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Chemically-assisted phytoextraction from metal(loid)s-polluted soil at a typical carlin-type gold mining area in southwest China

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ABSTRACT

Laowuchang (LWC) gold mine, located in Southwest China, is a typical Carlin-type gold deposit with the characteristic Au–As–Sb–Tl trace elements assemblage. This study focuses on the mobilization of arsenic (As), antimony (Sb), and thallium (Tl) in polluted soils collected from LWC mining sites and the influence of adding ammonium thiosulfate in different chelant to soil ratio (0, 0.5 and 2 g kg^{-1}) on the efficacy of As, Sb and T1 phytoextraction by *Brassica juncea*. The soluble metal(loid)s in the soils after harvesting plants was studied to understand the potential of phytoextraction in managing the risk of metal(loid)s dispersion. Results show that the soils contained relatively high concentration of soluble As (3.6 ~ 5.5 mg kg^{-1}) and low concentration of soluble Sb (40 ~ 128 μg kg^{-1}) and T1 (6.2 ~ 10 μg kg^{-1}). The level of soluble As in the soils is toxic to the environment according to the Chinese environmental quality standard for irrigation water (GB 5084-92). The key results from phytoextraction experiment were that addition of thiosulfate enhanced As and Sb accumulation in *B. juncea* compared to the control, and the maximum As and Sb uptake in the roots and shoots occurred in the treatment of 2 g thiosulfate kg^{-1} of soil. However, application of thiosulfate inhibited plant uptake of Tl. The concentration of soluble As in 2 g kg^{-1} thiosulfate treatment was decreased significantly compared with the control and initial soils. Compared with the initial soils, soluble Sb was increased both in non-treated (control) and treated soils. However, no noticeable difference was observed between the soils of non-treated and treated plants. Both plant growth and thiosulfate amendment increased the soluble T1 in the soil, when compared to the initial soil. We conclude that thiosulfate-assisted phytoextraction might be used to reduce the environmental risk apparent for LWC soil through reducing the soluble As fractions, while managing the soluble fractions of Sb (compared with control soil). Meantime, further studies are required to manage the T1 risk in the soils at LWC mining site.

1. Introduction

Arsenic (As), antimony (Sb), and thallium (Tl) have no known essential biological functions and are acutely toxic at low concentrations (Finkelman et al., 1999). Humans are exposed to As, Sb, and Tl via inhalation and ingestion and may suffer from acute and chronic intoxication (Jaishankar et al., 2014). Both natural and anthropogenic activities can cause metal(loid)s pollution in the environment. Anthropogenic sources include metal mining and smelting and/or refining, industrial processes, coal combustion, and waste incineration (Alloway, 2013). Once metal(loid)s enter the soils, they will persist in soils and may be transformed into more toxic forms under varying redox conditions (Francis, 1990). Countries in East Asia, such as China, India, and Pakistan, experience serious As pollution because of the geological enrichment of this metalloid in these areas (Rodríguez-Lado et al., 2013; Shrivastava et al., 2015; Podgorski et al., 2017). It is estimated that global Sb reserves are 4–5 million metric tons, of which nearly 85% distributes in China (Miao et al., 2014). The Sb and T1 pollution usually occurs in metal(loid)s mining sites and shooting ranges (Johnson et al., 2005; Lis et al., 2003; Xiao et al., 2004) as a consequence of the inadequate management of wastes generated by mining and refining or retorting activities. The pollution of soil with metal(loid) s is a serious environmental problem because they may be

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transported to the terrestrial food webs, posing a severe environmental risk to animals and humans who consume the polluted food products (Gall et al., 2015).

Although various physical and chemical technologies have been developed to remediate metal(loid)s polluted soils (Wang et al., 2012a; Antonkiewicz and Para, 2016), a number of polluted lands remains derelict due to its remediation is unsustainable and uneconomic using conventional technologies. Such situation is worse for the areas (*e.g.* LWC mining area) where are economically marginal for hard redevelopment (Cundy et al., 2013). Gentle remediation opinions (GROs), which refers to use of plant, fungi, and bacteria-based methods, with/without amendments for reducing pollutant transfer to food chain, are applicable to soft-end use for a site, and have lower implement costs than conventional remediation methods (Cundy et al., 2016). Phytoextraction uses plants to accumulate the soluble fraction of metal(loid)s from soils, is regarded as a GROs due to its lower impact on soil functions and effective for risk management (Cundy et al., 2013, 2016). The ability of plant to accumulate metal(loid)s and metal(loid)s bioavailability in soils are dominant factors affecting the phytoextraction efficacy (Gonzaga et al., 2006). The high phytoextraction yield can be achieved by growing high-biomass plants in conjunction with optimal chelants addition (Wang et al., 2011; Antonkiewicz et al., 2016). *Brassica juncea* is a ideal candidate for phytoextraction due to its high-biomass, tolerance of metal(loid)s, adaption of a wide range of climatic conditions, and commercial available (Clemente et al., 2005).

