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Chapter 6

PHYTOREMEDIATION OF MERCURY-CONTAMINATED MINE WASTES

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ABSTRACT

Although mercury (Hg) is one of the most toxic elements to living organisms, emission of Hg from natural sources worldwide has only a limited impact on life. However, over the past 150 years, anthropogenic Hg emissions have more than doubled the global Hg input from natural Hg sources (Hylander and Meili, 2003).

There have been sporadic episodes of human Hg poisoning from discharge of Hg-containing wastes to soil and water systems. Nevertheless, human activity still causes Hg releases into the environment. The main Hg pollution sources are atmospheric Hg discharges from coal-burning power plants, mining operations and incineration of urban and medical wastes (Morel et al., 1998).

The effect of Hg discharge into soils and aquatic systems is exacerbated by the transformation of inorganic Hg forms into methylmercury, which accumulates in living cells and tissues. It constitutes the greatest fraction of total mercury in living organisms and is quickly biomagnified in the aquatic and terrestrial food chains. Humans and wildlife can be exposed to methylmercury through ingestion of contaminated fish, which tend to accumulate high Hg concentrations in contaminated areas. Therefore,

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communities dependent on Hg-laden fish as a main food source frequently have elevated Hg levels in blood (Veiga and Hinton, 2002). Methylmercury is considered the sixth most toxic compound of a total of six million such toxins known to humankind (Malm, 2001).

The concept of using plants for environmental remediation has been intensively researched over the last 15 years. Phytoremediation embraces an array of low-cost plant-based technologies that could be potentially advantageous for remediation of Hg-polluted soils, especially in developing countries, where artisanal and small-scale mining for gold extraction has left a legacy of Hg pollution. Potentially, phytoremediation could remove Hg from the soil by growing successive crops of Hg-accumulating plants (phytoextraction). The Hg in the biomass could then be recovered, or stored in an area where it does not pose a risk to the environment. Alternatively, plants could be used to reduce the Hg flux in contaminated sites (phytostabilisation). This role relies on plants stabilising the soil surface, fixing Hg in the rootzone and returning water to the atmosphere via evapotranspiration. The reduction of Hg flux requires that the plants do not translocate large amounts of Hg into the shoots, where it may be consumed by herbivores.

Mercury in most soils is unavailable for uptake by plant roots. However, soil amendments can be used to increase the bioavailablility of Hg to plants. In this chapter, we examine the conditions by which the Hg concentration in the soil solution can be enhanced for plant uptake for three metal-contaminated mine sites where Hg pollution caused by anthropogenic or ASM activities are of concern.

In this chapter, we review the most important sources of Hg into the environment, the behaviour of Hg in soils, and describe plant Hg uptake experiments, with a view to elucidating the most viable strategies for the phytoremediation of Hg-contaminated sites worldwide. We describe laboratory and greenhouse experiments where the effect of total Hg concentrations, plant species, and humic acids was investigated on induced Hg solubilisation and plant-Hg accumulation. The results of a field-scale phytoextraction experiment in situ is also presented. Based on the soil Hg chemistry, thiol-containing ligands were tested for their potential to enhance Hg solubility for subsequent plant uptake. Research was conducted with mine wastes collected from Hg-contaminated sites in New Zealand, Brazil, and China.

The possibility of Hg (0) emissions to the atmosphere and/or Hg leaching to groundwater during the course of phytoextraction represents a threat to the health of humans and ecosystems and should not be overlooked. Therefore, we evaluated the generation of Hg-containing leachates and Hg vapour emissions from plant pots enclosed in gas-tight volatilisation chambers. These experiments provided a picture of Hg fate in the plant-soil-atmosphere continuum.

Finally, we discuss the role of phytostabilisation to reduce the flux of Hg in the environment.

INTRODUCTION

Artisanal and Small-Scale Mining (ASM)

Artisanal and small-scale mining (ASM) is any mining activity that does not follow the conventional technical approach adopted by organised mining companies. This means that the cycle of geological exploration, drilling, reserve proving and engineering studies are not part of ASM. Around 13 million miners are currently involved in ASM operations in approximately 55 countries. Between 80–100 million people worldwide directly or indirectly depend on this activity as a source of income (Veiga et al., 2006).

Environmental degradation and deplorable social conditions normally accompany ASM (Figure 1). The most profound impact of ASM is the pollution of the environment with metallic Hg. Mercury is a cheap, simple, and effective reagent for extracting alluvial gold, but its use results in environmental, social and human health problems (Veiga and Hinton, 2002).

There are two main processes for extracting gold from secondary gold ores (alluvial, colluvial or illuvial material): amalgamation and cyanidation. Most miners use amalgamation, which relies on the capacity of the two metals to interpenetrate each other in a deep sorption process (Veiga et al., 2006). Once formed, the dense Hg-Au amalgam grain can be separated from the rest of the sediment by gravitation. Gold extraction using Hg consists of four main steps: amalgamation, separation of the mineral portion, amalgam decomposition, and gold smelting.

Excess Hg, which is removed from the amalgam by squeezing the liquid Hg through a piece of fabric by hand, is re-bottled and used again. Amalgam decomposition frequently involves heating the amalgam, which usually contains 60% of gold, in open-air pans or shovels using a blowtorch. Gold-doré is the result of amalgam heating and contains about 20g of Hg per Kg of gold. The residual Hg is released from the gold-doré by smelting in gold shops. Environmental contamination due to ASM gold mining occurs mostly from atmospheric deposition of emitted Hg (0) as a result of amalgam decomposition and gold smelting. A further substantial fraction of Hg can also contaminate water and soils after discharge of the amalgamation tailings, which can contain between 200–500 mg/kg of residual Hg (Veiga et al., 2006). This practice has been implicated in the generation of Hg hot spots in adjacent soils and water bodies (Veiga and Hinton, 2002).

Figure 1. Artisanal gold mine site in the Brazilian Amazon.

The Hg problem due to ASM might be growing due to the gradual increase in gold price since 2003 (Veiga et al., 2006). As a result, Hg emissions from ASM may be increasing. It is estimated that between 80–100 tonnes are annually released to the environment due to ASM worldwide. Mercury emissions from China, the main source, comprise between 200–250 tonnes/annum. Indonesia releases 100–150 tonnes/annum, while Brazil, Bolivia, Colombia, Peru, Philippines, Zimbabwe and Venezuela each releases from 10 to 30 tonnes of Hg annually (Veiga et al., 2006). The number of artisanal mine sites worldwide is ca. 160,000, with some 2,000 abandoned artisanal gold mine sites in the Brazilian Amazon (Veiga and Hinton, 2002).

MERCURY IN SOILS

Most Hg that is released into soil is adsorbed onto the solid-phase of organic matter and onto soil minerals, such as sulphides and oxy-hydroxides of iron and aluminium (Evans, 1987). A substantial fraction, however, undergoes physical transformation (leaching, erosion, and volatilisation) and biochemical transformation (methylation, photochemical and biological reduction) (Morel et al., 1998). Mobilisation of Hg can occur through exchange reactions with sulphur-containing ligands and chloride ions, leading to enhanced Hg solubility in soil solution (Schuster, 1991). In weathered tropical soils, Hg bound to iron and aluminium oxy-hydroxides can be mobilised from the surface horizon through the erosion of deforested soils (Roulet et al., 2000). The removal of plant cover allows increased oblique runoff on slopes and subsequent depletion of iron oxy-hydroxides and Hg in the upper centimetres of the soil (Roulet et al., 1999). The mobilised Hg eventually forms complexes with dissolved organic constituents and reaches aquatic systems, where it can be exported to areas away from the pollution source (Oliveira et al., 2001; Veiga, 2004).

The strong affinity of Hg for organic matter influences Hg solid phase speciation more than inorganic mineral colloids. The abundance of functional groups in HS provides many mechanisms for Hg binding including chelation, ionic exchange, complex formation, adsorption, and precipitation. Because of these interactions, Hg adsorption onto the solid phase of clay minerals, silicates, and metal oxides/hydroxides will not profoundly affect Hg speciation in soils as it would do for metals such as Zn, Al, Cd and Ni (Wallschalger, 1998).

MERCURY REMEDIATION

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 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). Chemical stabilisation, electrolytic extraction and chemical leaching, although expensive, have proved effective for the clean up of Hg-contaminated media (Villas Boas, 1997; Hinton et al., 2003). Other methods, such as the use of impermeable plastic lines and the burying of contaminants will merely postpone clean up for a later date (Meagher et al., 2000).

Phytoremediation has been proposed as an environmentally friendly technology for the clean up of heavy metal polluted soils. It has attracted attention in recent years due to the low cost of implementation and environmental benefits (Robinson et al., 1998). These features make phytoremediation particularly welcome in developing countries, where there is a lack of financial incentive to remediate and rehabilitate heavy-metal polluted areas.

PHYTOREMEDIATION

Phytoremediation refers to any plant-based system that is used to improve contaminated media (Pilon-Smiths, 2005). Phytoremediation uses the sun's energy to cleanse contaminated soil and is therefore inherently cheaper than other remediation technologies. Plants contain, sequester, remove, or degrade contaminants as part of their normal physiological processes such as water uptake, inorganic ion uptake, evapotranspiration, root exudation and turnover, and the photosynthetic production of phyto-chemicals. Rhizosphere microbes stimulated by plant roots can biodegrade organic contaminants (Tsao, 2003). The application of these plant-rhizosphere processes has been used to treat contaminated soils, sediments, surface and ground waters. Treatable contaminants include hydrocarbons, chlorinated compounds, and nitroaromatics in the organic contaminant class, as well as heavy-metals, metalloids, radionuclide, and salts in the inorganic contaminant class.

PHYTOEXTRACTION

Inorganic soil contaminants can be phytoextracted from the soil subsurface through repeated cropping and safe storage of the harvested plant biomass following accumulation of metals in the plant aerial tissues. Incineration of the biomass can reduce up to 95% the material to be disposed of in landfills (Robinson et al., 1998) (Figure 2).

Phytoextraction can be applied to the soil removal either of those elements that are essential (e.g., B, Cl, Co, Cu, Fe, Mn, Mo, Ni, Zn) or non-essential (e.g., Cd, Pb, Cr, Hg and As) to plants (McGrath, 1998). Plant species suitable for phytoextraction can be divided in two classes according to metal accumulation capacity and biomass production. The first class is a group of plants known as metal hyperaccumulators. By definition, hyperaccumulator plant species are those that can accumulate metals to a concentration at least 100 times higher than "normal" plants growing in the same environment (Robinson et al., 1998).

