Journal of Hazardous Materials 186 (2011) 119–127

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Ammonium thiosulphate enhanced phytoextraction from mercury contaminated soil – Results from a greenhouse study

Jianxu Wang[a,b], Xinbin Feng[a,*], Christopher W.N. Anderson[c], Guangle Qiu[a], Li Ping[a], Zhengduo Bao[a,b]

[a] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, China
[b] Graduate University of Chinese Academy of Sciences, Beijing 100049, China
[c] Soil and Earth Sciences, Institute of Natural Resources, Massey University, Palmerston North 4442, New Zealand

ARTICLE INFO

Article history:
Received 9 July 2010
Received in revised form 1 October 2010
Accepted 23 October 2010
Available online 3 November 2010

Key words:
Ammonium thiosulphate
Mercury contaminated soil
Phytoextraction
Fractionation
Environmental risk

ABSTRACT

According to the 'hard and soft' acid-base principle, mercury is a 'soft metal' and will preferentially form soluble chemical complexes with sulphur-containing ligands. In this work mercury uptake by *Chenopodium glaucum* L. growing on mercury-contaminated soil was promoted using ammonium thiosulphate. The relative geochemical fractionation of mercury in the soil was subsequently investigated as a function of plant growth with and without thiosulphate amendment. The results indicate that the solubility of mercury is significantly increased through the application of thiosulphate to the soil. Substantially higher mercury levels were found in *C. glaucum* L. treated with 2 g kg^{-1} thiosulphate of soil when compared to the non-treated plants. Compared with initial soil, soluble and exchangeable fractions were increased both in planted and planted treated plants. However, no significant difference was observed between the soils of the planted and planted treated plants. The oxide-bound mercury concentration was significantly decreased for the planted soil (treated and non-treated) at the end of the experiment. Moreover, this fraction was highly correlated with the plant tissue mercury concentration. Taken together, thiosulphate assisted phytoextraction could be used to reduce environmental risk apparent for mercury-contaminated soil through reducing the oxide bound fractions, while managing the bioavailable fractions (compared with no treated plant).

1. Introduction

Mercury (Hg) pollution demands attention because of the toxicity, mobility and long residence period of the metal in the environment, and its ability to be transformed to methyl mercury (MeHg) in soil, a bioaccumulative compound that can readily cross the blood–brain barrier.

As a consequence of contamination from mercury mining and smelting activities, as well as excessive pesticide application and wastewater irrigation in agriculture, mercury pollution of soils is becoming a serious problem worldwide [1].

Many efforts have been undertaken to find methods of removing mercury from soil, such as stabilization/solidification and pyrolysis [2–4]. However, the application of these methods has been limited due to the associated damage to the soil matrix that can result, and to the expense of the use of these technologies. In contrast, the use of plant species to remove pollutants from soils, generally defined as phytoextraction, offers the great advantages of being inexpensive and beneficial to the soil matrix [5]. The main factor that influences the efficiency of phytoextraction is the ability of plants to extract pollutants from soil. For example, the fern *Pteris vittata* L. has been found to be more efficient in its ability to remove arsenic from soil than other species due to its strong ability to accumulate arsenic into its aboveground tissues [6]. This kind of plant is regarded as a hyperaccumulator plant.

No plant species have been identified as mercury hyperaccumulators. This is because all plants naturally accumulate low concentrations of mercury into their above-ground biomass. For example, the mercury concentration in the leaves of alfalfa has been shown to be less than 2.3 mg kg^{-1}, growing on soil with a mercury concentration as high as 2.1–97 mg kg^{-1} [7]. However, the addition of various chemical amendments to soil can increase the bioavailablity of mercury in the soil solution and enhance the uptake of mercury by plants (chemically-enhanced phytoextraction). For example, the addition of potassium iodide (KI) to mercury contaminated soil has been shown to increase the mercury concentration in the roots, branches and leaves of willows [8]. According to the 'hard and soft' acid-base principle, mercury is a 'soft metal' and will preferentially form chemical complexes with sulphur-containing ligands including thiosulphate [9]. Therefore, attention has focused on the use of sulphur-containing ligands to promote mercury phytoextraction. Ammonium thiosulphate, for example,

* Corresponding author. Tel.: +86 851 5891356; fax: +86 851 5891609.
E-mail address: fengxinbin@vip.skleg.cn (X. Feng).

0304-3894/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2010.10.097

Table 1
Sequential extraction procedure to separate mercury into different geochemical fractions.

Fractions	Extracting agents [14]	Extracting agents [18]	Extracting agents [19]
Water soluble	–	H_2O	H_2O
Exchangeable	0.11 mol L^{-1} CH_3COOH	0.5 M NH_4Ac–EDTA	1 mol L^{-1} CH_3COONH_4
Fe–Mn oxide-bound/humic bound	0.5 mol L^{-1} $NH_2OH{\cdot}HCL$	–	1 mol L^{-1} NH_4OH
Organic bound	8.8 mol L^{-1} H_2O_2 and 1 mol L^{-1} CH_3COONH_4	0.2 M NaOH and CH_3COOH 4% (v/v)	0.02 mol L^{-1} HNO_3 8.8 mol L^{-1} H_2O_2 (pH = 1.5, HNO_3) 1 mol L^{-1} CH_3COONH_4 (pH = 2, HNO_3)
Residual	Total Hg of substrate minus concentration in other fractions	HNO_3:H_2SO_4:$HClO_4$ 1:5:1 (v:v:v)	Aqua regia/HF

has been shown to significantly increase the soluble mercury concentration in tailings, and to subsequently enhance the uptake of mercury by the species *Brassica juncea Czern.* [10]. A comparison of published studies indicates that KI is less suitable for phytoextraction than ammonium thiosulphate due to its low transfer efficiency of mercury from root to shoot [8].

Soil total mercury concentrations can help us to understand the degree of mercury pollution in a particular soil, but provide limited information on the environmental risk of mercury in that soil. Knowledge of the fractionation or speciation of mercury can help us to understand the potential risk of mercury in soil. Various methods have been applied to investigate the trace element fractions in the solid phase and solution phase of soil, and these methods include X-ray absorption fine spectroscopy (XAFS), nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR), pyrolysis, as well as sequential extraction procedures (SEP) [11–17]. Among these methods, SEP is routinely used to separate trace elements into one of several operationally defined fractions according to different extractions that are sequentially used on a sample [18,19] (Table 1). However, few studies have used SEP to investigate the chemistry of mercury in soil. Studies that have been reported are not generally comparable due to differences in the chemicals used to extract mercury, and variably defined fractions of the metal in soil [20].

Numerous studies have reported that chemicals (chelates, chelants or ligands) can enhance the plant uptake of heavy metals from contaminated soil, but these studies seldom consider the effect of the chemicals on the fractionation of the heavy metals in soil, especially the potential transformation of insoluble fractions of metal to more-soluble fractions. In this study, a novel SEP which was modified from Jeyakumar [21], was defined and applied to study the change of mercury fractions that may be promoted through the application of chemically-enhanced phytoextraction.

