only 16.5%. The bio-available As decreased significantly when 10, 20 mmol/kg CaO₂ was added in the soil (12.4, 13.1%, respectively). But when 100 mmol/kg CaO₂ were added to the soil, the bio-available As (25.8%) was significantly higher than that of control. In the presence of Ca under highly oxidizing and moderate pH conditions, calcium hydrogen arsenate (CaHAsO₄) and calcium arsenate (Ca₃(AsO₄)₂) can precipitate and

reduce the mobility of As (Porter *et al.* 2004). But under acid or alkaline pH conditions, As was more mobile (Magalhães 2002).

Table 1. Effects of different CaO₂ concentration treatments on the pH and bio-available As of the soils.

| Concentration of CaO ₂ treatments (mmol/kg) | pH | Percentage of bio-available As to total As (%) |
|--|-------|--|
| 0 | 5.59e | 16.5b |
| 10 | 5.88d | 12.4c |
| 20 | 6.19c | 13.1c |
| 50 | 7.12b | 18.3b |
| 100 | 8.39a | 25.8a |

The different letter in the same column indicates a significant difference at $P < 0.05$ according to Duncan's multiple rang test.

CaO₂ application could alleviate As toxicity to celery. The biomass of celery shoots increased significantly while the As concentration in the shoots of celery decreased significantly with CaO₂ application (Figure 1). In comparison with the control, the supply of 375, 750 or 1500 kg/ha CaO₂ lead to an increase in fresh weight of celery shoots of 25%, 56% or 22%, respectively while lead to a decrease in As concentration of celery shoots of 33%, 75% or 53%, respectively. The optimal dose of CaO₂ application was 750 kg/ha, at this dosage, the celery was found with highest biomass and lowest As concentration. CaO₂, a strong oxidizer, could oxidize As (III) into the less toxic As form of As(V) and reduce the mobility of As. Furthermore, the supply of Ca²⁺ could also inhibit As desorption. It has been reported that the amount of soluble As is lower in the presence of Ca when treated with ferric oxides (Dutre and Vandecasteele 1995).

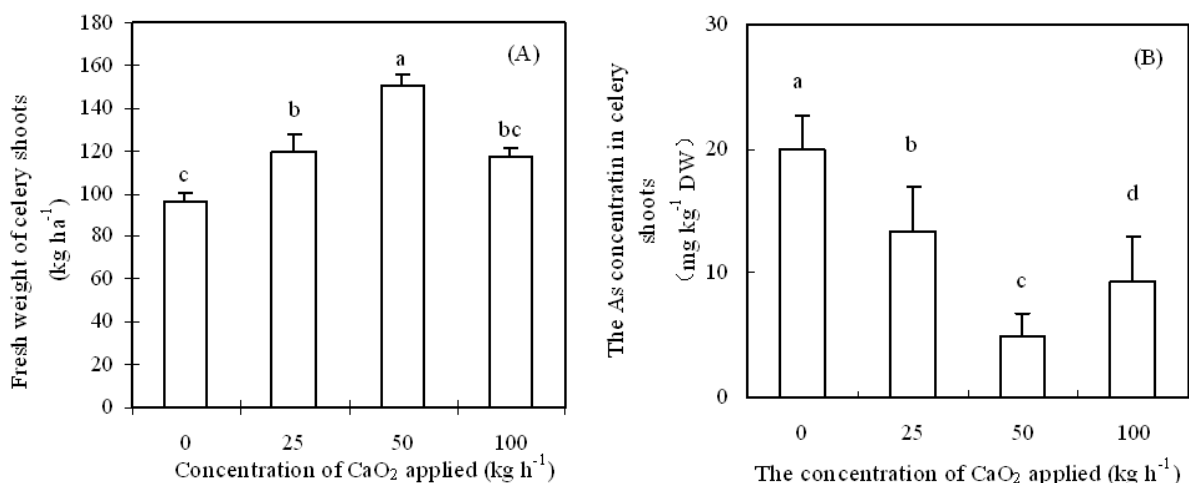


Figure 1. Effects of CaO₂ application on fresh weight (A) and As concentration (B) in celery shoots. (The different letter in the same column indicates a significant difference at $P < 0.05$ according to Duncan's multiple rang test).

