Phytoremediation of Mercury-Contaminated Mine Wastes

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ABSTRACT

Mercury (Hg) is a toxic heavy metal that is concentrated in organisms. Injudicious use of Hg and its compounds have resulted in widespread soil contamination. This study investigates the potential use of plants for the remediation of Hg-contaminated mine wastes. Plants can remove soil Hg via phytoextraction and phytovolatilisation. I investigated both of these strategies by focusing on a methodology for Hg analyses in plants and soils with a view to the determination of volatile Hg emitted from plants. Secondly, I determined the feasibility of Hg phytoextraction and phytovolatilisation from contaminated mine wastes.

An accurate method for the analysis of Hg in air, plant and various soil fractions was a key component of this study. I developed a hydride-generation atomic absorption spectroscopy method for total Hg analyses in digest and liquid matrices of the aforementioned samples. Quality assurance was ensured by comparing results with those of an external certified laboratory. The maximum discrepancy was 15 %.

To measure plant Hg-volatilisation, a method that captures Hg-vapour in solution for subsequent analyses was developed. Initially this system was used to trap Hg vapours released from the root system of *Brassica juncea* plants grown in hydroponic solutions. A subsequent study improved the Hg trapping system, allowing the capture of volatile Hg from both roots and shoots. Mercury recoveries from the whole plant system (traps + plant + solutions) averaged 90 % using this experimental apparatus.

In most contaminated substrates, plant Hg uptake is insignificant, possibly due to the low bioavailability of Hg. This represents an obstacle for effective remediation using phytoextraction. Geochemical studies were carried out in Hg-contaminated substrates to examine the potential of chemical agents to induce Hg solubility and subsequent plant uptake. These studies utilised Hg-contaminated mine tailings collected from three locations: the Tui base-metal mine, in the North Island of New Zealand, the Gold Mountain mine, in North-Central China and, the Serra Pelada artisanal mine site, in Northern Brazil. The results demonstrated that Hg solubility in all tested substrates is increased in the presence of sulphur-containing chemical ligands. The effectiveness of these ligands was influenced by site-specific geochemistry.

Plants species were able to accumulate up to 60 mg/kg of Hg in shoot tissues upon addition of sulphur-containing ligands to Tui and Gold Mountain substrates. The degree of plant-Hg accumulation was shown to be dependant on plant species and on the thioligand-induced soluble Hg fraction. Shoot Hg transport was inhibited for Gold Mountain substrate amended with 1.25g/kg of humic acid. The maximum Hg extraction yield for *B. juncea* plants growing in Tui field sites averaged 25 g per hectare following application of sodium thiosulphate.

Volatilisation of Hg vapour from barren substrates occurred as a result of biotic (microorganisms) and abiotic (chemical and photochemical reduction) processes. The presence of *B. juncea* plants in substrates enhanced the volatilisation process up to 23 fold. Phytovolatilisation was the dominant pathway responsible for between 75 to 99.5 % of the total Hg removed from substrates.

It was concluded that Hg removal from contaminated mine wastes can be accomplished by both thioligand-induced phytoextraction and phytovolatilisation. There are risks of groundwater contamination by Hg species mobilised after application of thioligands to substrates. Estimated Hg (0) emissions from plant-based operations at contaminated sites ranged between 1.5 to 3.6 kg of Hg/ha per year. Due to extensive atmospheric dilution, Hg emissions from small-scale phytoremediation operations would not cause serious harm to the local population or the regional environment. Phytoremediation combined with gold-phytoextraction can help to mitigate Hg-pollution in artisanal mine sites in the developing world.

DEDICATION

I dedicate this thesis to my parents Ivan Netto Moreno and Vilma Reichfeld Netto Moreno who have limitlessly supported my career.

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"The most beautiful thing
we can experience is the
Mysterious. It is the source
of all true art and science".

ALBERT EINSTEIN (1879-1955)

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CHAPTER 1
INTRODUCTION

1.1 Statement of the problem

Mercury (Hg) is among the most toxic elements to living organisms. The cycling of this element from natural emission sources over the Earth had not represented a threat to life for hundreds of thousands of years. Over the past 150 years, however, anthropogenic Hg emissions have at least doubled the global Hg input from natural Hg sources (Hylander and Meili, 2003). The high toxicity levels exhibited by some organic Hg forms limit the capacity for assimilation of this element by the ecosphere. Humankind has, therefore, witnessed deadly episodes of human Hg poisoning from discharge of Hg-containing wastes to soil and water systems. In spite of this, Hg contamination still continues worldwide. The main Hg pollution sources are related to atmospheric Hg discharges from coal-burning power plants, mining operations and incineration of urban and medical wastes. It is estimated that 60 % of these atmospheric Hg inputs ends up in soils (Morel et al., 1998). The use of Hg in agricultural pesticides, for the bleaching of paper and textile materials, and in energy and defence-related activities also results in Hg-contaminated environments. Thousands of square kilometres of land, sediments, lakes and rivers in the World have been contaminated with Hg as a result of accidents or lack of technical knowledge during production, use and storage of Hg (Meagher et al., 2000). This problem is potentially aggravated in the developing world (Veiga and Hinton, 2002). Conventional technologies for the remediation of Hg in soils are expensive and of questionabe effectiveness in the long term. The development of an inexpensive and environmentally friendly alternative for the remediation of Hg-contaminated soils is, therefore, urgently required. Developing nations that lack financial support and incentives to implement remedial procedures could benefit from a low-cost Hg-remediation technology.

1.2 Aims of the study

The broad aim of study was to investigate the possibility of using plants for the clean up of Hg-contaminated mine wastes. The specific aims of this work were to assess the efficiency of ligand-induced phytoextraction and phytovolatilisation for the removal of Hg from substrates. Research was conducted with mine wastes collected from Hg-contaminated mine wastes in New Zealand, Brazil and China.

1.3 Structure of the thesis

This thesis is composed of 10 chapters. Chapters 1, 2 and 10 are, respectively, the introduction, literature review and general conclusions. Chapters 3-9 contain the experimental results of the research. These chapters were written in the form of research articles that have been either published or submitted to scientific journals. Differences in the structure of the chapters are, therefore, the result of distinct manuscript styles adopted by these journals.

In order to present the research results in a logical and comprehensible style, this study was divided in two main sections:

A. the methodological approach utilised for the determination of Hg in plant and substrates and for the measurement of volatile Hg released from plants;

B. the phytoextraction and phytovolatilisation of Hg contaminated mine wastes.

The first section details the methods utilised for the analysis of total Hg in the different compartments that comprise the soil-plant-atmosphere *continuum*. This section also explains the technique used for trapping volatile Hg emitted from plants. The second section demonstrates the efficiency of phytoextraction and phytovolatilisation for the Hg removal from the contaminated substrates.

In order to provide basic information about the experiments, chapters 3-9 are briefly introduced at the beginning of each respective section. Critical aspects about the

methodology as well as about the relevance of the experiments to the overall objective of the work are also portrayed.

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CHAPTER 2
LITERATURE REVIEW

2.1 Mercury and its uses

Mercury's symbol, Hg, is derived from the Latin word *hydrargium*, which means liquid silver. This liquid metal is usually associated with cinnabar in its native form, where it takes the form of tiny drops or impregnations of a silvery-white colour (Prinz et al., 1978). Because of this, the metal is sometimes called quicksilver (i.e., living silver). Mercury is a heavy liquid metal that volatilises at a relatively low temperature (357°C) and solidifies at –39°C (Atkins and Jones, 1997). Being an excellent conductor of electricity and heat, elemental Hg is employed in hundreds of applications. Mercury is used in medical thermometers, as it expands uniformly with increasing temperature. It is also used in fluorescent light bulbs and in mercury lamps (street lighting). The latter application, however, has been replaced by the use of sodium vapour lamps, in view of the contamination of the environment when mercury lamps are broken. Perhaps the most important use of mercury is based on its ability to form amalgams, which are solutions or alloys with other metals. For instance, the solid "dental amalgam" used to fill cavities in teeth, or the amalgam of sodium and mercury in the chlor-alkali process, which is used to convert aqueous sodium chloride into chlorine and sodium hydroxide by electrolysis (Baird, 1995). The use of mercury amalgams to extract silver from ore dates back from 1570 during the colonisation of South America by the Spaniards (Oliveira et .al, 1999). Although this practice has been banned in developed nations, it is still employed in the developing world. It is estimated that between 10 to 15 million miners are currently involved in artisanal and small-scale gold mining operations in approximately 40 countries (Veiga, 2004).

2.1.1 Artisanal and small-scale mining

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 previous geological exploration, drilling, reserve proving and engineering studies are not part of ASM (Hinton, 2002).

These activities are also frequently accompanied by extensive environmental degradation and deplorable social conditions, both during operations and after mining activities have ceased (Figure 2.1) (Veiga and Hinton, 2002).

Figure 2.1. Artisanal gold mine site in the Brazilian Amazon.

Artisanal gold mining generally involves the extraction of metallic gold from secondary gold ores (alluvial, colluvial or illuvial material), by gravity processes (Hinton et al., 2002). This means that gold can be extracted by amalgamation or cyanidation processes without prior sulphide oxidation. The favourite practice chosen by most miners is amalgamation, and involves the large-scale use of Hg. Mercury is a cheap, simple and effective reagent for extracting alluvial gold but its use results in environmental, social and human health problems (Hinton et al., 2002; Veiga and Hinton, 2002). Alternative processes like cyanidation, oil or wax agglomeration and halide concentration, are neither competitive economically nor suitable for the types of operations usually employed with secondary ore deposits (Villas Bôas, 1997). Gold extraction using Hg consists of four main steps: amalgamation, separation of the mineral portion, amalgam decomposition and gold smelting. The amalgamation technique relies on the capacity of Hg to form an alloy with gold, which consists of a dense Hg-Au amalgam grain that can be separated from the rest of the sediment by gravitation.

Calculations for global anthropogenic Hg emissions vary from 1910 to 6000 tonnes per year (Hanisch, 1998; Pacyna and Pacyna, 2002). Fuel combustion, mostly coal, contributes with 75 % of this figure (Pacyna and Pacyna, 2001). Mine wastes can also contribute as point emission sources of atmospheric Hg. These emissions are influenced by substrate concentration, light, temperature, and precipitation (Gustin et al., 1997; Gustin et al., 2003). It has been calculated that mine wastes in Central Western Nevada (USA) release up to 0.4 tonnes per year of atmospheric Hg (Gustin et al., 1996). Artisanal and small-scale mining in South America, Russia and Asia are also an important source of anthropogenic emissions, releasing between 450 to 800 tonnes per year of metallic Hg into the environment (Lacerda, 2003; Veiga, 2004). From this total, 80 % is lost to the atmosphere as Hg (0) vapour during amalgam burning and bullion smelting and 20 % contaminates soils and aquatic systems via discharge of Hg-rich amalgamation tailings (Veiga and Hinton, 2002). It is estimated that ASM gold mining contributes 20 % of the total Hg atmospheric emissions from human activities (Lacerda, 2003). Airborne Hg emitted by mining and redeposited in forests can be transported long distances in association with forest fires (Veiga et al., 1994). Deforestation by slash and burning is a common agricultural practice in the Brazilian Amazon and is responsible for mobilising extensive amounts of Hg contained in the biomass of plants and in the organic fraction of forest soils. It is estimated that these practices emit between 8 to 80 tonnes of Hg per year to the atmosphere of the Amazon region (Veiga et al., 1994; Lacerda, 1995).

2.2.2. Natural sources

Mercury occurs in many types of rock, generally in the form of sulfide minerals. These consist essentially of cinnabar (HgS) or polymorphous HgS, and marcasite (FeS_2) and native sulphur (S). Mercury can be also found in the form of native Hg, or associated with native gold (Au), or to a lesser extent with copper (Cu) and silver (Ag) (Prinz et al., 1978). Mercury-enriched geological terrains constitute a long-term source of atmospheric Hg (0) (Gustin et al., 2001).

Atmospheric Hg emissions from cinnabar and other types of ore can range between 10-30 tonnes per year (Hylander and Meili, 2003). It has been suggested that around 6.5 tonnes of Hg per year can be released from degassing of cinnabar ore in a small area (< 1000 m^2) of the Almaden mine in Spain (Gustin, 2003). Geothermal areas and areas of recent volcanic activity are also Hg sources with atmospheric emissions that can reach around 100 tonnes per year (Nriagu and Decker, 2004). Plant communities have the ability to emit Hg (0) vapour from Hg pools in soils. It has been estimated that Hg emissions from vegetation and forested soils represent an input of around 1400 to 3200 tonnes of Hg per year to the atmosphere (Lindberg et al., 1998).

2.3 Mercury in Plants

2.3.1 A brief historical background

Siegel and Siegel (1979) reviewed early works on Hg toxicity and accumulation in plants from the end of 18th century to the mid 20th century. According to these authors, the first report on the effect of Hg on plant life appeared in 1797 as a result of work carried out by the Dutch researchers Deiman, Paats, Van Troostwyck and Lauwerenburgh. The toxicity of Hg was assessed in this early study by putting plants into direct contact both with Hg vapours and Hg oxides. Boussingault, in 1867, confirmed the toxic effects of Hg vapours to plant cells and suggested that sulphur compounds might be able to inhibit its phytotoxic effects. In 1934, Zimmerman reported discoloration and desiccation of flower buds as a symptom of Hg poisoning in roses grown in potting soils amended with $HgCl_2$. Ratsek, in the same year, reported abnormal accumulation of Hg in leaves after exposure to Hg vapours, an effect that was shown to be enhanced in the presence of light. Fuller and Gray, in 1942, observed that seed germination in the presence of the Hg vapours altered plant growth and morphology. During the 50's and 60's many authors recognized the ecological significance of Hg. The deadly effects of human methylmercury poisoning in Minamata in the 1950s urged the scientific community to understand the pathways that lead to Hg

biomagnification in the food chain. As a result of that, numerous reports on the Hg content in flora and fauna were carried out in order to identify Hg polluted ecosystems and the sources of contamination.

Several studies during the 1970s concluded that average background level of Hg in plants is not usually greater than 100 ng/g (ppb) (Kabata-Pendias and Pendias, 2000). In a survey examining the Hg content in plants grown in 912 soil samples throughout the United States, Shacklette (1970) concluded that plants would rarely contain Hg concentrations above 500 ng/g, unless growing in close proximity to mercury ore deposits or mines. Bache et al. (1973), in a pot trial study on the absorption of mercury from soils treated with 1 and 10 mg/g (ppm) of various Hg compounds, found less than 100 ng/g of total mercury in the edible plant portions of beans, cabbage, carrots, millet, onions, potatoes and tomatoes.

The 1980's saw increasing public concern regarding the presence of Hg in agricultural soils treated with Hg-containing fungicides, or Hg-rich organic composted materials. Composts were used as an alternative to commercial fertilisers to better maintain soil fertility and productivity (Weaver et al. 1984; Semu et al., 1985; Capon, 1987; Al-Attar et al., 1988). A study conducted with beans, lettuce and another 16 edible crop species grown on a garden plot treated with residential compost for 6 years showed methylmercury levels averaging 12.8 % of the total edible Hg content (Cappon, 1987). High biomass plants, such as wheat and beans, were also investigated for Hg uptake and showed high levels of Hg accumulation both at flowering and maturity. Straws of wheat treated either with the pesticide Aretan (2-methoxyethylmercury chloride) or $HgCl_2$ showed high levels of Hg uptake from soils before flowering. The Hg uptake pattern, according to the authors, resembled that of macronutrients, i.e., greater uptake in the early growth periods (Semu et al. 1984). The same authors demonstrated that Hg translocation for both plant species was enhanced for soils treated with $HgCl_2$ when compared to Aretan but no explanation was given to support this difference.

This study, therefore, indicated that Hg accumulation in plants might be influenced by the Hg species present in soils. Other variables that have been shown to affect Hg accumulation in plants are soil Hg concentration (Ellis and Eslick, 1997), Hg exposure route (Suszcynsky and Shann, 1995), plant species and tissue type (Cocking et al., 1995; Heeraman et al., 2001), organic matter and humic acid content (Mo et al., 1989; Wang et al., 1995; Heeraman et al., 2001), and rhizosphere microorganisms (DeSouza et al., 1999 a).

2.3.2 Toxicity and tolerance

The toxic effect of Hg on plants can arise from the inactivation of several enzymes or by the linkage of Hg to sulphydryl groups present in vital enzymes (Ferreira et al., 1989). Mercury also increases the activity of peroxide through generation of reactive oxygen species like superoxide (O_2^-), hydroxy radicals (OH^-) and hydrogen peroxide (H_2O_2) (Ali et al., 2000). The formation of these toxic free radicals disrupts the normal functioning of enzymes, which, in turn, brings about metabolic changes at the cellular level. In aquatic and terrestrial plants Hg toxicity has been shown to reduce biomass, photosynthetic activity, total chlorophyll, nitrogen as well as phosphorus and potassium contents (Ferreira et al., 1998). Decreased levels of potassium in root tips of Hg-exposed *Picea abies* seedlings was related to disruption of the root cell membranes (Boening, 2000). Further, increased Hg levels in tissues of maize plants provoked an increase in proline, an amino acid that is usually associated with stress adaptation and, consequently, Hg tolerance (Ferreira et al, 1998). Accumulation of Hg in tissues may also be accompanied by symptoms of oxidative stress (Ali et al., 2000). The aquatic plant *Potamogeton crispus* exposed to Hg at 10 μM showed increased levels of lipidic peroxidation and potassium leakage and a pronounced reduction in the total chlorophyll content. However, at lower Hg levels (0.1 to 0.25 μM), the oxidative damage in the plant was reduced. The authors related this to an increase in the content of some stress amino acids and peptides, such as cysteine and non-protein thiols (phytochelatins).

Phytochelatins comprise a class of metal chelating peptides that are synthesized by plants in response to heavy metal stress. These peptides present the structure of (γ-Glu-Cys)n – Gly (n=2-11) and have been described as the major component of heavy metal detoxification in higher plants (Grill et al., 1985; Rauser, 1995). Phytochelatins are also referred as non-protein thiols because they present a high content of sulphydril groups, lack a tertiary and quaternary proteic structure and have no genes encoding for their aminoacidic chain structure (Cobbett, 2000). Monocotyledons and dicotyledons plant species have been shown to synthesize phytochelatins in response to Cd exposure (Grill et al., 1987). An important study on phytochelatin synthesis in response to toxic metal exposure was carried out with cell cultures of *Rauvolfia serpentina.* In this study, phytochelatins were induced to various degrees in response to the presence of salts of Ni, Cu, Zn, Ag, Sn, Sb, Te, W, Au, Hg, Pb, and Bi and to the anions arsenate and selenate (Rauser, 1995). Comprehensive work on phytochelatin synthesis in response to Hg stress was carried out with the submerged plants *Hydrilla verticillata* and *Vallisneria spiralis* (Gupta et al., 1998). This study demonstrated that phytochelatins are synthesised by roots and leaves of both plant species in response to varied levels of Hg in the culture media. Although some authors consider the synthesis of phytochelatins by plants as a defence system in order to tolerate Hg toxicity (Punz and Sieghardt, 1993; Gupta et al., 1998; Ali et al., 2000), their participation in the detoxification of some excess metals has been questioned. Comparisons of the phytochelatin content in several populations of *Silene vulgaris* exposed to Cu, Zn and Cd were carried out to verify this hypothesis (Rauser, 1995). The concentrations of phytochelatins were shown to be the same in populations with small and large tolerance zones to these metals, thus indicating that synthesis of these peptides was not influential in plant metal tolerance.

2.3.3 Mobilisation

Since a large proportion of metals in soils are either bound to organic (humus) and inorganic (clay) soil constituents or present as insoluble precipitates, then mobilisation of metals into the soil solution is a fundamental prerequisite for plants to accumulate metals in their tissues. Three mechanisms have been proposed for describing how plant roots mobilise soil-bound metals into the soil solution: i) secretion of metal-chelating molecules (phytosiderophores) into the rhizosphere; ii) reduction of soil bound metal

ions by specific enzymes (e.g. metal reductases) and iii) acidification of the rhizosphere by extrusion of protons (Marschner, 1986; Raskin et al., 1994). The release of phytosiderophores by graminaceous species appears to be a response to Fe and Zn deficiency and can also mobilize Cu, Zn and Mn from soils (Marschner, 1991). Deficiency of some nutrients in soils also seems to be the main cause for soil acidification and for activation of metal reductases by Fe-deficient dicotyledonous plants (figure 2.3).

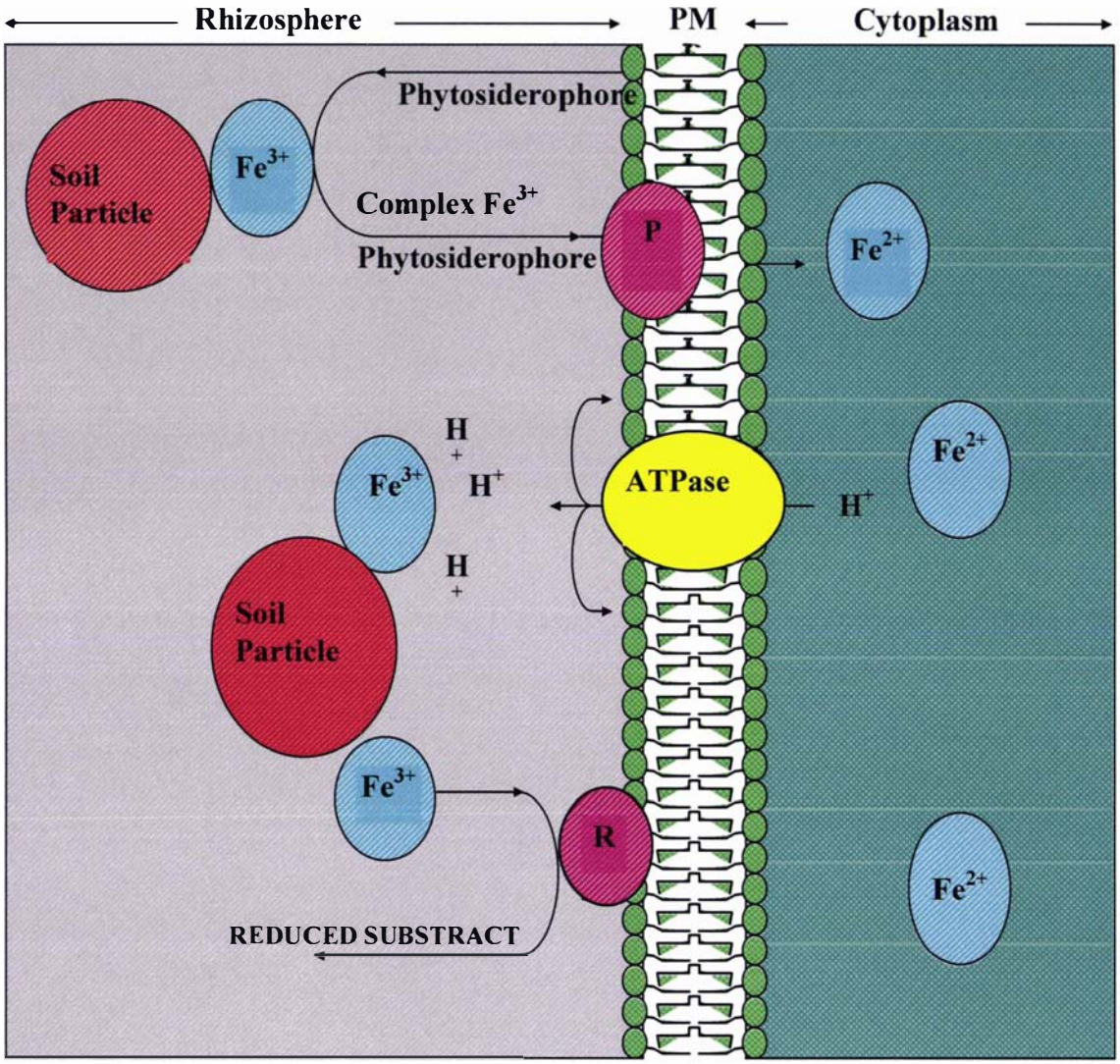

Figura 2.3. Schematic view of ion ferric mobilization by phytosiderophore excretion, rhizosphere acidification and plasma membrane bound metal reductases. Extrusion of protons outside of the cell occurs via membrane ATPases. PM = Plasma Membrane, P = Carrier for the complex Fe^{3+} phytosiderophore, R = Plasma membrane metal reductase (Redraw from Marschner, 1991).

Mycorrhizal fungi or root-colonizing bacteria might also perform all three of the above-mentioned processes. Shoot Zn accumulation by *Thlaspi caerulescens* is enhanced by

rhizosphere bacteria, which play an important role in increasing the availability of water-soluble Zn in soil (Whiting et al., 2001). Vesicular-arbuscular mycorrhizae (VAM) have been shown to alleviate the toxic effects of Zn on growth of several grass species (Dueck et al., 1986; Shetty et al., 1994) and to enhance the accumulation of this element in shoots of White clover (*Trifolium subterraneum*) (Bürkert and Robson, 1994). It has been reported that root uptake of Hg and Se for wetland plants is actively promoted by rhizosphere bacteria (DeSouza et al., 1999). For instance, when Saltmarsh bulrush (*Scirpus robustus*) and Rabbit-foot grass (*Polypogon monspelienses*) were grown in the presence of ampicillin, an antibiotic known to inhibit both gram-positive and gram-negative bacteria types, root accumulation for both metals was inhibited when compared to controls (plants not treated with ampicillin). This result was further confirmed by inoculating sterile saltmarsh bulrush plants with bacteria isolated from the rhizosphere of plants collected from the field; inoculated plants accumulated significantly more Hg and Se compared to sterile controls (DeSouza et al., 1999).

2.3.4 Uptake and transport

Solubilised metals enter cell roots via extra and intracellular pathways, depending whether the transport involves ion movement across the plasmatic membrane (symplast) or across the cell wall (apoplast) (Figure 2.4). Once inside the roots, metal ions can be either stored or transported upward to the shoot (Raskin, 1994). While the vacuole plays an important role in metal ion storage and detoxification, root-to-shoot transport of metal ions occurs via the conducting cells of the xylem (Salisbury and Ross, 1992). However, there is evidence that the phloem also participates in the distribution of metal ions in the plant (Clarkson and Lutge, 1989; Stephan and Scholz, 1993). Since both the xylem and the phloem contain metal ligand compounds such as organic acids, amino acids and peptides, movement of metals in these vessels are likely to occur in the form of complexes with these organic substances. For instance, analysis of xylem saps in Tomato revealed that xylem Cu is transported to shoots in the form of complexes with asparagine and histidine whereas Zn and Fe are transported in the form of complexes with citric acid (Clarkson and Luttge, 1989). Other studies have shown that Ni transport in the xylem sap of some hyperaccumulator species is associated either with carboxylic or amino acids (Brooks, 1998). The unusual amine nicotianamine has been linked to the phloem transport of chelated forms of Fe and,

possibly, of some other heavy metals (Stephan and Scholz, 1993). However, to my knowledge, there is no report relating organic substances to the transport and distribution of Hg in higher plants. The fact that phytochelatins are synthesised in leaves and roots in response to Hg stress (as mentioned before) suggests, nonetheless, that Hg transport in plant tissues might be associated with sulphur-containing peptides.

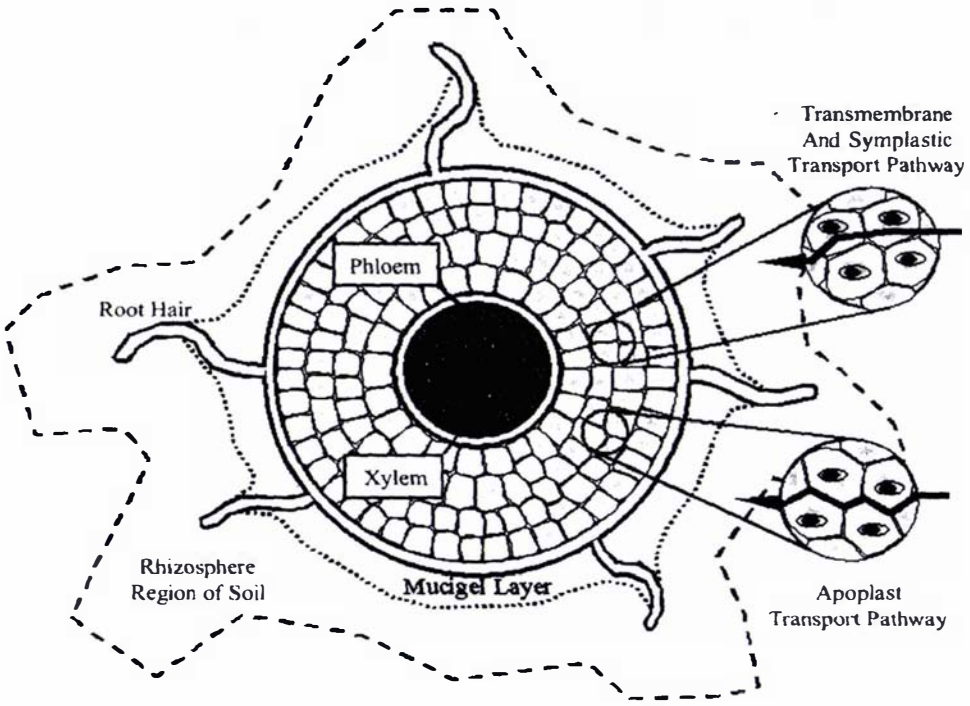

Figure 2.4. Schematic cross-section of a primary root. The symplastic pathway occurs through the root cells (upper bubble). The apoplastic pathway occurs between the cells (lower bubble). Source: Tsao, 2003.

2.3.5 Volatilisation

Early studies on the release of Hg (0) to the atmosphere from vascular plants appeared in the 1970s from work carried out by Siegel et al. (1974) with leaves of Garlic vine (*Pseudocallyma alliacium*), Avocado (*Persea americana*) and Haole-koa (*Leucaena glauca*). Although volatile Hg emissions could be trapped in HNO_3-$HClO_4$ solutions, the authors rejected the possibility of release of elemental Hg from the leaves. More recently, with the development of more accurate sampling and measuring methods for volatile Hg collections (e.g. gold-coated sand traps), it has been possible to conclude

that the elemental gas Hg (0) is the main Hg compound released from plants (Leonard et al, 1998 a and b, Lindberg et al, 1998).

Fluxes of Hg (0) from foliar surfaces of a variety of plants species have been characterised as bi-directional (Leonard et al, 1998 a, b; Schwesig and Krebs, 2003). That means that plants can be simultaneously a sink and a source for Hg (0). However, foliar uptake has been shown to be of lower magnitude compared to emissions from plants and soils (Lindberg et al., 1998). The Hg (0) fluxes from five plant species (*Fragaria vesca, Eucalyptus globulus, Lepidium latifolium, Artemisia douglasiana,* and *Caulanthus* sp.) collected from Hg-contaminated sites in Nevada (USA) with soil Hg concentration ranging between 450 to 1750 mg/kg were measured in a closed gas exchange system. The fluxes were shown to vary between 10 to 90 ng/m^2/h (Leonard et al., 1998 a). A tower-based micro-meteorological method has been developed for measuring Hg (0) fluxes over mature deciduous forest (hardwood stand comprised primarily of Oak [*Quercus* spp]) and young Pine plantation (*Pinus alba*) in Tennessee (USA) (Lindberg, et al., 1998). The same system was used for measuring Hg(0) fluxes over emergent macrophyte vegetation (*Typha domingenesis* and *Cladium jamaicense*) in the Florida Everglades (Lindberg et al., 2002). Emissions of Hg (0) from the canopy of the trees and from the foliar surfaces of macrophytes ranged from 10-300 and 10-40 ng/m^2/h, respectively. Vapour Hg (0) fluxes were influenced by temperature, solar radiation and atmosphere turbulence but were most strongly correlated with water vapour fluxes (Lindberg et al., 2002). A compartment model for describing the Hg movement in the soil-plant-atmosphere system has indicated, therefore, that Hg (0) is transpired from plants (Leonard et al., 1998 a). In this case, Hg (0) moves in the transpiration stream from roots to mesophill cells of the leaf interior and through the stomata. It has been further proposed that Hg (0) flux is stomatally controlled, thereby decreasing in the dark and under drought stress. Lindberg et al. (2002) has suggested that Hg (0) emissions originate from gaseous Hg (0) in soil pores, which enter the transpiration stream with, but not in equilibrium with, soil water. The source of an elemental Hg (0) pool in soils would be related to biotic (bacterial metabolism) and abiotic (photoreduction, electron transfer during reaction with humic substances) reduction processes happening on Hg species present in the soil surface.

2.4 Mercury in Soils

2.4.1 Speciation of soluble Hg in the soil

One of the most intriguing features of Hg in the soil environment is its ability to form complexes with Cl^-, OH^-, S^{2-} and sulphur-containing functional groups of organic ligands. This tendency is explained by the hard and soft acid-base principle (Pearson, 1963). According to this principle, small and not very polarizable metals (acids) such as Fe^{3+}, Al^{3+}, and Co^{3+} are preferentially bind with ligands (bases) that are also small and less polarizable (e.g. oxygen-containing functional groups). These acids and bases are called "hard". By contrast, metal ions such as Hg^{2+}, Ag^+, and Cu^+ tend to be larger and more polarizable and thus, they prefer larger and polarized ligands (e.g. N and S-containing anions or functional groups). These acids and bases are called "soft" (Cotton et al., 1987). As a result of coordination selectivity, Hg^{2+} will form complexes with soft ligands such as NH_3^+, CN^-, Cl^-, and S-donors. However, complexation of Hg^{2+} with the anions Cl^- and OH^- will depend on the composition of the soil solution, pH and chloride content (Andersson, 1979). Since the concentration of these ions is relatively high in most natural systems, it could be expected that the complexes $HgCl_2$, $Hg(OH)_2$ and HgOHCl would dominate over other Hg forms usually found in drainage water and soil solutions. Consequently, trace concentrations of these soluble Hg complexes can be found in most terrestrial soils (Schuster, 1991). In anoxic flooded soils and sediments at alkaline pH and in the presence of sulphide ions, on the other hand, Hg speciation will be completely dominated by sulphide and bisulphide complexes (Morel et al., 1998). The strong affinity of Hg for organic matter profoundly influences Hg speciation in the soil environment (Kabata-Pendias and Pendias, 2000). Humic substances (HS) represent 50 % of the natural organic matter in soils and contain a high proportion of sulphur-containing functional groups (Wallchlager et al., 1998 a). The soluble fraction of HS comprises fulvic and humic acids, which are known Hg ligands. Since Hg complexes with humic substances are stable over the entire pH range from 1-14 (Wallchlager, 1996), these complexes can be abundant in the solution of organic and mineral rich horizon soils (Evans, 1986).

2.4.2 Retention of Hg in the soil

The adsorption of Hg onto mineral surfaces has been thoroughly reviewed by Schuster (1991). According to this author, the chemistry of solid phase Hg might be explained by the same principles that govern Hg speciation in the soil solution. Since the dominant species of Hg^{2+} in solution are uncharged complexes, the mechanism for sorption onto the solid phase may be insoluble inorganic complex formation. In the case of mineral surfaces, Hg adsorption follows a pH-dependent pattern similar to other hydrolysable cations, such as Pb^{2+}, Cu^{+}, and Zn^{2+} (Evans, 1989). For instance, Hg adsorption on hydrous MnO_2 is increased between pH 2.5 and 3 (Schuster, 1991). In oxisol soils (consisting of quartz [SiO_2], kaolinite [$Al_2Si_2O_5(OH)_4$] and gibbsite [$Al(OH)_3$]), on the other hand, Hg adsorption is increased at a higher pH range, i.e., between 3 to 5 (Melamed et al., 1998). It is believed that the increase in the adsorption capacity is due to the formation of non-charged hydroxide complexes, thus implicating a non-eletrostatic type of interaction. Therefore, it has been proposed that hydroxide complexes are the main active species in the adsorption process. However, the presence of stronger ligands in solution can alter this behaviour. High concentrations of chloride ions in the system, for example, can reduce the Hg adsorption capacity as a result of Hg-chloro-complex formation (Schuster, 1991, Melamed et al., 1998).

The strong affinity of Hg for organic matter influences Hg solid phase speciation to a greater extent when compared to 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. As a result 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 a). Although organic matter has a higher adsorption capacity, there is also a pH-dependant pattern for this type of interaction. For instance, in acid soils (pH < 5), the Hg sorption process is clearly dominated by organic matter whereas in neutral soils iron oxides and clay minerals may be dominating (Andersson, 1979).

2.4.3 Mobility and transport of Hg

Humic substances are the naturally preferred binding partners for Hg and can exert a dominant influence on Hg transport and mobility in the environment. This is attributed to the tendency of Hg to complex with water-soluble OM under conditions of natural pH and salinity. For instance, Wallschager et al (1998 b) presented evidence that the interaction between Hg and soluble organic complexes is associated mainly with soluble HS fractions (a process that appears to be affected by the amount of Hg present), the quantity and quality of organic carbon in the environmental compartment, and other geochemical parameters. By investigating the contaminated sediments and floodplain soils of the river Elbe (Germany), these authors demonstrated that mobilisation of Hg was chemically and irreversibly coupled to large molecular weight humic acid molecules, thereby controlling longitudinal, horizontal and vertical transport processes. Mercury mobilisation and transport in the river systems of the Amazon region is also strongly influenced by the presence of organic matter (Lacerda and Solomons, 1992). The Amazonian dark water rivers are typified by relatively low pH (< 5), low content of particles in suspension, and high content of dissolved organic substances in the water column. A strong correlation has been found between Hg and the organic matter content in dark water systems far away from Hg emission sources (Lacerda and Solomons, 1992). Therefore, it is believed that metallic Hg from artisanal gold mining is transported over large distances in the acidic Amazon waters complexed with humic acids (Melamed et al., 2000). In this case, metallic Hg discharged to soils and sediments can become mobile via reaction with soluble organic acids in oxic environments (Veiga, 2004). The environmental mobility and transport of Hg in the abovementioned examples would be dependant on the concentration gradient and the mass flow of water. The latter process, in turns, is a function of the soil's water potential, soil porosity and long-distance transport through macropore flow (Bundt et al., 2000). Alternatively, Hg bound to iron and aluminium oxy-hydroxides mineral surfaces in weathered tropical soils can be mobilised through selective erosion of the soil profile (sandification) or through destruction of the soil clay matrix under acid conditions (podzolization) (Roulet et al., 1999, Roulet et al., 2000). The released Hg species can complex with the soluble fraction of soil organic matter and reach aquatic systems for further transport. This mechanism has been proposed to explain the transport of Hg in dark water systems in Hg-enriched areas of the Amazon basin where

there is evidence of intense podzolisation (Lacerda and Solomons, 1992; Veiga, 1994; Oliveira et al., 2001).

2.5 Mercury Pollution and Remediation

2.5.1 Pollution

Mercury from anthropogenic and natural sources exhibit a cyclic behaviour in the environment alternating between the atmosphere, soil and water compartments both in inorganic and organic forms (Figure 2.5). The primary compartment for this pollutant is the atmosphere, where gaseous elemental Hg (0) can undergo long-range transport and deposition to remote areas. Although, the residence time of Hg (0) in the atmosphere is longer than other inorganic and organic gaseous species, its deposition can be accelerated as a result of local existing hydrological cycles (Downey, 2004; Lacerda, 2003). Deposition of Hg back to the earth surface occurs via wet and dry deposition, after photochemical oxidation of Hg(0) to Hg(II) in the presence ozone (Morel et at., 1998). Mercury (II) deposited in surface soils is very reactive and can be incorporated in the solid phase of soil constituents (e.g. sulphides, oxy-hydroxides of iron, organic matter) or be transported in the form of soluble complexes to aquatic environments. Mercury in aquatic systems is readily absorbed to particulate matter and can be metabolised by microorganisms. Sulphate-reducing bacteria have been linked to the production of toxic methylmercury from Hg(II) in anoxic sediments. (Choi, 1994 a and b). However, abiotic methylation of Hg(II) in aquatic environments is also possible in the presence of methylcobalamin, methyltin compounds, and humic matter (Weber,1993). Once produced, methylmercury is bioconcentrated 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 (Morel et al., 1998). Humans and wildlife can be exposed to methylmercury through ingestion of contaminated fish, which are likely to have high Hg concentrations in impacted areas. Therefore, 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 ones known to humankind (Malm, 2001).

