AN INVESTIGATION INTO THE EFFECT OF METALS ON CHLOROPHYLL CONTENT AND PHOTOSYNTHESIS ACTIVITY OF THE WETLAND PLANT *PHRAGMITES AUSTRALIS* IN THE LOWER DIEP RIVER, MILNERTON, CAPE TOWN

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by

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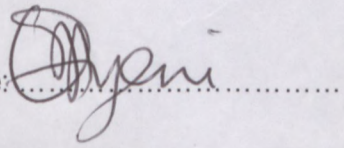
DECLARATION

I, Olutoyosi Olaide Ayeni, hereby declare that the work contained in this dissertation is my own original work that all sources used or quoted, have been indicated and acknowledged by means of complete references, and that this dissertation was not previously submitted by me or any other person at any other university for a degree award.

Signature:  Date 18/4/2011

ABSTRACT

A study involving a wetland plant, common reed *(Phragmites australis* L.) was carried out along the bank of the lower Diep River and the adjacent soil samples from four different sites (Milnerton Lagoon, Lower Estuary, Milnerton Bowling Club and Woodbridge Island), Cape Town, South Africa. The aim was to determine the extent of metal contamination and its impact on physiological indices.

Results showed that among the metals evaluated, Al and Fe were consistently higher in all the soil samples (from both river bank and the adjacent soil) followed by Zn, Mn, Pb, Cu, Cd, Co, Cr and Ni. The concentrations of Al in the river banks ranged between 1214.1 - 3176 $mg.kg^{-1}$ compared with the adjacent soils, where Al concentration ranged from 434.8 - 2445.4 $mg.kg^{-1}$. The Fe concentrations from the river bank values ranged from 1136.4 - 4897.2 $mg.kg^{-1}$ compared with Fe concentrations of the adjacent soil samples which ranged from 402.2 - 2459.8 $mg.kg^{-1}$. Generally, Zn ranged from 2.4 - 211.5 $mg.kg^{-1}$; Mn: 5.5 - 48.05 $mg.kg^{-1}$; Pb: 0.97 - 71.7 $mg.kg^{-1}$; Cu: 0.3 - 45.9 $mg.kg^{-1}$; Cd: 0.0 - 9.3 $mg.kg^{-1}$; Co: 0.2 - 2.7 $mg.kg^{-1}$; Cr: 0.3 - 2.1 $mg.kg^{-1}$; and Ni: 0.02 - 2.6 $mg.kg^{-1}$. Overall, Ni had the lowest concentrations in the ecosystem.

Results also showed that the abundance of metals from plant samples were in the order of Al > Pb > Cd > Co > Ni > Cr; and for micronutrients, Fe > Mn > Zn > Cu both in the shoots and roots sampled from all the sites investigated. The values of chlorophylls a, b and total chlorophyll as well as photosynthesis were significantly higher in the *P. australis* plant samples and from the adjacent soil compared with those from the river bank. These results suggest that contamination of soils and wetland ecosystem by metals over and above plant requirements may affect the chlorophyll and photosynthesis rate of the plant thereby undermining the physiological functioning of plants growing along river systems.

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DEDICATION

First and foremost, I would like to thank God Almighty, for providing me with the grace and insight to complete this dissertation (2 Corinthians 12:9a).

Secondly, I dedicate this work to my loved ones for their unwavering trust, support and motivation during these four years of study, namely:

- My children: Toluwanimi, Orejesu and Tofunmi.
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CHAPTER ONE

CHEMICAL, BIOLOGICAL AND PHYSIOLOGICAL INDICATORS OF METAL POLLUTION IN WETLANDS

1.1 GENERAL OVERVIEW

Large areas of land are contaminated with metals due to natural processes such as lithogenic, pedogenic and anthropogenic factors, the latter of which industrial activity like mining, sewage and traffic are examples, which arise as a result of rapid urbanization and industrialization (Stronkhorst et al., 2003; Agunbiade and Fawale, 2009). According to Grimm et al. (2008), urbanization alters both biotic and abiotic ecosystem properties within, surroundings, and even at great distances from urban areas. Urbanization is a globally important land-use change associated with pollution.

Freshwater pollution could also be due to continual release of wastewater into rivers (Hussein et al., 2001), which exerts adverse effects on the quality of soil and plants growing in it, as a result of increases in metal concentrations (Prasad, 1999). Other sources of metals in freshwater ecosystems include storm water runoff, runoff from agricultural and suburban lands, purified sewage returns, sewage discharges and runoff from hardened catchments; and irrigation water (Duffus, 2002; Sharma and Dube, 2005; Bragato et al., 2006; Madejón et al., 2006; Brown and Magoba, 2009).

Metal loads in freshwater ecosystems have increased in recent times. For example, studies conducted in China, involving numerous metal mines indicated that the process of metal mining has caused severe metal pollution (Wong and Bradshaw, 2002). Freshwater ecosystems are exposed to metals that are dissolved in water, associated with excessive runoff and deposited in bottom sediments. Thus, freshwater organisms can bioaccumulate certain metals to concentrations that greatly exceed those dissolved in water.

Pollution by metals is a serious problem because of their toxicity, ability to accumulate in biota, and non-biodegradability in the environment (Prica et al., 2008). The ability of living tissues to accumulate, magnify and transform pollutants in the environment has made them of great significance in environmental studies. Plants and animal tissues have been reported to be capable of accumulating and magnifying pollutants like metals to a level that is toxic to plants and human lives (Hopkin et al., 1999; Liu et al., 2005; Bragato et al., 2006; Cabrera et al., 2006; Madejon et al., 2006).

In aquatic ecosystems, the bioavailability and toxicity of metal ions depends strongly on the chemical form in which the metals occur (their "speciation") and in turn, speciation depends on solution conditions, especially pH and concentration of various ligands (Förstner and Wittmann, 1979; Otte et al., 1993; Veseley and Majer, 1994). For this reason, researchers cannot solely rely on metal concentrations in water and sediments but also need to include freshwater organisms in their studies.

Aquatic macrophytes are widely distributed in various wetland environments, from freshwater to salty water (Bragato et al., 2006). They have several characteristics that favour metal accumulation, for example, biomass, the above ground organs (leaf area) and below ground organs (the roots and rhizomes) (Stoltz and Greger, 2002; Bonanno and Lo Giudice, 2010). There is evidence that wetland plants can accumulate heavy metals in their tissues, such as duckweed (*Lemna minor*) (Zayed et al., 1998), Water hyacinth (*Eichhornia crassipes*) (Vesk et al., 1999), Salix (Stoltz and Greger, 2002), Cattail (*Typha latifolia*) and Common reed (*Phragmites australis*) (Ye et al., 1997; Yang et al., 2008).

According to the different capacity for metal uptake, species which are able to accumulate relatively high metal concentrations in the aboveground tissues could be good candidates for phytoextraction. Wetland species such as the common reed (*Phragmites australis*) (Ye et al., 1997; Meagher, 2000; Yang et al., 2008) could be very important in ecotoxicological assessment to clean up contaminated ecosystem. So far, however, little information is available on the profile of metal pollution in selected soils and plants within the Diep River, in Cape Town, South Africa.

1.2 LITERATURE REVIEW

1.2.1 INTRODUCTION

Metals are potentially toxic substances. Excessive concentrations in biological systems can destabilize ecosystems because of their bioaccumulation in organisms, with toxic effects on biota and even death in most living organisms (Alloway and Ayres, 1993; Nagel et al., 1996; Banaszak et al., 2000; Kaznina et al., 2005; Liphadzi and Kirkham,

2006; Andra et al., 2010). Bioaccumulation refers to the accumulation of contaminants by species in concentrations that are orders of magnitude higher than in the surrounding environment (Weigel 2004). This could involve the excessive accumulation of metals in plants. Some of the major sources of metals are irrigation water (when contaminated by sewage and industrial effluent), battery production, metal products, metal smelting, cable coating industries, brick kilns, automobile emissions, re-suspended road dust and diesel generator sets (Duffus, 2002; Sharma and Dube, 2005; Bragato et al., 2006; Madejón et al., 2006). Other sources can include unsafe or excessive application of pesticides, fungicides and fertilizers, and can also include sewage sludge (Murthy et al; 1989; Bart and Hartman, 2003; Silliman and Bertness, 2004; Weis and Weis 2004).

The term biomarker relates to the response within organisms to different levels of pollution. It refers to the capability of the organism to indicate the presence and amount of pollutants in the system (Nimis et al., 1993; Sloof et al., 1998). Biomarkers may provide both qualitative and quantitative information on levels of contamination in organisms such as plants (Prasad, 1997, 1999; Maksymiec and Baszynski, 1999a, b).

Similarly the term biomarker means any biological response to an environmental chemical below individual level, measured inside an organism or its products (urine, faeces, hairs, feathers, etc), indicating a departure from the normal status, that cannot be detected from the intact organism (Van Gestel and Van Brummelen, 1996). Biomonitors or bioindicators are any species that provides additional information about the health of an environment. In some cases, the assessment of pollutants in soil, sediment and water may not necessarily gives an indication of the state of the environment, without measuring the impact of such pollutant in the tissues of living organisms that are part of the food web. The assessment of the bioavailability of the pollutants in living tissues gives a strong indication of their toxicity (Wang et al., 1997; Mertens et al., 2006; Wang et al., 2009). For example, biomonitors in a soil environment are species that reveal the soil quality and other information which are difficult to measure by direct soil analyses (Madejón et al., 2004).

Plant parameters such as chlorophyll content, plant growth and biomass production, photosynthesis, transpiration, metal uptake and metabolism may be used to determine the level of toxic stress in plants (Breckle and Kahle, 1992; Csintalan et al., 1992). A

variety of plant species growing in heavily polluted areas may be used as bioindicators to determine the level of pollution. They may also be used in phytoremediation programmes. Phytoremediation can be defined as the efficient use of plants to remove, detoxify or immobilise environmental contaminants in a growth matrix (soil, water or sediments) through the natural biological, chemical or physical activities and processes of the plants (UNEP, 2010).

Plants are unique organisms equipped with remarkable metabolic and absorption capabilities, as well as transport systems that can take up nutrients or contaminants selectively from the growth matrix, soil or water. An understanding of such physiological responses of plants (e.g. the content of chlorophyll and photosynthesis processes) and metal bioaccumulation as a result of exposure to metals would be a step towards establishing how these physiological parameters could be used as biomarkers of pollution and serve as an early warning system.

1.2.2 ELEVATED CONCENTRATIONS OF METALS IN SOIL AS AN INDICATION OF POLLUTION

The rapid industrialization and urbanization in some of the developing countries of Africa have resulted in large-scale pollution of the environment and the enrichment of metals in the soil (Adeniyi, 1996; Sanderson et al., 2002; Osuji et al., 2005; Shuping, 2008; Agunbiade and Fawale, 2009). Environmental sustainability depends largely on a sustainable soil ecosystem, when the soil is polluted, the ecosystem is altered and plant growth is affected (Adriano et al., 1998; Quan et al., 2007). Soil has become a dumping site for industrial and municipal waste, most of which contain metals (Phillips 1999; Yusuf et al., 2003; Okunola et al., 2007).

Roadside soils have been shown to have considerable contamination owing to depositions of vehicles-derived metals (Adeniyi, 1996; Okunola et al., 2007; Agunbiade and Fawale, 2009). Crude oil and their products which are deposited on road surfaces or nearby soils, or in the water bodies have been shown to have elevated levels of metals, consequently becoming toxic to plants and micro-organisms (Odjegba and Sadiq, 2002; Merkl et al., 2005; Osuji et al., 2005; Wang, 2009). Crude oil spills may

also add metals to the soil, which become deleterious to soil biota and crop growth (Osuji et al., 2005).

Similarly, many reports from different parts of the world have shown soils and sediments were heavily polluted with a mixture of metals from industrial effluent and this ultimately affects the physiological parameters of organisms in the soil ecosystem (Table 1). Some of the examples include: Cd, Ni, Pb, As and Zn (Reeves and Baker, 2000; Kannavou et al., 2001; Jin et al., 2002; Xendis et al., 2003; An, 2004; Cui et al., 2004).

A study by Chen (2000) in Taiwan showed that Cd, Pb and Zn concentrations in soils contaminated with industrial effluent were at high levels, ranging from 175 - 378, 252 - 3145 and 100 mg/kg, respectively. In the study, more than 800 ha of land were contaminated with industrial effluent waters containing metals such as (Cd, Cr, Cu, Pb and Zn, which were then reflected in brown rice in different concentrations (Chen, 2000).

Extensive and continued monitoring of chemical concentrations of metals in areas with similar industrial activities is therefore important to alerting people on the presence of dangerous pollutants in the ecosystem. So far, little information is available on the extent of metal pollution in most of the developing world such as those found in Africa.

1.2.3 METAL CONCENTRATIONS IN PLANTS EXPOSED TO ELEVATED LEVELS OF METALS

Plants that grow in polluted environments may show stress symptoms due to bioaccumulation of metals through direct uptake by the plants' roots, stems or shoot (Godbold et al., 1984; Baker, 1987; Dahmani-Muller et al., 2000; Monni et al., 2001) (Table 2). In all plants, some metals are known to perform specific functions within the tissue systems, which may manifest different visual symptoms at elevated concentrations (Table 3). For example, the excessive accumulation of toxic levels of metals in plant tissues, above the established critical levels, may cause growth abnormalities such as leaf senescence, leaf chlorosis (Baker et al., 1994), dwarfism, wilting and death of the whole plant (Godbold et al., 1984; Baker, 1987; Monni et al., 2001; Liphadzi and Kirkham, 2006).

The toxic effect of metals on the physiological functioning within plants is connected to their accumulation in different plant tissues. Studies have shown that plant species will concentrate certain metals in their leaves, stems and roots to varying degrees and, hence, critical levels may vary among species (Breckle and Kahle, 1992; Baker et al., 1994; Liphadzi and Kirkham, 2006).

Studies have shown that the symptoms that are associated with elevated Mn concentrations (±300 µg/g in plants) include brown spots on mature leaves, interveinal chlorosis and necrosis, deformation of young leaves, growth retardation and leaf tip burning (Foy et al., 1978, 1988; Wissemeier and Horst, 1992).

Ecotoxicological studies have shown that plants that are subjected to elevated concentrations of metals in the rhizosphere may have difficulties in the translocation of these metals from the roots to the aerial parts, a situation which may facilitate root damage as a result of excessive metal bioaccumulation (Godbold et al., 1984; Baker, 1987; Baker et al., 1994; Crozier et al., 2000). In such instances, root and plant growth in general will be severely affected by the high concentrations of metals in the roots because roots are also the main routes of metal uptake and nutrients from soil into plants (Crozier et al., 2000).

Based on research findings on some plant species, toxic concentrations of selected metals are: Cd, 0.1; Ni, 10; and Pb, 30, Cu, 20; Fe, 100; Zn; 100 and Mn, 300 µg/g dry weight (Kirkham, 1975; Alloway, 1995; Fageria et al., 2002; Liphadzi and Kirkham, 2006). The bioavailability of these metals to plants depends on factors such as the biological parameters, physico-chemical properties of the metallic elements and their compounds (Duffus, 2002) as well as a range of various factors, such as soil pH, temperature, redox potential, chemical speciation, seasonal changes, sediment type, salinity and organic matter (Otte et al., 1993). At the elevated levels indicated above, certain plants may be well adapted and can employ several strategies in order to survive (Clijsters et al., 1999; Monni et al., 2001).

