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Acidification and trace metal mobility in soil and shallow groundwater on the Gnangara Mound, Western Australia

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Abstract
Soil and shallow groundwater on the Gnangara Mound, Western Australia, is acidifying partially as a result of acid deposition from air pollution onto the soil. Sampling in the upper part of the saturated zone revealed a reduced pH between 3.8 and 5 at 8 of 16 sites sampled. Depth profiles show a sharp acidification front, with a higher pH of 6 below the interface. As a consequence, aluminium and trace elements (As, Cd, Pb, Cu, Zn and Ni) were released at low pH values. In the acidified zone, the Al concentration reaches 8 mg/L. The sources and controlling mechanisms behind the observed geochemistry are explored by reactive transport modelling incorporating ion exchange, surface complexation and Al-hydroxide equilibrium. These simulations estimate that the progression of the acidification front has taken place over 100 years at an average rate of 5 cm/year. Al-hydroxide dissolution and ion exchange involving Al with adsorbed Ca and Mg attenuates the progression of the front. Low pH releases trace metals from surface complexation, which accumulate by re-adsorption at the front due to the buffering mechanisms at this interface. Acidification and trace metal accumulation potentially causes severe impacts in a number of groundwater-dependent wetlands on the Gnangara Mound.

Key Words
Acidification, trace metals, geochemistry, groundwater, reactive transport modelling, air pollution

Introduction
In Western Australia, acidification has become a prominent problem in coastal areas due to unsustainable management practices, drought conditions, partially due to regional climate change, and increased industrial activity (Appleyard and Cook 2008). Investigations have focused on mechanisms related to oxidation of high contents of sulfidic material often associated with peaty coastal wetland environments. These are exposed to oxidizing conditions due to water table decline as a result of reduced rainfall and increased groundwater abstraction. However, rainfall and dry deposition may also be a source of acid especially in areas of concentrated industrial activity and high traffic density. Atmospheric emissions of anthropogenic gaseous sulphur and nitrogen oxides may have a powerful acidifying potential. The problems associated with acidification are well detailed in the literature, causing: inhibited plant growth; infrastructure corrosion and; large fish kills (White *et al.* 1997) as well as adversely impacting large habitat areas such as wetlands and woodlands (Xu 2008; van Tol *et al.* 1998). Acidification may also impact the geochemical equilibrium in soils and aquifers (Hansen and Postma 1995; Kjøller *et al.* 2004; Appleyard and Cook 2008). Deposition of acid rain may result in the gradual acidification of sediment profiles when they have a low buffering capacity. Aquifers with low alkalinity levels are particularly prone to acidification (Edmunds and Kinniburgh 1986), particularly in areas where dewatering is taking place (Swedish EPA 2002). Once sediments are acidified, increased mobility of Al as well as toxic trace elements are likely to occur (Appelo and Postma 2005). These processes are a concern in the poorly buffered soils of the Perth region where according to Appleyard and Cook (2008) an acidification front is believed to have developed in the aquifer since the beginning of the 20th century as a consequence of the increased use of fossil fuels as well as higher traffic densities. The study site, the Gnangara Mound (**Error! Reference source not found.**) located on the Swan Coastal Plain, is underlain by sediments of the Quaternary age as well as Bassendean sands - soils that are known to have a limited capacity for neutralising acid (Bawden 1991; Appleyard and Cook 2008). The limited content of carbonates and the progressive leaching of base cations, such as Ca and Mg, characteristic of these sediments, render these soils prone to acidification. Further to this, the release of adsorbed trace elements under low pH conditions could cause concentrations to reach toxic levels, contaminating groundwater resources and connected surface water bodies. The aim of this paper is to investigate the effects of atmospheric acid deposition on sandy aquifers. In particular the rate of acidification and the mobility of trace metals.

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Methods

Installation of wells, the sampling and the chemical analysis is described in (Appleyard and Cook 2008). The Geochemical code PHREEQC (Parkhurst and Appelo 1999) was utilised for speciation calculations; for analysing the effect on trace metal mobility in batch titrations; and finally for 1D reactive transport simulations of the downward migration of the acidification front and the associated mobility of trace metals.

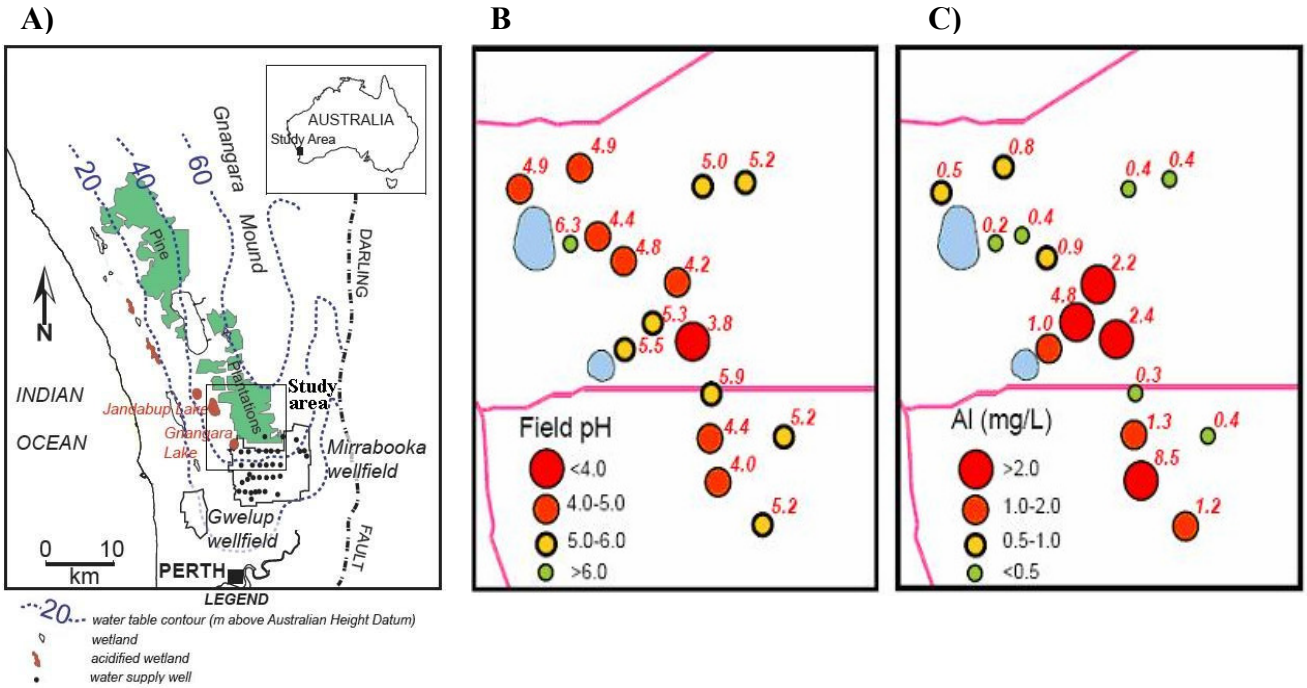

Figure 1. A) Location of the study area on the Gnangara Mound showing the position of water supply bores and pine plantations (green shading). Wetlands that have become periodically acidic are also depicted in red and distribution of acidity indicators at the water table near Lake Jandabup B) pH and C) dissolved Al (Appleyard and Cook 2008).

Results and Discussion

Water chemistry, acidification and buffering mechanisms

Shallow groundwater data, sampled in June 2008, reveals low pH levels ranging from 4-6 in the leached sands at the top of the Gnangara Mound aquifer (Figure 1B). The observed pH depth profiles indicate that the shallow groundwater is acidified to a depth of between 4 and 10 metres. Below this an abrupt increase in pH is delineating the acidification front (Hansen and Postma 1995; Kjøller *et al.* 2004; Appelo and Postma 2005) where geochemical processes cause a buffering of the low pH water. Marine derived salts appear to determine the major ion water chemistry at most sites as reflected by high Na and Cl levels constituting the highest proportion of the chemical composition. Sulphate is measured at relatively high values reaching a maximum of 75 mg/L at site 2. The high mass SO_4^{2-}/Cl ratio of 3 indicates a source of sulphur in addition to marine salts. This can be explained by a number of inputs such as pyrite oxidation, air pollution or other sources. Although nano-crystalline pyrite has been identified in illuvial horizons in podosol soil profiles on the Gnangara Mound (Prakongkep *et al.* 2009), preliminary calculations suggest that there may be insufficient pyrite to account for observed levels of acidification (Ward 2009). In the acidified zone Al concentrations are quite high, whereby data at site 2 reveal it to be the most abundant cation at 21% of the total composition, reaching a level of 8 mg/L (Figure 1C) - 40 times the permissible level for Al in drinking water. Detailed mineralogy suggests that the sources of the soluble Al are the dissociation of allophane-organic complexes in illuvial horizons (Prakongkep *et al.* 2009) and the dissociation of Al-oxyhydroxide minerals according to (1). Deeper in the profile the Al concentrations decline across the acidification front corresponding to a rise in pH as illustrated in Figure 2. Speciation calculations suggest that secondary mineral equilibria with Al-containing minerals, such as gibbsite or jurbanite are controlling the pH and Al distributions. In addition, ion exchange processes involving Al, Ca and Mg (2) at the acidification front causes a reduction in Al resulting in a further rise in the pH via the coupling of equations (1) and (2).

$$Al(OH)_3 + 3H^+ \Leftrightarrow Al^{3+} + 3H_2O \quad (1)$$

$$\tfrac{1}{3}Al^{3+} + \tfrac{1}{2}Ca - X_2 \Leftrightarrow \tfrac{1}{3}Al - X_3 + \tfrac{1}{2}Ca^{2+} \quad (2)$$

The concentration of dissolved trace elements (Pb, Ni, Fe, Zn, Cu, Cd and As) appears to be elevated in the acidified zone, although limited data below the acidified zone makes it difficult to assess the background levels. High trace metal concentrations were measured at a site where the acidification front is particularly distinct: Cu 65; Pb 12; Ni 26 and Zn 82 (all in µg/L). It appears that the trace elements, leached under low pH conditions, are accumulating at the acidification front due to the increased pH in what has been termed a geochemical trap (Kjøller *et al.* 2004).

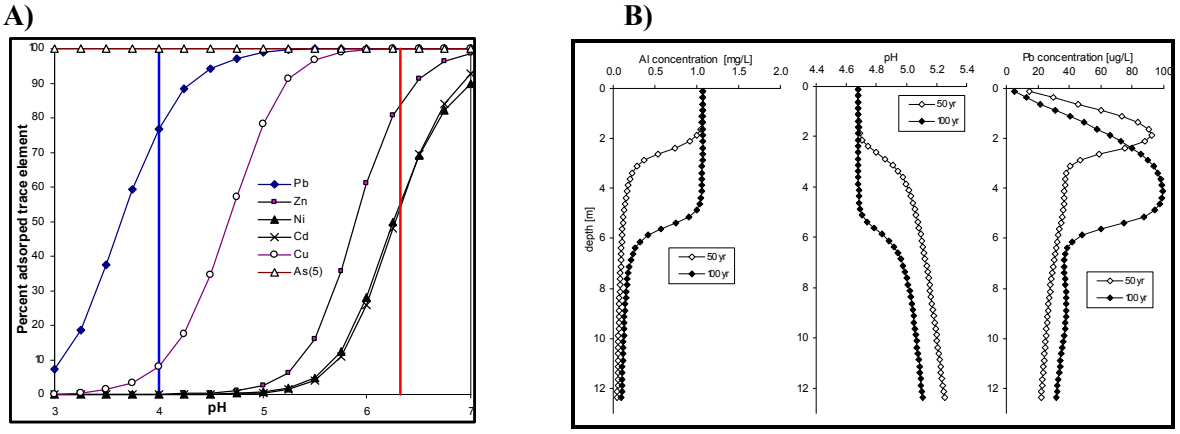

Figure 2. Results of the PHREEQC modelling. A) Simulated desorption of trace elements (Pb, Ni, Zn, Cu, Cd and As) in a batch titration of a neutral (pH = 6.32, red line) groundwater sample in equilibrium with a hydrous-ferric-oxide (hfo) surface (Dzombak and Morel 1990). B) Depth profiles of dissolved Al, pH and dissolved Pb from a 1D reactive transport model combining ion exchange, mineral equilibria (Gibbsite) and surface complexation.

Reactive transport modeling of acidification and mobilization of Al and trace elements

Simulations designed to explain the observed geochemistry at the Gnangara Mound were done in PHREEQC. Surface complexation and trace metal mobilization was investigated by equilibrating a neutral (pH = 6.32) sample with a hydrous-ferric-oxide (hfo) surface assuming the default surface oxide parameters given in PHREEQC (Dzombak and Morel 1990). Subsequently, this system is titrated from a pH of 6.32 to 3, replicating the change in pH due to acidification. This clearly illustrates almost total mobilization for Ni, Cd, Zn and Cu and significant increased mobility for Pb (Figure 2A). Arsenic (As(5)) on the contrary (assuming oxic conditions) remains completely adsorbed at these low pH values. However, Figure 2A also predicts that mobilized metals transported to the acidification front where pH increases would re-adsorb in this zone. Thus an accumulation of metals should happen with locally potentially high dissolved concentrations (Kjøller *et al.* 2004). To further investigate the progression of the acidification front into the aquifer and reactive transport of trace metals, a 1D transport model was produced. The simulations were designed to replicate the conditions on the Gnangara Mound incorporated ion exchange, mineral equilibria with Gibbsite ($Al(OH)_3$) and surface complexation. The vertical component of the groundwater flow was estimated to be 1 m/y (Appleyard and Cook 2008). Polluted precipitation with a pH of 4.3 infiltrated a model column extending to 12.5 m below the surface. Initial chemical conditions in the column were derived from uncontaminated (pH ~ 6) water below the acidification front. The sediment cation exchange capacity (CEC) was set to 0.8 meq/kg (Cook *et al.* 2006). Gibbsite equilibrium was achieved with a saturation index of 1.1 obtained from speciation calculations using PHREEQC. The model overall replicates the acidification front and the observed pH (Figure 2B). The results predict the acidification front progresses down into the aquifer at a rate of approximately 5 cm/y taking between 50 and 100 years to reach its current location. However, this estimate is highly uncertain, site specific and variable depending on the local pore water velocity, buffering capacity and acid load; parameters that are not well constrained at the site. Furthermore only the modelled pH can be compared with field data since depth specific profiles of Al and trace metals do not exist. Despite this, the modelling results demonstrate, at least conceptually, the expected behaviour of trace elements as exemplified by Pb in Figure 2B where Pb is released in the acidified zone and accumulated at the acidification front due to the increase in pH and re-adsorption.

Conclusions

Groundwater acidification at the Gnangara Mound, WA, appears to be caused by a combination of factors including pyrite oxidation, the dissociation of allophane-organic complexes and the leaching of stored acidity from historical air pollution. Modelling suggests that the pH is controlled by mineral equilibrium with unspecified Al-hydroxides whereas the progression of the acidification front is impeded by a combination of Al-hydroxide equilibrium and cation exchange involving Al with base cations (Ca and Mg). The data and the modelling indicates that the acidification causes desorption of trace elements under acidic conditions which are then accumulated at the front due to the buffering and increase in pH. The acidification and elevated trace metals not only threaten fragile eco-systems that exist on the Gnangara Mound such as wetland, woodland and lake systems, but also potential water resources for human consumption.

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Arsenic hyperaccummulation by ferns: A field study in northern NSW

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Abstract
Historical applications of arsenic-based pesticides to control cattle ticks has resulted in large expanses of As contaminated dip sites across Australia. A field experiment was conducted to evaluate the extraction of As using As hyperaccumulating ferns, *Pityrogramma calomelanos* (L.) Link var. *austroamericana* (Domin) Farw. (Gold dust fern) and *Pteris vittata* L. (Chinese brake fern), at a disused As contaminated cattle dip site at Wollongbar, in northern New South Wales (NSW), Australia. Arsenic concentrations in the fronds of *Pityrogramma calomelanos* var. *austroamericana* and *Pteris vittata* were 1262–3941 mg/kg and 775–2569 mg/kg dry weight (DW), respectively. Our results showed that both ferns successfully accumulated As under field conditions, however, As removal rate and bioaccumulation factor was higher in Gold dust fern (3–5) than in Chinese brake fern (1–3).

Key Words
Hyperaccumulation, cattle dip sites, phytoremediation, phosphate extractable, contamination

Introduction

Arsenic has been classified as a toxic and carcinogenic metalloid which exists in the environment in both organic and inorganic forms. The inorganic form of As is supposed to be more common in soils and found in two main oxidation states, arsenate (As^{V}) and arsenite (As^{III}), the later being more toxic and available than As^{V} (Masscheleyn *et al.* 1991). Both natural processes and anthropogenic activities are responsible for the release of As in soil and water bodies. Mining, smelting, use of pesticides and herbicides in agriculture, wood treatment with CCA and tanning operations are some of the major human activities causing As contamination in soil (Smith *et al.* 1998).

Arsenic-based pesticides have been widely used at the cattle dip sites throughout the world including Australia. From the early 1900s to 1955, prior to the introduction of dichlorodiphenyltrichloroethane (DDT), more than 1600 dips were constructed in northern NSW Australia, where arsenicals were used as the dipping solution to control the cattle ticks (Smith *et al.* 1998). Soil As contamination around such sites needs attention due to the presence of toxic and bioavailable forms of As.

Remediation of the contaminated soils includes the excavation, capping, chemical immobilization and phytoremediation or phytoextraction (Gonzaga *et al.* 2006). Phytoextraction of the As contaminated soils, using ferns as hyperaccumulators, has emerged as an effective remediation strategy, which provides a cost-effective and environmental friendly option as compared to other remediation methods (Kertulis-Tartar *et al.* 2006). The bioaccumulation factor (BF; ratio of the As concentration in fronds to total As in soil) and translocation factor (TF; ratio of As concentration in fronds to total As in roots) determine the capability and efficiency of the plants to remove the As from soil (Ma *et al.* 2001; Gonzaga *et al.* 2006).

Pteris vittata L. (Chinese brake fern) is a well known As hyperaccumulator that can survive in soil with As concentrations of up to 1500 mg/kg and accumulate As in fronds > 3000 mg/kg DW (Tu and Ma 2002; Kertulis-Tartar *et al.* 2006). Other *Pteris* and non-*Pteris* ferns (e.g. *Pteris longifolia*, *Pteris cretica* and *Pityrogramma calomelanos*) have also been identified to accumulate As (Francesconi *et al.* 2002; Wei *et al.* 2007). Recently, Kachenko *et al.* (2007) found that *P. calomelanos* var. *austroamericana* (Domin) Farw. (Pteridaceae) can accumulate As in fronds up to 16 415 mg/kg DW in fronds. To our knowledge, there has been no research on the phytoremediation capacity of Gold dust fern under field conditions. The study aims to (1) determine the capability of *P. calomelanos* var. *austroamericana* and *P. vittata* for As hyperaccummulation in the field (2) compare BFs of *P. calomelanos* var. *austroamericana* and *P. vittata* under field conditions.

Methods
Two fern species, *P. vittata* and *P. calomelanos* var. *austroamericana* were selected for the field experiment. *Pteris vittata* was obtained from the Randwick City Council Nursery, NSW while *P. calomelanos* var. *austroamericana* was propagated from spores under controlled glasshouse conditions. After eight months of growth, uniform ferns were ready for transplanting in the field.

The field site was located at the Environmental Centre of Excellence, Wollongbar in northern NSW, Australia. It is a disused cattle dip site, where As-based pesticides were used to control the cattle ticks (DIPMAC 1992). The soil around the dip site was contaminated with variable and high concentration of As from the dipping process and disposal of waste material from the dip.

The study area was selected on the basis of preliminary soil sampling at 0–10 cm depth for total soil As distribution in the area. The selected area was sprayed with Roundup® weed-killer and cleared mechanically prior to planting. In January 2009, fern species were transplanted into hand-excavated holes in two separate plots of 3.15 m^2 size each, keeping plant to plant distance of 30 cm to give 42 ferns per plot. The plants were watered twice a day using drip irrigation system. Black nylon weed mat was used to minimise the weed growth and a shade cloth was erected over the area to protect ferns from direct sun light exposure.

An intensive soil sampling was done in June 2009, to collect the soil samples at 0–20 cm, 20–40 cm and 40–60 cm depths using a hand-driven soil corer. After five months period, the ferns were harvested at the fresh fronds tip, thoroughly washed and dried in a fan-forced oven at 70 ºC for 48–72 h until a constant weight was obtained.

Soil samples were air dried and ground to obtain < 2 mm fraction which was used to determine the various soil properties as given in Table 1. Sub-samples of soil (< 200 µm) were used to determine the total soil As concentration. Soil samples were digested with a mixture of hydrofluoric and other mineral acids at 125 ºC and diluted with HCl and E-pure water (Huang and Fujii 1996). Fern samples were digested in a mixture (1:1) of nitric and perchloric acids at 120–180 ºC and diluted with E-pure water (Miller 1998). The digests were analysed for As using a Varian Vista AX CCD inductively coupled plasma atomic emission spectrometer.

Potassium dihydrogen phosphate solution (0.5 M KH_2PO_4) was used to extract the specifically sorbed (bioavailable) As pool in soil with a 1:25 soil to solution ratio and 4 h shaking time (Alam *et al.* 2007). The As concentration in the phosphate extracts was analysed using a Varian hydride-generation atomic absorption spectrometer.

Table 1. Properties of soil at the Wollongbar experimental site.

pH (1:5 $CaCl_2$)	EC (1:5) (dS/m)	CEC ($mmol_c/kg$)	Total carbon (%)	DCB Fe (g/kg)	DCB Al (g/kg)	Sand (%)	Silt (%)	Clay (%)
4.82	0.11	87.5	4.5	158.9	13	16	40	44

Results
During the course of the experiment, all the ferns survived without symptoms of As phytoxicity. In both species As concentration in fronds increased with the increasing levels of soil As, which indicated the capability of Chinese brake fern and Gold dust fern to grow and tolerate in soils with the high levels (313–1903 mg/kg) of As. The total As concentration in soil was in the range of 393 to 1903 mg/kg for the plot where Chinese brake fern was grown, while it varied between 313 and 1486 mg/kg for Gold dust fern (Table 2). Arsenic concentrations in fronds of the Chinese brake fern and Gold dust fern were in the range of 775–2569 mg/kg DW and 1262–3941 mg/kg DW, respectively (Table 2). Bioaccumulation factor (based on total soil As) for Chinese brake fern and Gold dust fern were between 1–3 and 3–5, respectively. Arsenic concentration in fronds as a function of total and extractable soil As illustrated higher accumulation in Gold dust fern as compared to Chinese brake fern (Figure 1). These results are consistent with the previous studies on Chinese brake fern grown under field or controlled conditions (Zhao *et al.* 2002; Wei and Chen 2006). The higher concentration of As and BF in Gold dust fern suggest that this species is more efficient in As extraction under field conditions. Our results are opposite to the earlier studies that have examined As

accumulation in these two species under glasshouse conditions. Kachenko *et al*. (2007) observed a higher As accumulation capacity for the Chinese brake fern with no toxicity symptoms at 100–500 mg/kg applied As, while Gold dust fern showed toxicity with As concentrations in fronds >3008 mg/kg DW. Similarly, Xu *et al*. (2009) compared the hyperaccumulation potential of the two fern species in four different soils in a pot experiment, and observed higher As concentrations for Chinese brake fern than the Gold dust fern. At the time of sampling growth of Gold dust fern was better than the Chinese brake fern. To compare the total biomass of both species, one average plant from each of species was harvested above soil. The total biomass of the Gold dust fern (80.04 g DW) was almost two times greater than the Chinese brake fern (39.49 g DW).

Table 2. Total and phosphate extractable As concentration in soil and total As concentration in fronds of Chinese brake fern and Gold dust fern.

Fern species	Total soil As (mg/kg)	Phosphate extractable As (mg/kg)			As in fronds (mg/kg)
		0–20 cm	20–40 cm	40–60 cm	
Gold dust fern					
Range	313–1486	21–117	29–104	13–53	1262–3941
Mean	753	51	54	28	2617
Median	734	45	52	25	2589
SD (±)	284	23	19	11	764
Chinese brake fern					
Range	393–1903	25–90	23–105	12–141	775–2569
Mean	909	51	53	42	1501
Median	878	52	45	34	1339
SD (±)	354	17	21	31	500

Wei and Chen (2006) observed that the As accumulation rate and BFs of the Chinese brake fern were relatively less than the Cretan brake fern (*Pteris cretica*) on a contaminated mining site, suggesting that As accumulation by Chinese brake fern in the field depends on the various soil properties. In this study, it is not immediately evident why As concentration in the Gold dust fern was higher than the Chinese brake fern, however, we cannot exclude the role of soil properties and field conditions (as compared to glasshouse) affecting the growth behaviour of ferns (Wei and Chen 2006; Xu *et al.* 2009).

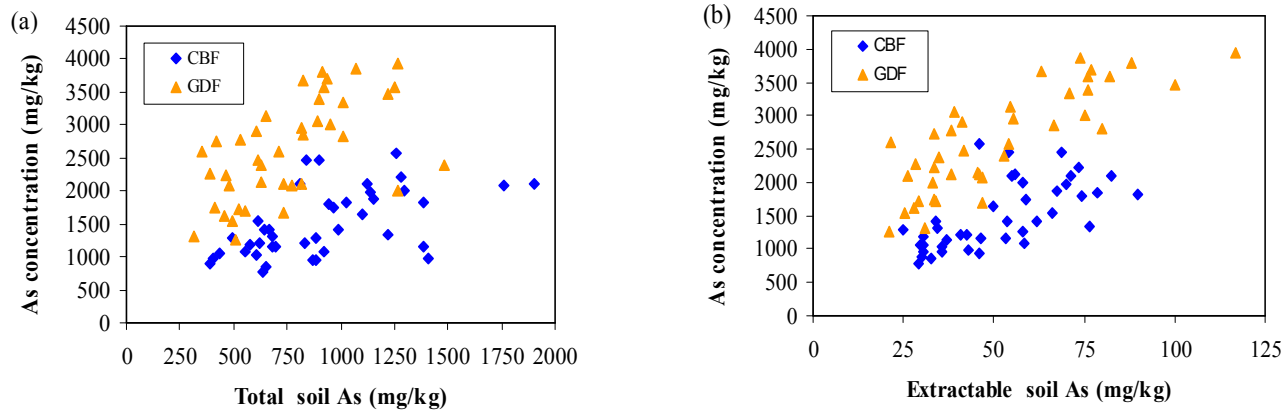

Figure 1. Total As concentration in Chinese brake fern (CBF) and Gold dust fern (GDF) as a function of (a) total soil As and (b) phosphate extractable soil As at 0–20 cm depth.

Arsenic concentration in the Gold dust fronds was better correlated with the total soil As ($r^2 = 0.59$) than the Chinese brake fern ($r^2 = 0.55$). Furthermore, there was a stronger relationship between the As concentration in the Gold dust fern and phosphate extractable soil As at 0–20 cm ($r^2 = 0.67$) and 20–40 cm ($r^2 = 0.53$) depths than the Chinese brake fern ($r^2 = 0.42$ and 0.14, respectively).

Conclusion

Both Gold dust fern and Chinese brake fern accumulated high levels of As in fronds under field conditions. On the basis of the total As concentrations in fronds and BFs, we conclude that the Gold dust fern is more efficient in As hyperaccumulation than the Chinese brake fern, and thus better suited for the remediation of As contaminated dip sites in NSW.

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Assessing the Phytotoxicity of Cr(III) and Cr(VI) in Cr(VI)-Spiked Soils by Using XANES and Resin Extraction Methods

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Abstract
Simultaneously determining the two species, Cr (III) and Cr (VI), under different organic matter contents and pH levels would be required for assessing Cr phytotoxicity in contaminated soils. Two acid soils (Neipu and Pinchen) were adjusted within three pH ranges (4-5, 5-6, and 6-7) and then treated with four Cr (VI)-spiked levels (0, 500, 1000, and 1500 mg/kg). The extent of Cr (VI) reduction was determined by using X-ray absorption near edge structure spectroscopy (XANES). Two selective exchange resins (Chelex 100 and DOWEX M4195) were used to extract Cr (III) and Cr(VI) separately from soil samples. And, wheat seedling experiment was conducted for illustration of Cr phytotoxicity. The results showed that in Neipu soil, Cr (VI) was mostly reduced to Cr (III) by the larger amount of organic matter. Chelex 100 extractable Cr (III) was decreased with raising pH and then the injury of seedlings was significantly reduced. Nevertheless, according to the results of XANES spectra, in Pinchen soil, Cr (VI) was not completely reduced to Cr (III). Dowex M4195 extractable Cr (VI) was increased with raising pH. And, injury of seedlings was increased with raising the Cr (VI)-added levels. Thus, Cr phytotoxicity in the injury of seedlings was dominated by Cr(VI) in Pinchen soil.

Key Words
Chelex 100, Dowex M4195, reduction.

Introduction
Chromium(VI) is carcinogenic due to its high mobility and toxicity, and exists in the environment as $Cr_2O_7^{2-}$ or CrO_4^{2-}; Cr(III) is less soluble and is considered to be less toxic than Cr(VI). Organic matter in soil acts an predominant electron donor which contributed to the reduction of Cr (VI) to Cr (III). The reaction is pH dependent and is enhanced with decreasing pH (Adriano 1986). It has been evidenced that the solubility of Cr(III) increases with decreasing pH, and the phytotoxicity of Cr(III) under acid condition is rising due to its high solubility (Han *et al*. 2004). It is assumed that the phytotoxicity of Cr in Cr (VI)-contaminated soil with various soil pH levels and organic matter content could be attributable to both Cr(VI) and Cr(III) or either of them. Therefore, the phytotoxicity of Cr(VI) and Cr(III) in Cr(VI) - contaminated soil need to be clarified. The XANES spectroscopy provide nondestructive measurement of oxidation states of chromium on the soil surface and is a useful tool to estimate the degree of Cr(VI) reduction (Bang and Hesterberg 2004; Lee *et al*. 2006). In this study, Dowex M4195 (Yu *et al*. 2004) and Chelex 100 resins (Chen *et al*. 2008), were used to assess the phytotoxicity of Cr(VI) and Cr(III) in Cr(VI)-contaminated soil with various soil pH levels and organic matter content condition. In addition, the wheat seedling growth experiment was used to determine the phytotoxicity effect of soil Cr.

Methods
Two major agricultural acid soils, Pinchen (pH = 4.3; organic matter = 27.3 g/kg; Fe_d (Dithionite-citrate-bicarbonate extractable iron) content = 27.7 g/kg) and Neipu (pH = 4.1, organic matter = 93.7 g/kg; Fe_d content = 13.2 g/kg), from Taiwan were used. Soil pH of Neipu and Pinchen soils were adjusted within three ranges for 4-5, 5-6 and 6-7 by different rate of $CaCO_3$ addition. Limed soils were mixed thoroughly and incubated at room temperature for 1 month. Chromium(VI) ($K_2Cr_2O_7$) were then spiked into soils to reach four levels of Cr(VI), and then underwent three wetting-drying cycles for three weeks at room temperature. The soil samples were ground and sieved through a 425 μm sieve for phytotoxicity experiment, a 177 μm sieve for resin Cr extraction and sequential extraction, and a 63 μm sieve for XANES analysis.

The DOWEX M4195 resin was saturated with 500 mg/L $CuCl_2$ and converter into Cu-saturated form. The

Cu-saturated resin was retained in 425 μm polypropylene (PP) bags. The Chelex 100 resin was saturated by 2 M $CaCl_2$ and converted into Ca-saturated form. Then, the Ca-saturated resin was retained in 177 μm PP bags. Ten gram soil samples were placed into 300 mL flasks, and then 100 mL distilled water was added. One gram of Chelex 100 resin and 2 g of DOWEX M4195 resin were added into separate flasks and shaken at 25 ℃ for 24 hrs separately. After Cr extracted, the Chelex 100 resin and the DOWEX M4195 resin were washed with distilled water and placed into 100 mL of 2 M H_2SO_4 and 100 mL of 10 % NaCl respectively to desorb the Cr adsorbed on resins. The Cr concentration in solution was determined by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy, Perkin Elmer, Optima 2000DV).

The XANES spectra were collected from National Synchrotron Radiation Research Center of Taiwan. The X-ray absorption Cr K-edge (5989 eV) spectra were obtained using a wiggler beam line, BL-17C1. The modified Neübauer method (Yu *et al*. 2004) was used to investigate the phytotoxicity of soil Cr to wheat seedlings (*Triticum Vulgare*, variety Taichuang select No. 34). The experiment was carried out for 25 days in the Phytotron of the National Taiwan University. A modified sequential fractionation scheme of Tessier *et al*. (1979) was used to clarify the distribution of Cr (III) reduced from Cr (VI) in Neipu soil.

Results

Although the highest pH level (pH = 6.4) was not effective in reducing Cr(VI) in Neipu soil, there was no detectable Cr(VI) peak in all rates of $CaCO_3$ addition (Figure 1 (a)). Thus, the Cr(VI) reduction was not regardless of soil pH. In the case of Pinchen soil, the degree of Cr(VI) reduction was relate to soil pH (Figure 1 (b)). The intensity of the characteristic Cr(VI) peaks in XANES spectrum increased with increasing soil pH.

The Dowex M4195 resin extractable Cr was not detected in Neipu soil in all Cr treatments at each pH level (Table 1), whereas generally increased with increasing Cr(VI) addition and pH levels in Pinchen soil. It is suggested that Cr(VI) was most probably reduced into Cr(III) by larger amount of organic matter in Neipu soil. Due to the organic matter content in Pinchen soil is lower, soil pH became the main factor that appreciably affected Cr(VI) reduction. Additionally, the greater amount of Chelex 100 extractable Cr was detected in Pinchen soil than that in Neipu soil, which increase with Cr(VI) addition and decreased with increasing soil pH (Table 1). It is indicated that the reduction of Cr(VI) increased soil pH due to the proton consumption reaction (Bolan and Thiagarajan 2001), and reduced the solubility of Cr(III) reduced from Cr(VI) in Neipu soil than that in Pinchen soil. Moverover, the reduced product Cr(III) was mainly associated with the organic bound and Fe-Mn oxide bound fractions in Neipu soil.

In Cr (VI)-spiked Neipu soil, plants were not injured in all Cr(VI) treatments for each pH level (Figure 2), whereas the plant height of wheat seedlings increased with increasing Cr(VI) addition due to the increase in pH facilitated the plan growth. In the case of Pinchen soil, the plant height of wheat seedlings approximately decreased with increasing Cr(VI) concentration and soil pH. It is suggested that the injury of plants could be attributed to both Cr(VI) and the Cr(III) reduced from Cr(VI). However, the amount of soil Chelex 100 extractable Cr was quite low and the larger amount of spiked Cr (VI) was not reduced to Cr(III). Thus, Cr(VI) was supposed to be responsible for phytotoxicity.

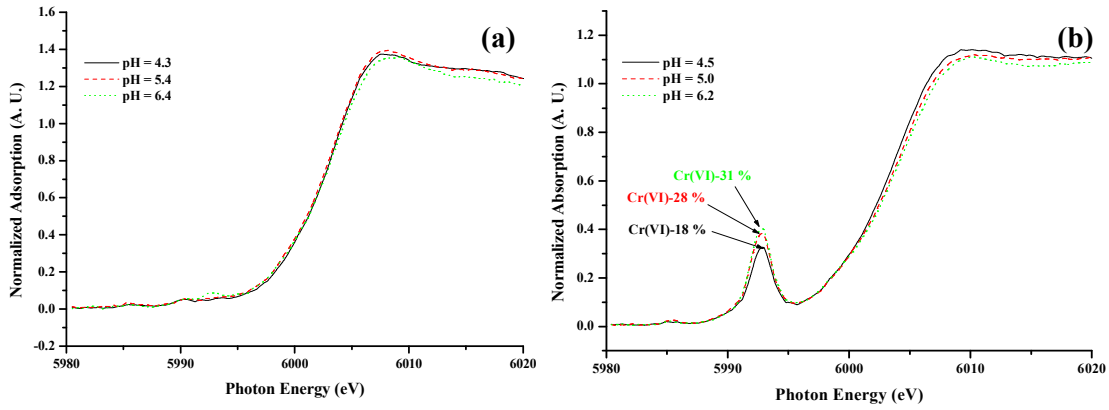

Figure 1. The XANES spectra of Cr(VI)-spiked soil (1500 mg Cr(VI)/kg soil) at different pH. (a) Neipu; (b) Pinchen soil.

Table 1. The amounts of Chelex 100 and Dowex M4195 resin extractable Cr in Cr(VI)-spiked soils with different soil pH.

Soils	Soil Cr(VI) (mg/ kg)	Resin extractable Cr (mg /kg)					
		pH =4.3		pH =5.4		pH = 6.4	
		Chelex 100	Dowex M4195	Chelex 100	Dowex M4195	Chelex 100	Dowex M4195
Neipu soil	Control	ND^{dAB}	ND	ND^{d}	ND	ND^{c}	ND
	500	5.1^{b}±0.9	ND	4.0^{b}±0.4	ND	2.5^{b}±0.7	ND
	1000	8.4^{a}±1.1	ND	8.2^{a}±1.2	ND	6.0^{a}±0.3	ND
	1500	8.6^{a}±2.3	ND	8.3^{a}±1.7	ND	6.1^{a}±1.8	ND
		pH = 4.5		pH = 5.0		pH = 6.2	
		Chelex 100	Dowex M4195	Chelex 100	Dowex M4195	Chelex 100	Dowex M4195
Pinchen soil	Control	ND^{f}	ND^{e}	ND^{d}	ND^{e}	ND^{d}	ND^{d}
	500	3.7^{c}±0.1	9.2^{c}±0.6	4.1^{c}±0.0	17.8^{c}±0.7	1.5^{c}±0.1	13.1^{c}±0.1
	1000	11.6^{b}±0.4	40.7^{b}±0.0	12.7^{b}±2.3	62.0^{b}±1.7	9.2^{b}±0.0	65.2^{b}±0.0
	1500	20.9^{a}±0.1	96.3^{a}±1.8	22.0^{a}±0.5	130.5^{a}±3.0	24.0^{a}±1.1	223.8^{a}±7.9

[A]Values followed by the same letter, within the same column and type of soil, are not significantly different (Duncan Multiple Range Test, p =0.05).
[B]Detection limit (≤ 0.05 mg/kg)

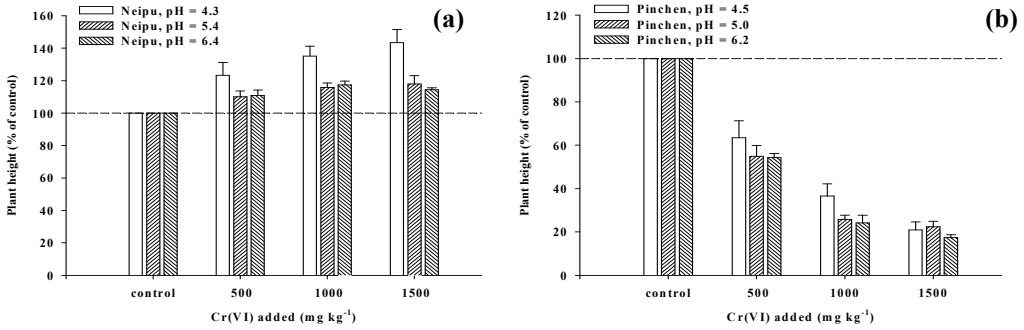

Figure 2. The plant height of wheat seedlings in Cr(VI)-spiked soils with different soil pH. (a) Neipu soil; (b) Pinchen soil.

Conclusion

Cr(VI) added into Neipu soil was mostly reduced to Cr(III) by the large amount of organic matter, and the Cr(VI) reduction was regardless of soil pH. The decrease of phytotoxicity of Cr(III) reduced from Cr(VI) due to the solubility of Cr(III) decreased while proton consumption reaction causing soil pH increasing and binding with organic matter and the formation of precipitation. In Pinchen soil, DOWEX M4195 resin extractable Cr increased with increasing pH, and the greater amount of Chelex 100 extractable Cr was detected than that in Neipu soil. The injury of wheat seedlings can be attributed to both Cr(VI) and Cr(III). However, the large amounts of available Cr(VI) was supposed to be dominant for phytotoxicity.

Acknowledgement

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Assessment of arsenic phytotoxicity of a contaminated Ferrosol using radish.

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Abstract
Arsenic (As) contaminated soil presents a potential risk to human and environmental health, especially when As contaminated sites are redeveloped for residential purposes. State Environmental Planning Policy 'No 55 – Remediation of Land' requires planning authorities to consider land contamination when assessing development applications. This study's aim was to directly assess As phytotoxicity and soil availability using a quick growing, common residential garden vegetable. The results of this study may be used to inform a review of the adequacy of the current phytotoxicity investigation level (PIL), 20mg Total As/kg soil. A phytotoxicity trial growing long scarlet radish (*Raphanus sativus*) revealed no significant difference in root elongation in soils contaminated with 10, 86, 169, 244, 315 and 656mg As/kg. The current PIL of 20mg/kg is excessive in this Ferrosol and remediation to the health investigation level (HIL) of 100mg/kg would be sufficient. The PIL should be specific to the remediation site and based on plant availability of As.

Key Words
contamination arsenic phytotoxicity Ferrosol remediation radish.

Introduction

Arsenic (As) based pesticides, used extensively in the early to mid 1900s, have caused contamination in excess of 1000mg As/kg at some sites throughout Australia (Smith *et al.* 1998). Many of these highly contaminated sites are decommissioned livestock dip sites and cropped areas such as abandoned banana farms. Some of these contaminated lands are adjacent to population growth areas and as such, there is significant pressure to redevelop this agricultural land for residential use. In New South Wales (NSW), State Environmental Planning Policy No 55 – Remediation of Land, (SEPP 55) (DUAP 1998), requires planning authorities to consider land contamination as a land use constraint and the redetermination of appropriate actions to be undertaken as part of the redevelopment.

Current guidelines for As site contamination assessment are based on Total As concentration. This is consistent with the National Environment Protection Measures (NEPM) for As. After testing the soil, a remediation action plan (RAP) is normally formulated to rehabilitate the contaminated site as part of the conditions of any redevelopment approval. Normally, the remediation target levels for residual soil As employ a health investigation level (HIL e.g. 100 mg As/kg for residential areas) and a phytotoxicity investigation level (PIL e.g. 20 mg As/kg) (NSW EPA 1998). The HIL is based on significant research and forms part of the Australia wide NEPM. However, a soil PIL at 20 mg As/kg is a value that may change significantly with the soil type and properties (pH, clay content and type, organic matter, iron and aluminium oxides, phosphate-sorptive characteristics) and plant species (As sensitivity, preferred uptake and accumulation characteristics).

As such, a single soil Total As concentration alone is an inappropriate indicator for assessing phytotoxic impacts at contaminated sites. In order to improve the assessment of As phototoxicity in soil, a direct plant based measure of soil As availability for any soil type is needed.

A phytotoxicity procedure already exists for assessment of potting mixes (Standards Australia 2002. *AS 3743 - Potting Mixes*). Using this procedure, a series of trials were undertaken to test the application in the assessment of the level of As phytotoxicity of a contaminated soil. This procedure tests the toxicity of potting mixes by germinating the seed of an indicator plant (usually Radish, *Raphanus sativus*), incubating the germinated seed for five days and measuring the length of the radicle in comparison with those grown in a known non-toxic potting mix. This is a simple trial that takes approximately five days to complete and yields a direct measurement of the toxic effects on seed germination and root extension.

The aim of this study was to assess As phytotoxicity of a Ferrosol for the purpose of remediation of a site by identifying the impact of soil As concentration on the germination of plants by growing radish (*Raphanus sativus*).

Methods

Soil site characteristics

The soil for testing was taken from a proposed redevelopment site at Bilambil heights on the North Coast of NSW. The predominant soil type was a Ferrosol. The soils were red-brown with a light to medium clay texture. Six soil samples were recovered and tested for Total As. The Total As concentrations were; 10mg/kg, 86mg/kg, 169mg/kg, 244mg/kg, 315mg/kg and 656mg/kg.

Germination Trial

The experimental trials were undertaken in two steps: a test procedure to validate the impact of soil As on plant root development and a direct test of the validated procedure on the trial site soils.

