Available online at www.sciencedirect.com
SCIENCE DIRECT®
Environmental Pollution 136 (2005) 341–352
ENVIRONMENTAL POLLUTION
www.elsevier.com/locate/envpol

Mercury volatilisation and phytoextraction from base-metal mine tailings

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

[a]*Institute of Natural Resources, Soil and Earth Sciences, Massey University, Private Bag 11222, Palmerston North, New Zealand*
[b]*HortResearch, Palmerston North, New Zealand*

Received 16 July 2004; accepted 22 November 2004

Mass balance studies indicated that volatilisation was a dominant pathway for mercury removal.

Abstract

Experiments were carried out in plant growth chambers and in the field to investigate plant-mercury accumulation and volatilisation in the presence of thiosulphate (S_2O_3)-containing solutions. *Brassica juncea* (Indian mustard) plants grown in Hg-contaminated Tui mine tailings (New Zealand) were enclosed in gastight volatilisation chambers to investigate the effect of ammonium thiosulphate ($[NH_4]_2S_2O_3$) on the plant-Hg volatilisation process. Application of $(NH_4)_2S_2O_3$ to substrates increased up to 6 times the Hg concentration in shoots and roots of *B. juncea* relative to controls. Volatilisation rates were significantly higher in plants irrigated only with water (control) when compared to plants treated with $(NH_4)_2S_2O_3$. Volatilisation from barren pots (without plants) indicated that Hg in tailings is subject to biological and photochemical reactions. Addition of sodium thiosulphate ($Na_2S_2O_3$) at 5 g/kg of substrate to *B. juncea* plants grown at the Tui mine site confirmed the plant growth chambers studies showing the effectiveness of thio-solutions at enhancing shoot Hg concentrations. Mercury extraction from the field plots yielded a maximum value of 25 g/ha. Mass balance studies revealed that volatilisation is a dominant pathway for Hg removal from the Tui mine site. A preliminary assessment of the risks of volatilisation indicated that enhanced Hg emissions by plants would not harm the local population and the regional environment.

Keywords: Volatilisation; Hg-phytoextraction; Induced plant-Hg accumulation; Ammonium thiosulphate ($[NH_4]_2S_2O_3$); Base-metal mine tailings

1. Introduction

Mercury (Hg) is a global pollutant that cycles between air, water and soil as a result of natural processes and anthropogenic activities. Although anthropogenic Hg emissions have been reduced by half since the 1980's (Pacyna et al., 2001), ongoing Hg contamination is still a worldwide problem. Depending on the Hg source and the form of discharge, Hg in soil may be present in concentrated hot spots or dispersed over large areas (Hinton and Veiga, 2001). Mobile Hg species in soils can leach to receiving waters where they can undergo methylation by anaerobes such as sulphate-reducing bacteria (Choi et al., 1994). Abiotic methylation of Hg (II) in waters is also possible in the presence of methylcobalamin and humic matter (Weber, 1993). Methylmercury is a developmental toxicant and readily crosses placental barriers. In cases of acute poisoning via

* Corresponding author. Tel.: +55 11 3277 54651; fax: +55 11 3277 5461.
E-mail addresses: fabionmoreno@terra.com.br (F.N. Moreno), chris@tiaki.co.nz (C.W.N. Anderson), r.b.stewart@massey.ac.nz (R.B. Stewart), brett.robinson@env.ethz.ch (B.H. Robinson).

0269-7491/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2004.11.020

ingestion of Hg-laden fish, muscular atrophy and neurological damage are prominent (Veiga and Hinton, 2002). Exposure to methylmercury during human gestation can delay the onset of walking and talking and diminish learning ability (US Environmental Protection Agency, 2004).

Although not all Hg-contaminated sites are vulnerable to methylmercury formation (Veiga, 2004), Hg in soils represent a serious threat and, in many circumstances, must be removed. Ex situ remediation technologies such as excavation, physical separation, and hydrometallurgical treatments are expensive, particularly if Hg contamination is spread over a large area or extends below the water table. Thermal treatment (soil heating combined with soil vapour extraction) can be effective for Hg removal from solid media but is technically complicated and also costly. Furthermore, soil heating releases Hg as a vapour into the environment and can have deleterious effects on the physical, chemical and biological properties of soils (Meagher et al., 2000; Hinton and Veiga, 2001). Phytoremediation uses plants to rehabilitate degraded environments. By using free services provided by nature (energy from the sun and CO_2 from the atmosphere), plants species can extract nutrients, accumulate heavy metals and radionuclides, and transform or degrade some organic contaminants more economically than current available chemical/physical technologies (Raskin et al., 1994; Schnoor et al., 1995; Robinson et al., 1998).

Two different approaches have been proposed to foster plant-based systems for the remediation of Hg-polluted soils. The first approach involves the use of plants encoding genes from Hg-detoxifying bacteria, which have increased Hg resistance and enhanced volatilisation capacity. These transgenic plants are able to extract Hg (II) and methylmercury from contaminated soils and sediments and to convert these forms to the less toxic and more volatile Hg (0) (Rugh et al., 1996; Bizily et al., 1999). The second approach uses non-toxic thio-containing solutions to induce Hg accumulation into above ground tissues of high-biomass plant species (Moreno et al., 2004a). Mercury has a strong affinity for thiol groups and can be readily complexed to the thiosulphate ion (Wilkinson et al., 1987). However, the kinetics of the formation/decomposition of Hg-thiosulphate complexes is poorly understood (D. Dreisinger, 2004, personal communication). In spite of that, ammonium thiosulphate has been used to induce *Brassica juncea* (Indian mustard) to accumulate 40 mg Hg/kg of shoot tissue from a lead-copper-zinc metal mine contaminated with Hg (Moreno et al., 2004a). This concentration is 40–400 times higher than average background levels of Hg in plants (Kirkham, 1977; Kabata-Pendias and Pendias, 2000). Mass balance studies, however, revealed that a substantial Hg fraction that could not be accounted for. This unaccounted fraction suggested that Hg (0) was volatilised from the substrate as a result of biological and chemical transformations. In this work we describe plant growth chamber and field studies on the volatilisation and thiosulphate-induced accumulation of Hg by *B. juncea* plants growing in an Hg-contaminated mine tailings.

2. Materials and methods

2.1. Substrate characterization

Substrate for the greenhouse research was collected from the tailings dam of the abandoned Tui base-metal mine, located on the NW flank on Mount Te Aroha, approximately 3 km from of the township of Te Aroha, North Island of New Zealand (Fig. 1). The site is contaminated with Hg at concentrations ranging from 1.3 to 4.5 mg/kg (Moreno et al., 2004c). The metal-bearing minerals present in the tailings include sphalerite (ZnS), galena (PbS), chalcopyrite ($CuFeS_2$), and pyrite (FeS_2) with minor amounts of Cd, Ag, and Au. The tailings have been have been left in contact with the air since closure of the mine in 1974. Oxidation of pyrite and other sulphide-bearing minerals has depressed the pH of surface tailings to as low as 2.3 and increased the bioavailability of the metals Cu, Fe, Pb, Mn and Zn. No native vegetation has colonised the site. The nearby Tui stream and the local ground water supply have been severely contaminated by acid mine drainage produced on site (Morrell et al., 1996).

