Speciation of antimony and arsenic in the soils and plants in an old antimony mine

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Abstract

The speciation changes of antimony (Sb) in soil–plant system are largely unknown as compared with those of arsenic (As). In this study, indigenous plants and associated soils were sampled at the Xikuangshan Sb mine (XKS), China. The Sb in the soils (441–1472 mg/kg) were far greater than As (32–354 mg/kg), and the Sb and As availabilities in the soils, were 5.5% and 3.9% in average, respectively. HPLC-ICP-MS revealed the presence of four species of Sb in the soils and plants, including Sb\textsubscript{III}, Sb\textsubscript{V}, TMSb and UnkSb (unknown). The use of XANES revealed that the UnkSb consisted of inorganic Sb in the form of Sb\textsubscript{V}. Inorganic Sb were prevalent in the soil and plant samples at the eight sites, whereas TMSb was observed in only a few of the rhizosphere soils, and, in plants at a few of the sites, primarily in the leaves and to a lesser extent in the stems. Arsenic was detected in the soils primarily as inorganic forms, while, DMA was detected in high proportions in all of the plant tissues at all of the sites. The methylation of Sb was far less than that of As in the indigenous plants at XKS. The results suggest that As and Sb differ in transformation characteristics in the soil–plant system in XKS.

1. Introduction

Antimony (Sb) and arsenic (As) are metalloids belonging to Group 15 of the periodic table (Wilson et al., 2010). Arsenic is a toxic and carcinogenic element that is widespread in the environment, and As contamination has been reported worldwide (Sharma and Sohn, 2009). High concentrations of Sb and As are often present simultaneously in sulfide ores (Filella et al., 2002), which have caused the co-contamination of As and Sb (Telford et al., 2009; Wang et al., 2011). Plants inhabited in the vicinity of As and Sb co-contaminated sites have been reported to accumulate high concentrations of As and Sb in their aerial parts. For example, at high contaminated acidic sites, Agrostis capillaris L. was found to accumulate up to 240 mg/kg As and 68 mg/kg Sb in the shoots (Bech et al., 2012). Corrales et al. found two fodder species of plant Trifolium pratense L. and Trifolium repens L. had high tolerance to Sb, and could accumulate as high as 770 mg/kg Sb in their shoots (Corrales et al., 2014). The enrichment of As and Sb in environments could potentially render health risks to humans via food chain, consequently the levels of As and Sb in the environment must be controlled.

Compared to As, very little is known regarding the behavior, ecotoxicology and the environmental distribution of Sb (Telford et al., 2009; Tighe et al., 2005). The chemical similarities between the two metalloids have prompted concerns regarding the enrichment of both metalloids in various environments (Filella et al., 2002; Krachler and Emons, 2001). Sb is often thought of as behaving similarly to As, although this generalization is not always with justification (Casiot et al., 2007; Wilson et al., 2010). Dimethylarsinic acid is very soluble in water, in contrast with dialkylstibinic acid, which is polymeric and relatively insoluble (Parris and Brinckman, 1976). Sb and As display the same range of oxidation states in the environment (−3 to +5) (Wilson et al., 2010). Both of their levels of toxicity in the environment strongly depend on their speciation (Filella et al., 2002; Gebel, 1997). In general, the order of toxicity of Sb species is as follows: antimonite(Sb\textsubscript{III}) > antimonate(Sb\textsubscript{V}) > organoantimonials (e.g., methylated species) (Gebel, 1997; He and Yang, 1999); the toxicity of As species exhibits a similar order: arsenites (As\textsubscript{III}) > arsenates (As\textsubscript{V}) > organoarsenicals (e.g., methylated species) (Wilson et al., 2010).
The speciation transformation process and corresponding mechanisms of As in plants have been well documented (Ma et al., 2001; Tu et al., 2003; Wang et al., 2002). However, those in the case of Sb are far to be resolved, despite that organic Sb have been occasionally detected in some studies. It appears that Sb can be methylated by bacteria and fungus (Filella et al., 2007). For example, biomethylation of Sb by the filamentous fungus Scopulariopsis brevicaulis was recently established (Jenkins et al., 1998). Nevertheless, the information on organic species of Sb in plants had rarely been reported, with most current studies on Sb speciation in plants only focusing on SbV and SbIII (Olkenhaug et al., 2011). Müller et al. used a chromatographic method to study the Sb speciation in Pteris vittata L. spiked with 16 mg/kg SbV, they found that SbV, SbIII, trimethylated SbV and an unidentified Sb compound in the plant (Muller et al., 2009). In their study, the speciation of Sb in the soils of rhizosphere and non-rhizosphere was not known and the relationship between the Sb speciation in soils and in plants was not identified either. It is thus important to identify the location and sources of Sb methylation in plants, which may favor the understanding of the mechanisms on speciation in Sb uptake and transformation in the soil–plant system.

