EFFECTS OF LOW MOLECULAR WEIGHT ORGANIC ANIONS ON THE RELEASE OF ARSENITE AND ARSENATE FROM A CONTAMINATED SOIL

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Abstract. Low-molecular-weight-organic-anions (LMWOAs) are important exudates of plants and may influence the mobility and bioavailability of metals or metalloids. In the present study the effects of selected LMWOAs, citrate, malate and oxalate, on the release of arsenite (As(III)) and arsenate (As(V)) in a contaminated soil were investigated. The organic anions have significant influence upon the release of arsenic from the soil, and a linear relationship exists between the released arsenic and the concentration of LMWOAs in the extractants. pH effects on the arsenite and arsenate adsorption were not significant over the range from 3 to 7. The amounts of arsenite and arsenate released were significantly correlated with the release of Fe, Mn and Al, suggesting that arsenic was mainly released from Fe-, Mn- and Al-oxides or hydroxides in soil. The ratio of released arsenite to arsenate was not influenced by LMWOAs.

Keywords: arsenite, arsenate, low-molecular-weight-organic-anions, release, soil

1. Introduction

Arsenic is ubiquitous in soils with the concentrations ranging from 1 to 40 mg/kg in uncontaminated soils. However, much higher concentration of arsenic is present in a very large number of places in the world (Mandal and Suzuki, 2002). Drinking of As-contaminated groundwater is perhaps the most common exposure pathway of humans to arsenic (Berg *et al.*, 2001). As a consequence more stringent limits for arsenic in drinking water have been recently proposed (European Union, 1998; Christen, 2000). Arsenic contamination in soil may be prevalent at mining and industrial sites (Brandestetter *et al.*, 2000). Leaching of arsenic from the contaminated soils could pose a potential risk to groundwater quality, as well as the mobility and bioavailability of arsenic in soil-water-plant systems (Barrow, 1974; Livesey and Huang, 1981). Therefore, it is necessary to investigate the potential mobilization of arsenic in soils, especially in the contaminated soils.

Mobilization of arsenic in soil has been intensively investigated and it is mainly affected by the soil biogeochemical factors, such as soil texture, organic matter, nature and constituents of minerals, pH, redox potential, competing ions (Adriano, 2001). Although arsenic has a high affinity to soil, when the environmental condition changes, the mobility and even speciation of arsenic may be changed. For instance, in natural ecosystems the rhizosphere processes always occur and lead to the exudation of some organic anion into soil. Such processes may have an impact on the mobilization of arsenic in soil and lead to the release of arsenic into ground and surface water. Therefore, it is important to have a better understanding of the effects of organic anions upon the mobility of arsenic. Generally, only the mobile fraction of toxic metals in the soil is easily available by plants. Such mobilization must also be taken into account when arsenic phyto-accumulation is assessed.

In the rhizosphere, plant roots can exude many organic compounds; among them low-molecular-weight-organic-anions (LMWOAs) are important. Through the effect of acidification, chelation and oxidation-reduction reactions in the rhizosphere, LMWOAs can influence metal solubility in soils and uptake by plants. Cieslinski *et al.* (1998) found that LMWOAs in rhizosphere soils could influence the solubilization of particulate-bound cadmium in soil solutions; Jones *et al.* (1994) indicated that organic acids mediated iron dissolution in the rhizosphere. Although the importance of arsenic pollution in soils is widely recognized, virtually little is known about the effect of LMWOAs in rhizosphere processes upon the mobilization of arsenic in soil.

It has also been reported that P-deficient plants could exude LMWOAs and result in enhanced availability (Hoffland, 1992; Neumann and Römheld, 1999; Kirk *et al.*, 1999). This response is thought to change soil pH, displace P from sorption sites, chelate metal cations that could immobilize P or to form soluble metal-chelate complexes with P (Kirk *et al.*, 1999). Arsenic and P belong to the same chemical group and have comparable dissociation constants for their acids and solubility products for their salts, and represent similar geochemical behavior in soil (Adriano, 2001). So, it is reasonable to predict that LMWOAs exudation could also play a role in the mobilization of As in soil. However, there is still no direct evidence to these speculations.

Several LMWOAs have been identified as exudation products in soil rhizosphere processes (Lundström, 1994; Jones and Darrah, 1994), of which citrate, malate and oxalate are the most important compounds. Therefore, in this study we investigate the influences of these three organic anions on the solubility of arsenite and arsenate in a contaminated soil. Effects of the LMWOAs concentration and pH were investigated. The relationship between arsenic mobility and the co-released Mn, Al and Fe was also investigated in order to explain the mechanism. Such knowledge of the fate of arsenic in the soil is particularly valuable for risk assessment of arsenic in environment.

