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EVALUATION OF MERCURY ACCUMULATION AND BIOTRANSPORTATION IN WETLAND PLANTS AFFECTED BY GOLD MINING AND INDUSTRIAL ACTIVITIES



Odwa Mbanga

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science

University of the Witwatersrand
Johannesburg
February 2017

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any university.



(Signature of candidate)

__22/May/2017_____

ABSTRACT

Six different plant species that grow in a natural wetland impacted by old gold mining and other industrial activities were randomly selected with surface sediments. These included: *Cyperus eragrostis* (Nutgrass), *Datura stramonium* (Jimson weed), *Melilotus alba* (White sweetclover), *Panicum coloratum* (Blue panicgrass), *Persicaria lapathifolia* (Pale smartweed) and *Phragmites australis* (Common reed). These were used to investigate the levels of mercury in the wet and dry seasons, as well as to evaluate which of the species could be utilized for the remediation of mercury contaminated areas.

The results obtained indicated that metal contamination could be determined from sediments and plant tissues. The pH values of the sediment samples were mostly neutral to slightly acidic and the redox potential was high in the wet season. On the other hand the dry season was characterised by very acidic and moderately oxidizing conditions. In summer all six plant species had higher concentration of HgT in sediments, whereas in winter the levels of HgT were elevated in the aerial tissues of the plants. The mercury accumulation patterns differed according to individual plant species and seasonality. Seasonal differences were significant but generally the MeHg concentrations in the wet season were higher in both surface sediments and plant tissues. Mercury methylation differed between species but concentration of MeHg was in general higher in plants with high concentration of mercury in sediments. The conversion of bioavailable HgT seemed more pronounced in tissues of the plants sampled in the wet season unlike those sampled in the dry season.

Generally bioaccumulation factors were less than 1 in both the wet and dry seasons for all the plant species indicating that Hg was mainly retained in sediments. The translocation factor values were greater than 1 meaning metals were accumulated fundamentally in aboveground tissues for the plants *D. stramonium, P. lapathifolia, P. coloratum* and *C. eragrostis* in both the wet and dry seasons. The small bioaccumulation factors combined with translocation

factor values greater than 1 were an indication that mercury present in the sediments was not the only source of mercury for the plant species growing in a contaminated environment. For *P. australis* the translocation of mercury was heavily influenced by seasonality, however this was not the case with *M. alba*.

All the selected plant species demonstrated the capacity to grow in a heavily contaminated area, where *P. australis* and *M. alba* seemed to have developed an exclusion strategy to deal with toxic heavy metals therefore suitable for phytostabilisation. *D. stramonium, P. lapathifolia, P. coloratum* and *C. eragrostis* on the other hand exhibited characteristics of plants that can be successfully used for phytoextraction and phytovolatilization.

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ABBREVIATIONS

AGM: Artisanal Gold Mining

AMD: Acid Mine Drainage

ANOVA: Analysis of Variance

BF: Bioaccumulation Factor

CE: *Cyperus eragrostis*

C_l: Metal concentration in leaves

C_r: Metal concentration in roots

CRM: Certified Reference Material

C_s: Metal concentration in sediments

DNA: Deoxyribonucleic acid

DS: *Datura stramonium*

EDS: Energy Dispersive Spectroscopy

Eh: Redox potential

ET: Evapotranspiration

FIMS 400: Flow Injection Mercury System

Hg: Mercury

HgT: Total mercury

IBM: International Business Machines

ISO: International Standards Organization

LOD: Limit of Detection

LOQ: Limit of Quantification

MA: *Melilotus alba*

MAE: Microwave Assisted Extraction

MeHg: Methylmercury

PA: *Phragmites australis*

PC: *Panicum coloratum*

PL: *Persicaria lapathifolia*

PTFE: Polytetrafluoroethylene

R^2: Coefficient of determination

RSD: Relative Standard Deviation

SA: South Africa

SD: Standard Deviation

SEM: Scanning Electron Microscopy

SPSS: Statistical Package for Social Sciences

SRB: Sulphate Reducing Bacteria

TEL: Threshold Effect Level

TF: Tailings Footprint

TFs: Translocation Factor

TSF: Tailings Storage Facility

USEPA: United States Environmental Protection Agency

Wits: Witwatersrand basin

CHAPTER 1: INTRODUCTION

Over the last decades an incredible amount of research has been done on mercury in the environment. From studies about ecological effects of mercury behaviour and its impact on wildlife and humans (Boening, 2000), mercury speciation in the aquatic habitat (Ullrich et al., 2001) to the proposal of strategies that can be used to remediate mercury pollution in aquatic habitats (Wang et al., 2004).

Mining plays a huge role in the economy of developed as well as developing countries. In about ninety countries in the world gold mining is practiced. South Africa (SA) was among the top producers of gold, together with China, Australia, Canada and the United States of America (Mudd, 2007). This has since changed in 2006. According to the latest information (US Geological survey, 2016) other countries gave surpassed South Africa, including China, Russia, the United States Canada, Peru and Australia. In SA mining mostly occurs in a region known as the Witwatersrand Basin, and 98% of South African gold is mined from this region. Initially, a mercury amalgam method was used for gold extraction (Alpers et al., 2005). The mercury amalgam method works through bringing the ore mined underground to the surface, milled into fine sand and then treated with a film of mercury spread on copper plates, resulting in formation of mercury gold amalgam (Naicker et al., 2003). For the recovery of gold, the mercury gold amalgam is scraped off and distilled. Once mineral concentrate is removed the residual mixture of finely milled ore and water left are tailings dumps (Tutu et al., 2005). The leftover processing chemicals are then transported and deposited to areas near the extraction plant and consequently form part of the mine tailings (Naicker et al., 2003). However, as mining operations reached deeper levels in the ground, miners encountered un-oxidised ore comprising of pyrite (FeS_2) and this interfered with the extraction of gold (Naicker et al., 2003). Due to the interference, the mercury amalgam method had to be phased out and replaced with a cyanidation method which was phased in during the 1890s. Gold cyanidation is used due to the selective dissolution of gold by weak cyanide solutions from other ore constituents (Lusilao, 2012). Once the gold has been dissolved in the cyanide,

it is precipitated with zinc dust and a 10% lead nitrate solution, resulting in the recovery of very fine gold precipitate on a precoat filter (Hilson and Monhemius, 2006). Both the mercury amalgamation and the cyanidation methods have a high selectivity for gold, as such other ore minerals were unaffected during the extraction process and reported to the tailings dams (Lusilao, 2012). Due to high intensive mining operations in the Witwatersrand (Wits) Basin by the end of 1972 (Forstner and Wittmann, 1976), there has been an increase in the number of tailing dams to approximately 240 (Tutu et al., 2005) in this region. This has resulted into acid mine drainage (AMD) distinguished by low pH values, elevated salinity levels, high amount of iron, sulphate, manganese and aluminium, high concentration of toxic heavy metals such as mercury. Poor monitoring of the tailing dams, inadequate design and neglect have exacerbated AMD (Wittmann and Forstner, 1976; Naicker et al., 2003). Oxygen from the atmosphere enables pyrite, and iron sulphide oxidation which further enhances AMD thus causing enormous environmental pollution of the surrounding watersheds (Tutu et al., 2005). Acidic waters aid in the dissolution and add to the solubility and mobility of heavy metals thereby becoming bioavailable to organism and the surrounding environment (Akcil and Koldas, 2006). Wittmann and Forstner, (1976); Naicker et al., (2003) have reported the existence of AMD at the Wits basin and the occurrence of high concentration of heavy metals such as mercury in the surface waters as well as sediments in this region (Lusilao, 2012).

Mercury (Hg) is a heavy metal released into the surrounding during gold mining and other industrial activities and it is amongst the most toxic contaminants to living organisms. Even though Hg occurs naturally in the environment, human activity has resulted into an enormous increase in the amount of its emission. The major sources of Hg that add to its elevated levels in the atmosphere have been identified to be gold mining and coal combustion from power plants (Pacyna et al., 2006). These are known as the main anthropogenic sources of Hg. Scientists have taken to task to explore the use of wetlands as a cheap alternative method for the remediation of heavily contaminated areas. In South Africa, wetlands are located near these anthropogenic sources yet biotransportation and Hg speciation is not entirely understood more especially in areas affected by mining. These

wetlands are connected to rivers which in turn serve as water sources for purposes of domestic, agricultural, recreation and industrial activities. As a result, living organisms and humans have experienced Hg poisoning from soil and water that has been affected by Hg contamination. Unfortunately Hg contamination perpetuates worldwide in spite of this. Conventional methods developed to remediate soils affected by Hg contamination are not economically friendly and their effectiveness in the long run becomes questionable. There is therefore a need to find an environmentally and economically viable alternative for the remediation of Hg contaminated wetland areas. This project was inspired by the lack of knowledge with regards to the use of wetland biota to clean-up mercury pollution emanating from gold mining and other industrial activities. Unfortunately there are very few long term records of mercury and methylmercury in wetland plants in semi-arid areas like SA. Moreover, no seasonal changes of the mercury loads in affected areas were reported until very recently (Lusilao-Makiese et al., 2014), thus establishing widespread baselines or current trends is presently difficult. Understanding the biotransportation and accumulation of mercury in wetland plants becomes important to predict and deal with mercury contamination. In addition, it also important not only to assess the impact of seasonality in terms of wetlands efficiency but also to determine how these seasonal changes will affect the Hg speciation in this type of ecosystems.

CHAPTER 2: LITERATURE REVIEW

2.1 Natural sources of mercury

Mercury by nature can be found in the environment. It naturally occurs in the form of insoluble sulphide minerals in many types of rock material. These include mercury sulphide (HgS), iron sulphide (FeS_2) and sulphur (S). Mercury can also be found in its uncharged form (Hg), forming a complex with gold (Au) or covalently bonded to copper (Cu) and silver (Ag) (Prinz et al., 1978). These geological sites serve as sources of elemental mercury (Hg^0) (Gustin et al., 2001). Mercury sulphide ore and other types of ores containing mercury contribute significantly to the amount of mercury emitted to the atmosphere per year (Hylander and Meili, 2003). Research has shown that in small areas such as those less than 1000 m^2 of Almaden mine in Spain, about more than 6 tonnes of Hg is emitted into the atmosphere per year due to degassing of mercury sulphide minerals (Gustin, 2003). Areas that have considerable amount of heat generated from earth's crust and those that have experienced recent volcanic activity are also sources of Hg which can be emitted to the atmosphere reaching levels of more than 99 tonnes per year (Nriagu and Decker, 2004).

2.2 Anthropogenic sources of mercury

There are several man-made sources that liberate mercury into the atmosphere. These comprise of incinerators for urban, medical and industrial wastes, industrial facilities that produce cement and chemicals, ore processing facilities, fossil-fuel fired power plants, caustic soda production plants, and industries that manufacture ferrous and non-ferrous metals. Pacyna et al., (2006); Dabrowski et al., (2008); Pirrone et al., (2010) identified gold mining and coal combustion to be prime factors responsible for high concentrations of Hg in the atmosphere. The main focal point of the current study was Hg pollution which emanates as a result of gold mining.

2.2.1 Mercury pollution due to Gold mining

Without any doubts gold mining contribute significantly to a country's economy as is the case in South Africa. However, the negative environmental impacts associated with gold mining (pollution) cannot be ignored which come in the form of huge amount of waste material generated throughout the duration of mining activities and ore processing. This pollution presents itself in the form of heavy metals that are often persistent in the air, water and soil. Ancient methods of gold mining involved the use of Hg to form an amalgam with gold for its recovery. During the formation of Hg-Au amalgam, elemental Hg gets lost to the environment and is usually found throughout regions of historic gold mining operations. It is noteworthy to state that, in illegal mining practises gold amalgamation is still utilized this is known as artisanal small-scale gold mining (AGM).

2.2.2 Artisanal small-scale gold mining

AGM can be described as an informal type of gold mining that involves no use of any technical procedure employed by organised mining industries. AGM is characterised by massive environmental deterioration throughout the duration of mining activities right through to even when the mine stops operating (Viega and Hinton, 2002) (Figure 1). Artisanal small-scale gold mining can be further explained as the removal of gold from secondary gold ores by gravity process through the use of amalgamation or cyanidation process (Hinton et al., 2002). Amalgamation is the method that is mostly used by miners and constitutes the extensive use of Hg which poses negative consequences to the environment, human health and social problems (Hinton et al., 2002; Viega and Hinton 2002).



Figure 1: Artisanal gold mining in Gauteng, South Africa

The emission of Hg form anthropogenic sources has been estimated to have risen per year to more than 5.900 tonnes (Hanisch, 1998; Pacyna and Pacyna, 2002). On an annual basis, natural sources account for 5207 Mg of mercury released to the global atmosphere, including the contribution from re-emission processes, which are emissions of previously deposited mercury originating from anthropogenic and natural sources, and primary emissions from natural reservoirs. Anthropogenic sources, which include a large number of industrial point sources, are estimated to account for 2320 Mg of mercury emitted annually (Pirrone et al., 15th ICHEMET, 2010). The greatest contributor is waste material coming from mining operations. Lacerda (2003) estimated that about 20% of Hg in the atmosphere comes from AGM and most of it ends up in water sources such as rivers and wetlands.

2.3 The biogeochemistry of mercury in wetlands

Wetlands are areas that are covered in water for the substantial part of the year which they receive from stream flows, water overflowing from rivers filled to capacity or connections with ground water. The types of wetlands in SA could be

pigeonholed as fens and swamps reason being that they receive water from rivers in the form of lateral inflows and from the atmosphere in the form of rainfall. There are many purposes that wetlands can be used for like the remediation of acid mine drainage (Perry and Kleinmann 1991). Such functions include but not limited to their capacity to act as areas that sink chemicals and pollutants released from human activities such as gold mining. Wetlands are used because they have the capacity to absorb huge amounts of toxic substances and nutrients (Gopal, 1999). They are characterized by sediments conditions such as water saturation throughout the duration of the year. Wetlands are also characterised by water-saturated sediments whose pore spaces are water filled. Consequently these ecosystems are largely anoxic as depth goes deeper to the bottom of the wetland because of the slow rate at which oxygen from the atmosphere diffuses into the wetland (Brinx 1994). The ability of wetland to act as chemical sinks is due to the presence of wetland plants. Mercury found in wetlands can either originate from the atmosphere or be transported from the watershed. In specific cases direct discharge of waste from industrial activities such as gold mining can supply mercury to the wetlands (Zillioux et al,. 1993).

Depending on the physical and chemical properties wetlands can change dramatically the concentration of heavy metal pollutants, and impact on the bioavailability of elements present in these systems. These environments are capable of transforming relatively small levels of inorganic mercury into methylmercury therefore they can be used in the intensive investigation of phytoremediation strategies that can be employed in areas heavily contaminated with Hg (Lacerda and Fitzgeral, 2001). An in-depth analysis of the biogeochemistry of Hg in wetlands and its impacts and availability to organisms living in water such as plants are crucial for the meaningful monitoring and remediation of contaminated areas. The biogeochemical cycling of Hg in wetlands is directly associated with the behaviour of Hg in the atmosphere and aquatic ecosystems.

2.3.1 Cycling of mercury in aquatic environment

The biogeochemical models developed for mercury cycling in both fresh and salty water environments are believed to be similar despite differences in the organic and inorganic ligands (Figure 2) (Hudson et al., 1994). The present study focused on mercury cycling in fresh waters. Mercury in fresh waters can be found in multiple physical and chemical forms such as elemental mercury (Hg^0), mercury bound to inorganic ligands (HgS, $HgCl_2$, $Hg(OH)_2$ etc.) and organo-mercury compounds such as monomethylmercury, dimethylmercury and ethylmercury (Ullrich, et al., 2001). In the aquatic ecosystems the toxicity, solubility and mobility of mercury is determined by its various forms (i.e. its speciation). In addition the speciation of mercury is greatly influenced by environmental factors such as redox potential, the acidity or alkalinity of the environment, the amount of dissolved and suspended carbon and sulphur (Kim et al., 2003).

Elemental mercury (Hg^0) is the predominant form of mercury in the atmosphere. Some portion of Hg^0 comes from the conversion of Hg^{2+} which is initiated by aquatic microorganisms in the presence of reducing conditions (Furukawa et al., 1969; Nelson et al., 1973; Mason et al., 1995). Hg^0 is volatile and relatively unreactive. In the presence of chloride ions, Hg^0 can be oxidized into Hg^{2+}, but under mildly reducing or oxidizing conditions elemental Hg is stable (Demagalhaes and Tubino, 1995; Yamamoto, 1996). Vandal et al., (1991) and Fitzgerald et al., (1994) suggested that during the wet season most surface waters have high concentration of Hg^0. However, due to its volatile nature Hg^0 evaporates from surface waters into the atmosphere. In summer the concentration of Hg in aquatic environment increases due to remobilization from sediments which has been removed from the bottom of the aquatic environment and enable it to enter the aquatic biogeochemical cycle again (Bratkič et al., 2013).

Bratkič et al., (2013) also stated that decreased oxygen concentrations due to higher organic material content and respiration rates leads to the production of methylmercury (CH_3Hg^+). The presence of organic and inorganic complexing agents, redox (Eh) and pH conditions influence the chemical forms of Hg in aquatic systems. CH_3Hg^+ tends to form complexes and these forms of mercury

have high affinity for soft ligands such as sulphur found in the wetland sediments (Yamamoto, 1996). In freshwaters the dominant forms of inorganic mercury are HgOHCl, $HgCl_2$, and $Hg(OH)_2$ (Kim et al., 2003). CH_3Hg^+ is the most toxic form of Hg and its formation is through the methylation of Hg^{2+} by sulphate reducing bacteria (SRB) or other methylating microorganisms present in anaerobic conditions at the bottom of wetland sediments (Kim et al., 2003). CH_3Hg^+ is neurotoxic characterized by bioaccumulation and biomagnification into food webs leading to high concentration, which may in turn result into adverse effects on reproduction and fetal development in mammals and fish (Zanker et al., 2003). Dimethylmercury albeit its toxicity has been observed to occur at extremely low concentrations in the aquatic habitat, in addition it has not been determined without reasonable doubt in fresh water (Harrison et al., 2007). Among the different mercury species, CH_3Hg^+ is of particular interest due to its high toxicity and to its high capacity to bioaccumulate in food chains (USEPA, 1997; Bloom and Watras, 1989; Brosset and Lord, 1995). For toxicological and biogeochemical studies the total concentration of mercury is of little value without knowledge of its chemical forms. Thus, it is of paramount importance to study mercury speciation and factors which influence its mobility, reactivity, and potential bioavailability more especially when dealing with mercury contaminated areas.

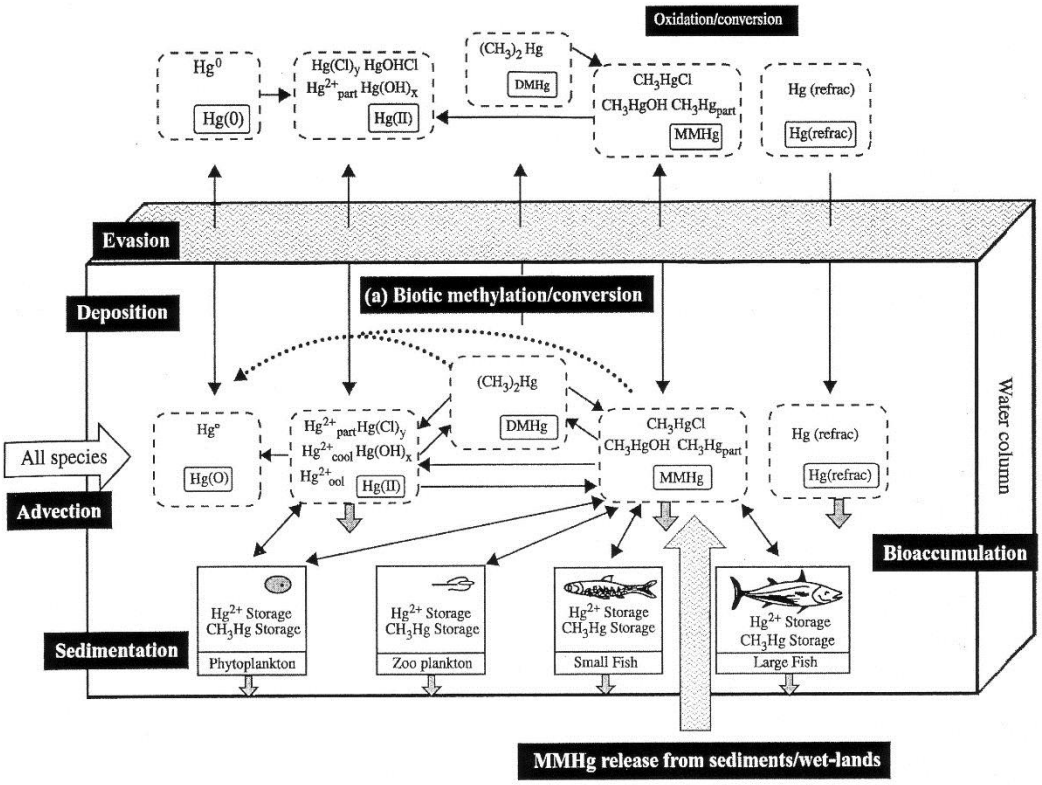


Figure 2: Broad representation of mercury biogeochemistry in the aquatic system (Hudson et al., 1994)

2.3.2 Methylation of mercury

Wetlands are often thought of as production point sources of methylmercury (MeHg). The toxicity of this form of Hg due to its non-polar character, ability to permeate rapidly and diffuse through the cell membranes, bioaccumulate and biomagnify in organisms (Selvendiran et al., 2008; Wood, 1980). Physical factors such as water saturation, temperature and chemical conditions like pH, redox potential, nutrient supply control the methylation of Hg. Microbial activity specifically SRB also plays a pivotal role (Gustin et al., 2006; Benoit et al., 2003). Under reducing conditions in waters a number of mercury sulphide complexes exist they are; HgS^0, $Hg(SH)_2{}^0$, $Hg(SH)^+$, $HgS_2{}^{2-}$ and $HgHS_2{}^-$ (Benoit et al., 2003). Scientists have hypothesised that these complexes might act as sources of inorganic mercury (Hg^{2+}) for microbial activity to convert to MeHg. The principal

area of methylation is aerobic/anaerobic interface, which is usually closer to the surface sediments in aquatic environments (Benoit et al., 2003).

The amount of dissolved organic content is another factor that influences Hg methylation and bioavailability. High methylation rates are usually noticed in surface sediments (Korthals and Winfrey, 1987) where the activity of microbes is greatest due to the input of fresh organic matter. Consequently, aquatic habitats that have elevated levels of organic matter production, such as wetlands may present significantly high rates of methylmercury production (Benoit et al., 2003).

Another factor the influences methylation is pH due to the acid-base chemistry involved in Hg forming complexes with thiols and sulphide groups. A negative correlation relationship between mercury in fish tissues and lake water pH has been noted in several studies (Benoit et al., 2003) demonstrating that pH greatly impacts methylation aquatic environments. In some studies done in freshwater habitats, it was observed that lower pH values corresponded to reduced methylation (Winfrey and Rudd, 1990). However other studies found that elevated levels of mercury methylation in surface sediments and epiliimnetic lake waters were associated with lower pH (Miskimmin et al., 1992; Ramlal et al., 1985; Xun et al., 1987).