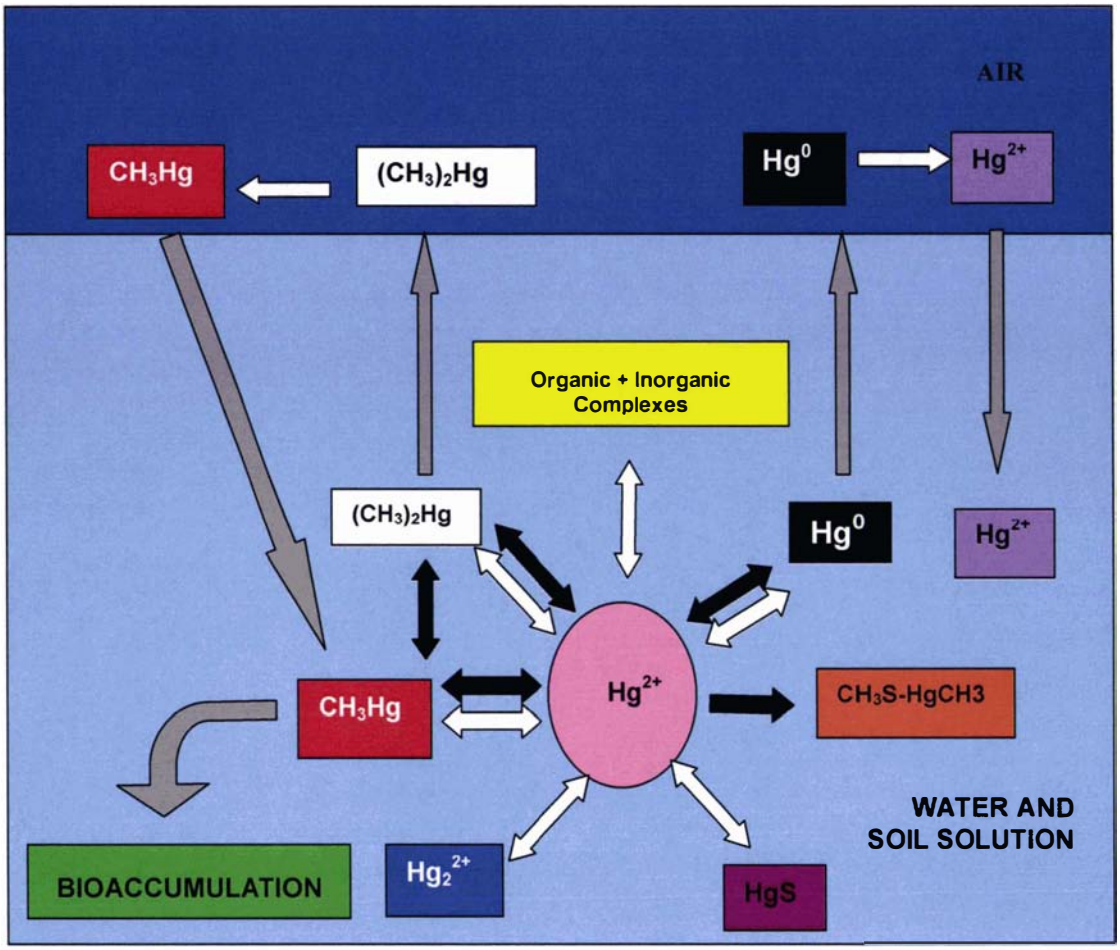

Figure 2.5. The mercury biogeochemical cycle. Black arrows – biological (microbially-mediated) transformations; white arrows – chemical transformations; grey arrows – transport processes; Hg^{2+} = mercuric ion; Hg_2^{2+}= mercurous ion; CH_3Hg = methylmercury; HgS = mercury sulphide; $(CH_3)_2Hg$ = dimethylmercury; Hg^0 = elemental mercury (adapted from Barkay et al., 1992).

Chronic exposure to moderate levels of methylmercury has a devastating effect on humans and animals. It has been reported that high levels of Hg from industrial sources in the Florida Everglades have affected the reproductive health of local birds and caused the death of panthers (Lindberg et al., 2002). Minamata disease, characterised by severe neurological disturbances, was attributed to human ingestion of methylmercury-contaminated fish. During the 1950's, a chemical plant employing Hg (II) as a catalyst in a process that produced polyvinyl chloride discharged Hg-containing wastes into Minamata Bay, in Japan. The methylmercury that subsequently formed then bioaccumulated in fish at concentrations up to 100 mg/kg. Mercury poisoning from this source affected thousands of people in the Minamata village (Baird, 1995). Intestinal adsorption of methylmercury from fish ingestion results in dysfunctions of the central nervous system. The main symptoms include numbness in

arms and legs, visual constriction, impairment of speech, loss of hearing and muscle coordination, and lethargy and irritability (Baird, 1995). Since methylmercury readily crosses placental barriers, women of childbearing age and their children are particularly susceptible to the methylmercury effects (Veiga and Hinton, 2002). Children born to mothers poisoned even slightly by methylmercury showed severe neurological damage, some to a fatal extent. Symptons in infants are similar to to those of cerebral palsy: mental retardation, motor disturbance and even paralysis (Baird, 1995).

2.5.2 Remediation

The discharge of Hg into soils and aquatic systems from anthropogenic activities is of great environmental concern due to the potential transformation of inorganic Hg forms into methylmercury. There are no available data describing how much Hg has been discharged into soils, sediments and waters worldwide. It has been guessed that thousands of square kilometres of land, rivers, lakes and estuaries in the USA are contaminated with millions of kilograms of Hg. Few of the Hg-contaminates sites in this country or elsewhere in the world have been remediated (Meagher et al., 2000). The Hg problem might be growing in developing countries, where Hg emissions from artisanal and small-scale gold mining have increased together with the rising price of gold in 2003 and 2004 (Veiga, 2004). It is estimated that around 160,000 artisanal mine sites are spread around the world. In the Brazilian Amazon, for instance, the figures point for around 2000 abandoned artisanal gold mine sites (Veiga and Hinton, 2002). 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 has been shown to be the most suitable technology among the physical remediation methods. Of the various chemical techniques, eletrolytic extraction and chemical leaching have demonstrated capacity for the clean up of Hg-contaminated media (Villas Bôas, 1997; Hinton et al., 2003). Other methods, such as the use of impermeable plastic liners 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. (Brooks, 1998; Terry and Bañuelos, 2000; Tsao, 2003). It has attracted attention in recent years due to the low cost of implementation and environmental benefits (Lombi et al., 2001). 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.

2.6 Phytoremediation

2.6.1 Definition

Phytoremediation is referred to any plant-based system that is used for the cleaning up of soils, sediments and groundwater systems contaminated with inorganic and organic pollutants (Schnoor et al., 1995; Robinson et al., 1998, Glass, 1999). Plant species can be an economic alternative to conventional remediation technologies because they rely uniquely on the sun's energy to do the remediation job. The concept of using plants to environmental remediation has been intensively researched over the last 15 years. Plants contain, sequester, remove, or degrade contaminants as a result of normal physiological processes such as water uptake, inorganic ion uptake, evapotranspiration, root exudation and turnover, photosynthetic production of phytochemicals. Rhizosphere microbes can effect biodegradation of organic contaminants that are stimulated by plant roots (Tsao, 2003). The application of these plant-rhizosphere processes has been used to treat contaminated soils, sediments, surface water and groundwaters. 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 (Schnoor, 1995).

Phytoremediation has been proven an effective strategy for site stabilisation. 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.

Additionally, wind blow dust or water borne wastes can be reduced or even eliminated by plant covers (McIntyre, 2003). The ability of plants to precipitate or immobilise metals in the organic fraction of soils, on root surfaces, or within root tissues, can prevent the migration of contaminants off site or into ground water systems. This latter application is referred to as phytostabilisation (Mench et al., 2000; Tsao, 2003). For instance, it has been demonstrated that plant roots can alter the soil pH by release of exudates such as organic acids and bases, therefore, facilitating the precipitation of metals in the rhizosphere (Marshner, 1986). Similarly, metals can be converted to precipitated forms as a result of changes in the soil redox potential by plant-derived chemicals or by rhizosphere microbes (Tsao, 2003).While phytostabilisation involves the containment of inorganic contaminants in the soils, phytovolatilisation and phytoextraction can actually remove metal elements and organic compounds from the contaminated media. These two applications have been explored for the removal of Hg from mine wastes and are described in detail in this chapter.

2.6.2 Phytovolatilisation

Certain organic and inorganic contaminants can be volatilised from the plant biomass to the atmosphere while present in a dissolved state in the transpiration stream (Brooks, 1998). This process will depend on the physical characteristics of the contaminant such as the Henry's Law gas constant and the compound's octanol-water partition coefficient (K_{ow}). These parameters have been applied to understand the uptake, transport and volatilisation of organic contaminants such as BTEX, TCE, MTBE and Ethanol (Burken and Schnoor, 1998; Corseuil and Moreno, 2000). Metalloids like Se and Hg also can be volatilised from plant tissues. The phytovolatilisation of a metal to the atmosphere can be advantageous because it removes the element completely from the local system, thereby minimizing entry of the contaminant into the food chain (Heaton et al., 1998). Studies on the role of plants and rhizosphere processes on phytovolatilisation are more advanced for Se than Hg. Although the volatilisation of Se compounds from plants was first reported in 1935 by Beath et al. (Zayed et al., 2000), the idea of using plants to decontaminate soil Se via volatilisation was only proposed in the 1990s by Duckart et al. (1992) and Terry et al. (1992). The volatile Se form released from plants is the organometallic dimethyl selenite (DMSe), which is

considered less toxic than Se present as selenate in soils (Brooks, 1998; Tsao, 2003). There are several factors that affect the rate of Se volatilisation from plants, including plant species (Terry et al., 1992), shoot removal (Zayed and Terry, 1994), chemical form of Se present in the media (Zayed et al., 1998), the presence of sulphate ions (Zayed and Terry, 1992), temperature, pH and light (Brooks, 1998) and microorganisms (Zayed and Terry, 1994; DeSouza et al., 1999 b). The microbial population in the root zone has received a great deal of attention because it can strongly influence Se volatilisation from plants. Antibiotic studies with selenate-supplied Broccoli (*Brassica oleracea*) and Indian mustard (*B. juncea*) have demonstrated that bacteria can enhance the rate of plant-Se volatilisation by 35 to 95 % from the contaminated media (Zayed et al., 2000). Recombinant DNA techniques have been also applied to the creation of plants with superior volatilising abilities for both Se and Hg (Zayed et al., 2000; Rugh et al., 1996; Heaton et al., 1998; Heaton et al., 2003). These techniques were used to insert the microbial *MerA* and *MerB* gene sequence into the genome of Tobacco (*Nicotiana tabacum*) and Brassica (*B. napus)* plants. The *MerA* genes have a sequence that encodes for the synthesis of a flavin-containing disulfide oxireductase (known as mercuric reductase) that is responsible for the microbial conversion of Hg (II) to Hg (0) (Barkay et al., 1992). The *MerB* genes have a sequence of genes that encodes for the synthesis of the enzyme organomercurial lyase that degrades organic Hg compounds into the ion form Hg (II). The *MerA* and *MerB* gene sequences are under the control of other DNA sequences called Operator/Promoter, which triggers the transcription of *MerA* and *MerB* upon the signal of transcriptional regulator (inducer/suppressor) proteins (*MerR1* and *MerR2*), whose genes transcription is self regulated and increases in the presence of Hg compounds. The genes specifying the various functions needed for reduction of Hg (II) and degradation of organic Hg compounds are organized in the mercury resistance (*Mer*) operon, which is mostly found in gram-negative bacteria (for more details see: Summers, 1992; Rugh et al., 1996; Brünker et al., 1996; Bizily et al., 1999;Brown et al., 2003).

The insertion of these gene sequences into the plant genome resulted in Tobacco and Brassica plants with increased Hg resistance. These plants were able to germinate in Hg-containing media that killed the wildtype controls (Rugh et al., 1996; Meagher et al., 2000). Transgenic plants are also able to transform root available Hg (II) to the less toxic Hg (0). These transformations happen inside plant tissues and elemental Hg (0) is

volatilised from the leaves to the atmosphere. The results for transgenic plants cultured in Hg-containing hydroponic medium indicate volatilising rates three to four-fold higher when compared to non-transgenic controls (Rugh et al., 1996). Yet, these authors were not able to replicate these results for transgenic plants grown in Hg-contaminated soils. The transgenic plants did show, however, superior biomass production and reduced tissue Hg concentrations when compared to wildtype controls for soil Hg concentrations ranging from 100 to 500 mg/kg (Heaton et al., 1998). The limitations for the application of Hg-phytovolatilisation technology are related to the potential impacts of Hg (0) release to the local and regional environment.

2.6.2 Phytoextraction

Inorganic soil contaminants can be phytoextracted from the soil subsurface through repeated cropping and safety storage of the harvested plant biomass following accumulation of metals in the plant aerial tissues. 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 (Brooks, 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 caerulescens*) but often do not produce high annual biomass (Robinson et al., 1998) (Figure 2.6). The second class is comprised of plants that have a relatively low metal concentration in plant tissues but can produce a substantial amount of biomass, as is the case of *B. juncea* (Nanda Kumar et al., 1995). So far, the metal hyperaccumulation trait has been observed for As (Ma et al., 2001), Ni, Cd, Co, Mn, Se, Tl, and Zn metals (Brooks, 1998) in more than 400 plant species. However, there are no reports of plants that can naturally hyperaccumulate the metals Au, Ag and Hg.

Figure 2.6. View of a metal-tolerant flora dominated by *Thlaspi caerulescens* over the base-metal mine waste of the Les Malines mining area (France). Source: Robinson et al., 1998.

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 (Anderson et al., 2000) (Figure 2.7). 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.

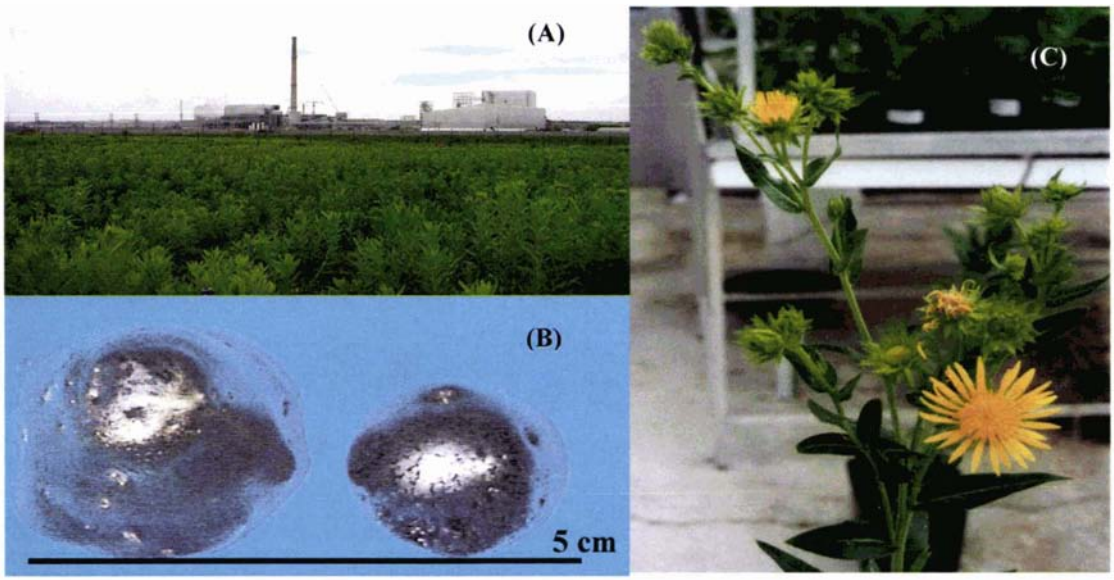

Figure 2.7. Phytoremediation of Ni-contaminated soils in Rustenburg, South Africa (A). The Ni-hyperaccumulator *Berkheya coddii* (B) was grown at a site near the refining facilities of the Anglo American Platinum Corporation (AMPLATS). Nickel ingots were recovered after harvesting and processing the Ni-rich plant biomass (C).

It is estimated that the overall cost for removing one meter of soil from a one acre site ranges between $0.6 to $2.5 million. In comparison, the costs for site preparation, planting and harvesting of plant material for the same acre site drop to between US$2000 and US$5000 (McIntyre, 2003). While phytoextraction appears to be cost effective, there are several limitations to the application of this technology on a commercial scale. Since most metal hyperaccumulator plants tends to be of slow growth and produce low biomass, several cropping seasons may be necessary for efficient metal removal. As a result, time requirements may be inhibitory for phytoextraction, as it can take years or even decades for site clean up (McGrath, 1998; Lombi et al., 2001). The bioavailability of target metals for plant uptake is another limiting factor for phytoextraction. Some metals such as Pb and Hg are so strongly adsorbed to the solid phase of the soil that only trace concentrations will be available in the soil solution. Induced or chemically- enhanced plant metal accumulation has been developed to deal with the metal insolubility and low biomass problems of phytoextraction. In this case, chelating agents such as EDTA, CDTA, DTPA, HEDTA, NTA, are amended to soil in order to induce the solubility and subsequent plant uptake of insoluble metals (Blaylock et al., 1997; Blaylock, 2000). By using this approach, high biomass plant species like maize (*Zea mays*) and *B. juncea* have been induced to accumulate relatively large amounts of insoluble metals such as Pb (Blaylock et al., 1997; Huang et al., 1997), Au (Anderson et al., 1998; Anderson et al., 2004[1] and Ag (Moreno et al., unpublished results). Field studies have demonstrated the effectiveness of the application of EDTA in combination with *B. juncea* for the phytoremediation of Pb-contaminated soils. (Blaylock, 2000). However, there is the concern about the leaching of metal-EDTA complexes to groundwater. This problem 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 after several weeks of EDTA treatment.

[1]This paper is described in the Appendix 1 of this thesis.

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SECTION A

METHODOLOGICAL APPROACH FOR THE ANALYSES OF MERCURY IN PLANTS AND SOILS AND FOR THE MEASUREMENT OF VOLATILE MERCURY EMITTED FROM PLANTS

This section is comprises Chapters 3, 4 and 5. Chapter 3 describes the method of hydride-generation atomic absorption spectroscopy (AAS) used in this research for the analyses of total Hg in soil and plant samples. The accuracy and precision of the method was assessed following a rigorous set of criteria, which was validated for quality control by an external certified laboratory.

Chapter 4 presents results from a preliminary experiment on the collection of volatile Hg from *B. juncea* plants cultured in Hg-containing solutions at concentrations ranging from 0 to 10 mg/L. This study involved the trapping of volatile Hg released from roots enclosed in a gastight compartment.

Chapter 5 describes a more advanced experimental apparatus for the collection of volatile Hg. This apparatus utilised a two-series trapping system designed for the collection of Hg vapours of inorganic and organic composition. The system allowed volatile Hg collection from both roots and shoots of a single *B. juncea* plant cultured in solutions containing Hg at 1 mg/L.

Two main reasons directed the utilisation of hydroponic media for the capture of volatile Hg in these preliminary experiments: 1) mass balance performed in this media allowed validation of the experimental apparatus designed for trapping volatile Hg emitted from plants, and 2) the bioavailability, speciation and concentration of soluble Hg in the hydroponic media is homogenous, thus reducing experimental variability. The high levels of reproducibility achieved in these experiments allowed the methodological approach to be adapted so that Hg vapours emitted from plants grown in the contaminated substrates could also be trapped. The reliability of the analytical techniques described in this section allowed for a reduction in the limit of detection for Hg vapours in the traps. Therefore, Hg vapours with values as low as 5 ng/mL were detected in the solution traps used in the subsequent volatilisation studies. These experiments are described in the Section B of this thesis.

CHAPTER 3

MERCURY ANALYSIS OF PLANT AND SOIL SAMPLES USING THE HYDRIDE-GENERATION AAS METHOD

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Abstract

Phytoremediation is the use of plants to improve degraded environments. Plants can be used to remove mercury (Hg) from contaminated soils through root uptake and translocation of this element into the above-ground tissues, and by volatilisation of elemental Hg(0) into the atmosphere. A key requirement of Hg-phytoremediation is the development of an accurate method for its analysis in plants and soil. The objective of this work was to develop the hydride-generation atomic absorption spectroscopy (AAS) method for the analysis of total Hg in liquid matrices of plants and plant-substrate digests. Total Hg in plant-substrates was extracted using either an *aqua regia* digest or ammonium thiosulphate ($[NH_4]_2S_2O_3$). Plant Hg was extracted using concentrated nitric acid (HNO_3) followed by acid evaporation in a water bath at 80°C for 1 hour. Linear calibration curves were obtained over the range of 125 to 1000 ng/mL of Hg. The detection limit for this method was based on two times the standard deviation of the blank (11.4 ng/mL). The reproducibility was better than 5% for a 1.0 mg/L mercury standard. Accuracy of the method was verified by analysing deionised water samples spiked with $HgCl_2$ and $HgNO_3$. This method was validated by analyses of a reference substrate as well as a comparative analysis of total Hg in samples by an external certified laboratory. Analysis of Hg-spiked and Hg-rich plant samples revealed a strong negative correlation between the volume of the sample added to the hydride generation reaction vessel and the final concentration of Hg determined in the plant tissues. Optimal analytical response of the equipment for Hg analysis of plant samples was achieved when sample volume added to the reaction vessel was 0.25 mL.

3.1 Introduction

Phytoremediation is a technology that uses plants to improve contaminated sites. The low-cost of this technology is one of its strongest potential advantages. By using the sun's energy as a driving force, plants can extract, degrade, transform or stabilize a large array of contaminants in media such as soil, water, sludge and sediments (Glass, 1999).

The phytoremediation of Hg-contaminated soil may be accomplished by phytoextraction (removal of Hg in the plant shoot biomass), phytostabilisation (immobilisation of Hg in the root-zone), phytovolatilization (release of Hg-vapour from roots and shoots into the atmosphere) or through a combination of these processes (Meagher et al., 2000; Moreno et al., 2003[2]; Moreno et al., 2004 a[3]). Whatever the plant process or the remedial scenario involved, the application of a correct method for the analysis of Hg in plant and soil samples is a *conditio sine qua non* for the development of this technology.

Here we describe a hydride generation method for the determination of total Hg in soil and plant samples. The performance of the method was evaluated using the following criteria:

1) The generation of linear calibration graphs;
2) The detection limit;
3) The recovery of Hg in Hg spiked deionized water and plant samples;
4) The determination of total Hg concentration in a tailings reference materials of known Hg concentration;
5) A comparative analysis of total Hg concentrations in solutions and Hg-rich plant samples by an external certified laboratory.

[2] This paper is described in Chapter 4 of this thesis.
[3] This paper is described in Chapter 7 of this thesis.

3.2 Material and Methods

3.2.1 Standards and reagents

All solutions were prepared using reverse osmosis (RO) water and the chemicals used were of analytical reagent grade. A Spectrosol Atomic Absorption (AA) solution of mercury nitrate $HgNO_3$ (1000 mg/L) was used as the primary standard. Four (4) working standard solutions were prepared during each analysis by appropriately diluting a 10 mg/L Hg solution in RO water.

Liquid samples containing Hg were added to the Hg vapour-generation flask along with 10 mL of 0.5 M hydrochloric acid (HCl). Sodium borohydride ($NABH_4$) was used as a reducing agent to generate elemental Hg in a 5% $NaBH_4$ + 1% potassium hydroxide (KOH) w/v solution.

3.2.2 Mercury-rich plant samples

Mercury-rich plant samples for analysis were obtained through the phytoextraction of this element into shoots of *Brassica juncea* plants grown under field conditions (Moreno et al., 2004 b[4]). A field plot with a dimension of 5 x 5 m was established on the Tui mine tailings, Te Aroha, NZ. The total Hg content of the substrate was 2.82 ± 0.31 mg/kg (Moreno et al., 2004 a). Seeding of the plot with *B. juncea* occurred after the substrate was fertilised with 5 g/L of Osmocote (slow release fertiliser). The pH was adjusted to 5.5 by addition of lime. Organic matter in the form of compost was also added at 3.2 L/m^2. Six weeks after substrate preparation, the plot was treated with sodium cyanide (NaCN) at 0.2 g/kg of substrate. The plant biomass was harvested one month after treatment.

[4] This paper is included in this thesis as Chapter 8.

3.2.3 Extraction procedures

3.2.3.1 Substrate samples

Total Hg from solid samples was measured using *aqua regia* digestion and ammonium thiosulphate ($[NH_4]_2S_2O_3$) extraction methods. For this purpose, one-gram substrate-samples were weighed into 50 mL polypropylene beakers 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 1 hour and the filtrates diluted to a final volume of 50 mL by adding RO water.

The ammonium thiosulphate extraction method involves ligand exchange reactions where Hg bound to the solid-phase of substrates is complexed with the sulphur binding site of the S_2O_3 ligand (Wilkinson et al., 1987). One gram samples of substrate were weighed into 50 mL polypropylene centrifuge tubes in triplicate. After addition of the $(NH_4)_2S_2O_3$ solution (20 mL at 2 g/L), the tubes were rotated in a shaker overnight at 45 rotations per minute (RPM) and the supernatant separated via centrifugation at 3000 RPM for 3 minutes.

3.2.3.2 Plant samples

Harvested shoot tissues were washed in tap water and placed in a drying oven at 70 °C until a constant weight was obtained. After grinding, subsamples (0.5 g) were accurately weighed into 50 mL plastic beakers before 15 mL of HNO_3 was added. The plant samples were left overnight then heated in a water bath at 80 °C for 1 hour. Subsequently, the digest solutions were transferred to 10 mL polythene tubes and diluted with RO water to make a final volume of 10 mL.

3.2.4 Reproducibility of the method

Reproducibility of the proposed method was assessed through Hg analysis of 1) deionized water samples spiked with mercuric ions $HgNO_3$ and $HgCl_2$ to give a final Hg concentration of 125, 500 and 1000 ng/mL and; 2) *B. juncea* plant samples digest (no Hg added) spiked with $HgNO_3$ to give a final Hg concentration of 500 ng/mL.

3.2.5 Quality control assessment

The quality assurance of the hydride generation method was obtained by:

1) Analysis of a tailings reference material obtained from the Department of Mining and Mineral Process Engineering (UBC, Vancouver, Canada) with a known Hg concentration of 100 mg/kg and;
2) ICP-MS analysis of total mercury concentrations in Hg-spiked solutions and Hg-rich plant samples by a external certified laboratory (Hill Laboratories, Hamilton, NZ).

3.2.6 Instrumentation

The apparatus consisted of a nitrogen source, a peristaltic pump, a sodium borohydride container, a mercury-vapour generation flask (75 mL pear-shaped flask), an absorption tube and a GBC900 atomic absorption spectrophotometer (Figure 3.1).

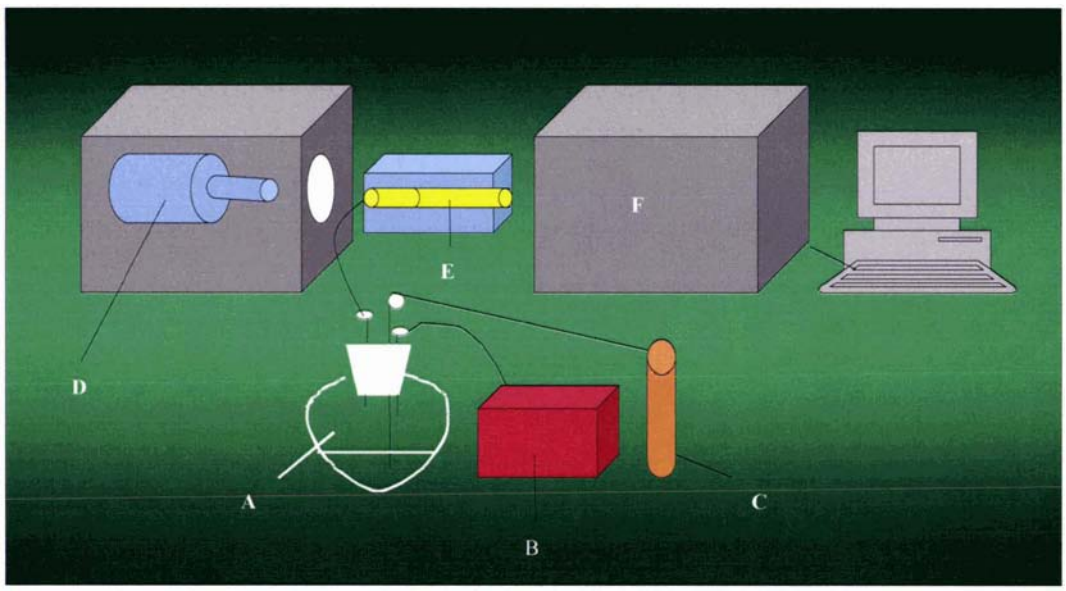

Figure 3.1. Schematic diagram of the hydride generation apparatus. A, mercury vapour generation flask; B, peristaltic pump; C, nitrogen source; D, mercury lamp; E, heating unit; F, absorption tube.

A peristaltic pump was used to inject a single burst of sodium borohydride into the mercury vapour generation flask at a flow rate of 15 mL/min. Nitrogen gas was used as a carrier to transport the mercury vapour to the atomic absorption tube.

The Hg vapour generation flask was a 75 mL pear-shaped glass flask mounted on a chassis. A Teflon bung with three inserted glass tubes sealed the vessel. Of the tubes, the first injects sodium borohydride, the second tube carries a continuous flow of low-pressure nitrogen gas into the sample. The last tube connects the reaction flask to the absorption tube. The absorption tube is a silica tube with an input tube at one end and an exit at the other. The tube is located at the path of the GBC 900 AAS. An ICI mercury hollow cathode lamp operating at a wavelength of 253.7 nm and a lamp current of 3 mA emitted light according to the spectral line for Hg. The GBC 900 instrument was set in the flame mode and quantification of mercury concentration was achieved by recording the peak height of the Hg signal over a period of 30 seconds. Interference by non-atomic absorption was minimised by a background correction system that measures absorbance from a broad-band output deuterium lamp (D_2).

3.3 Results and Discussion

3.3.1 Formation of Hg^0

Hydride generation is a technique developed for the determination of hydride-forming metals and metalloids (Atkins and Jones, 1997). There are several methods for hydride generation and collection and all have the following features in common: 1) a strong reducing agent to generate the hydride; 2) a heating device that decomposes the hydride, and 3) an atomic absorption measuring device which quantifies the amount of hydride produced (Robinson, 1994).

Various reducing agents and sources of nascent hydrogen have been suggested to convert the element of interest into its hydride. In the case of Hg, the elemental vapour Hg^0 can be generated by reaction the reaction of Hg^{+2} with either stannous chloride (SnCl) or sodium borohydride ($NaBH_4$). However, SnCl reduces only inorganic mercury. Total mercury determinations with this reducing agent, therefore, will be possible only after all organomercury species have been decomposed into inorganic Hg. On the other hand, $NaBH_4$ has the ability to reduce both inorganic and organic Hg in the same sample solution, thus making this reducing agent potentially advantageous over SnCl in the case of total Hg determinations (Oda and Ingle, 1981).

Hg (II) reacts with $NaBH_4$ to produce elemental Hg^0 according to the following equation:

$$Hg^{+2}_{(aq)} + 2NaBH_{4(aq)} + 6H_2O \longrightarrow Hg^0_{(g)} + 7H_{2(g)} + 2H_3BO_{3(aq)} + 2Na^+_{(aq)} \quad (1)$$

3.3.2. System calibration

The hydride generation system utilised a flow rate of 280 to 320 mL/min for nitrogen and 15 mL/min for $NaBH_4$. Under these conditions, linear calibration curves were obtained over the range of 0 to 1000 ng/mL of Hg (Figure 3.2).

The limit of detection (LOD) for mercury was calculated using the general definition proposed by Harris (1986). The LOD should correspond to the concentration of an element necessary to yield a net signal equal to two times the standard deviation of 10 blank measurements. For the Hg hydride generation method in this research the LOD found was 11.4 ng/mL. The reproducibility of the mercury signals (in terms of relative standard deviation) was 4.6%. This was determined by analysing 10 standards containing 1 mg/L of Hg.

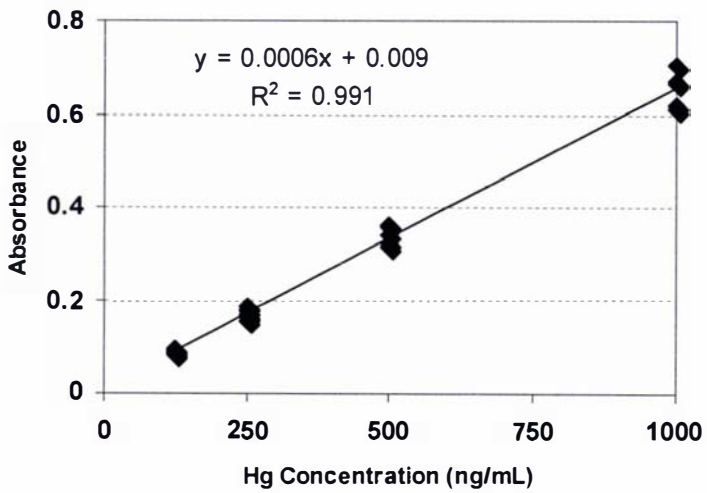

Figure 3.2.Calibration graph for the hydride-generation method.

3.3.3 System reproducibility

The reproducibility of the hydride-generation method for the analysis of water samples was investigated by spiking deionized water with two mercury species ($HgCl_2$ and HNO_3) at four different concentrations (125, 250, 500 and 1000 ng/mL) to assess any effect of Hg speciation on the reproducibility of analyses by the hydride method. Table 3.1 shows that the recovery values for total mercury ranged from 88.5 to 94.3% for both mercury species added.

Table 3.1. Determination of Hg^{2+} in deionized water samples.

Hg Form	Hg added (ng/mL)	Hg recovered (ng/mL)	Recovery %
$HgCl_2$	125	116.02	92.81
	250	224.11	89.64
	500	460.91	92.18
	1000	900.87	90.08
$HgNO_3$	125	110.63	88.50
	250	224.4	89.76
	500	452.13	90.42
	1000	943.21	94.32

The precision of the equipment for analysis of plant samples spiked with Hg at 500 ng/mL was examined when different volumes of sample were added to the reaction vessel. This was performed because elemental Hg generation by cold vapour methods may present errors if oxides of nitrogen, incompletely oxidized organic vapours, or smoke, having absorption lines at 253.7 nm, are present in the liquid matrix (Mitra, 1986). In the presence of such substances, a significant fraction of light emitted from the hollow cathode lamp is scattered and the absorbance can be substantially reduced (Msuya, 2000). Figure 3.3 shows a strong negative correlation between the volume of the sample added to the reaction vessel and the relative peak heights for Hg in the AAS ($r^2 = 0.99$, $p < 0.0001$). For instance, at 0.25 mL of sample volume, the relative peak for Hg is not significantly different from the control (488 ± 67 and 500 ± 29, respectively, $p > 0.05$). However, as the volume increases from 0.5 to 3 mL, the relative peak height for Hg in plant tissue sharply reduces from 403 ± 38 to 182 ± 31).

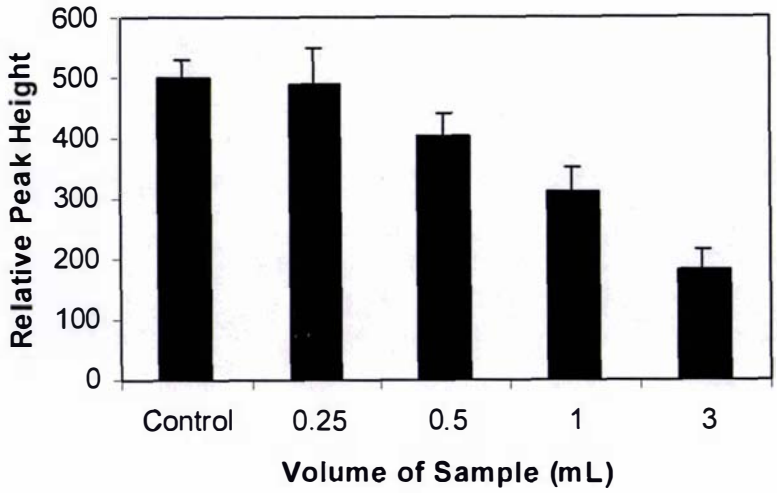

Figure 3.3. Relative peak heights for Hg in plant tissues as a function of the sample volume added to the reaction vessel. Bars denote ± 1 standard deviation from the mean of three replicates. The peak height is relative to the Hg content in plant tissues (in mg/kg DW). Controls refer to Hg-spiked samples without plant tissues.

3.3.4 Quality control assessment

The hydride generation method was validated by the analysis of total mercury concentrations in a reference tailings substrate, in Hg-spiked solutions and Hg-rich plant samples. The samples were analysed in triplicate and the results were compared with the total Hg analysis performed by an external certified laboratory. A comparative analysis using both the *aqua regia* and $(NH_4)_2S_2O_3$ procedures was performed to obtain total mercury concentrations in good agreement with the total Hg value present in the tailings reference substrate.

Figure 3.4 shows the potential of *aqua regia* and $(NH_4)_2S_2O_3$ to extract Hg from a non Hg-spiked tailings reference substrate containing a known Hg concentration of 100 mg/kg. The use of 2 g/L of $(NH_4)_2S_2O_3$ resulted in significantly higher Hg recoveries when compared to the HNO_3/HCl acid mixture (122.2 ± 9.4 mg/kg and 67.47 ± 11.2 mg/kg, respectively, t-test, p = 0.0056). The concentration of Hg recovered using the $(NH_4)_2S_2O_3$ procedure was in good agreement with the total Hg content of the reference substrate. These results indicate that $(NH_4)_2S_2O_3$ is suitable for total Hg measurements in high pH and low Eh substrates. Under these conditions highly stable thiosulphate complexes are expected to form (Anderson, 2000).

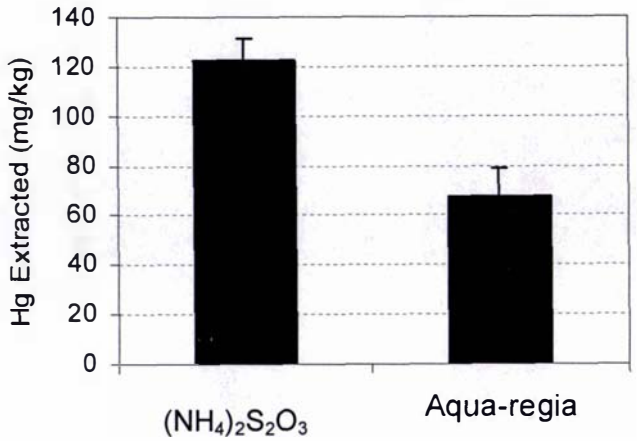

Figure 3.4. Comparison of the ability of two solutions to extract Hg from a tailings reference substrate containing 100 mg/kg of Hg. Bars denote ± 1 standard deviation from the mean of three replicates.

The comparative evaluation between the hydride generation method and ICP-MS analysis for the total Hg contents in spiked solutions and Hg-rich plant samples are shown in table 3.2.

Total mercury concentrations determined in solutions using the hydride generation AAS method were in good agreement with the Hg values found by the certified laboratory. Mercury recoveries ranged between 88 to 103%. Table 3.2 shows that Hg concentration found in the Hg-rich biomass agreed reasonably well with the Hg concentration detected by the ICP-MS method. Analysis by the hydride generation method reported 84 % of the Hg reported by analysis using ICP-MS. This difference was shown to be statistically significant (t-test, $p = 0.0069$).

Figure 3.5 shows the relative peak heights for Hg in the Hg-rich biomass of *B. juncea* as a function of the volume of sample added to the reaction vessel. The content of Hg is the same for all the samples. On average, the relative peak height decreased significantly from 4.95 ± 0.29 to 1.02 ± 0.17 as the sample volume increased from 0.25 to 3 mL ($r^2 = 0.979$). As demonstrated previously, the presence of oxidized plant tissues

in the liquid matrix significantly interfered with the analytical response of the hydride-generation method ($p = 0.0002$).

Table 3.2. Comparative analysis between the hydride generation and ICP-MS methods for the Hg determination in Hg-spiked solutions and Hg-rich plant samples.

Method	Hg added (ng/mL)	Hg Recovered (ng/mL)	Hg-rich plant samples (mg/kg DW)*
Hydride Generation	125 250 500 1000	110.63 224.4 452.13 943.21	4.95 ± 0.29#
ICP-MS	125 250 500 1000	107 248 474 1060	5.87 ± 0.10

*Mean and standard deviation of three replicates.
Value achieved at 0.25 mL of sample volume added to the reaction vessel.

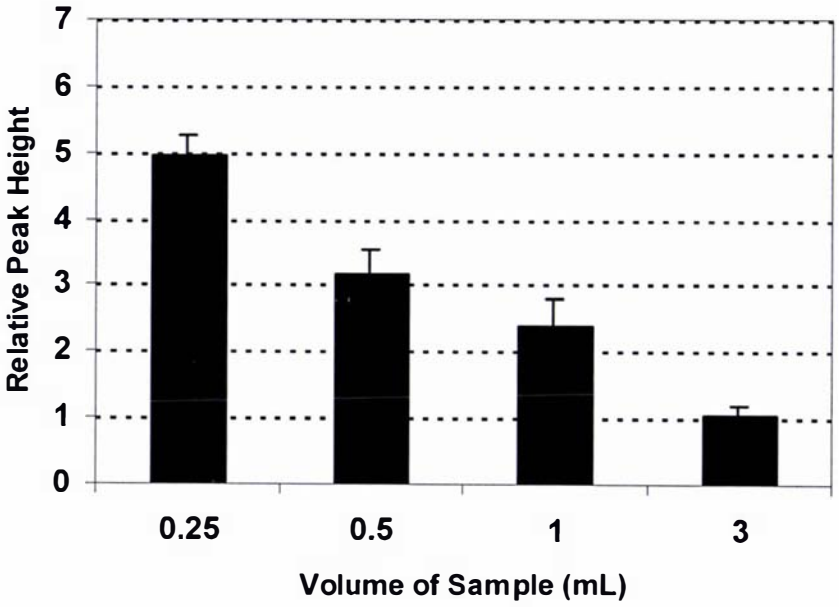

Figure 3.5. Relative peak heights in Hg-rich plant samples as a function of volume of sample added to the reaction vessel. Bars denote ± 1 standard deviation from the mean of three replicates. The peak height is relative to the Hg content in plant tissues (in mg/kg DW).

3.4 Conclusions

The hydride generation method has been proven to be accurate for the determination of Hg in an acid matrix of soil and plant samples with a limit of detection of 11.4 ng/mL and relative standard deviation of 4.6 %. Mercury recovered from Hg- spiked deionized water samples was greater than 88.5%. Mercury recovered from a tailing reference substrate after extraction with $(NH_4)_2S_2O_3$ validated this extraction procedure, and demonstrated the good performance of the analytical equipment for the determination of Hg in thiosulphate-amended solutions. The reproducibility of the hydride generation method for Hg analysis was confirmed by ICP-MS analysis. In the presence of oxidised plant tissues in the liquid matrix, however, analytical precision of the equipment decreased significantly as the volume of the sample added to the reaction vessel increased. In order to improve analytical precision of the equipment for Hg analysis in plant samples, we suggest that not more than 0.25 mL volume of this sample type should be added to the reaction vessel. Because Hg determination was done on plant samples with high Hg content, detection of Hg could be easily achieved at 0.25 mL of sample volume. However, for plant samples with low Hg content, the Hg signal could be within the limit of detection of the machine. Concentrating the Hg mass in the liquid matrix of plant sample digests (increasing the amount of plant tissue to be digested or reducing the dilution factor of the sample) may solve this problem.

