METAL BIOACCUMULATION, MEMBRANE INTEGRITY AND CHLOROPHYLL CONTENT IN THE AQUATIC MACROPHYTE *CERATOPHYLLUM DEMERSUM* FROM THE DIEP RIVER, WESTERN CAPE

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BY

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DECLARATION

I, Deborah Vivian Erasmus, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed D. Erasmus Date 26-09-2012

ABSTRACT

The Diep River is approximately 80 km in length and runs through agricultural land and urban parts of greater Cape Town, South Africa before entering the Atlantic Ocean, via an estuary. Generally, metal pollution in South African rivers is not well documented and using plants to monitor metal bioaccumulation is even less documented. The aim of this study was to investigate aluminium, iron, copper and zinc metal pollution in the Diep River and bioaccumulation of these metals in the leaves and stems of the submerged macrophyte *Ceratophyllum demersum* L. Furthermore, the effects of bioaccumulated metals on membrane integrity and chlorophyll content of these plants were investigated.

Site 1 was situated in the upper reaches of the river adjacent to agricultural land, while site 2 was in an urban area, where industrial activities predominate. *C. demersum* (from an uncontaminated source) were introduced into the river at the two sites and compared with one another on a fortnightly basis over a 12 week period. Plants at site 2 were also compared to existing plants that were naturally growing at the site. Comparisons were also made between leaves and stems of the plants, to establish the organ of preference regarding metal accumulation and storage.

Samples were digested with nitric acid and an ICP-MS was used to analyse metal concentrations in the water, sediment and plants. Chlorophyll extraction was done using dimethyl sulphoxide (DMSO) and the absorbance values determined using a spectrophotometer. Chlorophyll a, chlorophyll b and total chlorophyll contents were recorded and compared. Cell membrane integrity was determined by leaving plants for 24 hours in deionised water and measuring electrical conductivity and solutes (sodium, calcium, potassium and magnesium) before and after placement of the plants.

The results of the study showed that all metals in the water were generally higher than the Target Water Quality Range (TWQR) set out by the Department of Water Affairs and Forestry (DWAF, 1996). The pooled data for the entire sampling period, showed that only zinc concentrations in the water samples were significantly higher at site 1 compared to site 2 ($P<0.05$). For sediment though, all metals at site 1 were significantly higher than at site 2 ($P<0.05$). It is likely that zinc contamination from surrounding farms at site 1 contributed to these high levels of zinc in the water, originating most likely from pest management practices, fertilizers or animal food supplements.

The study found that *C. demersum* bioaccumulated metals rapidly and consistently over the 12 week study period, mostly in the leaves, probably due to the leaves having a larger surface area than the stems or being a detoxification strategy of the plant. The introduced plants at site 1 accumulated metals to higher concentrations than those at site 2 and all introduced plants accumulated metals to higher concentrations than the naturally occurring plants at site 2. These results are most likely due to higher bioavailability of metals at site 1 and the possibility of genetic adaptations in the existing plants at site 2.

The most solute loss (consisting of all possible solutes, not only the four ions tested) was experienced by the site 2 introduced plants, followed by the introduced plants at site 1. The reference plants (not exposed to a contaminated site) absorbed solutes, in contrast to all the sampled plants in the Diep River, indicating the loss of cell membrane integrity of all the *Ceratophyllum sp.* sampled from the Diep River. The reference plants gained sodium, potassium, calcium and magnesium after 24 hours. For sodium and potassium, the ions leaked out of the Diep River plants. But for calcium and magnesium, most results indicated that the plants actually absorbed ions from the water after the 24 hour period. If the responses of the plants are compared with the reference plants, only sodium and potassium had comparable results, probably due to their monovalent nature. These results can be attributed to the generally high levels of contaminants found in the Diep River and in the plants themselves, during the course of the study.

At site 2, where both existing plants and introduced plants could be compared, generally, when copper increased in the leaves of the plants, the chlorophylls also increased in the introduced plants but showed a decrease in the existing plants. There was no decline in chlorophyll production over the 12 week period for any of the naturally occurring plants at site 2, however the existing plants had significantly lower chlorophyll concentrations in the leaves compared to the introduced plants. It is possible that certain metals bioaccumulated over time by the existing plants may have contributed to the loss of chlorophyll production due to metal phytotoxicity and oxidative stress. Both introduced and existing plants at site 2 showed significant increases in all chlorophyll groups between the first and last sampling occasions, compared to site 1 where no significances between the first and last sampling occasions were recorded, from the beginning to the end of the study period. As the existing plants showed no marked fluctuations in chlorophyll concentrations over the study period, using chlorophyll readings as a biomarker for metal exposure in the environment needs further research before final conclusions can be drawn.

There was not enough evidence to conclude that *C.demersum* could be effectively used as a biomonitor species, however the study showed the importance of using submerged aquatic plants in the monitoring of metal pollution in rivers and particularly the Diep River, Western Cape. The study also emphasised the need to monitor water quality more closely in the upper reaches of the river and the realisation that agricultural activities have a severe impact on the aquatic environment of the Diep River.

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GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
Acute Effect Value (AEV)	The level of an ingredient at which there is expected to be a significant probability of acute toxic effects to up to 5% of the community. If this situation occurs frequently then this could result in the death of a species or community (DWAF, 1996).
Bioaccumulation	The increase in concentration of a substance in exposed organisms over time (usually increasing over time and with age) (Wright & Welbourn, 2002).
Bioindicator	An organism or part of an organism that contains information on the quality of its environment Passive bioindicators/biomonitors are organisms that already exist in the environment, while active bioindicators/biomonitors are organisms that are exposed to a certain environment for a defined period of time (Siebert et al., 1995).
Biomarker	Any biological response to an environmental chemical below individual level, measured inside an organism or in 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).
Biomonitor	An organism or part of an organism that quantifies the quality of its environment. This is done by reacting to certain changes in the environment which can be measured, like changes in its morphology or physiology of its metabolism. The organism reflects the exposure of the contaminant in the environment. Also refer to bioindicator. (Wright & Welbourn, 2002).
Chronic Effect Value (CEV)	The level of an ingredient at which there is expected to be a significant probability of measurable chronic effects that would influence up to 5% of a species in an aquatic community. If this situation occurs frequently then this could result in the death of individuals or sensitive species (DWAF, 1996).
Canadian Sediment Quality Guidelines (CSQG)	Guidelines for sediment quality set out by the Canadian government for the protection of aquatic life (CCME, 2001).

Phytoremediation	The removal of contaminants and toxic waste from the environment by plants. The plants can then be harvested and discarded or metals can be extracted for a specific use (Salt et al., 1995).
South African Water Quality Guidelines (SAWQG)	A document published by the Department of Water Affairs and Forestry setting out standards for water quality criteria in South Africa, first edition, 1996 (DWAF, 1996).
Target Water Quality Range (TWQR)	A range of concentrations within which no adverse effects are expected on the health of an aquatic ecosystem. These levels are determined by various quantitative and qualitative criteria and are set out by The Department of Water Affairs and Forestry (DWAF), as a means of determining a healthy aquatic ecosystem. DWAF strives to maintain the aquatic environment within the TWQR guidelines (DWAF, 1996).

CHAPTER ONE

INTRODUCTION & LITERATURE REVIEW

1.1 Metal pollution in rivers

Rivers are natural water channels that provide pathways for rainfall to be carried from an area of higher altitude to an outlet at a lower altitude, which in many cases is the ocean (Dobson & Frid, 1998). Water is one of the most important natural resources and many diverse demands are placed upon it. Skillful management of river systems is therefore of utmost importance in order to maintain a successful integrated aquatic environment that caters for all the modern demands that are placed on the water systems of our cities (Rashed, 2002). Rivers play an important part in our ecosystems, providing a habitat for plants and animals and play a vital part in various food chains. They provide a source of fresh water vitally important for life itself and offer places for recreational activities like canoeing or swimming, while being an aesthetically pleasing environment, offering peace and tranquillity (River Health Programme, 2003).

As an area becomes urbanised and the demand for fresh water exceeds its local supply and water is sourced from further afield, the rivers in that area change in nature and form, from being a supplier of fresh water to rather a means of removing unwanted wastewater (Brown & Magoba 2009). This often results in polluted river systems that struggle to maintain biodiversity.

Metals are metallic chemical elements in the environment that have a relatively high density, cannot be degraded or destroyed and at fairly low concentrations are deemed toxic or poisonous to both animal and plant life (Landner & Reuther, 2004). Metals are natural substances usually found in lower concentrations in sediment and river water according to the underlying bedrock of a specific river. In lower concentrations, some have nutritional value to plants, like copper, iron and zinc. These are also known as trace elements and are essential to maintain life but may become toxic if accumulated over a long period of time or introduced into the environment at high dosages (Smith, 1986). Non-nutrient metals like cadmium and lead have no function in maintaining life and are not required for plant life (Caussy et al., 2003). Although metals are natural components of the earth's crust and may naturally enter bodies via the air, water or food, in small quantities these metals may accumulate over a prolonged period and become lethal (Kucera et al., 2008). It is the non-nutrient forms of metals that pose the highest threat to the ecosystem but with most metals, it depends very much on the dosage, solubility and form of the metal

as to its degree of toxicity. It is human activities that are responsible for the dramatic increase in metals within river systems, to levels that are undesirable and highly toxic to most forms of life (Hill, 2010).

Many rivers have become contaminated with metals from unnatural sources such as industries (mining, paint and dye manufacture, leather tanning, rubber, textiles, paper factories), waste water treatment works and agricultural activities (fertilisers and pesticides). Other sources of metal contamination in rivers can be aligned to domestic wastewater that contains metal-containing cleaning products, as well as mining activities and acid rain. Natural sources like bedrock formations in the riverbed may also contribute to the metals being available in the sediment and water (Rashed, 2002).

As urbanisation occurs water flow in rivers increases during periods of high rainfall due to the impact that buildings and hard surfaces have on the water runoff which causes water flow to increase, often resulting in erosion and flooding. To control erosion and the increase in water volumes during periods of high rainfall, rivers are often canalised. For example, 75% of the length of the Diep River Western Cape is canalised (Brown & Magoba, 2009). Canalisation results in a dramatic decrease in naturally occurring riparian vegetation that results in rivers not being as affective in naturally filtering out pollutants, resulting in a reduction in water quality. Aquatic plants have been particularly well documented for their abilities to act as filters in reducing the impact of metal pollution in the river water by filtering out metals from the water (Dushenkov et al., 1995; Sawidis et al., 1995; Schneider & Rubio, 1999; Lee & Scholz, 2007).

1.2 The Diep River and metal pollution

The Diep River in the Western Cape, originates in the Perdeberg and Riebeek-Kasteel Mountains, to the north-east of Malmesbury and flows about 80 km to the Atlantic ocean near Milnerton, becoming a broad extensive vlei, called Rietvlei (Brown & Magoba, 2009, Water Institute of Southern Africa, 2009) (Figure 2.4). The Diep River has one main tributary, the Mosselbank River, originating in Durbanville Hills, then various other tributories, namely the Swart, Groen, Klein, Riebieek and Klapmuts rivers that all become part of the Diep River (DWAF, 2002; Brown & Magoba, 2009).

In 2008, an Estuary Management Plan for the Diep Estuary was prepared for the City of Cape Town and it highlighted the problems of pollution in the lower parts of the river stating that the main sources of pollution came from the various waste water treatment

works situated along the river, of which Malmesbury was not meeting the required standards at the time of the study. It also mentioned that storm water from urban areas, agricultural activities like fertilizer and pesticide runoff and cattle manure, as well as mining as being the main sources of pollution. Rapid urbanisation in Cape Town has led to an increase in farming, resulting in the building of more dams in the upper reaches and dredging and industrial activities have changed the characteristics of the Diep River over time and influenced its general makeup (Coastal & Environmental Consulting, 2011).

Shuping et al. (2011) found that metal concentrations of aluminium and zinc in the lower parts of the Diep River, were well over the Target Quality Guidelines for Aquatic Ecosystems (TWQR), set out by DWAF (DWAF, 1996) and concentrations of copper were also found to be high during summer. River pollution by metals, pesticides and industrial waste are a common problem in South African cities, and solutions need to be found to combat this problem before rivers become little more than a means of waste dispersal (DWAF, 2002; River Health Programme, 2003). The lower Diep River, Milnerton lagoon area, is directly affected by sewage effluent from the Potsdam Waste Water Treatment Works, which is situated close to the industrial area of Montague Gardens (Brown & Magoba, 2009).

In 1994, the former Department of Water Affairs and Forestry (DWAF) (now Department of Water and Environmental Affairs- DWEA), initiated the South African River Health Programme (RHP) which acknowledged the importance of protecting aquatic ecosystems by establishing a river monitoring system. In 2003 a 'State-of-Rivers Report' was released involving various organisations headed by DWAF. The report educates people on the condition of important South African rivers; included here is the Diep River, Western Cape. It reports on all environmentally important aspects of the rivers health, including water quality and river fauna and flora (River Health Programme, 2003).

Limited research has been done on metal concentrations in South African rivers (Okonkwo et al., 2005). Shuping et al. (2011) reported that much of the lower Diep River, Milnerton was contaminated with metals, in the water, sediments and in the roots of aquatic sedge, *Bolboschoenus maritimus*, a natural occurring sedge in the lower reaches of the river.

Human activities that impact on the increased concentrations of metals in a river are various and will depend on the industries or activities within the vicinity of the specific river. One source of metal pollution is mining. Metals are usually released into the atmosphere as particulates but can be deposited eventually onto water surfaces in this manner (Smol,

2002). Waste from mines often result in runoff that contain metals that end up in rivers after heavy rainfall or can be piped into the water system directly. Another source of metal pollution is from the burning of fossil fuels. When fossil-fuels like coal or petroleum are burnt, small amounts of metals are released into the air. As large quantities of coal are burned worldwide, these small quantities start to build up over time. The ash that is left after burning coal is more concentrated with metals and can easily be blown away or become part of runoff after rainfall and in this way enters the river system (Moss, 1988). Wastewater treatment plants pertaining to industrial or municipal waste are a major source of metal pollutants in waterways, whether it is from sewage treatment or the by-products of industry that are not required. The impact of these activities are very dependent on legislation, the adherement to the legislation, implementation of the legislation and the ability of the factory or municipality to maintain its equipment in good working order. A good reliable supply of electricity is also important to prevent any untreated effluent from entering the river (Coastal & Environmental Consulting, 2011).