Chenopodium glaucum L. (Oakleaved Goosefoot) is an annual herbaceous flowering plant, which occurs in almost all parts of the world but has rarely been used for phytoextraction. It is readily spread via the transport of seed by wind. From a phytoextraction perspective, it may be a good candidate as a remedial species due to its high survival rate, rapid growth, large biomass and resistance to adverse environmental conditions.

The objectives of this study were as follows: (1) to test the extent to which thiosulphate was able to solubilise mercury from soil collected from a Hg mining area; (2) to examine whether thiosulphate enhanced the phytoextraction of mercury by *Chenopodium glaucum* L.; and (3) to investigate how application of thiosulphate to soil can transform the relative partitioning of mercury in soil to specific geochemical fractions.

2. Materials and methods

2.1. Study area

Mercury contaminated soil was collected from the Wanshan (WS) mercury mining area, located adjacent to the city of Wanshan in eastern Guizhou province in China (Fig. 1). Mercury mining in some form has been conducted in this area since 221 BC [22]. Regulated and intensive mining finished in the area in 2001, leaving behind significant amounts of mercury contaminated mine-waste. The mercury contamination in the Wanshan district is well characterized [23–25]. Total mercury (THg) concentrations in the soil around Wanshan can reach as high as 790 mg kg^{-1} [24]. Permissible levels of THg in soil as regulated by the Chinese Ministry for the Environment are 1.5 mg kg^{-1} [26]. Background soil was collected from Qianxi County, in western Guizhou province, from an agricultural area that has not been subject to industrial pollution.

2.2. Experimental design

Research into the ability of thiosulphate to promote mercury solubility in soil was conducted in parallel through the use of a laboratory study and a greenhouse study.

2.2.1. The solubility of mercury in ammonium thiosulphate – lab study

One gram of soil was weighed into 50 ml polypropylene centrifuge tubes. Three levels of ammonium thiosulphate extractant solutions (0.05, 0.1 and 0.2 μM) were added to triplicate tubes for the Wanshan soils, while deionized water was used for the controls. The tubes were rotated in a shaker over night at 120 rpm per minute and the supernatant was separated after passing through a 0.45 μm microfilter.

2.2.2. Induced plant accumulation – greenhouse study

A pot culture experiment was conducted in the greenhouse during the summer of 2009. The soil was air-dried, and sieved (4 mm mesh) before use. A sub sample of homogenized soil (2.5 kg) was transferred into each of 12 plastic pots. The same mass of background soil was placed into a further three plastic pots. The pots with the Wanshan soil were assigned the following treatments, with three replicates per treatment: planted; planted + treated; non planted; non planted + treated. Seedlings of *C. glaucum* L. with four young leaves were sampled from a suburb of Guiyang city (background THg in these plants <10 ng g^{-1}) and three seedlings were planted into six pots of Wanshan soil and three pots of background soil. All pots were arranged in a randomized block design with an average diurnal temperature of 25–30 °C and humidity of 40–60%. Tap water was provided to the pots everyday to maintain a moisture level just below field water capacity to avoid the release of leachate from the pots. After two weeks, plants were thinned to leave one seedling in each pot. The plants were maintained for 60 days. At day 55, $(NH_4)_2S_2O_3$ solution was added to the pot at a treatment rate of 2 g of thiosulphate per kg of soil to six pots (planted + treated; non planted + treated) of Wanshan soil, while deionized water was added instead of $(NH_4)_2S_2O_3$ solution to the other Wanshan soil pots (planted; non planted). All treated pots received a treatment volume of 200 mL of solution. Five days after the addition of $(NH_4)_2S_2O_3$, all plants were harvested and divided into leaves, stems and roots, which were washed thoroughly with tap water followed by deionized water and then air dried. The

Fig. 1. Location of the Wanshan mercury mining area and soil sample site.

weight of plant tissue (dry weight) was recorded. Soil samples were also collected at the time of harvesting. All soil samples were air dried, ground in a ceramic disc mill and sieved to 150 mesh. The pots with background soil were not treated with thiosulphate, but were used to investigate whether the foliage absorbed Hg from air.

2.3. Sample analysis

The following soil sample properties were measured. The pH of the soil was measured with de-ionized water 1:2.5 (w/w) using a pH meter. Soil texture was determined using a Malvern Mastersizer 2000 (Malvern Ltd., UK) and organic matter (OM) was determined according to the potassium dichromate volumetric method [27]. Elemental mercury (Hg^0) in the soil samples was evaluated according to the method of García-Sánchez et al. [28]. Subsamples of soil (1 g) were placed in porcelain crucibles and heated at 180 °C for 24 h in an oven. Subsequently, these heated samples were analysed for their remaining mercury content. Hg^0 concentrations were derived from the difference between the analysed total mercury contents before and after the heating process. For THg analysis, soil samples were digested in a water bath (95 °C) using a fresh mixture of concentrated HCl and HNO_3 (3:1, v/v). Plant samples were digested with concentrated HNO_3. THg for soil samples collected from the planted and non-planted pots was measured by cold vapor atomic absorption spectrometry (CVAAS) using a F732-S spectrophotometer (Huaguang, China), while THg for plant samples and background soils was determined by the dual-stage gold amalgamation method and cold vapor atomic fluorescence spectrometry (CVAFS) using a Tekran 2500 (Tekran Ltd., Canada) [29].

2.4. Sequential extraction procedure

A new sequential extraction procedure based on the works of Jeyakumar et al. [21] (Fig. 2), allowing determination of five fractions, was proposed and developed for this study.

One gram of a soil sample was treated with four subsequent extractions using 1 M $Mg(NO_3)_2$, 1 M NaOAc (adjusted to pH 5 with HOAc), 0.4 M $NH_2OH.HCl$ (dissolved in 25% HOAc), and 30% H_2O_2 (adjusted to pH 2 with HNO_3), followed by oxidative digestion with *aqua regia* (Fig. 2). After each sequential extraction, the extracts were centrifuged at 3500 rpm per minute and the supernatant was separated after passing through a 0.45 μm microfilter. The residue was then washed with two volumes of deionized water (8 mL each volume) before the next extraction. Evaluation of the ability of this sequential extraction procedure to account for all soil mercury was carried out through correlation of the mercury concentration in the experimental soils as determined by single digestion, against that calculated as the sum of all fractions.