Conclusion

In present research, CaO₂ was applied to farm lands and used to reduce As accumulated in celery. Results showed that CaO₂ application increased the pH of soil significantly and the bio-available As was reduced with the low CaO₂ concentration application (10, 20 mmol/kg). The bio-available As was increased significantly, for the high concentration of CaO₂ (100 mmol/kg). In the field experiments, CaO₂ application could alleviate As toxicity for celery. The biomass of celery shoots increased significantly while the As concentration in the shoots of celery decreased significantly with CaO₂ application. The optimal dose of CaO₂ application was 750 kg/ha, at this dosage, the celery had the highest biomass and lowest As concentration.

References

- Akhter H, Cartledge FK, Roy A, Tittlebaum ME (1997) Solidification/stabilization of arsenic salts: effects of long cure times. *Journal of Hazardous Materials* **52**, 247-264.
- Boyle RW, Jonasson JR (1973) The geochemistry of arsenic and its use as an indicator element in geochemical prospecting. *Journal of Geochemical Exploration* **2**, 251-296.

- Duker AA, Carranza EJM, Hale M (2005) Arsenic geochemistry and health. *Environment International* **31**, 631-641
- Dutre V, Vandecasteele C (1995) Solidification stabilization of hazardous arsenic containing waste from a copper refining process, *Journal of Hazardous Materials* **40**, 55-68.
- Leist M, Casey RJ, Caridi D (2000). The management of arsenic waste: problems and prospects. *Journal of Hazardous Materials* **76**, 125-138.
- Liu, CP, Luo CL, Gao Y, Li FB, Lin LW, Wu CA, Li XD (2010). Arsenic contamination and potential health risk implications at an abandoned tungsten mine, southern China, *Environmental Pollution* **158**, 820–826
- Magalhães MCF (2002) Arsenic. An environmental problem limited by solubility. *Pure and Applied Chemistry* **74**, 1843-1850.
- Pantsar-Kallio M, Manninen PKG (1997) Speciation of mobile arsenic in soil samples as a function of pH. *Science of Total Environment* **204**, 193-200.
- Porter SK, Scheckel KG, Impellitteri CA, Ryan JA (2004) Toxic metals in the environment: thermodynamic considerations for possible immobilisation strategies for Pb, Cd, As, and Hg. *Critical Reviews in Environmental Science and Technology* **34**, 495-604.
- Williams PN, Price AH, Raab A, Hossain SA, Feldmann J, Meharg AA (2005) Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. *Environmental Science & Technology* **39**, 5531-5540.
- Yukselen MA, Alpaslan BJ (2001) Leaching of metals from soil contaminated by mining activities. *Journal of Hazardous Materials* **87**, 289-300.

Threshold toxic limits of Cd for leafy vegetables raised on a mollisol amended with varying levels of farmyard manure

Deepali Joshi ^A, Prakash Chandra Srivastava ^B and Prashant Srivastava ^C

^ADepartment of Soil Science, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263145, India.

^BDepartment of Soil Science, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263145, India, Email pcsriv@yahoo.com

^CCooperative Research Centre for Contamination Assessment and Remediation of the Environment, University of South Australia, Mawson Lakes, SA 5095, Australia, Email prashant.srivastava@crccare.com