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CHAPTER 4

IS VOLATILISATION A SIGNIFICANT PROCESS FOR MERCURY PHYTOREMEDIATION?

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Abstract

Mercury can be volatilised from planted substrates as a result of microbial and chemical transformations. This study evaluates the Hg volatilisation process for *B. juncea* plants grown in hydroponic solutions spiked with Hg at concentrations ranging from 0 to 10 mg/L. Volatile Hg vapours released from plant roots enclosed in a gastight root compartment were bubbled through a trapping system containing 5% $KMnO_4$ in 2N H_2SO_4. Volatile Hg released from shoots was estimated indirectly through mass balance calculations. Mercury recoveries using the trapping system averaged 81.5 % of the initial Hg mass added to controls. The volatile Hg mass released from roots increased significantly as the concentration of Hg in solution increased from 0.05 to 10 mg/L. The volatile Hg mass released from roots was significantly higher to controls for all tested concentrations. The Hg volatilisation process was correlated to the Hg mass accumulated in root tissues. There were no significant differences between the Hg mass values released from roots and shoots. The Hg mass released from the whole plant (shoots + roots) increased linearly from 50% to 80 % of the total Hg mass in the system, as the concentration in solution increased from 0.05 to 10 mg/L. The results from this study suggest that Hg losses from substrate may be plant-mediated and that rhizosphere microbes may contribute to the Hg volatilisation process.

4.1 Introduction

The last two decades have seen an increasing public awareness of the negative impacts of mercury (Hg) pollution. This public concern has focused political and scientific attention on the management of Hg–contaminated sites. Anthropogenic Hg contamination has spread mostly as a consequence of mercury's multiple use in industry (e.g., bleaching, pigment in paints, as a catalyst) and as an active component in agricultural pesticides. Other activities that also contribute to introduce Hg into the environment include coal burning power plants, urban waste disposal and mining operations (Nriagu, 1979). Once in the environment, Hg can be present in one of three oxidation states. The most reduced is elemental Hg^0, which is liquid at room temperature but can also volatilise to the atmosphere. The other two are ionic: the unstable mercurous ion (Hg^+) and, under oxidizing conditions especially at low pH, the stable mercuric ion (Hg^{2+}) (Siegel and Siegel, 1979). In addition to these three oxidation states, organic forms of Hg, such as methyl (CH_3Hg) and dimethyl ($[CH_3]_2Hg$) mercury, can be also found in soils as a consequence of biochemical transformations mediated by microbial activity, light and organic substances. These transformations will influence the retention and mobility of Hg in the soil environment. Additionally, all these processes will influence not only Hg distribution in both the soil material and the soil profile, but also its transference between neighbouring reservoirs and ecosystems, the transference process leading, ultimately, to Hg biomagnification in the ecological food chain (Andersson, 1979).

During the past decade there has been increasing interest in the possibility of using vegetation to remediate heavy-metal contaminated sites (phytoremediation). The use of plants represents a low-cost and environmentally friendly alternative to the traditional soil remedial procedures, which are quite often expensive and harsh to the environment (Anderson, 2000). Phytoremediation may play an important role for the decontamination of Hg-polluted mining sites in developing countries, where massive emissions of this element to the environment still occurs as a result of artisanal gold mining activities. Research demonstrated that addition of S-containing ligands (e.g.,ammonium thiosulphate) to Tui mine tailings mobilized Hg in substrates and

caused a substantial increase in the concentration of this element in shoots of Indian mustard (*Brassica juncea*) (Moreno et al., 2004 a[5]). However, mass balance calculations revealed that a significant fraction of Hg could not be accounted for by the final Hg mass in plant tissues, substrates and leachates. This unaccounted fraction suggested Hg was volatilised from the soil-plant system as elemental Hg. The objectives of this paper were (1) to investigate the effect of increasing Hg concentrations on accumulation and volatilisation of elemental Hg by roots and shoots of *B. juncea* and; (2) to evaluate the significance of the Hg volatilisation process for the phytoremediation of Hg-contaminated sites.

4.2 Material and Methods

4.2.1 Plant growth conditions

Seeds of *B. juncea* were germinated on silica-sand in a plant growth cabinet with temperature controlled to 22 ± 2 °C and photoperiod set to 14h. Seedlings were watered daily by adding 10 mL of ¼ strength Hoagland's solution. After one week, seedlings were gently washed to remove the sand and transferred to plastic pots containing 100 mL of ¼ strength Hoagland's solution (Hoagland and Arnon, 1950). The nutrient solution was kept aerated using a small pump and was replaced when the total volume was reduced by half. After a period of 12 days, the roots were washed with reverse osmosis (RO) water and immersed in 100 mL of deionized water containing Hg. The treatments comprised six different concentrations of Hg (0.05. 0.5, 1, 2.5, 5 and 10 mg/L) added as the soluble $HgCl_2$ salt.

4.2.2 Collection of volatile Hg

To collect elemental Hg from roots, the root system was isolated from the aerial portion by sealing the base of the stem with parafilm. The air circulating inside the root compartment was collected separately for each plant by bubbling it through a trapping system that consisted of a 10 mL vial containing 5% $KMnO_4$ in 2N H_2SO_4 (Kimura and

[5]This paper is included in this thesis as Chapter 7.

Miller, 1960) (Figure 4.1 and 4.2). Elemental Hg volatilisation from plant roots was collected for each one of the tested concentrations (3 replicates) over a period of 5 days. Assessment of the trapping system performance was monitored through collection of volatile Hg from controls (2 replicates) in a similar way. After collection of volatile Hg, the trap solution was transferred to 10 mL air-tight plastic vials and stored at 4°C until analysis. The precipitated fraction of the acid trap was redissolved with 10 mL of concentrated hydrochloric acid and the resulted solution was stored following the same procedure. The mass of volatile Hg collected for each replicate was, therefore, the sum of the Hg readings in the soluble and precipitated fractions of the acid trap.

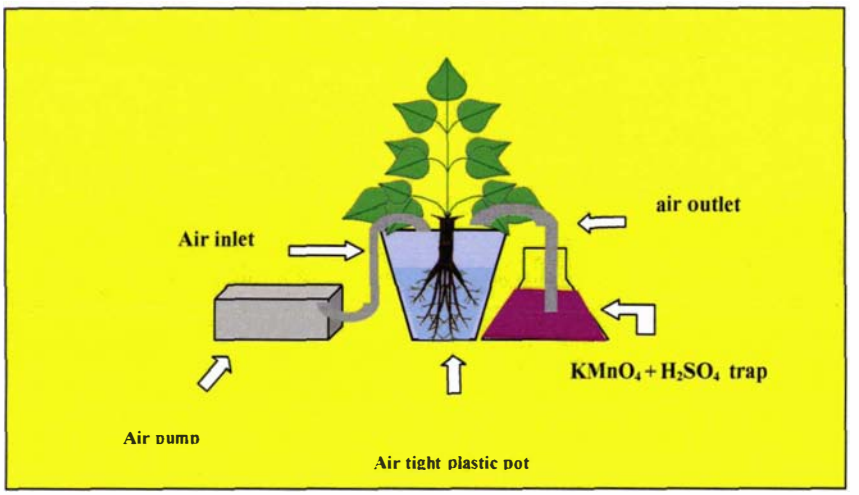

Figure 4.1. Experimental apparatus used for trapping volatile Hg released from *B. juncea* roots.

Figure 4.2. The experimental apparatus inside the temperature-controlled chamber.

4.2.3 Plant analysis

After the Hg collection period, shoots and roots were harvested and washed in tap water. Roots were separated from shoots and placed in a drying oven at 70 °C until a constant weight was obtained. Ground subsamples (0.1 g) were accurately weighed into 50 mL plastic beakers and digested with 15 mL of HNO_3. The plant samples were left overnight and, in the following day, were heated in a water bath at 80°C for 1 hour. Plant digests were transferred to 10 mL polythene tubes and diluted with reverse osmosis water to make a final volume of 10 mL.

4.2.4 Mercury analysis

Solution Hg concentrations were determined using hydride-generation atomic absorption spectroscopy. Sodium borohydride was used as a reducing agent to generate Hg vapour in a 5% $NaBH_4$ + 1% potassium hydroxide (KOH) w/v solution. The limit of detection (LOD) for mercury in solution was 11.4 ng/mL. Reproducibility, as determined through replicate analysis (n=10) of a standard solution containing 1 mg/L of Hg, was less than 5%. Reagent blanks were below detection limits in the solution. Linear calibration curves were obtained over the range of 125 to 1000 ng/mL of Hg using 4 standards prepared from a 10 mg/L mercuric nitrate ($HgNO_3$) spectrosol solution (May & Baker, AAS standard reagent solution). The analytical method was assessed for quality control by an external certified laboratory with agreements ranging from 85 to 103% for Hg-containing solutions and Hg-containing plant samples (Moreno et al. 2004 b[6]).

4.2.5 Mass balance calculations

Hg mass volatilised from shoots ($Hg_{volatile}$) was estimated indirectly according to the following equation:

$$Hg_{volatile} = Hg_{initial} - (Hg_{plant} + Hg_{solution} + Hg_{trap}) \quad [1]$$

[6]This paper is described in the previous Chapter.

Where Hg_{plant} is the total Hg mass accumulated in plant tissues (shoot + roots), $Hg_{initial}$ is the total Hg mass at the beginning of the experiment, $Hg_{solution}$ is the final Hg mass in solution and Hg_{trap} is the total Hg mass in the $KMnO_4 + H_2SO_4$ traps.

4.3 Results

Volatile Hg was successfully captured by the trap system. An average of 81.5 ± 5 % of the initial added Hg mass was recovered from controls. Figure 4.3 shows the total Hg mass volatilised from the root system at the end of the experiment. On average, the volatile Hg mass increased from 2.3 (± 0.9) μg to 314 (± 178) μg as the concentration of Hg in solution increased from 0.05 to 10 mg/L (r^2 =0.98, p<0.0001). It is important to mention that these means have 39 and 57 % of variation, respectively. The Hg mass volatilised from roots was significantly greater (p <0.05) than from controls for all tested concentrations (Figure 4.3).

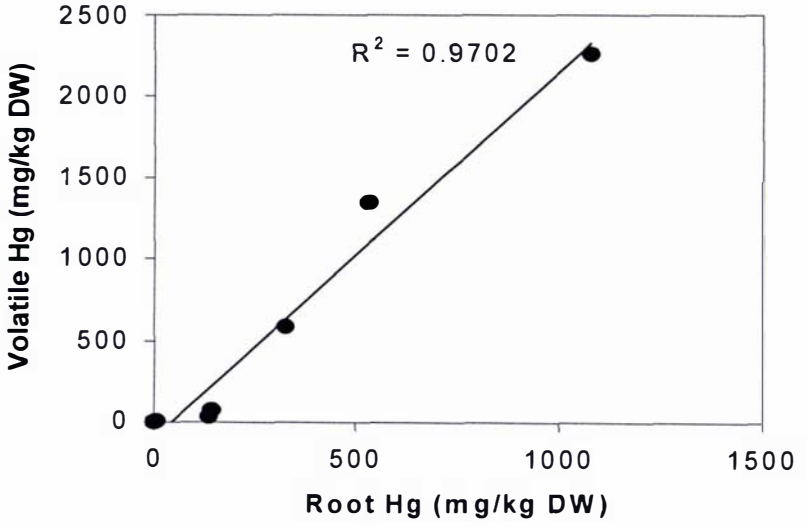

Figure 4.3. Hg mass (μg) volatilised from roots of hydroponically grown *B. juncea*. Bars denote ± 1 standard deviation from the mean of 3 replicates.

Figure 4.4 demonstrates that the root volatilisation process (expressed as the mass of Hg released per kilogram of dry weight tissue) was significantly and positively correlated to root Hg concentration (r^2=0.97, p<0.0001).

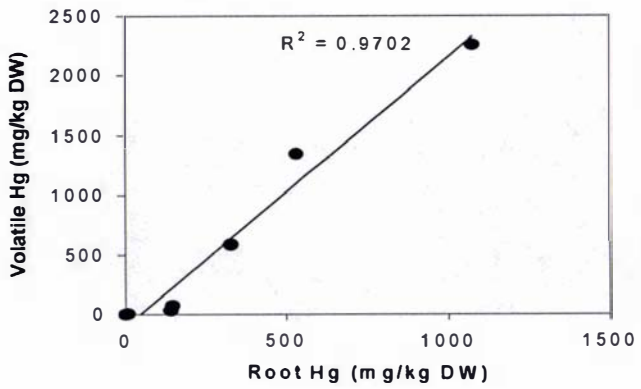

Figure 4.4. Relationship between Hg volatilisation (mg of Hg per kg of dry weight roots) and root Hg accumulation (mg/kg dry weight) by *B. juncea* plants.

The mass of volatilised Hg was calculated using Equation 1. The volatilisation results were expressed as the mass of Hg released per kg of dry weight tissue (Figure 4.5). The mass of Hg volatilised from roots was not significantly different to shoots ($p > 0.05$) for all tested concentrations, except at 2.5 mg/L ($p > 0.05$). This difference is related to a high content of volatile Hg mass collected at this concentration. Since volatile Hg emitted from shoots is calculated indirectely, the Hg mass volatilised from shoots will be a function of the total Hg content in the plant, traps and solution.

Figure 4.6 is a 100 % normalized picture of the mass balance for plant, solutions and volatilised Hg as a function of the added Hg treatments. Hg released from the whole plant (shoots + roots) increased linearly (r^2= 0.88; $p < 0.0001$) from around 50% to 80 % as the Hg concentrations in solution increased from 0.05 to 10 mg/L. Since physiological and nutritional responses of plants are affected by microbes living in association with plant roots (DeSouza et al., 1999), then it is possible that rhizosphere microorganisms also influenced Hg volatilisation from *B. juncea* plants. The fact that the mass of volatile Hg was significantly correlated to the mass of Hg accumulated in roots support this hypothesis.

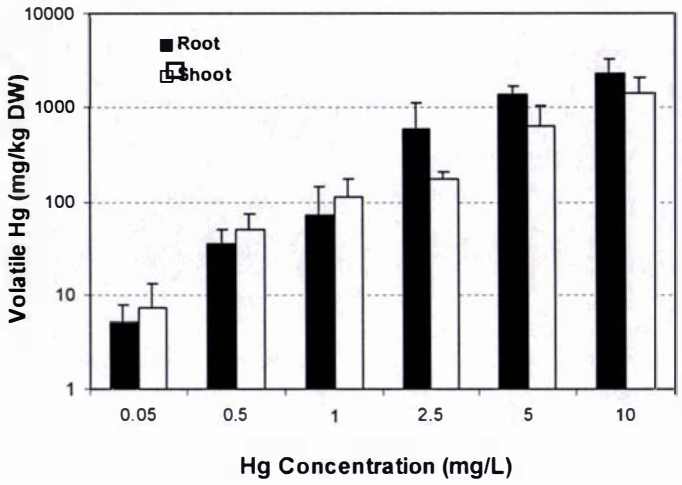

Figure 4.5. Comparison between Hg volatilisation from shoots (estimated) and roots (mg/kg dry weight). Bars denote ± 1 standard deviation from the mean of 3 replicates.

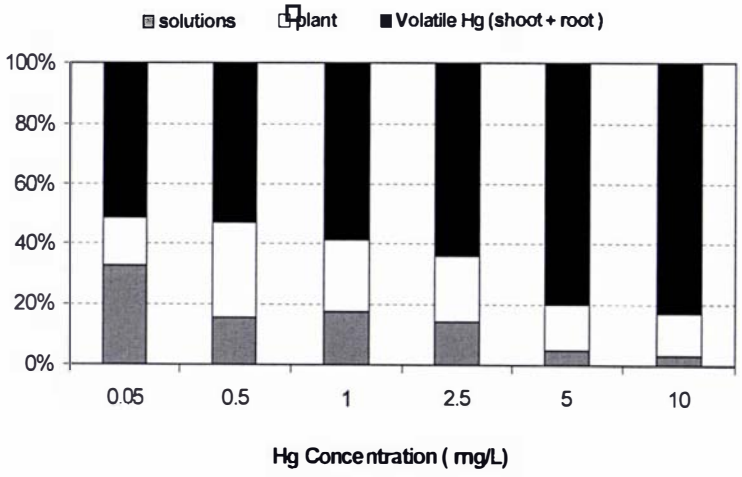

Figure 4.6. Normalized (100%) mass values for Hg accumulated in the plant, remained in solution and volatilised to the air for *B. juncea* grown in nutrient solution and treated with soluble Hg ($HgCl_2$). Note that 100% = initial Hg mass.

4.4 Discussion

The volatilisation of Hg (0) by plant leaves has been the subject of many studies that have attempted to investigate the behaviour of Hg at the soil-plant-atmosphere interface (Siegel et al., 1974; Siegel and Siegel, 1979; Leonard et al., 1998). Given that there is enough evidence to conclude that plants release Hg to the air, an important question becomes: Why use plants to remediate Hg-polluted sites if this is simply a process for the transference of Hg between the soil and air compartments?

To answer this question we must consider the form of Hg emitted to the atmosphere by *B. juncea* plants. The chemical solution we used for trapping Hg was design to capture elemental Hg (0) by oxidizing it to Hg (II), according to the following equation:

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

Therefore, it can be assumed that volatile Hg released by shoots and roots of tested plants was the inorganic vapour Hg (0).

According to the United States EPA, volatile forms of organic Hg that enter the atmosphere are removed or washed from that compartment at much faster rates than elemental Hg. This happens because organic Hg species like phenylmercury chloride or methylmercury chloride have a half-life in the atmosphere of a few weeks. Therefore, when these Hg species are made airborne, they rapidly return to Earth by dry deposition or are washed from the atmosphere to the surface by rainfall. In contrast, Hg (0) has a half-life in the atmosphere in the order of years. Consequently, vapours of Hg (0) released by plants to the atmosphere would have a tendency to remain airborne, becoming diluted within a large atmospheric pool dispersed over the Earth's surface (Meagher et al., 2000).

4.5 Conclusions

This study has demonstrated a system for trapping volatile Hg released from the roots of *B. juncea* plants grown in Hg-spiked solutions. The Hg mass released from roots increased significantly as the Hg concentration in solution rose from 0.05 to 10 mg/L. The Hg volatilisation process was significantly correlated to the mass of Hg accumulated in root tissues. Mass balance calculations indicated that volatile Hg was also released from shoot tissues. The amount of Hg volatilised from whole-plant tissues increased proportionally to the concentration of Hg in the hydroponic solutions. Rhizosphere microbes may have participated in the volatilisation process by transforming soluble Hg (II) to volatile Hg (0). Plants significantly enhanced volatile Hg mass emissions relative to controls. Therefore, any plant remediation strategy for the decontamination of Hg-polluted substrates should consider Hg losses from the soil-plant system by volatilisation.

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CHAPTER 5
MEASURING VOLATILE MERCURY RELEASED FROM PLANTS

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Abstract

During mercury (Hg) phytoremediation, Hg vapour losses may occur from the plant-soil system. These losses are due to Hg volatilisation from shoots and roots of plants. In this work we describe a method for quantifying Hg vapours released from Indian mustard (*Brassica juncea*) plants grown in Hg-containing hydroponic solution. Shoot and root Hg volatilisation was measured using a two-trap system in series. The experimental unit consisted of two small air pumps, one gastight acrylic volatisation chamber (3.6 L) and a set of four Hg traps. Mercury vapour released from shoots of a plant contained within the chamber were sequentially passed through organic and inorganic traps by continuous airflow (1 L/min). Inorganic Hg vapour was trapped using a solution containing 5% $KMnO_4$ dissolved in 2N H_2SO_4. Organic Hg vapour was trapped using a solution containing 5% Na_2CO_3 and 2.5% Na_2HPO_4. Root Hg volatilisation was measured using a similar set up where the incoming air (1 L/min) was bubbled into a gastight root compartment contained within the volatilisation chamber. Inorganic Hg volatilised from roots and shoots was successfully captured by the trapping system. Organic Hg vapours were below detection levels (11.4 ng/mL). Average Hg recovery for the whole plant system (traps + tissues + solutions) was close to 90%. Volatilisation of Hg from roots was higher than volatilisation from shoots. Mass balance results indicated that around 40 % of the total Hg added to the system was volatilised by plants; while Hg retention in plant tissues accounted for 50% of the total Hg mass in the system. Our research suggests that rhizobacteria also participate in the plant Hg volatilisation process.

5.1 Introduction

Anthropogenic sources of Hg pollution are related to the widespread use of mercury throughout history. Industrial (bleaching, paper, textiles), military (ballast in submarines, coolant in reactors), mining (in amalgams for the extraction of gold) and agricultural (pesticides) activities have contaminated large areas of soil, sediment and water around the world with Hg (Meagher et al, 2000; Hinton et al., 2002). Large amounts of Hg vapour are released into the atmosphere as a result of the burning of coal and fuel oil and the incineration of medical and domestic solid waste (Nriagu, 1979).

Mercury can be transformed to many inorganic and organic compounds by way of biogeochemical processes mediated by plants, microorganisms, light and organic substances. In particular, the conversion of divalent Hg to organic Hg (e.g. methylmercury) by abiotic and biotic pathways is an issue of environmental concern. Methylmercury is readily available to plants and animals and can biomagnify in the ecological food chain. Consequently, most of the Hg present in humans is in the form of methylmercury, and almost all methylmercury originates from fish in the food supply (Morel et al., 1998; Brabo et al., 2000).

Plant Hg tolerance mechanisms involve controlling metal levels inside tissues through the synthesis of phytochelatins and metallothioneins (Punz and Sieghard, 1993; Goldsbrough, 2000). These substances are small, cysteine-rich peptides capable of binding to heavy metal ions and are assumed to be involved in the accumulation, detoxification and metabolism of metal ions such as Cd^{+2}, Zn^{+2}, Pb^{+2}, Ag^{+2} and Hg^{+2} (Grill et al., 1987; Gupta et al., 1998; Goldsbrough, 2000). Prokaryotes, which comprise the various types of bacteria, have developed other metal tolerance strategies to heavy metal exposure. Among these strategies are included the ability to reduce, oxidize, or transform metal ions to less toxic metal species. For instance, direct enzymatic oxidation or reduction reactions by bacteria significantly alter the toxicity of a wide range of ions, such as Cr, Fe, Mn, Hg, NO_3-N, Se, and U (Lovley, 1994). For bacteria living in Hg-contaminated soil, enzymatic reduction of Hg(II) to Hg(0) has been shown to be an effective solution to Hg exposure, as elemental Hg is the least toxic and most volatile form of Hg (Meagher et al., 2000). Volatilisation of Hg from the

soil-plant environment, therefore, cannot be considered a process that occurs as a result of the metabolic transformations inside plant tissues. Scientific literature suggests a role of rhizosphere bacteria in the promotion of plant uptake and volatilisation of heavy metals including Zn, Se and Hg (DeSouza et al., 1999; Whiting et al., 2001). Particularly for Se, research has shown that rhizosphere bacteria and bacteria inside the roots (endophytic bacteria) may contribute partially or wholly to Se volatilisation by plants (Zayed and Terry, 1993).

Our current research is focused on the use of plants to extract, immobilise and/or volatilise Hg from contaminated soils. Phytoremediation of Hg could be an attractive remedial technology for the developing world, where many countries cannot afford the elevated costs associated with traditional soil decontamination technologies. Early studies have indicated that *B. juncea* plants grown in Hg-contaminated mine tailings will volatilise a substantial fraction of the total Hg mass present in the soil-plant system (Moreno et al., 2004 a[7]). In this paper, we demonstrate a method for quantifying both organic and inorganic Hg vapours released from the aerial and root portions of *Brassica juncea* plants immersed in Hg-containing hydroponic solution. We also perform a comparative study of the role of root and shoot tissues in the Hg volatilisation process and their contribution for the Hg mass balance in the plant system.

5.2 Material and Methods

5.2.1. Plant growth conditions

Seeds of *B. juncea* were germinated on silica-sand in a controlled environment cabinet with temperature and photoperiod set to 22 ± 2°C and 14h, respectively. Seedlings were watered every second day with 10 mL of ¼ strength Hoagland's solution (Hoagland and Arnon, 1950). After one week, seedlings were gently washed to remove sand and transferred to plastic pots containing 100 mL of ¼ strength Hoagland's nutrient solution. A small pump set at a flow rate of 1 L/min oxygenated the nutrient solution. The nutrient solution was replaced when the total volume was reduced by half. After a period of 12 days the plants were removed, washed with reverse osmosis (RO) water

[7] This paper is included in this thesis as Chapter 7.

and immersed in 100 mL of deionised water containing Hg at 1 mg/L added as the soluble $HgCl_2$ salt.

5.2.2 Collection of volatile Hg

A two-trap system in series was designed to capture inorganic and organic Hg vapours released from shoots and roots of *B. juncea* plants. The experimental unit consisted of two small air pumps, one gastight acrylic volatisation chamber (3.6 L) and a set of four Hg traps (Figure 5.1 and 5.2).

Hg vapours released from the shoots of each plant were contained within a volatilisation chamber. The vapour was then sequentially passed through inorganic and organic traps by continuous airflow (1 L/min). Inorganic Hg vapour (elemental Hg) was trapped in a 70 mL solution containing 5% $KMnO_4$ dissolved in 2N H_2SO_4 (T1) Organic Hg vapour was trapped in a 70 mL solution containing 5% Na_2CO_3 and 2.5% Na_2HPO_4 (T2) The efficiency of these trap solutions for the quantitative capture of metallic and organic Hg vapours was shown to vary between 95 to 99% (Kimura and Miller, 1960). Both trap solutions were contained within 125 mL Erlenmeyer flasks. The outlet of the inorganic trap was open to the atmosphere to maintain pressure equilibrium within the trap system.

Volatilisation from the root compartment was measured using a similar set up where the root system was isolated from the aerial portion in a gas tight root vessel (100 mL volume). Air was bubbled into the root compartment using continuous airflow (1 L/min). Outlet tubes carried air from the shoot and root compartments into the Hg traps. Hg volatilisation was measured in triplicate for both shoot and root compartments over a period of three days. Assessment of the trapping system performance was monitored through triplicate collection of volatile Hg from controls (experimental set up with no plants) in a similar way. After collection of volatile Hg, both T1 and T2 trap solutions were transferred to 100 mL air-tight plastic containers and stored at 4°C until analysis. The precipitated fraction of the acid trap (T1) was redissolved with 70 mL of concentrated hydrochloric acid and the resulted solution was stored following the same procedure. The mass of inorganic Hg vapours collected for each replicate was,

therefore, the sum of the Hg readings in the soluble and precipitated fractions of each acid trap (T1).

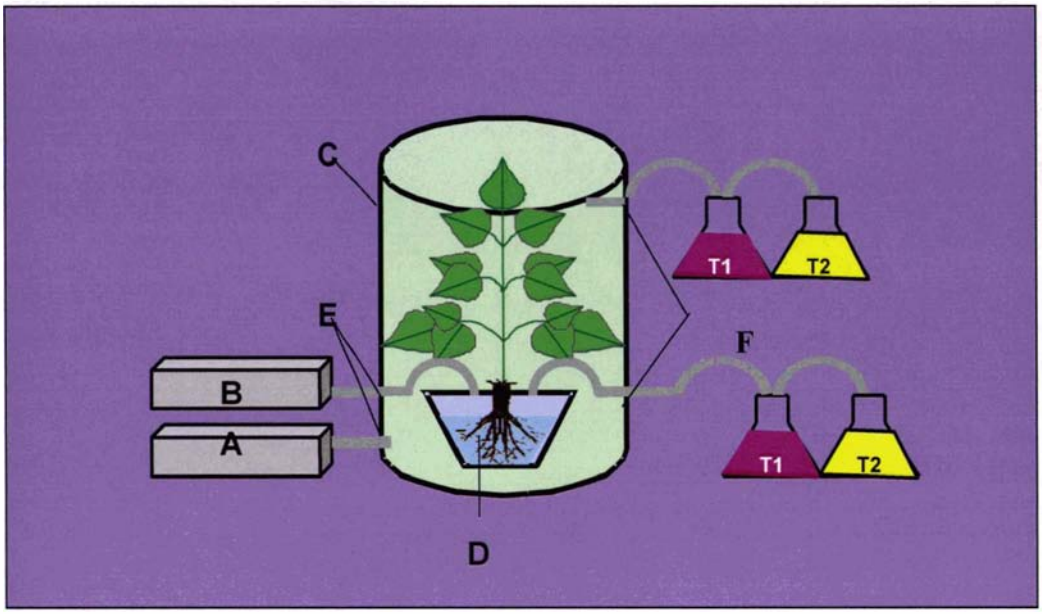

Figure 5.1. Experimental unit used for trapping Hg from roots and shoots of *B. juncea*. A and B, air pumps; C, gas tight plant chamber; D, gas tight root vessel; E, air inlets; F, air outlets; T1, inorganic Hg vapour trap; T2, organic Hg vapour trap.

Figure 5.2. View of the experimental apparatus inside the controlled environment cabinet.

5.2.3 Plant analysis

After the Hg collection period, shoots and roots were harvested and washed in tap water. Roots were separated from shoots and placed in a drying oven at 70°C until a constant weight was obtained. Ground subsamples (0.1 g) were accurately weighed into 50 mL plastic beakers and digested with 15 mL of HNO_3. The plant samples were left overnight and, in the following day, were heated in a water bath at 80°C for 1 hour. Plant digests were transferred to 10 mL polythene tubes and diluted with reverse osmosis water to make a final volume of 10 mL.

5.2.4 Mercury analysis

Solution Hg concentrations were determined using hydride-generation atomic absorption spectroscopy. Sodium borohydride was used as a reducing agent to generate Hg vapour in a 5% $NaBH_4$ + 1% potassium hydroxide (KOH) w/v solution. The limit of detection (LOD) for mercury in solution was 11.4 ng/mL. Reproducibility, as determined through replicate analysis (n=10) of a standard solution containing 1 mg/L of Hg, was less than 5%. Reagent blanks were below detection limits in the solution. Linear calibration curves were obtained over the range of 125 to 1000 ng/mL of Hg using 4 standards prepared from a 10 mg/L mercuric nitrate ($HgNO_3$) spectrosol solution (May & Baker, AAS standard reagent solution). The analytical method was assessed for quality control by an external certified laboratory with agreements ranging from 85 to 103% for Hg-containing solutions and Hg-containing plant samples (Moreno et al. 2004 b[8]).

5.3 Results and Discussion

The permanganate acid trap successfully captured inorganic Hg vapours released from shoots and roots of *B. juncea* plants. Organic Hg vapours, however, were not detected in the carbonate-phosphate traps (Hg values < 11.4 ng/mL). Efficiency for Hg recovery using the experimental apparatus was calculated at an average value of 89.8 ± 3.8 % of the initial Hg mass added to the plant-solution system (traps + plant tissues +

[8]Results from this paper were described in Chapter 3.

solutions). Since the quantitative capture of Hg in the permanganate acid solution involves oxidation of elemental Hg (0) to ionic Hg (II) (Moreno et al., 2003[9]), then we would assume that the Hg form released from *B. juncea* plants was the elemental vapour Hg (0).

Figure 5.3 shows the total Hg mass (μg) recovered from the plant and control traps at the end of the experiment. On average, the mass of Hg recovered in the plant traps significantly exceeded the Hg mass detected in control traps ($p < 0.001$).

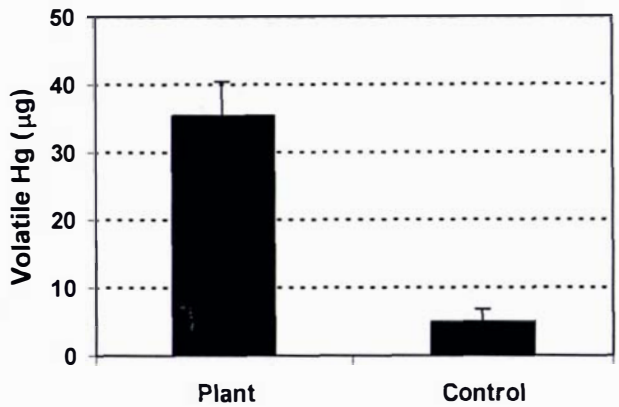

Figure 5.3. Total Hg mass (μg) volatilised from *B. juncea* plants and controls (no plants). Bars denote ± 1 standard deviation from the mean of 3 replicates.

The fact that a small proportion of the total Hg mass added in the controls (around 5%) was captured in the control traps indicates that Hg from the hydroponic media contaminated the permanganate trap solution. This Hg fraction may have reached the trap solution in the form of soluble $HgCl_2$ spilled out from the root compartment as an aerosol during oxygenation of hydroponic solution by the air pumping system.

Figure 5.4 (A and B) shows the total Hg volatile mass emitted from shoots and root of *B. juncea* at the end of the experiment. The Hg mass volatilised from roots was significantly greater than the Hg mass volatilised from shoots ($p < 0.05$) (Figure 5.4 A). This difference is amplified when the Hg mass values are expressed per kg of dry

[9] See chemical equation described in Chapter 4.

weight tissue ($p < 0.001$) (Figure 5.4 B). Since *B. juncea* roots constitute a smaller proportion of the total plant dry mass (38 %, on average), the amount of volatile Hg released from roots exceeded the amount of volatile Hg released from shoots by a factor of 60.

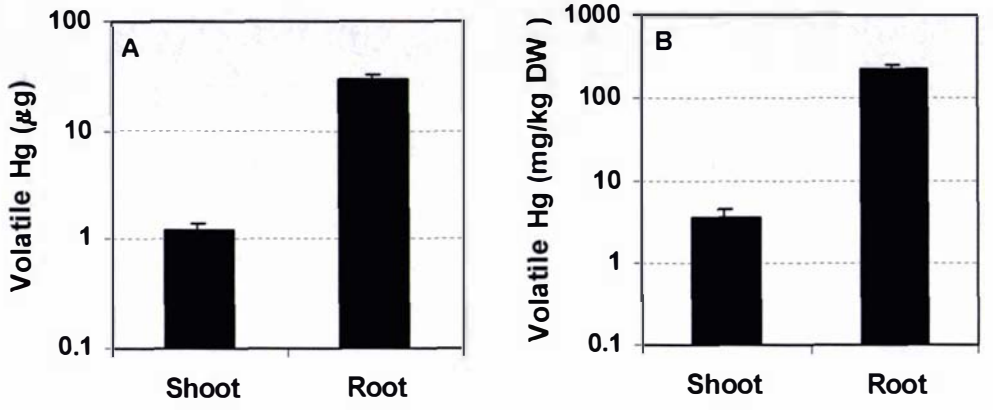

Figure 5.4. Volatilisation of Hg by shoots and roots of *B. juncea* plants. Values are expressed on (A) mass (µg) and (B) concentration (mg/kg dry weight). Bars denote ± 1 standard deviation from the mean of 3 replicates.

Since most of the Hg in our study was volatilised from the root system of *B. juncea*, then is it appropriate to consider the root tissues as the main site for Hg volatilisation from the plant. It is plausible to think that part of the Hg volatilised by the plant was the result of enzymatic transformation of Hg (II) to elemental Hg (0) by bacteria living at the root zone or inside root tissues. The plant volatilisation process, in this context, can be considered a beneficial result of the plant-rhizobacteria interaction in order to confer plant Hg tolerance in metal contaminated environments. Further soil enzymatic assays and soil microorganisms extraction and isolation experiments are required to support this hypothesis.

Figure 5.5 describes the Hg mass balance for the air, plant and solution compartments at time of analysis. The values are expressed as a percentage of the total Hg mass added at the beginning of the experiment and thus, 100% denotes 100 µg of Hg(II) added to 100 mL of hydroponic solution (1 mg/L). At the end of the experiment, only 10 % of the total Hg mass added to the system remained in solution. The bulk of the Hg mass

added was either volatilised from the root compartment (around 40 %) or retained in plant tissues (around 50%, mostly bound to root tissues). The chemistry of Hg in oxic waters suggest that at pH around 6 most of the Hg(II) in solution will be complexed to hydroxide ions (Morel et al., 1998). The uncharged complex $Hg(OH)_2$ is slightly hydrophobic and thus, will diffuse very slowly through the lipidic bilayers of cellular membranes (Morel et al., 1998). It is, therefore, possible that increased retention of Hg in root tissues was caused by the adsorption of $Hg(OH)_2$ complexes to the cell wall of *B. juncea* roots.

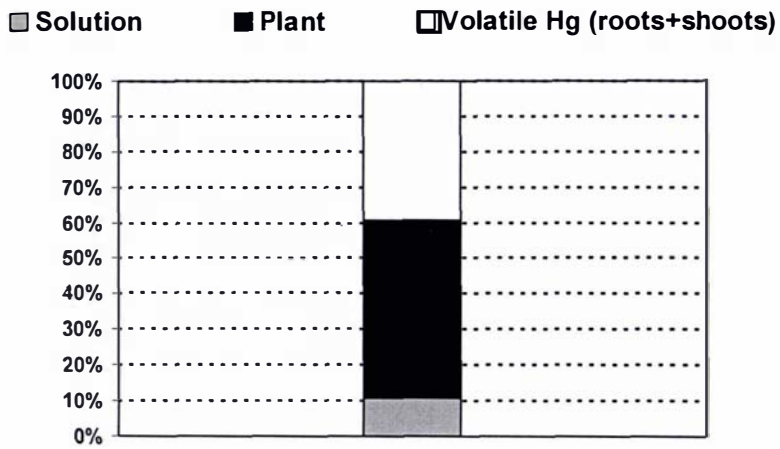

Figure 5.5. Mass balance for the Hg distribution between the air, plant and solution compartments. Note that 100 % denotes total Hg mass added at the beginning of the experiment (100 μg of Hg in 100 mL of hydroponic solution).

Transgenic Tobacco plants have been reported to volatilise 75 % of the Hg (0) from a 1 L hydroponic media initially containing 1000 μg Hg over a one-week period (Meagher et al., 2000). All of the remained 25 % Hg was still bound to roots. Conversely, we have shown non-transgenic *B. juncea* plants volatilised ca. 40% of the Hg(0) from a 100 mL solution containing 100 μg Hg after 3 days. The remaining 60% Hg was retained within the plant-solution system, most of it bound to root tissues. The main difference between transgenic and non-transgenic plants in this regard is related to the efficiency at which Hg is transferred from the plant to the atmosphere compartment. Upon extrapolation to mass balance terms, this would mean that transgenic plants held only 25 % of total added Hg mass inside the roots and thus, they are more efficient than

non-transgenic plants in the plant-assisted Hg-volatilisation process (phytovolatilisation).

5.5 Conclusions

In this study we have demonstrated a trapping system to capture volatile Hg emitted from the roots and shoots of *B. juncea*. The efficiency of the system was around 90% for Hg recovery in the permanganate (inorganic) traps, in plant tissues and solutions. Detection of Hg in the permanganate acid traps indicates the predominant Hg form emitted by *B. juncea* plants is the metallic elemental Hg (0). The fact that roots volatilised 60 times more Hg than shoots and that almost all of the plant Hg fraction was retained within the root zone suggests the participation of rhizosphere bacteria in the volatilisation process. Finally, our results demonstrate that Hg can be removed from polluted sites through plant-assisted volatilisation of elemental Hg vapour into the atmosphere. Subsequent studies will investigate the effect of chelating agents (e.g. ammonium thiosulphate) on the Hg-phytovolatilisation process from *B. juncea* plants growing in Hg-contaminated substrates.

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SECTION B

THE PHYTOEXTRACTION AND PHYTOVOLATILISATION OF MERCURY FROM CONTAMINATED MINE WASTES

This section comprises Chapters 6, 7, 8 and 9. Chapter 6 describes thioligand-induced plant-Hg accumulation for *Brassica juncea*, *Phaseolus vulgaris* and *Vicia villosa* grown in mine tailings collected from the Gold Mountain mine (Northern China). Since Hg retention in soils is affected by the presence of humic acid, experiments described in this chapter attempted to examine the effect of this substance on the Hg root uptake and shoot translocation for thioligand-treated *B. juncea* plants. The effect of substrate Hg concentration on thiosulphate-induced Hg uptake and transport was also tested for this plant species. Chapter 6 presents novel findings and provides strong argument for the influence of Hg complex speciation on Hg root retention and shoot transport.

Chapter 7 presents an account of the effect of soluble Hg species and thioligands on the plant-Hg accumulation for hyperaccumulator and non-accumulator plant species. *Berkheya coddii, Atriplex canescens, B. juncea* and *Lupinus* sp. were grown in Hg-contaminated tailings collected from the Tui mine in the North island of New Zealand. Soluble Hg (added in the form of $HgCl_2$) was applied at 0 to 10 mg Hg /kg for pots containing the first two species. Sulphur-containing ligands were applied at 2 mg/kg for the pots containing the last two species. These experiments were important for subsequent studies on phytovolatilisation because they provide an indirect account of the Hg losses from planted substrates.

Chapter 8 portrays a detailed study of thioligand-induced phytoextraction and phytovolatilisation for the Tui mine tailings. *Brassica juncea* was selected for this study because of the high shoot Hg values exhibited by this species in previous studies (Chapters 5 and 6). Pot experiments investigated the effect of thiosulphate on plant-Hg accumulation and volatilisation. An experiment conducted at the Tui mine site examined the thioligand-induced Hg-phytoextraction for *B. juncea* plants grown in the field. The potential of Hg phytoextraction was evaluated in this chapter taking into account 1) the *on site* biomass production on a per hectare basis, and 2) the Hg values accumulated into shoot tissues following application of thiosulphate solutions. The experiment carried out at the Tui mine site was a pioneering demonstration of

thioligand-induced Hg-phytoextraction under field-scale conditions. This research presents an important step towards and evaluation of the contribution of plant accumulation in the Hg-phytoremediation process.