The demonstration of a dose-response relationship is an essential criterion for establishing whether the metal is responsible for the effects measured. Plant exposure to metal does not necessarily indicate toxicity; it is only after the concentration is above

threshold level that toxicity is reached. Visible symptoms are detected only after a threshold is reached. Therefore, the use of plant biomarker is very significant in determining the biological effects of pollutants in the environment at different leaves and stages of growth.

Some of the common abnormalities and toxic levels in different plant species are shown in Tables 2 and 3. It is when such metals are present in excessive amounts in the soil that they have the potential to excessively be taken by plants and later become toxic (Monni et al., 2001).

Plants require essential micronutrients (Zn, Cu, Mn, and Fe) to grow and complete their life cycle (Durham and Snow, 2006). Both essential and non-essential, (Al, Pb, Co, Cr, Cd, Ni) elements are assimilated by plant even though not all are beneficial to plants. Under the deficient supply of an essential metal, an organism shows poor growth and low yield, whereas an excess supply leads to toxic effect and can sometimes be lethal (Godbold et al., 1984; Baker, 1987; Prasad, 1999; Monni et al., 2001; Davies et al., 2002, Shanker et al., 2004; Liphadzi and Kirkham, 2006).

Several cases of accumulation of heavy metals such as Zn, Cu, Pb, Cd, Ni and Cr, have been thoroughly studied in several wetland plant species, such as *Eichhornia crassipes*, *Typha latifolia*, *Spartan alterniflora* and *Phragmites australis* (Saltonstall, 2002; Bragato et al., 2006; Jayaweera et al., 2007). The accumulation capacity strongly depends on the plant's physiological properties (Liu et al., 2005), and inherent soil properties such as pH, organic matter content, texture, temperature, nutrient availability, etc (Sharma et al., 2007). Cd is particularly dangerous because plants growing in contaminated soils can absorb and accumulate Cd in edible tissues in large quantities without any visible signs, thereby introducing the metal into the food chain (Monteiro et al., 2008).

It is therefore important to know the amount of metals accumulated in plants at different levels of exposure so as to establish their potential effects in plant growth and development, as well as in phytoremediation programmes.

1.2.4 USE OF BIOMARKERS IN THE DETECTION OF METAL STRESS IN PLANTS

Ernst and Peterson (1994) have defined biomarkers as biochemical, physiological and morphological changes in plants, which measure their exposure to chemicals. The concept in plants may be linked to an "early-warning" signal of pollution induced stress. Metals can be found in the tissues and fluids of plants as a result of exposure to metals in air, soil and water (Bialonska and Dayan, 2000). At high exposure concentrations, such bioaccumulation of metals could be potentially toxic to plants. In general, enzyme induction in the photosynthetic pathway can be considered as an indirect reaction on toxic metal action (Papi et al., 2002; Tarpley et al., 2005). A set of parameters can be used for rapid screening of metals' toxicity relationship with changes in, for example, plant development, which include, chlorophyll content, mineral concentration, plant growth and photosynthetic activities in plant species (Rijstenbil et al., 1994; Ralph and Burchett, 1998; Ralph, 2000; Foyer et al., 2003).

The presence of elevated levels of metals may interfere with chlorophyll content and adversely affect the photosynthetic activity and plant enzymes, which invariably affect plant provision of nutrients for survival (Papi et al., 2002; Tarpley et al., 2005). Such biological responses in stressed plants may be useful in the monitoring of metal pollution in terrestrial and aquatic ecosystems. Such a biological approach makes it possible to determine whether the natural ecosystem is being altered by pollutants without relying on expensive techniques and conducting long term field experiments (Ernst and Peterson, 1994; Lagadic et al., 1997, 1998; Ferrat et al., 2003; Agunbiade and Fawale, 2009). This could then serve as early warning signs of specific or general stress at each biological level (Ernst and Peterson, 1994).

Understanding the concept of biomarkers and physiological responses of plants is thus critical to the long-term safety and conservation of ecosystems. The biomarkers based on the photosynthetic activity and antioxidative enzyme activities can offer fast and reliable indication of toxicity in polluted wetlands.

The content of chlorophyll is an important biomarker of plant exposed to metals. For example, chlorophyll a and chlorophyll b fluorescence were considered to be very sensitive biomarkers when the plant cellular system was exposed to herbicides and

heavy metals (Popovic et al., 2003). Metal toxicity has a direct effect on chlorophyll content, and may lead to visible symptoms of chlorosis and necrosis (Table 2), suggesting that a chlorophyll measurement may provide information on the plant physiological changes that are due to exposure to elevated levels of toxic metal concentration.

Elevated levels of mineral concentration in different plant organs (above and below ground) may give an indication of metal toxicity effects on plants as a biomarker. At higher toxic level, metals accumulate and damage the cellular components which then reflect as measurable parameter in the plant (Van Assche and Clijsters, 1990; Agunbiade and Fawale, 2009). Scientific evidence has shown that plant species will concentrate certain metals in their leaves, stems and roots to varying degrees and, hence, critical levels are normally used to quantify the toxicity level(s) in the plant organs (Breckle and Kahle, 1992; Baker et al., 1994; Dahmani-Muller, 2000; Liphadzi and Kirkham, 2006).

Several different parameters may be measured to characterize growth of a plant. The most accurate measure of biomass accumulation is plant dry weight. Other growth parameters include plant height, internode lengths, number of leaves, leaf areas, stem lengths, and stem diameters at prescribed time intervals during the growth period. Ecotoxicigical studies have shown that plants that are subjected to elevated concentrations of metals in the solution may have had difficulties in translocation of these metals from the roots to the aerial parts, a situation which may facilitate tissue damage (Baker, 1987; Baker et al., 1994; Crozier et al., 2000; Godbold et al., 1984) leading to reduction in biomass. In such instances, plant growth will be severely affected.

Measurement of photosynthesis through specialized equipment such as Fluorometer may be a parameter used in biomarker assessment, as the rate of photosynthesis is intimately linked to growth. Inhibition of photosynthesis by metals has been documented (Clijsters and Van Assche, 1985; Prasad and Strzalka, 2000 and Cronk and Fennessey, 2001; Kaznina et al., 2005; Guoa et al., 2007). Additionally, mechanisms involving enzyme induction in the photosynthetic pathway can be considered as an indirect reaction on toxic metal action (Papi et al., 2002; Tarpley et al., 2005). Plants with metal

levels above the threshold experience a decrease in photosynthesis rate (A), evapotranspiration (E) intercellular carbondioxide concentration (Ci) and stomatal conductance (Qs). Such parameters can be used as photosynthetic biomarkers in comparing plants growing in polluted and non-polluted environment.

1.2.5 CHLOROPHYLL DEGRADATION AS A BIOMARKER OF METAL EXPOSURE IN PLANTS

Chlorophyll is an important component in photosynthesis, which enables plants to convert carbon dioxide and water in the presence of energy from the sun to produce carbohydrates (Hopkin, 1993; Walker et al., 1996). This is used in all plants' essential growth and developmental processes, which gives rise to the plant's distinctive green colour. Any stress that interferes with this metabolic process may produce responses that could be detected by using specialized methods and equipment and such responses may possibly be used as biomarkers of stress (Vangronsveld and Clijsters, 1992). Against this background, it is possible to use chlorophyll concentration in plants as potential biomarker in ecotoxicological studies (Stoltzs and Greger, 2002; Bragato et al., 2006).

The elevated levels of most metals in plants will interfere with chlorophyll content and induce chlorosis (Padmaja et al., 1992; Yan-Hua et al., 2008). The effective method to estimate chlorophyll is through the extraction of the photosynthetic pigments in dimethyl sulfoxide (DMSO) and quantifies the ratio of optical density of chlorophyll a and b at wavelengths of 435 nm and 415 nm. This is a reliable method for an estimation of chlorophyll degradation due to pollution (Ronen and Galun, 1984). The advantages of DMSO as a solvent for the extraction of photosynthetic chlorophyll pigments are that the extraction is simple, rapid, and complete, and that the extract is easily stored at low temperatures without degradation under normal laboratory conditions (Hiscox and Istraelstam, 1979; Filbin and Hough, 1984). Another method used to estimate chlorophyll content in plants includes the chlorophyll fluorescence technique using the fluorometer. The fluorometer measures the light energy absorbed by chlorophyll molecules in a leaf, which is re-emitted as light-chlorophyll fluorescence (Sánchez-Rodríguez et al., 1999., Hura et al., 2007; Monteiro et al., 2008; Reyes-Diaz, et al., 2009; Mielke and Schaffer,

2010). The advantages of this method are that it is simple, non - destructive, non-invasive, rapid and sensitive (Maxwell and Johnson, 2000; Moradi and Ismail, 2007). Plants exposed to higher concentration of heavy metals such as Al, Pb, Zn, and Ni, showed decreasing chlorophyll contents as indicated by the decrease in light fluorescence (Moustakas et al., 1993; Banaszak et al., 2000; Macfarlane and Burchett, 2001). Decreases in pigments due to damage by elevated concentrations of metals suggest that chlorophyll fluorescence measurement can be used as biomarker to monitor chlorophyll contents alterations in polluted ecosystems.

Several cases of decreased chlorophyll content owing to metal toxicity have been reported in the plant kingdom growing in wetland ecosystems (Shalygo et al., 1999; Abdurakmanova et al., 2000; Schoefs, 2001; Cosio et al., 2004; Valavanidis et al., 2005). For example, decreased levels of chlorophyll were observed in *Paspalum distichum*, a wetland plant (Bhattacharya et al., 2009), due to application of contaminated sludge rich in Cr and Mn. In other studies involving rice (*Oryza sativa*), significant reduction in chlorophyll concentrations was recorded at elevated levels of Cd and Cu (Das et al., 1997; Ashan et al., 2007; Shao et al., 2007). A study by Stobart et al. (1985) on the effects of Cd on chlorophyll content in the leaves of barley revealed that this metal inhibited the biosynthesis of chlorophyll, total chlorophyll content and the chlorophyll a/b ratio. Therefore, this parameter offers an opportunity of detection of toxic stress in plants.

1.2.6 ALTERATIONS IN PHOTOSYNTHETIC ACTIVITY AS BIOMARKERS OF EXCESSIVE METAL EXPOSURE IN PLANTS

Photosynthesis is the vital metabolic energy-generation and storing process that takes place in chloroplasts in the presence of sunlight. Metals can affect plant physiological processes such as photosynthesis, which is an important process in plant growth and development (Clijsters and Van Assche 1985; Greppin and Strasser, 1991; Hopkin, 1993; Walker and Hopkin, 2006; Urban et al., 2007). Some visible photosynthesis symptoms associated with metal toxicity are shown in Table 1.2.

The photosynthesis process in plants exposed to metals is affected through several mechanisms. Photosynthesis is inhibited at several levels, such as: carbon dioxide

fixation, stomatal conductance, chlorophyll synthesis, electron transport and enzymes of the calvin cycle (Prasad and Strzalka, 2000; Monnet et al., 2001; Shanker et al., 2004). Singh et al. (2010) reported chlorosis and fragmentation of leaves with mucilaginous discharge in *Najas indica* plants exposed to excessive Pb. In other studies, excessive copper affected the oxidative enzymes in wheat, oat and beans leaves, thus affecting photosynthesis (Shainberg et al., 2001).

The presence of excessive concentrations of metals may interfere with the chlorophyll formation process, thus causing adverse effects on the photosynthetic activity, which therefore impairs plant growth (Padmaja et al., 1992; Jonak et al., 2004). Duckweed (*Lemna minor*) exhibited chlorosis and reduced growth at higher levels of metal concentrations (Fe, Cu, Cd, Hg, Pb, Ni, Zn and Mn) (Zayed et al., 1998). Similarly, Ibemesim (2010) reported chlorophyll reduction in *Paspalum conjugatum* (Sour Grass) indirectly through metal effects owing to crude oil exposure on the soil. Sun and Wu (1998) reported chlorosis of the leaves of water spinach supplied with higher levels of Ni owing to reduced photosynthesis and a decrease in chlorophyll concentration. Bibi et al. (2010) also reported negative effects of heavy metals (Cd, Cr, and Zn) on the freshwater macrophytic *Nitella graciliformis* J. by decreasing the chlorophyll content and manifesting poor plant growth.

In higher plants, the photosynthetic rates have been reportedly affected at higher levels of metal (Fe, Mn, Cu, Al, Cd, Zn, and Pb) concentrations (Belkhodja et al., 1998; Koukal et al., 2003; Sharma and Dubey, 2005; Zhang et al. 2007, Ali et al. 2008). In all plants, metals are known to perform specific functions within the tissue systems and each may manifest different visual symptoms at elevated concentration. The elevated concentrations of metals may affect the photosynthetic apparatus by acting and interfering with the carbon dioxide fixation at several levels (Prasad and Strzałka,1999) resulting in negative effects on the gas exchange site in plant tissue membranes (Baryla et al., 2001). This could ultimately impact the synthesis of starch due to a low translocation rate of photosynthesis in plants growing in polluted environments (Ericson, 1979).

Other studies have shown elevated starch levels in plants grown in Pb, Cd, Zn, and Cu-polluted areas compared to those found in clean sites (Breckle and Kahle, 1991; Smith

et al., 1996; Prasad and Strzałka, 1999). One possible mechanism proposed is the interference by these metals in the breakdown of starch to sucrose (Prasad and Strzałka, 1999). Under such circumstances, the net result of impaired photosynthesis is always reduced carbon fixation, which further reduces the plant growth at the whole-plant level. In other studies, which involved a combination of toxic concentrations of metals such as mercury, lead, cadmium and copper, using aquatic plants (*Potamogeton pectinatus* L., *Valisneria spiralis* L., and *Hydrilla verticilata*), it was shown that all combinations of the metal pollutants caused senescence in all three species by decreasing chlorophyll, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein and dry weight owing to decreased photosynthesis (Jana and Choudhuri, 1982; Juneau et al., 2002).

Studies conducted by Shanker et al. (2004) on the toxicity of Cr to wheat, peas, rice, maize, beans and sunflower plants revealed that the plants' physiological processes such as photosynthesis, growth and development were affected. The affected plants lacked a specific transport system for Cr and hence reduced their photosynthesis activities (Shanker et al., 2004). In this study, Cr toxicity in plants was observed at multiple levels, from reduced yield, through effects on leaf and root growth, to inhibition on enzymatic activities and mutagenesis. Nagel et al. (1996) studied algae *(Chlamydomonas reinhardtii)* and revealed that higher levels of Cr affected photosynthesis through decomposition of the absorption spectra in green pigments. Conclusively, Cr affects photosynthesis in terms of CO_2 fixation, electron transport, photo-phosphorylation and enzyme activities (Davies et al., 2002, Shanker et al., 2004).

In a study undertaken to investigate the effect of Cd on growth and photosynthesis of two tomato cultivars at the seedling stage, Dong et al. (2005) listed leaf necrosis, chlorosis, and reddish brown discoloration of the leaf blade as some of the symptoms observed when tomato plants were exposed to a high concentration of Cd. Moya et al. (1993), Prasad et al. (2000) and Hattab et al. (2009) also reported that photosynthesis, transpiration, carbohydrate metabolism and other metabolic activities were inhibited by elevated levels of Cd and Pb in tomato plants.