Abstract

Green house experiments were conducted during 2008-09 to evaluate the threshold toxic limits of Cd in leafy vegetables grown on a Mollisol receiving varying levels of farmyard manure (FYM). The treatments involved factorial combinations of three levels of FYM (0, 2.23 and 4.46 g/kg soil), seven levels of Cd (0, 5, 10, 25, 50, 100 and 150 mg Cd/kg soil) which were imposed on three plant species (amaranthus, Fenugreek and Buckwheat). Amaranthus was most susceptible to the toxicity of Cd as compared to fenugreek and buck wheat. The threshold toxic limits of Cd in plant tissue for 10 percent reduction in the relative yields of leafy vegetables at 0, 2.23 and 4.46 g FYM/kg soil were 7.3, 29.5 and 10.3 mg Cd/kg dry matter in amaranthus, 2.5, 3.6 and 4.9 mg Cd/kg dry matter in fenugreek and 6.0, 21.0, 15.3 mg Cd/kg dry matter in buckwheat, respectively. The threshold toxic limits of 0.1 N HCl extractable Cd in soil for 10 percent reduction in the relative yields of leafy vegetables at 0, 2.23 and 4.46 g FYM/kg soil levels were 1.4, 4.0 and 2.0 for amaranthus, 3.0, 6.7 and 20.5 mg Cd/kg soil for fenugreek and 2.8, 14.5 and 14.6 mg Cd/kg soil for buck wheat, respectively. The threshold toxic limits of 0.005 M DTPA (pH 7.3) extractable Cd in soil for 10 percent reduction in the relative yields of leafy vegetables at 0, 2.23 and 4.46 g FYM/kg soil levels were 1.3, 3.3 and 1.9 for amaranthus, 1.8, 3.4 and 9.0 mg Cd/kg soil for fenugreek and 1.6, 11.5 and 7.9 mg Cd/kg soil for buck wheat, respectively.

Key Words

Cadmium, farmyard manure, leafy vegetables, Mollisol, soil extractants, threshold toxic limits.

Introduction

Leafy vegetables are among the crop species that are most vulnerable to heavy metal pollution. Leafy vegetables accumulate higher amount of heavy metal like Cd due to their intense vegetative growth. Cadmium contaminated vegetables grown in wastewater irrigated soils may pose public health hazards. The increase in the accumulation of Cd in soils leads to increase in the uptake of metals, thereby, creating complex situation in soil-plant-animal-human system. The present investigation was carried out with the objective of evaluating the toxic concentrations of Cd for amaranthus (*Amaranthus spp.*), fenugreek (*Trigonalia foenum*) and buckwheat (*Crotalaria retusa*) grown in a mollisol receiving varying levels of farmyard manure.

Methods

Greenhouse experiments

Greenhouse experiments were conducted using a surface (0-15 cm) Mollisol having sandy loam texture, 7.05 pH and 0.128 dS/m electrical conductance in 1:2 soil water suspension, 11.1 g/kg organic C and 0.16 mg/kg DTPA extractable Cd/kg soil. The treatments imposed in triplicate to potted soil (2.5 kg/pot) were a factorial combination of three levels of FYM (0, 2.23 and 4.46 g/kg soil) and seven levels of Cd (0, 5, 10, 25, 50, 100 and 150 mg Cd/kg soil). The content of total Cd in FYM was 2.4 mg Cd/kg. Healthy Seeds of amaranthus (*Amaranthus spp.*), fenugreek (*Trigonalia foenum*) and buckwheat (*Crotalaria retusa*) were sown and raised near field capacity moisture regime. After 35 d growth, plants were harvested close to soil level and washed thoroughly in tap water, 0.1 N HCl and finally in distilled water. Plants were dried in an electric oven at 60°C and weighed to record dry matter yields. Percent relative dry matter yields were calculated for different treatments as:

Relative yield (%) = (Dry matter yield at a given level of Cd / Dry matter yield at 0 mg Cd/kgsoil) × 100

Soil extraction

After crop harvest, the soil samples under different treatments were extracted for 0.005 M DTPA (pH 7.3) extractable Cd (Korcak and Fanning, 1978) and 0.1M HCl method (Misra and Pande, 1974). Finely ground plant sample (1 g) was digested in di-acid (HNO₃: HClO₄, 3:1 v/v). Soil and plant extracts were analysed for Cd by atomic absorption spectrophotometry.