Plants that fall in this category can accumulate metals up to greater than 1% of the total plant dry weight (e.g the Zn hyperaccumulator *Thlaspi caerulensces*) but often do not produce high annual biomass (Robinson et al., 1998). So far, the metal hyperaccumulation trait has been observed for As, Ni, Cd, Co, Mn, Se, Tl, and Zn (Robinson et al., 1998; Ma et al., 2001) in more than 400 plant species. However, there are no reports of plants that can naturally hyperaccumulate Pb, Hg, Au and Ag.

A clear advantage of phytoextraction is the generation of metal-rich biomass that can be recyclable. This approach has been successfully used to recover Ni from the biomass of the South-African Ni-hyperaccumulator *Berkheya coddii* grown in a Ni-contaminated soil (Moreno and Sígolo, 2007). Incineration of the metal-rich biomass could reduce substantially the volume and mass of contaminant that would need to go to landfills.

Conventional approaches to the reclamation of metal contaminated sites (removal, isolation, and incineration) are expensive and vary as a function of the soil density, regional transportation and landfill costs. It is estimated that the overall cost for removing one meter of soil from a one-hectare site ranges between US$ 1.5 to 6.2 million. In comparison, the costs for site preparation, planting, and harvesting of plant material for the same hectare site drop to between US$ 5000 to US$ 125000 (McIntyre, 2003).

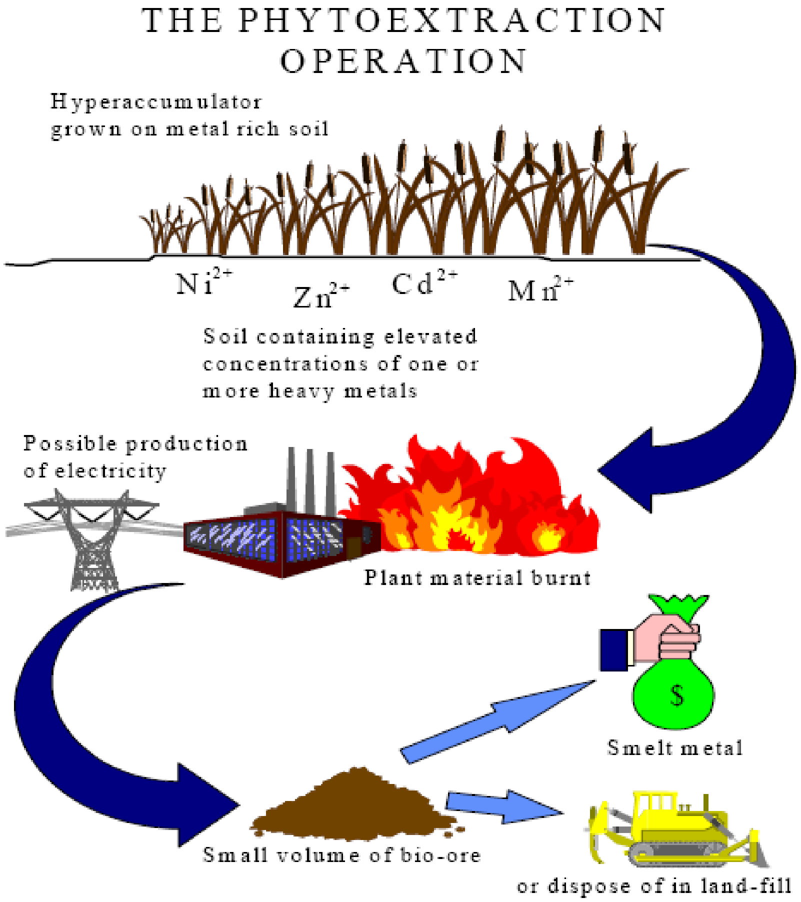

Figure 2. Basic concept of phytoextraction of metal-contaminated soils.

An important concern in phytoextraction is that the plant should translocate metals from the soil to the aerial parts allowing a considerable quantity of the metal to be removed from the soil with each crop (Robinson et al., 1998). The bioconcentration factor (concentration of the metal in dried plants divided by the soil content of the same element) is a measure of the ability of a plant to take up and transport metals to the shoots, which are the parts of the plant that can be harvested. Most plants have bioconcentration factors for metals of less than 1. Modeling has shown that for these plants, the number of crops required to reduce the concentration of the metal in the top soil by half is in excess of 100 (McGrath and Zhao, 2003). Phytoextraction is a realistic approach for plants with a bioconcentration factor above 5 and biomass production of at least 10 t/ha.

The limitations for phytoextraction relates thus to the long period required for site clean up, the restricted number of target metals that can be extracted, the lower depth of rooting systems, the low biomass production of most metal hyperaccumulator plant species, the phytotoxicity levels of metals in soils and insufficient knowledge about agronomic and management practices (Van Netel et al., 2007).

LIGAND-INDUCED PHYTOEXTRACTION

Some metals (e.g., Pb and Hg) are so strongly adsorbed to the solid phase of the soil that only trace concentrations will be available for plant uptake. Ligand-induced phytoextraction has been developed to deal with metal insolubility or with the low plant biomass problems of phytoextraction. In this case, chelates such as EDTA (ethylenediaminetetraacetic acid) are amended to soil in order to induce solubility and subsequent plant uptake of insoluble metals (Nowack et al., 2006). By using this approach, Pb has been shown to accumulate in shoot tissues of high biomass plant species (e.g., *Zea mays* and *B. juncea*) by factors of over 250 (Huang et al., 1997; Blaylock et al., 1997). The potential of ligand-induced phytoextraction for soil decontamination has been demonstrated in laboratory, greenhouse and field studies for Pb (Blaylock et al., 2000), Au (Anderson et al., 2005) and As (Tassi et al., 2001).

However, there is a concern about the leaching of metal-ligand complexes to groundwater. EDTA, for instance, is persistent in the environment being a common pollutant in almost all natural waters (Nowack et al., 2006). The implications of ligand-enhanced phytoextraction for groundwater pollution has been examined by Lombi et al. (2001), who found that EDTA complexes of Cu, Ni, Zn, Cd, and Pb metals were persistent in soil several weeks after EDTA treatment. Model studies have demonstrated that up to 90% of a chelant that is applied to soil is not taken up by plants and thus, can be leached down to groundwaters (Nowack et al., 2006). Biodegradable chelants such as EDDS (ethylenediaminedisuccinic acid) have been proposed as substitutes for EDTA in ligand-induced phytoextraction as metal-EDDS complexes have been shown to be easily biodegradable in soils (Tandy et al., 2006).

SUBSTRATE TYPES

Mercury-contaminated mine wastes from three different locations were investigated: the Tui base-metal mine, in the North Island of New Zealand; the Gold Mountain mine (GM), in North-Central China; and the Serra Pelada artisanal mine site (SP), in Northern Brazil. Selected geochemical characteristics of these substrates are presented in Table 1.

The Tui Base Metal Mine

The Tui base-metal mine is located on the NW flank on Mount Te Aroha, approximately 3 km north east of the township of Te Aroha, North Island of New Zealand (Figure 3). The cessation of activities in 1974 left a tailings dam containing 100,000 m^3 of sulphide-rich tailings. 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. Oxidation of pyrite and other sulphide-bearing minerals has depressed the pH of surface tailings and increased metal bioavailability. Organic matter levels in this substrate are virtually absent. The nearby Tui stream and the local ground water supply have been severely contaminated by acid mine drainage (AMD) produced on site (Morrell et al., 1996).

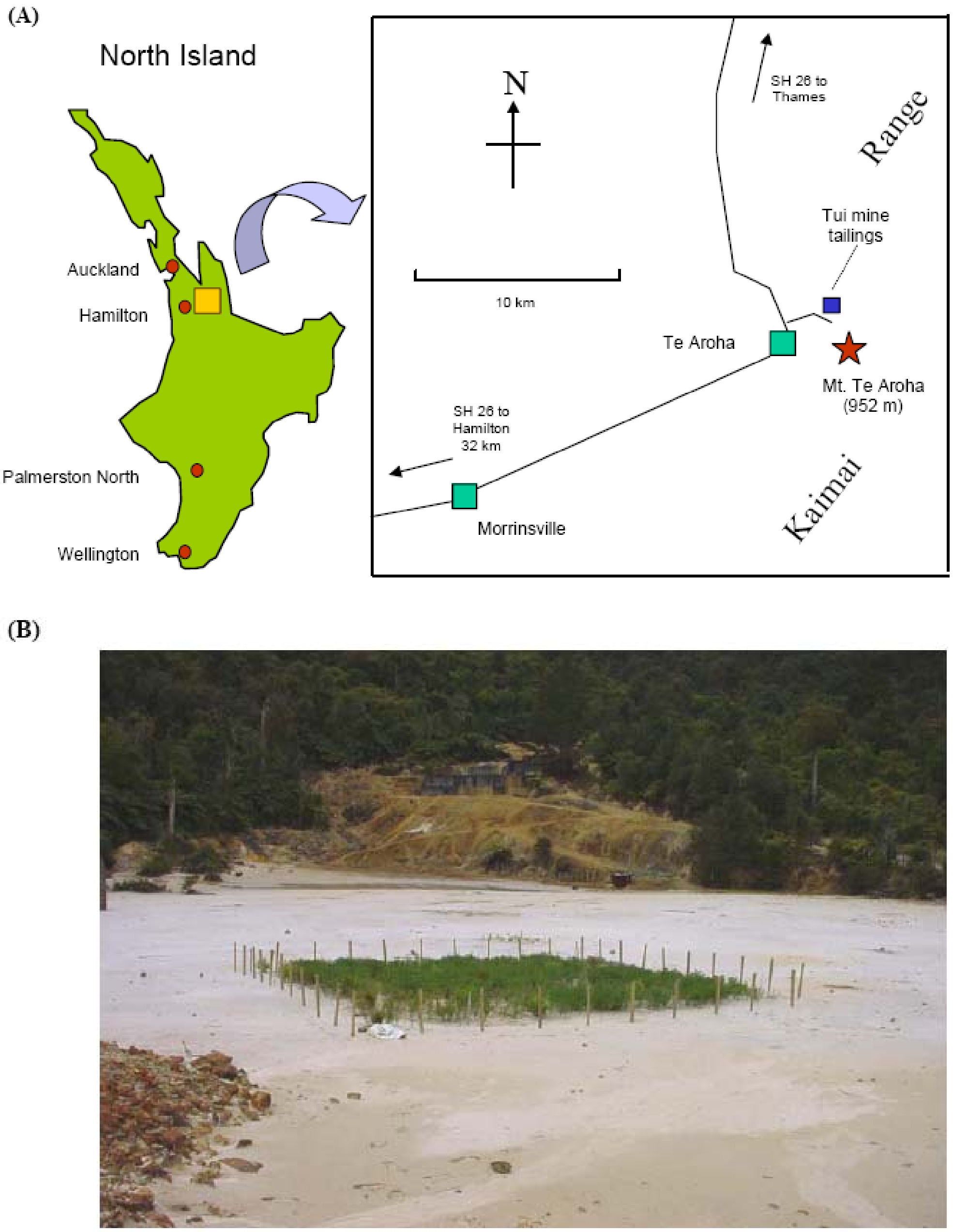

Figure 3. The Tui mine tailings. The tailings dam is located on the NW flank on Mount Te Aroha, approximately 3 km of the township of Te Aroha, North Island of New Zealand (A). Revegetation trials ongoing on the Tui mine tailings (B).