Chapter 9 describes the potential of phytoremediation for mitigating Hg-pollution at artisanal mine sites. Induced plant-Hg uptake and volatilisation experiments were carried out in substrate samples collected from mining areas located in Brazil and China. The methodological approach used in this study was similar to that described in the previous chapter. Gold phytoextration and Hg phytoremediation are presented as a potential solution for the environmental restoration of artisanal mine sites in developing countries.

The efficiency of thioligand-induced phytoextraction and phytovolatilisation for substrate Hg removal are thoroughly compared in Chapters 8 and 9. These two chapters provide an environmental assessment of the global, regional and local impacts of volatile Hg emissions from a local Hg phytovolatilisation operation.

CHAPTER 6

INDUCED PLANT UPTAKE AND TRANSPORT OF MERCURY IN THE PRESENCE OF SULPHUR-CONTAINING LIGANDS AND HUMIC ACID

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Abstract

The induced accumulation of mercury (Hg) by plants was investigated for the species *Phaseolus vulgaris* (Bush bean), *Brassica juncea* (Indian mustard), and *Vicia villosa* (Hairy vetch). All plants were grown in modified Hg-contaminated mine tailings and were treated with sulphur-containing ligands to induce Hg accumulation. The effects of varied substrate Hg concentration and humic acid (HA) level on the induced plant-Hg accumulation for *B. juncea* were examined. Thiosulphate salts (ammonium and sodium) mobilised Hg in the substrates and caused an increase in the Hg concentration of roots and shoots of all tested plant species. Root Hg accumulation was positively correlated to extractable Hg for $(NH_4)_2S_2O_3$-treated *B. juncea* plants grown in HA-amended substrates. However, shoot Hg translocation for this species was inhibited at 1.25 g of HA per kg of substrate. The results suggest that the plant-Hg accumulation process in the presence of $(NH_4)_2S_2O_3$ and HA is dependent upon plant root characteristics (e.g. root surface area) and Hg complex speciation (e.g. Hg-HA and Hg-thiosulphate complexes). Inhibition of shoot Hg transport in the presence of HA indicates that Hg phytoextraction may be inefficient for Hg-polluted soils with high organic matter content.

6.1 Introduction

Mercury (Hg) is one of the most toxic metals to living organisms. In the case of human Hg poisoning, the critically affected organ is the brain, with symptoms that vary from minor learning disabilities to extremely diminished mental capacity (Suzuki, 1979).

Despite this toxicity, Hg is extensively used in developing countries for small-scale gold (Au) mining (Veiga & Hinton, 2002).

Small-scale miners extract Au from ore through the Hg amalgamation method, which uses elemental Hg at an Au/Hg ratio of 1/70. A lack of technical knowledge, combined with lax regulations in developing countries leads to widespread Hg contamination, mostly through the release of Hg(0) to the atmosphe. A substantial fraction of Hg also contaminates water and soils after the discharge of amalgamation tailings (Veiga & Hinton, 2002). Heavy-mineral rich tailings, containing up to 200 mg/kg of elemental Hg, are often left exposed to the environment, where they are prone to weathering (e.g. leaching, erosion, and volatilisation) (Melamed & Villas Bôas, 1998; Roulet et al., 2000; Moreno et al., 2004 a) as well as biochemical transformations (methylation and biological reduction) (Morel at al., 1998).

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

Sulphur-containing solutions have been used to induce Hg accumulation into above ground tissues of high-biomass plant species (Moreno et al., 2004 a[10]). For instance, *B. juncea* was able to concentrate Hg to a level of 40 mg/kg in the shoot tissues, following application of ammonium thiosulphate ($[NH_4]_2S_2O_3$) to mine tailings contaminated with Hg at 2.8 mg/kg. Thioligand-induced plant-Hg accumulation has been, therefore, proposed as a potential strategy for the removal of Hg from contaminated substrates. Despite the potential environmental impact of chelate-enhanced phytoextraction (e.g. leaching of heavy metals to groundwater) (Lombi et al., 2001), complexing agents can be a useful tool for studying the uptake and transport of metals that have limited plant availability in soils. In the current study we investigate the effect of sulphur-containing ligands on the Hg availability and uptake for three plant species grown in Hg-contaminated mine tailings. We also examine the effects of substrate Hg concentrations and humic acid levels on the root-to-shoot transport of Hg for thiosulphate-treated *B. juncea* plants.

6.2 Material and Methods

6.2.1 Site description

The Hg-contaminated mine tailings used in this study were collected from the processing centre of the Gold Mountain mine, North-Central China. The exact location of the mine has been omitted so as to protect the local miner's community (A.J.Gunson, personal communication). The Gold Mountain mine is a small-scale mine that extracts gold (Au) from ore using the amalgamation method. An aqueous slurry of Au ore is ground with elemental Hg in locally manufactured mills. Gold liberated during rotation of iron wheels contacts Hg and forms an amalgam that may contain a 1:1 Au/Hg ratio. The Gold Mountain mine processes about 10 to 15 tonnes of ore daily and is responsible for annual emission of an estimated 70 tonnes of Hg into the regional environment (Gunson and Veiga, 2004). Mineralogical analysis (ACME Labs, Vancouver, BC, Canada) indicated the solid fraction of tailings to contain the following metals: Hg (100 mg/kg), Au (0.2 mg/kg), Cu (10,000 mg/kg), Ni (88 mg/kg), Fe (17 % w/w), As (14 mg/kg), Sb (63 mg/kg), and Te (4 mg/kg).

[10] This paper is described in Chapter 7 of this thesis.

6.2.2 Substrate preparation

In order to reduce background variability and maximise Hg uptake, all plant experiments were carried out in modified mine tailings. The modified substrate was prepared through dilution of the original Gold Mountain mine tailings (100 mg Hg/kg) with a 1:1 mixture of coarse and fine silica sand (fine fraction < 1000 microns) to give final Hg concentrations of 0, 1.25, 2.5 and 5 mg/kg. The substrate with Hg at 2.5 mg/kg was amended with commercially available humic acid (Aldrich, USA). The HA powder was mixed with the substrate to give (w/w) HA concentrations of 0, 0.125 and 1.25 g/kg of substrate. All substrates were supplemented with Osmocote (slow release NPK fertiliser) at 5 g/kg and left to equilibrate for one week prior to the initiation of the growth experiment. No lime was added, as the pH of substrate (around 8) was suitable for plant growth. A separate batch of substrate samples was sealed in plastic bags and shipped to New Zealand for Hg analysis.

6.2.3 Extractable Hg

Extractable Hg concentrations were determined for diluted and original tailings substrates. The extractants investigated were ammonium thiosulphate ($[NH_4]_2S_2O_3$), sodium thiosulphate ($Na_2S_2O_3$), and ammonium thiocyanate supplemented with hydrogen peroxide ($NH_4SCN + H_2O_2$). Extractable Hg concentrations for substrates amended with humic acid were measured using only $(NH_4)_2S_2O_3$ as an extractant. One gram of substrate was weighed into 50 mL polypropylene centrifuge tubes in triplicate. After addition of extractant solutions (20 mL at 2 g/L, unless otherwise stated), the tubes were rotated on an end-over-end shaker overnight at 45 rotations per minute (RPM) and the supernatant separated after centrifugation at 3000 RPM for 3 minutes. The pH and Eh of the extractant solutions were measured using a pH and Eh meter (Copenhagen Radiometer, PHM 83 Autocal pH meter).

6.2.4 Induced plant-Hg accumulation experiments

6.2.4.1 Effect of plant species x sulphur-containing ligands

Seeds of *B. juncea, Phaseolus vulgaris* and *Vicia villosa* were germinated in 400 mL plastic pots (8 x 8 cm) filled with the amended substrates containing Hg at 2.5 mg/kg. Two weeks after seeding each pot was thinned to leave one individual plant. The experiment was initiated 5 weeks after seeding. Three sulphur–containing ligands were investigated for their ability to induce Hg accumulation in the plants: ammonium thiosulphate ($[NH_4]_2S_2O_3$), sodium thiosulphate ($Na_2O_3S_2$) and ammonium thiocyanate supplemented with hydrogen peroxide ($NH_4SCN + H_2O_2$). The experiment utilised a two-factorial completely randomised design with plants species (*B. juncea*, *P. vulgaris*, and *V. villosa*) and sulphur-containing ligands ($[NH_4]_2S_2O_3$, $Na_2S_2O_3$, $NH_4SCN + H_2O_2$) as factors. The extractants were dissolved in 1 L of reverse osmosis (RO) water and 10 mL of the resulting solutions were added to plant pots to give a final concentration of 2g of chemical per kg of substrate (unless otherwise stated). Plants from each species that were treated with water were designated as control plants. Five replicates were used for each plant-chemical combination.

6.2.4.2 Effect of substrate Hg concentrations and humic acid levels

Seeds of *B. juncea* were sown in 400 mL plastic pots (8 x 8 cm) filled with the modified substrate contaminated with Hg at 0, 1.25, 2.5 and 5 mg/kg. Another batch of *B. juncea* seed was also sown in modified substrate contaminated with Hg at 2.5 mg/kg and amended with humic acid at 0, 0.125, and 1.25 g/kg of substrate. Two weeks after seeding, each pot was thinned to leave one individual plant. Ammonium thiosulphate solution was added after 5 weeks of plant growth at a rate of 2 g/kg of substrate. Plants that received only water were designated as controls plants. These two experiments utilised a completely randomised design with 5 replicates per each treatment level.

All thioligand–induced plant experiments were carried out over a 5 days period in a greenhouse with the ambient temperature set at 25 ^{o} C with no humidity control (Figure 6.1). The Pot positions were randomly changed on a periodic basis to equalise light exposure. The greenhouse facility was located at the campus of University of British

Columbia, Vancouver, CA. The maximum interval period between planting, treating and harvesting the plants was six weeks. The greenhouse experiments were carried out over the summer period and the plant pots were watered every day to field capacity. All plants were treated before the outset of flowering.

6.2.5 Plant harvest

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

6.2.6 Plant digestion

Ground shoots and roots were accurately weighed (0.1 g) into 50 mL plastic beakers. Concentrated HNO_3 (15 mL) was then added. The digest samples were left overnight and, in the following day, were heated in a water bath at 80°C for 1 hour. Digest solutions were transferred to 10 mL polythene tubes and diluted with reverse osmosis (RO) water to make a final volume of 10 mL. A blank reagent was used with all digestions.

6.2.7 Substrate digestion

The total Hg concentration in the original and Hg-amended substrates was determined through *aqua regia* digestions. One gram of substrate was weighed into 50 mL polypropylene pots in triplicate and a 15 mL solution of HNO_3 and HCl at 1:3 ratio was added. The samples were digested in water bath at 80°C for 1 hour and the filtrates diluted to a final volume of 50 mL with reverse osmosis (RO) water.

Figure 6.1. Pots with *B. juncea* plants inside the greenhouse. The picture shows plants growing on substrates before treatment with sulphur-containing ligands.

6.2.8 Mercury analysis

Total Hg concentrations in plant and substrate digests and in extractant solutions were analysed using the hydride-generation atomic absorption spectroscopy technique (Moreno et al., 2004 b[11]). The analysis was performed using a GBC 909A AAS (Victoria, Australia) operating in the flame mode. A sodium borohydride solution (5% $NaBH_4$ + 1% KOH) in combination with 10 ml of 0.5 M of HCl was used to generate the Hg vapour. The limit of detection (LOD) for mercury in solution was 10 ng/mL for plant digests and 5 ng/mL for soil digests and extractant solutions. Reagent blanks were below detection limits in the solution. Linear calibration curves were obtained over the range of 125 to 1000 ng/mL of Hg using 4 standards prepared from a 10 mg/L mercuric nitrate ($HgNO_3$) spectrosol solution (May & Baker, AAS standard reagent solution, England). The Hg readings obtained from the replicate analysis (n=10) of a standard solution containing 1 mg/L of Hg could be reproduced with less than 5% of variation. The analytical method was assessed for quality control by an external certified laboratory with agreements ranging from 85 to 103% for Hg-containing solutions and Hg-containing plant samples (Moreno et al., 2004 b).

[11] This paper was described in this thesis as Chapter 3.

6.2.9 Statistical analysis

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

a) control (water) x sulphur-containing ligands (ignoring plant species);
b) between the three sulphur-containing ligands (ignoring plant species);
c) between plant species (ignoring sulphur-containing ligands);
d) between plant species within each sulphur-containing ligand.

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

6.3 Results

6.3.1 Total and extractable-Hg concentrations

The total Hg concentrations in the *aqua regia* digests and the pH and Eh for original and diluted tailings samples are shown in Table 6.1. All amended substrates exhibited similar geochemical conditions with moderately alkaline pH (between 8.1 and 8.3) and mildly reducing conditions (Eh between –62 and -73 mV).

Table 6.1. Mercury concentrations and the pH and Eh of original and modified samples of Gold Mountain (GM) mine tailings.

Substrate Composition	Target Hg (mg/kg)	Measured Hg (mg/kg)[b]	pH[c]	Eh[c] (mV)
Original	100[d]	67.47 ± 11.25	9.45	-137
	1.25	1.65 ± 0.05	8.17	-62
Modified[e]	2.5	2.42 ± 0.07	8.24	-66
	5	3.38 ± 0.11	8.32	-72
HA 0			8.24	-66
Modified +HA 0.125	2.5	2.42 ± 0.07	8.19	-63
HA 1.25			8.34	-73

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

The extractable Hg concentrations for the original Gold Mountain mine tailings are shown in Figure 6.2. Mercury solubility in the mine tailings was significantly increased in the presence of $(NH_4)_2S_2O_3$. The total Hg concentration in $(NH_4)_2S_2O_3$. extracts reached 122 ± 9.4 mg/kg and was significantly higher than the concentration extracted using $Na_2S_2O_3$, $NH_4SCN + H_2O_2$ and water ($p < 0.001$, Figure 6.1). The concentration of Hg extracted using $(NH_4)_2S_2O_3$ was significantly higher than the Hg concentration found in *aqua regia* digests, as shown in Table 1 ($p < 0.01$, Table 6.1).

The effect of humic acid on the water and $(NH_4)_2S_2O_3$.-extractable Hg concentrations of diluted mine tailings is shown in Figure 2. Again, the Hg concentration was significantly higher in the presence of $(NH_4)_2S_2O_3$ relative to water ($p < 0.0001$). The concentration of water-soluble Hg was higher for substrates that had been amended with humic acid at rates of 0.125 and 1.25 g HA/kg ($p < 0.01$). This increase corresponded to a 40 % increment in the Hg soluble fraction relative to non-HA amended water-treated substrates. There were no significant differences within the $(NH_4)_2S_2O_3$ treatment for the Hg values between the control (no HA-amended substrates) and the two tested levels of humic acid ($p > 0.05$).

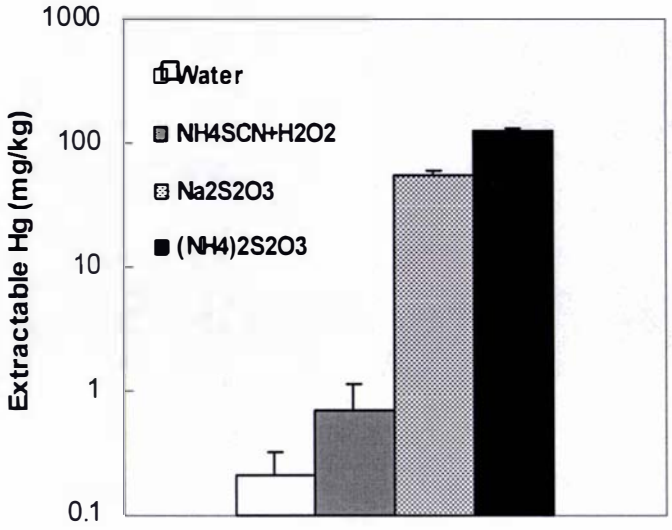

Figure 6.2. Effect of sulphur-containing ligands on extractable Hg concentrations of original Gold Mountain mine tailings. Bars denote ± 1 standard deviation from the mean of three replicates. NH_4SCN = ammonium thiocyanate; H_2O_2= hydrogen peroxide; $Na_2S_2O_3$= sodium thiosulphate; $(NH_4)_2\ S_2O_3$= ammonium thiosulphate; water = control.

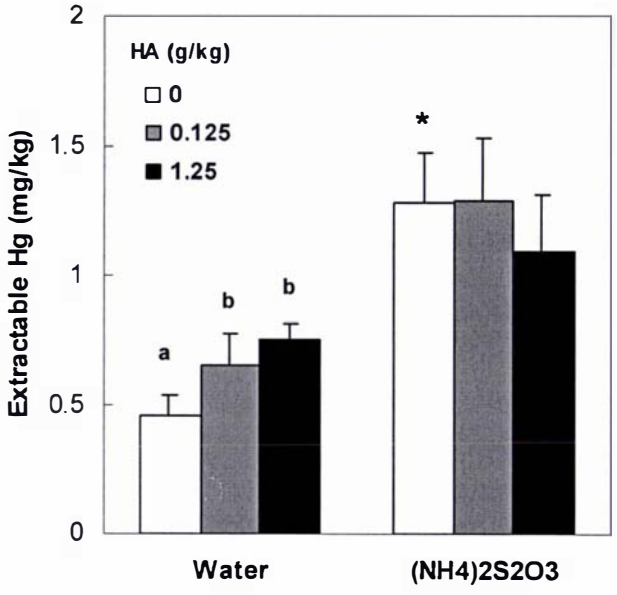

Figure 6.3. Humic acid-extractable Hg for modified substrates containing Hg at 2.5 mg/kg. Bars denote ± 1 standard deviation from the mean of three replicates. Letters compare treatments within each treatment level. Means with different letters are significantly different at $p < 0.05$ (Tukey's test). The symbol (*) indicates not statistically significant ($p > 0.05$). HA=humic acid; $(NH_4)_2S_2O_3$=ammonium thiosulphate, water=control.

6.3.2 Effect of plant species x sulphur-containing ligands

Although great care was taken to ensure that Hg was washed off from the root system, both the experimental protocol and the analytical techniques used in this study were not sufficient to distinguish between Hg that was adsorbed onto, or taken up into (absorbed) root cells. Therefore, we assume that the accumulation of Hg by the roots of the tested plants will contain both of these Hg fractions.

Table 6.2. Effect of sulphur-containing ligands and plant species on plant-Hg accumulation for plants growing in modified substrates containing Hg at 2.5 mg/kg. Values for Hg in roots and shoots are in mg/kg. The shoot:root ratio is the quotient of Hg content in shoots / Hg content in roots[a].

Treatment Description (Plant Species/chemical) [b]	Contrast[c] Number	Root	Shoot[d]	Shoot:Root Ratio[d]
VV/ Control	**1**	5.5 ± 0.8	-	-
VV/ $Na_2S_2O_3$	**2**	113 ± 10	9.5 ± 1.4	0.08 ± 0.04
VV/ $(NH_4)_2S_2O_3$	**3**	131 ± 13	14.8 ± 0.8	0.11 ± 0.015
VV/ $NH_4SCN + H_2O_2$	**4**	23 ± 2.6	-	-
BJ / Control	**5**	9.8 ± 1.7	-	-
BJ / $Na_2S_2O_3$	**6**	69 ± 4	15.2 ± 2.3	0.22 ± 0.03
BJ / $(NH_4)_2S_2O_3$	**7**	61± 6	16.4 ± 2.5	0.27 ± 0.04
BJ / $NH_4SCN + H_2O_2$	**8**	12 ± 1.4	-	-
PV / Control[e]	**9**	4.2 ± 0.3	-	-
PV / $(NH_4)_2S_2O_3$	**10**	28 ± 4.4	17.2 ± 1.8	0.62 ± 0.14

[a]Values are ± 1 standard deviation from the mean of five replicates.
[b]VV = Vicia villosa; BJ = Brassica juncea; PV = Phaseolus vulgaris, $NH_4S_2O_3$= ammonium thiosulphate, $Na_2S_2O_3$ = sodium thiosulphate, NH_4SCN= ammonium thiocyanate, H_2O_2= hydrogen peroxide.
[c] Relates a single plant-chemical combination to its respective treatment mean.
[d]Missing cells due to Hg below detection levels.
[e]PV/$Na_2S_2O_3$ and PV/$NH_4SCN + H_2O_2$ treatments were omitted due to absence of plant germination.

The Hg concentration in plant shoots and roots harvested at the end of the experiment, are shown in Table 6.2. The associated comparisons including their F-ratio and *p*-values are shown in Table 6.3. The application of $(NH_4)_2S_2O_3$ and $Na_2S_2O_3$ mobilised Hg in substrates and greatly increased root Hg accumulation relative to controls and to $NH_4SCN + H_2O_2$ ($p < 0.0001$, Table 6.2). The induced root Hg accumulation was significantly higher for *V. villosa* relative to all plant-chemical combinations ($p < 0.0001$, Table 6.3, see root contrasts). For example, in the presence of $(NH_4)_2S_2O_3$ and

$Na_2O_3S_2$, the root Hg concentration for this plant species increased to greater than 100 mg/kg dry weight relative to an average of 69 mg/kg recorded in the root tissues of *B. juncea*.

Table 6.3. Linear contrasts and the associated F-ratio and *p*-values for the comparison of plant/chemical treatment means. The contrasts were tested separately for roots, shoots, and the shoot:root ratio[a].

	Treatment Contrasts[b]	DF[c]	F- Value[c]	Pr > F[c]
	1&5&9 vs2&3&4&6&7&8&10	1	816.62	< 0.0001
	2&3 vs4&6&7&8&10	1	1276.03	<0.0001
Root	2&3 vs6&7&10	1	730.87	< 0.0001
	2&6vs 3&7&10	1	636.36	< 0.0001
	3 vs7&10	1	67.46	<0.0001
	7 vs10	1	22.18	<0.0001
	2 vs 6	1	121.31	< 0.0001
	2&3 vs6&7&10	1	15.35	0.0009
	2&6 vs3&7&10	1	15.35	0.0009
Shoot	3 vs7&10	1	2.60	0.1224
	7 vs10	1	0.40	0.5360
	2 vs6	1	21.02	0.0002
	2&3 vs6&7&10	1	84.10	<0.0001
	2&6 vs3&7&10	1	84.10	< 0.0001
Shoot:Root	3 vs7&10	1	54.63	<0.0001
Ratio	7 vs10	1	65.39	<0.0001
	2 vs 6	1	9.67	0.0055

[a]See contrast number in Table 6.2 for treatments description.
[b] The associated hypothesis for each contrast tested possible differences between the means of each plant/chemical contrast number; comparisons were not orthogonal.
[c]DF = degrees of freedom; Pr >F = probability level for rejecting or accepting the hypothesis associated with each treatment contrast at the $\alpha = 0.05$ level.

The Hg concentration in the shoot tissues of $(NH_4)_2S_2O_3$ and $Na_2S_2O_3$-treated plants was significant higher than in control plants and those treated with $NH_4SCN + H_2O_2$, which had Hg concentrations below detection levels (Table 6.2). These results indicate that Hg mobilised by $(NH_4)_2S_2O_3$ and $Na_2S_2O_3$ could be taken up into the roots and subsequently transported to the aerial tissues of the tested plant species. Although the Hg concentration in shoot tissues appears to be similar between plant species in the presence of these ligands (Table 6.2), there was a significant plant-chemical effect in the shoot Hg translocation ($p < 0.001$, see first two contrasts Table 6.3). For instance, Hg values in aerial tissues of $Na_2S_2O_3$-treated plants were significantly lower than those observed in the $(NH_4)_2S_2O_3$-treated plants ($p < 0.001$, see 1st contrast for shoots, Table 6.3). Also, Hg translocation to the aerial tissues of *V. villosa* was significantly reduced when compared to the other two species ($p < 0.001$, see 2nd contrast for shoots, Table 6.3). The results for Hg accumulation in shoot tissues indicate, on the other hand, that

shoot Hg translocation was similar between plant species within the $(NH_4)_2S_2O_3$ treatment (Table 6.2, see shoots). However, $(NH_4)_2S_2O_3$-treated plants showed variable efficiency for Hg transport from root to shoot tissues when the results were expressed by their respective shoot:root ratios (Table 6.2, see Shoot/Root Ratio). For example, the shoot:root ratio for *P. vulgaris* was 2 and 5 times higher after $(NH_4)_2S_2O_3$ treatment relative to *B. juncea* and to *V. villosa*, respectively ($p < 0.0001$, Table 6.2 and 6.3, see contrasts for shoot:root ratio). The shoot:root ratio for *B. juncea* was approximately twofold greater than the shoot:root ratio obtained for *V. villosa* in the presence of both $(NH_4)_2S_2O_3$ and $Na_2O_3S_2$ ($0.0001 < p < 0.01$, Table 6.2, see contrasts for shoot:root ratio).

6.3.3 Effect of substrate Hg concentrations and humic acids levels

The results in Figure 6.4 (a) shows that the Hg accumulation in shoot and root tissues of $(NH_4)_2S_2O_3$-treated *B. juncea* plants was significantly enhanced as a function of increasing Hg concentrations in substrates ($p < 0.05$). Control (water-treated) plants, on the other hand, had shoot Hg values below detection levels for all tested substrate Hg concentrations (data not shown). Plants grown in Hg-free substrates (0 mg/kg Hg) also had shoot Hg values below detection levels (Figure 6.4 a). These results suggest that foliar uptake of Hg (0) from the atmosphere that may have been released from plants and substrates was not a significant pathway for Hg accumulation in aerial tissues.

Although the root Hg concentration after $(NH_4)_2S_2O_3$ treatment appears to be increasing with regard to Hg concentration in substrates (Figure 6.4 a), a plot of the root Hg concentration versus the $(NH_4)_2S_2O_3$-extractable Hg concentration shows a significant asymptotic response (Figure 6.4 b, $r^2 = 0.7411$, $p < 0.001$). Consequently, it can be inferred that Hg uptake into the roots of *B. juncea* is physiologically regulated above certain concentrations and thus, not completely dependent on the amount of Hg available in the substrate. However, this is not true for shoots, whose Hg concentration showed a significant and linear response to the concentration of $(NH_4)_2S_2O_3$-extractable Hg in the substrate (Figure 6.4 b, $r^2 = 0.8324$, $p < 0.0001$).

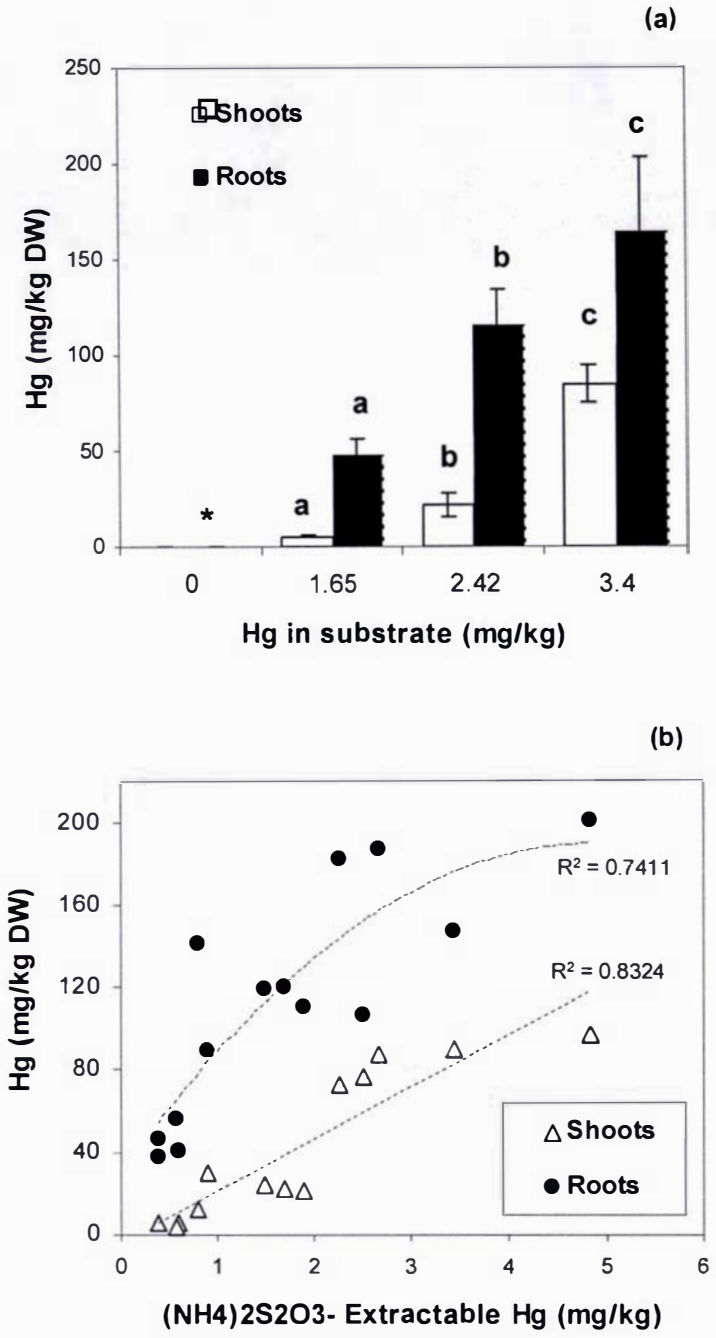

Figure 6.4. Accumulation of Hg in roots and shoots of $(NH_4)_2S_2O_3$-treated *B. juncea* plants as a function of total (a) and $(NH_4)_2S_2O_3$-extractable (b) Hg concentrations in modified substrates. Bars denote ± 1 standard deviation from the mean of five replicates. DW = dry weight, $(NH_4)_2 S_2O_3$=ammonium thiosulphate.

The effect of variable humic acid levels on the root Hg concentration in *B. juncea* after water (control) and $(NH_4)_2 S_2O_3$ treatment is shown in Figure 6.5. Addition of $(NH_4)_2S_2O_3$ to substrates greatly enhanced the root Hg concentration relative to the control treatment ($p < 0.0001$). The Hg concentration of both control and $(NH_4)_2S_2O_3$-treated plants was substantially affected by the amount of HA in the substrate. The concentration of Hg in the root tissues of control plants significantly increased from 9.8 to 13.7 mg/kg when the HA content in substrates was raised from 0 to 1.25 g/kg ($p < 0.05$). The root Hg concentration of $(NH_4)_2S_2O_3$-treated plants showed a similar pattern, increasing significantly from 61 to around 100 mg/kg over the same HA concentration range ($p < 0.05$). In contrast, the shoot Hg concentration of *B. juncea* was significantly decreased in the presence of HA ($p< 0.01$, Figure 6.6). Root to shoot transport, therefore, appears to be inhibited by humic acid.

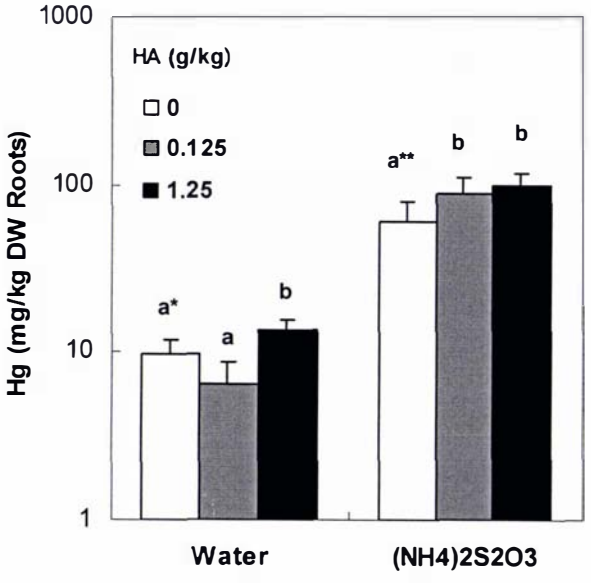

Figure 6.5. Accumulation of Hg in roots of water and $(NH_4)_2S_2O_3$-treated *B. juncea* plants as a function of the humic acid content (g/kg) in modified substrates containing Hg at 2.5 mg/kg. Bars denote ± 1 standard deviation from the mean of five replicates. Means with different letters are significantly different (Tukey's test). The symbol (*) indicates the significance level ($^{*}p < 0.05$, $^{**}p < 0.01$). HA=humic acid, $(NH_4)_2S_2O_3$=ammonium thiosulphate, water=control, DW = dry weight.

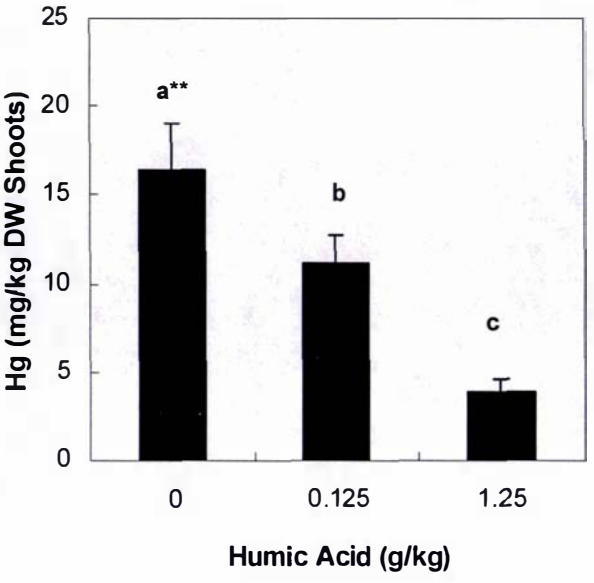

Figure 6.6. Accumulation of Hg in shoots of $(NH_4)_2S_2O_3$-treated *B. juncea* plants as a function of the humic acid content (g/kg) in modified substrates containing Hg at 2.5 mg/kg. Bars denote ± 1 standard deviation from the mean of five replicates. Means with different letters are significantly different at $p < 0.01$ (Tukey's test). .HA=humic acid, $NH_4S_2O_3$=ammonium thiosulphate, water=control. DW = dry weight.

The relationship between the root Hg concentration in *B. juncea* and the extractable Hg concentration of HA amended substrates is shown in Figure 6.7 (a). Both water and $(NH_4)_2S_2O_3$-treated plants showed strong evidence for a positive and linear relationship between the concentration of Hg in root tissues and the concentration of extractable Hg ($r^2 = 0.6893$, $p < 0.001$ and $r^2 = 0.6255$, $p < 0.001$, respectively). However, when the concentration of extractable Hg was plotted against the concentration of Hg in shoot tissues, the regression relationship was inverted, confirming the inhibition effect of HA on shoot Hg translocation ($r^2 = 0.74$, $p < 0.0001$, Figure 6.7 b). The relationship between the root and shoot Hg concentration as a function of extractable Hg was confirmed by correlation analysis ($r = -0.86$ for shoots; $r = 0.82$ for roots of water-treated plants, and $r = 0.86$ for roots of $[NH_4]_2S_2O_3$-treated plants).

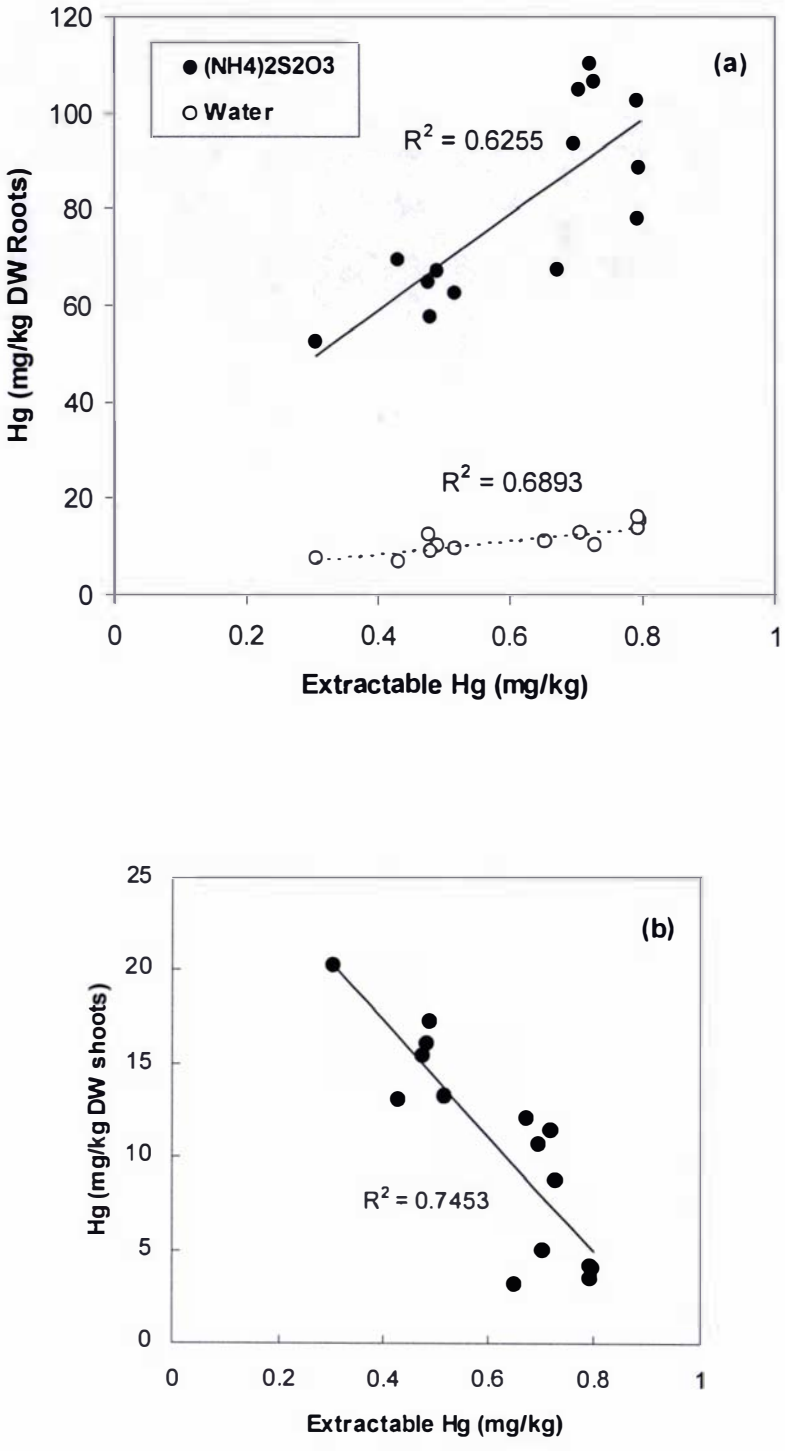

Figure 6.7. Accumulation of Hg in roots (a) and shoots (b) of control and $(NH_4)_2 S_2O_3$-treated *B. juncea* plants as a function of extractable Hg in humic acid amended substrates (1.25 g/kg) containing Hg at 2.5 mg/kg. DW=dry weight. Water treatment for shoots was below detection levels.

6.4 Discussion

6.4.1. Mercury speciation in the substrate

Given that the original tailings samples exhibited mild reducing conditions (-137 mV), high pH (9.45) (Table 6.1), the speciation of solid phase Hg in the tailings substrate may be in the elemental Hg (0) form (Brookins, 1988). In this reduction 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 (Figure 6.2) 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 ammonium and sodium thiosulphate salts may have, therefore, mobilised Hg because they are strong ligands and non selective to specific Hg species. The Hg solubilization process is possibly related to thiosulphate Hg-complex formation in the presence of the thiosulphate ion. Thiosulphate solutions have complexing abilities with a variety of metal ions from the IB and IIB classes of the periodical table (e.g. Ag, Au, Hg, Cu) and will dissolve many insoluble forms of Hg under alkaline conditions (Wilkinson et al., 1987; Molleman and Dreisinger, 2002).

The existence of a water-extractable Hg fraction in the original tailings samples (Figure 6.2) indicates that Hg in an oxidized form was available for exchange reactions with S-containing ligands in the substrate solution. Since there was no significant variation in the pH values between control and HA-amended substrates (Table 6.1), enhanced Hg solubility in the water extracts for HA-amended substrates can only be explained through the formation of Hg-HA complexes (Figure 6.2). The fact that the pH of diluted substrates was around 8 (Table 6.1) and that humic acid is soluble at alkaline conditions (Wallschläger et al., 1996) provides support for this statement. Considering that soluble Hg-HA complexes were present in both water and $(NH_4)_2S_2O_3$ extracts (Figure 6.3), we hypothesise that the total soluble Hg fraction of HA-amended substrates comprises a mixture of both Hg-HA and Hg-thiosulphate complexes.

6.4.2 Root Hg accumulation in relation to plant species

Results from the current study indicate that Hg accumulation might be dependent on the root specific features of plant species. For instance, in the presence of $(NH_4)_2S_2O_3$, *V. villosa* showed root Hg values that were inversely related to shoot:root ratio while the opposite was true for *P. vulgaris* (Table 6.2). The same pattern was also observed for *V. villosa* and *B. juncea* in the presence of $Na_2S_2O_3$. The explanation for this variability might be attributed to an interaction between plant roots and the components (i.e., diffusion and mass flow) that affect nutrient uptake and transport to root surfaces. (Marschner, 1986; Marschner, 1991). For example, the transport of nutrients to plant roots along a concentration gradient (i.e., diffusion) is closely related to plant factors such as root morphology and root surface area (Marschner, 1991). A field study conducted with the purpose of correlating Hg uptake with the characteristics of plant roots found a strong relationship between root Hg accumulation and root surface area (Cocking et al., 1995). Plant species with fine root systems (such as *Allium* sp.) and greater surface area exhibited elevated Hg concentration in roots, whereas an inverse relationship was observed for plants with a larger root size (smaller root surface area). Also, Hg plant uptake was shown to be strongly dependent on root length density of Zorro fescue (*Vulpia myuros* L.) grown in acidic Hg-contaminated mine soils (Heeraman et al., 2001). It was observed during harvesting that *V. villosa* produced more roots per unit of soil volume when compared to the other two species. This observation might have contributed to the superior root Hg concentrations exhibited by this species in the presence of S-containing ligands.