2.4 Mercury interaction with plants

There are various ways in which Hg can be transported in the environment they include; the exchange between the atmosphere and sediment surface, ocean, fresh water and vegetation (Figure 3). However, the modes of transport that have significant impact to human beings involve the exchange between soil vs vegetation as well as water vs vegetation. Once Hg accumulates in vegetation it may gain access to human diet. It can also be through the consumption of aquatic organisms such as fish or terrestrial organisms like birds and livestock. Moreover, the movement of Hg between the soil surface and vegetation provides a possibility to remove Hg from contaminated soil by plant uptake. Plants have the unique ability in that wherever they grow they develop mechanisms to remove a variation

of metals Hg included. Several researchers like Bersenyi et al., (1999); Kalac and Svoboda (2000); Coquery and Welbourn (1994) have demonstrated that mercury can be fundamentally accumulated in the root systems of plants growing in contaminated areas. Laboratory research work by these scientists (Beauford et al., 1977; Cavallini et al., 1999; Godbold and Hütterman, 1988) demonstrated that plants growing in solutions that have been polluted by mercury have a tendency to use their roots to absorb Hg and accumulate most of it in the roots than shoots. Volatile elemental mercury can be absorbed by plant leaves via the stomata (Browne and Fang, 1978; Cavallini et al., 1999; Du and Fang, 1982, 1983). High temperatures and mercury vapour concentration increase the potential of plant leaves to take up Hg^0 to a greater extent (Du and Fang 1982). Leaves are also capable of absorbing Hg particulate deposited on the leaf surface and release the volatile Hg^0 into the atmosphere (Siegel et al., 1974; Kozuchowski and Johnson, 1978).

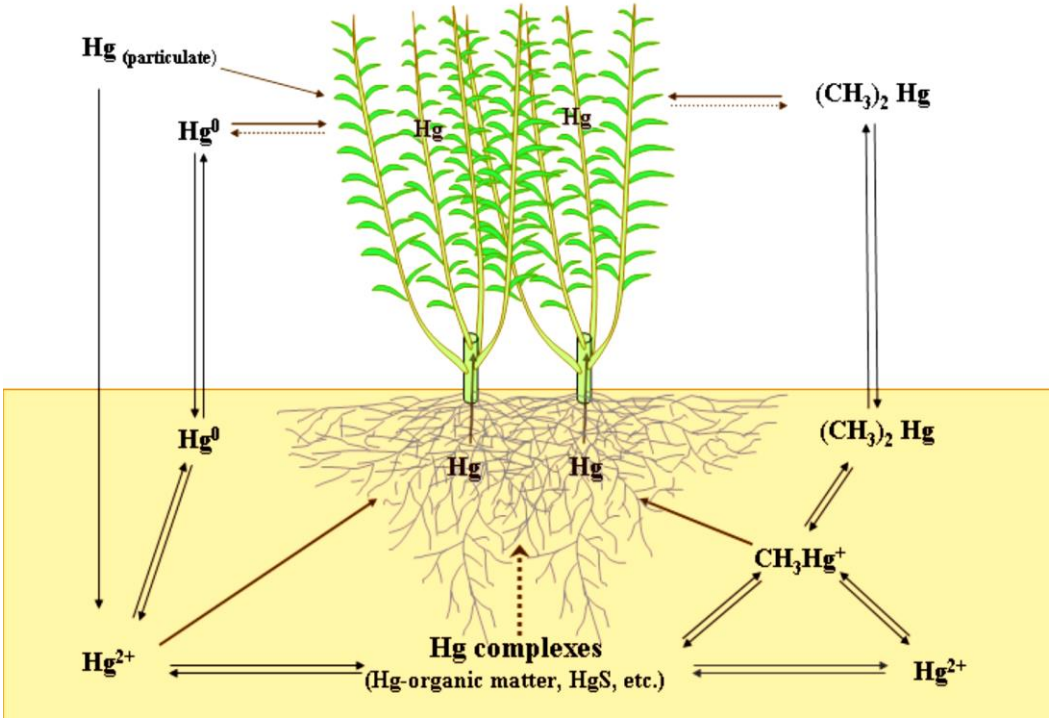


Figure 3: The cycling and interaction of mercury with wetland plants in different environmental media (Wang, 2004)

The biochemical and physiological processes of plants growing in Hg contaminated area might be affected by Hg (Patra and Sharma, 2000). For instance, in order for Hg^0 to interact with most plant biomolecules it must be oxidised to Hg^{2+}, and this conversion is catalysed by peroxidase or catalase (Du and Fang, 1983; Ogata and Aikoh, 1984). Mercury is class B metals therefore its positively charged species have a high affinity for sulphydryl (-SH) group. The fact that most proteins contain the –SH functional group mean that their structure and function can be easily disrupted by the interactions between mercury and the functional group (Clarkson, 1972; Liu et al., 1992; Bizily et al., 2000; Braeckman et al., 1998).

2.4.1 Mercury toxicity and tolerance

The negative impacts cause by Hg in plants can be observed in the deactivation of protein or by Hg bonding to sulphydryl functional groups of vital proteins thus rendering them non-functional (Ferreira et al., 1989). In addition, Hg enhances the production of reactive oxygen species such as superoxide radical ($\bullet O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\bullet OH$) (Ali et al., 2000). The generation of these species interrupts the standard function of proteins these changes also manifest at cellular level. Some of the physical changes associated with Hg toxicity in plants present themselves in the form of reduced biomass, disturbed photosynthetic activity, reduced chlorophyll, potassium, nitrogen and phosphorous contents (Ferreira et al., 1998). In a study conducted by Boening (2000) it was demonstrated that plant species growing in mercury contaminated environmental medium would have cellular defects which would manifest in the cell membranes of root system and result into low levels of potassium content. Moreover, high concentration of Hg in maize plants tissues triggered production of proline, an amino acid related to stress adaptation and ultimately mercury tolerance (Ferreira et al., 1998). Ali et al., (2000) stated that signs of oxidative stress are usually shown by plants that have accumulated Hg in their tissues. This was corroborated by an experiment where by Potamogeton crispus was exposed to 10 μM of Hg depicted high amount of lipidic peroxidation and potassium leakage and a significantly low chlorophyll content. But, the oxidative damage in the plant was lowered at smaller Hg concentration (0.1 to 0.25 μM). The authors attributed this to the presence of elevated levels stress amino acids and peptides like phytochelatins (non-protein thiols) and cysteine. Phytochelatins are oligomers with chelating properties manufactured by plants for heavy metal detoxification (Grill et al., 1985; Rauser, 1999). Grill et al., (1987) showed that monocotyledonous and dicotyledonous plants exposed to cadmium synthesised phytochelatins in response to heavy metal stress. In another study Gupta et al., (1998) demonstrated that plant species synthesised phytochelatins in roots and leaf tissues exposed to various levels of mercury. Therefore this mechanism is

thought of as a strategy plants employ to tolerate the toxicity of Hg (Gupta et al., 1998; Ali et al., 2000).

2.4.2 Mobilization

In soils/sediments metals exist in the non-bioavailable form due to being bound to humic substances and insoluble inorganic soil components or existing as non-soluble precipitates, then mobilization becomes very important in order for the metals to be accumulated by plants. Various mechanisms have been suggested for describing the mobilization of soil bound metals by the plant root system: a) excretion of metal-chelating molecules known as phytosiderophores into the root zone; b) reduction of metals bound to soil by metal reductases (enzymes); c) acidification of the root zone by secretion of protons (Marschner, 1986; Raskin et al., 1994). In and experiment conducted by Marschner (1991) it was observed that plants of the grass family secreted phytosiderophores in response to iron and zinc deficiency and also enhanced the mobility of copper, zinc and manganese from the soil. It was concluded that nutrient deficiency in soils is another aspect that results into soil acidification.

The presence of rhizosphere bacteria plays a significant role in the accumulation of heavy metals in wetland plants. In an experiments conducted by De Souza et al., (1999) it was observed that *Scirpus robustus* and *Polypogon monspeliensis* accumulated lower levels of Hg and Se when bacterial growth was prohibited with antibiotics. This is indicative of the crucial role these symbiotic bacteria play for efficient metal uptake. Mycorrhizae are fungi that grow in association with the roots of a plant in a symbiotic or mildly pathogenic relationship. These fungi act as a link between the root and sediments thereby increasing the surface area of the root hairs (Meharg and Cairney, 2000). Some researchers have proposed that fungi can protect plants by prohibiting any movement of heavy metals such that they are not taken up by the root system (Khan et al., 2000). On the other hand there are contradictory reports which have suggested that fungi like arbuscular mycorrhizae can help plants take up metals reaching toxic levels (Weissenhorn

and Leyval, 1995). In a study conducted by Lakatos et al., (1999) evidence was presented showing that periphyton present in the rhizosphere of *Phragmites australis* enhanced the ability of this plant to take up more and retain heavy metal.

2.4.3 Uptake and transport

The route that essential nutrients needed by plants enter the plants' system is the same channel that toxic metals use to enter plant cells. There are various ways in which plants get exposed to heavy metals, either via aboveground tissue or by their roots or both ways combined. Once in the system the concentration of heavy metal is governed by: (i) the amount of the metal in the soil available for uptake; (ii) the ability to migrate from sediments to the surface of root tissues; (iii) translocation into to the root system from the surface of the roots; and (iv) the translocation of the heavy metal to the aerial tissues from the roots (Patra et al., 2004). The movement of heavy metals in sediments is influenced by factors such as soil pH, dissolved organic content, the amount of the heavy metal itself present in the environment, the properties of the soil like clay, oxides and capability to exchange cations. If the heavy metal is present in large quantity in the soil and its bioavailable most of it will be taken up by the plant. If however, it is strongly adsorbed to the soil, the uptake will depend on the amount of root produced.

Soluble metals can be transported from the sediments rhizosphere to the root system through the route of extra and intracellular pathways, this is determined by whether the transport entails movement of metal ions across the cell wall (apoplast) or across the plasma membrane (symplast) (Figure 4). On occasion that metal ions gain access to the root system they can either accumulate in vacuoles or might be transported to the aboveground plant tissues (Raskin, 1994). The movement of metal ions from roots-to-shoots is made possible by conducting cells of xylem whilst the vacuole is responsible for storage and degradation of metals into less toxic forms (Salisbury and Ross, 1992). Some researchers have indicated that phloem also plays a pivotal role in the translocation of heavy metal ions in plants (Clarkson and Luttge, 1989; Stephan and Scholz, 1993). It has been proposed that these metal ions in the xylem and phloem probably exist as

complexes due to the presence of ligand compounds such as peptides, organic compounds with acidic properties and amino acids which can bind to metal ions. For instance, in an experiment conducted by Clarkson and Luttge (1989), where xylem saps in tomato were investigated, it was observed that xylem copper was predominantly translocated to the aerial tissues in the form of histidine and asparagine complexes whilst iron and zinc were distributed and formed complexes with citric acid. Brooks (1998) indicated that the xylem transportation of nickel in some hyperaccumulators can be linked to carboxylic or amine acids complexation. Other heavy metals and chelated species of iron can be transported in the phloem via complexation with amine nicotianamine (Stephan and Scholz, 1993). Researches relating to the impact organic substances have on the distribution and movement of mercury in plants is currently very rare at least to my knowledge. The notion that phytochelatins are produced in the roots and aerial plant tissues as a mechanistic response to Hg stress demonstrates that the movement of Hg in plants might be greatly influenced by proteins containing thiol functional groups.

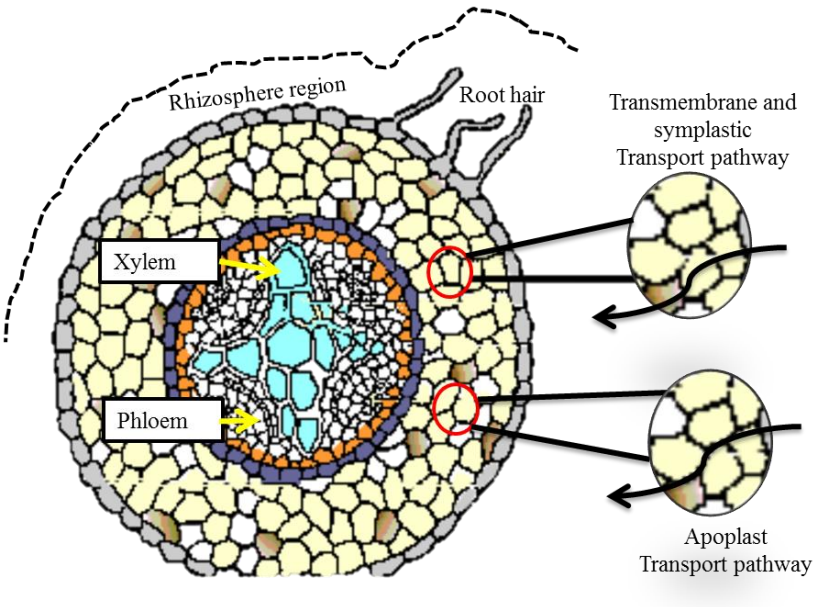


Figure 4: Cross-sectional root system showing movement through (upper bubble) and in between the root tissues (Tsao, 2003).

2.5 Mercury in soil

2.5.1 Soluble mercury speciation in soils

Mercury in soils can bind to ligands such as S^{2-}, Cl^{-}, OH^{-} and form complexes as well as with thiol groups on organic ligands. However, the amount of chloride ions present in a system, pH and the characteristic make-up of the soil determine to a large extent Hg^{2+} complexation with hydroxyl and chloride ions (Anderson, 1979). Generally in natural system (i.e. drainage water and soil solutions) these ions exist in elevated levels therefore the predominant complexes are $HgCl_2$, $Hg(OH)_2$ and HgOHCl. As a result, in most terrestrial environments even small levels of these soluble complexes of Hg can be found (Schuster, 1991). Under anaerobic conditions and high pH values (i.e alkaline environments), ligands that are present in high concentrations are sulphides and bisulphides which in turn influence Hg speciation and complexation (Morel et al., 1998).

Mercury also has a strong affinity for organic matter therefore this is another factor which greatly influences its speciation in terrestrial habitats
(Kabati-Pendias and Pendias, 2000). The composition of organic matter in soils is such that 50% of it is in the form of humic substances and contains high levels of thiol groups (Wallchlager et al., 1998a). The soluble portion of humic substances is made up of humic and fluvic acids and these can act as ligands biding to Hg resulting into Hg complexation. The abundance of Hg complexes with humic substances especially in mineral rich soils is due to the stability of these complexes over the entire pH range from 1 to 14 (Wallchlager, 1996).

2.5.2 Adsorption of mercury in the soil

A detailed review explaining the chemistry of Hg adsorption onto mineral surfaces was done by Schuster, (1991). As stated by this author, the insoluble inorganic species of mercury are the ones that get adsorbed onto the soil surfaces forming complexes since its predominant species in solutions are neutral complexes. The interaction of mercury with mineral surfaces is facilitated by pH

(Evans, 1998). For example, Schuster (1991) observed that under low pH conditions such as 2.5 and 3 the adsorption of Hg on the surfaces of MnO_2 increased. It was suggested that in the process of adsorption hydroxide complexes played a huge role even though this behaviour can be changed by the existence of stronger ligands in soil solutions. For example, soil solutions that have elevated levels of chloride ions translate into mercury-chloro-complex formation thus lowering the adsorption capacity of Hg to soils (Schuster, 1991; Melamed et al., 1998).

The speciation of Hg in soils is greatly influenced by the strong relationship that exists between Hg and organic matter. The presence of many functional groups in humic substances enables a lot of possible mechanism for the binding of Hg to soils, these include complex formation, chelation, ion exchange, precipitation and adsorption (Schuster, 1991). Even though the adsorption capacity of organic matter is high, pH is a factor that cannot be ignored as it plays a critical role in the interaction of Hg with organic matter. For example, Andersson (1979) observed that in neutral soils the sorption of Hg was largely influenced by clay material and ion oxides as opposed to acidic conditions (pH < 5) where the process was largely influenced by organic matter.

2.5.3 Mobility and transport of mercury

The transportation and movement of Hg in terrestrial environments is largely impacted by humic substances. This is because of the ability of Hg to form complexes with organic matter that is water soluble under conditions of natural salinity and pH. For instance, Wallchlager et al., (1989b) demonstrated that soluble fraction of humic substances is the main component found in Hg and organic complexes. These authors also indicated that humic acid molecules coupled to Hg largely controlled its mobilisation and transport. Aquatic environments that are characterised by low pH, low content of suspended particles and elevated levels dissolved organic matter will greatly enhance the mobilisation and transport of Hg even if the water system is located further from the Hg emission sources (Larceda and Solomons, 1992). It is therefore suspected that

mercury from gold mining activities can travel over long distances and upon encountering humic substances shall form complexes (Melamed et al., 2000). In instances where mercury is directly discharged to soils its mobility can occur through interaction with soluble organic acids in aerobic environments (Viega, 2004).

The following factors affect the mobility, transport and bioavailability of metals in soil/sediments and water

- Adsorption and binding to solid surfaces (like oxide ions, organic matter, and soil composition).
- Geochemical composition of sediments and soil (like redox conditions, pH, moisture).
- The material make-up of the soil and sediments, water, including complexing agents, pH, dissolved organic matter, and composition of interfering ions.
- Sequestration and binding in plants
- Species-dependent regulation mechanisms for uptake, excretion, and storage
- Uptake route and specific habitats of test species
- Metal speciation

Toxicity arises only when the bioavailable fraction of the metal enters the plant system. Since plants do not have a standard way of reaction when in contact with heavy metal, biosorption depends on the nature of heavy metals and environmental conditions (Figures 3 and 4).



Figure 5: Bioavailability as a function of exposure

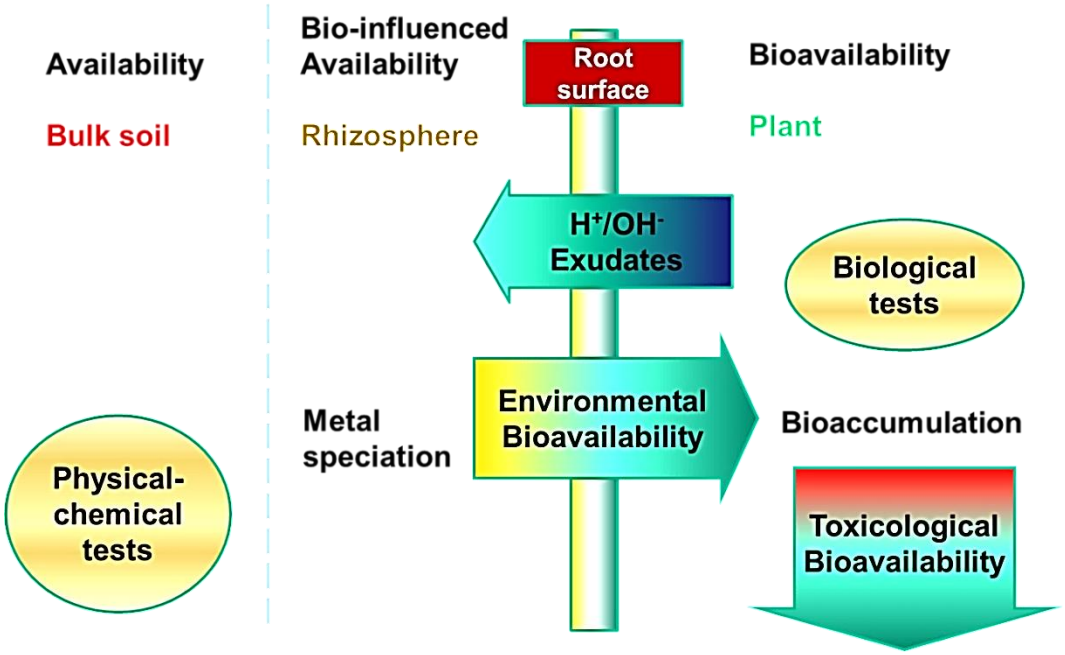


Figure 6: Bioavailability and toxicity of metals (Heaton et al., 1998).

Biogeochemical properties of an ecosystem influence tremendously the levels of contaminants by either increasing or reducing its amount regardless the original magnitude in source. Wetlands are quick to respond to pollutants such as Hg therefore they can be used in the intensive investigation of phytoremediation strategies that can be employed in areas heavily contaminated with Hg. These environments may not only concentrate elements, but in most cases, alter the biogeochemistry of metals and ultimately influence their bioavailability (Lacerda and Fitzgerald, 2001, ISO 17402, 2008) (Figure 6 & 7).

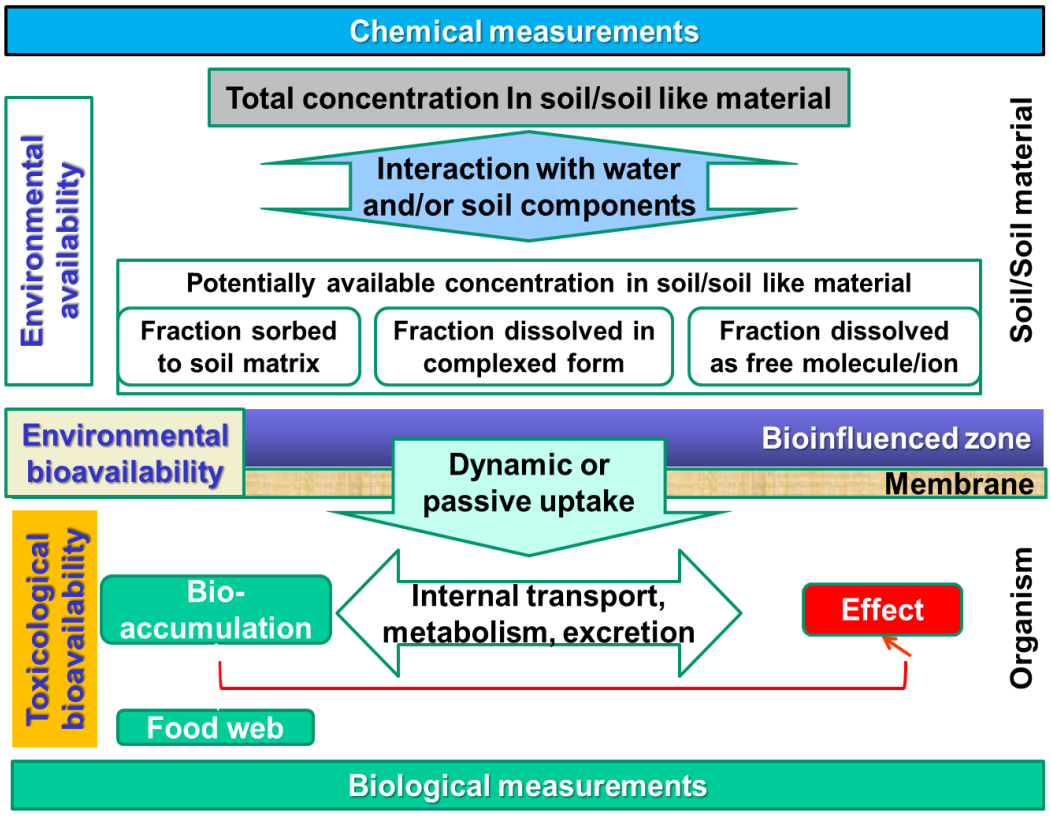


Figure 7: Schematic illustration of the concept of bioavailability (ISO 17402, 2008)

2.6 Remediation of mercury

There are numerous ways in which heavy metal contaminated soil can be remediated, these include but not limited to the chemical, biological and physical techniques. The conventional techniques are precipitation, ion exchange, neutralization, electro-winning, coagulation or membrane processes. However, physical and chemical remediation methods can negatively impact the characteristics of the soil, disrupt the diversity of plant and animal life and leave the soil permanently damaged and as a futile medium for plant growth (Heaton et al., 1998). These remediation techniques are, in general, characterized by high capital and operational costs, problems of residual metal sludge disposal and may lead to loss of mercury (Padmavathiamma and Li, 2007; Heaton et al., 1998; Tangahu et a., 2011). Therefore there is a need to establish a cost effective clean-

up method to manage pollutants from the soil leaving the soil intact and its fertility uncompromised. One such method is phytoremediation which can be defined as the use of plants to degrade, transfer, remove and stabilize contaminants in soil, sediment and water in order to clean contaminated environments (Padmavathiamma and Li, 2007). This strategy is advantageous because plants not only minimize soil erosion but also enhance soil structure. There is however drawbacks associated with these phytoremediation strategies some of which are the accessibility of mercury to plant roots which might limit phytoremediation. And the fact that mercury is not able to move from plant root to aerial tissues once inside the plant suggest that plants do not have to capacity to transfer viable amounts of mercury out of the soil/root system (Heaton et al., 1998).