The validation trial tested the germination and root extension of an As contaminated washed sand. The sand was treated with sodium arsenite to yield total sand As concentrations of; 0mg/kg, 100mg/kg, 200mg/kg, 300mg/kg, 500mg/kg and 800mg/kg. The sand was put into small pots and used a randomised complete block design with the six treatments and three replications. The phytotoxicity trial used *long scarlet* radish. Ten seeds were placed in each pot and moistened. The pots were then kept moist for five days and the radish harvested and the length of each radicle measured.

The soil test adopted a randomised complete block design using six treatments (soil As of 10mg/kg, 86mg/kg, 169mg/kg, 244mg/kg, 315mg/kg and 656mg/kg) with three replications. The soil treatment of 10mg/kg As was selected as the control treatment. Natural As concentrations in soil are usually less than 15mg/kg (Walsh *et al.* 1977). The initial soil moisture content was estimated for each soil sample. The gravimetric moisture content was then calculated and used to determine the amount of water required per gram of soil to reach field capacity. Soil samples were air-dried, ground and put through a 2mm sieve to eliminate structural variance and ensure maximum soil to root contact. The soils were then placed in pots, wet to field capacity, with each pot planted with ten *long scarlet* radish seeds. The pots were kept moist for five days and then the radish harvested and the length of each radicle measured.

Statistical Analysis

All statistical analysis was assessed using the 0.05 (α) level of significance for difference and only the resultant $R^2 > 0.70$ were reported. A t test on root length was conducted assuming equal and unequal differences to determine any significant difference in root elongation in the trial.

Results

Arsenic dosed-sand

The trial method was ratified using washed sand dosed with sodium arsenite in which bioavailability was uninhibited by any sorptive components. The addition of increasing concentrations of As^{iii} caused a significant decrease in root elongation. A statistical summary of t test on root length is shown in Table 1. A root elongation response curve is shown in Figure 1. The results for radish grown in dosed sand confirmed the viability of the test method. The validation trial shows a strong response to the addition to As to the sand. The initial test indicates that radish should respond to soil As by reducing radicle extension.

Arsenic contaminated soil

In terms of root elongation, no significant difference was observed in soils contaminated with up to 656mg As/kg, except for radish seedlings grown in soil containing 169mg As/kg compared with those grown in soil containing 244mg As/kg (where significant difference was noted). A summary of the t test on root length is shown in Table 2.

Table 1. Summary of t test on root elongation in six sand samples of validation trial (* 0.05, ^ 0.01).

	Equal variances							
	mg As/kg		0	100	200	300	500	800
		Mean root length (mm)	27	11	7	3	2	0
Unequal variances	0	27		**^8.926**	**^10.774**	**^12.848**	**^14.210**	**^15.684**
	100	11	**^8.926**		**^3.711**	**^7.789**	**^11.208**	**^16.099**
	200	7	**^10.774**	**^3.711**		**^3.912**	**^6.493**	**^10.273**
	300	3	**^12.848**	**^7.789**	**^3.912**		1.934	**^5.048**
	500	2	**^14.210**	**^11.208**	**^6.493**	1.934		**^4.273**
	800	0	**^15.684**	**^16.099**	**^10.273**	**^5.048**	**^4.273**	

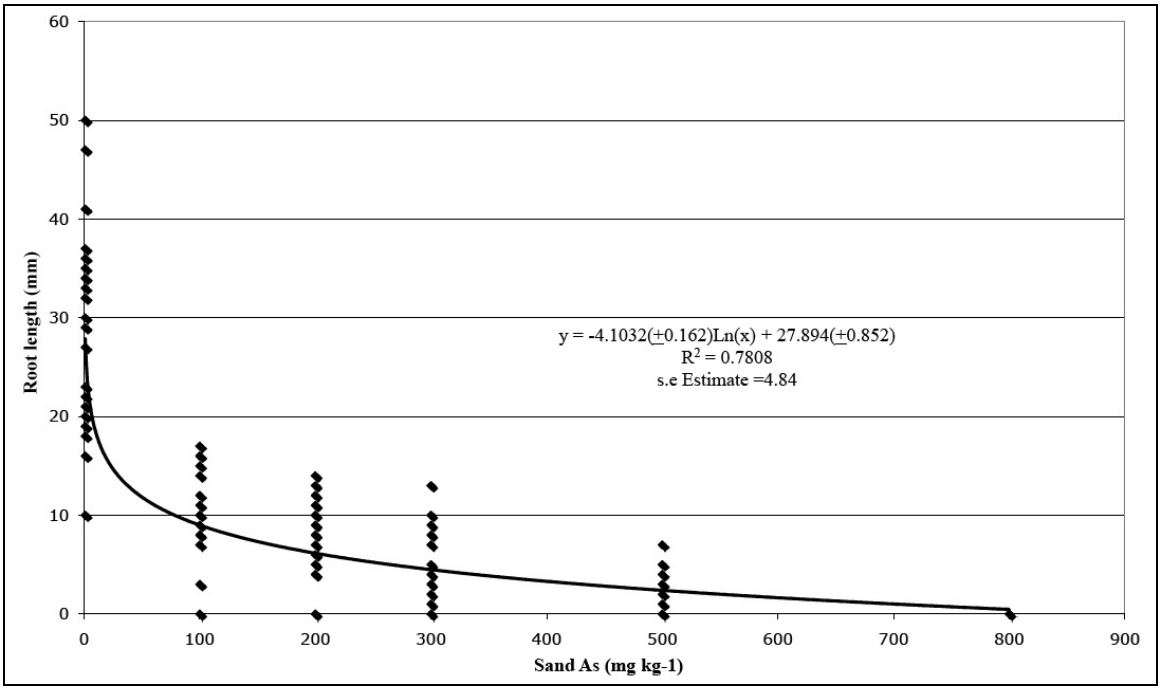

Figure 1. Root elongation with increasing sand arsenic concentration.

Table 2. Statistical summary of t test on root elongation in six Ferrosol soil samples following a phytotoxicity trial (* 0.05^ 0.01).

	Equal variances							
	mg As/kg		10	86	169	244	315	656
		Mean root length (mm)	83	87	82	93	92	87
Unequal variances	10	83		-0.566	0.156	-1.652	-1.520	-0.677
	86	87	-0.566		0.765	1.346	-1.183	-0.146
	169	82	0.156	0.811		1.884	-1.748	-0.915
	244	93	-1.669	1.346	***2.040**		0.163	1.018
	315	92	-1.536	-1.185	-1.890	0.163		-0.879
	656	87	-0.673	-0.155	-0.913	1.109	-0.955	

Discussion

The validation trial demonstrates the root extension of radish in sand is significantly affected by soil As especially when the As is readily available. The initial trial used similar soil Total As concentrations as those found on the contaminated Ferrosol and indicate that if the soil As was available to the plant, root extension would be reduced. The repetition of the trial method using the contaminated Ferrosol demonstrated that germination of radish was uninhibited by soil As and that the Total As concentrations of this soil are not phytotoxic to radish. The Ferrosol at this location was well drained, aerobic and exhibited pH's ranging from 5.5 to 6.4. Additionally, the clay component of this Ferrosol would include As sorptive components such as iron and aluminium oxides, hydroxides and oxyhydroxides. The presence of these compounds would be restricting the soil-plant availability of As (Masscheleyn *et al.* 1991). The As is strongly sorbed to the soil matrix.

While soil analysis showed concentrations of Total As greatly exceeding the HIL of 100mg As/kg, the phytotoxicity trial revealed that the As is not available to plants. In this context, the current PIL of 20mg As/kg is excessive at this site and remediation to the HIL is sufficient.

Conclusion

This direct method of determining phytotoxicity is both time and cost effective and may be applied to any soil type to assess As phytotoxicity at contaminated sites. A simple, rapid method such as this represents a suitable alternative to laboratory analysis and estimates of As availability based on soil properties such as pH and mineralogy. This method should be used to determine site-specific remediation values for other soil contaminants and for framing appropriate management measures to control soil contamination concentrations. Further work is required to assess the plant accumulation of As on sites that have been identified as not being phytotoxic.

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Bioavailability of barium to invertebrates and humans in soils contaminated by barite

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Abstract
The solubility, bioaccessibility and bioavailability (earthworm) of barium (Ba) was investigated in soils contaminated with barite. The results suggested barite contamination resulted in stress in the earthworm (*Eisenia fetida*) as indicated by concurrently increasing weight loss and barite loading in the soils. In this study, acid digestion estimates of Ba were at least as good an indicator of Ba induced stress in *E.fetida* as any bioaccessibility measure.

Introduction
Barium (Ba) is a group 2 element that shares several chemical characteristics with calcium (Ca). Barium, however, is not an essential nutrient to animals and plants but instead is known to cause several deleterious effects on most organisms. Barite ($BaSO_4$) is a highly insoluble mineral. It is insoluble in water, acid and bases, and on its own is unlikely to cause a risk to humans or the environment. Ba may be highly mobile in soil as Ba primarily associates with soil colloids by ion exchange. However, barite solubility is extremely important in the environment. The aim of this paper is to examine the bioaccessibilty and bioavailability of Ba from barite in contaminated soils.

Materials and methods
Soils
Six soil samples contaminated with barite plus a control were sampled in duplicate. Samples were sieved through 2-mm sieves for general soil characterisation and earthworm studies. Barium concentrations were measured using both microwave-assisted acid digestion and X-ray Fluorescence (XRF) methodologies. Mineralogy of the samples was determined in samples L7 and L10 (control) using X-ray Diffraction (XRD).

Earthworm bioavailability
Bioavailability of soil-Ba to earthworms was conducted according to the standard protocol. Experiments were conducted in triplicate. Each replicate consisted of 200 g of air-dry soil which was wet to below field capacity (typically 80% of soil field capacity). All experiments were carried out in environmental chambers at constant temperature of 18°C under light. To each 200g quantity of soil, 10 healthy adult earthworms (*Eisneia fetida*) were added. Before the bioavailability study, the earthworms were placed on moist filter paper in petri dishes and allowed to depurate for 24 h. Earthworms were then weighed and recorded before exposure to each soil. During the test, earthworm mortality was monitored daily (28 days). At the end of the 28 d incubation, the earthworms were removed from test vessels and allowed to depurate on moist filter paper again for 24 hour and weighted before being stored in a fridge at -18°C. Depurated earthworms were digested in concentrated HNO_3 using fresh weights. Chemical extractions to estimate bioavailability of Ba to earthworms was estimated by both water extraction and dilute salt extractions. Water extractability of Ba was measured by reacting 10g of air-dry soil (<2-mm) with water (18 Ω) for 16 hrs. In addition, extractable Ba was measured by reacting 2 g soil and 20 mL of 0.1 *M* $CaCl_2$ for 4 hrs. Both water and 0.1 *M* $CaCl_2$ extractable supernatants were separated by centrifugation (4000 rpm, 10 mins) and filtration (0.45 µm syringe driven filters).

In vitro gastrointestinal bioaccessibility
Gastric solution was prepared using the same recipe as outlined in Ruby et al. (1996): 1.25 g of pepsin (Sigma Chemical Co.), 0.5 g of sodium citrate, 0.5 g of malic acid, 420 µL of lactic acid and 500 µL of acetic acid was added to 1 L of DI water and mixed gently for approximately 1 min. The pH of the gastric solution reflected "fasting" conditions in the human stomach (Ruby et al. 1996). The small intestine pH was simulated at pH 7.

The gastric phase (100 mL) was added to the glass bottles and combined with 1 g of material. The mixture was allowed to stand for 10 minutes at 37 ^{o}C in a water bath. Each vessel was purged with Argon gas prior to addition of soil and solutions. Samples were taken from the suspension (1 mL) at 20, 40 and 60 mins. An equal volume was replaced from the stock solution of the appropriate gastric solution, to maintain the initial volume. After 60 mins suspensions were titrated to pH 7.0 using the $NaHCO_3$. At this stage, 70 mg of porcine bile salts and 20 mg of porcine pancreatin were added to the mixture to reflect the small intestine conditions. Samples were taken from all reaction vessels at 1 and 3 hours after titration to pH7.0. The % bioaccessible barium in the gastric (GBAc), intestinal (IBAc) phases and the average of gastric and intestinal (ABAc) were calculated using equation 1:

$$\%\text{Bioaccessible Ba} = \left(\frac{\text{Ba extracted, } {}^{mg}/_{kg}}{\text{Ba Acid Digestion, } {}^{mg}/_{kg}} \right) \times 100 \quad (1)$$

The acid digestion total measure was used since it was considered to represent the maximum concentration able to be accessed by living organisms.

Analysis

All soil samples were analysed using inductively coupled plasma- mass spectroscopy (ICP-MS) (Agilent 7500c) after appropriate dilutions. Quality control was monitored during analysis by addition of 50 µg/L check samples and blanks every 20 samples. Recovery of check samples was always between 90-110%. The Ba content in earthworms after avoidance test was conducted by digesting the frozen earthworm in 5 ml of concentrated nitric acid overnight and then heating it under programmed heating to 140°C to evacuate the acid to less than 1 ml. The remaining acid was diluted to 10 ml using Milli-Q water. The solution was filtered before analysis using ICP-MS.

Results

Barium concentrations estimated from acid digestion and XRF are presented in Table 1. Barium concentrations presented differ dramatically between the two total concentrations estimates. Acid digestion results are in all cases far below that determined by XRF. Although "total" concentrations estimates based on acid digestion methods are useful, differences in the two measures increased as the XRF concentration increased. The total Ba in controls using XRF was 500-700 mg/kg. Concentrations in contaminated soils determined by XRF were in the range of 1300 mg/kg to 29.2%. Samples in L7/1 and L7/2 had Barite concentrations of 45 % on wt basis.

Table 1. Total Ba concentrations estimated from acid digestion and XRF, proportion of $BaSO_4$, Gastric bioaccessibilty (GBAc %), Intestinal bioaccessibility (IBAc %) and the average of gastric and bioaccessible Ba (ABAc %), percentage weight loss in earthworms and tissue burden of Ba (mg/kg Fresh weight).

Soils	Total Ba Acid Digest (mg/kg)	Total Ba XRF (mg/kg)	Wt % $BaSO_4$	GBAc %	IBAc %	ABAc %	Weight loss (%)	Tissue Ba (mg/kg FW)
L10/1(Control)	120.0	700.0	0	91.8	51.7	71.8	-5.59	11.8
L10/2(Control)	123.0	500.0	0	74.6	34.6	54.6	-3.35	10.8
L1/1	483.0	1300	n.d	75.1	40.5	57.8	3.02	2.3
L1/2	500.0	1400	n.d	85.2	33.5	59.3	5.87	9.7
L3/1	1833	5300	n.d	34.5	17.7	26.1	12.71	2.8
L3/2	1867	7700	n.d	27.3	13.1	20.2	19.09	3.1
L4/1	2033	5700	n.d	30.7	16.1	23.4	7.94	118.9
L4/2	1667	10100	n.d	26.3	10.8	18.5	13.09	123.6
L6/1	3367	26.96%	n.d	35.0	21.4	28.2	42.54	6.6
L6/2	3300	29.20%	n.d	27.4	16.0	21.7	43.04	9.6
L7/1	4733	26.53%	45	26.3	17.2	21.8	37.20	4.0
L7/2	3633	24.99%	45	22.9	14.7	18.8	35.19	3.5
L9/1	2167	10100	n.d	39.1	21.9	30.5	42.41	18.3
L9/2	2367	6700	n.d	24.9	14.1	19.5	41.01	17.0

Barium solubility in the *in vitro* bioaccessibility measures test were shown to reach a maximum in the gastric phase at 60 minutes. Ba bioaccessiblity increased gradually to 60 minutes, then decreased with time as pH was increased to 7.0. The second phase with a pH of 7 is intended to simulate the small intestine. The magnitude of change in % bioaccessibility at pH 7 was highest in samples with lower total concentrations, which was also the samples which had the highest gastric bioaccessibility (control sample). The control

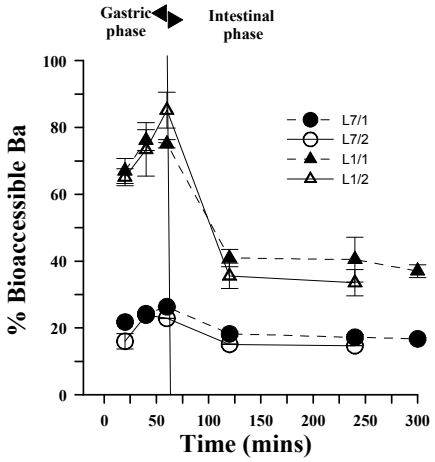

Figure 1. Bioaccessible Ba (%) in the gastrointestinal tract as a function of time estimated from the PBET test. Two soils are used to indicate trends. Line indicates where the 'gastric" and 'intestinal' phases begin and end.

samples have little to no barite contributing to the total concentrations, which may explain the higher bioaccessibility of Ba. It is unclear why Ba concentrations reduced at the higher pH, as $Ba(OH)_2$ phases are highly soluble. Body burdens of Ba in the earthworm did not correlate with any measures of bioaccessibility considered (see Table 1 for data). However, it was observed that weight loss occurred in barite contaminated soils. Furthermore, % weight loss correlated with all the measures of bioaccessibilty (human and ecological). Total Ba concentrations (acid digestion and XRF) were also correlated ($r > 0.5$). However, the highest correlation was observed with GBAc ($r = 0.85$), ABAc ($r = 0.85$) and acid digests ($r = 0.85$).

Summary

The results show that despite barite being a highly insoluble mineral in water and acid, barite contamination resulted in stress in the earthworm *E.fetida*, as indicated by increasing weight loss and barite loading. In this study acid digestion estimated Ba was at least as good an indicator of Ba induced stress in *E.fetida* as a bioaccessibility measure.

Bioavailability of metals and organic contaminants in soils

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Abstract
Recent advances in the understanding and prediction of the risk of metal toxicity in different soils which explicitly include the concept of bioavailability are reviewed, along with development of terrestrial biotic ligand models for metals. Both of these are aimed at assessing the "safe" concentrations of metals that can accumulate in soils without negative biological effects. For organic contaminants, the recent emphasis appears to be on the area of their partial extraction from soils, and the search for extraction regimes that mimic either their bioavailability or bioaccessibility in soils. This appears to be due to the focus in the organic arena not on toxicity *per se* but on the assessment and remediation of already contaminated sites.

Key Words
Zinc, copper, nickel, cobalt, polycyclic aromatic hydrocarbons.

Introduction
Concerns regarding the potential risks of existing chemicals in the environment gave rise to the European Union Directive (793/93/EEC) for the evaluation and control of risks posed by existing substances including metals and organic contaminants. New research has been carried out to include bioavailability in the assessment of potential toxic effects of metals and metalloids in different environmental compartments, including soils, by an international research group. These assessments do not use extractants in an attempt to describe bioavailability in different soils, but instead take an empirical approach of relating soil properties themselves to the degree of toxicity expressed in bioassays. A further development for metals has been the more mechanistic concept of the terrestrial biotic ligand model (tBLM), which seeks to explain the interactions that lead to toxicity in organisms. Toxicity risk assessments for a number of organic contaminants have been performed, but usually on the basis of desk studies, and often, because of a lack of data, large assessment factors are imposed. For organics, the emphasis has been on defining and attempting to characterize their bioavailability and bioaccessibility. Bioavailability is defined as the contaminant fraction "which is freely available to cross an organism's (cellular) membrane from the medium the organism inhabits at a given point in time". Whereas bioaccessibility encompasses what is actually bioavailable now plus what is "potentially bioavailable" (Semple *et al*. 2004). Extraction procedures that mimic or parallel bioavailability/bioaccessibility have been sought in order to assess exposure and bioremediation potential. Such procedures are often referred to as biomimetic techniques (Semple *et al*. 2007). It is likely that the dichotomy described above between metals and organic comes from an emphasis in the latter case not on toxicity, but on the degradation and remediation of contaminants over time.

Objectives and results
Risk assessments in the EU are done according to the Technical Guidance Document of the European Commission (2003), comparing the predicted environmental concentration (PEC) and predicted no-effect concentration on representative organisms (PNEC). Risk decisions are made if the PEC:PNEC ratio for a particular metal is >1. Assessment factors are also applied if few organisms have been tested for toxicity data for a particular substance. The main organisms considered for toxicity evaluation within the terrestrial ecosystem are soil microbes, invertebrates and plants; usually with three examples in each trophic level. However, gaps in knowledge and inconsistency in published data sets due to their frequent use of non-systematic and non-standard tests necessitated new research. The objectives for the metals Zn, Cu, Ni and Co were: 1) to account for the huge differences in toxicity between soils given the same doses of metals, using the same standard bioassays, and 2) to account or the differences in toxicity between laboratory and field experiments ("ageing"). Comprehensive testing can also reduce the size of assessment factors.

For organics, considerable effort has been directed towards developing non-exhaustive chemical techniques for the measurement of putative contaminant bioavailability (Semple *et al*. 2003). Non-exhaustive techniques are mostly based on the principle that bioavailability, in particular to microorganisms, is governed by

contaminant mass transfer mechanisms such as desorption from solid to aqueous soil phases (Bosma *et al*.1997). Several disadvantages or limitations of utilising mild solvent extraction have been outlined, such as (i) the type of solvent used (i.e. polar or non-polar solvent, e.g. methanol or hexane, respectively), ii) the nature of the extraction (e.g. soxhlet, shake, supercritical fluid) and iii) the impact of the extraction procedure on the physico-chemical properties of the soil (Semple *et al*. 2003). Subsequently, a range of non-exhaustive extraction techniques that are not dependent on organic solvents have been considered for bioavailability prediction such as solid phase extraction (Tenax beads (Cornelissen *et al*. 1997) and XAD resin (Cuypers *et al*. 2001)), supercritical fluid extraction (Hawthorne *et al*. 2000), cyclodextrin extraction (Reid *et al*. 2000) and persulphate oxidation (Cuypers *et al*. 2000).

Metals
Up to nineteen relatively uncontaminated soils were collected (depending on the metal studied) from Europe (and North America in the case of Ni) that ranged widely in soil pH, clay, organic C and content of amorphous oxides (Smolders *et al*. 2009). These were amended with soluble metal salts at sufficient doses to measure a dose-response to each metal individually. In a further series of tests on aged soils, known established metal gradients or long term field experiments were selected and sampled to be used either for direct toxicity tests or in parallel toxicity studies where the control (low metal) soils were amended and short term toxicity tested for direct comparison with the long-term contaminated soils. The tests used were usually OECD or ISO standard tests, or for some endpoints where standardized test did not exist, well documented published methodologies were used. Data were fitted to log-logistic curves for the dose-response to metals whether possible, and these were used to derive 10 or 50% effect concentrations (EC10 or EC50) expressed as added metals. No observed effect concentrations (NOEC) were determined using analysis of variance. Relationships between the degree of toxicity and soil properties were investigated using single and multiple regression techniques.

The following total numbers of chronic toxicity test data for the 4 metals were produced: plants (189), invertebrates (211), and microbial processes (270). Toxicity thresholds based on the free metal ion activity in soil solution were generally more variable than those expressed on total soil metal (Figure 1). Soil pH was generally a good predictor of metal solubility, but a poor predictor of metal toxicity across soils. The toxicity thresholds based on total soil metal concentrations were found to rise almost proportionally to a soil's effective cation exchange capacity. In general total metal as a percentage of soil eCEC was the best fit for many metals and biological endpoints. Total soil metal concentrations yielding 10% biological inhibition in *freshly amended* soils were up to 100 fold smaller (median 3.4 fold, n=110) than in corresponding *aged* soils or field-contaminated soils. The PNEC values for specific soil types were calculated using this information. Allowing for the modifying effects of soil properties and for ageing was shown to result in PNEC values that are above the natural background concentration range for soils.

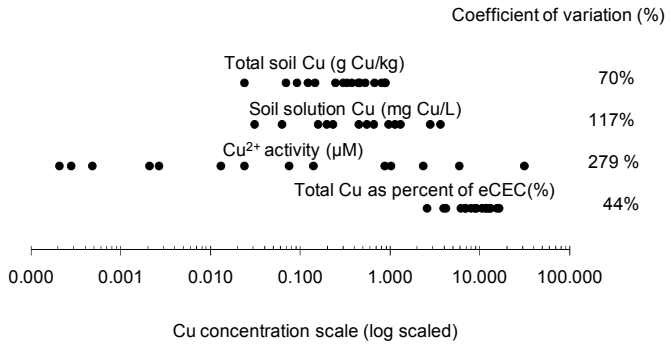

Figure 1. Four different expressions of the toxic EC50 concentrations to shoot growth of tomato in 19 freshly Cu amended soils (after Smolders et al 2009).

A tBLM which accounts for the effects of competition from protons, Ca and Mg on toxicity of the metals Cu and Ni was tested using the data (Thakali *et al*. 2006a,b).

Organics
Cyclodextrins are cyclic oligosaccharides, formed from enzymatic degradation of starch by bacteria, comprising a torus of α 1,4-linked glucose units. Cyclodextrin molecules have high aqueous solubilities, due to the array of hydroxyl functional groups on the exterior, but also possess a hydrophobic organic cavity to the interior which is approximately 6.5 Å in diameter for β-cyclodextrin. Unlike the portion of contaminants extracted by the use of organic solvents, cyclodextrins have been shown to correlate closely with key biological fractions, such as the portion of the contaminant that is mineralisable. Owing to their molecular structure they also contain a hydrophobic cavity. It is possible to form an inclusion complex between the cyclodextrin macrocycle and a hydrophobic organic molecule i.e. the cyclodextrin acts as a 'molecular bucket' (Reid *et al.* 2000).

Numerous studies have shown investigated the use of cyclodextrin extraction as a predictor for microbial degradation of PAHs in single contaminant-spiked soils, multiple PAH spiked soils and field contaminated soils. In each case, the cyclodextrin extraction directly predicted the extent to which the PAHs would be degraded. Two sediment samples were sequentially extracted with HPCD, Tenax and Triton-X and PAH removal during extraction was then compared with PAH removal during biodegradation. It was demonstrated that HPCD and the Tenax extractions closely followed biodegradation and removed primarily readily available PAHs, while the Triton X-100 over-predicted biodegradation endpoints (Cuypers *et al*. 2002).

The principle aim of developing a method that quantifies bioavailability/bioaccessibility is to provide practitioners with a tool that accurately predicts the rates and end points of bioremediation strategies that employ microbial degradation. Bioavailability/bioaccessibility is considered to be the primary factor affecting the success of any such clean up approach. Fractions removed using non-exhaustive extraction techniques have been successfully correlated with the extent of degradation after the application of bioremediation practices (Semple *et al*. 2003; 2007).

Conclusions
This series of metals projects resulted in calibrated bioavailability models that can be used to normalize toxicity across different soil types, and allow for the effects of ageing, which had previously resulted in many previous soil risk assessments producing PNEC values below those of typical background soils. The bioavailability and ageing factors have been accepted by EU regulators and have already been used as a first screening tier in the Ecological Risk Assessment Framework in the UK, and in the Flemish regulation on soil remediation and soil protection in Belgium. We used our data to calibrate tBLM models for Cu and Ni toxicity to plants, invertebrates and microbes and although these explain the expression of toxicity they are not predictive. More work is needed to develop robust predictive tBLM models for use with soils. In terms of organic contaminants, it is clear that chemical techniques routinely used and described in the literature, estimate the bioaccessible rather than the bioavailable fraction. Remediation scientists are more concerned with what is bioaccessible over time at a given site than what is bioavailable. But question: "can the bioavailable portion of substance X to species Y actually be measured?" remains to be answered (Semple *et al*. 2004).

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Bioavailability of metals in Australian biosolids.

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Abstract
The presence of metal contaminants in biosolids is one of the key factors restricting their use in agriculture. Moreover, the regulation of such activities is complicated by an incomplete understanding of the likelihood of long-term changes in metal bioavailability occurring following land application. This study seeks to address this knowledge gap by subjecting a wide range of Australian sewage sludge samples to a combination of standard chemical characterisation methods, isotopic dilution lability assessment, and advanced spectroscopic analyses. Key results and differences between these samples will be presented, providing insight into the variation in concentration and bioavailability of major metal contaminants in Australian biosolids. Discussion will focus on the role of key sorbent phases and mechanistic factors as identified through spectroscopic interrogation.

Key Words
Sewage sludge, Biosolids, Metals, Bioavailability, Fixation, Oxides.

Introduction
Sewage sludges are continuously produced as a by-product of municipal wastewater treatment and their subsequent disposal presents a major management issue for the wastewater industry. Among the disposal options for stabilised sewage sludges (biosolids) application to land as a soil conditioner is of major importance, particularly in countries such as Australia where soils are typically nutrient poor and low in organic matter. Nevertheless, the land application pathway has recently been subject to widespread scrutiny due to uncertainties regarding the long term fate and release of contaminants from sludge-derived products. The inevitable presence of metal contaminants in biosolids is of particular concern due to their accumulative and persistent nature in the environment. In fact, exceedance of regulatory metal limits currently presents one of the most common barriers to the beneficial reuse of biosolids in agriculture. Despite this, knowledge of the factors controlling metal bioavailability in biosolids and biosolids-amended soils remains far from complete. For instance, although it is well known that metal contaminants in sewage sludge can potentially be sorbed by both inorganic and organic constituents, the nature of the key metal sorbing phases is still a matter of debate (Basta *et al*. 2005). While some authors have suggested that organic matter is the dominant sorbent (Beckett *et al*. 1979; McBride 1995) others have indicated that inorganic constituents such as iron and manganese oxides play the dominant role (Chaney *et al*. 1999; Li *et al*. 2001; Hettiarachchi *et al*. 2003, 2006). How much this may vary between different biosolids products due to temporal and spatial variations in wastewater treatment plant (WWTP) influent characteristics and wastewater processing is not yet well understood but with the nature of the constituents controlling metal sorption having important repercussions in terms of long-term bioavailability it is important for such knowledge gaps to be addressed.

It has generally been postulated that metal chemistry and bioavailability in biosolids-amended soils will predominantly be controlled by the properties of the biosolids in the short to medium term but that soil properties will play an increasingly important role in the long term (Merrington *et al*. (2003). However the time frame over which this change is likely to occur is quite uncertain, with estimates in the literature ranging from weeks to years (Parkpain *et al*. 1998; Smith 1996). Moreover, the factors that govern this temporal change have not yet been determined (Merrington *et al*. 2003). If the dominant sorptive phase is indeed organic matter, mineralisation processes may lead to contaminants being released from the biosolids over time. This possibility has prompted various authors (Beckett *et al*. 1979; McBride 1995) to advance a 'time bomb' hypothesis by which a long-term increase in contaminant bioavailability is envisaged. On the other hand, if the contaminants are mainly sorbed by stable inorganic constituents it is feasible that substantial changes in their long-term bioavailability may not occur. To date, evidence in favour of either hypothesis is inconclusive (e.g. Hettiarachchi *et al*. 2003, 2006; Oliver *et al*. 2006). In fact, very little research has actually been conducted to systematically assess the partitioning of biosolids contaminants

between the organic and mineral sludge phases. Yet this knowledge would be invaluable for predicting the long-term effects of land application and associated risks, and could also potentially advance efforts to maximise stable associations of contaminants in sewage sludge derived end-products.

The primary objective of the study described here is to bring enhanced understanding of the mechanisms controlling the fixation of metal contaminants in biosolids. This will be achieved through the completion of a targeted laboratory programme combining conventional environmental research techniques such as total metal extractions, salt extractions and standard sample characterisation with the use of isotopic dilution for metal lability assessment and advanced spectroscopic techniques specifically suited to the analysis of key biosolids constituents and the interrogation of mechanistic factors.

Methods

Sewage sludge samples have been collected from over 40 Australian WWTPs. The samples have been sourced from all states and territories of Australia and comprise samples from a wide range of WWTPs with differing influent characteristics and network scales, as well as varying unit treatment processes and plant design. Samples have been obtained from both metropolitan and rural WWTPs. Detailed information regarding the wastewater treatment steps and sludge stabilisation methods at the individual plants have also been collected through the use of a WWTP process survey. Samples are being characterised for a range of parameters that are likely to affect the bioavailability and fixation of metal contaminants in the sludge matrix, for example, pH, EC, organic matter content, oxide content and form (e.g. crystalline, amorphous) etc. The materials will also be fractionated in order to assess whether key contaminants (e.g. Cd, Zn, Cu, Pb) are associated with the organic or mineral phases. Metal lability/chemical reactivity in the samples will be investigated using a $CaCl_2$ extraction in combination with a multilabelled isotopic dilution technique. These analyses will provide a measure of the extractable and isotopically exchangeable metals which will be correlated with the chemical characterisation of the biosolids described above. On the basis of results from these analyses, biosolids with particularly interesting characteristics will be selected for further investigation using advanced spectroscopic techniques. In particular, the nature of aluminium oxides present in the samples will be studied using ^{27}Al Nuclear Magnetic Resonance (NMR) spectroscopy and the nature of the iron oxides will be probed via Mössbauer spectroscopy.

Results and Discussion

A collection of over 40 sludge samples is currently undergoing detailed characterisation for a range of both standard and non-standard chemical and physical parameters. These samples have been sourced from a wide range of WWTPs with differing influent characteristics and network scales, as well as varying unit treatment processes and plant design. Key results and differences between samples from these systems will be presented and discussed, providing insight into the variation in concentration and bioavailability of key metal contaminants in Australian biosolids from different geographical regions and catchment types. Advanced spectroscopic techniques provide an ideal means of characterising such samples, facilitating the detailed inspection of sludge constituents and their role as sorbents.

There are many potential sludge treatment processes that can be applied prior to land disposal but in order for the suitability of these options to be properly assessed further understanding of the range in existing properties in both raw and stabilised sewage sludges is needed. Currently, there is a paucity of information showing the true variability in metal sorption and fixation among different biosolids and sewage sludge products even though this can be expected to be an important regulator of bioavailability. Furthermore, although it is increasingly well recognised that total metal concentrations are not an accurate determinant of bioavailability based risk, lack of understanding of the key mechanisms controlling metal bioavailability in biosolids amended soils, particularly in the long term, slow the adoption of more suitable bioavailability based guidelines. Even good quality biosolids contain substantial quantities of metal contaminants, and a thorough understanding of their long-term fate when added to soils is clearly a necessity if risk assessments and measures for environmental protection are to be effective.

Conclusions

A clear understanding of the factors controlling metal bioavailability in biosolids and the potential for long term changes is paramount to appropriate risk management. Further research combining detailed chemical analyses and powerful spectroscopic methods are needed to provide this knowledge.

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Bioavailability of soil organic carbon and Fe as influenced by forestry practices in a subtropical coastal catchment

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Abstract
Potential impacts of plantation forestry practices on soil organic carbon and Fe available to microorganisms were investigated in a subtropical coastal catchment. The impacts of harvesting or replanting were largely limited to the soil top layer (0–10 cm depth). The thirty-year-old *Pinus* plantation showed low soil moisture content (Wc) and relatively high levels of soil total organic carbon (TOC). Harvesting and replanting increased soil Wc but reduced TOC levels. Mean dissolved organic carbon (DOC) and microbial biomass carbon (MBC) increased in harvested or replanted soils, but such changes were not statistically significant ($P > 0.05$). Total dithionite-citrate and aqua regia-extractable Fe did not respond to forestry practices, but acid ammonium oxalate and pyrophosphate-extractable, bioavailable Fe decreased markedly after harvesting or replanting. Numbers of heterotrophic bacteria were significantly correlated with DOC levels ($P < 0.05$), whereas Fe-reducing bacteria and S-bacteria detected using laboratory cultivation techniques did not show strong correlation with either soil DOC or Fe content.

Keywords
Subtropical catchment; forestry management; soil carbon; Fe biogeochemistry; soil bacteria.

Introduction
Previous research suggests that forestry managements can affect rates of elemental cycling, nutrient availability, as well as microbial community structure in soils by changing soil moisture content (Wc) and clay content, as well as pH and other physicochemical factors (Buzek, Paces *et al.* 2009; Frey, Kremer *et al.* 2009; Kara and Bolat 2008). Labile soil organic carbon indicators such as dissolved organic carbon (DOC) and microbial biomass carbon (MBC) are often used to assess soil quality, particularly soil organic carbon (SOC) changes, as they provide a relatively short-term response to changes in soil management, as compared with soil total organic carbon (TOC). This study was conducted in a subtropical coastal catchment undergoing active plantation forestry practices, e.g. harvesting and replanting. We aimed to determine the microbiological bioavailability of SOC in response to forestry practices by examining DOC and MBC in different soils. A regional concern is that potentially enriched SOC may promote Fe dissolution and subsequent mobilisation, either chemically or microbially, from catchment soils into the estuary ecosystem. Enhanced elemental cycling in such habitats can be driven substantially by biogeochemical processes in which microbial activities are major catalysts (Stemmler and Berthelin 2003). To assess the potential for Fe release from soils into the surrounding waterways, Fe (active, bioavailable and dissolved forms) was extracted and related bacterial populations were enumerated using laboratory cultivation techniques.

Methods
Study area and soil sampling
The Poona Catchment (150 km^2) is on a relatively flat coastal plain, located ca. 300 km north of Brisbane on the Fraser Coast of SE QLD (Australia), discharging into the Great Sandy Strait, an environmentally-sensitive estuarine habitat of national significance, as well as a UNESCO-listed world heritage area. The catchment has a high summer and low winter rainfall due to the subtropical climate. The annual average rainfall is 1270 mm. The mean monthly maximum temperatures range from 30.2ºC in Dec. to 21.5ºC in July. Presently, the dominant land-use is *Pinus* plantation forestry (58 km^2). Forest soils were sampled from three sites (ca. 30-year-old forested, OF; newly-harvested, NH; and newly-planted, NP) in the upper-catchment in Dec., 2008. Intact duplicate 30 cm push-cores were collected at each site using PVC tubing, and were sealed for transportation to the laboratory within two days. Fresh cores were separated into three segments (0–10 cm, top layer; 10–20 cm, mid layer and 20–30 cm, bottom layer) and homogenized before analysis. Replicate subsamples were processed for microbial analysis within two weeks. The remaining subsamples were stored at 4ºC for soil physicochemical analysis.

Soil physicochemical and microbial analysis

Soil physicochemical properties were determined as follows: soil Particle Volume Distribution (PVD) by laser particle sizing; soil Wc, gravimetrically on drying (105ºC, 24h); pH, redox potential (Eh) and electron conductivity (Ec) in 1:5 soil:water suspension using a TPS multiple field analyser (90-FMLV); soil dissolved nitrate, phosphate, sulfate and Fe by 0.01M $CaCl_2$-extraction (Houba, Temminghoff *et al.* 2000); TOC by Loss-On-Ignition (500ºC, 4h); DOC by cold water-extraction (Robertson, Coleman *et al.* 1999); MBC by chloroform-fumigation-extraction (Vance, Brookes *et al.* 1987); and total reactive Fe by dithionite citrate-extraction (Fe_{DC}), aqua regia-extraction (Fe_{aqua}), acid ammonium oxalate-extraction (Fe_{AAO}) and pyrophosphate-extraction (Fe_{pyro}) (Courchesne and Turmel 2007). For Fe_{CaCl2} and Fe_{AAO} extraction, anaerobic techniques were used (Phillips and Lovely 1986). Extracts were analysed within two weeks of extraction. For cultivation and enumeration of bacteria potentially involved in Fe cycling, selective enrichment media were used (Table 1). Bacterial numbers per gram soil were determined by a 1:10 dilution-to-extinction method. R2A medium was used to enumerate heterotrophic bacteria (colony-forming units, CFU) by the plate count method (HPC). Cultures were collected after 4 weeks incubation.

Table 1. Selective enrichment media used for cultivation and enumeration of Fe cycle-related bacteria

Medium	pH	Target bacteria	Reference
9K	2.5	Acidophilic Fe(II)-oxidising (FeOB)	Eaton, Clesceri *et al.* 2005
Liquid gradient medium	4.8	Neutrophilic, microaerophilic FeOB	Hanert 2006
Semi-solid gradient medium	6.3	Neutrophilic, microaerophilic FeOB	Emerson and Floyd 2005
Fe(III)-NO_3 medium	7.0	Anaerobic, nitrate-dependent FeOB	Widdel and Bak 1992
Fe(III)-EDTA medium	7.0	Neutrophilic Fe(III)-reducing (FeRB)	Gould, Stitchbury *et al.* 2003
Sulfur medium	4.8	Sulfur-oxidising (SOB)	Eaton, Clesceri *et al.* 2005
Thiosulfate medium	7.8	Thiosulfate-oxidising (TOB)	Eaton, Clesceri *et al.* 2005
MP liquid medium	7.0	Sulfide-oxidising (MPB)	Eaton, Clesceri *et al.* 2005
API medium	7.5	Sulfate-reducing (SRB)	Eaton, Clesceri *et al.* 2005

Data analysis

Data were subjected to GLM ANOVA, correlation and regression analysis using SPSS 16.

Results

Soil physicochemical properties

Based on PVD analysis (Table 2), all soils were classified as loamy sand. Soil Wc was low in site OF, i.e. 3.97–5.10% in soil profile. But at site NH and NP, soil Wc increased to 6.56–7.56% and 9.18–13.13%, respectively. All samples were weakly-acidic. The top layer soil pH at site OF was ca. 6.2 which decreased to ca. 5.1 and 5.3 at site NH and NP. In contrast, the pH in mid and bottom layer soils did not vary between treatments as much as the top layer soil. The Eh remained at 288–336 mV for all soils, indicating a well-oxidised environment regardless of forestry practice. Soil Ec did not respond to forestry practice. Dissolved nutrient analysis revealed low soil fertility at site OF. By comparison, dissolved nitrate in NH soils, increased markedly, but appeared to decrease in NP soils, displaying similar levels to those in mature forested soils. Dissolved phosphate was seldom detected, while sulfate appeared not to be limiting in Poona catchment.

Table2. Soil physicochemical properties

Site	Depth	PVD (%)			Wc	pH	Eh	Ec	Soil dissolved nutrients (µg/g)		
	(cm)	Clay	Silt	Sand	(%)		(mV)	(µS/cm^2)	NO_3-N	PO_4-P	SO_4-S
OF	0–10	6.8	14.4	78.8	4.0	6.2	288	17.0	2	0	26
	10–20	8.2	15.6	76.3	4.5	6.1	301	8.1	2	0	18
	20–30	8.8	18.3	72.9	5.1	6.0	312	7.7	2	0	27
NH	0–10	4.7	14.0	81.3	6.6	5.1	336	19.0	7	0.3	38
	10–20	5.8	13.6	80.6	6.9	5.5	325	8.6	5	0.3	28
	20–30	4.8	12.8	82.4	7.6	5.8	319	7.9	5	0	22
NP	0–10	2.9	14.5	82.6	13.1	5.3	309	7.2	3	1.6	17
	10–20	5.1	12.8	82.0	8.6	5.8	296	4.4	3	0.05	20
	20–30	7.6	13.4	79.0	9.2	6.1	327	4.9	3	0	16

SOC and Fe analysis

Soil TOC (Table 3) at site OF was ca. 2.7–2.9% with a uniform distribution in soil profile. However, TOC from NH (1.0–1.5%) and NP (0.7–1.9%) soil was significantly lower ($P < 0.05$). Soil available organic carbon, i.e. DOC and MBC peaked in top layer soils, 50–70 µg/g and 167–246 µg/g, respectively. DOC was

higher in both NH and NP soils as compared with OF soils. In contrast, MBC was highest at site NP, particularly in top layer soils. A positive correlation between DOC and MBC was shown ($F = 0.641$, $P < 0.01$, $n = 18$).

Table 3. SOC and extractable Fe (µg/g dry soil)

Site	Depth (cm)	TOC (%)	DOC	MBC	Fe_{DC}	Fe_{aqua}	Fe_{AAO}	Fe_{pyra}	Fe_{CaCl2}
OF	0–10	2.9	50	193	5031	3316	1735	1791	0.403
	10–20	2.7	18	174	6967	3280	2273	2069	0.253
	20–30	2.8	4	100	9450	5999	3698	3190	0.469
NH	0–10	1.5	70	167	4696	2445	665	798	0.469
	10–20	1.0	21	55	10478	3526	684	586	0.372
	20–30	1.0	14	35	7306	2452	618	706	0.423
NP	0–10	1.9	64	246	4534	1169	530	469	0.911
	10–20	0.7	11	102	5885	1709	704	619	0.534
	20–30	1.0	6	24	6634	4434	1069	1116	0.271

Soil total active (fine crystal, poorly crystalline and organically-complexed) Fe extracted with dithionite citrate (Fe_{DC}) yielded the highest concentration, ca. 1.5–4 times that of Fe_{aqua}, yet results from the two methods showed correlation at significant level ($n=18$, $F = 0.77$, $P < 0.01$). Both Fe_{DC} and Fe_{aqua} increased with depth instead of responding to harvesting or planting, whereas microbially bioavailable Fe (poorly crystalline and organically complexed, Fe_{AAO} and Fe_{pyra}) was more sensitive to forest practices. For example, at site OF, Fe_{AAO} ranged from 1735 to 3698 µg/g soil in the profile, but decreased markedly in NH soils (618–665 µg/g) and NP soils (530–1069 µg/g). In contrast, total dissolved Fe (Fe_{CaCl2}) was distributed in a different way. Fe_{CaCl2} was increased particularly in site NP, but such a difference was not statistically significant.