6.4.3 Selectivity of mercury complexes to shoot transport

Literature indicates that plants are selective about the form of metal transported to aboveground tissues (Blaylock et al., 1997; Anderson et al., 1999; Moreno et al., 2004 a[12]). For example, the accumulation of Hg was preferentially confined within root tissues for plants species grown in Hg-contaminated mine tailings treated with soluble $HgCl_2$ at 1, 5 and 10 mg/kg (Moreno et al., 2004 a). The pH of this substrate was around 5.5 and the Ni-hyperaccumulator *Berkheya coddii* and the salt-tolerant *Atriplex*

[12] This paper is described as Chapter 7 in this thesis.

canescens showed no evidence of stress to Hg-exposure. However, translocation to the upper plant parts was restricted for all tested concentrations, yielding a maximum concentration factor (concentration shoot tissue/concentration in soil) of only 0.8 at the highest Hg concentration. By contrast, $(NH_4)_2S_2O_3$-treated plants grown on the highest substrate level (3.4 mg Hg/kg, Table 6.1) accumulated Hg in shoots at an average of 85 mg/kg (Figure 6.4 a), hence yielding a concentration factor of 25. Since thiosulphate complexes are stable at neutral to alkaline conditions (Bowell et al., 1993) and the pH of the modified substrates was around 8 (Table 6.1), it is plausible that Hg-S_2O_3 complexes were preferentially selected for shoot transport over other Hg species present in the substrate.

6.4.4 Effect of humic acids on root uptake

Despite the strong affinity of Hg for organic matter, few studies have examined the effect of these substances on the uptake and translocation of Hg in higher plants. Limited dissociation of Hg bound to soil organic components translates into low Hg availability in soils and, therefore, restricted Hg uptake by plants. For example, the addition of organic matter to Hg-contaminated mine spoil has been negatively correlated with the Hg tissue concentrations in Zorro fescue (*Vulpia myuros* L.) (Heeraman et al., 2001). Humic acid from decayed plant material suppressed Hg uptake for duckweed (*Lemna minor*) grown in a hydroponic medium containing Hg at 10 mg/L (Mo et al., 1989). Similarly, the root concentration factor (concentration root tissue/concentration in soil) of *Brassica chinensis* and *Lactuca sativa* was significantly decreased with increasing humic acid concentrations in two types of Hg-contaminated soils (Wang et al., 1997). The reasons for the decline in plant Hg concentrations were attributed to a decrease in the soluble Hg fraction due to Hg complexation with either organic matter or humic acid. In contrast, our studies showed that the Hg soluble fraction of modified substrates was significantly increased in the presence of HA due to Hg-HA complex formation (Figure 6.3). Furthermore, the discrepancy in substrates in the root Hg values between water and $(NH_4)_2S_2O_3$-treated plants (respectively, 13.7 and 99.8 mg/kg at 1.25 HA, Figure 6.5) was possibly linked to the uptake of Hg-HA and Hg-thiosulphate complexes by plants. Considering that Hg-HA complexes were soluble in the aqueous phase of the HA-amended substrates, then Hg root uptake would be

initially a function of the available Hg-HA complexes in the substrate solution. Subsequently, the application of $(NH_4)_2S_2O_3$ to substrates would have mobilised the unavailable Hg fraction through Hg-thiosulphate complex formation, enhancing Hg accumulation in root and shoot tissues. The significantly positive correlation between extractable Hg and root Hg accumulation (Figure 6.7 a) indicates that root tissues absorbed both Hg-HA and Hg-thiosulphate complexes. Furthermore, the evidence of negative correlation between extractable Hg and shoot Hg concentration indicates that Hg translocation to aerial tissues was severely restricted in the presence of HA (Figure 6.7 b). Our results indicate, therefore, that Hg-thiosulphate complexes were selectively translocated into aerial tissues to the detriment of Hg-HA complexes, which were probably adsorbed to root tissues.

6.5 Conclusions

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

extrapolated with care to field conditions. The inhibition of shoot Hg translocation in the presence of HA is a limiting factor for the application of this technology in organic–rich Hg-polluted soils. Research should be extended to investigate the effect of the currently tested parameters on the generation of Hg-containing leachates.

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CHAPTER 7

PHYTOREMEDIATION OF MERCURY-CONTAMINATED MINE TAILINGS BY INDUCED PLANT-Hg ACCUMULATION

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Abstract

In most contaminated soils and mine tailings, mercury (Hg) is not readily available for plant uptake. A strategy for inducing Hg mobilisation 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 non-metal accumulators *Brassica juncea* and *Lupinus* sp. was studied by pot trials containing mine tailings treated either with soluble Hg or S-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 (DW). The addition of ammonium thiosulphate ($[NH_4]_2S_2O_3$) to tailings mobilised Hg in substrates, as indicated by the elevated Hg concentrations in leachates from the pots of both species. Ammonium thiosulphate 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 Hg may indicate Hg volatilisation. The results indicate that there is potential for induced plant-Hg accumulation for phytoremediation of Hg-contaminated sites. Issues of Hg leaching and volatilisation, however, need to be addressed before this technology can be implemented in the field.

7.1 Introduction

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 ground water supplies with metal ions (Morrell et al., 1996; Holmtröm et al., 2001).

Implementation of remedial procedures for metal removal is, therefore, a first step towards 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 phytovolatilisation 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. Phytovolatilisation, on the other hand, uses plants to clean-up metal polluted sites through Volatilisation of the contaminant from the plant biomass into the atmosphere (Brooks, 1998; Zayed et al., 2000; Meagher et al., 2000).

Suitable plants for phytoextraction and phytovolatilisation 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 (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 of the Se-accumulator *Astragalus* sp. and the non-accumulator *Brassica juncea* (Robinson et al.,

1998; McGrath, 1998; Zayed et al., 2000). Finally, the third group includes transgenic plants containing the bacterial gene sequence responsible for expressing the mercury (Hg) ion reductase (*MerA*) and organomercury lyase (*MerB*) enzymes, which confers increased Hg resistance and enhanced volatilisation capacity (Rugh et al., 1996; Bizily et al., 1999). For example, transgenic *MerA-Brassica napus* 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 volatilise 1.5 ng of Hg(0) per milligram of root tissue per minute (Meagher et al., 2000).

The success of phytoextraction is dependent 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, be dependent 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 et al., 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 S-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). Additionally, 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 aim 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:

1. To test tolerance, uptake and translocation of increasing concentrations of soluble Hg ($HgCl_2$) by *Berkheya coddii* and *Atriplex canescens*;
2. To examine the chemical solubilization of Hg in the substrate in the presence of S-containing ligands; and
3. To investigate the enhanced Hg accumulation of *B. juncea* and *Lupinus* sp. in this substrate in the presence of selected S-containing ligands.

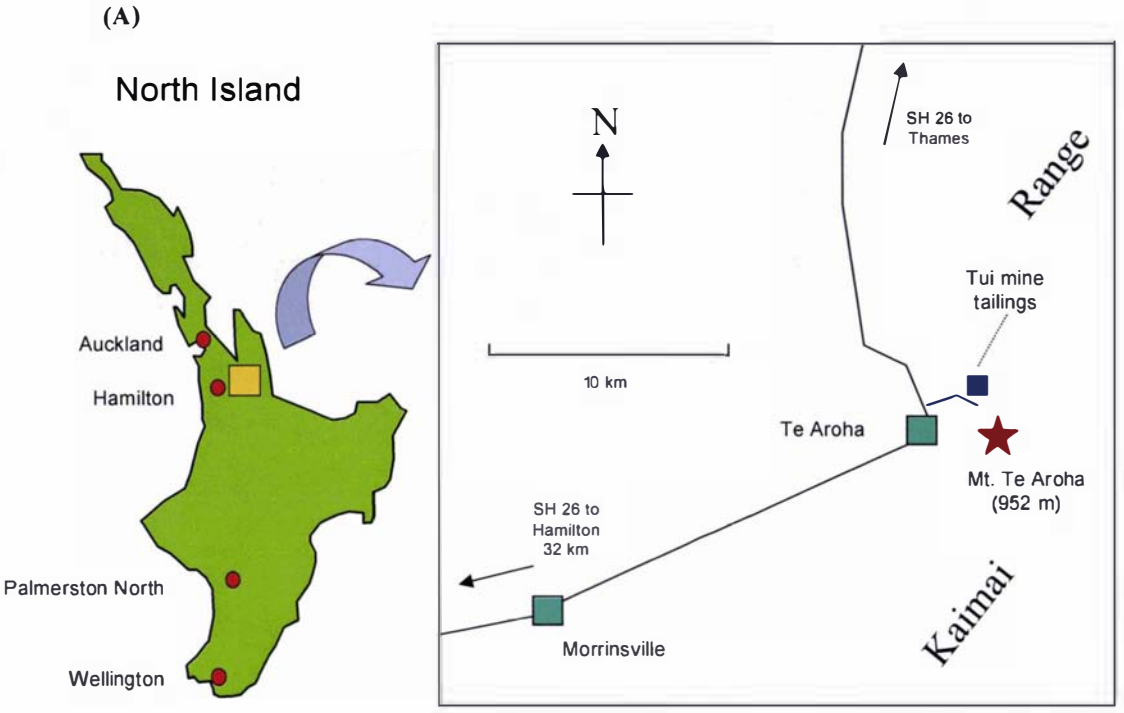
7.2 Material and Methods

7.2.1 Site description.

Substrate samples for the pot trials were collected from 12 locations within the tailings dam of the abandoned Tui base-metal mine, located on the NW flank on Mount Te Aroha, approximately 3 km of the township of Te Aroha, North Island of New Zealand (Figure 7.1 A and B). 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 Pb-Cu-Zn concentrate containing minor amounts of Hg-Cd-Ag-Au. 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).

7.2.2 Substrate characterization

The experiments described in this paper used samples collected from two different locations at the Tui mine site. The experiment investigating plant accumulation of added soluble Hg used a low Hg content sample (0.30 ± 0.03 mg/kg), whereas the experiments investigating the chemical solubilization of tailings and the induced plant-Hg accumulation used a high Hg- content sample (2.82 ± 0.31 mg/kg).



(B)

Figure 7.1. Location of Tui mine tailings. The tailings dam is located on the NW flank on Mount Te Aroha, approximately 3 km of the township of Te Aroha, North Island of New Zealand (A). Revegetation trials ongoing in the Tui mine tailings (B).

7.2.3 Extractable Hg

Seven chemical extractants were tested for their ability to estimate the concentration of chemically-solubilised 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 0.2 g/L, unless otherwise stated), the tubes were rotated on an end-over-end shaker overnight at 45 rotations per minute and the supernatant filtered. The following chemicals were investigated for their ability to extract mercury: ammonium thiocyanate (SCN), potassium thiocyanate (KSCN), thiourea (CH_4N_2S), humic acid (HA), ammonium thiosulphate ($[NH_4]_2S_2O_3$), sodium sulphide (Na_2S), hydrogen peroxide (at 0.27%), and hydrogen peroxide (at 0.27%) + ammonium thiocyanate (SCN + H_2O_2).

7.2.4 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, mercury 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 mercury in this particular sub-sample of Tui mine tailings showed average mercury levels of 0.3 mg/kg.

Plants from each species grown in untreated substrates (without added Hg) were designated as control plants. Pots with mercury 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.

7.2.5 Induced plant-Hg accumulation

The non-accumulator 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 grown for a further two weeks. After five weeks, each pot was thinned to leave only one individual plant. Three

chemicals, each with a different functional S-group, were investigated for inducing Hg accumulation in plants: thiourea, ammonium thiosulphate ($[NH_4]_2S_2O_3$) and ammonium thiocyanate supplemented with hydrogen peroxide at 0.27% ($SCN + H_2O_2$). The amount of chemical added was at 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 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 addition of extractant solutions all plants were harvested and leachates and substrates sampled.

The substrates used for investigating both soluble and induced Hg plant accumulation were fertilised with 5 g/L of Osmocote (slow release Fertiliser) and had their pH adjusted to 5.5 by the addition of lime. The experiments were carried out in a greenhouse with temperature controlled at 21^0C and under a natural sunlight flux. Both experiments utilized a two-factorial completely randomised experimental design (CRD) with plant species, mercury concentrations, and S-containing ligands as factors (accordingly to 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.

7.2.6 Plant harvest

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

7.2.7 Plant digestion

Ground shoots and roots were accurately weighed (0.1 g) into 50 mL plastic pots and digested with 15 mL of HNO_3. The plant samples were left overnight and, in the following day, were heated in a water bath at 80°C for 1 hour. Subsequently, the plant digests were transferred to 10 mL polythene tubes and diluted with reverse osmosis water (RO) to make a final volume of 10 mL.

7.2.8 Soil digestion

Total mercury determinations for all tailings substrates were obtained through *aqua regia* digestions of dried samples collected at the Tui mine site. For this purpose, one gram of substrate was weighed into 50 mL polypropylene pots in triplicate and a 15 mL solution of HNO_3 and HCl at 1:3 ratio was added. The samples were digested in a water bath at 80°C for 1 hour and the filtrates diluted to a final volume of 50 mL by adding RO water. Total Hg from substrates of both soluble and induced plant pot trials was extracted using exactly the same procedure.

7.2.9 Mercury analyses

All samples of plant material, tailings and leachates were analysed for Hg using hydride-generation atomic absorption spectroscopy (Moreno et al., 2004[13]). The analysis was performed using a GBC 909A AAS (Victoria, Australia) operating in the flame mode. Mercury-containing liquid samples were analysed along with 10 ml of 0.5 M HCl. Sodium borohydride was used as a reducing agent to generate Hg vapour in a 5% $NaBH_4$ + 1% potassium hydroxide (KOH) w/v solution. The limit of detection for Hg in solution was 11.5 ng/mL (i.e. 0.0115 mg/L). The Hg readings obtained from the replicate analysis (n=10) of a standard solution containing 1 mg/L of Hg could be reproduced with less than 5% of variation. Reagent blanks were below detection limits, i.e. < 5 ppb, in the solution. Linear calibration curves were obtained over the range of 125 to 1000 ng/mL of Hg using 4 standards prepared from a 10 mg/L mercuric nitrate ($HgNO_3$) AAS Reagent (M&B). The analytical method was assessed for quality control

[13] This paper was described in the Chapter 3 of this thesis.

by an external certified laboratory with agreements ranging from 85 to 103% for Hg-containing solutions and Hg-containing plant and soil samples (Moreno et al., 2004).

7.2.10 Statistical analysis

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 pair-wise comparison of means. Except for dry yield biomass, all plant data were lognormally distributed.

7.3 Results

7.3.1 Total and chemically solubilised Hg

Figure 7.2 shows total mercury concentrations for the 12 locations sampled along the site. Total Hg values between the sampled locations were significantly different, ranging from 0.5 ± 0.57 to 4.5 ± 0.43 mg/kg (p<0.001). The high within-location variability of Hg concentrations shows that mercury was not uniformly distributed in the Tui mine tailings.

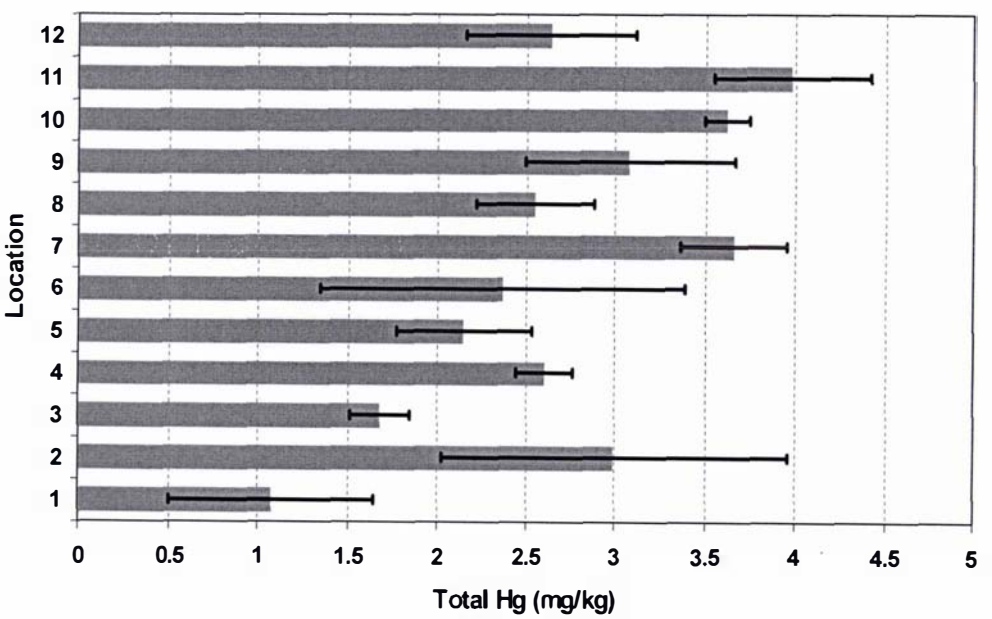


Figure 7.2. Total Hg concentrations for the 12 locations sampled along the Tui mine tailings. Bars denote ± 1 standard deviation from the mean of 3 replicates.

Soluble Hg concentrations as a function of chemical extractants added to Tui mine tailings are shown in Figure 7.3. Mercury solubility in Tui mine tailings was significantly increased in the presence of $(NH_4)_2S_2O_3$ and SCN + H_2O_2 when compared to the control (p<0.0001, Figure 7.3). Figure 7.4 shows that the concentration of soluble Hg was significant and positively correlated (r^2 =0.88, p<0.0001) to the concentration of $(NH_4)_2S_2O_3$ applied to the substrate. At the highest $(NH_4)_2S_2O_3$ concentration, the concentration of Hg in the extract reached 4.3 mg/kg, which is equivalent to the highest range for total mercury concentration found in the *aqua regia* digests (Figure 7.2). 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 $(NH_4)_2S_2O_3$ and involved extraction of Hg bound to the solid phase of sulfidic minerals possibly through the formation of a thiosulphate-Hg complex (Wilkinson et al., 1987). For the second process, hydrogen peroxide may have oxidised sulfidic Hg forms, allowing formation of an Hg-SCN complex.

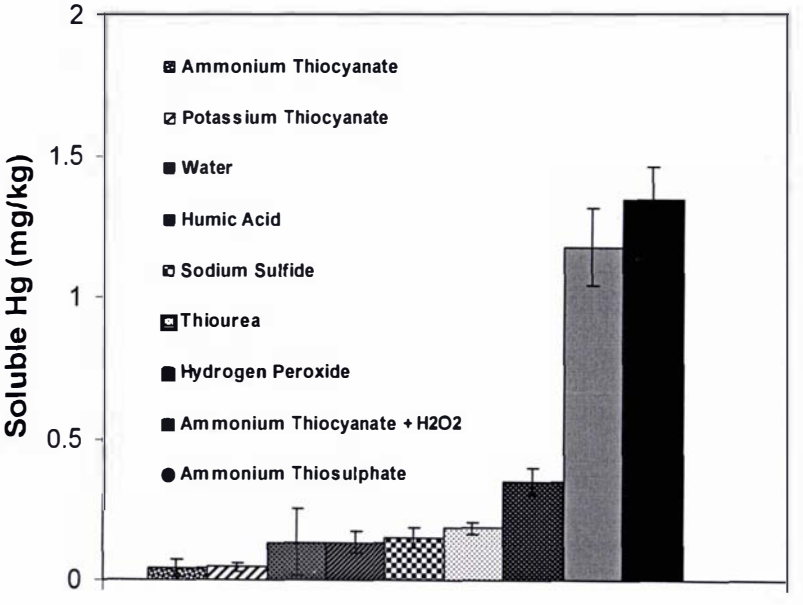


Figure 7.3. Chemically solubilised Hg as a function of S-containing ligands applied to Tui mine tailings at a 2g/L concentration, (except H_2O_2, [0.27%]). Bars denote ± 1 standard deviation from the mean of 3 replicates.

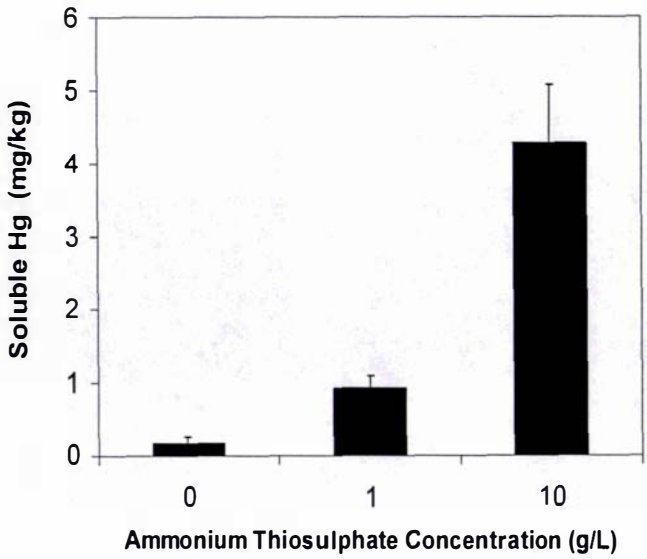


Figure 7.4. Chemically solubilised Hg as a function of ammonium thiosulphate applied to Tui mine tailings at the concentrations of 0, 1 and 10g/L. Bars denote ± 1 standard deviation from the mean of 3 replicates.

7.3.2 Soluble Hg plant accumulation

The accumulation of soluble Hg in roots and shoots of *B. coddii* and *A. canescens* is shown in Figures 7.5 A and B. On average, root concentration was an order of magnitude higher than shoot concentration. Roots of both species showed similar patterns for Hg accumulation as a function of Hg treatments, indicating that a physiological mechanism exists for regulating Hg uptake above certain total Hg concentrations.

Accumulation of Hg in roots, therefore, increased 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 7.5 A). In the case of shoots, *B. coddii* accumulated significantly more Hg than *A. canenscens* (Figure 7.5 B). At substrate additions of 10 mg/kg of Hg, for instance, the Hg average values for *B. coddii* and *A. canescens* in shoots were 8.7 and 2.47 mg/kg dry weight, respectively. Furthermore, *B. coddii* showed Hg shoot concentrations that were highly and positively correlated to increasing concentrations of Hg in substrates (r^2 = 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 7.1). Both plant species, therefore, showed evidence of some degree of tolerance to the presence of Hg in substrates.

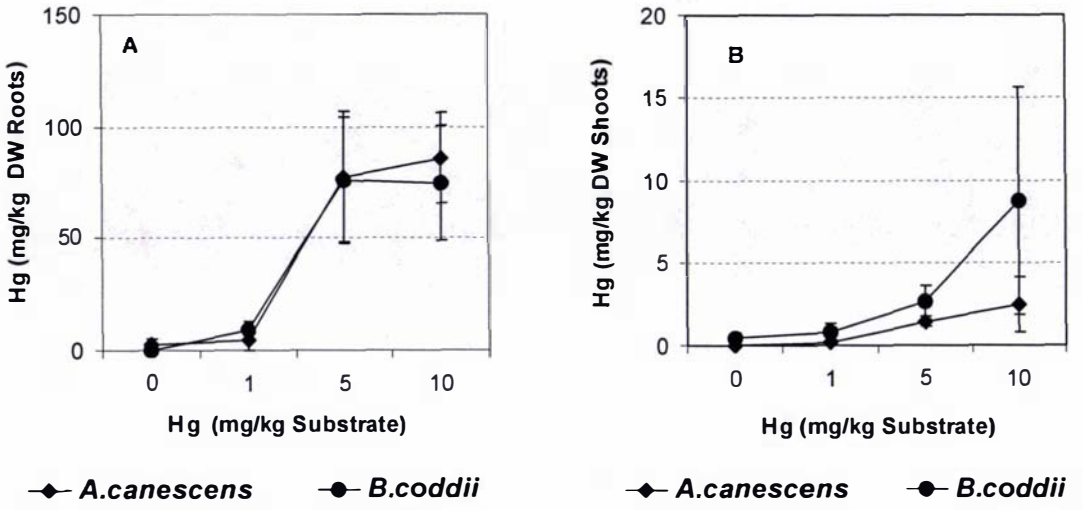


Figure 7.5. Accumulation of Hg in roots (A) and shoots (B) of *Atriplex canenscens* and *Berkheya coddii* grown in Tui mine tailings treated with 1, 5, and 10mg/kg of soluble Hg ($HgCl_2$). Bars denote ± 1 standard deviation from the mean of 5 replicates. DW = dry weight.

Table 7.1. 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 ($HgCl_2$). Values are the mean of 5 replicates ± 1 standard deviation.

Hg Treatment		**Plant Species**	
(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

Figure 7.6 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 ($0.05<p<0.0001$). Additionally, 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 present very limited availability for plant uptake.

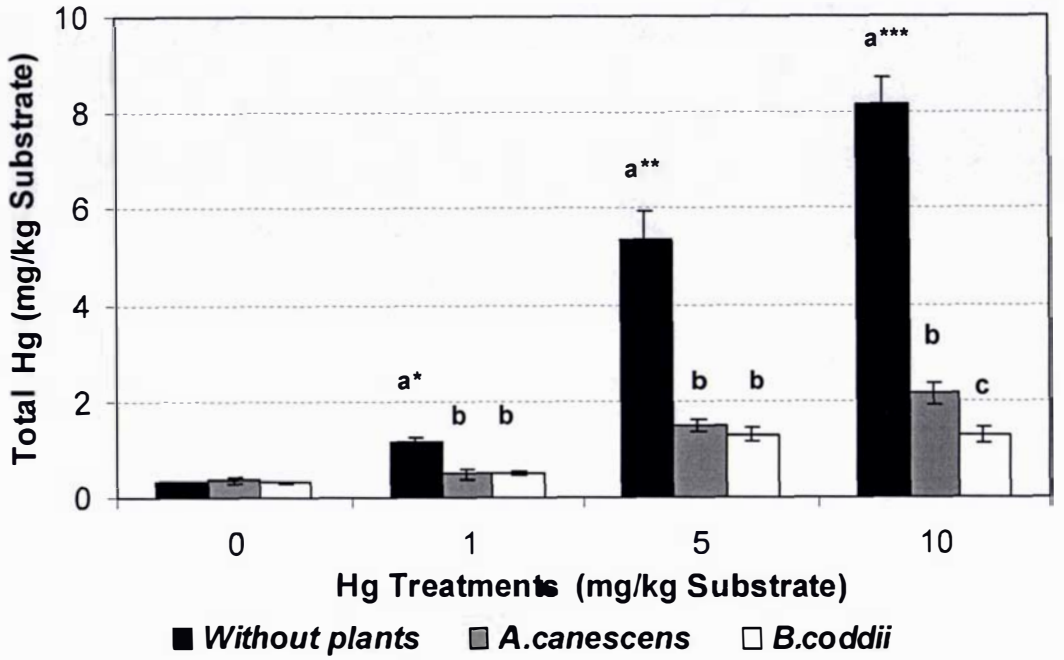


Figure 7.6. Total Hg concentrations in controls and planted substrates after application of 1, 5 and 10mg/kg of soluble Hg ($HgCl_2$) to pots. Bars denote ± 1 standard deviation from the mean of 3 replicates. Letters compare means within each Hg treatment level. Means with different letters are significantly different (Duncan's test). The symbol (*) indicates the significance level (α) for the test (* = 0.05, ** = 0.01, and *** = .0001).

7.3.3 Induced plant-Hg accumulation

The results for induced Hg accumulation by *B. juncea* and *Lupinus* sp. as a consequence of the addition of S-containing ligands to Tui mine tailings are shown in Table 7.2 and Figure 7.7. The application of $(NH_4)_2S_2O_3$ 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 $(NH_4)_2S_2O_3$ 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 of dry weight, respectively, after individual addition of $(NH_4)_2S_2O_3$ (Table 7.2).

Table 7.2. Root Hg concentrations (mg/kg) of *Brassica juncea* and *Lupinus* sp. after application S-containing ligands at 2 g/kg (unless otherwise stated) to Tui mine tailings. Values are the mean of 5 replicates ± 1 standard deviation. Letters compare treatments in the vertical. Means with the same letter are not significantly different (Duncan's test at a significance level ($\alpha = 0.01$).

Treatment	N#	Plant Species			
		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
NH_4SCN*	10	**c**	107.15 ± 23.71	**b**	194.93 ± 98.09
$(NH_4)_2S_2O_3$**	10	**c**	255.32 ± 96.68	**b**	104.13 ± 23.24

#Missing values due to below-detection limits for Hg concentrations in sample digests;
*Ammonium thiocyanate was supplemented with hydrogen peroxide at 0.27%.
**Ammonium thiosulphate.

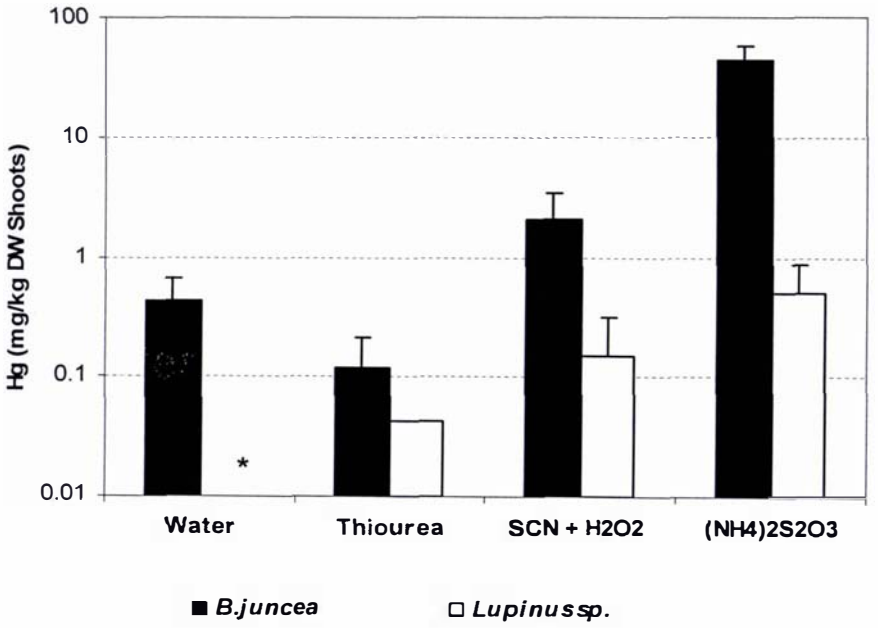


Figure 7.7. Shoot Hg concentrations of *Brassica juncea* and *Lupinus* sp. after application of S-containing ligands at 2g/kg to Tui mine tailings. Note that Water = control, SCN + H2O2 = ammonium thiocyanate + hydrogen peroxide (at 0.27%); $(NH_4)_2S_2O_3$ = ammonium thiosulphate. Bars denote ± 1 standard deviation from the mean of 5 replicates. The symbol (*) indicates Hg below detection levels. DW = dry weight.

Application of $(NH_4)_2S_2O_3$ to substrates also dramatically increased Hg accumulation in shoots of *B. juncea* relative to other treatments (Figure 7.7). Recorded values in aerial tissues of *B. juncea* averaged 43 mg/kg, with a single replicate reaching a value

of 61 mg/kg. By contrast, translocation of Hg to shoots of *Lupinus* sp. was substantially lower when compared to *B. juncea* shoots. The maximum value observed for the *Lupinus* sp. was 0.5 mg/kg in the presence of $(NH_4)_2S_2O_3$ (Figure 7.7). Additionally, the presence of $(NH_4)_2S_2O_3$ 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).

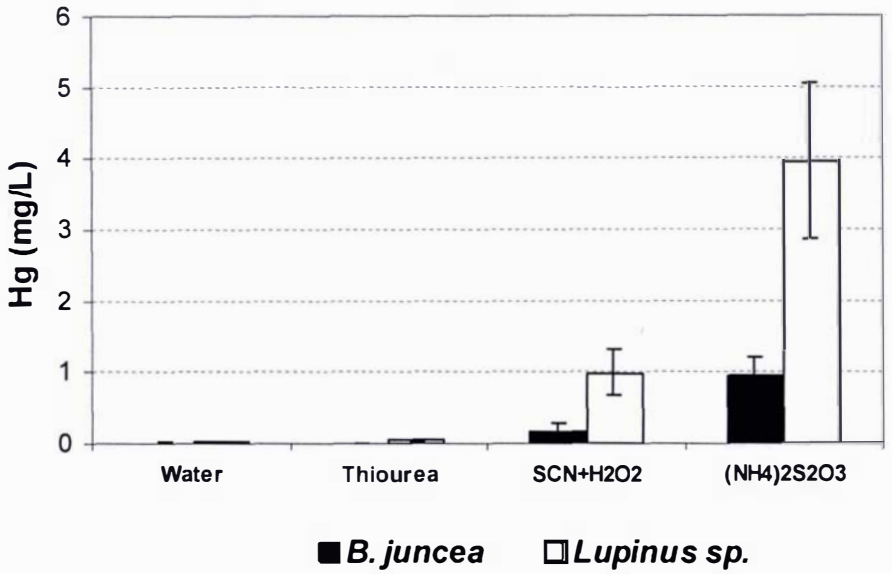


Figure 7.8. Hg concentrations in leachates collected from *Brassica juncea* and *Lupinus* sp. pots after application of S-containing ligands at 2g/kg to Tui mine tailings. Note that Water = control, SCN + H_2O_2 = ammonium thiocyanate + hydrogen peroxide (at 0.27%); $(NH_4)_2S_2O_3$ = ammonium thiosulphate. Bars denote ± 1 standard deviation from the mean of 3 replicates.

The Hg concentration in leachates was enhanced after addition of S-containing ligands to Tui mine tailings (Figure 7.8). Specifically, the application of $(NH_4)_2S_2O_3$ 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 $(NH_4)_2S_2O_3$, 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 7.9 shows a 100% normalized picture of mass balance for plant, soil, leachates, and unaccounted Hg for *B. juncea* and *Lupinus* sp. grown in Tui tailings and treated with $(NH_4)_2S_2O_3$. The unaccounted 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 $(NH_4)_2S_2O_3$, the Hg mass balance for both species was compared only within this treatment level.

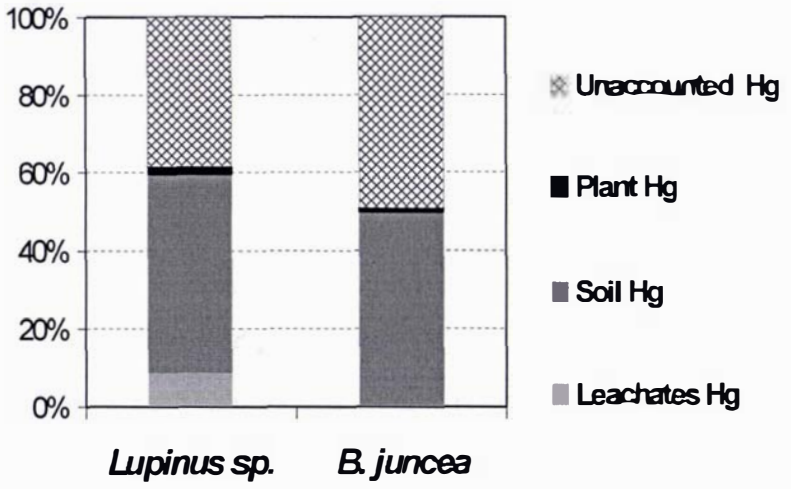


Figure 7.9. Normalised (100%) values of plant, soil, leachates and unaccounted Hg from *Brassica juncea* and *Lupinus* sp. after application of ammonium thiosulphate at 2g/kg to Tui mine tailings. Note that 100% = total Hg mass in control pots (564 μg).

The existence of an Hg fraction that could not be accounted for indicates the added Hg has been transformed to a form of Hg not measured after the addition of $(NH_4)_2S_2O_3$. Therefore, it is possible to infer that the unaccounted Hg fraction might be due to Hg (0) volatilisation from plant leaves or roots and their associated microbes (Barkay et al., 1992; Leonard et al., 1998, 1994; Meagher et al., 2000). Additionally, because photochemical and chemical reduction processes are significant mechanisms for Hg (0) emissions from contaminated sites (Andersson, 1979; Morel, et al., 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 7.6) provides support for this assumption. Finally, the existence

of a volatilised 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 7.6).

7.4 Discussion

The high sulfide content (>10%) and the presence of the sulfide-bearing minerals galena (PbS), sphalerite (ZnS), 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 Fe-sulfides. In addition, trace quantities of cinnabar have been shown to be present in the Tui ore (Morrel, 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). However, our studies showed the presence of significant amounts of water extractable Hg in the Tui tailings (Figure 7.3). 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 lead to acid mine drainage production. As a result, the surface tailings exhibit variable but low pH (2.76 – 3.85) and high concentrations of total sulfur (S) and sulfates (SO_4^{2-}) (Morrel, 1996). The presence of significant amounts of water-soluble Hg species in the Tui tailings indicates, 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 S-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 risks to the environment.

Average background levels of mercury in plants are usually not greater than 100 ng/g (ppb) dry weight (Kabata-Pendias and Pendias, 2000). Through addition of $(NH_4)_2S_2O_3$ to Tui mine tailings, *B. juncea* was able to accumulate over 400 times this value in the aerial tissues. Additionally, Hg analysis in New Zealand native plants grown on Tui Mine tailings showed Hg values below detection levels for above-ground plant tissues

(data not shown), thus highlighting the effect of $(NH_4)_2S_2O_3$ on shoot Hg accumulation. The greater ability of $(NH_4)_2S_2O_3$ to enhance Hg shoot accumulation over other S-containing ligands appears to be genotype 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 $(NH_4)_2S_2O_3$ under the moderately acidic conditions prevailing in Tui mine tailings effectively reduced Hg retention in cell wall of *B. juncea* roots, thus facilitating Hg translocation to aerial tissues.

The volatilisation of Hg by plants has been the subject of many studies that have attempted to investigate the behaviour of Hg at the soil-plant-atmosphere interface (Siegel et al., 1974; Siegel and Siegel, 1979; Leonard et al., 1998). Indeed, Leonard et al. (1998), in a carefully designed system for studying Hg plant 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 Hg fraction lost from the system (Figure 7.9). We have overcome this problem in subsequent studies by using gastight volatilisation systems carefully designed to account for Hg losses from hydroponically grow *B. juncea* plants (Moreno et al., 2003[14]). The results demonstrated that the volatilisation 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 rose 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 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 volatilisation.

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

[14] Paper described in the Chapter 4 of this thesis;

solubilising 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 $(NH_4)_2S_2O_3$ and SCN + H_2O_2 to substrates (Figure 7.8). 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 EDTA-assisted phytoremediation operation (Madrid et al., 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; Paterson and Schnoor, 1993; Robinson et al., 2003).

Another issue of concern is the potential toxicity of S-containing chemical ligands to fauna and flora. It has been demonstrated, however, that thio-complexes are readily broken down by soil microorganisms and that thiosulphate salts are only slightly toxic to plant and animals (Anderson et al., 2000). The lethal concentration (LD_{50}) of sodium thiosulphate in rabbits, for instance, is 4000 mg/kg, a value that indicates a toxicity level similar to that of sodium chloride in rats (LD_{50} = 3000 mg/kg) (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 prevails 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 Hg soil concentration by 0.25 mg/kg. Those calculations do not take into account Hg losses from the system as a result of leaching or volatilisation.

7.5 Conclusions

In this work, we have effectively induced plants to accumulate Hg from a contaminated metal waste by treating the substrate with S-containing complexing agents. 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 (CF = 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 volatilisation of Hg by plants established on site in conjunction with periodic removal of Hg-containing plant material after treatment with $(NH_4)_2S_2O_3$. The results of this preliminary study, however, should be validated under field conditions where Hg plant uptake 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 on Tui mine tailings, whereas additional plant pot trials using gastight volatilisation chambers will the address the effect of $(NH_4)_2S_2O_3$ on the plant-Hg volatilisation process.

Ethical issues around the use of volatilisation as a remediation technique are discussed in Chapter 10.

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CHAPTER 8

MERCURY VOLATILISATION AND PHYTOEXTRACTION FROM BASE-METAL MINE TAILINGS

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Abstract

Laboratory, greenhouse and field studies were conducted to investigate plant-mercury accumulation and volatilisation in the presence of thiosulphate-containing solutions. *Brassica juncea* (Indian mustard) plants grown in Hg-contaminated Tui mine tailings were enclosed in gastight volatilisation chambers to investigate the effect of ammonium thiosulphate ($[NH_4]_2S_2O_3$) on the plant-Hg volatilisation process. Application of $(NH_4)_2S_2O_3$ to substrates caused a significant increase in the Hg concentration in shoots and roots of *B. juncea*. Volatilisation rates were significantly higher in control plants than in $(NH_4)_2S_2O_3$-treated plants. Mercury volatilisation from control pots (without plants) was effected by biological (Hg-resistant bacteria) and chemical (photoreduction) transformations. Addition of sodium thiosulphate ($Na_2S_2O_3$) at 5 g/kg of substrate to *B. juncea* plants grown at the Tui mine site confirmed laboratory and greenhouse studies showing the effectiveness of thio-solutions at enhancing shoot Hg concentrations. Mercury extraction from the field plots yielded a maximum value of 25 g/ha. Mass balance studies revealed that spontaneous phytovolatilisation is a dominant pathway for Hg removal from the Tui mine site. A preliminary assessment of the risks of phytovolatilisation indicated that enhanced Hg emissions by plants would not harm the local and the regional environment.