2.2. Plant growth conditions

Non-sieved Tui substrates were fertilised with 5 g/kg of Osmocote (NPK slow release fertilizer) and amended with lime to adjust the pH to 5.5. Replicate plastic pots (6×6 cm, $n = 20$) were filled with the growth substrate and sown with seeds of *B. juncea* at a rate of ca. 20 seeds per pot. Pumice was added in a 3:1 ratio to improve drai age of the substrate. Plants were watered everyday to field capacity. Drainage collected from each pot was reapplied to the substrates. Five milliliters of 1/4 strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) was irrigated onto the pots every second day to supplement plant nutritional requirements. Two weeks after germination, each pot was thinned to leave only one individual plant. Plants were kept in a greenhouse with temperature ambient set at 15–25 °C with no humidity control. Pot positions were randomly changed on a periodic basis to equalize light exposure. All plants were treated before the outset of flowering. Plants reached around 20 cm of height after 5 weeks of growth.

2.3. Extractable Hg

Extractable Hg concentrations in Tui mine tailings were determined through the use $(NH_4)_2S_2O_3$ and

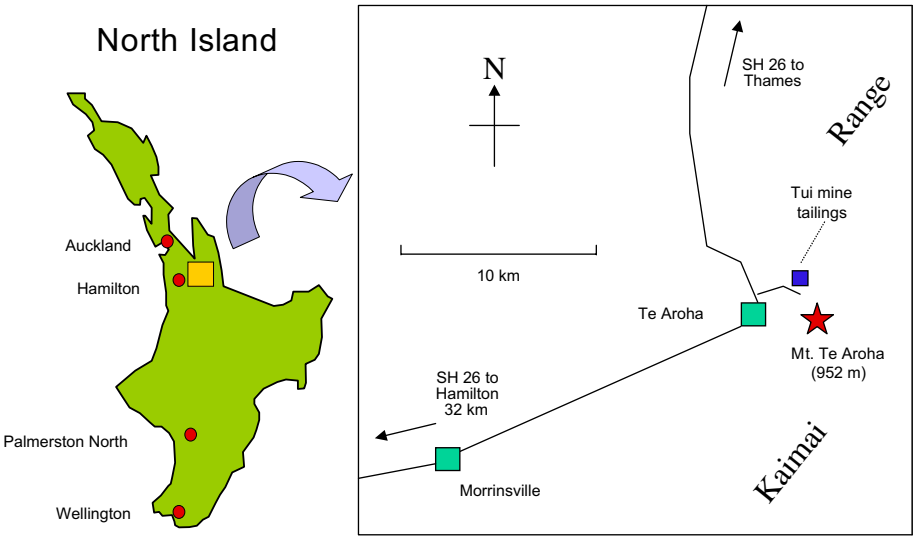

Fig. 1. Location of Tui mine tailings. The tailings dam is located on the NW flank on Mount Te Aroha, approximately 3 km of the township of Te Aroha, North Island of New Zealand.

$Na_2S_2O_3$ as chemical extractants (Moreno et al., 2004a). One gram of sieved tailings (<1000 microns) was weighed into 50 mL polypropylene centrifuge tubes in triplicate. After addition of 20 mL of extractant solutions (at 2 and 10 g/L), the tubes were rotated on an end-over-end shaker overnight at 45 rotations per minute (RPM) and the supernatant separated via centrifugation at 3000 RPM for 3 minutes.

2.4. Effect of ammonium thiosulphate on Hg volatilisation and accumulation

After 5 weeks of plant growth, the solution of $(NH_4)_2S_2O_3$ was applied to replicate pots at a rate of 1 g thiosulphate per kg of substrate ($n = 3$). Water was used as a comparison to the thiosulphate treatments ($n = 3$). Pots without plants (unplanted pots) were used as controls ($n = 3$). A single plant pot was enclosed within a gastight acrylic volatilisation chamber (3.6 L volume) immediately after the chemical treatment. Volatile Hg was captured in an acid trap solution containing 5% $KMnO_4$ dissolved in 2 N H_2SO_4 (Fig. 2). The efficiency of this trap solution to capture quantitatively Hg (0) has been shown to vary between 95 to 99% (Kimura and Miller, 1960). A continuous airflow was supplied to the volatilisation chamber using a small air pump. Mercury vapour released from plants and substrates was driven together with the incoming air into an Erlenmeyer flask containing 70 mL of the acid trap solution. The flow rate of the incoming air was monitored using an air flow meter (J&W, model AMD 1000, California, US) and was constantly held to 100 mL/min using a small clamp attached to the air outlets. The clamp was manually regulated. The outlet

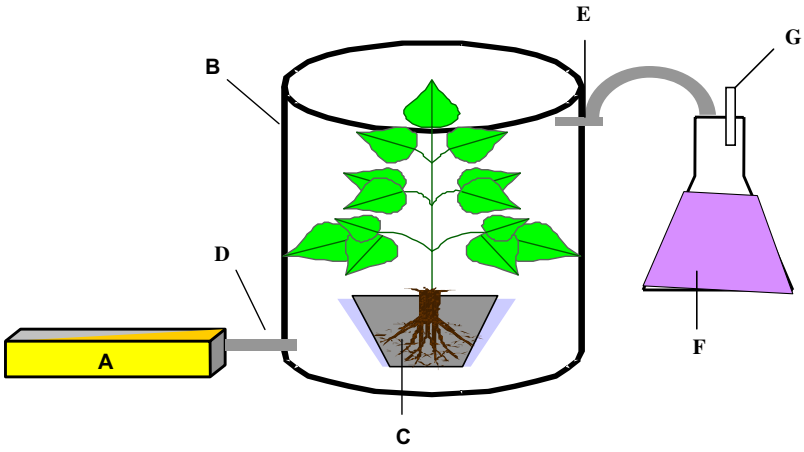

Fig. 2. Experimental unit used for trapping Hg released from plants. A, air pump; B, gas tight plant chamber; C, plant pot; D, air inlet; E, air outlet; F, inorganic Hg vapour trap; G, air outlet of the trap.

of the acid trap was open to the atmosphere to maintain pressure equilibrium within the trap system. A 10 mL syringe attached to the volatilisation chamber was used to water the plants during the period of volatile Hg collection. Watering was carefully performed to avoid losses of Hg by leaching. Volatilisation was measured over a 3 days period in a plant growth chamber with photoperiod set for 14 hours and temperature kept constantly at 22 °C. Collection of volatile Hg was done in triplicate for $(NH_4)_2S_2O_3$-treated plants, plants irrigated with water and control pots. The plant growth chamber has a capacity for 3 individual volatilisation chambers and, therefore, the experiment was repeated 3 times. At the end of the three days period, the acid trap solution was transferred to 100 mL air-tight plastic containers and stored at 4 °C until analysis. The precipitated fraction of the trap was redissolved in 50 mL of concentrated hydrochloric acid, and the resulted solution was preserved following the same procedure. The volatile Hg mass collected for each replicate was, therefore, the sum of Hg readings in the soluble and precipitated fractions of the acid trap. The use of this experimental apparatus has allowed Hg recoveries around 90% for *B. juncea* plants cultured in Hg-spiked solutions (Moreno et al., 2004b).

