# Phytoremediation of Mercury-Contaminated Mine Tailings by Induced Plant-Mercury Accumulation

Fabio N. Moreno, Chris W. N. Anderson, Robert B. Stewart, Brett H. Robinson

In most contaminated soils and mine tailings, mercury (Hg) is not readily available for plant uptake. A strategy for inducing Hg mobilization in soils to increase accumulation potential in plants was investigated to enhance Hg phytoremediation. Accumulation of Hg in the nickel hyperaccumulator *Berkheya coddii*, the salt-tolerant *Atriplex canescens*, and the nonaccumulators *Brassica juncea* and *Lupinus* sp. was studied by pot trials containing mine tailings treated with either soluble Hg or sulfur-containing ligands. Accumulation of Hg in shoots of *B. coddii* and *A. canescens* after addition of soluble Hg was lower than 10 mg/kg dry weight. The addition of ammonium thiosulfate ($(NH_4)_2S_2O_3$) to tailings mobilized Hg in substrates, as indicated by the elevated Hg concentrations in leachates from the pots of both species. Ammonium thiosulfate caused a significant increase in the Hg concentration in shoots of *B. juncea*. Conversely, Hg translocation to *Lupinus* sp. shoots was significantly reduced in the presence of this ligand. Mass balance calculations revealed a significant fraction of Hg was lost from the system. This unaccounted-for Hg may indicate Hg volatilization. The results suggest that there is potential for induced plant Hg accumulation for phytoremediation of Hg-contaminated sites. Issues of Hg leaching and volatilization, however, need to be addressed before this technology can be implemented in the field.

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In spite of the technological advances over the past few decades, soil degradation from mining activities is still occurring in developed and developing nations (Veiga and Hinton, 2002). Mining operations generally involve the displacement of thousands of tons of rock and the generation of large volumes of metal-contaminated waste. Among the environmental impacts associated with the presence of base-metal waste is the metal contamination of local soils and water systems. Metal contamination of surrounding soils is propagated via airborne dispersal of dust particles and wind erosion, whereas acid mine drainage emanating from tailings dams can pollute adjacent streams and groundwater supplies with metal ions (Holmström et al., 2001; Morrell et al., 1996).

Implementation of remedial procedures for metal removal is therefore a first step toward the rehabilitation and/or reclamation of heavy metal–polluted sites (Gupta et al., 2000). During the past decade there has been increasing interest in the possibility of using vegetation for remediating heavy metal–contaminated sites (phytoremediation). Plant-based remediation represents a low-cost and environmentally friendly alternative to traditional techniques such as soil removal and capping, which can be expensive and leave the site barren (Brooks, 1998).

Among the different areas embraced by the field of phytoremediation, special interest has been devoted to the phytoextraction and phytovolatilization of metals from contaminated soils. In the first case, metals are removed from soils by concentrating them in the aerial parts of the plant. Harvesting and disposal of shoot biomass allows the metal to be removed in significant quantities from the soil. Phytovolatilization, on the other hand, uses plants to clean up metal-polluted sites through volatilization of the contaminant from the plant biomass into the atmosphere (Brooks, 1998; Meagher et al., 2000; Zayed et al., 2000).

Suitable plants for phytoextraction and phytovolatilization can be divided into three groups. The first are the plants known as metal hyperaccumulators, which can accumulate unusually high levels of metals in their aerial tissues but quite often do not provide high annual biomass. The zinc

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(Zn) hyperaccumulator *Thlaspi caerulenscens*, for example, can contain up to 1% of this metal on a dry weight basis and falls within the low-biomass plant group. The second group consists of plants that have a relatively lower metal concentration in plant tissues but can produce a substantial amount of biomass. Plants in this group can also volatilize metals, as is the case with the selenium accumulator *Astragalus* sp. and the nonaccumulator *Brassica juncea* (McGrath, 1998; Robinson et al., 1998; Zayed et al., 2000). Finally, the third group includes transgenic plants encoding the bacterial mercury (Hg) ion reductase (merA) and organomercury lyase (MerB) genes, which have increased Hg resistance and enhanced volatilization capacity (Bizily et al., 1999; Rugh et al., 1996). For example, transgenic *Brassica napus* expressing the merA gene can germinate in media containing up to 50 mg/L Hg, while transgenic tobacco, cultured in Hg-containing hydroponics solution and expressing the same gene, can volatilize 1.5 ng of Hg(0) per milligram of root tissue per minute (Meagher et al., 2000).

The success of phytoextraction depends on the availability of the target metal in soil for plant uptake (Blaylock et al., 1997). For example, Hg, one of the most toxic pollutants, has limited solubility in soils, and thus low availability for plant uptake. In general, only trace concentrations of Hg are found in soil solution, mostly as uncharged complexes (Schuster, 1991). Plant availability and uptake of Hg will therefore depend on the ability to control the processes that enhance the concentration of this element in the soil solution (McLaren and Cameron, 1996). The coordination chemistry of Hg suggests that this element will be present mostly as a complex in soil solution. Therefore the partitioning of Hg from the solid phase into soil solution will occur as a consequence of coordinative reactions where Hg ions are exchanged with water molecules for some preferred ligands (Yaron, Calvet, and Prost, 1996). According to the hard and soft acid-base principle (Pearson, 1963), Hg is a soft metal and thus forms stronger complexes with soft ligands like $Cl^-$, $OH^-$, $S^{2-}$, and sulfur-containing functional groups of organic ligands. Consequently, in well-oxygenated soils, the uncharged and soluble species $HgCl_2$, Hg(OH)Cl, and $Hg(OH)_2$ tend to predominate over other aqueous species, depending on the presence of $Cl^-$ ions and soil pH. In mildly reduced environments and in the presence of other metal sulfides or sulfhydril groups, Hg will precipitate as insoluble cinnabar (HgS) (Barnett et al., 1997). In addition, the strong affinity of Hg for organic matter profoundly influences Hg solid phase speciation and is regarded as one of the major driving forces for Hg adsorption by soil particles (Kabata-Pendias and Pendias, 2000).