8.1 Introduction

Mercury (Hg) is a global pollutant that cycles between air, water and soil as a result of natural processes and anthropogenic activities. Although anthropogenic Hg emissions have been reduced by half since the 1980's (Pacyna et al., 2001), ongoing Hg

contamination is still a worldwide problem. Depending on the Hg source and the form of discharge, Hg in the environment may be present in concentrated hot spots or dispersed over large areas (Hinton and Veiga, 2001). *Ex situ* remediation technologies such as excavation, physical separation, and hydrometallurgical treatments are expensive, particularly if Hg contamination is spread over a large area or extends below the water table. Thermal treatment (soil heating combined with soil vapour extraction) can be effective for Hg removal from solid media but is technically complicated and also costly. Furthermore, soil heating releases Hg as a vapour into the environment and can have deleterious effects on the physical, chemical and biological properties of soils (Meagher et al., 2000; Hinton and Veiga, 2001). Phytoremediation, on the other hand, uses plants to rehabilitate degraded environments. By using free services provided by nature (energy from the sun and CO_2 from the atmosphere), plants species can extract nutrients, accumulate heavy metals and radionuclides, and transform or degrade some organic contaminants more economically than current available chemical or physical technologies (Raskin et al., 1994; Schnoor et al., 1995; Robinson et al., 1998).

The discovery of unusually high concentrations of nickel in the small Tuscan shrub *Alyssum bertolonii* in late 1940's (Minguzzi and Vergnano, 1948), eventually lead to the development of phytoextraction. This technology employs metal hyperaccumulator plant species (among others) to remove metals from soils by accumulating them in the aerial parts of the plant. By definition, hyperaccumulator plants accumulate one or more metals to concentrations at least a 100 times higher than 'normal' plants growing in the same environment (Brooks, 1998). The hyperaccumulation of metals by plants has been catalogued for Ni, Cd, Cu, Co, Mn, Se, Tl and Zn (Brooks, 1998). However, there are no reports of plants that naturally hyperaccumulate Hg, a toxic pollutant with low solubility in soil and limited availability for plant uptake. Two different approaches have been proposed to foster plant-based systems for the remediation of Hg-polluted soils. The first approach involves the use of plants in which gene sequences from Hg-detoxifying bacteria have been inserted into their genome, increasing Hg resistance and enhanced volatilisation capacity. These transgenic plants are able to extract Hg (II) and methylmercury from contaminated soils and sediments and to convert these forms to less toxic and volatile Hg (0) (Rugh et al., 1996; Bizily et al., 1999, Meagher. 2000). The second approach uses non-toxic thio-containing solutions to induce Hg accumulation into above ground tissues of high-biomass plant species (Moreno et al.,

2004 a[15]). Ammonium thiosulphate has been used to induce *B. juncea* to accumulate 40 mg Hg/kg of shoot tissue from mine tailings contaminated with 2.8 mg Hg/kg. Mass balance studies, however, revealed that a substantial Hg fraction that could not be accounted for. This unaccounted fraction suggested that Hg (0) was volatilised from the substrate as a result of biological and chemical transformations. In this work we describe laboratory, greenhouse and field studies on the thiosulphate-induced accumulation and volatilisation of Hg by *B. juncea* plants growing in an Hg-contaminated mine tailings.

8.2 Material and Methods

8.2.1 Substrate characterization

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

8.2.2 Plant growth conditions

Tui substrates were fertilised with 5 g/kg of Osmocote (slow release fertiliser) and amended with lime to adjust the pH to 5.5. Replicate plastic pots (250 mL) were filled with the growth substrate and sown with seeds of *B. juncea* at a rate of ca. 20 seeds per pot. Pumice was added in a 3:1 ratio to improve drainage of the substrate. Two weeks

[15] This paper is described in the previous chapter of this thesis.

after germination, each pot was thinned to leave only one individual plant. Hoagland's nutrient solution (5 mLs of ¼ strength) (Hoagland and Arnon, 1950) was irrigated onto the pots every second day to supplement plant nutritional requirements. Plants were kept in a greenhouse with temperature ambient set at 15-25°C with no humidity control. Unplanted substrates were used as controls. Pot positions were randomly changed on a periodic basis to equalize light exposure. Daily watering was carried out with a hand-held hose.

8.2.3 Extractable Hg

Extractable Hg concentrations in Tui mine tailings were determined through the use of $(NH_4)_2S_2O_3$ and $Na_2S_2O_3$ as chemical extractants. One gram of tailings was weighed into 50 mL polypropylene centrifuge tubes in triplicate. After addition of 20 mL of extractant solutions (at 2 and 10 g/L), the tubes were rotated on an end-over-end shaker overnight at 45 rotations per minute (RPM) and the supernatant separated via centrifugation at 3000 RPM for 3 minutes.

8.2.4 Effect of ammonium thiosulphate on Hg volatilisation

After 5 weeks of plant growth, 5 mL of $(NH_4)_2S_2O_3$ solution was applied to replicate pots to give a concentration of 1g thiosulphate per kg of substrate (n=3). Water was used as a control treatment for pots with and without plants. Each plant was enclosed within a gastight acrylic volatilisation chamber (3.6 L volume). Volatile Hg was captured in an acid trap solution containing 5% $KMnO_4$ dissolved in 2N H_2SO_4 (Figure 8.1). The efficiency of this trap solution to quantitatively capture Hg (0) has been shown to vary between 95 to 99% (Kimura and Miller, 1960). A continuous airflow was supplied to the volatilisation chamber using a small air pump. Mercury vapour released by a plant was driven together with the incoming air into an Erlenmeyer flask containing 70 mL of the acid trap solution. The flow rate of the incoming air was monitored using an air flow meter (J&W, model AMD 1000) and was constantly held to 100 mL/min using small clamps attached to the air outlets. The outlet of the acid trap was open to the atmosphere to maintain pressure equilibrium within the trap system. A 10 mL syringe attached to the volatilisation chamber was used to water the plants during the period of volatile Hg collection. Watering was carefully performed to avoid

losses of Hg by leaching. Volatilisation was measured over a three day period in a plant growth cabinet with temperature and photoperiod set to 22°C and 14 hours, respectively. Collection of volatile Hg was done in triplicate both for $(NH_4)_2S_2O_3$-treated and control plants and for control pots. At the end of this period, the acid trap solution was transferred to 100 mL air-tight plastic containers and stored at 4°C until analysis. The precipitated fraction of the trap was redissolved in 50 mL of concentrated hydrochloric acid, and the resulted solution was preserved following the same procedure. The volatile Hg mass collected for each replicate was, therefore, the sum of Hg readings in the soluble and precipitated fractions of the acid trap. The use of this experimental apparatus has allowed Hg recoveries around 90 % for *B. juncea* plants cultured in Hg-spiked solutions (Moreno et al., 2004 b[16]).

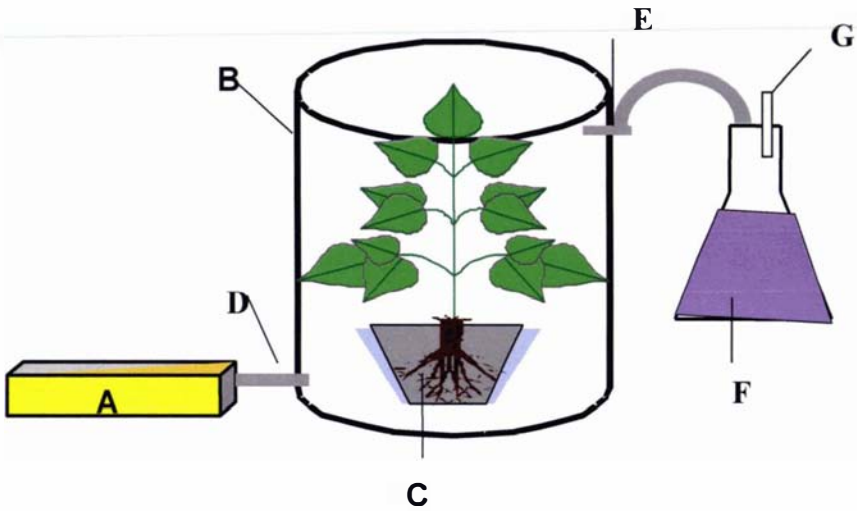

Figure 8.1. Experimental unit used for trapping Hg released from plants. A, air pump; B, gas tight plant chamber; C, plant pot ; D, air inlet; E, air outlet; F, inorganic Hg vapour trap; G air outlet of the trap.

8.2.5 Effect of ammonium thiosulphate levels on induced plant-Hg accumulation

An additional set of 5 week-old *B. juncea* plants growing in the Tui mine tailings was treated with $(NH_4)_2S_2O_3$ at application rates of 0, 1, 2, 5, and 10 g/Kg. The experiment utilized a completely randomised design with 4 replicates per treatment level. Plants that received only water were designated as controls. The experiment was carried out

[16] This paper is described in Chapter 5 of this thesis.

for 5 days in a greenhouse with temperature controlled at 21 °C under a natural sunlight flux.

8.2.6 Induced Plant-Hg Accumulation Field Trials

Three field plots with dimensions of 5 x 5 m were established at the tailings dam of the abandoned Tui base-metal mine. The plots were fertilized with NPK fertiliser at 75 g/m^2 and had their pH adjusted to 5.5 by addition of lime. Organic matter in the form of compost was added at a rate of 3.2 L/m^2. *B. juncea* seeds were planted in two rows of 5 m length (0.5 m width) after each plot was tilled to 15 cm depth. Collection of soil samples (n = 4) was carried out at the 0-15 cm depth for each plot just after seeding. After 6 weeks of plant growth, $Na_2S_2O_3$ in the form of a solution (w/v) was applied to the field plots at a concentration of 5 g/kg of substrate (unless otherwise stated). Plant samples that were collected from each plot prior to the treatment application were used as controls. Two weeks after the treatment, biomass from the plots was harvested and processed for Hg analyses.

8.2.7 Plant harvest

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

8.2.8 Plant digestion

Ground shoots and roots were accurately weighed (0.1 g) into 50 mL plastic beakers. Concentrated HNO_3 (15 mL) was then added. The digest samples were left overnight and, in the following day, were heated in a water bath at 80°C for 1 hour. Digest

solutions were transferred to 10 mL polythene tubes and diluted with reverse osmosis (RO) water to make a final volume of 10 mL. A blank reagent was used with all digestions.

8.2.9 Substrate digestion

The total Hg concentration in tailings sub samples was determined through *aqua regia* digestions of dried substrate. One gram of substrate was weighed into 50 mL polypropylene beakers 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 1 hour and the filtrates diluted to a final volume of 50 mL using RO water.

8.2.10 Mercury analysis

Total Hg in plant and tailings digests and in extractant and trap solutions was analysed using Hydride-generation atomic absorption spectroscopy (Moreno et al., 2004 c[17]). The analysis was performed using a GBC 909A AAS (Victoria, Australia) operating in the flame mode. A sodium borohydride solution (5% $NaBH_4$ + 1% KOH) was used to generate Hg vapour. The limit of detection (LOD) for mercury in solution was 10 ng/mL for plant digests and 5 ng/mL for soil digests and extractant and trap solutions. The Hg readings obtained from the replicate analysis (n=10) of a standard solution containing 1 mg/L of Hg could be reproduced with less than 5% of variation. Reagent blanks were below detection limits in the solution. Linear calibration curves were obtained over the range of 125 to 1000 ng/mL of Hg using 4 standards prepared from a 10 mg/L mercuric nitrate ($HgNO_3$) spectrosol solution (May & Baker, AAS reagent standard solution). The analytical method was assessed for quality control by an external certified laboratory with agreements ranging from 85 to 103% for Hg-containing solutions and Hg-containing plant samples (Moreno et al., 2004 c).

[17] This paper is described in the Chapter 3 of this thesis.

8.2.11 Statistical analysis

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

8.3 Results and Discussion

8.3.1 Volatilisation and induced plant-Hg accumulation pot trials

Figure 8.2 shows the daily Hg volatilisation rates for $(NH_4)_2S_2O_3$-treated and control *B. juncea* plants on a mass (A) and per kg dry weight (B) basis. In both cases, the Hg volatilisation rates from control plants significantly exceeded the Hg volatilisation rates from $(NH_4)_2S_2O_3$-treated plants by a factor 3 ($p < 0.05$). The Hg volatilisation rate from control pots (without plants) was significantly lower than from control plants and $(NH_4)_2S_2O_3$-treated plants ($p < 0.05$) (Figure 8.2A).

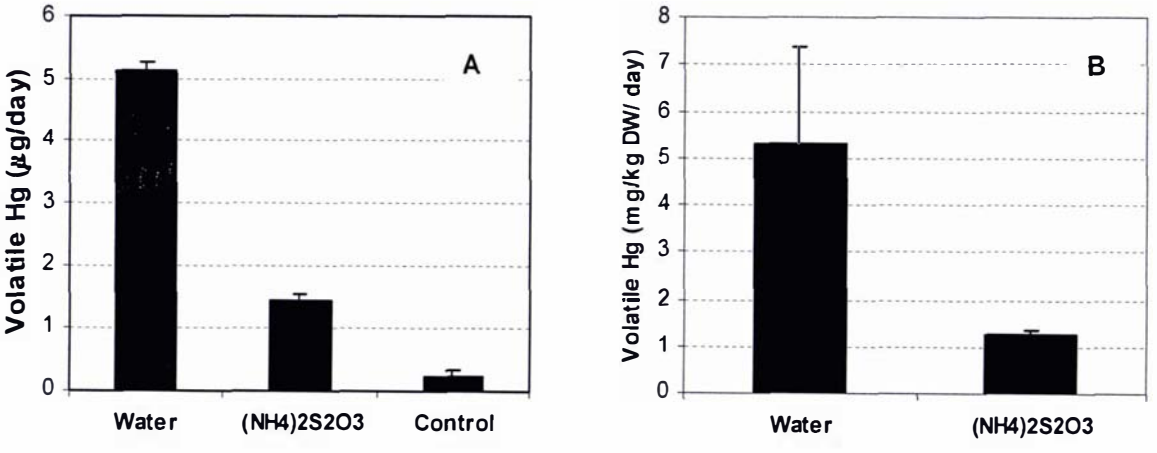

Figure 8.2. Volatilisation rates from *B. juncea* plants grown in Tui mine tailings treated with ammonium thiosulphate ($[NH_4]_2S_2O_3$) at 1 g/Kg. (A) Total Hg mass (µg/ day) emitted from plants and control pots (without plants) and (B) Total Hg mass emitted from plants per unit dry weight (kg) per day. Bars denote ± 1 standard deviation from the mean of 3 replicates. Note that water = control plants, control = pots without plants, DW = dry weight.

The Hg efflux from control pots was on average 23 times lower than the Hg efflux from control plants and around 6 times lower than $(NH_4)_2S_2O_3$-treated plants. These Hg emissions may be due to biological transformations (microorganisms) as well as to chemical reactions (photochemical and chemical reduction).

Figure 8.3 shows the Hg concentration in root (A) and shoot (B) tissues for both $(NH_4)_2S_2O_3$-treated and control plants. It is clear that the induced plant-Hg accumulation and volatilisation processes are inversely related to each other. For instance, $(NH_4)_2S_2O_3$-treated plants accumulated a significantly higher Hg concentration than control plants for both shoot and root tissues ($p < 0.05$). Recorded values for shoot Hg concentrations were 4.63 ± 1.4 and 1.14 ± 0.57 mg/kg for $(NH_4)_2S_2O_3$-treated and control plants, respectively (Figure 8.3 A). The difference between the treatments was amplified for root tissues, where Hg values for $(NH_4)_2S_2O_3$-treated plants were 6 times superior to control plants (Figure 8.3 B).

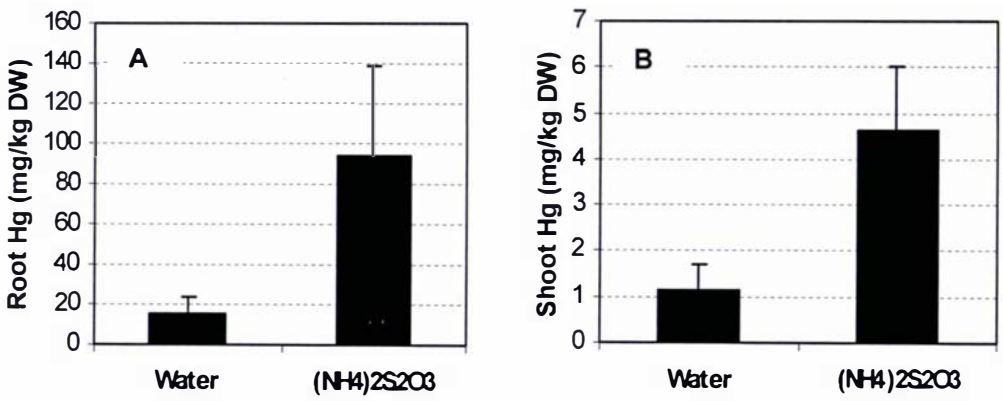

Figure 8.3. Root (A) and shoot (B) Hg concentrations of *B. juncea* plants after addition of ammonium thiosulphate ($[NH_4]_2S_2O_3$) at 1 g/kg to Tui mine tailings. Bars denote ± 1 standard deviation from the mean of 3 replicates. Note that water = control, DW = dry weight.

Figure 8.4 shows total (A) and extractable (B) Hg concentrations (mg/kg) in the Tui mine tailings after plant growth and $(NH_4)_2S_2O_3$ treatment. A lower total Hg concentration was found in the $(NH_4)_2S_2O_3$-treated substrate. However, this difference was not significant ($p > 0.05$, Figure 8.4 A). Conversely, there was a significant difference between the two treatments for the extractable Hg concentrations (p =

0.0032) (Figure 8.4 B). The control and $(NH_4)_2S_2O_3$-treated substrates had extractable Hg concentrations of 1.95 and 1.3 mg/kg, respectively.

Assuming the water-soluble Hg as the fraction available for plant uptake before application of $(NH_4)_2S_2O_3$ to tailings, then the Hg discrepancy between both substrates may be due to plant uptake of the sulfidic Hg fraction mobilised after application of $(NH_4)_2S_2O_3$ to pots.

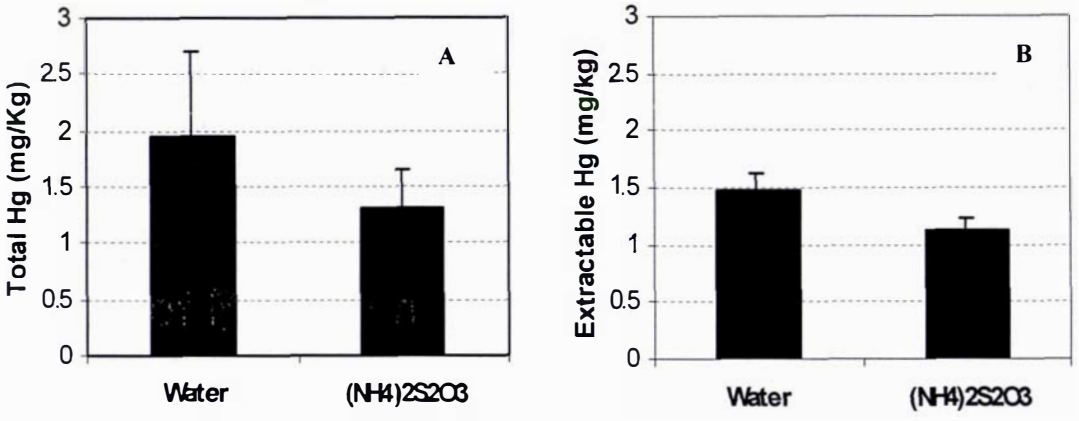

Figure 8.4. Total (A) and extractable (B) Hg concentrations in Tui mine tailings after growth of *B. juncea* plants and application of ammonium thiosulphate ($[NH_4]_2S_2O_3$) at 1 g/kg. Bars denote ± 1 standard deviation from the mean of 5 replicates. Note that water = control.

Figure 8.5 (A) describes the Hg distribution between the air (Hg trap), plant and substrate compartments for $(NH_4)_2S_2O_3$ and control treatments at the end of the experiment. The values are expressed as the percentage of the Hg mass in each compartment and thus, 100% is the total sum for the respective Hg fractions in substrate, plant and traps. The total Hg mass in the $(NH_4)_2S_2O_3$-treated substrate was about 13% lower compared to the control treatment. The Hg mass fraction accumulated in plant tissues comprised around 20 % of the total Hg mass for the $(NH_4)_2S_2O_3$-treated system whereas in the control system this Hg fraction corresponded to less than 5%. Conversely, the volatilised Hg fraction in the control system comprised around 8% of the total Hg mass whereas in the $(NH_4)_2S_2O_3$-treated system this fraction corresponded to 2%.

The effect of $(NH_4)_2S_2O_3$ on both Hg accumulation and volatilisation processes is emphasised in Figure 8.5 (B) by the comparison of the Hg mass distribution between roots, shoots and trap compartments at the end of the experiment. It is apparent that most of the Hg mass in the control system (around 75%) is preferably volatilised to the air in detriment to the plant-Hg accumulation process. After application of $(NH_4)_2S_2O_3$ to the substrates, however, this pattern changes dramatically with most of the Hg mass (around 80%) being retained in root tissues.

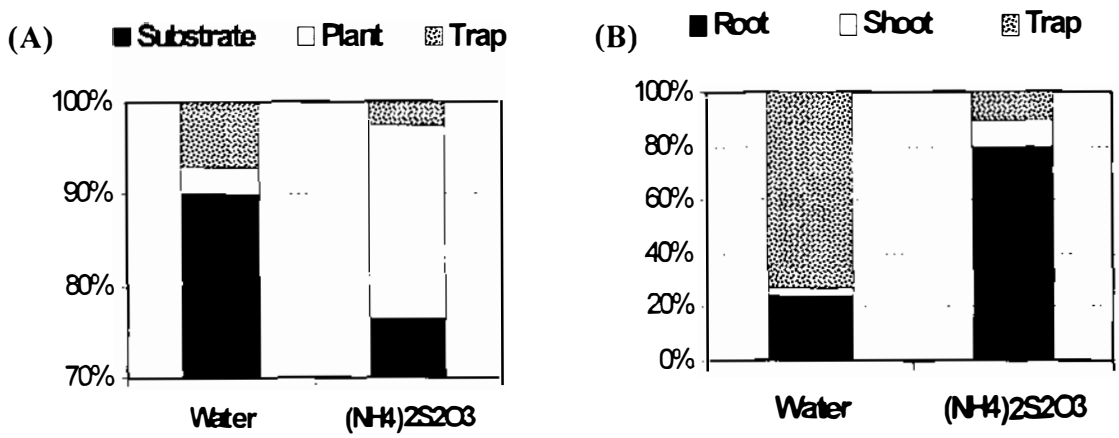

Figure 8.5. Mercury Distribution in the air-plant-soil system after application of ammonium thiosulphate ($[NH_4]_2S_2O_3$) at 1 g/kg to Tui mine tailings. Normalized values (100 %) represent the Hg mass in substrate, plant and acid trap (A) and the Hg mass partition between root, shoot and trap compartments (B). Note that 100% is the sum of Hg mass in substrates, plant (shoot + root) and acid trap.

The induced plant-Hg accumulation in the roots and shoots of *B. juncea* as a function of different concentrations of $(NH_4)_2S_2O_3$ added to substrates is shown in Figure 8.6 (A and B). The concentration of Hg in root tissues exceeded the Hg concentration in aerial tissues for all tested $(NH_4)_2S_2O_3$ concentrations. The Hg concentration in root tissues was greater at lower application rates of $(NH_4)_2S_2O_3$ and reached a maximum average of 243 ± 200 mg/kg at 1g/kg of $(NH_4)_2S_2O_3$ applied to substrates ($p < 0.05$). At 5 and 10 g/kg of $(NH_4)_2S_2O_3$, on the other hand, the Hg concentrations in root tissues dropped to around 50 mg/kg (Figure 8.6 A). The Hg accumulation pattern was different for shoot tissues, where maximum Hg concentrations were observed at intermediate application rates of $(NH_4)_2S_2O_3$. At application rates of 2 and 5 g/kg, for instance, shoot Hg concentrations averaged around 15 mg/kg, whereas at 1 and 10 g/kg of $(NH_4)_2S_2O_3$

shoot Hg values reduced significantly by a factor of 2 ($p < 0.05$) (Figure 8.6 B). The lack of a significant correlation between root and shoot Hg values ($p > 0.05$) in the presence of $(NH_4)_2S_2O_3$ indicates that translocation of Hg into shoots of *B. juncea* was not a function of the amount of Hg taken up by roots (data not shown). We, therefore, believe that reduced levels of Hg in shoot tisseus at higher thiosulphate concentration were due to the toxic effects of this sulphur-containing ligand.

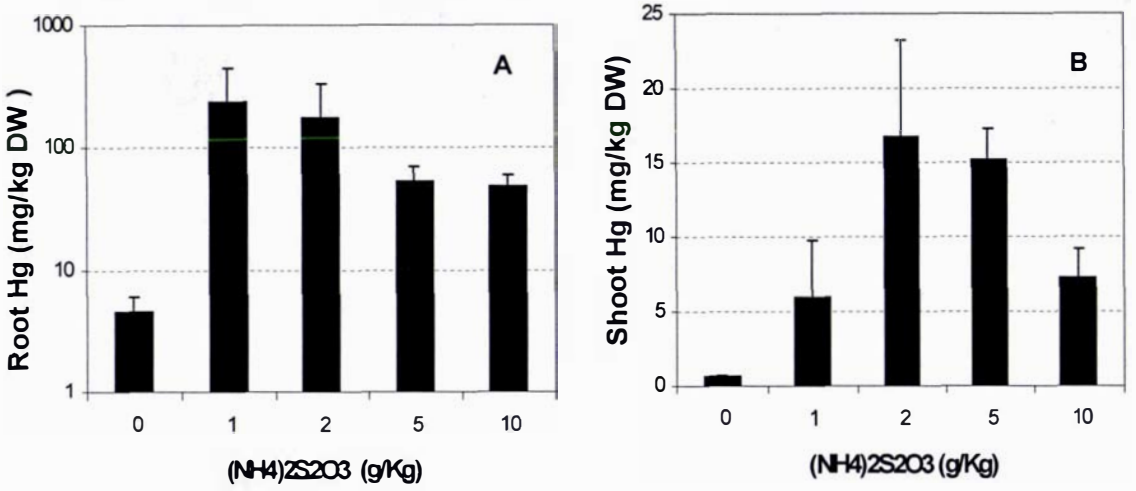

Figure 8.6. Root (A) and shoot (B) Hg concentrations of *B. juncea* plants after addition of increasing concentrations of ammonium thiosulphate ($[NH_4]_2S_2O_3$) to Tui mine tailings. Bars denote ± 1 standard deviation from the mean of 4 replicates. DW = dry weight.

8.3.2 Induced plant-Hg accumulation field trials

The results for the field experiment conducted at the three plots are described in Figure 8.7 and Table 8.1. The addition of $Na_2S_2O_3$ to substrates enhanced Hg solubility, leading to increased Hg accumulation in roots and shoots of *B. juncea*. The extractable Hg concentration rose proportionally to increasing concentrations of $Na_2S_2O_3$ added to the substrates (Figure 8.7). Thiosulphate treatment induced a significant increase in root and shoot Hg concentrations relative to control plants, which had shoot and root Hg values below detection levels. Root Hg concentrations between the plot locations ranged from 9 ± 0.8 to 17 ± 2.7 mg/kg for $Na_2S_2O_3$-treated plants. Mercury concentrations in shoots of *B. juncea* were higher in plot number 2, where the assayed value averaged 9.7 ± 1.25 mg/kg (Table 8.1). For plot number 3, the average Hg value

for shoots was unexpectedly low (2.9 ± 0.7 mg/kg) despite the elevated levels of soluble Hg recorded in the $Na_2S_2O_3$ extracts. The fact that the Hg content of roots was greater than shoots for all tested plots indicates that application of $Na_2S_2O_3$ to substrates increased the retention of Hg by the root tissues. This trend was particularly evident for plot number 3, where the roots accumulated almost 6 times more Hg than the shoots.

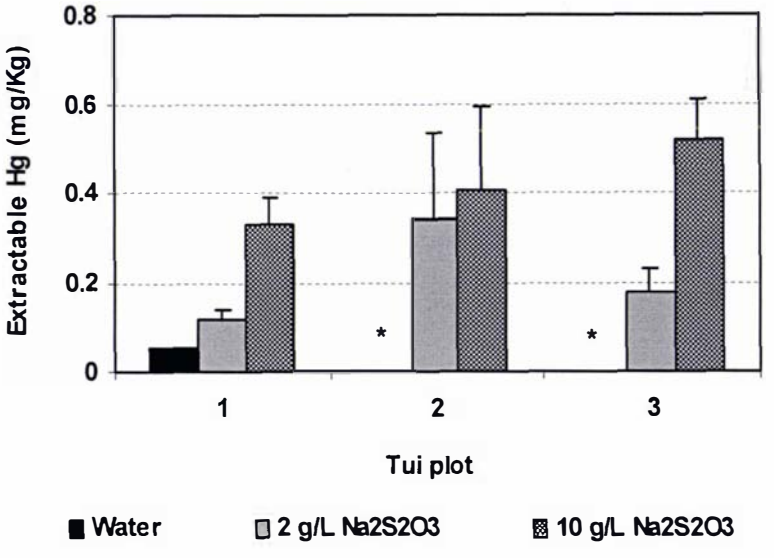

Figure 8.7. Extractable Hg concentrations as a result of sodium thiosulphate ($Na_2S_2O_3$) application to Tui mine tailings at concentrations of 2 and 10 g/L. Bars denote ± 1 standard deviation from the mean of 3 replicates. Note that (*) means Hg below detection levels.

Table 8.1. Phytoextraction results for the Tui mine tailings after application of sodium thiosulphate ($Na_2 S_2O_3$) at 5 g/kg (unless otherwise stated) to Tui tailings field plots*.

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

*Values are the mean ± 1 standard deviation from 5 replicates
[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.

The phytotoxic conditions that prevail in the substrates of the Tui base metal mine did not prevent plant growth on site (Figure 8.8). Estimates of biomass production indicated a maximum dry matter yield of around 2.5 tonnes per hectare (Table 8.1). Considering that plants on site had a relatively short growing season (6 weeks) and that the sulphide-rich tailings of the Tui mine exhibit high levels of the toxic metals Zn, Cu, Mn, Pb and Ag (Morrell et al., 1996), the low levels of plant biomass production on this site are unsurprising. As a result, Hg-extraction yields were also low. Due to its highest biomass production and shoot Hg concentration, the maximum Hg-extraction yield was achieved at plot number 2 with an average value of 24.3 ± 3.1 g/ha (Table 8.1). This Hg-extraction yield was over 30 times the maximum yield obtained for *Hordeum vulgare* (barley), which extracted 0.71 g Hg/ha from an Hg-contaminated site in Almaden, Spain (Rodriguez et al., 2003).

Figure 8.8. Experimental field plot at the Tui mine tailings (North Island, NZ) before application of sodium thiosulphate ($Na_2S_2O_3$) to the substrate. Biomass for *B. juncea* plants grown at the toxic tailings yielded a maximum of 2.5 t/ha.

Since the quantitative capture of Hg in the permanganate acid solution involves oxidation of elemental Hg (0) to ionic Hg (II) (Moreno et al., 2003[18]), then we would presume that the predominant Hg form released from *B. juncea* plants was the

[18] See chemical equation described in Chapter 4.

elemental vapour Hg (0). The Hg volatilisation from Tui mine tailings (as shown for control pots, Figures 8.2 A and 8.3A) may be the result of on site biological and sunlight-mediated reduction of Hg (see Morel et al., 1998 for a detailed explanation on Hg photoreduction). The role of bacteria on Hg volatilisation from Hg-contaminated environments has been well documented (Barkay, 1987; Barkay et al., 1991; Barkay et al., 1992; Saouter et al., 1994). The 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 disulfide oxireductase known as mercuric reductase (MR) that catalyses the reaction:

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

The MR removes Hg from stable thiol salts [$Hg(SR)_2$] by eletrochemical reduction in a NADPH-coupled redox reaction (equation 1). A small proportion of Hg (II) resistant bacteria are also resistant to organic Hg forms due to the activity of the organomercurial lyase (OL). The OL cleaves the carbon-Hg bond (R') by a protonolytic attack, as follows:

$$R'HgSR \longrightarrow Hg(SR)_2 + R'H \qquad [3]$$

The product of this reaction, $Hg(SR)_2$, is then reduced by MR to Hg (0). This strategy has been shown to be an effective solution to bacterial Hg-exposure, as elemental Hg is the least toxic and the most volatile form of Hg. The fact that planted substrates volatilised more Hg than the barren pots (Figure 8.2 A) indicates that plants can substantially increase the atmosphere Hg emissions from the Tui mine site. The enhanced Hg volatilisation can be explained as the result of biological Hg reduction carried out by Hg (II)-resistant bacteria living in the rhizosphere, the main site for Hg volatilisation from the plant (Moreno et al., 2004 b[19]). Alternatively, volatile Hg might have been emitted from volatilisation sites (mesophyll cell surfaces) inside the leaves (Gustin et al., 1997; Leonard et al., 1998). The interaction between Hg-resistant bacteria and plants may be considered as beneficial to plants, allowing them to grow in an Hg-contaminated environment. In return, plants stimulate microbial activity in the

[19] This paper is described in the Chapter 5 of this thesis.

root zone by releasing organic carbon and nutrients, a process that is called "rhizosphere effect" (Bowen and Rovira, 1991).

Previous studies have shown that the Tui mine tailings has an average water extractable Hg concentration of 0.13 ± 0.11 mg/kg of substrate (Moreno et al., 2004 a[20]). We hypothesize that the water-soluble Hg fraction of Tui tailings constitutes the available Hg pool for both plant uptake and microbial transformations. Geochemical conditions in the tailings profile have oxidised sulfidic Hg minerals such as cinnabar (Morell et al., 1996), thus releasing Hg (II) ions to this pool. Amendment of the substrate with $(NH_4)_2S_2O_3$ or $Na_2S_2O_3$ will extract Hg bound to the solid phase of sulfidic minerals and increase the concentration of soluble Hg (Figure 8.7).

According to Duckwart et al. (1992), when plant roots and microbes compete for the same pool of available selenium (Se), the rates of microbial transformation and plant uptake become dependent processes. When Se was added to the soil in the form of readily plant-available Na_2SeO_4, enhanced uptake of the metalloid by plants depleted the pool of soluble Se available for microbial Se volatilisation. In our experiments the addition of 1 g/kg of $(NH_4)_2S_2O_3$ mobilised the sulfidic-extractable Hg pool and enhanced plant Hg uptake and translocation to the detriment of microbial volatilisation rates. The fact that control plants did not change the sulfidic Hg fraction in substrates towards the end of the experiment (Figure 8.4 A and B) indicates that the water-extractable Hg fraction is a common pool for both plant uptake and Hg volatilisation processes. Therefore, the addition of $(NH_4)_2S_2O_3$ altered the Hg volatilisation pathway in Tui tailings to the extent that the Hg mass taken up by plants exceeded the volatile Hg mass produced by biological and chemical transformations.

Results from the thiosulphate-induced plant-Hg accumulation experiments described in this chapter indicate that Hg removal from the Tui mine tailings can be accomplished by both phytoextraction and phytovolatilisation. Using the highest values for biomass production and plant-Hg accumulation following application of $Na_2S_2O_3$ on site, the maximum Hg phytoextraction yield averaged around 25g Hg/ha (Table 8.1). However, since Hg phytovolatilisation occurs spontaneously from the plant-soil system before

[20] As discussed in Chapter 7 of this thesis.

and after amendment of the Tui substrate, we estimated the mass of Hg emitted to the atmosphere by *B. juncea* plants grown on site. Assuming an average volatilisation rate of 5.5 mg Hg/kg of plant tissue per day (Figure 8.2 B) and an average biomass of 2.5 tonnes (Table 8.1), then around 14 g Hg/ha were daily emitted to the atmosphere of the Tui mine site before the application of thiosulphate. Assuming that these Hg emissions occurred over a 30 days period, then, by extrapolation, an equivalent value of at least 420 g Hg/ha were emitted to the atmosphere of Tui mine tailings during the experimental period. If we include the plant-Hg volatilisation rates after the amendment was applied to the substrate (mean of 1.2 mg Hg/ kg plant tissue per day), then the total value increases to 500 g Hg/ha.

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

Given the fact that *B. juncea* plants volatilise significant amounts of Hg (0) to the air, the implementation of a phytoremediation system for the removal of Hg at the Tui site would require a rigorous assessment of the environmental fate of the volatilised Hg. It should not be forgotten, however, that bacterial reduction and photochemical and chemical reduction processes happening at the Tui site have been already emitting Hg to the atmosphere at an average efflux rate of 0.23 μg Hg/kg per day (Figure 8.2 A). Considering that the Tui tailings contains 364 kg of Hg in 100 000 m^3 and assuming that the modelled process in the top 30cm is true for the entire 10m depth profile, then it would take 2,426 years for this natural process to remove most of Hg from the tailings dam. Since revegetation of the Tui site supports three plant-growing seasons per year, the presence of *B. juncea* plants *on site* would enhance these emissions 23-fold, reducing the clean up process to 105 years. However, because the active zone for nutrient uptake is limited by root growth and *B. juncea* roots do not extend below 0.5 m depth, plants will only remediate surface soils. If we assume a total Hg mass of 20 kg in the 0-0.5 m depth profile of the Tui mine and constant volatilisation rates, then is feasible that plants will remove most of this Hg mass in around 13 years.

The 23-fold increase in Hg emissions caused by the plants would represent an efflux rate of 1.5 kg of Hg per year to the atmosphere. What would be the risks of this

technology to the local environment and human population in terms of Hg (0) exposure? To pose a significant risk, the level of atmospheric Hg (0) in the first 1 to 1.5 m above the revegetated area at the Tui site would have to be above the threshold limit value (TLV) for Hg vapour inhalation (1 $\mu g/m^3$) (World Health Organization, 1976). Since Hg atmospheric levels are not available for comparison purposes, we will address this issue reporting 0.004-0.01 $\mu g/m^3$ as the range for Hg (0) atmospheric levels in the Tui non-vegetated area (Lindberg et al, 1995). These Hg levels were measured in the first 1 to 1.5 m above the soil of an Hg-contaminated site in Oak Ridge, TN (US), which contains 80,000 kg of Hg in a 250 ha area (Meagher et al., 2000). Assuming that Hg emissions would be expected to increase by a factor of 23 due to the on site presence of *B. juncea* plants, the resulting Hg atmospheric levels would be still between 57 to 4 times below the TVL for Hg (0) exposure. It is, thus, very unlikely that the Te Aroha population would experience adverse health effects due to Hg vapours emitted from an Hg-phytoremediation operation at the Tui mine tailings.

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

Since plant Hg volatilisation can contribute to natural Hg emissions to the atmosphere, then plant Hg (0) emissions makes the use of plant-based systems for the remediation of Hg-contaminated sites questionable. Therefore, an assessment of the regional environmental impacts of this technology would be required prior to its systematic implementation in the field. If we target the Hg mass contained in 0- 0.5 m depth profile of the Tui mine and assume that 100 % of this Hg is released as Hg (0), then over the next 13 years, around 20 kg will be emitted to the global Hg atmospheric pool.

Considering that Hg world emissions from the plant kingdom can reach between 850 to 2000 x 10^3 kg of Hg per year (Lindberg et al., 1998) and that artisanal gold mining in South America, Russia and Asia can contribute with another 450 x 10^3 kg Hg per year (Lacerda, 2003), the Hg mass emitted by *B. juncea* plants at the Tui site (1.5 kg per year) would have an insignificant impact on this total. However, given that most of this Hg (0) emitted to the air is re-oxidized and returns back to the Earth's surface as particulate Hg, enhancing the emissions of Hg (0) could be potentially unsafe on a regional scale.

Once emitted to the atmosphere, Hg (0) is partitioned between vapour and particulate forms, which have a mean residence time of 233 and 30 days, respectively (Kvietkus and Sakalys, 2001). However, meteorological factors such as rain and snow-fall, and prevailing winds can accelerate the rate of deposition of both vapour and particulate Hg to the ground. Therefore, it is possible to speculate that the high rainfall regimes at the township of Te Aroha (around 1460 mm/ year) (NZMS, 1983), and the predominance of westerly winds in the region, would enhance the Hg deposition rates over the Coromandel Peninsula and the Bay of Plenty, both coastal areas of the North Island of New Zealand. The 1.5 kg of Hg annually redeposited over the next 13 years would be diluted over a much larger area in surface soils and waters. Mercury redeposition would, therefore, have minimum regional impact, as the mass of Hg in soil and water compartments is several orders of magnitude greater than that in the atmospheric Hg pool (Nriagu, 1979). Investigations carried out on the waters that cover the aquatic system of the Taupo Volcanic Zone, in the North Island of New Zealand, have revealed high levels of Hg, As and other elements. The source of this contamination is either from naturally occurring geothermal discharges or from geothermal power stations. However, research should be carried out to verify the reactivity of plant-emitted Hg and the potential for methylmercury formation in the coastal areas of the North Island of New Zealand. It is known that some lakes and river waters of the Taupo Volcanic Zone contain Hg levels that exceed the background level for surface waters and that trout collected from lake Rotorua have flesh methylmercury concentrations above WHO limits for Hg (0.5 μg/g DW) (Robinson, 1994).

8.4 Conclusions

One of the goals of this research was to study the accumulation of Hg into aboveground plant tissues via thiosulphate-induced Hg solubilization of Tui mine tailings. Mercury phytoextraction would allow off site transport and safe disposal of this contaminant. Application of sodium thiosulphate ($Na_2S_2O_3$) effectively induced Hg root uptake and shoot translocation in *B. juncea* plants grown in the field. The maximum extraction yield was around 25 g Hg/ha. Volatilisation studies demonstrated that water-soluble Hg in Tui tailings is transformed to Hg (0) and that plants significantly enhanced this process. Mass balance calculations revealed that spontaneous Hg phytovolatilisation is the dominant mercury pathway, accounting for 95 % of the total Hg mass removed from the Tui mine tailings during the experimental period. Therefore the results indicate that phytoremediation of the Hg-contaminated Tui base metal mine could be accomplished using both induced plant-Hg accumulation and phytovolatilisation techniques. A preliminary assessment indicates that the risk of Hg phytovolatilisation to the local and regional environment is minimal. However, there is a risk that Hg emitted by plants could be deposited in regional aquatic systems and further biomagnified.