Fe biogeochemistry-related bacterial populations

Bacteria are important catalysts of soil organic matter decay and elemental cycling. In Poona catchment forest soils, heterotrophic bacteria were found at ca. 10^6 CFU/g dry soil (Table 4). Bacterial cell numbers were significantly correlated with DOC ($F = 0.63$, $P < 0.05$, $n = 18$), indicating that soil available organic carbon is a major controlling factor in the distribution of heterotrophs. Previous research also showed that soil biota tend to increase in the first few years after clear cutting (Paul and Clark 1996). Factors such as availability of dead roots for decomposition, greater susceptibility of residual litter to decomposition due to more favourable soil Wc, temperature, and regrowth of herbs or shrubs all contribute to the increased SOC.

Table 4. Bacteria cultivated from forest soils (log_{10} CFU/g or cell/g dry soil)

Site	Depth (cm)	Heterotrophic bacteria	Fe-bacteria				S-bacteria			
			$FeOB_3$[a]	$FeOB_7$[b]	$FeOB_{NO3}$[c]	FeRB	MPB	SOB	TOB	SRB
OF	0–10	6.1	ND[d]	ND	ND	6.5	3.5	1.0	1.5	ND
	10–20	6.0	ND	ND	ND	7.0	1.5	1.0	1.0	ND
	20–30	5.8	ND	ND	ND	7.5	2.0	0.5	1.0	ND
NH	0–10	6.6	ND	ND	ND	7.0	2.5	3.5	1.5	ND
	10–20	6.1	ND	ND	ND	7.0	1.5	2.5	1.0	ND
	20–30	6.4	ND	ND	ND	6.5	2.0	1.5	1.0	ND
NP	0–10	6.2	ND	ND	ND	9.0	2.0	4.5	1.5	ND
	10–20	5.8	ND	ND	ND	6.0	1.0	3.0	2.5	ND
	20–30	5.6	ND	ND	ND	5.5	1.0	2.5	2.0	ND

a. acidophilic FeOB; b. neutrophilic, microaerophilic FeOB; c. anaerobic, nitrate-dependent FeOB; d. not detected.

As the soil pH ranged from 5.1–6.2, it was not surprising that no acidophilic FeOB were detected through laboratory cultivation. However, neither neutrophilic FeOB (microaerobic or anaerobic, nitrate-dependent) were detectable in soils using a variety of selective enrichment media. In contrast, neutrophilic FeRB ($> 10^5$ cell/g dry soil) were found in all soils, suggesting that over the duration of the sampling period, microbial Fe(III) reduction coupled with chemical Fe(II) oxidation dominates Fe biogeochemistry. FeRB numbers in top layer soils followed the order NP > NH > OF (Table 4), but did not respond to forestry practices in mid or bottom layer soils. No significant correlation was found between FeRB and extractable Fe of any kind. As the soil pH was weakly acid (ca. 5.5 or less), the Fe(III) concentration may not be limiting to FeRB. A differentiation of Fe species and bacterial quantification by molecular techniques would help to further describe FeRB distribution.

Fe turnover in oxic-anoxic transition zones with circumneutral pH may be highly active. In our case, dissolved Fe(II) in near neutral pH soil solutions may be rapidly, abiotically oxidised, while subsequently-produced amorphous Fe(III) compounds can be utilised immediately by FeRB as a preferred substrate. S-bacteria, particularly SRB, are often involved in Fe cycling in natural habitats (Lovley 2006). Surprisingly, three groups of S-bacteria capable of oxidising sulfide, sulfur or thiosulfate were detected in many soils but no SRB were detected (Table 4). Since sulfate appeared not to be limiting in this area, the absence of SRB may be attributed to the highly oxidised soil conditions. The coexistence of FeRB and diverse SOBs in the absence of FeOB or SRB implies a complex soil-Fe-bacteria ecosystem in which abiotic and biotic processes are interacting. Further DNA-based analysis (in progress) is required to identify the specific organism associated with these cultures.

Conclusion

In Poona catchment, harvesting and replanting of *Pinus* plantation forests affects soil pH, Wc and other physicochemical properties. Such impacts are largely limited to the top soil layer. Soil TOC was decreased in NH and NP soils, whereas soil DOC and MBC were increased. In contrast, total active Fe (Fe_{DC} and Fe_{aqua}) did not respond to forestry practices, while bioavailable Fe (Fe_{AAO} and Fe_{pyra}) decreased markedly in NH and NP soils. The numbers of heterotrophic bacteria were correlated with soil DOC content, but neither culturable Fe-reducing bacteria nor S-bacteria were correlated with SOC or extractable Fe of any kind. Nevertheless, NH or NP soils appeared to harbour higher numbers of these bacteria, indicating a more active soil-Fe-bacteria ecosystem. Our study detected changes in the bioavailability of SOC and Fe due to forestry practices, which further affected microbial populations potentially involved in Fe biogeochemistry.

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Bioavailability of trace metals in sediment cores from sunderban mangrove wetland, india: An urgent need for bioremediation

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Abstract
The paper attempts to identify the enrichment pattern of acid leachable trace metals (ALTMs) such as Fe, Mn, Cr, Cu, Ni, Pb, Cd, Co, Ag and As and their relationship with sediment quality parameters (pH, organic carbon and texture) in core sediments (<63µm particle size) from Indian Sunderban mangrove wetland, India. The results indicate that the change in pH values causes coagulation and precipitation of ALTMs. Fe and Mn have fairly close distribution patterns of enrichment in surface layers which might be ascribed to early diagnetic processes. The most prominent feature of ALTMs is the enrichment of Fe, Mn, Cr, Cu, Ni and Pb in the surface-subsurface layers in the sediment cores, which is mainly attributed to the intense industrial and agricultural activities as well as drainage of domestic sewage to this coastal region. The ALTMs also indicate their association with organic carbon and Fe-Mn oxyhydroxides. The enrichment is well- supported by the correlation, grouping and clustering of ALTMs in statistical analyses. Anthropogenic Factor (AF) values indicated ALTMs enrichment for all heavy metals due to intense anthropogenic activities. The result suggests the urgent need for phytoremediation using mangrove plants as remediation strategy to decontaminate the sediments contaminated with inorganic contaminants.

Key Words
Acid leachable, phytoremediation, enrichment, Indian Sunderban

Introduction
Sediments are the important component of ecosystem in which toxic compounds accumulate through complex physical and chemical adsorption mechanisms depending on the properties of the adsorbed compounds and the nature of the sediment matrix (Leivouri 1998). The leaching of metals provides an accurate measure of the bioavailable metals in any aquatic environment which are often readily available to organisms affecting them directly. Hence assessment of trace metal enrichment in sediments on the acid leachable (non-residual) elements is of prime interest, as it often yields more data on the extent of trace metal enrichment than the total sediments, which include the residual or non-residual fraction, and so may mark the relationships sought.

Methods
Selection of Sampling sites, collection of samples and preservation
The Indian Sunderban ($21^0$31'6" to $22^0$12' 14" N and $88^0$11' 28"to $89^0$05'53" E), formed at the estuarine phase of the Hugli river of an area of ~ 9600 km^2, is a mangrove wetland belonging to the low-lying coastal zone. Six sampling sites were selected, namely, Lot 8 (S_1), Gangasagar (S_2), Jharkhali (S_3), Gosaba (S_4), Canning (S_5) and Dhamakhali (S_6). The sites have diverse human interferences with a variable degree of exposure to heavy metal and trace organic contamination. Core samples were collected from the six selected sites with the help of a steel corer (40 cm in length and 5 cm in diameter) which is gently pushed into the sediments and retrieved back in sealed position. They were transported in frozen conditions (- 4° C) to the laboratory. The samples were oven dried (40° C) and were disaggregated using an agate mortar and pestle, sieved through 63µm sieve, which was stored in pre-cleaned inert polypropylene bags for further chemical analyses.

Analytical methods
The extraction of acid leachable metals was done by weighing 5 g of dry sediment sample in a 100 ml plastic bottle in which 75 ml of 0.5 N HCl was added and after mechanically shaking for 16 h it was filtered with Whatman 'A' filter paper. The final filtered solution was analyzed for ALTMs in ICP-MS. High purity standards (NIST, USA) were used and standard solutions were prepared. The accuracy of the analysis was determined by standard addition method and the recovery of elements was 75-97%. A standard reference material MAG1 was used to ensure the quality control and accuracy of the analysis. All statistical analyses were carried out by the software package STATISTICA 6.0.

Results

Regarding textural composition, the four stations (S_1 to S_4) show variable admixture of sand, silt and clay with an overall size range from sandy to clayey very fine. This wide array of textural differences may be attributed to vigorous estuarine mixing, suspension-resuspension and flocculation-deflocculation processes. The pH values of core samples are mainly basic in nature (pH from 8.1 to 8.9). Organic carbon (OC) values are very low (0.18% - 3.52 %) which might be due to the mixing processes and marine sedimentation at the sediment water interface, where the rate of delivery, as well as the rate of degradation by microbial-mediated processes, can be high (eg. Canuel and Martens 1993). Fe and Mn have fairly close distribution patterns of enrichment in surface/subsurface layers (~0-8 cm) in sediment cores (Fe: 3937-5201 mg/kg; Mn: 300-615 mg/kg) at all the stations (excepting S_2 and S_5) which might be due to the early diagnetic processes as well as the strong association to the geochemical matrix between the two elements. The distribution pattern of seven ALTMs (Cr, Cu, Ni, Pb, Ag and As) exhibits variations between sites and depths in the core samples which is ascribed to the metal deposition in mangrove sediments through natural processes as well as anthropogenic activities. Peak values of Cu, Ni, Pb, Ag and As in Dhamakhali (S_3) (at 32-36 cm depth) indicate scavenging of trace metals by Fe and Mn oxyhydroxides and are deposited as metal sulphides with a common source of (Prohic and Kniewald 1987. Distribution of Cr concentrations in the sediment core indicates higher values in top layers (0-8 cm) at all six sites which suggest that it is present as Cr (VI), which is readily adsorbed by Fe and Mn oxides (Davis *et al.* 1996), is relatively mobile and after release in the pore waters, they migrate downward into the reducing zone and precipitates again as Cr $(OH)_2$ (Shaw *et al.* 1990). Vertical profiles of Cu indicate the relatively high acid-leachable values in the top layer in (S_1) (31.5 to 48.6 mg/kg), (S_4) (36.7 to 51.6 mg/kg) and (S_6) (48.9 to 69.8 mg/kg). This is due to the presence of humic-copper complexes and indicates the presence of anthropogenic input under reducing conditions . Like Cu, a similar trend of enrichment of Ni was also observed in the top layer (0-8 cm) at same three sites. This indicates that it is also due to the presence of a mobile fraction of these metals in sediments successively bound to humic acids in the mangrove sediments. Profiles of Pb in the surface layers (0-8 cm) show relatively higher concentrations at Canning (S_6) (19.6 to 21.2 mg/kg) than Jharkhali (S_4) (16.5 to 17.3 mg/kg) and are attributed to the local redox conditions, which allowed Pb to be co-precipitated with Mn during Mn-oxide formation in the surficial sediment. Likewise, the levels of Cd, Ag do not vary greatly in the core profiles of all the six stations which might be due to homogenous input of this metal in the wetland system. The sources of As stem from anthropogenic activities like intense exploitation of ground water, application of fertilizers and insecticides as well as burning of coal for domestic purposes. The concentration of As and Cu exceeded the Effects-Range Low (ER-L) values of (8.2 and 34 µg/g respectively, Long *et al.* 1995) indicating that there may be some ecotoxicological risk to the benthic organisms. The cluster diagram based on the linear pair coefficient pair of correlation between different variables of six different core samples forms two different clusters (as shown in Fig. 1) : elemental association with sand and organic carbon (cluster I) and association with mud, carbonate and pH (cluster II). The association of Ni, Pb, Cu, Mo, Ba, As (contaminant elements) in cluster I clearly suggests that they have a common origin in the aquatic environment. The higher values of anthropogenic factors (AFs) (>1) in all the core samples indicate that the area is affected by the heavy input of industrial effluents from the industries situated in the upstream side of the feeding rivers in the mangrove region.

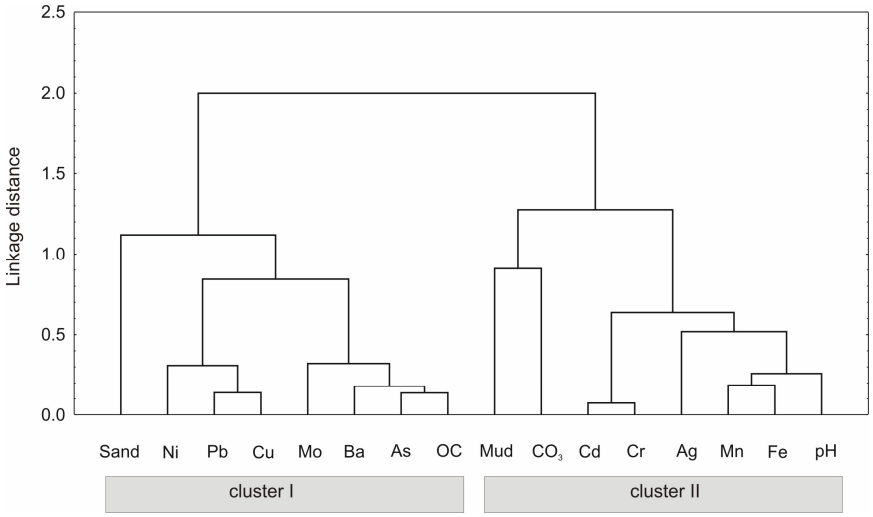

Figure 1. Results of cluster analysis based on complete linkage method for core samples in Sunderban wetland.

Conclusion

The work presents comprehensive data base of ALTMs in core sediments of Sunderban mangrove wetland highlighting the geochemical processes concerned with the differences in distribution patterns. The results indicate that ALTMs are trapped in the mangrove sediments due to the change in pH conditions at various sites and the reduction of organic carbon and carbonates in the mangrove region. The down core profile distribution of ALTMs also suggests that Fe, Mn enrichment is due to the diagnetic behaviour of the metal. The authors strongly recommend phytoremediation as potential strategy using mangrove plants as excluder species for nonessential metals and regulators of essential metals.

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Bioavailability, causation and correlation: can we really conclude the herbicide diuron resulted in mangrove dieback in river estuaries of Central Queensland?

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Abstract
Statistically significant correlations in the form of dose-response relationships can provide valuable evidence to support the case that a particular stressor has caused an environmental impact. However, the selection of a parameter representing the dosage requires careful consideration if meaningful assessments of causation are to be made. Application of the concept of bioavailability can provide a valuable tool for parameter selection. This approach is applied to the proposition that the herbicide diuron is implicated in mangrove dieback in river estuaries of Central Queensland.

Key Words
Bioavailability, Correlation, Causation, Herbicide, Sediment, Mangrove.

Introduction

Runoff of agricultural chemicals in catchment areas adjacent to the Great Barrier Reef (GBR), particularly pesticides (Lewis *et al*. 2009) and nutrients (O'Reagain *et al*. 2005) has been a major concern over the past decade. These contaminants have been associated with detrimental impacts on various organisms, particularly mangroves (Duke *et al*. 2003, 2005), seagrass (Haynes *et al*. 2000) and coral (Jones *et al*. 2003). Herbicide residues have been detected in waterways of the GBR catchment area (Mitchell *et al*. 2005) as well as in intertidal and subtidal sediments (Haynes *et al*. 2000). However, it is wrong to conclude that the mere presence of a chemical in the environment causes harmful biological impacts to organisms. This paper demonstrates the importance of considering basic knowledge of bioavailability to avoid erroneous conclusions regarding causation.

Causation and correlation

Causation does not necessarily follow from a correlation between an observed biological impact and the presence of a particular stressor in the environment (Beyers 1998). Nevertheless, correlations are often useful indicators of causation, particularly when used in combination with other criteria (Adams 2003). Where chemical stressors are implicated, the correlations examined will usually take the form of a dose-response relationship. This in turn requires careful consideration of the concept of bioavailability.

Bioavailability, and selecting an appropriate dose parameter

A generalised representation of bioavailability processes in a soil or sediment is shown in Figure 1. This can be applied to the case where the putative environmental stressor is an organic contaminant (X). Process A represents partition of X between solid soil/sediment surfaces and its dissolution in the aqueous phase. Process B represents direct uptake of the sorbed X by the organism from the solid, whereas process C represents direct uptake of dissolved X from solution. Bioavailability of X will depend on the organism, and the appropriate dose parameter will depend on whether process B, or C or a combination of B and C are important. This is illustrated by considering uptake of X by three different organisms – (i) soil microbes; (ii) earthworms; and (iii) plants, in the context of processes illustrated in Figure 1.

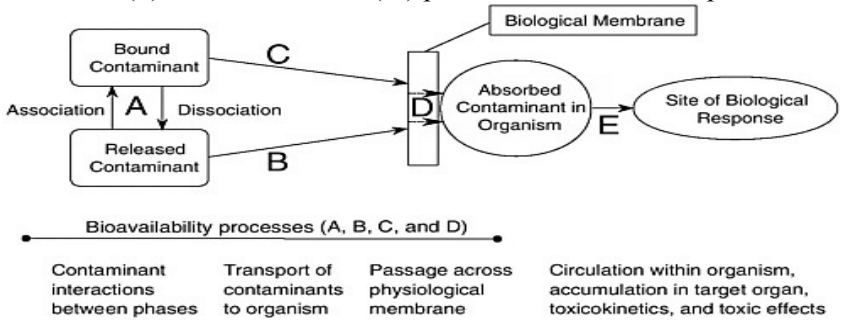

Figure 1. Bioavailability processes in soil or sediment. (Committee on Bioavailability (2003). Note – 'Bound Contaminant' in this reproduced figure is equivalent to "sorbed contaminant' as discussed in the text.

Soil-sorbed organic contaminants are generally considered unavailable for microbial biodegradation without prior desorption (Ogram *et al*. 1985; Yang *et al*. 2006). However, evidence suggests that some soil-sorbed contaminants can be degraded by specific microorganisms or at least that desorption into bulk solution is not a prerequisite for biodegradation (Guerin *et al*. 1992; Tang *et al*. 1998). Consequently, for microbes, process C is generally dominant for uptake of X, but process B can also be important in exceptional cases.

A distinction can be made between "bioavailability" and "bioaccessibility" (Semple *et al*. 2004). Bioavailability is defined as material freely available to cross an organsm's cellar membrane from the medium an organism inhabits at a given time. Where a constraint is imposed in time/space, the material may be considered bioaccessible but not bioavailable.

Only the chemical dissolved in the soil solution is thought to be environmentally bioavailable to the earthworm for dermal uptake (Belfroid *et al*. 1996). However, earthworms can also ingest soil with X sorbed on solid surfaces, subsequently subjected to the chemical conditions present in the animal's gastrointestinal tract (Lanno *et al*. 2004). Therefore both processes B and C will generally be important for earthworms.

For plants, it has long been established that the bioavailability of organic chemicals associated with soils and sediments, including many common herbicides, depends primarily on the uptake of the dissolved organic molecule in the aqueous phase in the root zone (Pillay and Tchan 1971; Boesten 1993). This has been demonstrated for uptake of simazine by oat and cotton seedlings (Sheets 1961), uptake of atrazine by wheat plants (Walker 1972), and for carrot, parsnip, lettuce, and turnip seedlings (Walker and Featherstone 1973), and more recently for accumulation of atrazine within the shoots of rice seedlings (Su *et al*. 2007). Consideration of bioavailability therefore shows process C, but not process B, will be important for uptake of X by the root system of a plant. This, in turn, tells us that the concentration of X in solution, not the concentration of X sorbed on solid surfaces of a soil or sediment should be used to represent the dose parameter.

A case study – diuron and mangrove dieback

Mangrove dieback has been reported in the river estuaries of Central Queensland, following major flooding events in 1998 in the Pioneer River and 2008 in the Fitzroy River, mainly affecting the species *Avicennia marina*. These events were widely reported in the media, with some researchers suggesting that the cause of the dieback was related to the herbicide diuron, used in production of sugar cane. However, when dose-response relationships are examined, as illustrated in Figures 2A and 2B, there is no statistically significant correlation. Using this dose parameter, based on consideration of bioavailability, there are no correlations to support the claim that diuron has caused the dieback. Furthermore, it is interesting that Wake's data deals with much higher concentrations of diuron reporting 100% healthy trees at 60 ng/litre diuron, but Duke's maximum concentration is less than 15 ng/litre. This finding is in contrast to the conclusions previously drawn where bioavailability was not considered, and only process B was considered relevant (Duke *et al*. 2003). This is illustrated in Figure 3 where the dose parameter applied was the concentration of diuron sorbed on the sediment.

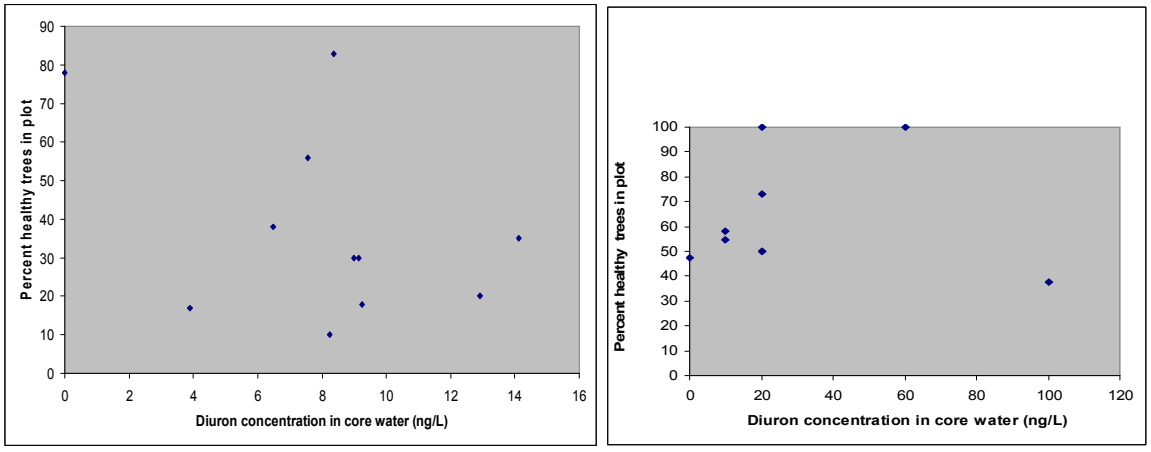

Figure 2. Percentage of healthy mangroves plotted against concentration of diuron in root zone core water at sites in CQ river estuaries. A: in 2002 (Duke *et al.* 2003) (r = -0.401); B: in 2004 (Wake 2005) (r = 0.025).

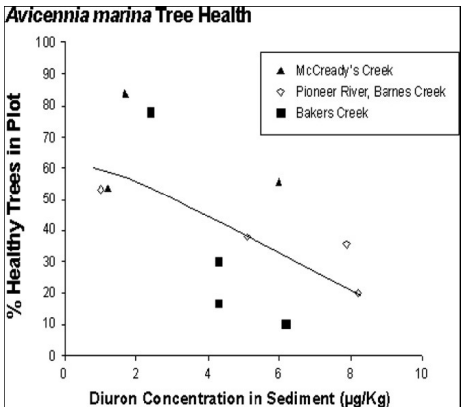

Figure 3. Percentage of healthy mangroves plotted against concentration of diuron sorbed on sediments at sites in Central Queensland river estuaries in 2002. Reproduced from Duke (2008) (r = -0.6544).

The correlation reported by Duke (Duke *et al*. 2003, 2008; McKillup 2008) is not useful for establishing causation. The dose parameter does not take account of the dominant process whereby diuron uptake occurs via the mangrove roots. There is no evidence in the literature that mangroves can directly uptake sorbed diuron, as in the case of microbes (Guerin *et al*. 1992; Tang *et al*. 1998), nor ingest sediments as in the case of earthworms (Lanno *et al*. 2004). Moreover, it is important to understand that there is no direct proportionality between diuron concentration in root zone solution and herbicide sorbed on sediments where samples from different locations are considered, as in Figure 3. Differences in sediment organic content, (Walker 1972) mineral composition (Gilchrist 1993) and moisture content (Lambert 1966) at different locations are well documented for mangrove sediments (Duke *et al*. 2003; Wake 2005) and rule out any simple concentration proportionality.

Conclusion

A consideration of bioavailability is important before selecting a relevant dosage concentration parameter if the objective is to examine correlations for dose-response relationships in order to support or refute evidence for causation. For the case study presented, application of this principle shows that causation of mangrove dieback by Diuron in the river estuaries of Central Queensland is not supported.

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Biological indicators of soil quality in area of lead mining and metallurgy, Paraná State, Brazil.

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Abstract
This work aimed to determine heavy metal contents in soil and native plants and to evaluate microbial and fauna activities in soils of the Pb mining and metallurgy area, to establish the biological indicators of these soils qualities. The collections were made at five different locations (sites 1, 2, 3, 5, 6), in four seasons. Fertility and physical analysis and total (HF, HNO_3, H_2O_2 microwave extraction) and bioavailable (HNO_3 0.5 mol/L boiling extraction) Pb, Cu, Ni and Zn contents were measured for soil samples. Samples of native plants were collected and after nitric-perchloric digestion, total heavy metal contents were determined. The microbial and mesofauna studies in the soil were done for the depth of 0 to 5 cm. Total heavy metal contents were determined in Formicidae group (HNO_3 microwave digestion). The maximum total and bioavailable soil Pb contents were at site 3 (25,930 and 15,370 mg/kg, respectively). The alteration of organisms of the soil's mesofauna were more evident than for microbiology activity: site 1 (reference - low and natural soil Pb levels) presented the highest mesofauna diversity. The Pb levels in the Formicidade group and in plants were consistent with the contents of Pb in the soils.

Key Words
Heavy metals, soil bacteria and fungi, soil microbiology metabolism, soil mesofauna diversity, plant toxicity.

Introduction

In Adrianópolis, Paraná State, Brazil, after mining and Pb metallurgy for almost 60 years, about 177,000 t of rejects were left on the soil. In previous studies, the dissemination of contamination was observed through the high metal levels in the blood of people that lived around the area (Cunha 2003). These Pb mining and metallurgy activities caused visible impacts on the environment, such as intense erosion and great volume of rejects dispersed on the soil.
Microorganisms are the most numerous organisms in the soil biological fraction and are dependent on changes in the quantity contaminants and metabolism relations due to environmental changes, such as pollutant transportation to the soil (Doelman *et al.* 1994). The influence of soil management or contaminant addition to the soil, usually induce a quicker response from soil mesofauna than for other pedogenic attributes, making these organisms good environmental quality indicators (Lanno *et al.* 2004).

Methods

Physical and chemical assessments
Heavy metal content and some physical and chemical characteristics (fertility, field capacity and texture) were determined (Lim and Jackson 1986, with adaptations), to assess soil quality, through the chemiometric Principal Component Analysis (PCA). Soils (0 to 5 and 5 to 10 cm) from 5 locations, in 4 seasons of the year, were sampled in Adrianópolis, Paraná State, Brazil, with the following characteristics in regard to the contamination forms (Figure 1): site 1 – reference (native vegetation); site 2 – incorporated residue in the profile; site 3 – next to one of the factory's chimneys, with potential transport of the particulate matter; site 5 – greater reject volume on the soil; site 6 – similar conditions to site 3, but with sandy textured soil. The total Pb, Cu, Ni and Zn contents were determined through ICP-AES, after digestion of the soil samples with concentrated HNO_3, HF and H_2O_2 in microwave. To extract the heavy metals in bioavailable forms, a boiling HNO_3 0.5 mol/L solution was used.

Microbiological assessments
The following soil microbiology parameters were estimated (Wollum II 1982): Total Bacteria (TB); Sporulating Bacteria (SB); percentage of Sporulating Bacteria comparing to TB (SB%); Fungi (FG); ratio between FG/TB; Microbiology Respiration in the time 1 (5 days) (MR1) and 2 (10 days) (MR2); Microbiology Carbon Biomass (MCB); Microbiology Carbon Biomass percentage comparing to the soil total

organic carbon (MCB%); Metabolic Quotient in 5 days (qCO_2a) and in 10 days (qCO_2b). Three methods were used in statistical analysis i) determination of an Environmental Quality Index regarding to microbiological parameters (EMQI); ii) differentiation of the sites through Principal Component Analysis (PCA); and iii) simple correlation analysis.

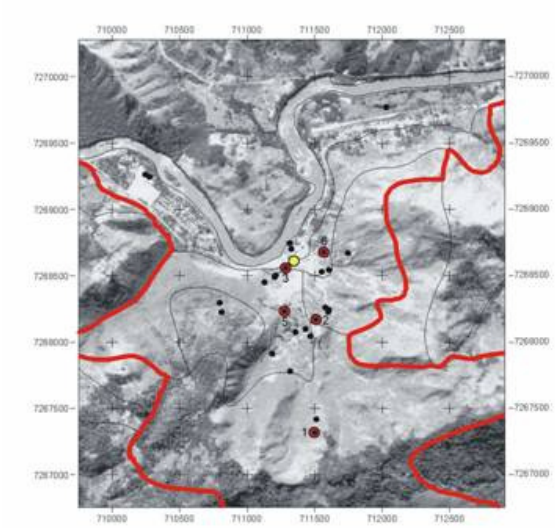

Figure 1. Aerial photo with the sampled points (1, 2, 3, 5 and 6 - red) and waste (yellow) locations (the red line delimits the mining influence area). Note that the Ribeira River is close to the wastes.

Mesofauna and heavy metals in native plants assessments

Soil samples were collected with Berlese funnel the depth 0 to 5 cm (20 funnels x 5 sites x 1 depth x 4 seasons = 400 samples). After the mesofauna separation, the selection and identification of the organisms (21 distinct groups) were accomplished. Individuals of the Formicidae group were digested with concentrated HNO_3 in microwave and the Pb, Cu, Ni and Zn contents were determined through ICP-AES. Plants from *Poaceae* family were collected in all sites and after digestion by nitric-perchloric method, the Pb, Cu, Ni and Zn contents were also determined. The same statistical treatments were employed.

Results

The maximum total and bioavailable soil Pb contents were, respectively, 25,930 and 15,370 mg/kg. The reject incorporation to the soil profile elevated Pb solubility, causing a lesser difference between the total and the bioavailable content in site 2 than in site 5. The sites 3 and 5 showed higher contamination risk: site 5 - samples with higher total Pb contents, associated to the pronounced area declivity and high Ribeira river water contamination risk by erosive process; site 2 - samples with higher bioavailable Pb contents, associated with the proximity to the hydrostatic level and leaching contamination risk. Site 5 present plants growing restrictions, where the soil samples were grouped (PCA) due to the low fertility and clay content, that can potentially cause erosion in this area. The high capacity of exchangeable of soil capacity and pH in water (above 7.5) and the high clay content reduced the solubility Pb forms in site 3.

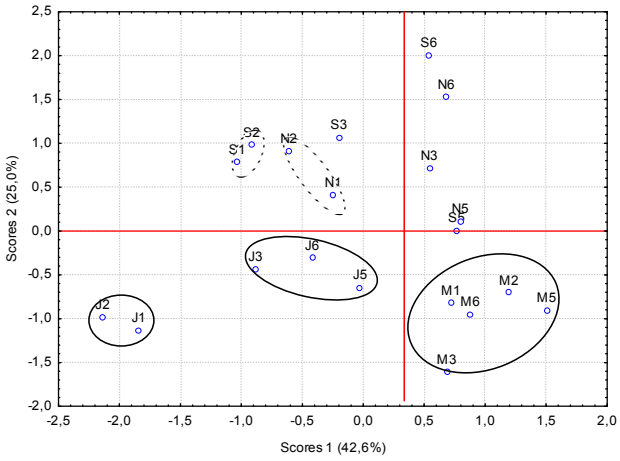

Figure 2. Principal Component Analysis (PCA) of the soil samples (0 - 5 cm), based on the microbiological characteristics, indicating similar sample groups. The notation represents the sampling month and the site number.

The most favorable temperature and humidity conditions, associated with the high soil fertility, caused the heavy metals prejudicial effects to have less effect on the microorganisms. However, in the month with the highest climatic stress to the organisms (May – lowest temperatures), the soils with the highest Pb content showed reduced populations and microbiology activity (Table 1), meaning that in these conditions the bacteria and fungi were good soil quality indicators. The highest Sporulation Bacteria (SB) proportion in the most Pb contaminated soils in the coldest month (0.95* correlation for May) can be interpreted as a resistance mechanism of these organisms. The Environment Microbial Quality Index (EMQI) in the 0 to 5 cm soil layer (Table 2) was more efficient than PCA in distinguishing the locations with heavy metal contamination. The climatic conditions (sampling month) were more important in soil grouping in the PCA graphic (Figure 2). Excepting site 6 (low soil fertility and clay content), the EMQI values decreased in the opposite sense to the increasing soil Pb content. However, in the 5 to 10 cm layer, the PCA was more efficient to this purpose. The total organism number from the 21 identified groups and the Environmental Mesofauna Quality Index were not good indicators of the soil heavy metal contamination level. The quantity and distribution of isolated species were more efficient to this purpose. The best environmental quality from site 1 was evidenced by the major diversity of organism groups and occurrence of the Pseudoscorpiones, Mollusca and Isopoda groups only in this soil. The Arachinida and Psocoptera groups were also considered good environmental indicators, with an increase of their populations in sites with higher heavy metals content (sites 2, 3 and 5), possibly because of the lesser occurrence of competitors/predators organisms. The heavy metal content in the individuals from the Formicidae group (site 1 - 11.5 mg/kg; site 2 - 70.5 mg/kg; site 3 - 84.6 mg/kg; site 6 - 13.4 mg/kg) had a direct relation to the soil's bioavailable Pb content. In regard to the accumulation of heavy metals in native species, with the exception of site 1 (Table 3), all plants showed the phytotoxic effect of Pb, which suggests the prohibition of pasture as a land use in the area. The higher Pb levels were detected in the roots, especially in location 3, which presented the higher bioavailable Pb contents in the soil.

Table 1. Microbiology analysis of 0 - 5 cm soil samples (the terms are explained in the Methods section. FCU - Formation Colonies Units, C - carbon. nd - noun determined parameter by analytical problems)

Sampling month	Site	Microbiology count					Microbiology respiration		MCB	MCB%	qCO_2a	qCO_2b
		TB	SB	FG	SB%	FG/TB	MR1	MR2				
		FCU/g of soil					mg C-CO_2/ kg/h		mg C/kg			
May	1	9.52	2.78	0.89	2.92	0.009	0.79	0.58	nd	nd	nd	Nd
	2	0.80	0.74	0.05	9.25	0.006	0.71	0.46	nd	nd	nd	Nd
	3	2.70	8.94	0.24	33.11	0.009	1.31	0.78	nd	nd	nd	Nd
	5	0.76	2.16	0.24	28.42	0.032	0.13	0.14	nd	nd	nd	Nd
	6	6.66	4.64	1.70	6.97	0.026	0.51	0.34	nd	nd	nd	Nd
Sep.	1	15.86	1.17	0.69	0.74	0.004	1.49	1.19	571.6	0.98	2.60	2.08
	2	6.79	1.06	1.26	1.56	0.019	1.29	1.05	426.6	0.83	3.03	2.46
	3	3.58	0.20	1.53	0.56	0.043	0.71	0.55	366.6	0.73	1.94	1.49
	5	0.91	1.60	0.29	17.58	0.032	0.17	0.14	257.2	0.97	0.67	0.53
	6	0.42	0.02	1.30	0.48	0.310	0.16	0.13	86.9	0.44	1.84	1.54
Nov.	1	1.97	1.07	0.73	5.43	0.037	1.11	0.84	873.0	1.63	1.27	0.96
	2	2.07	0.66	2.91	3.18	0.141	1.37	1.06	803.0	1.60	1.70	1.32
	3	0.27	0.34	0.34	12.39	0.124	0.37	0.31	296.5	0.60	1.25	1.06
	5	1.51	1.08	0.52	7.16	0.034	0.07	0.07	383.0	2.77	0.18	0.17
	6	0.88	0.06	2.75	0.65	0.314	0.12	0.12	181.2	1.12	0.67	0.67
Jan.	1	34.53	64.94	23.54	18.81	0.068	1.67	1.44	1158.4	1.42	1.44	1.24
	2	56.72	77.05	10.70	13.58	0.019	1.84	1.64	877.1	2.08	2.10	1.87
	3	37.37	15.08	9.52	4.03	0.025	0.75	0.61	690.4	1.20	1.08	0.88
	5	10.30	14.60	1.15	14.17	0.011	0.29	0.25	903.2	4.65	0.32	0.28
	6	23.96	9.54	8.72	3.98	0.036	0.36	0.31	521.6	1.95	0.69	0.59

Table 2. Environmental Quality Index based on microbiological parameters (EMQI) (Numbers in parenthesis in the last column represent the median soil total Pb contents (mg/kg) of the 4 months)

Local	May	September	November	January	Media
1	3.3	26.9	32.0	55.4	29.4 (654.4)
2	1.5	21.0	30.8	52.3	26.4 (6,889.3
3	3.8	16.0	11.9	32.3	16.0 (15,437.5)
5	0.7	9.7	13.1	33.3	14.2 (20,949.3)
6	2.5	5.4	7.1	23.0	9.5 (845.8)

Table 3. Pb contents (mg/kg) of the native plants (sites 1 and 3 - *Panicum maximum*; sites 2 and 5 - *Paspalum notatum*; site 6 - *Pennisetum purpureum*)

Sampling month	Site				
	1	2	3	5	6
	Shoot				
September	16.89	182.04	171.39	575.53	47.15
November	19.86	90.02	126.05	512.16	102.55
January	nd	3.06	13.28	211.35	nd
	Root				
September	22.65	99.80	939.96	376.07	164.90
November	12.67	122.90	733.68	420.88	129.96
January	24.14	74.04	414.72	436.45	168.17

Conclusion

In the months with the highest climatic stress to the organisms (May and September – lowest temperatures), the soils with the highest Pb content showed lesser population and microbial activity, meaning that in these conditions, the bacteria and fungi were good soil quality indicators. The highest sporulating bacteria proportion in the most Pb contaminated soils in May is attributed to the resistance mechanism of these organisms. The best environmental quality from site 1 (natural Pb levels - reference soil) was evidenced by the major diversity of organism groups and the occurrence of the Pseudoscorpiones, Mollusca and Isopoda. The first group is recognized in the literature as sensitive to heavy metal in soil.

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Changes in Cd bioavailability in metal spiked soils amended with biosolids: results from a wheat seedling bioassay

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Abstract
Three contrasting soils were collected from the Canterbury region in New Zealand and treated with a one-off application of Cd (1, 5 and 10 mg/kg applied as sulphate), in the presence and absence of biosolids applied at a rate equivalent to 400 kg N/ha. Soils were then incubated for two weeks and 24 weeks at a constant temperature of 25 ± 2 °C in the absence of light. A seedling bioassay, using wheat (*Triticum aestivum*) was undertaken to assess changes in plant Cd concentrations and soil solution Cd chemistry during incubation. Six measurements of Cd availability were also employed to determine which test yielded the strongest correlation with plant Cd concentrations. Overall, Cd concentrations in shoots were significantly reduced on average by 30% in plants grown in Cd spiked soils amended with biosolids compared to unamended soils, even though no significant changes were detected in measures of Cd solubility. Of the six methods examined, $Ca(NO_3)_2$ extraction yielded the strongest correlation with plant Cd, and a comparison between relationships determined for biosolids amended and unamended soils revealed no significant difference ($p = 0.625$). Consequently, this result provides evidence that Cd bioavailability as measured by $Ca(NO_3)_2$ is not altered in the presence of biosolids.

Key Words
Plant uptake, Cd availability, soil amendments.

Introduction
In New Zealand, biosolids are applied to land as an alternative means of waste disposal providing many benefits including increased soil fertility and productivity. Additionally, biosolids have been applied to metal-contaminated soils to assist in metal sequestration, rendering the metals less available to plants and other soil biota. For plants and soil biota, metal availability is controlled by numerous biogeochemical processes. These processes are strongly influenced by soil properties such as amounts of organic matter present, soil pH, clay and oxide content. For many soil biota the bioavailability of a metal depends on the concentration and chemical form (species) in the soil solution, particularly simple metal ion concentrations, (e.g. Cd^{2+}). To be able to accurately assess metal bioavailability has been the focus of much research, although few studies have compared metal bioavailability across soils in presence and absence of biosolids amendments. This study attempts to compare the effects of soil amendments, such as biosolids has on the validity of potential measures of Cd bioavailability, using contrasting soil types spiked with increasing amounts of Cd salts.

Methods
Three contrasting soils were collected from the Canterbury region in New Zealand and then treated with a one-off application of Cd (1, 5 and 10 mg/kg, applied as sulphate), in the presence and absence of biosolids applied at a rate equivalent to 400 kg N/ha. These treated samples were wetted to field capacity and then incubated for two weeks and 24 weeks at a constant temperature of 25± 2 ° C in the absence of light. A seedling bioassay, using bread wheat (*Triticum aestivum*) was undertaken to assess changes in plant Cd concentrations and soil solution Cd chemistry during the 24 weeks of incubation. Six measures of Cd solubility, total-Cd, EDTA and $Ca(NO_3)_2$-extractable Cd, soil solution Cd, effective solution concentration (DGT-DIFS) and Cd^{2+} activity modelled using WHAM 6.0., were compared with plant Cd concentrations to determine which method gave the best predictive measure of Cd bioavailability. A linear regression with grouped data was also performed to determine the effects of biosolids amendment on the validity of these potential measures of bioavailability.

Results and discussion

Comparisons between biosolids amended and unamended metal spiked soils revealed significant increases in concentrations of dissolved organic carbon (DOC), soil solution salinity, and Ca^{2+} and Mg^{2+} ions in soil solution as a result of the addition of biosolids, irrespective of metal treatment concentrations (Table1). Overall, the increased length of soil incubation time resulted in a significant decrease in soil and soil solution pH, as well as an increase in salinity levels of the soil solution after 24 weeks (Table 1).

The presence of biosolids had no significant effect on total, EDTA, $Ca(NO_3)_2$ extractable Cd or soil solution Cd, effective concentration of Cd, or Cd^{2+} activity (Table 2). In contrast, the longer soil incubation time of 24 weeks resulted in significant increases in the availability of Cd compared to an incubation period of two weeks (Table 2). These results showed that amending soils with biosolids did not affect the solubility of Cd, soil incubation time however, significantly increased the solubility of Cd.

Table 1. Significance levels for comparisons of general soil parameters between biosolids amended and unamended metal spiked soils (n = 180), and between all treated soils sampled after two weeks and 24 weeks of incubation Differences were considered significant at *P*<0.05 and significant differences are highlighted in bold in the table below.

Soil variable	**Biosolids amended vs unamended Cd spiked soils**	***2 weeks vs 24 weeks incubation***
Soil pH	0.768	**< 0.001**
Soil solution pH	0.196	**0.009**
DOC (mg/L)	**0.001**	0.24
Salinity of soil solution (ΣSO_4^{2-}, Cl^-, Na^+) (mg/L)	**0.002**	**< 0.001**
Soil solution Ca concentration (mg/L)	**0.027**	0.65
Soil solution Mg concentration (mg/L)	**0.001**	0.35

Table 2. Significance values for comparisons of six potential measures of Cd bioavailability between biosolids amended and unamended metal spiked soils (log transformed data, n = 180). Differences were considered significant at *P*<0.05 and highlighted in bold in the table below.