The goal of this study was to investigate the phytoextractive potential of different plant species with a view to remediating Hg-contaminated mine tailings. The specific aims, which involved combined studies on the geochemistry of Hg in the tailings with simple plant pot trials, are detailed below:

To test tolerance, uptake, and translocation of increasing concentrations of soluble Hg ($HgCl_2$) by *Berkheya coddii* and *Atriplex canescens*;

To examine the chemical solubilization of Hg in the substrate in the presence of sulfur-containing ligands; and

To investigate the enhanced Hg accumulation of *B. juncea* and *Lupinus* sp. in this substrate in the presence of selected sulfur-containing ligands.

## Materials and Methods

### Site Description

Substrate samples for the pot trials were collected from the tailings dam of the abandoned Tui base-metal mine, located on the northwest flank on Mount Te Aroha, approximately 3 km northeast of the township of Te Aroha, North Island of New Zealand (Figure 1). The most recent period of mining activity occurred between 1967 and 1974 with the extraction and processing of up to 100 tons of ore a day, yielding up to 10 tons of lead-copper-zinc concentrate containing minor amounts of mercury, cadmium, silver, and gold. The cessation of activities in 1974 left a tailings dam containing 100,000 $m^3$ of sulfide-rich tailings with high levels of heavy metals. The main metal-bearing minerals present in the Tui ore are sphalerite (ZnS), galena (PbS), chalcopyrite ($CuFeS_2$), and pyrite ($FeS_2$), which have been oxidized in contact with the air, producing acid mine drainage (Morrell et al., 1996).

### Substrate Characterization

The experiments described in this article utilized two types of samples collected from the Tui mine site. The experiment investigating plant accumulation of added soluble Hg used a low Hg content sample (0.3 ± 0.003 mg/kg), whereas the experiments investigating the chemical solubilization of tailings and the induced plant Hg accumulation utilized a high Hg content sample (2.82 ± 0.31 mg/kg).

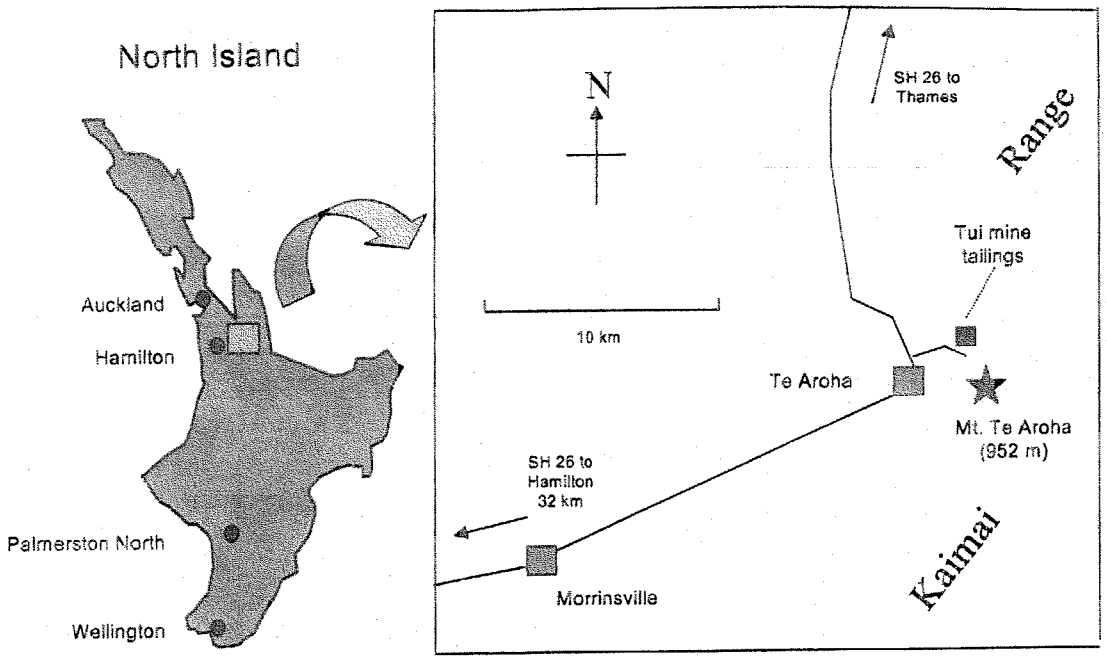


**Figure 1.** Location of Tui mine tailings. The tailings dam is located on the northwest flank on Mount Te Aroha, approximately 3 km northeast of the township of Te Aroha, North Island of New Zealand.

### Chemically Solubilized Hg

Seven chemical extractants were tested for their ability to estimate the concentration of chemically solubilized Hg in the Tui tailings. One gram of tailings was weighed into 50 ml polypropylene centrifuge tubes in triplicate. After addition of extractant solutions (20 ml of 2 g/L, unless otherwise stated), the tubes were rotated in a shaker overnight and the supernatant filtered. The following chemicals were investigated for their ability to extract Hg: ammonium thiocyanate (SCN), potassium thiocyanate (KCN), thiourea, humic acid (HA), ammonium thiosulfate ($NH_4S_2O_3$), sodium sulfide ($Na_2S$), hydrogen peroxide (at 0.27%), and ammonium thiocyanate (SCN + $H_2O_2$) plus hydrogen peroxide (at 0.27%).