Any construction site within close proximity to a river or even a tarred surface may contribute to the addition of metals in a river through water runoff. Dumped metal waste, as well as certain fertilisers, pesticides and animal waste can increase the natural metal content of waterways. Natural metal sources, volcanoes, veld fires and even sea spray may contribute to increased metal presence, however human activities are still the main cause for the unnaturally high load of metals that are found in rivers today (Hill, 2010).

In the upper reaches of the Diep River, north of Malmesbury, where farming activities like grain production, grape and dairy farming are prevalent, the river seems less polluted than in the lower regions (personal observation). Here, forms of pollution like fertilisers, pesticides and animal waste are expected to be the main contributing factors to river pollution. In this region many dams have been built to assist farmers in irrigating crops. Dams impact on the water flow of the river, allowing for less flushing out of pollutants. Dams have a negative impact on rivers by increasing water temperatures and decreasing the levels of oxygen in the water, resulting in poor water quality which often results in fish diseases and the deaths of certain invertebrate species (River Health Programme, 2003). Another possible contributing factor to pollution in the upper regions of the Diep River is a large wine co-operative, just outside Malmesbury on the riverbank, which could possibly add its waste to the river (personal observation).

The town of Malmesbury has a waste water treatment works which contributes to the river system in treated waste water. Malmesbury is a main town in the region, so it has a

certain percentage of that metal being available for an organism to take up (Connell et al., 1999). When the water pH is low (acidic), more metals become soluble and are easily taken up by organisms. If water pH is alkaline with a pH above 7, the total concentrations of metals in the water are inaccurate and tend to be underestimated (Smith, 1986).

Temperature and salinity in river water also plays a role in metal availability. This can be established by pH readings which relate to the bioavailability of metals to plants. An increase in temperature will lower pH slightly but the variation in temperature would need to be substantial for even a 0.5 point decrease. As for salinity, sea water has a pH reading of between 8-8.3, so if diluted with freshwater of a low pH, it could influence the pH and thus the bioavailability of metals (Smith, 1986).

If polluted water is added via pipes directly into the river without being properly treated or naturally filtered through aquatic plants, this may have detrimental effects on all the plants and animals associated with that water, resulting in eutrophic conditions which occurs when an excess of organic matter or nutrients is discharged into a slow flowing or stagnant part of a river. Eutrophication promotes excessive plant growth, especially that of algae which ultimately results in decay and excessive depletion of oxygen in the water. As a result, other organisms like fish die due to insufficient oxygen (Hargraves, 1991). Certain plant species flourish to the detriment of less prolific species often becoming extinct in the ecosystem since eutrophication disrupts the normal functioning of the ecosystem (Hill, 2010). Damming up of water in the upper reaches for irrigation purposes, negatively impacts the dilution and potential flushing out of the polluted water downstream and further contributes to the problem.

All metals are persistent and cannot be destroyed (Hill, 2010). The impact of high dosages of metals on human health can have immediate or a delayed detrimental effect on human health. Metal pollution in freshwater river systems has been actively monitored and researched (Gümgüm et al., 1994; Okonkwo, 2005; Meck et al., 2006; Ntengwe & Maseka, 2006).

1.4 Metals in sediment

Sediment can be a major source of metal pollutants in the aquatic ecosystem (Walker et al., 2006). Metals accumulate in both water and river sediment at different levels, due to varying levels of water pH (metals are more available in acid waters than alkaline waters) and the underlying geological rock formations also play an important role in the presence

of metals. Due to fluctuations of water discharge and the unknown time that the pollutant will reside in a specific water mass, it is always wise to analyse both water and sediment samples to determine metal concentration. Sediment analysis is thus an excellent, reliable way to interpret metal concentrations and thus the health of a river (Singh et al., 1997). The investigation and analysis of sediment to determine the impact that human activities have had on a river system with regards to metal pollution is well documented (Gümgüm et al., 1994; Singh et al., 1997; Samecka-Cymerman & Kempers, 2001; Gaynor & Gray, 2004; Chen et al., 2009).

It is recommended that sediment samples be taken at different times of the year due to the influences that pH, salinity, redox potential and organic chelators often have on the sediment in a river, as the metals may not be in a form that is readily available to aquatic organisms at a certain time of the year due to variance of the above mentioned factors (Soares et al., 1999). Metal concentrations in water and sediments can vary from week to week and do not indicate the bioavailability of the metals to aquatic organisms (Adekoya et al., 2006). A low concentration of a certain metal in sediment or water does not necessarily mean that an aquatic organism would also show a low concentration of that metal in their tissues and vise-versa (Connell et al., 1999). Bioavailability of metals released from sediment and bedrocks is complex and is interrelated with various chemical, environmental and biological processes (John & Leventhal, 1995). Analysis of data would be incomplete without the analysis of living organisms to establish a true indication of metal concentrations and bioavailability in the aquatic environment. The bioavailability of a metal is essential to the toxicity of that metal (John & Leventhal, 1995; Caussy et al., 2003).

1.5 Metal bioaccumulation in aquatic plants

Submerged aquatic plants (macrophytes) provide oxygen to organisms within the water body. Other aquatic plants such as reeds filter out pollutants in the water (Lee & Scholz, 2007). Some water plants are a source of food and provide shelter to certain organisms. Aquatic plants play an important role in the aquatic ecosystem. The aquatic environment is forever changing due to the pressures asserted on it by water pollutants which include metals and waste. Invasive aquatic plant species increase in number due to increased levels of nitrates and phosphates being introduced into the river system from organic waste. Some aquatic plants are known to be metallophytes, able to tolerate high levels of metals within their organs and are able to live in waters and sediments with high concentrations of metals. They either resist the metals by not absorbing them or tolerate the metals by detoxifying them or bioaccumulating them in their organs (Shrestha, 2003).

Metallophytes are thus potentially effective bioindicators of metal pollution (Pajević et al., 2008).

Many studies have been conducted on aquatic plants, with results indicating that often the aquatic plant accumulates far higher concentrations of metals than the surrounding medium. For example, Soares et al. (2008) reported on *Salvinia auriculata* (a floating aquatic plant), that it has the capacity to bioaccumulate large amounts of chromium in its leaves. Demirezen & Aksoy (2006) found that many aquatic plants bioaccumulate more metals in their organs than what is found in the surrounding environment.

Research into bioaccumulation of metals by aquatic plants has been well documented for various metals (Samecka-Cymerman & Kempers, 2001; Aksoy et al., 2005; Kara, 2005; Marques et al., 2007; Deng et al., 2008; Peng et al., 2008; Hu et al., 2010). In most of these studies it was found that the roots of the aquatic plants were able to bioaccumulate metals more readily than that of the leaves. Research involving submerged macrophytes as bioaccumulators of metals within their tissues (Cardwell et al., 2002.; Duman et al., 2006; Fritioff & Greger, 2006; Deng et al., 2008; Peng et al., 2008) only tested leaves, as in many cases aquatic macrophytes do not display true roots.

1.6 *Ceratophyllum demersum* L. as a bioindicator of metal pollution in the Diep River

A bioindicator is an organism that contains information on the quality of the environment (Markert et al., 1999). *C. demersum* is a fast growing aquatic macrophyte, common throughout rivers and lakes of the world. It is a submerged free floating plant and is found in the lower parts of the Diep River where the water is stagnant or very slow moving. Studies have shown that certain aquatic plants like mosses (Siebert et al., 1996) and aquatic macrophytes may be used as bioindicators of metal pollution in the aquatic environment (Grasmück et al., 1995). *C. demersum* was established by Robinson et al. (1995) to be a bioindicator of arsenic pollution in aquatic environments and Robach et al. (1996) studied aquatic macrophyte communities (which included *C. demersum*) to determine if they could be used as bioindicator species of eutrophication in a river system in France. *C. demersum* was also one of the bioindicator species used in the study by Kaglyan et al. (2005) to establish radionuclide contamination in aquatic systems within the Chernobyl nuclear reactor exclusion zone.

1.7 C*eratophyllum demersum* L. as a biomonitor in freshwater ecosystems

Many studies have specifically focused on heavy metals in *C. demersum*, as this plant has been proven to be a bioaccumulator of metals such as lead, cadmium, copper and zinc (Gupta & Chandra, 1996; Keskinkan et al., 2004; Kumar & Prasad, 2004; Mishra et al., 2006). *Ceratophyllum* spp. and other aquatic macrophytes are ideal biomonitors of aquatic pollution as they accumulate metals in their organs and reveal the health of the environment through metal bioaccumulation. Aquatic macrophytes are therefore ideal biomonitoring organisms of metal pollution in the aquatic environment as they are visible, plentiful, sedentary, and easy to collect and as they bioaccumulate metals in their organs are able to tolerate high concentrations of metals within their systems which reflect the contamination of the river that they reside in (Milan et al., 2006).

A biomonitor is an organism that holds information on the quantitative details of the quality of that environment over an extended period of time (Markert et al., 1999). Ramadan (2003) used various aquatic plants as biomonitors of metal pollution in Lake Manzala in Egypt and compared results with the sediments.

Using biomonitors to analyse pollution gives a more accurate account of the polluted environment over time. Biomonitoring may be performed actively (known as active biomonitoring), when organisms are bred specifically in a laboratory or taken from an unpolluted site and placed in a polluted area for a predetermined period and then later analysed or be done passively, when organisms in a polluted area reside in that environment and are analysed for metals or contaminants (Markert, et al., 1999). A combination of active and passive biomonitoring of a polluted site would only add to comparative analyses and broaden the scope of research. Active biomonitoring has been applied in research in freshwater environments (Wepener et al., 2005) and in other applications for many years (Wegener et al., 1992). *C. demersum* has proven to be a successful biomonitor of metal pollution due to its excellent capacity to bioaccumulate metals over long periods of time (Butterworth et al., 2000; Milan et al., 2006; Pajević et al., 2008) and its adaptability to polluted aquatic environments.

1.8 Metal toxicity in plants

Metals are introduced into the aquatic environments due to weathering of soils and rocks, volcanic activity and human activities that involve metals. Metals are unable to be broken down into less harmful components and may be absorbed by plants through their roots

(via sediment or soil) or leaves (via water through stomata). Some metals are required by plants in small quantities as nutrients to sustain metabolic processes within the plant, however at large dosages these metals become toxic to the plant and have detrimental effects on plant growth. Different plant species are able to acquire different nutrients from various environments, where one plant will suffer nutrient deficiencies or even die, another will thrive or be able to sustain a healthy existence, and this is due to genetic adaptations (Lambers et al., 2008).

Aquatic plants are good bioaccumulators of metals in the aquatic environment and absorb metals and nutrients via soil, sediment or water or often through more than one route. Aquatic macrophytes bioaccumulate metals at a much higher concentration than that of their surroundings and some species of plants have adapted to harsh polluted conditions by doing this (Zurayk et al., 2001).

Certain metals are not required by plants for normal healthy development and plants that grow in certain soils do not discern between heavy metals and their required elements in their mineral uptake and tend to accumulate metals within the plant tissues (Hill, 2010). It has been well documented that certain plants, including many aquatic plants, exhibit cell membrane damage as a result of metal accumulation (Quartacci et al., 2001; Souza-Santos et al., 2001; Tripathi et al., 2003; Kumar & Prasad, 2004; Sinha et al., 2005; Liu et al., 2007; Panda, 2007). Several researchers have found that accumulated metals, in plants, could induce lipid peroxidation (e.g. Sinha et al., 1997; Souza-Santos et al., 2001; Tripathi et al., 2003; Sinha et al., 2005; Liu et al., 2007). This leads to lowered membrane integrity (Souza-Santos et al., 2001; Panda, 2007) which in turn, results in the leakage of essential ions such as potassium, from the plant cells (Sinha et al., 1997; Quartacci et al., 2001). Kumar & Prasad (2004) documented that there was a significant increase in electrical conductivity (EC) of the water medium holding the aquatic plant *C. demersum* when 2.5 µM lead was added to the water. Concentrations of sodium, potassium and calcium ions were recorded which indicated that the increase in EC was due to ion leakage caused by membrane damage resulting from the lead exposure. As the concentrations of lead were increased, the EC and leakage of sodium, potassium and calcium became higher.

Lipid peroxidation as a result of metal accumulation, has also been shown to affect stages in the biosynthesis of chlorophyll (Tripathi et al., 2003), thus, resulting in significantly lowered chlorophyll contents in such plants (Sinha et al., 1997; Tripathi et al., 2003; Sinha et al., 2005; Liu et al., 2007). Particularly in aquatic plants it has also been documented

that they too experience reduced chlorophyll production (Sinha et al., 1997; Sinha et al., 2005; Shakya et al., 2008; Ebbs & Uchil, 2008) due to metal toxicity. Sinha et al. (2005) documented that the chlorophyll content in *Pistia stratiotes* L. (a floating aquatic plant) decreased with increases in chromium concentrations and exposure periods.

1.8.1 Aluminium

Aluminium (Al) is an abundant metal in the earth's crust but it is toxic to plants if absorbed, as it has no known biological function (Wright & Welbourn, 2002). Aluminium enters rivers and freshwater systems, if not naturally through erosion of rock then via industries and mines that process or use aluminium. Aluminium is more available in acidic soils and if absorbed by the plant, causes potential injury to the cell wall, plasma membrane, signal-transduction pathways, root cytoskeleton and DNA (Lambers et al., 2008). Ryan et al. (1994) discovered that aluminium toxicity adversely affected root elongation in plants. Aluminium also prevents the uptake of calcium (Ca) and magnesium (Mg) by the plant due to blockages that it causes in the plasma membrane, preventing the uptake of these vital microelements. Calcium is required by the plant during cell division thus, aluminium prevents the cells from dividing (Kochian et al., 2005). According to these authors, by preventing the uptake of magnesium, aluminium causes the plant to exhibit typical symptoms of magnesium deficiency like chlorotic leaves with brown spots.

Aluminium toxicity in aquatic plants also poses a threat to organisms that consume these plants and other organisms higher up in the food chain, resulting in health problems. Aluminium may also negatively affect the availability of phosphates in the environment to other aquatic organisms (DWAF, 1996).