Advantages and disadvantages of phytoremediation

Macek et al. (2000) gave a comprehensive review of the advantages and disadvantages of phytoremediation. The main advantages of phytoremediation are:

• Low operating costs
• Far less disruptive to the environment
• In situ application avoids excavation.
• Large-scale clean-up operations
• A relatively easy process with available equipment and supplies generally used in agriculture
• High probability of public acceptance

Like any other method of environmental remediation, phytoremediation has its ***disadvantages***:

• Slower than some other alternatives to restore an area
• Limit of the climatic and geological conditions of the contaminated site, e.g. temperature, altitude, soil type, and accessibility to agricultural equipment
• Biological methods are not capable of 100% reduction of contaminants
• Formation of vegetation may be limited by extremes of environmental toxicity
• Need to take care of the accumulators after remediation to avoid reemission

2.6.1 Classes of phytoremediation

There are various factors which govern the type of phytoremediation method which will be employed at a specific site, they include the type of contaminants, conditions of the site, the amount of clean-up that are needed and the types of plants. Phytoimmobilization and phytostabilisation are techniques specifically used for contaminant containment as opposed to phytoextraction and phytovolatilization which are used for removal of contaminants (Padmavathiamma and Li, 2007). To categorically define various plant-based techniques of phytoremediation with each unique mechanism of action for remediating environments that have been prone to metal pollution: (1) phytostabilization where by metal contaminant is stabilized by plant roots within the rhizosphere as opposed to being removed from the soil; (2) phytofiltration where plants are used to clean aquatic environments; (3) Phytovolatilization in which metals from the soil are extracted by a plant then released into the atmosphere by volatilization; (4) phytoextraction in which metals from the soil are absorbed by a plant then translocated to the harvestable aboveground tissue where they accumulate (Padmavathiamma and Li, 2007).

2.6.2 Phytostabilization

This phytoremediation technology takes advantage of plant species to restrict contaminants and keep them in the soil, through absorption and accumulation by plant roots such that a contaminant is adsorbed onto the roots or it precipitates within the rhizosphere only (Padmavathiamma and Li, 2007). This mechanism minimizes the movement of pollutants and inhibits movement to groundwater and air as indicated in Figure 8. It is best demonstrated in fine textured soils that have high levels of organic matter (Padmavathiamma and Li, 2007). Phytostabilization is characterized by plants that have a generation of high root biomass capable of minimizing the mobility of pollutants via uptake, precipitation and storage in roots rather than transfer to aboveground tissue.

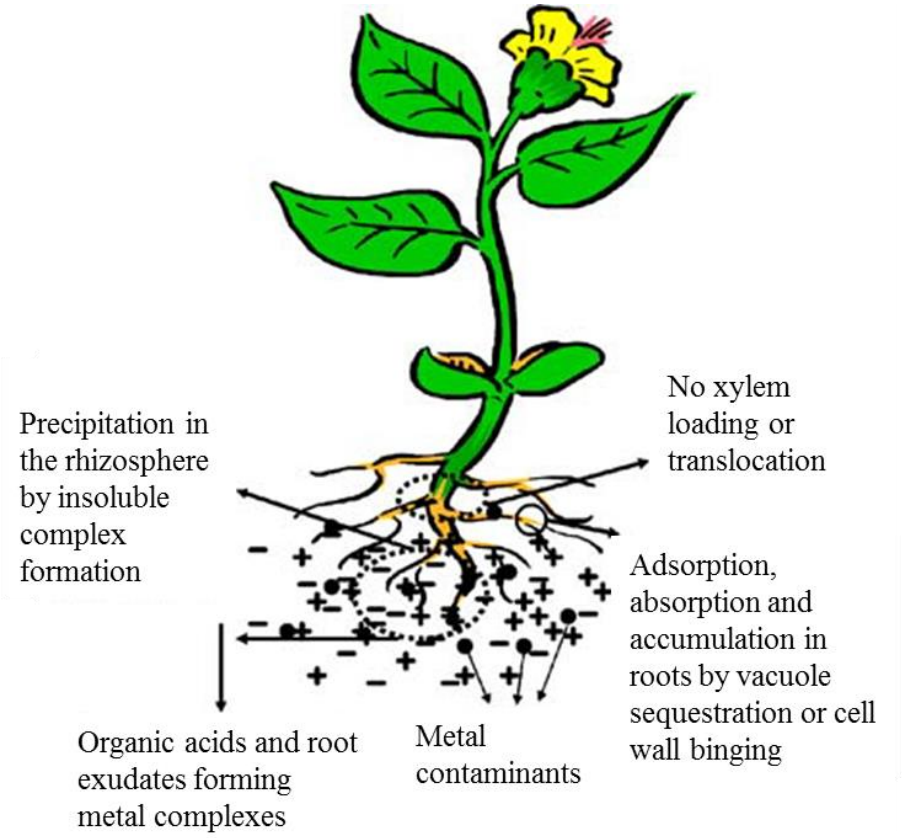


Figure 8: Diagrammatic illustration of phytostabilisation (Padmavathiamma and Li, 2007)

Out of the 17 plant species sampled from a heavily polluted site, Yoon et al., (2006) demonstrated that those with elevated levels of pollutants in the belowground tissues compared to soil coupled with highest concentration in roots relative to shoots were more than capable for phytostabilization. Adriano et al., 2004; Berti and Cunningham 2000; Cunningham et al., 1997 proposed that there are mechanisms such as generation of non-soluble metal complexes around the rhizosphere this in turn prevents the mobility and movement of a metal thus restriction from entering a plant system. Plant species with the potential to be used for phytostabilization take up low levels of metal contaminants, therefore can be thought of as potent tools to attain stabilization of tailings with low possibility of affecting the food chain (Padmavathiamma et al., 2007).

2.6.3 Phytofiltration

Prasad and Freitas, (2003) defined this method as those plants that utilize their root system to concentrate, adsorb and precipitate pollutants (metals) mainly from aquatic environments. Plants used in pyhtofiltration employ various mechanisms to achieve the aforementioned strategies which include: complex formation within the root zone, ion exchange and chemisorption (Gardea-Toresdey et al., 2004). Precipitation of metals in the root zone is facilitated by the production of root exudates and this may alter the pH within the area. Dushnekov and Kapulnik, (2000) contend that in order for plants to be used for phytofiltration they should possess qualities such as the ability to accumulate reasonable amount of the metal(s) of interest, significant generation of root biomass and must be easy to handle as they require harvest from time to time.

2.6.4 Phytovolatilization

This remediation method takes advantage of the fact that some plants can uptake contaminants from the soil, convert them into evaporative forms which will eventually be transported into the atmosphere (Padmavathiamma and Li, 2007). The strategy of this remediation strategy is shown in Figure 9. Bizily et al., (1999) demonstrated that plants whose DNA material has been modified such that these plants express *mer* A and *mer* B genes were capable of transforming organo-mercury and Hg^{2+} to Hg^{0} which easily evaporates into the air and less toxic. This was corroborated by Rugh et al., (1996, 1998) stating that plants have the ability to take up ionic as well as organic mercury through their root system and this gets transported to the aboveground plant tissues. Little information is known about the subsequent volatilization of this metal once in the leaves of plant species albeit the high vaporization rates of Hg in general. Hg accumulated on the leaves of plant species volatilizes and escapes to the atmosphere via the stomata (the greater the surface area of the leaves the higher the chances of Hg volatilization). However, it is noteworthy to state that elemental mercury can still return and be

deposited back into the water sources and soils, thus methylation can re-start all over again.

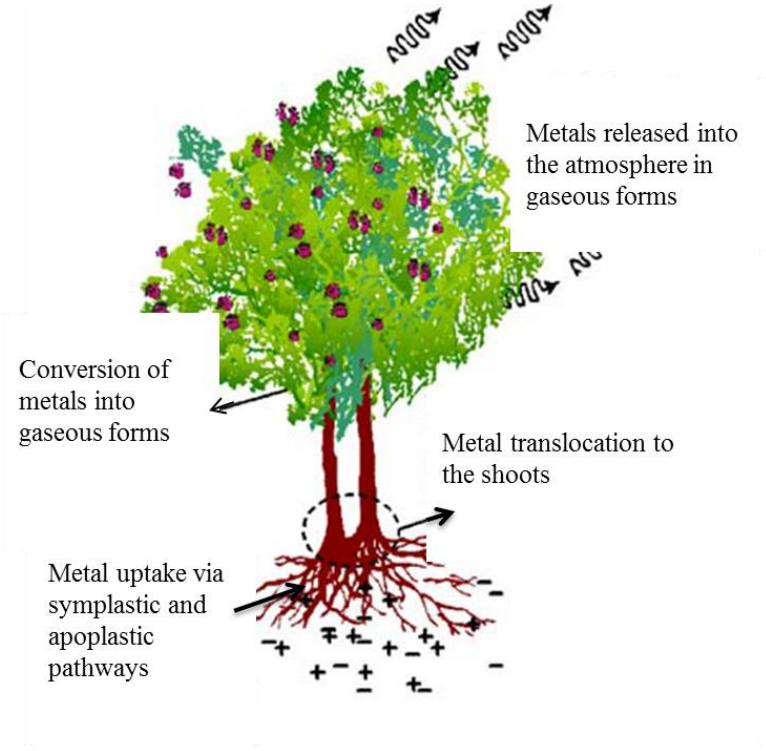


Figure 9: Illustration of phytovolatilization (Padmavathiamma and Li, 2007)

2.6.5 Phytoextraction

This is the technique whereby plant species capable of accumulating pollutants take up metals from contaminated environments and store them in the aerial tissues (see Figure 10) (Salt et al., 1995). Plants under this category are characterized by high translocation factors, high accumulation and tolerance of metal, production of high root biomass and minimal release of pollutants into the atmosphere (Padmavathiamma et al., 2007). These plants are usually termed hyper-accumulators (McGrath and Zhao, 2003). More than 399 plant species have been recognized as hyper-accumulators of metals, for instances, Reeves and Baker, (2000) indicated that up to 31000 μg g^{-1} dry weight of nickel could be

accumulated by *Thlaspi spp* and 43710 µg g^{-1}dry weight of zinc. Unfortunately hyper-accumulators of mercury were still yet to be found.

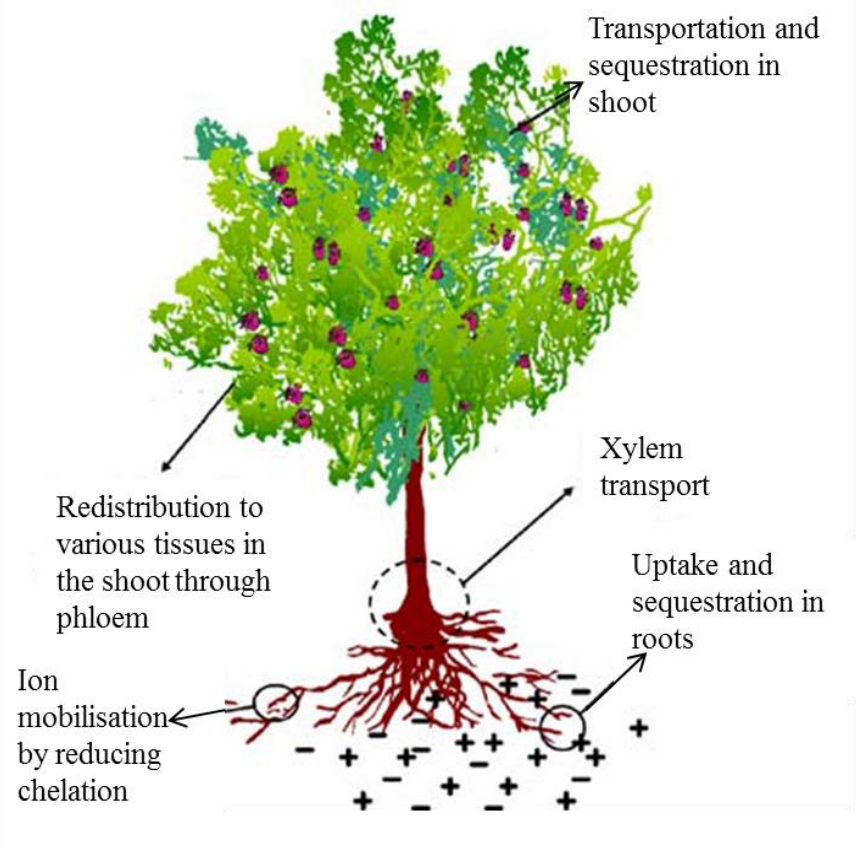


Figure 10: Mechanistic presentation of phytoextraction (Padmavathiamma and Li, 2007)

2.7 Statistical tools used for data analysis

2.7.1 Statistical package for social sciences

Statistical Package for Social Sciences (SPSS) is a software package that can be used to perform comprehensive statistical analysis on research data. It was developed by Norman H. Nie, Hadlai C. Hull and Dale H. Bent at the University of Stanford in 1975. As of 2009 IBM bought SPSS, it is now fully incorporated into the IBM Corporation Business Analytics Software portfolio. SPSS can fulfil a variety of statistical functions but in this study it was specifically used for test for normality, analysis of variance, correlation and linear regression and principal

component analysis. All of these functions were performed on version 23 IBM SPSS.

2.7.2 Normality test

In research before any comparison is done, an assessment of the normality of data is needed to test the distribution of each continuous variable in the research data. The distribution of data whether is normal on not normal will determine whether parametric or non-parametric tests can be employed to make inferences about the data. In order for some statistical procedures such as analysis of variance, correlation, regression and t tests (these are known as parametric tests) to be used the data analysed has to be normality distributed (Ghasemi et al., 2012). Normality test should be treated with the seriousness that it deserves, for when the assumption does not hold it becomes very difficult to make reliable and accurate inferences about the research data. In the present study because the sample size was less than 50, to minimize statistical errors it was of utmost importance to assess data distribution, in which case a Shapiro-Wilk test was used to test for normality (Ghasemi et al., 2012) (Table 1). In the aforementioned test, sample data are compared to a normally distributed data with the same mean and standard deviation (Ghasemi et al., 2012). The null hypothesis for this test is that the data are normally distributed, this is rejected if the p value is below 0.05. In SPSS output the p value is labeled as Sig circled in red (Table 1). In this hypothetical example both the p values for HgT and MeHg are above 0.05, thus the null hypothesis is kept. Therefore in terms of the Shapiro-Wilk test it can be assumed that the data are normally distributed.

Table 1: Hypothetical example of a normality test on SPSS

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
HgT	.152	10	.200*	.970	10	.894
MeHg	.169	10	.200*	.960	10	.782

2.7.3 One way analysis of variance

The one way analysis of variance (ANOVA) is a parametric test used in statistics to establish if there exist any statistically significant distinctions between the population means of two or more independent groups (Green and Salkind, 2003; Morgan et al., 2004). This particular research study dealt with two population groups therefore an independent samples t-test was used to ascertain statistically significant differences between the population means of the two groups. Both ANOVA and independent t-test are known as significance tests and are widely used in analytical chemistry to evaluate experimental data (Miller and Miller, 2000). The importance of the independent samples t-test is that population means between two independent groups on the same continuous variable are compared. In a significance test the truth of a null hypothesis is tested, often the null hypothesis is that there exists no significant difference between the population means of groups being compared aside from that which can be accounted for by random variation (Miller and Miller, 2000). If the null hypothesis is true, the probability that the observed difference between the population mean of the two groups comes from random errors can be calculated on SPSS (Green and Salkind, 2003). If the calculated probability is low the null hypothesis is unlikely to be true. Under normal conditions the null hypothesis will be rejected if the probability also known as the p value is less than 0.05 (Miller and Miller, 2000). In such instances at 5% confidence interval the difference is said to be significant.

2.7.4 Correlation

Correlation is a unitless measure of the strength of a relationship between two variables. The Pearson product- moment coefficient of correlation, r (Pearson's correlation for short) is the mostly used model of correlation. The ranges within which r values span are from -1 to +1. A correlation coefficient of 0 is indicative of no association between the two variables. A value of +1 is a perfect positive relationship and a correlation value of -1 demonstrates a perfect negative correlation (Crawford, 2006, Figure 11).

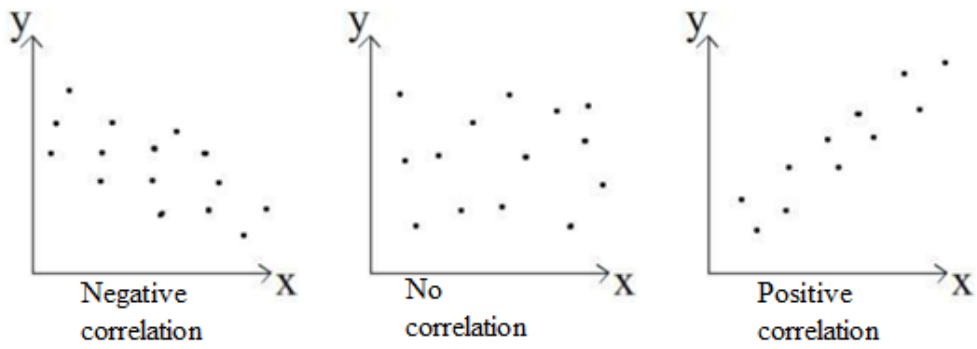


Figure 11: Examples of correlational relationships

Correlation data is usually presented in the (x; y) form however, none of the variables is thought of as a predictor or an outcome because they are treated equally (Crawford, 2006). Graphically correlation data is usually shown in the form of a scatter plot (Figure 11). A regression line which best fits the data is plotted accompanied by an equation which best describes the relationship between the two variables. From the calculated regression equation the nature and the strength of the relationship can be determined.

2.8 Data processing

2.8.1 Bioaccumulation factor

Bioaccumulation factor (BF) can be defined as the concentration of a metal in the root system divided by the concentration present in the sediments and it indicates accumulation behaviour of a plant (Majid et al., 2014). BF is largely used to establish the extent of a plants' ability to uptake heavy metals from polluted sediments into the root tissues. BF is appropriately calculated as:

$$BF = \frac{C_r}{C_s} \qquad (1)$$

where: C_r is the metal concentration in roots (µg kg^{-1}) and C_s representing metal concentration in sediment samples (µg kg^{-1}). If the BF is greater than 1 then a plant has a higher uptake capacity of metals and the BF that is less than 1 is indicative of plants with very little ability to transfer contaminants from the sediments to roots (Radulescu et al., 2013).

2.8.2 Translocation factor

Translocation factor (TF) refers to the ratio of the concentration of a metal in plant leaves to that found in the root system (Majid et al., 2014). TF assists in the determination of the ability of different plants to take up toxic metals from sediments and translocate them to the aerial tissues (Yoon et al., 2006). The equation used to calculate TF is:

$$TF = \frac{C_l}{C_r} \qquad (2)$$

where: C_l represents the metal concentration in the leaves (µg kg^{-1}) and C_r being the metal concentration in the root system (µg kg^{-1}). Plants characterised by TF exceeding 1 are grouped as high-efficiency plants suitable for phytoextraction because of the effectiveness in translocating metals from roots to shoots (Majid et al., 2014).

2.9 Analytical validation parameters

When any analysis is done on any analytical instrument more especially when dealing with trace elemental analysis, the aim is always to get precise, reliable, accurate and consistent data. Analytical validation methods become pivotal in attaining this goal. The results obtained from method validation can shed light into the consistency, quality and reliability of analytical results. In this present study limit of detection (LOD), limit of qualification (LOQ), linearity and reproducibility & repeatability were parameters of focus for the determination of method validation.

2.9.1 Limits of detection and quantification

Limit of detection (LOD) can be define as smallest amount of an analyte in a sample that can be detected by an analytical instrument but not certainly quantified as an exact value (Huber, 2010). An LOD can be based on the sensitivity of an instrument used in a particular analysis (instrument based detection) or on the method used to determine the amount of an analyte in a sample (method based detection). The detection limit based on an instrument informs an analyst about the sensitivity of an instrument to detect an analyte in a sample without any interference. The method based detection on the other hand determines how much analyte is needed to distinguish the signal of an analyte from the intrinsic noise that might be present. The detection limit based on the method takes into consideration both the sample preparation technique used to prepare the analyte as well as the minimal response given by the instrument upon the detection of the analyte. LOD can be estimated using various methods but in this study the focus was on the calculation from standard deviation of the blank solution (Shrivastava and Gupta, 2011).

The minimum amount of an analyte in a sample whose quantity can be measured with suitable precision and accuracy is known as the limit of quantification (LOQ) (Huber, 2010).

The blank determination calculation method is utilised to evaluate LOD and LOQ on condition that the analysis of blank solutions yields an instrument response with a standard deviation that is not zero (Shrivastava and Gupta, 2011). The mathematical expression of LOD is such that the mean value representing the concentration of the analyte solution corresponding to the blank is added to standard deviation of the blank multiplied by three, whereas LOQ is expressed as concentration of the analyte corresponding to the blank solution added to standard deviation of the blank multiplied by ten as presented in the equations below:

$$LOD = mean_{blank} + 3SD_{blank} \quad (3)$$

$$LOQ = mean_{blank} + 10SD_{blank} \quad (4)$$

This method is fast and easy to apply. The disadvantage is however that uncertainty associated with analytes that have low concentration whether they will yield signal response that are different from a blank sample (Shrivastava and Gupta, 2011).

2.9.2 Linearity

The coefficient of determination often denoted as R^2 or r^2 is used in statistics to evaluate how well the observed data are to the fitted regression line. It is a statistic tool utilised in simple linear regression and it provides information about whether the fraction by which the variance of the errors is less than the variance of the dependent variable. The coefficient of determination is denoted R^2 because in a simple linear regression model it is just the square of the correlation between the dependent and independent variables, which is commonly denoted by r. In an instance where the regression line is presented in the form of an equation and the y intercept is specified, the coefficient of determination is often denoted as r^2. Both r^2 and R^2 range from 0 to 1, an R^2 of 0 means that the dependent variable cannot be predicted from the independent variable. Whereas an R^2 of 1 indicates that the dependent variable can be estimated without error from the independent variable (Miller and Miller, 2000).

2.9.3 Reproducibility and repeatability

Reproducibility refers to the ability to repeat an experimental procedure using the same method under different conditions and producing independent results which are close and similar. Repeatability is the difference of measurement an analyst gets by repeatedly measuring the same item multiple times (Slezák and Waczulíková, 2011). These are essential when monitoring precision and accuracy of analytical results from an instrument and play a very crucial role in method validation.

CHAPTER 3: AIMS AND OBJECTIVES

3.1 Aims

The study aimed to establish temporal trends in mercury speciation and the influence of environmental changes on its accumulation and bio-transportation through the use of wetland biota in order to identify potential, cost effective remediation measures that could be employed in contaminated areas.

3.2 Objectives

The above aim was addressed by the following specific objectives:

- To assess the potential impacts of mercury contamination in wetlands and riverine systems.
- To determine the influence of seasonal changes on mercury speciation by studying its accumulation and biotransformation using wetland biota.
- To indicate the best biota combination for effective trapping and removal of mercury in contaminated wetlands.

3.3 Key questions

The research attempted to answer the following questions:

- Most wetlands undergo seasonal changes in saturation which are characterised by periods of flooding in summer and drying out during winter in South Africa. How does this seasonality affect mercury uptake by wetland plants?
- Some wetland plants can convert mercury to other forms, some have protective systems which prevent them from taking up mercury. Other plants store mercury on their leaves. Which of these plants will accumulate and retain large amounts of mercury?

