Induced plant uptake and transport of mercury in the presence of sulphur-containing ligands and humic acid

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Summary

• The induced accumulation of mercury (Hg) by plants was investigated for the species *Phaseolus vulgaris* (Bush bean), *Brassica juncea* (Indian mustard), and *Vicia villosa* (Hairy vetch).
• All plants were grown in modified Hg-contaminated mine tailings and were treated with sulphur-containing ligands to induce Hg accumulation. The effects of varied substrate Hg concentration and humic acid (HA) level on the induced plant-Hg accumulation for *B. juncea* were examined.
• Thiosulphate salts (ammonium and sodium) mobilised Hg in the substrates and caused an increase in the Hg concentration of roots and shoots of all tested plant species. Root Hg accumulation was positively correlated to extractable Hg for $(NH_4)_2S_2O_3$-treated *B. juncea* plants grown in HA-amended substrates. However, shoot Hg translocation for this species was inhibited at 1.25 g HA kg^{-1} of substrate.
• Mercury–thiosulphate complexes could be translocated and accumulated in the upper parts of the plants up to 25 times the Hg concentration in the substrate. We conclude that shoot Hg accumulation in the presence of thiosulphate salts is dependent upon plant species characteristics (e.g. root surface area) and humic acid content.

Key words: Hg complex speciation, Hg availability, humic acid, induced plant-Hg accumulation, mine tailings, sulphur-containing ligands.

Abbreviations

Introduction

Mercury (Hg) is one of the most toxic metals to living organisms. In the case of human Hg poisoning, the critically affected organ is the brain, with symptoms that vary from minor learning disabilities to extremely diminished mental capacity (Suzuki, 1979). Despite this toxicity, Hg is extensively used in developing countries for small-scale gold (Au) mining (Veiga & Hinton, 2002).

Small-scale miners extract Au from ore through the Hg amalgamation method, which uses elemental Hg at an Au : Hg ratio of 1 : 70. A lack of technical knowledge, combined with lax regulations in developing countries leads to widespread Hg contamination, mostly through the release of Hg (0) to the atmosphere. A substantial fraction of Hg also contaminates water and soils after the discharge of amalgamation tailings (Veiga & Hinton, 2002). Heavy-mineral rich tailings, containing

up to 200 mg kg^{-1} of elemental Hg, are often left exposed to the environment, where they are prone to weathering (e.g. leaching, erosion, and volatilisation) (Melamed & Villas Bôas, 1998; Roulet *et al.*, 2000; Moreno *et al.*, 2004a) as well as biochemical transformations (methylation and biological reduction) (Morel *et al.*, 1998).

Mercury that is released to surface soils is generally retained in the solid phase through adsorption onto sulphides, clay particles, and organic matter (Evans, 1989). These Hg forms are insoluble, and therefore, relatively immobile. However, exchange reactions can occur in the soil solution, leading to increased Hg solubility and mobility in soil. Chloride (Cl$^-$) and hydroxide (OH$^-$) ions occur naturally in soils and the soluble $HgCl_2$, $Hg(OH)Cl$, and $Hg(OH)_2$ complexes are the predominant Hg species in well-oxygenated environments (Schuster, 1991). Mercury has a strong affinity for thiol groups and Hg speciation under anoxic conditions is completely dominated by sulphide and bisulphide complexes (Morel *et al.*, 1998). Humic substances (HS) represent 50% of the natural organic matter in soils and contain a high proportion of S-containing functional groups (Wallschläger *et al.*, 1998a). The soluble fraction of HS comprises fulvic and humic acid (HA), which are known Hg ligands (Wallschläger *et al.*, 1996). Therefore Hg–HA complexes are mobile in soils (Wallschläger *et al.*, 1998b) and HA has been demonstrated to enhance both Hg bioavailability in soils and Hg uptake by organisms (Hinton, 2002).

Sulphur-containing solutions have been used to induce Hg accumulation into above ground tissues of high-biomass plant species (Moreno *et al.*, 2004a). For instance, *Brassica juncea* was able to accumulate 40 mg kg^{-1} of Hg in the shoot tissues following application of $(NH_4)_2S_2O_3$ to mine tailings contaminated with Hg at 2.8 mg kg^{-1}. Thioligand-induced plant-Hg accumulation has been therefore proposed as a potential strategy for the removal of Hg from contaminated substrates. Despite the environmental impact of chelated-enhanced phytoextraction (e.g. leaching of heavy metals to groundwater) (Lombi *et al.*, 2001), complexing agents can be useful tools for studying the geochemistry and the uptake of pollutants that have limited plant availability in soils. In the current study we investigate the effect of sulphur-containing ligands on the Hg availability and plant Hg uptake for three plant species grown in Hg-contaminated mine tailings. We also examined the effects of substrate Hg concentrations and humic acid levels on the root-to-shoot transport of Hg for ammonium thiosulphate-treated *B. juncea* plants.

Materials and Methods

Site description

The Hg-contaminated mine tailings used in this study were collected from the processing centre of the Gold Mountain mine, north-central China. It was requested that the exact location of the mine be omitted to protect the local mining community (A.J. Gunson, pers. comm.). The Gold Mountain mine is a small-scale mine that extracts gold (Au) from ore using the amalgamation method. The aqueous slurry of Au ore is ground with elemental Hg in locally manufactured mills. Gold liberated during rotation of iron wheels contacts Hg and forms an amalgam that may contain a 1 : 1 Au : Hg ratio. The Gold Mountain mine processes about 10–15 tonnes of ore daily and is responsible for annual emission of an estimated 70 tonnes of Hg into the regional environment (Gunson & Veiga, 2004). Mineralogical analysis (ACME Laboratories, Vancouver, BC, Canada) indicated the solid fraction of tailings to contain the following metals: Hg (100 mg kg^{-1}), Au (0.2 mg kg^{-1}), Cu (10 000 mg kg^{-1}), Ni (88 mg kg^{-1}), Fe (17%), As (14 mg kg^{-1}), Sb (63 mg kg^{-1}), and Te (4 mg kg^{-1}).