Method	**Biosolids amended vs unamended Cd spiked soils**	***2 weeks vs 24 weeks incubation***
Total metal	0.090	*
EDTA	0.190	0.98
$Ca(NO_3)_2$	0.760	0.28
Soil solution	0.320	**0.002**
DGT	0.320	**<0.001**
Free ion activity	0.320	**0.001**

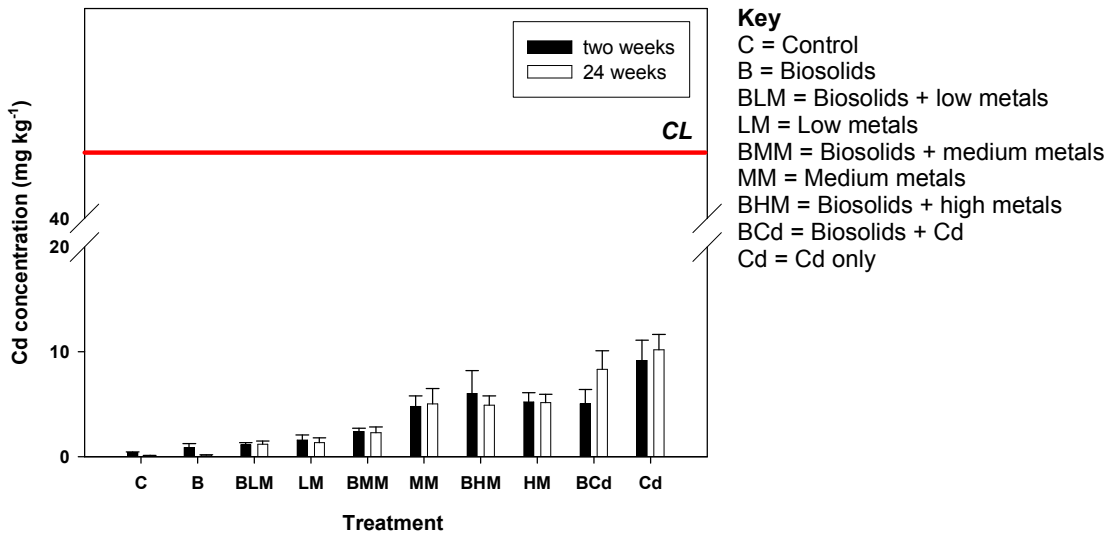

Figure 1. Mean Cd concentrations in wheat shoot tissue (mg/kg dry weight) grown in biosolids amended and unamended metal spiked soils incubated for two weeks and 24 weeks. The critical level (CL) for Cd as depicted by the red line is 43 mg/kg. Bars denote the SEM, n = 89 for both trials.

Comparisons of shoot Cd concentrations in plants grown in biosolids amended metal spiked soils with unamended metal spiked soils revealed a significant decrease in shoot concentrations in plants grown in biosolids amended soils compared to unamended soils for both soil incubation times ($p = 0.003$ and $p = 0.042$ for two and 24 weeks respectively). Additionally, this significant decrease ($p < 0.001$) in shoot concentration in the presence of biosolids was observed across all levels of Cd soil concentrations (Figure 1), furthermore, a linear regression analysis of the grouped data revealed that for Cd, the addition of biosolids significantly reduced shoot concentration on average by 32% ($p = 0.023$).

Calcium nitrate extractable Cd yielded the strongest correlation of the six bioavailability estimates ($r^2 = 0.63$ for pooled soil and wheat data), while soil solution Cd yielded the poorest correlation ($r^2 = 0.30$). There was no significance difference in relationships determined between available Cd and shoot Cd concentrations in the presence or absence of biosolids (Figure 2). Consequently this result provides evidence that Cd bioavailability as measured by $Ca(NO_3)_2$ is not dependent on the presence or absence of biosolids and is a robust measure of bioavailability.

While the findings in this study appear to support the retention of Cd in biosolids amended soils, limitations in the experimental set-up and analyses make it difficult to accurately attribute the mechanism(s) by which Cd retention is occurring. Studies on the availability and uptake of Cd in plants have identified pH and DOC as the major influential factors controlling soil availability (McLaughlin *et al*. 2006; Collins *et al*. 2003; Gray 1999). However, pH did not significantly change with the addition of biosolids, and therefore can not be directly attributed to the reduction in Cd shoots concentrations in this study. From these results two possible mechanisms may be responsible for this effect: (1) DOC sourced from biosolids that is reducing the availability of Cd in soil solution to the plants via complexation of free Cd^{2+} and weakly sorbed Cd and, (2) addition of contaminants and salts in biosolids (i.e. Zn^{2+}, Ca^{2+}) which competitively inhibit the uptake of Cd by plants.

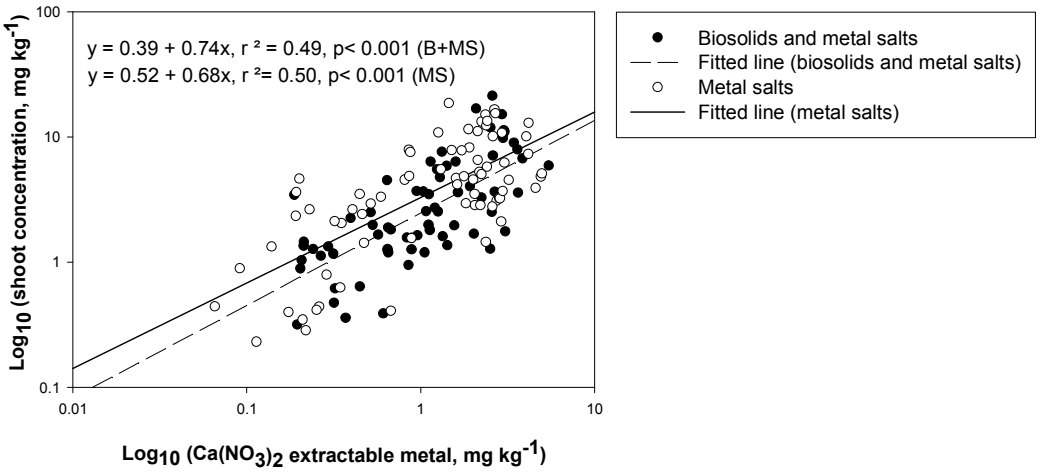

Figure 2. Effect of biosolids amendment on the relationship between shoot Cd concentration and Cd bioavailability as estimated by $Ca(NO_3)_2$ extraction, n = 71 (Biosolids + Cd salts) and n = 72 (Cd salts).

Conclusions

While, the addition of biosolids did not alter the solubility of these metals, soil incubation time did significantly increase the availability of Cd, which may be attributed to the decrease in soil and soil solution pH that was also observed. Overall, Cd concentrations in shoots were significantly reduced in plants grown in metal spiked soils amended with biosolids compared to unamended metal spiked soils. However, no strong relationship between measured soil variables (pH, DOC), and Cd concentrations in shoots were obtained. Statistical analyses also revealed that Cd bioavailability as measured by $Ca(NO_3)_2$ is not dependent on the presence or absence of biosolids and is a robust measure of bioavailability.

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Do soil enzyme activities generate good endpoints for assessing heavy metal toxicity in soils?

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Abstract
Three contrasting field soils were amended with increasing concentrations of Cu and Zn and an assessment made over two years (three annual samplings) of the efficacy of soil phosphatase, sulphatase and urease activities as biological endpoints to measure heavy metal toxicity. Generally, EC_{50} values generated from these relatively simple assays were similar to those from soil biological and plant growth properties that are usually regarded as more relevant indicators of soil health and function. In addition, for the enzyme activity results there were fewer data sets that, either did not fit the sigmoidal dose-response model used to produce EC_{50} values, or fitted poorly with low R^2 values and/or large 95% confidence intervals, than were found from the data of the other methods used. All results suggest that the soil limit concentrations in Australasian guidelines for Cu and Zn are likely to be protective of these soil properties.

Key Words
Soil biochemical properties, heavy metals, dose-response, EC_{50}, biosolids, guidelines

Introduction

Despite soil heavy metal limits being an integral part of rules and guidelines for biosolids re-use, regulators, the farming industry and the wider community remain cautious about land application, partly because the metals, once in the soil, are there in perpetuity. This is especially true in New Zealand – whose economy is strongly dependent on the export of agricultural produce – where there is a fear of damaging our so-called "clean green" reputation. This situation is not helped by there being no clear origin for the actual heavy metal soil limit concentrations in the various guidelines that were developed from the EC Directive of 1986 (CEC, 1986; e.g., NZDoH 1992; NZWWA 2003; EPA Victoria 2004).

In recent times, attempts have been made to derive EC_{20} and EC_{50} values (concentrations causing a 20% or a 50% decline of microbial numbers or activity) for heavy metal effects on microbial and biochemical properties of soils exposed to biosolids (Speir *et al.* 2007). Soil enzyme activities were included among the endpoints for this research because they are regarded as sensitive indicators of soil health and biological functioning and because they are easy to measure. Conclusions were that definitive EC values could not be calculated because the data sets were too variable and the maximum heavy metal concentrations were not high enough. The variability issue arises because biosolids enhances soil biological activity though its nutrient and organic matter contents and because it is almost impossible to make an heterogeneous mix of biosolids and soil. The metal concentration issue arises because biosolids is not sufficiently contaminated to raise soil metal concentrations enough to severely inhibit biological activities. Many applications of biosolids would be required to obtain enough metal to determine definitive EC values.

As a result of the problems encountered using biosolids, we established field trials where soils were amended with heavy metal salts (Cu and Zn), duplicating the range of concentrations used by the researchers in the Australian National Biosolids Research Program. A range of soil biochemical properties were measured, including the activities of phosphatase, sulphatase and urease. Effects were related to metal concentration and EC_{50} were determined using a sigmiodal dose-response model. The usefulness of the enzyme activity-derived EC values was assessed in comparison to those from other biological measures of soil function.

Materials and Methods

Field trials

Three field trials were established on contrasting soils in different regions of New Zealand; on Waihou silt loam near Hamilton, on Tahunanui sand near Nelson and on Templeton fine sandy loam near Lincoln. The soils were all under pasture. Each trial comprised 30 randomly-assigned plots (1 m x 1 m) receiving $CuSO_4$ at seven dosages, $ZnSO_4$ at seven dosages, and an undosed control, all duplicated. The amounts of Cu applied were sufficient to raise soil concentrations by 5 – 2000 mg kg^{-1}, and of Zn to raise soil concentrations by 10 – 3000 mg kg^{-1}. The soils were amended by removing the top 10 cm from each plot,

coarsely sieving to remove herbage, roots and stones, mixing with the appropriate amount of salt using a concrete mixer, and finally returning to the same location in the field. Following amendment the plots were left for two weeks prior to first sampling. After sampling, the plots were sown with ryegrass/clover seed.

Sampling and analysis
Approximately 1 kg soil was taken from each plot (25 – 30 soil cores, 0-10 cm depth and 2 cm diameter) two weeks after amendment and 1 year and 2 years later. Soil was sieved on returning to the laboratory and a subsample air-dried for heavy metal analyses and for other chemical properties. The remainder was maintained at 4° C for biochemical analyses, which were carried out as soon as possible.

Total Cu and Zn concentrations were determined by XRF of pressed discs of finely ground soil. Phosphatase and sulphatase enzyme activities were measured as described by Speir *et al*. (2007), based on the methods of Tabatabai and Bremner (1969, 1970). Urease activity was measured as described by Searle and Speir (1976), except that the colorimetric analysis was done manually. The other biological and biochemical assays included microbial biomass C (MBC) (Vance *et al.* 1987) and respiration (Sparling and Zhu 1993), substrate-induced nitrification (SIN) (OECD 2000) and above- and below-ground wheat seedling biomass in a plant germination test (Smart *et al.* 2004).

Data analysis
Mean biological data from each treatment in each trial were related to mean total metal concentration using the sigmoidal dose-response program developed by CSIRO, Australia (Barnes *et al.* 2003), based on the model developed by Haanstra *et al*. (1983). Values of EC_{50} were calculated, along with their 95% confidence intervals.

Table 1. EC_{50} values for enzyme activities and total soil Cu.

Property	Soil	Sampling	EC_{50} (mg kg–1)	95% confidence interval (mg kg–1)	Slope	R2
Phosphatase	Waihou	Two weeks	2880	1190 – 6980	-1.63	0.93
		One year	1090	630 – 1890	-2.57	0.96
		Two years	1380	930 – 2040	-3.80	0.95
	Tahunanui	Two weeks	920	400 – 2130	-2.11	0.93
		One year	580	280 – 1200	-3.13	0.93
		Two years	590	390 – 900	-2.72	0.98
	Templeton	Two weeks	1220	330 – 4450	-1.45	0.91
		One year	670	610 – 740	-3.40	0.998
		Two years	630	490 – 820	-2.57	0.99
Sulphatase	Waihou	Two weeks	13300	3410 – 52400	-1.55	0.93
		One year	2140	1400 – 3270	-3.00	0.95
		Two years	1860	1430 – 2400	-4.68	0.96
	Tahunanui	Two weeks	720	290 – 1780	-1.84	0.96
		One year	310	180 – 530	-2.79	0.98
		Two years	280	100 – 770	-2.29	0.97
	Templeton	Two weeks	390	60 – 2320	-1.67	0.94
		One year	210	50 – 830	-1.98	0.96
		Two years	310	170 – 550	-2.52	0.99
Urease	Waihou	Two weeks	4850	3350 – 7030	-2.74	0.98
		One year	2950	1680 – 5200	-3.10	0.91
		Two years	2670	1740 – 4090	-4.76	0.91
	Tahunanui	Two weeks	410	40 – 4370	-1.88	0.88
		One year	360	20 – 6470	-2.16	0.87
		Two years	730	380 – 1380	-6.22	0.86
	Templeton	Two weeks	1130	750 – 1690	-3.24	0.97
		One year	720	500 – 1050	-4.99	0.96
		Two years	870	740 – 1030	-6.11	0.99

Results

The soil enzyme EC_{50} values for Cu are shown in Table 1. In all but one instance (urease at Tahunanui) EC_{50} values declined markedly between the two week and one year samplings and the slope of the sigmoidal curve always increased over this period. Increased slope, coupled with a high R^2 value, always results in a smaller 95% confidence interval and generally the smallest confidence intervals occurred after two years. The soil enzyme EC_{50} values for Zn are shown in Table 2. The temporal trends are less distinct for Zn, but again, slopes are usually less initially. Overall, for Zn, R^2 values are lower than those for Cu, indicating a poorer fit of the data and this is reflected in larger 95% confidence intervals.

Table 2. EC_{50} values for enzyme activities and total soil Zn.

Property	Soil	Sampling	EC_{50} (mg kg−1)	95% confidence interval (mg kg−1)	Slope	R2
Phosphatase	Waihou	Two weeks	20000	100 – 5 x 106	-1.75	0.42
		One year	2730	1720 – 4320	-2.27	0.96
		Two years	2180	1450 – 3290	-3.34	0.94
	Tahunanui	Two weeks	2190	1180 – 4080	-6.07	0.73
		One year	2490	150 – 42000	-5.68	0.34
		Two years	2800	160 – 48000	-3.44	0.43
	Templeton	Two weeks	1330	600 – 2960	-2.21	0.95
		One year	1180	760 – 1820	-3.52	0.93
		Two years	1480	920 – 2380	-3.16	0.92
Sulphatase	Waihou	Two weeks	15800	1600 – 156000	-1.88	0.83
		One year	3090	1630 – 5850	-3.43	0.86
		Two years	2160	1420 – 3270	-3.38	0.93
	Tahunanui	Two weeks	3700	990 – 13800	-1.58	0.85
		One year	1100	510 – 2370	-3.01	0.88
		Two years	860	220 – 3420	-2.74	0.74
	Templeton	Two weeks	1880	650 – 5480	-2.14	0.88
		One year	760	530 – 1100	-6.09	0.93
		Two years	1020	850 – 1220	-8.25	0.97
Urease	Waihou	Two weeks	26000	300 – 2.2 x 106	-1.59	0.71
		One year	3820	2670 – 5460	-2.61	0.97
		Two years	2620	1760 – 3900	-3.94	0.93
	Tahunanui	Two weeks		No fit to model	-22	0.07
		One year		No fit to model	-6.04	0.08
		Two years	1360	380 – 4940	-6.39	0.33
	Templeton	Two weeks	1240	70 – 23000	-1.36	0.82
		One year	1020	280 – 3730	-2.19	0.91
		Two years	1370	30 – 56000	-1.38	0.71

Discussion

The greatest advantages of enzyme activity assays over other biological measures of soil health and function are simplicity of the methods and ease of analysis. The assay methods used here require little soil, short incubation periods, few chemicals and simple instrumentation. In most instances, the enzymes have proved to be sensitive to increasing soil metal burden and the data have fitted the sigmoidal dose-response model extremely well. Even when the three data sets for each enzyme were grouped (data not shown), R^2 values remained similar to those shown and EC_{50} values were similar to one year and two year values.

The enzyme data compared very favourably with results gained using the more time-consuming assays – MBC, basal respiration, SIN, and seedling shoot and root biomass (data not shown). For Cu, phosphatase EC_{50} values were usually similar to those generated from MBC and SIN, although the SIN curves had steeper slopes and, consequently, smaller confidence intervals. Except in the Waihou soil, sulphatase activity was more sensitive (lower EC_{50} values) than the other enzymes and MBC and SIN. Seedling shoot dry weight gave inconsistent results, whereas root dry weight gave similar EC_{50} values to those for phosphatase in Waihou and Tahunanui soils, but closer to those for sulphatase in the Templeton soil.

For Zn, MBC, respiration and SIN gave inconsistent results (greater variability and some very large 95% confidence intervals), but acceptable EC_{50} values were similar to those for phosphatase or sulphatase. Seedling shoot and root dry weights also gave inconsistent EC_{50} values.

Overall, our results suggest that soil enzyme activities can be used to generate useful endpoints for assessing heavy metal toxicity to soil health and function and that their EC_{50} values are not dissimilar to those from more direct measures of soil microbial function and plant performance. It is also clear that these properties are unlikely to be seriously adversely affected in these soils at the maximum Cu and Zn concentrations recommended in Australasian guidelines (100 mg kg^{-1} for Cu and 200-300 mg kg^{-1} for Zn). Our data also highlight the need for appropriate controls when conducting experiments of this type, since all soil biological and biochemical properties can be influenced by factors other than heavy metals (e.g., soil pH, fertiliser application).

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Effects of clay microstructure and compost quality on chlordecone retention in volcanic tropical soils: consequences on pesticide lability and plant contamination

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Key Words
Fractal structure, chlordecone, organic matter, allophane, contamination, French West Indies.

Abstract
The scientific and economic context of our study is related to the pollution of the soils, fresh and marine water by a persistent organochlorine pesticide (chlordecone) in a tropical context (French West Indies). The former application of chlordecone results today in a diffuse pollution in agricultural soils, which are sources of contamination for cultivated roots, tubers, vegetables and terrestrial and marine ecosystems. Chlordecone is a very though and stable molecule (considered as a POP), it is mainly present in solid phase and has a strong affinity with organic matters. To prevent consumers and ecosystems exposure, it is thus necessary for us to evaluate the factors that influence chlordecone migration in the environment. In our research, we studied the impacts of clay microstructure on the chlordecone retention, comparing allophanes (amorphous clays present in andosols) and halloysite clays (type 1/1). We showed that allophane aggregates had a greater ability to trap chlordecone mainly due to their fractal structure. We also measured the effects of added composts on soil microstructure and on chlordecone lability and transfer rate from soil to plant 3 and 6 months after incorporation. The intensity and persistence of these effects were related to the initial quality and richness of the added composts.

Introduction
Chlordecone is a very though pesticide which was used for 2 decades in both Guadeloupe and Martinique (from 1971 to 1993) mainly for the control of the banana black weevil. Chlordecone has a great soil and organic matter affinity (in the literature Koc varies from 17500 L/kg according to Kenaga (1980) to 2000-2500 L/kg according to ATSDR (1995) but a low water solubility (Dawson *et al.* 1979). Thus, chlordecone mainly diffuses in environment through water elution (Cabidoche *et al*. 2009), soil erosion and transport of clay particles. In Martinique, andosols and nitisols are the most frequent polluted fields and are considered as the pollution reservoirs (Cabidoche *et al*. 2006).

Volcanic soils like andosols contain amorphous clays (allophanes), issued from the transformation of volcanic materials (Colmet Daage and Lagache 1965). These amorphous clays present very different structures and physical properties compared to usual clays (Woignier *et al*. 2007). Allophanes aggregates develop a fractal structure in andosol which leads to peculiar physical features: large pore volume and pore size distribution, a high specific surface area and very large water content (Chevalier *et al*. 2008). These soils have been strongly polluted and the clay microstructure should be an important physico-chemical characteristic governing the fate of the pesticide in the environment.

Methods
In our research we studied the lability of chlordecone by laboratory lixiviation experiments on two contaminated soils: an andosol and a nitisol (respectively containing two different type of clays: allophane and halloysite) and we characterized the changes of clays microstructure induced by two contrasted composts (Table 1) (Li *et al.* 2003). The incubation was realized at 28-30°C, 90% of maximal retention capacity (to preserve the physical structure of allophane) and 5% (w/w) of compost was added. We also studied their effects on plant contamination (i) a radish after 3 months of incubation and (ii) a lettuce after 6 months.

Table 1. Characteristics of the two commercial composts used

	Biogwa	Vegethumus
Water content	47 %	24.8 %
Organic matter content	20.6 %	46.6 %
Humic yield (CBM)	49 kg C/t of fresh product	577 kg C/t of fresh product
Humic potential (K_1)	0.11	0.70

Results

Effect of the clay microstructure on the lability of chlordecone

Figure 1 shows the evolution, during the incubation time, of water extractible chlordecone after compost addition. The results demonstrate that chlordecone amounts extracted by water decrease according to incubation time for both soil types and composts.

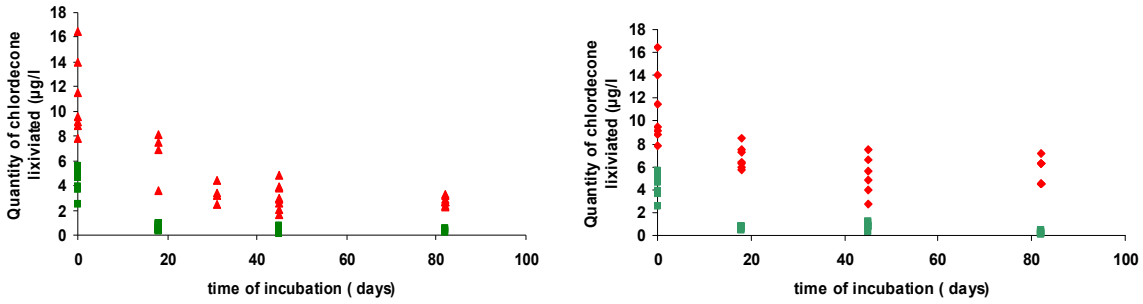

Figure 1. Quantity of lixiviated chlordecone (µg/l) for different incubation times from andosol (red dots) and nitisol (green dots) after compost addition (left: Vegethumus, right: Biogwa).

Vegethumus appears to be more efficient in the reduction of chlordecone lability than Biogwa, probably due to its higher organic matter content and K_1. This is confirmed by measuring the effect of composts on soil N mineralization (Figure 2), which shows that Vegethumus provides significantly greater amounts of mineral N in both soils.

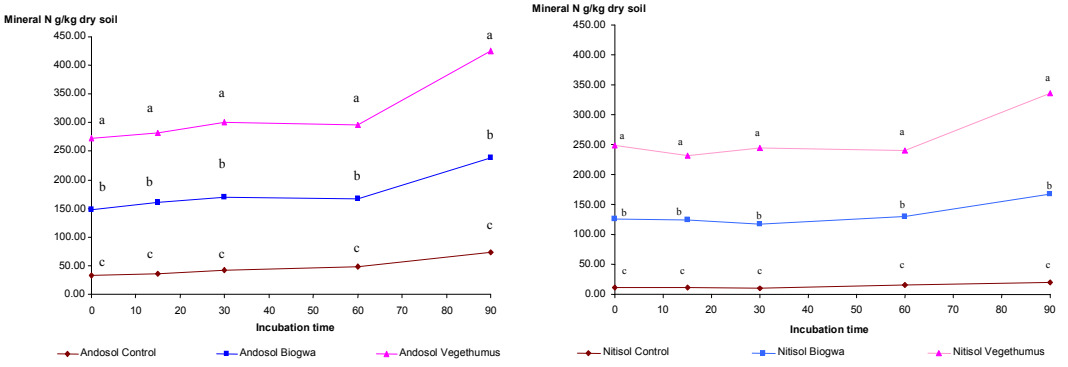

Figure 2. Effect of compost addition on production of total mineral N on andosol (left) and nitisol (right). For each date, means with different letters are significantly different ($p < 0.001$).

Considering the effect of composts on the poral volume (Figure 3), the pore size distribution shows that the porosity of the andosol is greatly reduced with the incubation time by the addition of organic matter and that this effect is more important in the case of Vegethumus. This effect is also associated with the reduction of specific area. In the case of nitisol, the effect of compost addition is not noticeable. But compost addition induces a dramatic reduction of microporosity in the case of andosol where allophanes develop a fractal structure. This phenomenon could explain the lower lability of chlordecone by retaining the molecule in this very tortuous and closed porosity. Thus, in the case of nitisol, the impact of compost addition could be more related to the high affinity of chlordecone for organic matter, as the modification of the porosity is poorly influenced.

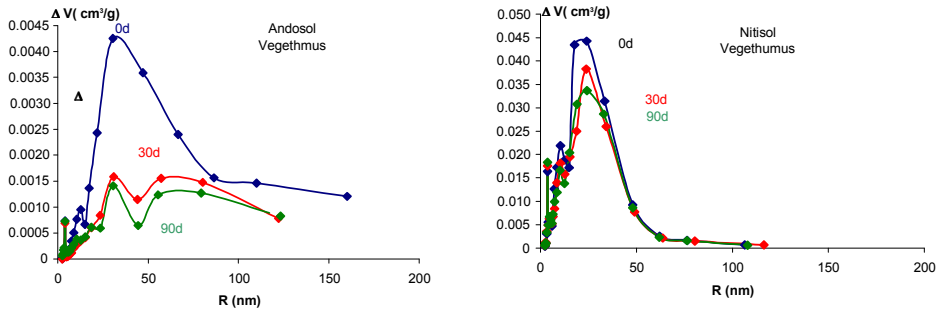

Figure 3. Evolution of the distribution of size pores according to the incubation time (0 day : blue ; red : 30 days ; green : 90 days) after addition of Vegethumus (left : andosol, right : nitisol)

Effect of the added composts on plant contamination after 3 months of incubation
Three months after the incorporation of compost, radishes were seeded in the incubated soil. Chlordecone contamination was measured in small roots, main root (edible part) and leaves at harvest time.

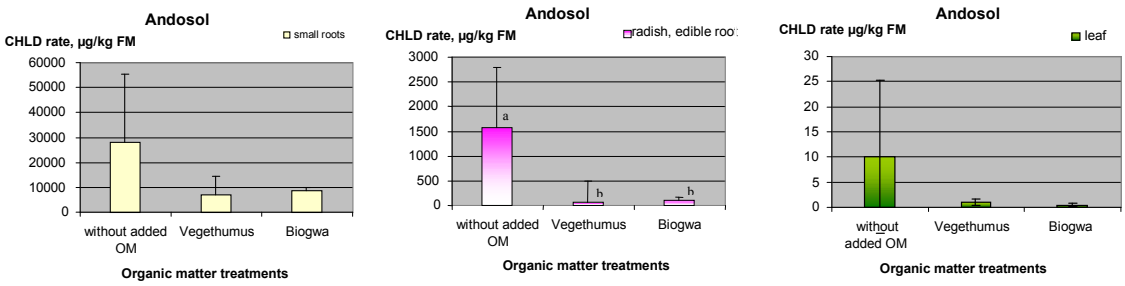

Figure 4. Chlordecone content (µg/kg of fresh material) of radish (in yellow : small roots ; in pink : main root ; in green : leaves) cultivated on contaminated andosol according to the different added composts. Means with different letters are significantly different ($p < 0.05$).

Figure 4 shows that plant contamination transfer decreases drastically between small roots, main root and leaves, even in the control. Nevertheless, the addition of compost reduces noticeably, in both cases, the contamination of plant tissues. The direct contact between roots and soil chlordecone seems to be the main factor of plant contamination as the diffusion in the plant is similar for all the treatments.

Effect of the added composts on plant contamination after 6 months of incubation
After six months of incubation, effects of added composts start to differ noticeably. Contamination of lettuce is significantly increased (4-fold) with Biogwa, compared to Vegethumus where the contamination remains very low (figure 5). This could be explained by the important differences of quality and biochemical composition of the two composts. Biogwa has the characteristics of an organic fertilizer, mineralized after a short period and contributing poorly to soil organic matter. At the opposite, Vegethumus has the characteristics of an organic amendment, with a slow mineralization dynamic (20% after 6 months) and with an important contribution to soil organic matter content (70% of added C integrates the soil organic matter).

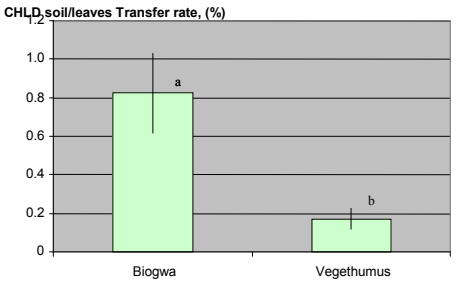

Figure 5. Effect of compost after 6 months of incubation on chlordecone transfert (%) from the andosol to the plant (lettuce). Means with different letters are significantly different ($p < 0.05$).

Conclusion

Andosol (allophane clay) and nitisol (halloysite clay) behaviour differed in the processes involved in chlordecone retention. The structural properties and the spatial arrangement of allophane aggregates constitute a trap for chlordecone molecule, thus mechanically retained. Compost addition modified the andosol porosity (poral volume and specific area) according to the compost quality and highly reduced chlordecone lability. In the case of nitisol, the retention of the molecule seems more directly affected by added organic matters such as composts, leading to a chemical retention of chlordecone. The intensity of the compost effects is driven by its initial richness (C and dry matter) while the persistence could depend of the complexity of its biochemical composition, conditioning its decomposition kinetic. Andosol is able to retain pesticides stronger than nitisol, combining physical trapping and chemical retention. As a consequence, andosol could be highly polluted but less contaminant for crops and environment because of these effects. Further investigations should be realized to confirm these results at a larger scale before making recommendations for the management of polluted fields.

Acknowledgments

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Energy relationships between constant charge soils and cations

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Abstract
This paper described the relationships of energies between the constant-charge soils (yellow-brown, and black soils) and five kinds of cations (K^+, NH_4^+, Ca^{2+}, Cd^{2+}, Cr^{3+}) as inferred from the Wien effect measurements in suspensions. The results showed that the mean free binding energies, ΔG_{bi}, of K^+ and NH_4^+ to the two soils were in the range of 6.3 to 7.0 kJ/mol, the binding energies of K^+ being larger than those of NH_4^+. The range of binding energies of divalent cations to both soils was 7.2-9.4 kJ/mol. As for the same cation, the binding energies were of the order yellow-brown soil < black soil. The mean free binding energies of Cr^{3+} to both soils were smaller than those of all divalent cations. The mean free adsorption energies, ΔG_{ad}, of monovalent cations to both soils at the same field strength, such as 150 kV/cm, showed that the adsorption energies of K^+ and NH_4^+ to yellow-brown (0.88-0.90 kJ/mol) were near the same, but the adsorption energy of NH_4^+ for black soil was more than that of K^+ by 0.1 kJ/mol. The range of adsorption energies of divalent cations to both soils was 1.4-2.2 kJ/mol.

Key words
Electrical conductivity, field strength, mean free energy

Introdution

Chemical phenomena in soils are the appearance of various forms of energies and their transformation (Yu 1976). Soil chemists usually explain the interactions between various ions and clay minerals/soil particles in terms of the affinity parameter of a best-fitted Langmuir isotherm, whether the assumptions behind the originally derived Langmuir model are applicable or not (Ajwa and Tabatabai 1997). Since the fifties of last century, to investigate the nature of soil chemical phenomena on basis of the energy relationship became an active research field (Yu 1976) because the energies between ions and soil particles can be used for characterizing interactions of ions with soil particles, which is very interesting and useful for the investigations of plant nutrition and soil environmental protection. Critter and Airoldi (2003) experimentally determined the ion-exchange equilibrium on cationic latosol soils/aqueous solution interface and calculated the Gibbs free energy by linearization of the Langmuir equation. However, the investigation of energy relationship between cations and soil particles in the second half of the 20th century was based on indirect deduction, rather than on direct measurement. Since there is no break-through in methods to determine the binding energy of cations to soil particles (Yu 1963; 1976), up to now the experimental results in the aspect have been little reported, and there is still no conspicuous advance.

Recently, a novel method for evaluating the interactions of ions with charged colloidal particles, based on measurements of the Wien effect in suspensions, has been developed (Li *et al.* 2005). The binding and the adsorption energies of cations with soil particles can be determined on basis of the new method. In the present report we applied the new method to investigate the energy relationships between two constant charge soils (yellow-brown and black soils) and five kinds of cations (K^+, NH_4^+, Ca^{2+}, Cd^{2+}, and Cr^{3+}).

Materials and methods

Soil Samples

The tested yellow-brown soil (Alfisol) and black soil (Mollisol), which were expected to carry only negative charge, were collected from a depth of about 1m in Nanjing and Haerbin, respectively. The organic matter content of yellow-brown and black soils was 5.4, and 13.6g/kg, respectively. The clay fraction of <2 μm in diameter was separated by sedimentation, dried, and ground. The positive and negative charge densities of the clay fractions of the two soils at different pH values are presented in Figure 1.

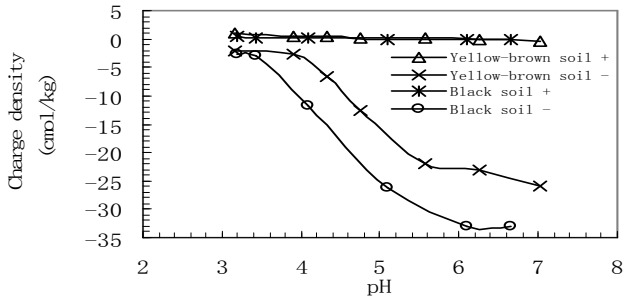

Figure 1. Variation of negative and positive charge densities of the soils with pH

Preparations of Homoionic Soil Samples and Suspensions

The clay fractions of the two soils were saturated with various cations by three sequential equilibrations with 1 mol/L solutions of chlorides of these cations. After the free chlorides contained in the clay samples were thoroughly removed, the chloride-free clay fractions were dried and ground. Suspensions were prepared by adding deionized water to soil samples in plastic bottles to achieve a solid concentration of 10g/L. The suspensions were allowed to stand for about 7-10 days prior to the Wien effect measurements.

Wien Effect Measurement Procedure

The procedure of Wien effect measurements was described in a previous paper (Li *et al.* 2005). The data presented in Figure 2 are the means of two sets of measurements; the standard errors were usually smaller than the symbols on the graphs. All the reported measurements were made at a constant temperature of 25°C.

Equations evaluating binding and adsorption energies

The equation evaluating the binding energy (Li *et al.* 2005) is given by

$$\Delta G_{bi}=RT\ln(2\cdot CEC\cdot C_p\cdot\lambda/EC_0) \quad (1)$$

The adsorption energy evaluation is according to the following equation

$$\Delta G_{ad}=RT\ln(EC/EC_0) \quad (2)$$

The application of equation (2) to a series of Wien effect measurements may provide a spectrum of the cation adsorption energies. All binding/adsorption energies will be assigned positive signs in this paper.

Results and discussion

Mean Gibbs free binding energy

The parameters needed for calculating mean Gibbs free binding energy according to equation (1) are presented in Table 1 along with the mean free binding energies. The mean free binding energies were of the order $Ca^{2+} > Cd^{2+} > K^+ > NH_4^+ > Cr^{3+}$, and $Ca^{2+} > Cd^{2+} > Cr^{3+} > K^+ = NH_4^+$ for yellow-brown and black soils, respectively. The binding energy of K^+ to yellow-brown soil calculated from K^+ ion activity in the suspension of pH 5.33, which was determined with potassium ion-selective electrode by Xuan *et al*. (1965), was 7.25 kJ/mol, that is closed to the value (6.71 kJ/mol) calculated from EC_0. The binding energies of divalent cations are larger than those of monovalent cations by 0.45~2.40 kJ/mol. The sequence of mono- and divalent cations is reasonable (Diatta 2004), but it was somewhat unexpected that the mean free binding energy of Cr^{3+} for both soils was smaller than those of all divalent cations. This may be due to that the high degree of hydrolysis of Cr^{3+} in water resulted in its transformation into divalent $Cr(OH)^{2+}$ and monovalent $Cr(OH)_2^+$, together with H^+ cations. The hydrolysis constant (p*K*) of Cr^{3+} (3.9) is much smaller than those of Ca^{2+} (12.5) and Cd^{2+} (9.7) (Wen *et al.* 1977). It is notable that the ΔG_{bi} values of yellow-brown soil were smaller than those of black soil, probably because of the higher organic matter content in the black soil.

Table 1. The parameters necessary to evaluation of mean Gibbs free binding energies (ΔG_{bi})

	Yellow-brown soil					Black soil				
	K^+	NH_4^+	Ca^{2+}	Cd^{2+}	Cr^{3+}	K^+	NH_4^+	Ca^{2+}	Cd^{2+}	Cr^{3+}
pH	5.35	5.29	4.97	4.60	4.24	6.44	6.39	5.74	5.38	4.53
CEC (mol/kg)	0.199	0.192	0.156	0.103	0.0504	0.333	0.334	0.311	0.288	0.181
λ (mS·L/cm·mol)	73.52	73.55	59.50	54.00	67.0	73.52	73.55	59.50	54.00	67.0
$CEC\cdot C_p\cdot\lambda$ (mS/m)	0.146	0.141	0.0928	0.0556	0.0338	0.245	0.246	0.185	0.156	0.121
$EC_0/100$ (mS/cm)	1.95	2.26	0.693	0.621	0.769	2.90	2.91	0.828	0.796	0.823
ΔG_{bi} (kJ/mol)	6.71	6.26	8.16	7.16	5.39	7.01	7.01	9.42	9.10	8.39

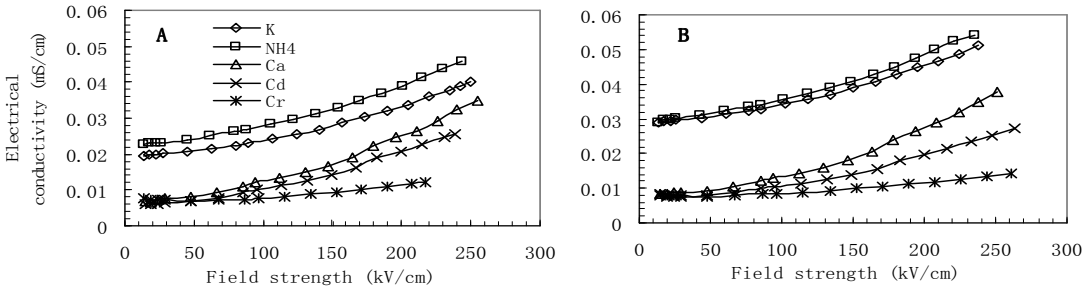

Figure 2. Dependence on field strength of electrical conductivities of suspensions (10 g/L) of yellow-brown soil (A), and black soil (B) saturated with different cations in deionized water

EC-Field strength relationships

The effects of field strength on the electrical conductivities of the suspensions of yellow-brown and black soils saturated with various cations are shown in Figure 2A and B, respectively. With weak fields (about 15 kV/cm), the EC values of the suspensions ranged from 0.006 to 0.029 mS/cm, and the EC values of various suspensions increased with increase in field strength. The changes in EC values of suspensions of the two soils with cations at the same field strength are in the same order $NH_4^+ > K^+ > Ca^{2+} > Cd^{2+} > Cr^{3+}$.

Mean Gibbs free adsorption energy

The mean Gibbs free adsorption energies of all cations released at a given applied field, evaluated via equation 2, are presented in Figure 3. In the range of low field of 15~80 kV/cm the adsorption energies of mono- and divalent cations were not different, but as field strength is more than 100 kV/cm, the ΔG_{ad} values of divalent cations is obviously larger than those of monovalent cations. The ΔG_{ad} values among various cations descend in the order $Ca^{2+} > Cd^{2+} > NH_4^+ > K^+ > Cr^{3+}$. It is not well known that ΔG_{ad} values of Cr^{3+} to the soil particles are lower than those of mono- and divalent cations, and this will be further investigated.

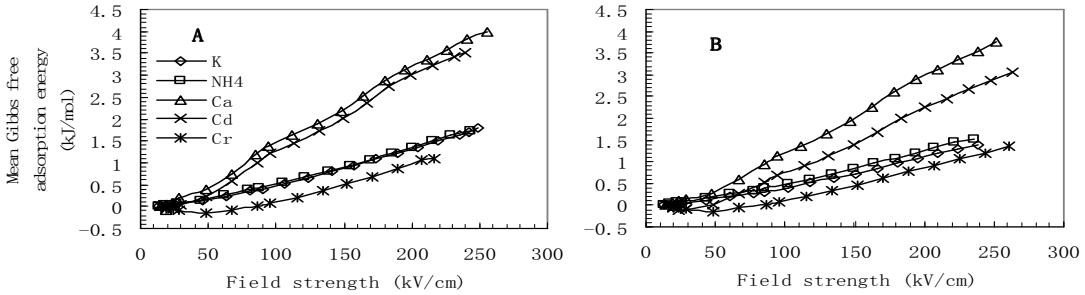

Figure 3. Mean Gibbs free adsorption energies as a function of field strength for yellow-brown soil (A), and black soil (B) saturated with different cations in deionized water (10 g/L)

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Enhancement of Cd solubility and bioavailability induced by straw incorporation in a Cd-polluted rice soil

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Abstract
Of the factors affecting migration and bioavailability of toxic metals in heavy metal contaminated soil, dissolved organic carbon (DOC) provides binding sites for metal cations and reduces the fixation and adsorption of heavy metals by the soil solid phase. Elevation of DOC level due to the direct incorporation of crop residues may lead to enhanced accumulation of toxic metals in crops grown in polluted farmland. In this study, an incubation experiment and a pot experiment were conducted respectively to investigate the effects of wheat straw incorporation on DOC level, cadmium availability, and Cd accumulation in rice plants, and to establish the relation between Cd solubility and DOC level. A Cd-contaminated rice soil was used and incorporated with different rates (0, 0.5 and 1%) of wheat straw in both experiments. Results showed that the change of Cd in soil solution was very similar to that of DOC level. Wheat straw addition significantly elevated Cd and DOC level in soil solution while NH_4NO_3-extrated Cd was not affected. There existed a close linear correlation between soluble Cd and DOC level. Enhanced Cd accumulation in rice plants, grown in a Cd contaminated soil, induced by wheat straw incorporation was observed in this study.

Key Words
Cadmium, bio-availability, dissolved organic carbon, crop straw, rice soil

Introduction

The pollution of soil and agricultural farmland has received worldwide attention. In China, about one fifth of arable land has been polluted by heavy metals, due to acceleration of industrialization and urbanization, heavy input of chemical fertilizers and pesticides, and rapid development of transportation system (Chen 1996; Gu *et al.* 2003). However, the environmental risk of heavy metals in polluted soil largely depends on their mobility and availability which are affected by many factors and closely related to the composition of the solution (Bümmer *et al.* 1986). Dissolved organic carbon (DOC) provides binding sites for metal cations and regulates the fixation and adsorption of heavy metals by soil solid phase. DOC in soil solution may affect the toxicity or bioavailability of heavy metals to plants (Inaba and Takenaka 2005; Hernández-Allica *et al.* 2007). Rice provides a staple food source for over 60 percent of the population in China. Apart from other sources of heavy metal pollution, rice field may receive more heavy metals due mainly to the use of untreated or not well treated sewage water discharged from nearby industries as irrigation water (Wang 1997). Cadmium (Cd) is one of the main pollutants in rice paddy soil near industrial areas. Cd is highly toxic to rice growth and development (Chien and Kao 2000) and has strong interference with the uptake of other metal cations by rice plant (Liu *et al.* 2003). Cd-contaminated milled rice (> 1mg Cd/kg) once appeared in the markets in many regions in China and became a potential threat to human health (Wang 1997). In wheat-rice rotation systems, directly incorporating wheat straw into rice field is becoming an alternative way to replace open-field burning (Singh *et al.* 2008). Our previous study indicated that wheat straw incorporation significantly increased DOC level in rice field (Lu *et al.* 2006) and elevated the concentration of organic acids, an important source of DOC, in soil solution (Shan *et al.* 2008). An earlier study showed that there existed a close link between Cd dissolution and DOC level in different soils (Chen and Chen 2002). The objective of our present work was to investigate the relationship between Cd availability and DOC level in a Cd-contaminated rice soil with different rates of wheat straw incorporated, and to clarify the question whether wheat straw incorporation may induce enhanced Cd accumulation in rice plant.