2.5. Induced plant-Hg accumulation field trials

Three field plots with dimensions of 5×5 m were established at the tailings dam of the abandoned Tui base-metal mine. The plots were fertilized with commercially available NPK fertilizer at a rate of 75 g/m^2 and had their pH adjusted to 5.5 by addition of lime. Organic matter (mushroom compost) was added at a rate of 3.2 L/m^2. Around 75 grams of *B. juncea* seeds were planted in two rows of 5 m length $\times$0.5 m width $\times$0.15 m depth. Collection of soil samples ($n = 4$) was carried out at the 0–15 cm depth for each plot just after seeding. After 6 weeks of plant growth, $Na_2S_2O_3$ in the form of a solution (w/v) was applied to the field plots at a concentration of 5 g/kg of substrate. This application rate was calculated based on the mass of substrate in each planted row (0.6 tonnes) and, thus 6 kg of sodium thiosulphate were applied per plot. The sodium salt was chosen for the field study because bulk quantities of the ammonium salt were not commercially available. Previous laboratory experiments showed, however, that Hg extractability in the presence of the sodium salt was not significantly different to the ammonium salt ($P > 0.05$). Plant samples that were collected from each plot prior to the treatment application were used as controls. Two weeks after the treatment, biomass from the plots was harvested and processed for Hg analyses.

2.6. 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. The intact root system could be harvested from the substrates 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 soaking process was carried out for one hour and was done separately for each plant-chemical treatment The roots were further washed several times with tap water to remove residual substrate. Plant tissues were placed into individual paper bags and dried at 70 °C. After drying, all plant samples were ground and sealed in plastic bags for subsequent Hg analysis.

2.7. Plant digestion

Ground shoots and roots were weighed accurately (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 hour. Digest solutions 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.

2.8. Substrate digestion

The total mercury concentration in tailings sub samples was determined through *aqua regia* digestions of dried substrate. One gram of substrate was weighed into 50 mL polypropylene pots in triplicate and a 15 mL solution of HNO_3 and HCl at 1:3 ratio was added. The samples were digested in water bath at 100 °C for 1 hour and the filtrates diluted to a final volume of 50 mL using RO water.

2.9. Mercury analysis

Total Hg in plant and tailings digests and in extractant and trap solutions was analysed using Hydride-generation atomic absorption spectroscopy (AAS) (Moreno et al., 2004c). The analysis was performed using a GBC 909A AAS (Victoria, Australia) operating in the flame mode. A sodium borohydride solution (5% $NaBH_4$ + 1% KOH) was used to generate Hg vapour. The limit of detection (LOD) for mercury in solution was 10 ng/mL for plant digests and 5 ng/mL for soil digests and extractant and trap solutions. 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. Reagent blanks were below detection limits in the solution. Linear calibration curves were obtained over the range

of 125 to 1000 ng/mL of Hg using 4 standards prepared from a 10 mg/L mercuric nitrate ($HgNO_3$) spectrosol solution (May & Baker, AAS reagent standard solution). Solutions with Hg concentration over the 1000 ng/ml range were diluted with RO. The analytical method was assessed for quality control by an external certified laboratory with agreements ranging from 85 to 103% for Hg-containing solutions and Hg-containing plant samples (Moreno et al., 2004c).

2.10. Statistical analysis

A copy of SAS PC version 8e was used for statistical analyses (SAS Inst, 1988). Treatment differences were examined through the *t*-test, assuming equality of variances between two treatment means. Differences among three means were assessed through one-way analyses of variance (ANOVA). Tukey's test was used for pair-wise comparison of means at 0.05 and 0.01 significance levels. Data was log-transformed to achieve a normal distribution.

3. Results

3.1. Effect of ammonium thiosulphate on Hg volatilisation and accumulation

Fig. 3 shows the daily Hg volatilisation rates for *B. juncea* plants and control pots (without plants) on a Hg mass basis (A) and per kg dry weight (B) basis. In both cases, the Hg volatilisation rates from water-treated plants significantly exceeded the Hg volatilisation rates from $(NH_4)_2S_2O_3$-treated plants by a factor 3 ($P < 0.05$). The Hg volatilisation rate from control pots (without plants) was significantly lower than from water-treated plants and $(NH_4)_2S_2O_3$-treated plants ($P < 0.05$) (Fig. 3A). The Hg efflux from control pots was in average 23 times lower than the Hg efflux from water-treated plants and around 6 times lower than $(NH_4)_2S_2O_3$-treated plants. We believe these Hg emissions to be due to biological transformations and photoreduction processes occurring in the substrate.

Table 1 shows the Hg concentration (mg/kg) in plant and substrates after addition of $(NH_4)_2S_2O_3$ to Tui mine tailings. It is clear that the induced plant-Hg accumulation and volatilisation processes are inversely related to each other. For instance, $(NH_4)_2S_2O_3$-treated plants accumulated between 4 to 6 times the Hg concentration found in water-treated plants for both shoot and root tissues, respectively ($P < 0.05$, Table 1). The induced plant-Hg accumulation was, however, more pronounced for roots, where recorded Hg values were 20 times superior to shoots (Table 1). It is noteworthy that water-treated plants accumulated around 1 mg/kg of Hg in shoots. This concentration is 10–100 times higher than

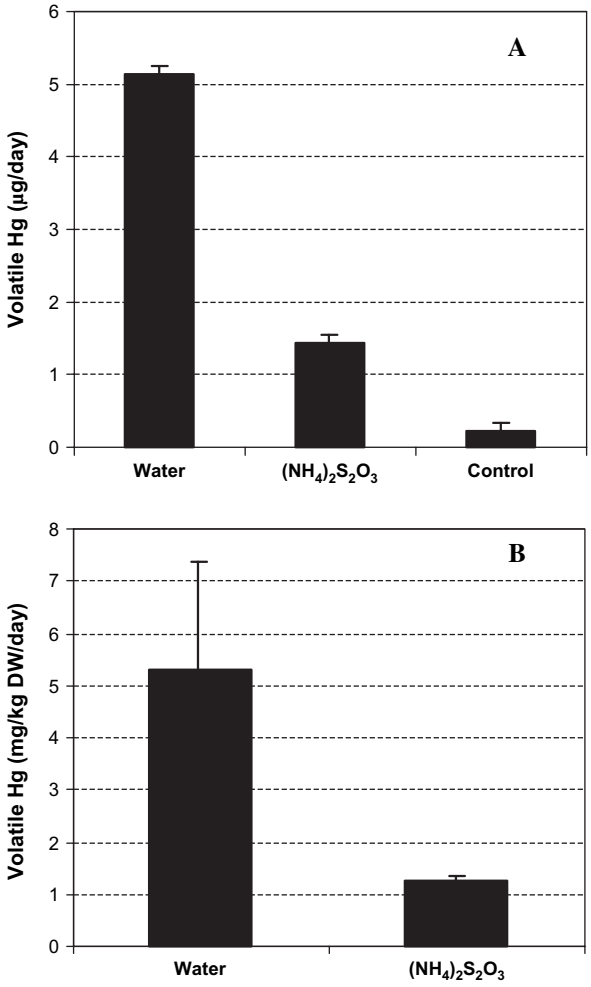

Fig. 3. Volatilisation rates from *B. juncea* plants grown in Tui mine tailings treated with ammonium thiosulphate ($[NH_4]_2S_2O_3$) at 1 g/kg. (A) Total Hg mass (µg/day) emitted from plants and control pots (without plants) and (B) Total Hg mass emitted from planted substrates per unit dry weight (kg) per day. Bars denote ±1 standard deviation from the mean of 3 replicates. DW = dry weight.