There is evidence that As is detoxified via methylation in biological systems; however, little similar evidence regarding similar processes involving Sb is available (Gebel, 1997). Like As, previous studies also found widespread Sb methylation in biological media. Cullen et al. found, for the first time, the presence of methyl antimony in freshwater fish (Cullen and Reimer, 1989), and Duester et al. reported that monomethylated Sb and As were the dominant species in agricultural and garden soils (Duester et al., 2005). However, due to limited information regarding Sb species and transformation within and between the environment and biological systems, the mechanisms of Sb speciation are still largely unknown (Corrales et al., 2014; Jenkins et al., 2002; Wehmeier and Feldmann, 2005), in contrast with the well documented speciation of As.

The co-existence of As and Sb may affect each other on the uptake by plants. Our previous results showed that the uptake of Sb in Pteris cretica L. (Cretan brake fern) was enhanced by increasing As levels, but As was suppressed by high levels of Sb, accompanied with decreased As and enhanced Sb levels in the cytosol fraction (Feng et al., 2011). However, we did not know whether the co-presence of As and Sb in growth media would affect their speciation transformation, as well as whether and where Sb and As methylation occur in the soil–plant system.

Previous studies of Sb speciation primarily involved soils with relatively low levels of Sb (Filella et al., 2007), whereas little work has focused on media with high levels of Sb. In addition, the limited reports of high Sb speciation were based on analyses of only samples of certain types, which may not allow for insights into the speciation and transformation of Sb in the environment and biological systems, e.g., the soil–plant system, and reveal the effect of such bio-factors as rhizospheres.

It is known that heavy metal toxicity depends strongly on the speciation of these elements in the environment. Therefore, knowledge of the distribution, speciation, and transformation of Sb and As in the soil–plant system of the XKS mine area is important for understanding its geochemical and biological cycling and for evaluating the environmental and human health risks. Despite the significance of quantitative speciation, there is only limited information regarding Sb in the soil–plant system (De Gregori et al., 2007; Dodd et al., 1996; Lintschinger et al., 1997, 1998a, 1998b; Nash et al., 2000; Ulrich, 1998a, 1998b; Ulrich et al., 2000). At present, studies of the XKS mine area have primarily involved the total concentrations of Sb and As, and investigations of the Sb and As species in the environment focused only on the inorganic species, particularly in the soil–plant system. It is also unknown whether Sb and As methylation occurred in the soil or plants and whether a rhizosphere environment promotes Sb or As methylation.

Our previous studies have shown that Xikuangshan mine (XKS) is an area of enrichment in Sb and As in the soil–plant system. This situation provides the prerequisite conditions for tracing the Sb species and their translocations from the soils to the plants and making comparisons with the corresponding characteristics of As. Consequently, this study was performed with the goal of estimating the Sb and As speciation, distribution and transformation characteristics in the soil–plant system in the mining and smelting areas of XKS. The methylation of Sb and As in soil and plant extracts was also investigated in this study. The findings are expected to contribute to a better understanding of the behavior of Sb in soils and plants.

2. Materials and methods

2.1. Site description

XKS Sb mine is located in Lengshuijiang County, Hunan province, in south-central China (Fig. 1). The climate in this area is characterized by a typical subtropical continental monsoon, with an average temperature of 16.7 °C and annual rainfall of 1354 mm. The average year-round relative humidity is 53.1%. The XKS Sb mine is the largest Sb mine in the world and measures 70 km². The mine consists of two mining areas, i.e., the north mine and south mine, which are accompanied by several Sb smelters in the central area (Fu and Wei, 2013). Antimony mining in this area began in 1897, and the present annual production of Sb is 55,000 tons of ore and 40,000 tons of Sb products (Wang et al., 2011). The long-term, large-scale Sb mining and smelting activities have resulted in Sb, As and other heavy metal contamination of the local environment (Fu and Wei, 2013; He, 2007; Liu et al., 2010; Wang et al., 2011; Wei et al., 2011). The concentrations of Sb in the soils have been measured at 100.6–5045 mg/kg; soil near the Sb mine also contains high concentrations of As (Fu and Wei, 2013; Wei et al., 2011).

