Available online at www.sciencedirect.com
ScienceDirect
Environmental and Experimental Botany 62 (2008) 78–85
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Phytofiltration of mercury-contaminated water: Volatilisation and plant-accumulation aspects

Fabio N. Moreno [a,*], Christopher W.N. Anderson [b], Robert B. Stewart [b], Brett H. Robinson [c]

[a] *Institute of Geosciences, University of São Paulo, R. do Lago, 562, Cidade Universitária, CEP 05508-080 São Paulo, SP, Brazil*
[b] *Soil and Earth Sciences, Institute of Natural Resources, Massey University, Private Bag 11222, Palmerston North, New Zealand*
[c] *Institute of Terrestrial Ecosystems, Swiss Federal Institute of Technology (ETH), Universitätstrasse 16, CH-8092 Zürich, Switzerland*

Received 12 July 2007; accepted 25 July 2007

Abstract

Phytofiltration may be a cost-effective approach for treating Hg-contaminated wastewater. We investigated the removal of Hg from solutions by Indian mustard [*Brassica juncea* (L.) Czern.] grown in hydroponic conditions with solutions containing Hg concentrations from 0 to 10 mg/L. Plants were enclosed in gastight volatilisation chambers to assess the effect of Hg concentrations on plant transpiration, accumulation and volatilisation. We also determined the speciation and site of origin of volatilised Hg. Solution Hg concentrations of 5 and 10 mg/L detrimentally affected transpiration. Roots concentrated Hg 100–270 times (on a dry weight basis) above initial solution concentrations. The plants translocated little Hg to the shoots, which accounted for just 0.7–2% of the total Hg in the plants. Volatilisation from planted vessels increased linearly as a function of Hg concentrations in solutions. Most Hg volatilisation occurred from the roots. Volatilised Hg was predominantly in the Hg(0) vapour form. Volatilisation was dependant on root uptake and absorption of Hg from the ambient solution. Production of Hg(0) vapour in the solutions may result from the activity of root-associated algae and Hg-resistant bacteria. Phytofiltration effectively removed up to 95% of Hg from the contaminated solutions by both volatilisation and plant accumulation. However, Hg(0) vapours released from living roots may have unforeseen environmental effects.

Keywords: Mercury; Translocation; Root absorption; Hg(0) vapour; Phytoremediation

1. Introduction

Although developed nations have long banned the use of metallic mercury (Hg) for gold (Au) extraction, miners from the developing world still employ it in artisanal and small-scale gold mining (ASM) operations. In developing countries, ASM may release up to 450–800 tonnes per year of metallic Hg into the environment (Lacerda, 2003; Veiga, 2004). This figure corresponds to 10–20% of global Hg anthropogenic emissions. Veiga (2004) estimated that Brazil and China together contribute with as much as 300 tonnes per year of metallic Hg because of ASM.

Gold extraction using metallic Hg consists of four main steps: amalgamation, separation of mineral portion, amalgam decomposition and gold melting (Veiga and Hinton, 2002). Environmental contamination due to ASM mostly occurs from atmospheric deposition of emitted Hg(0) because of amalgam decomposition and gold smelting. Mercury also contaminates water bodies and soils after the discharge of water used to wash away amalgam residues (Veiga and Hinton, 2002). The amalgam residues may contain up to 450 mg/kg of residual Hg, whereas the Hg concentration in the wastewater varies from 0.5 to 3 mg of Hg per kg of suspended matter (Oliveira et al., 2004).

Organic acids oxidise the metallic Hg deposited in surface soils and waters, thus forming soluble Hg(II) complexes, which may be transported to areas away from the pollution source (Veiga, 2004). Owing to the toxicity and biomagnification potential of methylmercury in the food chain, Hg(II) methylation is a critical step in the Hg biogeochemical cycle. Biotic and abiotic methylmercury pathways include enzymatic transference of CH_3 radicals from methylcobalamin to Hg(II) via sulphate-reducing bacteria (Barkay et al., 2003) and methylation in the presence of alkylated and methyl tin compounds (Cerrati et al.,

* Corresponding author. Tel.: +55 11 3277 5461; fax: +55 11 3277 5461.
E-mail address: fabionmoreno@terra.com.br (F.N. Moreno).

0098-8472/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2007.07.007

1992; Weber, 1993), carboxylic acids (Falter, 1999) and humic and fulvic acids (Weber, 1993), respectively.

Phytoremediation describes the use of plants to improve degraded environments. Developing nations that lack financial support and incentives to implement remedial procedures could benefit from cost-effective plant-based systems to remove toxic metals from soil and water. Phytofiltration employs aquatic and terrestrial plants with the ability to remove metals, metalloids and radionuclides from polluted groundwater, surface water and wastewater (Dushenkov et al., 1995). Plants such as As-hyperaccumulating ferns (*Pteris vittata*, *Pteris cretica*), Indian mustard (*Brassica juncea*), sunflower (*Helianthus annuus*) and water hyacinth (*Eichornia crassipes*) may remove As, Cu, Pb, Ni, Zn, Cr, Cd, Pu, U and Hg from aqueous solutions by precipitating, adsorbing or concentrating these elements in their roots or shoot tissues (Dushenkov et al., 1995, 1997; Lytle et al., 1998; Riddle et al., 2002; Axtell et al., 2003; Huang et al., 2004).

We aimed to investigate the phytofiltration potential of Hg by *B. juncea* plants with a view for treating Hg-contaminated wastewater. Since previous works have reported Hg losses from the plant–soil system by Hg(0) volatilisation (Moreno et al., 2005a,b), we hypothesize that this terrestrial plant species could remove Hg from water by both volatilisation and plant-accumulation processes. Specifically we sought to evaluate the effect of Hg concentrations on plant transpiration, accumulation and volatilisation and to examine speciation and site of origin of volatilised Hg produced in the air–plant–solution system.