3.4 Justification

Biogeochemical models for cycling of mercury in wetlands affected by gold mining have been developed in Europe and North America. These systems are unique to those found in South Africa (SA) particularly because, it is a semi-arid region and most wetlands are river-fed and therefore undergo seasonal changes in saturation which are distinguished by phases of flooding in the wet season and drying out during dry season. Summer is characterised by high temperatures which can lead to high evapotranspiration rates as is the case in the interior of the country, this can also result in the concentration of pollutants to very high levels. While almost all heavy metals are cumulated in wetlands due to precipitation (after pH rise- liming), mercury in anaerobic sediments is either reduced to elemental mercury and or organomercury species (Lusilao-Makiese 2012). SA was reported to be the second emitter of mercury in the world contributing more than 10% of the global mercury emission (Pacyna, 2006). This poses a concern since inadequate research work has been done on the effects that mercury has on the environment. Both large scale and artisanal mining has had an effect on the emissions of mercury, and there appears to be very limited information regarding bio-transformations and bioavailability of mercury. Mining in the east and central rand was dominant between 1886 and early 1970s. This has led to a significant increase in pollution in a form of acid mine drainage resulting from tailings dumps (Naicker et al., 2003, Tutu et al., 2008). There is therefore a need to gain a thorough understanding of the processes involved in mercury formation/emissions and the effects these have on the environment and what potential risks this poses on. Previous research on the Hg distribution in the Witwatersrand (Wits) Goldfields has demonstrated a drastic change in Hg speciation with seasonal changes. It is therefore, important not only to assess the impact of seasonality in terms of wetlands efficiency but also to determine how these seasonal changes will affect the Hg speciation in this type of ecosystems (Lusilao, 2012). This project was inspired by the paucity of research on the behaviour of mercury and methylmercury in wetland biota growing in areas that have been affected by mining and other industrial activities. Unfortunately there are very few long term

records of mercury and methylmercury in wetland plants in South Africa, thus establishing widespread baselines or current trends is presently difficult. Understanding the bio-transportation and accumulation of mercury in wetland biota is therefore necessary in order to predict the potential impacts and hazards associated with mercury contamination, and ultimately find an alternative cost effective method for the remediation of contaminated areas.

CHAPTER 4: RESEARCH METHODOLOGY

4.1 Chemicals and reagents

Listed below are analytical grade acids and chemicals used in sample preparation and they were purchased from Merck chemicals (Pty) Ltd (Johannesburg, South Africa). Nitric acid (HNO_3), Hydrochloric acid (HCl), Hydrofluoric acid (HF), Hydrogen peroxide (H_2O_2), Boric acid (H_3BO_3) in powder form, Toluene and liquid nitrogen.

Hydrobromic acid (HBr), L-cysteine and these ultra-pure acids and chemicals HCl, HNO_3, Hydrogen sulphate ($H_2SO_{4)}$, Tin chloride ($SnCl_2$) were purchased from Sigma-Aldrich (Johannesburg, South Africa). Deionised water (d-H_2O) used for dilution and preparation of standard solutions was purified from a Milli-Q-RO_4 system (Millipore, Bedford, MA, USA).

4.2 Instruments

A multiprobe GPS Aquameter^{TM} (Aquaread, England) was used to record field parameters during sampling. Once samples were brought to the laboratory, a porcelain knife (lassar, South Africa) was utilised to separate and cut plant samples into their tissues (roots, stem and leaves). A FreeZone^{6} freeze dryer system from (Labcono, Kansas city, USA) was utilised to remove any moisture from the samples. The moisture content was monitored through the use of an analytical balance (Precisa 180A, Switzerland), to determine the percentage of water in a sample by drying the sample to a constant weight. All samples that required weighing were weighed using this analytical balance with a precision of 10^{-4} g. This research dealt with solid samples that needed to be converted into liquid form prior analysis of which a closed microwave assisted extraction (MAE) system (Multiwave 3000, Anton Paar, Johannesburg South Africa) was used for sample preparation. In the case of the determination of organic species of mercury a DLAB MX-S vortex mixer (CC Imelmann (Pty) Ltd, Johannesburg South Africa) and a (Hettich Lab Technology, Germany) centrifuge were used. All the

samples were analysed using the Flow Injection Mercury System coupled to a Cold-Vapor Atomic Absorption Spectrometry (FIMS 400, PerkinElmer, Johannesburg South Africa).

4.3 Cleaning procedure

Presented below is a cleaning method adopted from (Monperrus et al., 2005):

- All the containers involved in the study were soaked in a water bath containing 2% of biocide detergent for half an hour. They were thoroughly washed through the use of a brush, and then rinsed with tap water.
- An acid bath containing 10% of HNO_3 by volume was prepared into which all the vessels were soaked for 48 hours. Containers were then rinsed with deionized water with an electrical resistivity of 18.2 MΩ cm.
- A clean paper towel was used to dry all the vessels which were kept free from contamination in sealed polyethylene bags until use.

4.4 Sampling protocol

4.4.1 Scope of the study

Germiston is a heavily industrialised area which is part of the greater Johannesburg, located in the east of Johannesburg characterised by a history of intensive gold mining activities. Some of these activities are artisanal gold mining, tailings storage facility (TSF) that are undergoing reprocessing (Figure 13), cement production and industries involved in the manufacturing of fuel, petroleum, chemicals and rubber products. Thus this area can be considered to have a number of pollution point sources whose pollutants can be transported in large amount to a natural wetland found in this area. This natural wetland is further prone to pollution as heavy metals, organic compounds, suspended matter and a large amount of nutrients are transported from the surrounding areas into the wetland.

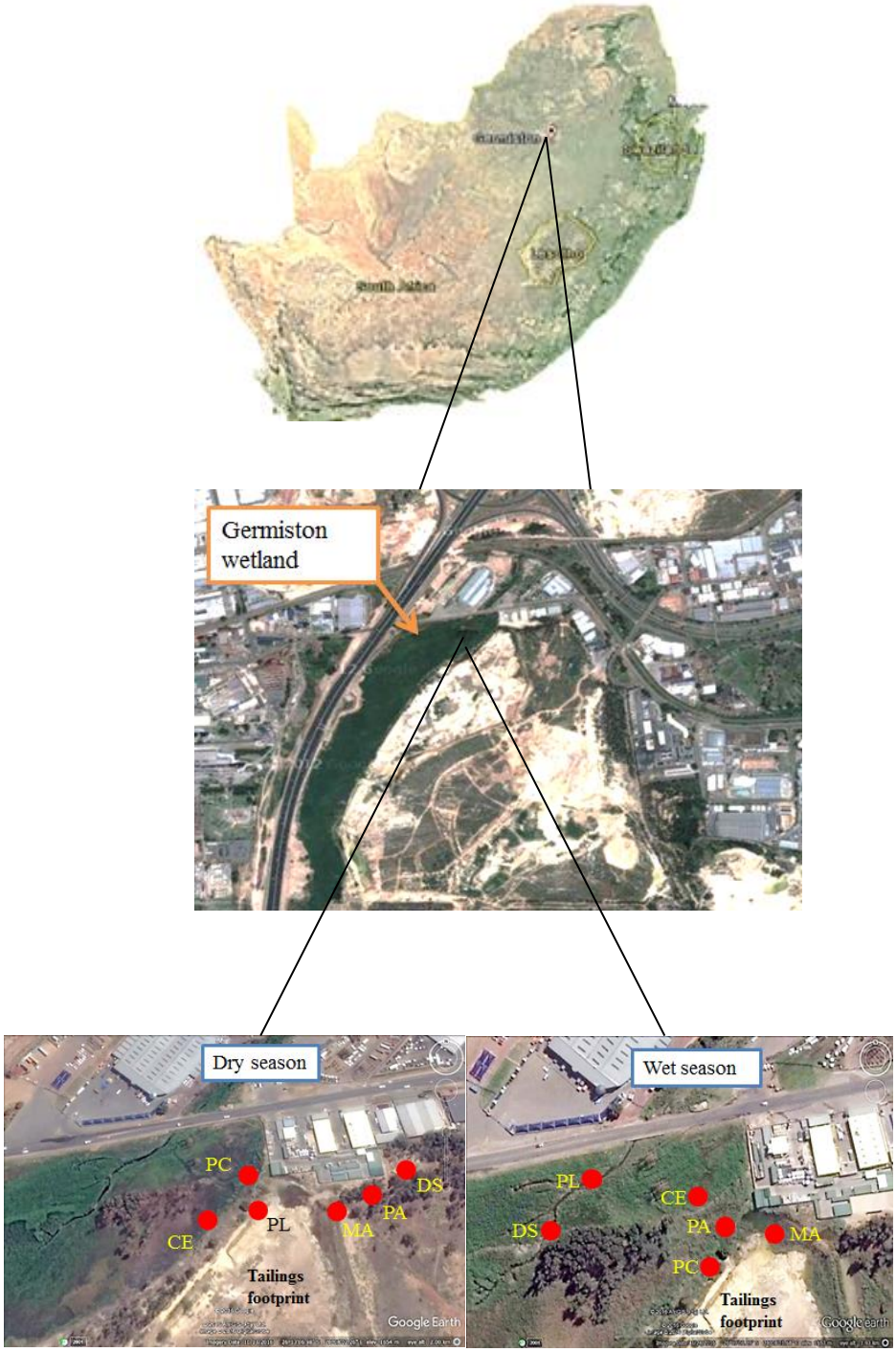


Figure 12: Location of the Germiston sampling site with sampling points

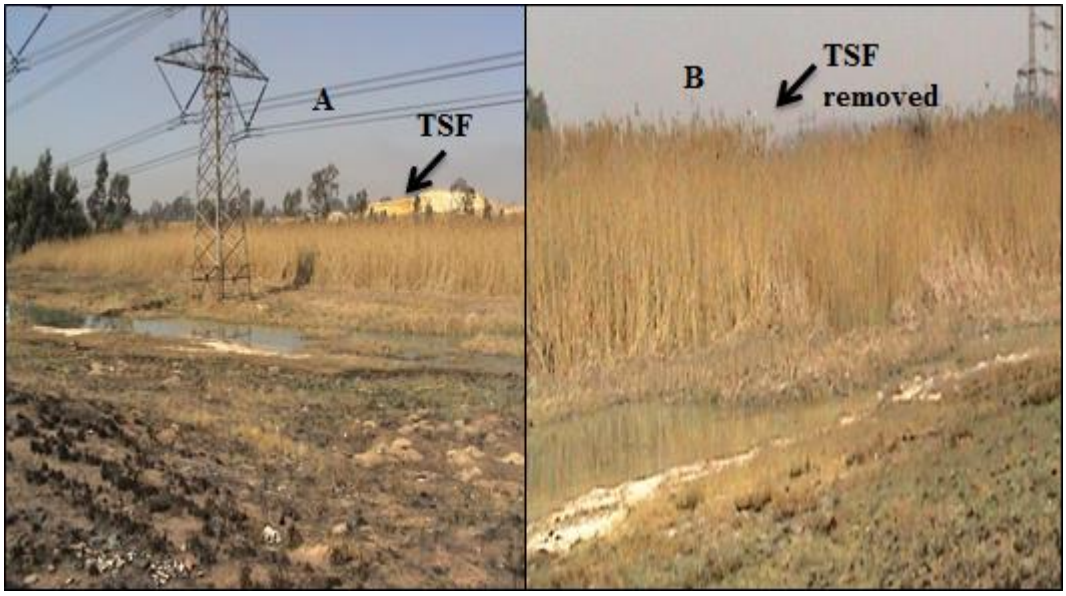


Figure 13: Study site of the Germiston wetland surrounded by gold tailings storage facility (A) and after tailings dumps have undergone reprocessing (B)

The sampling site is adjacent to the tailings footprint (TF) (Figure 12). Thus, metals from the TF can be washed to the sampling site via fluvial transportation and erosion. In addition, neglected TSF also found in the area are subjected to water and wind erosion this might lead to heavy metals to be distributed to water systems and the surrounding areas. This might also result in the formation of acid mine drainage. Connected to the wetland is Natal Spruit River (Figure 14) that flows into the Vaal River. Furthermore the Vaal River serves as a water source for purposes of domestic, agricultural, recreation and industrial activities in the Vaal region. This poses health hazards and negative impacts on the environment at large.

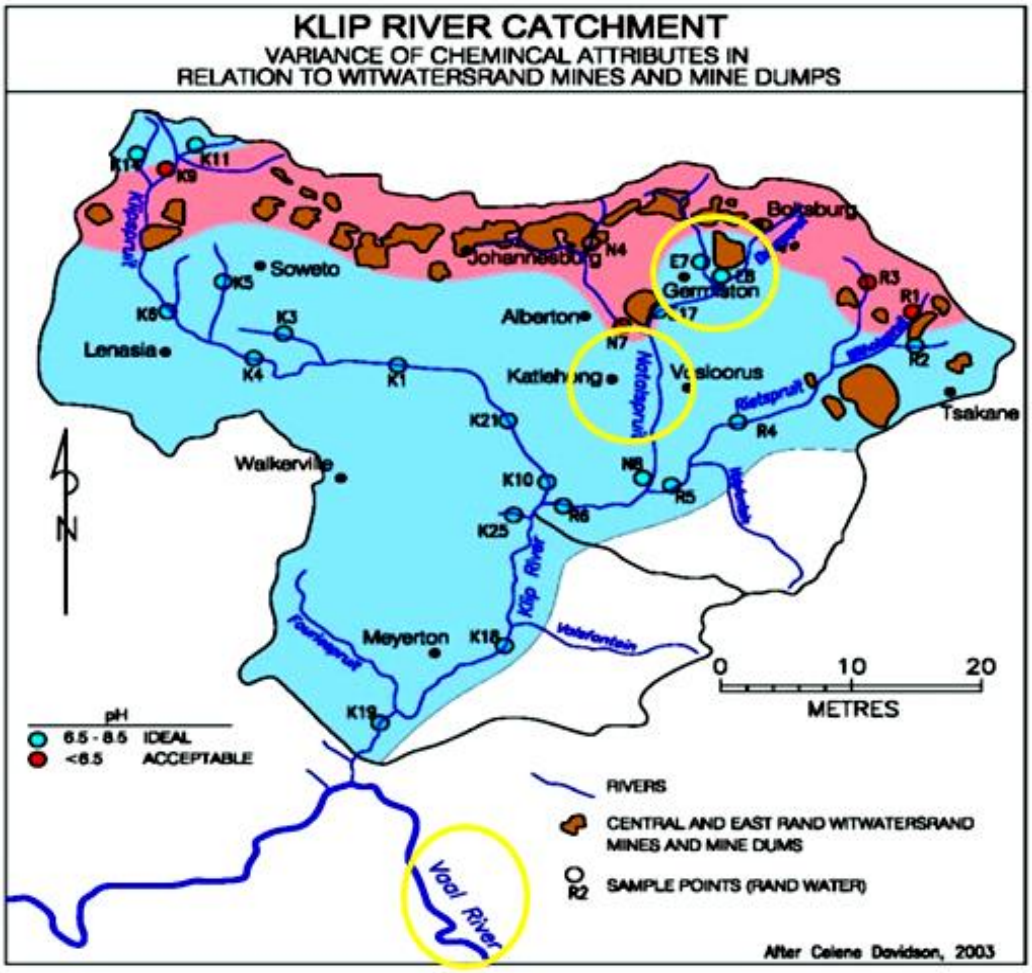


Figure 14: The Klip river catchment showing the connection between the wetland and the rivers

The main concerns from an environmental perspective are the impacts of pollution on downstream impoundment and on users of this water source. The downstream communities, which are exposed to polluted streams and rivers, face serious pollutions consequences.

Extensive research has been initiated by the Environmental Analytical Chemistry Research group at the University of the Witwatersrand. Its main focus was on providing preliminary information on total mercury (HgT) contamination in wetlands associated with gold mining activities and seasonal trends of the Hg

loads have also been determined. This helps in assessing the effect of prolonged exposure to polluted discharges emanating from the Wits mining complex.

4.4.2 Collection of samples

Plant samples

It was of utmost importance to be mindful of the fact that laboratory analysis is a small scale representation of what might be happening in the environment. Sampled plant species were obtained from a relatively large area of land, therefore to minimize errors it was ensured that samples reflected a true representation of all the plant population in the field. This was done by practically taking as many plants as possible in triplicates to ensure reproducibility of results, sampling the entire aboveground tissues of the plant material together with the roots and sediments from where the plant grew.

The study aimed to understand the effect of seasonality, thus the first round of sampling happened towards the end of the wet season, this was in March 2015 and the second session happened towards the end of the dry season July 2015. The wet and dry seasons sampling was motivated by the need of understanding the seasonal impact on the Hg transport and distribution in the semi-arid area. Sampling points were selected based on the availability of the plant species during each season. Plant samples were randomly sampled from the wetland. Vegetation samples consisted of six different plant species together with the surface sediments from which the plants grew. Nitrile gloves were worn at all time to minimize the risk of contamination, samples were kept in polyethylene plastic bags.

Later, the plant material was cut into smaller pieces and appropriately sorted out into categories of roots, stem, leaves and seeds. Vegetation samples were then frozen and lyophilized at -40°C (Oritz et al., 2002) for 48 hours. Lyophilized samples were ground into fine homogenous powder using a pestle and a mortar with the aid of liquid nitrogen. These were kept in cleaned polystyrene bottles in the dark, to prevent photodegradation (Yu and Yan, 2003).

4.4.3 Description of plant samples

Table 2: Description of the selected macrophytes

Reference image	Description of plant species
 *Datura stramonium* (DS) Common name "Jimson weed"	An herbaceous annually growing plant that grows in various locations including disturbed soils (excavated lands, fields, waste ground etc). This plant is usually found in permeable and aerobic damp soils (like clay and loam soils). This plant has adapted to grow under drier climate conditions up to a height of about 1 m. Its roots are long, stem is often strong and thick whilst the leaves are large & soft.
 *Phragmites australis* (PA) Common name "Common reed"	A perennial (dormant in winter and grows in summer) reed/grass that grows in all soil types provided there is sufficient moisture. Can also be found in fresh and marine habitats. Can reach 5 m in height, reasonably large roots capable to survive under anaerobic conditions, leafy stems and long & wide leaves. This is the major plant in wetlands.
 *Persicaria lapathifolia* (PL) Common name "Pale Smartweed"	An annual herbaceous plant that grows in damp clay and loamy soils with organic matter. Usually found in terrestrial and freshwater environments. Has a preference for partial or full sunlight, can grow to 1.2 m in height.

 *Melilotus alba* (MA) Common name "White sweetclover"	This herbaceous species grows biennially (needs two years to finish growth cycle) and is stimulated by sunlight. This plant has adapted and grows under moderately moist to dry soil conditions which have clay, loam and gravel characteristics. It can reach 3 m in height, rough stem and trifoliate leaves on both sides of the stem.
 *Panicum coloratum* (PC) Common name "Blue panicgrass"	A grass species that grows during warm seasons (perennial) under dry or water saturated soils such as clay sediments or sandy soils in river beds & drainage courses. This plant basically grows in a very broad type of soil environment. It can tolerate drought conditions and can grow up to 10-150 cm in height. It is characterised by fibrous roots, firms stem and long leaf blades.
 *Cyperus eragrostis* (CE) Common name "Nutgrass"	This perennial sedge grows in moist soils such as clay and loam. Also grows in moist but well-drained soils. It can grow to the height of 0.9 m. Prefers roots to be permanently submerged in water. Stems are tri-angular shaped and eaves appear to be grass-like. Can tolerate acidic, alkaline and neutral pH.

4.5 Sample preparation

Sample preparation has been recognised as the most crucial step and the ultimate source of error in the development of modern analytical method. Solid samples need to be solubilised through the use of appropriate dissolution method depending on the sample composition in order to be analysed. There are various factors that need to be considered when dealing with solid samples such as plants, so as to minimize uncertainty and to achieve objectives of the analysis. Included in these factors are sample type, sample matrix composition responsible for the degree of difficulties during sample preparation and analyte determination. Consequently, good choice of sample treatment becomes crucial in ensuring reliable data.

The forms of mercury investigated in the study were total mercury (HgT) and methylmercury (MeHg) for reasons explained under the section of literature (Lusilao-Makiese et al., 2012).

4.5.1 Determination of HgT

The method employed for plant sample treatment was acquired from an existing sample pre-treatment method developed by the United States Environmental Protection Agency (USEPA, 1996; Mangum, 2009). Aliquots of homogenised plant and sediments samples were weighed (0.25 ± 0.005 g). Samples were weighed in PTFE-TFM liners to which acid reagents were added and these were digested using a closed microwave assisted extraction system. For the sediment samples, the digestion was carried out at 800 W for 45 minutes using 3 ml HNO_3, 9 ml HCl and 1 ml HF. In order to neutralize the damaging nature of hydrofluoric acid, 6 ml of concentrated boric acid H_3BO_3 was added to each sample after digestion. For plants samples 8 ml HNO_3 was used together with 2 ml H_2O_2. The temperature within the extraction containers was maintained at 170°C. The digested samples were stored in centrifuge tubes and diluted to 50 ml through the use of deionised water and kept safely at 4°C until analysis.

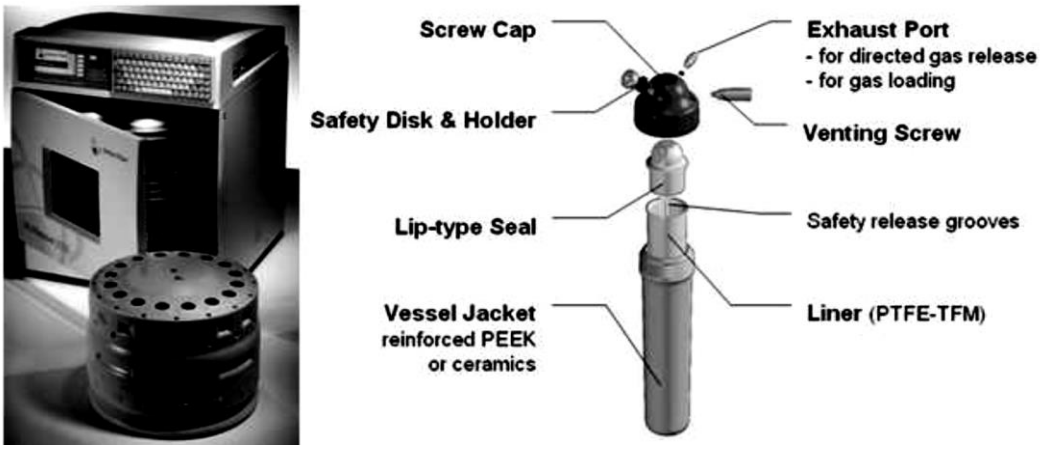


Figure 15: Presentation of the Multiwave 3000 Microwave assisted extraction system and the design of the vessel

Presented on the tables below are conditions under which the microwave was used for the extraction of mercury from surface sediments and plant samples.

Table 3: Microwave programme for extraction of mercury from sediment samples

Phase	Power (W)	Ramp (min)	Hold (min)	Fan
1	800	10:00	10:00	1
2	600	10:00	10:00	1
3	0	05:00	05:00	3

Sample weight: 0.250 g; Reagents: (3 ml) HNO_3; (9 ml) HCl; (1 ml) HF; (6 ml) H_3BO_3

Table 4: Microwave programme for extraction of mercury from plant samples (biological tissues).

Phase	Power (W)	Ramp (min)	Hold (min)	Fan
1	600	10:00	10:00	1
2	0	05:00	05:00	3

Sample weight: 0.100 g; Reagents: (8 ml) HNO_3; (2 ml) H_2O_2

4.5.2 Determination of MeHg

The procedure used for the determination of MeHg was an existing method developed by (Calderón et al., 2013). This procedure was based on liquid-liquid extraction using two liquid phases' hydrobromic acid (HBr) and toluene solvent. HBr is an acidic aqueous solution that was used to solubilise MeHg from the sample into the aqueous phase. Once MeHg was transferred to the aqueous phase toluene was then used to transfer MeHg into the organic phase using the principle of like dissolves like. Briefly 0.2 g of lyophilised sample was weighed into centrifuge tubes. 10 ml of HBr was added to the sample which was manually shaken to mix the contents. 20 ml of Toluene was added and the contents were vigorously shaken for 2 minutes using a vortex. This mixture was centrifuged for 10 minutes at 3000 rpm. 15 ml aliquot of the upper organic layer was transferred into another centrifuge tube containing 6 ml L-cysteine solution. HBr is an acidic aqueous solution that was used to hydrolyse the sample. This technique was based on liquid-liquid extraction (LLE). Thus, two phases were needed: aqueous (HBr) and organic (Toluene). MeHg needed to be soluble in the aqueous phase hence the use HBr, because H_2O would not do anything and also to take the advantage of the fact that MeHg has a good affinity with halides such as Br^-. When your MeHg had been solubilised in the aqueous phase, L-Cysteine was added the solution (Hg has high affinity for the SH functional group present in L-Cysteine) to transfer MeHg into it and separate it from other inorganic Hg compounds that are not soluble in organic solvents. A second extraction was performed and the remaining organic layer was again transferred into the centrifuge tube containing L-cysteine solution. Samples were stored at 4°C until analysis.