There is an increasing demand for available, abundant, applicable, and economical chelants to use as solubilizing amendments for the promotion of metal(loid)s phytoextraction from contaminated soils. Several studies reported the use of EDTA, EDDS, and $S_2O_3^{2-}$ to improve the phytoextraction efficacy of lead (Pb), zinc (Zn), copper (Cu), and mercury (Hg) (Bagga and Peterson, 2001; Tassi et al., 2004; Meers et al., 2008; Barbafieri et al., 2000). Ammonium thiosulfate is a common liquid sulfur (S) and nitrogen (N)-containing fertilizer, and has the sulfur and nitrogen content of 26% and 12% respectively. This fertilize has been used in As phytoextraction due to its effectiveness in promoting As accumulation by plants, easily available, environmentally friendly, and cost-effective (Grifoni et al., 2015).

Arsenic usually occurs in the presence of Sb and T1 in environment, being them close chemical relatives. However, the effectiveness of chelants for the immobilization and/or solubilization of Sb and T1 in soils remains undocumented. Laowanchang (LWC) gold mine is located in the south of Qinlong County in Southwest China (Fig. 1). The ore is a Carlin-type gold deposit with the characteristic Au—As—Sb—Tl trace elements assemblage. At this mine, a large quantity of waste generated by gold refining activity is dumped into the surrounding environment without any treatment. The wastes contain high levels of metal(loid)s (Li et al., 2015). However, knowledge on the potential risk of the dispersion of the metal(loid)s in the soils, and remediation efforts for this polluted soil are relatively scarce.

Therefore, this study aimed to assess the risk of metal(loid)s dispersion in LWC soils by analyzing the soluble metal(loid)s concentrations, and to use ammonium thiosulfate as an chelant to assist *B.juncea* to remediate the polluted soils. Such knowledge is vital to assess the hazard of metal(loid)s to the environment and to evaluate the potential of phytoextraction to remediate the metal(loid)s polluted soils.

2. Materials and methods

2.1. Soil sampling

The surface soil samples (0—20 cm depth) were collected from a polluted site near an abandoned gold refinery in LWC gold mine (Fig. 1). About 100 kg of soils were randomly collected from an area of 100 m^2. Soil material was homogenized, air-dried, finely ground, and passed through a 4-mm nylon sieve before use.

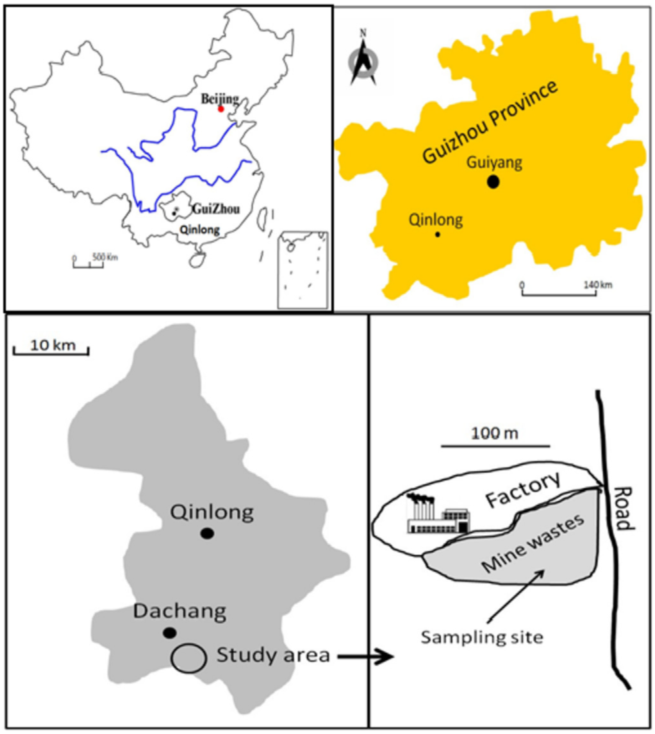

Fig. 1. Soil sampling site.

2.2. Soil incubation test

Aiming to study the mobility of As, Sb and T1 in the soils, a laboratory incubation experiment was conducted. Briefly, about 450 g of air-dried soils were filled into three plastic pots, which were moistened to field capacity (60%) with distilled water and kept for 7 days to reach equilibrium. The pot was maintained in the laboratory under room temperature for 61 days. About 4 g composite soil samples were taken from each pot at the day 1, 3, 7, 15, 30, 59 and 61 respectively after equilibrium. All the pots were weighted every two days, and the distilled water was supplied to each pot to reach a constant moisture content of 60%. During the incubation period, the room temperature was 22 ± 3 °C. After each sampling campaign, soils were immediately subjected to extraction and analysis.