Nickel is an essential micronutrient for plants (Eskew et al., 1983), with its toxicity negatively affecting photosynthesis, mineral nutrition, sugar transport and water relation

(Pandey and Sharma 2002; Parida et al., 2003; Nakazawa et al., 2004). The influence of Ni on photosynthesis is that it damages the photosynthetic apparatus on almost every level of its organization, from reduction of leaf chlorophyll concentration and damage of chloroplasts (Madhava Rao and Sresty, 2000; Molas, 2002; Vinterhalter and Vinterhalter, 2005). Similarly, higher levels of Cobalt in plant (Co) alter the structure and number of chloroplasts per unit area of leaf leading to inhibition of the PS2 activity and Hill reaction, which invariably lower the rate of photosynthesis (Plekhanov and Chemeris, 2003). Therefore, photosynthesis is an important physiological plant parameter, and may be used as a biomarker to assess the toxicity of metals in different ecosystems.

1.2.7 CONCLUSION

In conclusion, this chapter strongly recommends the use of simple biomarkers in assessing aquatic plants exposed to heavy metals. The qualitative and quantitative determination of pollution in wetland ecosystems is dependent on the metal loads present in the system. Their deleterious effects could easily be monitored by scientists and ecotoxicologists using different plant species as biomonitors.

1.3 BACKGROUND TO THE RESEARCH PROBLEM

Several researchers found that various wetland plants accumulate metals (Cosio et al., 2004; Valavanidis et al., 2005; Bhattacharya et al., 2009; Shuping et al., 2010). The lower Diep River, Milnerton was previously found to be significantly contaminated with metals (Shuping et al., 2010). There is however a research gap on the toxicity of metals to the plant species which grow in this area. If bioaccumulation of metals in plants is above certain critical levels, metals may have significant effects on the physiological processes such as those that regulate photosynthesis and chlorophyll content (Kukkola et al., 2000; Myśliwa-Kurdziel and Strzatka, 2002).

1.4 STATEMENT OF THE RESEARCH PROBLEM

The Western Cape is the fastest growing region in South Africa and is characterized by an increasing population and economic sector (CCT, 2006). Cape Town, in particular, and its suburbs, has a history of environmental pollution owing to higher industrial and automobile activities. Such activities may be associated with high levels of metals that are present in soils, waters and plants, which become contaminated. The concentration of metals such as lead (Pb), cadmium (Cd), zinc (Zn), manganese (Mn), iron, (Fe) and copper (Cu), nickel (Ni), chromium (Cr) and mercury (Hg) in water, surface soil, aquatic plants and animals, as well as land plants, should be ascertained and quantified. At present, there exists little information on the profile of these pollutants in the ecosystems of selected areas in Cape Town. Metal pollution, above certain critical levels, may have significant effects on physiological parameters in plants and other organisms that occur in the ecosystem. In order to address this problem, this research assessed the extent of metal effects in the wetland plants in the Diep River, Cape Town.

1.5 AIM OF THE RESEARCH

The aim of this research was to elucidate the effects of exposure to metals on a wetland plant, *Phragmites australis,* through the measurement of physiological parameters such as chlorophyll content and photosynthesis. An understanding of how the physiological processes such as chlorophyll production and photosynthesis would be affected, as a result of exposure to metals, would be a step towards establishing biomarkers, for use in biomonitoring and environmental health assessment.

1.6 SPECIFIC OBJECTIVES OF THE STUDY

- To evaluate chlorophyll content, photosynthesis and metal concentrations in *P. australis* plants and to validate if there is a significant movement of pollutants from the river banks and to the adjacent soil.

- To determine the concentrations of metals (Al, Cd, Cr, Pb Co, Ni, Zn, Fe, and Mn) in a wetland plant species, *Phragmites australis* that grows along the banks of the contaminated lower Diep River.

- To determine the impact of the metals on physiological indices (photosynthesis and chlorophyll content) of this plant species.

1.7 SIGNIFICANCE OF THE RESEARCH

Shuping (2008) did a survey on metal contamination in the lower Diep River, Milnerton and found the river to be contaminated with some metals. This river has been subjected to deterioration in water quality over decades due to bad farming practices and other land uses (Brown and Magoba, 2009). Land use in upper reaches of the river is predominantly agricultural, while in the lower parts it is largely residential (formal and informal settlements) as well as industrial. The common reed, *Phragmites australis* has been found to be one of the species that inhabit this wetland (Shuping, 2008).

Phragmites australis is described as being tolerant of metals and organic compounds found in contaminated environments and it is favoured as a bioindicator for toxicity in both the aquatic and terrestrial ecosystems (Gorsuch et al., 1991; Yang et al., 2008). The common reed can provide a large quantity of biomass that can remove considerable amounts of metals that accumulated in its leaves. While, the roots that has high accumulation of metals can be collected and disposed of (Bonanno and Lo Giudice, 2010).

Table 1: Elevated toxic concentration in sediment and soils exposed to heavy metals

Metal	Normal Concentration	Status	Reference
Cadmium	0.6 mg/kg 0.14 – 2.5 mg/kg 0.07–1.10mg/kg 0.02 – 2 mg/kg	Sediments Sediments Soil Soil	Canadian Council of Ministers of the Environment (1999a) Forstner and Wittmann, 1979 Kabata-Pendias and Pendias, 2001 Alloway, 1995
Nickel	34- 55 mg/kg 1 – 20 mg/kg 43 – 126 mg/kg 60 mg/kg 1 mg/kg	sediments Soil Soil Soil Soil	Forstner and Wittmann, 1979 Kabata-Pendias and Pendias, 2001 Chen et al., 1994 Chen et al., 1992c Temmerman et al., 1984
Lead	53.3 mg/kg 10 – 70 mg/kg 50 mg/kg	Sediment Soil Soil	Canadian Council of Ministers of the Environment (1999c) Kabata-Pendias and Pendias, 2001 Temmerman et al., 1984
Cobalt	0.1 – 74 mg/kg 5 – 15 kg/kg	Sediment Soil	Forstner and Wittmann, 1979 Temmerman et al., 1984
Chromium	37.3 mg/kg 5 -120 mg/kg	Sediment Soil	Canadian Council of Ministers of the Environment (1999b) Kabata-Pendias and Pendias, 2001
Aluminium	4.1 - 5.5 mg/kg	Sediment Soil	Peverill et al., 1999
Manganese	390–6700 mg/kg 500– 800 mg/kg	Sediment Soil	Forstner and Wittmann, 1979 Temmerman et al., 1984

Table 1: Elevated toxic concentration in sediment and soils exposed to heavy metals

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Manganese	390–6700 mg/kg 500– 800 mg/kg	Sediment Soil	Forstner and Wittmann, 1979 Temmerman et al., 1984

Table 2: Visible symptoms associated with metals stress in plants (after Bonnet et al, 2000; Kukkola et al, 2000)

Metal	Toxic Symptoms
Cadmium	Chlorosis, necrosis, purple colouration
Lead	Dark green leaves
Nickel	Decrease in leaf area, chlorosis, necrosis, stunting (Nakazawa et al., 2004)
Chromium	Alterations in the germination process, stunted growth, reduced yield, mutagenesis (Shanker et al., 2004)
Cobalt	leaf fall, inhibition of greening, discolored veins, premature leaf closure, and reduced shoot weight
Aluminum	Stunting, yellowing and death of the leaf tips, inhibition of root elongation
Zinc	Stunting, reduction of leaves elongation
Copper	Chlorosis, yellow colouration, inhibition of of root growth, less branched roots
Iron	Dark green foliage. Thickening of roots, brown spots on leaves.

Table 3: Elevated toxic concentration of heavy metals in different plant species

Name of the plants	Plant Part	Metal	Toxic Concentration Mg.kg-1	Reference
Spinach	Shoot	Cr Cd Pb Ni Zn	1.08 - 5.40 0.01- 3.42 0.05 - 1.93 0.01- 0.05 1.95 -2.73	Sharma et al., 2005 Qishlaqi et al., 2007
Wheat	Shoot	Cr Cd Pb Ni Zn	0.93 0.01 - 0.43 0.09 - 25.45 0.53 - 20.33 35.53 - 41.96	Qishlaqi et al., 2007
Lettuce	Shoot	Cr Cd Pb Ni Zn	0.17 0.03 - 2.01 0.03 - 0.65 0.01 - 0.03 2.17- 3.75	Qishlaqi et al., 2007
Celery	Shoot	Cr Cd Pb Ni Zn	0.01 - 0.05 0.01 - 2.19 0.05 -0.09 0.01 - 0.21 1.11- 2.97	Qishlaqi et al., 2007
Brown Rice	Shoot	Cr Cd Pb Ni Zn Co	0.16 0.07 0.43 0.54 39.2 2.48	Lin, 1991

CHAPTER TWO

METAL CONTAMINATION OF SOILS COLLECTED FROM FOUR DIFFERENT SITES ALONG THE LOWER DIEP RIVER, CAPE TOWN, SOUTH AFRICA

2.1 INTRODUCTION

The increasing consumption and exploitation of the earth's raw materials (fossil fuel and minerals) coupled with the exponential population growth over the past years have resulted in environmental degradation and build up of waste products of which metals is of great concern (Kabata Pendias and Pendias, 1992, Diagomanolin et al., 2004). Rapid industrialization and urbanization in developing countries like South Africa, has resulted in large-scale pollution of the environment, resulting in the enrichment of metals in the soil (Tong and Che Lam, 2000; Sanderson et al., 2002; Ren et al., 2006).

Soil is unconsolidated minerals and organic material found on the immediate surface of the earth that serves as a natural medium for plants growth (Brady and Weil, 2008). It is also a key component of natural ecosystems. Environmental sustainability depends largely on a sustainable soil ecosystem, because when the soil is polluted the ecosystem is altered and agricultural activities affected (Adriano et al., 1998; Heaney et al., 1999; Schiff et al., 2002; Hankard et al., 2004). In spite of the importance of soil to support different kinds of life, it has become a dumping place for several industrial and municipal wastes, most of which contain metals (Phillips, 1981; 1999; Yusuf et al., 2003). Metals are among the major contaminants found in both contaminated lands and natural soils. Their presence in excess quantities in the ecosystems threatens the life in both terrestrial and aquatic environments (Fatoki and Awofolu, 2003; Rengel, 2004; Zhang et al., 2007).

Roadside soils have been shown to have considerable contamination owing to deposits of vehicle-derived metals (Adeniyi, 1996; Sutherland and Tack, 2000; Swaileh et al., 2004; Okunola et al., 2007). Crude oil and their products which are deposited on road surfaces or nearby soils, or in water bodies have been shown to have elevated levels of metals, consequently becoming toxic to plants and micro-organisms that are present in those areas (Odjegba and Sadiq, 2002; Merkl et al., 2005; Osuji et al., 2005; Nabulu et al., 2006).

Previous studies carried out by Reinecke et al. (1997; 2000) have shown that variety of metals accumulate in South Africa's urban soils. Studies conducted in Taiwan have shown that more than 800 ha of land were contaminated with industrial effluent

containing metals such Cd, Cr, Cu, Pb and Zn, which were then reflected in brown rice (Chen, 2000).

Many sites around the world have been found to be polluted with metals, such as Cd, Ni, Pb, As and Zn (Reeves and Baker, 2000; Kannavou et al., 2001; Jin et al., 2002; Xendis et al., 2003; An, 2004; Cui et al., 2004). Such developments in different parts of the globe have made environmentalists and ecotoxicologists to be increasingly interested in toxicity and environmental degradation.

According to Shuping (2008) the lower Diep River has been subject to deterioration in water quality over decades due to bad farming practices and other land uses. Land use in the upper catchment is predominantly agriculture, while in the lower catchment it is largely residential (formal and informal settlements) and industrial. Jackson et al. (2009) found the lower Diep River to be polluted with a variety of metals. The concern is that industrial and household effluents could be discharging appreciable quantities of metals into the Diep River which may be detrimental to wetland plants, microorganisms, human health and ecosystem health in general.

The objective of this study was to determine the extent of metal contamination in the river banks and adjacent soil along a section of the lower Diep River.

2.2 MATERIALS AND METHODS

2.2.1 THE STUDY AREA

The study was conducted along the banks of the lower Diep River, in the following sites: 1) Milnerton lagoon: latitude 33° 52.499'S, and longitude 18° 29.548; 2) Lower estuary: latitude 33° 52.329'S, and longitude 18° 29.714'E; 3) Milnerton bowling club: latitude 33° 52.183'S, and longitude 18° 29.750'E, and 4) Woodbridge Island: latitude 33° 53.486'S, and longitude 18° 29.127'E (Fig. 1). The Diep River originates from the Kasteel Mountain, Malmesbury and flows in a south westerly direction towards Table Bay, where it flows into the Atlantic Ocean (Brown and Magoba, 2009).

2.2.2 SITE SELECTION

The sampling sites were selected based on their location near residential and industrial areas, which may emit a cocktail of metal pollutants to some distances in the environment. This include: Milnerton lagoon (site 1), Lower estuary (site 2), Milnerton bowling club (site 3) and the lower side of the Woodbridge Island (site 4) (Fig.1).

2.2.3 COLLECTION OF SOIL SAMPLES

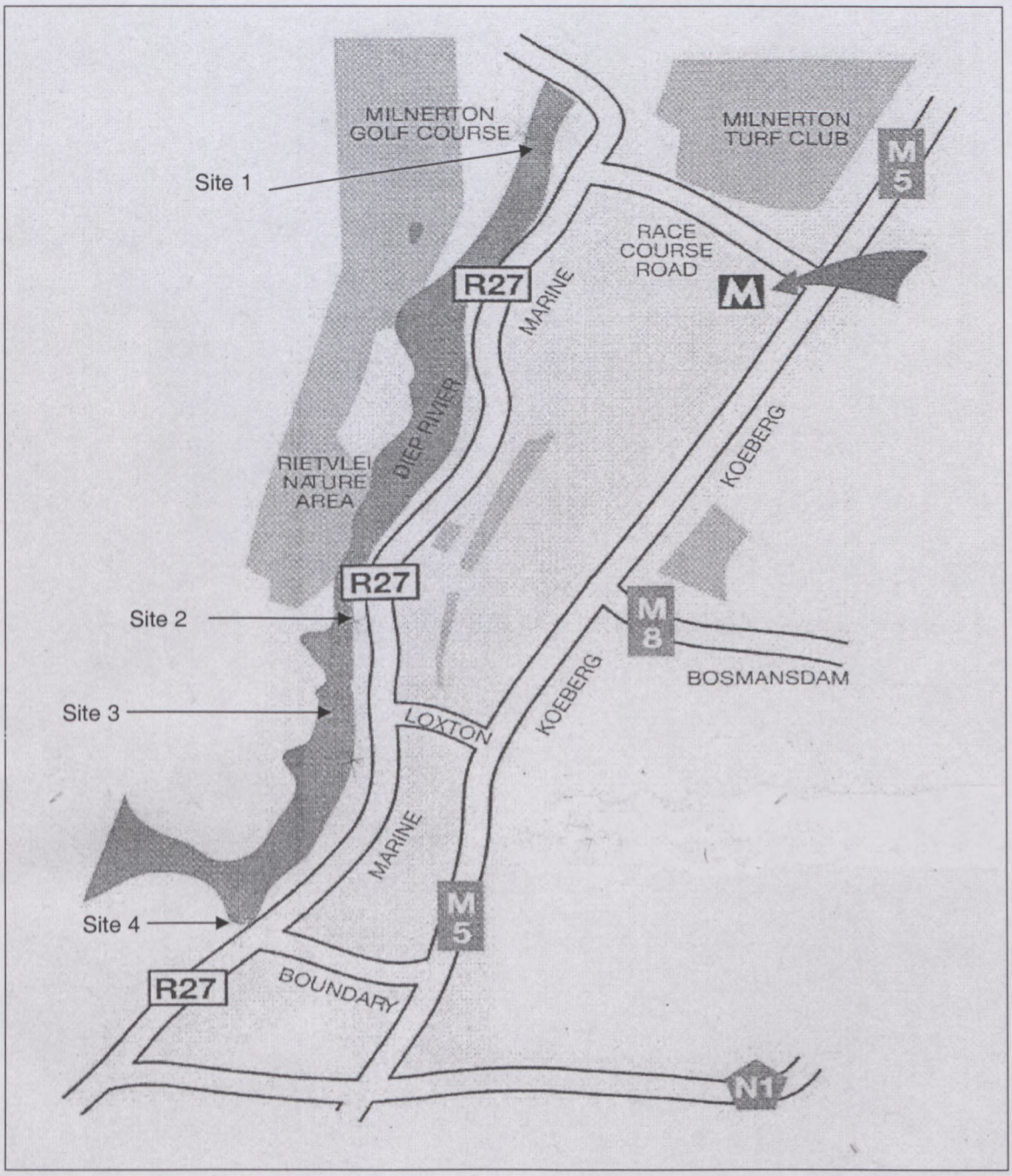
For each site, four soil samples were taken from the river bank and from adjacent soil in the same vicinity (2 m apart). The soil surface was first cleared of debris using a gloved hand. Four entire plants of *Phragmites australis* including roots were dug up. The soil in close association with the roots was carefully removed and taken for analysis. The collected soil samples were put into polythene bags, sealed, appropriately labeled and transferred to the laboratory.