### Soluble Hg Plant Accumulation

The nickel hyperaccumulator *B. coddii* and the salt-tolerant *A. canescens* were grown from seeds in flat trays. They were transplanted individually into 250 ml plastic pots filled with 1:1 mixture of Tui mine tailings and pumice as one-week-old seedlings. The pumice was used to improve drainage of the fine-textured tailings. After five weeks, Hg was added as aqueous $HgCl_2$ to achieve total Hg concentrations of 1, 5, and 10 mg/kg in each growing pot. Previous analysis for total Hg in this particular subsample of Tui mine tailings showed average Hg levels of 0.3 mg/kg. Plants from each species grown in untreated substrates (without added Hg) were designated as control plants. Pots with Hg added at 1, 5, and 10 mg/kg of Hg but without plants were designated as control pots. All plants were watered daily. Forty-four days after $HgCl_2$ addition, all plants were harvested and substrates sampled.

### Induced Plant Hg Accumulation

The nonaccumulator plants *B. juncea* and *Lupinus* sp. were seeded directly into pots filled with the high Hg content Tui mine tailings. No pumice was added, as drainage was adequate for plant growth. In each pot, approximately 20 seeds of *B. juncea* and two seeds of *Lupinus* sp. were sown and the seedlings then grown for two more weeks. After five weeks each pot was thinned to leave only one individual plant. Three treatments, each with a different functional sulfur group, were investigated for inducing Hg accumulation in plants: thiourea, ammonium thiosulfate ($NH_4S_2O_3$), and ammonium thiocyanate supplemented with hydrogen peroxide at 0.27% (SCN + $H_2O_2$). The amount of chemical added was 2 g/kg of substrate, unless otherwise stated. Plants from each species grown in untreated substrates (without addition of ligands) were designated as control plants. Pots treated with ligands but without plants were designated as control pots. All plants

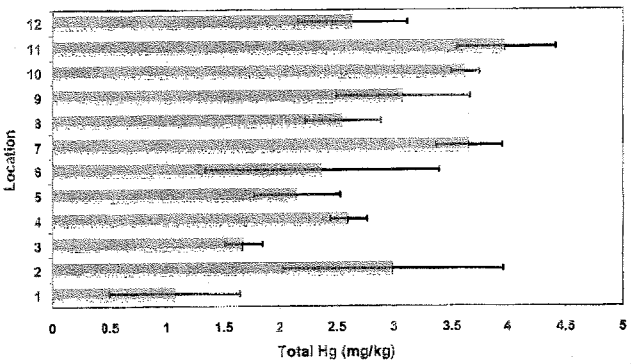


**Figure 2.** Total Hg concentrations for the 12 locations sampled along the Tui mine tailings. Bars denote ±1 standard deviation from the mean of three replicates.

were watered daily. A system for leachate collection was set up by putting the individual pots on the top of funnels and connecting them to 100 ml Erlenmeyer flasks. Five days after the addition of extractant solutions, all plants were harvested and leachates and substrates sampled.

The substrates used for investigating both soluble and induced plant Hg accumulation were fertilized with 5 g/L of Osmocote (slow-release fertilizer) and had their pH adjusted to 5.5 by the addition of lime. The experiments were carried out in a greenhouse with the temperature controlled at 21° C and under a natural sunlight flux. Both experiments utilized a two-factorial completely randomized experimental design (CRD), with plant species, Hg concentrations, and sulfur-containing ligands as factors (accordingly for each group of experiments). At least five pots were used as replicates for comparing plant treatment means (unless otherwise stated). Leachates and substrates were collected in triplicate. Except for dry yield biomass, all plant data were log-normally distributed.

### Soil and Plant Digestions

Total Hg determinations for all tailings substrates were obtained through *aqua regia* digestions of dried samples collected from 12 locations at the Tui mine site. For this purpose, 1 g 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 a water bath at 80° C for one hour and the filtrates diluted to a final volume of 50 ml by adding reverse osmosis water. Total Hg from substrates of both soluble and induced plant pot trials was extracted using exactly the same procedure.

Plants to be analyzed for total Hg were prepared in the following manner. Shoots and roots were washed in tap water and placed in a drying oven at 70° C until a constant weight was obtained. Subsamples (0.1 g) were accurately weighed into 50 ml plastic pots and digested with 15 ml of $HNO_3$. The plant samples were left overnight, and the following day were heated in a water bath at 80° C for one hour. Subsequently the plant digests were transferred to 10 ml polythene tubes and diluted with reverse osmosis water to make a final volume of 10 ml.

### Mercury Analyses

All samples of plant material, tailings, and leachates were analyzed for Hg using hydride-generation atomic absorption spectroscopy (Godden and Thomerson, 1980). Mercury-containing liquid samples were analyzed along with 10 ml of 0.5 M HCl. Sodium borohydride was used as a reducing agent to generate Hg vapor in a 5% $NaBH_4$ + 1% KOH wt/vol solution. The limit of detection for Hg in solution was 11.5 ng/ml (i.e., 0.0115 mg/L). Reproducibility was obtained through replicate analysis of 10 standards containing 1 mg/L of Hg and variation was less than 5%. Reagent blanks were below detection limits, that is, less than 5 ppb, in the solution. Linear calibration curves were obtained over the range of 125 to 1000 ng/ml of Hg using four standards prepared from a 10 mg/L mercuric nitrate ($HgNO_3$) AAS reagent (M&B). 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 and soil samples.