2.2. Samples collection and preparation

Initially, we intended to collect samples of the same plant species at various sites and investigate their variations in Sb and As concentrations. However, this goal could not be realized due to the great variation in the vegetation in the mining and smelting areas around XKS. Ultimately, the typical indigenous plant species at XKS were individually sampled at eight sites around the south mine, north mine and central smelters (Fig. 1). Descriptions of the sampling sites and plant species are summarized in Table 1. Samples of the associated rhizosphere and non-rhizosphere soils were also collected. Soils attached to plant roots were carefully collected and labeled as rhizosphere samples, and soils approximately 30 cm from the corresponding plant roots (down to a depth of 20 cm) were collected and labeled as non-rhizosphere samples.

The soil samples were prepared by removing stones and plant debris, were freeze-dried and were then ground and passed through a no. 20 mesh sieve (0.85-mm mesh openings). A 20-g portion of the soil sample was further ground using a mechanical agate grinder to produce fine powders for As and Sb speciation analysis (<150-μm particle size). The plant samples were washed with tap water followed by several rinses with deionized water. Each plant sample was separated into three parts (roots, stems and leaves), freeze dried and cut into pieces using ceramic scissors. The samples were then pulverized using a stainless steel mill to produce homogenous powders. The powdered soils and plants were stored in polyethylene packages at 4 °C prior to the concentration and speciation analyses for Sb and As.
2.3. Chemical analysis

The soil pH was measured using a 1:2.5 ratio of soil to deionized water. The amount of organic matter was determined using the Walkleye–Black method (Nelson and Sommers, 1982). Total nitrogen was assessed using the method of Kjeldahl distillation (Bao, 2000). Available phosphorus (P) was extracted using 1 mol/L (pH 4.8) ammonium acetate, and available As and Sb were extracted using 0.5 M NaHCO3 (Woolson et al., 1971). An aliquot (0.1 g) of soil sample was transferred to a 50-mL flask for digestion. The digestion procedure was performed by soaking the material in 5 mL of aqua regia for 12 h. The flasks were heated to 120 °C on an electric hot plate for approximately 3 h. The plant samples were digested using concentrated HNO3 and HClO4. Specifically, an aliquot (0.2 g) of fine powder of each plant sample was transferred to a 50-mL flask containing 3 mL of concentrated HNO3 for 12 h, which was then heated to 90 °C for approximately 3 h. The flasks were cooled to room temperature when the volumes of the sample solutions were approximately 1.5 mL, which was followed by addition of 1 mL of H2O2 and heating for approximately 1 h; H2O2 was repeatedly added three times. The digestion liquid of both the soil and plant samples was transferred into 25-mL tubes and was made up to 25 mL using deionized water (18.2 MΩ cm) water. The total Sb and As concentrations were measured using an Inductively Coupled Plasma Mass Spectrometry (HPLC-ICP-MS) (PE, USA) and the P concentration was determined using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) (PE, USA). The detection limits for Sb and As were 0.01 μg/L. Certified soil and plant reference materials (GBW07418 for soils and GBW07603 for aspen leaves, by the National Institute of Metrology, China) were used to check the accuracies of the analyses. The recoveries of Sb and As were within the ranges of 78–121% and 78–119%, respectively.