Methods

Soil and straw used

Cd-contaminated soil (top 20 cm) was sampled and air-dried at room temperature immediately after wheat harvest in a rice-wheat rotation system (31°17.6N, 119°53.7E) adjacent to Taihu Lake, China. The cropping system had been irrigated by untreated sewage water discharged from surrounding small industries for years and total Cd content in the sampled soil was 7.4 mg/kg. The soil was a moderately acid loam with pH 5.8, and clay, silt and sand content was 19%, 47 %and 34%, respectively. Other properties of the soil were: CEC

11.4 cmol/kg, total C 13.5 g/kg, total N 1.5 mg/kg, bicarbonate-extractable P 18 mg/kg and exchangeable K 0.09 cmol/kg. The straw used in this study was obtained from the matured wheat cultivar *Yangmai 5* (*Triticum aestivum L.*) which was grown in a neat field free from Cd contamination. The straw sample was oven-dried at 60 °C and cut into pieces (approximately 1cm in length) before use. The straw had a C/N ratio 82.4 and the contents of soluble sugar, cellulose, hemicellulose and lignin in the straw were 12.1%, 35.2%, 37.3% and 15.4%, respectively.

Incubation experiment
An incubation experiment with 3 duplicates was conducted in this study to investigate the response of DOC to straw incorporation and to understand the relation between Cd solubility and availability and DOC in flooded condition. 100 g soil (dried base) was put into a set of plastic beakers and three rates, 0%, 0.5% and 1%, of straw incorporation were implemented by adding 0, 0.5 and 1g wheat straw, respectively, into the beakers. Then 150 ml deionized water was added to each beaker (soil: water= 1:1.5) and the water layer was approximately 2 cm thick. After thoroughly mixed using glass rod, the open beakers were incubated at 30°C and the water layer was maintained by promptly adding deionized water to the beaker. At 1, 3, 5, 7, 10, 20, 40 and 60 d of incubation, the content in the beaker was moved to centrifuge tube and centrifuged at 4000 rpm for 20 min. The supernatant solution was filtered through a 0.45 μm membrane filter (Millex-HV, PVDF, Warsaw, Poland) for determination of DOC and Cd, while the centrifugal sediment was extracted using 1 mol/L NH_4NO_3 for Cd determination only.

Pot experiment
3 kg Cd-contaminated soil was mixed thoroughly with 0, 15 and 30 g wheat straw (straw incorporation rate was 0, 0.5 and 1%, respectively), and the mixture was put into a plastic pot (5L in volume). 3 rice (*Oryza Sativa L.*) seedlings, developed in a nursery bed for 20 d, for each pot was transplanted one day later after 4.5L solution containing 2g urea and 1g KH_2PO_4 as basal fertilizer was added to the mixture. The experiment duplicated 3 times. The rice plants were sampled at maturity stage and divided into root, straw, hull and milled rice. All the plant materials were oven-dried at 80°C for 48h and milled after dry weights were recorded. For Cd determination, milled samples were digested using a mixture of $HNO_3/HClO_4$ 85:15 (v/v).

Measurements
DOC was determined by a total carbon analyser (TOC-V CSN, Japan) and value of DOC was obtained by subtracting inorganic carbon (IC) from total carbon (TC) in the solution; Cd concentration was measured by an atomic absorption spectrometer (PEM2100, Germany).

Statistical analysis
Statistical analysis for the data obtained in this study was performed using SPSS (v.15.0, SPSS Inc. USA). Duncan's SSR-test was used to detect significant difference among the means at $p<0.05$.

Results
DOC concentration in soil solution increased in the early stage of incubation and the peak concentration in the treatments without and with straw incorporation appeared at 3th and 5th d, respectively, after flooding (Figure 1). Then DOC concentration in all treatments declined gradually with incubation time. Straw incorporation significantly elevated DOC concentration in soil solution, and as shown in Figure 1, the peak concentration in treatments with 0.5% and 1% straw incorporation was approximately 2 and 3 times, respectively, higher than that in the treatment without straw incorporation. Much higher DOC concentration in the treatments with straw incorporation was maintained even at the end of incubation, compared to the treatment without straw incorporation (Figure 1).

The change pattern of Cd concentration in soil solution under different rates of straw incorporation (Figure 2) was very similar to that of DOC (Figure 1) and the peak concentration of Cd and DOC in soil solution occurred simultaneously. The response of Cd concentration to straw addition was also close to that of DOC. Straw incorporation significantly increased Cd concentration in soil solution and such effect was enhanced by higher rate of straw incorporated (Figure 2).

The similarity in change pattern with incubation time and response to straw incorporation indicated that Cd solubility, expressed by Cd concentration in soil solution in this study, might be closely associated with DOC level in the polluted soil. Statistical analysis showed that there existed a significant linear correlation

between Cd and DOC concentration (Figure 3). R-square value (R^2) of the fitted linear equation was 0.8865, which meant that approximately 90% variation of Cd solubility could be explained by the change of DOC level in Cd-contaminated soil. Based on the equation in Figure 3, 100 mg C increase of DOC would predict an increase of 36.9 μg Cd in soil solution.

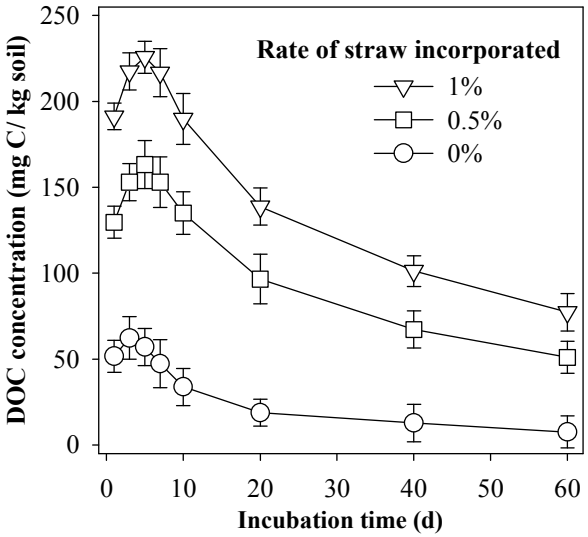

Figure 1. Change of DOC concentration in soil solution under different rates of straw incorporation. Vertical bars indicate standard errors.

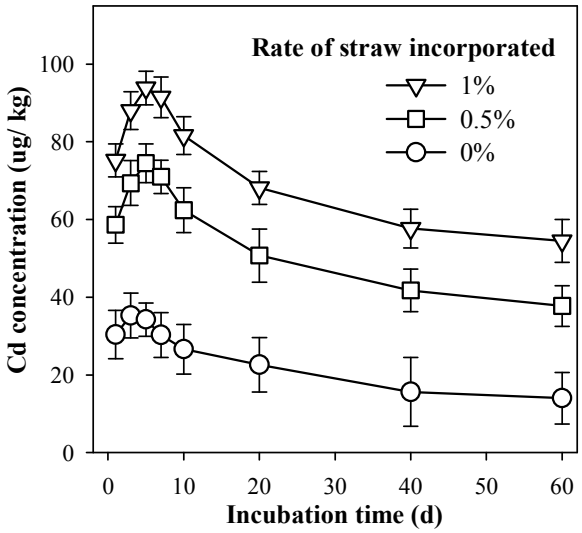

Figure 2. Change of Cd concentration in soil solution under different rates of straw incorporation. Standard errors are shown as vertical bars.

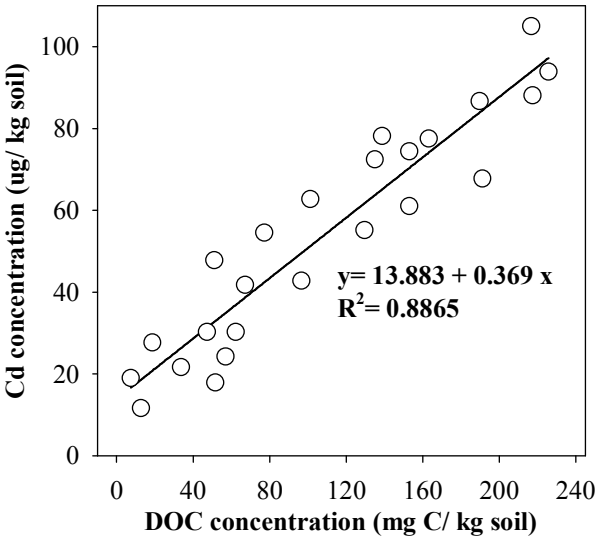

Figure 3. Correlation between Cd concentration (y) and DOC concentration (x) in soil solution. 24 data points in the figure represent the means calculated from 3 straw incorporation rates at 8 incubation times.

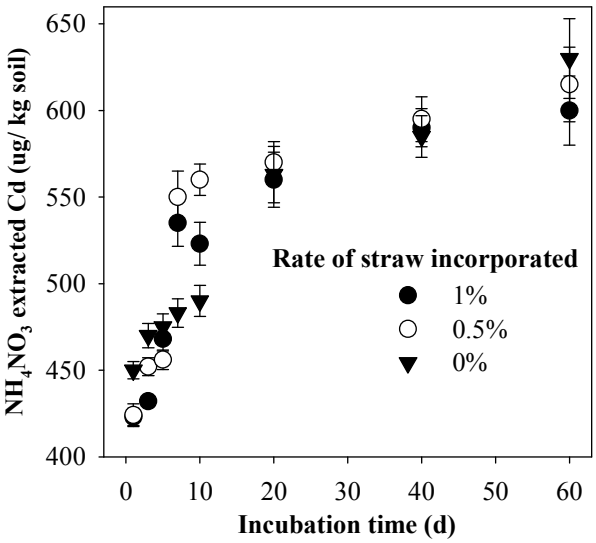

Figure 4. Change of NH_4NO_3 extracted Cd concentration in soil solution under different rates of straw incorporation. Vertical bars represent standard errors.

Cd extracted by 1 mol/L NH_4NO_3 tended to increase with the incubation time (Figure 4) and the tendency may partly attribute to the flooded soil condition. Unlike Cd in soil solution, however, NH_4NO_3-extracted Cd did not apparently respond to straw incorporation (Figure 4).

The data obtained from pot experiment was shown in Table 1. Although a slight increase in biomass accumulation due to the beneficial effect of straw incorporation on soil fertility was observed, the difference in biomass accumulation between straw incorporation treatments was not statistically significant (Table 1), which indicated that enhanced Cd solubility by straw incorporation produced no detrimental influence on rice growth. However, Cd accumulation in both root and shoot of rice plant was significantly elevated by straw incorporation. Compared with the treatment without straw incorporation, Cd content in milled rice, straw, root and hull increased 382.4%, 279.9%, 279.3% and 35.5%, respectively, when 1% straw was incorporated into the polluted soil (Table 1). Since NH_4NO_3-extracted Cd, did not respond to straw

incorporation (Figure 4), Cd in soil solution may contribute to the enhancement of Cd accumulation in rice plant induced by wheat straw incorporation. Of all plant parts tested, Cd accumulation in milled rice was most sensitive to straw incorporation while root had the highest Cd content (Table 1). Therefore, straw incorporation in Cd contaminated rice soil increased the risk of Cd into food chain through milled rice.
In wheat–rice rotation systems in China, the incorporation rate of wheat straw seldom exceeds 5 t/ ha (approximately 0.5% of straw application in plow soil), which is lower than the rate in this study. However, high accumulation of Cd in rice plant induced by straw incorporation in Cd-contaminated soil may still occur due to uneven incorporation of the wheat straw in some regions lack of labour or relevant machinery.

Table 1. Effect of straw incorporation on biomass accumulation and Cd content in different parts of rice plant at maturity.

Rate of straw incorporated (%)	Biomass accumulation (g DW/pot)	Cd content (mg/ kg DW)			
		root	straw	hull	Milled rice
0	76.5a	0.405a	0.209 a	0.076 a	0.242 a
0.5	81.4a	0.985b (143.2)	0.557 b (166.5)	0.089 ab (17.1)	0..779 b (219.0)
1	78.3a	1.536c (279.3)	0.794 c (279.9)	0.103 b (35.5)	1.167 c (382.4)

Different letters in the same column indicate significant difference detected by SSR-test at $p<0.05$. Biomass accumulation is the total dry weight (DW) of root, straw, hull and milled rice.The figure between brackets shows increase percentage over the treatment without straw incorporation.

Conclusion

Based on the results obtained in this study, we can conclude that wheat straw incorporation significantly enhanced Cd accumulation in rice plants in Cd-contaminated soil. This effect was induced by higher DOC level under wheat straw application which elevated Cd solubility and bioavailability. In heavy metal contaminated soil, direct incorporation of crop residue, especially in large amount, may not be recommended, to avoid hazardous accumulation in crop products.

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Factors Influencing the Availability of Copper in Australian Vineyard Soils

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Abstract
The regular use of copper-based fungicides in vineyards results in an accumulation of copper (Cu) in the surface soils which may be harmful to soil organisms and plants and have implications for the long-term management of affected land. This study investigated the factors (e.g. soil characteristics) influencing the availability of Cu in vineyard soils from 10 different regions of Australia. Concentrations of 0.01 M calcium chloride extractable Cu measured in surface soils collected from 98 different vineyards ranged from <0.1 to 0.94 mg/kg and accounted for 0.10 to 1.03% of the total Cu concentrations in the soils. Differences in the calcium chloride extractable Cu concentrations were related to the total Cu concentration of the soil. The extractable Cu was also related to soil properties, including pH, cation exchange capacity, exchangeable K, clay, silt, and calcium carbonate. The information generated from this study may prove useful in devising strategies to reduce the availability and toxicity of Cu in agricultural soils.

Key Words
Copper, vineyard, soil, fungicide, availability, risk assessment

Introduction

Copper-based fungicides (e.g. copper sulphate, copper oxychloride) have been used in vineyards throughout the world, including Australia, for many decades to protect against downy mildew (*Plasmopara viticola*). However, their use results in an accumulation of Cu in surface soils which can potentially impact on the biological health of the soil and have implications for the long-term management of affected land. For instance, Cu concentrations in the surface soils of vineyards with histories of Cu-fungicide use have been reported to range from 130 to 1280 mg/kg in European vineyards and to generally be in the range of 24 to 159 mg/kg but as high 249 mg/kg in Australian vineyards (Wightwick *et al.* 2008). It has been reported that earthworm populations decreased and the structure of microbial communities changed, following applications of Cu-based fungicides (Maboeta *et al.* 2002; 2003; Ranjard *et al.* 2006).

Research is needed to understand the risks posed by Cu-fungicide residues in different soils/conditions and to devise appropriate risk management strategies if needed. However, it is not possible to make generic conclusions regarding the likely risks posed by Cu in vineyard soils based on total Cu concentration, since the availability of Cu in soil and toxicity to soil organisms and plants is known to vary greatly across soils with different physical-chemical properties. The availability of Cu in the soil is controlled by the total Cu concentration of the soil and by soil characteristics which influence the extent and strength of Cu adsorption in soil (e.g. pH, cation exchange capacity, clay content, and organic matter content). Accounting for differences in the availability of Cu presents a particular challenge from an Australian perspective, as there are approximately 60 different viticultural production regions with a wide variety of soil types (e.g. in terms of pH, texture, organic matter content). The objectives of this study were to:

- Determine the environmental availability of fungicide derived Cu in the surface soils of vineyards in 10 different viticultural regions of Australia.
- Investigate the factors correlated with differences in Cu availability between regions.

Methods
This study used surface soil (0 - 10cm) samples previously collected from 98 vineyards in 10 different grape-growing regions across five different states of Australia (Victoria, South Australia, New South Wales, Western Australia and Tasmania). Surface soil samples were also collected from areas of remnant vegetation in each region to act as "reference sites" that had not received any artificial inputs of Cu. The environmentally available Cu concentrations of these soils were determined using the 0.01 M calcium chloride ($CaCl_2$) soil extraction method. This method was selected over other approaches (e.g. EDTA extraction, free Cu^{2+} measurements, diffuse gradients in thin films) as it represents an acceptable immediate for determining freely available Cu in terms of ease of use, suitability for a range of different soils, and applicability to biological response. Copper and other elements in soil extracts were analysed by inductively coupled plasma emission spectrometry (ICP-ES). The soil samples had previously been analysed for total Cu and a range of different physical-chemical soil properties. Simple and multiple stepwise regression analysis was used to determine significant relationships between Cu availability and other parameters including total Cu concentration of the soil, physical-chemical soil properties, concentrations of other elements in the soil, and history of Cu-fungicide use.

Results
The $CaCl_2$ extractable Cu concentrations in the vineyard soils ranged from <0.1 to 0.94 mg/kg and accounted for 0.10 to 1.03% of the total Cu concentrations in the soils. With the exception of one site, $CaCl_2$ extractable Cu was not detected in the soil from the reference sites. Differences in the $CaCl_2$ extractable Cu concentrations were generally related to the total Cu concentration of the soil ($R^2 = 0.48$). This regression could be improved by including pH, clay, silt, exchangeable K, and calcium carbonate ($R^2 = 0.70$), with pH and clay content being the greatest contributors to the regression. Using simple linear regression only a weak relationship ($R^2 = 0.06$) could be found between pH and the concentration of $CaCl_2$ extractable Cu. However a polynomial regression indicated a U-shaped trend ($R^2 = 0.39$) suggesting that the $CaCl_2$ extractable Cu concentration increased with increasing soil acidity (pH < 7.5) and to a lesser extent with increasing soil alkalinity (pH > 8.5). Although there is uncertainty around the increase with increasing alkalinity as only 13 out of 62 data points were above pH 8.

Conclusion
The results from this study highlight the wide variation in Cu availability across different soils and the difficulties in adopting a one-size fits all approach to risk assessment and management in an Australian context. This study has identified the key soil properties that appear to be controlling Cu availability in Australian vineyard soils. Information on the influence of soil properties on Cu availability may also prove useful in devising strategies to reduce the availability and toxicity of Cu in agricultural soils, if deemed necessary. For example, controlling soil pH may prove to be a cost effective in-situ remediation strategy, as the pH of agricultural soils can be relatively easily manipulated through the use of lime. Further research underway is investigating the effects of accumulated Cu on soil microbial activity and the extent to which such effects on soil organisms are influenced by soil properties and how closely this is related to the available Cu concentration.

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Isolations and consortia of PAH-degrading bacteria from the rhizosphere of four crops in PAH-contaminated field

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Abstract
Polycyclic aromatic hydrocarbon (PAH)-degrading bacterial consortia were enriched from the rhizosphere of four crops in PAH-contaminated field using phenanthrene, pyrene and benzo[a]pyrene as the sources of carbon and energy. The PAHs concentration in rhizosphere was lower than that in bulk soils, whereas, the extracted DNA amount was greater. Thirteen isolations belonged to five genera of *Firmicutes, Betaproteobacteria*, and *Gammaproteobacteria*. Eleven bands cut from DGGE gel profiles belonged to nine genera of *Firmicutes, Bacteroidetes*, *Alpha proteobacteria, Betaproteobacteria*, and *Gammaproteobacteria*. The bands of DGGE showed that the diversity of PAH-degrading bacteria was greater in rhizosphere. *Stenotrophomonas maltophilia* was a major population in either rhizosphere or bulk soil under maize, soybean and rape, while *Acidovorax avenae* was promoted exclusively in rhizosphere. *Lysinibacillus sphaericus* as a PAH-degrading bacterium was reported for the first time. This study indicated that the rhizosphere of vegetation in contaminated field contains higher diversity of population of PAH-degrading bacteria, and some exhibit the potential for biodegradation or bioremediation in rhizosphere.

Key Words
Bacterial communities, DGGE, smelting factory, PAH-degrading strains, rDNA

Introduction
Soil is an important pool for polycyclic aromatic hydrocarbons (PAHs) as its high partition coefficient between air and soil (Wilcke *et al.* 1996). PAHs in surrounding posed considerable health risk to human beings because of its persistence and carcinogenicity (Ma *et al.* 2009a). Rhizoremediation is proposed as the most potential approach for PAH remediation in soil (Ma *et al.* 2009b). Soil microflora play vitally important role during rhizoremediation of xenobiotics (Johnsen *et al.* 2005; Semple *et al.* 2007). The interaction among microbial degrader, plant and PAHs in soil might be regulated through rhizosphere processes (de Carcer *et al.* 2007). For investigating the effect of rhizosphere processes on PAH-degrading bacterial community, great efforts have been done in recent years. But many previous studies were implemented using spiked soils (Joner *et al.* 2001), in which the effect of rhizosphere on PAH-degrading bacterial community is largely different from that in natural contaminated field. In this study, rhizosphere soils were sampled from a PAH contaminated field near a small smelting factory. We attempted to detect bacteria related to PAH degradation and evaluate the role of rhizosphere in PAHs remediation in soil.

Methods
Soil sampling
The soil samples were collected at a crop garden in Wuxi of Jiangsu province, China (31.6041N, 120.4759E), where suffered from the pollution of PAHs due to the coal combusion from a surrounding smelting factory. Various plots with rape, soybean, maize and oat were chosen separately. Rhizosphere samples were collected by removing the soil adhering to the plant roots after gently shaking. The falling soils collected by shaking were treated as bulk soils. Soils were lyophilized and passed through a 2-mm sieve. The extraction and cleanup scheme was based on US EPA 3550C and 3630C, respectively. The quantification method was according to the US EPA 8270D.

Enrichment of PAHs-degrading consortia and isolation of PAHs-degraders
About 5 g soils were added to 50 ml of mineral medium, and supplied with 0.5 ml of PAH mixture solution. The PAH mixture of phenanthrene, pyrene and benzo[a]pyrene was dissolved in acetone and filtered through a 0.22 μm pore film and added at final concentrations of 0.2, 0.1 and 0.02 g/L respectively. The solvent was allowed to evaporate on a rotary shaker before adding the sample or inoculation. Enrichment was conducted at 25°C and 120 rpm and kept from light for about 1 month. The enrichment cultures were transferred with 1 ml inoculums to 50 ml fresh medium with PAH spiked, and repeated two times every 2 weeks. About 10^{-4}

dilutions were spread on M8 agar plates and incubated at 25°C. Colonies that were different in morphology from each consortium were streaked onto fresh LB plates to obtain pure culture. Finally, 13 PAHs degrading consortia were obtained.

PCR amplification of the 16S rDNA genes and sequencing
Genomic DNA of isolated bacteria was prepared with the bacteria genome DNA extraction kit (Generay, Shanghai). The 16S rDNA genes were amplified from genomic DNA using the universal primer set 27f and 1502r. The thermal cycling parameters were a 5 min hot start at 94°C, followed 32 cycles of denaturation for 30s at 94°C, annealing at 53.5°C for 30s, and extension for 2 min at 72°C, with a final extension of 20 min at 72°C. The PCR products were cloned into pEASY-T1 simple clone vector (TransGen Biotech, Beijng) and sequenced using M13 prime by Shanghai Invitrogen.

DGGE analysis of the structure of the bacterial communities
Total DNA in soil and enrichment consortia were extracted with FastDNA SPIN Kit for soil (Qbiogene) and genome DNA extraction kit (Generay, Shanghai), respectively. PCR was performed with the total DNA of the consortia or the isolates as templates. Primers F338GC and R518 were used to amplify the variable V3 region of bacterial 16S rDNA genes for DGGE analysis (Muyzer *et al.* 1993). The PCR procedure was as follows: an initial cycle of 5 min at 95°C, followed by 20 cycles of 45 s at 94°C, 1 min at 63.5°C, with a touchdown of 0.5°C per cycle and 45 s at 72°C, then followed by 10 cycles of 45 s at 94°C, 45 s min at 53.5°C and 45 s at 72°C, with a final extension of 10 min at 72°C. Electrophoresis was performed at 60°C in an 8% (w/v) polyacrylamide gel with a denaturant gradient ranging from 40% to 60% for 15 min at 30 V, then 280 min 165V in 1× Tris-acetate-EDTA buffer with Dcode Universal Mutation Detection system (Bio-Rad Labo-ratories, Hercules, CA). After electrophoresis, the gel was stained by SYBR Green I and the pictures were captured by an Tanon Imaging System and analyzed by ImageJ 1.42q (http://rsb.info.nih.gov/ij). The cluster analysis was carried out with R. Bands of interest on the DGGE gel were excised and cloned into the pEASY-T1 simple clone vector (TransGen Biotech, Beijng) and sequenced using M13 prime by Shanghai Invitrogen.

Phylogenetic analysis
The 16S rDNA and 16S rDNA V3 region sequences were aligned with published sequences from the GenBank database using the ClustialX program (Thompson *et al.* 1997). Phylogenetic trees were constructed by the neighbor-joining method using the ape package of R software. Nearly full length 16S rDNA sequences of most phylogenetically related strains were selected from GeneBank database as reference strains.

Results
PAHs and DNA concentrations in rhizosphere
The sum of PAH concentrations in detected bulk soils ranged from 16 μg/g to 33 μg/g. Under all four crops, total PAHs concentration in rhizosphere was significantly lower than in bulk soils ($p<0.05$). Particularly under soybean, total PAHs concentration in rhizosphere was 40% less than in bulk soils (Figure 1. a). The extracted DNA amount from rhizosphere was greater than that from bulk soils (Figure 1. b).

Isolation and identification of PAH-degrading bacteria
Bacteria in enrichment consortia were isolated with M8 plate medium, purified with LB plate medium, and identified by sequencing of 16S rDNA (about 1500bp). Finally 13 strains of different 16S rDNA sequences were obtained and were identified belonging to five genera of the *Firmicutes* and *Proteobacteria* (including *Beta-*, and *Gammaproteobacteria*). All seven strains closely related to *Bacillus* genera (99%) have been proven to be PAH-degraders. Three *Proteobacteria* strains, which were most closely related to *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans,* and *Serratia marcescens* respectively, have all been reported for PAHs degradation. However, two *Lysinibacillus* strains were the first time being testified as PAH degrader in the present study. The Genbank accession numbers of isolations S1 to S11 were from GQ889238 to GQ889248.

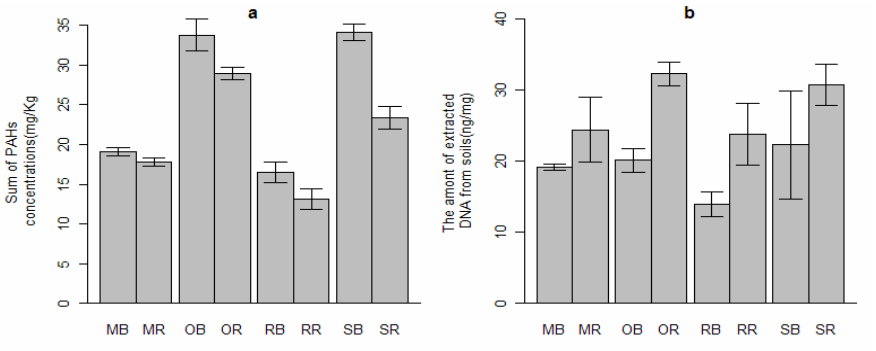

Figure 1. The sum of PAHs concentrations (a) and the extracted DNA amounts (b) in the tested rhizosphere and bulks soils. MB, OB, RB and SB represent the bulk soil in gardens of maize, oat, rape and soybean respectively; MR, OR, RR and SR represent the rhizosphere of maize, oat, rape and soybean respectively.

Table 1. Sequence analysis of bands retrieved from the 16S rDNA DGGE profiles.

Strains (Genbank Accession No.)	Closest type strains in Genbank data base (Accession No.)	Length of fragment (bp)	Similarity (%)
B1(GQ 889238)	*Bacillus subtilis* FQ06 (GQ360038.1)	197	100
B2(GQ 889239)	*Caulobacter sp.* 3-3 (FJ605177.1)	172	100
B4(GQ 889240)	*Bacillus pumilus* NAPCC-1 (FJ458437.1)	195	98
B5(GQ 889241)	*Bacillus sp.* CAt2NG (GQ272359.1)	198	100
B6(GQ 889242)	*Stenotrophomonas maltophilia* (GQ287630.1)	197	100
B7(GQ 889243)	*Erythromicrobium ramosum* (GQ284449.1)	172	98
B8(GQ 889244)	*Acidovorax avenae* (AY512827.1)	197	98
B9(GQ 889245)	*Labrys sp.* LLQ-6 (FJ549002.1)	172	100
B10(GQ 889246)	*Rhizobium sp*. D255c (AB480418.1)	172	100
B11(GQ 889247)	*Burkholderia sp*. lxb-13 (GQ249223.1)	197	100
B12(GQ 889248)	*Cytophaga sp.* SSL03 (EU395843.1)	192	100

DGGE analysis of the bacterial structure

The diversity of enriched PAH-degrading bacterial consortia, presented as the bands of DGGE gel profile, was sharply lower than that of bacterial community diversity in bulk soil. There were dozens of bands in each lane of the DGGE gel profile for bacterial community both in rhizosphere and bulk soils. About ten bands were, however, observed in each lanes of the DGGE gel profile of PAH-degrading bacterial consortia. The bacterial community diversity was different between rhizosphere and bulk soils, except under rape. Among the bands, *Stenotrophomonas maltophilia* (B6) was stronger than other bands in all lanes. *Acidovorax avenae* (B8) tended to be stronger in rhizosphere under all crops (Table 1). According to the results of cluster analysis, the discrepancies of PAH-degrading bacterial community diversity among crop speices were obvious, and were much less than those between rhizosphere and bulk soils.

Bacterial identification by band sequencing

All strong bands in DGGE gel profile were subjected to DNA sequencing. Eleven sequenced bands covered nine genera of *Firmicutes, Bacteroidetes*, and *Proteobacteria* (including *Alpha-*, *Beta-*, and *Gammaproteobacteria*). The Genbank accession numbers of sequenced bands were from GQ889249 to GQ889261. The isolated strains did not match the strong bands in DGGE gel profile, except that both B1 and S1 were identified as *Bacillus pumilus* with similarity of 100% and 99%, respectively. The species in PAH-degrading enriched consortia were much broader than that of the isolated strains. Among the bands in consortia, B1, B4 and B5, belonging to *Firmicutes,* were closely related to *Bacillus subtilis* FQ06 (100%), *Bacillus pumilus* NAPCC-1 (98%), and *Bacillus cereus* HB59 (100%), respectively. B2, B7, B9 and B10, four genera of *Alphaproteobacteria,* were closely related to *Caulobacter sp.*3-3 (100%), *Erythromicrobium ramosum* THWCS10 (98%), *Labrys sp.* LLQ-6 (100%), and *Rhizobium sp*. D255c (100%), respectively. Two *Betaproteobacteria* bands*,* B8 and B11, were closely related to *Acidovorax avenae* and *Burkholderia sp. lxb-13* with similarities of 98% and 100% respectively. Band B6 was closely related to *Stenotrophomonas maltophilia* of *Gammaproteobacteria,* and band B13 was closely related to *Cytophaga sp.* SSL03 of *Bacteroidetes.* Most of corresponding species for each band have been widely reported to be PAH degrader or be isolated from PAHs polluted environment. With the sequences of 16S rDNA of all isolated strains and DGGE bands, a rooted phylogenetic tree was constructed. The biggest group was *Firmicutes,*

which was composed of ten strains and three bands, and included ten *Bacillus* and three *Lysinibacillus sphaericus*. The second biggest group was *Alphaproteobacteria,* which was composed of four bands as *Caulobacter sp., Erythromicrobium sp.*, *Labrys sp.*, and *Rhizobium sp*. respectively. *Betaproteobacteria* included two bands and one strain, *Gammaproteobacteria* group included one band and two strains, and *Bacteroidetes* group only had one band. Expect *Lysinibacillus* strains, all other bacteria species have been reported for PAH degrading or resisting capacity.

Conclusion

This study indicated that the rhizosphere of vegetation in contaminated field contains higher diversity of population of PAH-degrading bacteria. And some of the identified PAH-degrading bacteria have the potential for PAHs bioremediation in rhizosphere, among which two *Lysinibacillus* strains were reported as PAH degrader for the first time in the present study.

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Microbial extracellular enzymes and natural and synthetic polymer degradation in soil: current research and future prospects

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Abstract
Bacteria and fungi encounter complex organic matter in soil that is a potential source of the energy, carbon, and nutrients that are required for cell maintenance and growth. Cellulose and lignin are two of the most abundant biopolymers in detritus. However, bacteria and fungi do not have the ability to transport these macromolecules into the cytoplasm. Instead they depend on the activity of extracellular enzymes which generate soluble low molecular mass compounds that are recognized by cell wall receptors and transported into the cell. Many organic pollutants in soil are polymeric and poorly soluble (e.g. PAHs, PCBs) or toxic and these also require extracellular catalysis prior to uptake and metabolism. The complexity and diversity of extracellular enzymes and the macromolecules that they degrade will be reviewed and the many locations and multiple fates of these enzymes once they have left the cytoplasm illustrated. The many ways in which extracellular enzymes overcome the destructive or inhibitory properties of the soil matrix and the various strategies that microbes adopt for effective substrate detection and utilization will be described.

Key Words
Extracellular enzymes; cellulose; lignin; xenobiotics, microbial ecology**.**

Introduction

Enzyme synthesis and secretion is an energetically expensive process and regulatory mechanisms and ecological strategies have evolved to ensure the efficient capture of the products of substrate catalysis. However, soil is a hostile environment for extracellular enzymes because, once they leave the cell, they are subject to denaturation, degradation, adsorption and dilution. The locations and functions of enzymes in soil have been researched and discussed for decades (Burns 1978; Burns 1982; Caldwell 2005; Nannipieri *et al*. 2002; Wallenstein and Weintraub 2008) but recent advances in molecular, microscopic, and analytical techniques coupled with imaginative thinking (Allison 2005; Bouws *et al*. 2008; Wallenstein and Weintraub 2008) have begun to provide new insights. Developments have been motivated by the need to understand how the activities of enzymes contribute to a large number of industrial, medical and environmental processes.

Substrate and enzyme diversity in soils

Extracellular enzymes in soils catalyse the degradation of plant, animal and microbial macromolecules in addition to many potentially polluting xenobiotics. The most common plant polymers, cellulose and lignin, depend on the simultaneous and/or sequential activities of a large number of enzymes produced by a diverse community of bacteria and fungi; it is likely that more than fifty different extracellular enzymes are involved in the breakdown of a plant leaf prior to the low molecular mass carbon products entering the cell. Basidiomycete and ascomycete fungi are major degraders of cellulose, employing a battery of extracellular hydrolytic enzymes including endo-1,4-β- glucanases, cellobiohydrolases and β-glucosidases. The best known cellulose degrader, *Trichoderma reesei*, has thirty or more glycosyl hydrolases including seven endo-glucanases, and a secretome containing greater than one hundred proteins. Lignin, with which cellulose is usually associated, is a chemically complex phenylpropanoid that is degraded by a suite of oxidative enzymes containing laccases, manganese peroxidases and lignin peroxidases (Baldrian 2006; Osono 2007). The involvement of Fenton chemistry in the process demands the input of enzymes generating hydrogen peroxide as well as Fe^{2+} and Mn^{2+}. The white rot fungus, *Phanerochaete chrysosporium,* has more than 85 genes for glycosyl hydrolases, in excess of 100 for 'ligninases' and a secretome of almost 800 proteins. The feasibility of using *P. chrysosporium* (and many other fungi) for the oxidative degradation of organic pollutants, such as PAHs, PCPs, dioxins and many pesticides has been much studied (Rubilar *et al*. 2008; Husain *et al*. 2009).

Regulation, location and ecology of extracellular enzymes

In some cases, microbes produce small amounts of extracellular enzymes, regardless of substrate availability,

as a mechanism to detect substrate. If the substrate is present, these constitutive enzymes generate signals that induce additional enzyme synthesis. The synthesis *de novo* of many celluloytic extracellular enzymes is stimulated only in the presence (or sometimes absence) of a suitable substrate or other inducer whereas, in contrast, many 'ligininases' respond to stress factors such as redox potential, ionic strength, Fe^{2+}, CO_2, light, sulphide and sulfate and oxalic acid.

In broad terms, extracellular enzymes are contained within the periplasmic space (Gram-negative bacteria), associated with the outer cell wall, or released into the soil. However, the multiple functional locations of extracellular enzymes, combined with the capacity of the microbial community to detect potential substrates, suggest that there are many ways in which macromolecular organics can be transformed into soluble matter.

Enzymes that are retained on the cell wall are likely to be configured such that their active sites are exposed and the zones that are susceptible to attack by proteases are protected. Other cell-bound extracellular enzymes include those contained within a multicellular biofilm and others that are protected by specialized structures attached to the cell wall. The latter are the cellulosomes and contain cellulases (as well as hemicellulases and pectinases) arranged on a scaffold that facilitates efficient cleavage of polysaccharides (Bayer *et al*. 2008). Cells with bound enzymes must be in contact with their substrate.
Once enzymes have diffused away from their parent cell they may be sorbed, denatured and degraded. However, some extracellular enzymes are more stable than their intracellular counterparts because they are glycosylated, have disulfide bonds: modifications providing thermo-stability, a broad pH range for activity, and some resistance to proteases. In addition, some enzymes become stabilized through interactions with clay minerals and humic acid and retain a proportion of their activity (Allison 2006; Quiquampoix and Burns 2007). Stabilized soil enzymes represent a reservoir of potential activity and may represent the first catalytic response to changes in substrate availability in soils as well as serving as the originator of signal molecules for the microbial community. Even if the enzymes survive, the substrate may not be found and, even if it is, the correct combination of enzymes in the right sequence must be present for catalysis to occur.

A way to overcome some of these constraints is suggested by a mechanism that involves microbes 'sensing' both the substrate and their own population numbers. In this way gene function is connected to cell density and enzymes are only synthesizing and/or secreted when cell numbers are high enough to have a major impact. This is a process known as quorum sensing and has been well-described for many phytopathogens especially *Erwinia* species (Barnard and Salmon 2007). Quorum sensing in the rhizosphere is believed to be an important controlling process for all sorts of microbial interactions (DeAngelis *et al*. 2008).

Once in contact with their substrate many polysaccharases have a number of ways in which they not only maintain their stability but also increase their activity. One mechanism relates to the all-important substrate binding moiety (Wilson 2008) which, in the case of cellulase, anchors the enzyme to the substrate at appropriate points for the enzyme's catalytic domain to cleave the β-1,4-linkages. Few microorganisms secrete all the necessary enzymes and must rely on other microbes to successfully generate the soluble products. This observation reinforces the idea of a community-driven process.

However they are generated, the products of extracellular organic matter breakdown may be intercepted by other microbes which, although not investing any resources in enzyme production, will benefit (Allison 2005). Some microbes employ antibiotics and enzymes to reduce this 'cheating', others rely on the activities of predators to control their rivals. Of course, what might appear as cheating may be part of a complex and poorly understood microbial community synergy: the so-called cheaters provide some direct or indirect benefit to the cheated. Or it may be that the benefits of a successful extracellular depolymerization far outweigh the disadvantages derived from some of the products being high-jacked.

Conclusion
An enhanced knowledge of extracellular enzyme function will have many practical applications, including manipulating the soil for bioremediation, biocontrol, plant nutrient generation and availability, aggregate stability, and C cycling and sequestration. There are also implications for plant pathology, food quality and storage, biofuel production, and the impacts of climate change on enzyme activities and the humic matter pool. One of the greatest challenges in soil biology is to link the functional and ecological aspects of microbial extracellular enzyme activities to organic matter degradation. We are now equipped with the analytical (electrophoretic, chromatographic, mass spectrometric), microscopical (fluorescence, scanning probe, atomic and ultrasonic force, confocal laser, differential interference), molecular (genomics,

proteomics, metabolomics, secretomics, metagenomics) and bioinformatic tools to achieve these objectives (Wallenstein and Weintraub 2008). Are the activities of microbial enzymes in soil an example of organized chaos, ongoing selection processes or the expression of an advanced and stable degradative community? The next few years will answer many of these questions.

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Modelling the chemical influences on bioavailability of geogenic arsenic in soils

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Abstract
Arsenic contaminated soils are a major problem worldwide and understanding how soil affects the fate of arsenic is important in determining the risk to humans who use the land. Most studies have investigated the influence of individual soil physico-chemical variables on arsenic mobility, but they are not readily extrapolated to predict the fate of arsenic in more complex soil systems. In this study the chemical controls on arsenic mobilisation were investigated using a geogenic arsenic rich soil from an allotment site in Northern England. Investigations of the distribution of arsenic suggested that the dominant factor (>70%) influencing arsenic mobility in the soils was the presence of iron oxide and hydroxide minerals. Bioavailability of arsenic in the soils was highest in the top 30 cm of the soil. Multiple regression analysis of the dataset suggested that the presence of phosphate ions increased the bioavailability of arsenic. Moreover, calcium ions increased arsenic sorption to the soil surface thereby reducing bioavailability. These findings have important implications for current land-use where applications of phosphate fertilisers may mobilise arsenic and increase its bioavailability.

Key Words
Brownfield, sequential extraction procedure, ICP-MS, principle component analysis.

Introduction

Arsenic contamination of soils is a worldwide problem. Research has been undertaken to study the human health risks associated with anthropogenic arsenic while geogenic arsenic (i.e. from natural sources) has been largely overlooked. To meet the demands for the ever increasing global population, and the need to development land for housing and agriculture, contaminated land that has previously been seen as unfit for human inhabitation (e.g. contaminated land) is being utilised. However, such land uses may increase the risk to humans from exposure to toxic elements such as arsenic. Remediation of arsenic contaminated land is currently not feasible and so risk assessments must be undertaken to determine potential land use for contaminated sites before development can take place. Risk assessments have previously been based on the total metal loading of the soil with a concentration of 20 mg/kg soil generally considered the maximum safe limit for residential soils (UK EPA 2004). However, recent research suggests total arsenic is not necessarily a reliable measure of risk from contaminated soils and other factors such as speciation (Ge *et al.* 2000), bioaccessability (Palumbo-Roe *et al.* 2005) and the fractional distribution of arsenic in soils (McLaren *et al.* 1998) may represent a more accurate measure of potential risk.

Arsenic distribution in soils is variable (both spatially and temporally) and is dependent on the soil's physical, chemical and biological properties. In order to predict risk from exposure to contaminated soils it is important to model the effects of these different properties on the cycling of arsenic. Soil parameters including pH and redox potential (Masscheleyn *et al.* 1991), organic matter (Gustafsson *et al.* 2003), calcium and phosphate ion concentration (Davenport and Peryea 1991) and iron and aluminium oxides/hydroxides (Cances *et al.* 2005) have all been found to affect the biogeochemical cycle of arsenic.
Often these studies investigated the effects of soil properties in isolation and may not represent the behaviour of arsenic in complex media such as soils. Soil is a heterogeneous matrix and it is important to model the soil as a whole system in order to fully understand the risks posed to humans and the environment from arsenic contaminated land. The aim of this project was to identify the chemical parameters that influence the adsorption and desorption of arsenic from a geogenic arsenic rich soil.

Methods

Study site and sampling regime
The location selected for this study was Buckingham Avenue allotment site in Scunthorpe (53°36'N,

0°39'E), North Lincolnshire, UK (see Figure 1). The soil on the site was formed on Jurassic Ironstone which has been found to be naturally rich in arsenic (Palumbo-Roe *et al.* 2005). One area of the site that was chosen for sampling has been under "natural" vegetation for over 15 years, with no recorded anthropogenic activity. Soil sampling was carried out at this site in January 2006, and consisted of a series of topsoil samples taken at 5 m increments along a 25 m transect. In addition a soil pit (dimensions: (w) 1 m x (l) 2 m x (d) 0.7 m) was dug in the centre of one of the allotment plots, and samples taken from the soil profile at 5 cm increments to a depth of 70 cm. These samples were taken to determine spatial variability both across the site and with depth in the soil.