2.2.4 PREPARATION OF SOIL SAMPLES

Soil samples were transferred to Petri dishes and dried at 60°C for 48 h. The samples were removed from the oven and allowed to cool to room temperature then ground with a mortar and pestle. Each sample was sieved using a nylon sieve (2 mm pore size) and then stored in plastic containers.

2.2.5 DIGESTION OF SOIL SAMPLES

This was carried out as described by Odendaal and Reinecke (1999). For the digestion process 10 ml of 55% HNO_3 was added to each soil sample (1 g) in test tubes and stirred properly using a glass rod. Each of the mixture was then heated on a dry block heater in a fume cupboard at 40°C for 1 h, then at 120 °C for 3 h. The samples were then removed and allowed to cool to room temperature. Each of the cooled solution was made up to 20 ml with distilled water and filtered using cellulose nitrate filter paper (0.45 µm). Each of the filtrate obtained were further diluted to a final volume of 100 ml each with distilled water, and appropriately labeled for analysis of metals.



Map 2.1: Map of the lower Diep River showing the study sites

2.2.6 ANALYSIS OF SAMPLES FOR METAL CONTAMINATION

The digested soil samples were analyzed for the presence of metals using the Inductively Coupled Plasma-Mass Spectrophotometer (ICP - MS, Agilent 7500ce. England).

To obtain the soil metal concentrations, the ICP values were converted using the formula:

$$SMC = \left(\frac{ICP_{Reading} - C_{Reading}}{WSS} \right) * DF$$

Where: SMC = Soil metal concentration (mg kg^{-1}); ICP = Inductively Coupled Plasma values; C = Blank; DF = Dilution factor; WSS = Weight of soil sample (g). The soil metal concentration data were statistically analyzed using the STATISTICA software package 2009 (StatSoft Inc., Tulsa, OK, USA).

2.3 RESULTS

Distribution of metals in soil from the different sites

Aluminum (Al)

Table 4 shows the results of metal concentrations from both the river bank and adjacent soil from all the four sampling sites. The concentrations of Al in river banks ranged between 1214.1 - 3176 $mg.kg^{-1}$. In adjacent soils Al concentration ranged from 434.8 - 2445.4 $mg.kg^{-1}$. Generally, the river bank, samples had significantly highest concentrations of Al compared with adjacent sites (Table 4).

Chromium (Cr)

In this study, Cr concentrations were significantly higher in samples collected from the river bank compared with those from the adjacent sites (Table 4). The concentrations of Cr

in river banks ranged between 0.4 - 2.1 $mg.kg^{-1}$. In adjacent soils Cr concentration ranged from 0.3 - 1.7 $mg.kg^{-1}$. The highest Cr concentration was found at site 3 (Table 4).

Cobalt (Co)

Table 4 shows significant differences in Co concentration when comparing river bank and adjacent soil samples in sites 2, 3 and 4. Cobalt levels from adjacent soils ranged from 0.2 - 1.9 $mg.kg^{-1}$. The highest (1.9 $mg.kg^{-1}$) Co values from adjacent soil were found in site 4. The concentrations of Co in river banks ranged between 0.8 - 2.7 $mg.kg^{-1}$, with site 4 having the highest value.

Nickel (Ni)

For the river bank samples, Ni concentrations ranged between 0.02 - 2.6 $mg.kg^{-1}$, and for the adjacent soil, it ranged from 0.02 - 0.1 $mg.kg^{-1}$. There was a slight variation in the concentration of Ni in all the sites, except in site 1. It was found that the highest concentration in the River bank soil was at site 3 (2.6 $mg.kg^{-1}$). There were statistically significant differences between sites 2, 3 and 4, while site 1 had no statistically significant difference. Ni had the lowest concentration of 0.02 mg kg^{-1} for the adjacent soil and 0.03 $mg.kg^{-1}$ for river bank soil samples (Table 4).

Cadmium (Cd)

Generally, the river bank soil samples in all sites had significantly highest concentrations of Cd in river bank soils compared with adjacent sites (Table 4). Cd concentrations in the river banks ranged from 0.3 - 9.3 $mg.kg^{-1}$. The highest concentration (9.3 $mg.kg^{-1}$) of Cd in the river bank soil was recorded at site 2 and the lowest concentration (0.3 $mg.kg^{-1}$) was found at site 3. No Cd was detected in the adjacent soil samples at site 4.

Lead (Pb)

Table 4 show that significant differences in Pb concentration were found only in sites 1, 3 and 4 when comparing river bank and adjacent soil samples. Pb had the highest

concentrations of 71.7 mg.kg^{-1} in the river bank at site 3, followed by 49.6 mg.kg^{-1} at site 4. Similarly, the highest concentration in the adjacent soil was also recorded at site 3 (49.6 mg.kg^{-1}). This implies that site 3 had the highest Pb metal load.

Manganese (Mn)

The comparison of Mn between the adjacent soil and the river bank soil samples was significantly different at each site. Mn in the river bank soil samples ranged from 6.7 - 48.05 mg.kg^{-1}, whereas for the adjacent soil sites it ranged from 5.4 mg.kg^{-1} (site 1) to 27.02 mg.kg^{-1} (site 4) (Table 5).

Iron (Fe)

Table 5 Indicated that Fe had the highest concentration in all the 4 sites investigated. The river bank soil samples had significantly highest concentrations of Fe compared with adjacent sites. For instance, in site 1, the concentration of Fe was 402.2 mg.kg^{-1} (adjacent soil) and 1136.4 mg.kg^{-1} (river bank).

Copper (Cu)

Table 5 showed that across the 4 sites, the river bank soil samples had significantly highest concentrations of Cu compared with adjacent sites. Site 4 of the river bank had the highest concentration of Cu (45.9 mg.kg^{-1}), followed by site 3 (34.7 mg.kg^{-1}) and site 1 (5.6 mg.kg^{-1}), while the lowest value was at site 2 (2.56 mg.kg^{-1}). With the adjacent soil, the concentrations were recorded in a decreasing order of 12.56 mg.kg^{-1} (site 3), 5.40 mg.kg^{-1} (site 4), 0.5 mg.kg^{-1} (site 1), and 0.3 mg.kg^{-1} (site 2) respectively.

Zinc (Zn)

The concentrations of Zn are given in Table 5. Overall, the river bank soil samples in sites 2, 3 and 4 had significantly highest concentrations of Zn compared with adjacent sites. Highest Zn value in river bank (211.5mg.kg^{-1}) and adjacent soil (53.9 mg.kg^{-1}) were found at site 4; followed by site 3; river bank (164.5 mg.kg^{-1}), adjacent soil (52.3 mg.kg^{-1}) and site 2 river bank (11.6 mg.kg^{-1}) and adjacent soil (2.6 mg.kg^{-1}) respectively.

2.4 DISCUSSION

Aluminium and Fe had the highest concentration followed by Zn, Mn, Pb, and Cu while Cr and Ni had the lowest concentrations. The concentrations of Al from all sites in the river bank and adjacent soils were above the level recommended by Peverill et al. (1999). The recommended and acceptable concentration for Al is 4.1 - 5.5 $mg.kg^{-1}$ for soil (Peverill et al., 1999). In this study, the concentrations obtained were 434.8, 527.2, 2445.4 and 2279.2 $mg.kg^{-1}$ (adjacent soil) and 1214.1, 1532.8, 2988.6 and 3176.0 $mg.kg^{-1}$ (river bank) for sites 1, 2, 3 and 4 respectively. These concentrations all exceeded the established guidelines recommended by Peverill et al. (1999). Such high levels are undesirable as they may cause poor root growth and proliferation in the soil and hinder other forms of life (Sammut et al., 1995; Vuori, 1996). Research evidence elsewhere suggests that toxic levels of Al in the soil resulted into shallow rooting due to Al toxicity (Kochian, 1995; Foy et al., 1999; Kinraide, 2003), a condition which could also be present in the study area. Other forms of life may also be impeded by high Al concentrations in the ecosystem. Similar to our results, Jackson et al. (2009) also reported higher levels of Al in selected areas along the Diep River.

The concentrations of Cr in the river bank soil ranged from 0.4 - 2.1 $mg.kg^{-1}$ whereas in the adjacent soil, it ranged from 0.3 - 1.7 $mg.kg^{-1}$. These values are far below the recommended value of 64 $mg.kg^{-1}$ in Canada (CCME, 1999), implying that they were within the accepted limits for the growth and development of different organisms such as plants and micro-organisms (Kimbrough et al., 1999; Segura-Muñoz et al., 2004). This justifies that the industrial discharges close to study sites into the ecosystem does not contain significant concentration of Cr and the reported values could sustain life of different organisms in the study area.

With regard to Co, research reports suggest that cobalt is not considered a harmful metal in soil (Shuhaimi-Othman, 2008). In this study, Co levels ranged from 0.2 - 2.7 $mg.kg^{-1}$. These levels are below the proposed toxic levels of between 4.1 and 14.0 $mg.kg^{-1}$ in different ecosystems (Choueri et al., 2009). Therefore, Co levels in the study sites cannot be linked to causing adverse biological effects to different organisms growing in these habitants.

The comparison of Ni concentrations from all the 4 sites provided evidence that this metal was not a threat in the environment. Nickel concentration ranged from 0.02 - 2.6 $mg.kg^{-1}$, values far below the Canadian soil quality guideline of 50 $mg.kg^{-1}$ (CCME, 1999).

Considering the established guideline, toxic levels were observed for Cd at site 1 (3.5 $mg.kg^{-1}$ in the adjacent site and 7.1$mg.kg^{-1}$ in the river bank) and site 2 (6.19 $mg.kg^{-1}$ in the adjacent site and 9.3 $mg.kg^{-1}$ in the river bank). Results for Cd in site 3 (0.11 $mg.kg^{-1}$ in the adjacent site and 0.3 $mg.kg^{-1}$ in the river bank) however, were lower than 1.4 $mg.kg^{-1}$ as recommended in the Canadian soil quality guidelines (CCME 1999), while at site 4, no Cd was detected in the adjacent site, whereas in the river bank high value (7.2 $mg.kg^{-1}$) was recorded. Considering the established guidelines, it can be suggested that Cd could jeopardize the life and health of certain plants and other forms of life at sites 1, 2 and 4.

The concentrations of Pb ranged from 0.97 - 49.6 $mg.kg^{-1}$ in adjacent soil and from 1.5 - 71.7 $mg.kg^{-1}$ in the river bank soil. The proposed interim soil quality guideline for Pb in Canada is 70 $mg.kg^{-1}$ for agricultural soil (CCME 1999). Out of the 4 sites, elevated levels were observed in site 3 (river bank and adjacent soil) and 4 (river bank soils) suggesting a probable Pb contamination which could be associated with adverse biological effects on organisms living in the ecosystem and possible negative effect on the ecosystem health.

Studies on the concentrations of Mn, Fe, and Zn showed that these metals exceeded the recommended concentrations in the soil (Viets and Lindsay, 1973; Lindsay and Norvell, 1978; Silanpää, 1982; Temmerman et al., 1984; Lindsay and Cox, 1985). Concentrations of Mn ranged between 6.71 and 48.05 $mg.kg^{-1}$ for the river bank samples, and 5.3 - 27.0 $mg.kg^{-1}$ for the adjacent soil samples in all the sites. Values recorded from both sites exceeded the recommended standard of 2.0 - 5.0 mg. kg^{-1} (Silanpää, 1982).

The high concentration of Fe in the soil (402.2 - 2459.8 $mg.kg^{-1}$) for adjacent soil and (1136.4 - 4859.1 $mg.kg^{-1}$) for the river bank samples gives a clear indication that the sites are heavily polluted with this metal and may cause hazardous effects to plants and

other organisms. The recommended concentration of Fe in the soil for the cultivation of plants range from 0.30 - 10 $mg.kg^{-1}$ (Lindsay and Cox, 1985).

Zinc, an essential micronutrient of many enzymes systems, such as respiration enzymes activators, and the biosynthesis of plant growth hormones, is required in concentration not exceeding 200 $mg.kg^{-1}$ for agricultural soil according to Kabata-Pendias (2001) and the Canadian soil quality guideline (CCME, 1999). The concentration beyond this may becomes toxic to plants and other organisms. However, results from this study revealed that concentration of Zn in the soil samples ranged between 2.4 - 53.9 for the adjacent soil and 2.9 - 211.5 $mg.kg^{-1}$ for river bank soil samples (Table 2). The results of this study suggest that the contamination would likely be in site 4 as their values are higher than what can be tolerated by most plants.

Cu levels in the adjacent soils ranged from 0.3 - 12.5 $mg.kg^{-1}$ and in the river bank from 2.56 - 49.9 $mg.kg^{-1}$. These results showed that all 4 sites had values which are less than the Canadian soil quality guideline of 63 mg. kg^{-1} for agricultural soil (CCME, 1999). Hence Cu is not a threat in this ecosystem.

Results of this study revealed that most sites investigated were generally contaminated with the metals studied. This was due to the fact that the study sites were located in an area with industrial manufacturing activities as well as residential areas. Some of the effluents from these industries are emitted directly into the river. Furthermore, other types of runoff from the streets drain in the river. It is therefore logical to assume that contaminants in these effluents find its way into the river and get deposited into the river bank soil. The study sites were also close to a major road characterized by heavy traffic, which could also be contaminated directly from pollutants from the vehicles.

2.5 CONCLUSION

Results of this study showed some degree of high metal contamination in soil collected from river bank compared with lowest concentration in the adjacent soil in all sites investigated. The concentrations of some of these metals exceeded the established guidelines in the soil. Site 3 and 4 were more polluted than site 1 and 2 respectively. The high level of industrial activities and the close proximity of the study area to a highway

may be largely responsible for the high level of pollution observed. This data may act as an important guideline for future studies in this area. An integrated approach between both the industrial and environmental scientist for the control of pollution in this area is required (Ayeni et al., 2010). Future studies should focus on the impact of these metals on different organisms found in this ecosystem as some may find their way into food chain and cause harmful effects to plants, animals and ecosystems at large. Recommended for further studies are quality standard guidelines for sediment in this wetland.