1.8.2 Copper, iron and zinc

Copper (Cu), iron (Fe) and zinc (Zn) are considered as heavy metals but also essential plant micronutrients. As plants only require these three metals in very small quantities, larger concentrations would be toxic to the plant (Lambers et al., 2008). Copper is found naturally in the environment and is a sought after metal in industry and agriculture, thus it is released both naturally and from human activities into the environment. Copper is associated with mines, industry, landfills and waste disposal. Most water–soluble copper is due to agricultural runoff, as copper is an important ingredient of many fungicides. Copper does not break down and accumulates in plants and other organisms. It decreases the biodiversity of flora in an area contaminated by excess copper. It negatively affects micro-

organisms and earthworms in the soil (Walker et al., 2006). It has been proven that many aquatic plants bioaccumulate copper and are ideal candidates for reducing the amount of copper in wastewater treatments (Kara & Zeytunluoglu, 2007), as copper in drinking water, is highly toxic in high concentrations to both animals and humans. Other studies by Hu et al. (2010) Sánchez-Viveros et al. (2010) and Samecka-Cymerman & Kempers (2004), all found aquatic plants to bioaccumulate copper. Sánchez-Viveros et al. (2010) also established that high concentrations of copper affected membrane permeability in two *Azolla* species (floating aquatic plants), resulting in electrolyte leakage from cells. Copper toxicity was also reported in *Lemna gibba*, an aquatic floating duckweed (Megateli et al., 2009) when excessive copper caused a reduction in growth. Similarly, Babu et al. (2003) found that high concentrations of copper resulted in a decreased growth rate, caused by a reduction in chlorophyll production which also resulted in leaf necrosis.

A study by Devi & Prasad (1998) on *C. demersum* (an aquatic macrophyte) established that copper toxicity increased lipid peroxidation and decreased the chlorophyll content in the cells. Although copper is essential in plants as it forms a component of enzymes (Salisbury & Ross, 1985), high dosages have detrimental effects on the plant (Devi & Prasad, 1998).

Iron is the most useful of all metals as its alloy is steel, which is a highly versatile metal, used in the manufacturing of cars, food containers, cargo ships and paper staples (DWAF, 1996). Due to its abundant usage, it is expected that iron would constitute a large proportion of metal pollution in the environment. In humans iron forms an essential part of haemoglobin in the blood (Maton et al., 1993). In plants, iron is involved in chlorophyll synthesis (Salisbury & Ross, 1985).

Research by Gallego et al. (1996) found that high concentrations of iron in young sunflower leaves resulted in oxidative damage to the leaves as well as decreased chlorophyll production. Although some studies have proven that aquatic plants generally are bioaccumulators of iron (Demirezen & Aksoy, 2006), evidence by Babovic et al. (2010) concluded that *C.demersum* accumulated the highest amount of zinc, copper and iron in its tissues compared to other macrophytes used in the study of a fishpond in Serbia. Rashed (2002) also found in a study of three aquatic plants from the Nile River, that *C. demersum* accumulated most of the metals that were tested and was considered to be an excellent biomonitor of metal pollution.

Zinc has many applications in industry from galvanised steel to plastics and wall paper. It is the 23rd most abundant metal on earth (DWAF, 1996). Zinc contamination of water bodies is associated with the wastewater from mining and industry where zinc is a component of the end product. Zinc has a tendency to increase the acidity of waters which could cause some plant species to die and often soils polluted with zinc have little plant diversity. Zinc negatively impacts on soil micro-organisms and earthworms, causing the process of organic matter breakdown to be hindered (Landner & Reuther, 2004). In plants, zinc plays an important role in the activation of enzymes (Salisbury & Ross, 1985), an essential micronutrient and a stabilizer of proteins, membranes and DNA-binding proteins (Aravind & Prasad, 2004).

Zinc is bioaccumulated in aquatic macrophytes, as discussed by various researchers (Fritioff & Greger, 2006; Nyquist & Greger, 2007; Khellaf & Zerdaoui, 2009; Megateli et al., 2009). Aquatic plants are able to survive very high concentrations of zinc, showing little or no visual signs of toxicity. At 4 mg/L zinc, *Lemna gibba* (a floating duckweed) showed a reduction in growth and although chlorophyll production was reduced at 30 mg/L, no visual signs of toxicity were evident, proving the high threshold that this plant has for zinc concentrations within its tissues (Megateli et al., 2009). It has also been proven that zinc plays an important role in reversing cadmium toxicity in *C. demersum* (Aravind & Prasad, 2005).

Certain plant species employ specific mechanisms to counteract the toxicity of specific metals. Ultimately each plant species will endure the stress that metal toxicity exerts upon it, up to a certain point and every plant species will be able to cope with different levels of metal toxicity according to various interacting factors within the environment, before it can no longer tolerate the situation and dies (Hall, 2002).

1.9 Using toxic responses of plants as biomarkers

A biomarker is any biological response to a chemical (or metal) at the individual level, demonstrating a departure from normal status. This is usually restricted to responses at the level of organisation of the whole organism or below (Walker et al., 2006). Certain responses of plants to their environment often indicate the health and quality of that environment. Biomarkers are responses that can be measured in various organs of the plant (Walker et al., 2006). A biomarker should exhibit certain valuable characteristics like being specific, sensitive, and easy to use and have non-destructive sampling procedures (Walker, 1995; Ferrat et al., 2003).

Responses of plants to metals on a suborganismal level are increasingly being used as biomarkers to indicate the health of an environment and the degree of metal pollution in that environment (Frankart et al., 2002; Li & Xiong, 2004; Mukherjee et al., 2004; Dewez et al., 2005; Wepener et al., 2005; Pavlikova et al., 2008). Wepener et al. (2005) studied a freshwater mollusc and fish, Dewez et al. (2005) an alga and Pavlikova et al. (2008) spinach. The first three authors (Frankart et al., 2002; Li & Xiong., 2004; Mukherjee et al., 2004) used duckweed (*Lemna* spp.), a floating aquatic plant to conduct their biomarker studies.

Toxins affect organisms' integrity at the biochemical level and individual level which results in poor growth, reproduction and survival which ultimately affects the ecosystem (Wepener et al., 2005). Important research was carried out by Ferrat et al. (2003) on the use of biomarkers in aquatic plants in order to evaluate environmental quality. Aspects that affected aquatic plants on the organismal level were: modification of photosynthetic activity, which included chlorophyll fluorescence and photosynthetic pigment concentration; enzymatic activities related to nutrients; heat shock proteins; phenolic compounds; oxidative stress biomarkers which included oxidative burst, lipid peroxidation and antioxidant enzymes and molecules and lastly biomarkers of detoxication which included phytochelatins, biotransformation enzymes, phase I and II enzymes. Three responses that researchers often use as biomarkers in aquatic plants are: photosynthetic activity, stress protein synthesis and oxidative stress (Butterworth et al., 2000).

1.9.1 Photosynthetic activity

An increase in chlorophyll should have a positive influence on photosynthesis, which in turn is associated with plant growth and well being (Haberlandt, 1914), however there are conflicting opinions regarding the relationship between chlorophyll content and photosynthesis, as reported by Benedict (1972), who found that several flowering plant mutants had lower chlorophyll readings than the same green varieties but, had higher photosynthetic rates. There is also proof that photosynthetic rate depends on plant species and that this variation is not related to chlorophyll content (Hesketh, 1963).

A decrease in chlorophyll is often associated with metal pollution in aquatic environments (Sinha et al., 1997; Devi & Prasad, 1998; Babu et al., 2003; Sinha et al., 2005) and can be used as a biomarker for metal presence in the water. By measuring chlorophyll

fluorescence, researchers are able to establish the biochemical and physiological state of the plant (Ferrat et al., 2003).

Lipid peroxidation as a result of metal accumulation, has also been shown to affect stages in the biosynthesis of chlorophyll (Tripathi et al., 2003), thus resulting in significantly lowered chlorophyll contents in such plants (Sinha et al., 1997; Tripathi et al., 2003; Sinha et al., 2005; Liu et al., 2007). It has been documented that many aquatic plants have reduced chlorophyll production due to metal toxicity (Sinha et al., 1997; Sinha et al., 2005; Ebbs & Uchil, 2008; Shakya et al., 2008). Sinha et al. (2005) documented that the chlorophyll content in *Pistia stratiotes* L. (a floating aquatic plant) decreased with increases in chromium concentrations and exposure periods.

Aquatic macrophytes serve as convenient models for the assessment and monitoring of toxic metals (Prasad et al., 2001). Devi & Prasad (1998) proved that copper accumulation in *Ceratophyllym demersum* L. plants caused increased oxidative stress. Kumar & Prasad (2004) used *C.demersum* (which is a submerged, free–floating rootless aquatic macrophyte that grows worldwide in fresh water systems) as a model species for laboratory toxicity bioassays.

1.10 Statement of the research problem

The Diep River, Western Cape, originates in the Perdeberg and Riebeek-Kasteel Mountains, to the east and north of Malmesbury and flows in the region of 65 km to the Atlantic Ocean near Milnerton (Brown & Magoba, 2009). Due to rapid urbanisation that required ways of waste removal and increased farming activities that required additional irrigation water (building of dams), the Diep River is unfortunately no longer a vision of beauty but rather a polluted river system in most parts. The estimated ecological health of the Diep River in the year 2002/2003 was considered to be good in the upper reaches and poor in the lower reaches (River Health Programme, 2003). Due to increased urbanisation, eight years later these results may not be considered valid. Shuping et al. (2011) found the Lower Diep River sediment to be highly polluted with metals and tests conducted on *Bolboschoenus maritimus*, a naturally growing sedge, showed high concentrations of metals in its roots. As the water in the river in the lower reaches is very alkaline and as most metals are bioavailable to organisms mostly at a low pH, one cannot take water sampling alone as a reliable way of measuring metals in the river. It is important to test aquatic plants for metal concentrations, as they reflect the health of the water body more accurately (Grasmück et al., 1995; Siebert et al., 1995; Ramadan, 2003). Although aquatic

plants have been well documented to accumulate metals, not much research has been conducted on aquatic plants in river systems in South Africa and how metal toxicity affects them. Even fewer studies have been conducted on aquatic plants of the Diep River, Western Cape. *C. demersum* has a worldwide distribution and is well documented as being a bioaccumulator of metals (Gupta & Chandra, 1996; Devi & Prasad, 1998; Cardwell et al., 2002; Aravind & Prasad, 2005). *C. demersum* was considered a possible ideal choice of plant species for metal pollution studies in the Diep River, as it occurs naturally in the stagnant waters of the lower reaches and bioaccumulation studies have not been performed on this plant in the Diep River. The identification of reliable, quick, easy and effective biomarkers of metal exposure is important, as is the identification of reliable test species. These may in future be used as part of standard toxicity assessment studies.

1.11 Research objectives

The objectives of this study are outlined as follows:

- To determine the degree of metal contamination in water, sediments and introduced *C. demersum* at two different sites in the Diep River.
- Comparing existing *C. demersum* growing in the river with introduced plants to determine metal bioaccumulation.
- To determine the organ of bioaccumulation in *C. demersum* ie. stems or leaves.
- To determine the effect of accumulated metals on membrane integrity of *C. demersum*.
- To determine the effect of accumulated metals on chlorophyll content of *C. demersum*.
- To ultimately determine whether *C. demersum* is an effective biomonitoring species for metal pollution in the Diep River and whether the above-mentioned responses can be used as biomarkers of metal exposure.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Site selection and plant choice

The Diep River originates in the Perdeberg and Riebeek-Kasteel Mountains north east of Malmesbury near Riebeek Kasteel in the South Western Cape, South Africa, The river flows for about 80 km in a southwesterly direction, passing through the town of Malmesbury, suburb of Table View and Rietvlei Wetland Reserve before forming an estuary, known as Milnerton Lagoon bordering the Atlantic Ocean (Figure 2.4). Various tributaries join the Diep River including the Klein, Swart, Platklip, Groen, Sout and Mosselbank Rivers (River Health Programme, 2003; Brown & Magoba, 2009; Water Institute of Southern Africa, 2009).

The upper parts of the Diep River are surrounded by predominantly grain and grape farms but dairy and sheep farms are also evident (personal observation). Vissershok, a large landfill site that receives hazardous waste, is located a short distance from the river south of Malmesbury. There are three Wastewater Treatment Works adjacent to the river in Malmesbury, Kraaifontien and Table View that discharge treated effluent into the river (River Health Programme, 2003). The lower parts of the Diep River are surrounded by residential areas, informal settlements and industries that include a large oil refinery (personal observation).

An inspection of the upper Diep River was undertaken, near its source in the Kasteel Mountains, to establish which plants were common to both the upper and lower regions of the river. A suitable plant species was required that could be tested as a possible model for biomarker studies, with reference to chlorophyll production and possible cell membrane damage resulting from metal pollutants within the water. It was important that the chosen sites were perennial sources of water as some parts of the upper regions of the river flowed only seasonally and were very shallow and narrow. Jackson et al. (2009) experienced problems relating to certain parts of the Diep River drying up during part of their study.

C. demersum was observed growing in the lower reaches of the Diep River, near Gill Road, Table View, behind the McPhearson's Garden Centre in a relatively slow moving to stagnant waterbody. This part of the Diep River was considered to have above average

levels of zinc, aluminium and iron in the water and high levels of metals in the sediments (Shuping, 2008; Shuping et al., 2011). As *Ceratophyllum* spp. naturally occur in slow moving waterbodies, this plant did not occur in the faster flowing, upper reaches of the river north of Malmesbury (site 1), so it was deemed necessary, as part of the study, to introduce the plant in a controlled manner to an area in the upper reaches of the river, with fewer possible sources of metal pollution and to a site in the lower reaches (site 2) in Table View, in order for data to be collected and compared.