2. Materials and Methods

2.1. SOIL

The soil heavily contaminated with arsenic was collected in Xingren county, Guizhou province in China. The sampling site was in farmland and close to a thallium ore deposit. The soil sample was air-dried and ground to pass through a 2-mm sieve, homogenized and analyzed. Soil pH was measured in aqueous slurry of 1:1 soil and water mixture. Organic matter in soil was determined by the Walkley-Black method (Nelson and Sommer, 1982). Cation exchange capacity (CEC) of the soil was determined by the method described by Rhoades (1982). Selected properties of the soil sample and metal concentrations are presented in Table I.

2.2. EXTRACTION

Triplicate 2.00 g of the soil sample were weighed into 50 ml polypropylene tubes and 20 ml of extraction solution was added. The samples were then shaken at 20 °C for 16 hours. pH of the suspension was kept at pH 5.5 adjusted with 0.1 M HCl or NaOH with less than 2% of the volume changed. The extracted samples were then centrifuged and filtered through a filter of 0.45-mm pore size and diluted before analysis.

TABLE I
Selected physical and chemical characteristics
of the soil

Property	
Particle size (%)	
>200 μm	55.66
20–200 μm	39.36
2–20 μm	4.91
<2 μm	0.07
pH (1:1 in H_2O)	6.37
CEC (c mol/kg)	15.2
OM (%)	6.73
As, mg kg^{-1}	302.2 ± 16.1
Fe, g kg^{-1}	52.2 ± 2.1
Al, g kg^{-1}	25.9 ± 1.2
Mn, g kg^{-1}	1.1 ± 0.1

2.3. DETERMINATION OF As

Arsenic concentrations were determined by hydride generation atomic fluorescence spectrometry (HG-AFS, AFS-230 model atomic fluorescence spectrophotometer, Beijing Haiguang Instrumental Company, Beijing, China). An automatic intermittent hydride generation device was used to generate the arsenic hydride. Argon gas was used as the carrier gas for the transposition of arsenic hydride from the hydride generator to the atomizer. Fluorescence measurements for samples were interpolated using the calibration line with As(III) standards. A calibration plot was obtained from 0 to 30 $\mu\text{g L}^{-1}$ As measured in the same conditions as those for the samples. This solution was continuously feed at a carrier flow of 6 ml min^{-1} and mixed with a flow of the reducing solution (NaBH_4 3.0% w v^{-1} in NaOH 0.5 w v^{-1}). The AsH_3 generated was transferred to fluorescence spectrometry by using an argon flow rate of 400 ml min^{-1} .

Inorganic arsenic species were selectively determined by exploiting the generation of As(V) from As(III) at low acidic medium while arsenate does not produce any signal. Total arsenic was determined by hydride generation under strong acidic condition after the As(V) completely reduced to the trivalent state with 10 g L^{-1} thiourea according to the method proposed by Han and Wang (1985). Then As(V) was calculated from the difference between As(III) and the total arsenic concentration.

Arsenic stock solutions were obtained from National Research Center for Certified Reference Materials, Beijing, China. All the reagents used were of analytical reagent grade or better.

All the data obtained by three replicate analyses. Statistical analysis was performed by use of the Office XP-Excel and Origin 6.0.

3. Results and Discussion

3.1. DETERMINATION OF ARSENIC SPECIATION

Inorganic As is the predominant form of arsenic in soil (Cullen and Reimer, 1989; Garcia-Manyes *et al.*, 2002). We first analyzed the arsenic speciation in the soil extracts by HPLC-HG-AAS. The chromatograms displaying a multispecies solution containing 10 $\mu\text{g L}^{-1}$ of As(III), As(V), monomethylarsonate (MMA), dimethylarsinate (DMA) and the soil extract by 5mM citrate are given in Figure 1. No MMA and DMA were detected in the soil extract. Therefore, in this work only inorganic species were further investigated.

In order to make sure that As(III) could be selectively determined without interference from arsenic (V) and As(V) could be totally reduced to As(III) by thiourea for the total As determination, a series of solution with 10 $\mu\text{g L}^{-1}$ of As(III) and different concentrations of As(V) were analyzed. The results are listed in Table II.

Figure 1. Chromatograms of arsenic speciation obtained from the HPLC-HG-AAS analyses; (A) standard solution ($10 \mu\text{g g}^{-1}$ As(III), As(V), MMA and DMA); (B) soil solution extracted by 5 mM citric acid at pH 5.5).