Table 4: Concentrations of metals in soil samples from different sites along the Diep River, Cape Town, South Africa.

Site	Site Status	Concentrations of metals (mg. kg^{-1})					
		Al	Cr	Co	Ni	Cd	Pb
1	Adjacent soil	434.8±19.1b	0.3±0.01b	0.7±0.07a	0.02±0.002a	3.5±0.10b	0.97±0.14b
	River bank	1214.1±31.2a	0.4±0.01a	0.8±0.09a	0.02±0.003a	7.12±0.25a	2.6±0.3a
	F Statistics	455***	81.55***	0.03NS	0.67NS	192.12***	27.2***
2	Adjacent soil	527.2±21.9b	0.3±0.01b	0.2±0.13b	0.02±0.002b	6.19±0.17b	1.5±0.11a
	River bank	1532.8±60.9a	0.5±0.02a	0.9±0.10a	0.03±0.001a	9.3±0.47a	1.5±0.2a
	F Statistics	241.55***	73.58***	21.61***	6.08***	40.19***	0.02NS
3	Adjacent soil	2445.4±182.9b	1.7±1.53b	1.4±0.22b	0.05±0.05b	0.11±0.01b	49.6±3.2b
	River bank	2988.6±513.6a	2.1±0.74a	1.9±0.07a	2.6±0.7a	0.3±0.05a	71.7±19.2a
	F Statistics	2.69*	323.85***	179.54**	1743.52***	333949.5*	1.06*
4	Adjacent soil	2279.2±179.5b	0.3±181.4b	1.9±0.5b	0.1±0.01b	0.00±0.00b	4.6±0.4b
	River bank	3176.0±244.3a	0.7±244.3a	2.7±0.2a	0.4±0.03a	7.2±19.2a	49.6±3.2a
	F Statistics	11.52***	81.35***	3663.96*	1308914*	34.06*	7110.61*

Mean ± SE followed by dissimilar letter in the same column are significant at $P \leq 0.05$ according to Fischer LSD. (*: $P \leq 0.05$; **: $P \leq 0.01$, ***: $P \leq 0.001$; NS = Not significant).

Table 5: Concentrations of essential micronutrients in the soil samples from different sites along the Diep River, Cape Town, South Africa.

Site	Site Status	Concentrations of essential micronutrients (mg. kg^{-1})			
		Mn	Fe	Cu	Zn
1	Adjacent soil	5.4±0.1b	402.2±13.3b	0.5±0.03b	2.4±0.2a
	River bank	6.7±0.2a	1136.4±23.7a	5.6±2.3a	2.9±1.4a
	F Statistics	33.5***	730***	5.13***	0.12NS
2	Adjacent soil	6.6±0.2b	659.7±28.0b	0.3±0.3b	2.6±1.2b
	River bank	11.5±0.4a	4596.9±418.8a	2.56±0.16a	11.6±2.3a
	F Statistics	132.54***	87.97***	50.43***	11.51***
3	Adjacent soil	25.6±3.3b	1444.8±185.1b	12.56±0.9b	52.3±5.5b
	River bank	39.2±6.9a	2685.4±221.9a	34.7±6.1a	164.5±24.9a
	F Statistics	9.33**	13.61**	21.49***	9.72***
4	Adjacent soil	27.02±4.5b	2459.8±199.1b	5.40±0.6b	53.9±3.8b
	River bank	48.05±3.5a	4897.2±411.1a	45.9±3.2a	211.5±14.8a
	F Statistics	18.44***	20.08***	127.73***	53.81***

Mean ± SE, followed by dissimilar letter in the same column are significant at $P \leq 0.05$ according to Fischer LSD. (**: $P \leq 0.01$, ***: $P \leq 0.001$; NS = Not significant).

CHAPTER THREE

ASSESSEMENT OF METAL CONCENTRATIONS, CHLOROPHYLL CONTENT AND PHOTOSYNTHESIS IN *Phragmites australis* ALONG THE LOWER DIEP RIVER, CAPE TOWN, SOUTH AFRICA.

3.1 INTRODUCTION

The management of rivers requires an understanding of the linkage of management actions to biological response (Laing et al., 2003). In recent times, the use of plants for soil restoration and phytoremediation has increased due to the natural capacity of plant species to accumulate various metals (Arora et al., 2006; Bragato et al., 2009). Metal pollution is of significant environmental concern because they are non-degradable, have long half-lives in soil and have adverse effects on ecosystems (Jayaweera et al., 2007; Nymazal et al., 2007).

A cornerstone of ecotoxicology is the ability to demonstrate a relationship between the exposure of metals and the physiological responses of plants (such as on chlorophyll content, photosynthesis activities and metal accumulation in tissues). The demonstration of a metal exposure-response relationship is an essential criterion for establishing that the metal is responsible for the effects measured. For most metals, exposure to low levels does not lead to any observable effect, but it is only after a threshold is reached that an effect can be detected (Kabata-Pendias and Pendias, 1992; Rodríguez et al., 2007).

Metal toxicity is one of the common stresses that limit plant growth and development (Gallego et al., 1996; Liphadzi and Kirkham, 2006). Scientific evidence has shown that plant species which grow in polluted environments may be stressed in various ways. For example, elevated bioaccumulation of toxic concentrations of metals (through direct uptake by the plants' roots, stems or shoots) results in malfunctioning of their physiological systems (Dahmani-Muller et al., 2000; Monni et al., 2001; Plekhanov and Chemeris, 2003; Liphadzi and Kirkham, 2006).

The established threshold concentrations of metals in different plants are shown in Table 3. Elevated levels above these thresholds may interfere with the physiological functioning of plants such as chlorophyll and photosynthesis activities. However, whether the bio-accumulation patterns in plants (such as *P. australis*) growing at different pollution gradients differ significantly, is a subject that warrants further investigation.

Some metals are required by plants for their normal growth and developmental activities. However, when present in excess in plant tissues, they may be extremely toxic to plant cells (Gallego et al., 1996; Cronk and Fennessey, 2001; Bragato et al., 2006). If a plant is stressed, changes in the chlorophyll content may occur before any physical signs of stress are evident. Several cases of decreased chlorophyll synthesis and metabolism due to metal toxicity have been reported in the plant kingdom (Abdurakmanova et al., 2000; Schoefs, 2001; Kucuk et al., 2003; Calheiros et al., 2007; Baldantoni et al., 2009; Bragato et al., 2009; Bonanno and Lo Giudice, 2010).

Zayed et al. (1998) observed duckweed plants exhibiting chlorosis and reduced growth at higher levels of metals such as, Fe, Cu, Cd, Hg, Pb, Ni, Zn and Mn. Similarly, studies by Stobart et al. (1985) and Dong et al. (2005) on the effects of cadmium on chlorophyll content in barley revealed inhibited biosynthesis, reduction in total chlorophyll content and the chlorophyll a/b ratio.

Chlorophyll concentration may fundamentally influence the functioning of the photosynthetic apparatus and thus affect whole plant metabolism (Clijsters and van Assche, 1985; Sun and Wu, 1998; Prasad and Strzalka, 2000). For example, Rye grass *(Lotium perenne,* cv.S-23) leaves turned yellowish when treated with Ni. Plants became necrotic and suffered interveinal chlorosis (Khalid and Tinsley, 1980). Sheoran et al. (1989) reported a reduction in photosynthesis and enzyme activities in pigeon pea (*Cajanus cajan*) indirectly by reporting a decrease in chlorophyll content due to elevated levels of cadmium and nickel in the leaves. In higher plants, studies have shown that growth and photosynthetic activities were significantly affected by cadmium (Nagel et al., 1996).

Photosynthesis provides plants with their main building material, carbohydrates and the energy necessary to thrive and prosper in their environment. Metal stress is considered to be a major environmental factor limiting plant growth and yield (Kalavrouziotis et al., 2007). It induces many physiological, biochemical and molecular responses, in which photosynthesis is one of the primary physiological targets (Kalavrouziotis et al., 2007). Generally, in metal stressed plants, stomatal limitation is responsible for the decline of photosynthesis (Cornic, 2000). In a study on the effects of excess copper on the photosynthesis of barley plants, Vassilev et al. (2003) revealed reduced photosynthesis

and other associated visual symptoms such as growth reduction, interveinal foliar chlorosis, wilted leaves and necrotic leaf tips and attributed it to chronic copper phytotoxicity.

Most of the published data concerning toxicity testing of metals has focused on single metal effects. However, metal pollution of plants growing in polluted environments in nature has been shown to be due to the presence of cocktail of several metals (Walker et al., 2003; Rodríguez et al., 2007). They may exhibit toxicity simultaneously and interactively at different levels (Vázquez et al., 2006). It is therefore, worthwhile to assess metal toxicity induced to plants through expression of biomarkers such as those related to chlorophyll synthesis and photosynthetic activity.

The extent to which physiological parameters in *P. australis* such as chlorophyll content, rate of photosynthesis and metal accumulation in shoots and roots growing at different sites along a pollution gradient in Diep River in Cape Town, still remain unravelled. Therefore, this study was aimed at evaluating chlorophyll content, photosynthesis and metal concentrations in *P. australis* plants to validate if there is a significant movement of pollutants from the river banks and to the adjacent soil.

3.2 MATERIALS AND METHODS

3.2.1 DESCRIPTION OF STUDIED SPECIES

P. australis is a clonally grass species with woody hollow culms which can grow up to six meters in height. Leaves are lance late, 20 - 40 cm long and 1 - 4 cm wide. Flowers develop by mid summer and are arranged in tawny spike lets with many tufts of silky hair. It is an invasive grass commonly abundant along the Atlantic coast, and also found in wetlands around the world (Cronk and Fennessy, 2001).

P. australis is an emergent macrophyte, cosmopolitan in distribution and the most productive natural plant population in the biosphere (Wetzel, 1995; Cronk and Fennessy, 2001; Saltonstall, 2008). It grows perennially, with a constant turnover of population members that are senescing as new cohorts.

Reeds (*P. australis*) are the focus of this study because it is the most abundant plant species lining the river banks along the Diep River. Furthermore, it is widespread along South African rivers and commonly occurs in monospecific stands (Laing et al., 2003; Saltonstall, 2008).

3.3 SITE SELECTION

The study was conducted along the banks of the lower Diep River (Map 1). This river is located in Cape Town. The sampling sites were: 1) Milnerton lagoon: latitude 33° 52.499'S, and longitude 18° 29.548; 2) Lower estuary: latitude 33° 52.329'S, and longitude 18° 29.714'E; 3) Milnerton bowling club: latitude 33° 52.183'S, and longitude 18° 29.750'E, and 4) Woodbridge Island: latitude 33° 53.486'S, and longitude 18° 29.127'E were selected based on their location near both the refinery and landfill site, which may emit a cocktail of pollutants to some distance in the environment.

The Diep River originates from the Kasteel Mountain, Malmesbury and flows in a south-westerly direction towards Table Bay, where it flows into the Atlantic Ocean (Brown and Magoba, 2009).

3.4 COLLECTION OF PLANT SAMPLES

For each of the site, plant samples were taken from the river bank and adjacent soil in the same vicinity. Four plant samples were collected from each site. The soil surface was first cleared of debris using a plastic shovel. Whole plants from the cleared area were completely uprooted with the entire root system and were then separated into shoots (stems and leaves) and roots. The plant samples were put into separate paper bags, sealed, appropriately labelled and transferred to the laboratory.

3.4.1 PREPARATION AND ANALYSIS OF PLANT SAMPLES

The plant samples were divided into shoots (leaves and stems) and roots, washed thoroughly with distilled water to remove any soil particulate matter and then dried in the oven at 60°C for 48 h. The samples were removed from the oven and allowed to cool to room temperature then ground with a mortar and pestle. Each of the sample were sieved using a nylon sieve (2mm pore size) and thereafter stored in plastic containers.

3.4.2 DIGESTION OF PLANT SAMPLES

This was carried out as described by Odendaal and Reinecke (1999). For the digestion process 10 ml of 55% HNO_3 was added to each plant sample (1 g) in test tubes and stirred properly using a glass rod. Each of the mixture was then heated on a dry block heater in a fume cupboard at 40°C for 1 h, then at 120 °C for 3 h. The samples were removed and allowed to cool to room temperature. Each of the cooled solution was made up to 20 ml with distilled water and was filtered using cellulose nitrate filter paper (0.45 µm). Each of the filtrate obtained were further diluted to a final volume of 100 ml each with distilled water, and appropriately labeled and stored in the refrigerator.

3.4.3 ANALYSIS OF SAMPLES FOR METALS

The digested plant samples were analyzed for the presence of metals using the Inductively Coupled Plasma - Mass Spectrophotometer (7500CE, Agilent, England). To obtain the plant metal concentrations, the ICP values were converted using the formula:

$$PMC = \frac{(\text{ICP reading} - \text{blank reading}) \times \text{dilution factor (20)}}{\text{WPS (mg)}}$$

Where: PMC = Plant metal concentration (mg kg^{-1}); ICP = Inductively Coupled Plasma values; C = Blank; DF = Dilution factor; WPS = Weight of plant sample (g). The plant metal concentration data were statistically analyzed using the STATISTICA software package 2009 (StatSoft Inc., Tulsa, OK, USA).

3.4.5 DETERMINATION OF PHOTOSYNTHESIS IN PLANT LEAVES

Measurements were taken on-site using the Infra-red gas analyzer (IRGA, Pharmacia, LKB. Ultraspec.11E, Blockrom, England). Photosynthetic parameters in the intact plant leaves were assessed in the morning between 8am and 11am, according to the manufacturer's instructions. Parameters measured included photosynthesis (A), rate of evapo-transpiration (E), intercellular carbon dioxide concentration (Ci) and stomata conductance (Gs).

Readings were recorded at 20 minutes intervals between each plant sample on both river bank and adjacent soil sites. The carbon dioxide assimilation rate was expressed as the amount of carbon - dioxide assimilated per unit leaf area and time µmol carbon dioxide was consumed.

3.4.6 DETERMINATION OF CHLOROPHYLL CONTENTS IN PLANT LEAVES USING SPECTROPHOTOMETRY

The plant leaf on which photosynthesis reading was conducted was neatly detached using a gloved hand and placed into a polythene bag, sealed, appropriately labeled and put in a cooler. Samples were then transported to the laboratory. The leaves were cut into pieces using a clean scalpel, macerated in a crucible and then used for chlorophyll contents analysis as described by Hiscox and Istraestam (1979). To 100 mg of macerated leaves, 7 ml dimethylsulphoxide (DMSO) was added in a vial and incubated at 4°C for 72 h. After incubation, the extracts obtained were diluted with 10 ml DMSO; out of which 3 ml of the diluted extract was withdrawn, transferred into curvets and the absorbance determined at 645 and 663 nm. A pure solution of dimethylsulphoxide (DMSO) was used as blank. Total chlorophyll contents (Chlorophyll a and b) were

determined using the equation below as described by Arnon (1949) and results expressed as mg L^{-1}.

$$\text{Chlorophyll a: Chl a} = 12.7D_{663} - 2.69\ D_{645}$$

$$\text{Chlorophyll b: Chl b} = 22.9D_{645} - 4.68D_{663}$$

$$\text{Chlorophyll Total: (Chl.}\tau\text{)} = 20.2\ D_{645} + 8.02\ D_{663}$$

3.5 RESULTS

3.5.1 METALS IN PLANT SHOOTS AND ROOTS

Results of metal concentrations in the shoots and roots of *P. australis* collected from both the river bank and the adjacent soil from site 1 to site 4 are shown in Tables 4 - .