### Statistical Analyses

A copy of SAS PC version 8e was used for statistical analyses. Possible interactions between the main factors were addressed performing a two-way analysis of variance (ANOVA) on the logs of the data. Duncan's multiple range test was used for pairwise comparison of means.

## Results

### Total and Chemically Solubilized Hg

Figure 2 shows total Hg concentrations for the 12 locations sampled along the site. Total Hg values between the sampled locations were significantly different, ranging from 1.1 ± 0.57 to 3.95 ± 0.43 mg/kg ($p < 0.001$). The high within-location variability of Hg concentrations shows

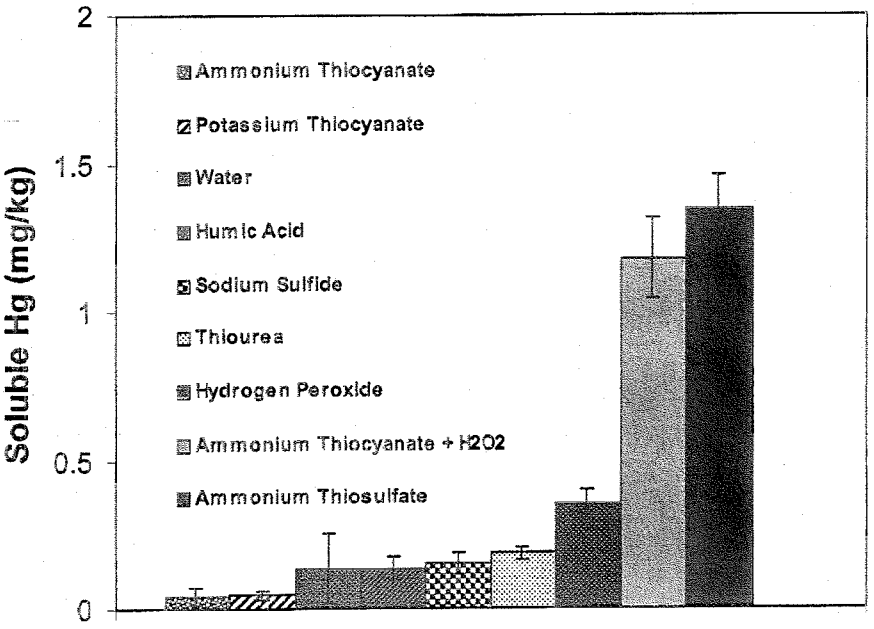


Figure 3. Chemically solubilized Hg as a result of sulfur-containing ligands applied to Tui mine tailings at a 2 g/L concentration [except $H_2O_2$, (0.27%)]. Bars denote ±1 standard deviation from the mean of three replicates.

that Hg was not uniformly distributed in the Tui mine tailings.

Soluble Hg concentrations, as a function of chemical extractants (specifically, sulfur-containing ligands) added to Tui mine tailings, are shown in Figure 3. Mercury solubility in Tui mine tailings was significantly increased in the presence of $NH_4S_2O_3$ and $SCN + H_2O_2$ when compared to the control ($p < 0.0001$) (Figure 3). Figure 4 shows that the concentration of soluble Hg was significant and positively correlated ($r = 0.88$, $p < 0.0001$) to the concentration of $NH_4S_2O_3$ applied to the substrate. At the highest $NH_4S_2O_3$ concentration, the concentration of Hg in the extract reached 4.3 mg/kg, which is equivalent to the highest range for total Hg concentration found in the *aqua regia* digests (Figure 2). We believe therefore that two main processes increased Hg solubility under the acidic conditions that prevail in the Tui mine tailings. The first process occurred in the presence of $NH_4S_2O_3$ and involved extraction of Hg bound to the solid phase of sulfidic minerals, possibly through the formation of a thiosulfate-Hg complex. For the second process, hydrogen peroxide may have oxidized sulfidic Hg forms, allowing formation of a Hg-SCN complex.

## Soluble Hg Plant Accumulation

The accumulation of soluble Hg in roots and shoots of *B. coddii* and *A. canescens* is shown in Figure 5. On average, root concentration was an order of magnitude higher than shoot concentration. Roots of both species showed very similar patterns for Hg accumulation as a function of Hg treatments, indicating that a physiological mechanism exists for regulating Hg uptake above certain concentrations. Accumulation of Hg in roots therefore increased drastically up to 5 mg/kg of Hg in substrates, keeping a steady concentration in the root tissue of around 75 mg/kg with no significant increase beyond this level (Figure 5A). In the case of shoots, *B. coddii* accumulated significantly more Hg than *A. canescens* (Figure 5B). At substrate additions of 10 mg/kg of Hg, for instance,

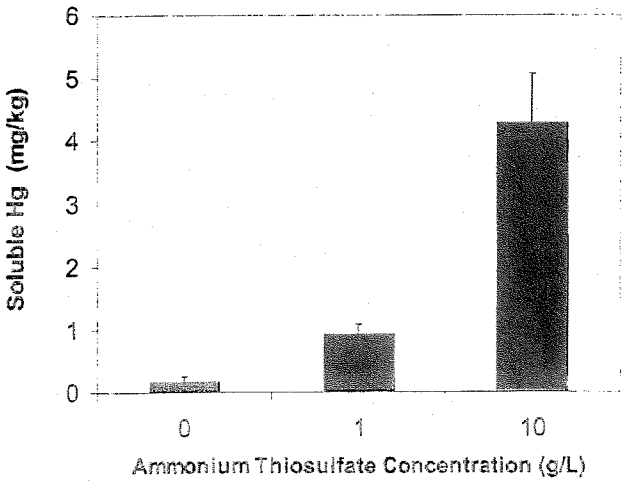


Figure 4. Chemically solubilized Hg as a result of ammonium thiosulfate ($NH_4S_2O_3$) applied to Tui mine tailings at the concentrations of 0, 1, and 10 g/L. Bars denote ±1 standard deviation from the mean of three replicates.