2. Material and methods

2.1. Plant growth culture

Plastic pots (100 mL) were filled with silica-sand and sown with seeds of Indian mustard (*B. juncea*) at a rate of ∼10 seeds per pot ($n = 20$). The pots were watered and placed inside a plant growth chamber with the temperature maintained at $22 \pm 2\,^{\circ}C$ and a photoperiod of 14 h. All seeds germinated after 1 week. Hoagland and Arnon (1950) solution (one-fourth strength, 10 mL) was added every day to provide the plant's nutritional requirements. After the second week, the seedlings were gently washed to remove the sand and transferred to plastic pots containing 100 mL of one-fourth strength Hoagland's solution at pH 6. The nutrient solution, replaced weekly, was aerated using a small pump. The seedlings were used for the experiments 12 days after transplantation into the nutrient solutions.

2.2. Effect of Hg concentration on plant transpiration, accumulation and volatilisation

The treatments consisted of dissolution of $HgCl_2$ in deionised water to give the following Hg concentrations: 0, 0.05, 0.5, 1, 2.5, 5 and 10 mg/L. The effect of Hg concentrations on plant transpiration, root and shoot accumulation and volatilisation was measured for each treatment. The plant roots were washed with reverse osmosis (RO) water and then immersed in 100 mL of Hg-containing solutions. The solution pH was maintained at 6 by adding either NaOH or HCl solutions (0.01 M). Transpiration, determined gravimetrically using a four-figure balance, was measured at the beginning of the experiment (day 0) and thereafter at 24 h intervals. Mercury volatilisation was quantified by enclosing the root system in a gastight root compartment (100 mL plastic vessel). Outlet tubes carried air from the root compartments into a trapping system that consisted of a 10 mL vial containing 5% $KMnO_4$ in 1 M H_2SO_4 (Moreno et al., 2005a,b). The vial was open to the atmosphere to maintain pressure equilibrium within the trap system. Plant transpiration, accumulation and volatilisation were measured in triplicates ($n = 3$) over a period of 5 days.

2.3. Speciation and site of origin for volatilised Hg

The assessment for volatilised Hg speciation and site of origin was performed for plants exposed to 1 mg/L of Hg (as $HgCl_2$) in the ambient solution. A two-trap system in series captured inorganic and organic Hg vapours released from shoots and roots of the *B. juncea* plants. The experimental unit consisted of two small air pumps, two gastight volatilisation chambers (3.6 L and 100 mL of volume) and a set of four Hg traps (Fig. 1). Trap solutions used in this work followed the protocols of Yannai et al. (1991), Devars et al. (2000) and Moreno et al. (2005a,b). Mercury vapour released from the shoots of each plant was contained within the larger volatilisation chamber. The vapour then passed sequentially through inorganic and organic traps by continuous airflow (1 L/min). Inorganic Hg vapour [elemental Hg(0)] was trapped in a 70 mL solution containing 5% $KMnO_4$ dissolved in 1 M H_2SO_4 (T1). Organic Hg vapours were trapped in a 70 mL solution containing 5% Na_2CO_3 and 2.5% Na_2HPO_4 (T2). Both trap solutions were contained within 125 mL Erlenmeyer's flasks. Outlet tubes carried air from the shoot and root compartments into the Hg traps. The outlet of the inorganic trap (T2) was open to the atmosphere to maintain pressure equilibrium within the trap system. Mercury vapour released from the root system was contained using a gastight root compartment (as described above). The gastight root compartment, a 100 mL plastic vessel, was placed inside the larger gastight shoot com-

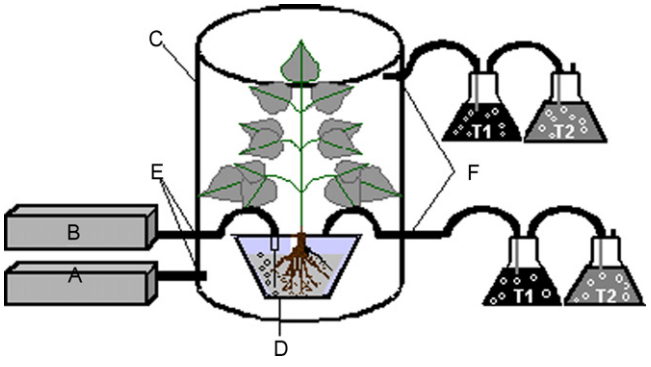

Fig. 1. Experimental apparatus used for capturing volatilised Hg from roots and shoots of *B. juncea* plants cultured in solution containing Hg at 1 mg/L. Controls comprised of the same unit without plants. (A and B) Air pumps; (C) gastight shoot compartment (3.6 L); (D) gastight root compartment (100 mL) aerated by a syringe needle; (E) air inlets; (F) air oulets; (T1) permanganate acid trap; (T2) carbonate-phosphate trap.

partment (3.6 L) (Fig. 1). Volatile Hg speciation and site of origin were measured in triplicates ($n = 3$) over a period of 3 days.

Both experiments occurred inside a plant growth chamber with the temperature controlled at $22 \pm 2\,^{\circ}C$ and photoperiod set to 14 h. All the measured parameters were monitored by the use of controls ($n = 3$, unless otherwise stated), which were comprised of the same experimental set up without plants. Aluminium foil was wrapped around the root compartment to prevent algae growth and/or photochemical reactions. Air was bubbled into the root compartment using continuous airflow (1 L/min). The flow rate of the aeration system was monitored using an air flow meter (J&W, model AMD 1000) and was adjusted to 100 mL/min using small clamps attached to the air outlets. After collection of volatilised Hg, trap solutions were transferred to airtight plastic containers and stored at 4 °C. The precipitated fraction of the permanganate acid trap was redissolved with concentrated hydrochloric acid and the resulted solution was stored following the same procedure.