Phytoremediation offers advantages for the rehabilitation and remediation of the Tui mine tailings. The establishment of a vegetative cover is a better option than taking no action, as Hg is naturally emitted from the site and AMD has severely contaminated local surface and groundwater supplies. Plant roots would stabilise the substrate and reduce leaching of metals. A fraction of soil Hg could be sequestered to aboveground tissues and removed off site after harvesting for safe disposal. However, the suitability of phytoremediation for cleaning up of Hg from Tui mine tailings should be compared with other existing technologies. The environmental impacts of this technology should be the subject of discussion between researchers, environmental professionals and the community before implementation in the Tui mine site.

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CHAPTER 9

MERCURY PHYTOEXTRACTION AND PHYTOVOLATILISATION FROM Hg-CONTAMINATED ARTISANAL MINE SITES

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Abstract

Mercury (Hg) phytoextraction and phytovolatilisation were investigated for mine tailings collected from artisanal gold mines in Brazil (the Serra Pelada mine) and China (the Gold Mountain mine). *Brassica juncea* plants grown in mine tailings were enclosed in gastight volatilisation chambers to investigate the effect of sulphur-containing solutions and substrate type on plant-Hg accumulation and volatilisation. Mercury volatilisation from planted substrates was compared to Hg emissions from barren mine tailings. Volatilisation from plants was not affected by the application of sulphur-containing ligands to either Gold Mountain (GM) or Serra Pelada (SP) mine tailings. Mercury volatilisation from planted substrates was significantly higher than from barren substrates. Mercury accumulation was enhanced in the presence of thiosulphate for plants grown in GM tailings. There was no significant increase in the plant-Hg accumulation after application of thiocyanate to the SP tailings. There was a significant relationship between the Hg mass emitted by plants and the Hg concentration in the plant and in the substrates. Our results indicate that Hg could be removed from mine tailings through phytoextraction and plant-Hg volatilisation. Mass balance studies revealed that Hg volatilisation is the dominant pathway for Hg removal using *B. juncea* plants. Mercury emissions from phytoremediation would have limited impact on local and regional environments due to a many-fold dilution in the atmosphere. Phytoremediation could, therefore, help to alleviate Hg-pollution at artisanal mine sites.

9.1 Introduction

Artisanal and small-scale mining (ASM) is characterised by limited planning and the use of simple techniques to extract metals from primary and secondary ore bodies (Hinton et al., 2002). Artisanal mining is a livelihood adopted by rural communities because it represents the most promising source of income. It is, therefore, a central activity to at least 10 million people from the developing world, including emerging economies such as China and Brazil. In general, these small-scale mines have a negative effect on the environment, causing deforestation, soil erosion, river diversion and river silting (Hinton et al., 2002). However, the most profound impact of ASM is the pollution of the environment with metallic Hg (Veiga and Hinton, 2002). It has been estimated that between 450 to 800 tonnes of metallic Hg (i.e. around 20 % of total anthropogenic Hg emissions) are released annually into the worldwide environment as a result of artisanal and small-scale gold mining operations. Brazil and China contribute with around 40 % of this total (Lacerda, 2003; Veiga, 2004).

Small-scale gold miners are driven by survival and a need to support their family. Consequently, little consideration is paid to the toxic effects of Hg or to the dangerous consequences of Hg release into the environment. Metallic Hg is freely discharged in soils and water in the form of amalgamation tailings that contain up to 500 mg/kg residual Hg (Veiga and Hinton, 2002).

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 (leaching, erosion, and volatilisation) (Melamed et al., 1998) and biochemical transformations (methylation and biological reduction) (Barkay et al., 1992; Morel at 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 (Lacerda and Solomons, 1992; Veiga, 1994; Oliveira et al., 2001). The transformation of inorganic Hg into toxic methyl Hg can occur through the action of methylating bacteria on soluble Hg species (i.e., free Hg ions or Hg complexed to organic acids) under anoxic conditions. Once formed, methyl Hg is biomagnified and, in top predators such as fish, it can exceed safe levels for human consumption (Southworth et al., 2004).

In some scenarios, phytoremediation is a low-cost technology for the remediation of metal-contaminated sites. Plant roots can stabilise a substrate, reduce leaching and contribute to the build up of organic carbon in soils, thereby rehabilitating degraded land (Robinson et al., 1998). Plants can extract nutrients, accumulate heavy metals and radionuclides, and transform or degrade some organic contaminants (Schnoor et al., 1995). It is, therefore, logical to propose a plant-based system for the remediation of Hg-polluted soils. It has been suggested that terrestrial plants can function both as a source and sink of atmospheric Hg (Leonard et al., 1998 a and b; Lindberg, 1998; Lindberg, 2002). Further, Hg-phytovolatilisation promoted by Hg-resistant transgenic plants is a promising tool for the removal of inorganic and organic Hg forms from contaminated soils and sediments (Meagher et al., 2000, Heaton et al., 2001; Heaton et al., 2003). As an alternative, phytoextraction of Hg from contaminated soils is proposed based on evidence for enhanced Hg accumulation in harvestable plant tissues following substrate treatment with $NH_4S_2O_3$ (Moreno et al., 2004 a[21]). A strategy for Hg removal from low to moderately contaminated soils would involve periodic removal and safe storage of Hg-containing plant biomass after soil treatment with non-toxic chemical solutions.

In this work we aim to determine the volatilisation and induced Hg accumulation for *B. juncea* plants grown in Hg-contaminated mine tailings, with a view to determining the feasibility of phytoremediation on artisanal gold mine sites in Brazil and China.

[21] This paper is described in Chapter of 7 of this thesis.

9.2 Material and Methods

9.2.1 Substrate type

Mercury-contaminated substrates from two locations were investigated in this work: 1) mill tailings collected from the processing centre of the Gold Mountain (GM) artisanal mine, North-Central China, and 2) mine tailings collected at the Serra Pelada (SP) artisanal gold mine site, State of Pará, Brazil (Figure 9.1 and 9.2). Selected geochemical characteristics of the two substrates are presented in Table 9.1.

Table 9.1. Selected geochemical characteristics of the Serra Pelada (SP) and Gold Mountain (GM) mine tailings. Units for metal concentrations are in mg/kg, unless otherwise stated.

Tailings Type	Ph	Eh (mV)	Hg mg/kg	Au mg/kg	Cu mg/kg	Mn mg/kg	Fe (%)	As mg/kg
SP[1]	5.4	93	0.675**	0.09	1338	188.4	1.3	5.0
GM[2]	9.4 (8.2)*	-137 (-63)*	100 (2.5)	1.58	9356	n.a.	4.48	13.8

[1]Source: Cabral et al., 2002.
[2]ACME Labs, Vancouver, B.C., Canada. Tailings were modified for plant experiments.
*pH and Eh and Hg concentrations for the modified GM mine tailings used in this study are shown inside the brackets.
**Hg value in the mine wastes of Serra Pelada.
n.a.= not analysed

9.2.2 Sample collection

Tailings from the SP mine were collected by the first author during a field survey of the mine during June 2003. Tailings from the GM mine were provided by University of British Columbia (Vancouver, B.C., Canada). Both substrates were sealed in plastic bags and shipped to New Zealand for Hg analysis.

Figure 9.1. Location of the Serra Pelada artisanal mine site. The red star in the upper map indicates the Carajás Mineral Province, which hosts Brazil's most important mineral deposits including iron, aluminium, copper, nickel, manganese and gold.

Figure 9.2. View of a tailings area within the artisanal gold mine site of Serra Pelada (June 2003). A sluice riffle box used for amalgamation of gold-rich gravels is visible in the background. A patch of shrubs that has succeeded in colonising the area is visible at the centre of the figure.

9.2.3 Substrate preparation for plant growth

Due to the phytotoxic Hg concentration of the Gold Mountain (GM) tailings (Table 9.1), plant growth was carried out in a diluted GM tailings substrate. The modified substrate was prepared by adding silica sand to the GM tailings to give a final Hg concentration of 2.5 mg/kg. The SP tailings were used for plant growth experiments without dilution. Both types of substrates were fertilised with 5 g/kg of Osmocote slow-release fertiliser. The SP substrate was amended with lime to adjust the pH to 5.5. No lime was added to the GM substrate as the pH of substrate (around 8) was suitable for plant growth. Each substrate was left to equilibrate for one week prior to seeding. Plastic pots (250 mL) were filled with each substrate and sown with seeds of *B. juncea* at a rate of ~20 seeds per pot (n = 20 for each substrate type). Two weeks after germination, each pot was thinned to leave one individual plant. Hoagland's nutrient solution (5 mL of ¼ strength) (Hoagland and Arnon, 1950) was irrigated onto the pots every second day to supplement the plant's nutritional requirements. Plants were kept in

a greenhouse with ambient temperature set to vary diurnally from 15-25 °C without humidity control. Unplanted substrates for each type of mine tailing were used as controls. Pot positions were randomly changed on a periodic basis to equalize light exposure. Daily watering was carried out with a hand-held hose.

9.4 Extractable Hg

Extractable Hg concentrations were determined for the SP and both the original and modified GM mine tailing samples. Two sulphur-containing chemical ligands were tested for their ability to extract Hg: ammonium thiosulphate ($[NH_4]_2S_2O_3$), and ammonium thiocyanate (NH_4SCN) supplemented with 0.3% hydrogen peroxide (H_2O_2). One gram of substrate was weighed into 50 mL polypropylene centrifuge tubes (n = 3). After addition of the extractant solution (20 mL at 2 g/L, unless otherwise stated), each tube was rotated on an end-over-end shaker overnight at 45 rotations per minute (RPM) and the supernatant separated by centrifugation at 3000 RPM for 3 minutes. The pH and Eh of the extractant solutions were measured using a pH and Eh meter (Copenhagen Radiometer, PHM 83 Autocal pH meter).

9.5 Plant-Hg volatilisation and accumulation experiments

B. juncea plants growing in each substrate were treated either with $(NH_4)_2S_2O_3$ or NH_4SCN. A volume of 5 mL for each thioligand solution was applied to give a concentration of 2 g of chemical per kg of substrate. After 5 weeks of growth, the effect of plants, sulphur-containing ligands and substrate type on volatile Hg emissions (n=3) was assessed. Water was used as a control treatment for pots with and without plants. Immediately after treatment, both pots and plants were individually enclosed within a gas-tight acrylic volatilisation chamber (3.6 L volume). Volatile Hg released from the soil-plant system was captured in two successive trap solutions containing 5% $KMnO_4$ dissolved in 2N H_2SO_4. The efficiency of this trap solution to quantitatively capture Hg has been shown to range from 95 to 99 % (Kimura and Miller, 1960). A continuous airflow was supplied to the volatilisation chamber using a small air pump. Mercury vapour released by the plants was driven by with the incoming air into two Erlenmeyer flasks, each containing 70 mL of the acid solution. The flow rate of the incoming air was monitored using an air flow meter (J&W, model AMD 1000) and was adjusted to

100 mL/min using small clamps attached to the air outlets. The outlet of the second acid trap was open to the atmosphere to maintain pressure equilibrium within the trap system. A 10 mL syringe attached to the volatilisation chamber was used to water the plants during the period of volatile Hg collection. Watering was carefully performed to avoid any possible loss of Hg through leaching. Plant-Hg volatilisation in the presence of the thioligands was measured over three days inside a plant growth chamber with temperature and photoperiod set to 22°C and 14 hours, respectively. Collection of volatile Hg was done in triplicate for both plants and control pots. After collection of volatile Hg, the trap solutions were each transferred to a 100mL airtight plastic container and stored at 4 °C until analysis. The precipitated fraction of the acid traps was redissolved using 50mL of concentrated hydrochloric acid, and the resulting solution was stored using the same procedure. The mass of volatile-Hg collected for each replicate was, thus, the sum of the Hg readings in the soluble and precipitated fractions of both acid traps. The use of this experimental apparatus has allowed an average Hg recovery of 90 % for *B. juncea* plants cultured in Hg-spiked solutions (Moreno et al., 2004 b[22]).

9.6 Plant harvest

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

[22] This paper is described in Chapter 5 of this thesis.

9.7 Plant digestion

Ground shoots and roots were accurately weighed (0.1 g) into 50 mL plastic beakers. Concentrated HNO_3 (15 mL) was then added. The digest samples were left overnight and, on the following day, were heated in a water bath at 80°C for 1 hour. Digest solutions were transferred to 10 mL polythene tubes and diluted with reverse osmosis (RO) water to make a final volume of 10 mL. A blank reagent was used with all digestions.

9.8 Substrate digestion

The total mercury concentration was determined through *aqua regia* digestion of dried and sieved (<1000 micron) SP and GM growth samples (original and modified). One gram of substrate was weighed into 50 mL polypropylene beakers 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 1 hour, and the filtrates diluted to a final volume of 50 mL using reverse osmosis (RO) water.

9.9 Mercury analysis

Total Hg in the plant and substrate digests, and in the extractant and trap solutions was determined using hydride-generation atomic absorption spectroscopy (Moreno et al., 2004 c[23]). The analysis was performed using a GBC 909A AAS (Victoria, Australia) operating in the flame mode. A sodium borohydride solution (5% $NaBH_4$ + 1% KOH) in combination with 10 ml of 0.5 M of HCl was used to generate the Hg vapour. The limit of detection (LOD) for mercury in solution was 10 ng/mL for plant digests and 5 ng/mL for soil digests and extractant and trap solutions. Reagent blanks were below detection limits in the solution. Linear calibration curves were obtained over the range of 125 to 1000 ng/mL of Hg using 4 standards prepared from a 10 mg/L mercuric nitrate ($HgNO_3$) spectrosol solution (May & Baker, AAS standard reagent solution, England). The Hg readings obtained from the replicate analysis (n=10) of a standard solution containing 1 mg/L of Hg could be reproduced with less than 5% of variation.

[23] This paper is described in Chapter 3 of this thesis.

The analytical method was assessed for quality control by an external certified laboratory with agreements ranging from 85 to 103% for Hg-containing solutions and Hg-containing plant samples (Moreno et al., 2004 c).

9.10 Statistical analyses

In order to study changes in Hg volatilisation as a function of sulphur-containing solutions (NH_4SCN and $[NH_4]_2S_2O_3$), substrate type (SP and GM) and plants (planted and unplanted substrates), a completely randomised experimental design was used. The analyses of variance (ANOVA) were performed in single treatments with the following one-way structure: planted/control/SP, planted/NH_4SCN+H_2O_2/SP, planted/control/GM, planted/$(NH_4)_2S_2O_3$/GM, unplanted/SP, unplanted/GM. Linear contrasts were then performed for the following comparisons:

a) Unplanted x Planted (ignoring substrate type and sulphur-containing ligands);
b) Planted/SP x Planted/GM (ignoring sulphur-containing ligands);
c) Unplanted/SP x Planted/SP (ignoring NH_4SCN+H_2O_2);
d) Unplanted/GM x Planted/GM (ignoring $(NH_4)_2S_2O_3$);
e) Planted/NH_4SCN+H_2O_2/SP x Planted/Control/SP; and
f) Planted/$(NH_4)_2S_2O_3$/GM x Planted/Control/GM.

A copy of SAS PC version 8e was used for statistical analyses (SAS Inst, 1988). The t-test was performed for comparing two treatment means, assuming equality of variances. Linear and polynomial regression models were used to provide the relationships between two variables. The significance of the fitted regression was assessed through the ANOVA and the coefficient of determination (r^2). The linearity and homogeneity of the regression model was examined through the plot of residuals against the fitted values. The normality of the data was assessed through the Shapiro-Wilk test. Where necessary, log-transformation was used to transform data to a normal distribution.

9.3 Results

9.3.1 Total and extractable Hg in substrates

Of the chemical extractants used, $(NH_4)_2 S_2O_3$ released the highest concentration of Hg from SP tailings (Figure 9.3). The extracted Hg concentration was not significantly different from the soluble Hg concentration after aqua regia digest ($p > 0.05$). Ammonium thiosulphate released the highest concentration of Hg from both the original and modified GM substrate ($p < 0.0001$, Figure 9.3). The concentration of Hg extracted using $(NH_4)_2 S_2O_3$ was greater than that made soluble after *aqua regia* digests for the original samples ($p < 0.01$) but not for the modified substrate ($p > 0.05$, Figure 9.3).

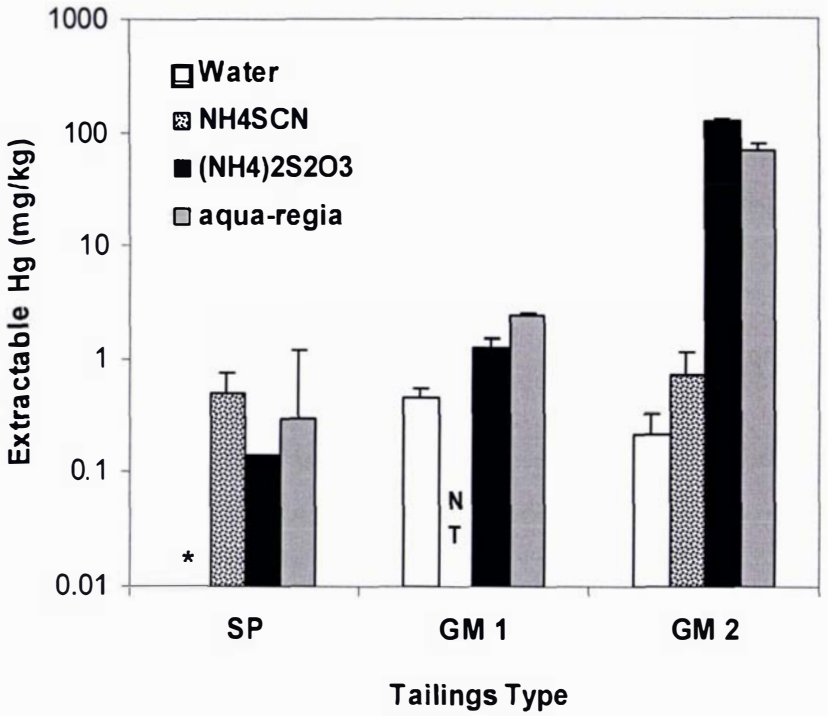

Figure 9.3. Effect of sulphur-containing ligands (applied at 2 g/kg substrate) and *aqua regia* on extractable Hg concentrations of Serra Pelada (SP) and Gold Mountain (GM) substrates. Bars denote ± 1 standard deviation from the mean of three replicates. The symbol (*) denotes Hg below detection levels. GM 1 and GM 2 = modified and original GM substrates, respectively. $(NH_4)_2 S_2O_3$ = ammonium thiosulphate; NH_4SCN = ammonium thiocyanate supplemented with hydrogen peroxide (H_2O_2) at 0.3%; NT = not tested.

9.3.2 Effect of plants, substrate type and sulphur-containing ligands on plant-Hg volatilisation and accumulation

Table 9.2 shows the volatile Hg mass captured in the acid permanganate traps for planted (ligand-treated and controls) and unplanted SP and GM substrates after the end of the experiment. The associated contrasts and their F-ratio and *p*-values for these treatments are shown sequentially in Table 9.3. The presence of *B. juncea* plants significantly enhanced Hg volatilisation relative to barren tailings ($p < 0.0001$, Table 9.3). The mass of volatile Hg released from planted GM substrates was increased by a factor of 12 when compared to barren GM substrates ($p < 0.0001$, Table 9.2 and 9.3). The mass of Hg emitted from planted SP substrates was not significantly different to the Hg mass emitted from unplanted substrates ($p > 0.05$, Table 3).

Table 9.2. Mercury mass (μg) volatilised from planted and unplanted Serra Pelada (SP) and Gold Mountain (GM) substrates. Planted substrates were treated with sulphur-containing ligands at 2 g/kg (unless otherwise stated) prior to Hg collection in the acid permanganate traps.

			Hg Mass (μg)		
Tailings Type	Treatment	N[¶]	Trap 1	Trap 2	Total Hg Mass (μg)[*]
SP	Unplanted	6	0.58 (1/3)	0.10 (1/3)	0.68
	Planted/Control	6	1.16 ± 0.87 (3/3)	0.52 ± 0.23 (3/3)	1.68 ± 1.07
	Planted/NH_4SCN[§]	6	0.38 ± 0.17 (2/3)	0.67 (1/3)	0.71 ± 0.65
GM	Unplanted	6	0.165 (1/3)	BDL	0.16
	Planted/Control	6	3.78 ± 2.25 (3/3)	2.86 ± 1.46 (3/3)	6.65 ± 3.67
	Planted/$(NH_4)_2S_2O_3$	6	4.85 ± 1.01 (3/3)	1.90 ± 0.57 (3/3)	6.75 ± 0.68

In the brackets are shown the frequency for the detectable Hg mass per number of analysed replicates.
[*]Total Hg mass is the arithmetic mean of three replicates for the Hg mass collected in two acid traps.
[§]NH_4SCN treatment was supplemented with hydrogen peroxide at 0.3 %.
[¶] N=3 for each one of the traps.
BDL= Hg below detection levels.
Ammonium thiocyanate = NH_4SCN; Ammonium thiosulphate = $(NH_4)_2S_2O_3$.

However, we found a significant plant effect for the Hg mass emitted when control (not thioligand-treated) substrates were compared to barren substrates ($p < 0.05$, Table 9.3). In this case, Hg emissions from planted substrates were increased 2.5 times relative to emissions from barren SP substrates (Table 9.2). The Hg mass emitted from planted SP substrates was significantly lower than the Hg mass released from planted GM

substrates, suggesting a substrate Hg concentration effect in the volatilisation process ($p < 0.0001$, Table 9.3). The addition of sulphur-containing ligands did not significantly affect plant Hg emissions from either SP or GM substrates ($p > 0.05$, Table 9.3).

Table 9.3. Linear contrasts and the associated F-ratio and *p*-values for the effect of *B. juncea* plants, sulphur-containing ligands and substrate type on volatile Hg emissions from Serra Pelada (SP) and Gold Mountain (GM) substrates.

Contrasts[#]	DF[¶]	F- ratio	Pr > F[¶]
Unplanted x Planted	1	38.92	< 0.0001
Planted/SP x Planted/GM	1	34.77	< 0.0001
Unplanted/SP x Planted/SP	1	2.57	0.1257
Unplanted/SP x Planted/Control/SP	1	5.64	0.0336
Unplanted/GM x Planted/GM	1	53.60	< 0.0001
Planted/NH_4SCN/SP x Planted/Control/SP	1	0.95	0.3417
Planted/$(NH_4)_2S_2O_3$/GM x Planted/Control/GM	1	0.00	0.9846

[#]The associated hypothesis for each contrast tested possible differences between the means of single treatments; comparisons were not orthogonal.
[¶]DF = degrees of freedom; Pr >F = probability level for rejecting or accepting the hypothesis associated with each linear contrast at the $\alpha = 0.05$ level.
Ammonium thiocyanate (NH_4SCN) was supplemented with hydrogen peroxide at 0.3 %.
Ammonium thiosulphate = $(NH_4)_2S_2O_3$.

Figure 9.4 shows the Hg concentration in *B. juncea* plants after substrate treatment with either thiosulphate or thiocyanate solution. Application of $(NH_4)_2S_2O_3$ to GM substrates significantly increased Hg accumulation in the plants (roots + shoots) relative to controls ($p < 0.05$, Figure 9.4 a). Recorded values in whole plant tissues average 46.5 and 4.1 mg/kg dry weight, respectively for $(NH_4)_2S_2O_3$-treated and control plants. The plant-Hg accumulation effect was noticeably high for aerial tissues. The Hg concentration in the shoots of $(NH_4)_2S_2O_3$-treated plants reached around 42 mg/kg dry weight. In contrast, shoot Hg values for control plants were below detection levels (data not shown). The application of NH_4SCN to SP mine tailings induced a slight increase in the plant-Hg concentration. However, this increase was not significant relative to the controls ($p = 0.059$, Figure 9.4 b). The limited effect of NH_4SCN on plant-Hg accumulation was possibly related to low levels of total and therefore potentially soluble Hg in the SP substrates.

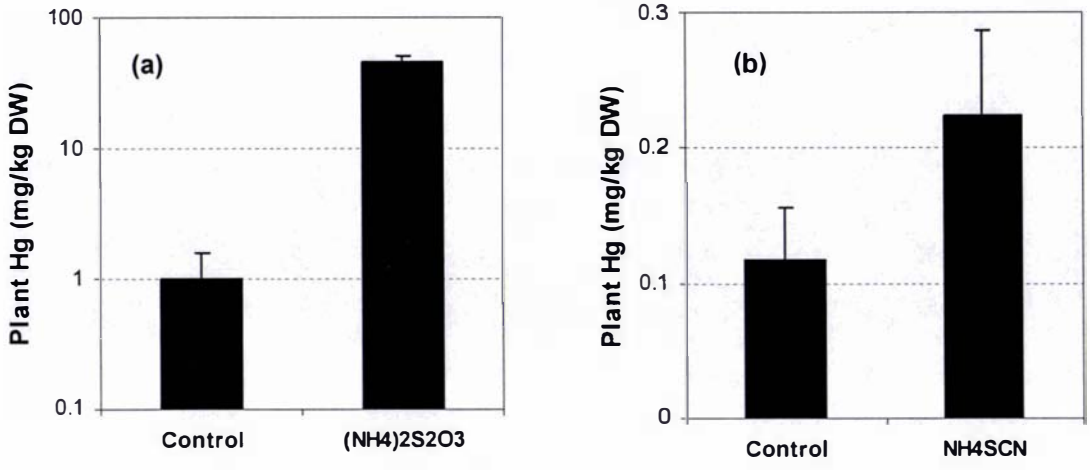

Figure 9.4. Whole plant-Hg concentrations in *B. juncea* after application of sulphur-containing ligands at 2 g/kg to Gold Mountain (a) and Serra Pelada (b) substrates. Bars denote ± 1 standard deviation from the mean of three replicates. Ammonium thiocyanate (NH_4SCN) was supplemented with hydrogen peroxide at 0.3 %, $(NH_4)_2S_2O_3$ = ammonium thiosulphate, DW = dry weight.

3.3 Soil-plant trends in the Hg volatilisation from planted substrates

Figure 9.5 shows the total (a) and extractable (b) Hg concentrations (mg/kg) for the GM substrate at the end of the experiment.

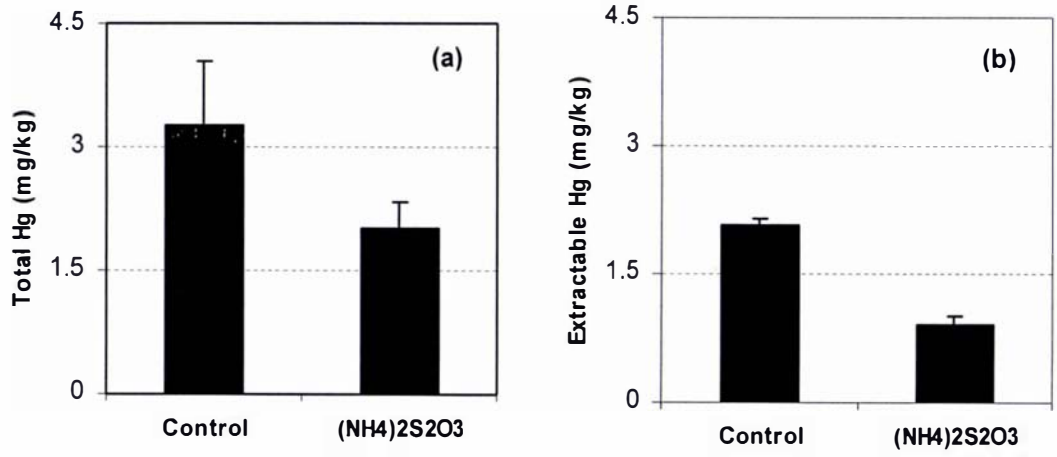

Figure 9.5. Total (a) and extractable (b) Hg concentrations in Gold Mountain substrates after growth of *B. juncea* plants and application of ammonium thiosulphate ($[NH_4]_2S_2O_3$) at 2 g/kg. Bars denote ± 1 standard deviation from the mean of 5 replicates. Ammonium thiocyanate (NH_4SCN) was supplemented with hydrogen peroxide at 0.3 %.

The total Hg concentration in the $(NH_4)_2S_2O_3$-treated substrates was significantly lower than the Hg concentration in the control substrates ($p < 0.05$, Figure 9.5 a). This difference was most pronounced with respect to extractable Hg concentrations ($p < 0.0001$, Figure 9.5 b). Because plant-Hg accumulation is proportional to the dissolved Hg concentration (Moreno et al., 2004 a[24]), the concentration discrepancy shown in Figure 9.5 (b) is possibly related to the plant uptake of an insoluble Hg fraction made soluble after application of $(NH_4)_2S_2O_3$ to the substrates. As there was limited plant Hg uptake from the SP tailings, no significance difference in the total and extractable Hg fractions were observed between NH_4SCN-treated and control substrates ($p > 0.05$) (data not shown).

The effect of $(NH_4)_2S_2O_3$ on extractable, plant and volatile Hg fractions for the GM substrate is summarized in Figure 9.6. The values are expressed as a percentage, and therefore 100% is the sum of the total Hg mass in the extractant solutions, plant (root + shoot) digests and permanganate acid traps at the end of the experiment.

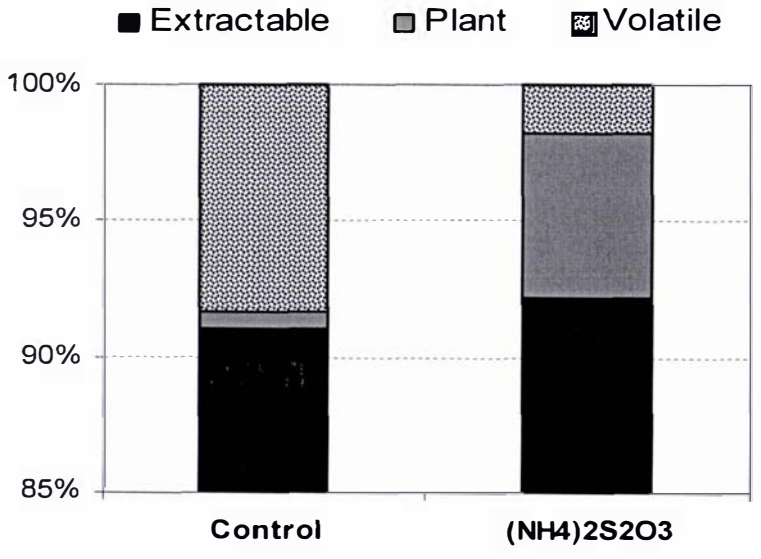

Figure 9.6. Mercury distribution in the air-plant-soil system after application of ammonium thiosulphate ($[NH_4]_2S_2O_3$) at 2 g/kg to Gold Mountain substrates. Values represent the Hg mass in extractable, plant and acid traps. Note that 100% is the sum of Hg mass in the extractant solution, plant digests (shoot + root) and acid traps.

[24] This paper is described in Chapter 7 of this thesis.

The Hg distribution within the air-plant-substrate system was based on the assumption that plant uptake and volatilisation processes share the same pool of soluble Hg in substrates (Moreno et al., 2004 d[25]). The total Hg mass depicted in Figure 9.6 is, therefore, relative to the water and $(NH_4)_2S_2O_3$-extractable Hg fractions present in control and $(NH_4)_2S_2O_3$-treated substrates, respectively. Around 9.1 % of the water-soluble Hg fraction (72.64 μg) in control pots was volatilised from the system, whereas 0.73 % of this total was accumulated into plant tissues. Conversely, around 1.8 % of the $(NH_4)_2S_2O_3$-extractable Hg fraction in the $(NH_4)_2S_2O_3$-treated pots (329 μg) was volatilised from the system, while the Hg mass accumulated in plant tissues increased to around 6.5 % of this total. These results indicate that plant-Hg accumulation was increased at the expense of the Hg volatilisation after application of $(NH_4)_2S_2O_3$ to the substrates.

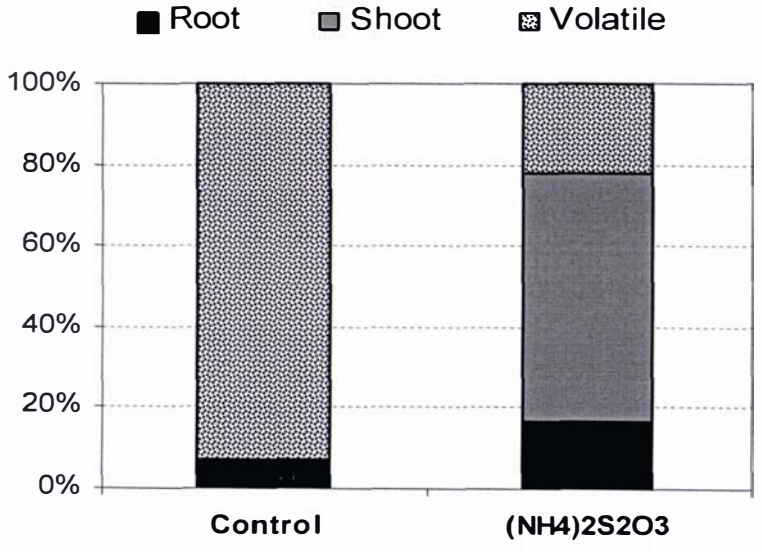

Figure 9.7. Mercury mass partition between root, shoot and trap compartments after application of ammonium thiosulphate ($[NH_4]_2S_2O_3$) at 2 g/kg to Gold Mountain substrates. Values represent the Hg mass in root, shoot and acid traps. Note that 100% is the sum of Hg mass in the extractant solution, plant digests (shoot + root) and acid traps.

The effect of $(NH_4)_2S_2O_3$ on the Hg accumulation and volatilisation processes for plants grown in the GM substrate is emphasised in Figure 9.7. Again, the values are expressed as a percentage and 100% is the sum of the total Hg mass distributed between roots, shoots, and permanganate acid traps at the end of the experiment.

[25] This paper is described in Chapter 8 of this thesis.

Mercury for the control treatment was preferentially volatilised to the air with less than 10 % remaining in the root system at the end of the experiment. In contrast, shoot accumulation accounted for most of the Hg mass for the $(NH_4)_2S_2O_3$-treated substrates (around 60 %). The remaining Hg mass was either retained in roots (around 20 %) or emitted to the air (around 20 %).

We have examined the relationship between 1) the volatile Hg mass released from planted substrates and the Hg concentration in *B. juncea* tissues (Hg concentration in root + shoot) and; 2) the volatile Hg mass released from planted substrates and the Hg concentration in the substrates (Figures 9.8 and 9.9). In order to highlight these soil-plant trends, current Hg mass emissions from planted SP and GM substrates were combined with data on volatile Hg mass emissions recorded from *B. juncea* planted Tui mine tailings (Moreno et al., 2004 d[26]). Figure 9.8 shows a significant asymptotic response for the volatile plant-Hg mass as a function of the Hg concentration in plant tissues (r^2= 0.64, p = 0.0013). The same asymptotic relationship occurs for the plot of volatile plant-Hg versus total Hg concentrations in the substrates (r^2= 0.62, p = 0.0044, Figure 9.9).

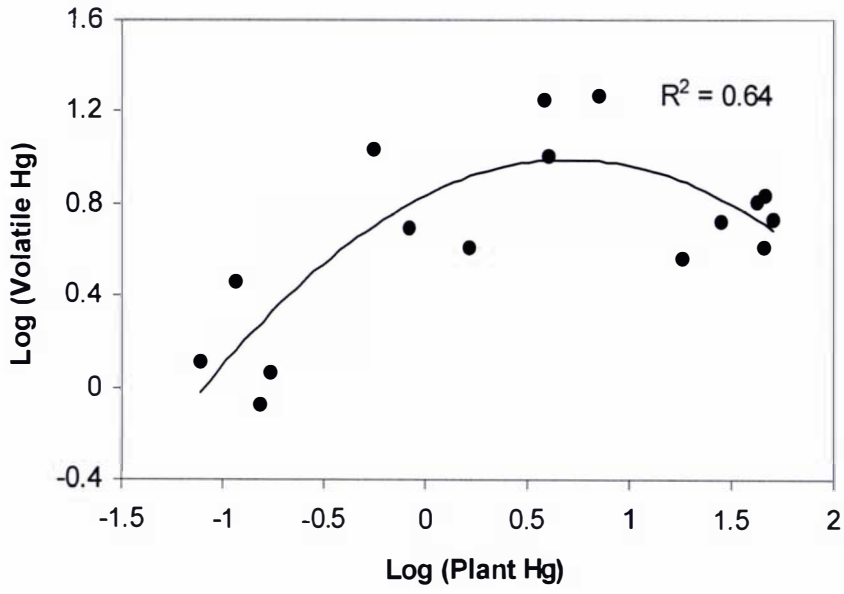

Figure 9.8. Volatile Hg mass (μg) emitted from *B. juncea* plants as a function of the Hg concentration in plant tissues (mg/kg DW). Data shown in the plot was logarithmically transformed.

[26] This paper is described in Chapter 8 of this thesis.

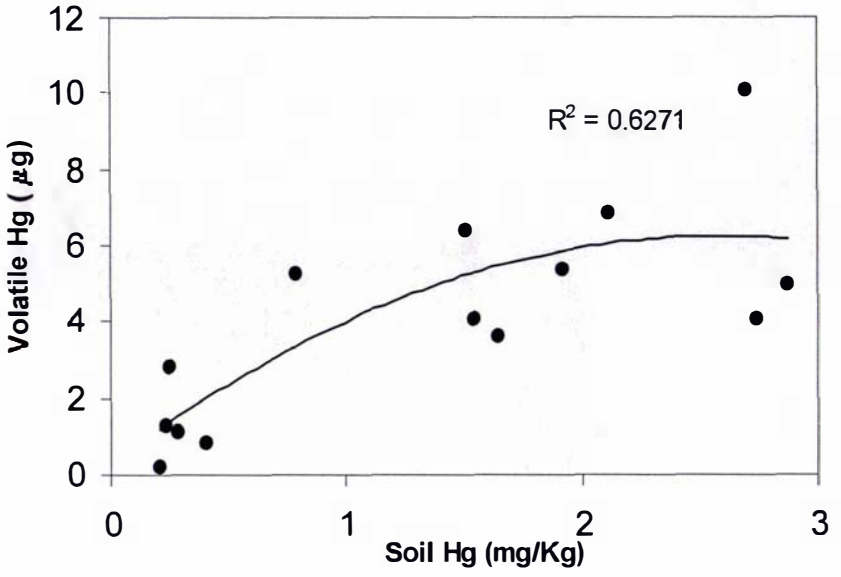

Figure 9.9. Volatile Hg mass (µg) emitted from *B. juncea* plants as a function of the total Hg concentration in the substrates (mg/kg).

Examination for linearity and variance homogeneity using a plot of residuals versus fitted values indicates the appropriateness of the data for fitting the quadratic curves between the variables (data not shown). These results suggest that substrate Hg concentration and plant uptake of Hg may limit Hg volatilisation from planted mine tailings substrates.

9.4. Discussion

9.4.1 Speciation and solution geochemistry of Hg complexes in the mine tailings

Schuster (1991) uses an Eh-pH diagram fro Hg to indicate that the free metal Hg (0) is a potentially stable form of Hg at pH > 5, under reducing to moderately oxidizing conditions (Figure 9.10). Table 9.1 shows that SP mine tailings exhibit moderately oxidising conditions and pH 5.4, whereas GM tailings has a slightly alkaline pH (8.2 - 9.4) and mild reducing conditions. Therefore, Hg dispersed in each of these substrates during artisanal gold mining was likely to be stable in the elemental Hg (0) form (Figure 9.10). 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. However, given that

goethite and manganese oxides are abundant in SP tailings (Cabral et al., 2002), and that Fe accounts for 4.8% of total weight of the GM talings (Table 9.1), it is likely that another Hg fraction was bound to the solid phase of Fe and Mn oxide minerals. The extraction of Hg from the solid phase of these minerals to the substrate solution was, therefore, the result of complexation reactions involving the added sulphur-containing ligands (Figure 9.3).

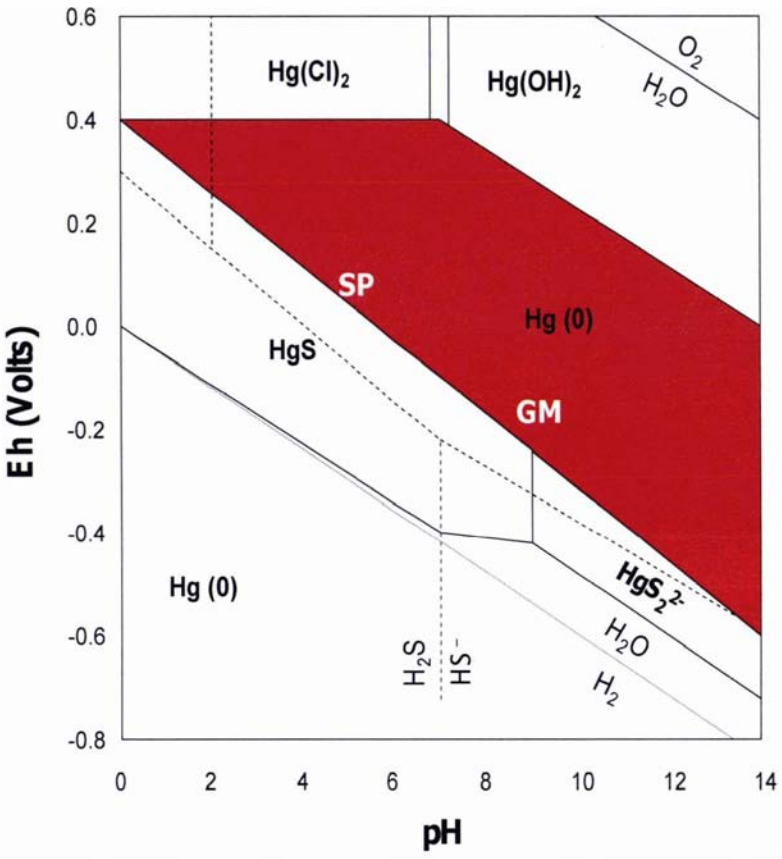

Figure 9.10. Eh-pH diagram for part of the system Hg-O-H-S-Cl. Mercury speciation for Serra Pelada (SP) and Gold Mountain (GM) substrates occurs within the field of native Hg (red-shaded area). Assumed activities for dissolved Hg species are: Hg = 10^{-8}, Cl = $^{-35}$, S = 10^{-3}. Redraw from Schuster (1991).