2.3. Greenhouse pot experiment

The pot experiment was conducted in a greenhouse located at the Institute of Geochemistry, Chinese Academy of Sciences. Approximately 0.7 kg of air-dried soil and 0.3 g of urea and 0.4 g of KH_2PO_4 were placed in each plastic pot (1 L). Approximately 10 ~ 15 *B. Juncea* seeds were sown into each pot. After 2 weeks, plants were thinned, leaving three seedlings in each pot. The pots were divided into three groups (control, 0.5 g thiosulfate kg^{-1} of soil and 2 g thiosulfate kg^{-1} of soil), and each group contained three replicates. The purified water was added regularly to the pots to maintain a soil moisture content of 50 ~ 60%. All pots were arranged in a randomized block design with an average diurnal temperature of 25 ~ 30 °C and humidity of 40 ~ 60%. The plants were maintained for 85 days. At day 80, ammonium thiosulfate solution was added to the pots at a treatment rate of 0.5 g chelant kg^{-1} of soil or 2 g chelant kg^{-1} of soil, while purified water was added to the control pots (Wang et al., 2011). Five days after the addition of $(NH_4)_2S_2O_3$, all plants were harvested and divided into roots and shoots, which were washed thoroughly with purified water followed by deionized water and then dried in an electric heating air-blowing drier at 60 °C for 72 h. Thereafter, all plant matters were homogenized with an electrical grinder. Soil samples from each pot were collected after plant harvest, which were air-dried and homogenized, and a subsample of each pot was obtained following a quartering protocol. The subsample was ground into fine powder (200 mesh) using an agate mortar.

2.4. Water-soluble metal(loid)s extractions

Soil samples weighing approximately 2 g were placed in 50-mL centrifuge tubes (Labcon, Labcon Co.), and 20 mL of deionized water was added. For each sample, the mixture was shaken in a reciprocal shaker for 2 h; the filtrate was separated by passing it through a 0.45-μm microfiltration membrane and subsequently acidified with 1 mL of sub-boiling distilled nitric acid (HNO_3).

2.5. Sample analysis

The pH of the soils was measured using a pH meter at a ratio of 2.5:1 (w/w) de-ionized water to the soil suspension. Organic matter content was determined according to the potassium dichromate volumetric method (Lu, 2000). For total trace elements determination, soil samples weighing 0.05–0.1 g were placed in 15-mL Teflon tubes and digested in an electric blast drying oven at 160 °C for 15 h using a mixture of sub-boiling distilled HNO_3 and hydrofluoric acid (HF) (15:1, v/v). The digested solution was heated until the acid was reduced to a volume of 0.5–1 mL, which was reconstituted to 10 mL with 20% HNO_3. About 0.05–0.1 g of plant powders was weighed in 15-mL Teflon tubes and digested in an electric blast drying oven at 150 °C for 16 h with 3 mL of sub-boiling distilled HNO_3. After digestion, 1 mL of 30% H_2O_2 was added to the tube, and the mixture was heated in a heater at 80–90 °C until the solution was reduced to a volume of 0.5–1 mL, which was reconstituted to 15 mL with 20% HNO_3. Arsenic concentration was analyzed by atomic fluorescence spectrometry (AFS-920; Beijing Jitian Instrument Co.), while the Sb and Tl concentrations in solutions were analyzed using inductively coupled plasma–mass spectroscopy (ICP-MS; Element, Finnigan MAT Co.).

2.6. Quality control and quality assurance

The standard reference material orange foliage GBW10020 was used for the plant analytical quality control. The measured average total As, Sb, and Tl concentrations of the reference material were 1.2 ± 0.4, 0.21 ± 0.05, and 0.07 ± 0.02 mg kg^{-1} respectively ($n = 5$), which were comparable to the certified values of 1.1 ± 0.2, 0.2 ± 0.06, and 0.06 ± 0.01 mg kg^{-1}, respectively. The relative percentage difference between the sample replicates was <9%.

2.7. Data analysis

Difference in plant biomass, metal(loid)s concentrations in the plants, soluble metal(loid)s concentrations in the soils among the different treatments were examined by one-way ANOVA followed by LSD (Equal Variance Assumed) or Tamhane's T2 (Equal Variance not Assumed) test as available in the SPSS *17.0* statistical package (SPSS Inc, Chicago).

The bioaccumulation factor ($BAF_{soluble}$) and translocation factor (TF), which reveal the capacity of plant to accumulate soluble metal(loid)s from soils and transport metal(loid)s from roots to shoots, were calculated using the following formula according to Wang et al. (2011).