average background levels of Hg in plants (Kirkham, 1977; Kabata-Pendias and Pendias, 2000). This result indicates that foliar uptake of volatile Hg was a possible entrance pathway for plants enclosed in the gastight volatilisation chamber.

Table 1 also shows the total and extractable Hg concentrations (mg/kg) after plant growth and $(NH_4)_2S_2O_3$ treatment. A lower total Hg concentration was found in the $(NH_4)_2S_2O_3$-treated substrate although this difference was not significant ($P > 0.05$). Conversely, there was a significant difference between the two treatments for the extractable Hg concentrations ($P = 0.0032$). Assuming that water-soluble Hg was the only fraction available for plant uptake before application of $(NH_4)_2S_2O_3$ to tailings, the Hg discrepancy between both substrates may be due to plant uptake of the sulfidic Hg fraction mobilized after application of $(NH_4)_2S_2O_3$ to pots.

Table 1
Plant and substrate Hg concentrations (mg/kg) for water and ammonium thiosulphate-treated substrates at the end of the experiment

Treatment	Plant Hg (mg/kg)[a]		Substrate Hg (mg/kg)[b]	
	Shoot	Root	Total	Extractable
Water	1.1 ± 0.5 a	14.8 ± 9 a	1.95 ± 0.7 a	1.47 ± 0.15 a
$(NH_4)_2S_2O_3$[c]	4.6 ± 1.4 b	93.5 ± 45 b	1.30 ± 0.3 a	1.12 ± 0.09 b

Ammonium thiosulphate was applied to Tui mine tailings at a rate of 1 g/kg of substrate. Values are the means of at least 3 replicates ±1 standard deviation. Letters compare treatments within each column. Means with different letters are significantly different at $\alpha=0.05$ (Tukey's test).

[a] Plant values are the mean ±1 standard deviation of 3 replicates.

[b] Substrate values are the mean ±1 standard deviation of 5 replicates.

[c] $(NH_4)_2S_2O_3$=ammonium thiosulphate.

3.2. Mass balance for Hg in the soil-plant system

Fig. 4A describes the Hg distribution between the air (Hg trap), plant and substrate compartments for $(NH_4)_2S_2O_3$ and control treatments at the end of the experiment. The values are expressed as the percentage of the Hg mass in each compartment and thus, 100% is the total sum for the respective Hg fractions in substrate, plant and traps. It should be pointed out that no leaching occurred over the period of volatile Hg collection. The total Hg mass in planted $(NH_4)_2S_2O_3$-treated substrates was about 13% lower compared to substrates that received only water. The Hg mass fraction accumulated in plant tissues comprised around 20% of the total Hg mass for the $(NH_4)_2S_2O_3$-treated system whereas in the water-treated system this Hg fraction corresponded to less than 5%. Conversely, the volatilised Hg fraction in the water-treated system comprised around 8% of the total Hg mass whereas in the $(NH_4)_2S_2O_3$-treated system this fraction corresponded to 2%.

The effect of $(NH_4)_2S_2O_3$ on both Hg accumulation and volatilisation processes is emphasised in Fig. 4B by the comparison of the Hg mass distribution between roots, shoots, and trap compartments at the end of the experiment. It is apparent that most of the Hg mass in the water-treated system (around 75%) is preferably volatilised to the air in detriment to the plant-Hg accumulation process. After application of $(NH_4)_2S_2O_3$ to the substrates, however, this pattern changes dramatically with most of the Hg mass (around 80%) being retained in root tissues.

3.3. Induced plant-Hg accumulation field experiment

The results for the field experiment conducted at the three plots are described in Fig. 5 and Table 2. The

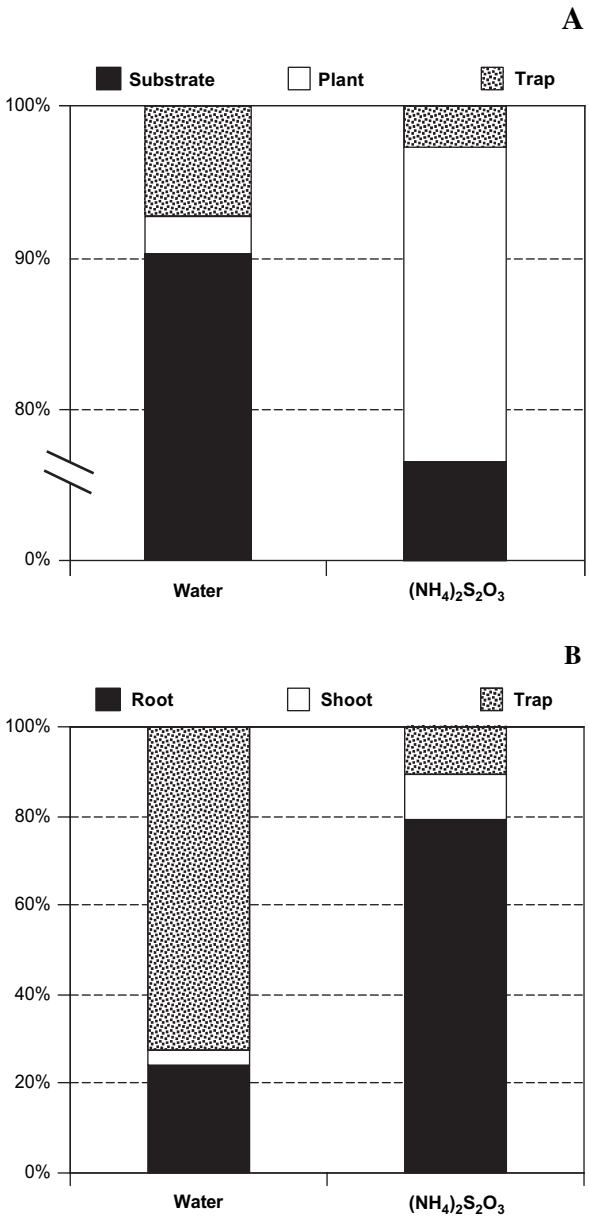

Fig. 4. Mercury Distribution in the air-plant-soil system after application of ammonium thiosulphate ($[NH_4]_2S_2O_3$) at 1 g/kg to Tui mine tailings. Normalized values (100%) represent the Hg mass in substrate, plant and acid trap (A) and the Hg mass partition between roots, shoots and trap compartments (B). Note that 100% is the sum of Hg mass in substrates, plant (shoot + root) and acid trap.