Substrate preparation

In order to reduce background variability and maximise Hg uptake, all plant experiments were carried out in modified mine tailings. The modified substrate was prepared through dilution of the original Gold Mountain mine tailings (100 mg Hg kg^{-1}) with a 1 : 1 mixture of coarse and fine silica sand (fine fraction < 1000 microns) to give final Hg concentrations of 0, 1.25, 2.5, and 5 mg kg^{-1}. The substrate with Hg at 2.5 mg kg^{-1} was amended with commercially available humic acid (catalogue n° H1, 675–2, Aldrich Chemical Company, USA). The chocolate-brown, dust-like powder was mixed with the substrate to give (w/w) HA concentrations of 0, 0.125, and 1.25 g kg^{-1} of substrate. All substrates were supplemented with Osmocote (slow release NPK fertiliser) at 5 g kg^{-1} and left to equilibrate for 1 wk before the initiation of the growth experiment. No lime was added, as the pH of substrate (around 8) was suitable for plant growth. The preparation of substrates was carried out in the laboratory facility of the Department of Mining and Mineral Processing Engineering at the University of British Columbia (UBC), Vancouver, BC, Canada. A separate batch of substrate samples was sealed in plastic bags and shipped to New Zealand for Hg analysis.

Extractable Hg

Extractable Hg concentrations were determined for diluted and original tailings substrates. The extractants investigated were ammonium thiosulphate ($[NH_4]_2S_2O_3$), sodium thiosulphate ($Na_2S_2O_3$), and ammonium thiocyanate supplemented with hydrogen peroxide ($NH_4SCN + H_2O_2$). Extractable Hg concentrations for substrates amended with humic acid were measured using only $(NH_4)_2S_2O_3$ as an extractant. One gram of substrate was weighed into 50 ml polypropylene centrifuge tubes in triplicate. After addition of extractant solutions (20 ml at 2 g l^{-1}, unless otherwise stated), the tubes were rotated in a shaker overnight at 45 rotations per minute (rpm) and the supernatant separated after centrifugation at 3000 rpm for 3 min. The pH and Eh of the extractant solutions were

measured using a pH and Eh meter (Copenhagen Radiometer, PHM 83 Autocal pH meter).

Effect of plant species × sulphur-containing ligands

Seeds of *Brassica juncea* (Indian mustard), *Phaseolus vulgaris* (Bush beans), and *Vicia villosa* (Hairy vetch) were germinated in 400-ml plastic pots (8×8 cm, $n = 60$) filled with the amended substrates containing Hg at 2.5 mg kg^{-1}. Two weeks after seeding each pot was thinned to leave one individual plant. The experiment was initiated 5 wk after seeding. Three sulphur–containing ligands were investigated for their ability to induce Hg accumulation in the plants: ammonium thiosulphate ($[NH_4]_2S_2O_3$), sodium thiosulphate ($Na_2S_2O_3$), and ammonium thiocyanate supplemented with hydrogen peroxide ($NH_4SCN + H_2O_2$). The experiment utilised a two-factorial completely randomised design with plants species (*B. juncea*, *P. vulgaris*, and *V. villosa*) and sulphur-containing ligands ($[NH_4]_2S_2O_3$, $Na_2S_2O_3$, and $NH_4SCN + H_2O_2$) as factors. The extractants were dissolved in 1 L of reverse osmosis (RO) water and 10 ml of the resulting solutions were added to plant pots to give a final concentration of 2 g of chemical per kg of substrate (unless otherwise stated). Plants from each species that were treated with water were designated as control plants. Five replicates were used for each plant-chemical combination.

Effect of substrate Hg concentration and humic acid level

Seeds of *B. juncea* were sown in 400-ml plastic pots (8×8 cm) filled with the modified substrate contaminated with Hg at 1.25, 2.5, and 5 mg kg^{-1} ($n = 30$). *B. juncea* seeds sown in substrates without added Hg were used as control for monitoring the shoot uptake of Hg via the atmosphere pathway ($n = 5$). Another batch of *B. juncea* seed was also sown in modified substrate contaminated with Hg at 2.5 mg kg^{-1} and amended with humic acid at 0, 0.125, and 1.25 g kg^{-1} of substrate ($n = 40$). Two weeks after seeding, each pot was thinned to leave one individual plant. Ammonium thiosulphate solution was added after 5 wk of plant growth at a rate of 2 g kg^{-1} of substrate. Plants that received only water were designated as controls plants. These two experiments utilised a completely randomised design with five replicates per each treatment level.

The thioligand–induced plant experiments were carried out over a 5-d period in a glasshouse with the ambient temperature set at 25°C with no humidity control. All the experiments were carried out together and plants received the chemical treatments in the same day. The pot positions were randomly changed on a periodic basis to equalise light exposure. The glasshouse facility was located at the campus of UBC, Vancouver, BC, Canada. The maximum interval period between planting, treating and harvesting the plants was 6 wk. The glasshouse experiments were carried out over the summer period and the plant pots were watered every day to field capacity. All the plants were treated before the outset of flowering.

Plant harvest

At the end of the experiments, plants were harvested and washed in tap water. Shoots were excised from roots by using a steel blade. Due to the sandy composition of the substrates, the intact root system could be harvested from the pots by soaking the bulk roots with the adhering substrate in a bucket filled with water. The buckets were acid washed and the water was fully replaced after each soaking period. The roots were further washed several times with tap water to remove residual substrate and HA particles. The soaking process was carried out for one hour and was done separately for each plant-chemical treatment. Plant tissues were placed into individual paper bags and dried at 70°C. After drying, all plant samples were sealed in plastic bags and shipped to New Zealand for Hg analysis.

Plant digestion

Ground shoots and roots were accurately weighed (0.1 g) into 50-ml plastic pots. Concentrated HNO_3 (15 ml) was then added. The digest samples were left overnight and, in the following day, were heated in a water bath at 100°C for 1 h. Digest solutions were transferred to 10 ml polythene tubes and diluted with RO water to make a final volume of 10 ml. A blank reagent was used with all digestions.