$$BAF_{soluble} = [Metal(loid)s]_{shoot} / [Soluble - Metal(loid)s]_{soil}$$

$$TF = [Metal(loid)s\,]_{shoot} / [Metal(loid)s\,]_{root}$$

3. Results and discussion

3.1. Physico-chemical properties of the soils

The general physicochemical properties of the soils are presented in Table 1. The soil was alkaline, contained low levels of organic matters, but high content of Fe_2O_3. Thus the alkalinity and high Fe_2O_3 content were expected to affect the metal(loid)s mobility in the soils. The Zn, Cu, nickel (Ni), and chromium (Cr) concentrations in the soils were lower than the corresponding maximum allowable values according to the soil quality standards of the Chinese government, while the concentration of As was several orders of magnitude higher than the standard (CNEPA, 1995; GB15618-1995). The As concentration (3122 mg kg^{-1}) recorded in LWC soil was overwhelmingly higher than that in a paddy field (range: 3.1 ~ 42.5 mg kg^{-1}) in Bangladesh, which is known for high As pollution (Meharg and Rahman, 2003). The total concentrations of Sb and Tl in LWC soil were comparable to the polluted soils collected from the Xikuangshan Sb mine and Lanmuchang Tl mine in China, respectively (He et al., 2012), and exceeded the threshold limit values of 37 and 1 mg kg^{-1} recommended by the Netherlands government and Environment Canada, respectively (Canadian Council of Ministers of the Environment, 1999; Leeuwen and Aldenberg, 2012). The high concentration of As, Sb, and Tl in the soils was due to the mixture of tailings enriched in As, Sb, and Tl (Li et al., 2015).

3.2. Mobility of metal(loid)s in the soils

The concentration of soluble As, Sb and T1 in the soils ranged from 3.6 to 5.5 mg kg^{-1}, 40–128 ng g^{-1}, and 6.2–10.0 ng g^{-1}, respectively during the incubation period (Fig. 2). The concentration of As greatly exceed the toxic level (0.1 mg L^{-1}) for irrigation water allowed by the Ministry of Environmental Protection of China (CNEPA, 1992; GB5084-92). The high pH (pH = 8.4) may explain the elevated soluble As concentration in the soils, because the positive surface charge of the soil particles was decreased under alkaline condition, enabling desorption of negatively charged and soluble arsenate species (Masscheleyn et al., 1991). The soluble concentrations of Sb and Tl were one order of magnitude lower than As. Although no Chinese standards for Sb and Tl in irrigation water was set, the soluble Sb and Tl concentration in the soils were within the recorded values observed at shooting ranges (soluble Sb: 19 ~ 349 ng mL^{-1}) and naturally Tl polluted soils (soluble T1: 8 ~ 870 ng g^{-1}), respectively in Switzerland (Okkenhaug et al., 2011; Voegelin et al., 2015). Overall, As can be considered as the major environmental concern with respect to the soils at the LWC mining site. The adequate remedial methods should be applied to minimize the dispersion risk and toxicity of As to the environment. The significant positive correlation between the soluble concentration of As and Sb or T1 in the soils indicates that As is accompanied with Sb and T1 during the solubilization processes (Fig. 3). Besides As, therefore, it is necessary to simultaneously monitor the mobility of Sb and T1 during the remedial process.

Table 1
Physicochemical properties of the LWC soils.

Properties	pH	OM %	Fe_2O_3%	Total metal(loid)s concentrations, mg kg^{-1}							
				As	Sb	T1	Zn	Cu	Ni	Mn	Cr
Qinglong Soil	8.38 (0.05)	0.24 (0.04)	10 (0.2)	3122 (320)	332 (47)	15 (1)	160 (15)	78 (14)	129 (20)	1125 (107)	232 (17)
Standard[a]				40	–	–	500	400	200	–	300

[a] Chinese Environmental quality standard for soils (level III); Data in bracket indicates the standard deviation of three replicates;- means there is no limitations set for these metal(loid)s; OM means organic matter.

3.3. Plant biomass

Generally, As can inhibit the growth of plants by both affecting biomass accumulation and compromising plant reproductive capacity. The level of soluble As in LWC soil was within the previously reported EC_{50} values of 1.5 ~ 20.3 mg kg^{-1} for plants (Romero-Freire et al., 2014). Thus we initially expected a significant reduction of plant biomass due to the toxicity of As to plants. However, the biomass (dry weight) of *B. juncea* roots and shoots from each pot ranged from 0.05 to 0.14 g and from 1.2 to 2.0 g (Fig. 4), respectively, which were similar with those growing in non-polluted soils (González et al., 2017). This indicates that *B. juncea* can tolerate high levels of As without significant reduction in plant biomass under our experimental conditions. Therefore, *B. juncea* is a potential candidate for phytoremediation at LWC mining site.

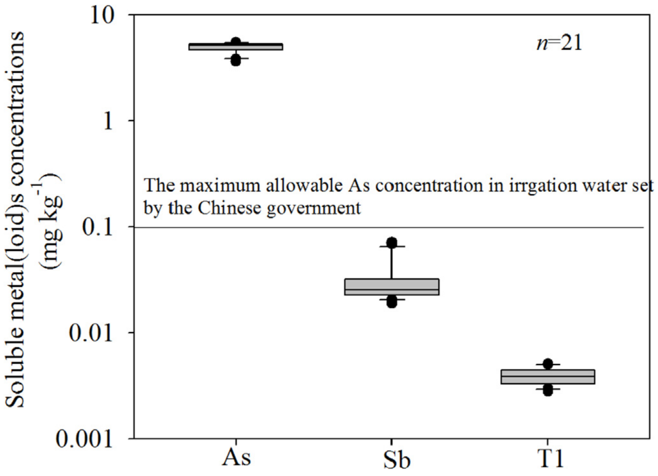

Fig. 2. The concentration of soluble As, Sb and T1 in the soils during the laboratory incubation ($n = 21$).