Table 1. Selected geochemical characteristics of Gold Mountain, Tui and Serra Pelada mine tailings used in this chapter

	Substrate Type		
	Gold Mountain[1]	Tui[2]	Serra Pelada[3]
Total Concentration			
Hg (mg/kg)[4]	100	2.82 ± 0.31	0.27 ± 0.06
Au (mg/kg)	1.58	0.5	0.09
Cu (mg/kg)	9356	na	1338
Mn (mg/kg)	na	na	188.4
Cd (mg/kg)	na	26	na
Zn (mg/kg)	na	5.400	na
Pb (%)	na	1.15	na
Fe (%)	4.48	Na	1.30
Other Characteristics			
PH[5]	9.4	3.9	5.4
Eh (mV)[5]	-137	400	93
Total Carbon (%)[6]	0.7	na	0.1
Total Nitrogen (%)[6]	0.07	na	0.02
OM (%)[6]	1.3	na	0.3
C:N[6]	10	na	5
Soil Volume (g/ml)[6]	1.42	1.5	1.30

[1] Analysis of total Au, Cu, Mn and Fe for the original mine tailings were carried out by ACME Labs, Vancouver, BC, Canada.

[2] Source: Moreno et al., 2004.

[3] Source: Cabral et al., 2002.

[4] Total Hg concentrations in the samples were determined through *aqua-regia* digestions; values are the mean of three replicates.

[5] The pH and Eh values are the mean of three measurements.

[6] Analyses of these soil parameters were carried out by Fertilizer and Lime Research Centre, Palmerston North, NZ.

Na= not analysed.

The Gold Mountain Mine

The Gold Mountain mine is a small-scale mine located in North Central China that extracts Au from ore using the amalgamation method. The exact location of the mine has been omitted so as to protect the local mining community. An aqueous slurry of the Au ore is ground with elemental Hg in locally manufactured mills. Gold liberated during rotation of iron wheels contacts Hg and forms an amalgam that may contain a 1:1 Au/Hg ratio. The Gold Mountain mine processes about 10 to 15 tonnes of ore daily and is responsible for discharge of an estimated 70 tonnes per year of Hg into the regional environment (Gunson and Veiga, 2004).

The Serra Pelada Artisanal Mine

The Serra Pelada mine is located within the Carajás mineral province, approximately 100 km southwest of Marabá in the State of Pará, Brazil (Figure 4).

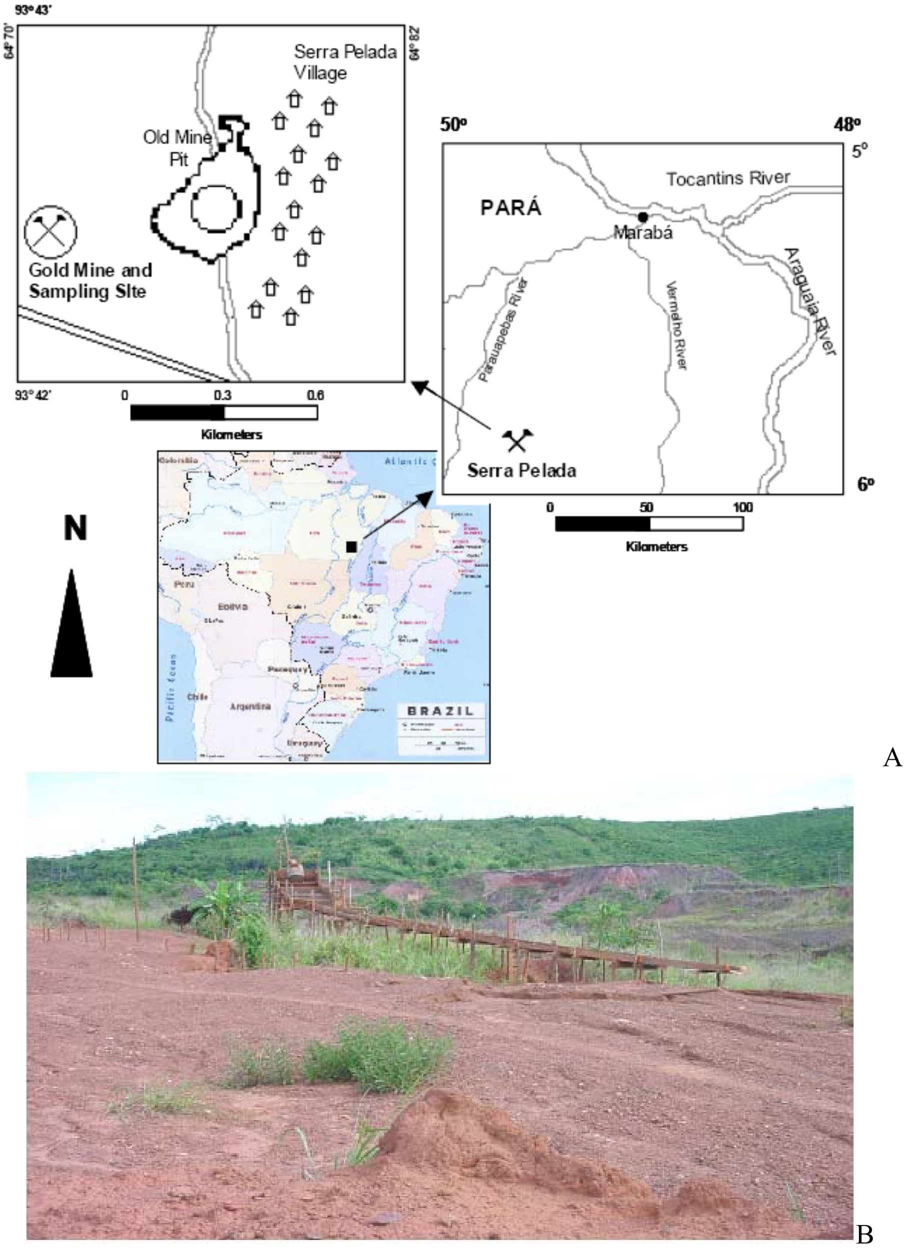

Figure 4. Location of the sampling site at the Serra Pelada artisanal gold mine, State of Pará, Brazil (A). View of a tailings area within the artisanal gold mine site of Serra Pelada (B). A sluice riffle box used for amalgamation of gold-rich gravels is visible in the background.

The mine attracted 20,000 miners within only a few months after gold was discovered, in January 1980. By 1990, the main pit was flooded and most of the gold miners had moved on. The Serra Pelada mine was responsible for officially producing 43 tons of gold in around 10 years. As the amalgamation method was the preferred method employed by local miners, there are many Hg-polluted spots in the 835ha of the Serra Pelada area. Deforestation is also prominent, as many hectares of native forest were destroyed in order to find exploitable ores. Loss of plant cover increased erosion and this has lead to a reduction in soil fertility. As the mineral resources in Serra Pelada were depleted, the community became increasingly dependant on agricultural activities for subsistence, and additional deforestation and soil erosion have occurred.

MERCURY SPECIATION IN SUBSTRATES

The Tui Base Metal Mine

The high sulphide content (>10%) and the presence of the sulphide-bearing minerals galena (PbS), sphalerite (ZnS), and chalcopyrite ($CuFeS_2$) in the Tui ore indicate that Hg in the tailings might be present as sulphidic Hg forms such as cinnabar, metacinnabar, Hg-polysulphides or Hg associated with Fe-sulphides. Trace quantities of cinnabar have been shown to be present in the Tui ore (Morrel, 1996). These Hg forms are insoluble, not easily altered, and seldom found as detrital material (Kabata-Pendias, 2000). Because of their insolubility, sulphidic forms of Hg are said to be unavailable and/or immobile in the environment.

The Gold Mountain Mine

Given that the original tailings samples exhibited mild reducing conditions (-137 mV), high pH (9.45) (Table 1), the speciation of solid phase Hg in the tailings substrate may be in the elemental Hg (0) form. In this oxidation state, Hg is volatile and thus some Hg may have been lost from the system due to volatilisation. However, it is possible that dissolved Hg species in the tailings were adsorbed to iron oxy-hydroxides, as it has been demonstrated for soils with neutral to alkaline conditions (Andersson, 1979). The fact that the mine tailings has an alkaline pH and contains 17 % Fe provides support to this assumption.

The Serra Pelada Artisanal Mine

Table 1 shows that SP mine tailings exhibit moderately oxidising conditions and pH 5.4. Therefore, Hg dispersed in this substrate during artisanal gold mining was likely to be stable in the elemental Hg (0) form. As the vapour pressure of Hg in the elemental state is high, some Hg fraction might have escaped from the system in the gaseous form. The SP tailings contain 1.15 % of Fe and 188 mg/kg of Mn, probably in the form of goethite (α-FeOOH) and manganese oxides, as shown in Table 1. Therefore, it is likely that another Hg fraction was bound to the solid phase of these Fe and Mn oxide minerals.

ENHANCEMENT OF HG AVAILABILITY IN MINE WASTES

The success of phytoextraction is dependent on the availability of the target metal in soil for plant uptake (Blaylock et al., 1997). Mercury 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, be dependent on the ability to control the processes that enhance the concentration of this element in the soil solution (Moreno et al., 2004).

In this section we describe the results for induced Hg solubility assays with the tested tailings in the presence of different chemical ligands. The effect of humic acid-amended substrates on induced Hg solubility was investigated only for the modified GM mine tailings. This substrate was prepared through dilution of the original GM mine tailings (100 mg/kg) with a 1:1 mixture of coarse and fine silica sand to give final Hg concentrations of 0, 1.25, 2.5 and 5 mg/kg. The humic acid (HA) powder was mixed with the substrate to give (w/w) HA concentrations of 0, 0.125, and 1.25 g/kg of substrate.

Effect of Ligand Type

The coordination chemistry of Hg indicates 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 (Moreno et al., 2004).