Substrate digestion

The total Hg concentration in the original and Hg-amended substrates was determined through *aqua regia* digestions. One gram of substrate was weighed into 50 ml polypropylene pots in triplicate and a 15-ml solution of HNO_3 and HCl at a 1 : 3 ratio was added. The samples were digested in water bath at 100°C for 1 h and the filtrates diluted to a final volume of 50 ml with RO water.

Mercury analysis

Total Hg concentrations in plant and substrate digests and in extractant solutions were analysed through the hydride-generation atomic absorption spectroscopy technique (Moreno *et al.*, 2004b). The analysis was performed using a GBC 909 A atomic absorption spectrophotometer (AAS, Victoria, Australia) operating in the flame mode. A sodium borohydride solution (5% $NaBH_4$ + 1% KOH) in combination with 10 ml of 0.5 M of HCl was used to generate the Hg vapour. The limit of detection (LOD) for mercury in solution was 10 ng ml^{-1} for plant digests and 5 ng ml^{-1} for soil digests and extractant solutions. Reagent blanks were below detection limits in the solution. Linear calibration

curves were obtained over the range of 125–1000 ng ml^{-1} of Hg using four standards prepared from a 10 mg l^{-1} mercuric nitrate (HgNO$_3$) spectrosol solution (AAS standard reagent solution, May & Baker, Dagenham, UK). Solutions with Hg concentration over the 1000 ng ml^{-1} range were diluted with RO. The Hg readings obtained from the replicate analysis (n = 10) of a standard solution containing 1 mg l^{-1} of Hg could be reproduced with less than 5% of variation. The analytical method was assessed for quality control by an external certified laboratory and the maximum discrepancy was 15%.

Statistical analysis

A copy of SAS PC version 8e was used for statistical analyses (SAS Institute, 1988). Due to poor germination rates for *P. vulgaris* and Hg readings below detection limits, some plant-chemical combinations were missing. As a result of this, the two factorial structure of the plant-Hg induced experiment became unbalanced. The analyses of variance (ANOVA) for the effect of plant species × sulphur-containing ligands was, therefore, performed in one-way structures with each plant-chemical combination regarded as a single treatment. Linear contrasts were then performed for comparing the treatment means separately for roots, shoots, and the *shoot : root* ratio. The following comparisons were performed:

control (water) × sulphur-containing ligands (ignoring plant species);

between the three sulphur-containing ligands (ignoring plant species);

between plant species (ignoring sulphur-containing ligands);

between plant species within each sulphur-containing ligand.

Differences between three or more treatment means in the remaining experiments were performed through one-way analysis of variance (ANOVA). Tukey's test was used for pairwise comparison of means at 0.05 and 0.01 significance levels. The *t-test* was used to compare two treatment means assuming equality of variances. Simple linear and polynomial regression models were used to interpret the relationships between two variables. The significance of the fitted regression was assessed through the ANOVA and the coefficient of determination (r^2). Correlation analysis was used to assess the positive and negative dependence between two variables. The ANOVA for testing the effects of sulphur-containing ligands, plant species, substrate Hg concentration, and humic acid on Hg uptake and transport was carried out in log-transformed data.

Results

Total and extractable Hg concentrations

The total Hg concentrations in the *aqua-regia* digests and the pH and Eh for original and diluted tailings samples are shown in Table 1. All amended substrates exhibited similar geochemical

Table 1 Mercury concentrations[a] and the pH and Eh of original and modified samples of Gold Mountain (GM) mine tailings.

Substrate composition	Target Hg (mg kg^{-1})	Measured Hg (mg kg^{-1})[b]	pH[c]	Eh[c] (mV)
Original	100[d]	67.47 ± 11.25	9.45	−137
	1.25	1.65 ± 0.05	8.17	−62
Modified[e]	2.5	2.42 ± 0.07	8.24	−66
	5	3.40 ± 0.11	8.32	−72
HA 0			8.24	−66
Modified + HA 0.125	2.5	2.42 ± 0.07	8.19	−63
HA 1.25			8.34	−73

[a]Total Hg concentrations in the samples were determined through *aqua regia* digestions.
[b]Values are the mean and standard deviation of at least three replicates.
[c]pH and Eh values are the mean of three measurements.
[d]Analysed by cold vapour atomic absorption spectrometry, ACME labs, Vancouver, BC, Canada.
[e]The modified substrate was prepared through dilution of the original GM mine tailings with a 1 : 1 mixture of coarse and fine silica sand.
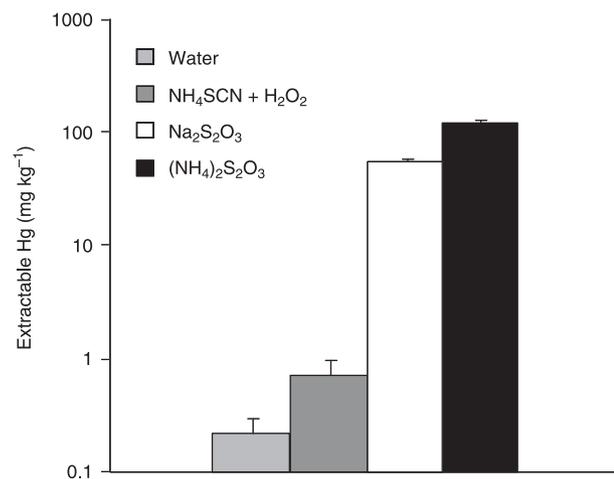

Fig. 1 Effect of sulphur-containing ligands on extractable Hg concentrations of original Gold Mountain mine tailings. Bars denote ±1 SD from the mean of three replicates. NH$_4$SCN, ammonium thiocyanate; H$_2$O$_2$, hydrogen peroxide; Na$_2$S$_2$O$_3$, sodium thiosulphate; (NH$_4$)$_2$S$_2$O$_3$, ammonium thiosulphate; water, control.

conditions with moderately alkaline pH (between 8.1 and 8.3) and mildly reducing conditions (Eh between −62 and −73 mV).