addition of $Na_2S_2O_3$ to substrates enhanced Hg solubility, leading to increased Hg accumulation in roots and shoots of *B. juncea*. The extractable Hg concentration rose proportionally to increasing concentrations of $Na_2S_2O_3$ added to the substrates (Fig. 5). The thiosulphate treatment induced a significant increase in root and shoot Hg concentrations relative to control plants, which had shoot and root Hg values below detection levels. Root Hg concentrations between the plot locations ranged from 9.02 ±0.8 to 17.07 ±2.7 mg/kg for $Na_2S_2O_3$-treated plants. Mercury concentrations in

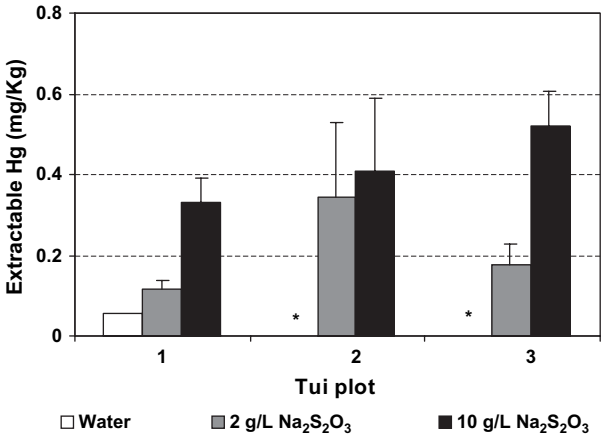

Fig. 5. Extractable Hg concentrations as a result of sodium thiosulphate ($Na_2S_2O_3$) application to Tui mine tailings at concentrations of 2 and 10 g/L. Bars denote ±1 standard deviation from the mean of 3 replicates. Note that (*) means Hg below detection limits (5 ng/mL).

shoots of *B. juncea* were higher in plot number 2, where the assayed value averaged 9.77 ±1.2 mg/kg (Table 2). For plot number 3, the average Hg value for shoots was unexpectedly low (2.99 ±0.7 mg/kg) despite the elevated levels of soluble Hg recorded in the $Na_2S_2O_3$ extracts. The fact that the Hg content of roots was greater than shoots for all tested plots indicates that application of $Na_2S_2O_3$ to substrates increased the retention of Hg by the root tissues. This trend was particularly evident for plot number 3, where the roots accumulated almost 6 times more Hg than the shoots.

The phytotoxic conditions that prevail in the substrates of the Tui base metal mine did not prevent plant growth, as average plant height on site was around 25 cm (Fig. 6). One week after treatment with sodium thisosulphate solutions, however, phytotoxic symptoms (e.g., wilting and chlorosis) were evident. Estimates of biomass production indicated a maximum dry matter yield of around 2.5 tonnes per hectare. Considering that plants on site had a relatively short growing season (6 weeks) and that the sulphide-rich tailings of the Tui mine exhibit high levels of the toxic metals Zn, Cu, Mn, Pb and Ag (Morrell et al., 1996), the low levels of plant biomass production on this site are unsurprising. As a result, Hg-extraction yields were also low. Due to its highest biomass production and shoot Hg concentration, the maximum Hg-extraction yield was achieved at plot number 2 with an average value of 24.3 ± 3.1 g/ha (Table 2). This Hg-extraction yield was over 30 times the maximum yield obtained for *Hordeum vulgare* (barley), which extracted 0.71 g Hg/ha from an Hg-contaminated site in Almaden, Spain (Rodriguez et al., 2003).

4. Discussion

4.1. Speciation and possible origin of volatile Hg released from barren and planted substrates

The quantitative capture of volatile Hg in the potassium permanganate solution under acid conditions can be written by the following equation:

$$2KmnO_{4(aq)} + 3Hg(0)_{(g)} + 4H_2SO_{4(aq)} \rightarrow 2MnO_{2(prec.)} + 3HgSO_{4(prec.)} + K_2SO_{4(aq)} + 4H_2O \quad (1)$$

Since elemental Hg (0) is oxidized by potassium permanganate, then we would presume that the predominant Hg form released from barren and planted substrates was the inorganic vapour Hg (0). This statement can be validated by early gastight volatilisation experiments with *B. juncea* plants grown in Hg-spiked solutions containing 1 mg/L of Hg (Moreno et al., 2004b). Potassium permanganate and carbonate-phosphate solutions were used for trapping plant-released inorganic and organic vapours, respectively. Mercury recovery averaged around 90% of the initial Hg mass added to the plant solution (traps + plant tissues + solutions). However, Hg values in the carbonate-phosphate traps were below detection levels (Moreno et al., 2004b).

The Hg volatilisation from Tui mine tailings (as shown for control pots, Fig. 3A) may be the result of on site biological and sunlight-mediated reduction of Hg (see Morel et al., 1998 for a detailed explanation on Hg

Table 2
Phytoextraction results for the Tui mine tailings after application of sodium thiosulphate ($Na_2S_2O_3$) at 5 g/kg (unless otherwise stated) to Tui tailings field plots*

Tui Plot	Root Hg (mg/kg DW)	Shoot Hg (mg/kg DW)	Harvested biomass (kg, DW)	Equivalent Biomass (t/ha)[a]	Hg Extraction yield (g/ha)[b]
1[c]	9.02 ± 0.8	2.99 ± 0.5	0.19	0.38	1.14 ± 0.2
2	15.39 ± 2.1	9.77 ± 1.2	1.24	2.49	24.39 ± 3.1
3	17.07 ± 2.7	2.93 ± 0.7	1.16	2.33	6.84 ± 1.7

* Values are the mean ±1 standard deviation from 5 replicates (1 replicate = 0.1 g of ground roots and shoots digested in 15 ml HNO_3, See material and methods section).
[a] Calculated on a basis of 5 m^2 area for each plot.
[b] Hg values for extraction yields (g/ha) are the product of shoot Hg concentrations (mg/kg DW) and equivalent plant biomass production (t/ha).
[c] $Na_2S_2O_3$ applied at 2.5 g/kg.