Site 1 was located 2 km off the R45 road at a turnoff to a B&B on the Riebeeks River Rd (The Roundstone Guesthouse) off the road to Riebeek Kasteel from Malmesbury, GPS co-ordinates S 33° 22' 42.0" & E 18° 49' 52.7" (Figure 2.1 & Figure 2.2). It is approximately 200 m above mean sea level and about 8 km from the source of the river (Figure 2.4) (Water Institute of Southern Africa, 2009). This is in an agricultural farming area of mostly grain crops and grapes but sheep and dairy farming also occurs. The water is visually clean, slightly murky and fast flowing. There is riparian vegetation present that shades the river in parts. A few exotic invader plant species do occur amongst the riparian vegetation (eg. *Ricinus communis*, *Quercus robur*). The river at this point is about 3 meters wide and strongly flowing. Its depth is very uneven as at some points it is up to 1m in its deepest parts and just a few centimetres in the shallow areas. Sand banks are evident along the sides where no water flows, indicating that the river level varies greatly.

The second site (called site 2) was located at the end of Gill Road, Table View, behind the McPhearson's Garden Centre where *C. demersum* grows abundantly (GPS co-ordinates S 33° 56' 20.3" & E 18° 31' 01.9"). It is approximately 5-10 m above mean sea level and approximately 8 km from the ocean (Figure 2.4) (Water Institute of Southern Africa, 2009). This site is a known contaminated site according to Shuping (2008) (Figure 2.1 & Figure 2.3). Visually the existing *C. demersum* in the river seem healthy and well established, even though the water is very murky. One cannot see the bottom of the river as the water is very dark in colour. The flow of the river is very slow (personal observations).

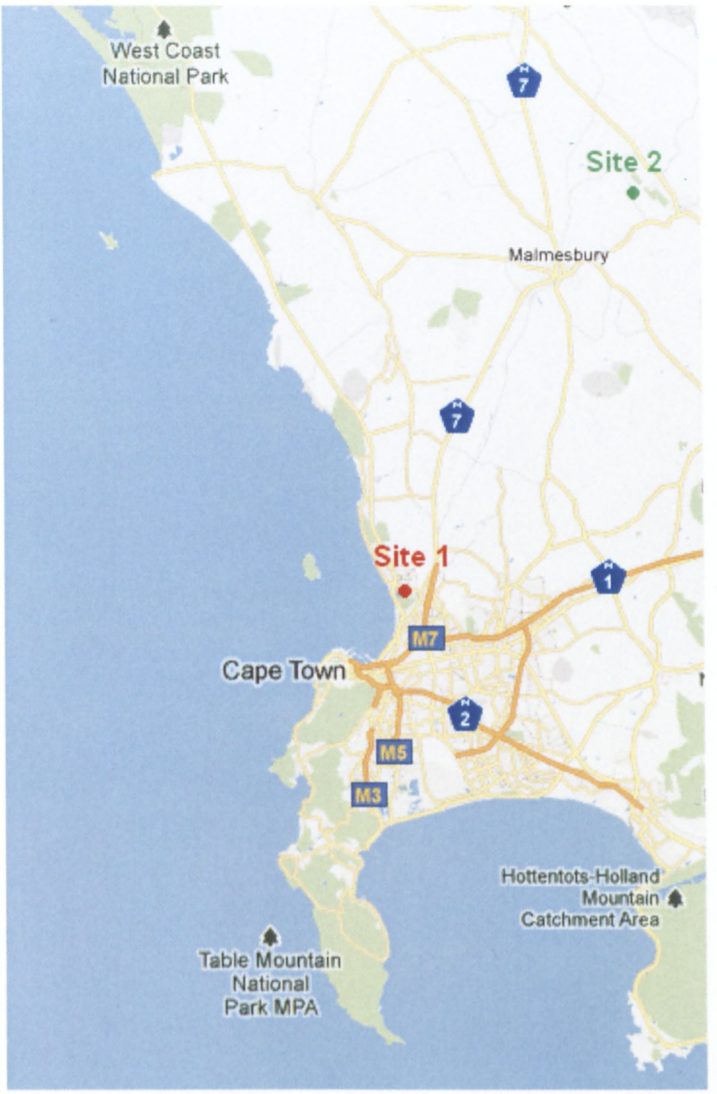

Figure 2.1: Map of Western Cape showing the positions of site 1 and site 2 in the Diep River (Tele Atlas, 2011).

Figure 2.2: Map of Riebeeck Kasteel region (north east of Malmesbury) showing the location of site 1 (Tele Atlas, 2011).

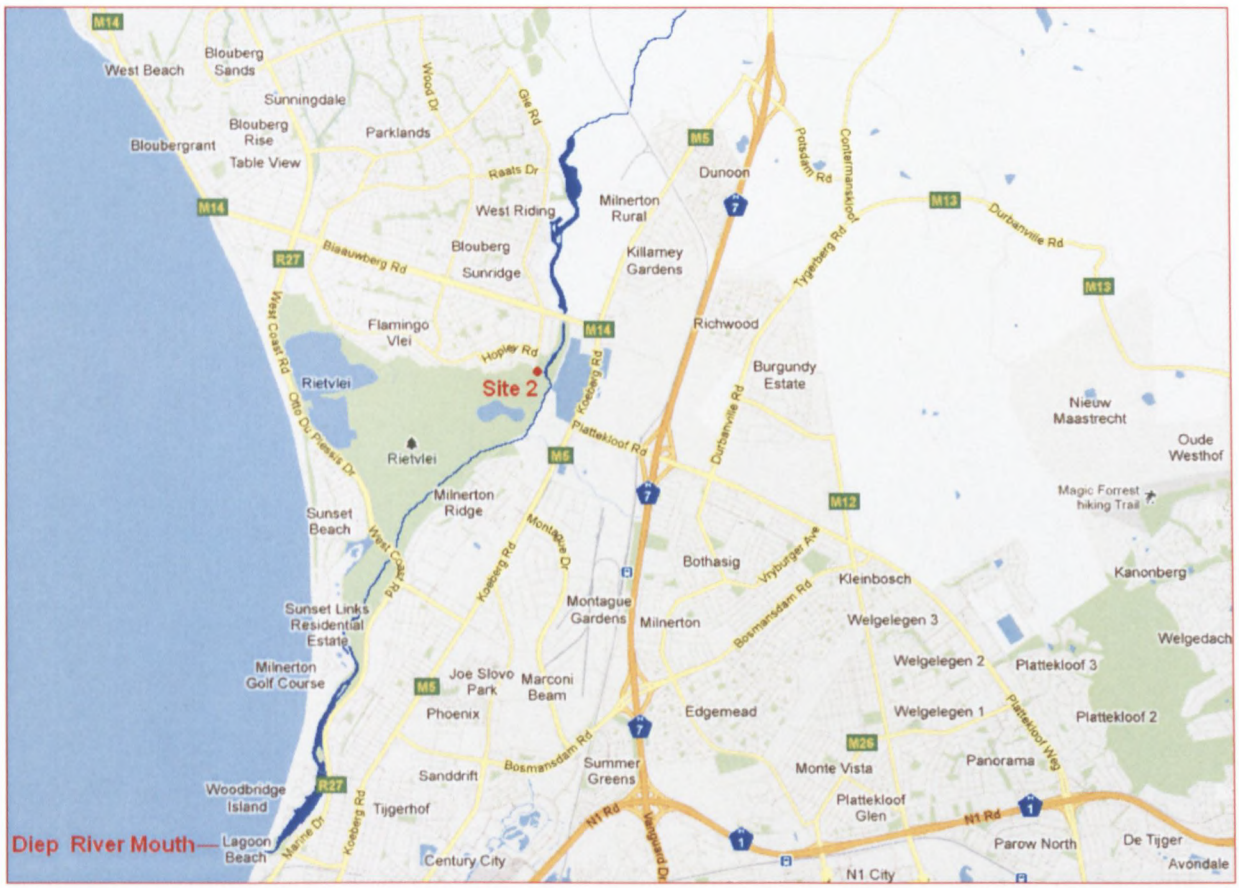

Figure 2.3: Map of Table View region (Milnerton) showing location of site 2 (Tele Atlas, 2011).

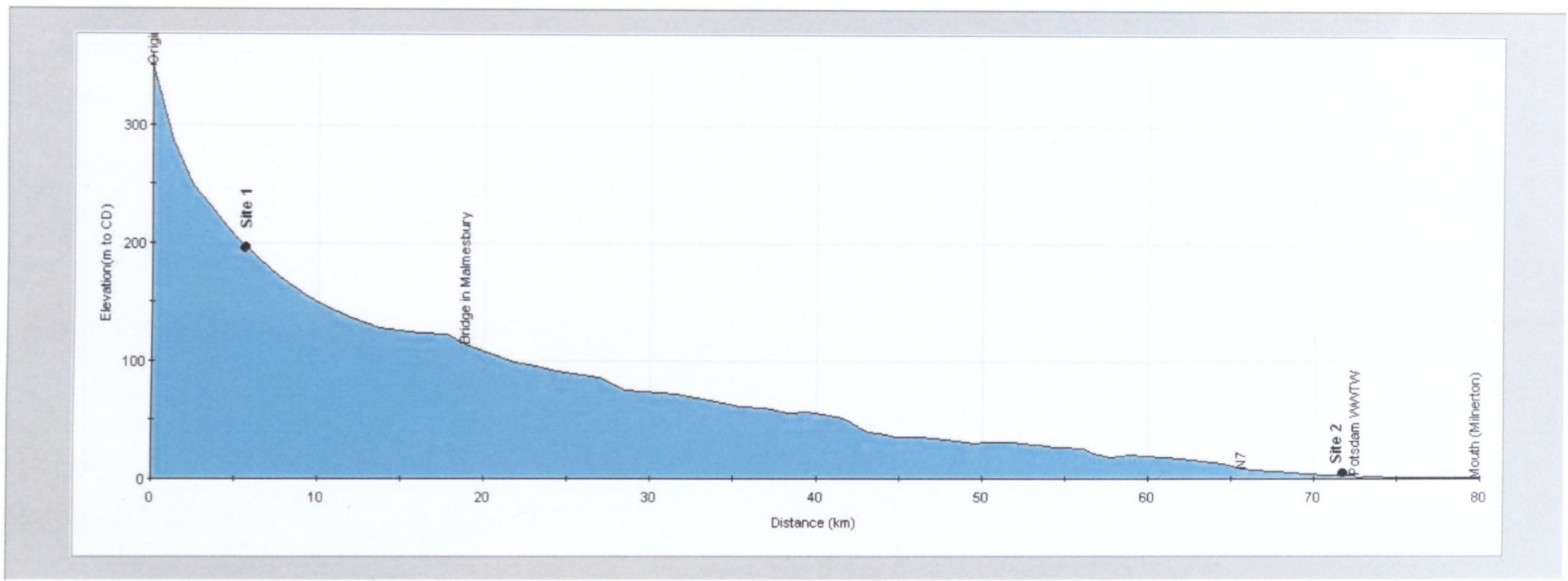

Figure 2.4: The profile of the Diep River, Western Cape (Water Institute of Southern Africa, 2009).

Mother stock of *C. demersum* were collected from the relatively clean and unpolluted fishpond at the Glasshouse Nursery, situated on the Cape Town campus of the Cape Peninsula University of Technology, in District Six, where it thrives in a pond community with other aquatic plants, fish and insects. This plant appears healthy and is abundant in the pond, often inhabiting much of the available pond space. No known pollutants are fed into this pond. The plants were analysed for metals and called the reference plants.

2. 2 Climate and rainfall of the region

The Diep River occurs in the winter rainfall region of the South Western Cape, South Africa (Grindley and Dudley, 1988). The area experiences a Mediterranean climate with warm, dry summers and cool, wet winters. The total monthly rainfall (mm), during the sampling period for the two different sites, is shown in Table 2.1 and Figure 2.5. For site 2, information was obtained from the Cape Town Portnet weather station situated near Paarden Eiland and for site 1, rainfall data was obtained from Lelyfontein weather station which is situated between Malmesbury and Riebeek Kasteel. Unfortunately data for Portnet weather station was incomplete for the 10 years prior to the study, so it was not used in the rainfall data comparisons.

Table 2.1: Total monthly rainfall (mm), recorded for the Diep River area during the sampling period (September 2009 to January 2010)

Months	Rainfall (mm)	
	Site 1	Site 2
September 2009	41.0	55.8
October 2009	4.9	15.04
November 2009	107.9	51.2
December 2009	1.0	6.8
January 2010	0.1	3.0

Source: South African Weather Services.

During the sampling period, the rainfall for November was particularly high and unseasonal, as the average rainfall for Cape Town in November is 22.43 mm and during the study period 51.2 mm fell over site 2 and 107.9 mm fell over site 1 (South African Weather Services) (Table 2.2 & Figure 2.5). This information has important relevance due to the unconventionally high rainfall received in November during the study period (Table 2.1 & Figure 2.5).

Table 2.2: Average monthly rainfall (mm) for Cape Town (Lelyfontein station), situated close to site 1, over a 10 year period prior to the present study (1999-2008).

Months	Rainfall (mm)
September	32.27
October	21.98
November	22.43
December	11.63
January	9.44

Source: South African Weather Services.

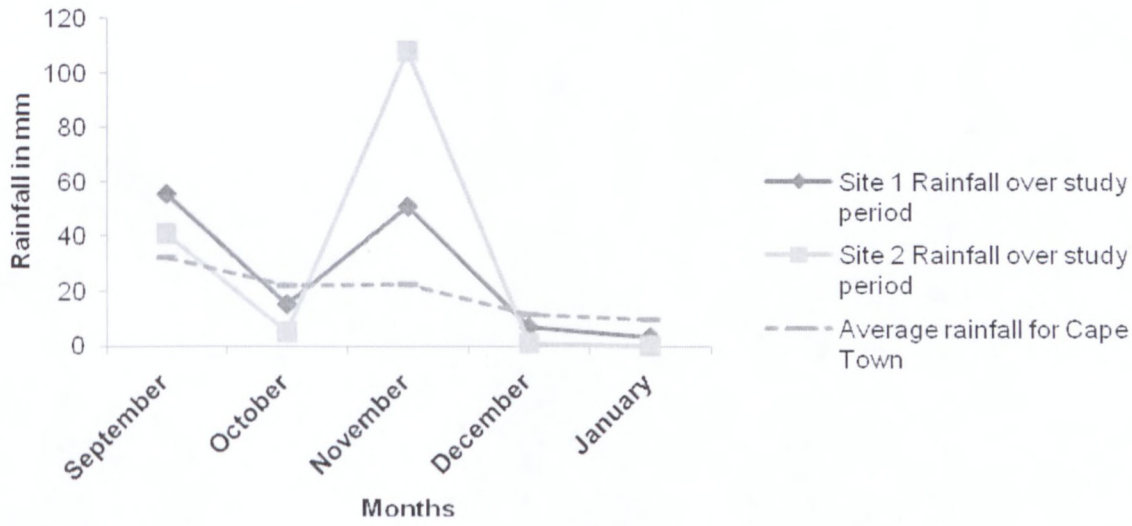

Figure 2.5: Rainfall data for study period September 2009 – January 2010 at sites 1 and 2 of the Diep River. Average rainfall based on data 10 years prior to present study at Lelyfontein weather station, Cape Town.