4.5.3 Analytical procedure

Both HgT and MeHg were analysed through the use of an automated Flow Injection Mercury System coupled to a Cold-Vapor Atomic Absorption Spectrometry (FIMS 400, Perkin-Elmer) using a solution of $SnCl_2.2H_2O$ in 3%

HCl (v/v) as a reducing agent and 3% (v/v) of HCl in de-ionised water as a carries solution.

4.6 Preparation of stock and standard solutions

The FIMS 400 mercury analyser requires a carrier and reductant solutions. Both these solutions were prepared on the day of analysis because they become unstable after two days. The 1 L carrier solution was prepared by 30 ml of HCl in a 1 L borosilicate bottle which was then filled with deionised water up to the mark. A 1 L reductant solution was prepared by dissolving 11 g of $SnCl_2$ in 30 ml of HCl which was then filled with deionised water up to the mark of a 1 L borosilicate bottle.

A stock solution of 100 μg L^{-1} was always prepared on the day of analysis in a 25 ml volumetric flask by transferring 250 μL from 1 mg L^{-1} (concentrated Hg standard) into a volumetric flask containing ultra-pure HNO_3 and H_2SO_4. This stock solution was then further diluted to prepare Hg standard solution.

Five standard solutions with Hg concentration ranging from 1 μg L^{-1} to 10 μg L^{-1} were used in constructing a calibration curve. For the purposes of quality assurance the mercury analyser was set up in such a way that it measures each sample five times. Parameters such as detection limit and quantification limit of the method were reported as well as the coefficient of determination. The calculation of standard deviation was also done which was subsequently used as error bars in any event where data were reported graphically.

4.7 Method validation used for mercury determination

Certified reference materials (CRMs) were used to evaluate the analytical performance of the measurements of HgT and MeHg in sediments and plant tissues as wells as to validate research methodologies employed in this study. For sediments LGC6187 (River sediments) was used for the evaluation of the method used to quantify HgT, whereas for plant tissues BCR-482 (lichens) was used. BCR-463 (tuna fish) was used for the validation of the method used in the

determination of MeHg. All of these CRM_S were purchased from the European Community Bureau of Reference (Brussels, Belgium). The aforementioned sample preparation procedure was followed in which each CRM was prepared in triplicate.

Other validation methods such as LOD and LOQ were used to probe whether the method used in the determination of both HgT and MeHg performed satisfactorily. This was done using the blank calculation method where by the mean and standard deviation of the analyte solution corresponding to the blank sample wass used to estimate LOD and LOQ as per equation 3 and 4 respectively. The relationship between the instruments' response and the concentrations of standard solutions was used to test for linearity. Reproducibility of the CRMs was also calculated to ascertain whether the research methodologies yielded good recoveries.

CHAPTER 5: RESULTS AND DISCUSSION

5.1 Instrument calibration

Shown in Table 5 are the calibration results obtained from the FIMS-400 upon the analysis of five standard solutions whose Hg concentration ranged from 0.0 to 10 µg L^{-1}. An outstanding linearity was obtained denoted by the coefficient of determination (R^2) for both the calibration of HgT and MeHg ranging from 0.9967 to 0.9999 respectively (Figure 16 and 17). This means the chosen method could be appropriately used for the quantification of HgT and MeHg in plant samples and surface sediments.

Table 5: FIMS 400 calibration results for HgT and MeHg

Parameter	HgT	MeHg
LOD (µg L^{-1})	0.0220	0.0245
LOQ (µg L^{-1})	0.0285	0.0257
R^2	0.9967	0.9999

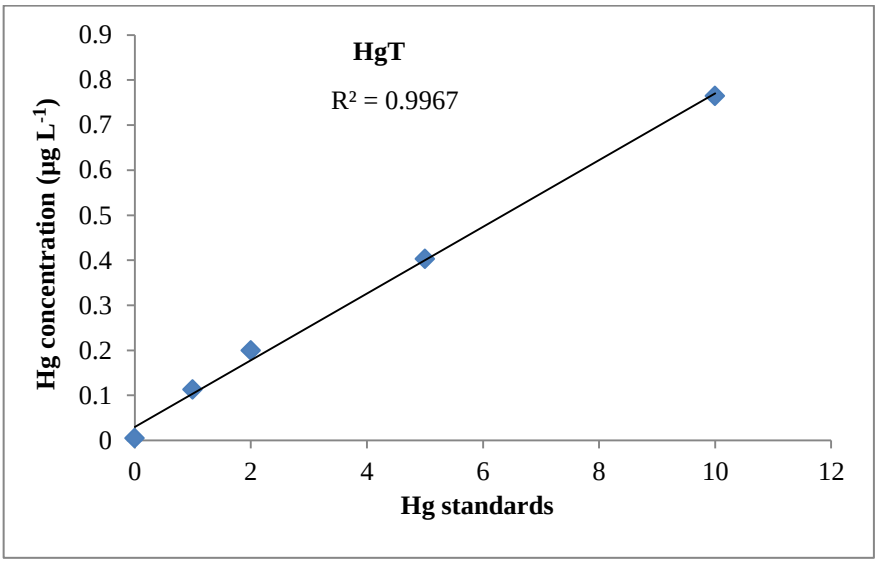


Figure 16: FIMS 400 calibration curve of HgT

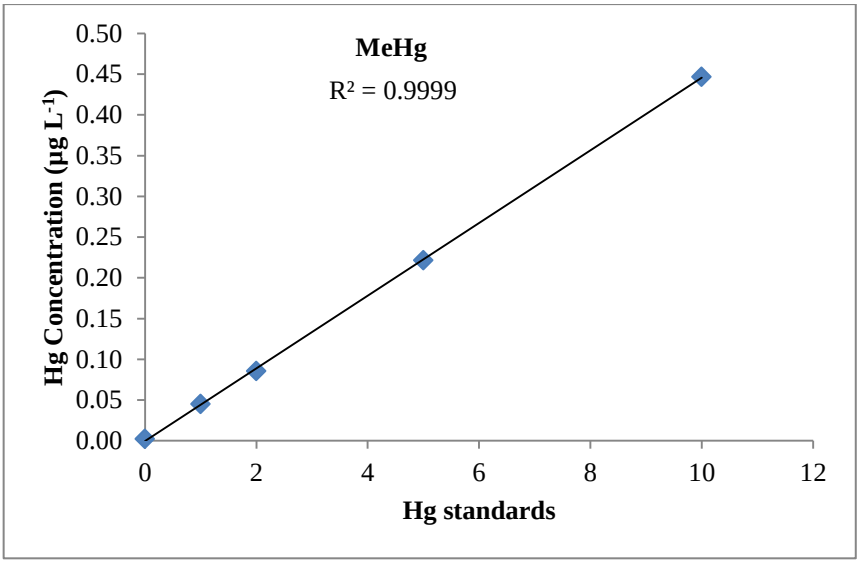


Figure 17: FIMS 400 calibration curve of MeHg

The LOD and LOQ were fairly low as shown in Table 5 and this makes the chosen method appropriate to be used for the determination of mercury and its species in biological samples in this case plant species.

5.2 Validation of methods utilised in the quantification of mercury

Tabulated in Table 6 are the externally verified values of CRMs used in this study shown with mean and standard deviation. The efficiency of the research method used for the quantification of HgT in plant samples and the toluene-extraction-L-cysteine extraction based method of quantifying MeHg was done by comparing the concentration values obtained in the study with the certified values.

Table 6: Verified amounts of HgT and MeHg in CRMs and specifically determined amounts in the present research study

CRM		Certified (n±SD.µg kg^{-1})		Determined (n±SD.µg kg^{-1})		Recovery (%)
Type	**Name**	**HgT**	**MeHg**	**HgT**	**MeHg**	
BCR-482	Lichens	480±20	-	474±10	-	98
LGC6187	Sediments	1400±100	-	1370±88	-	98
BCR-463	Tuna fish	-	3030±160	-	2762±120	91

It is evident from Table 6 that very good precision and accuracy were achieved for both HgT and MeHg since good recoveries close to 100% were observed for all the material with the exception of BCR-463 which showed a lower recovery of 91%. This could be accounted for by the notion that this material possess large amount of lipid content which might greatly impact the separation of the different phases during the liquid-liquid extraction of MeHg by combining and forming an emulsion as a result leading to sample loss (Maggi et al., 2009). Overall these results not only demonstrate the efficiency of the used sample preparation protocol but also the performance of the analytical techniques since no major contamination and only a small loss of mercury was observed.

5.3 Field measurements

Table 7 shows the field parameters measurements obtained from the wetland's surface sediments in the wet and dry seasons. The pH values of the sediment samples were mostly neutral to slightly acidic and the redox potential exhibited a uniform trend varying from (0.42 to 0.55 V) in the wet season. A different trend was observed in the dry season characterised by very acidic conditions and redox potential ranging from 0.26 to 0.49 V. Compared to the dry season both pH and redox potential values were higher in the wet season. It could be inferred that sediments from the dry season were characteristic of a system affected by AMD with lower pH values and low redox potential indicative of anaerobic conditions

due to the absence of oxygen. In addition the lower pH values observed in dry season could be evidence of the acidification of the area through pyrite oxidation (Lusilao-Makiese et al., 2014) however, further investigation is required to support this claim. The pH value at collection point PC in the wet season was low with high Eh denoting the existence of AMD in the area which could contribute to the release of Hg and other heavy metals into the water (Tutu et al., 2008). The low pH and high Eh at this sampling point also implies that the localized surface accumulation of mercury could be from recently deposited particles and leached from the tailings footprint. The reductive conditions observed in the dry season enable the reduction of sulphate to sulphide anion which has a high affinity for metals thereby binding and immobilizing them.

Table 7: Field measurements of surface sediments collected in the wet and dry seasons

	Wet season		**Dry season**	
Sample ID	**pH**	**Eh (V)**	**pH**	**Eh (V)**
DS	7.3	0.42	6.0	0.26
PA	7.3	0.42	4.1	0.38
PL	7.3	0.42	4.1	0.38
MA	7.3	0.42	6.0	0.49
PC	4.2	0.55	6.4	0.38
CE	7.3	0.42	4.1	0.39

The pH affects metal speciation, solubility from mineral surfaces, transport and bioavailability of metals in aqueous solutions. Generally solubility of metal hydroxide minerals and adsorption-desorption processes are affected by pH. Under pH conditions in natural water, metal hydroxides have very low solubilities. The activity of hydroxide ion is directly influenced by pH, therefore solubility of metal hydroxides minerals increases with decreasing pH, and more dissolved metals become potentially available for incorporation into biological processes as pH decreases (John and Leventhal, 1995). The impact of the pH on the bioavailability of Hg as it relates to plants will be further explored in the

sections to follow, this background information lays a foundation for the basis of what this research study tries to argue.

5.4 Mercury concentration in sediments and plants

The annual total rainfall for the year 2015 was 403 mm and this according to the South African Weather Service was the driest year in over 111 years in SA, with rainfall below the mean in each of the last four years.

Concentrations of HgT and MeHg in surface sediments and tissues of plants (wet weight) collected at the wetland in the wet and dry seasons are shown in Figure 18. Concentrations expressed on the dry weight basis are provided in the appendix Figure A1. Total mercury concentration in surface sediments raged from 437 to 692 µg kg^{-1} in wet season and varied significantly in the dry season from 360 to 1005 µg kg^{-1}. Evaluation of Hg concentration in sediments has been determined by the United State Environmental Protection Agency (USEPA) using various criteria. These categories are: the threshold effect level (TEL) with a value of 174 µg kg^{-1} (MacDonald, Ingersoll, and Berger 2000). TEL represents the suggested minimum limit for Hg contamination effect on biota, above which there is potential for observable effects. There is also Hg probable effect level of 486 µg kg^{-1} representing the concentration of Hg above which adverse effects of contamination are expected to occur frequently. Finally the toxic effect threshold concentration of 1000 µg kg^{-1}, where sediments are considered to be heavily polluted (MacDonald, Ingersoll, and Berger 2000 and references therein). Most of the analysed sediments fell out of the Hg probable effect level of 486 µg kg^{-1} with a few exceptions. These sediments can therefore be considered heavily polluted.

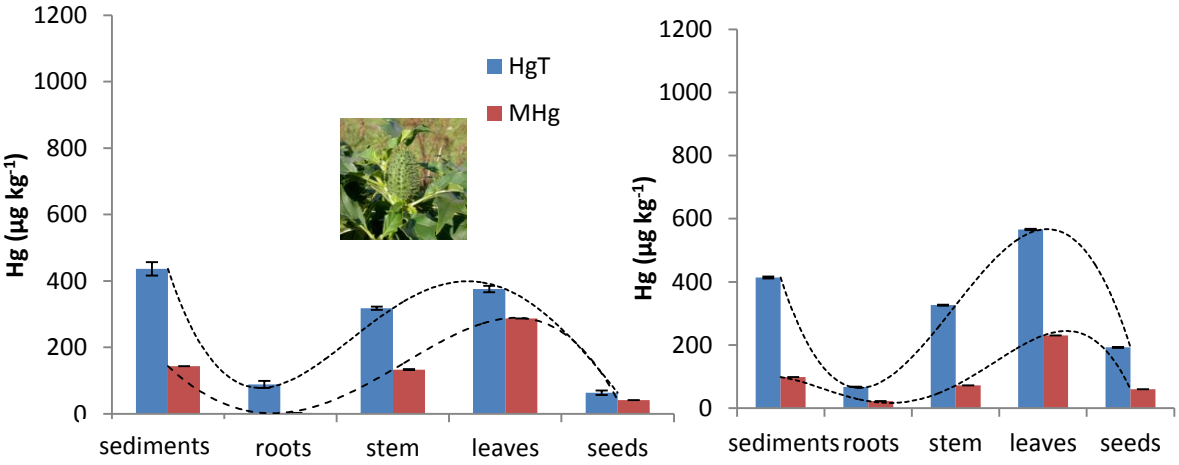
The levels of HgT among these sediment PA, PC and MA collected in the wet season were similar perhaps due to the fact that these sampling points were located next to each other adjacent to the TF (Figure 12). For sediments located further from the TF (PL and CE), high levels of HgT were observed in the wet season. These observations could be explained by the probable migration of

leached mercury away from the TF or the surrounding polluted sediments due to run-off during the rainy season. Furthermore, a study conducted by De Lacerda and Salomons (2012) focused on investigating important physico-chemical factors that affect concentrations of mercury in water and suspended particles. This study was particularly interested in aquatic habitats that get drainage from tailings during storm events. It was observed that an increase in the redox potential corresponded with high levels of mercury in suspended particles. These results suggested that there was a probable transportation of contaminated particles from tailings which were eventually deposited and accumulate in sediments alongside drainage pathways. Generally these high HgT concentration levels observed in these surface sediments are indicative of a pollution problem occurring at the site, probably from the surrounding TF (Figure 12). The oxidative conditions demonstrated by high Eh values of sediments collected in the wet season encourage metal remobilisation this could explain high levels of HgT in observed surface sediments.

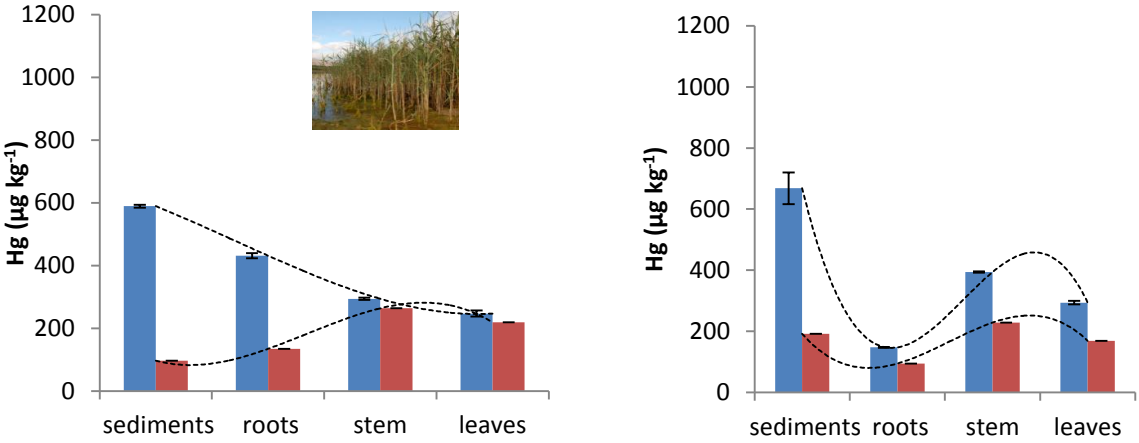
Surface sediments collected in the dry season revealed acidic pH values and slightly anoxic conditions in all studied sites. The lowering of the sediments pH (acidification) during the dry season is a factor that encourages the solubilisation of heavy metals such as mercury, thus increasing mercury bioavailability. This could explain the high HgT concentration of 1005 µg kg^{-1} observed in MA and this was the most polluted sample. A different trend was observed in the dry season where by these sediments (MA and PL) collected directly on the edge of the TF sowed elevated concentration of HgT (Figure 12). This could be the result of the discharge of contaminated particles from tailings footprint to the surrounding areas. In addition, the enrichment of mercury in sediments alongside to the old TF could be the direct consequence of historical loads of mercury in tailings and seepage from the facilities. Similar results were obtained from unpublished work carried out by Lusilao-Makiese et al., (2015) which focused on determination of mercury in sediments from the same site as the current study (see Table A3 on the appendix).

Wet **Dry**

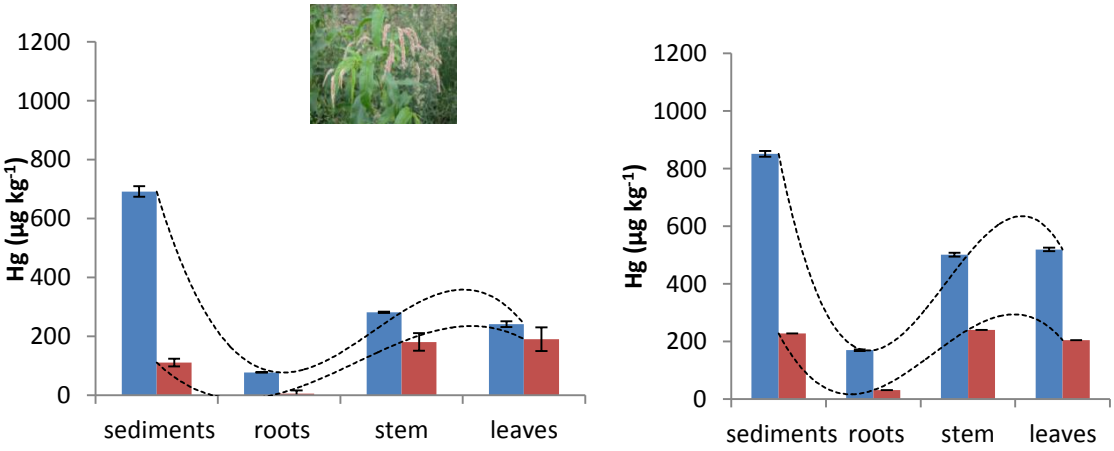
Datura stramonium



Phragmites australis



Persicaria lapathifolia



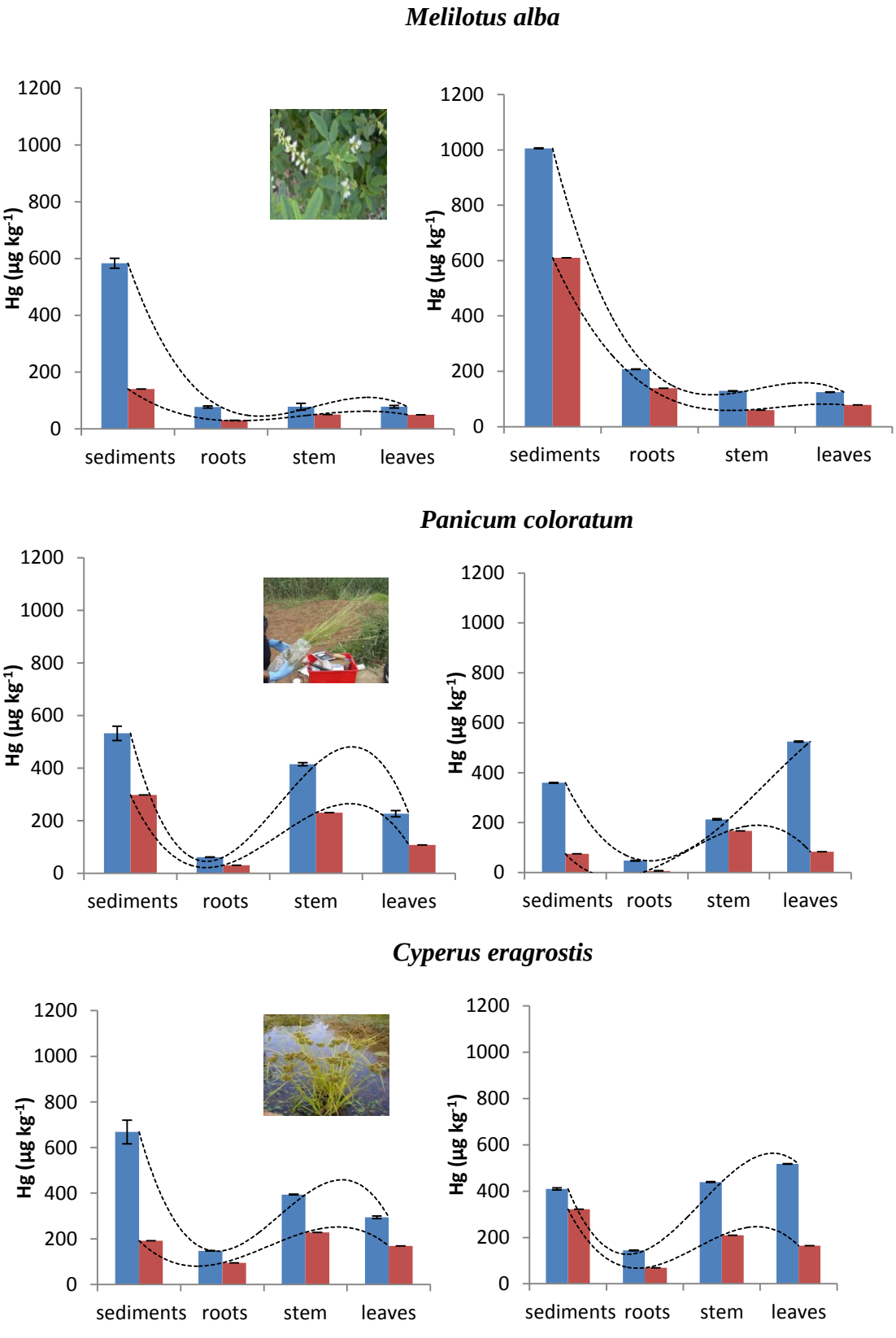


Figure 18: The concentration of HgT and MeHg in the fresh plant tissues and sediments collected in the wet and dry seasons

The concentrations of HgT and MeHg in plant tissues collected at the wetland are shown in Figure 18. Huckabe et al., (1983) suggested the range of normal or background concentration of total mercury in plants to be between 80 to 100 µg kg^{-1} but information with regards to the permissible levels of methylmercury was not available. The high levels of HgT observed in current study suggest that the area from which the selected plants grow is highly contaminated. From the six different plant species collected four of them namely *D. stramonium, P. lapathifolia, P. coloratum and C. eragrostis* exhibited similar behavioural pattern in both the wet and dry season, in the sense that HgT was mostly accumulated in the above ground plant tissues (Figure 18). Furthermore, in *D. stramonium* the highest level of HgT (566 µg kg^{-1}) was obtained in the leaves from the dry season than any other plant species collected (Figure 18). This could be attributed to the notion that plants growing in mercury contaminated areas might get Hg from the sediments which is in turn released to the atmosphere, this is however not the case in areas with low levels of HgT (Ericksen and Gustin, 2004). Schroeder and Munthe, (1998) observed that HgT released from polluted soils may lead to increased concentration in the atmosphere. Therefore, this adds to the elevated levels of HgT in the aerial tissues of these plants due to foliar adsorption.