Fig. 6. Experimental field plot at the Tui mine tailings (North Island, NZ) before application of sodium thiosulphate ($Na_2S_2O_3$) to the substrate (march/2004). Biomass for *B. juncea* plants grown at the toxic tailings yielded a maximum of 2.5 tonnes/ha after 6 weeks of planting.

photoreduction). The role of bacteria on Hg volatilisation from Hg-contaminated environments has been well documented (Barkay, 1987; Barkay et al., 1991; Barkay et al., 1992; Saouter et al., 1994). The enzymatic reduction of Hg (II) to Hg (0) is carried out by Hg-resistant bacteria (both gram positive and negative) via a flavin-containing disulfide oxireductase known as mercuric reductase (MR):

$$Hg(SR)_2 + NADPH + H^+ \rightarrow Hg(0) + NADP^+ + 2RSH \quad (2)$$

The MR removes Hg from stable thiol salts ($Hg[SR]_2$) by electrochemical reduction in a NADPH-coupled redox reaction (Eq. (1)). The resulting Hg (0) is rather volatile [Henry's constant (H) = 0.3] and escapes from the organism (Barkay et al., 1992). This strategy has been shown to be an effective solution to bacterial Hg-exposure, as Hg (0) is less toxic than methylmercury compounds (Meagher et al., 2000). The fact that planted substrates volatilised more Hg than the barren pots (Fig. 3A) indicates that there is a plant-mediated factor in the Hg volatilisation from substrates. The enhanced plant Hg (0) emissions may have occurred from both roots and shoots of *B. juncea*. Root-induced Hg volatilisation could be the result of biological Hg reduction carried out by Hg-resistant bacteria living at the rhizosphere or inside the roots. Experiments with *B. juncea* plants grown in Hg-containing solutions have shown that around 95% of the total Hg mass volatilised from the plant was originated from the root compartment, thus implicating the role of rhizosphere processes in the plant-Hg volatilisation (Moreno et al., 2004b). Alternatively, a small fraction of volatile Hg might have been emitted through open stomata located in the mesophyll cell surfaces of foliar tissues (Gustin et al., 1997; Leonard et al., 1998). Lindberg et al. (1998) suggested that a gaseous Hg (0) pool in soil pores could originate from abiotic and biotic reduction of Hg (II) compounds. The measured levels of gaseous Hg (0) in this pool (on the order of 200 ng Hg/m^3) indicate that Hg (0) can be found dissolved in the soil water. Dissolved Hg (0) could, therefore, be taken up in the transpiration stream and subsequently transported to leaves where it may be released as a gas along with water vapour.

4.2. Mercury uptake versus volatilisation

The high sulphide content and the presence of sulphide-bearing minerals in the Tui ore indicates that a fraction of Hg in the tailings may be present as sulphides such as cinnanbar (HgS), Hg polysulfides or Hg associated with iron sulphides (Moreno et al., 2004a). These Hg forms are insoluble, and thus, they are said to be relatively unavailable for chemical and biological transformations (Kabata-Pendias and Pendias, 2000). However, extractability studies showed the presence of significant amounts of Hg in the water extractable fraction of Tui mine tailings (Moreno et al., 2004a). We hypothesize that the water-soluble Hg fraction of Tui tailings constitutes the available Hg pool for both plant uptake and biological transformations. Geochemical conditions in the tailings profile (e.g. acid mine drainage) oxidised sulfidic Hg minerals such as cinnabar (Morrell et al., 1996), thus releasing Hg (II) ions to this pool.

According to Duckart et al. (1992), when plant roots and microorganisms compete for the same pool of available selenium (Se) the rates of microbial

transformation and plant uptake become dependent processes. When Se was added to the soil in the form of readily plant-available Na_2SeO_4, enhanced uptake of the metalloid by plants depleted the pool of soluble Se available for microbial Se volatilisation. In our experiments the addition of 1 g/kg of $(NH_4)_2S_2O_3$ mobilised the sulfidic-extractable Hg pool and enhanced plant Hg uptake and translocation to the detriment of volatilisation rates. The fact that water-treated plants did not change the sulfidic Hg fraction in substrates towards the end of the experiment (Table 1) indicates that the water-extractable Hg fraction is a common pool for both plant uptake and Hg volatilisation processes. Therefore, it is possible to conclude that addition of $(NH_4)_2S_2O_3$ may have altered the Hg volatilisation pathway in Tui tailings to the extent that the Hg mass taken up by plants exceeded the volatile Hg mass produced by biological transformations.

4.3. Mass balance calculations

Results from the thiosulphate-induced plant-Hg accumulation experiments described in this paper indicate that Hg removal from the Tui mine tailings can be accomplished by both phytoextraction and volatilisation. Using the highest values for biomass production and plant-Hg accumulation following application of $Na_2S_2O_3$ on site, the maximum Hg phytoextraction yield averaged around 25g Hg/ha (Table 2). However, since Hg volatilisation spontaneously occurs from the plant-soil system before and after amendment of the Tui substrate, we estimated the mass of Hg emitted to the atmosphere by *B. juncea* plants grown on site. Assuming an average volatilisation rate of 5.5 mg Hg/kg of plant tissue per day (Fig. 3B) and an average biomass of 2.5 tonnes (Table 2), then around 14 g Hg/ha were daily emitted to the atmosphere of the Tui mine site before the application of thiosulphate. Assuming that these Hg emissions happened over a 30-day period, then, by extrapolation, an equivalent value of at least 420 g Hg/ha were emitted to the atmosphere of Tui mine tailings during the experimental period. If we include the plant-Hg volatilisation rates after the amendment was applied to the substrate (mean of 1.2 mg Hg/kg plant tissue per day), then the total value increases to 500 g Hg/ha. Assuming that the site supports three growing seasons per year, the Hg emissions caused by the plants (Fig. 3A) would represent an efflux rate of 1.5 kg of Hg per year to the atmosphere. Considering that the Tui area contains 364 kg of Hg in 100 000 m^3 of tailings, then it would take around 243 years to clean up the whole area through plant-enhanced Hg volatilisation. However, because the active zone for nutrient uptake is limited by root growth and *B. juncea* roots extends not below 0.5 m depth, plants will only remediate surface soils. If we assume a total Hg mass of 20 kg in the 0–0.5 m depth profile of the Tui mine, then is feasible that plants will remove most of the Hg mass in the 50 cm of the tailings in less than 15 years.

4.4. Considerations about the environmental impacts of plant Hg (0) emissions

Given the fact that *B. juncea* plants volatilise significant amounts of Hg (0) to the air, the implementation of a phytoremediation system would require a rigorous assessment of the impacts of this technology. The main concern would be, therefore, related to the risks of Hg (0) exposure to the human population. However, to pose a significant risk to humans the level of atmospheric Hg (0) in the first 1 to 1.5 m above the revegetated area at the Tui site would have to be above the threshold limit value (TLV) for Hg vapour inhalation (1 $\mu g/m^3$) (World Health Organization, 1976). Since Hg atmospheric levels are not available for comparison purposes, we will address this issue reporting 0.01 $\mu g/m^3$ as the value for Hg (0) atmospheric levels in the Tui non-vegetated area (Lindberg et al., 1995). This is the maximum Hg level found in the first 1 to 1.5 m above the soil of an Hg-contaminated site in Oak Ridge, TN (US), which contains 80 000 kg of Hg in a 250 ha area (Meagher et al., 2000). Assuming that Hg emissions would be expected to increase by a factor of 23 due to the on site presence of *B. juncea* plants (Fig. 3A), the resulting Hg atmospheric levels would be still 4 times below the TLV value for Hg (0) exposure. It is, thus, very unlikely that the Te Aroha population would experience adverse health effects due to Hg vapours emitted from a Hg-phytoremediation operation at the Tui mine tailings.