We can see that there is no interference from As(III) for the determination of As(III) and the As(V) could be quantitatively reduced to As(III) with thiourea.

Quality assurance was carried out by the determination of total arsenic in soil certified reference materials (GBW 07408 and GBW 08302 purchased from the National Research Center for Certified Reference Materials, Beijing, China). Seven replicated analyses were made for each sample. The results obtained were

TABLE II
Determination of arsenic in the mixture
solution of As (V) and As (III) ($\mu\text{g g}^{-1}$)

Added		Detected	
As (III)	As (V)	As (III)	As (V)
10.0	0	10.1	0
10.0	4.0	10.0	4.1
10.0	8.0	10.1	7.8
10.0	12.0	10.1	11.9
10.0	16.0	10.1	16.3
10.0	20.0	10.0	20.2

$12.1 \pm 1.0 \mu\text{g g}^{-1}$ and $4.0 \pm 0.3 \mu\text{g g}^{-1}$ for GBW 07408 and GBW 08302, respectively. The certified values are $12.7 \pm 1.7 \mu\text{g g}^{-1}$ and $3.8 \pm 0.4 \mu\text{g g}^{-1}$ for GBW 07408 and GBW 08302, respectively. Good agreements were achieved between the data obtained by the present method and the certified values.

3.2. EFFECT OF THE ORGANIC ACIDS UPON THE RELEASE OF ARSENITE AND ARSENATE

It has been reported that concentrations of organic acids in soil solution are in the range of 1×10^{-3} to 4×10^{-4} M (Stevenson and Ardakani 1972; Stevenson, 1994). Higher concentrations ($>10^{-3}$ M) of LMWOAs would be expected to be produced in localized zones where biological activity is intense, such as the rhizosphere (Rovira, 1969; Stevenson, 1991) and near decomposing plant residues (Bruckert, 1970; Bruckert and Kacquin, 1969). Therefore, dissolution of As in the contaminated soil by citrate, malate and oxalate in the concentration range from 0 to 10 mM was investigated. The release curves for total As, As(III) and As(V) in different concentration of citrate, malate and oxalate are presented in Figures 2A–C. The total concentration of As in the soil is 302.2 mg kg^{-1} and the dissolved As by water is about $0.5 \mu\text{g g}^{-1}$, representing only 0.17% of the total As in the soil, which suggests that a relative low soluble As representing in the contaminated soil in mining areas and there should be no threat upon the groundwater quality. Similar results were also reported in the As contaminated soils in southwestern England by Abedin *et al.* (2002). However, in the presence of organic anions, enhanced As solubility was observed. Linear relationships ($p < 0.01$) exist between the dissolved As and the concentrations of LMWOAs in the extractants and the R^2 values are from 0.9695 to 0.9982, as shown in Figures 2A–C. We should pay more attention to such phenomenon because the LMWOAs exudation always happens in the rhizosphere process. The observation of LMWOAs enhancing the arsenic solubility

Figure 2. Dissolve of As with different concentrations of LOMWAs at pH 5.5 $\diamond$: Total As, $\circ$: As(V) and Δ : As(III).

TABLE III

Concentration of co-released Fe, Mn and Al by different concentration of low molecular weight organic acids ($\mu\text{g g}^{-1}$)

Conc. of LMWOA (mM)	Citrate			Oxalate			Malate		
	Fe	Mn	Al	Fe	Mn	Al	Fe	Mn	Al
0	19.4	0.44	14.2	18.6	0.50	7.9	4.7	0.38	6.7
1	35.9	1.2	34.8	14.9	0.73	11.8	5.7	0.46	6.3
2	63.4	3.9	27.1	40.5	2.9	61.3	6.2	0.77	6.9
4	92.2	22.1	77.4	75.1	3.0	53.0	8.9	1.7	10.2
6	114.6	35.7	78.6	105	3.4	62.3	10.9	2.7	9.4
8	189.8	49.4	122.0	134.2	4.2	67.5	46.7	24.1	38.1
10	347.7	101.6	207.4	203.2	10.0	252.0	75.4	37.2	44.3

indicates that such process will consequently increase the mobility of arsenic in soil.