The soil-air-foliar exchange occurs when sediments particles suspended in the air eventually descend down onto the surfaces of vegetation. This is mostly pronounced in plants near soil surface, therefore this could explain the high concentrations of HgT in the aerial tissues of the aforementioned plant species.

A slightly different pattern was observed for *P. australis* in the wet season with the highest HgT (432 µg kg^{-1}) concentration in the root tissues (Figure 18). This was not the case for this species sampled in the dry season as higher HgT levels were determined in the aboveground tissues relative to the roots (Figure 18). Seasonal variation in growth characterises *P. australis* in that during the wet season this plant displays rapid growth characterised by the development of high leaf area unlike the dry season where this plant is dormant (Dye et al., 2008). Schierup and Larsen (1981) observed low concentration of Hg in plant leaves produced later in the growing season and high concentration in those produced earlier. It was proposed that this might be the direct consequence of the age of the

leaves, such that the freshly developing leaves have small amount of accumulated metals and as they undergo photosynthesis and develop during the growing season, they slowly build up huge amount of metals until senescence. Therefore aboveground plant tissues might have high metal concentration as leaves get older because of the continuous transportation of metals into leaf tissues and constant exposure to elevated levels of metals (Weis and Weis, 2004). These results were corroborated by Drifmeyer and Redd (1981). Verkleij and Schat (1990) suggested that the migration of metals into mature leaves is a coping mechanism plants employ to get rid of a portion of their metal burden. This could explain the high concentration of HgT in the leaves and stem of this plant observed in the dry season.

M alba in both the wet and dry seasons showed that mercury concentrations in various tissues investigated could be arranged in the descending order of magnitude such that sediments> roots> stem> leaves (Figure 18). This indicates the ease with which mercury could be determined in plant tissues or sediments. The lowest concentrations of mercury were obtained in tissues of *M. alba* compared to all the plants sampled in the wet season, but the highest concentration in sediments was observed (Figure 18). This shows the high availability of mercury to *M. alba* and restricted movement from sediments to the root tissues and once inside the plant. These results were corroborated by (Deng et al., 2004; Keller et al., 1998; Núñez et al., 2011; Taylor and Crowder 1983; Ye et al., 1997). It is has been reported that plants that have a generation of high root biomass are capable of minimising the mobility of contaminants through uptake, precipitation and retention in roots rather than transfer to aboveground tissue. This is through formation of insoluble complexes that limit the mobility of metals.

Table 8: Bioaccumulation factor (roots/sediment) and Translocation factor (leaves/roots) of the investigated plant species

	Wet season		**Dry season**	
Plant species	**BFs**	**TFs**	**BFs**	**TFs**
DS	0.20	4.26	0.61	8.30
PA	0.73	0.57	0.22	1.99
PL	0.11	3.10	0.20	3.07
MA	0.13	0.54	0.21	0.60
PC	0.11	3.70	0.13	10.94
CE	0.22	1.99	0.35	3.60

Generally BFs were less than 1 in both the wet and dry seasons for all the plant species indicating that Hg was mainly retained by sediments (Table 8). The plant species that had BF closer to 1 were PA (0.73) in the wet season and DS (0.61) in the dry season indicating a reasonable uptake capacity of Hg though the HgT root concentration was less than the level of HgT in the sediments of these plants (Figure 18). According to the TFs, metals were accumulated fundamentally in aboveground tissues (TFs are greater than 1) for the plants DS, PL, PC and CE as seen in the wet and dry seasons (Table 8). Exceptions occurred for PA species in the dry season in that the TF value was 1.99 which is greater than 1 (Table 8). MA exhibited the same behaviour in both season with low BF and TF values indicating that this species does not really accumulate much mercury in the aerial tissues.

The concentration of HgT in plant tissues (roots, stem and leaves) sampled in the dry season was higher for most plant species than in the wet season with the exception of *P. coloratum* that had higher concentration of HgT in stem and leaves in the wet season 414 and 226 μg kg^{-1} respectively (Figure 18). This could be as a result of the movement of heavy metals form the tailings footprint to the plant tissues in the form of surface run-off and the effect of rainfall which may facilitate the leaching of the soil and contributes to the dilution of soil solution during the wet season. The dry season is characterised by strong winds. Heavy metal contaminated particles from the atmosphere might be deposited on the

exposed surfaces of plant tissues such as leaves and get incorporated into the plants' system through foliar absorption. Furthermore, evaporation of moisture from the plants combined with evapotranspiration form the sediments leads to heavy metal pre-concentration thus increasing the metal concentration in the roots and leaves.

Oxidation of sediments encourages the remobilization of metals. At the bottom of the wetland there is a region known as the anaerobic zone where metals mostly occur in the reduced state. However, plants are capable of transporting oxygen from the above tissue through aerenchyma tissue to the roots thus oxidizing the sediments. As a result of this oxidative process metal contaminants might be remobilized thus increasing their bioavailability in the wetland (Wies and Wies 2004). Under reductive conditions metals are generally nonbioavailable. Plant can release exudates which might lead to the acidification of the root zone thus remobilizing metals this might be seen by the decrease in pH and increase in the concentration of (soluble) Hg in sediments. This might explain the elevated levels of HgT in plant tissues observed in the dry season (see Table 7 where in the dry season sediments pH is low and moderately oxidizing conditions). In a study conducted by Ravit et al., (2003) it was observed that the extent to which *S. alterniflora* oxidized its rhizosphere was greater than that of *P. australis* because the former species possess a lager root system and a bigger number of fine roots.

Generally concentrations of metals increase in standing dead plant biomass and in detritus this might explain the high concentration of mercury in winter. In winter the sampled plants appeared dry and dead standing.

5.5 Methylmercury concentration in sediments and plants

Figure 18 shows the determined levels of MeHg in the surface sediments and plant tissues sampled from the wetland in the wet and dry seasons. The concentration of MeHg in the surface sediments sampled in the wet season ranged from 97 to 298 µg kg^{-1} and between 75 to 610 µg kg^{-1} in the dry season. Harrison et al., (2007) state that the concentration of MeHg in sediments should be about 3% of HgT. This was however not the case in this study as the levels of MeHg in

surface sediments range from 16 to 56% of HgT in the wet season and between 21 and 61% of HgT in the dry season. In the dry season PL, MA and CE sampled along the edge of the tailings footprint showed MeHg enrichment ranging from 27, 61 and 78% of HgT (Figure 12). A similar trend was also observed for PC sampled in the wet season with a MeHg level of 56%. It has been reported that methylation of mercury is controlled by pH, temperature and redox potential because these determine the availability of Hg^{2+} whilst sulphate reducing bacteria controls the activity of Hg methylation. From the study it appears that most sediments and plants sampled from the dry season showed enrichment in MeHg levels at sites corresponding to low pH and moderately oxidizing conditions (PL, MA and CE in the dry season) and PC in the wet season (Table 7). This trend was corroborated by (Lusilao-Makiese et al., 2014; Hines, Brezonik, and Engsteom 2004). This trend could be explained by higher temperatures might enhance the activity of SRB thus leading to effective conversion of bioavailable Hg^{2+} under low pH and moderately oxidizing conditions. Wood (1980) suggested that aquatic habitats characterised by low pH values and positive redox potential would favour the conversion of Hg^{2+} to methylmercury. Low pH and aerobic conditions are enabling factors for the oxidation of sulphide into sulphate. This increases Hg^{2+} solubility and hence a greater availability of Hg^{2+} for methylation (Fagerström and Jernelöv 1971; Robinson and Tuovinen 1984). Generally the elevated levels of MeHg could be related to the pollution occurring in the area and seepage of leached mercury from the tailings footprint which eventually migrates to the surroundings areas.

The lowest concentration of MeHg was obtained in the root tissues compared to all the plant tissues evaluated, with concentrations ranging from 2 to 135 µg kg^{-1} in the wet season and between 7 to 139 µg kg^{-1} in the dry season (Figure 18). Plant roots promote Hg methylation due to the presence of microbes at the rhizosphere. The presence of rhizosphere bacteria plays a significant role in the accumulation of heavy metals in wetland plants. In an experiment conducted by De Souza et al., (1999) it was observed that *Scirpus robustus* and *Polypogon monspeliensis* accumulated lower levels of Hg and Se when bacterial growth was prohibited with antibiotics. This is indicative of the crucial role these symbaitoc

bacteria play for efficient metal uptake. Mycorrhizae are fungi that grow in association with the roots of a plant in a symbiotic or mildly pathogenic relationship. These fungi act as a link between the root and sediments thereby increasing the surface area of the root hairs (Meharg and Cairney, 2000). Some researchers have argued that fungi perform a preventative role in defence of plants by prohibiting plants from accumulating metals by restricting the movement of metals in the fungal tissues (Khan et al., 2000). This could explain low levels of MeHg in the root tissues of the sampled plant species.

In the wet season the MeHg contents in the leaves of the six plant species varied from 50 to 230 µg kg^{-1} and between 60 to 240 µg kg^{-1} in the dry season (Figure 18). It appears from the results that plants species that showed enrichment in MeHg were those collected from sampling points corresponding to higher pH 7.3 and highly oxidizing conditions 0.42 V as shown in Table 7 and Figure 18, this was particularly the case with most plants sampled in the wet season *D. stramonium, P. australis, P. lapathifolia, P. coloratum* and *C. eragrostis*. *M. alba* was an exceptional case which showed higher MeHg contents in the dry season notice the high pH 6.0 and high redox potential 0.49 V (Table 7). Mercury methylation for all the plant species seemed to occur mostly in the aerial tissues of the plants in both seasons. It could be that some of the MeHg in the aboveground plant tissues could be coming from the root zone, and due to the mobility of MeHg it gets transported via xylem to the areal tissues of the plants, notice the high TFs for DS, PL, PC and CE in both wet and dry season and PA in the dry season (Table 8). This would explain high concentration of MeHg in leaves and stem of the aforementioned plants. Intra methylation of Hg in plants could also explain high levels of MeHg generally. The elevated levels of MeHg in the aerial tissues of these plants demonstrate the role played by atmospheric pollution because the sampling site is adjacent to the tailings footprint.

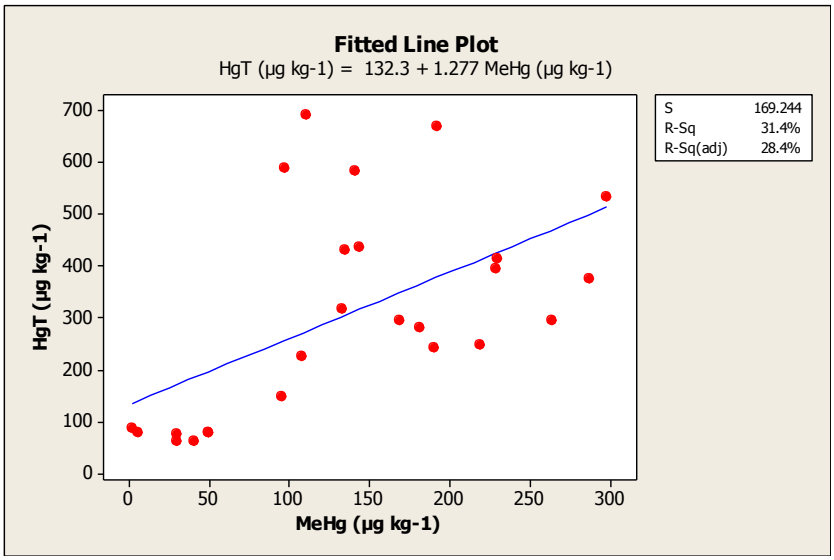


Figure 19: Correlation between MeHg and HgT in plant samples collected in the wet season

No significant relationship was observed though the p value was 0.04 the R^2 value was as low as 31.4%. This poor correlation could be due to the complex mechanisms involved in the uptake of HgT and MeHg by plants, these results were corroborated by (Qiu et al., 2008). In addition, it could be that the MeHg in these plants collected in the wet season could not be as a consequence of the bioconversion of HgT into MeHg Figure 19. Instead this mercury could be coming from the atmosphere because (Ericksen and Gustin 2004) reported that plants growing in areas with elevated levels of Hg get it from the soil as well as the atmosphere. Therefore plants are exposed to multiple ways in which Hg can enter into the systems.

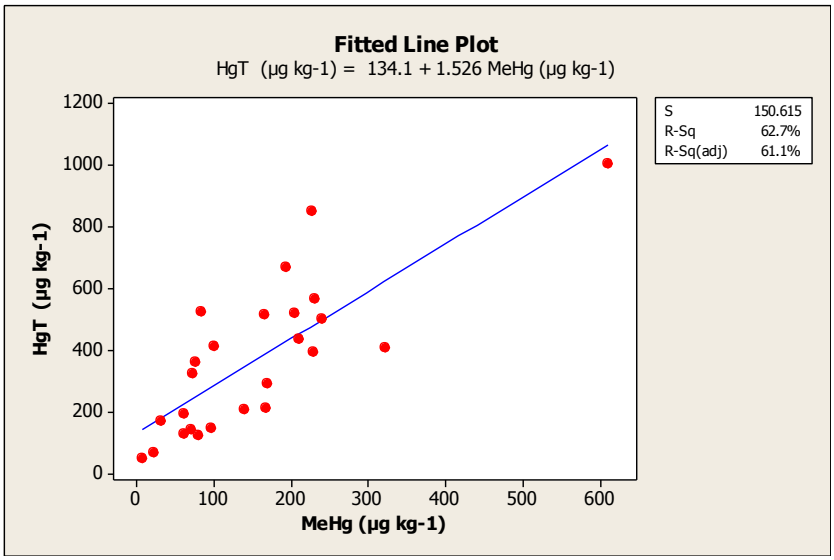


Figure 20: Correlation between MeHg and HgT in plant samples collected in the dry season

At 0.05 significance level, a p value of 0.000 was obtained corroborated by the R^2 of about 62.7% and this is indicative of a fairly strong relationship between MeHg and HgT. This could probably reflect the biotransformation and conversion of some amount of HgT into MeHg or intra-methylation within plant species Figure 20.

Correlation graphs were generated to investigate the extent to which the bioavailable portion of HgT gets converted to MeHg in the various plant tissues.

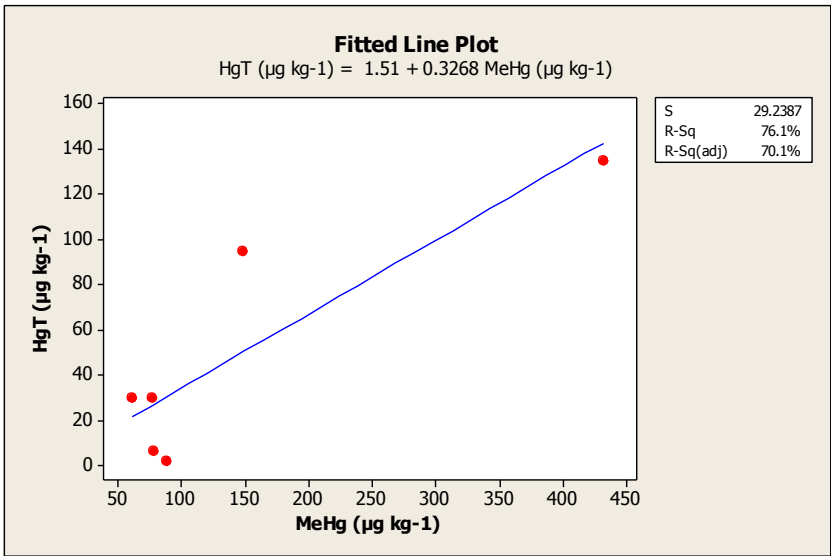


Figure 21: Relationship between HgT and MeHg of the roots all plants sampled in the wet season

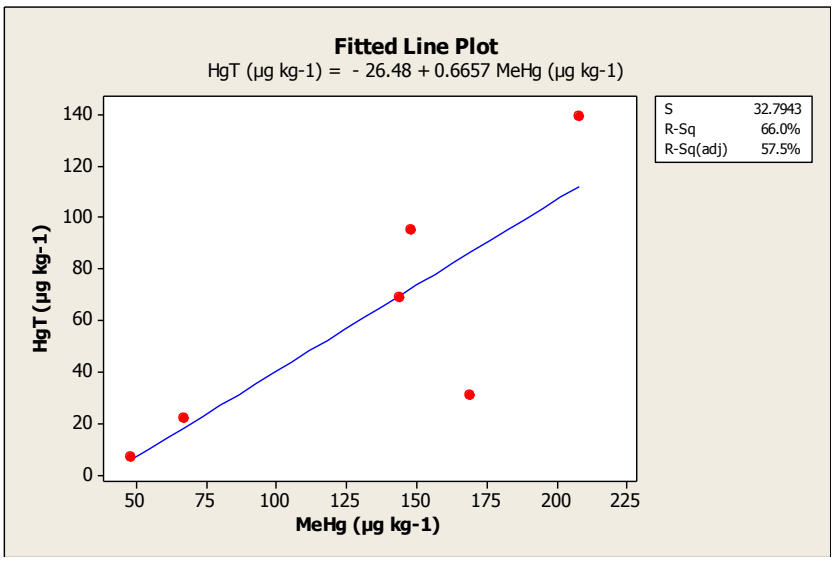


Figure 22: Relationship between HgT and MeHg of the roots all plants sampled in the dry season

The R^2 value obtained for the root tissues of the plants collected in the wet season was observed to be higher 76.1% (Figure 21) compared to that obtained in the dry season 66.0% (Figure 22). This implies that the conversion of the bioavailable

portion of HgT into MeHg was more pronounced in the wet season than the dry season. This will be further explained in the section to follow.

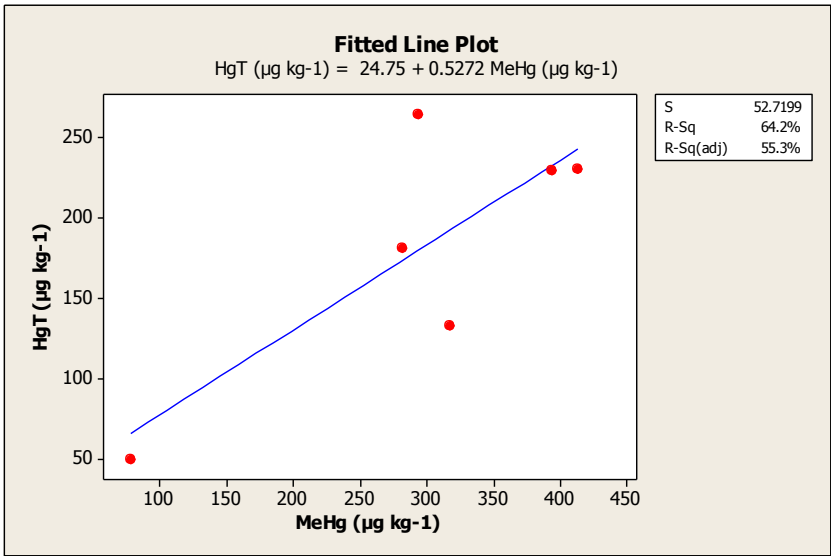


Figure 23: Correlation between HgT and MeHg of the stem tissues of all plants sampled in the wet season

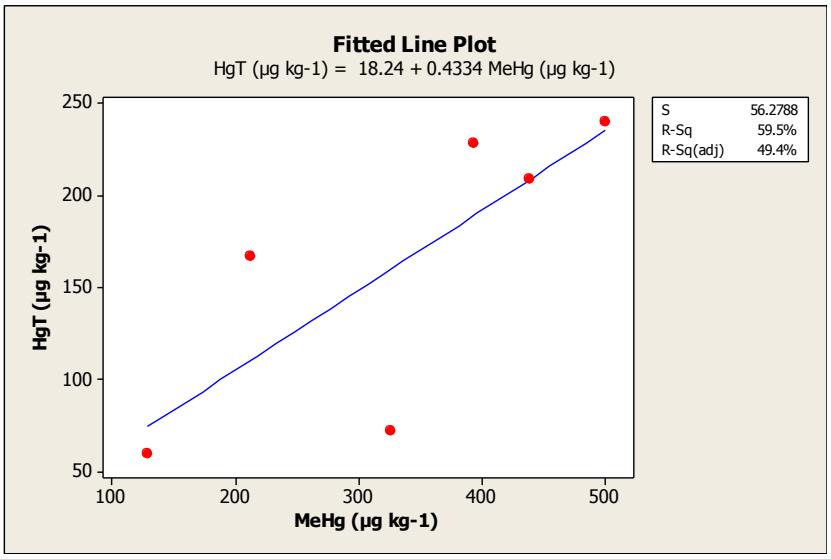


Figure 24: Correlation between HgT and MeHg of the stem tissues of all plants sampled in the dry season

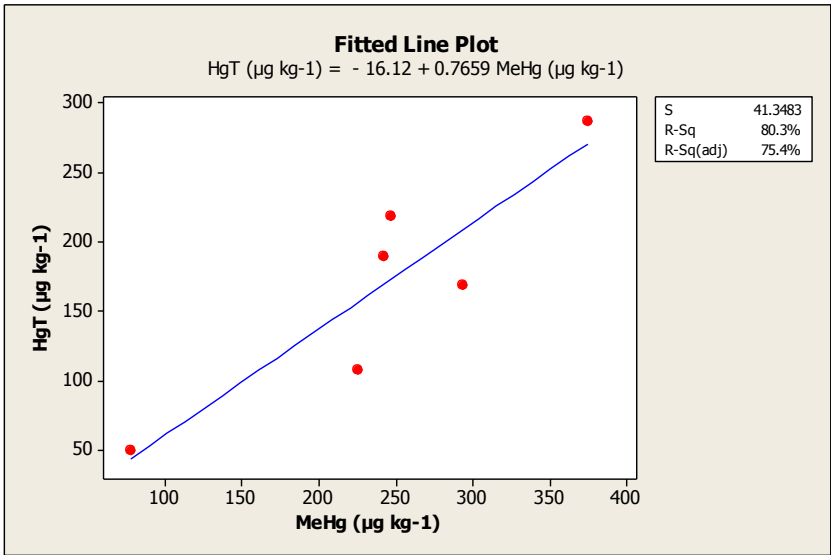


Figure 25: Association between HgT and MeHg concentrations in the leaves of all plant species collected in the wet season

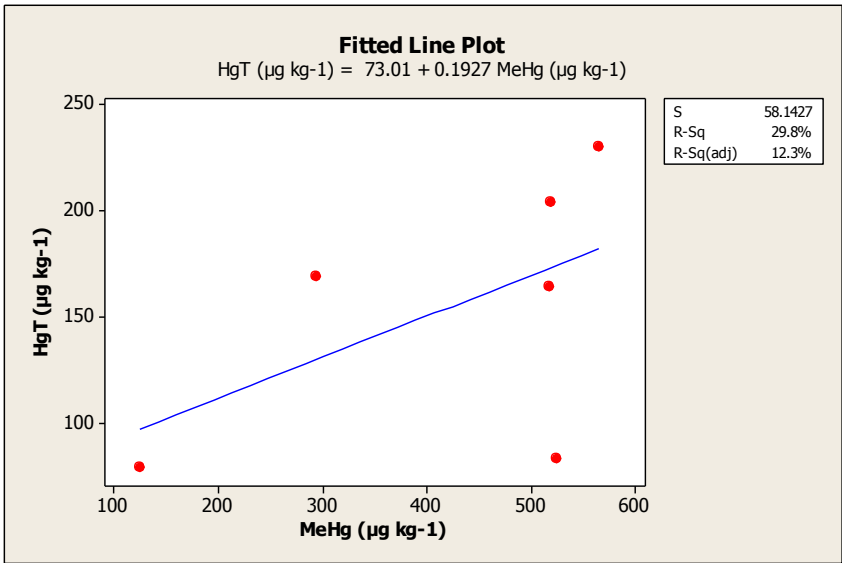


Figure 26: Association between HgT and MeHg concentrations in the leaves of all plant species collected in the dry season

A significant positive correlation was observed between MeHg and HgT in the leaves of the plants sampled in the wet season with an R^2 value of about 80.3%

(Figure 25) whereas in the dry season no positive correlation was observed, the R^2 was as low as 29.8% (Figure 26).