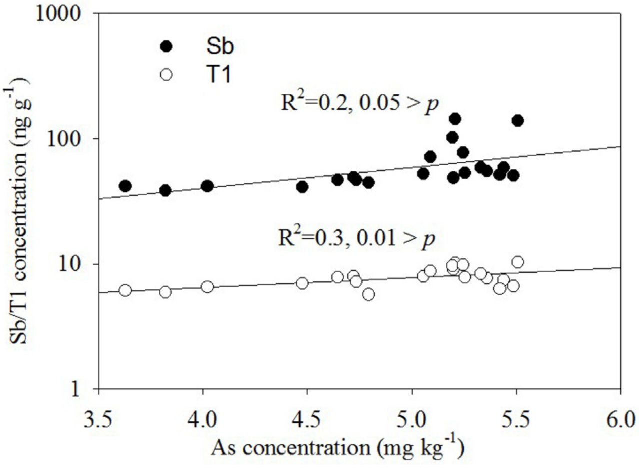

Fig. 3. The relationship between the soluble As and soluble Sb or T1 in LWC soils.

3.4. Metal(loid)s in the plants

The concentrations of As, Sb, and Tl in *B. juncea* are shown in Table 2. The average concentration of As and Sb in the roots and shoots of untreated *B. juncea* (control) were 96 and 16 mg kg^{-1}, 3.3 and 0.5 mg kg^{-1}, respectively. Application of thiosulfate to soils significantly increased both As and Sb concentration in the tissues of *B. juncea*, increased the $BAF_{soluble}$ 45- and 2.8-fold for As (2 g thiosulfate kg^{-1} of soil) and Sb (0.5 g thiosulfate kg^{-1} of soil) (Table 3), respectively. The result is comparable with previous studies which reported that thiosulfate could promote Hg and As accumulation in the roots and shoots of plants (Wang et al., 2011; Grifoni et al., 2015). Unlike As and Sb, thiosulfate decreased the Tl concentration in the roots of *B. juncea*, and the $BAF_{soluble}$. The difference in shoot Tl concentration between the control and thiosulfate treatment was non-significant ($p > 0.05$). There is little information on the interactions between thiosulfate and As, Sb, and Tl in plants. Prior studies reported that thiosulfate combined with Hg^{2+}/Ag^{+} to form $Hg(S_2O_3)^{2-}$/$AgS_2O_3^-$ complexes, which could be preferentially accumulated by the plants (Campbell et al., 2002; Wang et al., 2012b). It is less likely for As and Sb to combine with thiosulfate to form As-thiosulfate or Sb-thiosulfate complexes

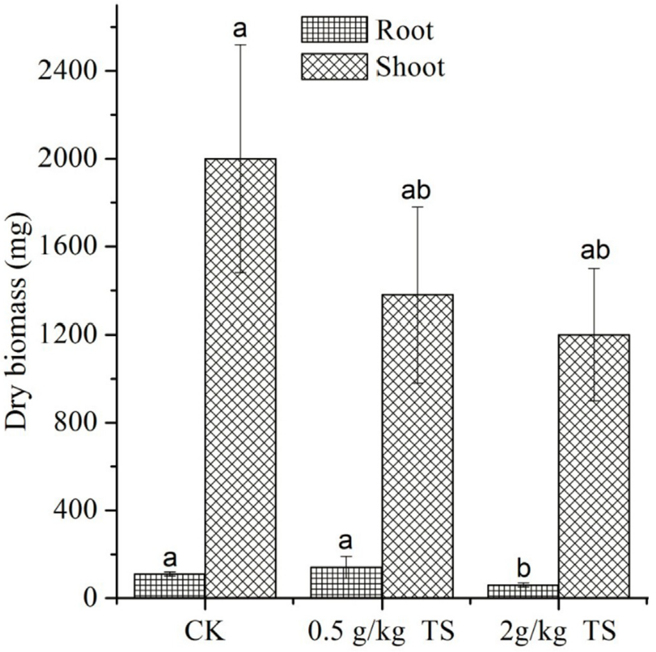

Fig. 4. The dry biomass of the Indian mustard, the error bars represent the standard deviation of three replicates. Significant differences between control and thiosulfate treatments are indicated by different small letters ($p < 0.05$); CK: Control; TS: thiosulfate.

Table 2
The concentrations of As, Sb and T1 in the roots and shoots of *B.juncea* both in the control and thiosulfate treatments.

Treatments	As (mg kg^{-1})		Sb (mg kg^{-1})		T1 (mg kg^{-1})	
	Root	Shoot	Root	Shoot	Root	Shoot
CK	96 ± 14 a	16 ± 0.6 a	3.3 ± 0.5 a	0.5 ± 0.05 a	27.2 ± 10.5 a	8.3 ± 2.5 a
0.5 g/kg TS	190 ± 21 b	24 ± 2.0 a	5.3 ± 0.7 b	1.4 ± 0.4 b	16.5 ± 3.8 b	8.8 ± 0.4 a
2 g/kg TS	231 ± 66 b	74 ± 11 b	7.5 ± 0.6 c	1.4 ± 0.2 b	3.2 ± 0.7 c	9.0 ± 0.3 a

Significant differences between control and thiosulfate treatments are indicated by different small letters ($p < 0.05$); CK: Control; TS: thiosulfate.