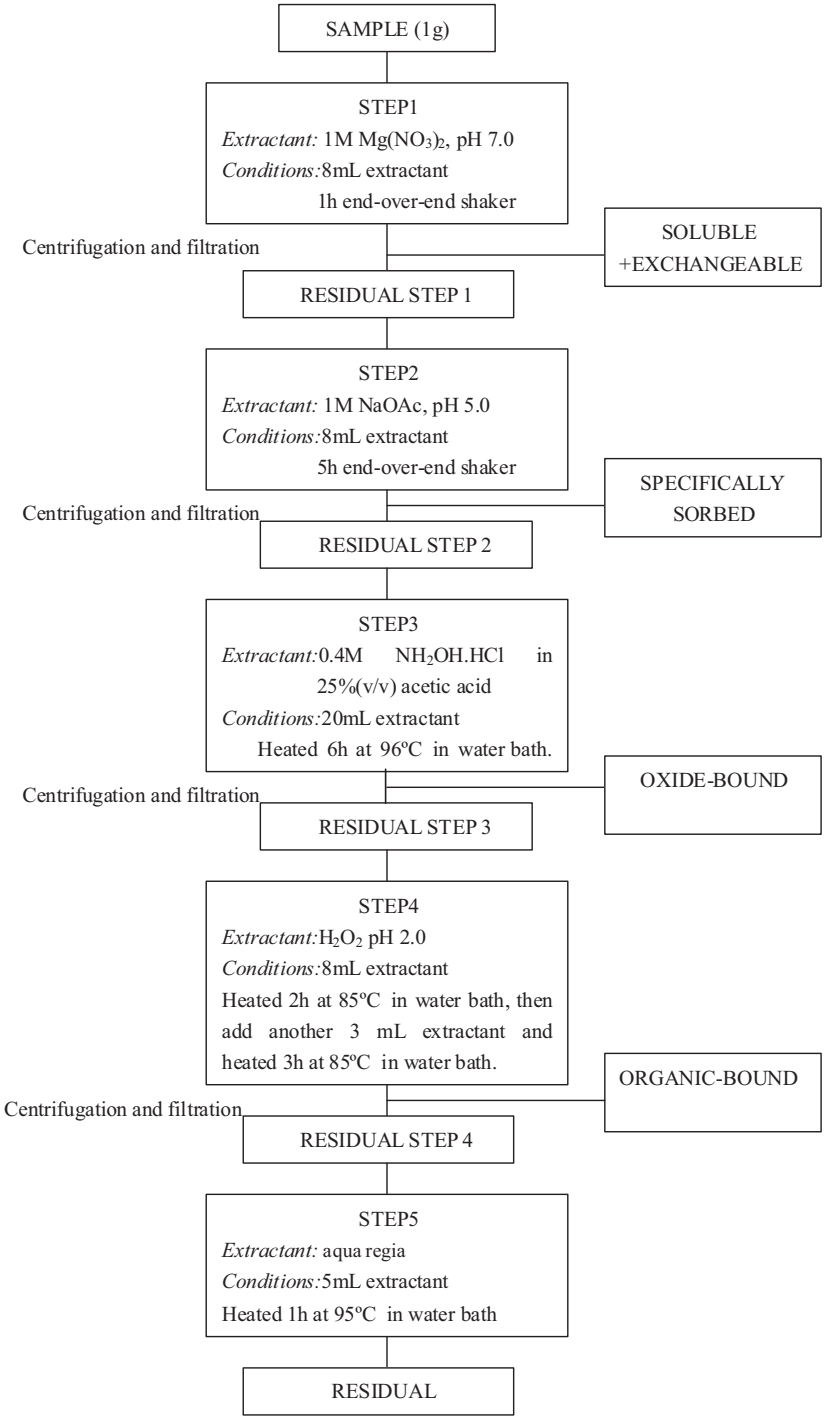

Fig. 2. The sequential extraction procedure used to fractionatate mercury forms in the Wanshan soil.

2.5. Quality control and quality assurance

The standard reference materials GSS-5 (GBW07405), GBW(E)070009 and GSV-3 (manufactured by the Institute of Geophysical and Geochemical Exploration, China) were used for soil and plant analytical QC, respectively. The THg recovery for soil standard reference materials was in the range of 92–101%, and the relative percentage difference of sample duplicates was <6%. The THg recovery for plant standard reference materials was in the range of 82–92%, and the relative percentage difference of sample duplicates was <9%.

2.6. Data analysis

Statistical analysis was carried out with *SPSS 17.0* for windows. *Sigmaplot 10.0* was used to fit a curve to modeled data.

3. Results and discussion

3.1. Physico-chemical properties of soil

The physico-chemical properties of the studied soil are summarized in Table 2. Soils collected from the Wanshan mining district

Table 2
Physico-chemical properties of the Wanshan soil (mean ± sd, $n = 3$).

Soil parameters		
pH (1:2.5)		6.88 ± 0.04
EC ($\mu s\,cm^{-1}$)		304.0 ± 0.02
OM ($g\,kg^{-1}$)		49.4 ± 5.8
Soil density ($g\,cm^{-3}$)		1.17 ± 0.01
Particle size distribution	Sand % >0.05 mm	15.36 ± 0.81
	Silt % 0.002–0.05 mm	55.96 ± 0.14
	Clay % <0.002 mm	28.67 ± 0.51
Total mercury ($mg\,kg^{-1}$)		151.13 ± 5.30
Element mercury ($mg\,kg^{-1}$)		27 ± 11

were classified as pH neutral (ranging from 6.88 to 7.04). The elemental form of mercury constituted 18% of the total amount of mercury in the soil. This figure is high relative to that reported for soils at an abandoned cinnabar mining area located in the South-West of Spain (1–8% [28]), but low relative to figures reported for the Idrija mercury mine region in Eastern Europe (30–60% of the total Hg in the vicinity of mining and smelting works [30]). The THg concentrations in the soil ranged from 144 to 153 $mg\,kg^{-1}$, which is nearly 100 times higher than the maximum upper limit for mercury content (1.5 $mg\,kg^{-1}$) in agriculture soils in China [26] and the soil can therefore be regarded as heavily polluted. The THg concentrations in background soil ranged from 0.12 to 0.14 $mg\,kg^{-1}$.

3.2. Solubility of mercury by $(NH_4)_2S_2O_3$

The extractable concentration of mercury as a function of the concentration of ammonium thiosulphate is shown in Fig. 3. The extractable concentration of mercury increased with the concentration of the extractant. Mercury solubility was significant increased by 300% in 0.2 μM thiosulphate relative to deionized water. The soluble concentration of mercury for lower concentrations of extractant was not significantly different from the control.

3.3. Induced plant accumulation

The THg concentration in the roots, stems and leaves of *C. glaucum* L. after thiosulphate treatment is summarized in Fig. 4. The THg concentration was increased 1100%, 600% and 200% in roots, stems and leaves of *C. glaucum* L. for the thiosulphate-treated pots, respectively, relative to the non-treated control. The results are similar to previous studies. Moreno et al. [10] reported that the mercury concentration in the roots and leaves of thiosulphate treated *B. juncea* was nearly 500% and 4000% higher than the control. Plant roots may be able to select the Hg–S_2O_3 complex and transport this to shoots in preference to other mercury complexes in soil [31]. Nowack et al. [32] reported that the addition of chelants to the soil could change the primary route of plant metal-uptake from the symplastic to the apoplastic pathway and subsequently increase the transport of metals from root to shoot. Thio-groups, small PCs (phytochelatins) and certain DNA families are believed to play an important role in the accumulation of mercury in plant tissues [33–35].