2.3 *Ceratophyllum demersum L.*

C. demersum is a fast growing aquatic macrophyte, common throughout freshwater rivers and lakes of the world, preferring stagnant to very slow moving water bodies. It is commonly referred to as "Water Hornwort" or "Coontail." It is a submerged free floating plant that is regarded as being a good water oxygenator and provides a protective

environment for fish eggs and an ideal habitat and food source for small snails and insects. It is also a food source for certain water fowl (Figure 2.6).

2.3.1 Classification

The name, *Ceratophyllum*, from the Greek 'keras' meaning 'horn'; and 'fullon' meaning 'leaf'; hence 'horn-like leaf' describes the leaves of the plant. 'Demersum' is taken from the latin 'demersus' meaning 'under water', referring to the plants habitat. The common names 'Horn' refers to the actual shape of the leaf as previously mentioned and 'wort' from the Anglo-Saxon meaning for 'plant'. The common name of "Coontail" refers to the plant's growth habit, resembling that of a racoon's tail (Rook, 2009). The taxonomic classification of *C. demersum* is tabled below (Table 2.3).

Table 2.3: Classification of *Ceratophyllum demersum* L. (Rook, 2009).

KINGDOM	Plantae
DIVISION	Magnoliophyta, the Angiosperms (flowering plants)
CLASS	Magnoliopsida, the Dicotyledons
SUBCLASS	Magnoliidae
ORDER	Nymphaeles
FAMILY	Ceratophyllaceae, the Hornworts
GENUS	*Ceratophyllum*
SPECIES	*demersum*

2.3.2 External morphology

Ceratophyllum demersum L. is a rootless submerged water plant. It has very long smooth stems that are easily broken and are free branching, reaching 2 - 3 m long. The stems vary in length according to the depth of the water and will sometimes anchor itself into the mud if necessary using modified leaves that resemble roots. Leaves of *C. demersum* are bright green, stiff and coarse textured. The sessile leaves occur in whorls of 5 - 12 at each node and are once or twice forked. They are 1.5 – 4 cm long and are serrated along one margin. The internode lengths on the stem differ throughout the stem length, depending on the light intensity and most healthy leaves are found crowded together at nodes closely packed towards the branch tips. The stem seems to make more branches near the water

surface thus exposing the densely packed leaves at the branch tips to as much sunlight as possible (Huxley et al., 1999; Rook, 2009).

Figure 2.6: *Ceratophyllum demersum* L. commonly known as Hornwort or Coontail growing in a fishpond where reference plants were harvested (Author, 2009).

C. demersum is monoecious, having separate male and female flowers occurring on the same plant. The flowers are inconspicuous, tiny, solitary and sessile, occuring at the leaf bases. Both male and female flowers consist of a perigone of between 8 - 15 green segments. The male flower consists of 10 - 20 stamens and has no sepals or petals present. The female flower has a single unicarpellary pistil with a superior ovary. Sepals and petals are absent. The fruit is an achene measuring just 4 - 7 mm, having just one seed. It has one spine at the fruit tip and two spines at the base which is a distinguishing factor when it comes to species identification (Huxley et al., 1999).

2.3.3 Habitat

C. demersum is a common aquatic plant worldwide in inland and coastal ponds and slow moving rivers and streams. It is an amazing tough plant adapting to many different

environments but does not tolerate being out of water for even a short period of time (Robinson, 2003). In nutrient rich waters it forms dense masses of plant growth and will easily adapt to living with algae which is very common in polluted water bodies. *C. demersum* is known to combat algal growth in freshwater ponds as it consumes nutrients that build up due to sewage or agricultural pollutants in the water body. It secretes substances that counteract the algal growth which is beneficial in maintaining a natural environment (Gross et al., 2003). Algal growth will often build up excessively and detrimentally influence the aquatic ecosystem. Although thriving in warmer climates, Hornwort is able to tolerate cold water (-10° to -15° C) and shade which inhibits many other aquatic plant species (Buczacki, 1995).

C. demersum tolerates both very high and very low light intensities. It is able to grow in water with high calcium content and tolerates a pH of between 6-9 and water temperatures that vary between 10-28 °C (Hiscock, 2003). It grows rapidly and with very little demands and can be considered an opportunistic species. Opportunistic aquatic plants are able to flourish in biologically disturbed environments (Henderson & Cilliers, 2002). They may have negative influences, if they grow in dense stands, as so often *C. demersum* does, in nutrient rich water bodies, where it is known to hinder water flow and interfere with fishing and water sports. Positive attributes include oxygenation of the water body, being a protective environment for fish eggs and other small aquatic animals and insects and a food source to snails and some water fowl (Friends of Rietvlei, 2008).

2.3.4 *Ceratophyllum demersum L.* in the Diep River

C. demersum occurs naturally in the lower Diep River in the vicinity of Gill Road, Table View. It grows abundantly in the stagnant and slow moving part of the river which widens and forms many enclaves of water bodies. It grows in thick dense bodies that occur along the shoreline for meters at a time, depending on the water depth. Much of the plant is lost when the water level which is extremely variable, becomes seasonally lower (personal observation).

Observations during the period of field work from September to December 2009 showed the water level of the river drop substantially and many plants died due to exposure. This however had little effect on the massive quantities of hornwort still growing and thriving in the eutrophic water conditions. It was decided to use *C. demersum* as the plant species for this study as it was an existing inhabitant of the Diep River and was not a declared

weed species according to the "Conservation of Agricultural Resources Act" (Act 43 of Republic of South Africa 1983). Like many river systems in South Africa, the Diep River is also home to invasive aquatic plants like *Eichhornia crassipes* (Water hyacinth) that flourishes in the summer months in the lower river regions but as the chosen plant had to be introduced temporarily to another section of the river, it was imperative that the chosen plant was not a problem plant. *C. demersum* through observations along the river, past Malmesbury, does not occur in the upper reaches, most likely due to seasonally heavy water flow, as hornwort prefers a still water body to thrive.

Another reason for choosing *C. demersum* for this study is that fresh healthy plant material was available from the fishpond at the Cape Peninsula University of Technology's Glasshouse Nursery on the Cape Town campus. These plants were growing in a relatively unpolluted environment and stock was plentiful. The plants from this fishpond were used as part of the fieldwork study.

2.4 Placing of the introduced plants in the river

One hundred and sixty *C. demersum* were removed from the fishpond at the Glasshouse Nursery at the Cape Peninsula University of Technology, Cape Town campus. The fishpond does not seem to be polluted and is a habitat for aquatic plants and other organisms including frogs and insects. An overflow pipe from the irrigation system leads into the pond. No fertilizers are used in the irrigation system.

Ceratophyllum sp. was weighed using a scale to two decimal points to obtain plants of equal weight. Due to the branching habit of the stems, it was not possible to measure the length of the plants (Figure 2.6). One hundred and sixty plants (9.11 ±0.13 g) were weighed over a two day period. The plants were carefully removed from the fishpond and gently washed in deionised water to remove any duckweed and debris that could be attached to the plants. In some cases eggs had to be removed from the leaves. Excess water was shaken off the plant before they were weighed. Small portions of the plant were pinched off by hand until an ideal weight was achieved. They were then stored overnight in large plastic containers containing deionised water and two air stones that were attached to an air pump.

After all 160 plants were weighed, they were carefully placed in 8 buckets containing 20 plants each, ready to be transferred to baskets and placed in the river. Plants were transported in 8 baskets measuring 270 mm x 270 mm x 120 mm containing multiple

holes of 20 mm x 12 mm on all sides. The lid was fastened with cable ties to facilitate easy access to the plants. Twenty plants were placed in each basket and 4 baskets were destined for each site in the river (80 plants per site in total). The holes in the basket would allow water to flow through the basket and provide for light to penetrate allowing the plant to photosynthesize. If the baskets were to turn over the plants would still have access to light and water flow would not be hindered.

Figure 2.7: Site 1 baskets of introduced *Ceratophyllum demersum* L. placed in the upper parts of the Diep River, near the Kasteelberg, Riebeek Kasteel (Late September 2009) (Author, 2009).

The exact location chosen to place the baskets at site 1 were relatively out of site from passers-by and the depth of the river was at its deepest (about 0.6 m deep). The baskets were tied to a metal rod and placed in the water. The baskets floated on the surface, the lid being flush on the water surface and the basket being submerged, this allowed the plants to receive sufficient sunlight being close to the water surface (Figure 2.7 & Figure 2.8). At site 2, the baskets were placed in the water, attached by nylon cord, to a metal rod, 2-3 m from the pond edge (Figure 2.9 & Figure 2.10).

Figure 2.8: Site 1 in the upper Diep River, near the Kasteelberg, Riebeeck Kasteel. Baskets are covered in leaf debri (November 2009) (Author, 2009).

Figure 2.9: Site 2, Lower Diep River behind Mc Phearson's Garden Centre, Gill Road, Table View. Existing *Ceratophyllum demersum* L. are growing in the water in the foreground but not easily visible (Late September 2009) (Author, 2009).

Figure 2.10: Site 2 in the Lower Diep River. Algal growth evident in the foreground with baskets of introduced plants visible off centre. The existing *Ceratophyllum demersum* L. are growing in the foreground amongst the algae (November 2009) (Author, 2009).

2.5 Sampling procedures

Samples were taken for the study during the late spring and summer seasons of 2009, as the rainfall of the region mainly falls in winter and the river flows well and is accessible during spring and summer.

Six visits were made to each site with two week intervals between each visit and 12 plants were removed from each batch. The plants were transported in the river water from the specific site, in separate plastic containers with lids and transported to the laboratory for further analysis. At each site visit, samples of water and sediment were also taken for further analysis pertaining to metals.

Any visually significant factors like water colour, water depth, silt within the water, leaves on the water surface, shade from overhanging plants, algal growth and living organisms within the immediate environment of the plants, were also noted for possible later referral.

During each sampling occasion, a handheld Multi-Parameter (PCS Testr 35) was used to record the EC of the water in millisiemens per centimeter (mS/cm). Water pH, salinity and temperature were also recorded, using this meter. Also, during each sampling occasion,

water and sediment samples were taken for metal analysis. Water samples were taken from one meter from the river edge and placed in a plastic water bottle and labelled, while the sediment sample was scooped up using a container on the end of a wooden rod and placed in a clean plastic container. These samples were labelled and stored in a freezer until all sampling occasions had been completed.

A sediment sample from each site was sent to Bemlab (a SANAS accredited testing laboratory) where the sediment was classified mechanically into sand, silt and clay percentages. All sediment samples were taken from the top 15 cm of the river bed approximately one meter from the river edge.

2.6 Establishing the degree of cell membrane damage to plant samples

After two weeks of acclimatising to the conditions in the river, *C. demersum* were harvested from the two sites. A random sample was also harvested from existing *C. demersum* growing in the second site. Twelve *Ceratophyllum sp.* were carefully removed from one of the placed baskets at site 1 and another twelve plants from a basket at site 2. The plants were then placed in a labelled bucket with the existing river water from the site.

The samples of existing *C. demersum* consisted of eight portions of visually healthy looking specimens. It was noticeable that these plants had many eggs attached to the leaves and organisms were thriving within the confines of the small micro-environment that these plants provided.

The procedure in the laboratory was to establish whether any cell membrane damage occurred to the plants whilst in the river and to compare this data from the two different sites as well as the findings of the introduced plants compared with the existing plants at site 2. Six plants from each site were placed individually in 600 ml glass beakers, filled up to the 500 ml line with deionised water. The new glass beakers were first rinsed in deionised water before the plants were placed in them. The EC, water temperature and pH of the deionised water in the beakers was first measured and documented. Each plant was weighed using a Radwag 2008 balance and excess water was shaken off before weighing them.

The procedure described by Kumar & Prasad (2004) was followed to establish the degree of membrane leakage. The plants were rinsed in a solution of 10 m*M* EDTA for 5 minutes to remove any adsorbed metal or nutrient ions that may be attached to the plant surface.

They were then transferred to a bucket of distilled water and gently rinsed for 5 minutes, before they were individually placed in beakers of deionised water. The beakers were left covered in the laboratory for 24 hours and stirred gently on occasions with a plastic rod. Care was taken not to damage any plant material. At 24 hours the plants were stirred using the magnetic stirrer at a slow speed for 3 minutes. After 24 hours the plants were removed from the beakers and a portion of the beaker water from each beaker was frozen for future nutrient analysis of sodium, calcium, potassium and magnesium. The EC, water temperature and pH readings were taken from each beaker after the plants were removed.

2.7 Determination of chlorophyll contents in the leaves and stems of *Ceratophyllum demersum* L.

The chlorophyll content of *C.demersum* leaves and stems were measured together using dimethylsulphoxide (DMSO), as described by Hiscox and Israelstam (1979). The method of DMSO chlorophyll extraction allows the chlorophyll to be extracted from the leaf tissue without any maceration of the tissue occurring (Hiscox and Israeltam, 1979).

Six plants were sampled from site 1 and six from site 2 and a random sample of six specimens were sampled from the existing *Ceratophyllum sp.* at site 2 for this purpose.

Leaf samples were taken from half of the plants, while the other half of the plant was kept for metal analysis. A 0.1 g subsample of leaves from each plant was taken and placed in a glass vial containing 7 ml DMSO (Dimethyl Sulphoxide). Four replicates were taken from each plant sample and leaves were excised from the same region on the plant for each replicate. The young, slightly mature leaves were chosen, as many of the older leaves had discoloured. Only the leaves on the growing ends of the plants appeared healthy. In total 24 samples were taken from site 1 (from the six plants) and 24 samples were taken from site 2 (from the six plants). Six replicates were taken from the random sample of existing plants growing at site 2. The existing *Ceratophyllum sp.* had many eggs attached to them and care was taken not to include any eggs in the sample.