Table 3
$\text{BAF}_{\text{soluble}}$ and TF of *B.Juncea* for As, Sb and T1(mean ± sd, $n = 3$).

Treatments	As		Sb		T1	
	$\text{BAF}_{\text{soluble}}$	TF	$\text{BAF}_{\text{soluble}}$	TF	$\text{BAF}_{\text{soluble}}$	TF
CK	5.1 ± 0.7	0.17 ± 0.02	4 ± 0.5	0.16 ± 0.03	1606 ± 568	0.36 ± 0.22
0.5 g/kg TS	8.2 ± 1.0	0.12 ± 0.02	11.6 ± 3.8	0.28 ± 0.10	950 ± 198	0.55 ± 0.11
2 g/kg TS	229 ± 85	0.33 ± 0.09	11.3 ± 1.2	0.18 ± 0.02	374 ± 88	2.99 ± 0.79

$\text{BAF}_{\text{soluble}}$ = bioaccumulation factor; TF = transfer factor; CK: Control; TS: thiosulfate.

under the recorded pH in LWC soils (pH = 8.4) because the reactions are thermodynamically unfavorable. We hypothesize that the mechanism by which thiosulfate-promoted As and Sb accumulation in *B.juncea* may be related to the damage of the plasma membrane of the roots. The significant decrease of the concentration of T1 in the thiosulfate treated roots is an indication of the Tl leakage. In *B. juncea*, Tl is presented mainly as Tl$^+$ (Krasnodebska-Ostrega et al., 2008) which could be taken up by roots via cation transporters (*e.g.*, Na$^+$/K$^+$ pumps) due to the similar radii of K$^+$ and Tl$^+$ (Siegel and Siegel, 1975). Cation transporters require an electrochemical gradient of protons inside the plasma membrane to drive cation transportation. External environmental stress (*e.g.* chemicals, drought, *etc.*) can damage the plasma membrane, causing a loss of the electrochemical gradient and subsequent leakage of electrolytes and metal(loid)s ions (Luo et al., 2006). We propose that addition of thiosulfate to the soils might damage the plasma membrane, resulting in the leakage of Tl ions. A significant negative correlation ($p < 0.05$) between T1 and As/Sb in roots demonstrates that T1 leakage is accompanied with As and Sb accumulations in the roots (Table 4). Therefore, plasma membrane damage is also proposed to be a main pathway of plant uptake of metal(loid)s assisted by thiosulfate because soluble metal(loid)s ions (As and Sb) may enter the root via the damaged plasma membrane. The previously recorded close linkage between the cell electrolytic leakage of roots and the concentration of metal in the tissues of plant provides the direct evidence supporting our hypothesis (Luo et al., 2006).

Thiosulfate promoted the translocation of metal(loid)s from the roots to the shoots, as demonstrated by both the higher TF values and the significant positive correlation between the roots and shoots metal(loid)s concentrations ($p < 0.05$) (Tables 3 and 4). The possible reason accounting for this promoted translocation is that chelant may induce the change of the primary route of metal(loid)s uptake in plants from the symplastic to the apoplastic pathway, which is more efficient in transporting metal(loid)s to aboveground tissues (Nowack et al., 2006). Furthermore, the addition of thiosulfate to soils may enhance the plant tolerance to metal(loid)s. Pickering et al. (2000) used X-ray absorption near edge structure (XANES) spectroscopy to study the speciation of As in *B. juncea*, and found that As was mainly associated with thiolate complexes (*e.g.* glutathione) in the roots. Similarly, Ji et al. (2017) used XANES spectroscopy to study the speciation of As in *Lolium perenne* L, and found that over 60% of Sb in the tissues of plant was complexed by thiol complexes (*e.g.* glutathione and phytochelatins). It is probable that complexation of As/Sb with thiolate complexes is the main mechanism by which *B. juncea* detoxifies and sequesters the metal(loid)s. Application of thiosulfate to soils enhanced the sulfur accumulation in the plants (Wang et al., 2012b), which might in turn mediate the synthesis of more organic thiolate compounds (*e.g.* glutathione), strengthening the plant tolerance to metal(loid)s. Thus, plants treated with thiosulfate are expected to accumulate more As and Sb (Grifoni et al., 2015).

Table 4
Pearson's correlation matrix, giving the linear correlation coefficients (r) between the metal(loid)s concentration (As, Sb and T1) in the roots and shoots of *B.juncea* in the control and thiosulfate treatments ($n = 9$).