The extractable Hg concentrations for the original Gold Mountain mine tailings are shown in Fig. 1. Mercury solubility in the mine tailings was significantly increased in the presence of (NH$_4$)$_2$S$_2$O$_3$. The total Hg concentration in (NH$_4$)$_2$S$_2$O$_3$ extracts reached 122 ± 9.4 mg kg^{-1} and was significantly higher than the concentration extracted using Na$_2$S$_2$O$_3$, NH$_4$SCN + H$_2$O$_2$, and water ($P < 0.001$, Fig. 1). The concentration of Hg extracted using (NH$_4$)$_2$S$_2$O$_3$ was significantly higher than the Hg concentration found in *aqua regia* digests, as shown in Table 1 ($P < 0.01$, Table 1).
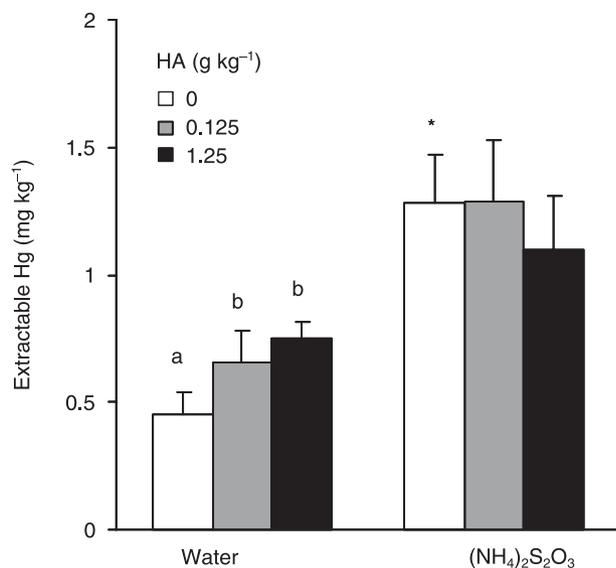
Fig. 2 Humic acid-extractable Hg for modified substrates containing Hg at 2.5 mg kg^{-1}. Bars denote ±1 SD from the mean of three replicates. Letters compare treatments within each treatment level. Means with different letters are significantly different at $P < 0.05$ (Tukey's test). An asterisk indicates not statistically significant ($P > 0.05$). HA, humic acid; $(NH_4)_2S_2O_3$, ammonium thiosulphate; water, control.

The effect of humic acid on the water and $(NH_4)_2S_2O_3$-extractable Hg concentrations of modified mine tailings is shown in Fig. 2. Again, the Hg concentration was significantly higher in the presence of $(NH_4)_2S_2O_3$ relative to water ($P < 0.0001$).

The concentration of water-soluble Hg was higher for substrates that had been amended with humic acid at rates of 0.125 and 1.25 g HA kg^{-1} ($P < 0.01$). This increase corresponded to a 40% increment in the Hg soluble fraction relative to non HA amended water-treated substrates. There were no significant differences within the $(NH_4)_2S_2O_3$ treatment for the Hg values between the control (no HA-amended substrates) and the two tested levels of humic acid ($P > 0.05$).

Effect of plant species × sulphur-containing ligands

Although great care was taken to ensure that Hg was washed off from the root system, neither the experimental protocol nor the analytical techniques used in this study was sufficient to distinguish between the Hg that was adsorbed onto, or taken up into (absorbed) root cells. Therefore, we assume that the accumulation of Hg by the roots of the tested plants will contain both of these Hg fractions.

The Hg concentrations in plant shoots and roots, harvested at the end of the experiment, are shown in Table 2. The associated comparisons including their F-ratio and P-values are shown in Table 3. The application of $(NH_4)_2S_2O_3$ and $Na_2S_2O_3$ mobilised Hg in substrates and greatly increased root Hg accumulation relative to controls and to $NH_4SCN + H_2O_2$ ($P < 0.0001$, Tables 2 and 3). The induced root Hg accumulation was significantly higher for *V. villosa* relative to all plant-chemical combinations ($P < 0.0001$, Table 2). For example, in the presence of $(NH_4)_2S_2O_3$ and $Na_2S_2O_3$, the root Hg concentration for this plant species increased to more than 100 mg kg^{-1} dry weight relative to an average

Table 2 Effect of sulphur-containing ligands and plant species on Hg accumulation for plants growing in modified substrates containing Hg at 2.5 mg kg^{-1}

Treatments Description (Plant Species/chemical)[b]	Contrast[c] Number	Root	Shoot[d]	Shoot : root ratio[d]
VV/Control	1	5.5 ± 0.8	BDL	NA
VV/Na$_2$S$_2$O$_3$	2	113 ± 10	9.5 ± 1.4	0.08 ± 0.04
VV/(NH$_4$)$_2$S$_2$O$_3$	3	131 ± 13	14.8 ± 0.8	0.11 ± 0.015
VV/NH$_4$SCN + H$_2$O$_2$	4	23 ± 2.6	BDL	NA
BJ/Control	5	9.8 ± 1.7	BDL	NA
BJ/Na$_2$S$_2$O$_3$	6	69 ± 4	15.2 ± 2.3	0.22 ± 0.03
BJ/(NH$_4$)$_2$S$_2$O$_3$	7	61 ± 6	16.4 ± 2.5	0.27 ± 0.04
BJ/NH$_4$SCN + H$_2$O$_2$	8	12 ± 1.4	BDL	NA
PV/Control[e]	9	4.2 ± 0.3	BDL	NA
PV/(NH$_4$)$_2$S$_2$O$_3$	10	28 ± 4.4	17.2 ± 1.8	0.62 ± 0.14

Values for Hg in roots and shoots are in mg kg^{-1}. The *shoot : root* ratio is the quotient of Hg content in shoots/Hg content in roots[a].
[a]Values are ±1 SD from the mean of five replicates.
[b]VV, *Vicia villosa*; BJ, *Brassica juncea*; PV, *Phaseolus vulgaris*; $(NH_4)_2S_2O_3$, ammonium thiosulphate; $Na_2S_2O_3$, sodium thiosulphate; $(NH_4)_2S_2O_3$, ammonium thiocyanate; H_2O_2, hydrogen peroxide.
[c]Relates a single plant-chemical combination to its respective treatment mean.
[d]Missing cells due to Hg below detection limits.
[e]PV/Na$_2$S$_2$O$_3$ and PV/NH$_4$SCN + H$_2$O$_2$ treatments were omitted due to absence of plant germination.
BDL, below detection limits; NA, not applicable.