A total of 56 vials were placed in the fridge with their lids on, at 4°C for 110 hours. After refrigeration, an additional 3 ml of DMSO (Dimethyl Sulphoxide) was decanted into each vial to make up a 10 ml solution. Due to the nature of the tiny leaf material it was deemed unnecessary to remove the tiny portions of leaf material from the vials as they had sunken

to the bottom of the vial and did not pose a problem when pouring out the solution into the cuvettes. A 3 ml sample of chlorophyll extract was transferred into a small cuvette for absorbance determination. A spectrophotometer (UV/Visable Spectrophotometer, Pharmacia LKB. Ultrospec II E) was used to determine the absorbance values at 645 and 663 µM. This information was then used in an equation proposed by Arnon (1949), to determine the total leaf chlorophyll contents against the DMSO blank, expressed as $mg.L^{-1}$. The wavelength was set to 645 and the reading for cell 1 (the blank) was zeroed. Readings were then taken for the four replicates stationed at cell 2 to 5. These readings were recorded. The wavelength was then adjusted to 663 µM and the process was repeated. To determine Chlorophyll a (Chla) and Chlorophyll b (Chlb), the following formulae were used: Chla = 12.7D663 – 2.69 D645; Chlb = 22.9 D645 – 4.68 D663. To calculate the total chlorophyll content, Chla was added to Chlb and expressed in milligrams per litre (mg/L).

2.8 Determination of metal concentrations in the river water, sediment, water solutions and stems and leaves of *Ceratophyllum demersum* L.

Water samples in the river from both sites were tested for aluminium, iron, copper and zinc concentrations. These metals were selected as they were the most prominent metals measured by Shuping (2008) in the Lower Diep River. According to the method described by Shuping (2008), for all water samples, five ml of 55% nitric acid was added to each 10 ml water sample. A 5 ml nitric acid blank was also prepared. The samples were then heated in a Grant UBD dry block heater in a fume cabinet, at 40°C for 1 hour. After this, the temperature was increased to 120°C for a further 3 hours. After acid digestion, the samples were left to cool and diluted with distilled water to obtain a 100 ml sample. The samples were then filtered using 0.45 µm cellulose nitrate membrane filter paper. Samples were then poured into polyethylene plastic containers and stored in the refrigerator until ICP-MS analysis.

From the six plant specimens removed from each site, at each sampling occasion, half of the plant was used for chlorophyll determination and the other half was used for metal analysis. The sampling procedure and methods set out by Shuping (2008), using nitric acid digestion was followed. Plant specimens (one of leaves and one of stems) were made up into samples, using a XB 220A Precisa balance. There were six replicates of each stem and six replicates for each leaf sample. All the empty petri dishes were numbered and weighed. Thawed *C demersum* L. plants (having been stored in a freezer)

from both sites, as well as the existing plants on the second site, were carefully separated into leaves and stems using a sharp pointed scissors and weighed in the petri dishes after thawing. The samples were dried for 48 hours at 60°C to obtain the dry weight.

Plant samples, as well as sediment samples were supplemented with 10 ml of 55% nitric acid. A 10 ml nitric acid blank was also prepared. Samples were digested following the same procedure as for the water samples. They too were left to cool and then diluted with distilled water to make up 100 ml samples. Filtration was done using a small funnel and Whatman number 6 filter paper to remove any slurry. Further filtration was done using 0.45 µM cellulose nitrate membrane filter paper. The samples were then poured into labelled polyethylene plastic containers and stored in a refrigerator until ICP-MS analysis..

Metal concentrations were determined using the ICP-MS (Inductively Coupled Plasma-Mass Spectrophotometer) at the University of Stellenbosch. ICP results were then converted using the following formula:

For plants and sediments: $$\frac{(\text{ICP reading} - \text{Blank}) \times 100}{\text{mass (g)}}$$

For water samples: [ICP Readings – Blank] X 10

The plant metal and sediment metal concentrations were expressed as mg / kg and all water metal concentrations as mg / l.

2.9 Statistical analyses

The Kruskal–Wallis One Way Analysis of Variance on Ranks, followed by multiple pairwise comparisons (Tukey's test or Dunn's Method) were carried out for all statistical pairwise comparisons, using SigmaStat 3.5 software package.

CHAPTER THREE

RESULTS AND DISCUSSION: WATER

3.1 RESULTS

3.1.1 Physico-chemical parameters

The conductivity, pH, salinity and temperature of the water were measured at each sampling site during each sampling occasion. These parameters are tabulated in Table 3.1.

Table 3.1: Conductivity, pH, salinity and temperature, measured at the two Diep River sampling sites, during each sampling occasion.

Sampling occasion	Conductivity (mS/cm)	pH	Salinity (ppm)	Temperature (°C)
1				
Site 1	0.61	8.15	435	17.5
Site 2	2.35	8.02	1190	23
2				
Site 1	0.42	8.81	199	16
Site 2	2.55	8.62	1300	25
3				
Site 1	0.65	8.68	316	19
Site 2	2.87	9.18	1480	24
4				
Site 1	1.04	8.36	510	17
Site 2	2.46	8.66	1270	21
5				
Site 1	0.38	7.43	180	18
Site 2	1.54	9.97	773	24
6				
Site 1	0.59	7.87	282	18.7
Site 2	2.60	9.28	1340	23.5

3.1.2 ALUMINIUM

Aluminium was detected in all the samples of water taken from the Diep River at site 1 and site 2 on all sampling occasions.

3.1.2.1 Comparisons of aluminium concentrations between consecutive sampling occasions, per site

Site 1

There were no significant differences in aluminium concentrations in the water samples at site 1 between the consecutive sampling occasions ($P>0.05$) (Table 3.2 & Figure 3.1).

Site 2

There were no significant differences in the concentrations of aluminium in the water at site 2 between the sampling occasions except between the first and last sampling occasion where there was a significant decrease over the sampling period ($P<0.05$).(Table 3.2 & Figure 3.1).

3.1.2.2 Comparison of aluminium concentrations between sampling sites 1 and 2 on the same sampling occasion

There were no significant differences between the two sites per sampling occasion ($P>0.05$) (Table 3.2 & Figure 3.1).

3.1.3 IRON

Iron was detected in all the samples of water from the Diep River at site 1 and site 2 on all sampling occasions.

3.1.3.1 Comparisons of iron concentrations between consecutive sampling occasions, per site

Site 1

The concentrations of iron in the water at site 1 were significantly higher at the first sampling occasion compared to the last sampling occasion ($P<0.05$). There were no

significant differences between any consecutive sampling occasions during the sampling period (P>0.05) (Table 3.3 & Figure 3.2).

Site 2

The concentrations of iron in the water at site 2 were significantly higher during the first sampling occasion compared to the second (P<0.05). There was also a significant decrease in iron concentrations from the first sampling occasion compared to the last sampling occasion (P<0.05). There were no significant differences in any of the other consecutive sampling occasions during the sampling period (P>0.05) (Table 3.3 & Figure 3.2).

3.1.3.2 Comparisons of iron concentrations between sampling sites 1 and 2 on the same sampling occasion

There were no significant differences between the two sites during the sampling period (P>0.05).

3.1.4 ZINC

Zinc was detected in all the samples of water from the Diep River at site 1 but only on the third and fourth sampling occasions at site 2.

3.1.4.1 Comparisons of zinc concentrations between consecutive sampling occasions, per site

Site 1

There were no significant differences in the concentrations of zinc in the water at site 1 (P≥0.05) (Table 3.4 & Figure 3.3).

Site 2

There were no significant differences in the concentrations of zinc in the water at site 2 between each consecutive sampling occasion (P>0.05) (Table 3.4 & Figure 3.3).

3.1.4.2 Comparisons of zinc concentrations between sampling sites 1 and 2 on the same sampling occasion

There were no significant differences between the two sites per sampling occasion but there were significantly higher concentrations of zinc in the water samples at site 1 than at site 2 ($P<0.05$) (Table 3.4 & Figure 3.3).

3.1.5 COPPER

Copper was detected in all the water samples from the Diep River at site 1 and site 2 except for the second sampling occasion at site 1.

3.1.5.1 Comparisons of copper concentrations between consecutive sampling occasions, per site

Site 1

There were no significant differences in copper concentrations in the water during the consecutive sampling occasions at site 1 ($P>0.05$) (Table 3.5 & Figure 3.4).

Site 2

There were no significant differences in copper concentrations in the water during the consecutive sampling occasions at site 2 ($P\geq0.05$) (Table 3.5 & Figure 3.4).

3.1.5.2 Comparisons of copper concentrations between sampling sites 1 and 2 on the same sampling occasion

There were no significant differences between the two sites during the sampling period ($P\geq0.05$) (Table 3.5 & Figure 3.4).

Table 3.2: Mean (±SD) aluminium concentrations (mg/L), measured in water from two Diep River sampling sites, per sampling occasion. Sample size: per sampling occasion: n = 6.

Sampling occasion	Site 1	Site2
1	0.325 ± 0.0756	~1.004 ± 0.129
2	1.036 ± 1.770	0.201 ± 0.0479
3	0.868 ± 0.529	0.295 ± 0.334
4	0.480 ± 0.0919	0.279 ± 0.187
5	0.380 ± 0.0781	0.320 ± 0.312
6	0.0749 ± 0.0787	~0.0307 ± 0.0687
Pooled data for entire sampling period (n=30)	0.5273 ± 0.3590	0.2738 ± 0.0514

~ = Significant difference between the first and last sampling occasions per site.

Table 3.3: Mean (±SD) iron concentrations (mg/L), measured in water from two Diep River sampling sites, per sampling occasion. Sample size: per sampling occasion: n = 6.

Sampling occasion	Site 1	Site2
1	~0.896 ± 0.335	~1.657 ± 0.266
2	0.402 ± 0.216	# 0.697 ± 0.137
3	1.605 ± 0.792	1.230 ± 0.515
4	1.077 ± 0.326	0.899 ± 0.408
5	0.281 ± 0.191	0.796 ± 0.494
6	~0.00406 ± 0.00908	~0.202 ± 0.0961
Pooled data for entire sampling period (n=30)	0.7108 ± 0.5912	0.9135 ± 0.4940

= Significant difference from the proceeding sampling occasion per site.
~ = Significant difference between the first and last sampling occasions per site.

Table 3.4: Mean (±SD) zinc concentrations (mg/L), measured in water from two Diep River sampling sites, per sampling occasion. Sample size: per sampling occasion: n = 6.

Sampling occasion	Site 1	Site2
1	0.0489 ± 0.0631	0.000 ± 0.000
2	0.0424 ± 0.0739	0.000 ± 0.000
3	0.00901 ± 0.0125	0.0845 ± 0.189
4	0.0149 ± 0.00983	0.0521 ± 0.0671
5	0.0156 ± 0.0349	0.000 ± 0.000
6	0.0323 ± 0.0284	0.000 ± 0.000
Pooled data for entire sampling period (n=30)	0.0272 ± 0.0164¤	0.0228 ± 0.0367

¤ = Significant difference between sites.

Table 3.5: Mean (±SD) copper concentrations (mg/L), measured in water from two Diep River sampling sites, per sampling occasion. Sample size: per sampling occasion: n = 6

Sampling occasion	Site 1	Site2
1	0.00843 ± 0.00967	0.00263 ± 0.00400
2	0.000 ± 0.000	0.000835 ± 0.00187
3	0.00142 ± 0.00227	0.000655 ± 0.00146
4	0.00305 ± 0.00506	0.00108 ± 0.00180
5	0.00104 ± 0.00177	0.00143 ± 0.00320
6	0.000438 ± 0.000978	0.0209 ± 0.0395
Pooled data for entire sampling period (n=30)	0.0024 ± 0.0031	0.0046 ± 0.0080

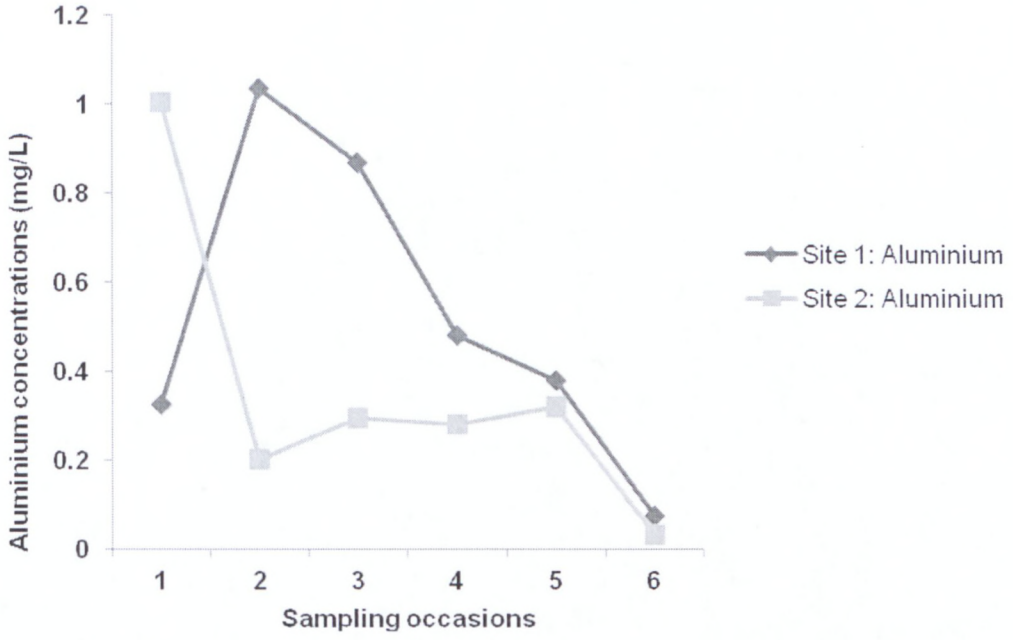

Figure 3.1: Mean aluminium concentrations (mg/L), measured in water from two Diep River sampling sites, per sampling occasion.