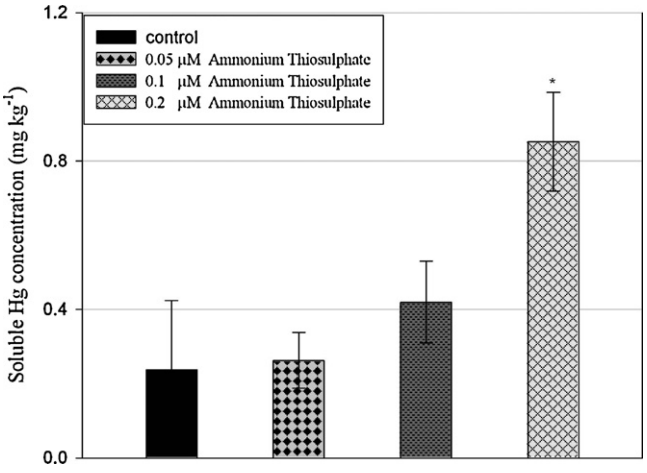

Fig. 3. The concentration of mercury chemically solubilized by ammonium thiosulphate. Bars denote standard deviation from means of three replicates; significant differences among different molar ammonium thiosulphate solution are indicated by asterisks ($p < 0.05$).

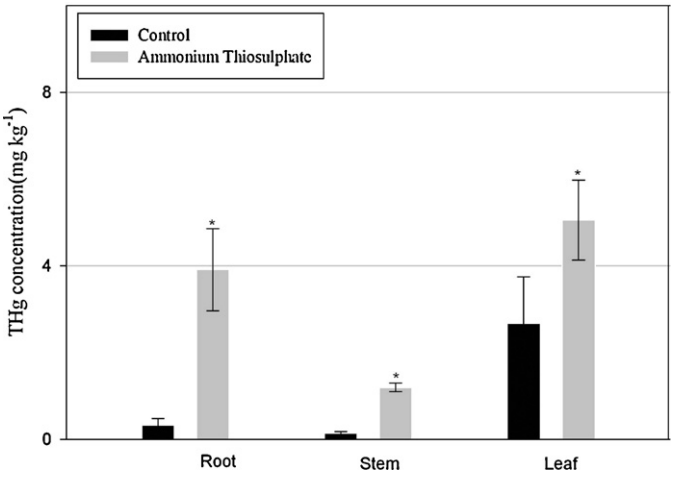

Fig. 4. The THg concentration in root, stem and leaf in control and ammonium thiosulphate treated pots (dry weight). Bars denote standard deviation from means of three replicates; significant differences among control and ammonium thiosulphate are indicated by asterisks ($p < 0.05$).

The mercury concentration in the leaves of *C. glaucum* L. grown in background soil was 0.15 ± 0.05 $mg\,kg^{-1}$, which was significantly lower than both the control and $(NH_4)_2S_2O_3$ treated pots for the Wanshan soil ($p < 0.05$). This indicates that foliar absorption of mercury from the air was not a significant contribution in this experiment.

Although mercury uptake by the plant was promoted after addition of thiosulphate to the soil, the potential for risk caused by the judicious application of chemicals to the environment to achieve this aim should be considered [32]. Van Nevel et al. [36] reported that the use of chelators could increase the risk of contaminant leaching, limiting the use of this technique in the field. In order to avoid the leaching of heavy metals, biodegradable chelators such as EDDS have been suggested in place of less degradable chemicals such as EDTA [36]. In this study the application of thiosulphate solution was controlled to ensure no leaching and thus no vertical movement of potential contaminants to below the root-zone of plants. In any field application of the technique, consideration of the rate of breakdown of thiosulphate, and water use efficiency of the plants would need to be considered to mitigate any potential risk.

3.4. Change of soil mercury fractions between time of planting and harvest of plant

There was a good agreement between the sum of the mercury fractions and the single total mercury digestion; the total mercury recovery rate ranged between 84% and 116% (Table 3). This demonstrated that the sequential extraction technique was able to account for mercury in this geochemical system. The concentration of mercury associated with each fraction in the soil at the beginning of

Table 3
The evaluation of the sequential extraction method (mean ± sd, $n = 3$).

	Total Hg (mg kg^{-1})	Summation of each fractions (mg kg^{-1})	Recovery rate (%)
Initial soil	151.13 ± 5.3	135.36 ± 8.35	90 ± 10
Planted	121.16 ± 8.5	117.76 ± 17.82	98 ± 9
Planted + treated	121.57 ± 4.16	110.59 ± 5.86	91 ± 3
Non-planted	136.52 ± 4.30	126.12 ± 10.54	108 ± 13
Non-planted + treated	135.87 ± 6.18	123.41 ± 4.91	111 ± 10

Table 4
The mercury concentration of each fraction in soil before and after the experiment (mean ± sd, $n = 3$).

	Soluble and exchangeable (mg kg^{-1})	Specifically sorbed (mg kg^{-1})	Oxide bound (mg kg^{-1})	Organic bound (mg kg^{-1})	Residual (mg kg^{-1})
Initial substrate	0.001 ± 0.0001	0.001 ± 0.0001	4.61 ± 0.36	54.82 ± 10.62	75.92 ± 2.34
After harvest					
Planted	0.012 ± 0.01	0.01 ± 0.002	1.74 ± 0.18	34.80 ± 1.10	81.64 ± 18.99
Planted + treated	0.02 ± 0.01	0.01 ± 0.01	0.82 ± 0.19	38.43 ± 3.15	71.29 ± 1.60
Non-planted	0.003 ± 0.002	0.01 ± 0.001	3.65 ± 0.08	50.74 ± 7.88	71.72 ± 8.65
Non-planted + treated	0.012 ± 0.003	0.015 ± 0.001	3.28 ± 0.01	51.12 ± 1.10	68.99 ± 6.62

Table 5
The dry weight and total mercury mass of the leaf, stem and root of a single plant (mean ± sd, $n = 3$).

	Leaf		Stem		Root	
	Dry weight (g)	THg mass (μg)	Dry weight (g)	THg mass (μg)	Dry weight (g)	THg mass (μg)
Control	3.36 ± 0.82	8.43 ± 1.55	5.09 ± 0.87	0.70 ± 0.12	1.57 ± 0.08	0.51 ± 0.22
Thiosulphate treated pots	4.68 ± 0.73	19.66 ± 1.62	3.70 ± 0.72	3.06 ± 0.76	1.40 ± 0.09	4.54 ± 2.61

the experiment (initial soil) and at the end of the experiment (after harvest of the plants) is summarized in Table 4.