Critical toxic limits of Cd in mollisol and plants

A method proposed by Bingham *et al.* (1975) was employed to calculate the critical toxic concentration of Cd in soil and plants. Percent relative dry matter yields were plotted against extractable content of Cd in soil or content of Cd in plant tissues. Critical toxic limits of Cd in soil and plants were determined for 10 percent reduction in dry matter yield.

Results

Cadmium levels significantly influenced the dry matter yields of all the three tested leafy vegetables (Table 1). In comparison to control (0 mg Cd/kg soil) decreased dry matter yields were observed at 5 to 150 mg Cd/kg soil for amaranthus, at 50 to 150 mg Cd/kg soil for fenugreek and at 25 to 150 mg Cd/kg soil for buckwheat. In general, FYM application did not influence the dry matter yields of amaranthus and fenugreek however, in the case of buckwheat higher level of FYM slightly decreased the dry matter yield in comparison to no application of FYM. The interaction effect of Cd and FYM levels influenced the dry matter yield of only amaranthus significantly. In general, at 0 and 4.46 g FYM/kg soil all the levels of Cd (5 to 150 mg Cd./kg soil) caused a decrease in dry matter yield of amaranthus. However at 2.23 g FYM/kg soil levels, only Cd levels from 10 to 150 mg/kg soil decreased the dry matter yield of amaranthus, significantly in comparison to control.

Table 2. Effect of cadmium and FYM application on dry matter yield (g/pot) of leafy vegetables.

| Cd levels (mg/kg soil) | FYM levels (g/kg soil) | | | Mean |
|---------------------------|------------------------|-----------|-----------------|------|
| | 0 | 2.23 | 4.46 | |
| A. Amaranthus | | | | |
| 0 | 9.49 | 6.07 | 6.38 | 7.31 |
| 5 | 4.07 | 5.62 | 4.12 | 4.60 |
| 10 | 5.65 | 3.03 | 2.74 | 3.81 |
| 25 | 2.06 | 2.69 | 2.61 | 2.45 |
| 50 | 1.20 | 1.08 | 1.46 | 1.25 |
| 100 | 0.55 | 0.70 | 0.41 | 0.55 |
| 150 | 0.29 | 0.30 | 0.21 | 0.26 |
| Mean | 3.33 | 2.78 | 2.56 | 2.89 |
| Effect | FYM levels | Cd levels | FYM × Cd levels | |
| CD (P≤0.05) | NS | 1.00 | 1.73 | |
| B. Fenugreek | | | | |
| 0 | 1.28 | 1.18 | 0.91 | 1.12 |
| 5 | 1.11 | 1.15 | 1.26 | 1.17 |
| 10 | 1.09 | 0.99 | 0.97 | 1.02 |
| 25 | 1.04 | 0.87 | 1.35 | 1.08 |
| 50 | 0.96 | 0.71 | 0.63 | 0.76 |
| 100 | 0.63 | 0.69 | 0.46 | 0.59 |
| 150 | 0.47 | 0.42 | 0.84 | 0.88 |
| Mean | 0.94 | 0.86 | 0.84 | 0.88 |
| Effect | FYM levels | Cd levels | FYM × Cd levels | |
| CD (P≤0.05) | NS | 0.20 | NS | |
| C. Buckwheat | | | | |
| 0 | 2.70 | 2.30 | 2.10 | 2.37 |
| 5 | 2.30 | 2.83 | 1.94 | 2.35 |
| 10 | 2.20 | 2.25 | 1.95 | 2.13 |
| 25 | 1.90 | 2.10 | 1.50 | 1.83 |
| 50 | 1.60 | 1.53 | 1.33 | 1.48 |
| 100 | 1.13 | 1.35 | 1.10 | 1.19 |
| 150 | 0.93 | 0.75 | 0.66 | 0.78 |
| Mean | 1.82 | 1.87 | 1.51 | 1.78 |
| Effect | FYM levels | Cd levels | FYM × Cd levels | |
| CD (P≤0.05) | 0.23 | 0.36 | NS | |

As shown in Figure 1, addition of increasing levels of Cd significantly increased the concentration of Cd in leafy vegetables, the magnitude of increase was highest in amaranthus followed by buckwheat and fenugreek. As regards the main effects of FYM levels, application of 4.46 g FYM/kg soil increased the Cd concentration significantly only in amaranthus over no application of FYM (0 g FYM/kg soil). The interaction effect of Cd and FYM levels also influenced the concentration of Cd in leafy vegetables significantly.