	Shoot Sb	Shoot T1	Root As	Root Sb	Root T1
Shoot As	ns	ns	0.72^{*}	0.91^{**}	-0.82^{**}
Shoot Sb		ns	0.84^{**}	0.69^{*}	ns
Shoot T1			ns	ns	ns
Root As				0.80^{**}	-0.77^{*}
Root Sb					-0.93^{**}

Significant differences are indicated by asterisks (*$p < 0.05$; **$p < 0.01$); ns means not significant.

The mass of As, Sb and T1 in the tissues of *B.juncea* is displayed in Fig. 5. The roots and shoots of *B. juncea* treated with 0.5 g kg^{-1} and 2 g kg^{-1} thiosulfate accumulated higher amounts of As, with average mass of 26 μg and 81 μg, respectively. The average mass of Sb in the roots of plant treated with 0.5 g kg^{-1} thiosulfate was significantly higher than that in the control. In contrast to As and Sb, application of thiosulfate decreased the mass of Tl in the roots and shoots of *B. juncea*.

3.5. Soluble metal(loid)s in the soils

The mobility of As, Sb and Tl in the soils was evaluated by analyzing their water-extractable fractions after plants harvest. The results are shown in Fig. 6. The concentration of soluble As in the treated soils (2 g thiosulfate kg^{-1} of soil) was approximately 0.4 mg kg^{-1}, which was significantly lower than in the control and initial soils (approximately 3.8 mg kg^{-1}). This result is different from a previous study which reported that adding thiosulfate increased the soluble As fraction in soil, and attributed this increase to competitive binding between sulfate/thiosulfate and arsenate to soil particles, by which arsenate was released into soil solutions (Grifoni et al., 2015). Assuming that As in the plants was mainly accumulated from the pool of soluble As in the soils, the mass of As in the treated *B.juncea* should account for the decreased mass of

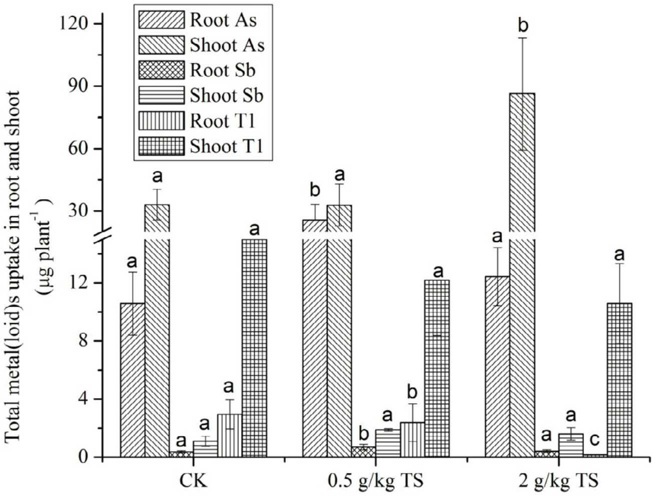

Fig. 5. The mass of As, Sb and T1 in the tissues of *B.juncea*. Significant differences between control and thiosulfate treatments are indicated by different small letters ($p < 0.05$); CK: Control; TS: thiosulfate.

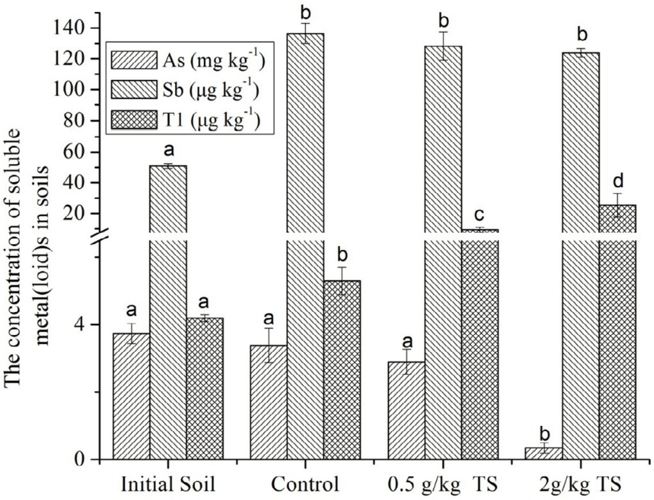

Fig. 6. The soluble concentration of As, Sb and T1 in the initial, control, thiosulfate treated soils after harvest of plant. Significant differences among initial, control and thiosulfate treated soils are indicated by different small letters ($p < 0.05$); TS: thiosulfate.

soluble As in the soils. In fact, the mass of As in the plants only accounted for less than 10% of the missed soluble As in the soils. The leaching of As from pot was expected to be minor due to the careful management of watering. We propose that an immobilization process of As may have occurred in the thiosulfate treated soils. The interaction between sulfur and arsenic in soil is complex (Langner et al., 2011) and the dynamic transformation of thiosulfate in soil may affect the mobility of As. After adding thiosulfate to soil, majority of which quickly reacts to form tetrathionate, which can be transformed to hydrogen sulfide (H_2S) by iron-oxidizing bacteria (Ng et al., 2000). The reaction of H_2S with ferric (oxyhydr) oxides can produce pyrite (FeS_2), which is an adsorbent for arsenate (Lohmayer, 2015). In addition, the bacteria in the soil have been reported to be able to simultaneously use arsenate and thiosulfate as electron acceptors and subsequently produce As_2S_3 precipitate (Drewniak et al., 2015). This bacteria-mediated As immobilization can occur within a short period of time. Further studies are needed to explore the interaction between As and thiosulfate in the soils.