2.4. Plant digestion

At the end of the experiments, shoots and roots were harvested and washed in tap water. Roots were separated from shoots and placed in a drying oven at 70 °C until a constant weight was obtained. After the total dry weight of root and shoots was recorded, the samples were ground. Subsamples (0.1 g) were then accurately weighed into 50 mL plastic pots and digested with 15 mL of HNO_3. The plant samples were left overnight and, in the following day, were heated in a water bath at 80 °C for 1 h. Plant digests were transferred to 10 mL polythene tubes and diluted with reverse osmosis (RO) water to make a final volume of 10 mL. A blank reagent was used with all digestions.

2.5. Mercury analysis

The total Hg concentrations of the plant digests, hydroponic solutions and Hg traps were determined using hydride-generation atomic absorption spectroscopy (Moreno et al., 2004) using a GBC 909A AAS (Victoria, Australia). 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 Hg in solution was 5 ng/mL. Reagent blanks were below detection limits in the solution. Linear calibration curves were obtained over the range of 125–1000 ng/mL of Hg using four standards prepared from a 10 mg/L mercuric nitrate ($HgNO_3$) Spectrosol solution (May & Baker, AAS standard reagent solution, England). The Hg readings obtained from the replicate analysis ($n = 10$) of a standard solution containing 1 mg/L of Hg could be reproduced with less than 5% of variation. An external certified laboratory assessed the quality of the analytical method. The maximum discrepancy between the results was 15% (Moreno et al., 2004).

2.6. Statistical analysis

A copy of SAS PC version 8e was used for statistical analyses (SAS Inst, 1988). The Student's *t*-test was used to compare two means. Differences between three or more means were performed by a one-way analysis of variance (ANOVA). Duncan's test was used for pair-wise comparison of means at 0.05 and 0.01 significance levels. Linear and polynomial regression models were used to provide 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. Log-normally distributed data were log-transformed.

3. Results

3.1. Effect of Hg concentration on plant transpiration, accumulation and volatilisation

Mercury significantly reduced plant transpiration (Fig. 2). Increasing Hg concentrations in the solutions reduced transpiration rates by 1.4–5.1-fold when compared to the control (0 mg/L) ($P < 0.05$). In spite of this, Hg-exposure did not affect dry matter yields ($P > 0.05$) (Table 1) and plants exhibited no visible sign of chlorosis at the end of the experiment.

We expressed the plant Hg accumulation in terms of bioconcentration factors (BF). The BF is defined here as the concentration in plant tissues/concentration in the ambient solution. Table 1 shows the plant Hg-accumulation and BFs for *B. juncea* grown in solution containing various Hg concentrations. The Hg concentrations in roots and shoots increased linearly as a function of Hg treatments (roots, $r^2 = 0.80$, $P < 0.001$; shoots, $r^2 = 0.85$, $P < 0.001$). Root Hg concentrations were at least an order of magnitude higher than shoot Hg concentrations. The greater ability of roots to concentrate Hg was also shown by root BF values, which were around 130–340-fold higher than shoot BF values. Although tissue Hg concentrations were higher at the

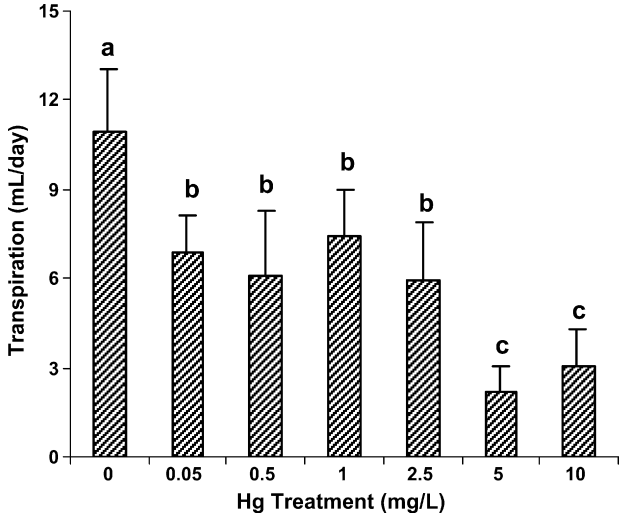

Fig. 2. Transpiration rates for *B. juncea* plants cultured for 5 days in solutions containing Hg at concentrations ranging from 0 to 10 mg/L. Values are the means of three replicates ± 1 standard deviation. Letters compare the treatment means. Means with the same letters are not statistically different (Duncan's test at $\alpha = 0.05$).

Table 1
Dry weights, plant Hg concentrations and the bioconcentration factor (BF) for *B. juncea* plants cultured for 5 days in solutions containing Hg at concentrations ranging from 0 to 10 mg/L

Hg (mg/L)	Dry weight (mg)		Plant Hg (mg/kg DW)		BF[a]	
	Root	Shoot	Root	Shoot	Root	Shoot
0	150 ± 08	382 ± 53	BDL	BDL	NA	NA
0.05	136 ± 28	496 ± 50	5 ± 01	BDL	100	NA
0.5	119 ± 30	316 ± 19	136 ± 36	0.52 ± 0.14	272	1.04
1	162 ± 15	445 ± 79	142 ± 07	1.07 ± 0.08	142	1.07
2.5	162 ± 24	424 ± 17	327 ± 68	1.56 ± 1.18	130	0.62
5	144 ± 11	345 ± 78	532 ± 110	1.56 ± 1.16	106	0.31
10	125 ± 33	443 ± 55	1076 ± 572	3.56 ± 2.21	107	0.35

Values are the means of three replicates ± 1 standard deviation. BDL, Below detection limits; NA, not applicable.
[a] The bioconcentration factor was calculated according to the equation: total Hg concentration in root and shoot tissues (mg/kg DW)/initial Hg concentration in solutions (mg/L).

upper Hg range, maximum BF values occurred at intermediate Hg concentrations (Table 1).