Mercury is described as a “soft metal” and has a tendency to form complexes with soft ligands such as NH_3^+, CN^-, Cl^- and S-donors. This affinity can be more pronounced when SCN^- and $S_2O_3^-$ anions are present in the soil solution. The solution geochemistry of metal-thiocyanate complexes favours stable complex formation under moderately acidic and oxidizing conditions (Bowell et al., 1993). Conversely, stable Hg-thiosulphate complexes are likely to form in neutral to alkaline pH conditions (Wilkinson et al., 1987).

The geochemistry of GM and SP mine tailings (Table 1) indicates that Hg may form stable complexes with SCN^- and $S_2O_3^-$ anions present in the soil solution. The GM tailings is alkaline (pH 9.4) whereas the SP tailings exhibits more acidic (pH 5.4) and oxidizing conditions (Eh 93), thus supporting the formation of stable Hg-thiosulphate and Hg-thiocyanate complexes, respectively. Figure 5 A shows that the increase in the soluble Hg concentration relative to water in these substrates was possibly due to complex formation in the presence of the respective ammonium salts. For the Tui mine tailings, the soluble Hg concentrations were superior in the presence of the ammonium salts of thiosulphate ($[NH_4]_2S_2O_3$) and thiocyanate (NH_4SCN) plus hydrogen peroxide (Figure 5 B). The increased Hg solubility from the Tui mine tailings was likely due to two processes. The first occurred in the presence of $(NH_4)_2S_2O_3$ and likely involved extraction of Hg bound to the solid phase minerals (mostly sulphidic) possibly through the formation of a thiosulphate-Hg complex. For the second process, hydrogen peroxide may have oxidised sulphidic Hg forms, allowing formation of a stable Hg-SCN complex.

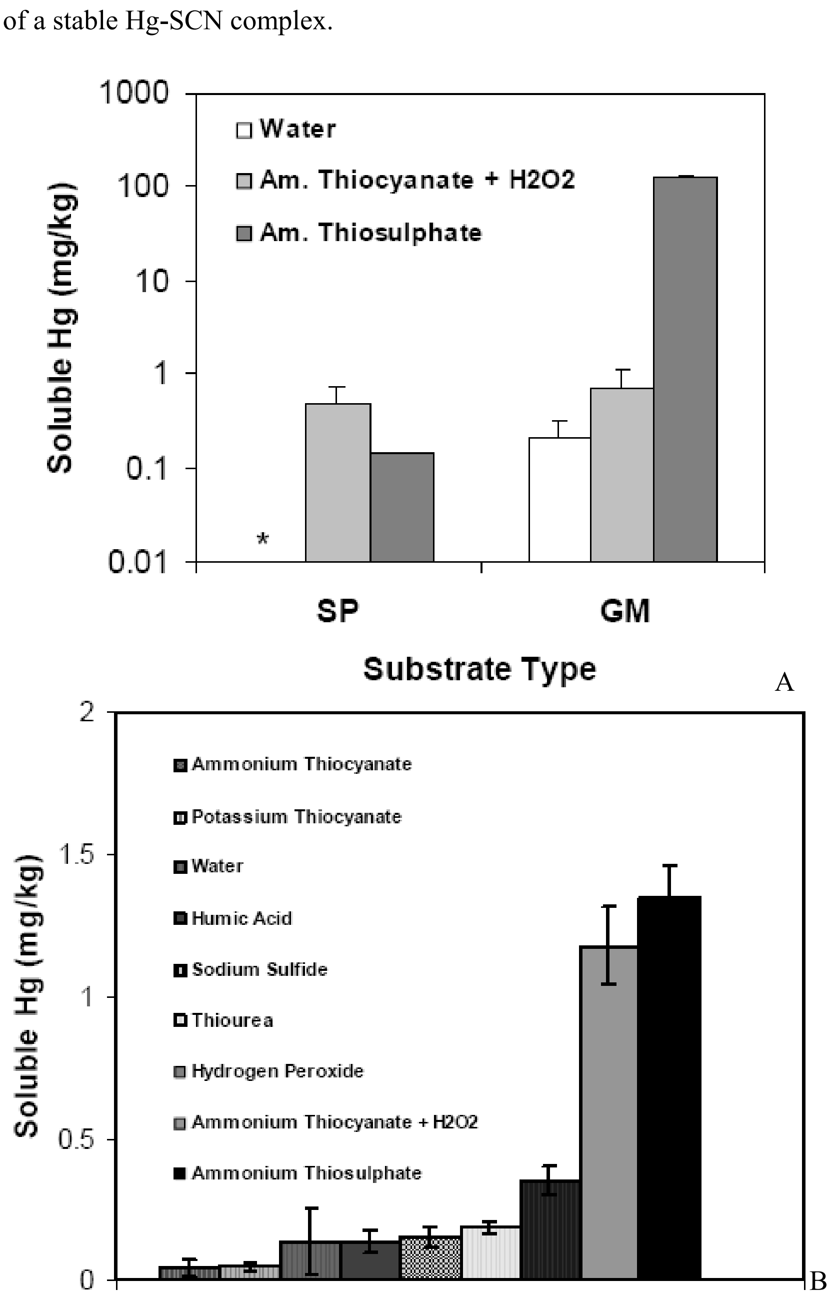

Figure 5. Effect of thioligands (applied at 2 g/L concentration) on soluble Hg concentrations of Serra Pelada (SP) and original Gold Mountain (GM) (A) and Tui (B) substrates. Bars denote ± 1 standard deviation from the mean of three replicates. The symbol (*) indicates Hg below detection limits (5ng/mL in the extract solutions). Ammonium thiocyanate was supplemented with hydrogen peroxide (H_2O_2) at 0.3%.

The kinetics of the formation/decomposition of Hg thiosulphate complexes is unclear. However, Bowell et al. (1993) modelled the stability fields for the gold thiosulphate system in a pH-Eh diagram. These authors demonstrated that the formation of a stable Au-thiosulphate complex would be expected in the pH range of 3 to 12. As both Hg and Au exhibits similar geochemistry, then is possible that the acidic conditions that prevail in the Tui mine tailings were not restrictive to the formation of a stable Hg-thiosulphate complex.

Effect of Humic Acids

Humic substances (HS) represent 50% of the natural organic matter in soils and contain a high proportion of sulphur-containing functional groups (Wallchläger et al., 1998 a). The soluble fraction of HS comprises fulvic and humic acids (HA), which are known Hg ligands. Since Hg complexes with humic substances are stable over the entire pH range from 1-14 (Wallchläger, 1996), these complexes can be abundant in the solution of organic and mineral rich horizon soils (Evans, 1986). Therefore, Hg-HA complexes are mobile in soils (Wallchläger et al., 1998 b) and HA have been demonstrated to enhance both the Hg availability in soils and Hg uptake by organisms (Veiga and Hinton, 2002).

The effect of Ha on the solubility of Hg in the GM mine tailings is shown in Figure 6. The Hg concentration in the extracts was almost two fold higher in the presence of $(NH_4)_2S_2O_3$ relative to water and averaged over 1 mg/kg. The Hg concentrations increased significantly with increasing Hg concentration in the water-soluble fraction of the HA-amended substrates. Conversely, humic acids had no significant effect in the Hg solubility in the presence of the thiosulphate ion in the soil solution.

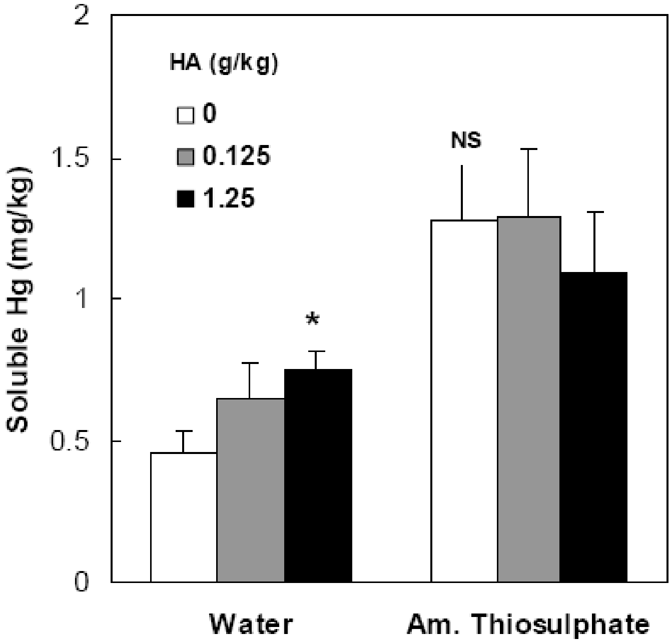

Figure 6. Effect of humic acids (HA) on soluble Hg concentrations for modified GM substrates containing 2.5 mg/kg of Hg. The substrate was treated with 2 g/L of ammonium thiosulphate. Bars denote ± 1 standard deviation from the mean of three replicates. The symbol (*) indicates statistically significant ($p < 0.05$). NS = not significant ($p > 0.05$). Water = control.

The enhanced Hg solubility in the water extracts for HA-amended substrates can only be explained through the formation of Hg-HA complexes. The fact that the pH of the modified GM substrates was around 8 and that humic acid is soluble at alkaline conditions (Wallschläger et al., 1996) provides support for this statement. Considering that soluble Hg-HA complexes were present in both water and $(NH_4)_2S_2O_3$ soluble fractions (Figure 6), we hypothesise that the total soluble Hg fraction of HA-amended substrates comprises a mixture of both Hg-HA and Hg-thiosulphate complexes. This result will have strong implications for the uptake of Hg by plants during the course of phytoextraction.

INDUCED PLANT-HG ACCUMULATION

Metal transport from the soil solution to plant roots can occur by diffusion as well as by convection with the transpiration water stream. Therefore, increasing the dissolved metal concentration by adding ligands or chelants to soils can enhance the diffusive and convective flux toward roots. Metals can be then delivered to the plant shoots via the xylem through the apoplastic pathway, an interconnected system that includes all cell walls in the root cortex (Nowack et al., 2006). The formation of Hg-complexes in the soil solution is thus, a prerequisite to induce the accumulation of Hg in aerial plant tissues. However, there is still a debate whether metal complexes are taken into the plant or not. It is believed that metal-complexes disrupt cells in the root cortex allowing the free passage of metal complexes into the xylem vessels, therefore facilitating shoot metal accumulation (Nowack et al., 2006).

In this section, we show the role of plant species and sulphur-containing ligands, substrate Hg concentration, substrate type and humic acids in the induced plan-Hg accumulation process. For plant growth, all substrates were supplemented with Osmocote (slow release fertiliser) at 5 g/kg. The acidic pH of Tui mine tailings was adjusted to 5.5 by addition of lime. Due to the phytotoxic Hg concentration (100 mg/kg) of the original GM mine tailings, the plant experiments on this substrate were carried out on a diluted substrate containing Hg at 1.25, 2.5 and 5 mg/kg, as explained before. Sulphur-containing ligands were applied at 2 g/kg rate in the form of solutions. All experiments were run for five days under greenhouse conditions.