Treatment contrasts[b]	df[c]	*F*-Value	Pr > F[c]
Root 1 & 5 & 9 vs 2 & 3 & 4 & 6 & 7 & 8 & 10	1	816.62	< 0.0001
2 & 3 vs 4 & 6 & 7 & 8 & 10	1	1276.03	< 0.0001
2 & 3 vs 6 & 7 & 10	1	730.87	< 0.0001
2 & 6 vs 3 & 7 & 10	1	636.36	< 0.0001
3 vs 7 & 10	1	67.46	< 0.0001
7 vs 10	1	22.18	< 0.0001
2 vs. 6	1	121.31	< 0.0001
Shoot 2 & 3 6 & 7 & 10	1	15.35	0.0009
2 & 6 vs 3 & 7 & 10	1	15.35	0.0009
3 vs 7 & 10	1	2.60	0.1224
7 vs 10	1	0.40	0.5360
2 vs 6	1	21.02	0.0002
2 & 3 6 & 7 & 10	1	84.10	< 0.0001
2 & 6 vs 3 & 7 & 10	1	84.10	< 0.0001
Shoot : root 3 vs 7 & 10	1	54.63	< 0.0001
Ratio 7 vs 10	1	65.39	< 0.0001
2 vs 6	1	9.67	0.0055

Table 3 Linear contrasts and the associated F-ratio and P-values for the comparison of plant/chemical treatment means

The contrasts were tested separately for roots, shoots, and the *shoot : root* ratio[a].
[a]See contrast number in Table 2 for treatments description.
[b]The associated hypothesis for each contrast tested possible differences between the means of each plant/chemical contrast number; comparisons were not orthogonal.
[c]df, degrees of freedom; Pr > F, probability level for rejecting or accepting the hypothesis associated with each treatment contrast at $\alpha = 0.05$.

of 69 mg kg^{-1} recorded in the root tissues of *B. juncea* (Table 2).

The Hg concentration in the shoot tissues of $(NH_4)_2S_2O_3$ and $Na_2S_2O_3$-treated plants was significantly higher than in control plants and those treated with $NH_4SCN + H_2O_2$, which had Hg values below detection limits (Table 2). These results indicate that a fraction of Hg mobilised by $(NH_4)_2S_2O_3$ and $Na_2S_2O_3$ could be taken up into the roots and subsequently transported to the aerial tissues of the tested plant species. Although the Hg concentration in shoot tissues appears to be similar between plant species in the presence of these ligands (Table 2), there was a significant plant-chemical effect in the shoot Hg translocation ($P < 0.001$, see first two contrasts Table 3). For instance, Hg values in aerial tissues of $Na_2S_2O_3$-treated plants were significantly lower than the ones observed in the $(NH4)_2S_2O_3$-treated plants ($P < 0.001$, see 1st contrast for shoots, Table 3). Also, Hg translocation to the aerial tissues of *V. villosa* was significantly reduced when compared to the other two species ($P < 0.001$, see 2nd contrast for shoots, Table 3). The results for Hg accumulation in shoot tissues indicate, however, that shoot Hg translocation was similar between plant species within the $(NH_4)_2S_2O_3$ treatment (Table 2, see shoots). Furthermore $(NH_4)_2S_2O_3$-treated plants showed variable efficiency for Hg transport from root to shoot tissues when the results were expressed by their respective *shoot : root* ratios (Table 2, see *shoot : root* ratio). For example, the *shoot : root* ratio for *P. vulgaris* was two and five times higher after $(NH_4)_2S_2O_3$ treatment relative to *B. juncea* and to *V. villosa*, respectively ($P < 0.0001$, Tables 2 and

3, see contrasts for *shoot : root* ratio). The *shoot : root* ratio for *B. juncea* was approximately two-fold greater than the *shoot : root* ratio obtained for *V. villosa* in the presence of both $(NH_4)_2S_2O_3$ and $Na_2S_2O_3$ ($P < 0.0001$ and $P < 0.01$, respectively, Table 2, see contrasts for *shoot : root* ratio).

Effect of substrate Hg concentration and humic acid level

The results shown in the Fig. 3(a) demonstrates that Hg accumulation in shoot and root tissues of $(NH_4)_2S_2O_3$-treated *B. juncea* plants was significantly enhanced as a function of increasing Hg concentrations in substrates ($P < 0.05$). Control (water-treated) plants, on the other hand, had shoot Hg values below detection limits for all tested substrate Hg concentrations (data not shown). Plants grown in substrates without added Hg also had shoot Hg values below detection limits (Fig. 3a). These results suggest that foliar uptake of Hg (0) released from planted substrates was not a significant pathway for Hg accumulation in the aerial tissues of tested plants under glasshouse conditions.