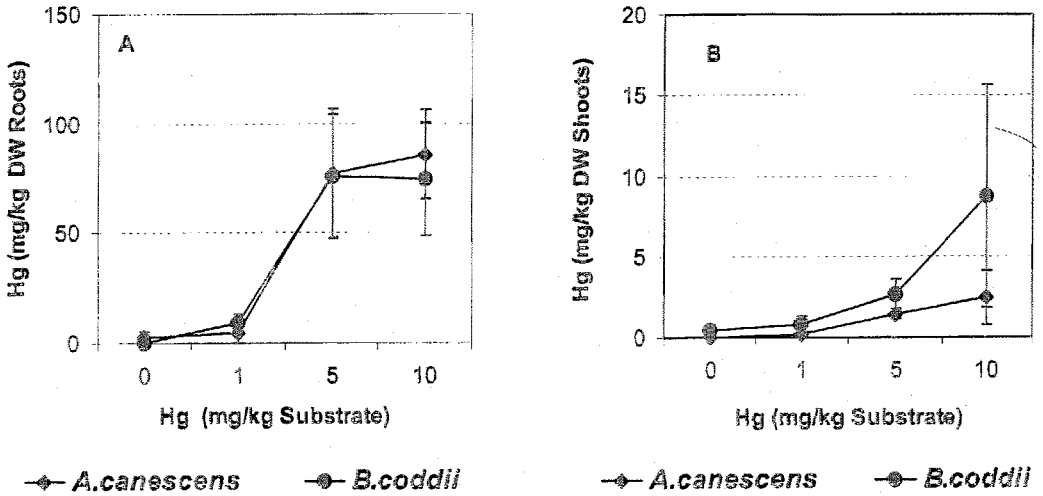


**Figure 5.** Accumulation of Hg in roots (A) and shoots (B) of *Berkheya coddii* and *Atriplex canescens* grown in Tui mine tailings treated with 1, 5, and 10 mg/kg of soluble Hg ($HgCl_2$). Bars denote ±1 standard deviation from the mean of five replicates. DW = dry weight.

the average Hg values for *B. coddii* and *A. canescens* in shoots were 8.7 and 2.47 mg/kg dry weight, respectively. Furthermore, *B. coddii* showed shoot Hg concentrations that were highly and positively correlated to increasing concentrations of Hg in substrates ($r = 0.76$, $p < 0.0001$). Mean values for dry matter yield were not significantly different, neither between plant species nor between levels of Hg treatments (Table 1). Therefore both plant species showed evidence of some degree of tolerance to the presence of Hg in substrates.

Figure 6 compares Hg concentrations (in mg/kg) between planted and unplanted substrates within each treatment level of Hg at the end of the experiment. The Hg concentrations found in control substrates were significantly higher than in planted substrates for all Hg-treated substrates ($0.05 > p > 0.0001$). In addition, there was no significant difference between control and planted pots in relation to the initial concentration of Hg present in untreated substrates (0.3 mg/kg, on average). These results indicate the naturally occurring Hg forms present in the tailings provide very limited availability for plant uptake.

**Table 1.** Dry matter yield (g/pot) of *Berkheya coddii* and *Atriplex canescens* in Tui mine tailings treated with 1, 5, and 10 mg/kg of soluble Hg ($HgCl_2$)

| | | Plant species | |
|---|---|---|---|
| Hg treatment (mg/kg) | *N* | *A. canescens* | *B. coddii* |
| 0 | 14 | 0.23 ± 0.12 | 0.31 ± 0.12 |
| 1 | 14 | 0.46 ± 0.28 | 0.30 ± 0.07 |
| 5 | 14 | 0.41 ± 0.21 | 0.38 ± 0.15 |
| 10 | 14 | 0.31 ± 0.11 | 0.38 ± 0.16 |

Values are ±1 standard deviation from the mean of five replicates.

## Induced Plant Hg Accumulation

The results for induced Hg accumulation by *B. juncea* and *Lupinus* sp. as a consequence of the addition of sulfur-containing ligands to Tui mine tailings are shown in Table 2 (roots) and Figure 7 (shoots). The application of $NH_4S_2O_3$ and SCN + $H_2O_2$ solubilized Hg in the substrates and substantially increased Hg uptake by the roots, as well as translocation to shoots. Overall, root accumulation in the presence of $NH_4S_2O_3$ and SCN + $H_2O_2$ was significantly higher ($0.05 > p > 0.0001$) among all plant-ligand combinations. Mercury concentrations in the root tissues of *Lupinus* sp. and *B. juncea* averaged 255 and 104 mg/kg dry weight, respectively, after individual addition of $NH_4S_2O_3$ (Table 2). Application of $NH_4S_2O_3$ to substrates also dramatically increased Hg accumulation in shoots of *B. juncea* relative to other treatments (Figure 7). Recorded values in aerial tissues of *B. juncea* averaged 43 mg/kg, with a single replicate reaching a value of 61 mg/kg. In contrast, translocation of Hg to shoots of *Lupinus* sp. was substantially lower when compared to *B. juncea* shoots. The maximum value observed for the former species was 0.5 mg/kg in the presence of $NH_4S_2O_3$ (Figure 7). In addition, the presence of $NH_4S_2O_3$ induced a more equal distribution of the Hg mass between shoots and roots of *B. juncea*, whereas for *Lupinus* sp. the great majority of the total plant Hg mass was retained in the root tissues (data not shown).