3.4.1. Soluble & exchangeable and Specifically sorbed fractions

The concentration of mercury in the soluble and exchangeable (X_1) fraction was significantly ($p < 0.05$) increased in the soil sampled at the end of the experiment for the planted, planted + treated and non-planted + treated pots, relative to the initial soil. There was no significant difference between the initial soil and non-planted control at the end of the experiment. However, the differences between the planted, planted + treated and non-planted + treated pots at the end of the experiment were statistically insignificant. The data indicate that the effect of thiosulphate treatment on the concentration of mercury associated with the soluble and exchangeable fraction is of the same magnitude as the effect of the plant on this fraction (no difference between planted and non planted + treated). The concentration of mercury associated with the specifically sorbed fraction (X_2) was similarly increased for the soils sampled after harvest, but no significant difference was observed between the four treatments.

The observed change in the soluble and exchangeable mercury fraction demonstrates that plant growth may enhance the transformation of mercury fractions in soil. Ko et al. [37] found that *Brassica juncea* increased the concentration of plant-available As when grown on arsenopyrite gold mine tailings. Similarly, the exchangeable concentration of Cd, Cu and Pb in the rhizosphere of *Echinochloa crus-galli* grown with root exudates was greater than the control (without root exudates) [38]. Root metabolism could release some organic compounds such as low molecular weight organic acids, which are widely reported to have an impact on metal bioavailability [39].

No difference was observed in the concentration of mercury associated with the X_1 fraction between the planted and planted + treated pots for the soils sampled after harvesting. A similar result has been reported in other studies. Kim et al. [38] described that the addition of root exudates could increase the uptake of Cd, Cu and Pb by *Echinochloa crus-galli*, but no significant difference was observed in the exchangeable metal concentration between the control plants and plants treated with root exudates. In our work, the phenomenon could be attributed to thiosulphate treatment, as follows. The speciation of mercury in soil solution is unknown, but is likely to be Hg^{2+} or complexes with water-soluble organic matter [40]. Under the conditions of pH recorded for the Wanshan soil, and an oxic redox potential, thiosulphate could be a stable counter ion [41]. Thus, in the treated pots, thiosulphate could combine with mercury and form soluble thio-Hg complexes (likely to be Hg–S_2O_3). If uptake is a function of mass flow, and directly proportional to the soluble mercury concentration in soil solution, plant uptake may rapidly reduce the concentration of mercury in the X_1 fraction of the thiosulphate treated pots to an equilibrium level that is apparent for the soil of the planted, but not thiosulphate treated pots (X_1: 0.02 mg kg^{-1} (0.01%) in the treated pots and 0.012 mg kg^{-1} (0.01%) in non treated pots). This could account for the lack of difference in the X_1 fraction for the planted and planted + treated pots at the end of the experiment.

Mercury uptake by plants is mediated by a counter ion on Hg^{2+}: the plant will restrict uptake if the correct counter ion (anion) is not present [31]. For the non-treated planted pots, the mercury concentration in X_1 increased relative to the initial soil. This phenomenon may be explained through the presence of plant exudates or natural ligands in the soil solution that promote mercury solubility. However, the plant, to some extent, may be able to restrict uptake of these soluble complexes. So, despite the presence of an increased concentration of soluble mercury in the plant-available fraction, as a result of plant growth, uptake is limited. When thiosulphate is added, a new ligand is present that can form a stable complex with mercury in X_1. The plant has limited ability to restrict uptake of this complex, so that the mercury concentration in the plant increases. In summary, we believe that both the addition of thiosulphate to the soil, and plant growth, can increase the soluble concentration of mercury in soil. But without the appropriate combination of plant plus chemical ligand, mercury cannot be removed through phytoextraction relative to the control.

3.4.2. Oxide-bound fraction

In the planted pots (both non treated and thiosulphate-treated pots) the mercury concentration in the oxide fraction significantly decreased from 3.41 mg kg^{-1} before the experiment to 1.50 and 0.74 mg kg^{-1} for the not treated and treated pots, respectively. Given the apparent lack of change in the percentage of mercury associated with the X_1 and X_2 fractions in the soil between treatments, and the recorded increase in mercury uptake by the plants as a result of thiosulphate treatment of the soil, we propose that mercury accumulated by the plants was transferred from the X_3 fraction to the X_1 fraction, and subsequently taken up under the correct conditions of plant plus suitable ligand. This therefore indicates that the potentially plant-available pool of mercury in the Wanshan soil is predominantly associated with the oxide fraction. To further explain this theory, the plant tissue mercury concentration was plotted against the concentration of mercury in the oxide-bound fractions for both the thiosulphate-treated and the non-treated soils (Fig. 5). The relationship between the THg concentration in each of roots, stems and leaves was significant correlated with the concentration of mercury in the oxide bound fraction (X_3) ($p < 0.05$, $p < 0.01$ and $p < 0.01$, respectively). No significant correlation was observed between the THg concentration in the plants and mercury in any other geochemical fraction. This firmly demonstrated that the oxide-bound mercury fraction may represent a pool of potentially bioavailable mercury which could be transformed into a more available form (complexed) by plant and thiosulphate, and taken up by *C. glaucum* L.

3.4.3. Organic bound and residual fractions

There was no significant difference in the concentration of mercury associated with the organic bound fraction and the residual fraction for the planted pots at the end of the experiment relative to the initial soil. However, the decrease of the average values of organic bound fractions demonstrated that the growth of the plant could also transform the mercury from organic fractions to other geochemical fractions. Zhang et al. [42] mentioned that the growth of *Brassica napus* could enhance the transport of the organic bound and oxide bound Chromium fractions to bioavailable fractions. Mercury attributed to the residual fractions was relatively stable due to the low solubility of sulphur bound mercury [43].

3.4.4. Total mercury concentration (THg)

The THg concentration was significantly ($p < 0.01$) reduced in the soils at the end of the experiment for the planted pots (treated and non treated), relative to the initial soil, but not changed ($0.05 < p$) for the non-planted pots (Table 3). The small amount of mercury accumulated by the plants, however, could not account for this degree of reduction in the THg concentration in the soil at the end of the experiment (Table 5), demonstrating that the majority of mercury may have been lost through other pathways. Due to the strict controls placed on watering to limit the leaching of mercury through the bottom of the pot, we propose that mercury may have been lost into the air.

The high concentration of elemental mercury in the soil (27 mg kg^{-1}) may have led to a high rate of mercury volatilization from the soil. Many studies have proved that the emission of mercury from soil to the air is related to the THg concentration in soil and vegetation. Wang et al. [44] reported that the flux of mercury from soil to the air could reach 8385 ± 6770 ng m^{-2} h^{-1} for a soil THg concentration at 743.5 mg kg^{-1}. Moreno et al. [45] mentioned that vegetation could enhance mercury volatilization from the Tui base-metal mine tailing in New Zealand. These authors stated that mercury volatilization was mainly related to biological transformations and photoreduction processes occurring in the substrate. Future research will investigate the potential for plant mediated volatilization of mercury from the Wanshan soil.