Effect of Sulphur-Containing Ligands and Plant Species

Average background levels of mercury in plants are usually not superior to 0.1 mg/kg dry weight (Kabata-Pendias, 2000). By adding $(NH_4)_2 S_2O_3$ to Tui mine tailings, *B. juncea* was able to accumulate over 400 times this value in the aerial tissues, as shown in Figure 7 A. Recorded values in aerial tissues of *B. juncea* averaged 43 mg/kg, thus reaching a bioconcentration factor around 15 (Plant Hg 43/ Soil Hg 2.8, values in mg/kg). Induced plant-Hg accumulation for plant species grown in the modified GM mine tailings (Hg at 2.5 mg/kg) was also effective in the presence of thiosulphate salts (ammonium and sodium) (Figure 7 B). However, shoot Hg values were not as pronounced as those observed for *B. juncea* grown in the Tui substrate.

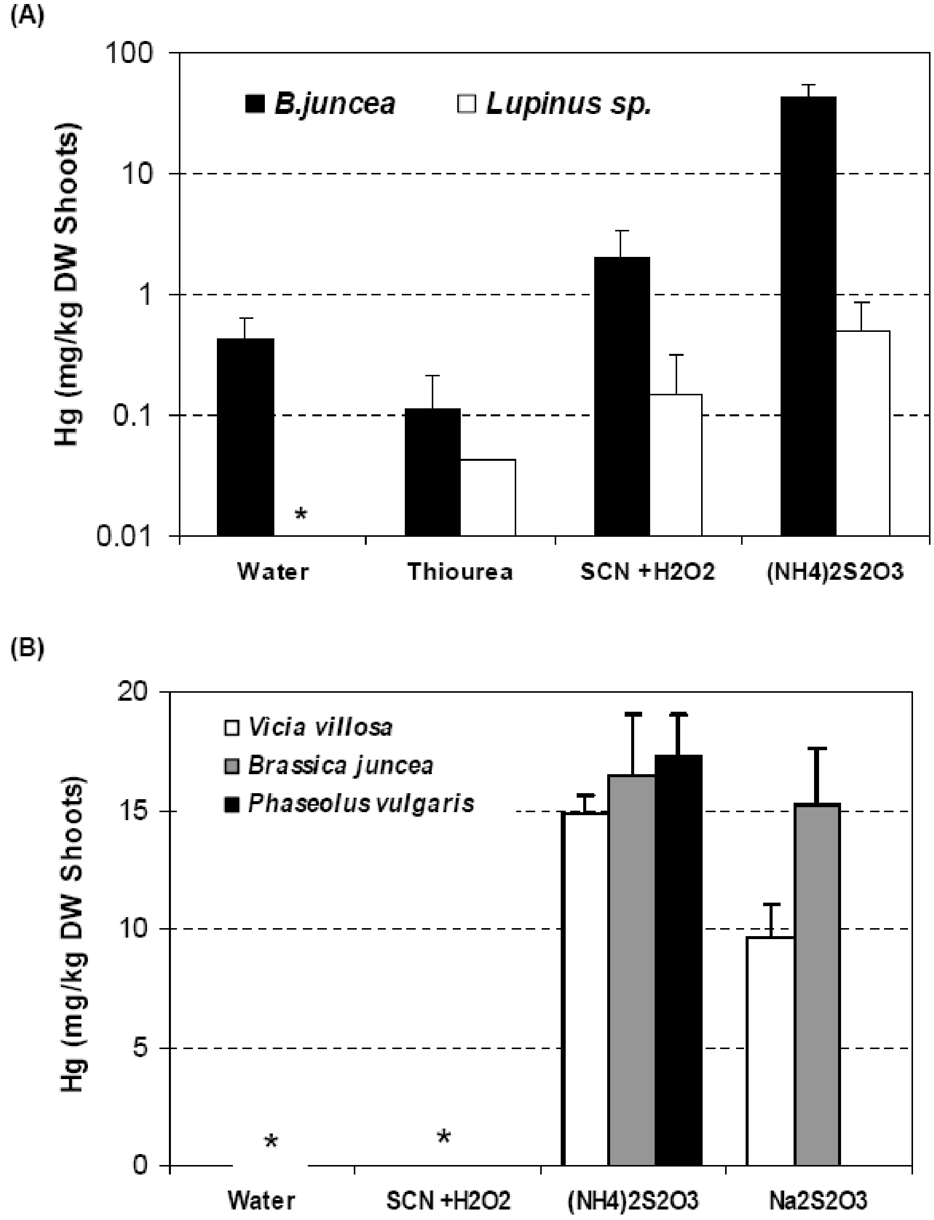

Figure 7. Shoot Hg concentrations in different plant species after application of sulphur-containing ligands at 2g/kg to Tui (a) modified GM mine tailings (b). Note that Water = control, SCN + H_2O_2 = ammonium thiocyanate + hydrogen peroxide (at 0.27%); $(NH_4)_2S_2O_3$ = ammonium thiosulphate. Bars denote ± 1 standard deviation from the mean of 5 replicates. The symbol (*) indicates Hg below detection levels (5 ng/mL in the plant digests). DW = dry weight.

Overall, plants species grown in the modified GM substrate accumulated over 15 mg/kg in shoot tissues in the presence of the ammonium salt, hence yielding a bioconcentration factor around 6. The thiosulphate-induced plant-Hg accumulation process was, therefore, effective both under the moderately acidic (pH 5.5) and the slightly alkaline (pH 8.2) conditions existing in the Tui and modified GM substrates, respectively.

Application of other sulphur-containing ligands, such as thiourea or thiocyanate, to the Tui or GM substrates did not induce Hg levels to the same extent. For the modified GM tailings, Hg shoot accumulation was below detection levels in the presence of other ligands (Figure 7 A and B).

The translocation of soluble Hg species was not only a function of ligand type but also of plant species. For example, application of thiosulphate to *Lupinus* sp. and *Vicia villosa* grown respectively in Tui and modified GM substrates did not enhance Hg accumulation in aerial tissues as it did for *B.juncea* and *Phaseolus vulgaris* under similar conditions. This discrepancy may result from intrinsic plant species characteristics such as root morphology, root surface area, and plant transpiration rates.

Effect of Substrate Type

In general, Hg has a low solubility regardless of the natural geochemical conditions that prevail in substrates. The application of thiosulphate substantially increased Hg solubility and subsequent shoot accumulation for *B. juncea* grown in Tui and Gold Mountain substrates, as shown in Figure 8. Thiocyanate was also effective for inducing Hg solubility in SP substrates (Figure 5 A) and, to some extent, shoot-Hg accumulation. Although shoot tissues accumulated around 2.3 times more Hg than controls, this difference was not statistically significant. This is possibly due to the low levels of total and hence soluble Hg in the SP substrate (Table 1), which limited the effect of NH_4SCN on induced plant-Hg accumulation. Therefore, it is plausible that thioligand-induced plant-Hg accumulation will work only above a threshold value for total Hg in substrates.

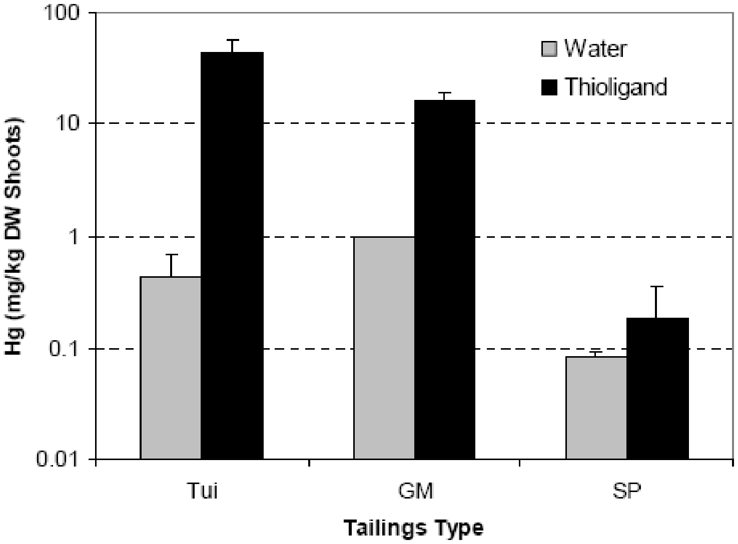

Figure 8. Shoot Hg concentrations for *B. juncea* plants after application of sulphur-containing ligands to mine tailings substrates. Tui and modified Gold Mountain (GM) mine tailings were treated with ammonium thiosulphate at 2 g/kg. Serra Pelada substrates (SP) was treated with ammonium thiocyanate at 2 g/kg supplemented with hydrogen peroxide at 0.3 %. Bars denote ± 1 standard deviation from the mean of at least 3 replicates.

Effect of Substrate Hg Concentration

There is still no evidence of a physiological role for Hg in living organisms. Therefore, Hg is a non-essential element and can be toxic to higher plants. Reduced levels of biomass and plant transpiration rates are among the primary effects of Hg toxicity to aquatic and terrestrial plants (Moreno et al., 2008). As metal removal by plants relies on biomass production and metal-accumulating capacity, these toxic effects of Hg can limit the effectiveness of phytoextraction. Conversely, if soil Hg concentrations are not a limiting factor for plant growth, then increasing soil Hg concentration can increase the Hg content of plants (Figure 9 A).