Although the root Hg concentration after $(NH_4)_2S_2O_3$ treatment appears to be increasing with regard to Hg concentration in substrates (Fig. 3a), a plot of the root Hg concentration vs the $(NH_4)_2S_2O_3$-extractable Hg concentration shows a significant asymptotic response (Fig. 3b, $r^2 = 0.7411$, $P < 0.001$). Consequently, it can be inferred that Hg uptake into the roots of *B. juncea* is physiologically regulated above certain concentrations and thus, not completely dependent on the amount of Hg available in the substrate. However, this is not true for
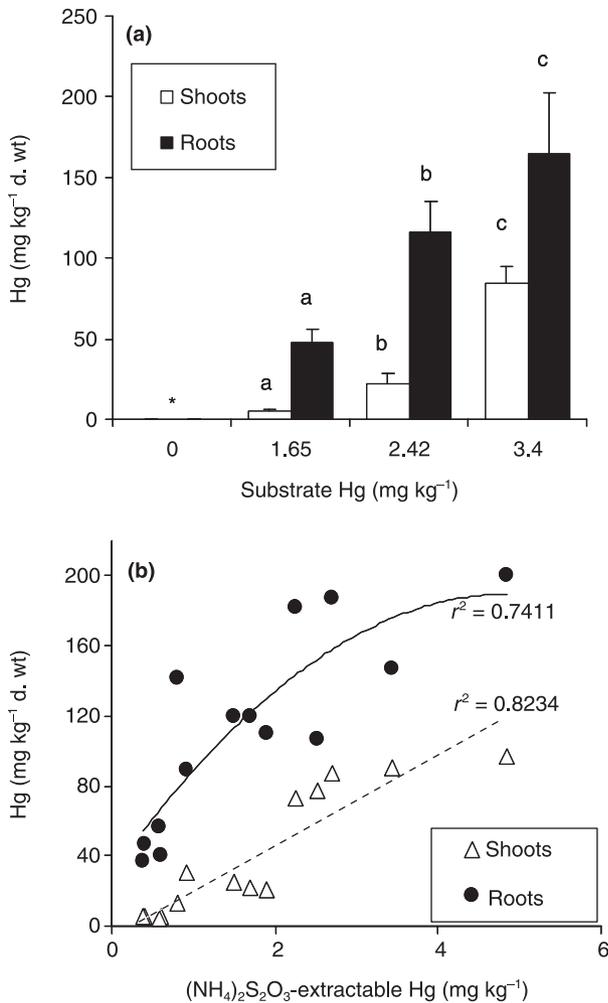
Fig. 3 Accumulation of Hg in roots and shoots of $(NH_4)_2S_2O_3$-treated *Brassica juncea* plants as a function of total (a) and $(NH_4)_2S_2O_3$-extractable (b) Hg concentrations in modified substrates. Bars denote ±1 SD from the mean of five replicates. Letters compare means between Hg levels. Means with different letters are significantly different at $P < 0.05$ (Tukey's test). $(NH_4)_2S_2O_3$, ammonium thiosulphate; DW, dry weight. An asterisk indicates Hg below detection limits.
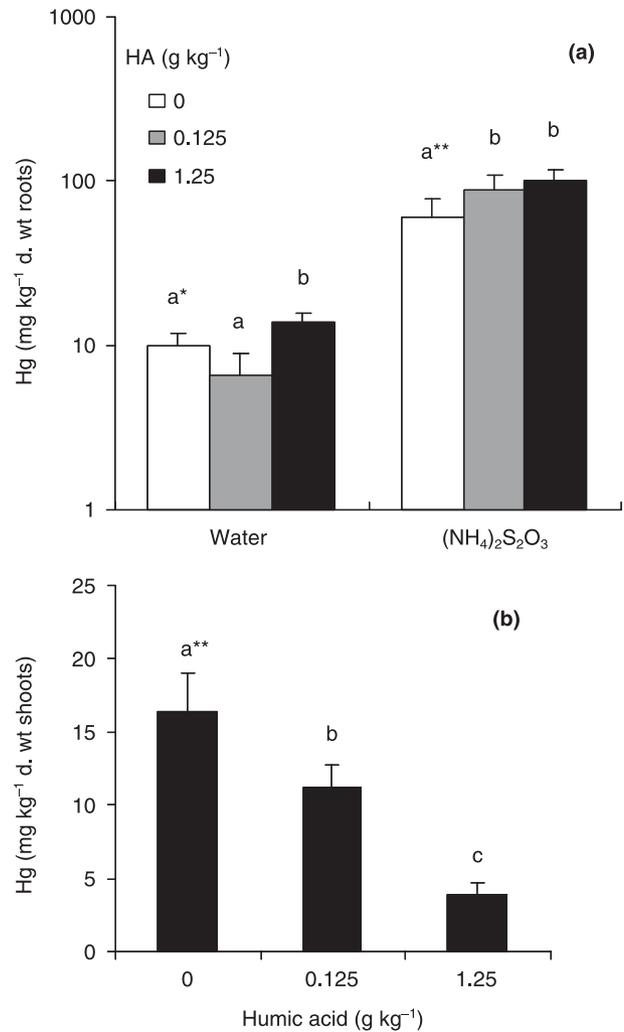
Fig. 4 Accumulation of Hg in roots (a) and shoots (b) of water and $(NH_4)_2S_2O_3$-treated *Brassica juncea* plants as a function of the humic acid content (g kg^{-1}) in modified substrates containing Hg at 2.5 mg kg^{-1}. Bars denote ±1 SD from the mean of five replicates. Means with different letters are significantly different (Tukey's test). Significance level: *, $P < 0.05$; **, $P < 0.01$. HA, humic acid; $(NH_4)_2S_2O_3$, ammonium thiosulphate; water, control; d. wt, dry weight.

shoots, whose Hg concentration showed a significant and linear response to the concentration of $(NH_4)_2S_2O_3$-extractable Hg in the substrate (Fig. 3b, $r^2 = 0.8324$, $P < 0.0001$).

The effect of variable humic acid levels on the root Hg concentration in *B. juncea* after water (control) and $(NH4)_2S_2O_3$ treatment is shown in Fig. 4(a). Addition of $(NH_4)_2S_2O_3$ to substrates greatly enhanced the root Hg concentration relative to the water treatment ($P < 0.0001$). The Hg concentration of both water and $(NH_4)_2S_2O_3$-treated plants was substantially affected by the amount of HA in the substrate. The concentration of Hg in the root tissues of control plants significantly increased from 9.8 to 13.7 mg kg^{-1} when the HA content in substrates was raised from 0 to 1.25 g kg^{-1} ($P < 0.05$). The root Hg concentration of $(NH_4)_2S_2O_3$-treated plants

showed a similar pattern, increasing significantly from 61 to around 100 mg kg^{-1} over the same HA concentration range ($P < 0.05$). By contrast, the shoot Hg concentration of *B. juncea* was significantly decreased in the presence of HA ($P < 0.01$, Fig. 4b). Root to shoot transport therefore appears to be inhibited by humic acid.