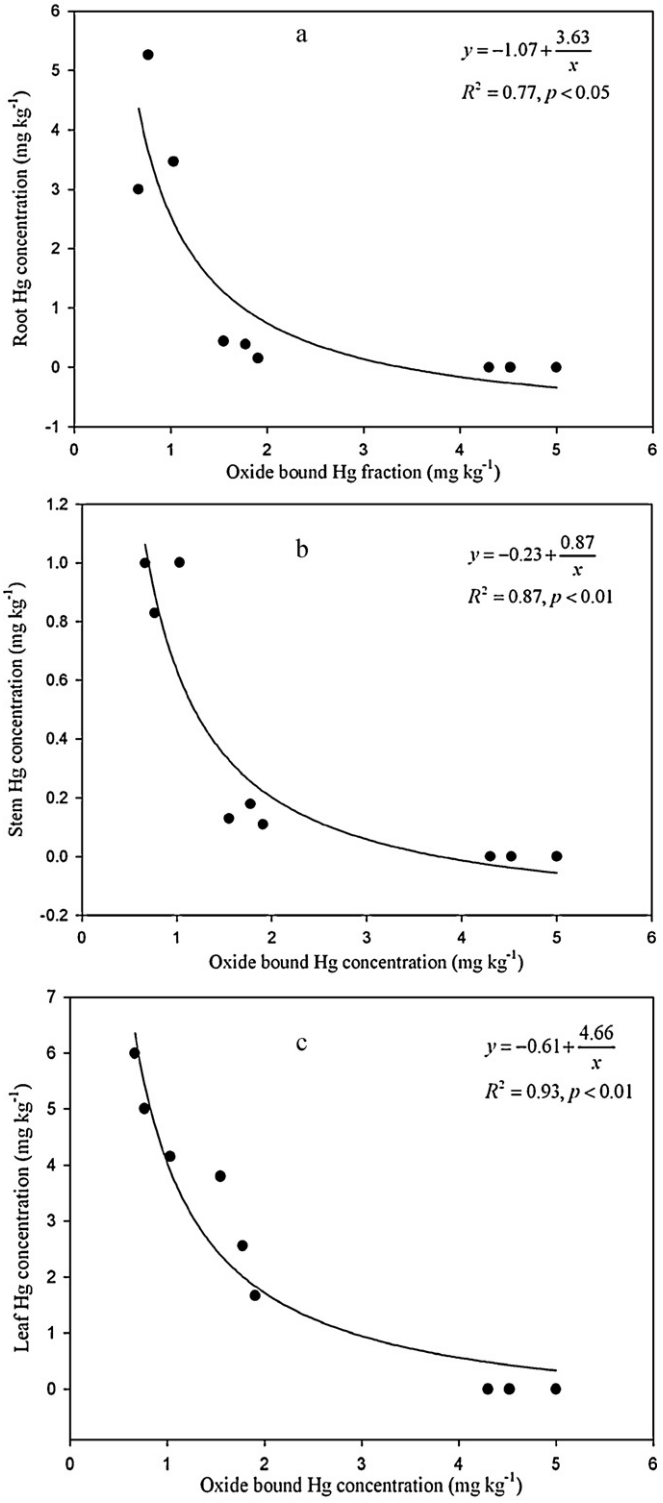

Fig. 5. The relationship between the concentration of mercury in the oxide bound mercury fractions and the plant tissue mercury concentrations.

3.5. Bioaccumulation factors (BAF)

The bioaccumulation factor (BAF) of mercury by *C. glaucum L.* is defined in this work using the following equations (after [46]):

$$\text{Bioaccumulation factor}(\text{BAF}_{\text{Total}}) = \frac{\text{THg concentration in plant shoot}}{\text{THg concentration in soil}}$$

Table 6
Bioaccumulation factors of *C. glaucum* L. (mean ± sd, $n = 3$).

	Control			Ammonium thiosulphate		
	Root	Stem	Leaf	Root	Stem	Leaf
BAF						
$[Hg]_{tissue}/[Hg]_{Avail}$	21 ± 14a	8 ± 3a	171 ± 124a	94 ± 21b	24 ± 9b	132 ± 66a
$[Hg]_{tissue}/[Hg]_{total}$	0.003 ± 0.001a	0.001 ± 0.001a	0.023 ± 0.007a	0.04 ± 0.01b	0.01 ± 0.005b	0.05 ± 0.01b

$[Hg]_{avail}$ means $X_1 + X_2$. $[Hg]_{total}$ means $X_1 + X_2 + X_3 + X_4 + X_5$. Significant differences among control and ammonium thiosulphate are indicated by different small letters ($p < 0.05$).

$$\text{Bioaccumulation factor}(BAF_{Avail}) = \frac{\text{THg concentration in plant shoot}}{\text{Available Hg concentration in soil}}$$

The BAF derived using the total mercury concentration in the soil was higher in root, stem and leaf (Table 6) for thiosulphate-treated plants relative to the control. This supports the observation that thiosulphate can enhance the uptake of mercury by *C. glaucum* L. However, when the BAF is derived using the available concentration of mercury in the soil at the end of the experiment (defined as the concentration of mercury in $X_1 + X_2$ fractions), the value for the thiosulphate-treated soil was higher than the non-treated control, with the single exception of the ratio of $Hg_{[leaf]}/Hg_{[avail]}$, where the values for both thiosulphate treatment and the control were statistically the same. This fact further supports the observation that thiosulphate treatment can cause the plant to absorb solubilized mercury into the root and transport this mercury to the stem. However, the observation that there was no difference in $Hg_{[leaf]}/Hg_{[avail]}$ between the thiosulphate-treated and the non-treated plants demonstrates that the accumulation efficiency of mercury by leaf of *C. glaucum* L. is not dramatically increased after thiosulphate treatment.

3.6. Practical potential for phytoextraction

Assuming an average shoot biomass (dry weight) production for *C. glaucum* L. of 1676 Kg ha^{-1} (figure extrapolated from the pot experiment), and the average shoot mercury concentration (leaf + stem) obtained in this study (4.85 mg kg^{-1}), the thiosulphate assisted mercury phytoextraction yield was 8 g ha^{-1}. Given that the THg concentration in soil is over 100 mg kg^{-1}, phytoextraction as demonstrated in this research is not a viable technology to decrease the THg concentration of the Wanshan soil to a safe level. However, mercury in the bioavailable (X_1) and potentially available (X_3) soil fractions was significantly affected through the action of growing plants on the soil. Mercury in the soluble or exchangeable fraction presents the highest environmental risk, as this is the fraction of soil mercury that can be readily transformed into more toxic complexes such as MeHg. The growth of plants on the Wanshan soil increased the percentage of mercury in the bioavailable fraction (planted but not treated). This point should be considered in the revegetation of Hg-contaminated mine waste. The process of growing plants may increase the potential for mercury risk. However, application of thiosulphate to soil caused a reduction in the amount of mercury in the oxide fraction, while not-affecting the relative amount of mercury in the bioavailable fraction, when compared to the non-planted control. This effectively resulted in a significant reduction of the total amount of mercury associated with the bioavailable and potentially available fractions of the soil. Thiosulphate treatment of Wanshan soil may transport mercury from the oxide fraction to the soluble and exchangeable fraction, which is then taken up by plants. However, the fate of this transformed mercury needs to be further investigated. Specifically, the potential affect of plants and thiosulphate treatment on mercury volatilization must be considered. Moreno et al. [47] proposed that thiosulphate treatment of soil could, in fact, be a route to reduce the volatilization of mercury into the environment that is mediated or facilitated by plants. In their work they showed a reduction in mercury volatilization by *B. juncea* after treatment of soil with thiosulphate. Considering our results, we believe that thiosulphate-assisted mercury phytoremediation could potentially be used to manage the level of contamination in the bioavailable pools of soil mercury, effectively reducing the amount of mercury in the potentially-available pools of soil metal, and could therefore be used to reduce environmental risk in an acceptable time frame. Any operation where chemicals are applied to soil to promote metal solubility must, however, be conducted and managed with care. Future research will investigate the possibility of loss of mercury out of the root zone of plants growing on Wanshan soil.