Table 2 shows the effect of Hg treatments on root and shoot Hg accumulation, translocation and the shoot/root Hg concentration quotient (shoot:root ratio). There was no significant correlation between the Hg mass accumulated and the solution Hg concentration. The highest values occurred at intermediate Hg concentrations and significantly decreased at solution Hg concentrations of 5 and 10 mg/L. This was not true for shoots, whose effective Hg accumulation values decreased significantly as a function of Hg treatments ($r^2 = 0.34$, $P < 0.05$). For each treatment, roots accumulated around 12–30% of the total Hg mass added whereas the effective accumulation values for shoots were never above 0.5%. Table 2 also indicates that only a small fraction, between 0.7 and 2%, of the Hg mass taken up by roots was translocated to the shoots. For all solution concentrations, shoot concentrations ranged from 0.3 to 0.75% of the total Hg taken up by roots, as shown by the shoot:root ratio. This distribution pattern indicates restricted transport of soluble Hg into plant shoots.

Table 2
Effective Hg accumulation in plant tissues (roots and shoots), translocation and the shoot:root ratio for *B. juncea* plants cultured for 5 days in solutions containing Hg at concentrations ranging from 0.05 to 10 mg/L

Hg (mg/L)	Hg in roots[a] (%)	Hg in shoots[a] (%)	Translocation[b] (%)	Shoot:root ratio[c] (%)
0.05	14.4 ± 2.0	NA	NA	NA
0.5	31.1 ± 5.5	0.32 ± 0.05	1.14 ± 0.11	0.41 ± 0.24
1	22.9 ± 1.7	0.47 ± 0.05	2.02 ± 0.05	0.76 ± 0.06
2.5	21.5 ± 6.8	0.26 ± 0.19	1.51 ± 1.61	0.54 ± 0.52
5	15.1 ± 1.9	0.10 ± 0.03	0.73 ± 0.32	0.30 ± 0.07
10	12.1 ± 3.0	0.16 ± 0.11	1.22 ± 0.22	0.31 ± 0.08

Values are the means of three replicates ± 1 standard deviation. NA, Not applicable.
[a] Effective accumulation in roots and shoots was calculated according to the equation: total Hg mass in roots and shoots (μg)/total Hg mass (μg) in the solutions at the beginning of the experiment × 100.
[b] Effective translocation was calculated according to the equation: total Hg mass in shoots (μg)/total Hg mass in the plant (μg) × 100.
[c] The shoot:root ratio was calculated according to the equation: total Hg concentration in shoots (mg/kg DW)/total Hg concentration in roots (mg/kg DW).

Fig. 3 shows the mass of Hg volatilised from the root compartment and captured in the acid permanganate traps as a function of Hg treatments after the end of the experiment. The detection of minor amounts of Hg, about 0.5 μg, in the control treatment indicated contamination of the trap by Hg species in the air. The Hg emissions from planted vessels increased linearly from 0.625 to 314 μg at the 0–10 mg/L range ($r^2 = 0.76$, $P < 0.0001$) and were significantly greater than controls for all tested concentrations ($P < 0.05$) (Fig. 3). The discrepancy between plants and controls in relation to Hg mass emissions was greater at the upper Hg range. Planted vessels volatilised 35–42 times more Hg than the unplanted controls at 5 and 10 mg of Hg/L of solution.

3.2. Speciation and site of origin of volatilised Hg

Table 3 shows the mass of volatilised Hg captured from shoot and root compartments in the acid permanganate (T1) and

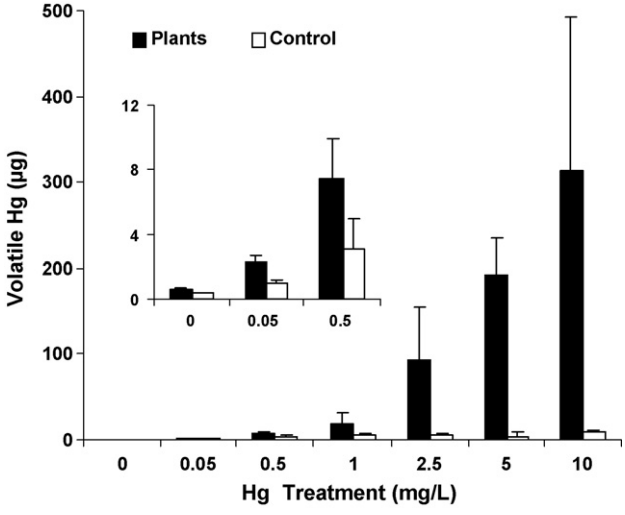

Fig. 3. Mercury mass (μg) captured in permanganate acid traps for *B. juncea* plants cultured for 5 days in solutions containing Hg at concentrations ranging from 0 to 10 mg/L. Controls comprised of Hg-treated solutions without plants. Values are the means of three replicates ± 1 standard deviation (S.D.), except for controls ($n = 2 \pm 1$S.D.). The inset displays the volatilised Hg mass (μg) for the first three treatments.

Table 3
Mercury mass (μg) captured in acid permanganate (T1) and carbonate-phosphate (T2) traps for *B. juncea* plants cultured in solutions containing Hg at 1 mg/L for 3 days

Treatment/compartment	N[a]	Hg mass in traps (μg)		Total Hg mass[b] (μg)
		T1	T2	
Plant/roots	6	33.85 ± 5.5 A	0.94	34.16 ± 5.2 A
Control/roots	6	5.60 ± 1.7 Ba	0.51 ± 0.01 b	6.11 ± 1.8 B
Plant/shoots	6	1.76 ± 0.29 Aa	0.60 ± 0.22 Ab	2.17 ± 0.66 A
Control/shoots	6	0.90 ± 0.33 Ba	0.54 ± 0.04 Aa	1.45 ± 0.37 A

Controls comprised of Hg-treated solutions without plants. Values are the means of three replicates ± 1 standard deviation. Upper and lower-case letters compare treatment means in the vertical and in the horizontal, respectively. Means with the same letters are not statistically different (Duncan's test at $\alpha = 0.05$).