Sequential Extraction Procedure
Fractionation of arsenic in the soil samples was carried out using a modified sequential extraction procedure (SEP) (Wenzel *et al.* 2001), with the final step in the procedure changed to a nitric acid digestion method taken from Castlehouse *et al*. (2003). The SEP identified five operationally defined phases for arsenic sorption in soils (i) non-specifically sorbed; (ii) specifically sorbed (exchangeable); (iii) sorbed to amorphous iron oxyhydroxides; (iv) sorbed to crystalline iron oxyhydroxides; and (v) residual. The concentration of arsenic in the fractions was analysed by ICP-MS. Speciation of arsenic in the non-specifically sorbed phase was carried out by HPLC-ICP-MS.

Chemical characterisation
Total metals in the soils were extracted by nitric acid digestion and analysed by ICP-MS (Castlehouse *et al.* 2003). Soil waters were extracted using a 1:10 soil solution ratio and the 0.45µm filtered extracts analysed for soluble cations and anions by ion chromatography and total inorganic and total organic carbon using a carbon analyser. Other analyses on the soil included total carbon, hydrogen and nitrogen, pH and total organic carbon by loss on ignition.

Statistics
Statistical analyses were carried out on the data using Minitab® v14. One-way and two-way ANOVA were used to determine any significant trends in the data. Posthoc testing using Fishers individual error rate was carried out to enable more detailed interpretation of the datasets. Multiple linear regression analysis was carried out on the whole dataset using principal component analysis (PCA) to determine any relationships between parameters.

Results
The results from the analysis of topsoil samples taken along the transect found that there was no variability in any of the soil chemical properties with location on the site (data not shown). This is consistent with the soil being under natural vegetation for a number of years. No variability in concentrations of total elements in the soil was found with changes in depth. However, results for the soluble ions and arsenic fractionation were found to have significant trends.

The distribution in arsenic amongst the five operationally defined phases was found to vary significantly with depth in the soil ($P<0.001$). The majority of arsenic at all depths in the soil profile was found to be sorbed to iron oxyhydroxides phases in the soil (Figure 2) with >70% of the total arsenic in the soil sorbed within these phases. The bioavailable fraction of arsenic in the soil, represented by the non-specifically sorbed phase was also found to vary significantly with depth ($P<0.001$) with the highest concentration (0.154 mg/kg) measured in the top 5 cm of the soil. Concentrations of non-specifically sorbed arsenic decreased between 5 and 35 cm and were thereafter less than the detection limit. The results for the specifically sorbed arsenic were also found to vary significantly in the depth profile however there was no consistent trend in the dataset. Residual arsenic was not found to vary with changes in depth.

Results of the chemical characterisation showed a number of parameters varied significantly with depth. Phosphate ($P<0.001$), nitrate ($P<0.001$), potassium ($P<0.001$) and fluoride ($P<0.001$) were found to decrease with depth in the soil with the highest concentrations measured in the top 30 cm. Calcium ($P<0.001$) and sulphate ($P<0.001$) were also found to have significant trends with concentrations increasing with depth. The results for both chemical characterisation and arsenic fractionation analyses were subject to principal component analysis to determine any relationships within the dataset. Phosphate was found to have a significant positive correlation with non-specifically sorbed arsenic (Figure 3). A positive correlation was also found between calcium ions and specifically sorbed arsenic. No other relationships were found in the data.

Figure 1. Location of research site in Scunthorpe, NE England (53°36'N, 0°39'E).

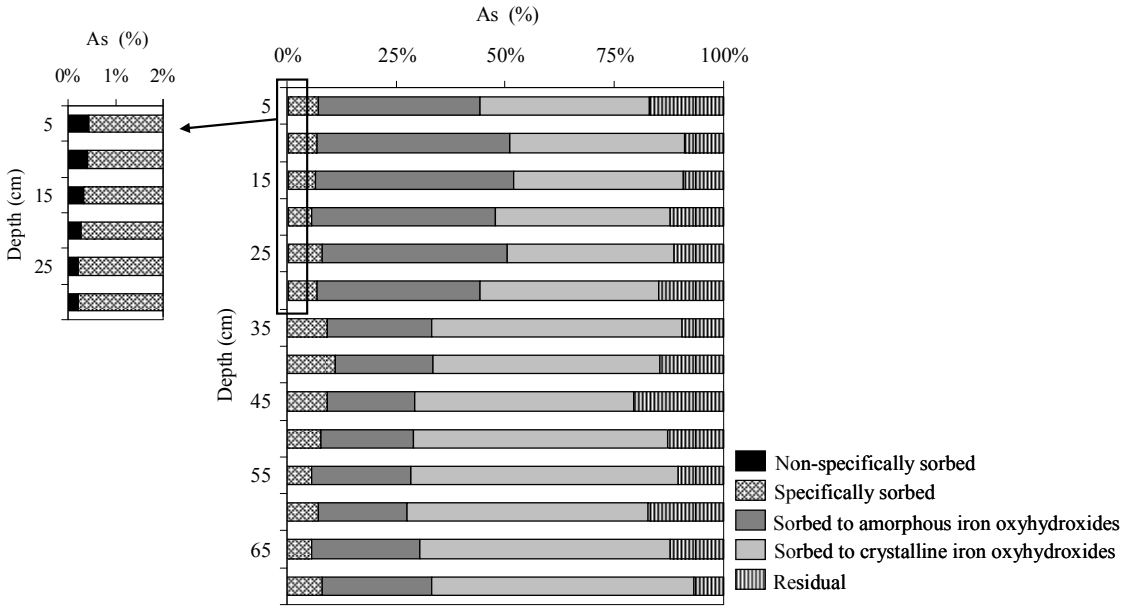

Figure 2. Fractional distribution of arsenic amongst five operationally defined phases as a function of depth. Inset: enlargement of the non-specifically sorbed arsenic phase.

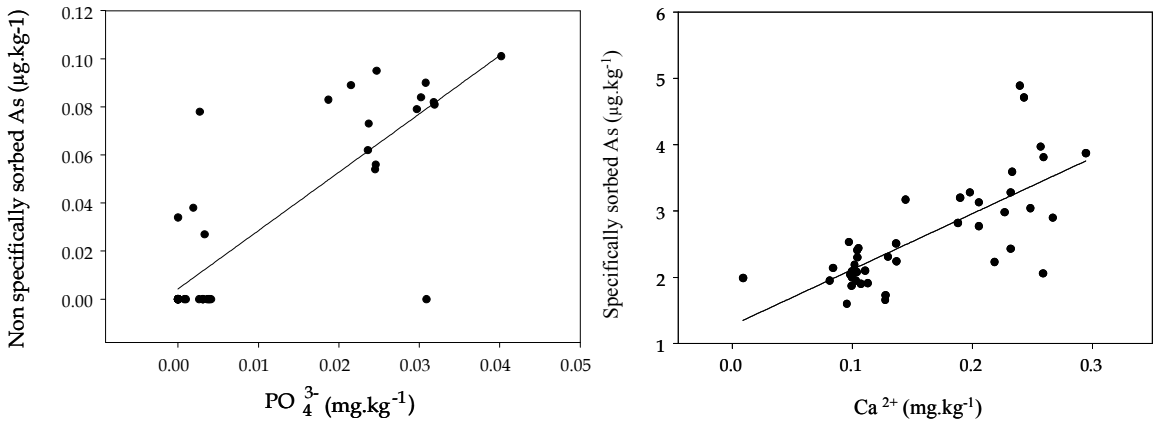

Figure 3. Regression analysis of (Left): phosphate ion concentration and non-specifically sorbed arsenic (r^2 = 0.695; *P*<0.001) (Right): calcium ion concentration and specifically sorbed arsenic (r^2 = 0.542; *P*<0.001).

Conclusion

This study found that most of the arsenic in the soil was bound to crystalline and amorphous iron oxyhydroxides (>70%). These phases are generally not bioavailable and therefore suggest that the majority of the arsenic in the soil is low risk for leaching and uptake by plants under current conditions.
The arsenic in the non-specifically sorbed and specifically sorbed phases was found to vary significantly with depth in the soil. Regression analysis of the data suggested phosphate may increase arsenic mobility by increasing the concentration of non-specifically sorbed arsenic. In contrast calcium may have increased sorption of arsenic to the soil with a corresponding increase in the concentration of specifically sorbed arsenic. These results suggest that the application of soil amendments such as phosphate fertilisers and lime may have a significant impact on the fate of arsenic in these soils. This has major implications for the current land use on the site and highlights the need for further investigation into the biogeochemistry of arsenic.

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Phytotoxicity assay of selected plants to Pyrene contaminated soil

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Abstract
Three selected plants (Alfalfa, oilseed rape and perennial ryegrass) were tested for their ability to germinate and grow in a soil contaminated with a typical polycyclic aromatic hydrocarbon (PAH), Pyrene, at two concentrations: 50 mg/kg or 100 mg/kg dry soil. When compared to control plants grown in uncontaminated soil, pyrene did not inhibit the germination of the three tested species even at the highest concentration (100 mg/kg), with a germination percentage of alfalfa, oilseed rape and ryegrass of 95, 90 and 86%, respectively. The shoot and root lengths of the three plants were also unaffected by the presence of pyrene at both concentrations. Based on these results, the three tested species can be considered as pyrene tolerant plants, and can be used in phytoremediation experiments. Among the tested species, oilseed rape seemed to be the most suitable plant, probably because of the greater root length. Greenhouse studies are in progress to evaluate the development of these plants in the advanced growth stages, and their effect on pyrene level in soil.

Key Words
PAH, pyrene, phytoremediation, alfalfa, oilseed rape, perennial ryegrass.

Introduction
Recently, the PAHs contribute to the environmental matrix has gained an increased attention by the research community (Henner *et al*. 1997). PAHs are a class of very hazardous pollutants that accumulated increasingly in the environment. They consist of two or more conjugated aromatic rings ranging from a simple two-ring compound to more complex six-ring compounds. The US Environmental Protection Agency (EPA) has indicated 16 PAHs as priority pollutants for carcinogenic risk **(**Agency for Toxic Substances and Disease Registry**,** ATSDR).

In general, the commonly used remediation technologies for PAHs contaminated soils are very expensive, and sometimes can damage the natural soil structure and texture. (Lundstedt *et al.* 2006). On the opposite, the use of plants, their associated microflora and agronomical techniques to lower soil toxicity is considered to be a safe, efficient, eco-friendly and economic means of removing pollutants from contaminated soil, as proved by the numerous phytoremediation studies carried out in the last years (Venkata *et al*. 2006).Several evidences show that the concentration of PAHs can be strongly reduced by the presence of plants, which enhance their removal when compared to bulk soils.

Numerous plant species were tested for their ability to remediate soils contaminated with pyrene. In particular, the PAHs degradation is enhanced in the rhizosphere compartment, where rhizodeposition stimulates the growth of microorganisms, characterized by great densities and activities than those of surrounding soil (Fan *et al*. 2008). For example, the advantage of choosing legumes is due to their ability to fix atmospheric nitrogen, whose availability in PAH-contaminated soils is often limited (Hutchinson *et al.* 2001). Similarly, the benefit of choosing grasses is due to their extensive fibrous root system, which would provide a larger surface for colonization by soil microorganisms than a taproot (White *et al*. 2006). Anyway , an initial phytotoxicity bioassays can be a useful and effective screening tool to eliminate plants which are sensitive to the contaminants found in soil and reduce the number of plants for pot or greenhouse phytoremediation studies (Kirk *et al.* 2002).

Pyrene was selected among the 16 EPA priority PAHs as the model compound since it exhibits intermediate toxicity, hydrophobicity and environmental persistence , and represents the dominant PAH produced by incomplete combustion of oil and oil-byproducts. Pyrene is a four-ringed PAH, colorless, and biodegradable (Kanaly and Harayama 2000). According to the current legislation (D.Lgs.152-2006, All.4), the pyrene level in sites of private and public residential use and of industrial use should not exceed 5 mg/kg and 50 mg/kg, respectively.

Materials and methods
An uncontaminated soil (no previously history of contamination with pyrene) was collected from a farm in Turi, South Italy. It has a clay loam texture, pH 7.81, organic carbon content 25 mg/kg, total nitrogen 2.4 g/kg, available phosphorous 40.7 mg/kg and exchangeable potassium 626 mg/kg. Before use the soil was dried in greenhouse to constant weight.

Three plant species were chosen (Alfalfa (*Medicago sativa* L), Perennial ryegrass (*Lolium perenne L.*) and oilseed rape (*Brassica napus)*) according to their endemic spread (indigenous species) and their nature (legumes and grass). Seeds were purchased by N. Sgaravatti and C. Sementi S.P.A., Italy.

The general phytotoxicity assay described by Henner *et al*. (1999) and Kirk *et al*. (2002) was followed, with few modifications. For each experiment, 40 g of dried soil were added to a plastic Petri dish in 4 replicates. A stock solution of pyrene (98% purity, SIGMA-ALDRICH) in acetone was added to soil to reach two concentrations, 50 and 100 mg/kg. The spiked soil was carefully mixed and air-dried under fume hood for more than 24 hour, until the smell of acetone had disappeared. Simultaneously, a blank was performed by adding to the soil the same quantity of acetone in order to observe the possible solvent effect on tested plants, and a control with an equivalent amount of water instead of the acetone.

The seeds of Alfalfa, Perennial ryegrass and oilseed rape were immersed in water for 1 hour and then sown in the pyrene spiked soil using 7 seeds per Petri dish. The dishes were arranged in completely randomized design at room temperature and under natural sunlight. To make up for the water loss, each plate has been added with 10–20 ml of water daily. After 14 days, the number of seeds germinated for each treatment was counted and plants were removed to measure shoot and root lengths. Data were statistically analysed using the one-way analysis of variance (ANOVA), and comparisons of means were carried out using the Duncan's test.

Results
The germination data indicate that pyrene did not inhibit the germination of the tested plants (Figure 1). In particular, the germination of the three tested plant species is not significantly affected by the presence of pyrene at both concentrations. These results agree with those of Smith *et al.* (2006), who reported that germination of seven plants (grasses and legumes) is not affected by PAH contamination in soil, whereas dry foliage yield was significantly reduced. The same conclusion was reached by Sverdrup *et al.* (2007), who showed that PAHs have no influence on seed germination of *L. perenna T. pretense* and *Brassica alba*, whereas the growth of these plants, in terms of plant dry weight, is reduced. Huang *et al.* (1996) reported that pyrene induced chlorosis in *Brassica napus* L., but this symptom was not found in this study.

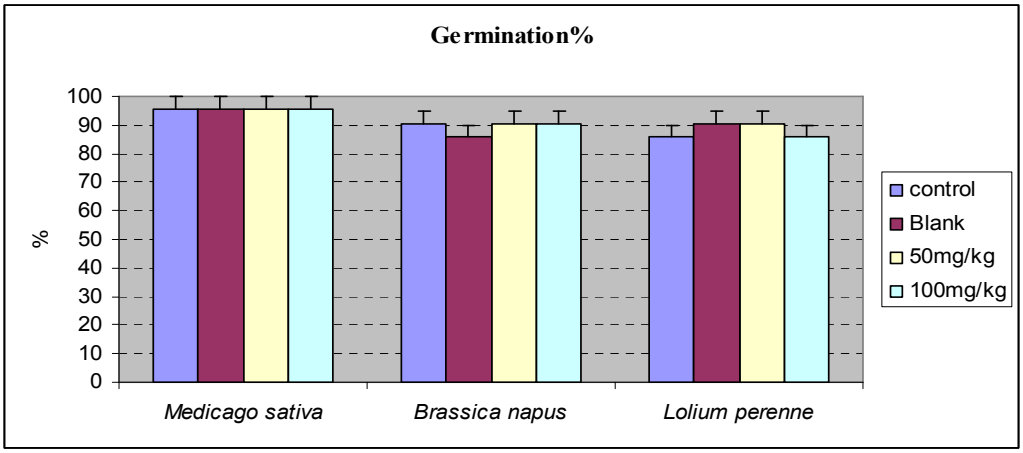

Figure 1. Germination percentage of the three tested plant species.

Similarly, the shoot and root lengths of control alfalfa, oilseed rape and ryegrass plants grown in the presence of pyrene, even at the highest concentration, were not significantly different from those grown in the absence of pyrene (Figures. 2 and 3).

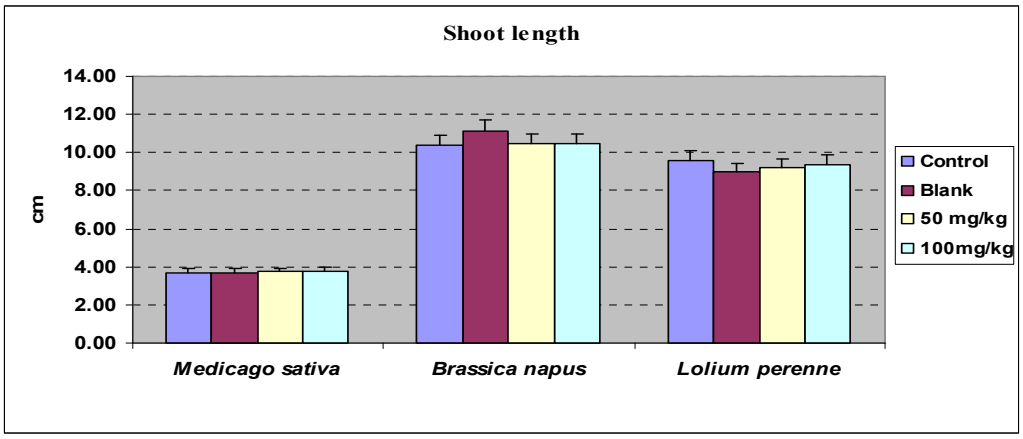

Figure 2. Shoot length of the three tested plant species.

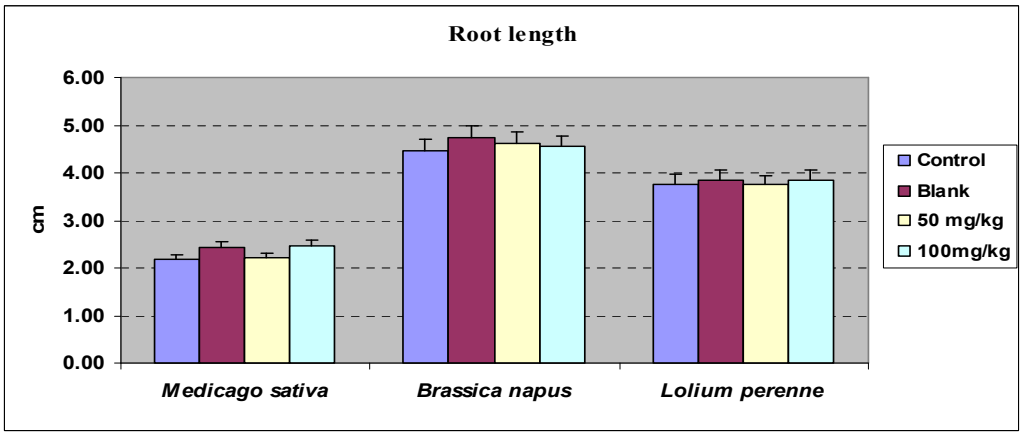

Figure 3. Root length of the three tested plant species.

In this study, we evaluated the extent of seed germination and seedling growth over a relatively short period of time (14 day). Obviously, seed germination represents the first important step to perform an effective phytoremediation. In fact, young seedlings may be particularly susceptible to PAH contaminants, since early periods of seedling growth are extremely important for root development and, consequently, for phytoremediation. Thus, the assessment of a good seedlings growth in PAH-contaminated soils may provide an important indication of the potential value of these plants in phytoremediation.

On the other hand, it is well known that for an effective phytoremediation, it is desirable to have tolerant plants with a root apparatus well distributed in soil, being the root length a parameter to assess the phytoremediation ability of different plant species. Longer roots may increase the rhizosphere area and thereby enhancing the ability to support soil microorganisms as compared to shorter ones (Harvey *et al*. 2002). Based on these considerations, the three tested species can be considered potentially as pyrene tolerant plants. Considering that longer root system is preferred for plants selected for use in phytoremediation, *Brassica napus* should appear to be the most promising plant.

Conclusion

The three plant species tested in this study germinate and grow well in pyrene contaminated soil. They show a good germination pattern, and no negative impact of pyrene has been observed in their shoot and root development. The general response of all tested plant species to pyrene is similar in all cases, both as germination percentage and as shoot and root growth, and no negative effects have been induced by pyrene, also at the highest concentration.

Brassica napus with the longest root seems to be the most suitable plant when grown in pyrene contaminated soil. Although roots of *Medicago sativa* seemed to be shorter than those of *Brassica napus*, the value of nitrogen fixing bacteria in stimulating pyrene remediation in roots of *Medicago sativa* should not be ignored.

PAHs generally do not kill plants, but would slow down or inhibit growth by decreasing plant biomass or

elongation. PAHs are also known to induce plant genetic mutation, retard growth, and increase the sensitivity of the plant to other stresses (Maliszewska-Kordybach and Smreczak 2000). Depending on these considerations, further greenhouse studies are in progress to evaluate the growth and the development of the three plant species in successive advanced stages by observing the effect of pyrene on their foliage yield, plant biomass and dry weight, in addition to their phytoremediation ability to decrease the pyrene level in soils.

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Plant availability of arsenic and cadmium as influenced by biochar application to soil

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Abstract
Biochar has gained significant importance due to its ability to increase long-term soil carbon pool and crop productivity. A pot experiment was conducted to investigate the influence of biochar on the availability of arsenic (As) and cadmium (Cd) to maize. An activated wood biochar was applied at three rates (0, 5 and 15 g/kg) in factorial combinations with three rates (0, 10 and 50 mg/kg) each of As and Cd separately to a sandy soil. After 10 weeks of growth, crop was harvested and dry matter yield and concentration of As and Cd were determined. The soil was analysed for extractable trace elements after the plant harvest. The results showed that the addition of wood biochar to soil did not have any significant influence on the dry matter yield of maize shoot, even at the highest application rate. However, application of As and Cd significantly reduced the dry matter yield by 93 and 27%, respectively. Biochar application decreased the concentration of both As and Cd in maize shoots. However, the concentrations of extractable As increased with biochar treatment and the effect of biochar on DTPA extractable Cd in soil was inconsistent. The results show that biochar application can significantly reduce the bioavailability of As and Cd to plants and suggest that biochar application may have potential for remediating contaminated soils.

Key Words
Trace elements, charcoal, adsorption, bioavailability, heavy metals.

Introduction
Biochar is a product of thermal decomposition of biomass produced by the process called pyrolysis. Biochar has been found to be biochemically recalcitrant as compared to un-charred organic matter and possesses considerable potential to enhance long-term soil carbon pool (Lehmann *et al.* 2006). Biochar has been shown to improve soil structure and water retention, enhance nutrient availability and retention, ameliorate acidity, and reduce aluminium toxicity to plant roots and soil microbiota (Glaser *et al.* 2002). Research has demonstrated that biochar application to soil can substantially raise the productivity of field crops (Chan *et al.* 2007; Lehmann *et al.* 2003; Rondon *et al.* 2007; Rondon *et al.* 2006). Biochar possesses organic functional groups on its surfaces and the negatively charged organic functional groups increase over time during its oxidation in soil (Cheng *et al.* 2008). The formation of surface functional groups and adsorption sites on biochar could influence its cation exchange capacity (CEC) (Cheng *et al.* 2006; Liang *et al.* 2006) and consequently the capacity of biochar-amended soils to form complexes with metal ions. This research was conducted to investigate the influence of an activated wood biochar on the availability and uptake of As and Cd by maize.

Materials and methods
A glasshouse experiment was conducted using three levels of biochar (i.e.; 0, 5 and 15 g/kg soil) combined factorially with three rates (i.e.; 0, 10 and 50 mg/kg soil) each of As and Cd separately. The required amount of biochar was thoroughly mixed with 1 kg soil. Each of the pots was fertilized with a basal dose of N, P and K at 100, 40 and 50 mg/kg, respectively. Hybrid maize (cv 31H50) were sown in each pot, and the germinated plants were later thinned to keep three plants per pot. After 10 weeks of growth, the aboveground biomass of the maize plants was harvested. The dry matter yield was recorded and the dried samples were digested in a mixture of nitric and percholoric acids. The digests were analysed for As and Cd with a Varian Vista AX CCD inductively coupled plasma atomic emission spectrometer. After the harvest of plants, soil was air dried, well mixed and passed through a 2-mm sieve. Soil pH and electrical conductivity (EC) were measured. The available Cd in soil was extracted by the DTPA extractant (Lindsay and Norvell 1978) and solutions were analysed for available Cd with a Varian 220FS flame atomic absorption spectrometer. Available soil As was determined by the phosphate extraction method (Alam *et al.* 2001) and the extracts were analysed for As with a Varian 220Z hydride generation atomic absorption spectrometer. All data were analysed by the generalized linear model analysis of variance using Genstat v10 (VSN International Ltd, UK 2007).

Results and discussion

The shoot dry matter yield of maize was not significantly affected by biochar application at different rates (0, 5 and 15 g/kg) in the absence of trace elements (Figure 1); these results are consistent with the results of a study by Hartley et al (2009), where biochar application did not show any significant effect on the dry matter yield of *Miscanthus*. Since the nutrient-poor sandy soil used in this study received adequate amounts of essential nutrients through basal fertilizers in all treatments, a positive response in biomass yield to nutrient addition through biochar application was not expected. Furthermore, the response of dry matter yield to biochar application may be more pronounced at levels higher than used in the present study. Arsenic was found to have the more significant adverse effect on the dry matter yield of maize (Figure 1).

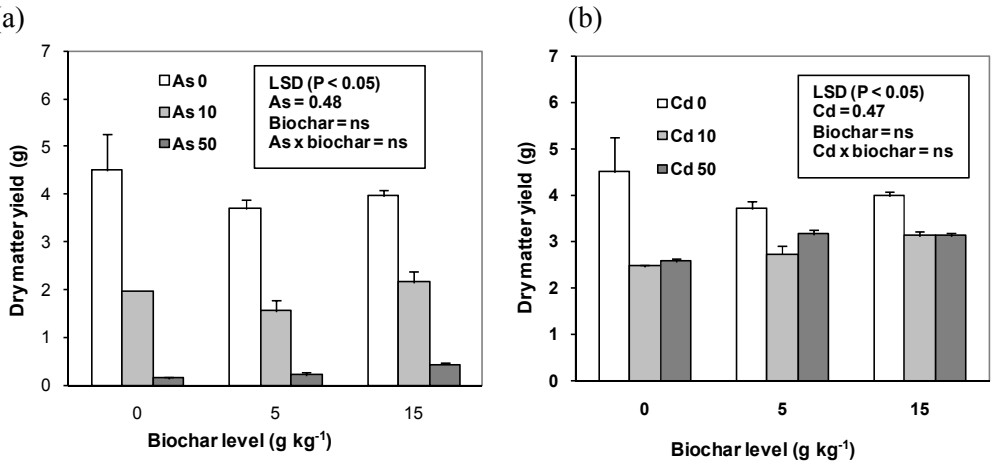

Figure 1. Maize shoot yield as influenced by biochar application to soil in combination with (a) As and (b) Cd.

Addition of biochar to soil was found to reduce shoot concentration of As (Table 1), possibly due to strong binding of As on the surface functional groups of biochar, making it less available to the plants. Cao and Ma (2004) found that addition of compost reduced As accumulation in carrot and lettuce, which they attributed to strong adsorption of As by organic matter. It is well established that trace elements can readily bound to organic matter in soil; however, organic amendments may enhance trace element mobility and bioavailability if the complexes are formed with soluble components of the amendments (Kumpiene *et al.* 2008), or by increasing supply of organic anions that may compete with certain trace elements for adsorption sites in soil (Dobran and Zagury 2006). The increase in extractable As in biochar-amended soil, as observed at the highest rate of biochar application (Table 1), may be attributed to the fact that biochar possesses and develops negatively-charged functional groups (Cheng *et al.* 2006), which may limit adsorption sites for As and hence contribute to the slight increase in extractable As in soil where biochar was added at the highest level. It is also possible that As accumulated in maize roots, which were not removed from soil, may have been extracted by the extractant used to measure available As.

Significant reduction in the concentration of Cd in maize shoots in biochar-amended soil (Table 1) can be attributed to the formation of stable metal-organic complexes (Kumpiene *et al.* 2008) and adsorption of the trace elements to organic matter (Elliott *et al.* 1986). The increase in pH caused by biochar application may also have enhanced the Cd adsorption to biochar. The development of carboxylic-C and aromatic-OH functional groups on biochar surfaces during their oxidation (Liang *et al.* 2006) could also increase CEC of soil (Cheng *et al.* 2006) and possibly increased Cd exchange capacity of soil. The increased concentration of extractable Cd in soil after plant harvest, in 10 and 50 mg/kg Cd treatments combined with at 5 g/kg biochar application, may be attributed to reduced plant Cd uptake in these treatments and thus more available Cd being left in soil. However, the decrease in concentration of soil Cd at the highest rate of biochar application can be attributed to increase in biochar bound Cd, which is not available to plants and is also not extractable by DTPA. Another explanation for the decreased concentration of available Cd at the highest biochar level could be due to its precipitation at high pH (Kabata-Pendias 2000), since at this rate biochar addition significantly increased soil pH compared with the other treatments.

Table 1. Arsenic and Cd in maize shoot in response to biochar, As and Cd application to soil. Phosphate extractable As and DTPA extractable Cd in soil after the pot experiment are also given. LSD values for trace element, biochar, and their interaction effects are presented at P<0.05.

Biochar (g/kg)	Trace element (mg/kg)	Shoot concentration (mg/kg)		Phosphate extractable (mg/kg)	DTPA extractable (mg/kg)
		As	Cd	As	Cd
0	0	0.15 ± 0.02	0.20 ± 0.04	0.12 ± 0.03	0.28 ± 0.04
5	0	0.11 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.36 ± 0.02
15	0	0.24 ± 0.01	0.22 ± 0.02	0.22 ± 0.03	0.42 ± 0.03
0	10	4.38 ± 0.30	6.78 ± 0.26	2.80 ± 0.07	6.07 ± 0.39
5	10	6.48 ± 1.43	5.00 ± 0.36	2.54 ± 0.21	7.30 ± 0.12
15	10	3.36 ± 0.63	4.59 ± 0.37	2.79 ± 0.17	6.78 ± 0.12
0	50	29.11 ± 1.74	31.26 ± 0.94	12.66 ± 0.29	28.77 ± 0.56
5	50	26.47 ± 1.94	23.24 ± 0.92	12.77 ± 0.36	34.44 ± 0.46
15	50	18.87 ± 2.75	12.80 ± 0.99	14.86 ± 1.01	27.79 ± 1.76
LSD	Biochar	2.4	1	0.7	1.1
(P < 0.05)	Trace element	2.4	1	0.7	1.1
	Biochar × trace element	4.1	1.7	1.1	1.9

Conclusions

This study has shown that the application of wood biochar to soil possesses the potential to reduce the availability of As and Cd to plants. The concentration of As and Cd in maize shoots decreased with the application of biochar to soil. Biochar application increased extractable As, whereas biochar application had inconsistent effects on extractable Cd. This study highlights the need for detailed mechanistic investigation of biochar-trace element interactions in order to generalize conclusions regarding whether biochar application can reduce trace element availability to plants. Field experiments evaluating long-term benefits of soil biochar addition using higher application rates and different types of biochar in relation to the bioavailability of toxic trace elements in contaminated soils are required.

Acknowledgements

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Possible toxicity of aluminium-humus complexes in Andosols

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Abstract
The nature and origin of the aluminum (Al) toxicity of allophanic Andosols is still unclear. We cultivated Al-sensitive plants and an Al-tolerant plant using Andosols having various properties. Burdock (*Arctium lappa*) and barley (*Hordeum vulgare*) were used as Al-sensitive plants and buckwheat (*Fagopyrum esclentum*) as an Al-tolerant plant. We measured the root lengths of the burdock and barley after a 4-day culture, and determined the Al concentrations of buckwheat plants after a month long culture. Typical non-allophanic soils showed the high toxicity of Al to roots of burdock and barley. Although Al toxicity was not observed in the typical allophanic soils in the Al-sensitive plants, acidic allophanic soils did show the toxicity as observed in the non-allophanic soils. Reflecting the toxicity (bioavailability) of these soils, the Al concentrations in buckwheat plants grown in the non-allophanic soils were much higher (2.6-4.3 mg/kg) than those in the typical allophanic soils (0.4-1.4 mg/kg). However, those concentrations of buckwheat in the acidified allophanic soils were comparable (2.7-4.0 mg/kg) to those in the non-allophanic soils. These allophanic Andosols contained a few 2:1 type minerals, therefore it is assumed that the Al^{3+} adsorbed into the permanently charged sites of the minerals is not abundant. Therefore, we concluded that Al-humus complexes play important roles in Al toxicity (availability) in the acidified allophanic Andosols as well as non-allophanic Andosols.

Key Words
Andisols, Kurobokusoils.

Introduction
Non-allophanic Andosols often show Al toxicity in Al-sensitive plant roots. The origin of the toxic Al has been considered to be primarily Al^{3+} adsorbed into the permanently charged sites of 2:1 type minerals (Saigusa *et al*. 1980; Shoji *et al*. 1993; Dahlgren *et al*. 2004). However, it was suggested that Al-humus complexes are also one of the pools of toxic Al (Takahashi *et al*. 1995, 2003, 2007; Ito *et al*. 2009). In contrast, typical allophanic Andosols rarely show Al toxicity to plant roots although allophanic soils also contain Al-humus complexes. With strong acidification, allophanic Andosols then come to possess toxic Al which causes injury plant roots (Takahashi *et al*. 2008). The origin of the toxic Al in the allophanic Andosols is still unclear. The aim of this study is to clarify the origin of toxic (bioavailable) Al in Andosols using the cultivation of Al-sensitive plants and an Al-tolerant plant.

Methods
Soil samples
Nine A horizon soil samples were used in this study (Table 1): two typical non-allophanic soils (Kawatabi 08 and Kawatabi 07), their limed soils, two typical allophanic soils (Morioka and Tsukuba), and three acidic allophanic soils (Utsunomiya, Yunodai and Tsutanuma). In addition, commercial pumice (Kanuma pumice) was used for comparison.

Table 1. Properties of soil samples

	pH(H_2O)	KCl-Al $cmol_c/kg$	Si_o g/kg	Al_p g/kg	Al_p/Al_o
Kawatabi 08	4.7	4.63	1.8	16.4	0.82
Kawatabi 07	4.4	5.95	1.8	16.2	0.80
Utsunomiya	4.6	4.62	16.3	11.8	0.26
Yunodai	5.3	0.72	9.9	9.5	0.31
Tsutanuma	5.4	0.06	16.4	6.0	0.15
Morioka	5.7	0.19	15.4	7.5	0.19
Tsukuba	7.0	0.07	20.7	3.2	0.08
Kanuma pumice	5.8	0.13	49.8	2.9	0.04

KCl-Al: 1M KCl extractable Al Al_o, Si_o: acid-oxalate-extractable Al and Si
Al_p: pyrophosphate-extractable Al

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Al-toxicity to Al-sensitive plants

We cultivated the burdock and barley in the soil samples (50 mL beakers). The seedlings were cultured at 25 ^{o}C. After 4-days, the plants were harvested and the length of the roots was measured (total length of the fibrous root system for barley and maximum length of the main root for burdock).

Al-availability to an Al-tolerant plant

Buckwheat is highly resistant to Al stress and known to be an Al-accumulator. Five seedlings were grown in soil samples (2 L pots) in a greenhouse kept at 25^{o}C with an appropriate water supply. After a growth period of a month, the plants were harvested and the Al concentrations of the shoots were determined.

Results

The typical non-allophanic soils (Kawatabi 08 and Kawatabi 07) showed the high toxicity of the Al to roots of burdock (Figure 1) and barley (Figure 2). The toxicity was mitigated in the limed soils (Kawatabi 08 (lime) and Kawatabi 07 (lime)). Although the Al toxicity in the Al-sensitive plant was not observed in the typical allophanic soils (Morioka and Tsukuba), the acidic allophanic soils (Utsunomiya, Yunodai and Tsutanuma) did show the toxicity as observed in the non-allophanic soils.

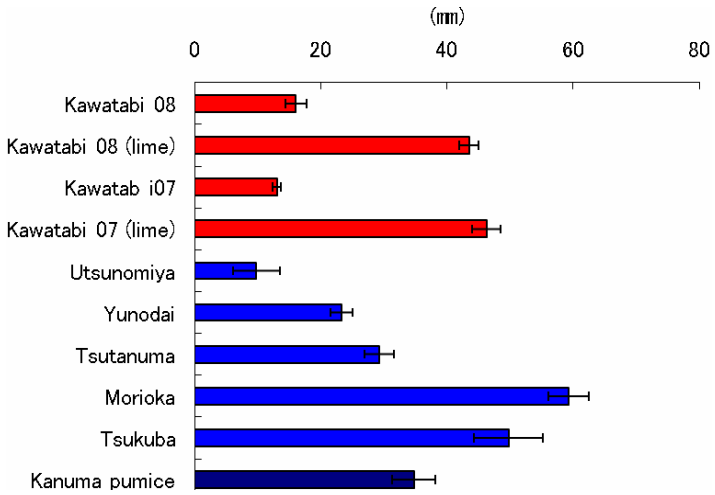

Figure 1. Length of main root of burdock

Reflecting the toxicity (bioavailability) of these soils, the Al concentration in buckwheat plants grown in the non-allophanic soils was much higher (2.6-4.3 mg/kg) than those in the typical allophanic soils (0.4-1.4 mg/kg) (Figure 3). However, the concentrations of buckwheat in the acidic allophanic soils were comparable (2.7-4.0 mg/kg) to those in the non-allophanic soils.

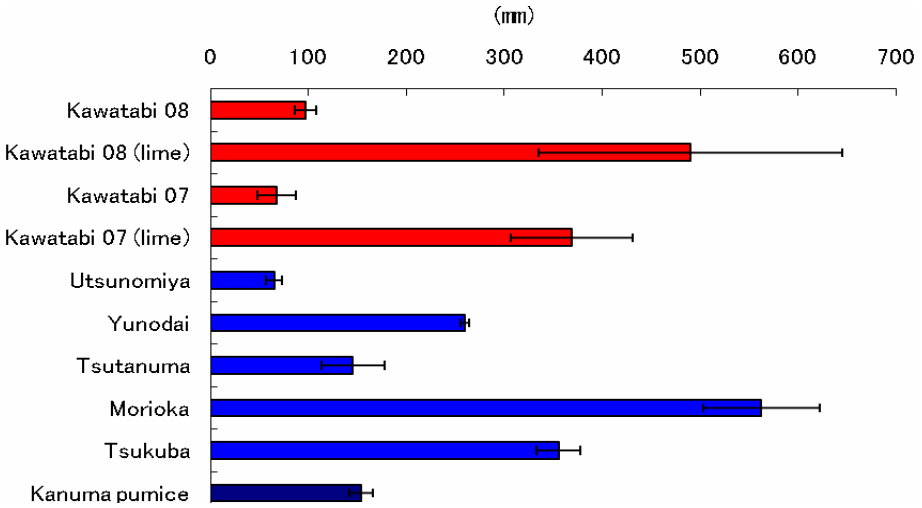

Figure 2. Total length of fibrous roots of barley

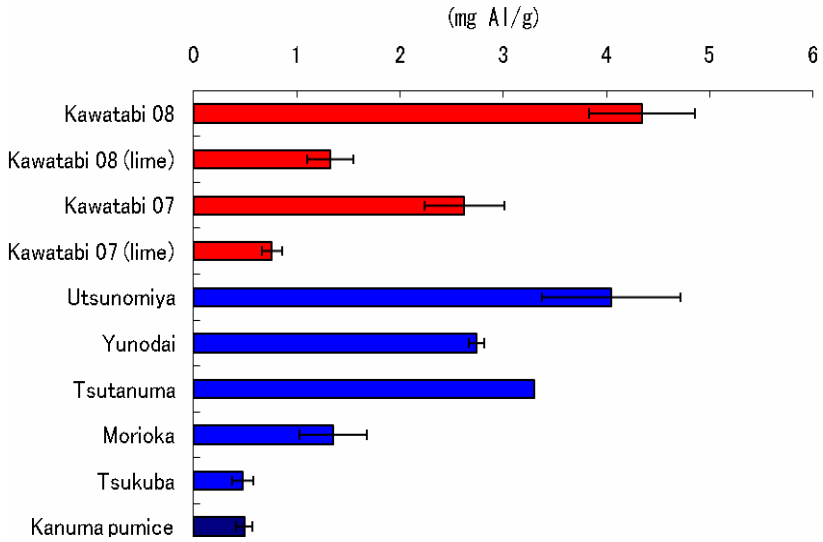

Figure 3. Al concentration in shoot of buckwheat

Figure 4 shows the relationships between the Al concentrations of buckwheat and the root length of burdock or barley. As expected, significant negative correlations were observed between the Al concentrations and the root lengths. Thus, the Al concentrations of buckwheat reflect the strength of the Al toxicity of soils to Al-sensitive plants.

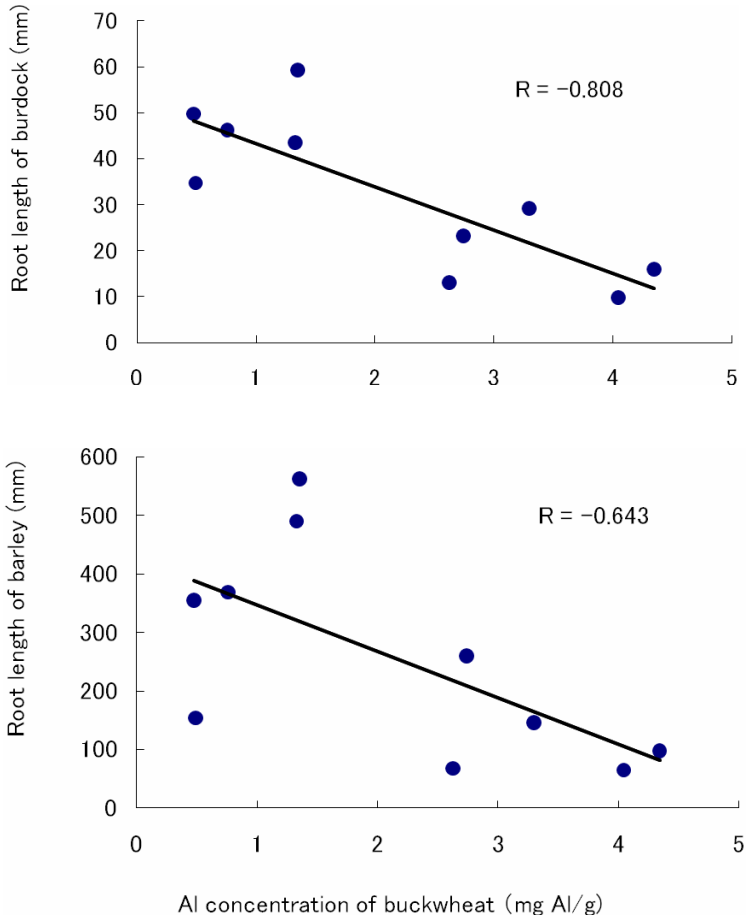

Figure 4. The relationship between Al concentrations of burdock and root lengths of burdock or barley

The Tsukuba and Morioka soils exhibited the typical properties of allophanic Andosols, such as high soil pH values, low concentrations of Al-humus complexes (low Al_p values) and low Al_p/Al_o ratios (Table 1). In contrast, the Utsunomiya and Yunodai soils possessed lower soil pH values, higher Al_p values and higher

Al_p/Al_o ratios. These properties are closer to those of non-allophanic Andosols. These allophanic Andosols contain a trace of 2:1 type minerals, so it is assumed that the Al^{3+} adsorbed into the permanently charged sites of the minerals is not abundant. Yagasaki *et al*. (2006) and Takahashi *et al*. (2008) revealed that, by acidification of the allophanic Andosols, the solubility of the soils can be controlled by the Al-humus complexes. Therefore, it is likely that the Al-humus complexes play important roles in the Al toxicity (bioavailability) in allophanic Andosols as well as non-allophanic Andosols.

Conclusion

The allophanic Andosols having lower soil pH values also showed Al toxicity (availability) as well as non-allophanic Andosols. We considered that the Al-humus complexes are closely related to the Al-toxicity in both the allophanic and non-allophanic Andosols.