From the results above it appears that the R^2 values of the correlation graphs of plant tissues sampled in the wet season were higher than those of the dry season, therefore it can be inferred that the conversion of bioavailable HgT seemed more pronounced in tissues of the plants sampled in the wet season compared to those collected in the dry season. The results suggest that in the wet season a strong positive correlation between MeHg and HgT in plant tissues was prominent. This could mean that the MeHg in plants sampled in the wet season could be as a result of the biotransformation of some of the bioavailable HgT in plants as such methylation was more favourable in the wet season than the dry season. This trend could be explained by the effect of temperature on the methylation of mercury.

Researchers have demonstrated evidence showing that during summer the rate at which mercury gets methylated increases (Bubb et al., 1993; Jackson et al., 1982). These results were corroborated by Wright and Hamilton (1982) who observed that at low temperatures such as 4°C MeHg released from sediments accounted for only 50 to 70% compared to that which was released at 20°C. This was explained by significantly reduced rates of microbial growth and metabolic activity that usually occurs under low temperatures which often occur in the dry season (Ullrich et al., 2001). Other researchers have observed that low temperatures that characterise the dry season in SA promote demethylation, while higher temperatures enhance mercury methylation, as a result increasing the rate at which methylmercury is produced in summer (Bodaly et al., 1993; Ramlal et al., 1993). During the wet season wetland waters might have higher amount of organic matter this might increase the concentration of mercury in plants. From a study conducted by Zillioux et al., (1993) suggested that disturbed wetlands produced more methylmercury as opposed to undisturbed. The mobility of organic content associated with mercury increases when a wetland is flooded, that is during the wet season. It was concluded that the disturbance of wetland systems through flooding leads to remobilization of mercury deposited from natural and anthropogenic sources.

Another factor which influences methylmercury production is the pH. On Table 7 it can be noted that the sediments collected in the dry season were quite acidic. Researchers have argued that acidic conditions have a potential to interfere microbial activity thereby reducing methylation rates (Ulrich et al., 2001). A study conducted by Connell and Patrick (1968) suggested that the activity of sulphate reducing bacteria was significantly reduced under acidic pH range. The possible explanation was under acidic conditions the distribution of the methylating vs demethylation bacteria favoured the latter to an extent that at low pH values demethylation became dominant. This therefore might explain the poor correlation between MeHg and HgT in plant tissues collected in the dry season.

Redox potential also plays a pivotal role as a factor that influences mercury methylation. Both anaerobia and aerobic conditions favour methylation (Ulrich et al., 2001). However at the bottom of a wetland in deeper sediment layers metals exist in the reduced form, and because in this region reducing conditions dominate, Hg is often strongly adsorbed onto suhphides forming the insoluble HgS this limits the bioavailability of Hg for methylation (Ulrich et al., 2001). Though redox conditions are oxidizing and moderately oxidizing in summer and winter respectively (Table 7), perhaps the extent to which methylation occurs in summer is more favourable than in winter. This could be explained by that during the wet season temperatures are high thus the rate at which organic matter is decomposed and primary production increases significantly. This is believed to stimulate and enhance the bacterial methylating activity (Ulrich et al., 2001).

5.6 Seasonality of the mercury biogeochemical cycle

5.6.1 Seasonal accumulation of mercury

The main focus was to evaluate whether there were significant differences in the accumulation of total and methylmercury in all the plants selected for the study in summer and winter. SPSS as a statistical tool package was used to statistically analyze data. A normality test was first performed to determine whether the data were normally distributed and this determined if parametric or non-parametric test

could be used. The sample size of interest was less than fifty therefore a Shapiro-Wilk test was considered appropriate to test for normality of variables. As indicated by Table 9 both variables HgT and MeHg were found to be normally distributed since the p-values for all the plant species were greater than 0.05 significance level. Therefore a parametric statistical technique of comparing means of two groups was considered to be appropriate in testing if the accumulation of HgT and MeHg varied seasonally between each of the six species studied, thus the independent t-test.

Table 9: The p values obtained from SPSS test of normality

Shapiro-Wilk test		
	Level of significance (p value)	
Plant species	**HgT**	**MeHg**
DS	0.894	0.782
PA	0.283	0.057
PL	0.362	0.519
MA	0.617	0.081
PC	0.387	0.684
CE	0.443	0.484

An independent t-test was performed in order to investigate if statistical significance difference existed in the way plants accumulate both HgT and MeHg in the different season namely summer and winter. The evaluation was done independently for each plant species. The data were not transformed as the test for normality succeeded. Thus the statistical analysis was performed on untransformed data. The significance level was set at $p< 0.05$ such that test results were deemed significant provided that the calculated p-value was less or equal to 0.05. The results are presented in the Table 10, the full sets of SPSS tables are provided in the appendix from Table A4 to A9.

Table 10: p-values of seasonal accumulation of HgT and MeHg in plant species

	t- test for equality of means	
	Level of significance (p value)	
Plant species	**HgT**	**MeHg**
DS	0.599	0.975
PA	0.189	0.015
PL	0.156	0.116
MA	0.040	0.013
PC	0.587	0.143
CE	0.963	0.264

From Table 10 it can be observed the seasonal accumulation of mercury in DS, PL, PC and CE showed no statistical significance difference, this is in agreement with the graphical representation shown in Figure 18. A different observation was made for plant species PA in that a statistical significance difference was evident as the p-value was 0.015, this mean that the accumulation and biotransportation of methylmercury differs significantly between winter and summer. This confirms the observed pattern of mercury accumulation shown in Figure 18 where in the wet season most mercury seemed to be largely concentrated in sediments and roots tissues whereas in the dry season it was concentrated in the stem and leaf tissues. Interestingly MA had p-values less than 0.05 for both HgT and MeHg 0.040 and 0.013 respectively meaning that this plant undergone seasonal changes in accumulation and biotransformation of mercury though graphically there seemed to be no difference (Figure 18). Seasonality is one of the factors that influence accumulation of heavy metals in plant tissues. This section shall focus on the effect of evapotranspiration as a factor that influences the interaction between plants and toxic heavy metals.

Seasonality affects how the plant takes up a metal and how the plant gets rid of the Hg through evapotranspiration (ET). Evapotranspiration is the process by which water from an object or organism is lost to the atmosphere via the combination of two simultaneously occurring processes namely evaporation and transpiration. ET is affected by the seasonality which meaning a change in the

weather conditions brings about a change in the rate of ET which in turn affects metal accumulation in plants. ET is dependent on the solar radiation which causes the water from the plant to evaporate more easily. In this study, during the wet season, the temperatures were much higher compared to the dry season therefore high ET rate in summer because of the availability of solar radiation (Hanson, 1991). Due to the volatile nature of MeHg it could be that in summer this compound is easily lost into the atmosphere and this could explain the statistical significance difference observed for MA (Table 10). Hg accumulated on the leaves of plant species volatilises and escapes to the atmosphere via the stomata (the greater the surface area of the leaves the higher the chances of Hg volatilisation). However, during the dry season, solar radiation is much less and plants close up their stomata to conserve water in order to survive therefore the rate of ET gets reduced (Dye et al., 2008). If a plant is conserving water and nutrients during the dry season as a matter of survival, the Hg in any form that exists in the plant will also be conserved which in turn increases the concentration of Hg during the dry season. Therefore these are some of the possible explanations for the significant differences observed in the accumulation of HgT and MeHg in PA and MA. The increase in rainfall during the wet season also dilutes the pollutants decreasing its concentrations in the plants.

In a study conducted by Siegel et al., (1987) it was observed that plant species collected from old mining sites, though from different countries exhibit similar trends in mercury accumulation as opposed to plants collected from areas where mining operation are active. It was then concluded that local weather conditions and other environmental elements greatly influence the accumulation and biotransportation of mercury in plants though the content of mercury in soil is relative.

5.6.2 Wet season

It was observed in summer that all six plant species selected for the study showed higher concentration of HgT in sediments (Figure 18). This could be attributed to the strategy that plants growing in contaminated areas develop as a coping

mechanism. The exclusion of metals from the root tissues has been suggested as a metal tolerance strategy (Taylor and Crowder 1983). The mechanism is such that a metal precipitates within the rhizosphere only. These plant species have mechanisms that enable the formation of insoluble complexes of mercury which results to lower bioavailability, thus reducing the uptake by the roots. The low BF and TF values of *P. australis* and *M. alba* suggest that these plants are suitable for phytostabilisation (Table 8). Two plant species, *H. hirta* and *Z. fabago* are indigenous plants that grow in mine tailings in South-East Spain, which have been found to be suitable for metal stabilization due to their ability to retain high levels of metal concentrations in their rhizospheres (Padmavathiamma et al., 2007). However a significantly different trend was observed for *D. Stramonium, P. lapathifolia, P. coloratum and C. eragrostis* in that the concentration of HgT in stem and leaves was greater than in the roots. In addition, the TF values for these plant species were greater than 1 showing that the small amount of mercury found in the roots was translocated to the leaves in these species. It could be inferred that these species have the ability to oxidise sediments in the rhizosphere. This oxidation occurs through the movement of oxygen from the aboveground tissues of a plant through a spongy tissue with large air spaces found between the cells of the stems and leaves to the root zone. This leads to remobilisation of metal contaminants therefore increasing their bioavailability (Weis and Weis 2004). Lacerda et al., (1992) observed a similar trend where *Avicennia* species of mangroves were found to oxidize the rhizosphere, thus reducing sulphides and enhancing metal concentrations in the exchangeable form.

The TF values of *P. australis* and *M. alba* in the wet season were 0.57 and 0.54 respectively meaning the Hg was predominantly concentrated in the roots compared to leaves (Table 8). This could indicate limited mobility of mercury once inside the plants. Similar results were reported for rooted species (Deng et al., 2004; Taylor and Crowder 1983). It can be suggested that generally, only a small amount of HgT taken up by roots was transported to the shoots. It could also be that root tissues exhibited a higher tolerance capacity than shoots. Chaney (1993); Loneragan and Webb (1993) argued that there are mechanisms that plants use to minimize the mobility of metals to shoots thereby enhancing metal

tolerance. These mechanisms include the interaction between anionic charge in cell walls of root tissues and cationic metal pollutants, formation of insoluble complexes as a result of the interaction between toxic metals and plant exudates, metals getting chelated as they interact with phytochelatin followed by accumulation in storage vacuoles. The present study has demonstrated that the selected plant species have the capacity to grow in areas with high heavy metal concentrations in sediments. In addition, because HgT concentrations greatly exceeded the stipulated normal mercury levels in plants (100 μg kg^{-1}) it can be concluded that these plants have a high tolerance to mercury contamination. All the selected plants showed the pattern of increasing levels of MeHg from roots to stem leaves during the wet season.

5.6.3 Dry season

Low water levels are in the dry season was observed. This is characteristic of the seasonal variation for the semi-arid climate of South Africa. In general the levels of HgT in the aerial tissues of the plants were higher in the dry season than in the wet season. The level of mercury total cumulated in sediments serves as a reservoir for production of organomercury under anaerobic conditions. There are three ways in which vascular plant can accumulate mercury: from the soil through to the roots this is induced by ionic interactions, through a minute opening in stem and leaves also known as the stomata from the atmosphere (via atmospheric deposition in the form of elemental and inorganic mercury) and by the retention of particulate mercury. Lindberg et al., (1979) proposed that the highest amount of mercury present in the above ground tissues of plants could be due to the atmospheric deposition of mercury which then gets converted in the plant system via some mechanisms into methylmercury. Plants sampled form the wetland were expected to have high levels of mercury due to old mining site with reprocessed tailings. The sampling site is adjacent to the (TF). Thus, metals from the tailings footprint can are washed down to the wetland via fluvial transportation and erosion.

Highest concentration of HgT in stem and leaves compared to roots was determined in most plant species with the exception of *M. abla* (Figure 18). The concentration of MeHg was lower in the dry season but for *M. alba* higher mercury methylation was observed. Surface roughness could be one of the reasons as to why leaves and seeds of *D. stramonium* accumulated more mercury because roughness might trap mercury particulate.

Mercury has the ability to be transported over long-range distances in the atmosphere, can also be distributed from the mine tailings by vehicular activity and be carried by wind, therefore its concentration in the leaves of the plants could not only be coming from the roots but also from atmospheric deposition. The aerial plant tissues are exposed to the atmosphere, this allows for mercury to be easily deposited on the stem and leaves tissues as a result get incorporated into the plants' system through foliar absorption. The absence of rainfall in the dry season means that particles of mercury on the plant leaves will not be washed off therefore foliar absorption becomes more pronounced. Furthermore, Zillioux et al., (1993) stated that mercury gets deposited directly to the plant leaves, such that after leaf fall, areas that do not contain trees and shrubs will have a lower concentration of mercury. Areas that contain leaf litter will definitely have higher concentration of mercury. During the dry season heavy metals from the wetlands are leached out. This may also contribute to increasing metal concentration in sediments and plant tissues during dry season. Consequently, evaporation and absence of rainfall in dry season can lead to elevated levels of HgT. These results were similar to those presented in a study carried out by Oluyemi et al., 2008.

As has been stated earlier the pH of the sediments collected in the dry season was significantly lower relative to that in the wet season. Generally, at low pH levels metals are more soluble in the sediments, hence more bioavailable to plants. Hence, toxicity problems are more severe in acidic sediments than in alkaline sediments. This could be one of the reasons why greater concentrations of HgT were observed in plants sampled in the dry season. In SA, summer is characterised by heavy rainfall creating surface runoff. The runoff effect is capable of washing away heavy metals from the above ground tissues of the plants and the effect of rainfall may facilitate the leaching of the sediments and

this might contribute to the dilution of the concentration of mercury during the wet season.

5.7 Distribution of mercury in plant tissues

Phragmites australis is one of the most widely distributed species on earth. It is commonly found in areas characterised by shallow or still water saturation at/or near the surface for the substantial part of the year. This plant is hailed for its ability to resist harsh environmental conditions. This includes the presence of pernicious contaminants such as Hg, Cd and Zn (Ye at al., 1998). *P. australis* has been used extensively in constructed wetlands for the treatment of waste water from industry. This specie can tolerate a very low pH levels and have been found growing under field conditions in pH as low as 2 to 4.4 (Ye at al., 1998) and can be very tolerant of environments that have high salinity. In the present study this species exhibited a different behavioural pattern between summer and winter. It is for these reasons that it was chosen to study the distribution of mercury in the roots, stem and leaves from the wet season. Shown in Figures 27 are the results obtained from the Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). The images were taken at 1000X magnification and samples were coated with gold and palladium. SEM images depicted square shaped in roots crystals, longitudinal sheet shaped crystals in stem, and irregular shaped crystal with a smooth surface in leaves. However, no indication of Hg was observed. It could be inferred that perhaps this heavy metal was below detection limit and that due to its volatile nature (MeHg and Hg^0) it might have evaporated and lost into during sample preparation which included sample coating with gold and palladium.

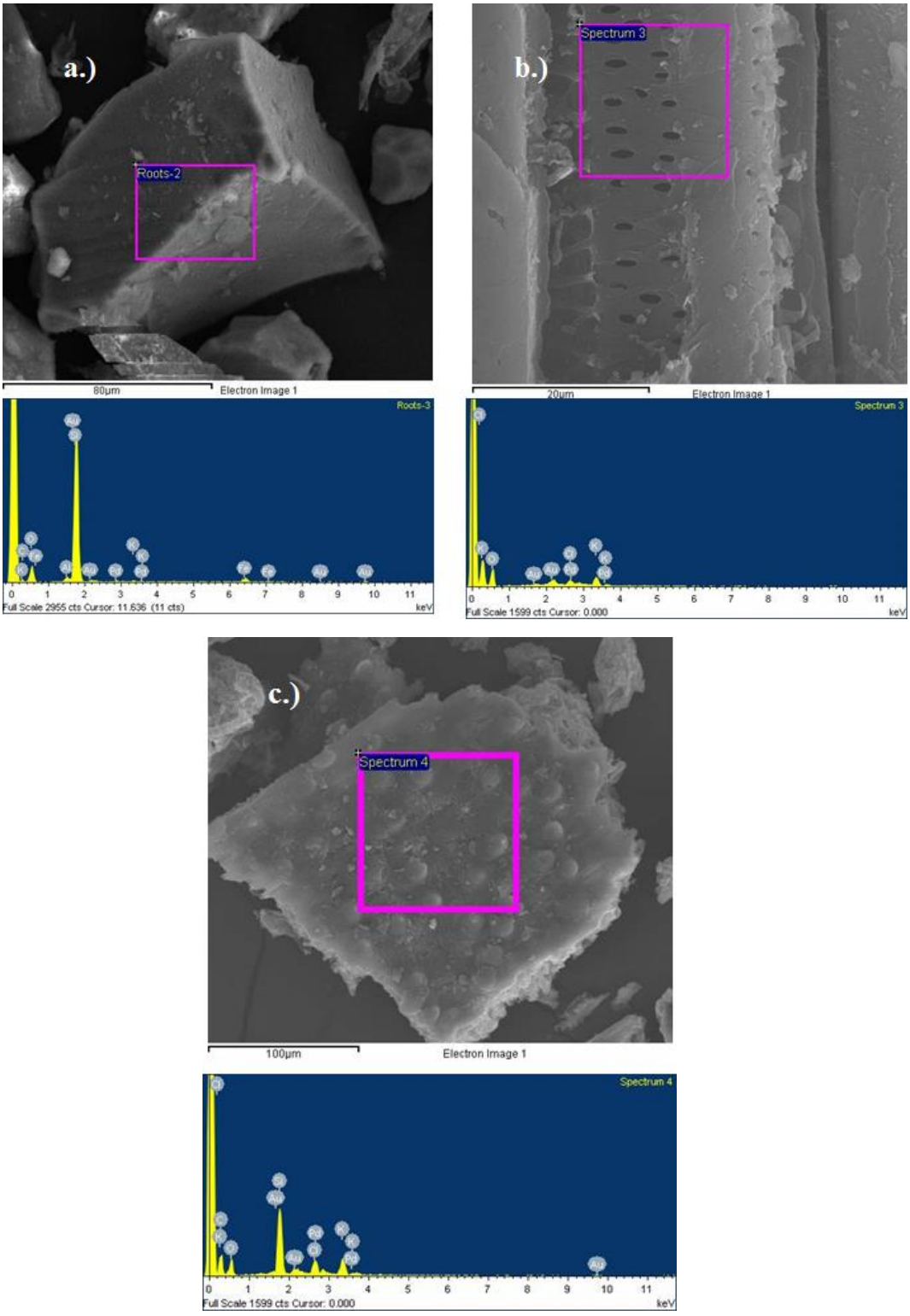


Figure 27: SEM images of *P.australis* and EDS spectra showing metal distribution in a.) Roots; b.) Stem and c.) Leaves

5.8 Spatial distribution of mercury at the study site

The concentrations of total mercury in the six surface sediments samples from the Germiston natural wetland were measured. Surfer software was used to generate contour maps to elucidate the influence of pollution from the tailings footprint adjacent to the sampling site, thus the spatial distribution of mercury within the surrounding area. Results are shown in Figure 28 and 29 for the wet and dry seasons respectively. In the wet season levels of HgT seemed to be increasing in the direction away from the tailings footprint where the highest HgT concentrations 692 and 668 μg kg^{-1} were obtained in PL and CE respectively (provided in the appendix Table A1). The location of the tailings footprint is such that it is adjacent to the sampling site. Therefore during the wet season surface run-off flows from the tailings footprint with contaminants towards the direction of the sampling site. In addition, tailings become subjected to water and wind erosion and this leads to distribution of heavy metals like mercury to water systems and surrounding areas. Thus, metals from the TF can be washed down to the sampling site via fluvial transportation and erosion. Migration of leached mercury from the TF or the surrounding polluted soil through runoff could explain the observed contamination. This explains the increasing mercury concentration in sediments located further from the tailings footprint.

A different trend was observed in the dry as shown in Figure 29. The surface sediments located adjacent to the tailings footprint showed the highest levels of total mercury and these were MA 1005 μg kg^{-1} followed by PL 851 μg kg^{-1} and PA 668 μg kg^{-1}. The enrichment of mercury in these sediments adjacent to the old TF might be due to historical loads of mercury in tailings and seepage from the facilities. It is worth mentioning that these were the sites (PA and PL) with lower pH values (Table 7). The release of non-bioavailable metals from sediments is highly favourable under low levels of pH. This result into the mobility and solubility of bioavailable mercury and this explains the levels of mercury observed.