2.4. The extraction procedure for soil and plant samples during analyses for Sb and As speciation

The extraction of Sb and As followed the procedures that we developed in two earlier studies. Briefly, a solution of 100 mmol/L citric acid at pH 2.03 was used for extraction of Sb from the soil and plant samples (Ge and Wei, 2013). A solution of 2 mol/L trifluoroacetic acid was used for As extraction from the plant samples, and a mixture of 1 mol/L phosphoric acid and 0.1 mol/L ascorbic acid.
acid was added to the soil samples to extract the As (Wang and Wei, 2010). An aliquot of 0.2 g of fine soil or plant powder was mixed with 10 mL of the respective extractor in a 50-mL centrifuge tube. The tube was shaken for 1 h at 300 rpm in a shaker at room temperature, then centrifuged at 8000 × g for 10 min in a high-speed refrigerated centrifuge (Jouan, France), and then filtered using 0.22-mm cellulose acetate membrane filters. The supernatant was transferred to a 25-mL graduated test tube. These procedures were repeated three times, and the supernatants were combined for analysis. The average extraction efficiencies of the Sb and As from the soils were 53% and 60%, respectively. The extraction efficiencies of the Sb and As from the plant tissues were remarkably varied, possibly due to the heterogenous properties of the plant powders of the samples, causing the extraction efficiencies for Sb species to total Sb much greater than 100% which were unconvincing, instead, we used percentage to extracted Sb in plants at such cases.

2.5. Analysis of Sb and As speciation using HPLC-ICP-MS

Standard solutions of antimonate (Sb\textsuperscript{V}), antimonite (Sb\textsuperscript{III}), and trimethylantimony (TMSb) were prepared by dissolving appropriate amounts of potassium hexahydroxiantimonate (K\textsubscript{3}Sb(OH)\textsubscript{6}, Sigma–Aldrich), potassium antimony tartrate trihydrate (K\textsubscript{2}Sb\textsubscript{2}C\textsubscript{8}H\textsubscript{8}O\textsubscript{12}, Sigma–Aldrich) and trimethylantimony dibromide (TMSbBr\textsubscript{2}, Sigma–Aldrich) in deionized water, respectively. The stock solutions of arsenite (As\textsuperscript{III}), arsenate (As\textsuperscript{V}), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) were purchased from the National Institute of Metrology, China. The working standard solutions of Sb and As were prepared daily by appropriately dilution of the stock solutions.

Speciation analysis was performed using an HPLC-ICP-MS system (PE, USA). The chromatographic system (PerkinElmer Series 200) was equipped with an auto-sampler and a quaternary pump that was coupled via a peek capillary to a cross nebulizer of an ICP-MS system. The conditions for the analyses of the speciation of Sb and As were previously developed by our group (Ge and Wei, 2010; Wang and Wei, 2010) and are summarized in Table 2. The pH values in the mobile phases were adjusted using formic acid or ammonia solutions. The detection limits for Sb\textsuperscript{III}, Sb\textsuperscript{V} and TMSb were 0.03, 0.02 and 0.05 mg/L, respectively; those for As\textsuperscript{III}, As\textsuperscript{V}, MMA and DMA were 0.41, 0.30, 0.66 and 0.56 μg/L, respectively. Standard solutions of various As and Sb species were added in soils and plants powders before the extraction performances. The spiked extraction recoveries of Sb\textsuperscript{III}, Sb\textsuperscript{V} and TMSb were 88%, 11% and 92% in the soil samples and 101%, 75% and 117% in the plant samples, respectively; those of As\textsuperscript{III}, As\textsuperscript{V}, MMA and DMA were 75%, 83%, 79% and 86% in the soil samples and 56%, 53%, 79% and 82% in the plant samples, respectively.

2.6. Analysis of Sb speciation using X-ray absorption near edge structures (XANES technique)

The K-edge XANES spectra of Sb were collected at the 14W1 beam line using a Si(3 1 1) double-crystal mono-chromator at the Shanghai Synchrotron Radiation Facility (SSRF). The energy storage ring provided a beam current of 200 mA and 3.5 GeV. The results from the soil samples were recorded in fluorescence mode using a Lytle detector, whereas those of the reference materials were recorded in transmission mode. The reference materials include antimony (Sb, Sinopharm Chemical Reagent Beijing Co. Ltd., SCRB), antimony(III) oxide (Sb\textsubscript{2}O\textsubscript{3}, SCRB), antimony(III) chloride (SbCl\textsubscript{3}, Sigma–Aldrich), potassium antimony tartrate trihydrate (K\textsubscript{2}Sb\textsubscript{2}C\textsubscript{8}H\textsubscript{8}O\textsubscript{12}, Sigma–Aldrich), trimethylantimony dibromide (TMSbBr\textsubscript{2}, Sigma–Aldrich), potassium pyroantimonate (K\textsubscript{2}H\textsubscript{3}SbO\textsubscript{4}, Sigma–Aldrich) and potassium hexahydroxiantimonate (K\textsubscript{3}Sb(OH)\textsubscript{6}, Sigma–Aldrich). The standard reference materials were ground and evenly spread on 3 M tape, whereas the soil samples were pressed into flakes for XANES analysis. The adsorption edge of the XANES was used to distinguish between Sb\textsuperscript{III} and Sb\textsuperscript{V} (Takaoka et al., 2005). Linear combination fitting analysis (LCF) was used to fit a combination of model compounds to unknown spectra using the Athena program in the IFEFFIT package. This approach is commonly used to determine the relative proportions of model compounds present in heterogenous samples (Fawcett et al., 2009). An energy range of −100 to 150 eV from the K-edge of Sb was used to fit the soil sample with respect to standard references. The goodness of the fits was expressed as an R-factor (R), which is a statistical measurement of the accuracy of the fit (Eq. (1)):