Aluminium (Al)

Al concentration in all sites was significantly higher in shoots collected from the river bank compared with adjacent soil. The mean values from samples collected from adjacent soil ranged from 8.7 -16.9 $mg.kg^{-1}$, whereas those from the river bank ranged from 23.6 - 237.9 $mg.kg^{-1}$. The highest Al concentration in plant tissues were found in samples collected from site 4 and 3 "respectively" (Table 4).

The Al concentration in the roots ranged from 1.1 - 313 $mg.kg^{-1}$ (Table 8). Three out of 4 sites had Al levels in the roots which were significantly different from samples collected from the river bank relative to those from adjacent soil. A higher level of Al was detected in roots taken from sites 4, 2, 3 and 1 "respectively".

Chromium (Cr)

Significant differences were observed in Cr concentrations between plant samples of *P. australis* collected along the river bank and from the adjacent soil at sites 1, 2 and 3 (Table 4). Cr was not detected in shoots sampled from site 4. The concentrations of Cr

in shoots collected from the adjacent soil were in the range of 0.0 - 0.8 $mg.kg^{-1}$. From the river bank, the shoot concentration of Cr varied from 0.1 - 0.8 $mg.kg^{-1}$.

In the roots, the Cr values were obtained in 2 sites out of the 4. The values from the river bank ranged from 0.1 - 488.6 $mg.kg^{-1}$, while from adjacent soil the concentration ranged from 0.0 - 12.5 $mg.kg^{-1}$ (Table 6). Site 3 had roots with higher concentrations of Cr as compared with site 2.

Cobalt (Co)

Shoot concentration of Co in *P. australis* plants from the river bank ranged from 0.1 - 55.9 $mg.kg^{-1}$ (Table 6), while those from the adjacent soil had values ranging from 0.0 - 16.9 $mg.kg^{-1}$. Three out of the 4 shoot samples collected from the river bank had Co which was significantly higher than those collected from adjacent site (Table 6).

Roots from the river banks had Co concentrations ranging from 0.2 - 25.6 $mg.kg^{-1}$, and the lowest values ranging from 0.0 - 0.2 $mg.kg^{-1}$ were reported from the adjacent soil investigated. All roots from the river bank from the 4 sites contained Co which was significantly higher than those in adjacent soil (Table 8).

Nickel (Ni)

For *P. australis* shoot samples analyzed from the river bank, their Ni concentration was consistently higher in all 4 sites with values ranging from 0.3 -1.9 $mg.kg^{-1}$. Ni was lowest in shoots sampled from adjacent soil with values ranging from 0.01 - 0.6 $mg.kg^{-1}$ (Table 6). Similarly, roots sampled from the river bank had significantly highest Ni values ranging from 0.6 - 2.9 $mg.kg^{-1}$ when compared with 0.0 - 0.3 $mg.kg^{-1}$ detected in roots sampled from adjacent soil (Table 8).

Cadmium (Cd)

Data collected from 3 out of the 4 sites showed that Cd shoot concentrations of *P. australis* were significantly different between those sampled from the river banks and adjacent soil. Cadmium in shoots ranged from 0.01 - 56.6 $mg.kg^{-1}$ compared with 0.01-

2.3 $mg.kg^{-1}$ recorded in shoot samples from adjacent soil (Table 6). In the roots, significantly differences were seen in Cd in samples from river banks in site 2, 3 and 4 as compared with those from adjacent soil (Table 5).

Lead (Pb)

Pb in shoots of *P. australis* analyzed from the river bank in all sites was significantly higher than their counterparts collected from the adjacent soil. From the river bank, Pb values ranged from 1.5 - 23.6 $mg.kg^{-1}$ (Table 4). It was found that the highest concentration of Pb in the river bank was at site 3 (23.6 $mg.kg^{-1}$). Generally, low contents of Pb were found in tissues from adjacent soil (Table 4).

The Pb content in roots was significantly different in three out of the 4 sites. The concentration of Pb in roots from the river bank ranged between 0.1 - 5.6 $mg.kg^{-1}$ with site 3 recording the highest Pb concentration (5.6 $mg.kg^{-1}$). Most of roots sampled from the adjacent soil had the lowest Pb concentration ranging from 0.1 -1.9 $mg.kg^{-1}$ (Table 8).

Manganese (Mn)

The available Mn in shoots ranged from 1.6 - 97.3 $mg.kg^{-1}$ and 0.8 - 35.9 $mg.kg^{-1}$ in samples from river bank and the adjacent soil respectively. Highest Mn values were found in site 4, followed by sites 3, 2 and 1, respectively (Table 7).

The root analysis result for Mn showed that 3 out of 4 sites had significantly varying values of Mn in *P. australis* plant collected from river bank compared with those from adjacent soil. As observed in shoots, more Mn in roots was recorded in plant samples from the adjacent soil. Root values for Mn from river bank ranged from 0.8 - 33.7 $mg.kg^{-1}$ (Table 9) and the values from plants adjacent soil ranged from 0.4 -10.3 $mg.kg^{-1}$. Compared with the values in shoots, highest Mn values in roots were found in sites 4, 3, 2 and 1, respectively.

Iron (Fe)

The mean value of Fe in shoot of *P. australis* samples collected from the 4 sites was significantly different from each other when data from the river bank and adjacent soil were compared. Levels of Fe from river bank samples ranged from 26.3 - 56.6 $mg.kg^{-1}$. The adjacent soil concentration of Fe in shoots ranged from 6.3 - 26.2 $mg.kg^{-1}$. The greatest Fe concentrations were also found in samples from the river bank compared those from adjacent soils shoots (Table 5).

The root accumulation of Fe in *P. australis* followed similar trend. Results indicated that Fe levels in most of the studied sites were greater in roots than shoots (Table 7 and 9). For instance, the highest concentration of 1164.4 $mg.kg^{-1}$ Fe in the roots were recorded in site 4 but the shoot value in the same plant was only 56.6 $mg.kg^{-1}$.

Copper (Cu)

The shoot and root concentrations of Cu in *P. australis* from the river bank were significantly higher than those from adjacent soil. Results showed that Cu values in shoots from the river bank ranged from 2.6 - 6.0 $mg.kg^{-1}$, but were significantly less in samples from adjacent soil, with values between 0.0 and 2.4 $mg.kg^{-1}$ (Table 5).

Concentration of Cu in roots of *P. australis* were significantly different in 3 out of the 4 sites sampled (Table 8). Cu concentration was significantly higher in roots from the river bank than those from the adjacent soil. Comparison across the sites showed that higher levels of Cu in roots were found at sites 4 and 3 (Table 9).

Zinc (Zn)

Zn in shoots of *P. australis* which were growing in the river banks had significantly higher amounts of Zn when compared with those growing in the adjacent soil in all sites. Concentration of Zn in shoots from the river bank and adjacent soil ranged from 3.8 - 50.6 $mg.kg^{-1}$ and 0.0 -10.1 $mg.kg^{-1}$ respectively (Table 7). Shoots sampled from sites 4 and 3 accumulated more Zn than those sampled from site 2 and 1.

Available Zn in roots of *P. australis* from the river bank and from the adjacent soil ranged from 6.2 -15.8 $mg.kg^{-1}$ and 0.4 -14.1 $mg.kg^{-1}$ respectively (Table 9), with significant quantities between adjacent and river bank observed in site 4 and followed by site 3, 2 and 1, respectively.

3.5.2 CHLOROPHYLL MEASUREMENTS IN *P. australis* GROWING IN THE RIVER BANKS AND ADJACENT SOIL.

Results of chlorophyll content showed that chlorophylls a, b and a+b were present in all the leaves examined from all the study sites (Fig. 3.1 – 3.4). For the adjacent soil leaves, site 1 exhibited the values of 14.1 $mg.L^{-1}$ (Fig. 3.1), site 4 values of 12.7 $mg.L^{-1}$ (Fig. 3.4), site 3 recorded the values of 11.4 $mg.L^{-1}$ (Fig. 3.3), for both chlorophyll a + b, a and b, while leaves from site 2 had the lowest value for chlorophyll (a + b) 9.1 $mg.L^{-1}$ (Fig. 3.4).

However, in the case of the river bank sites, the leaves from site 3 and 1 (7.9 and 8.8 $mg.L^{-1}$) recorded very low values for chlorophyll a + b (Fig. 3.3 and 3.1). However, the leaves from adjacent soil sites had greater values for chlorophyll a, b and a+b compared to those from river bank sites (Fig. 3.1).

3.5.3 MEASUREMENT OF PHOTOSYNTHESIS IN *P. australis* GROWING IN THE RIVER BANK AND ADJACENT SOIL

Results showed that the photosynthesis varied in plant leaves on both river bank and adjacent soil from site to site (Fig. 3.5 - 3.8). Photosynthesis rate (A) ranged between 1.0 - 10.6 µmol with plants from the river bank soil in site 1 exhibiting the highest rate of photosynthesis followed by plants from the other sites in the order 1 > 4 > 2 > 3. This implies that the most affected from the river bank soil is site 3, while site 1 is least affected, that is the least contaminated with most metals (Table 4).

Similarly, in the adjacent soil, the values for the photosynthesis (A) ranged from 6.5 - 13.4 µmol in an decreasing order of 1> 4> 2 > 3 which implies that the site mostly affected is site 3 and the least affected is site 1. The high values of photosynthesis observed in the adjacent soil site suggest that metals presence is minimal, while the low

values of the river bank site agreed with various researchers about metals affecting physiological parameters such as photosynthesis (Kalavrouziotis et al., 2007).

Results also showed that rate of evapotranspiration (E) was significant as the values ranged between 2.1 - 2.3 µmol for plants sampled in sites 1 and 4, while site 2 and 3 had the lowest values of 1.3 - 1.7 µmol (Fig. 3.6 and Fig. 3.7). For both the river bank and adjacent soil sites, the marginal difference in the values for evapotranspiration (E) is minimal, indicating that stomatal function is insignificant.

3.6 DISCUSSION

Results of this study revealed that all the sites investigated were generally contaminated with the metals. Al contamination was the single highest metal measured in the shoots (Table 4) and roots of *P. australis* (Table 6), and this was followed by Pb, Ni and Cr in shoots. Cr, Co and Pb were also significantly higher in the roots samples in the river bank in some of the sites studied (Table 6).

The levels of concentrations of Al in shoots of *P. australis* sampled from the entire river bank sites were in the range 23 7 - 237.9 $mg.kg^{-1}$. These were above the optimum recommended concentration level of 15 -18 $mg.kg^{-1}$ (Dobermann and Fairhurst, 2000) in a closely related wetland plant species. Shoot samples of *P. australis* from adjacent soil site had Al concentration ranging from 8.8-16.9 $mg.kg^{-1}$, which were below the recommended optimum of 18 $mg.kg^{-1}$, thus suggesting that *P. australis* growing in such environment may not be physiologically affected by Al toxicity. The Al concentration from *P. australis* roots collected from three out of four river bank were on the higher levels, whereas in the adjacent soil sites, elevated levels were only reported in one out of four sites (Table 6). Similar to our study, the presence of excessive Al in the plant parts is known to cause toxicity effects and reduce photosynthetic activities (Zhang et al., 2007).

Concentrations of Cr in shoots of *P. australis* sampled from the river bank sites were in the range 0.1-1.2 $mg.kg^{-1}$, while in the root samples it ranged from 0.1 - 488.6 $mg.kg^{-1}$. The optimum recommended concentration level for plants is 0.03 - 0.29 $mg.kg^{-1}$ (Burke et al., 2000), and for brown rice 0.16 $mg.kg^{-1}$ (Lin, 1991) as well as Lettuce 0.17 $mg.kg^{-1}$ (Qishlaqi et al., 2007). Cr is completely absent in the roots of samples from both sites 1

and 4 and in the shoot of site 4, the concentration were all within the critical limit for both shoot and root in all the studied sites, except in the root of site 3 (488.6 $mg.kg^{-1}$). This (488.6 $mg.kg^{-1}$) was the highest value obtained supporting literature that most wetland plants retain higher amounts of metals in their root than in leaf tissue (Keller et al., 1998; Karpiscak et al., 2001; Stoltz and Greger, 2002).

Cobalt was another important metal that was found to accumulate in excessive concentrations (16.9 - 55.9 $mg.kg^{-1}$) in *P. australis* plant shoots collected from the river bank and adjacent soil in site 4. Although small amounts were recorded in sites 1, 2 and 3, their levels (0.00 - 0.35 $mg.kg^{-1}$) were within the acceptable limits in plant tissues (Gough et al., 1979). At site 4 the observed tissue concentrations ranged from 16.9 - 55.9 $mg.kg^{-1}$, which were above the critical levels of 19 - 32 $mg.kg^{-1}$ (Gough et al., 1979) in a closely related Gramineae, the Sudan grass. The root levels across all sites ranged from 0.0 - 25.6 $mg.kg^{-1}$. However, highest values (25.6 $mg.kg^{-1}$) were recorded only in plant roots collected at river bank 4. This conforms to several wetland studies that the greatest metal accumulations occur in the roots (Karpiscak et al., 2001; Stoltz and Greger, 2002).

Ni concentrations found in our study ranged from 0.01 - 1.9 $mg.kg^{-1}$ in the shoot and 0.0 - 2.9 $mg.kg^{-1}$ in the roots. The established threshold of Ni in different plant species ranged from 5 - 100 $mg.kg^{-1}$ above which, plant physiological activities have been reportedly affected (Podlesakova et al., 2002). A comparison between the recorded Ni concentrations in our study and established threshold guidelines from the literature shows that they are below the established standards and may therefore not pose any toxicity threat to *P. australis* plants in the studied ecosystems (Table 4 and 6).

In this study, Cd concentration in both shoots and roots ranged 0.2 - 56.6 $mg.kg^{-1}$ and 0.0 - 35.3 $mg.kg^{-1}$, respectively (Table 6), while the critical levels suggested by Kabata-Pendias and Pendias (1992) is 0.5 - 0.7 $mg.kg^{-1}$.

Shoots sampled along the river bank showed that in sites 1 (9.07 $mg.kg^{-1}$) and 2 (56.67 $mg.kg^{-1}$) had Cd values significantly higher but site 3 values (0.27 $mg.kg^{-1}$) were lower than the critical level of 0.5 - 0.7 $mg.kg^{-1}$ proposed by Kabata-Pendias and Pendias

(1992). Comparatively, shoots from the adjacent soil in sites 2, 3 and 4 had low Cd values which were below the critical limit except in site 1 (2.3 $mg.kg^{-1}$).

Roots sampled from the river bank in sites 1, 3 and 4 had low values of Cd below the critical levels whereas site 2 value (35.3 $mg.kg^{-1}$) was above the recommended optimum. Compared to the adjacent soil, root values from site 3 and 4 were higher and above the established guidelines. Site 1 data of 0.1 $mg.kg^{-1}$ was far below the optimum recommended (Table 6).

A comparison between the Cd concentrations reported in our study and the established threshold guidelines from the by Kabata-Pendias and Pendias (1992) is 0.5 - 0.7 $mg.kg^{-1}$. This results shows that shoot from the river bank (75%) and adjacent soil (25%) sites had Cd values higher than the established standards by Kabata-Pendias and Pendias (1993) is 0.5 - 0.7 $mg.kg^{-1}$, indicating that Cd toxicity was a significant pollutant in the ecosystem. These might have resulted into the negative results reported in similar sites in chlorophyll synthesis and photosynthesis in this study (Fig.3.1- 3.4) and (Fig. 3.5 - 3.8)

According to Burke et al. (2000), the critical concentration of Pb in plants range from 0.12 - 0.5 $mg.kg^{-1}$. The concentration of Pb in the shoots sampled from the river bank ranged from 1.5 - 23.6 $mg.kg^{-1}$ (Table 4). All these values were above the established critical value. Shoots from the adjacent soils had Pb values ranging from 0.5 - 1.4 $mg.kg^{-1}$. Seventy five percent of the sites had elevated Pb values above the recommended optimum (Table 4). Taken together, results from this study suggest that the sites are heavily contaminated with Pb and may affect the growth of *P. australis* and other organisms found in the vicinity.