4. Conclusions

The data presented in this work suggest that ammonium thiosulphate can increase THg solubility, and thereby enhance the uptake of mercury by plants. This can be achieved through the addition of thiosulphate at a rate of 2 g of chemical per kg of soil, five days before the harvest of the plants. The amount of mercury in the soluble and exchangeable and specifically sorbed soil fractions was increased at the end of the experiment (planted pots), but no significant difference was found between the amount of mercury distributed in these fraction for the thiosulphate treated and non treated pots. The amount of mercury associated with the oxide bound fractions was significantly decreased for the planted experimental units (thiosulphate treated and non treated) through the course of the experiment. Furthermore, mercury in the oxide fraction was closely related to the plant tissue mercury concentration. In our opinion, this indicates that plants may take up the $Hg–S_2O_3$ preferentially over other mercury complexes. Although the THg content was reduced in the soil at the end of the experiment relative to the initial soil, we believe this was more related to mercury volatilization than plant accumulation. From the point of view of environmental risk, thiosulphate-assisted phytoextraction by *C. glaucum* L. was able to decrease the amount of soil mercury associated with the oxide bound fractions, while not-affecting the relative amount of mercury in the soluble and exchangeable fractions (compared with planted pots). This effectively reduced the total amount of mercury associated with the bioavailable and potentially-available soil fractions of the metal. The results of our research indicate that thiosulphate-assisted phytoextraction of mercury could be a viable technique to manage mercury risk in the Wanshan soil, although issues such as the leaching of mercury and other metals out of the root zone of the plants have yet to be investigated.

Acknowledgements

We thank Drs. Sarah Rothenberg for comments on the previous version of our manuscript. This research was financed by the National High-tech Research and Development Program of China (2008AA06Z335).