The dramatically higher soluble Tl concentration in the control soils (planted) relative to the initial soils suggests that plant growth promoted the solubilization of Tl in the soils. It has been reported that organic acids exuded by the root of white mustard (*Sinapis alba* L) could solubilize Tl in the rhizosphere (Vaněk et al., 2010a). The main mechanism of this solubilization has been interpreted as the complexation of organic ligands with major cations originating from various soil phases (*e.g.*, carbonates and oxides), resulting in the desorption of adsorbed Tl ions from soil particles (Vaněk et al., 2010a, 2010b). Thiosulfate enhanced soluble Tl concentration in the soils. This solubilization is likely related to the dissolution of Fe/Mn oxides by thiosulfate (Yang et al., 2005; Wang et al., 2011), leading to the release of the associated T1 from the Fe/Mn oxides.

The soluble Sb concentration in the control and treated soils was significantly higher than in the initial soils. Root activities might be again responsible for Sb mobilization in the control soils (Hockmann, 2014). The concentration of soluble Sb in the thiosulfate-treated pots was similar to that in the control pots. This result indicates that the effect of thiosulfate treatments on the soluble fractions of Sb in the soils is of the same order of magnitude as the effect of the plants on this fraction. Application of thiosulfate to soil did not significantly increase the soluble fractions of Sb in the soils. This unexpected result may be attributable to the dominant fraction of Sb in LWC soil (>96%) being in a mineral form (Sb_2S_3), and where the Fe/Mn oxide-associated Sb fraction only represented a minor fraction of the total Sb (Chen et al., 2014). Moreover, even though a minor fraction of Sb in the soil was solubilized by thiosulfate, they would be efficiently accumulated by the plants, as demonstrated by the higher Sb concentration in the treated plants relative to control.

3.6. Implications for managing the risk of metal(loid)s at LWC mining site

Thiosulfate notably promoted As and Sb accumulations in the tissues of *B.juncea*, and can be used in chemically-assisted phytoextraction of As and Sb. The soluble concentration of As in the soils treated by 2 g kg^{-1} thiosulfate was decreased down to 0.4 mg L^{-1}, which is close to the maximum allowable As concentration (0.1 mg L^{-1}) in irrigation water set by the Chinese government. This reduction may be attributable to the plant uptake and thiosulfate-induced As immobilization in the soils (Ng et al., 2000; Lohmayer, 2015). The repeated application of thiosulfate-assisted phytoextraction is recommended to further decrease the soluble As concentration at LWC mining site. The capital cost for implementing this phytoextraction is acceptable to public due to the plants and chelants are commercial available, and commonly used in agronomy activities.

The growth of plants in the LWC soil increased the concentrations of both Sb and T1 in the soluble fractions. This point should be considered in the re-vegetation of LWC mine wastes. The process of growing plants may increase the potential for Sb and T1 risk. However, adding thiosulfate enhanced the Sb accumulation in the plants, while not-affecting the relative amount of Sb in the soluble fraction, when compared to the control plants. Therefore, it is possible to use thiosulfate-assisted phytoextraction to reduce the soluble As pool, while managing the level of soluble Sb in the soils. Importantly, care should be taken regarding Tl pollution when using thiosulfate, because this chelant enhanced T1 mobilization. Without thiosulfate treatment, *B.juncea* (control) showed a high capacity of T1 accumulation, with $BAF_{soluble}$ of 1606. The soluble T1 in soil may be removed by growing *B.juncea*. Crop rotation has been introduced in phytoremediation sectors with the aim of removing multiple pollutants and producing environmental or economic benefits (Yang et al., 2017). It may be necessary to combine thiosulfate-assisted phytoextraction using *B.juncea* by crop rotation to remediate As, Sb, and Tl pollution at LWC mining site.

4. Conclusions

This was the first study to examine ammonium thiosulfate-assisted phytoextraction of As, Sb, and Tl polluted soils. Thiosulfate enhanced As and Sb, but inhibited T1 accumulation in *B. juncea*. Application of 2 g chelant kg^{-1} of soil achieved the highest concentration of As in the roots and shoots of *B. juncea*, as well as the highest As mass in the shoots. Addition of thiosulfate decreased the soluble As fraction in the soils, and this reduction may be related to both the plant uptake and thiosulfate-induced As immobilization. The Sb and T1 were solubilized by plant growth; thiosulfate has little effect on Sb mobilization, but enhances T1 mobilization. In summary, ammonium thiosulfate-assisted phytoextraction can be used to remove soluble As, while manage the pool of soluble Sb in soil. However, care should be taken regarding Tl mobilization when using thiosulfate.

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