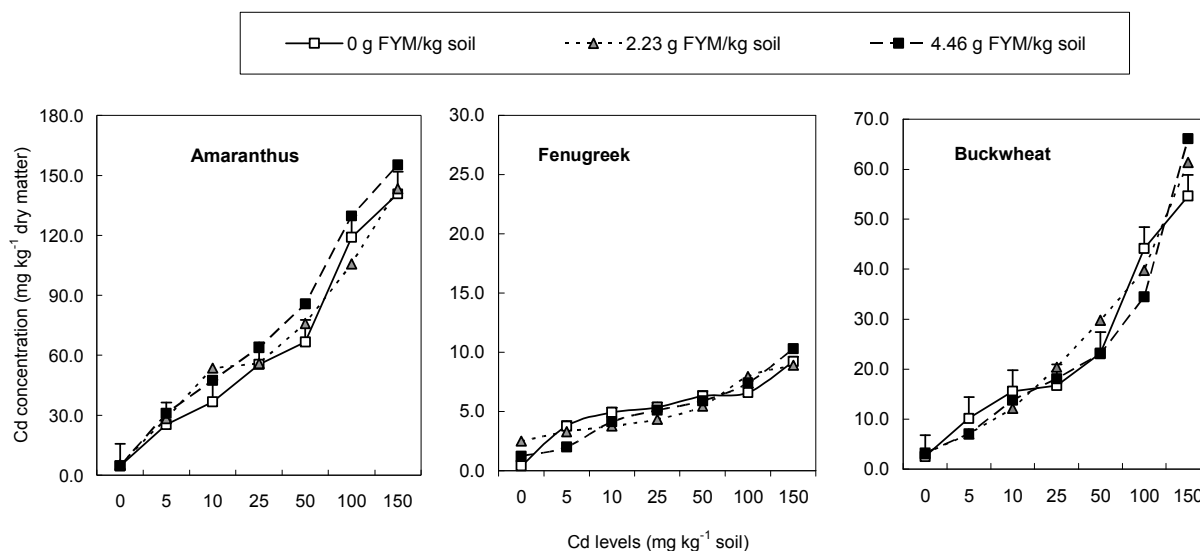


Figure 1. Effect of different levels of Cd on Cd concentrations (mg/kg dry matter) in leafy vegetables raised on a mollisol fertilized with varying levels of FYM. The vertical bars indicate critical difference at $P \leq 0.05$.

The threshold toxic limits of Cd for 10 percent reduction in the yields of leafy vegetables at 0, 2.23 and 4.46 g FYM/kg soil were 7.3, 29.5 and 10.3 mg Cd/kg dry matter in amaranthus, 2.5, 3.6 and 4.9 mg Cd/kg dry matter in fenugreek, and 6.0, 21.0, 15.3 mg Cd/kg dry matter in buckwheat, respectively (Table 2), respectively. In general, application of FYM led to relatively higher threshold toxic limits of Cd in plant tissue in comparison to no application of FYM. The effect could be ascribed to the fact that the magnitude of reduction in dry matter due to Cd levels was lower under FYM treatments than under no application of FYM.