\[
R = \frac{\sum (\text{data} - \text{fit})^2}{\sum \text{(data)}^2}
\]

3. Results

3.1. Total concentrations of Sb and As in soils and plants

The Sb concentrations in the soils were in the range of 229–1472 mg/kg at the eight sites; these values greatly exceeded those of As, which were 36.8–464 mg/kg. The availabilities of Sb in the soils were 5.5% on average, which also exceeded those of As, which were 3.9% on average (Table 3). Slight but insignificant differences were observed between total and available Sb and As concentrations in the rhizosphere and non-rhizosphere soils at the eight sampling sites at XKS.

Because the Sb levels in the soils were much higher than those of As, the roots and stems of the indigenous plants at XKS accumulated a great deal more Sb than As, except for the roots of Eriogonum annuum at site S5 and the stems of E. annuum and P. vittata at sites S5 and S8. However, the As concentrations exceeded those of Sb in the leaves of E. annuum, Fagopyrum dibotrys and P. vittata at sites S5, S7 and S8 (Table 4). The Sb concentrations varied greatly within and between plants. The Sb levels displayed a general pattern of roots > stems > leaves. The highest Sb concentration, 1421 mg/kg, was recorded in the roots of the fern Dryopteris erythrosora at site 3, and an Sb concentration of 107 mg/kg in the leaves of this fern was also the highest among the eight indigenous plants. The As level also varied among the plants and their tissues. In general, higher
Table 3
The physico-chemical properties and available P, Sb and As concentrations in the soil of the sampling sites (n = 8).

<table>
<thead>
<tr>
<th></th>
<th>Total (mg/kg)</th>
<th>Available (mg/kg)</th>
<th>Available to total (%)</th>
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<tbody>
<tr>
<td></td>
<td>As</td>
<td>P</td>
<td>Sb</td>
</tr>
<tr>
<td><strong>Rizosphere</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>161</td>
<td>693</td>
<td>623</td>
</tr>
<tr>
<td>SD</td>
<td>155</td>
<td>369</td>
<td>349</td>
</tr>
<tr>
<td><strong>Non-rhizosphere</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>155</td>
<td>665</td>
<td>518</td>
</tr>
<tr>
<td>SD</td>
<td>114</td>
<td>296</td>
<td>226</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>158</td>
<td>679</td>
<td>570</td>
</tr>
<tr>
<td>SD</td>
<td>131</td>
<td>323</td>
<td>289</td>
</tr>
</tbody>
</table>

Table 4
Total concentrations of Sb and As in soil and plant samples in Xikuangshan (mg/kg).

<table>
<thead>
<tr>
<th>Site</th>
<th>Soils</th>
<th>Roots</th>
<th>Stems</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sb</td>
<td>As</td>
<td>Sb</td>
<td>As</td>
</tr>
<tr>
<td>S1</td>
<td>493 ± 23</td>
<td>32 ± 5</td>
<td>31.1 ± 9.15</td>
<td>1.02 ± 0.11</td>
</tr>
<tr>
<td>S2</td>
<td>1472 ± 125</td>
<td>272 ± 12</td>
<td>438 ± 68.6</td>
<td>18.1 ± 2.04</td>
</tr>
<tr>
<td>S3</td>
<td>648 ± 55</td>
<td>65 ± 9</td>
<td>1421 ± 132</td>
<td>3.59 ± 0.13</td>
</tr>
<tr>
<td>S4</td>
<td>469 ± 18</td>
<td>101 ± 10</td>
<td>141 ± 52.4</td>
<td>4.84 ± 0.30</td>
</tr>
<tr>
<td>S5</td>
<td>472 ± 36</td>
<td>354 ± 18</td>
<td>53.8 ± 16.6</td>
<td>144 ± 35.1</td>
</tr>
<tr>
<td>S6</td>
<td>510 ± 21</td>
<td>149 ± 15</td>
<td>55.3 ± 13.7</td>
<td>2.11 ± 0.48</td>
</tr>
<tr>
<td>S7</td>
<td>479 ± 33</td>
<td>201 ± 13</td>
<td>1072 ± 108</td>
<td>18.9 ± 9.17</td>
</tr>
<tr>
<td>S8</td>
<td>441 ± 32</td>
<td>62 ± 6</td>
<td>107 ± 26.2</td>
<td>65.8 ± 16.8</td>
</tr>
</tbody>
</table>