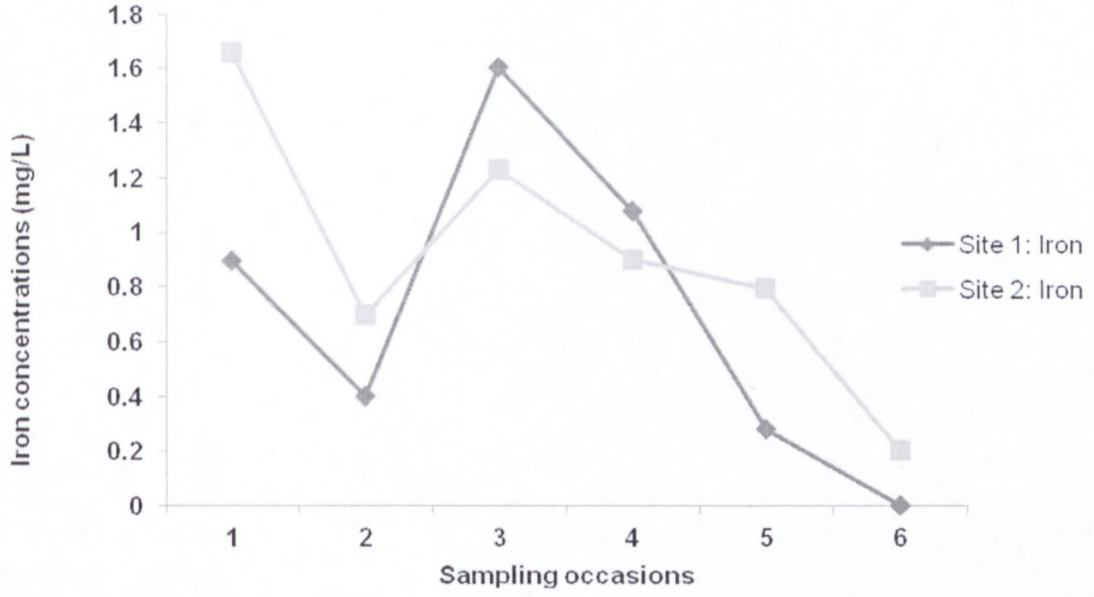

Figure 3.2: Mean iron concentrations (mg/L), measured in water from two Diep River sampling sites, per sampling occasion.

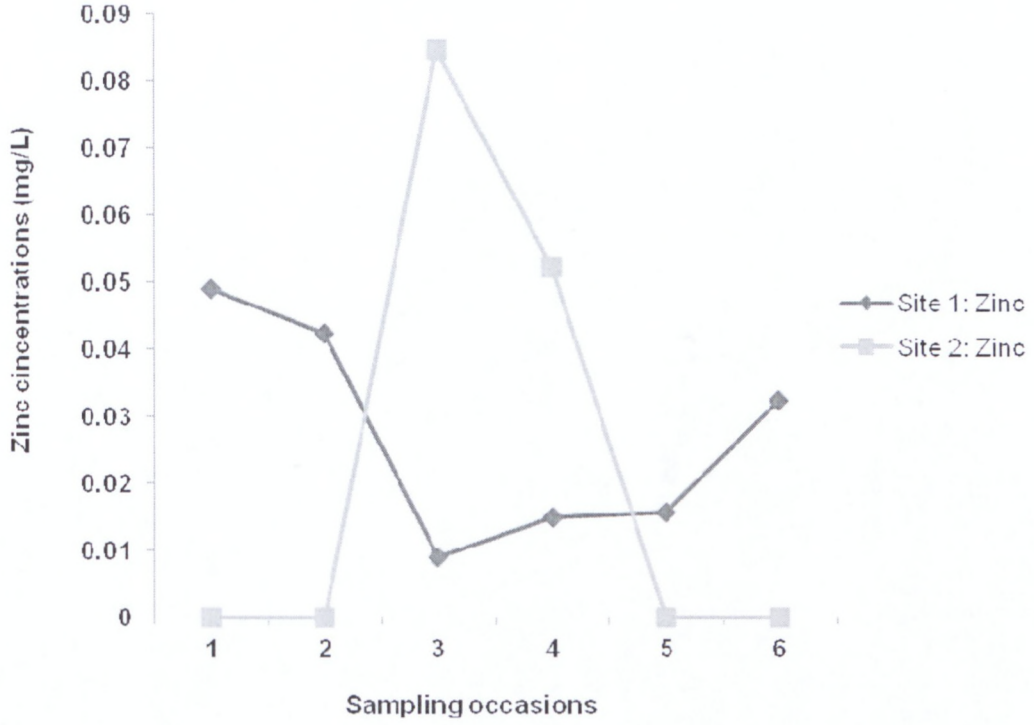

Figure 3.3: Mean zinc concentrations (mg/L), measured in water from two Diep River sampling sites, per sampling occasion.

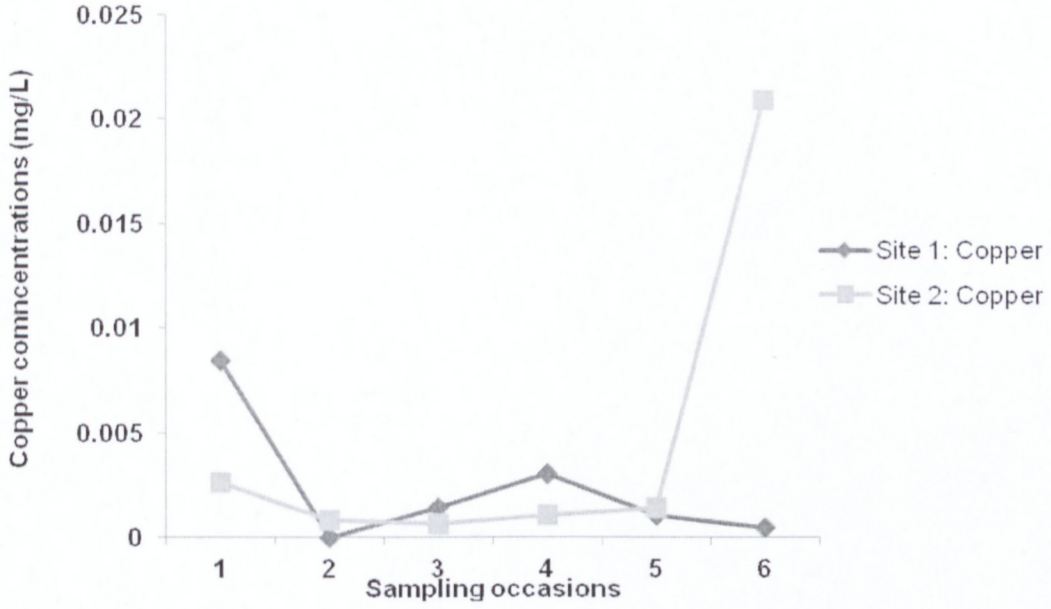

Figure 3.4: Mean copper concentrations (mg/L), measured in water from two Diep River sampling sites, per sampling occasion.

3.2 DISCUSSION

Water quality, as defined by the South African Water Quality Guidelines (SAWQG), set out by the Department of Water Affairs and Forestry, is the chemical, physical, biological and aesthetic properties of water that establish its suitability for a variety of uses and for the safeguarding of aquatic ecosystems (DWAF, 1996).

Visually, site 2 seemed to be a more polluted site than site 1, merely by looking at the water colour and smelling the water. The water at site 2 was visually darker in appearance and had an unpleasant odour. This may have resulted from organic matter present in the water resulting from the Potsdam Waste Water Treatment Works located close by. Sewage sludge is 7% nitrogen compared with cattle manure being 2% nitrogen in composition. Nitrogen is a vital fertilizer component for plants and enhances plant growth (Mauseth, 2003).

At site 1, where the area is surrounded by grape farming, farmers follow rigorous pest control methods to ensure a healthy crop. Several diseases affect grapes and no one fungicide is able to control all of them. Grapes are deciduous plants that lie dormant during winter but are sprayed routinely to prevent fungus outbreaks that attack leaves and young flowering buds. There are also diseases and insect pests that attack grape vines that require control (Longstroth, 2002). The season of this study coincided with late spring and early summer when pest control measures would most likely be high, with metal-based fungicides often sprayed as protective measures prior to pathogen infection, early on in the growing season. This occurs often several times at spray intervals of between 7-10 days (Longstroth, 2002; Poh et al., 2009). Farmers are reluctant to divulge any information regarding crop spraying due to the sensitive nature of the topic, so it is unlikely that proper facts will be established in this regard.

As all the metals tested may be an ingredient in metal-based fungicides that could have possibly been used on vineyards and crops in the vicinity (Table 3.6), it can only be assumed that these metals present in the fungicides could have possibly contaminated the water via runoff at site 1. Also, the unseasonably high rainfall during November of 2009 in the vicinity of site 1, could have contributed to more excessive fungicide spraying, as warm weather and rain contribute to the increase of fungal spores and high rainfall would also increase runoff into the river (Table 2.1).

The following table illustrates an example of fungicide that is manufactured containing one of the studied metals as an active ingredient (Table 3.6).

Table 3.6 Examples of some pesticides using aluminium, iron, zinc & copper as active ingredients.

Ingredient/ Metal	Name of fungicide/insecticide	Purpose	Reference
Aluminium	Fosetyl-aluminium	Fungicide	Alibaba (1999;2012)
Aluminium	Aluminium-phosphide	Insecticide & Rodent control (Fumigant)	Kingtai chemicals (2012)
Iron	Actino-Iron (A.I. Iron 21.9%)	Biological fungicide for soil pathogens	Naturalindustries (1992;2011)
Zinc	BravoZn	Fungicide	Syngenta (2012)
Copper	Copper liquid fungicide	Fungicide	Bonide (2012)

A study conducted by Poh et al. (2009) investigated the sources of micronutrients found in common fungicides and how they related to supplementation of the plant's micronutrient requirements. This illustrates the link between fungicides and the micronutrients that they contain and how they could possibly contribute to high concentrations of such metals in the environment.

Grain crops are predominantly winter crops (unlike grape farming) and grain pest control measures are thus carried out during the winter months, while the crop is growing. In July 2011 a crop spraying aircraft was seen spraying grain crops just south of Malmesbury and tractor based spraying activities were a common site along the N7 freeway approaching Malmesbury (personal observation). This too could have an impact on metal contamination in the Diep River and only reiterates the necessity for monthly sampling throughout the year to establish accurate results.

3.2.1 pH

According to the South African Water Quality Guidelines (SAWQG), most fresh waters, in South Africa, should be more or less neutral, with a pH of between 6 and 8 (DWAF, 1996). During the sampling period the pH at site 1 ranged between 7.43 and 8.81, slightly higher than the pH stipulated by DWAF (1996). At site 2 the pH was even higher, ranging from 8.02 and 9.97 over the sampling occasions (Table 3.1). Biological activities, like the photosynthesis of submerged aquatic plants, usually result in elevated pH values (DWAF,

1996). At site 2 submerged *C. demersum* were growing in the almost stagnant water but were absent at site 1. Generally biological activity was higher at site 2 than at site 1 which may explain the differences in pH. At site 2 there were a greater number of possible pollution sources in the vicinity, relating to industry, manufacturing and sewage treatment works. The high pH values at site 1 could most probably not have been the result of biological activities as the fast flowing water contained no plant growth and fish and other organisms were not evident. These high pH values could be due to localised pollution as a result of runoff from the surrounding farms, fertilizers and by products used in pest management programmes (Schulz, 2001; Longstroth, 2002) or from animal feed supplements that often contain addititives and metals, resulting in unusually high pH values (Li et al., 2005).

According to the Target Water Quality Range (TWQR) set out by the SAWQG (DWAF, 1996), pH values in river water should not vary more than 0.5 of a pH unit or by more than 5 % for a specific site per day. The sampling occasions were at two week intervals and the pH values remained relatively constant and fell within these parameters at both sites.

High pH readings are typical of eutrophic water systems caused by algal blooms that contribute to an increase in pH due to by-products of photosynthesis (Wright & Welbourn, 2002). The water at site 2 was visually a cloudy, green/brown colour with algal growth amongst the aquatic plants (personal observation), all typical of a eutrophic water body (Wright & Welbourn, 2002).

According to DWAF (1996), limited information is available on the effects of elevated pH, however small changes in pH values are able to change the concentrations of available metallic complexes which can result in noteworthy increases in the availability and toxicity of metals.

3.2.2 Temperature

Although the water temperature was generally 6 °C higher at site 2 than at site 1 over the six sampling occasions (Table 3.1), this increase in water temperature does not affect pH readings according to DWAF (1996). The higher temperature readings of the water at site 2 were most likely due to the stagnant water and much larger water surface area exposed to the sun. At site 1 the water was moving, the surface area of the water was much

smaller and there was also more shade over the river due to riparian vegetation (personal observation).

According to the Target Water Quality Range (TWQR) set out by the SAWQG (DWAF, 1996) water temperature for aquatic ecosystems should not vary more than 2°C or 10 % from the normal temperature ranges in that specific environment. As the normal water temperature for the Diep River sites is unknown a comparison could not be made. At site 1, the temperature of the water ranged from 16 to 19°C and at site between 21 and 25°C over the study period of 12 weeks (Table 3.1).

3.2.3 Conductivity

Electrical conductivity (EC) was measured at each site over the sampling period at each sampling occasion and ranged from 0.38 to 1.04 mS/cm at site 1 and almost double at site 2 from 1.54 to 2.87 mS/cm (Table 3.1). In order to compare the data with that of the Target Water Quality Range (TWQR) set out by the SAWQG (DWAF, 1996) the TDS measurements (Total Dissolved Solids) were used to compare the data with these guidelines. The TDS concentration is directly proportional to the electrical conductivity (EC) of water (DWAF, 1996).

River water will contain varying concentrations of Total Dissolved Solids (TDS), depending on underlying geological formations, the composition of minerals in the rocks and the quantity of decomposing plant material that the water is exposed to (Smith, 1986). As the information pertaining to the underlying rock formations at site 1 and site 2 are unknown, one cannot relate the TDS readings given in the SAWQG (DWAF, 1996) according to the parameters set out for the underlying geological formations that influence TDS readings.