The relationship between the root Hg concentration in *B. juncea* and the extractable Hg concentration of HA amended substrates is shown in Fig. 5(a). Both water and $(NH_4)_2S_2O_3$-treated plants showed strong evidence for a positive and linear relationship between the concentration of Hg in root tissues and the concentration of extractable Hg ($r^2 = 0.6893$, $P < 0.001$ and $r^2 = 0.6255$, $P < 0.001$, respectively). However, when the concentration of extractable Hg was plotted against the
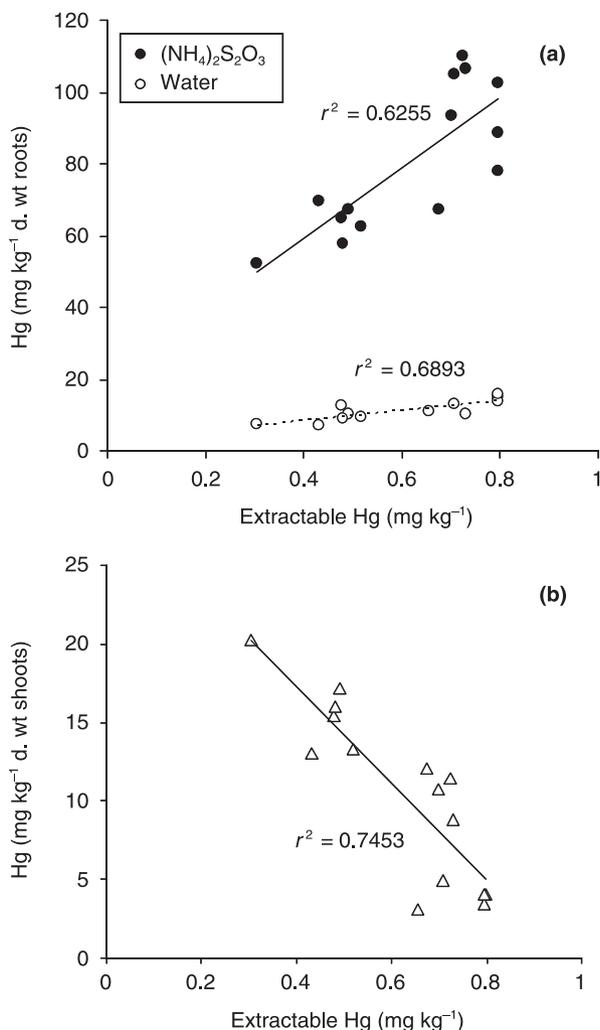
Fig. 5 Accumulation of Hg in roots (a) and shoots (b) of water and (NH$_4$)$_2$S$_2$O$_3$-treated *Brassica juncea* plants as a function of extractable Hg in humic acid amended substrates (1.25 g kg^{-1}) containing Hg at 2.5 mg kg^{-1} d. wt, dry weight.

concentration of Hg in shoot tissues, the regression relationship was inverted, confirming the inhibition effect of HA on shoot Hg translocation (r^2 = 0.7453, P < 0.0001, Fig. 5b). The relationship between the root and shoot Hg concentration as a function of extractable Hg was confirmed by correlation analysis (r = −0.86 for shoots of [NH$_4$]$_2$S$_2$O$_3$-treated plants; r = 0.82 for roots of water-treated plants, and r = 0.86 for roots of [NH$_4$]$_2$S$_2$O$_3$-treated plants).

Discussion

Mercury speciation in the substrate

Given that the original tailings samples exhibited mild reducing conditions (−137 mV) and high pH (9.45) (Table 1), the speciation of solid phase Hg in the tailings substrate may be in the

elemental Hg (0) form (Brookins, 1988). In this oxidation state, Hg is volatile and thus some Hg may have been lost from the system due to volatilisation. However, it is possible that dissolved Hg species in the tailings (Fig. 1) were adsorbed to iron oxy-hydroxides, as it has been demonstrated for Hg-contaminated soils with neutral to alkaline conditions (Andersson, 1979). The fact that the mine tailings have an alkaline pH and contain 17% Fe provides support to this assumption (Table 1). The ammonium and sodium thiosulphate salts may have, therefore, mobilised Hg because they are strong ligands and nonselective to specific Hg species. The Hg solubilization process is possibly related to thiosulphate Hg-complex formation in the presence of the thiosulphate ion. Thiosulphate solutions have complexing abilities with a variety of metal ions from the IB and IIB classes of the periodical table (e.g. Ag, Au, Hg, Cu) and will dissolve many insoluble forms of Hg under alkaline conditions (Wilkinson *et al.*, 1987; Molleman & Dreisinger, 2002).

The existence of a water-extractable Hg fraction in the original tailings samples (Fig. 1) indicates that Hg was available for exchange reactions with S-containing ligands in the substrate solution. Since there was no significant variation in the pH values between control and HA-amended substrates (Table 1), enhanced Hg solubility in the water extracts for HA-amended substrates can only be explained through the formation of Hg–HA complexes (Fig. 2). The fact that the pH of diluted substrates was around 8 (Table 1) and that humic acid is soluble at alkaline conditions (Wallschläger *et al.*, 1996) provides support for this statement. Considering that soluble Hg–HA complexes were present in both water and (NH$_4$)$_2$S$_2$O$_3$ extracts (Fig. 2), we hypothesise that the total soluble Hg fraction of HA-amended substrates comprises a mixture of both Hg–HA and Hg–thiosulphate complexes.