Note: n = 3 for repetitions in each sample for analysis.

concentrations of As were measured in the roots than in the stems and leaves; however, the highest As concentration, 217 mg/kg, was recorded in the leaves of *F. dibotrys* at site S7, and the As concentrations in various tissues of *P. vittata* at site S8 were essentially equivalent. These great variations in Sb and As levels within and between the plants and tissues reflect the corresponding concentrations in the soils and the abilities of the plant species to take up and translocate the two elements.

3.2. Analysis of Sb speciation in soils and plants using HPLC-ICP-MS

Three Sb species, including SbIII, SbV and an unknown Sb (UnkSb) were identified in the rhizosphere, non-rhizosphere soil and plant extracts at the eight sites (Fig. 2). Except for SbIII, SbV and UnkSb, TMSb was detected in the rhizosphere soils at sites S3, S6, S7 and S8 (Fig. 3a). In the rhizosphere soils at the eight sites, UnkSb was the major species of Sb, followed by SbIII, SbV and TMSb (Fig. 3a). In contrast, SbIII rather than UnkSb was the primary Sb species in the non-rhizosphere soils at sites S5, S6 and S8 (Fig. 3b). Everywhere (at all of the sites), SbIII was present in higher proportions than SbV in both the rhizosphere and non-rhizosphere soils (Fig. 3).

Among the Sb extracted from the roots of the majority of the plants, UnkSb was the major Sb species (Fig. 4a), whereas SbIII was dominant in the stems and leaves (Fig. 4b and c). In various tissues of all of the plants, the SbIII proportions were always higher than those of SbV, particularly in the leaves and stems. Specifically, the

![Fig. 2. Results of chromatography of Sb speciation in a rhizosphere soil sample (a) and a plant sample (leaf tissue) and (b) extracted using 100 mmol/L citric acid at pH 2.03. The symbols SbV, SbIII, TMSb and UnkSb denote antimonate, antimonite, trimethylantimony and unknown Sb, respectively.](image-url)
proportions of Sb\textsuperscript{III} in the stems were 31–85%, whereas those of Sb\textsuperscript{V} were only 0.6–35% (Fig. 4).

### 3.3. Analysis of As speciation in soils and plants using HPLC-ICP-MS

Only inorganic As species (As\textsuperscript{III}, As\textsuperscript{V}) were identified in the non-rhizosphere soils, whereas, in addition to inorganic As, organic As species, such as MMA or DMA, were identified in rhizosphere soils at a few of the sites. At all of the sites, the proportions of As\textsuperscript{V} in the rhizosphere and non-rhizosphere soils were always higher than those of As\textsuperscript{III} (Fig. 5).

In the roots of the majority of the plants, As\textsuperscript{III} was identified in only a few samples and in much lower proportions than As\textsuperscript{V}. In the stems and leaves, As\textsuperscript{III} was prevalent and present in much higher proportions than As\textsuperscript{V}. DMA was detected in significant proportions in the roots, stems and leaves of all of the plants, whereas MMA was rarely detected and only in small proportions (Fig. 6).

### 3.4. Analysis of Sb speciation using XANES

Because an unknown Sb(UnkSb) was detected in a number of the soil and plant extracts (Fig. 2), it was important to analyze its possible components and structure. One rhizosphere and one non-rhizosphere soil sample collected at each of sites S3, S6 and S7 were selected for further analysis of the Sb speciation using the XANES technique. The adsorption edge was used to distinguish the speciation of Sb in the samples. As shown in Fig. 7, both the adsorption edges of the rhizosphere and non-rhizosphere samples were equivalent in terms of their K\textsubscript{2}Sb(OH)\textsubscript{6} and K\textsubscript{2}H\textsubscript{2}Sb\textsubscript{2}O\textsubscript{7} concentrations (R-factor 0.006), suggesting that the Sb in those samples were of an inorganic Sb\textsuperscript{V} (Sb\textsuperscript{V}) form.