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Potential availability of fertiliser selenium in soils during flooding and subsequent aeration

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Abstract
The partitioning coefficient (K_d) and potential availability of selenite (SeO_3^{-2}) or selenate (SeO_4^{-2}) as a percentage of Se applied (% E_{add} values) were measured using isotopic dilution techniques in a study under variable redox conditions. Both Se species were added to a soil subjected to prolonged (30 d) submergence (low redox) followed by 7 d oxidising conditions (high redox). Even though applied Se had no effect on the SeO_3^{-2} or SeO_4^{-2} K_d values, time had a significant effect. Selenate K_d values were always lower than SeO_3^{-2} K_d values. Applied Se species had a significant effect on the SeO_3^{-2} $\%E_{add}$ values. For both forms of applied Se <20% of applied Se was initially in the available pool as SeO_3^{-2}. However, after 14 d of submergence $\%E_{add}$ values for SeO_3^{-2} increased to 80%. Then availability decreased and did not increase during oxidation. Applied Se species, time and their interaction had a significant effect on the SeO_4^{-2} $\%E_{add}$ values. Selenate applied soils initially had more than 80% in available pool but this decreased with time and did not increase during oxidation. These results clearly show that irrespective of the species of Se applied to the soil, speciation changes depend on the changes in soil redox and pH. Adsorbed SeO_3^{-2} and SeO_4^{-2} were released into the soil solution with the reduction of Fe oxides and we possibly reduced to Se (0). Since there was no increase in the available pool of either Se species during the oxidation phase we can assume that fixed Se, as Se(0), did not oxidise readily during the 7 day oxidation period.

Key Words
Redox, fortification, isotopic dilution, adsorption.

Introduction
Sorption and fixation of fertiliser and native selenium (Se) determine the availability for plant uptake. Sorption and desorption of Se in soil is mainly determined by the oxidation-reduction potential, pH, microbial activity, mineralogy and organic matter, and other anions (Dhillon and Dhillon 2000; Goh and Lim 2004). However, most of the information on Se was derived from experiments in aerobic soil systems, and the behaviour of Se in anaerobic soil systems, or those with fluctuating redox, hass not been extensively investigated. Chemical thermodynamic data can be used to predict the most stable Se species in a particular soil environment, but kinetics of the chemical transformations of Se in soils are not well understood (Mikkelsen *et al.* 1989). Moreover it is further complicated by different management practices and type of crops grown. The rate of transformation of fertilizer Se in soil into sorbed or fixed forms is very important for Se accumulation by crops. For paddy rice production systems, in which soils are subjected to variable redox conditions, the kinetics of transformation of fertilizer Se in soil will govern availability for crop uptake. In order to fill this research gap a controlled microcosm study was undertaken with the following objectives:

Materials and methods
This experiment utilized a controlled-atmosphere (N_2 and air) stirred flask technique Patric *et al.* (1973) to gain an understanding of the influence of redox conditions and applied Se species (selenite and selenate) on the availability (potential availability) of fertilizer Se in a rice growing soil. The redox conditions in the cell were periodically adjusted to simulate soil conditions exposed to rice plants. An uncontaminated paddy field soil was spiked with Se (1 mg of Se/kg) in the form of SeO_3^{-2} and SeO_4^{-2}. The soils were incubated for 30 d under reduced redox conditions followed by incubation for 7 d under oxidised conditions. Soil suspensions collected at 0, 14, 30, and 37 days were used to measure Se partitioning (K_d) and potential Se availability (E values) using an isotopic dilution technique. The apparent isotopic K_d values for SeO_3^{-2} or SeO_4^{-2} were calculated using the following equations:

$$K_d\ (L/kg) = \frac{R-r}{r} \times \frac{v}{m} \quad (1)$$

where, R is the total activity of $^{75}SeO_3^{-2}$ or $^{75}SeO_4^{-2}$ added to samples in Bq; r is the activity of $^{75}SeO_3^{-2}$ or $^{75}SeO_4^{-2}$ remaining in solution after equilibration in Bq; v is the volume in L and m is mass of soil in kg. The isotopically exchangeable pool of SeO_3^{-2} (*E* SeO_3^{-2} value) or SeO_4^{-2} (*E* SeO_4^{-2} value) in the soil was calculated using the equation:

$$E\ \text{value (mg/kg)} = \frac{S}{r} \times R \times \frac{v}{m} \quad (2)$$

where, S is the concentration of SeO_3^{-2} or SeO_4^{-2} in solution after equilibration (mg/L); r is the activity of $^{75}SeO_3^{-2}$ or $^{75}SeO_4^{-2}$ remaining in solution after equilibration (Bq); R is the total activity of $^{75}SeO_3^{-2}$ or $^{75}SeO_4^{-2}$ spiked into samples (Bq); v is volume in L and m is mass in kg. Results were expressed as a percentage of Se added (%E_{add} values) by comparing E values in control and Se-amended soils, and dividing by the rate of Se added.

Results and discussion

Redox and pH had an inverse relation during the incubation time (Figure 1). Redox decreased with time in the reduced phase and stabilized around -150 and -200 mV and increased quickly with oxidation. On the other hand pH had an increasing trend over the time in reduced soils and decreased within few hours in the oxidized phase before increasing and returning to the initial pH. There was no conversion of $^{75}SeO_3^{-2}$ to $^{75}SeO_4^{-2}$ during the shaking and equilibration time. Applied Se had no effect on the $SeO_3^{-2}$and SeO_4^{-2} K_d values indicating that SeO_3^{-2} and SeO_4^{-2} adsorption was still in the linear range of the sorption isotherm (adsorbed SeO_3^{-2} *versus* equilibrium SeO_3^{-2} concentration) at this rate of applied Se. However time had a significant effect, decreasing the SeO_3^{-2} and SeO_4^{-2} K_d values and 7 d after oxidation the soil had the lowest K_d values. Selenate K_d values were always lower than SeO_3^{-2} K_d values as observed by others (Collins *et al.* 2006).

Table 1. Characteristics of soil used for the experiment.

	Bentota soil (Thionic Histosol)
pH (water)	5.4
Clay (%)	17.7
Organic C (%)	4.3
Oxalate Fe (mg/kg)	6935
Oxalate Al (mg/kg)	1590
Total Se (µg/kg)	118

Applied Se species had a significant effect on the SeO_3^{-2} %E_{add} values. In both SeO_3^{-2} and SeO_4^{-2} treatments, soil initially had a very low % (<20%) of applied Se in the available SeO_3^{-2} pool . After 14 d of submergence, both Se treatments had much higher percentages (>80%) in the available SeO_3^{-2} pool. However, with time availability decreased and even after 7 d of oxidation availability did not increase. Applied Se species, time, and their combination effect had a significant effect on the SeO_4^{-2} %E_{add} values. Soil amended with SeO_4^{-2} initially had more than 80% in the available SeO_4^{-2} pool. In the soil amended with SeO_4^{-2}, labile SeO_4^{-2} decreased with time in the reduced phase and did not increase during the subsequent oxidation period. The pH and redox changes during the reduced phase evidently caused significant changes in Se speciation. By day 14, SeO_3^{-2} was the major Se species in both treatments. These results clearly

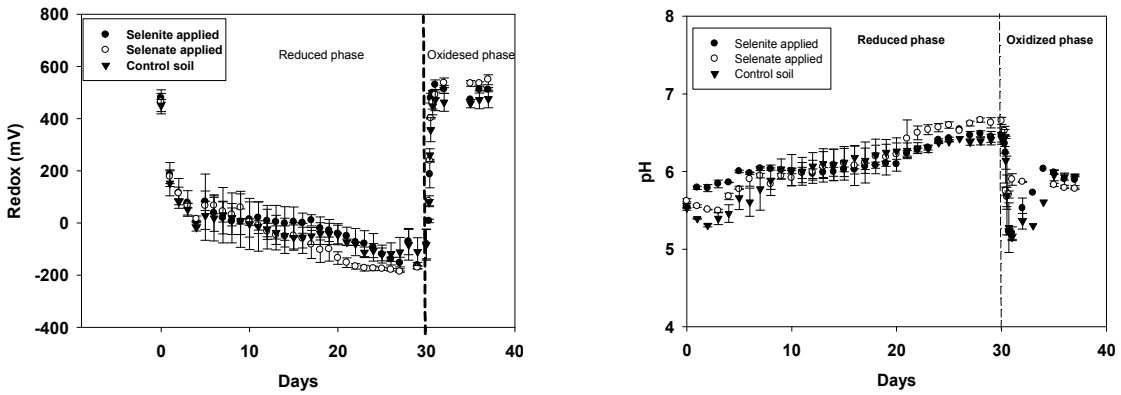

Figure 1. Redox and pH during the experiment.

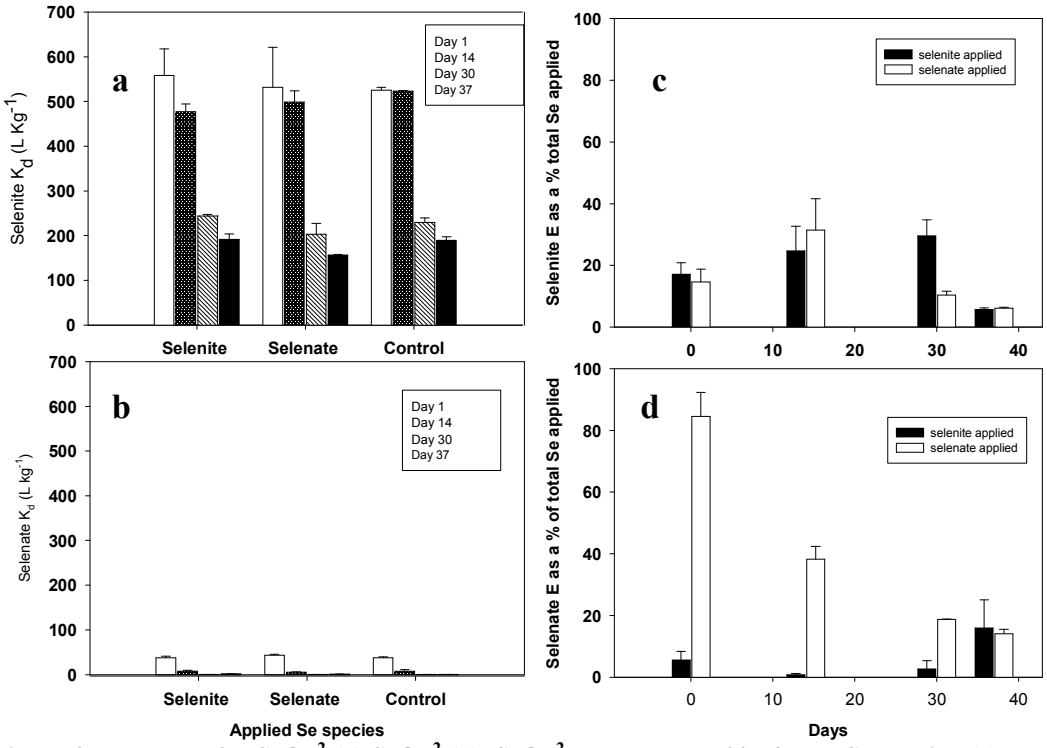

Figure 2. K_d values for SeO_3^{-2} (a) SeO_4^{-2} (b), SeO_3^{-2} *E* values as a % of total Se applied (c) and *E* value of SeO_4^{-2} as a % of total Se applied (d).

show that irrespective of the species of Se applied in to the soil, speciation changes depend on the interaction of soil redox and pH conditions. It is highly likely that the Reason for the decrease of available SeO_3^{-2} and SeO_4^{-2} with time was the reduction of these species to Se(0). Since there was no increase in the labile SeO_3^{-2} and SeO_4^{-2} during the oxidation phase, we can assume that the Se(0) formed did not oxidise readily when soils were aerated for one week.

We have shown that if we add Se at pre planting as either SeO_3^{-2} or SeO_4^{-2}, by the time of transplanting (14 d after submergence) significant amounts of fertilizer Se would be available for plant uptake as SeO_3^{-2}. However, the availability of both fertilizer Se species declined rapidly with time and did not increase on re-oxidation, suggesting that it is not a good practice to add fertilizer Se prior to planting rice, as availability goes down with submergence. However since rice roots have the special ability to oxidise their rhizosphere (Kirk *et al.* 1993) added Se may remain in forms that the plant can absorb. Experiments are underway to investigate the accumulation of Se forms in rice grains under variable redox conditions.

Conclusions

Irrespective of the species of Se added to soil, partitioning and availability of Se in soil was controlled by soil redox and pH. Long term submergence significantly reduced the availability of fertilizer Se applied possibly by reduction to Se(0). Oxidation of this Se(0) was insignificant.

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Predicting the relative bioavailability of arsenic, cadmium and lead via the incidental soil ingestion pathway using in vitro techniques

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Abstract
In this study, the bioaccessibility and relative bioavailability of soil-borne contaminants were compared to determine whether a simple rapid, inexpensive in vitro assay may be used to predict *in-vivo* relative bioavailability for human health exposure assessment. Arsenic, cadmium and lead bioaccessibility in contaminated soil was assessed using a variety of *in-vitro* assays (SBRC, IVG, PBET and DIN) incorporating gastric (G) and intestinal phases (I) while *in-vivo* relative bioavailability was determined using mouse or swine assays. When linear regression models were developed in order to determine the suitability of *in-vitro* assays for predicting arsenic, cadmium and lead relative bioavailability, the correlation between bioaccessibility and relative bioavailability varied depending on the methodology used. While arsenic, cadmium and lead relative bioavailability could be accurately predicted using SBRC-G, PBET-I and Rel-SBRC-I respectively, a single *in-vitro* method was not suitable for predicting relative bioavailability for all three contaminants.

Key Words
Arsenic, Bioaccessibility, Bioavailability, Cadmium, Lead, Human Health Exposure Assessment.

Introduction

Incidental ingestion of contaminated soil is a major non-dietary exposure pathway for many inorganic contaminants. In order to more accurately quantify exposure to inorganic contaminants via soil ingestion, determination of contaminant bioavailability is required. It has been established that arsenic (As), cadmium (Cd) and lead (Pb) bioavailability may be less that 100% as a result of mineralogy, the influence of soil properties and contaminant-soil residence time (ageing) (Ruby *et al*. 1996; Rodriguez *et al*. 1999; Basta *et al*. 2001; Juhasz *et al*. 2007a; 2007b). As a result, exposure and therefore risk to human health may be overestimated if a conservative bioavailability approach is adopted (i.e. 100%).

In-vivo assays using a variety of animal models (e.g. primate, swine, dog, rabbit, rodent) have been used to quantify the relative bioavailability of contaminants in soil (Freeman *et al.* 1993; Groen *et al*. 1994; Ng *et al.* 1998; Roberts *et al.* 2002; Juhasz *et al.* 2007b). However, given the time and cost requirements, in addition to ethical issues, there is great demand for an appropriate *in-vitro* assay for estimating relative contaminant bioavailability. *In-vitro* assays are simple, rapid and inexpensive and numerous methods have been applied for the determination of contaminant bioaccessibility (Rodriguez *et al*. 1999; DIN 2000; Oomen *et al.* 2002; Kelley *et al.* 2002). However, before these assays can act as a surrogate measure for relative bioavailability, correlation between *in-vitro* bioaccessibility and *in-vivo* relative bioavailability is a mandatory prerequisite for regulatory as well as scientific acceptance. This paper discusses the development, assessment and validation of *in-vitro* assays for predicting the *in-vivo* relative bioavailability of soil contaminated with As, Cd and Pb.

Methods

Contaminated soils
Arsenic, Cd and Pb contaminated soils used in this study were collected from regional areas where the soil type, source and contaminant-soil residence time varied. Soils were air dried then sieved and the <250 µm particle size retained for chemical characterisation and bioaccessibility / relative bioavailability assessment.

Assessment of bioaccessibility
Arsenic, Cd and Pb bioaccessibility was determined using four different *in-vitro* methods incorporating both gastric and intestinal phases. In vitro methods included:

1. SBRC (Kelley *et al*. 2002)
2. IVG (Rodriguez *et al.* 1999)
3. PBET (Wragg *et al*. 2007)
4. DIN (DIN 2000)

Arsenic, Cd and Pb concentrations in soil digests or *in-vitro* solutions were determined using ICP-MS. Certified reference materials were included in all analysis to ensure internal quality assurance, quality control practices.

Assessment of relative bioavailability
In-vivo As and Pb relative bioavailability was determined using a swine model according to Rees *et al.* (2009). Bioavailability was calculated using pharmacokinetic analysis encompassing area under the blood-concentration time curve following zero correction and dose normalisation. When relative As and Pb bioavailability was determined, the area under the blood-concentration time curve for the respective reference dose (sodium arsenate or Pb acetate) oral treatment was used for comparison. *In-vivo* Cd bioavailability studies were conducted with mice. Cadmium acetate (reference dose) or Cd contaminated soil was incorporated into formulated mice pellets and 10 g of the respective feed mix supplied to individual animals once daily over a 15 day exposure period. At the end of the exposure period, animals were euthanized and the kidneys and liver collected for Cd determination. Following tissue digestion, samples were diluted to 20 ml with 0.1% HNO_3 then filtered (0.45 μm) for analysis by ICP-MS.

Comparison of bioaccessibility and bioavailability
Arsenic, Cd and Pb relative bioavailability, derived from in vivo mouse or swine assays, was compared to bioaccessibility data determined using SBRC, IVG, PBET and DIN methods. Bioaccessibility-relative bioavailability best fit models were determined using stepwise multiple regression (SPSS 16.0.1).

Results
For all four *in-vitro* methods, As, Cd and Pb bioaccessibility was greater when gastric phase values were compared to the intestinal phase. Due to the low pH environment of the gastric phase, release of As, Cd and Pb from the soil matrix occurred as a result of dissolution processes which are dependent on mineralogy in addition to the gastric phase pH of the in vitro method. Generally the gastric phase of the SBRC assay produced the highest bioaccessibility results presumably due to the differences in pH values of the four *in-vitro* methodologies (1.5 versus 1.8, 2.0 and 2.5). Increasing the pH from gastric to intestinal phase conditions resulted in a significant decrease in As, Cd and Pb bioaccessibility presumably due to co-precipitation with and/or sorption to iron via surface complexation or ligand exchange.

Relative bioavailability varied significantly between contaminated soils ranging from 6.9 ± 5.0% to 74.7 ± 11.2% for As, 10.1 ± 0.4% to 92.1 ± 7.3% for Cd and 10.1 ± 8.7% to 19.1 ± 14.9% for Pb. When linear regression models were developed in order to determine the suitability of *in-vitro* assays for predicting As, Cd and Pb relative bioavailability, the correlation between bioaccessibility and relative bioavailability varied depending on the methodology used. While As, Cd and Pb relative bioavailability could be accurately predicted using SBRC-G, PBET-I and Rel-SBRC-I respectively (Table 1), a single *in-vitro* method was not suitable for predicting relative bioavailability for all three contaminants.

Table 1. Best fit linear regression models for predicting in vivo As, Cd and Pb relative bioavailability using *in-vitro* assays.

Contaminant	In vitro assay/phase	*In-vivo* – *in-vitro* predictive model	Pearson correlation
As (n=12)	SBRC-G	RBA (%) = 0.992*SBRC-G (%) + 1.656, r^2 = 0.754	0.868
Cd (n=7)	PBET-I	RBA (%) = 1.091*PBET-I (%) – 5.140, r^2 = 0.835	0.914
Pb (n=5)	Rel-SBRC-I[a]	RBA (%) = 0.580*Rel-SBRC-I (%) + 1.980, r^2 = 0.530	0.730

[a] Relative Pb bioaccessibility in the intestinal phase of the SBRC assay was calculated by adjusting the dissolution of Pb from contaminated soil by the solubility of Pb acetate at the corresponding pH value (pH 6.5).

Conclusion

When *in-vivo* As, Cd and Pb relative bioavailability data was compared to bioaccessibility data, simple, rapid, inexpensive *in-vitro* assays could accurately predict relative bioavailability. However, relative bioavailability - bioaccessibility correlations demonstrated that the selection of an appropriate *in-vitro* assay for predicting relative bioavailability is contaminant specific and that one *in-vitro* methodology may not presently be suitable for all inorganic contaminants.

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Regression model for prediction availability of essential heavy metals in soils

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Abstract
The aim of paper was to compare relationshipsof total and plant available essential heavy metals in soils considering soil acidity and humus impacts. Regression models should provide additional data (EDTA-extractable fraction) from available data (heavy metal total content, soil pH). The 60 soil samples were analysed for pH, humus, phosphorus, potassium, and additionally for essential heavy metals (Fe, Mn, Zn, Cu, Ni) by extraction by aqua regia and by ethylenediaminetetraacetic acid (EDTA). The heavy metal with highest total content was iron, then manganese, zinc, nickel, and copper regardless of soil pH. The order of EDTA-extracted elements changed with copper jumping from last to third place, but the percentage of total content extracted by EDTA was quite opposite: Cu, Ni, Zn, Mn, and Fe. Soil pH impacted on EDTA-extractable fraction and the regression models can predict the EDTA-extractable fraction of Zn, Cu and Ni with acceptable average errors, but not for Fe and Mn. The obligate input data for models are total soil content of certain heavy metal and pH. Model errors decreased using humus content as additional input data. The lowest model errors were for copper in acid soils and nickel in calcareous soils.

Key Words
Aqua regia, EDTA, iron, manganese, zinc, copper, nickel.

Introduction

Heavy metals importance for plants differs since there are essential micronutrients heavy metals (Fe, Mn, Zn, Cu, Ni, Mo), and also beneficial (Co) or nonbeneficial toxic heavy metals (Cd, Cr, Pb, Hg). The concentration of heavy metals in soils is influenced by activities such as agricultural practices and industrial activities and is associated with biological and chemical cycles. Intensification of agriculture production pointed out the role of micronutrients. Increase of fertilization may cause some negative effects because of changes in essential heavy metal availability (Györi 2006). For Most micronutrients availability is impacted by soil pH. Boron and molybdenum become toxic in high alkaline soils, and iron and manganese become toxic in highly acidic soils. Osztoics *et al.* (2005), also reported that a high Mn concentration was found in extremely acidic soils. Soil extraction techniques to measure the status of available micronutrients for plants are important in the diagnosis of deficiency or toxicity (Garcia *at al.* 1997). The aqua regia microwave acid digestion techniques produced the fastest, safest and accurate analytical results with precision better than 5% for determination of microelements in soil (Melaken *et al.* 2005) and this analysis is obligate in Croatia for determination of soil quality in term of organic agriculture and protection of agricultural soils. However, aqua regia digestion does not identify the plant available fraction of soil heavy metals. Hence, this method is not appropriate for determination of soil heavy metal impact on environment or on plant nutrition. On the other hand, soil extraction with EDTA is the most common method in Croatia regarding the determination of soluble or plant available fraction of essential heavy metals. EDTA or similar methods are not obligate in Croatia, either considering organic agriculture or conventional fertilization recommendations. Furthermore, the relation of total and soluble content of heavy metals in Croatian soils is not adequately investigated, although some results were published and the difference of fraction extracted by different methods was pointed out (Lončarić *et al.* 2008). The aim of this paper was to compare relationships of total and plant available essential heavy metals in soils considering possible soil acidity and humus content impacts on total and available essential heavy metals fractions. Also, the aim of regression models was to provide additional data (EDTA-extractable fraction) from other available data (total heavy metal content and soil pH).

Methods

The 60 soil samples were collected from arable soils from the depth 0-30 cm in continental part of Croatia. All the samples were analysed for basic chemical properties such as soil pH_{H2O} and pH_{KCl} (ISO 10390), soil organic matter by sulfochromic oxidation (ISO 14235) and plant available P and K extracted by ammonium-lactate (Egner *et al*. 1960). In addition to basic analyses, the essential heavy metals (Fe, Mn, Zn, Cu and Ni) were extracted by aqua regia (ISO 11466), and by ethylenediaminetetraacetic acid (EDTA).The fraction

extracted by aqua regia was considered as soil total content, and the EDTA-extractable fraction as plant available content. The concentrations of extracted heavy metals were measured by the AA flame method and by ICP-OES. The statistical analyses and regression models were done using PC applications Microsoft Excel and SAS.

Results

The pH_{H2O} of analysed soils varied from 4.45 to 8.83 and pH_{KCl} values were 3.61 to 7.88. The average difference between pH_{H2O} and pH_{KCl} was 1.05 pH units (6.63 vs. 5.58). The pH_{KCl} of 56 % samples was lower than 6 and hydrolytic acidity of these samples varied from 1.7 to 6.8 cmol/kg. The carbonate content in the rest of the samples for, 44% samples with pH_{KCl} higher than 6, was 0.84 to 40.9 % $CaCO_3$. The humus content of analysed soils was in a wide range 0.61 to 4.60 % with an average 1.75 %

Total essential heavy metals extracted by aqua regia

The total essential heavy metal concentrations in soils extracted by aqua regia were in expected order (Table 1): Fe was extracted in highest concentration, followed by Mn, Zn, Ni and Cu. Jurković *et al.* (2006) reported for acid Croatian soils highest level of available Fe and Mn and significantly lower Zn and Cu.

Table 1. Minimum, maximum and average concentrations (mg/kg) extracted by aqua regia.

	Fe	Mn	Zn	Ni	Cu
Minimum	17058	997	38	12	4.0
Maximum	45123	9757	99	58	41
Average	27217	6254	70	31	21

Maximum concentrations of zinc, copper and nickel as potentially toxic essential heavy metals were not higher than thresholds allowed for agricultural soils in Croatia (300, 100, 60 mg/kg, respectively).
The correlations between extracted total concentrations of analysed elements were very significant with manganese as an exception (Table 2) regardless of soil acidity and nickel in calcareous soils. However, soil acidity did not impact significantly on Fe, Mn and Zn total concentrations, but total concentration of copper was 15% and nickel 20% higher in calcareous than in acid soils (on average).

Table 2. Correlation coefficients for extracted elements in all soils, and acid and calcareous soils separately.

	All soils				Acid soils				Calcareous soils			
	Fe	Mn	Zn	Ni	Fe	Mn	Zn	Ni	Fe	Mn	Zn	Ni
Mn	ns				ns				ns			
Zn	0.81	ns			0.82	ns			0.83	ns		
Ni	0.50	ns	0.50		0.92	ns	0.85		ns	ns	ns	
Cu	0.68	ns	0.66	0.58	0.73	ns	0.58	0.78	0.83	ns	0.85	ns

Available essential heavy metals extracted by EDTA

Heavy metals extracted by EDTA should be the fraction available for plants. Hence, these amounts of heavy metals are significantly lower than total amounts in soils (Table 3), and percentage of total amounts extracted by EDTA differ among elements: copper was extracted in the highest relative amount (19%), than nickel (4.5%), zinc (3.5%) manganese (0.6%) and iron (0.3%). However, iron was extracted in highest absolute amounts, followed by manganese, than copper, zinc and nickel in lowest amounts. Similar results were published earlier (Lončarić *et al.* 2008) with the same relationships for iron, copper and zinc, rather lower for nickel, and significantly higher for extractable Mn.

Table 3. Minimum, maximum and average concentrations (mg/kg) extracted by EDTA

	Fe	Mn	Zn	Ni	Cu
Minimum	6.9	5.2	0.6	0.3	1.6
Maximum	453.8	112.5	7.7	3.8	10.1
Average	80.6	40.9	2.4	1.4	4.1
% of total amount	0.3%	0.6%	3.5%	4.5%	19.3%

Only few correlations among elements extracted by EDTA are significant, more for calcareous soils (Fe-Cu, Mn-Cu, Ni-Cu) than acid soils (Ni-Cu) and these relationships are not strong enough for prediction of analysed elements concentrations using regression models. However, it is well known that soil pH has a significant impact on heavy metal availability which is decreased by raising soil pH. For the analysed soils

considering Fe, Mn and Ni were extracted in lower propotions of total content from calcareous soils than from acid soils (75% lower, 46% and 14%, respectively). The extracted fractions of Zn and Cu were in slightly lower percentages of total amounts for acid soils than for calcareous soils (6 and 12%, respectively).

Regression model

The very significant impact of soil pH on the extractable fraction of heavy metals was used to generate regression models for prediction of EDTA-extractable amount of analysed heavy metals. Two regression models are described in this paper (Table 4):

1. TA model for prediction EDTA-extractable fraction of heavy metal, where total (T) heavy metal content extracted by aqua regia (in mg/kg) and soil pH_{KCl} (A) were used as input data,
2. TAH model - using all the data as TA model, plus humus (H) content (in %) as additional input data.

Table 4. Regression parameters in relation Y (= EDTA-extractable metal) = $TX_1 + AX_2 + HX_3$ (in mg/kg).

	Acid soils				Calcareous soils			
Model	Total metal (T)	pH_{KCl} (A)	humus (H)	r	Total metal (T)	pH_{KCl} (A)	humus (H)	r
TA (Zn)	0.015	0.259	-	0.82	0.054	-0.178	-	0.89
TA (Cu)	0,040	0,604	-	0,97	0,125	0,268	-	0.93
TA (Ni)	0.044	0.026	-	0.92	0.041	-0.0004	-	0.90
TAH (Zn)	0.017	-0.178	1.041	0.84	0.060	-0.163	-0.297	0.89
TAH (Cu)	0.068	0.095	1.003	0.96	0.106	0.036	1.171	0.95
TAH (Ni)	0.053	-0.252	0.580	0.94	0.029	-0.061	0.498	0.93

Although soil pH impacted significantly on the fraction of extracted Fe and Mn, the regression models TA and TAH were not good enough for prediction of EDTA-extractable Fe or Mn using total Fe or Mn content, soil pH_{KCl} and humus content as input data. The reason could be that extremely low fraction of Fe (0,3%) and Mn (0,6%) extracted by EDTA and variations of input data were too high. On the other hand, the impact of soil pH on the extractable fraction of Zn, Cu and Ni was rather low, but the fractions were higher (3,5; 19,3 and 4,5%, respectively) and very significant correlation enabled quite useful regression models.

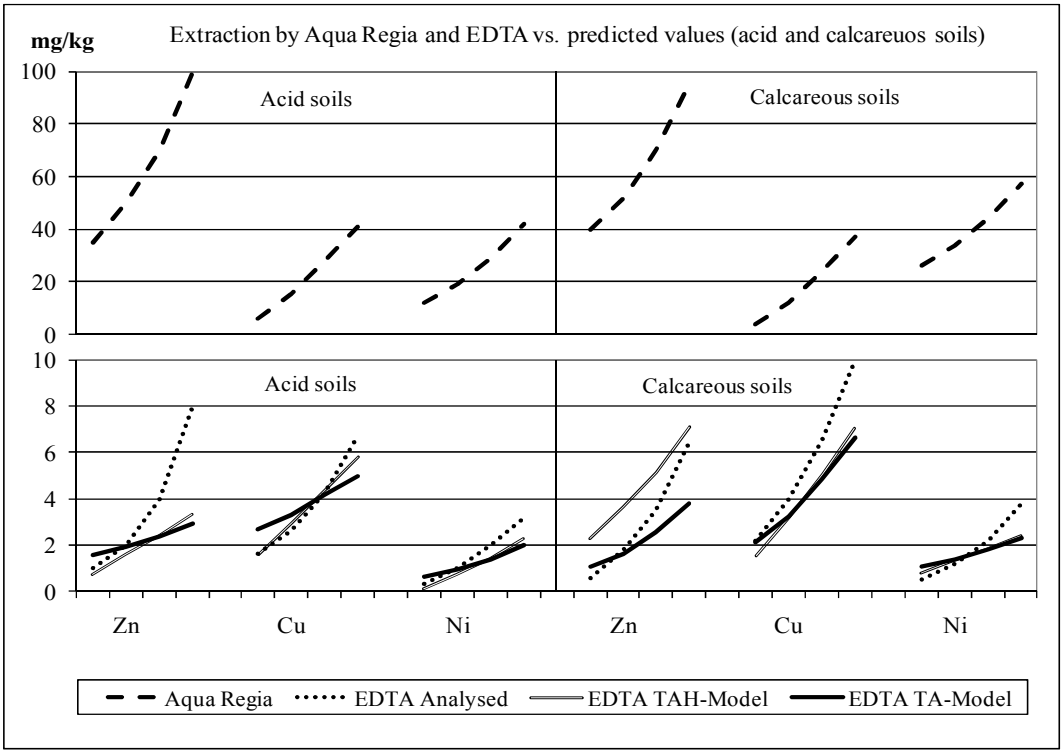

Figure 1. The comparison of total soil heavy metal contents, EDTA-extractable analytical values from acid and calcareous soils, and EDTA-extractable values predicted by TA and TAH models.

The model with highest error (28 and 34% for TAH and TA models) was the zinc prediction model for acid soils, and model error increased with increasing total soil Zn content, pH and humus content. For zinc, there is significant difference between TA and TAH models for calcareous soils with almost equal errors (around

30%), but the TA model predicts lower and TAH model higher values than actually measured (Figure 1). Also, the TA model is better for lower total soil zinc contents, and TAH model for highest contents. The most precise model was TAH copper model for acid soils with average error only 4.3% and 12.6% for calcareous soils. TA model had average error for acid soils 5,1%, but copper prediction is too high at low total copper contents and too low at higher total copper contents. The basic properties of all nickel models are average model errors in the range 17-29%, low difference between TA and TAH models, slightly lower error of the TAH model, and higher model accuracy at lower total soil nickel contents.

Conclusion

The heavy metal with highest total content in analysed agricultural soils was iron, followed by manganese, zinc, nickel and copper regardless to soil pH. The total contents correlated significantly with exception of manganese. The order of EDTA-extracted elements was almost the same with copper jumping from last to third place, but, the percentage of total content extracted by EDTA was quite opposite in order Cu, Ni, Zn, Mn and Fe. Soil pH impacted significantly on EDTA-extractable fractions and regression models for prediction of EDTA-extractable fraction in soil can be used for Zn, Cu and Ni with acceptable average errors (up to 34; 12,6 and 29%, respectively), but not for Fe and Mn. The obligate input data for models are total soil content of heavy metal and soil pH_{KCl} value, and model errors are rather lower using humus content as additional input data. The lowest model errors were for copper in acid soils and nickel in calcareous soils. Regression model can be successfully used for prediction extractable fraction of Zn, Cu and Ni (analyses is not obligate by bylaws in Croatia) using total soil contents of Zn, Cu and Ni (analyses obligated by bylaws in Croatia).

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Soil properties affecting parameters obtained from two-site reaction modelling of cadmium sorption kinetics

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Abstract
This study investigated the applicability of a two-site reaction model to characterize the dynamic sorption process of Cd in different Chilean cropland soils. Using these results and a set of data previously reported, some soil properties that affect dynamic sorption parameters were established through regression analysis. The two-site reaction model fitted very well data from batch sorption experiments, showing that is possible to simplify the reactions involving the Cd dynamics combining an instantaneous equilibrium process of adsorption and a slow first-order kinetic process of immobilization/dissolution in the soil. It was not found effect of either soils'cation exchange capacity or clay content on the sorption behaviour of Cd. The linear partition coefficient and forward reaction rate constant (immobilization) were positively correlated to the soil pH, and the forward/backward (immobilization/dissolution) rate constants ratio was highly and positive correlated to the Cd in the adsorbed phase and the pH. Sorption parameters can be estimated from the soil pH and the Cd in the adsorbed phase (%) and can be further used in modelling assessment to predict the fate of Cd introduced into cultivated soils through fertilization practices.

Key Words
Reaction rate constants, partition coefficient, immobilization/dissolution, batch experiments, chemical fractioning

Introduction

Cadmium is one of trace elements of major environmental concern due to its toxicity and mobility in the soil-plant-water system. This element is found naturally in the soil, but it can also be incorporated by human agricultural activities like land application of P fertilizers, sewage sludge (SS), and other organic amendments (Chang and Page 2000). The former is the most important input source of Cd to the soil in developing countries. In Chile, for example, the Cd concentration in SS is 10 to 50 times lower than that found in P fertilizers and amounts of up to 288 mg of Cd per kilogram of P can be incorporated annually through the application of P fertilizers (Molina *et al.* 2009). In the receiving soil, Cd is distributed in an aqueous phase and the solid phase represented by the soil's colloids. The solution and solid phases are in dynamic equilibrium through complex chemical reactions that take place at the solid-solution inter-phase. Chen *et al.* (2006) used a two-site model to characterize the dynamic sorption process of Cd in the soil. The model, which combines a linear reaction equilibrium model and a first-order reaction kinetics model, allowed establishing differences between the reaction rates involved in the equilibrium of the solid-solution phase of two California cropland soils.

The objectives of this work were (i) to study the dynamic sorption process of this element in different soils using the two-site model mentioned above, and (ii) to establish relationships between some soil properties and the sorption parameters, using the results obtained in this study and the data previously reported.

Methods

Modelling approach (Chen et al. 2006)
In the model, the different sorption reactions are ascribed to different soil phases: i) Adsorbed phase, the chemically reactive portion of Cd in soil, which includes the Cd in soil solution and electrostatically adsorbed Cd and/or Cd weakly adsorbed on organic and inorganic soil components, and ii) Mineral phase associated to trace elements that are strongly bind to different solid phases with slow reaction rate in the soil. They are occluded by or co-precipitated with metal oxides (Fe, Al and Mn oxides), clay minerals, carbonates or phosphates and other secondary minerals. Assuming the water-to-soil ratio is R (L/kg) and the initial Cd

concentration in the solution phase is C_0 (μg/mL), the mass balance equation in a Cd sorption experiment can be expressed by:

$$Ct \cdot R + K_a \cdot Ct + MP_t = C_0 \cdot R \quad (1)$$

where K_a is the linear adsorption constant (L/kg) for Cd distribution between the solution and the adsorbed phase, and C_t (μg/L) and MP_t (μg/kg) are the Cd concentration in the solution phase and in mineral phase, respectively, at specific equilibration time (t). The slower reaction kinetics of the immobilized mineral phase can be described by the following first-order reaction equation:

$$\frac{\partial MP}{\partial t} = k_f \cdot (R + K_a) \cdot C_t - k_b \cdot MP \quad (2)$$

where k_f and k_b are the forward and backward reaction rate constants (h^{-1}) corresponding to the immobilization (in the mineral phase) and dissolution processes, respectively. Combining Eq. (1) and (2), the time-dependent change of Cd in the solution phase and mineral phase is given by:

$$MP_t = \frac{k_f \cdot R \cdot C_0}{k_f + k_b} \cdot (1 - e^{-(k_f + k_b)t}) \quad (3)$$

$$Ct = \left(\frac{C_0 \cdot R}{R + K_a} - \frac{k_f \cdot C_0 \cdot R}{(k_f + k_b) \cdot (R + K_a)} \right) + \frac{k_f \cdot C_0 \cdot R}{(k_f + k_b) \cdot (R + K_a)} \cdot e^{-(k_f + k_b)t} \quad (4)$$

If t is fixed, the right-hand side of Eq. (3) and (4) may be reduced to a linear form in terms of C_0:

$$MP_t = a_1 \cdot C_0 \qquad a_1 = \frac{k_f \cdot R}{k_f + k_b} \cdot \left(1 - e^{-(k_f + k_b)t}\right) \quad (5)$$

$$C_t = a_2 \cdot C_0 \qquad a_2 = \frac{R}{R + K_a} - \frac{k_f \cdot R}{(k_f + k_b) \cdot (R + K_a)} \cdot \left(1 - e^{-(k_f + k_b)t}\right) \quad (6)$$

The Cd concentration in the solution phase (C_t) and in the mineral phase (MP_t) at specific *t* may be obtained for various C_0, and *Ct* vs. t would be a straight line. When C_0, is fixed, the right-hand sides of Eq. [3] and [4] may be reduced to an exponential form:

$$C_t = \frac{C_0 \cdot R}{R + K_a} - a_1 \ (1 - e^{-bt}) \quad (7)$$

$$MP_t = a_2 \cdot (1 - e^{-bt}) \quad (8)$$

$$with \quad a_1 = \frac{k_f \cdot C_0 \cdot R}{(k_f + k_b) \cdot (R + K_a)} \quad b = k_f + k_b$$

$$and \quad a_2 = \frac{k_f \cdot R \cdot C_0}{k_f + k_b}$$

Soils and correlation analysis

Composed soil samples were obtained from the plow layer (0-25 cm) of five different Chilean cultivated soils varying in their physicochemical characteristics. The samples were passed through a 2-mm sieve, air-dried, and characterized for texture by the Bouyoucos hydrometer method, pH and electrical conductivity (soil-to-water ratio of 1:1), organic carbon by the Walkey-Black method, exchangeable bases by using 1 M ammonium acetate extraction at pH = 7.0, and $CaCO_3$ equivalent by a gravimetric method.

A regression and correlation analysis between some soils' characteristics and the Cd sorption and kinetics parameters was performed considering the results of the present study and the results reported by Chen et al. (2006) for the two cultivated soils of California. Parameters included were the K_d, and k_f, k_b (dependent variables). Soil properties as pH, cation exchange capacity (CEC), and clay and organic matter content were considered (independent variables). The $CaCO_3$ content was not included because this characteristic has similar values among soils. In the case of the kinetic parameters, the amounts of Cd present in the adsorbed and mineral phases obtained by the sequential extraction procedure were also considered in the correlations.

Sorption experiments and chemical fractioning procedure
Batch sorption isotherms were carried out at a solution-to-soil ratio of 20:1. Different Cd solutions were applied into centrifuge tubes containing one gram of dry soil and equilibrated for 48 h (fixed time) in a reciprocating shaker. The initial Cd solution concentrations were in the range of 50 to 2000 μg/L in 0.01M $NaNO_3$ background. After the equilibrating period, the supernatant was separated by centrifugation for Cd determination and the solid underwent a chemical fractioning procedure (Chen et al. 2006) to obtain the adsorbed and mineral phase of Cd. A second set of sorption experiments were conducted at initial solution concentration of 100 μg/L (fixed concentration) and using equilibrating times from two hours to 10 d. In all samples, the Cd concentration was determined by GF-AAS.

Results
The model fit very well the experimental data of instantaneous adsorption and kinetic equilibrium for all soils (R^2>0.900). Figure 1 shows the sorption isotherms of Cd and its distribution between the adsorbed (AD) and the mineral (MP) phases for two soils. The isotherms had a linear behaviour within the concentration range studied (50 to 2000 μg/L), which agrees with the theoretical description (Eqs. (5) and (6)) when the equilibration time (48 h) is fixed.

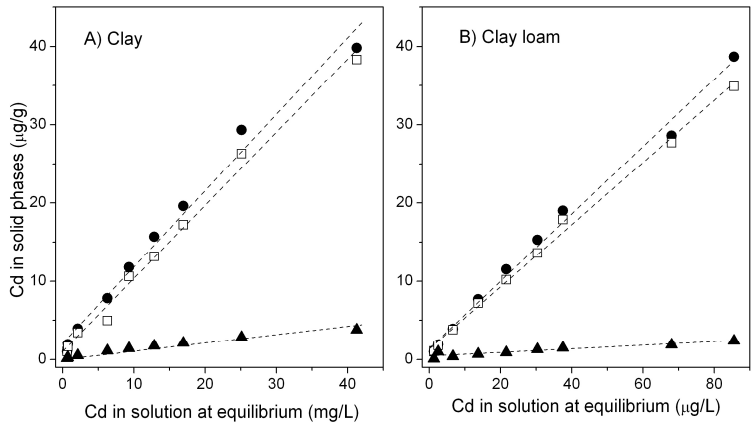

Figure 1. Sorption isotherms for a A) clay (pH=5.6) and B) clay loam (pH= 5.9) soil. Total Cd (●), adsorbed phase (□) and mineral phase (▲).

The results of sorption experiments at fixed initial concentration and at different equilibrium times for two soils are given in Figure 2. As predicted by Eqs. (7) and (8), while the amount of Cd in the solution phase dropped, the concentration in the mineral phase raised, exponentially with equilibrium time. In the evaluated soils, k_f values were higher than those for k_b.

In Table 1, the results of the correlation analysis are shown. The pH of the soil accounted for more that 90% of the variability seen in the values of K_d. It was found neither correlation with other soil properties nor an improvement in the fit of the regression when a second independent variable was added. Our results are in agreement with those reported in other sorption studies in which soil pH was the factor that had the largest effect on the values of K_d in natural and contaminated soils.