References

[1] P. Li, X.B. Feng, G.L. Qiu, L.H. Shang, Z.G. Li, Mercury pollution in Asia: a review of the contaminated sites, J. Hazard. Mater. 2–3 (2009) 591–601.
[2] T.C. Chang, J.H. Yen, On-site mercury-contaminated soils remediation by using thermal desorption technology, J. Hazard. Mater. 128 (2006) 208–217.
[3] J. Zhang, P.L. Bishop, Stabilization/solidification (S/S) of mercury-containing wastes using reactivated carbon and Portland cement, J. Hazard. Mater. 92 (2002) 199–212.
[4] X.Y. Zhang, Q.C. Wang, S.Q. Zhang, X.J. Sun, Z.S. Zhang, Stabilization/solidification (S/S) of mercury-contaminated hazardous wastes using thiol-functionalized zeolite and Portland cement, J. Hazard. Mater. 168 (2009) 1575–1580.
[5] X.C. Zhang, S.R. Zhang, X.X. Xu, T. Li, G.S. Gong, Y.X. Jia, Y. Li, L.J. Deng, Tolerance and accumulation characteristics of cadmium in Amaranthus hybridus L, J. Hazard. Mater. (2010) 303–308.
[6] L.Q. Ma, K.M. Komar, C. Tu, W. Zhang, Y. Cai, E.D. Kennelley, A fern that hyperaccumulates arsenic, Nature 409 (2001) 579–1579.
[7] S.E. Lindberg, D.R. Jackson, J.W. Huckabee, S.A. Janzen, M.J. Levin, J.R. Lund, Atmospheric emission and plant uptake of mercury from agricultural soils near the Almaden mercury mine, J. Environ. Qual. 8 (1979) 572–578.
[8] Y.D. Wang, M. Greger, Use of iodide to enhance the phytoextraction of mercury-contaminated soil, Sci. Total Environ. 1 (2006) 30–39.
[9] R.G. Pearson, Hard and soft acids and bases, J. Am. Chem. Soc. 85 (1963) 3533–3539.
[10] F.N. Moreno, C.W.N. Anderson, R.B. Stewart, B.H. Robinson, Phytoremediation of mercury-contaminated mine tailings by induced plant-mercury accumulation, Environ. Pract. 02 (2004) 165–175.
[11] H. Biester, M. Gosar, G. Müller, Mercury speciation in tailings of the Idrija mercury mine, J. Geochem. Explor. 65 (1999) 195–204.
[12] M. Delnomdedieu, A. Boudou, D. Georgescauld, E.J. Dufourc, Specific interactions of mercury chloride with membranes and other ligands as revealed by mercury-NMR, Chem. Biol. Interact. 81 (1992) 243–269.
[13] C.S. Kim, G.E. Brown, J.J. Rytuba, Characterization and speciation of mercury-bearing mine wastes using X-ray absorption spectroscopy, Sci. Total Environ. 261 (2000) 157–168.
[14] Y.H. Li, L.S. Yang, Y.F. Ji, H.F. Sun, W.Y. Wang, Quantification and fractionation of mercury in soils from the Chatian mercury mining deposit, southwestern China, Environ, Geochem. Health 31 (2009) 617–628.
[15] A.V. Piskunov, I.A. Aivaz'yan, V.K. Cherkasov, G.A. Abakumov, New paramagnetic N-heterocyclic stannylenes: an EPR study, J. Organomet. Chem. 691 (2006) 1531–1534.
[16] X. Feng, Y.J. Lu, D.C. Grégoire, Y. Hao, C.M. Banic, W.H. Schroeder, Analysis of inorganic mercury species associated with airborne particulate matter/aerosols-method development, Anal. Bioanal. Chem. 380 (2004) 683–689.
[17] J. Weber, A. Karczewska, Biogeochemical processes and the role of heavy metals in the soil environment, Geoderma 122 (2004) 105–107.
[18] C.M. Neculita, G.J. Zagury, L. Deschenes, Mercury speciation in highly contaminated soils from chlor-alkali plants using chemical extractions, J. Environ. Qual. 34 (2005) 255–262.
[19] R.T. Giulio, E.A. Ryan, Mercury in soils, sediments, and clams from a North Carolina peatland, Water Air Soil Pollut. 33 (1) (1987) 205–219.
[20] P.M.V. Nirel, F.M.M. Morel, Pitfalls of sequential extractions, Water Res. 24 (1990) 1055–1056.
[21] P. Jeyakumar, P. Loganathan, S. Sivakumaran, C.W.N. Anderson, R.G. Mclaren, Copper and zinc spiking of biosolids: effect of incubation period on metal fractionation and speciation and microbial activity, Environ. Chem. 5 (2008) 347–354.
[22] X.B. Feng, G.L. Qiu, Mercury pollution in Guizhou, China-an overview, Sci. Total Environ. 400 (2008) 227–237.
[23] M. Horvat, N. Nolde, V. Fajon, V. Jereb, M. Logar, S. Lojen, R. Jacimovic, I. Falnoga, L.Y. Qu, J. Faganeli, D. Drobne, Total mercury, methylmercury and selenium in mercury polluted areas in the province Guizhou, China, Sci. Total Environ. 1–3 (2003) 231–256.
[24] G.L. Qiu, X.B. Feng, S.F. Wang, L.H. Shang, Mercury and methylmercury in riparian soil, sediments, mine-waste calcines, and moss from abandoned Hg mines in east Guizhou province, southwestern China, Appl. Geochem. 3 (2005) 627–638.
[25] X.B. Feng, P. Li, G.L. Qiu, S.F. Wang, G.H. Li, L.H. Shang, B. Meng, H.M. Jiang, W.Y. Bai, Z.G. Li, X.W. Fu, Human exposure to methylmercury through rice intake in mercury mining areas, Guizhou Province, China, Environ. Sci. Technol. 1 (2008) 326–332.
[26] CNEPA, (Chinese National Environment Protect Agency), Environmental quality standard for soils (in Chinese), GB15618-1995, pp. 1–6.
[27] P. Li, X.B. Feng, L.H. Shang, G.L. Qiu, B. Meng, P. Liang, H. Zhang, Mercury pollution from artisanal mercury mining in Tongren, Guizhou, China, Appl. Geochem. 8 (2008) 2055–2064.
[28] A. García-Sánchez, A. Murciego, E. Alvarez-Ayuso, I. Regina, M. Rodríguez-González, Mercury in soils and plants in an abandoned cinnabar mining area (SW Spain), J. Hazard. Mater. 168 (2009) 1319–1324.
[29] USEPA, Mercury in water by Oxidation, Purgeand Trap, and Cold Vapor Atomic Fluorescence Spectrometry (Method 1631, Revision B), USEPA, 1999.
[30] D. Kocman, M. Horvat, J. Kotnik, Mercury fractionation in contaminated soils from the Idrija mercury mine region, J. Environ. Monit. 6 (2004) 696–703.
[31] F.N. Moreno, C.W.N. Anderson, R.B. Stewart, B.H. Robinson, M. Ghomsher, J.A. Meech, Induced plant uptake and transport of mercury in the presence of sulphur-containing ligands and humic acid, New Phytol. 166 (2005) 445–454.
[32] B. Nowack, R. Schulin, B. Robinson, Critical assessment of chelant-enhanced metal phytoextraction, Environ. Sci. Technol. 40 (2006) 5225–5232.
[33] M. Gupta, R.D. Tripathi, U.N. Rai, P. Chandra, Role of glutathione and phytochelatin in Hydrilla verticillata (I.f.) royle and Valusneria spiraus L. under mercury stress, Chemosphere 4 (1998) 785–800.
[34] S. Iglesia-Turino, A. Febrero, O. Jauregui, C. Caldelas, J.L. Araus, J. Bort, Detection and quantification of unbound phytochelatin 2 in plant extracts of brassica napus grown with different levels of mercury, Plant Physiol. 2 (2006) 742–749.
[35] A. Cavallini, L. Natali, M. Durante, B. Maserti, Mercury uptake, distribution and DNA affinity in durum wheat (Triticum durum Desf.) plants, Sci. Total Environ. 243 (1999) 119–127.
[36] L. Van Nevel, J. Mertens, K. Oorts, K. Verheyen, Phytoextraction of metals from soils: how far from practice? Environ. Pollut. 150 (2007) 34–40.
[37] B.G. Ko, C.W.N. Anderson, N.S. Bolan, K.Y. Huh, I. Vogeler, Potential for the phytoremediation of arsenic-contaminated mine tailings in Fiji, Aust. J. Soil Res. 46 (2008) 493–501.
[38] S. Kim, H. Lim, I. Lee, Enhanced heavy metal phytoextraction by Echinochloa crus-galli using root exudates, J. Biosci. Bioeng. 109 (2010) 47–50.
[39] S. Tao, W.X. Liu, Y.J. Chen, F.L. Xu, R.W. Dawson, B.G. Li, J. Cao, X.J. Wang, J.Y. Hu, J.Y. Fang, Evaluation of factors influencing root-induced changes of copper fractionation in rhizosphere of a calcareous soil, Environ. Pollut. 129 (2004) 5–12.
[40] D. Wallschläger, M.V.M. Desai, M. Spengler, R. Wilken, Mercury speciation in floodplain soils and sediments along a contaminated river transect, J. Environ. Qual. 27 (1998) 1034–1044.
[41] R.J. Bowell, R.P. Foster, A.P. Gize, The mobility of gold in tropical rain forest soils, Econ. Geol. 88 (1993) 999–1016.
[42] M.X. Zhang, B. Huang, M. Cheng, L. Feng, Research oil bioavailability of chromium in soil to cole plant, Nonferrous Met. 59 (2007) 95–99 (in Chinese).
[43] J.J. Rytuba, Mercury from mineral deposits and potential environmental impact, Environ. Geol. 3 (2003) 326–338.
[44] S.F. Wang, X.B. Feng, G.L. Qiu, X.W. Fu, Z.Q. Wei, Characteristics of mercury exchange flux between soil and air in the heavily air-polluted area, eastern Guizhou, China, Atmos. Environ. 41 (2007) 5584–5594.
[45] F.N. Moreno, C.W.N. Anderson, R.B. Stewart, B.H. Robinson, Mercury volatilisation and phytoextraction from base-metal mine tailings, Environ. Pollut. 136 (2005) 341–352.
[46] E. Moreno-Jiménez, R. Gamarra, R. Carpena-Ruiz, R. Millán, J. Pe Alosa, E. Esteban, Mercury bioaccumulation and phytotoxicity in two wild plant species of Almadén area, Chemosphere 11 (2006) 1969–1973.
[47] F.N. Moreno, C.W.N. Anderson, R.B. Stewart, B.H. Robinson, R. Nomura, M. Ghomsher, J.A. Meech, Effect of thioligands on plant-Hg accumulation and volatilisation from mercury-contaminated mine tailings, Plant Soil 275 (2005) 233–246.