Table 3. Threshold limits of Cd in plants for 10 percent reduction in relative yields.

| FYM level (g/kg soil) | Cd (mg/kg dry matter) | | |
|-----------------------|-----------------------|-----------|-----------|
| | Amaranthus | Fenugreek | Buckwheat |
| 0 | 7.3 | 2.5 | 6.0 |
| 2.23 | 29.5 | 3.6 | 21.0 |
| 4.46 | 10.3 | 4.9 | 15.3 |

The threshold toxic limits of 0.1 N HCl extractable Cd in soil for 10 percent reduction in the relative yields of leafy vegetables at 0, 2.23 and 4.46 g FYM/kg soil levels were 1.4, 4.0 and 2.0 for amaranthus, 3.0, 6.7 and 20.5 mg Cd/kg soil for fenugreek, and 2.8, 14.5 and 14.6 mg Cd/kg soil for buck wheat, respectively (Table 3). The threshold toxic limits of 0.005 M DTPA (pH 7.3) extractable Cd in soil for 10 percent reduction in the relative yields of leafy vegetables at 0, 2.23 and 4.46 g FYM/kg soil levels were 1.3, 3.3 and 1.9 for amaranthus, 1.8, 3.4 and 9.0 mg Cd/kg soil for fenugreek, and 1.6, 11.5 and 7.9 mg Cd/kg soil for buck wheat, respectively.

Table 4. Threshold limits of extractable Cd in soil for 10 percent reduction in relative yields.

| Soil Extractant | FYM level (g/kg soil) | Cd (mg/kg soil) | | |
|-----------------------|-----------------------|-----------------|-----------|-----------|
| | | Amaranthus | Fenugreek | Buckwheat |
| 0.1 N HCl | 0 | 1.4 | 3.0 | 2.8 |
| | 2.23 | 4.0 | 6.7 | 14.5 |
| | 4.46 | 2.0 | 20.5 | 14.6 |
| 0.005 M DTPA (pH 7.3) | 0 | 1.3 | 1.8 | 1.6 |
| | 2.23 | 3.3 | 3.4 | 11.5 |
| | 4.46 | 1.9 | 9.0 | 7.9 |

Both, DTPA (pH 7.3) and 0.1 N HCl extractable Cd had significant ($p \leq 0.01$) positive correlation with Cd concentration in plant tissue of all the three leafy vegetables (Table 4). For amaranthus and buckwheat, 0.1 N HCl had higher r values with Cd concentration in the plant tissue as compared to DTPA extractable Cd. However, in the case of fenugreek slightly higher value was obtained with DTPA (pH 7.3) extractant.

Table 5. Relationship between extractable soil Cd and concentration and uptake of Cd by different leafy vegetables.

| Crop | Soil Extractant | Cd concentration | Cd uptake |
|----------------|-----------------|------------------|-----------|
| Amaranthus spp | DTPA | 0.916** | 0.317 |
| | 0.1 N HCl | 0.957** | 0.472* |
| Fenugreek | DTPA | 0.928** | 0.375 |
| | 0.1 N HCl | 0.923** | 0.210 |
| Buckwheat | DTPA | 0.922** | 0.833** |
| | 0.1 N HCl | 0.963** | 0.820** |

**significant at $P \leq 0.01$; *significant at $P \leq 0.05$

The uptake of Cd by amaranthus and buckwheat showed a significant positive correlation with 0.1 N HCl extractable Cd. The uptake of Cd by buckwheat also showed a significant positive correlation with DTPA (pH 7.3) extractable Cd.

Conclusion

Increasing levels of Cd significantly reduced the dry matter production of leafy vegetables. Among leafy vegetables, the concentration of Cd was the highest in amaranthus followed by buckwheat and fenugreek. Application of FYM increased the threshold toxic limits of Cd in the tissues of leafy vegetables and also in terms of 0.1 N HCl or 0.005 M DTPA (pH = 7.3) extractable Cd in Mollisols.

References

- Bingham FT, Page AL, Mahler RJ, Ganje TJ (1975) Growth and cadmium accumulation of plants grown on a soil treated with a cadmium enriched sewage sludge. *Journal of Environmental Quality* **4**, 207-211.
- Korcak RF, Fanning DS (1978) Extractability of cadmium, copper, nickel and zinc by double acid versus DTPA and plant content at excessive soil levels. *Journal of Environmental Quality* **7**, 506-512.
- Misra SG, Pande P (1974) Evaluation of suitable extractant for available nickel in soils. *Plant and Soil* **41**, 697-700.