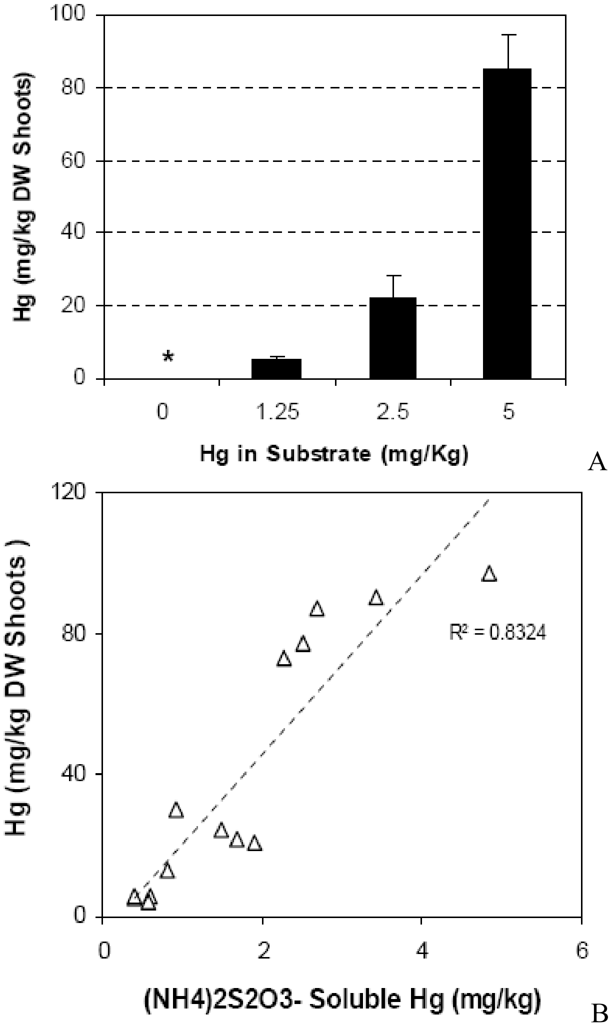

Figure 9. Accumulation of Hg in shoots of $NH_4S_2O_3$-treated *B.juncea* plants as a function of total (A) and $(NH_4)_2\,S_2O_3$-soluble (B) concentrations in modified GM substrates containing 2.5 mg/kg of Hg. Bars denote ± 1 standard deviation from the mean of five replicates. DW = dry weight, $(NH_4)_2\,S_2O_3$ = ammonium thiosulphate. An asterisk indicates Hg below detection levels (5 ng/mL in the plant digests).

For example, Figure 9 (A) shows that Hg accumulation in the shoots of $(NH_4)_2S_2O_3$-treated *B. juncea* plants was significantly enhanced as a function of increasing Hg concentrations in the modified GM substrates. At the highest substrate level (5 mg/kg), Hg was accumulated in shoots at an average of 85 mg/kg, hence yielding a concentration factor of 17. The plot of the shoot Hg concentration versus the $(NH_4)_2S_2O_3$-extractable Hg concentration (Figure 9 B) confirms this trend as it shows a positive and linear response of Hg in shoots to the concentration of available Hg in the substrates.

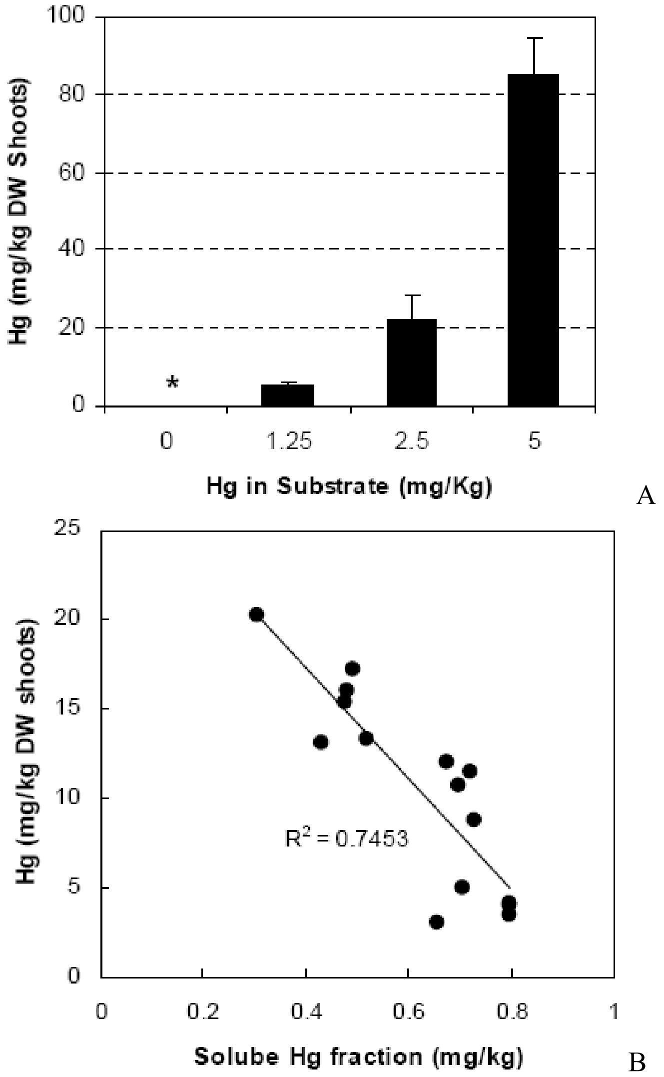

Figure 10. Accumulation of Hg in shoots of $(NH_4)_2S_2O_3$-treated *B.juncea* plants as a function of the humic acid content (g/kg) (A) and the $(NH_4)_2S_2O_3$-soluble Hg fraction (B) for modified GM substrates containing 2.5 mg/kg of Hg. Bars denote ± 1 standard deviation from the mean of five replicates. DW = dry weight, $(NH_4)_2 S_2O_3$ = ammonium thiosulphate.

Effect of Humic Acid

Some studies have shown that organic matter limits the dissociation of Hg bound to soil organic components. This effect translates into low Hg availability in soils and, therefore, into restricted Hg plant uptake as demonstrated by soil and hydroponic studies with *Vulpia myuros* L. (Zorro fescue), *Lemna minor* (Duckweed), *Brassica chinesis* and *Lactuca sativa* (Heeraman *et al.*, 2001, Mo *et al.*, 1989, Wang *et al.*, 1997). The reason for the decline in the plant Hg concentrations was attributed to a decrease in the soluble Hg fraction due to Hg complexation with either organic matter or humic acid. Figure 10 (A) shows that the shoot Hg concentration of *B. juncea* was significantly decreased in the presence of HA thus corroborating with previous studies. Root to shoot transport, therefore, appears to be inhibited by humic acid. These results were confirmed by plotting the plant Hg concentration in *B. juncea* as a function of the soluble Hg concentration of HA-amended substrates, as shown in Figure 10 (B). It is possible that that Hg-S_2O_3 complexes were preferentially selected for shoot transport over other dissolved Hg species present in the substrate such as HA-Hg complexes, which were probably adsorbed by root tissues.

Ligand-Induced Phytoextraction Field Trials

Three field plots with dimensions of 5 x 5 m were established on the tailings dam of the abandoned Tui base-metal mine. The plots were fertilised with NPK fertiliser at 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 in order to supplement plant nutritional needs. Around 75 grams of *B. juncea* seeds were planted in two rows. After 6 weeks of plant growth, $Na_2S_2O_3$ was applied to the field plots at a concentration of 5 g/kg of substrate. Two weeks after the treatment, biomass from the plots was harvested and processed for Hg analyses. Results for ligand-induced phytoextraction on the Tui tailings are shown in Figure 11 and Table 2. The addition of $Na_2S_2O_3$ to substrates enhanced Hg solubility, leading to increased Hg accumulation in the shoots of *B. juncea*. The extractable Hg concentration rose proportionally to increasing concentrations of $Na_2S_2O_3$ added to the substrates.

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	Shoot Hg (mg/kg DW)	Harvested Biomass (kg, DW)	Equivalent Biomass (t/ ha)[a]	Hg Extraction Yield (g/ ha)[b]
1[c]	2.99 ± 0.5	0.19	0.38	1.14 ± 0.2
2	9.77 ± 1.2	1.24	2.49	24.39 ± 3.1
3	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,).

[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.

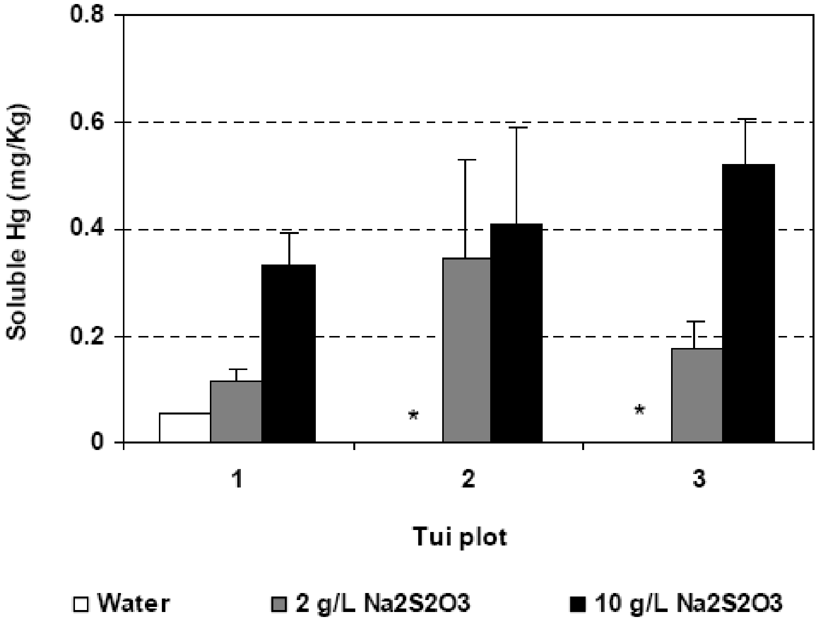

Figure 11. Extractable Hg concentrations as a result of sodium thiosulphate ($Na_2S_2O_3$) application to Tui mine tailings at concentrations of 2 and 10 g/L. Bars denote ± 1 standard deviation from the mean of 3 replicates. An asterisk indicates Hg below detection levels (5 ng/mL in the extract solutions).

The thiosulphate treatment induced a significant increase in the shoot Hg concentration relative to the control treatments, which had Hg values below detection levels (data not shown). Mercury concentrations in shoots of *B. juncea* were higher in plot number 2, where the assayed value averaged around 10 mg/kg (Table 2), thus reaching a bioconcentration factor above 3 (plant Hg 9.77 /soil Hg 2.82, values in mg/kg).

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 (Figure 12). 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 no surprising. 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 of around 25 g Hg/ha (Table 2). The addition of organic matter to Tui substrates might also have played a negative effect on shoot Hg translocation, as highlighted by the shoot Hg concentrations for $(NH_4)_2S_2O_3$-treated *B. juncea* plants grown in the humic acid amended substrates.

However, one should be bear in mind that the maximum Hg-extraction yield obtained in this experiment was over 30 times the maximum yield obtained for *Hordeum vulgare* (barley), which extracted 0.71 g Hg/ha from a Hg-contaminated site in Almadén, Spain (Rodriguez et al., 2003) without the application of synthetic chelates. Overall, ligand-induced phytoextraction increased the Hg removal efficiency by plants grown *in situ* thus encouraging more detailed studies under field conditions.