Table 6 indicated that 3 out of 4 sites from the river bank had elevated Pb levels ranging from (0.9 - 5.6 $mg.kg^{-1}$). From the adjacent soils, Pb levels in the roots ranged from (0.1 - 1.9) with 50% possessing values above the established limits. Similar to Pb shoot concentrations (Table 4), these results indicates that Pb also accumulated sufficiently in the roots (Table 6).

Available Mn in *P. australis* tissues ranged from 0.9 - 97.3 $mg.kg^{-1}$ in shoots (Table 5) and 0.4 - 33.7 $mg.kg^{-1}$ in roots (Table 7). The proposed threshold level for Mn in plants varies from 50 - 500 $mg.kg^{-1}$ (Allen, 1989). In this study the highest Mn concentrations (97.3 $mg.kg^{-1}$) was found in shoots sampled from river bank in site 4. This is the only site which had excessive Mn that could lead to toxicity in the *P. australis* plants.

Available Fe in the shoots and roots ranged from 6.3 - 56.6 mg kg^{-1} and 0.0 - 1164 $mg.kg^{-1}$ respectively (Table 5 and 7). The established guideline for Fe content in different plant parts at which toxicity is expected is between 1100 - 1600 mg kg^{-1} for most wetland plants (Marschner, 1995). More Fe accumulated in roots than in shoots. Generally, plant tissues contained Fe below this recommended threshold value, indicating that Fe was not higher enough to cause toxicity symptoms in the *P. australis*.

Available Cu in the shoots ranged from 0.0 - 6.0 mg kg^{-1} (Table 5) and corresponding values in roots ranged from 0.0 - 7.2 $mg.kg^{-1}$ (Table 7). The normal Cu concentration for plant growth in plant tissues is 5 - 20 $mg.kg^{-1}$ (Kabata-Pendias and Pendias, 1992). However, Marschner (1995) established that at 20 - 30 $mg.kg^{-1}$ the Cu toxicity is likely to occur in shoots. All plant organs sampled from all sites in this study had the lowest concentration of Cu.

Available Zn in shoots and roots of *P. australis* ranged from 0.0 - 50.6 $mg.kg^{-1}$ (Table 5) and 0.4 - 15.8 $mg.kg^{-1}$ (Table 7), "respectively". Previous studies reported Zn phototoxic levels for plants to vary from 100 - 1500 $mg.kg^{-1}$ (Marschner, 1995; Chaney, 1989). However, our study obtained values below 100 - 1500 $mg.kg^{-1}$, as other factors may have played a role in the accumulation capacity of the studied plant (Hussein et al., 2004).

Significant differences were observed in the values of Chl a, Chl b, and total chlorophyll (Chl a+b) in *P. australis* and higher Chl.T values obtained in *P. australis* plants growing outside the river sediment compared with those measured in the river banks (Figures 3.1 - 3.4). Similarly, there was a decrease in photosynthesis rate (A), evapotranspiration (E) intercellular carbondioxide concentration (Ci) and stomatal conductance (Qs) in plants from the river bank as compared to those measured in adjacent soil (Figures 3.5 - 3.8). The interference with the photosynthetic apparatus in plants leaves measured from the

river bank was probably due to elevated levels of certain metals in the soil (Tables 4 and 5) which caused excessive metal accumulation in the tissues (Table 4 - 7), thus interfering with chlorophyll synthesis and photosynthesis. This is consistent with previous studies which reported that excessive metals (such as Zn, Cd, Ni, Al, Cu) in the plant tissue negatively affected chlorophyll synthesis and photosynthesis process (Godbold, 1984; Rai et al., 1991b; Hussein et al., 1991; Vangronsveld and Clijsters 1992; Kahle 1993; Krupa et al., 1993a, b; Monni et al., 2001; Kalavrouziotis et al., 2007).

3.7 CONCLUSION

The higher concentration values of the metals in shoots and roots of *P. australis* sampled from the river bank as compared to those taken from the adjacent soil obtained in this study may be attributable to contamination of the soil by industrial effluents emanating from the industries sited in the area (Ayeni et al., 2010). This was reflected in the reduced photosynthesis and chlorophyll synthesis in plants which were growing close to the river bank.

Table 6: Concentrations ($mg.kg^{-1}$) of metals present in shoots growing on both river bank and adjacent soil site

Site	Status of the Sampled Site	Al	Cr	Co	Ni	Cd	Pb
		---------- mg. kg^{-1} ----------					
1	Adjacent soil	8.8±1.1b	0.0±0.0b	0.1±0.0a	0.01±0.0b	2.3±1.9b	0.6±0.0b
	River bank	23.7±8.7a	0.1±0.01a	0.1±0.0a	0.3±0.2a	9.0±8.9a	2.4±0.8a
	F Statistics	2.91*	0.24***	0.36NS	26.43*	10.64**	5.85***
2	Adjacent soil	8.7±1.0b	0.01±0.0b	0.0±0.0b	0.03±0.0b	0.01±0.0b	0.5±0.0b
	River bank	23.6±4.5a	0.2±0.11a	0.1±0.0a	0.5±0.1a	56.6±53.7a	1.5±0.6a
	F Statistics	10.43***	2.45*	63.28***	39.38***	0.73*	2.68*
3	Adjacent soil	16.9±6.2b	0.8±0.8b	0.0±0.0b	0.3±0.3b	0.01±0.0b	1.4±0.3b
	River bank	56.6±53.7a	1.18±1.07a	0.35±0.18a	1.9±0.4a	0.2±0.0a	23.6±2.2a
	F Statistics	0.40*	537.63	29.7*	10.60***	1.71*	0.60*
4	Adjacent soil	10.1±8.9b	NA	16.9±6.2b	0.6±0.6b	0.0±0.0a	1.3±1.3b
	River bank	237.9±21.8a		55.9±53.6a	0.8±0.8a	0.0±0.0a	73.9±3.0a
	F Statistics	0.56*		0.40*	10.8**	2.25NS	23.47***

Mean ± SE, followed by dissimilar letter in the same column are significant at $P \leq 0.05$ according to Fischer LSD. (*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$; NS = Not significant).

Table 7: Concentrations ($mg.kg^{-1}$) of essential micronutrients in plant shoots from four soil sites

Site	Status of the Sampled Site	Mn	Fe	Cu	Zn
		mg. kg^{-1}			
1	Adjacent soil	0.8±0.7b	20.4±11.7a	0.2±0.2b	0.0±0.0b
	River bank	1.6±1.1a	26.3±20.7a	6.0±1.9a	3.8±2.8a
	F Statistics	566.55**	1.05NS	9.09***	121.26***
2	Adjacent soil	2.9±0.6b	23.6±2.2b	0.0±0.0b	5.1±1.4b
	River bank	12.1±3.4a	43.4±17.7a	2.6±0.4a	11.7±3.8a
	F Statistics	73.57***	1.23*	44.25***	2.67*
3	Adjacent soil	18.5±6.6b	26.2±3.0b	2.4±1.2b	5.3±0.6b
	River bank	33.4±5.0a	33.8±5.4a	3.19±0.8a	18.7±4.6a
	F Statistics	3.21*	1.49*	439.00***	8.70***
4	Adjacent soil	35.9±5.4b	6.3±6.3b	0.3±0.3b	10.1±2.60b
	River bank	97.3±5.7a	56.6±53.7a	3.9±1.1a	50.6±6.6a
	F Statistics	60.59***	0.59*	773.46*	25.97***

Mean ± SE, followed by dissimilar letter in the same column are significant at $P \leq 0.05$ according to Fischer LSD. (*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$; NS = Not significant).

Table 8: Concentrations (mg.kg^{-1}) of metals in plant roots sampled in four different soil sites

Site	Status of the Sampled Site	Al	Cr	Co	Ni	Cd	Pb
		mg. kg^{-1}					
1	Adjacent soil	1.0±0.0a	NIL	0.2±0.0b	0.0±0.0b	0.0±0.0a	0.1±0.0a
	River bank	2.0±0.0a		0.5±0.1a	2.9±1.3a	0.0±0.0a	0.1±0.0a
	F Statistics	0.00NS		6.90*	5.57*	0.01 NS	3.64NS
2	Adjacent soil	5.4±2.3b	0.0±0.0b	0.1±0.0b	0.3±0.0a	3.5±1.1b	0.5±0.1b
	River bank	26.9±6.1a	0.1±0.1a	0.2±0.0a	0.6±0.2a	35.3±16.8a	0.9±0.1a
	F Statistics	21.35***	6.2**	8.82***	2.2*	3.57*	4.64**
3	Adjacent soil	5.5±1.7b	12.5±6.8b	0.0±0.0b	0.1±0.1b	0.01±0.0b	1.9±0.3b
	River bank	25.6±4.0a	488.6±167.8a	0.7±0.7a	0.6±0.3a	0.12±0.0a	5.6±1.3a
	F Statistics	46.71***	8.04*	2.13**	9.5**	5.91*	7.91*
4	Adjacent soil	89.8±19.7b	NIL	5.5±1.7b	0.0±0.0b	0.01±0.0b	1.2±0.1b
	River bank	313.0±46.1a		25.6±4.0a	1.9±0.5a	0.30±0.0a	1.7±0.8a
	F Statistics	19.81***		46.77***	4.64 **	0.71*	1.39 **

Mean ± SE, followed by dissimilar letter in the same column are significant at $P \leq 0.05$ according to Fischer LSD. (*: $P \leq 0.05$,**: $P \leq 0.01$, ***: $P \leq 0.001$; NS = Not significant).

Table 9: Concentrations (mg. kg^{-1}) of essential micronutrients in plant roots from four different soil sites.

Site	Status of the Sampled Site	Mn	Fe	Cu	Zn
		---------- mg. kg^{-1} ----------			
1	Adjacent soil	0.4±0.3a	0.0±0.0b	1.6±0.3b	1.1±0.7b
	River bank	0.8±0.4a	14.5±7.7a	2.9±0.5a	6.2±2.4a
	F Statistics	0.74NS	16.11***	2.66 **	4.27 **
2	Adjacent soil	3.6±0.9b	22.9±3.2b	0.0±0.0b	0.4±0.4b
	River bank	5.3±1.0a	49.6±11.4a	2.6±0.6a	8.9±2.5a
	F Statistics	4.89**	5.11*	15.84***	11.38**
3	Adjacent soil	4.6±0.9b	1.2±1.0b	0.7±0.2b	14.1±1.1b
	River bank	6.9±1.3a	37.7±11.6a	3.1±0.7a	15.4±2.4a
	F Statistics	36.740*	9.71**	1.82**	99.84***
4	Adjacent soil	10.3±1.2b	248.3±46.8b	0.3±0.3b	15.2±1.7b
	River bank	33.7±2.7a	1164.4±123.5a	7.2±1.3a	15.8±2.5a
	F Statistics	61.88***	48.16***	25.49***	76.86*

Mean ± SE, followed by dissimilar letter in the same column are significant at $P \leq 0.05$ according to Fischer LSD. (*: $P \leq 0.05$,**: $P \leq 0.01$, ***: $P \leq 0.001$; NS = Not significant).

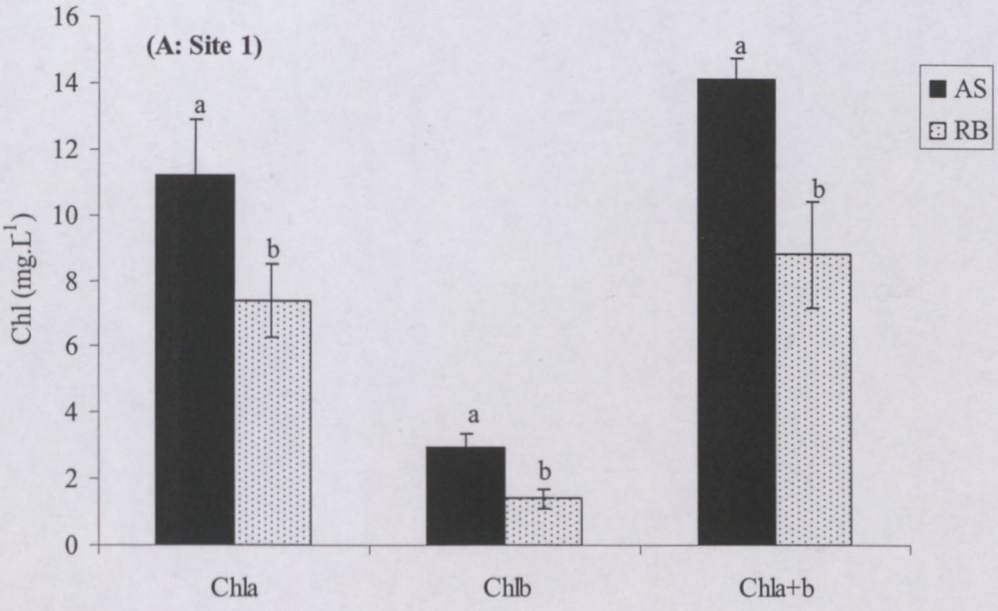


Fig. 3.1: Effects of metal contamination on Chl concentrations in plants sampled at Site 1 along the lower Diep River, Milnerton, in the Western Cape Province. AS: Adjacent soil, RB: River bank. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P \leq 0.05$.

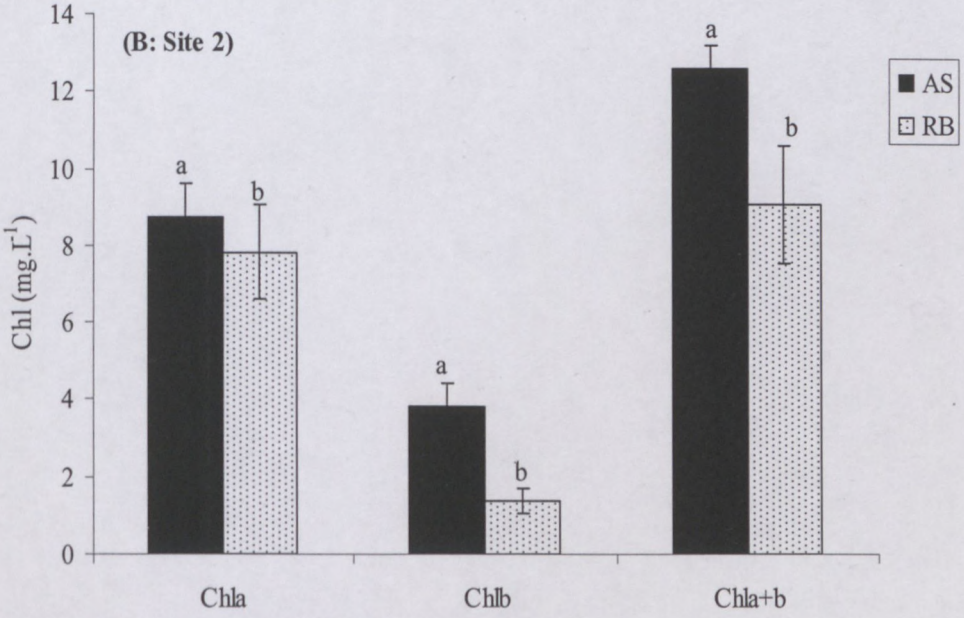


Fig. 3.2: Effects of metal contamination on Chl concentrations in plants sampled at Site 2 along the lower Diep River, Milnerton, in the Western Cape Province. AS: Adjacent soil, RB: River bank. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P \leq 0.05$.