### 4. Discussion

#### 4.1. Accumulation of As and Sb by plants at XKS

The present study was conducted to explore the variations in Sb and As species in the soils and plants at XKS with the aim of discerning the common and contrasting patterns of the speciation of the two elements. The concentrations of Sb and As in
The proportions of various species of As in the rhizosphere (a) and non-rhizosphere (b) soil extracts using HPLC-ICP-MS (%) at the eight sampling sites at XKS. The symbols AsV, AsIII, MMA and DMA denote arsenate, arsenite, monomethylarsine and dimethylarsine, respectively.

Fig. 5. The proportions of various species of As in the rhizosphere (a) and non-rhizosphere (b) soil extracts using HPLC-ICP-MS (%) at the eight sampling sites at XKS. The symbols AsV, AsIII, MMA and DMA denote arsenate, arsenite, monomethylarsine and dimethylarsine, respectively.

Several plants in this study exhibited a high ability to accumulate Sb in their tissues, particularly in their roots, such as D. erythrosora at site S3, F. dibotrys (D. Don) at site S7, Rumex patientia L. at site S2, Oplismenus undulatifolius (A) Bea at site S4 and P. vittata at site S8. Boehmeria nivea has reportedly accumulated levels of Sb as high as 5579 mg/kg in its roots at XKS (Okkenhaug et al., 2011). In this study, however, such levels were not observed, which may reflect the great variation in plant accumulations of Sb from site to site. Only E. annuus L. Pers (both in the roots and leaves) at site S5, F. dibotrys (D. Don) at site S7 (in the leaves) and P. vittata at site S8 (both in the roots and leaves) accumulated relatively high levels of As in their tissues. Unexpectedly, the As hyperaccumulator P. vittata did not exhibit its excellent ability to accumulate As in its leaves in this investigation. The higher concentrations of Sb and As in the roots than those in the aboveground parts of the majority of the plants suggested that these plants displayed a limited ability to transfer Sb and As.

4.2. Speciation of Sb in the soils and plants at XKS

In this study, speciation of Sb using HPLC-ICP-MS indicated that SbIII, SbV and UnkB (unknown Sb) were the three major Sb species in the majority of the soil and plant extracts (Figs. 3 and 4), whereas TMB was observed in only a few of the plant extracts at certain sites, primarily in the leaves and stems (Fig. 4b and c). The level of Sb was very high, its mobility is relatively high at XKS (Table 2), and the extracted SbV species in soils might be more complex in structure than the K5b(OH)6 standard, which may have caused a delay in the appearance of the SbV peak in the chromatography, as indicated by the very close proximity and the same shapes of the peaks of the SbV and UnkB in the soil and plant extracts from XKS (Fig. 2).
The appearance of Sb\textsuperscript{III} in HPLC-ICP-MS detection for soil and plant samples should be reasonable, since we had got convinced spiked recoveries for Sb\textsuperscript{V}, Sb\textsuperscript{III} and TMSb in soil samples for the extraction and determination.

However, when XANES was performed on selected soil samples, the results indicated that this unknown Sb species was the same in both the rhizosphere and non-rhizosphere soils: an inorganic Sb\textsuperscript{V} form, with no Sb\textsuperscript{III} being detected (Fig. 7). The inconsistency between the results of HPLC-ICP-MS for soil extracts and XANES for corresponding whole soil samples might be complicated. First, Sb\textsuperscript{III} was relatively in small proportions as compared with Sb\textsuperscript{V} in soil samples, which could not be detected by XANES; second, the quite heterogeneity and disturbanc of organic matter might blurred the detection of Sb\textsuperscript{III} in soil samples; third, due to limitation of machine-hours at Shanghai Synchrotron Facility, we only successfully tested several soil samples, which thus could not thus cover the real state of other samples as measured by HPLC-ICP-MS, consequently the existence of TMSb could not be ruled out. Nevertheless, the UnkSb species in the plants, observed in the chromatographic analysis, remains unidentified. Indeed, we also performed XANES analyses for Sb in the plant samples but failed to obtain sound data due to severe signal interference from the plant media. Therefore, whether the UnkSb species in the XKS plants is an inorganic or other form of Sb remains unresolved.