It was reported that As adsorption in soils is most controlled by surface complexation reactions with the oxides/hydroxides of Al, Mn and especially Fe (Inskeep *et al.*, 2002). Therefore in this study the co-released Fe, Mn and Al were investigated. The results are listed in Table III. Increasing concentrations of Fe, Al and Mn were observed with the increase of the LMWOAs concentration. Statistical analysis shows that As released is significantly correlated with the co-released contents of Fe, Al and Mn (Table IV), the correlation coefficient (r) ranges from 0.7794 to 0.9920. Citrate, malate and oxalate all have a high affinity for trivalent metals such as Al^{3+} and Fe^{3+} and bivalent Mn^{2+} (Jones, 1998), as shown in Table V. The effect of LMWOAs upon the increasing solubility of arsenic could have contributed to the dissolution of Fe, Mn and Al by LMWOAs. The dissolved As by different LMWOAs is in the order: citrate>oxalate>malate, which is consistent with the order of the stability constants of Fe-, Al- and Mn-organic ligand complexes.

TABLE IV

Correlation coefficient for As released in soil with the co-released Fe, Mn and Al (R^2)

Organic acid	Fe	Mn	Al
Citrate	0.9205*	0.9342*	0.9588*
Oxalate	0.9920*	0.8305**	0.9001*
malate	0.8985*	0.7794**	0.8166**

*Significant at 0.01 probability level.

**Significant at 0.05 probability level.

TABLE V
Stability constants of organic acid-metal complexes
(Martell and Smith, 1976–1989)

Metal	Citrate	Oxalate	Malate
Fe ³⁺	11.50	7.74	7.1
Al ³⁺	7.87	6.1	6.0
Mn ²⁺	2.93	1.38	1.39

The physiological and toxicological effects of arsenic depend on its chemical form (Cullen and Reimer, 1989). As(III) is much more toxic, soluble and mobile than As(V). We can see from Figures 2A–C that no matter what kind of LMWOAs was present, the extracted As(V) was higher than that of As(III). The ratio of As(V) to As(III) extracted was almost constant. Without organic acid, the ratio was 1.32, and in the presence of 5 mM of citric, oxalic and malic acid, the ratio is 1.45, 1.45 and 1.35, respectively. The results give us a hint that there is no preferential release of arsenic species in the presence of organic anions.

3.3. pH DEPENDENCE OF THE RELEASE ARSENITE AND ARSENATE IN THE PRESENCE OF LMWOAs

It is generally known that rhizosphere pH may considerably differ from that in the bulk soil. Depending on plant and soil factors pH differences can be up to two units. Therefore, the effect of solution pH on the release of arsenite and arsenate in the presence of LMWOAs was studied and the results are shown in Figure 3A–C. The pH of the solution appeared to have weak effect on the amount of As(III) and As(V) released. Previous studies (Manning and Goldbery, 1997; Smith, 1999) using soil and pure Fe hydroxides to investigate the pH-dependent arsenic desorption revealed that As(V) solubility increased upon the increasing pH in range from 3 to 8, whereas As(III) tended to follow the opposite pattern. Masschelyn *et al.* (1991) also observed that soluble As concentration was three times larger at pH 8 than at pH 5, and the authors contributed the results to the decrease of positive surface charge at high pH. However, in this research we found that both the total dissolved As and As(V) decreased slightly with the increasing pH. The released As(III) increased slightly in the presence of malate. In the presence of citrate or oxalate, the released As(III) slightly decreased first and then increased when the pH was higher than 6. The differences of this work from previous results of pH-dependent As desorption could be attributed to the contribution of LMWOAs. LMWOAs have a strong affinity with soil particles. But as the pH of the suspension increases, the soil surface becomes more negative. A greater repulsive interaction between the organic anions and the mineral surface tends to lower the affinity of LMWOAs for soil. In this situation, LMWOAs complex less Fe, Al and Mn, leading to less

Figure 3. Dissolve of As in the presence of 5 mM LOMWAs at different pH. $\diamond$: Total As, $\circ$: As(V) and Δ : As(III). A: citrate, B: oxalate, C: malate.

arsenic being released. The presence of LMWOAs made the desorption mechanism of arsenic more complicated.

4. Conclusion

The results of this study indicate that in the presence of citrate, malate and oxalate, the solubility of arsenic is significantly increased. Linear relationships exist

between the dissolved As(III) and As(V) and the concentrations of LMWOAs in the extractant. However, the pH of the solution has a weak effect on the amount of As(III) and As(V) released. As(III) and As(V) released are significantly correlated with the co-released concentrations of Fe, Al and Mn in the solution. The mechanism could be the dissolution of Fe, Mn and Al from soil by LMWOAs, increasing released arsenic. There is no preferential release between As(III) and As(V) in the presence of LMWOAs. LMWOAs always exist in soil, their effect upon the mobility of arsenic should be considered for the environmental risk assessment.

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