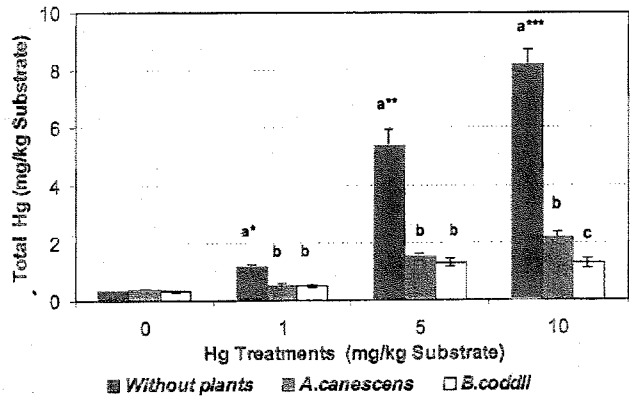


Figure 6. Total Hg concentrations in controls and planted substrates after application of 1, 5, and 10 mg/kg of soluble Hg ($HgCl_2$) to pots. Bars denote ±1 standard deviation from the mean of three replicates. Letters compare means within each Hg treatment level. Means with different letters are significantly different (Duncan's test). The symbol (*) indicates the significance level ($\alpha$) for the test (* = 0.05, ** = 0.01, and *** = 0.0001).

The Hg content in leachates was enhanced after addition of sulfur-containing ligands to Tui mine tailings (Figure 8). Specifically, the application of $NH_4S_2O_3$ promoted a significant increase ($p < 0.0001$) in the Hg concentration present in leachates collected from *Lupinus* sp. pots among all other plant-ligand combinations. After the addition of $NH_4S_2O_3$, the Hg concentration in leachates collected from *Lupinus* sp. and *B. juncea* pots increased by 4 and 1 mg/L, respectively, whereas Hg concentrations in controls were an order of magnitude lower.

Figure 9 shows a 100% normalized picture of mass balance for plant, soil, leachates, and unaccounted for Hg for *B. juncea* and *Lupinus* sp. grown in Tui tailings and treated with $NH_4S_2O_3$. The unaccounted for Hg fraction was obtained for each plant species by subtracting the Hg mass found in plants (shoots + roots), leachates, and substrates at harvest from the average Hg mass found in control pots (without plants) at the end of the experiment. Because Hg accumulation in roots and shoots was superior in the presence of $NH_4S_2O_3$, the Hg mass balance for both species was compared only within this treatment level.

The existence of a Hg fraction that could not be accounted for indicates that the added Hg has been transformed to a form of Hg that could not be measured after the addition of $NH_4S_2O_3$. Therefore it is possible to infer that the unaccounted-for Hg fraction might be due to Hg(0) volatilization from plant leaves or roots and their associated microbes (Barkay et al., 1992; Leonard et al., 1998; Meagher et al., 2000). In addition, because photochemical and chemical reduction processes are significant sources of Hg(0) emitted from contaminated sites (Andersson, 1979; Morel, Kraepiel, and Amyot, 1998), it is also possible that these abiotic factors have contributed to the volatile Hg fraction. The fact that Hg-treated substrates without plants exhibited up to 20% losses from initial Hg concentrations (Figure 6) provides support for this assumption. Finally, the existence of a volatilized Hg fraction offers an explanation for the discrepancy observed between planted pots and controls in relation to the Hg concentrations found in substrates at the end of the experiment (Figure 6).

Table 2. Root Hg concentrations (mg/kg) of *Brassica juncea* and *Lupinus* sp. after application of sulfur-containing ligands at 2 g/kg (unless otherwise stated) to Tui mine tailings

| | | Plant species | | | |
|---|---|---|---|---|---|
| Treatment | $N^a$ | *Lupinus* sp. | | *B. juncea* | |
| Control | 9 | a | 1.05 ± 0.77 | a | 18.97 ± 15.20 |
| Thiourea | 10 | b | 14.51 ± 9.60 | a | 36.58 ± 13.42 |
| $SCN + H_2O_2$[b] | 10 | c | 107.15 ± 23.71 | b | 194.93 ± 98.09 |
| $NH_4S_2O_3$[c] | 10 | c | 255.32 ± 96.68 | b | 104.13 ± 23.24 |

Values are ±1 standard deviation from the mean of five replicates.
Letters compare treatments in the vertical; means with the same letter are not significantly different [Duncan's test at a significance level ($\alpha$) of 0.01].
[a] Missing values due to Hg concentrations in sample digests below detection limits.
[b] Ammonium thiocyanate + hydrogen peroxide at 2 g/kg and 0.27%, respectively.
[c] Ammonium thiosulfate.

## Discussion

The high sulfide content (>10%) and the presence of the sulfide-bearing minerals galena (PbS), sphalerite (ZnS), pyrite ($FeS_2$), and chalcopyrite ($CuFeS_2$) in the Tui ore indicate that Hg in the tailings might be present as sulfidic Hg forms such as cinnabar, metacinnabar, Hg polysulfides, or Hg associated with iron sulfides. In addition, trace quantities of cinnabar were shown to be present in the Tui ore (Morrell et al., 1996). These Hg forms are very insoluble, not easily altered, and seldom found as detrital material (Kabata-Pendias and Pendias, 2000). Because of their insolubility, sulfidic forms of Hg are said to be unavailable and/or immobile in the environment. This means that they do not liberate Hg ions in water and thus their mobility for aqueous transport and transformation in the environment is low (Wallschäger et al. 1998). Our studies showed, however, the presence of significant amounts of water-extractable Hg in the Tui tailings