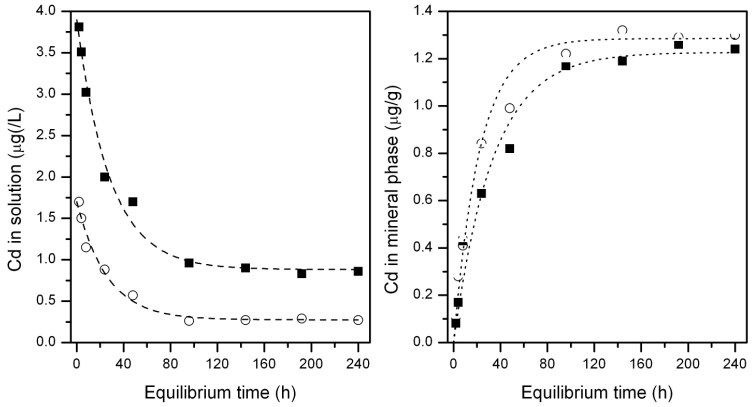

Figure 2. Cadmium in soil solution (left) and in mineral phase (right) for a clay (○)and clay loam (■) soil after different equilibrium times.

For example, Christensen (1989) working with Cd in low concentrations in 63 soils found that pH was the factor that most affect the K_d, and found no relationship of this parameter with the CEC. The correlation analysis showed that also k_f depended mainly on the pH of the soil (R=0.987). There was no effect of pH on k_b, but there was a certain tendency for it to increase with the Cd present in the MP. In turn, the k_f/k_b ratio was significant and positively affected by the amount of Cd present in the AD (R=0.984), and negatively affected by both the organic matter content (R=-0.999) and the Cd present in MP (R=0.967). The k_f/k_b ratio was highly correlated to Cd in the AD (%) and the soil pH.

Table 1. Correlation analysis between the average values of K_d, k_f, k_b, and k_f/k_b ratio and selected soil properties.

Independent	K_d		k_f		k_b		k_f/k_b ratio	
variable	R	p	R	p	R	p	R	p
pH	0.9299	0.010	0.9287	0.022	-	ns	0.8757	0.052
Clay (%)	-	ns[¶]	-	ns	-	ns	-	ns
Clay+Silt (%)	-	ns	-	ns	-	ns	-	ns
OM (%)[§]	0.9590	0.010	-	ns	0.8997	0.038	-0.9995	<0.001
CEC (cmol/kg)	-	ns	-	ns	-	ns	-	ns
AD (%)[†]	-	-	-	ns	-	ns	0.9848	0.002
MIN (%)[‡]	-	-	-	ns	0.8679	0.056	-0.9674	0.007
AD and pH	-	-	0.9667	0.033	-	ns	0.9999	<0.001

[§]OM = organic matter, [†]AD: Cd in adsorbed phase, [‡]MIN: Cd in mineral phase.
[¶]ns: non-significant at p<0.05.

Conclusion

For all the soils evaluated, it was possible to fit adequately the experimental data from batch sorption experiments to the two-site reaction model. Sorption parameters can be estimated from the soil pH and the Cd present in the adsorbed phase, and can be further used in modelling assessment to predict the fate of Cd introduced into cultivated soils through the application of P fertilizers and other soil amendments.

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Transport of naphthoic acids in metal-contaminated soil columns: Experimental study and modeling approach

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Abstract
Transport of two naphthoic acids (1-hydroxy-2-naphthoic acid (HNA) and 2-naphthoic acid (NA)) was studied in soil-packed columns. Firstly, the aqueous transport was described with surface complexation model incorporated in PHREEQC, implemented with the hydrodynamic parameters and by adjusting the sorption constants of solute breakthrough in column packed with an artificial soil. The experimental breakthrough curves of solutes in a metal contaminated soil were then determined and compared with those calculated using the sorption parameters derived from synthetic soil experiments. This modeling approach was used as tool to understand the mobility of HNA vs NA under convective-dispersive flow in contaminated soil. The effect of solute injection on the mobilisation of Pb, Zn and Cu from metal contaminated soil was also monitored. This study has important implications for the fate and transport of both carboxylated aromatic contaminants and heavy metals in mixed contaminated soils.

Key Words
PAH metabolites, metal, soil minerals, contaminated soil, modelling

Introduction

Soils contaminated by mixtures of heavy metals and Polycyclic Aromatic Hydrocarbons (PAHs) are frequently found in polluted industrial sites and along roads. Naphthoic acids are primary products of the biodegradation of PAHs composed of three rings such as phenanthrene and anthracene. They also are metabolic end-products in the biodegradation of the four-ring PAHs (Gibson 1984). The environmental fate of PAH metabolites is prompted by their ubiquitous distribution and their potentially deleterious effects on human health. Naphtoic (NA) and hydroxynaphthoic (HNA) acids have been also identified as intermediates of PAH natural attenuation in soil studies (Gibson 1984; Burgos et Pisutpaisal 2006). These compounds may have distinctly different sorption affinities and reactivities toward environmental surfaces than their parent compounds (Parikh *et al.* 2004). Due to their higher mobility in porous media, the fate of naphthoic acids in the environment is of significant interest. Therefore, the potential risk of groundwater pollution associated with the transfer of these compounds should be considered through their interactions with main soil constituents. While there are numerous studies on the transport of low-molecular-weight organic acids, the mobility of these carboxylate compounds in soils has been scarcely explored. For such compounds, specific interactions with mineral surfaces may dominate their fate in soils (Hanna 2007). In the present study, the mobility of two naphthoic acids (HNA and NA) was studied under column flow-through conditions in two soils: a lab-synthetic soil representative of natural soils and a contaminated roadside soil. The first objective was to develop a transfer model to simulate the breakthrough curves (BTC) of the naphthoic acids in the synthetic soil. Therefore, the model was applied to the roadside soil and discussed. The effect of naphthoic acids on the mobility of the main heavy metals from the contaminated soil was finally studied by measuring the metal aqueous concentrations in column eluates.

Methods

Preparation and characterization of soil samples

Synthetic Fe-Al-SiO_2 coating mineral was used as a model mineral soil due to abundance in the environment. In particular, ferrihydrite and gibbsite particles were deposited on quartz sand (Fontainebleau, France), with a grain size range of 150-300 µm. The final composition was 90% of sand, 4 % of ferrihydrite and 6% of gibbsite. The roadside soil was sampled along a major rural highway near Paris (France). An average representative sample of the soil was generated from the surface layer (0-2 cm). Experiments were carried out on the particle size fraction under 200 µm. Some mineralogical and physico-chemical parameters are summarized in Table 1 (Hanna *et al.* 2009). Cation exchange capacity (CEC) and heavy metal concentrations are reported in Table 2.

Table1. Characteristics of the contaminated soil

Parameter	
Natural pH_{H2O}	7.7
Organic carbon (%)	7.2
Clay (particles <2 µm) (%)	0.4
CaCO3 (%)	1.3
Quartz (%)	67
HAO (%)	2.2
HFO (%)	1.8
Soil particle density (g/cm3)	2.38
Specific area (m^2/g)	1.3

Table 2. Major elements and trace metals in contaminated soil.

Metals (mg/kg)	
Pb	2580±150
Zn	720±30
Cu	200±20
Cationic exchange capacity (meq/100 g)	
Total	16
Calcium	11.2
Sodium	3.96
Magnesium	0.60
Potassium	0.14

Column experiments

Solid materials were dry packed into glass chromatographic columns with 5 cm in diameter. The porous bed had a length of 20 ± 0.5 cm and a dry mass of 433 ±3 g. After packing to a uniform bulk density (1.10 ±0.01g.cm^{-3} for the roadside soil and 1.73±0.1g.cm^{-3} for the synthetic material), the columns were wetted upward with a background electrolyte solution (NaCl, 8.10^{-2} mol/L) at a constant flow rate (3 ml.min^{-1}). Bromide tracer experiments (8.10^{-2} mol/L in a pulse mode) were performed to identify the flow characteristics through the columns. Bromide concentrations were measured by ionic chromatography and BTC analyzed by using the method of moments and the model MIM (LTHE, Grenoble). The pore volume of soil column is V_p = 118 ± 2 mL for the roadside soil and 92 ±2 mL for the synthetic soil. Different synthetic soil columns sets were then fed with HNA (0.25 mmol/L) and NA (0.25 mmol/L) solutions at pH 6.6 ± 0.1 in a continuous mode at a constant flow rate (3 ml min^{-1}, Darcy velocity (q) = 0.15 cm min^{-1}). Roadside soil columns were also submitted to similar injection experiments. High Performance Liquid Chromatography (HPLC) was used to measure HNA and NA concentrations used in column eluates. Then, the trace element release from the contaminated soil was studied through the injection of HNA / NA mixture at the same flow rate and the HNA / NA transfer was characterized by UV-Vis online detection (wavelength = 254 nm). Dissolved iron and metal concentrations in the collected fractions were measured by ICP-AES. As the collected volumes were dedicated to metal analyses and the absorbance increases were observed at the same time as for single component injections, the variations of absorbance were related to naphthoic acids transfer without further HPLC analyses.

Modeling approach

The MINTEQA thermodynamic database incorporated in PHREEQC-2 formed the core to which surface complexation parameters were added. Modeling was based on the assumption that only one single type of reactive site exists on each mineral sorbent. Various physical and chemical properties of the solid adsorbent material are required before surface complexation modeling can be applied to experimental adsorption data: (i) the number of binding sites (mol of sites/ m^2 of pore volume); (ii) the specific area of adsorbent material (m^2/g) and (iii) the mass of reactive material (g/L). Surface characteristics parameters for quartz, for hydrous ferric oxide HFO and for HAO (including specific surface area, surface site density, and surface acidity constants for iron oxide) were taken from published data (Dzombak and Morel 1990). Binding sites ≡SOH, which is responsible for all surface complexation reactions, can accept and release hydrogen ions and take part in complexation reactions with anions. The surface sites were denoted by ≡SiOH for quartz, by ≡FeOH for iron oxides and by ≡AlOH for aluminium oxides. In this study, a 1:1 stoechiometry (mononuclear surface complexes) was postulated in accord with the SCM approach developed for organic anion adsorption.

Results

The transport experiments were firstly conducted in column packed with synthetic soil. The breakthrough curves of NA and HNA were modeled with PHREEQC by fitting the surface sorption constants. One surface reaction was assumed with each of the main sorbent phases of synthetic soil (SiO_2, HFO and HAO). The corresponding surface complexation constants adjusted using SCM incorporated in PHREEQC2 are: log K_{int} = 0.05, log K_{int} = 1.1 and log K_{int} = 3.2 for ≡SiHL, ≡AlHL and ≡FeHL surfaces complexes (HNA as HL^-), and log K_{int} = 0.02, log K_{int} = 0.4 and log K_{int} = 1.5 for ≡SiL, ≡AlL and ≡FeL surfaces complexes (NA as L^-). The breakthrough curves of NA and HNA through the column packed with the roadside contaminated soil are shown in Figure 1. For NA, the point of breakthrough was at 4 V/V_p, while for HNA, breakthrough started at about 17 V/V_p and then completed at about 30 V/V_p.

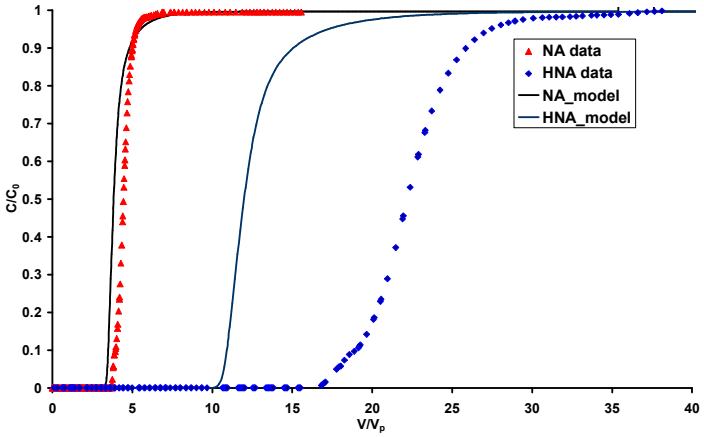

Figure 1. Experimental (symbols) and calculated (lines) breakthrough curves of HNA and NA for the roadside soil column (relative aqueous concentration of solute against the number of pore volumes (V/V_p)). Inflowing solution with C_0 = 0.25 mmol/L, pH= 6.6 ± 0.1; T=20°C.

In a first approach, the acid transport was modeled with PHREEQC, implemented with the hydrodynamic parameters defined by the Br^- tracer breakthrough experiment and the adjusted sorption constants derived from naphthoic acids breakthrough experiment in synthetic soil-packed column. The agreement between the experimental breakthrough curve of NA and that calculated using the sorption parameters derived from previous experiments is relatively good (solid lines in Fig. 1). The predicted breakthrough however underestimated HNA sorption in the column system by a factor of about 3 (Figure 1). The implementation of kinetic limitations did not improve the prediction of experimental HNA elution, which seems to indicate that the kinetics may be not the cause of such discrepancy. The best fit of experimental breakthrough curves of NA in the roadside contaminated soil with sorption parameters derived from synthetic soil, led us to believe that NA may interact preferentially with polar soil mineral surfaces such as HFO and HAO. In contrast, the underpredictions observed for HNA possibly relates to sorbent fractions which are not considered in the modeling approach of breakthrough experiment of synthetic soil (e.g. organic matter fractions). Therefore, 1-Hydroxy-2-naphthoic acid can sorb to the soil mineral surfaces but also to organic matter via hydrophobic interactions. This indicated that HNA may have specific retention on soil surfaces, which dominate its mobility through soil column. The model predictions were based on model parameters derived in the laboratory for well-characterized sorbents, while the conditions met in natural and heterogeneous soils may be very different from these model systems. Moreover, the lack of local geochemical equilibrium in the column could describe the inability of the model simulation to describe outflow concentrations.
In addition, the mobilization of metals through roadside soil packed column upon HNA and NA mixture transport was monitored (Fig. 2). A slight increase of metals was observed after the elution of two pore volumes, in relation with an increase of optical density, but no significant breakthrough of mobilized metal from soil was observed as the absorbance increases at V/Vp =15. The first front could be attributed to NA breakthrough and the second one to HNA elution, the retardation factor between the two compounds being close to 5, as shown in Figure 1.

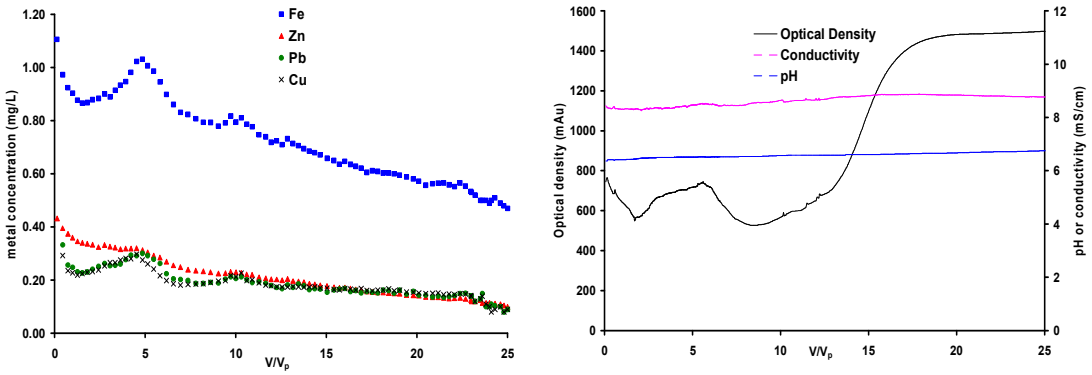

Figure 2. Experimental metal breakthrough curves (Fe, Pb, Zn and Cu) and pH, conductivity and optical density (254 nm) of the leachates from roadside contaminated soil upon injection of HNA and NA mixture.

The pH of the leachates increased slightly and then stabilized between 6.7 and 6.8. The eluted mass of trace metals, Fe, Zn, Pb and Cu remains very low by comparison with the initial content. The leaching of metal upon HNA/NA sorption is low relative to that mobilized using acetic acid (pH5) or EDTA (pH7) (Delmas *et al.* 2002; Hanna *et al.* 2009). This difference in metal leaching could be due to the stronger molecular chelation of EDTA towards cationic metals.

Conclusion

The multisurface modeling approach would be able to predict the transfer of organic ligands from contaminated soils and to a better understanding of the relationships between leaching and oxides surfaces properties in contaminated soils. The retention of NA in dynamic conditions (columns) could be successfully predicted through coupling aqueous transport (convection and dispersion) and retention parameters derived from synthetic soil experiment. The underpredictions observed for HNA possibly relates to sorbent fractions which are not considered in the present modeling approach (e.g. organic matter fractions). Due to its chemical structure, HNA has distinctly different sorption process toward environmental reactive surfaces than NA. In addition, the mobilization of metals through soil packed column upon HNA and NA mixture transport was found to be very low and less than that observed when weak acid solution such as acetic acid was injected. Further study is required to include a model for other important soil fractions such as soil organic matter into multisurface modeling approach.

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Using rhizosphere biogeochemistry to increase understanding of heavy metal bioavailability

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Abstract
A greater understanding of rhizosphere biogeochemistry is likely to improve our understanding of the factors that drive bioavailability of heavy metals in growth substrates. A glasshouse study was conducted that investigated the impacts of three growth substrates (topsoil, oxidised mining waste and unoxidised mining waste) and four species of New Zealand trees on the rhizosphere concentrations of arsenic, copper and zinc. In many of the substrate by species treatments the concentration of heavy metals was lower in the rhizosphere than in the bulk substrate. In addition, for a given plant species, where rhizosphere concentrations were significantly different to the bulk concentration, the rhizosphere concentration correlated better with plant shoot concentrations (i.e. bioavailability) than they bulk concentrations of heavy metals. In particular, the rhizosphere concentrations were good predictors of plant copper concentrations.

Key Words
Rhizosphere, mine rehabilitation, heavy metals, plant nutrition, soil formation.

Introduction
Researchers have been attempting to develop a robust method of assessing heavy metal bioavailability for decades. A number of potential methods have been suggested but as yet, none have been demonstrated to have wide applicability. In response, a growing body of research is now investigating rhizosphere biogeochemistry to increase our knowledge of the factors affecting heavy metal bioavailability and thus the development of robust soil bioavailability tools. While many researchers have investigated the impacts of microbes on heavy metal dynamics in the rhizosphere there have been fewer investigations into the direct impact of plants on rhizosphere biogeochemistry. In addition, most research has focused on soil with little emphasis on other growth substrates such as mining wastes. The following study investigated the effects of a four New Zealand tree species on the rhizosphere concentrations of arsenic, copper and zinc in soil and mining wastes.

Methods
The experiment was conducted as a pot trial in a glass house at Lincoln University, New Zealand. Three growth substrates were trialed, *viz.*, stockpiled topsoil, oxidized mining waste and un-oxidised mining waste (Table 1). All growth substrates were collected from the Globe-Progress Mine, New Zealand. The substrates were air dried and passed through a 1.5 cm sieve before use in the experiment. Plastic pots were lined with a plastic bag and filled with air-dry substrate (2.1 kg topsoil, 2.6 kg oxidised mining waste, 2.5 kg unoxidised mining waste). Fertiliser was added as the dry analytical reagent grade compound at a rate of 150 kg/ha N (as NH_4NO_3) and 50 kg/ha P (as KH_2PO_4) and thoroughly mixed into each pot.

Table 1. Description of topsoil and mining wastes collected from Globe-Progress Gold Mine, New Zealand.

Parameter	Topsoil	Oxidised waste	Un-oxidised waste
pH (1: 5 substrate: H_2O)	4.8	4.4	5.1
Electrical conductivity (1: 5 substrate: H_2O) (µS/m)	100	40	58
Gravimetric water content at field capacity (g/g)	0.31	0.13	0.16
Specific surface area (m^2/g)	0.525	0.563	0.811
Clay (%)	1.9	2.1	3.1
Silt (%)	64.3	54.7	77.9
Sand (%)	33.8	43.2	19.1

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Four New Zealand tree species used in mining restoration were trialed, viz. *Nothofagus truncata* (red beech), *Leptospermum scoparium* (manuka), *Aristotelia serrata* (wineberry) and *Griselina littoralis* (broadleaf). Seedlings were collected from an unmined area on the Globe Progress Site, and transported bare-rooted and wrapped in moist sphagnum moss to Lincoln University. One seedling was planted per pot with five replicate pots of each species by substrate combination. Approximately 275 g of white polythene beads (5 mm diameter) were placed on the surface of each pot to minimise evaporation. The pots were watered with deionised water to 90 % field capacity and maintained at 90 % field capacity throughout the experiment by regular watering with deionised water. The pots were placed in a randomised block design to counterbalance environmental variation within the glasshouse. Every four weeks each block was cycled one place through the glasshouse and the pots within a block re-randomised.

On the 105th day after transplant the experiment was harvested. Plant shoots were cut at the surface of the growth substrate and rinsed sequentially in tapwater, 2 % Decon in tapwater and 2 deionised water rinses before placing in paper bags and drying at 60 ºC for 48 h. Plant tissues were ground and digested in 70 % HNO_3 before analysis for As, Cu and Zn by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). Samples of the bulk substrate were collected using a 1 cm diameter auger. Roots were then gently removed from the substrate and rhizosphere substrate was collected using the method of Gobran *et al*. (1998). Growth substrates samples were air dried before analysis. Soluble As, Cu and Zn was determined by extracting the growth substrate in 0.05M $Ca(NO_3)_2$.

Statistical analyses (Paired t-tests and Pearson correlation coefficient) were performed using MINITAB (Minitab 1995). Data was considered significant when $P<0.1$.

Results

The concentrations of arsenic, copper and zinc showed a range of responses when comparing bulk to rhizosphere concentrations (Figure1). In particular, copper concentrations tended to be lower in the rhizosphere for all species and soil concentrations (Figure 1b, e, h, k). For the few species by substrates combinations where the rhizosphere was not significantly different to the bulk concentration of copper, the concentration appeared to be trending lower in the rhizosphere. Arsenic concentrations were the most variable with difference between bulk and rhizosphere concentration varying from bulk>rhizosphere to no significant difference to bulk<rhizosphere (Figure 1a, d, g, j). Rhizosphere zinc concentrations were the least likely to be differentiated from the bulk substrate concentration of the heavy metals tested (Figure 1c, f, I, l). The only substrate where rhizosphere zinc was consistently significant different to bulk concentrations was in the oxidized waste rock where the bulk>rhizosphere. However the trend was bulk>rhizosphere for zinc concentrations in a number of other species by substrate combinations.

The rhizosphere was a better predictor of plant heavy metal concentrations than the bulk substrate concentration, and thus of heavy metal bioavailability, for each species by metal combination where more than one substrate had significant differences between bulk and rhizosphere concentrations and the change in concentration was in the same direction. In particular, rhizosphere concentrations gave consistently better relationships with shoot copper concentrations. For manuka, wineberry and broadleaf the difference between using bulk and rhizosphere copper concentrations as a predictors of bioavailability was the difference between nonsignificant and significant correlations.

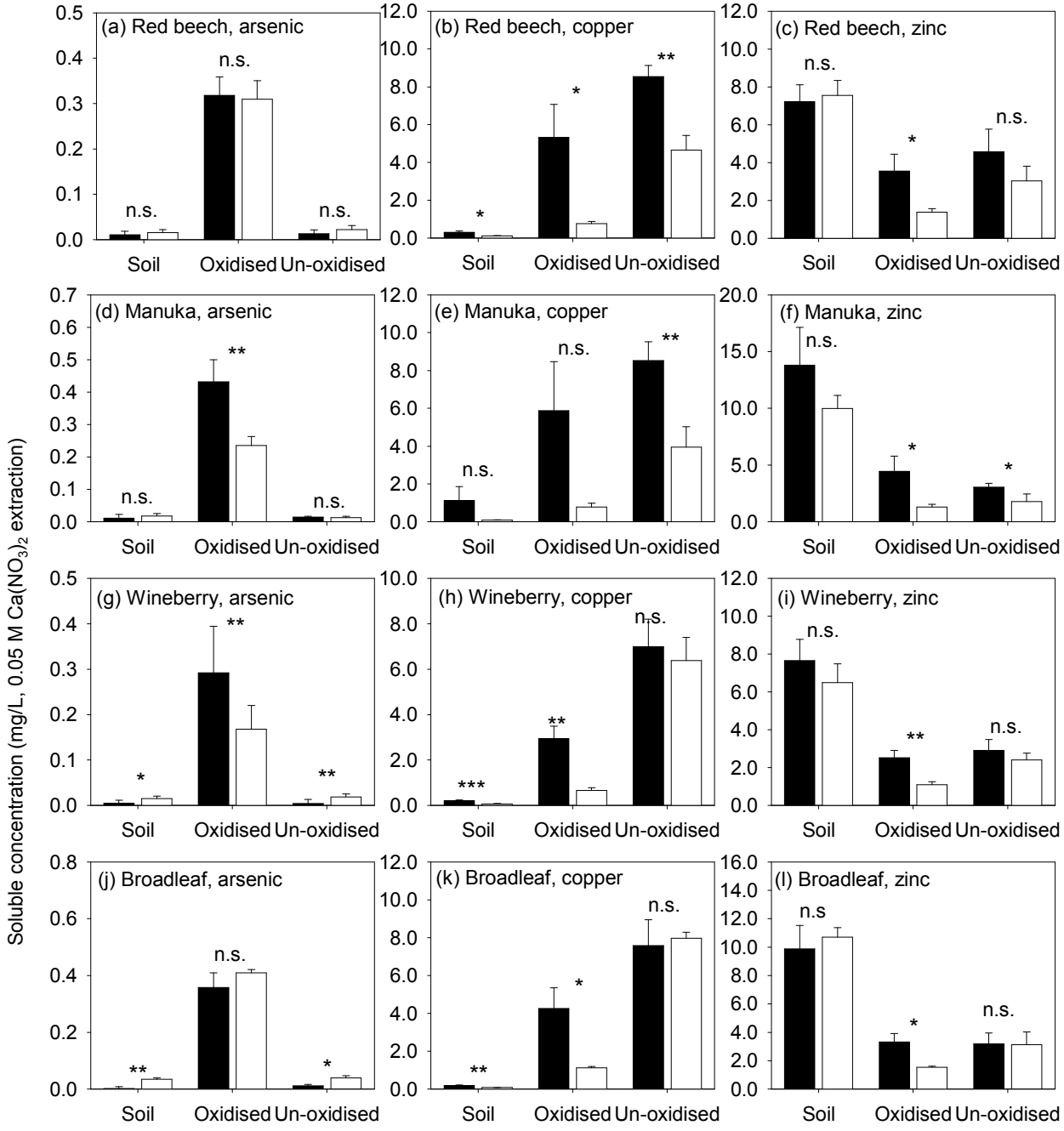

Figure 1. Relationship between the concentration of 0.05 M $Ca(NO_3)_2$ extractable arsenic, copper and zinc in the bulk substrate (■) and rhizosphere (□) for four New Zealand plant species grown in topsoil and two mining wastes (oxidised and un-oxidised) collected near Reefton, New Zealand. Values above paired columns correspond to the statistical significance of paired t-tests (P<0.01 ***, P<0.05, P<0.1 *, P>0.1 n.s.)**

Table 2. Pearson correlation coefficients between bulk and rhizosphere concentrations for four New Zealand tree species. The plants were grown in three substrates with results combined for this analysis. Shaded squares correspond to treatments where at least two of the substrates had significantly different rhizosphere to the bulk concentrations and the direction of change was consistent between substrates (Figure 1).

Species	Arsenic		Copper		Zinc	
	Bulk	Rhizosphere	Bulk	Rhizosphere	Bulk	Rhizosphere
Red beech	r=0.691	r=0.732	r=0.516	r=0.814	r=0.691	r=0.688
	P=0.009	P=0.004	P=0.071	P=0.001	P=0.009	P=0.009
Manuka	r=0.623	r=0.764	r=0.220	r=0.570	r=0.806	r=0.880
	P=0.017	P=0.001	P=0.450	R=0.033	P<0.001	P<0.001
Wineberry	r=0.774	r=0.654	r=-0.380	r=-0.481	r=0.860	r=0.841
	P=0.001	P=0.008	P=0.163	P=0.070	P<0.001	P<0.001
Broadleaf	r=0.525	r=0.757	r=0.288	r=0.645	r=0.856	r=0.885
	P=0.054	P=0.002	P=0.317	P=0.013	P<0.001	P<0.001

Discussion

In contrast to the current study (Figure 1), rhizosphere concentrations of heavy metals are commonly found to be higher than the corresponding bulk concentration (Hinsinger *et al*. 2009). One reason for the difference between this and other studies may be the prevalence of microbes in different substrates. Most previous studies have used soils that have long histories of plant growth, and thus, are likely to have high concentrations of microbes even in cases where the soil was moderately contaminated with heavy metals. In contrast, the mining wastes used in the current study had no previous history of plant growth and the soil had been stockpiled, and thus, were all likely to have low microbial activity. Therefore, the findings from the current study may be more indicative of direct plant impacts on the rhizosphere such as the rhizosphere representing a zone of depletion from absorbed heavy metals. In addition, the rhizosphere enrichment of arsenic for wineberry and broadleaf in the soil and un-oxidised waste (Figure 1 g, j) may indicate that these species were able to rapidly encourage a microbial population in their rhizosphere in these substrates. Wineberry is a pioneer species in New Zealand ecosystems (Salmon 1996) and thus may have evolved properties to encourage the rapid colonisation of its roots by appropriate microbes. These findings highlight the need to investigate the role of rhizosphere biogeochemistry in a number of substrates and situations to inform our understanding of heavy metal bioavailability.

The research in the current study has illustrated that rhizosphere concentrations of arsenic, copper and zinc are often better predictors of substrate bioavailability than the corresponding bulk concentration. These results confirm the importance of the rhizosphere for our understanding of bioavailability and suggest, in particular, that an understanding of rhizosphere biogeochemistry has a role in a risk-based approach to mining remediation

Acknowledgements

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What soil constituents contribute to the accumulation of fertilizer-derived U?

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Abstract
Phosphate fertilizer contains trace amounts of uranium (U) as an impurity and is a cause of concern as a possible source of U in agricultural soils. We collected soil samples from agricultural and unfarmed fields in Japan and compared U concentrations in surface and sub-surface soils. The ratio of U concentration in surface to that in sub-surface soils was around 0.87 in unfarmed fields and 1.36 in agricultural fields, suggesting that U accumulates in the surface layer of agricultural soils due to the long-term application of phosphate fertilizer. In order to investigate what soil constituents contribute to the accumulation of U, we determined pyrophosphate and acid oxalate-extractable U as a measure of U associated with soil organic matter and poorly crystalline Al/Fe minerals in soil, respectively, by using the surface soils taken from the long-term soil fertility experimental fields with and without application of phosphate fertilizer. Most of phosphate fertilizer-derived U was incorporated into either soil organic matter or poorly crystalline Fe/Al minerals in the agricultural surface soils. The contribution of soil organic matter as a pool of U in the soil appeared to be more important in upland and pasture soils, whereas that of poorly crystalline Fe/Al minerals was more important in paddy soils with alternating changes in redox conditions.

Key Words
Long-term soil fertility experimental fields, uranium, phosphate fertilizer, soil organic matter, poorly crystalline Fe/Al minerals.

Introduction

Phosphate fertilizer contains appreciable amounts of uranium (U) as an impurity (McBride and Spiers 2001). The amount of U introduced into soil from phosphate fertilizer during a single cultivation period is relatively low compared to that of U naturally present in soil. However, many researchers have noted that the continuous application of phosphate fertilizer causes an elevated concentration of U in agricultural soils but the concentration rarely exceeds the naturally occurring concentration (Rothbaum *et al.* 1979; Takeda *et al.* 2006; Taylor 2007). During a 10-year period in a paddy field of Japan, the increase of U in soil due to the application of calcium superphosphate fertilizer (60 kg/ha/y as P_2O_5) was estimated to be 5.3% of the total U in the soil (Tsumura and Yamasaki 1993). Taylor (2007) showed that the annual increase in the rate of accumulation of U in four New Zealand soils, 0.015 to 0.047 μg/g/yr, is linearly related with the application rate of phosphate fertilizer. He also indicated that the U increase rate in the acidic New Zealand soils, which are rich in carbon, oxide, and oxy-hydroxide, is higher than that in the neutral-to-slightly calcareous UK soil reported by Rothbaum *et al.* (1979), 0.003 to 0.014 μg/g/yr, although the U concentration in the phosphate fertilizer used by Taylor (2007) was lower than that used by Rothbaum *et al.* (1979). It is clear that soil properties are important factors in determining the accumulation rate of U in soil in addition to the amounts of U anthropogenically applied to soil. Takeda *et al.* (2006) compared U speciation in agricultural soil with that in adjacent unfarmed soil and showed that fertilizer-derived U is mainly associated with organic substances and Fe oxide in the Andosols of upland fields in Japan. The amounts of organic matter and Fe oxide are likely to be key factors in controlling the accumulation of U in soil.

In order to estimate which soil components are the most important contributors to the pool of phosphate fertilizer-derived U, it would be useful to compare the U speciation of agricultural soils with and without the application of phosphate fertilizer by keeping cultivation conditions other than phosphate fertilization constant. In Japan, long-term soil fertility experiments were launched in the 1920s to investigate the effects of different agricultural practices on crop yields and soil properties. The objectives of this study were to assess the major soil components that act as a pool of phosphate fertilizer-derived U and to evaluate the effects of different agricultural practices on the distribution of U in soil. The amounts and chemical speciation of U in agricultural soil will provide important information when considering the potential threat of U to human health through farmed foods.

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Methods

Soil samples from agricultural and unfarmed fields in Japan

Surface and sub-surface soil samples were taken from 15 pedons of paddy fields, 12 pedons of upland fields, and 10 pedons of unfarmed fields in Japan. The soil samples were passed through a 2-mm sieve, air-dried, and stored in plastic bottles until analysis.

Soil samples from long-term soil fertility experimental fields

Surface soil samples were taken from four long-term soil fertility experimental fields. Sites A and B were periodically submerged paddy fields; site C, an upland field with corn cultivation, and site D, a pasture. The soil types, cultivation practices, cultivated crops, fertilization regime, and relevant soil properties are listed in Table 1. To clarify the effects of phosphate fertilizer and compost application on the accumulation site of fertilizer-derived U in soil, fields under three different fertilizer regimes were selected, i.e., with N and K but without phosphate fertilizer (control), with N, P, and K fertilizer (NPK), and with compost in addition to N, P, and K fertilizer (NPK + compost). At site D, superphosphate was used, whereas a fused phosphate fertilizer used at other sites. Each cultivation experiment with a different fertilizer regime was repeated in 2 sets of fields at site B and 4 sets of fields at site D; however, a repeated field set was not established at sites A and C. At site I, all the soil from the experimental fields had been transferred to a concrete column (100 cm in internal diameter and 100 cm in depth) buried in the soil in 1974, and each cultivation experiment was continued. The soil samples were passed through a 2-mm sieve, air-dried, and stored in plastic bottles until analyses.

U analyses

Total U concentrations were determined for soils taken from agricultural and unfarmed fields in Japan, whereas total, pyrophosphate-extractable, and acid oxalate-extractable U in soil were determined for soils taken from long-term soil fertility experimental fields. To determine the total concentrations of U (U_t) in soil, 0.2 g of air-dried soil was digested with a mixture of HNO_3, $HClO_4$, and HF in a Teflon beaker with heating at 393 K or using a microwave digester (Multiwave3000, PerkinElmer). Pyrophosphate-extractable U (U_p) was extracted from 0.2 g soil with 10 mL of 0.1 mol/L sodium pyrophosphate adjusted to pH 10. Acid oxalate-extractable U (U_o) was extracted from 0.2 g soil by adding 10 mL of a mixed solution containing 0.1 mol L^{-1} oxalic acid and 0.175 mol/L ammonium oxalate (pH 3.3) and keeping the soil–solution mixture in a hot-water bath at 353 K for 1 h. The uranium concentrations in the extractant were determined using an inductively coupled plasma mass spectrometry system (SPQ-8000A, SII NanoTechnology Inc.). In the determination of the total and acid oxalate-extractable U concentrations, the matrix-induced signal suppression and the drift of the instrument response were compensated using the mass intensity of the 1 μg L^{-1} Bi internal standard. The uranium concentration in the pyrophosphate extract was determined using an external calibration standard. The accuracy of the analyzed data using the external standard was confirmed by the standard addition method with U concentrations of 2 and 10 μg/L added to selected samples of pyrophosphate extracts.

Results

U concentrations of agricultural and unfarmed fields in Japan

The U concentrations in soils taken from the surface layer of paddy, upland, and unfarmed soil pedons were 1.7 ± 0.47, 2.0 ± 0.75, and 1.0 ± 0.23 mg/kg, respectively (Figure 1); those concentrations were within the range of background concentration of U in Japanese soil, 0.08 to 14 mg/kg, reported by Yamasaki *et al.* (2001). The ratio of U in surface soil to that in subsurface soil was significantly higher for upland and paddy field soils than for unfarmed soils (Figure 1), suggesting that agricultural practices resulted in increased concentration of U in the surface soil of paddy and upland fields.

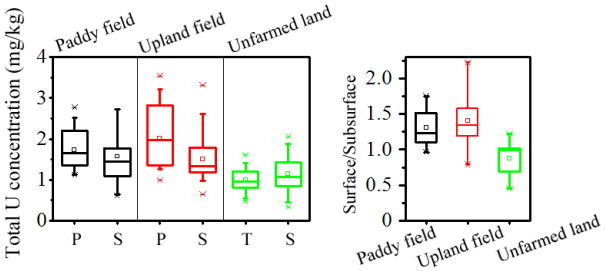

Figure 1. U concentration (a) and the ratio of U in surface to subsurface soils taken from agricultural and unfarmed fields in Japan. P: plowed layer, S: subsurface layer, T: top layer.

U concentrations of long-term soil fertility experimental fields

In the long-term soil fertility experimental fields, U_t, U_p, and U_o in soils with phosphate fertilization (NPK, NPK + compost) were higher than those in soils without phosphate fertilization (control) (Figure 2).

To evaluate the contribution of the U_p or U_o increase to the U_t increase, the ratio of ΔU_p or ΔU_o to ΔU_t, (R_p) was calculated using the following equation:

$$R_{p(o)} = \frac{\Delta U_{p(o)}}{\Delta U_t} = \frac{U_{P(o)}(NPK\ or\ NPK + compost) - U_{P(o)}(control)}{U_t(NPK\ or\ NPK + compost) - U_t(control)}$$

The calculated R_p and R_o are shown in Figure 2 by the numbers on the bar graph. In upland soil and pasture (sites C and D), R_p was larger than 0.8, showing that most of the increased U_t corresponded to the increased U_p. In the upland and pasture soils, soil organic matter is expected to be a major pool for phosphate fertilizer-derived U. R_p exceeded 1 at site A (paddy soils with a low TC concentration) and in the NPK field at site B (paddy soils). In the process of alternating changes in redox conditions, it is possible that the U associated with organic matter was redistributed to other fractions.

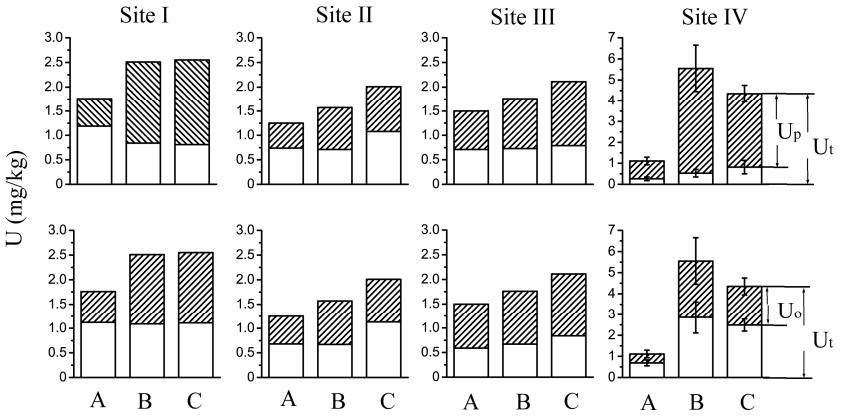

Figure 2. Total (U_t), pyrophosphate extractable U (U_p), and acid-oxalate extractable U (U_o) in the long-term fertility experimental fields A: control, B: NPK, C: NPK + compost (Yamaguchi *et al.* 2009).

R_o was nearly 1 for NPK and NPK + compost soils at site A and NPK soils at site B. This indicated that an increase in concentration of U_t was equivalent to an increase in U_o; in other words, most of the fertilizer-derived U was stored in an acid oxalate-extractable fraction in paddy soils. R_o ranged from 0.44 to 0.68 at sites C and D, where R_p exceeded 0.8. In upland and pasture soils, soil organic matter was more important as a pool of U than acid oxalate-extractable soil components.

U concentrations of agricultural and unfarmed fields in Japan

By comparing soils collected from long-term fertility experimental fields with and without the application of phosphate fertilizer, we showed that most of the fertilizer-derived U in soil was distributed to soil components dissoluble by either pyrophosphate or acid oxalate reagents. Part of U associated with Al complexed with humic substances, amorphous Al hydroxide, allophone, and imogolite was dissolved by both pyrophosphate and acid oxalate reagents. Therefore, the sum of U_p and U_o exceeded U_t in some fields. Incorporation of U into an Al-bearing mineral would not be a major mechanism for U accumulation in soil, as also suggested by the lack of any relationship between Al_o and U_o (data was not shown).

The contribution of ΔU_p to ΔU_t was more prominent than that of ΔU_o to ΔU_t (Figure 3), indicating the importance of soil organic matter as a binding site for fertilizer-derived U. Nonetheless, the application of compost in addition to phosphate fertilizer did not always cause larger amounts of U accumulation than those observed in the fields without compost application. Less-mature organic matter added to soil as compost might have lower capacity to hold U in the soil solid phase. In addition, dissolved organic matter derived from compost may complex with U and the organo-U complexes may be leached out. An increase in U_t due to the application of phosphate fertilizer is also attributed to the increase in U_o. The contribution of ΔU_o to ΔU_t was larger in paddy soils than in upland and pasture soils. The most pronounced differences between upland and paddy soils were the redox condition caused by a different water management regime. Alternating changes in the redox condition and subsequent cycle of dissolution and precipitation of Fe-

bearing minerals should influence the metal distribution in paddy soil. In the paddy fields we investigated (Sites A and B), ΔU_p exceeded ΔU_t; in other words, more U was found to be associated with soil organic matter than the amounts of U added to soil by the application of phosphate fertilizer. Under submerged conditions, the soil pH increases concomitant with a decrease in the redox potential and dissolution of Fe. The carbonate concentration of water in contact with paddy soil should also increase (Kyuma 2004). The higher pH and carbonate concentration contribute to the increased solubility of soil organic matter and U in the soil solid phase, respectively. Under submerged conditions, therefore, U associated with organic matter may be dissociated or dissolved. On the other hand, under reduced conditions, U forms UO_2, which has low solubility. When paddy soil is drained and is under oxic conditions, UO_2 is solubilized again as UO_2^{2+}, and then U may be redistributed among soil components. The behavior of U in paddy soil is complex due to the alternating changes under redox conditions. Although we could suggest that the accumulation characteristics of U for upland and paddy soils are different, the details of the mechanisms explaining the differences remain uncertain.

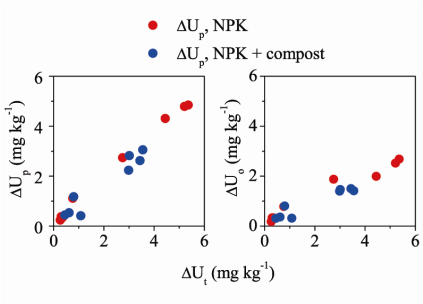

Figure 3. Relationships between increased U_t (ΔU_t) and increased U_p (ΔU_p) or U_o (ΔU_o) influenced by application of phosphate fertilizer (Yamaguchi *et al.* 2009).

Soil organic matter and Fe/Al minerals prevented fertilizer-derived U from leaching out to aquifers surrounding agricultural fields; therefore, they caused accumulation of U in the soil surface. However, it is noteworthy that even under exceptionally intensive use of phosphate fertilizer in the experimental plots, the U concentration in agricultural soil did not exceed the range of background concentration of U in Japanese soils.

Conclusion

Long-term application of phosphate fertilizer increases the concentration of U in agricultural soils in Japan. The fertilizer-derived U is either incorporated into soil organic matter, adsorbed, or precipitated with poorly crystalline Fe/Al-bearing minerals in agricultural surface soils. The contribution of soil organic matter as a pool of U in soil appears to be more important in upland and pasture soils, whereas that of poorly crystalline Fe minerals is more important in paddy soils that had undergone alternating changes in redox conditions.

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