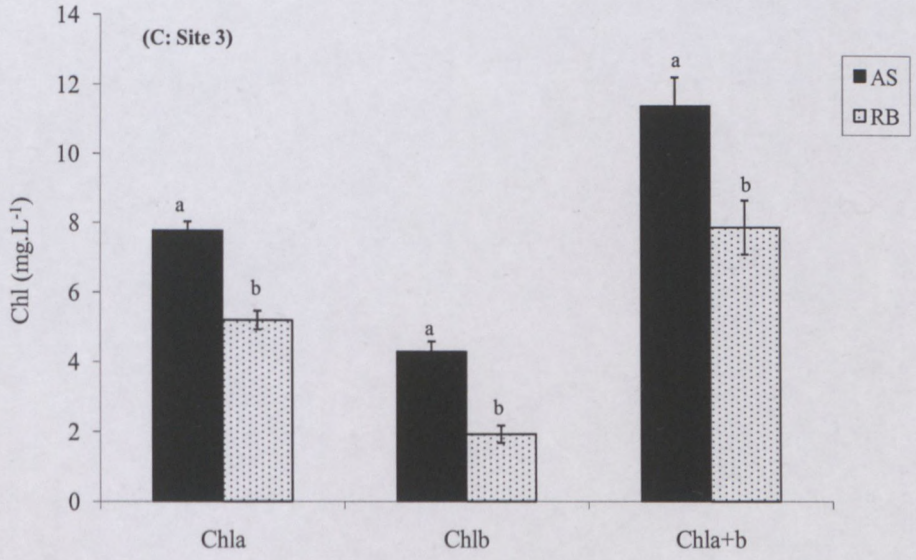


Fig. 3.3: Effects of metal contamination on Chl concentrations in plants sampled at Site 3 along the lower Diep River, Milnerton, in the Western Cape Province. AS: Adjacent soil, RB: River bank. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P \leq 0.05$.

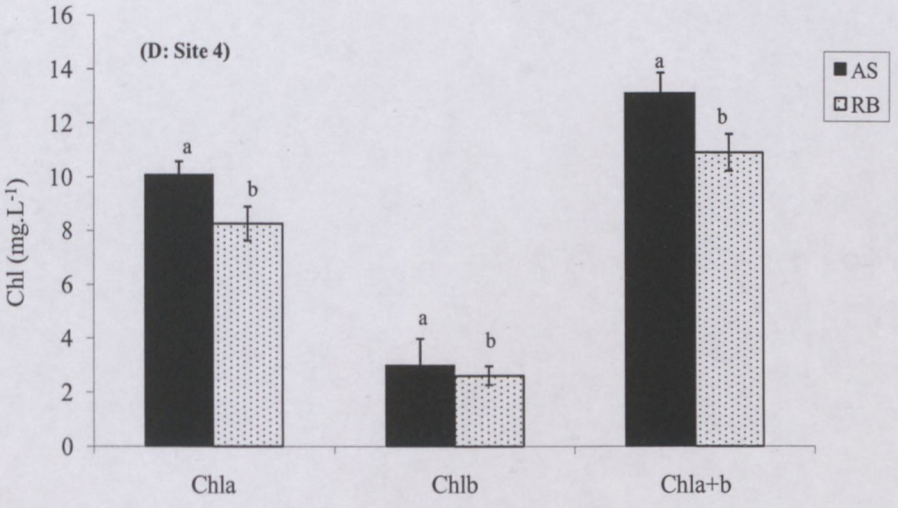


Fig. 3.4: Effects of metal contamination on Chl concentrations in plants sampled at Site 4 along the lower Diep River, Milnerton, in the Western Cape Province. AS: Adjacent soil, RB: River bank. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P \leq 0.05$.

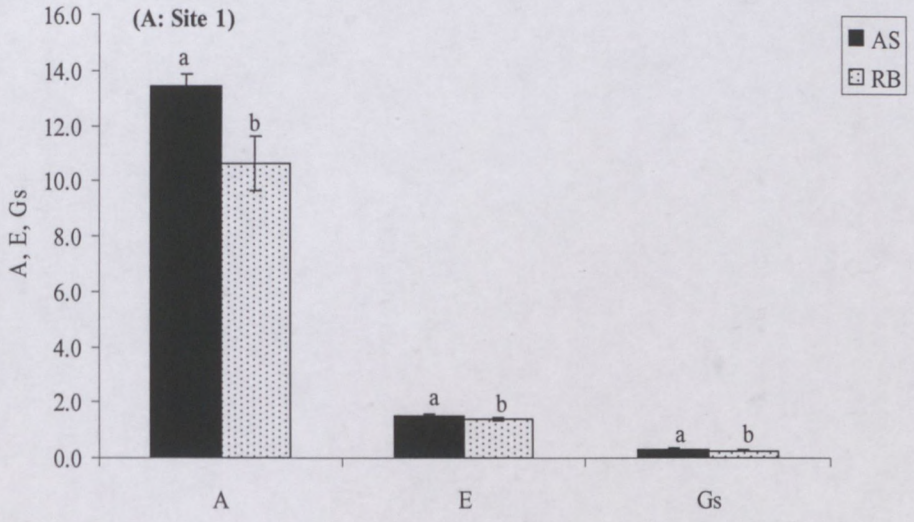


Fig. 3.5: Effect of metal contamination on photosynthesis (μmol $CO_2.m^{-2}.s^{-1}$), evapotranspiration (mmol $H_2O.m^{-2}.s^{-1}$), and stomata conductance ($mmol.m^{-2}.s^{-1}$) in plants sampled at site 1 along the lower Diep River, Milnerton in the Western Cape Province. AS: Adjacent soil, RB: River bank. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P \leq 0.05$.

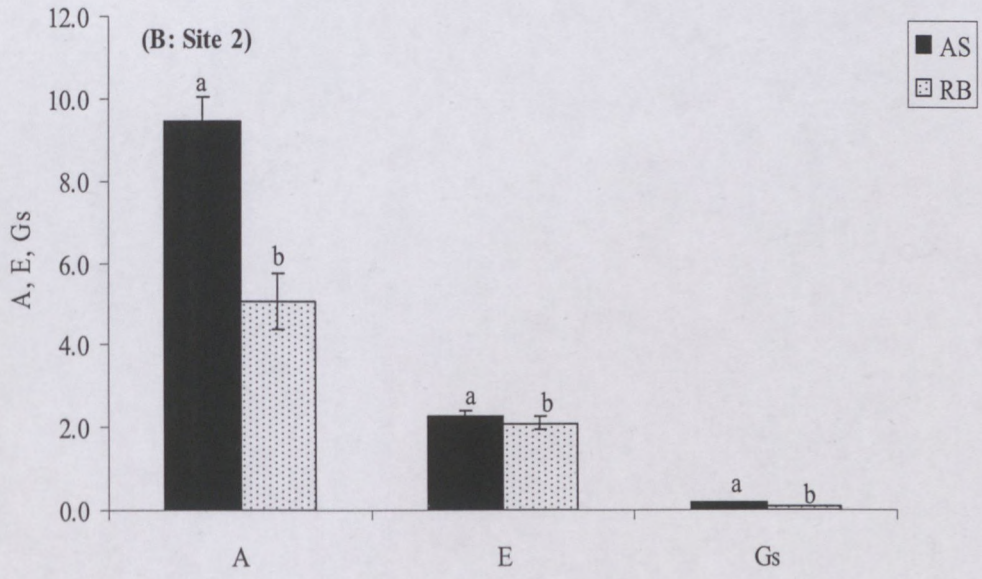


Fig. 3.6: Effect of metal contamination on photosynthesis (μmol $CO_2.m^{-2}.s^{-1}$), evapotranspiration (mmol $H_2O.m^{-2}.s^{-1}$), and stomata conductance (mmol.$m^{-2}.s^{-1}$) in plants sampled at site 2 along the lower Diep River, Milnerton in the Western Cape Province. AS: Adjacent soil, RB: River bank. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P \leq 0.05$.

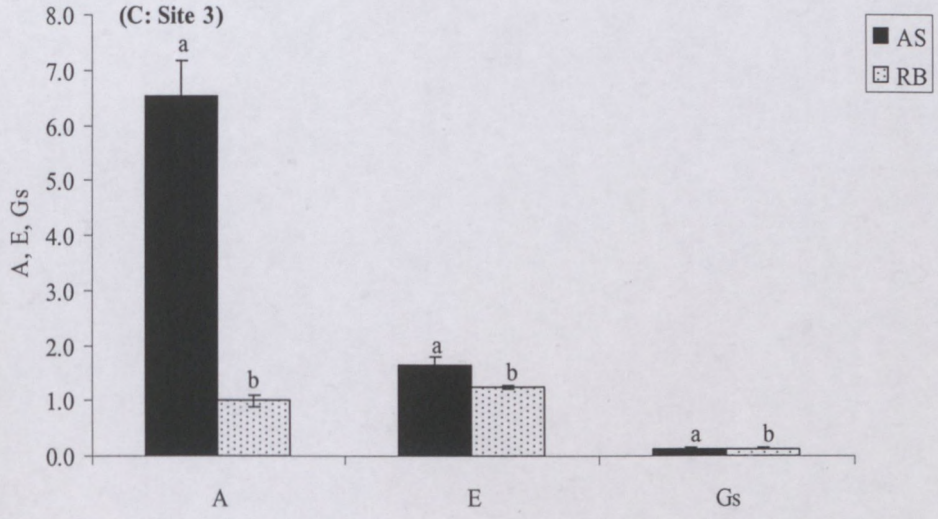


Fig. 3.7: Effect of metal contamination on photosynthesis (μmol $CO_2.m^{-2}.s^{-1}$), evapotranspiration (mmol $H_2O.m^{-2}.s^{-1}$), and stomata conductance ($mmol.m^{-2}.s^{-1}$) in plants sampled at site 3 along the lower Diep River, Milnerton in the Western Cape Province. AS: Adjacent soil, RB: River bank. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P \leq 0.05$.

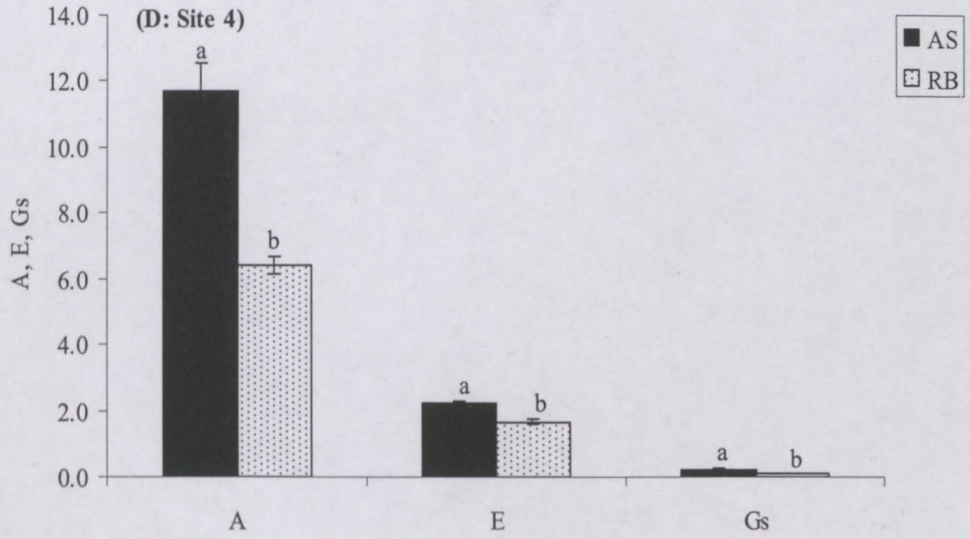


Fig. 3.8: Effect of metal contamination on photosynthesis (μmol $CO_2.m^{-2}.s^{-1}$), evapotranspiration (mmol $H_2O.m^{-2}.s^{-1}$), and stomata conductance ($mmol.m^{-2}.s^{-1}$) in plants sampled at site 4 along the lower Diep River, Milnerton in the Western Cape Province. AS: Adjacent soil, RB: River bank. Bars followed by dissimilar letter are significantly different by Fisher Least significant difference (LSD) test at $P \leq 0.05$.

CHAPTER FOUR

CONCLUSIONS

4.1 CONCLUSIONS

- In conclusion, the use of simple biomarkers in assessing aquatic plants exposed to heavy metal is recommended.
- Results of this study showed some degree of high metal contamination in soil collected from river bank compared with lower concentration in the adjacent soil in all sites investigated.
- The concentration of some essential micronutrients also exceeded the established guidelines in the soil. Site 3 and 4 were more polluted than site 2 and 3, respectively.
- The high level of industrial activities and the close proximity of the study area to a highway may be largely responsible for the high level of pollution observed.
- Future studies should focus on the impact of these metals on different organisms found in this ecosystem as some may find their way into food chain and cause harmful effects to animals and ecosystems at large.
- The higher concentration values of the metals in shoots and roots of *P. australis* sampled from the river bank as compared to those taken from the adjacent soil obtained in this study may be attributable to contamination of the soil by industrial effluents emanating from the industries sited in the area.
- In the current study, increased metal loads were found to be accompanied by decreasing chlorophyll concentrations and photosynthetic rate as indicated in previous studies that these parameters as indicators of metal stress (Kukkola et al., 2000; Maxwell and Johnson, 2000; Myśliwa-Kurdziel and Strzatka, 2002).
- The plants sampled from the river bank had much higher concentrations of metals (such as Cd in the shoot) and hence, a minimal photosynthesis suggesting that there is low translocation of the metal to the shoot. While, plants that grows on the adjacent soil had higher value of chlorophyll and photosynthesis.

4.2 FUTURE RESEARCH NEEDS

Research investigations reviewed here have mainly centered on few sites in wetland area of lower Diep River of Milnerton and *Phragmites australis*. There is considerable evidence that the ecological/physiological status in other similar wetland plant species could give a conclusive effect of metals. Similarly, this study could not establish a quantitative relationship between contaminant concentration in the environment and contaminant in the tissue of *P. australis* because of the seasonal nature of reducing soil condition in wetland, which implies that data collected, are for qualitative assessment only.

There is a continued need for three types of study.

Experiments with two species, under controlled conditions give the clearest view of what is likely to happen to plants as metals in its environment change. Laboratory studies with tracers are needed to follow the pathways of metals during and after uptake-how much is released into the ecosystems?

The second type of necessary study includes more comparisons of wide ranges of wetlands or lakes, so that the current niches of species can be defined. These studies need more detail than is usually measured at present; sediment metals need to be speciated, rhizospheric conditions need to be defined, and fallout of metals should be monitored as well as the rhizophere effects on metal mobility and availability in wetland plant, which can provide ecologically relevant sensitive diagnostics monitoring tool.

The third type of research that is desirable should add the dimension of time; thorough surveys should be repeated and historical and palaeo-ecological data, about both the environment and plant communities, should be compared with present trends. It is therefore advised that further studies on factors affecting metal uptake under conditions where wetlands undergo seasonal changes from extended periods should be carried out.

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PUBLICATIONS THAT RESULTED FROM THIS RESEARCH

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