[a] $N = 3$ for each T1 and T2 traps.

[b] Total Hg mass is the arithmetic mean of three replicates for the sum of the Hg mass captured in T1 and T2 traps.

carbonate-phosphate (T2) traps for plants exposed at 1 mg/L of Hg. For the T1 traps, volatilisation from root and shoot compartments were significantly higher than controls ($0.001 < P < 0.05$). Within the plant treatment, the mass of Hg in T1 traps was an order of magnitude greater than the mass in T2 traps ($P < 0.01$). The total Hg emission from the root compartment was 34.16 μg, representing around 94% of the total Hg mass captured by the T1 and T2 traps. Mercury volatilisation from the shoot compartment was not significantly different from the control ($P > 0.05$), indicating that volatilised Hg is not released from plant shoots.

3.3. Mercury removal from solution

Fig. 4 shows the Hg removal by *B. juncea* as a function of solution Hg concentration. Mercury concentrations in planted treatments were significantly lower than in controls for all Hg concentrations ($P < 0.05$), except at 0.05 mg/L ($P > 0.05$). The removal of Hg from solution (phytofiltration) was most efficient at intermediary concentrations, where plants removed between 80 and 95%. The Hg concentration in solution as a function of time for plants exposed at 1 mg Hg/L (Fig. 5) shows that the Hg concentration decreased 80-fold to 0.0125 mg/L in just 48 h. Equilibrium occurred at 0.061 mg/L.

Fig. 6 shows the contribution of plant accumulation and volatilisation mechanisms to the overall removal from solutions for each Hg solution concentration. Although both mechanisms were active in the removal process, their contribution to Hg extraction varied over the tested range, with plant tissues containing the highest proportion of Hg in solutions containing 1 mg/L of Hg. The volatilisation contribution increased as the solution concentrations diverged from 1 mg/L (Fig. 6). Thus, volatilisation depends on the root uptake of Hg from solutions. The significant and positive correlation found between volatilisation and root-Hg accumulation supported this relationship ($r = 0.98$, data not shown).

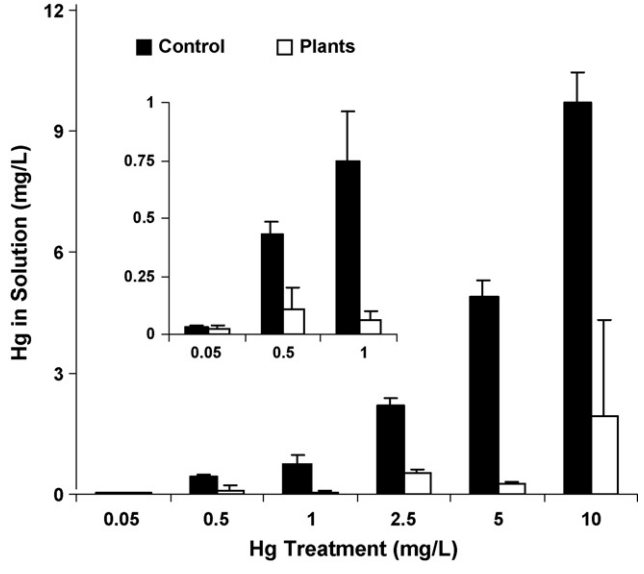

Fig. 4. Phytofiltration potential of *B. juncea* plants cultured for 5 days in solutions containing Hg at concentrations ranging from 0.05 to 10 mg/L. Controls comprised of Hg-treated solutions without plants. Values are the means of three replicates ± 1 standard deviation (S.D.), except for controls ($n = 2 \pm 1$S.D.). The inset displays the phytofiltration potential for the first three treatments.

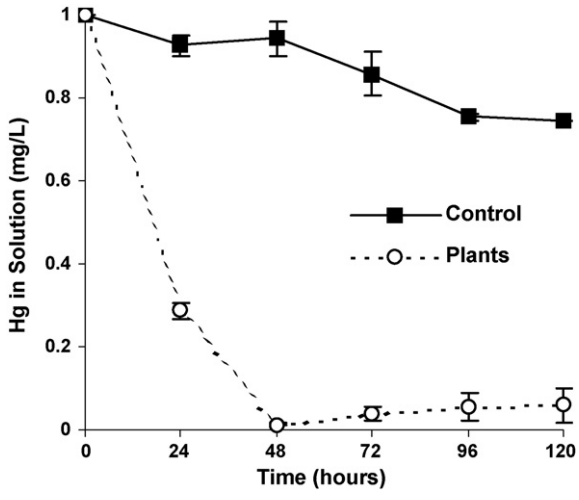

Fig. 5. Phytofiltration potential of *B. juncea* plants cultured in Hg-containing solutions at 1 mg/L. Controls comprised of Hg-treated solutions without plants. Values are the means of three replicates ± 1 standard deviation (S.D.), except for controls ($n = 2 \pm 1$S.D.).

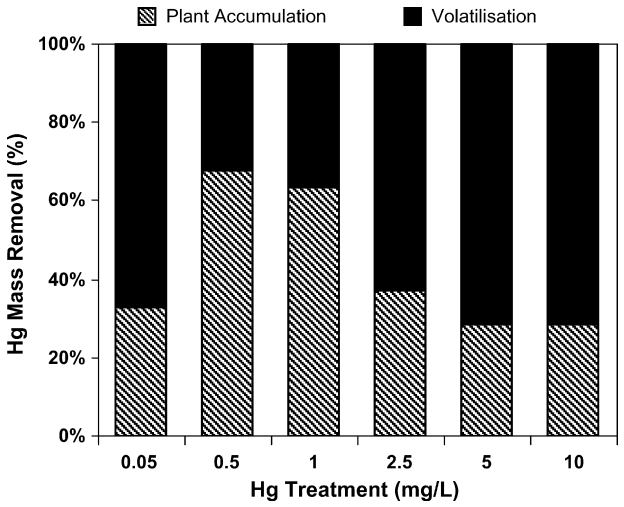

Fig. 6. Contribution of plant accumulation and volatilisation processes to the Hg mass removal by phytofiltration. *B. juncea* plants were cultured for 5 days in solutions containing Hg at concentrations ranging from 0 to 10 mg/L. Note that 100% = sum of total Hg mass removed by both plant accumulation (root + shoots) and volatilisation.