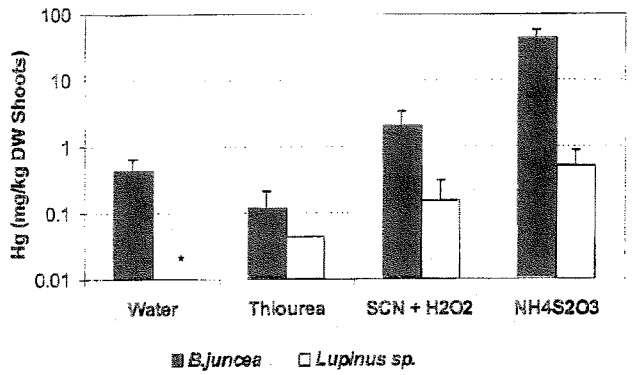


Figure 7. Shoot Hg concentrations of *Brassica juncea* and *Lupinus* sp. after application of sulfur-containing ligands at 2 g/kg to Tui mine tailings. Note that water = control, SCN + $H_2O_2$ = ammonium thiocyanate + hydrogen peroxide (at 0.27%); $NH_4S_2O_3$ = ammonium thiosulfate. Bars denote ±1 standard deviation from the mean of five replicates ($n$ = 1 for thiourea/*Lupinus* sp. treatment). The symbol (*) indicates Hg below detection levels. DW = dry weight.

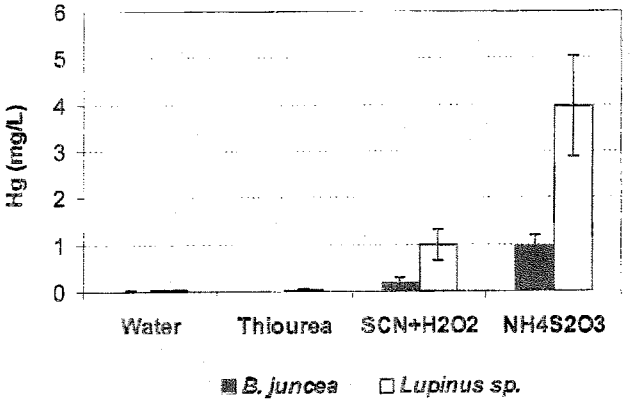


Figure 8. Hg concentrations in leachates collected from *Brassica juncea* and *Lupinus* sp. pots after application of sulfur-containing ligands at 2 g/kg to Tui mine tailings. Note that water = control, SCN + $H_2O_2$ = ammonium thiocyanate + hydrogen peroxide (at 0.27%); $NH_4S_2O_3$ = ammonium thiosulfate. Bars denote ±1 standard deviation from the mean of three replicates.

(Figure 3). Because the tailings have been left to weather since the closure of the mine in 1974, oxidation of pyrite and other sulfide-bearing minerals has led to acid mine drainage. As a result, the surface tailings exhibit variable but low pH (2.76–3.85) and high concentrations of total sulfur and sulfates ($SO_4^{2-}$) (Morrell et al., 1996). The presence of significant amounts of water-soluble Hg species in the Tui tailings suggests therefore that Hg was released to solution complexed to $SO_4^{2-}$ or $S^{2-}$ ligands, a reaction that has been shown to occur if significant levels of sulfur-containing functional groups are present in well-oxygenated solutions (Schuster, 1991). Consequently we cannot assume that the sulfidic Hg forms present in Tui mine tailings are able to retain Hg in an environmentally safe form and that they do not represent a risk to the environment.

Average background levels of Hg in plants are usually not greater than 100 ng/g (ppb) dry weight (Kabata-Pendias and Pendias, 2000). Through addition of $NH_4S_2O_3$ to Tui mine tailings, *B. juncea* was able to accumulate more than 400 times this value in the aerial tissues. In addition, Hg analysis in New Zealand native plants grown on Tui mine tailings showed Hg values below detection levels for aboveground plant tissues (data not shown), thus highlighting the effect of $NH_4S_2O_3$ on shoot Hg accumulation. The greater ability of $NH_4S_2O_3$ to enhance shoot Hg accumulation over other sulfur-containing ligands appears to be species specific, as only very small concentrations of Hg were found in the aerial tissues of *Lupinus* sp. Assuming that Hg retention in roots occurs at ion exchangeable sites on the cell wall (Blaylock et al., 1997), then $NH_4S_2O_3$ under the moderately acidic conditions prevailing in Tui mine tailings effectively reduced Hg retention in cell walls of *B. juncea* roots, thus facilitating Hg translocation to aerial tissues.

The volatilization of Hg by plants has been the subject of many studies that have attempted to investigate the behavior of Hg at the soil-plant-atmosphere interface (Leonard et al., 1998; Siegel, Puerner, and Speitel, 1974; Siegel and Siegel, 1979). Indeed, Leonard et al. (1998), in a carefully designed system for studying plant Hg emissions to the atmosphere, accumulated sufficient evidence to conclude that, in a biogeochemical context, plants work as biological conduits for Hg transfer from the geosphere to the atmosphere. Although great care was taken to minimize Hg losses, both the experimental procedure and the analytical techniques used in these experiments were not sufficient to determine the reasons for the unaccounted-for Hg fraction lost from the system (Figure 9). We have overcome this problem in subsequent studies by using gastight volatilization systems carefully designed to account for Hg losses from hydroponically grown *B. juncea* plants (Moreno et al., 2003). The results demonstrated that the volatilization process was significantly enhanced by plants in relation to controls and that it increased progressively from 40% to approximately 80% as the Hg concentrations in solutions increased from 0.05 to 10 mg/L. Therefore we can infer with a certain degree of confidence that plants and their associated microbes may have played an important role in the overall mass balance