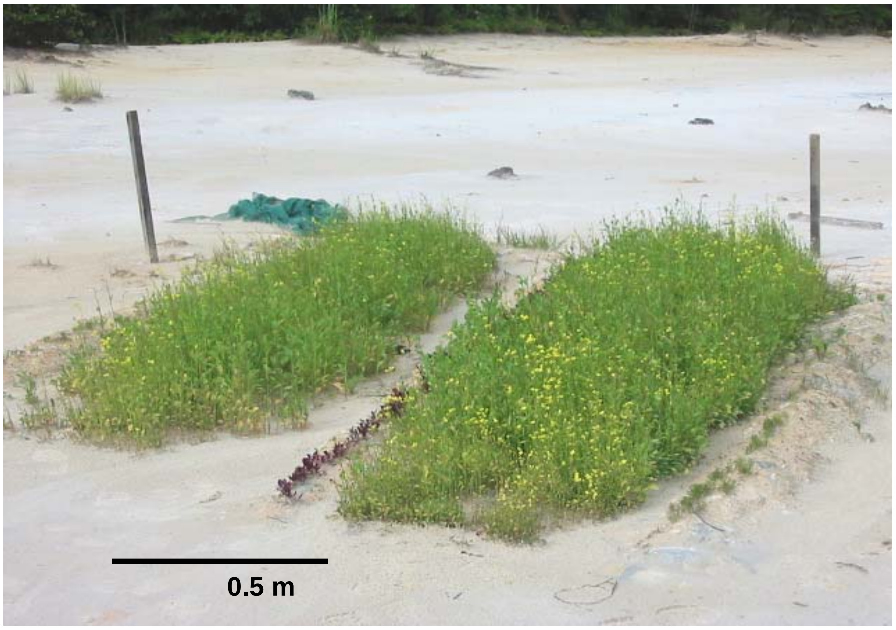

Figure 12. Experimental field plot at the Tui mine tailings (North Island, NZ) before application of sodium thiosulphate ($Na_2S_2O_3$) to the substrate (March/2004). Biomass for *B. juncea* plants grown at the toxic tailings yielded a maximum of 2.5 tonnes/ha after 6 weeks of planting.

LEACHING

The application of chelants to soils to induce a higher metal concentration in plants raises environmental concerns due to the potential contamination of groundwater via the leaching of mobilised metals. Leaching can occur when the rates of solubilising agents applied to the soil are above the levels of metals that the plants can remove from soil solution (Moreno et al., 2004). This excess metal may leach below the root zone into groundwater, a process exacerbated by macropore flow (Bundt et al., 2000). Modelling calculations for the application of EDTA revealed that 90% of the applied chelant is not taken up by plants during the course of ligand-induced phytoextraction (Nowack et al., 2006). This explains the enhanced generation of Hg-containing leachates after the addition of $(NH_4)_2S_2O_3$ to substrates, as shown in Table 3. The generation of leachates also appeared to be affected by a plant-chemical factor. However, the experimental protocol utilised in the experimental method did not allow for explanation of discrepancies resulting from plant species variability.

Given the potential for leachate generation, ligand-induced phytoextraction may pose an unacceptable environmental risk unless the link to receiving waters can be broken. In this case, the operation could be carried out ex situ. Geo-textiles and subirrigation drainage systems would intercept leachates and pump it back to the irrigation supply, thus preventing leaching and the potential for ground water contamination. Mercury-leachate generation also 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 (Moreno et al., 2004). Additional research should be carried out to examine the potential for chemical and biological degradation of Hg-thiosulphate complexes in revegetated mine substrates.

Table 3. Mercury concentrations in leachates (μg/L) collected from plant pots after application of ammonium thiosulphate ($[NH_4]_2S_2O_3$) at 2 g/kg to Tui and modified GM mine tailings[*]

			Treatment	
Substrate	Plant Species	Rep.	Water[a]	$(NH_4)_2S_2O_3$[b]
Tui[c]	*B. Juncea*	2	9.1 ± 11.2	953 ± 246
	Lupinus sp.	3	20.8 ± 6.6	3963 ± 1063
GM[d]	*B. Juncea*	3	BDL	2762 ± 506
	P. vulgaris	3	1.3 ± 0.5	1068 ± 332
	V. villosa	3	BDL	1868 ± 492
GM + HA[e]	*B. juncea*	3	3 ± 3.4	2592 ± 132

[*] Values are the mean ± 1 standard deviation from 3 replicates unless otherwise stated.

[a] Water = control;

[b] $(NH_4)_2S_2O_3$ = ammonium thiosulphate;

[c] Tui mine tailings, 2.82 mg Hg/kg of substrate, Table 1;

[d] GM, modified Gold Mountain mine tailings, 2.5 mg Hg/kg of substrate;

[e] GM + HA, modified GM substrates amended with humic acid at 1.25 g/kg.

BDL = below detection levels (5 ng/mL in the aqueous solutions)

VOLATILISATION

Mass balance calculations for greenhouse experiments investigating induced plant-Hg accumulation revealed a substantial Hg fraction in the substrates that could not be accounted for (Moreno et al., 2004). This fraction suggested that Hg (0) was volatilised from the substrate as a result of chemical and biological transformations. We, therefore, carried out a volatilisation study for ligand-induced plant-Hg accumulation using *B. juncea* plants grown in the Hg-contaminated mine tailings. Each plant pot was enclosed in a gastight acrylic chamber and these were subsequently placed inside a plant controlled light and temperature growht chamber (Figure 13). Volatile Hg was driven by air into two-successive trap solutions containing 5% $KMnO_4$ dissolved in 2N H_2SO_4 The use of this experimental apparatus has allowed Hg recoveries of around 90% for *B. juncea* plants cultured in Hg-spiked solutions (Moreno et al., 2008).

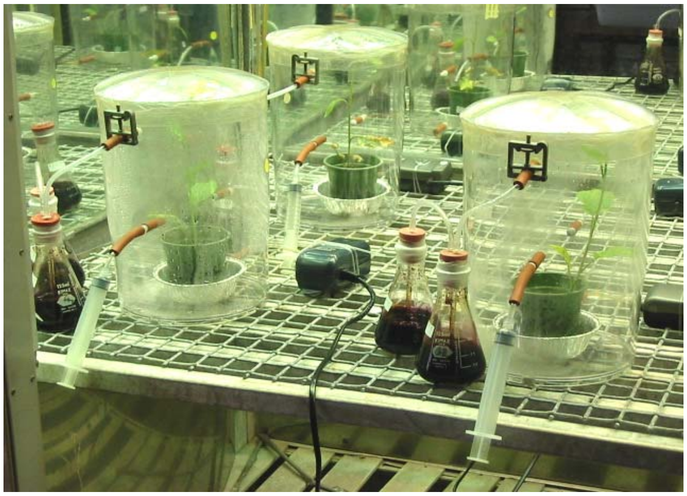

Figure 13. Gastight volatilisation chambers used for trapping volatile Hg released from the plant-substrate system. *B. juncea* plants grown on Tui, modified Gold Mountain (GM) and Serra Pelada (SP) substrates were treated either with $(NH_4)_2S_2O_3$ or NH_4SCN and immediately enclosed within the chambers.

Figure 14 shows the daily Hg volatilisation rates for thioligand and water-treated *B. juncea* plants and control pots (without plants) on a mass basis. Mercury volatilisation was ubiquitous regardless of the substrate type, existence of vegetation, or application of ligands. Overall, the presence of plants considerably enhanced the Hg volatilisation rates over unplanted (barren) mine tailings.

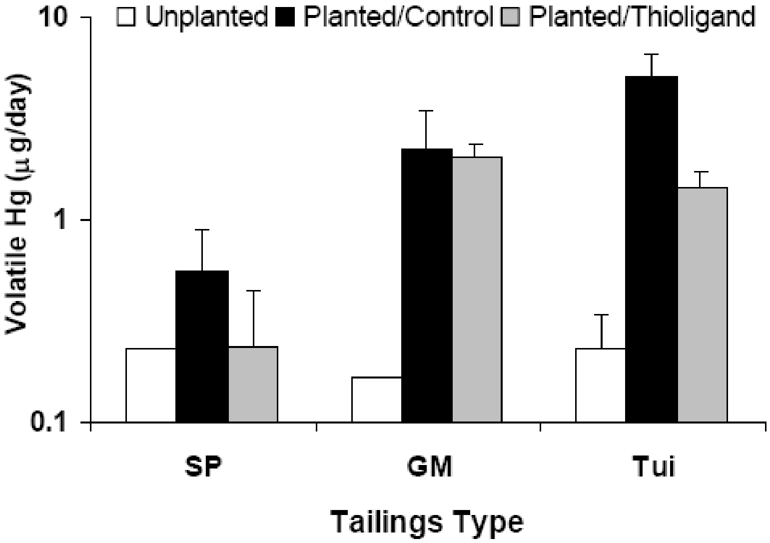

Figure 14. Volatilisation rates from *B. juncea* plants after application of sulphur-containing ligands to mine tailings. Tui and modified Gold mountain (GM) mine tailings were treated with ammonium thiosulphate at 2 g/kg. Serra Pelada substrates (SP) were treated with ammonium thiocyanate at 2 g/kg supplemented with hydrogen peroxide at 0.3%. Water was used as a comparison to thioligand treatments (planted/control). Pots without plants (barren pots) were used as controls. Bars denote ± 1 standard deviation from the mean of 3 replicates.

For Tui and SP mine tailings, Hg volatilisation was more pronounced for water-treated plants. For these substrates, the Hg volatilisation rate exceeded the Hg volatilisation rates from thioligand-treated plants at least by a factor of 2. Volatilisation rates between water and $(NH_4)_2S_2O_3$-treated plants grown in the modified GM substrate, on the other hand, were not statistically different. Application of thioligands did not cause any increase in the Hg volatilisation from substrates and in fact, for two substrates, there was a trend for a reduced Hg flux. A final point to note is that Hg volatilisation from barren substrates indicates that barren mine tailings appear to be inherently prone to biological or chemical transformations.

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

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

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). The Hg volatilisation from tested mine tailings, therefore, 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 photoreduction).

The role of bacteria on Hg volatilisation from Hg-contaminated environments has been well documented (Barkay et al., 2003). The reduction of Hg (II) to Hg (0) as well as the degradation of organomercury is carried out by Hg (II) resistant bacteria (both gram positive and negative). Bacteria resistant to Hg (II) produce a flavin-containing disulphide oxireductase known as mercuric reductase (MR) that catalyses the reaction:

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

The MR removes Hg from stable thiol salts [$Hg(SR)_2$] by eletrochemical reduction in a NADPH-coupled redox reaction (equation 2). The resulting Hg (0) is rather volatile [Henry's constant (H) = 0.3] and escapes from the organism (Barkay et al., 2003). 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 (Figure 14) indicates that there is a plant-mediated factor in the volatilisation of Hg from substrates. The enhanced Hg (0) emissions from planted substrates could be the result of biological Hg reduction carried out by Hg (II) resistant bacteria living in the rhizosphere or inside the roots. Experiments with *B. juncea* plants grown in Hg (II)-containing solutions have shown that around 95% of the total Hg mass volatilised from the plant originated from the root compartment, thus implicating the role of rhizosphere processes in Hg volatilisation (Moreno et al., 2008). Alternatively, increased soil moisture due to air-H_2O emissions from plants inside the gas chamber could have enhanced the photoreduction of Hg (II) from the substrate surface (Gustin et al., 2004). Further experiments should be carried out to assess the contribution of biological and photochemical transformations to the volatilisation of Hg from planted substrates.

