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

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Abstract
The mobility and toxicity of As species in soils is different. Therefore, understanding As species distribution is very important for evaluating the mobility and phytotoxicity of As in As-contaminated soils. In this study, the distribution of As species in solutions of a geogenic As-contaminated soil and three As(V)-spiked soils after flooding was determined and the toxicity of As to rice plants was investigated. Results showed that before flooding incubation, the major As species in solutions of tested soils was As(V). However, after flooding incubation, As(III) concentrations in solutions of tested soils increased with incubation time, as As(V) was reduced into As(III) under the flooding condition. Among the relationships between As species concentration and rice seedling growth, the soil NaH_2PO_4-extractable total As and height and dry weight of rice seedlings has the best fits for the dose-response mode and thus it is proposed for use as an index for the assessment of phytoavailability and phytotoxicity of As in As-contaminated paddy soils.

Key Words
As-contaminated paddy soils, As phytotoxcity, As speciation, HPLC-ICP-MS.

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

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

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

Soil flooding incubation and soil solution analysis
Put 20 g studied soil and 20 mL distilled water in a 50 mL centrifuge tube (three replicates). After shaking for 30 minutes and incubating at 25ºC for 0, 3, 6, 12, 24, and 42 days respectively, soil solutions were collected. One part of the solutions was preserved in 0.01 M H_3PO_4 immediately and used for determining As species, As(III), DMA, MMA and As(V), by using HPLC-ICP-MS. One part was preserved immediately in 5% HNO_3 and used for determining Fe, Mn, Si and P by using ICP-OES. Finally, the pH of soil solutions was also measured. All of the analyses were completed in one day.

Soil NaH_2PO_4-extractable As (Huang *et al.* 2006)
Put 2 g studied soil and 30 mL 0.5 M NaH_2PO_4 in a 50 mL centrifuge tube. After shaking for 2 hrs, soil solutions were collected and preserved with H_3PO_4 immediately. Then arsenic species [As(III), MMA, DMA, and As(V)] were determined by HPLC-ICP-MS.

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

Results and Discussion

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

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

	As(III)	DMA	MMA	As(V)	Total As
	(---------------------------		μg/L		--------------------------)
Without H_3PO_4	35.7 ± 2.7	1.28 ± 0.24	ND	2.84 ± 0.03	39.8 ± 2.9
With H_3PO_4	145 ± 9	1.46 ± 0.05	0.50 ± 0.02	11.6 ± 0.7	159 ± 9

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

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

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

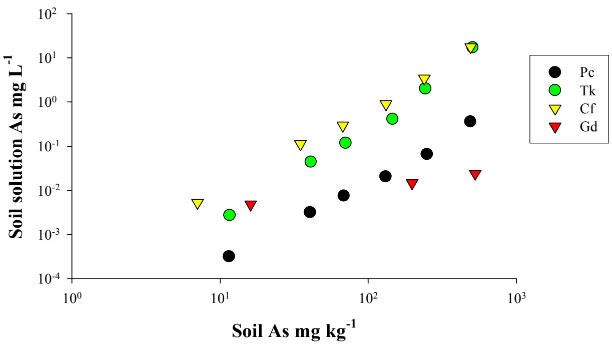

Figure 1. The relationships between the As content in studied soils and the total As concentration in soil solution before flooded incubation

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

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

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

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

Arsenic phytoavailability (phytotoxicity) to rice seedlings grown on soils

The relationships between the concentrations of total As, As(V) and As(III) in soil solutions (before flooding and after 42 days flooding) and rice seedling growth (plant height and dry weight) all were fitted well with the dose-response model (significant at $P = 0.01$). Among the dose-response relationships, the soil NaH_2PO_4-extractable total As with the heights and dry weights of rice seedlings has the best fit for the model and the effective toxicity concentration of decreasing 20% of rice plant heights (EC_{20}) was 78.7 mg As /kg (Figure 2). Therefore, soil NaH_2PO_4-extractable total As could be used as an index for the assessment of phytoavailability and phytotoxicity of arsenic in paddy soils. The results of the total As uptake in shoots and roots of rice seedlings were shown in Figure 3. It shows that As uptake ratio (root: shoot) is high which can be explained by the formation of iron plaque. The iron plaque was found on the roots of the rice seedlings grown in the tested soils, especially in the As-contaminated Gd soil. The iron plaque has high affinity for adsorbing As. That is why As uptake ratio (root : shoot) of As-contaminated Gd soil was higher than those of other studied soils. Therefore, the iron plaque may act as a barrier for uptake of As by rice plants.

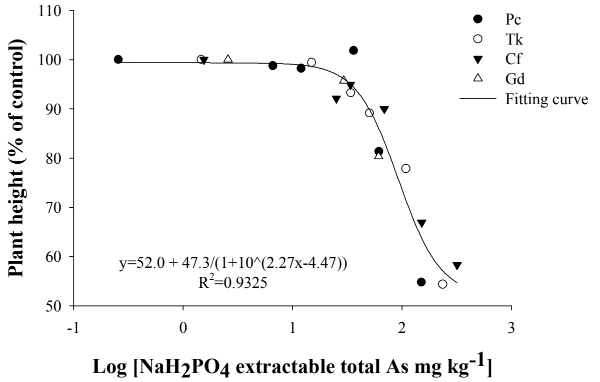

Figure 2. The dose-response relationship between the amounts of NaH_2PO_4-extractable total As and the plant heights of rice seedlings.

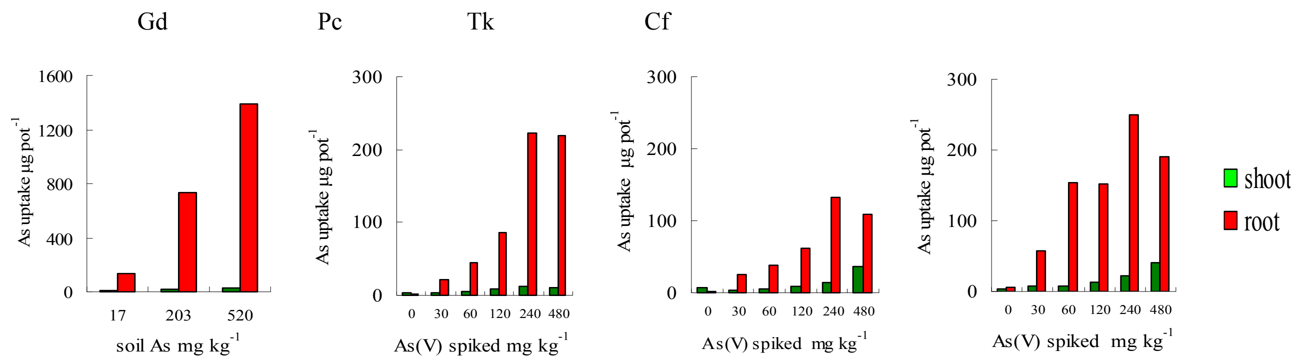

Figure 3. Total As uptake in shoots and roots of rice seedlings.

Conclusion

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

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