Transgenic *MerA*-Tobacco plants were tested for their phytoremediation potential in Hg (II)-spiked soils (Heaton et al., 1998). These plants can transform root-available Hg (II) to the less toxic Hg (0), therefore, volatilising it to the atmosphere. Estimates of the volatilisation capacity suggest *MerA*-expressing transgenic plants could increase 400-fold the spontaneous Hg efflux rates from an Hg-contaminated site. After careful consideration of the processes that govern the Hg (0) mass distribution between the atmosphere, soil and water compartments, the authors concluded that the release of Hg (0) by transgenic plants would have little global impact if this technology is applied to all available Hg-contaminated sites in the US (Meagher et al., 2000).

Due to the fact that Hg volatilisation from plants can actively contribute to the natural Hg emissions to the atmosphere, critics could agree that volatilisation makes questionable the use of plant-based systems for the remediation of Hg-contaminated sites. Therefore, an assessment of the regional environmental impacts of this technology would be required prior to its systematic

implementation in the field. If we target the Hg mass contained in 0–0.5 m depth profile of the Tui mine and assume that 100% of this Hg is released as Hg (0), then over the next 15 years 22.5 kg will be emitted to the global Hg atmospheric pool. Considering that Hg world emissions from the plant kingdom can reach between 850 to 2000×10^3 kg of Hg per year (Lindberg et al., 1998) and that small-scale gold mining in South America, Russia and Asia can contribute with another 450×10^3 kg Hg per year (Lacerda, 2003), the Hg mass emitted by *B. juncea* plants at the Tui site (1.5 kg per year) would have an insignificant impact on this total. Additionally, in some circumstances volatilisation may present some environmental advantages over thioligand-induced phytoextraction. For instance, it minimizes the potential for biomagnification, as Hg volatilised from plant tissues would not represent an exposure route to wildlife (Heaton et al., 1998). Also, unlike chelate-enhanced phytoextraction, volatilisation avoids the risk of ground water contamination by mobilised Hg. The Hg concentration in leachates has been shown to rise up to 4 fold after $(NH_4)_2S_2O_3$ amendment to planted Tui mine tailings (Moreno et al., 2004a).

4.5. Fate of plant-Hg (0) emissions in the North Island of New Zealand

Once emitted to the atmosphere, Hg (0) is partitioned between vapour and particulate forms, which have a mean residence time of 233 and 30 days, respectively (Kvietkus and Sakalys, 2001). However, meteorological factors such as rain and snow-fall, and prevailing winds can accelerate the rate of deposition of both vapour and particulate Hg to the ground. Therefore, it is possible to speculate that the high rainfall regimes at the township of Te Aroha (around 1460 mm/year) (NZMS, 1983), and the predominance of westerly winds in the region, would enhance the Hg deposition rates over the Coromandel Peninsula and the Bay of Plenty, both coastal areas of the North Island of New Zealand. The 1.5 kg of Hg annually redeposited over the next 15 years would be diluted over a much larger area in surface soils and waters. Redeposition would have minimal regional impact as the mass of Hg in the soil and water compartments are several orders of magnitude greater than the global atmospheric Hg pool, which has been estimated to be about 1×10^6 kg (Nriagu, 1979). For instance, investigations carried out in the waters that cover the aquatic system of the Taupo Volcanic Zone (TVZ), in the North Island of New Zealand, revealed high levels of Hg, As and other elements. The source of this contamination is either from naturally occurring geothermal discharges or from geothermal power stations. However, research should be carried out to verify the reactivity of plant-emitted Hg and the potential for methyl mercury formation in the coastal areas of the North Island of New Zealand. It is known that some lakes and river waters of the TVZ contain Hg levels that exceed the background level for surface waters and that trout fish collected from lake Rotorua have flesh methylmercury concentrations above WHO limits for Hg (0.5 μg/g DW) (Robinson, 1994).

5. Conclusions

The primary goal of the research was to study the accumulation of Hg into aboveground plant tissues via thiosulphate-induced Hg solubilization of Tui mine tailings. Application of sodium thiosulphate ($Na_2S_2O_3$) effectively induced Hg root uptake and shoot translocation in *B. juncea* plants grown in the field. The maximum extraction yield was around 25 g Hg/ha. Volatilisation studies demonstrated that Hg in Tui tailings is transformed to Hg (0) and that plants significantly enhanced this process. Mass balance calculations revealed that Hg volatilisation removed around 500 g Hg/ha during the experimental period. This value accounts for 95% of the total Hg mass removed from the Tui mine tailings and, thus Hg volatilisation is the dominant Hg removal pathway for the Tui tailings. A preliminary assessment indicated the risk of Hg volatilisation to the local and regional environment is minimal. However, there is a risk that Hg emitted by plants could be deposited in regional aquatic systems and further biomagnified in the ecological food chain.

There are environmental and economical advantages that favour volatilisation over thioligand Hg-phytoextraction. For instance, (1) volatilisation can remove Hg from substrates without the help of solubilising agents; (2) The plant-mediated Hg (0) reduction pathway substantially reduces the potential for Hg biomagnification, as Hg volatilised from plant tissues would not represent an exposure route of Hg to wildlife; (3) The cost of the remediation operation would be reduced as plant-mediated Hg (0) volatilisation would not require chemical amendments and site maintenance (harvesting); (4) There are no costs involved in transport and processing of the Hg-rich biomass, since Hg is not accumulated in shoot tissues. Finally, the environmental costs of atmospheric Hg (0) emissions from plants could be offset by the absence of Hg-containing leachates generated as a by-product of the induced plant-Hg accumulation strategy. However, the suitability of phytoremediation for cleaning up of Hg from Tui mine tailings should be compared with other existing technologies. The environmental impacts of this technology should be the subject of discussion between researchers, environmental professionals, and the community before implementation in the field.

Acknowledgments

We gratefully acknowledge the National Council for Scientific and Technological Development (CNPq/Brazil) for providing a scholarship to the first author. We also thank M.B. Kirkham for the helpful comments on the manuscript.

References

Barkay, T., 1987. Adaptation of aquatic microbial communities to Hg^{2+} stress. Applied and Environmental Microbiology 53, 2725–2732.

Barkay, T., Turner, R.R., VandenBrook, A., Liebert, C., 1991. The relationship of Hg (II) volatilisation from a freshwater pond to the abundance of *mer* genes in the gene pool of the indigenous microbial community. Microbial Ecology 21, 151–161.

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

Bizily, S.P., Rugh, C.L., Summers, A.O., Meagher, R.B., 1999. Phytoremediation of methylmercury pollution: MerB expression in *Arabdopsis thaliana* confers resistance to organomercurials. Proceedings of the National Academy of Sciences (USA) 96, 6808–6813.

Choi, S.C., Chase, J.T., Bartha, R., 1994. Metabolic pathways leading to mercury methylation in *Desulfovibrio desulfuricans* LS. Applied and Environmental Microbiology 60, 4072–4077.

Duckart, E.C., Waldron, L.J., Donner, H.E., 1992. Selenium uptake and volatilisation from plants growing in soil. Soil Science 53 (2), 94–99.