All tested substrates showed some degree of Hg (0) volatilisation with and without the presence of plants suggesting that this process is likely to happen spontaneously from any barren, revegetated, or phytoremediated Hg-contaminated mine site. Although important from an environmental point of view, the scaling up of the volatilisation data to a field-scale scenario would be unrealistic as Hg (0) emissions may be related to biotic/abiotic factors that could not be controlled under the conditions utilised in the experimental method. The significance of Hg (0) emissions from a phytoremediation operation would depend, therefore, on the elucidation of the biological/chemical pathways by which Hg (II) is reduced in substrates and how plant roots affect this process.

Bacterial resistance to inorganic and organic mercury compounds is found in a wide diversity of Gram-positive and Gram-negative bacteria (Barkay et al., 2003). This fact has motivated the University of Georgia (USA) to conduct research on the development of transgenic Hg-tolerant plants able to volatilise Hg (0) to the atmosphere. At the present time, the microbial *mer*A and the *mer*B genes, expressing mercuric ion reductase and organomercury lyase enzymes respectively, have been inserted into herbaceous (Brassica sp. and Tobacco), trees (Poplar trees), and wetland (Rice) species (Meagher and Heaton., 2005). These Hg-resistant plants are, therefore, capable of phytoremediating soils and sediments polluted with inorganic and organic Hg species. Two issues that may retard the large-scale application of transgenic plant-based systems is negative public perception of the release of genetically modified plants into the environment and concerns regarding the release of plant-Hg (0) vapours to the atmosphere.

PHYTOSTABILISATION

The ability of plants to precipitate or immobilise metals in the organic fraction of soils, onto root surfaces, or within root tissues, can be harnessed to prevent the migration of contaminants off site or into ground water systems. This latter application is referred to as phytostabilisation (Tsao, 2003). Its primary purpose is to reduce metal mobility and bioavailability hence minimising the associated ecological (food chain entrance) and environmental (metal distribution to other compartments) risks. Phytostabilisation can occur through sorption, precipitation, complexation, or metal valence reduction. For instance, the enzymatic reduction of toxic and more soluble Cr (VI) to inert and less soluble Cr (III) has been demonstrated to occur in the fine lateral roots of *Eichornia crassipes* (Lytle et al., 1998).

Phytotoxicity levels of metals in substrates may limit plant growth development thus requiring the application of chemical-immobilising agents. Besides improving soil physical properties, water holding capacity and water infiltration, the application of peat, biosolids and other organic amendments can increase cation exchange capacity, provide a source of carbon and protons and stimulate microbial activity. Reduction of metal bioavailability is achieved by adsorption, complexation and redox reactions thus allowing development of plant covers (Adriano et al., 2004; Moreno and Sígolo, 2007).

The main advantages of phytostabilisation are the following: (1) it eliminates the necessity of toxic material/biomass disposal; (2) it is an effective system to the natural dispersal of wind blow dust or water borne wastes. Additionally, soil erosion can be prevented as root systems aggregate soil into large clumps. Organic carbon in barren soils can be built up as a result of root exudation and the deposition of organic material that sloughs off from root tissues, thus enhancing soil biodiversity. The limitations of phytostabilisation include: contaminant remaining in the site, application of extensive fertiliser or chemical amendments, mandatory monitoring is required and the immobilisation of contaminants may be largely due to chemical amendments (Adriano et al., 2004).

Mercury Retention by the Root System

Successful phytostabilisation of Hg-contaminated substrates demands the use of plants with efficient root-Hg accumulation capacity and low Hg translocation to the shoot (Wang, 2005). Our studies show that untreated plants grown in mine tailings retained Hg preferentially in root tissues with minimal translocation to shoots, regardless of the substrate type and tested plant species. In fact, the analytical equipment did not detect Hg accumulation in shoots for most tested species (Table 4). In addition, the accumulation of Hg by roots was enhanced in the presence of both low and high levels of humic acids, as shown for *B. juncea* plants grown in the humic-acid amended modified GM substrate. Further experiments showed that the root-Hg accumulation pattern was positive and linearly correlated to the concentration of Hg in the water-soluble phase of humic–acid amended substrates (Figure 15). These results, consequently, indicate the strong affinity of roots for Hg, whose accumulated fraction may contain Hg adsorbed onto and/or taken up (absorbed) into root cells.

Table 4. Plant-Hg concentrations for untreated plant species grown in Tui, modified Gold Mountain (GM) and Serra Pelada (SP) mine tailings for 5 weeks. Values are mean of 5 replicates (unless otherwise stated) ± 1 standard deviation.

			Plant Hg (mg/kg)	
Substrate	Plant Species	Rep.	Root	Shoot
Tui[c]	*B. Juncea*	5	18.9 ± 15	0.4 ± 0.2
	Lupinus sp.	5	1.05 ± 0.7	BDL
	B. Juncea	5	9.8 ± 1.7	BDL
GM[d]	*P. vulgaris*	5	4.2 ± 0.3	BDL
	V. villosa	5	5.5 ± 0.8	BDL
GM + HA 0.125[a]	*B. juncea*	5	6.5 ± 0.8	BDL
GM + HA 1.25[b]	*B. juncea*	5	13.7 ± 1.1	BDL

[a] Modified GM mine tailings amended with humic acid at 0.125 g/kg;
[b] Modified GM mine tailings amended with humic acid at 1.25 g/kg;
BDL = below detection leveles (5 ng/mL in the plant digests).

The results for plant species grown in the contaminated mine tailings corroborate with previous experiments with *B.juncea* plants exposed to Hg at concentrations ranging from 0.05 to 10 mg/L in hydroponics solutions, where almost all Hg is bioavailable (Moreno et al., 2008). For all solution concentrations, roots accounted for 98–99 % of the total plant Hg, thus demonstrating the ability of plant roots to retain Hg and control its transport to shoots. Cavalinni et al. (1999) demonstrated that most Hg taken up by roots of *Triticum durum* cv.

Creso after 25 days exposure to ^{203}Hg-spiked solutions was found on cell walls at the external layers of the cortical cylinder. The binding of Hg to specific sites on the cell wall can restrict transport to sensitive sites in shoots and was suggested, therefore, as an important factor to Hg tolerance in plants (Cavallini et al., 1999).

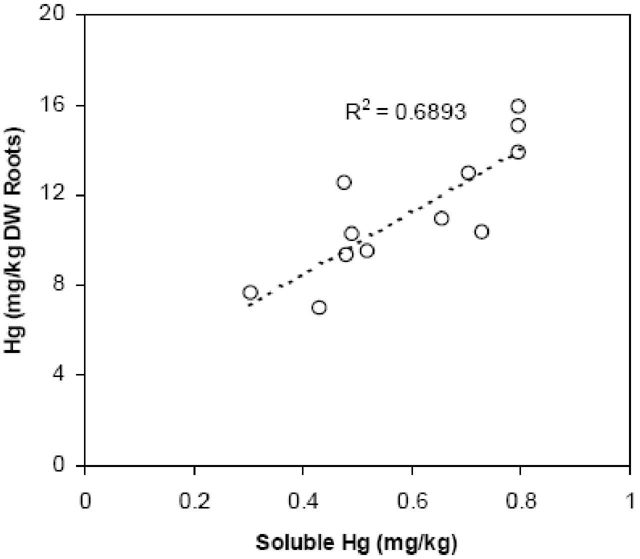

Figure 15. Accumulation of Hg in roots of untreated *B. juncea* plants as a function of the water-soluble phase in Gold Mountain substrate amended with humic acid at 1.25 g/kg containing 2.5 mg/kg of Hg. DW = dry weight.

Although preliminary, the results of this investigation provide conspicuous evidence that the root-Hg accumulation process may be used to phytostabilise Hg in contaminated mine tailings where phytoextraction is not possible or feasible. Further research should be carried out to investigate the role of plant roots and organic amendments on the reduction of the Hg bioavailable and mobile fractions in the soil profile.

CONCLUSION

Laboratory, greenhouse and field results from this research revealed that plants can remove Hg from mine tailings through ligand-induced phytoextraction. Providing that metal levels in substrates are non-phytotoxic and support biomass growth, this simple technique would allow for off-site transport and safe disposal of this contaminant. Although greenhouse studies indicated that single *B. juncea* crops could be induced to extract between 500 to 850 g of Hg per hectare (10 tonnes/ha x 50–85 mg of Hg/kg of shoots DW), the maximum yield for this species in the Tui field was around 25 g Hg/ha. To be economically feasible, phytoextraction yields at least 20 times higher would be needed to reduce considerably soil Hg concentrations. In order to maximise plant-Hg uptake, attention should be given to the ligand-irrigation system and plant water demands. The possibility of leaching soluble Hg species after ligand application requires such an operation to be performed ex situ where measures (e.g., geomembrane lining) can be implemented to avoid groundwater contamination. Risk analysis should be performed to quantify the risks of shoot-accumulated Hg contaminating the food chain.

Phytoremediation offers advantages for the management of Hg-polluted mining areas. The establishment of a vegetative cover is a better option than inaction, as Hg (0) is spontaneously emitted from any barren or naturally-revegetated mine site, and AMD can severely contaminate local surface and groundwater supplies. Phytostabilisation could be used in situ to prevent Hg flow to other compartments. Plant roots would stabilise the substrate, reduce the Hg bioavailable fraction and Hg leaching. Ligand-induced phytoextraction could be used ex situ to remove Hg from substrates and to generate a low volume disposal residue. After reaching acceptable soil Hg levels, the substrate would allow new land uses. Additionally, a plant-based remedial system could be coupled to profit-making operations (forestry, carbon credits generation or biomass energy programs).

In spite of its apparent limitations, there are some examples of successful commercial applications of phytoextraction for Pb- and As-contaminated soils (Edenspace, 2008). The scaling up of ligand-induced Hg phytoextraction or phytostabilisation to a commercial scale, however, will require time and multidisciplinary research efforts. This would include the contribution of expertise from a wide array of disciplines, such as soil science, geology, genetics, biochemistry, microbiology, botany, environmental engineering, and human health. It is imperative that scientific knowledge be disseminated to the public in comprehensible form, as phytoremediation is strongly dependant upon their support.

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