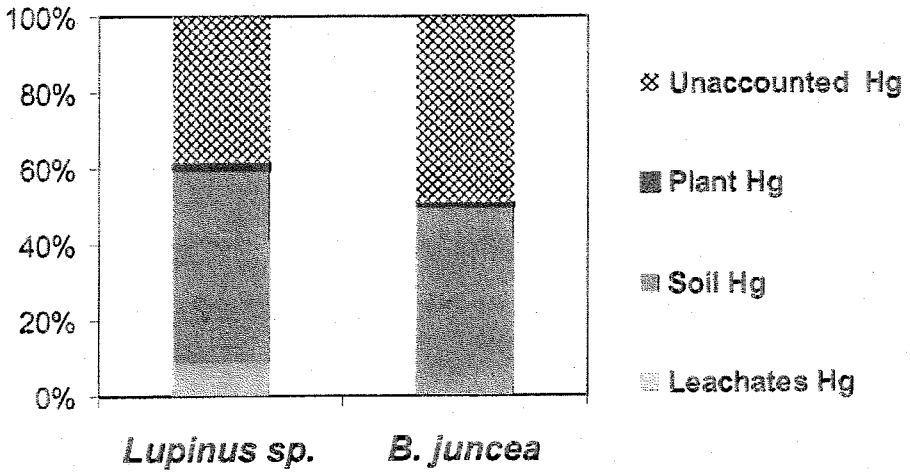


Figure 9. Normalized (100%) values of plant, soil, leachates, and unaccounted-for Hg from *Brassica juncea* and *Lupinus* sp. after application of ammonium thiosulfate at 2 g/kg to Tui mine tailings. Note that 100% = total Hg concentration in control pots (2.82 ± 0.31 mg/kg).

for Hg in Tui mine tailings. In this way, any plant-based strategy that aims to remediate Hg-contaminated soils should take into consideration the possibility of Hg losses from the system by volatilization.

The application of chelating agents to soils to induce a higher metal concentration in plants raises environmental concerns due to the potential contamination of groundwater via the leaching of mobilized metals. Leachates can be produced when rates of solubilizing agents applied to the soil are above the levels of metals in the soil solution that can be effectively taken up by plants. This excess metal may leach below the root zone into groundwater, a process exacerbated by macropore flow (Bundt et al., 2000). This explains the generation of Hg-containing leachates after the addition of $NH_4S_2O_3$ and $SCN + H_2O_2$ to substrates (Figure 8). Mercury leachate generation could be managed by using plant species with high transpiration rates, such as willows and poplars, or by intensive cropping practices and careful management of the irrigation system. The use of dual-pipe subirrigation-drainage systems has been proposed to collect metal-enriched drainage from an ethylenediaminetetraacetic acid (EDTA)-assisted phytoremediation operation (Madrid, Liphadzi, and Kirkham, 2003). In this system, water intercepted by drain tubes was pumped back to the irrigation supply in order to reduce leaching and the potential for groundwater contamination. This method could be optimized if a natural barrier of deep-rooted poplar or willow trees is established on site to help reduce downward movement of contaminants into the unsaturated zone, as has been proposed for leaching control of pesticides, nitrates, and heavy metals such as arsenic, boron, copper, and chromium (Paterson and Schnoor, 1992, 1993; Robinson et al., 2003).

Another issue of concern is the potential toxicity of sulfur-containing chemical ligands to fauna and flora. It has been demonstrated, however, that thio- complexes are readily broken down by soil microorganisms and that thiosulfate salts are only slightly toxic to plants and animals. The lethal concentration ($LD_{50}$) of sodium thiosulfate in rabbits, for instance, is 4000 mg/kg, a value that indicates a toxicity level very similar to that of sodium chloride in rats ($LD_{50}$ = 3000 mg/kg) (Anderson, Brooks, and Stewart, 2000; Strecher et al., 1968).

The feasibility of a phytoremediation operation using induced plant Hg accumulation was assessed for a *B. juncea* crop grown under the phytotoxic conditions that prevail in the Tui base-metal tailings. Assuming a conservative value of 10 tons per hectare (ha) of biomass production and an average Hg value of 50 mg/kg (dry weight) in shoot tissues, a single crop of *B. juncea* would remove 0.5 kg/ha of Hg from the Tui mine tailings. Following this approach and assuming a soil bulk density of 1.3, each crop of *B. juncea* would reduce the soil Hg concentration by 0.25 mg/kg. These calculations do not take into account Hg losses from the system as a result of leaching or volatilization.

## Conclusions

In this work we have effectively induced plants to accumulate Hg from contaminated metal waste by treating the substrate with sulfur-containing chelates. By doing this, *B. juncea* plants concentrated 43 mg/kg of Hg in shoot tissues from a substrate Hg concentration of 2.8 mg/kg, thus giving a concentration factor (concentration in plant tissue/concentration in soil) of 15.3. This achievement might have

some positive implications for the remediation of sites with low to moderate levels of Hg in substrates, where availability of some Hg forms limits Hg uptake and accumulation by plants. A possible phytoremediation strategy for the removal of Hg from the contaminated tailings therefore would be to promote volatilization of Hg by plants established on site in conjunction with periodic removal of Hg-containing plant material after treatment with $NH_4S_2O_3$. The results of this preliminary study, however, should be validated under field conditions where plant uptake of Hg and root growth can occur without the physical limitations imposed by simple pot trials. The logical sequence of this work will investigate plant Hg accumulation with New Zealand native species growing directly on Tui mine tailings, whereas additional plant pot trials using gastight volatilization chambers will address the effect of $NH_4S_2O_3$ on the plant Hg volatilization process.

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