Mercury accumulation in relation to plant species

Results from the current study indicate that root Hg accumulation might be dependent on the root specific features of plant species. For instance, *V. villosa* showed the highest root Hg values and the lowest *shoot : root* ratio while the opposite was true for *P. vulgaris* (Table 2). The same pattern was also observed for *V. villosa* and *B. juncea* in the presence of Na$_2$S$_2$O$_3$. The explanation for this variability might be attributed to an interaction between plant roots and the components (i.e. diffusion and mass flow) that affect nutrient uptake and transport to root surfaces. (Marschner, 1986; Marschner, 1991). For example, the transport of nutrients to plant roots along a concentration gradient (i.e. diffusion) is closely related to plant factors such as root morphology and root surface area (Marschner, 1991). A field study conducted with the purpose of correlating Hg uptake with the characteristics of plant roots found a strong relationship between root Hg accumulation and root surface area (Cocking *et al.*, 1995). Plant species with fine root systems (such as *Allium* sp.) and

greater surface area exhibited elevated Hg concentration in roots, whereas an inverse relationship was observed for plants with a larger root size (smaller root surface area). Also, Hg plant uptake was shown to be strongly dependent on root length density of Zorro fescue (*Vulpia myuros* L.) grown in acidic Hg-contaminated mine soils (Heeraman *et al.*, 2001). It was observed during harvesting that *V. villosa* produced more roots per unit of soil volume when compared to the other two species. This factor might have contributed to the superior root Hg values exhibited by this species in the presence of S-containing ligands.

Selectivity of mercury complexes to shoot transport

Literature indicates that plants are selective to the form of metal transported to above-ground tissues (Blaylock *et al.*, 1997; Anderson *et al.*, 1998; Moreno *et al.*, 2004a). For example, the accumulation of Hg was confined within root tissues for plants species grown in Hg-contaminated mine tailings treated with soluble $HgCl_2$ at 1, 5 and 10 mg kg^{-1} (Moreno *et al.*, 2004a). The pH of substrates was around 5.5 and the Ni-hyperaccumulator *Berkheya coddii* and the salt-tolerant *Atriplex canescens* showed no evidence of stress to Hg-exposure. However, translocation to the upper plant parts was restricted for all tested concentrations, yielding a maximum concentration factor (concentration shoot tissue/concentration in soil) of only 0.8 at the highest Hg concentration. By contrast $(NH_4)_2S_2O_3$-treated plants grown on the highest substrate level (3.4 mg Hg kg^{-1}, Table 1) accumulated Hg in shoots at an average of 85 mg kg^{-1} (Fig. 3a), hence yielding a concentration factor of 25. Since thiosulphate complexes are stable at neutral to alkaline conditions (Bowell *et al.*, 1993) and the pH of the modified substrates was around 8 (Table 1), it is plausible that $Hg-S_2O_3$ complexes were selected for shoot transport over other dissolved Hg species present in the substrate.

Effect of humic acid on root-to-shoot transport

Despite the strong affinity of Hg for organic matter, few studies have examined the effect of these substances on the uptake and translocation of Hg in higher plants. Limited dissociation of Hg bound to soil organic components translates into low Hg availability in soils and, therefore, restricted Hg uptake by plants. For example, the addition of organic matter to Hg-contaminated mine spoil has been negatively correlated with the Hg tissue concentrations in Zorro fescue (*Vulpia myuros* L.) (Heeraman *et al.*, 2001). Humic acid from decayed plant material suppressed Hg uptake for duckweed (*Lemna minor*) grown in a hydroponic medium containing Hg at 10 mg l^{-1} (Mo *et al.*, 1989). Similarly, the root concentration factor (concentration root tissue/concentration in soil) of *Brassica chinesis* and *Lactuca sativa* was significantly decreased with increasing humic acid concentrations in two types of Hg-contaminated soils (Wang *et al.*, 1997). The reasons for

the decline in plant Hg concentrations were attributed to a decrease in the soluble Hg fraction due to Hg complexation with either organic matter or humic acid. By contrast, our studies showed that the Hg soluble fraction of modified substrates was significantly increased in the presence of HA due to Hg–HA complex formation (Fig. 2). Furthermore, the discrepancy in the root Hg values between water and $(NH_4)_2S_2O_3$-treated plants (respectively, 13.7 and 99.8 mg kg^{-1} at 1.25 HA, Fig. 4a) was possibly linked to the uptake of Hg–HA and Hg–thiosulphate complexes by plants. Considering that Hg–HA complexes were soluble in the aqueous phase of the HA-amended substrates, then Hg root uptake would be initially a function of the available Hg–HA complexes in the substrate solution. Subsequently, the application of $(NH_4)_2S_2O_3$ to substrates would have mobilised the unavailable Hg fraction through Hg–thiosulphate complex formation, enhancing Hg accumulation in root and shoot tissues. The significantly positive correlation between extractable Hg and root Hg concentration (Fig. 5a) indicates that root tissues absorbed both Hg–HA and Hg–thiosulphate complexes. Furthermore, the evidence of negative correlation between extractable Hg and shoot Hg concentration indicates that Hg translocation to aerial tissues was severely restricted in the presence of HA (Fig. 5b). Our results indicate therefore that Hg–thiosulphate complexes were translocated into aerial tissues to the detriment of Hg–HA complexes, which were probably adsorbed to root tissues.

Conclusions

The results of this study show that Hg availability and Hg plant uptake are interrelated processes that appear to be controlled by plant species, the presence of sulphur-containing ligands, substrate Hg concentration, and humic acid content. Mercury solubility was significantly enhanced in the presence of thiosulphates and HA and may be related to the formation of Hg–thiosulphate and Hg–HA complexes. The Hg content in root and shoot tissues was significantly enhanced in the presence of thiosulphates but the Hg accumulation pattern was markedly different between plant species. For example, in the presence of $(NH_4)_2S_2O_3$, root Hg accumulation was greater for *V. villosa*, whereas shoot Hg translocation was superior for *P. vulgaris*. We believe that plant species characteristics played an important role in the enhanced root uptake and transport for Hg in the presence of thio-solutions. The uptake and translocation of Hg in *B. juncea* plants was shown to be dependent on the speciation of the Hg complex. In the presence of $(NH_4)_2S_2O_3$, shoot Hg translocation was increasingly promoted as a function of substrate Hg concentration. The Hg accumulation in shoot tissues in the presence of this ligand increased up to 25 times at the highest Hg level in the substrate. However $(NH_4)_2S_2O_3$-treated plants showed suppression of Hg translocation in the presence of HA. It is therefore plausible that Hg–thiosulphate complexes were transported to shoot

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tissues to the detriment of Hg–HA complexes, which were retained in root tissues.

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