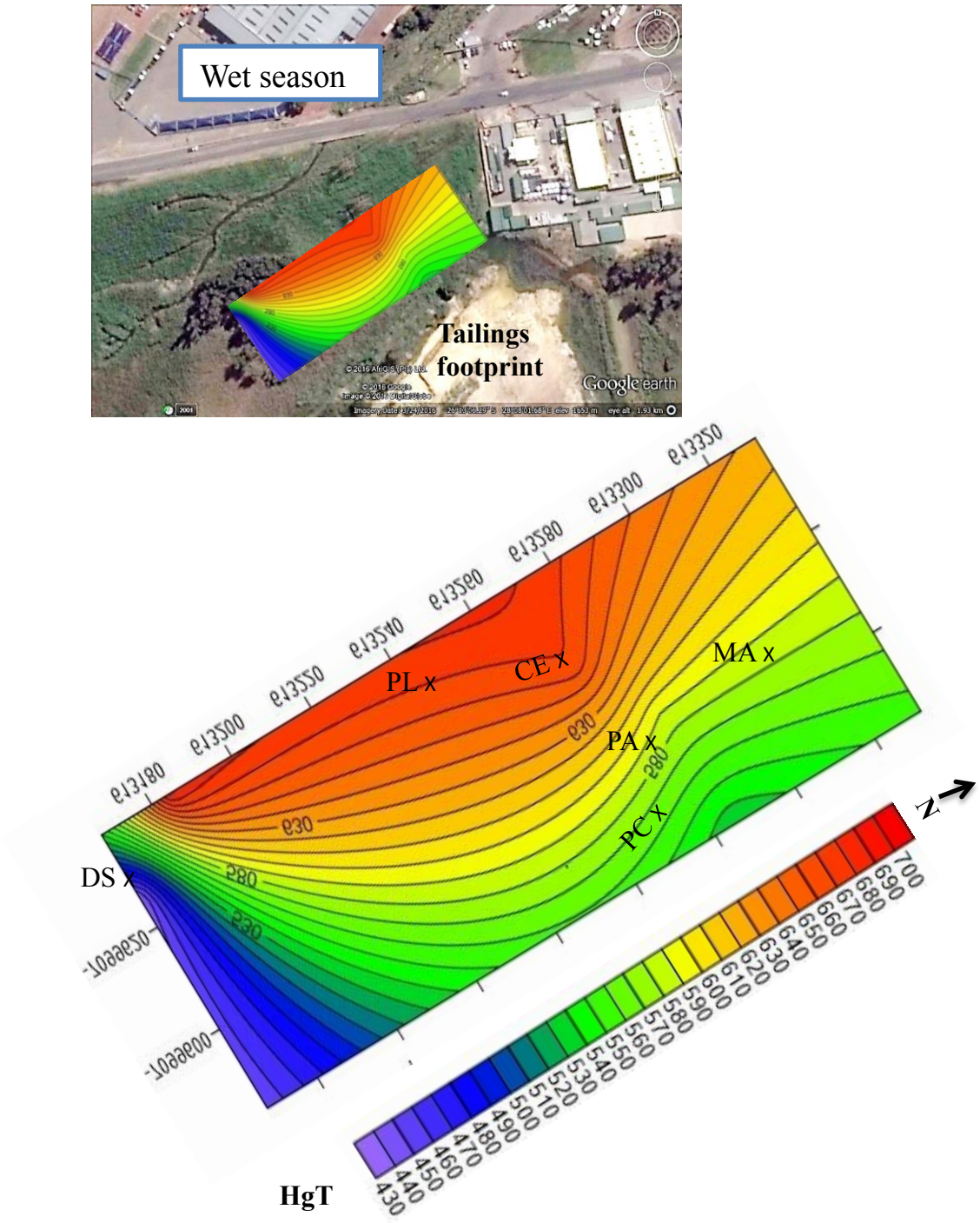


Figure 28: Spatial distribution of mercury in surface sediments collected in the wet season

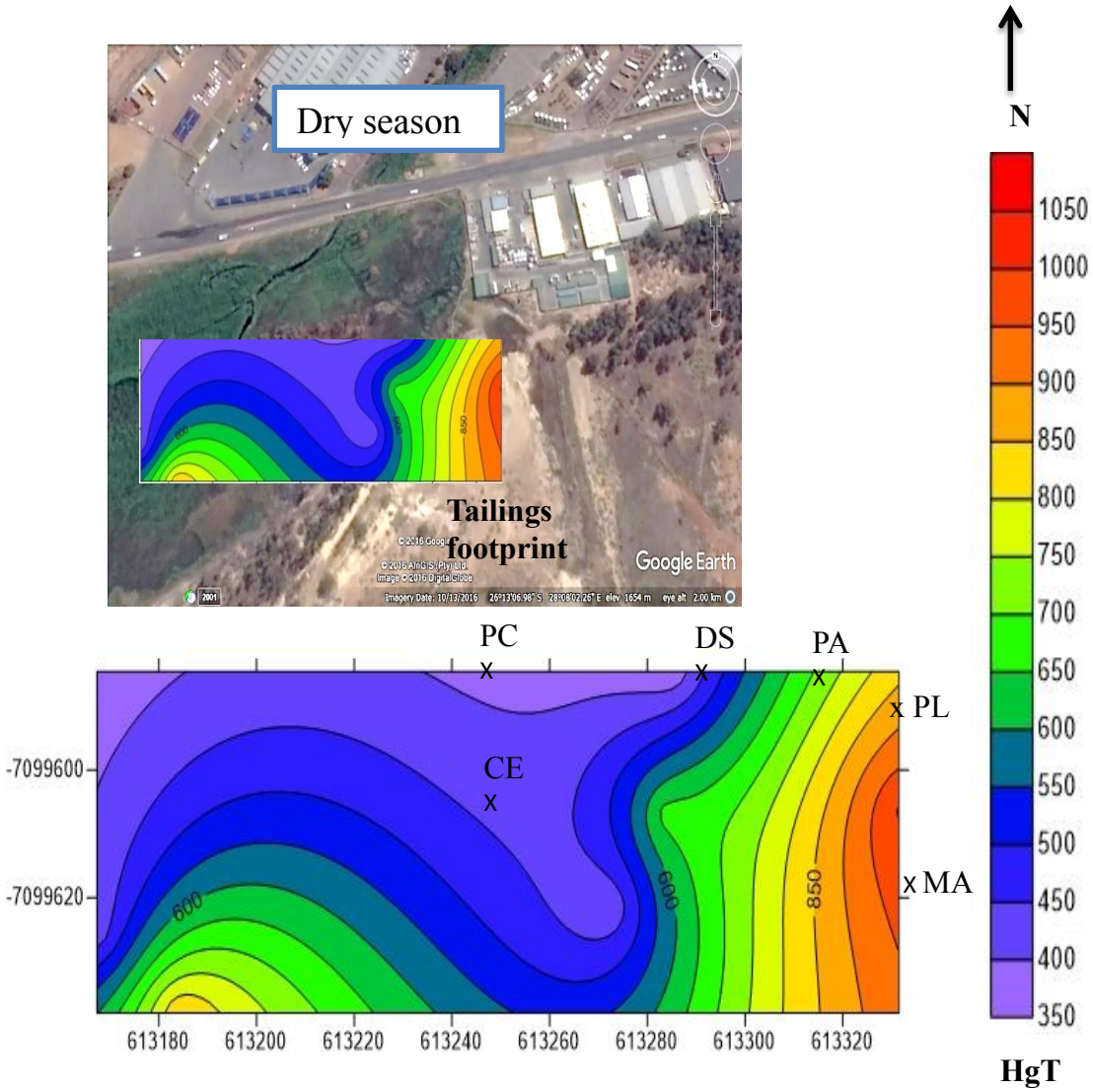


Figure 29: Spatial distribution of mercury in surface sediments collected in the dry season

CHAPTER 6: CONCLUSIONS

The study revealed that wetland plants can grow and uptake mercury from a contaminated area. Most of the analysed sediments fell out of the Hg probable effect level and the concentrations of mercury in the plant species was above the normal background value suggested by other researchers. Mercury bioaccumulation from sediments to the root tissues appeared to be hindered in some species, and these plants showed the ability to immobilize mercury and store it in the rhizosphere. However, other plant exhibited the ability to transport oxygen from the aboveground tissues to the rhizosphere thus making mercury more bioavailable for uptake. Mercury translocation into the stem and leave tissues appeared to characterise some wetland plants. Foliar adsorption seemed to be another important source of mercury especially in the aboveground plant tissues more pronounced in the dry season. In other species the translocation of mercury from sediments to the above ground tissues seemed unfavourable. This can be viewed as a positive characteristic as mercury would not be passed in the food chain through herbivores. The strong positive correlation between the conversions of the bioavailable total mercury into methylmercury in the wet season indicated that a combination of factors such as temperature, pH and redox potential should be taken into consideration when investigating plants to be used for phytoremediation.

Translocation factor gives an idea whether a plant can sufficiently take up metals from the sediments to the aerial tissues. A plant with a good translocation factor is good for phytoremediation. Besides their metal uptake capacity, plant species investigated developed mechanisms to cope with elevated levels of mercury in the wetland and this enhances their phytoremediation capacity. *D. stramonium, P. lapathifolia, P. coloratu* and *C. eragrostis* showed properties of plants that can be used for phytoextraction therefore being useful species to be utilized in constructed wetlands for the treatment of industrial effluents. *P. australis* and *M. alba* on the contrary exhibited properties of plants than can be used for phytostabilization. It is noteworthy to state that the behaviour of *P. australis* was heavily influenced by seasonality.

The highest metal concentration in the roots was obtained in *P. australis* in the wet season and *M. alba* in the dry meaning these species adopted an exclusion strategy for metal tolerance. The concentration of heavy metals in plants does not only depend on the metal concentration in sediments but also on other factors such as: plant species, the growth stage of a plant and element characteristics which control absorption, accumulation and translocation of metals. Mercury has the ability to be transported over long-range distances in the atmosphere; therefore its concentration on the leaves of the plants could not only be coming from the roots but also from atmospheric deposition. This was demonstrated in the translocation factor values of *D. stramonium, P. lapathifolia, P. coloratu* and *C. eragrostis* in both the wet and dry season being greater than one. Therefore seasonality and the amount of mercury present in the atmosphere have also been observed to play critical roles in mercury accumulation and biotransportation.

Therefore the different uptake and speciation patterns suggest that the most effective wetlands (including constructed wetlands) should include few different plant species working together because synergistic action is important to achieve effective trapping and removal of heavy metal pollutants.

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APPENDIX

Table A1 Concentrations of HgT and MHg in the tissues of plant species sampled in the wet season.

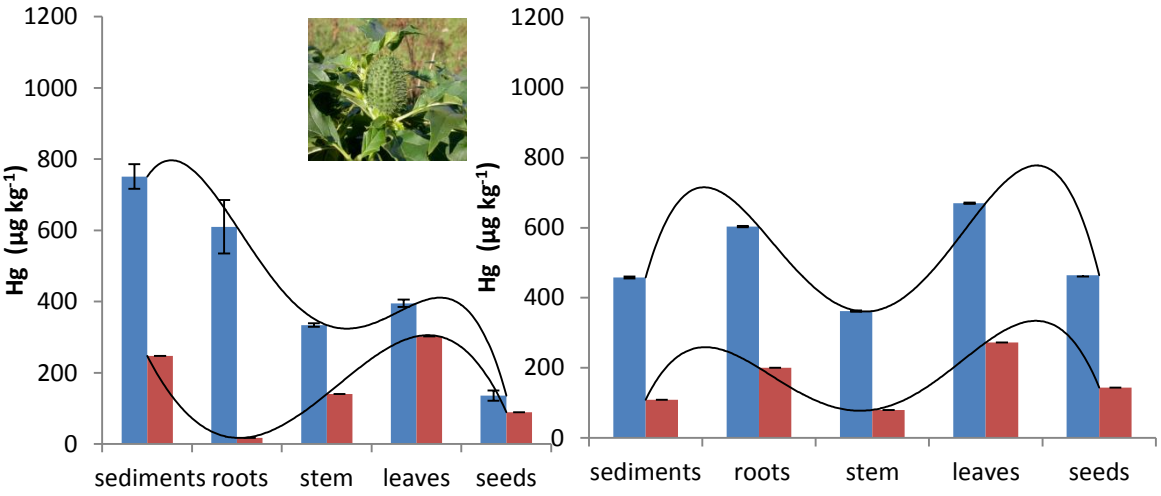
Sample ID	HgT ($\mu g\ kg^{-1}$)	MHg ($\mu g\ kg^{-1}$)	SD	RSD %	%MHg
D. stramonium					
Sediments	437	144	0.01	2.8	33
Roots	88	2	0.10	7.4	3
Stem	318	133	0.07	2.4	42
Leaves	375	287	0.11	6.9	76
Seeds	63	41	0.09	7.1	65
P .australis					
Sediments	589	97	0.03	2.4	16
Roots	432	135	0.07	4.3	31
Stem	294	264	0.09	4.9	90
Leaves	247	219	0.07	4.3	89
P. lapathifolia					
Sediments	692	111	0.07	0.9	16
Roots	78	6	0.03	3.0	9
Stem	282	181	0.08	4.7	64
Leaves	242	190	0.03	0.9	79
M. alba					
Sediments	583	141	0.10	0.2	24
Roots	77	30	0.09	6.8	39
Stem	78	50	0.07	2.7	64
Leaves	78	50	0.11	11.9	64
P. coloratum					
Sediments	532	298	0.06	7.0	56
Roots	61	30	0.08	11.3	50
Stem	414	230	0.09	6.1	55
Leaves	226	108	0.12	9.5	48
C. eragrostis					
Sediemnts	668	192	0.01	1.3	29
Roots	148	95	0.10	12.0	64
Stem	394	229	0.02	3.0	58
Leaves	294	169	0.05	5.5	57

Table A2: Concentration of HgT and MHg in the dried tissues of plant species sampled in the dry season.

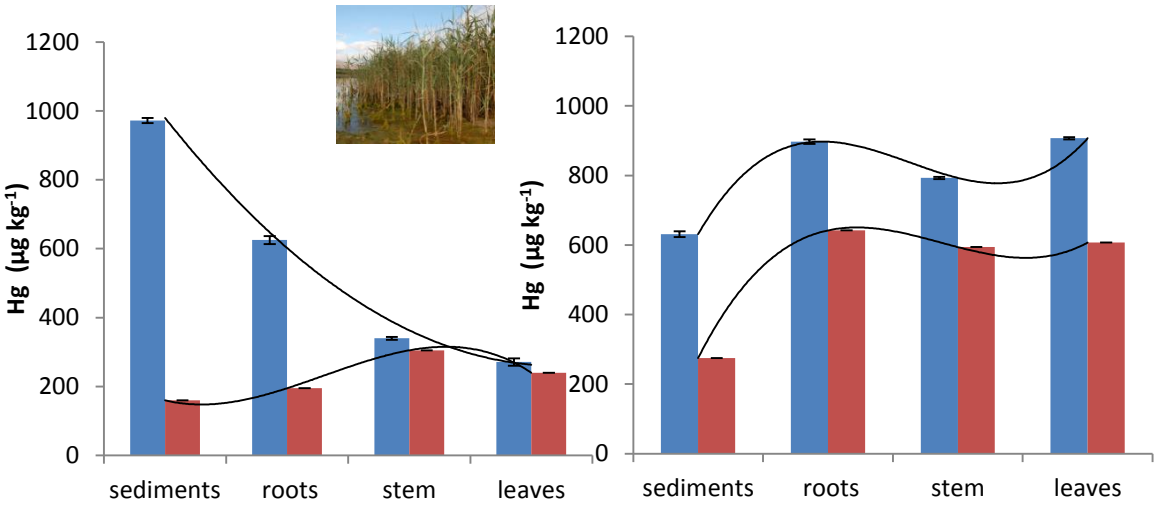
Sample ID	**HgT ($\mu g\ kg^{-1}$)**	**MHg ($\mu g\ kg^{-1}$)**	**SD**	**RSD %**	**%MHg**
D. stramonium					
Sediments	414	99	0.01	3.7	24
Roots	67	22	0.01	2.4	33
Stem	326	72	0.01	1.7	22
Leaves	566	230	0.01	2.6	41
Seeds	193	60	0.01	1.8	31
P. australis					
Sediments	668	192	0.05	1.3	29
Roots	148	95	0.01	3.2	64
Stem	394	229	0.01	6.2	58
Leaves	294	169	0.01	2.5	57
P. lapathifolia					
Sediments	851	227	0.02	2.2	27
Roots	169	31	0.01	3.3	18
Stem	501	240	0.01	3.6	48
Leaves	519	204	0.00	4.0	39
M. alba					
Sediments	1005	610	0.04	3.5	61
Roots	208	139	0.01	0.6	67
Stem	129	60	0.02	3.1	47
Leaves	125	79	0.01	3.0	63
P. coloratum					
Sediments	360	75	0.02	4.3	21
Roots	48	7	0.02	2.6	14
Stem	213	167	0.05	2.1	78
Leaves	525	83	0.01	2.4	16
C. eragrostis					
Sediments	410	322	0.01	3.2	78
Roots	144	69	0.01	2.5	48
Stem	439	209	0.00	1.9	48
Leaves	518	164	0.02	2.5	32

Wet season **Dry season**

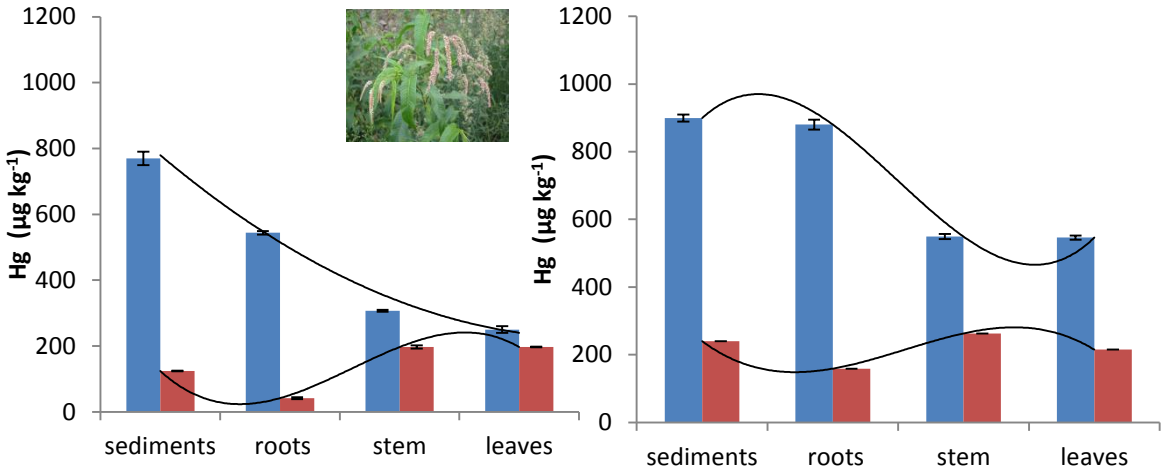
Datura stramonium



Phragmaties australis



Persicaria lapathifolia



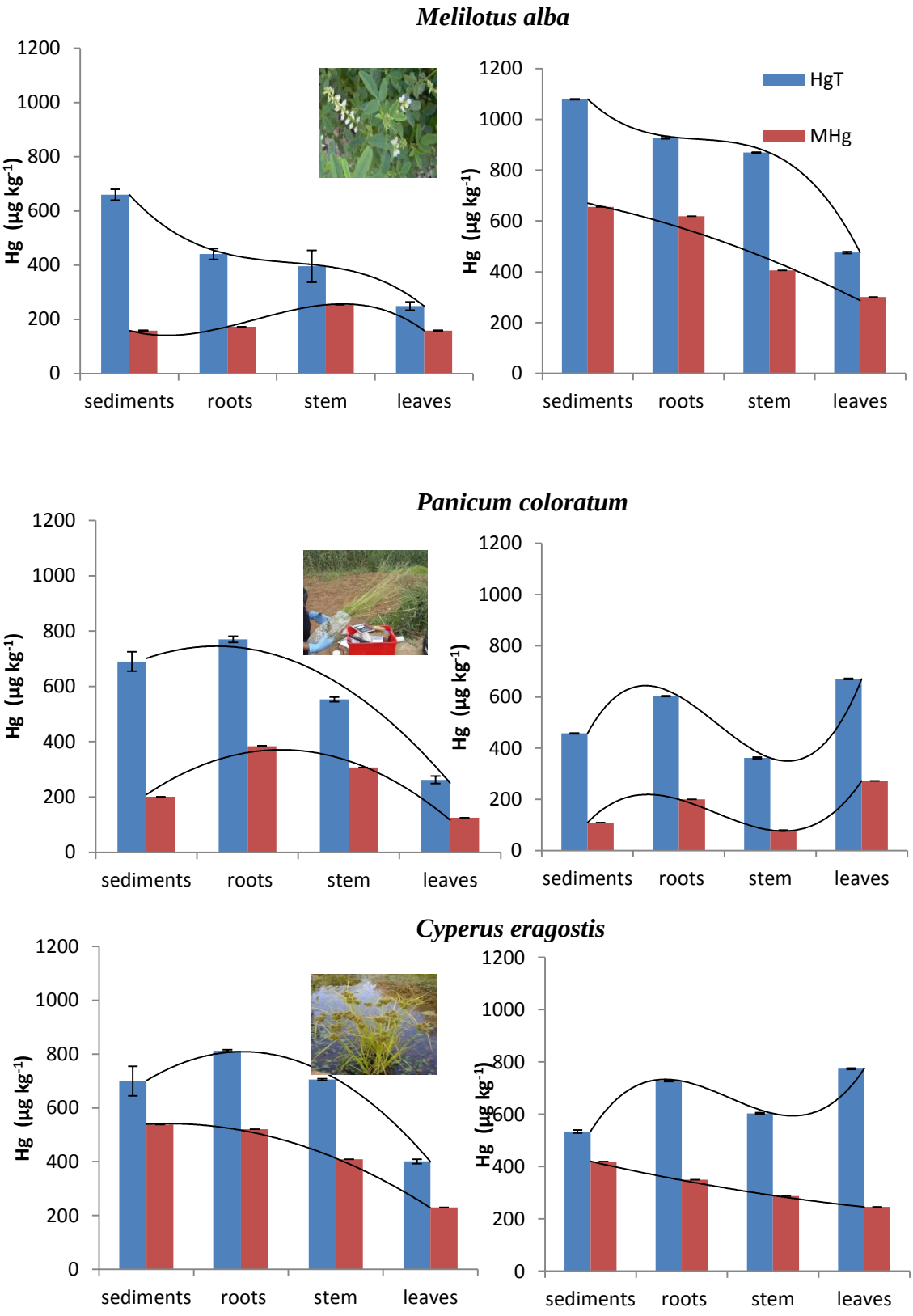


Figure A1: Concentration of HgT and MHg concentrations dry weight in the sediments and plant tissues of selected macrophytes.

Table A3: Field parameters and mercury concentrations in sediment profiles.

Sample Profile	Depth (cm)	pH	T/ ºC	ORP/ mV	Ec/ µS cm^{-1}	HgT/ µg kg^{-1}	%RSD (n=7)
A	0-20	7.2	13.9	458	283	169	1.2
	20-40	7.1	14.2	469	204	180	1.7
	40-60	7.0	14.3	151	360	148	4.2
	60-80	7.3	14	200	244	104	0.4
B	0-20	6.7	13.9	436	223	542	5.2
	20-40	7.2	12.6	308	221	139	0.6
	40-60	7.2	12.5	156	270	179	0.3
	60-80	7.3	12.2	-10	397	332	2.1
	80-100	7.3	13.6	-28	531	296	1.9

Table A4: Results of the test for normality and the independent-t test for the concentration of HgT and MeHg obtained for *D.stramonium.*

D. stramonium

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
HgT	.152	10	.200*	.970	10	.894
MHg	.169	10	.200*	.960	10	.782

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
HgT	Equal variances assumed	2.396	.160	-.548	8	.599	-66.200	120.857	-344.896	212.496
	Equal variances not assumed			-.548	5.973	.604	-66.200	120.857	-362.254	229.854
MHg	Equal variances assumed	1.286	.290	-.032	8	.975	-2.000	62.054	-145.097	141.097
	Equal variances not assumed			-.032	6.932	.975	-2.000	62.054	-149.025	145.025

Table A5: Results of the test for normality and the independent-t test for the concentration of HgT and MeHg obtained for *P. australis.*

P. australis

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
MHg	.265	8	.104	.828	8	.057
HgT	.171	8	.200*	.899	8	.283

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
MHg	Equal variances assumed	2.987	.135	-3.347	6	.015	-304.500	90.987	-527.136	-81.864
	Equal variances not assumed			-3.347	3.791	.031	-304.500	90.987	-562.719	-46.281
HgT	Equal variances assumed	3.636	.105	-1.483	6	.189	-255.000	171.975	-675.808	165.808
	Equal variances not assumed			-1.483	3.943	.213	-255.000	171.975	-735.226	225.226

Table A6: Results of the test for normality and the independent-t test for the concentration of HgT and MeHg obtained for *P. lapathifolia*

P. lapathifolia

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
MHg	.222	8	.200*	.930	8	.519
HgT	.197	8	.200*	.911	8	.362

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
MHg	Equal variances assumed	1.415	.279	-1.836	6	.116	-79.250	43.157	-184.852	26.352
	Equal variances not assumed			-1.836	4.938	.126	-79.250	43.157	-190.610	32.110
HgT	Equal variances assumed	.146	.715	-1.620	6	.156	-250.750	154.805	-629.543	128.043
	Equal variances not assumed			-1.620	5.801	.158	-250.750	154.805	-632.717	131.217

Table A7: Results of the test for normality and the independent-t test for the concentration of HgT and MeHg obtained for *M. alba.*

M. alba

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
MHg	.203	8	.200*	.843	8	.081
HgT	.208	8	.200*	.941	8	.617

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
MHg	Equal variances assumed	17.855	.006	-3.514	6	.013	-309.000	87.932	-524.161	-93.839
	Equal variances not assumed			-3.514	3.431	.032	-309.000	87.932	-569.959	-48.041
HgT	Equal variances assumed	.528	.495	-2.605	6	.040	-401.500	154.098	-778.565	-24.435
	Equal variances not assumed			-2.605	5.204	.046	-401.500	154.098	-792.989	-10.011

Table A8: Results of the test for normality and the independent-t test for the concentration of HgT and MeHg obtained for *P. coloratum*.

P. coloratum

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
MHg	.221	8	.200*	.947	8	.684
HgT	.191	8	.200*	.915	8	.387

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
MHg	Equal variances assumed	.898	.380	1.683	6	.143	120.000	71.286	-54.429	294.429
	Equal variances not assumed			1.683	5.551	.147	120.000	71.286	-57.905	297.905
HgT	Equal variances assumed	.048	.834	.573	6	.587	80.750	140.863	-263.929	425.429
	Equal variances not assumed			.573	5.631	.589	80.750	140.863	-269.485	430.985

Table A9: Results of the test for normality and the independent-t test for the concentration of HgT and MeHg obtained for *C.eragostis.*

C.eragrostis

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
MHg	.148	8	.200*	.926	8	.484
HgT	.249	8	.156	.922	8	.443

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
MHg	Equal variances assumed	1.310	.296	1.233	6	.264	99.000	80.317	-97.529	295.529
	Equal variances not assumed			1.233	4.579	.277	99.000	80.317	-113.302	311.302
HgT	Equal variances assumed	.466	.520	-.048	6	.963	-5.000	104.203	-259.974	249.974
	Equal variances not assumed			-.048	5.034	.964	-5.000	104.203	-272.319	262.319