Gustin, M.S., Taylor Jr., G.E., Maxey, R.A., 1997. Effect of temperature and air movement on the flux of elemental mercury from substrate to atmosphere. Journal of Geophysical Research 102, 3891–3898.

Heaton, A.C.P., Rugh, C.L., Wang, N.J., Meagher, R.B., 1998. Phytoremediation of mercury- and methylmercury-polluted soils using genetically engineered plants. Journal of Soil Contamination 7 (4), 497–509.

Hinton, J.J., Veiga, M.M., 2001. Mercury contaminated sites: a review of remedial solutions. In: Proceedings of the National Institute for Minamata Disease Forum (NIMD), Minamata, Japan, March 19–20.

Hoagland, D.R., Arnon, D.I., 1950. The water culture method of growing plants without soil. California Agriculture Experimental Station, Circular 347.

Kabata-Pendias, A., Pendias, H., 2000. Trace Elements in Soils and Plants. CRC Press, Florida, USA, 413 pp.

Kimura, Y., Miller, V.L., 1960. Vapor phase separation of methyl or ethylmercury compounds and metallic mercury. Analytical Chemistry 32, 420–424.

Kirkham, M.B., 1977. Trace elements in sludge on land: effects on plants, soils, and ground water. In: Loehr, R.C. (Ed.), Land as a Waste Management Alternative. Ann Arbor Science, MI, USA, pp. 209–247.

Kvietkus, K., Sakalys, J., 2001. Lifetime of gaseous and particulate mercury in the atmosphere. Proceedings of the 6th International Conference on Mercury as a Global Pollutant. Minamata, Japan, October 15–19 (Abstract).

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

Leonard, T.L., Taylor, G.E., Gustin, M.S., Fernandez, G.C.J., 1998. Mercury and plants in contaminated soils: 1. Uptake, partitioning, and emission to the atmosphere. Environmental Toxicology and Chemistry 17 (10), 2063–2071.

Lindberg, S.E., Kim, K.H., Munthe, J., 1995. The precise measurement of concentration gradients of mercury in air over soils: a review of past and recent measurements. Water, Air, and Soil Pollution 80, 383–392.

Lindberg, S.E., Hanson, P.J., Meyers, T.P., Kim, K.H., 1998. Air/exchange of mercury vapour over forests – the need for a reassessment of continental biogenic emissions. Atmospheric Environment 32 (5), 895–908.

Meagher, R.B., Rugh, C.L., Kandasamy, M.K., Gragson, G., Wang, N.J., 2000. Engineering phytoremediation of mercury pollution in soil and water using bacterial genes. In: Terry, N., Bañuelos, G. (Eds.), Phytoremediation of Contaminated Soil and Water. Lewis Publishers, United States, pp. 201–219.

Moreno, F.N., Anderson, C.W.N., Stewart, R.B., Robinson, B.H., 2004a. Phytoremediation of mercury-contaminated mine tailings by induced plant-mercury accumulation. Environmental Practice 6 (2), 165–175.

Moreno, F.N., Anderson, C.W.N., Robinson, B.H., Stewart, R.B., 2004b. Measuring volatile Hg released from plants. In: Currie, L.D., Hanly, J.A. (Eds.), Tools for Nutrient and Pollutant Management: Applications to Agriculture and Environmental Quality. Occasional Report N°.17. Fertilizer and Lime Research Centre, Palmerston North, New Zealand, pp. 233–240.

Moreno, F.N., Anderson, C.W.N., Robinson, B.H., Stewart, R.B., 2004c. 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: Applications to Agriculture and Environmental Quality. Occasional Report N°.17. Fertilizer and Lime Research Centre, Palmerston North, New Zealand, pp. 425–433.

Morrell, W.J., Stewart, R.B., Gregg, P.E.H., Bolan, N.S., Horne, D., 1996. An assessment of sulfide oxidation in abandoned base-metal tailings, Te Aroha, New Zealand. Environmental Pollution 94 (2), 2176–2225.

Morel, F.M.M., Kraepiel, A.M.L., Amyot, M., 1998. The chemical cycle and bioaccumulation of mercury. Annual Reviews in Ecological Systems 29, 543–566.

Nriagu, J.O., 1979. The Biogeochemistry of Mercury in the Environment. Elsevier, New York, pp. 23–41.

NZMS, 1983. Summaries of climatological observations to 1980. New Zealand Meteorological Service, Wellington, New Zealand.

Pacyna, E.G., Pacyna, J.M., Pirrone, N., 2001. Atmosphere mercury emissions from anthropogenic sources in Europe. In: Proceedings of the 6th International Conference on Mercury as a Global Pollutant. Minamata, Japan, October 15–19 (Abstract).

Raskin, I., Kumar, N.P.B.A., Dushenkov, S., Salt, D.E., 1994. Bioconcentration of heavy metals by plants. Current Opinions in Biotechnology 5, 285–290.

Robinson, B.H., 1994. Pollution of the Aquatic Biosphere by Arsenic and other elements in the Taupo Volcanic Zone. Master thesis, Massey University, Palmerston North, New Zealand, 127 pp.

Robinson, B.H., Leblanc, M., Petit, D., Brooks, R.R., Kirkman, J.H., Gregg, P.E.H., 1998. The potential of *Thlaspi caerulencens* for phytoremediation of contaminated soils. Plant and Soil 203, 47–56.

Rodriguez, L., Lopez-Bellido, F.J., Carnicer, A., Alcade-Morano, V., 2003. Phytoremediation of Hg-polluted soils using crop plants. Fresenius Environmental Bulletin 12 (9), 967–971.

Rugh, C.L., Wilde, H.D., Stacks, N.M., Thompson, D.M., Summers, A.O., Meagher, R.B., 1996. Mercury ion reduction and resistance in transgenic *Arabdopsis thaliana* plants expressing a modified bacterial merA gene. Proceedings of the National Academy of Sciences (USA) 93, 3182–3187.

Saouter, E., Gillman, M., Turner, R., Barkay, T., 1994. Development and field validation of a microcosm to simulate the mercury cycle in a contaminated pond. Environmental Toxicology and Chemistry 14 (2), 69–77.

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

Schnoor, J.L., Licht, L.A., McCutcheon, S.C., Wolfe, L.N., Carreira, L.H., 1995. Phytoremediation of organic and nutrient contaminants. Environmental Science and Technology 29 (7), 318–323A.

US Environmental Protection Agency, 2004. Mercury: Frequent Questions. US Environmental Protection Agency Web site, Washington, DC. <http://epa.gov/mercury/information1.htm>.

Veiga, M.M., 2004. Mercury pollution: revealing sources and suggesting solutions. Environmental 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. Natural Resources Forum 26, 15–26.

Weber, J.H., 1993. Review of possible paths for abiotic methylation of mercury (II) in the aquatic environment. Chemosphere 26 (11), 2063–2077.

Wilkinson, G., Gillard, R.D., McCleverty, J.A. (Eds.), 1987. Comprehensive Coordination Chemistry, vol. 2. Pergamon Press, UK, 1179 pp.

World Health Organization, 1976. Mercury: Environmental Health Criteria, vol. 1. World Health Organization, Geneva.