In this study, TMSb was identified only in the samples of rhizosphere soils from half of the tested sites, whereas no TMSb was detected in the non-rhizosphere soils from any of the sites. This finding indicates that the rhizosphere may favor Sb methylation in soils similar to the As methylation in the rhizosphere (Jia et al., 2013; Zheng et al., 2013). Similarly, methylantimony has been identified in soils involved in various land uses, including in situ farm land and garden soils, which suggests that plant roots have an effect on the methylation of Sb in soils (Amerelih et al., 2005; Duester et al., 2005; Murciego et al., 2007). Unfortunately, it was not clear from this study whether the presence of the TMSb in the rhizosphere soils is solely due to the plants or is aided by microorganisms in the rhizosphere.

TMSb was found in the leaves of plants at 5 sites and stems at 1 site, whereas no TMSb was identified in the roots of the plants at all 8 sites (Fig. 4), suggesting that Sb can be methylated within the plants, primarily in the leaves. The presence of TMSb in the rhizosphere soils at 4 sites and a lack it in the corresponding plant roots (Figs. 3a and 4a) suggest that the plants at XKS were unable to directly take up and maintain the presence of TMSb in their roots.

4.3. Speciation of As in soil–plant system in XKS

Organic As as DMA was detected in the rhizosphere soils at two sites, whereas no organic As was identified in the non-rhizosphere soils at any of the sites (Fig. 5). This finding is consistent with the distribution of Sb observed in this study, suggesting that certain plant species may promote the methylation of As and Sb in the soils. This observation is also consistent with the findings of Francesconi et al., who found traces of MMA and DMA in certain ferns growing in As-contaminated sites (Francesconi et al., 2002). These trends indicate that the transformations of As species will occur during the transport of As within plants. DMA was identified only in the rhizosphere soils of two sites, whereas DMA was identified in all types of plant tissues from all of the sampling sites (Fig. 6), which indicates that the plants growing at XKS may methylate As themselves rather than taking it up from the soils. The fewer identifications of DMA (at 2 sites) than TMSb (at 4 sites) in the rhizosphere soils in contrast with the higher frequency of DMA (all types of plant tissues at all 8 sites) than TMSb (in leaves at 5 sites and stems at 1 site) at XKS suggest that the plants growing at XKS tend to methylate As in greater quantities than Sb.

The proportions of As\textsuperscript{III} in the leaves and roots were far greater than those of As\textsuperscript{V}, whereas the opposite pattern was generally observed in the stems, suggesting that the As\textsuperscript{V} may have been converted to As\textsuperscript{III} in the stems, after which it most likely produced organic As via methylation during As translocation from the roots to the leaves (Fig. 6). Several studies also confirmed that the As in P. vittata and Pityrogramma calomelanos, which are two As hyperaccumulating ferns, was present primarily as As\textsuperscript{III} in the fronds 11–15]. The wide distribution of DMA in various plant tissues suggests that the plants growing at XKS can effectively bimethylate As during the translocation of As from the roots to the leaves. In this study, MMA was not identified in the majority of the plant tissues, which indicates that MMA was completely consumed during the conversion to DMA.

5. Conclusions

This study represents an attempt to determine the speciation of As and Sb in rhizosphere and non-rhizosphere soils and to explain the conversions of As and Sb species in a soil–plant system using HPLC-ICP-MS and XANES techniques. The plants growing at XKS were able to accumulate high levels of Sb and lesser levels of As. Both the inorganic species Sb\textsuperscript{III} and Sb\textsuperscript{V} were detected in the plant tissues, and Sb\textsuperscript{III} was present in greater proportions than Sb\textsuperscript{V}, particularly in the leaves and stems. Both organic As and Sb were detected in the plant tissues; however, the organic Sb was present only in the leaves, whereas As was found in various plant tissues, suggesting that Sb methylation in the plants at XKS occurred only in the leaves. Although TMSb was observed in the rhizosphere soils at a few of the sites, no TMSb was detected in the plant roots and stems, which suggest that these plants are limited in their ability to take up TMSb from the soils at XKS.