4. Discussion

4.1. Effect of Hg on plant transpiration

The toxic effect of Hg on plants can arise from enzyme inhibition by the linkage of Hg to sulphydryl groups (Ferreira et al., 1998). Such a breakdown of the normal –SH system affects the germination and growth of embryos because these tissues are particularly rich in SH-groups (Patra and Sharma, 2000). Ferreira et al. (1998) reported the primary effects of Hg toxicity in aquatic and terrestrial plants to be reduced levels of biomass, photosynthetic activity, total chlorophyll, as well as lower contents of nitrogen, phosphorus and potassium. Conversely, peroxidase activity, malondialdehyde levels and induction of thiol-enriched molecules increase in response to Hg exposure (Gupta et al., 1998; Ali et al., 2000; Moreno-Jiménéz et al., 2006). In our study, we observed reduced plant transpiration rates at the lowest level of Hg (0.05 mg/L) in solution (Fig. 2). This effect may be linked to disruptions in the structure and function of the plasma membrane, altering its permeability to water and nutrient uptake (Patra and Sharma, 2000). Exposure to $HgCl_2$ may have damaged the membrane of cells, thus resulting in decreased water uptake by *B. juncea*. This effect was more pronounced at high Hg concentrations in the solutions, as shown in Fig. 2.

4.2. Mercury retention by the root system

Since roots accounted for 98–99% of the total plant Hg (Table 2), it is possible that this Hg retention process was a physicochemical reaction enhanced by the hydroponic conditions. The dichloro-mercuric complex, $HgCl_2$, is relatively non-polar and rather lipid-soluble. The octanol–water partition coefficient (K_{ow}) for $HgCl_2$ is 3.3, indicating that this complex readily crosses the lipid bilayers of the plasma membrane. However, the chemistry of Hg in oxygen-containing waters indicates that at pH around 6, the Hg(II) in solution should be complexed to hydroxide ions (Morel et al., 1998). The K_{ow} for the uncharged mercuric hydroxide complex, $Hg(OH)_2$ (=0.5), is lower than $HgCl_2$ indicating that this complex is slightly more hydrophobic and will diffuse only slowly through the lipidic bilayer of the plasma membrane. Therefore, the increased retention of soluble Hg to roots may be caused by adsorption of $Hg(OH)_2$ and $Hg(OH)^+$ complexes to binding sites on the cell wall. Experiments with ^{203}Hg-spiked solutions demonstrated that most Hg taken up by roots of *Triticum durum* cv. Creso after 25 days exposure was found on cell walls at the external layers of the cortical cylinder (Cavallini et al., 1999). Root Hg accumulation by *B. juncea* plants may relate to the movement of soluble Hg complexes in the apoplast, an interconnected system that includes all cell walls in the root cortex (Salisbury and Ross, 1992). The binding of Hg to specific sites on the cell wall can restrict transport to sensitive sites in shoots and is an important factor to the Hg tolerance in plants (Cavallini et al., 1999).

In spite of the limited translocation of Hg to shoot tissues even at high Hg concentrations (Table 2), there was a significant and positive correlation between Hg translocation and transpiration ($r = 0.82$) (data not shown). This indicates that simple diffusion and mass flow of water would have delivered Hg to the plant shoots via the xylem. Uptake and upward transport of Hg may have occurred across the plasma membrane via the symplast pathway. Since a range of metals, including Hg, can induce phytochelatin synthesis (Rauser, 1995), movement of Hg in the symplast was likely in the form of Hg-thiol complexes.

4.3. Speciation and site of origin for volatilised Hg

The higher recovery rate of Hg in the permanganate acid solution and the lack of evidence for the contribution of shoots in the volatilisation process (Table 3) indicate that the root system of *B. juncea* releases Hg predominantly as the elemental Hg(0) vapour form. A mass balance of Hg in the air–plant–solution system (Table 4) validates these results. The efficiency for Hg recovery using the trapping system averaged more than 90% with no significant differences between plant and control treatments ($P > 0.05$) (Table 4). Our results, therefore, corroborate with Greger et al. (2005), who demonstrated the absence of Hg transpiration from shoots of six terrestrial plants, including oil-seed rape (*Brassica napus* L. cv Paroll).

Bacterial reduction of Hg(II) to Hg(0) via mercuric-reductase is a recognized pathway for the volatilisation of Hg from contaminated environments (Barkay et al., 1992). This pathway is carried out by a diversity of Hg-resistant bacteria (both Gram positive and negative) living in soil and water (Barkay et al., 2003). In the aquatic environment, some algae are able to produce Hg(0) vapours either in the light or in the dark (Ben-Bassat and Mayer, 1978; Devars et al., 2000). Volatilisation of Hg(0) by *Euglena gracilis* is a light-independent process mediated by metabolites capable of reducing Hg(II) (Devars et al., 2000). Neither the nutrient media nor the Hg-containing solutions, however, were pre-treated with algaecides or bactericides.