Mercury is described as a "soft acid" and has a tendency to form strong complexes with sulphur-containing ligands (Wallschläger, 1998), including the SCN^- and $S_2O_3^{2-}$ anions. 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 the tested mine tailings

(Table 9.1) suggests, therefore, that Hg will form stable complexes with SCN^- and $S_2O_3^{2-}$ anions present in the soil solution of the SP and GM substrates, respectively.

9.4.2 Origin and possible role of plant-Hg emissions from contaminated substrates

As the quantitative capture of Hg in the permanganate acid solution involves oxidation of elemental Hg (0) to ionic Hg (II), we can assume that the predominant Hg form released from unplanted and planted substrates was the elemental vapour Hg (0). Therefore, it can be inferred that Hg volatilisation from planted and unplanted substrates was partially due to vapour phase emissions of elemental Hg (0), as both types of mine tailings have a recent historical record of metallic Hg contamination. In addition, chemical processes (e.g. photo-reduction) might have contributed to Hg emissions from substrates (Gustin et al., 2003). However, our results also suggest that there is a plant-mediated factor in the Hg volatilisation from substrates. These plant-Hg emissions occurred from both control and thioligand-treated substrates (Table 9.2), most likely from a common pool of soluble Hg in the rhizosphere (Lindberg et al., 2002; Moreno et al., 2004 d). We therefore believe that plant transpiration and rhizosphere processes might be related to Hg volatilisation from the substrates.

The rhizosphere contains a large microbial population with high metabolic activity (Bowen and Rovira, 1991). Rhizosphere bacteria have been demonstrated to enhance root and shoot accumulation of Hg and Se for the wetland plants Saltmarsh bulrush (*Scirpus robustus* Pursh) and Rabbit-foot grass (*Polypogon monspelienses* [L.] Desf.) (DeSouza et al., 1999 a). Zayed et al. (2000) and DeSouza et al. (1999 b) reported that bacteria in the rhizosphere of Indian mustard (*B. juncea*) and Broccoli (*B. oleracea*) can account for between 35 to 95% of plant Se volatilisation, respectively. A great percentage of bacteria living in Hg-contaminated environments have Hg-resistant systems, and thus are able to catalyse the enzymatic reduction of Hg (II) to Hg (0) (Barkay et al., 1992; Meagher et al., 2000). Therefore, Hg-resistant rhizosphere bacteria might have contributed to the volatilisation of Hg from SP and GM substrates. Additional data supporting this hypothesis has come from hydroponics experiments with Hg-exposed *B. juncea* plants enclosed in gastight root and shoot volatilisation compartments. Quantification of the Hg mass volatilised from both compartments

revealed that around 95 % of total plant-Hg emissions from a *B. juncea* plant originated from the root system (Moreno et al., 2004 b[27]).

Literature shows that Hg (0) flux from soils and aquatic systems is associated with the transpiration of plants. The leaf-to-atmosphere path for Hg (0) has been reported for a number of plant species grown in Hg-contaminated soil including taxa from the *Brassicaceae* family such as *Lepidium latifolium* and *Caulanthus* sp. (Leonard et al., 1998 a and b). Diurnal Hg (0) vapour fluxes have been demonstrated for aquatic macrophyte communities growing in the Florida Everglades (Lindberg et al., 2002). The foliar mesophyll cells and the stomata have been suggested as the site for vapour Hg (0) emissions from plants (Leonard et al., 1998 a; Lindberg et al., 1998). The Hg (0) flux may be stomatally controlled by plants, thereby decreasing in the dark and under drought stress. Lindberg et al. (2002) proposed that Hg (0) emissions originate from gaseous Hg (0) in soil pores that enter the transpiration stream with soil water. The source of an elemental Hg (0) pool in soils would be related to biotic (bacterial metabolism) and abiotic (photoreduction, electron transfer during reaction with humic substances) reduction processes acting on Hg species present in the soil surface.

Mercury (0) is the least toxic form of Hg. Therefore, plant-mediated Hg volatilisation processes may be beneficial to plants, by alleviating the toxic effects of Hg (II) on plant roots. This might explain why control *B. juncea* plants grown in SP and modified GM mine tailings were shown to preferentially volatilise Hg rather than accumulate it into plant tissues. For instance, the volatile plant-Hg mass for control plants grown in the modified GM substrate significantly exceeded the accumulated plant-Hg mass by a factor of 12.5 (p = 0.0019, Figure 9.6). The *volatilised/accumulated Hg ratio* was markedly higher for plants grown in SP mine tailings, reaching a value around 30 (p < 0.0001, data not shown). However, after $(NH_4)_2S_2O_3$ was added to the modified GM substrate, non-soluble Hg forms were mobilised and the accumulated Hg mass was significantly enhanced to the detriment of the volatile Hg fraction (p = 0.0021, Figure 9.6). As result, the *volatilised/accumulated Hg ratio* for $(NH_4)_2S_2O_3$-treated plants dropped to 0.28. A similar behaviour was observed for *B. juncea* plants grown in base

[27] Paper described in Chapter 5 of this thesis.

metal Hg-contaminated mine tailings (Moreno et al, 2004 d[28]). In this case, the *volatilised/accumulated Hg ratio* dropped from 2.64 to 0.12 after $(NH_4)_2S_2O_3$ was applied to the substrates.

9.4.3 Implications for the phytoremediation of Hg-contaminated artisanal mine sites

The levels of mercury volatilisation reported in this chapter (Table 9.2), and the possibility for induced translocation of mercury from roots to shoots (Figure 9.7), suggest that plants could be used for the clean up of Hg-contaminated soil (phytoremediation). A field assessment of Hg phytoremediation using *B .juncea* has been described by Moreno et al., (2004 d). In this example, the maximum Hg phytoextraction yield averaged 25 g Hg/ha following $(NH_4)_2S_2O_3$ application, while an estimated 500 g Hg/ha was emitted to the atmosphere by the end of one growing season. Mercury volatilisation represented 95 % of the total Hg mass removed from the mine site during the experimental period. In the current study, Hg volatilisation accounted for around 75 % of the total Hg mass removed from the modified GM substrates. The remaining mass was either sequestered into shoot tissues (around 20 %) or accumulated in the roots (around 5%) following $(NH_4)_2S_2O_3$ treatment (Figure 9.7). Due to the lower Hg values in plant tissues, volatilisation was substantially magnified for the SP mine tailings and accounted for around 99.5 % of the total Hg mass removed from the substrate. However, this is a preliminary experiment and we believe that induced plant-Hg accumulation could contribute substantially more to the Hg removal process for SP substrates with a higher total Hg content.

Phytovolatilisation can be an efficient tool for Hg removal from contaminated sites, but its application may be hampered by concerns regarding the emission of Hg (0) to the atmosphere. The environmental risks of Hg volatilisation have been examined for transgenic plants applied to the clean up of sites heavily polluted with Hg in the USA (Heaton et al., 1998; Meagher et al., 2000). Transgenic *MerA*-Tobacco plants can volatilise 1.5 mg Hg (0) per kg of root tissue per minute from hydroponics solution. Based on comparisons of the natural Hg (0) efflux from barren contaminated sites, Meagher et al. (2000) suggested that a 400-fold increase in the atmospheric Hg (0)

[28] Paper described in Chapter 8 of this thesis.

levels from a phytovolatilisation operation using transgenic plants would not significantly impact local animals and plants or the global environment. The environmental risks of Hg phytovolatilisation have been also assessed for an Hg-contaminated site in NZ (Moreno et al., 2004 d[29]). Annual Hg emissions produced by *B. juncea* were estimated to be about 1.5 kg/year, based upon a biomass of 2.5 t/ha (measured in the field) and daily Hg volatilisation rates of 5.5 mg Hg/kg of plant tissue. This level of Hg volatilisation was judged to be of no harm to the regional environment. Additionally, phytovolatilisation may present some environmental advantages over thioligand-induced phytoextraction in some circumstances. For instance, it minimizes the potential for biomagnification, as Hg volatilised from plant tissues would not represent an exposure route to wildlife (Heaton et al., 1998). Also, unlike chelate-enhanced phytoextraction, phytovolatilisation avoids the risk of ground water contamination by mobilised Hg. The Hg concentration in leachates has been shown to rise up to 4 fold after $(NH_4)_2S_2O_3$ amendment to planted Tui mine tailings (Moreno et al, 2004 a[30]).

Although scaling up the results of our experiments to the field situation requires many un-proven assumptions, it is important to examine the significance of a Hg-phytovolatilisation operation on a local and regional scale. Assuming that 1 % of the 835 ha of the SP mining area must implement remediation (based upon evidence for bioaccumulation occurring [Hinton and Veiga, 2001]), and that the average Hg concentration in the 0-0.5 m surface profile of soils is 0.675 mg/kg (Table 9.1), then around 36.6 kg of Hg is potentially available for phytoremediation (uptake and volatilisation). Mass balance calculations reported in this work estimate that *B .juncea* plants could volatilise around 2 mg Hg/kg of plant tissue (dry weight) per day. Assuming that *B. juncea* produces 10 t/ha of dry biomass over a crop cycle and that the site supports 6 growing cycles per year (30 days period each one), then around 3.6 kg Hg/year would be emitted to the atmosphere of the SP mine. In addition, our results indicate that Hg could be accumulated into above ground tissues to a concentration of 40 mg Hg/kg dry weight following application of thio-solutions to substrates (Figure 9.4 a). Therefore, at the end of the first year around 6 kg of Hg could be potentially

[29] This paper is described in Chapter 8 of this thesis.
[30] Paper described in Chapter 7 of this thesis.

removed from the substrate through phytoextraction and spontaneous (not-induced) Hg volatilisation. Assuming constant rates for both processes, the remediation of the site would be feasible in 6 years. Considering that global Hg atmospheric emissions from anthropogenic activities (mainly fossil fuel combustion) are calculated in 1900 tonnes per year (Pacyna and Pacyna, 2002) and that artisanal and small-scale mining contributes with 20 % of this figure (Lacerda, 2003), the 3.6 kg of Hg mass annually emitted from a local phytoremediation operation would have an insignificant impact on this total. Similarly, if we assume 1000 ha as the average area for each of the 2000 Hg-contaminated sites in the Amazon region (Veiga and Hinton, 2002), then we can estimate that Hg contamination is spread over a 2 million ha area. In the unlikely and extreme scenario that phytoremediation is simultaneously applied to 1 % of this area, and assuming an average of 20 g Hg emitted per ha of planted area, around 0.4 tonnes Hg/year would be directly emitted to Amazon atmosphere via plant volatilisation. The plant-emitted Hg (0) mass would still be 2-3 orders of magnitude below the estimated annual Hg emissions from natural sources and anthropogenic activities.

It can be argued that phytoremediation through volatilisation is merely a process for the Hg transference between compartments and that it is not a long-term solution to the Hg-pollution problem. However, it should be pointed out that chemical transformations at the SP mine tailings appears to have been already emitting Hg (0) to the atmosphere at a rate of 1.51 mg Hg/ton of substrate per day (Table 9.2). Although the vapour of Hg (0) has a tendency to remain airborne (mean residence time of 233 days in the atmosphere) (Kvietkus and Sakalys, 2001), meteorological factors can accelerate its rate of deposition back to the Earth. Additional complications that may limit the application of the technology on a large-scale result from the susceptibility of atmospherically deposited Hg to methylation pathways (Hintelmann et al., 2002). Nevertheless, Hg vapour emissions from a local phytoremediation operation, being of a much smaller scale than natural and anthropogenic Hg emissions, would appear to be negligible in regard of the huge amounts of Hg stored in surface soils as a result of past and recent anthropogenic activities (Roulet et al., 1999; Oliveira et al., 2001).

9.4.4 Gold phytoextraction as a strategy for Hg remediation of artisanal mine sites

Since the amalgamation method used at artisanal mines is not 100 % efficient for gold extraction, tailings are frequently discharged with high amounts of gold (Au). Discharge of the Hg-rich amalgamation tailings into soil creates hot spots with exceedingly high levels of Hg and, possibly, with Au. Gold concentrations of up to 4 mg/kg have been found at artisanal Hg-contaminated sites in the Brazilian Amazon (Veiga and Meech, 1995). From a geochemical point of view, Au is very similar to Hg and can be made soluble in the presence of sulphur-containing ligands. Induced metal solubility is a pre-requirement for induced plant uptake and Au values as high as 57 mg/kg have been reported for NH_4SCN-treated B.*juncea* plants grown in low grade Au ores (Anderson et al., 1998). Gold-phytoextraction could be economically feasible at plant Au concentrations of 100 mg/kg or higher. Using greenhouse and field experiment data, Anderson et al. (2004[31]) generated a general model to quantify the relationship between substrate and plant Au concentrations. According to the model, a substrate Au concentration of at least 2 mg/kg is required to yield a crop with an Au concentration of 100 mg/kg. Assuming 10 t/ha for a dry biomass production and an average Au value of 100 mg/kg (dry weight) in shoot tissues, a single *B. juncea* crop can yield 1 kg Au/ha.

Technologies currently available for the remediation of Hg-polluted sites find limited application due to environmental and economic issues. We believe that Hg-phytoremediation coupled to Au-phytoextraction could help to alleviate the environmental degradation in artisanal Hg-contaminated mine sites. The idea is to use the value of Au in the harvested crop to pay for the costs of Hg remediation in areas that warrant an urgent remedial solution. If recovery and sale of the Au-rich biomass generates some cash revenue, then remediation and rehabilitation of Hg-contaminated areas would be encouraged. Miners and their families could carry out the whole operation after appropriate education and training. Local miner's cooperatives, non-governmental organisations or regional governmental institutions could manage the generated income from this activity. These funds could be applied for the environmental restoration (remediation, site stabilisation and erosion control) of

[31] This paper is shown in the Appendix 1 of this thesis

degraded areas and for the social upgrading (basic infrastructure, sanitation, schooling, health care) of the miner's community.

9.5 Conclusions

Our research has demonstrated that plants enhance Hg volatilisation from two contaminated substrates. Plants preferentially volatilised Hg from both substrates over accumulation. Mercury accumulation in plant tissues was significantly increased through $(NH_4)_2S_2O_3$ treatment of the GM substrate. There was no evidence for enhanced plant-Hg accumulation after addition of NH_4SCN to SP tailings. The Hg mass volatilised from planted substrates was related to the Hg concentration in plant tissues and to the Hg concentration in the substrate. These results suggest that Hg volatilisation from planted substrates involves an interaction between biotic (plant transpirations and rhizosphere processes) and abiotic (vapour-phase emissions, chemical transformations and photo reduction) factors. Mercury volatilisation was the main removal pathway accounting for 75 and 99.5 % of the Hg mass removed from modified GM and SP mine tailings, respectively. Plant accumulation accounted for 20 % of Hg removal after treatment with $NH_4S_2O_3$ for the GM tailings.

Induced plant-Hg accumulation and spontaneous plant-Hg volatilisation could be used for the remediation of Hg-contaminated mine tailings. Mass balance calculations show that the Hg (0) mass released by a localised phytoremediation operation would have a minor impact on the global Hg (0) mass emissions. However, there are concerns regarding the fate of Hg released to the atmosphere. Combined Au-Hg phytoextraction and Hg phytovolatilisation could offer an alternative technology for the rehabilitation of Hg-contaminated sites, and provide for an alternative livelihood to impoverished communities that rely on mining for subsistence. The environmental impacts of the phytoremediation technology should be discussed in depth by researchers, environmental professionals and the public.

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CHAPTER 10
CONCLUSIONS

10.1 Conclusions and implications from this study

This study has focused on 1) the methods of Hg determinations in plant systems and; 2) the volatilisation and plant uptake of Hg for the purpose of remediating Hg-contaminated mine wastes. Several conclusions can be drawn from the findings of the research:

Plants volatilise Hg as the gaseous Hg (0)

The fact that Hg values in the carbonate-phosphate traps were below detection limits indicates that inorganic Hg (0) is the main volatile form emitted by plants. These results have implications regarding the environmental impacts of Hg vapour emitted from a phytoremediation operation. As discussed in the Chapter 2, Hg (0) vapours have a larger residence time in the atmosphere than organic Hg vapours. Therefore, Hg (0) emitted by plants will have tendency to remain airborne, becoming diluted in larger Hg pool in the atmosphere.

Roots are the main site for Hg volatilisation from a B. juncea *plant*

The methodological approach utilised for the hydroponic experiments allowed capture of volatile Hg released from both roots and shoots of plants. The fact that around 95 % of the Hg mass emitted from the plant originated from the root compartment indicates that roots are the main site for Hg volatilisation from *B. juncea.* These results open some questions regarding the ability of plant roots to transform soluble Hg (II) to the volatile gas Hg (0). It is proposed that rhizosphere bacteria participate in this process. In this case, Hg (II) retained in the rhizosphere of plant roots could be reduced to Hg (0) as a result of cometabolic transformations carried out by Hg-resistant bacteria. Hydroponic experiments amended with antibiotics should be carried out to validate this hypothesis.

Shoot Hg translocation is dependant upon Hg solubility in the substrates

Under the natural geochemical conditions that prevail in substrates, Hg uptake was restricted to the root tissues for all the plant species tested in this study. This was related to the poor solubility of Hg in all tested substrates. The application of sulphur-containing ligands substantially increased Hg solubility and subsequent shoot accumulation for all plant species grown in Tui and Gold Mountain substrates, except for *Lupinus* sp. Although Hg solubility in Serra Pelada substrates was substantially increased in the presence of thiocyanate, uptake and transport to shoot tissues of *B. juncea* was not significantly increased relative to controls. Apart from mineralogy and pH and Eh, the main difference between the SP and the other two substrates was related to total the Hg concentration, as determined through *aqua regia* digestion. Shoot Hg accumulation in the pot trials was effectively enhanced after addition of thiosulphate to modified GM and Tui substrates, which consistently presented mean Hg concentrations in *aqua regia* digests greater than 1 mg/kg. Conversely, the mean Hg concentration in *aqua regia* digests for the SP substrates was below 0.3 mg/kg. Therefore, it is plausible that thioligand-induced plant-Hg accumulation will work only above a threshold value for total Hg in substrates. Caution should be observed when predicting induced plant uptake based only on soluble Hg concentrations.

Plant species and soluble Hg speciation influence root uptake and shoot transport

My research demonstrated that uptake and translocation of soluble Hg species were affected by differences in plant families and species. Application of thiosulphate to Tui and GM substrates enhanced root Hg retention in *Lupinus* sp. and *V.villosa,* respectively. However, *B. juncea* and *P. vulgaris* grown in the same substrates showed increased shoot Hg transport in the presence of the thisosulphate salts. This discrepancy is attributed to intrinsic plant species characteristics (e.g. root morphology and root surface area).

The hydroponic and pot trial experiments carried out in this study also demonstrated the importance of soluble Hg-thiosulphate complexes in the process of root uptake and shoot transport. Under slightly acid conditions (pH = 6) and in the presence of soluble $HgCl_2$ complexes in the hydroponic solution, uptake of Hg was restricted to the root

tissues of *B. juncea*. Increasing the Hg concentration in the solution did not result in higher shoot Hg levels as evidenced by a maximum concentration factor (concentration shoot tissues/concentration in the solution) of 0.6 at 10 mg Hg/L. Similarly, *B. coddii* and *A. canescens* grown in Tui substrate with the pH adjusted to 5.5 and treated with $HgCl_2$ yielded a maximum concentration factor of 0.8 at 10 mg Hg/kg of substrate. On the other hand, plants species grown in Tui and GM substrates treated with thiosulphate salts under similar pH conditions showed concentration factors ranging between 4 and 25 at substrate Hg concentrations ranging between 1.25 to 5 mg /kg.

Further evidence of the influence of soluble Hg speciation on Hg uptake and transport came from the pot trial study with humic acid-amended GM substrates. In the presence of higher levels of HA, shoot translocation of soluble Hg species was suppressed for $(NH_4)_2S_2O_3$-treated *B. juncea* plants, but not root uptake. Mercury Hg-thiosulphate complexes may be selectively transported to shoots over Hg-HA complexes, which were retained in root tissues. These results have a negative implication for the phytoextraction of Hg from organic-rich substrates, where Hg translocation to harvestable tissues might be precluded by Hg adsorption to root tissues.

Mobilisation of Hg in substrates enhances leachate generation

The Hg concentration in leachates collected from plant pots increased between 1 to 4 fold after treatment of Tui substrate with sulphur-containing solutions. There were significant differences in the leachate Hg concentration as a function of the thioligand used to promote Hg solubility. Mercury mobilisation and leachate generation was substantially higher in the order thiosulphate > thiocyanate > water. The generation of leachates also appeared to be significantly affected by a plant-thioligand factor. The experimental protocol utilised in this study did not, however, allow for explanation of discrepancies resulting from plant species, genus or family variability.

The potential for leachate generation represents one the major drawbacks in the application of thioligand-induced Hg- phytoextraction. Ionic and complexed Hg species present in the substrate solution, apart from being mobile, might be potentially reactive. The leaching of Hg species out of the root zone as a result of thioligand-induced Hg solubilisation might pose a risk to groundwater systems. The phytoextraction of metals

has been designed for the removal of contaminants from the impacted media via harvesting of metal-rich biomass. If there is substantial Hg leaching generation, then the main purpose behind this environmental friendly plant-based technology may be questioned. Research should be carried out to examine the potential for chemical and biological degradation of Hg-thiosulphate complexes in revegetated mine substrates. Further research will indicate if these Hg species pose a real risk to groundwater systems.

Phytovolatilisation is the predominant pathway for Hg removal from substrates

The Hg (0) mass captured in the permanganate traps increased up to 10 fold due to the presence of *B. juncea* plants in the SP, GM and Tui mine tailings. The mass balance calculations also indicated substantial volatile Hg losses from Tui mine tailings planted with *Lupinus* sp., *B. coddii*, and *A. canescens.* Since planted substrates can considerably enhance Hg (0) volatilisation rates from contaminated substrates, then Hg phytovolatilisation can be a tool for the remediation of Hg-contaminated land. The mass of Hg captured in the Hg traps was between 75 to 99.5 % higher than the mass of Hg accumulated in plant tissues after thioligand treatment of SP, GM and Tui substrates.

These results show that phytovolatilisation is the main Hg removal pathway from Hg-contaminated substrates. Mercury extraction yields from the Tui field experiment were also indicative of the dominant role of plant Hg (0) emissions in the phytoremediation process. Further research on the quantification of Hg (0) emissions from plants growing in the field would confirm the contribution of phytovolatilisation for the Hg removal from contaminated sites.

Phytovolatilisation of Hg (0) is not a major threat to the environment

Although gaseous Hg (0) might represent a threat to humans and biota, the toxicity of Hg (0) gas is six orders of magnitude lower than the toxicity of methylmercury. Additionally, the mass of Hg (0) released from a phytovolatilisation operation would be tremendously diluted in the air and, thus, would cause no harm to the local environment. The volatilised Hg would be uniformly deposited over the surface of soils

and waters after it returns to Earth. Since the Hg pools in these compartments are several orders of magnitude greater than that in the atmosphere, the Hg (0) contribution from a phytovolatilisation operation to regional soil and water Hg inventories would be negligible. However, research should be carried out to investigate the potential methylation pathways for atmospheric Hg (0) emitted from plants. Mercury phytovolatilisation raises an environmental dilemma that has been a subject of intense controversy among the scientific community and the public. These issues as well as the environmental impacts of this technology need to be discussed by researchers, environmental professionals and the public.

Consideration of phytovolatilisation vs phytoextraction

This research showed that *B. juncea* plants can remediate Hg-contaminated land without the help of solubilizing agents. The plant-mediated Hg (0) reduction pathway offers a simple but effective way of removing Hg from contaminated substrates. There are environmental and economical advantages that justify phytovolatilisation as a more suitable strategy than phytoextraction. For instance, 1) phytovolatilisation substantially reduces the potential for Hg biomagnification, as Hg volatilised from plant tissues would not represent an exposure route of Hg to wildlife; 2) The cost of the remediation operation would be reduced as plant-mediated Hg (0) volatilisation would not require chemical amendments and site maintenance (harvesting); 3) There are no costs involved in transport and processing of the Hg-rich biomass, since Hg is not accumulated in shoot tissues. The environmental costs of atmospheric Hg (0) emissions from plants could be offset by the absence of Hg-containing leachates generated as a by-product of the induced plant-Hg accumulation strategy.

Gold-phytoextraction combined to Hg-phytovolatilisation could help to alleviate environmental degradation in Hg-polluted artisanal mine sites

Gold phytoextraction and Hg-phytoremediation could be applied for the clean up of Hg-polluted artisanal gold mine sites (Figure 10.1). Many developing countries cannot afford the elevated costs involved in the environmental restoration of sites degraded by artisanal gold mining activity. After depletion of ore reserves, gold mine sites are abandoned and miners and their families who remain have to survive in Hg-polluted

environments under extreme conditions of poverty. The main source of protein for these communities is fish caught from rivers that received Hg-laden tailings from past and current mining operations. In some artisanal mine locations, governmental policies for regional development have distributed plots for house construction in illegal areas, or in areas situated atop old mine tailings in former pits. People living in these areas are exposed to Hg from different pathways and, thus, there is an urgent need to mitigate Hg-contamination. The initial establishment of plant covers with proved enhanced volatilisation capacity could remove most of surface Hg contamination. A further step in this remediation strategy would allow Au and Hg accumulation into harvestable plant tissues after thioligand treatment of substrates. Lining the substrate with impermeable geo-textiles would prevent migration of Hg-containing leachates towards local groundwater supplies. The key factor in this process is the recovery and sale of the Au-rich biomass to generate some cash revenue (Figure 10. 1). Miners and their families could carry out the whole operation after appropriate education and training. Local miner's cooperatives, non-governmental organisations or regional governmental institutions could invest the generated income from this activity in the restoration of polluted mine sites as well as in the social upgrading of the community.

Environmental implications of Hg phytovolatilisation

The primary goal of my research was to develop a cheap, effective and safe Hg remediation strategy by using plants. The fact that Hg can be volatilised from substrates during a phytoremediation operation (as shown in chapters 8 and 9) raises ethical questions regarding the environmental risks of plant Hg (0) emissions. In the core of the problem is the potential methylation of plant-emitted Hg. Methylmercury is the 6th most toxic compound known to humankind and comprises 10 and 2 % of the total forms of Hg in waters and soils, respectively. The relatively small Hg mass available for plant uptake and volatilisation processes (20 kg) at the Tui site indicates that phytovolatilisation might be a suitable remediation strategy for the Tui mine tailings. The assessment carried out in chapter 8 indicates that the 1.5 kg of Hg annually emitted by plants would be redeposited over much larger areas in land and water and thus, would be tremendously diluted. Under this scenario, the contribution of phytovolatilisation to the methylation potential of soils and waters would be negligible. That does not imply, however, that the large-scale application of phytovolatilisation

will be environmentally safe on a regional and global scale. Increasing the mass of volatile Hg enhances the potential for Hg methylation in soils and waters. As a result, large-scale phytovolatilisation operations applied simultaneously worldwide could have deleterious effects in humans if a fraction of plant-emitted Hg is redeposited in sea and biomagnified in fish. Therefore, I only advocate the utilisation of this technology for small-scale operations and under circumstances where there is an urgent need for Hg removal.

Artisanal mine sites represent the most severe example of Hg polluted environments. The continuous discharge of Hg-rich tailings into soils and watercourses exacerbates the potential for incorporation of Hg into the food chain and poses an eminent risk to the health of people living in artisanal mining communities. The levels of Hg in fish from polluted areas have been reported to be in excess of safe levels for human consumption. Also, high levels of Hg in hair and blood of artisanal miners have been related to high levels of Hg in consumed fish. The results presented in this thesis have indicated phytoremediation as a potential remedial solution for Hg-contaminated artisanal mine sites. However, there is a growing concern regarding the environmental impacts of plant-emitted Hg. It should not be forgotten, however, that barren and revegetated Hg-contaminated sites also represent a source of Hg to the atmosphere. Results presented in chapter 7, 8 and 9 indicated that Hg volatilisation occurred independently of the mine tailings composition and the presence of plant community. All tested plant species grown in the contaminated substrates showed some degree of Hg volatilisation suggesting that this process is likely to happen spontaneously from any revegetated mine site. However, I have demonstrated that it is possible to reduce the volatilisation process by treating the substrates with thioligands (Chapter 8). By using thiosulphate-induced plant-Hg accumulation a fraction of soil Hg that otherwise would be volatilised to atmosphere was immobilised into root and shoot tissues. The possibility of recovering Au and Hg off plant tissues after thioligand treatment is also interesting from an ecomical and social point of view. It is, therefore, not pointless to propose phytoremediation as a remedial solution to artisanal mining sites providing the plant-emitted Hg mass will not affect the methylation rates in soils and waters.

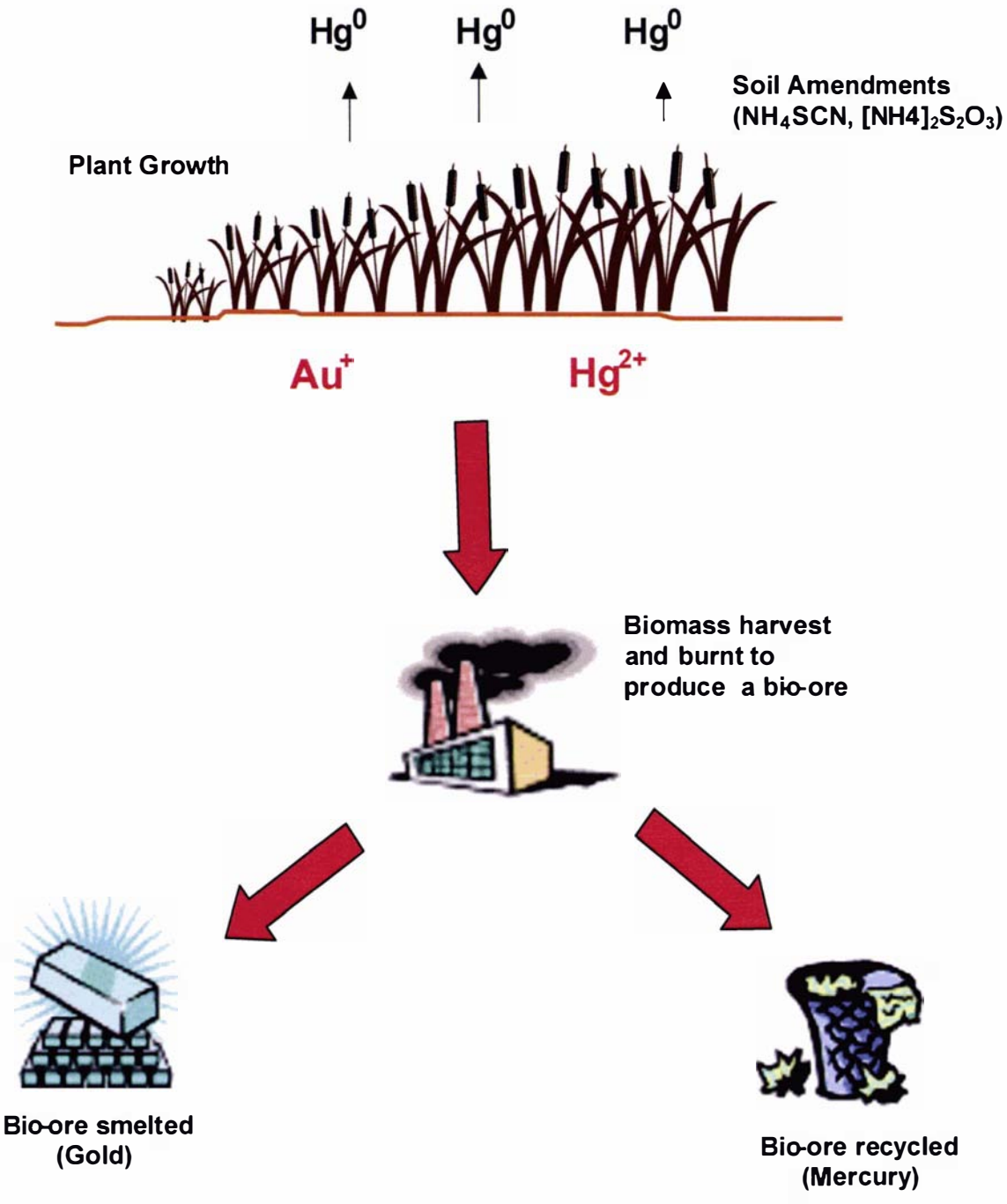

Figure 10.1. Proposed strategy for the remediation of Hg-contaminated soils in artisanal mine sites. The selling of the smelted Au-rich bio-ore could generate an income for the environmental restoration and social upgrading of abandoned artisanal mine communities.

10.2 The future of Hg-phytoremediation

The need to improve plant performance has motivated scientists from the Department of Genetics and Forest Resources from the University of Georgia (USA) to conduct research on the development of transgenic Hg-tolerant plants able to volatilise copious amounts of Hg (0) to the atmosphere. At the present time, microbial *MerA* and *MerB* genes have been inserted into the genome of obligate (Brassica sp. and Tobacco) and facultative (Poplar trees) upland, and obligate wetland (Rice) species. These plants are, therefore, potentially capable of phytoremediating soils and sediments polluted with inorganic and organic Hg species. An issue that may retard the large-scale application of transgenic plant-based systems is public concern over the release of genetically modified plants into the environment as well as of plant-Hg (0) vapours to the atmosphere. Plant pot trials carried out in the soil have, nonetheless, confirmed the enhanced tolerance of these plants. However, volatilisation appears to be rate-limited by low availability of mercurial compounds in the soil. Consequently, greenhouse and field-scale research on the volatilisation ability of transgenic plants in the presence of sulphur-containing solutions could provide valuable information towards the optimisation of this technology.

In vitro cell and tissue culture research is another approach that could be used for the creation of Hg-tolerant plants for phytoremediation purposes. Experiments with increasing levels of Hg in the culture media could lead to the selection of Hg-tolerant cell lines from a variety of plant species. Genomic combination of Hg-tolerant cells with high-biomass plant cells via protoplast fusion and other *in vitro* hybridisation techniques could generate high biomass plants with enhanced Hg tolerance.

The role of rhizosphere microorganisms in Hg plant uptake and volatilisation is another area where research should be focused. Theses studies could explore the association between plants and vesicular-arbuscular micorrhizae (VAM) with the purpose of increasing Hg tolerance and plant performance in the field.

Constructed wetlands is another exciting area that could be exploited for the immobilisation of water soluble Hg. Despite the potential of this environment to convert Hg species to methylmercury, these reducing systems have been shown to

precipitate Hg as the insoluble sulphide in the presence of gypsum. Also, methylmercury generation could be minimized in the system by the addition of elemental sulphur. These passive remediation systems could be employed to immobilise soluble Hg species present in acid mine drainage, thus preventing further Hg mobilisation into aquatic systems.

The discovery that Hg retention in root tissues is increased in the presence of humic acid deserves to be investigated in depth. Experiments with plants growing in the presence of humic substances should verify the immobilisation potential of root adsorbed Hg with the purpose of developing an Hg-phytostabilisation strategy.

10.3 Concluding remarks

Despite more than 8 years of intensive research on Hg volatilisation by transgenic plants, Hg phytoremediation technology is not yet commercially available. Therefore, it is precocious to say that Hg transgenic plants represent the apotheosis of Hg-phytoremediation research. Laboratory, greenhouse and field results from this research have demonstrated that non-transgenic plants can remove Hg from substrates through both phytovolatilisation and thioligand-induced phytoextraction. Given the potential for leachate generation, thioligand-induced phytoextraction may pose an unacceptable environmental risk unless the link to receiving waters can be broken. It has been demonstrated that the migration of metal contaminants in the unsaturated zone has been controlled by planting deep-rooted phreatophytic trees in metal-contaminated sites (Chapter 7). In some scenarios, these high water-use trees can eliminate leaching, thus mitigating the risk of groundwater contamination. Therefore, it would be desirable to field test thioligand-induced Hg phytoextraction in combination with phreatophytic trees.

No matter how theoretically attractive a scientific innovation may appear, there will always be barriers to commercial application. The optimisation of Hg-phytoremediation will, therefore, require multidisciplinary efforts to overcome these barriers. This would include not only the contribution of expertise from the traditional disciplines of soil science, genetics, biochemistry, microbiology, botany, environmental engineering, and human health, but also from the humanities areas such as philosophy, economics and

social development. It is imperative that scientific knowledge be disseminated to the public in a comprehensible form, as phytoremediation is strongly depend on their support.

We live in a culture where much is good, more is better, and too much may not be enough. Therefore, it may not be possible for Hg phytovolatilisation and phytoextraction to fulfil 100 % of the prerequisites expected for an environmentally friendly green technology. However controversial and ambiguous, the Hg-phytoremediation/Au-phytoextraction process may represent a valuable perspective for land restoration in less affluent nations. The benefits and environmental costs of this idea should be carefully weighed and discussed among the different sectors of our society.

APPENDICES

Appendix 1: Gold phytoextraction field trials

In 2003, a collaborative program was initiated between the Soil and Earth Sciences group from Massey University, and the Centre for Environmental Research in Minerals, Metals and Materials (CERM3) from University of British Columbia (UBC), Vancouver, B.C., Canada. The objective of this collaboration was to equip and establish a plant-based remediation and mining facility (termed phyto-reclamation) in the Department of Mining and Mineral Processing Engineering at UBC. The phyto-reclamation facility was set up between April and July of 2004 and allowed UBC to develop research activities on the phytovolatilisation and induced phytoextraction of mercury from contaminated soils (Chapters 6 and 9). As the principal researcher involved with the set up of this phyto-reclamation facility, I spent 4 months of my doctoral studies at UBC in Vancouver.

One aim of the phyto-reclamation facility was to demonstrate the feasibility of the gold and mercury induced phytoextraction through a field-scale phyto-reclamation trial. Collaboration was set up with Companhia Vale do Rio Doce (CVRD), from Brazil, to carry out the field research. Unfortunately no Hg-contaminated artisanal mine site was accessible within the mining company facilities to conduct a Au-Hg phytoextraction trial. We, therefore, decide to carry out the experiment at the at the Fazenda Brasileiro gold mine, located in the city Teofilândia, State of Bahia, Brazil.

Appendix 1 presents a publication, co-authored by myself, that is to be published in the journal Minerals Engineering. This publication describes the set-up, outcomes and conclusions of the field demonstration for gold phyto-reclamation technology.

Appendix 2: Plant References

Botanical Name		Common Name
Allium sp	L.	Lily spp.
Alyssum bertolonii	Desv.	
Arabidopsis thaliana	L.	Thale Cress
Astragalus sp.	L.	Locoweed
Artemisia douglasiana	L.	Mugwort
Atriplex canescens	Pursh	Fourwing saltbush
Berkheya coddii	Roessl.	
Brassica chinensis	L.	Bok Choi
Brassica juncea	(L.) Czern.	Indian Mustard
Brassica oleracea	L.	Broccoli
Brassica napus	L.	Rape
Caulanthus sp	L.	Wild Cabbage
Cladium jamaicense	(L.) Benth. ex Kurz	Sawgrass
Eucalyptus globulus	L.	Tasmanian bluegum
Fragaria vesca	Labill.	Wild strawberry
Hordeum vulgare	L.	Barley
Hydrilla verticillata	L.	Hydrilla
Lactuca sativa	L.	Lettuce
Lemna minor	L.	Lesser duckweed
Lepidium latifolium	Bess.	Broadleaved pepperweed
Leucaena glauca	L.	Haole-koa
Lolium perenne	L.	Perennial ryegrass
Lupinus	L.	Lupin spp
Mansoa alliacea	P. Mill. (Syn. *Pseudocallymna alliacium*)	Garlic vine
Nicotiana tabacum	L.	Tobacco
Persea americana	auct. non (L.) Benth.	Avocado
Phaseolus vulgaris	L.	Bush bean
Picea abies	(L.) Karst	Norway spruce
Pinus alba	S. Wats.	Western white pine
Polypogon monspelienses	(L.) Desf.	Rabbits-foot grass
Potamogeton crispus	L.	Curly pondweed
Quercus	Pers.	Oak spp.
Rauvolfia serpentina	(L.f.) Royle	Snakeroot
Scirpus robustus	(L.) Desf.	Saltmarsh bulrush
Silene vulgaris	J.C. & R. Presl.	Bladder campion, Maidens tears
Solanum lycopersicum	(L.)	Tomato
Thlaspi caerulenscens	L.	Alpine pennycress
Trifolium subterraneum	Pursh	White clover
Typha domingensis	Crantz.	Cattail
Vallisneria spiralis	(Moench) Garcke	Tape grass
Vicia villosa	Roth	Hairy vetch
Vulpia myuros	L.	Zorro fescue
Zea mays	L.	Maize

Appendix 3: Thesis data set

The complete data set for this thesis is saved on the accompanying CD-ROM, or can be obtained from the Soil and Earth Sciences Group at Massey University.