Table 4
Balance sheet for the Hg mass in the air–plant–solution system at the end of the experiment[a]. *B. juncea* plants were treated with soluble Hg at 1 mg/L for three days. Controls comprised of Hg-treated solutions without plants. Values are the means of three replicates ± 1 standard deviation. Letters compare treatment means in the vertical. Means with the same letters are not statistically different (Duncan's test at $\alpha = 0.05$)

Treatment	Volatilised Hg[b] (μg)	Plant Hg[c] (μg)	Solution Hg (μg)	Total Hg[d] (μg)
Plant	36.05 ± 5.71 A	44.84 ± 5.8	9.79 ± 1.3 A	90.81 ± 3.32 A
Control	7.56 ± 2.09 B	NA	84.39 ± 4.4 B	91.96 ± 2.33 A

NA, Not applicable.
[a] Note that the total Hg mass at the beginning of the experiment was 100 μg of Hg in 100 mL of solution.
[b] Volatilised Hg is the arithmetic mean for the sum of the Hg mass captured in root and shoot compartments for both T1 and T2 traps.
[c] Plant tissue Hg is the arithmetic mean for the sum of the Hg mass in root and shoot tissues.
[d] Total Hg is the arithmetic mean for the sum of the Hg mass in the air, plant and solution compartments.

This indicates the participation of root-associated algae or Hg-resistant bacteria in the volatilisation process.

4.4. Implications for the phytofiltration of Hg-contaminated wastewater

Living roots of *B. juncea* plants were able to reduce 2–18 times the initial Hg concentrations in the contaminated solutions (Fig. 4). At 1 mg/L, for example, the Hg concentration dropped to the Brazilian regulatory discharge levels of 0.01 mg/L (CONAMA, 2005) within 48 h (Fig. 5). Phytofiltration has the potential to treat low levels of Hg-contaminated wastewaters from ASM before discharge into local water bodies. Further Hg reductions to drinking water standards (0.001 mg/L) (Casarini, 2001) may be attainable by wastewater flow through several connected phytofiltration units, as has been proposed for uranium and arsenic contaminated waters (Dushenkov et al., 1997; Elless et al., 2005). Reductions in soluble Hg correlated well with increasing concentrations of Hg in roots ($r = 0.84$). Therefore, each additional treatment unit should double the Hg removal efficiency. The amount of water and the target final concentration will dictate the number of treatment units needed. In our experiments, one single plant with around 20 mL of roots was used to treat 100 mL of water (1:5, v/v). Therefore, 1000 plants of this size could treat 5000 L of Hg-contaminated water in just 48 h.

Due to Hg(0) volatilisation, between 30 and 70% of the extracted Hg was lost to the air at the end of the experiment (Fig. 6). Although important from an environmental point of view, the scaling up of the volatilisation data to a water treatment scenario would be unrealistic as the removal of Hg from solution was poorly correlated to the Hg(0) mass volatilised from planted vessels ($r = 0.13$). Furthermore, there is evidence that Hg removal by volatilisation is related to biotic factors that could not be controlled under the experimental conditions used in this study. It thus might be possible to decrease biological volatilisation by the addition of antibiotics to the Hg-contaminated water. More studies, however, are needed to elucidate the biological pathways by which Hg(II) is reduced in the system and how plant roots affect this process.

5. Conclusions

When exposed to varied range of Hg concentrations, *B. juncea* can concentrate Hg in roots 100–270 times higher than the initial Hg solution concentrations. Mercury was more toxic to plants at 5 and 10 mg/L. Accumulation of Hg by roots peaked at 0.5 and 1 mg/L of Hg in the solutions. Thus, removal of Hg from solutions by plant accumulation was dominant at these intermediate Hg concentrations. Volatilisation was dependant on the roots absorption of Hg. In view of the Hg chemistry in solution, root retention is probably due to adsorption of $Hg(OH)_2$ and $HgOH^+$ complexes to binding sites on the cell wall. Production of Hg(0) vapour occurred at the root interface, possibly augmented by algal and bacterial activity. The remediation of Hg-contaminated wastewater from ASM by phytofiltration is promising but the remediation relies on Hg losses from the system by Hg(0) volatilisation.

Acknowledgement

We gratefully acknowledge the National Council for Scientific and Technological Development (CNPq/Brazil) for providing a post-doctoral scholarship to the first author.

References

Ali, M.B., Vajpayee, P., Tripathi, R.D., Rai, U.N., Kumar, A., Singh, N., Behl, H.M., Singh, S.P., 2000. Mercury bioaccumulation induces oxidative stress and toxicity to submerged macrophyte *Potamogeton crispus* L. Bull. Env. Cont. Toxicol. 65, 573–582.

Axtell, N.R., Sternberg, S.P.K., Claussen, K., 2003. Lead and nickel removal using *Microspora* and *Lemna minor*. Biores. Technol. 89, 41–48.

Barkay, T., Turner, R., Saouter, E., Horn, J., 1992. Mercury biotransformations and their potential for remediation of mercury contamination. Biodegradation 3, 147–159.

Barkay, T., Miller, S.M., Summers, A.O., 2003. Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol. Rev. 27, 355–384.

Ben-Bassat, D., Mayer, A.M., 1978. Light-induced Hg volatilization and O_2 evolution in *Chlorella* and the effect of DCMU and methylamine. Physiol. Plant. 42, 33–38.

Casarini, D.C.P., 2001. Relatório de Estabelecimento de Valores Orientadores para Solos e Águas Subterrâneas no Estado de São Paulo. Cetesb, São Paulo (in Portuguese).

Cavallini, A., Natali, L., Durante, M., Maserti, B., 1999. Mercury uptake, distribution and DNA affinity in durum wheat (*Triticum durum* Desf) plants. Sci. Tot. Env. 233/234, 119–127.

Cerrati, G., Bernhard, M., Weber, J.H., 1992. Model reactions for abiotic mercury(II) methylation: kinetics of methylation of mercury(II) by mono-, di-, and tri-methylation in seawater. Appl. Organomet. Chem. 6, 587–595.

Conselho Nacional do Meio Ambiente (CONAMA), 2005. Resolução No. 357. Available at: http://www.mma.gov.br/port/conama/res/res05/res35705.pdf (in Portuguese).

Devars, S., Avilés, C., Cervantes, C., Moreno-Sánchez, R., 2000. Mercury uptake and removal by *Euglena gracilis*. Arch. Microbiol. 174, 175–180.

Dushenkov, V., Nanda Kumar, P.B.A., Motto, H., Raskin, I., 1995. Phytofiltration: the use of plants to remove heavy metals from aqueous streams. Env. Sci. Technol. 29, 1239–1245.

Dushenkov, V., Vasudev, D., Kapulnik, Y., Doloressa, G., Fleisher, D., Ting, K.C., Ensley, B., 1997. Removal of uranium from water using terrestrial plants. Env. Sci. Technol. 31, 3468–3474.

Elless, M.P., Poynton, C.Y., Willms, C.A., Doyle, M.P., Lopez, A.C., Sokkary, D.A., Fergunson, B.W., Blaylock, M.J., 2005. Pilot-scale demonstration of phytofiltration for treatment of arsenic in New Mexico drinking water. Water Res. 39, 3863–3872.

Falter, R., 1999. Experimental study on the unintentional abiotic methylation of inorganic mercury during analysis. Part 1. Localisation of the compounds effecting the abiotic mercury methylation. Chemosphere 39, 1051–1073.

Ferreira, C., Lopes Vieira, C., Azevedo, H., Caldeira, G., 1998. The effects of high levels of Hg on senescence, proline accumulation and stress enzymes activities of maize plants. Agrochimica 42 (5), 209–218.

Greger, M., Wang, Y., Neuschütz, C., 2005. Absence of Hg transpiration by shoot after Hg uptake by roots of six terrestrial plant species. Env. Pollut. 134, 201–208.

Gupta, M., Tripathi, R.D., Rai, U.N., Chandra, P., 1998. Role of glutathione and phytochelatin in *Hydrilla verticillata* (i.f.) Royle and Vallisneria spiralis L. under mercury stress. Chemosphere 37 (4), 785–800.

Hoagland, D.R., Arnon, D.I., 1950. The water culture method of growing plants without soil. California. Agric. Exp. Stat. Circular 347.

Huang, J.W., Poynton, C.Y., Kochian, L.V., Elless, M.P., 2004. Phytofiltration of arsenic from drinking water using arsenic-hyperaccumulating ferns. Env. Sci. Technol. 38, 3412–3417.

Lacerda, L.D., 2003. Updating global Hg emissions from small-scale gold mining and assessing its environmental impacts. Env. Geol. 43 (3), 308–314.

Lytle, C.M., Lytle, F.W., Yang, N., Qian, J.-H., Hansen, D., Zayed, A., Terry, N., 1998. Reduction of Cr(VI) to Cr(III) by wetland plants: potential for in situ heavy metal detoxification. Env. Sci. Technol. 32, 3087–3093.

Morel, F.M.M., Kraepiel, A.M.L., Amyot, M., 1998. The chemical cycle and bioaccumulation of mercury. Ann. Rev. Ecol. Syst. 29, 543–566.

Moreno, F.N., Anderson, C.W.N., Robinson, B.H., Stewart, R.B., 2004. Mercury analysis of plant and soil samples using the hydride-generation AAS method. In: Currie, L.D., Hanly, J.A. (Eds.), Tools for Nutrient and Pollutant Management. Occasional Report No. 17. Fertilizer and Lime Research Centre, Palmerston North, New Zealand, pp. 425–433.

Moreno, F.N., Anderson, C.W.N., Stewart, R.B., Robinson, B.H., 2005a. Mercury volatilisation and phytoextraction from base-metal mine tailings. Env. Pollut. 136, 341–352.

Moreno, F.N., Anderson, C.W.N., Stewart, R.B., Robinson, B.H., Nomura, R., Gomshei, M., Meech, J.A., 2005b. Effect of thioligands on plant-Hg accumulation and volatilisation from mercury-contaminated tailings. Plant Soil 275, 233–246.

Moreno-Jimenéz, E., Gamarra, R., Carpena-Ruiz, R.O., Millán, R., Peñalosa, J.M., Esteban, E., 2006. Mercury bioaccumulation and phytotoxicity in two wild plant species of Almadén area. Chemosphere 63, 1969–1973.

Oliveira, L.J., Hylander, L.D., Castro e Silva, E., 2004. Mercury behavior in a tropical environment: the case of small-scale gold mining in Poconé, Brazil. Env. Practice 6 (2), 121–134.

Patra, M., Sharma, A., 2000. Mercury toxicity in plants. Bot. Rev. 66 (3), 79–422.

Rauser, W.E., 1995. Phytochelatins and related peptides. Plant Physiol. 109, 1141–1149.

Riddle, S.G., Tran, H.H., Dewitt, J.G., Andrews, J.C., 2002. Field, laboratory, and X-ray absorption spectroscopic studies of mercury accumulation by water hyacinths. Env. Sci. Technol. 36 (9), 1965–1970.

Salisbury, F.B., Ross, C.W., 1992. Plant Physiology, fourth ed. Wadsworth Publishing Company, California, USA, 682 pp.

SAS Institute Inc., 1988. SAS/STAT User's Guide, Release 8e ed. Cary, NC, USA.

Veiga, M.M., 2004. Mercury pollution: revealing sources and suggesting solutions. Env. Practice 6 (2), 97–98.

Veiga, M.M., Hinton, J.J., 2002. Abandoned artisanal gold mines in the Brazilian Amazon: a legacy of mercury pollution. Nat. Resour. Forum 26, 15–26.

Weber, J.H., 1993. Review of the possible paths for abiotic methylation of mercury(II) in the aquatic environment. Chemosphere 26, 2063–2077.

Yannai, S., Berdicevsky, I., Duek, L., 1991. Transformations of inorganic mercury by *Candida albicans* and *Saccharomices cerevisae*. Appl. Env. Microbiol. 57 (1), 245–247.