The TDS readings taken in the water at site 1 ranged from 269 ppm to 740 ppm and at site 2 between 1750 ppm and 2030 ppm over the sampling period of four to five months (Table 3.1). According to the TWQR, TDS readings should not differ more than 15% from what is considered a normal cycle of the water (DWAF, 1996). At site 1 the TDS readings varied between 19% and 66% while at site 2 the variations in TDS readings varied between 20% and 106%. As the sampling occasions occurred over a period of four to five months and samples were not taken daily it is difficult to conclude if the changes in TDS were gradual or sudden and if they fall outside the TWQR.

Solutes accumulate as water moves downstream and are added by industrial effluent discharges and surface runoff from industrial areas (DWAF, 1996). Nearby Montague Gardens, Chevron Oil Refinery and Potsdam Wastewater Treatment Works, are examples of the types of sources that may have contributed to increased TDS concentrations at site 2. Evaporation, which also contributes to higher TDS readings, was also more prevalent at site 2 than at site 1 with a higher exposed water surface area and a noticeably changing water level during the sampling period (personal observation).

There was a considerably higher TDS reading at the fourth sampling occasion at site 1 compared with the other sampling occasions which could possibly be explained by the very high, unseasonal rainfall experienced at site 1 during this time (Table 2.1). Heavy rainfall often has the effect of increasing contaminants to river systems, as rainfall brings potential pollutants as runoff into the river from adjacent areas (Schulz, 2001; Dabrowski et al., 2002). Runoff from the surrounding agricultural farmlands and/or dairy farms at site 1 could have contributed to this higher reading through the addition of manure and pesticide residue. If there was no contaminated runoff, the rainfall would have had a diluting effect on the TDS of the water but as farming occurs alongside the river and the gradient is sloping, this was not the case.

3.2.4 Salinity

Salinity is the amount of salt in water. It is expressed as parts per thousand (ppt) or parts per million (ppm) and is typically 35 ppt or 35000 ppm in the ocean (Brown & Magoba, 2009). In the Diep River at site 1 salinity readings ranged from 0.180 ppt to 0.510 ppt and at site 2 from 0.773 ppt to 1.480 ppt (Table 3.1). There was clearly more salt in the water at site 2 than at site 1 probably due to the proximity of site 2 to the ocean and also the high evaporation rates that occur at site 2 that according to Brown & Magoba (2009) can influence salinity.

Freshwater generally has a salinity of 0.5 ppt, while water between 0.5 ppt and 17 ppt are considered to be brackish. Brack water is typical of river water that meets the oceans and estuaries (Smith, 1986). Thus, the salinity at site 1 is comparable to freshwater conditions, while the salinity at site 2 is only slightly higher but falling into the brackish water category. Higher salinity values in freshwater have detrimental effects on certain plants and organisms and limit the biodiversity of an environment. Salinity can also affect the accumulation of metals in plants, higher salinity levels increase the accumulation of

metals, as e.g. in *Aster tripolium* (Fitzgerald et al., 2003) and in *Bolboschoenus maritimus* (Shuping et al., 2011).

3.2.5 Aluminium

The bioavailability of aluminium in water is strongly pH dependent. At a neutral pH (6.5 – 7.5), aluminium is least soluble in water and most soluble and toxic in acidic waters (pH<4.0). Above pH 6.5, aluminium is generally insoluble in the form of aluminium hydroxide (DWAF, 1996).

The TWQR for acid-soluble aluminium in water is 10 µg per litre where a pH value is > 6.5 (DWAF, 1996). The Diep River sites were both alkaline in nature (site 2 being more alkaline than site 1, Table 3.1). All readings for aluminium over the sampling occasions were well over the recommended TWQR of 10 µg (Table 3.2 & Figure 3.1).

The Chronic Effect Value (CEV) of acid-soluble aluminium in water with a pH value > 6.5 is 20 µg and the Acute Effect Value (AEV) is 150 µg (DWAF, 1996). At site 1 all the sampling occasions showed aluminium levels that were higher than the recommended CEV ratings set out by the WQGR, meaning that the toxicity levels of aluminium at site 1 for the first five sampling occasions were too high. The aluminium concentrations in the water samples at site 1 for the AEV (0.15 mg/l) were all above the recommended WQGR rate, except for the last sampling occasion. At site 2 aluminium levels in the water were also over the CEV and the AEV results were also over the prescribed limit, except for the last sampling occasion which fell within the guidelines (DWAF, 1996).

Aluminium concentrations were also compared with two other studies that measured aluminium concentrations in the water near to site 2 of the present study (within 500m). Shuping (2008) detected 0.04 ±0.00 mg/l, 0.80 ±0.65 mg/l and 0.25 ±0.04 mg/l aluminium respectively in the water over a 5 month period during 2004, taking samples at two-monthly intervals. These aluminium concentrations were similar to that of the present study, both being well over the recommended TWQR of 10 µg (Table 3.2). A study conducted by Jackson et al. (2009) on metal contamination in the Diep River, also detected high concentrations of aluminium (well over the TWQRs) in the water samples taken from January 2005 to September 2005 at the Potsdam Wastewater Treatment Works, near to the present study site 2. Only during March 2005 was there no aluminium detected in the water. As most of these months do not correspond with the months of the

present study, this information cannot be accurately compared with the present study and is included to illustrate that prior to this study, the water in the Diep River in the vicinity of the Potsdam Wastewater Treatment Works was contaminated with high concentrations of aluminium. There were no comparative sites in the two other studies to compare data for site 1 as there were no sampling sites north of Malmesbury. The upper parts of the Diep River, north of site 1 is generally dry during summer (personal observation) and that the metal contamination could not have emanated from the river source but rather from surrounding areas near site 1. The sediment does not contain high clay content (Table 4.1), so fluctuations in aluminium concentrations cannot be as a result of cation exchange from the sediment (Smith, 1986) and has probably thus emanated from the surrounding environment.

A possible reason for the steady decrease in aluminium concentrations at site 1 after the second sampling occasion (where a spike in aluminium concentrations occurred) could be attributed to the unseasonably high rainfall during November 2009 which diluted the metal concentrations in the water (Table 2.1 & Figure 2.5). Another possibility could be the seasonal spraying of aluminium containing fungicides (Table 3.6) that may have contributed to the initial high concentration of aluminium at site 1, adjacent to the farming area, following fungicide application and runoff from the high rainfall experienced. At the end of the study period, when fungicides are no longer required on the grape crops, this could have been the reason for the decrease in aluminium concentrations. It is difficult to confirm, as farmers are generally reluctant to discuss spraying procedures and chemicals. Another possible reason for the higher aluminium concentrations in the water at site 1 could be the exposed rock outcrops that were evident in the riverbed (personal observation) and the constant weathering that could be occurring. The constant friction of water over bedrock could weather aluminium deposits from the rocks into the water (Moss, 1988). Another possible reason why the aluminium concentrations were generally higher in the water at site 1 could be that by the time the water reaches site 2 some of the aluminium had been filtered out by the abundant *Phragmites australis* reeds that are visible in the river. *Phragmites australis* is able to bioaccumulate metals including aluminium (He & Yongfeng, 2009).

At site 2 the initial sampling occasion showed significantly higher concentrations of aluminium in the water compared with the last sampling occasion. Aluminium concentrations showed a trend of decreasing over time at site 2 which could have resulted from an initial unknown localised contamination point possibly from surrounding industry. It

is assumed that the high levels of aluminium in the water is due to industries like panel-beaters, chemical manufacturers, aluminium factories and cold storage facilities that may contribute to the aluminium pollution via their wastes of pigments, metal alloys, batteries and solders that are considered to be primary sources of aluminium pollution in the environment. These industries are located close to site 2 of the current study and the Potsdam Wastewater Treatment Works (Jackson et al., 2009).

3.2.6 Iron

Iron has limited toxicity and bioavailability, depending on what form it is in. It is classified as a non-critical element and is required in small quantities by plants and animals for metabolism (DWAF, 1996).

According to the TWQR set out by the SAWQG, the iron concentrations in an aquatic ecosystem should not vary more than 10% of the background dissolved iron concentration for a particular site at a specific time (DWAF, 1996).

There were varied amounts of iron detected in the water at both sites with no noticeable differences between the sites as they followed a similar trend at both sites showing an initial higher concentration of iron that decreased on the second sampling occasion, spiking on the third sampling occasion and then over time becoming significantly lower at the final sampling occasion (Table 3.3 & Figure 3.2). The increase at the third sampling occasion at both sites could have been be due to increased runoff from the unseasonably high rainfall (Table 2.1 & Figure 2.5) that was experienced during November 2009 that could have brought iron containing contaminants into the river (possibly fungicide related at site 1 and industry related at site 2). All iron concentrations from consecutive sampling occasions differed above 10% per sampling occasion at site 1 but as the background iron concentrations are unknown for the Diep River, these fluctuations in iron levels are most likely unnatural. As two weeks occurred between sampling occasions it is not clear if this change in concentration occurred gradually or suddenly, only between the fourth and fifth sampling occasion at site 2 was the difference just over 10%.

There is insufficient data to derive a CEV or an AEV for iron in aquatic ecosystems according to the SAWQG document (DWAF, 1996).

Iron concentrations in the water of the Diep River at site 2 were also compared with two other studies conducted in 2004 and 2005 pertaining to metal concentrations in water of the Diep River. As there was no comparative site north of Malmesbury, site 1 was not compared with any of the data from these two studies. Shuping (2008) measured iron concentrations in the Diep River within 500 m of the present study's site 2 and over the comparative months of September, November and January only found iron in the water on the second and third sampling occasion of 0.97 ±1.07 mg/l and 0.05 ±0.09 mg/l respectively. This represents a more than 10% fluctuation in the iron concentrations at any given time set out by the TWQR set out by the SAWQG, DWAF (1996). Jackson et al. (2009) also established that the levels of iron in the water at the sampling site close to Potsdam Wastewater Treatment Works fluctuated over the 10% rate permitted by the TWQR set out by the SAWQG, DWAF (1996). As the sampling months did not correspond with the present study, the information assists in establishing excessively high concentrations of iron in the Diep River at site 2 during the months of April 2005 and June 2005 and then fluctuations of iron exceeding the 10% variation in concentration rate set out by the TWQR of the SAWQG (DWAF, 1996). Site 2 generally had more iron in the water than site 1, probably due to the influence of iron related industries within close proximity.

3.2.7 Zinc

The TWQR for zinc in water is 2 µg (0.0002 mg) per litre (DWAF, 1996). At site 1 all zinc concentrations in the water were above the TWQG (Table 3.4 & Figure 3.3). In most natural waters zinc exists as a divalent cation, which is a potentially toxic form, however It is highly dependent on low pH values to become bioavailable and thus toxic (DWAF, 1996). The result of fertilizer or insecticide runoff could have contributed to the high zinc concentrations in the water sampled at site 1, as site 1 is adjacent to agricultural farmlands. Zinc is an ingredient of certain fungicides and an additive to dairy feed (Ash & Ash, 2004). As fungicide spraying would occur on several occasions throughout the study period in early summer, it is highly likely that zinc could have entered the river as runoff from the pesticide spraying of grape vines as found by Longstroth (2002) and from cow manure as found by Li et al. (2005). At site 2, zinc was only detected at the third and fourth sampling occasions in the water samples and was over the TWQG limit of 0.002 mg/l (DWAF, 1996) (Table 3.4 & Figure 3.3). These results could be due to the higher rainfall that occurred at this time (Figure 2.5) and that site 2 is downstream from site 1 and all runoff would accumulate in the lower reaches of the river after heavy rainfall, however

the most likely cause of the sudden spike in zinc concentrations at site 2 could have occurred due to point – source pollution from the surrounding industries that may have contributed to the rapid increase from zero to concentrations well over the TWQG limits (DWAF, 1996).

The CEV of zinc is 3.6 µg and the AEV is 36 µg (DWAF, 1996). At site 1, all concentrations of zinc detected in the water samples were over the TWQR CEV ratings of 0.0046 mg/l. At site 2 other than the first two and last two sampling occasions when no zinc was detected in the water, the third and fourth water samples had concentrations over the CEV recommended rates.

The AEV of zinc at site 1 was over the limit at the first, second and last sampling occasions while at site 2 only at the third and fourth sampling occasions when zinc was detected in the water were the concentrations over the limit set out for the AEV (Table 3.4 & Figure 3.3).

Zinc concentrations in the water were also compared with two recent studies by Shuping (2008) and Jackson et al. (2009) conducted on metals in the water of the Diep River. As no study site corresponded with the present study site 1, only site 2 results were compared. Shuping (2008) established that during September 2004 and January 2005 no zinc was detected in the water samples taken at the sampling site within 500 m of the present study site 2 but 0.33 ±0.17 mg/l was detected in the water during November month which was over the TWQR for zinc in water of 2 µg (0.0002 mg) per litre set out by DWAF (DWAF, 1996). It is also over the acceptable CEV and AEV rates (DWAF, 1996).

Jackson et al. (2009) detected high concentrations of zinc in the water near the Potsdam Wastewater Treatment Works, close to the present study site 2 during all sampling occasions between the months of January 2005 and September 2005, well over the TWQR for zinc of 2 µg in water and the CEV and AEV rates set out by DWAF (1996). This information confirms that the site near Potsdam Wastewater Treatment Works had a high level of zinc in the water during the months of January and September 2005 but over the summer months during the present study and over November 2004 (Shuping, 2008), the zinc concentrations in the water varied substantially from no traces to quantities over the TWQR set out by DWAF (1996). This variation in zinc concentrations could probably be attributed to local industrial runoff due to the location of associated industries within close proximity to the sampling area (see aluminium discussion on industrial influences). Zinc concentrations at site 1 were more constant throughout the study period. This may

possibly be due to contaminants from runoff, from either dairy farms or vineyard pesticide spraying (Table 3.6). In a recent study conducted on dairy feed in the USA, Li et al. (2005) found that the main source of heavy metals (Zn and Cu included) were dairy feed and mineral supplements fed to cows and that half the farmers surveyed were feeding their animals over the recommended rates. These metals are excreted and the manure ends up in rivers. This scenario could have happened at site 1 due to the close vicinity of dairy farms to the river, the gradient of the slope and high rainfall experienced during November 2009 (Table 2.1 & Figure 2.5), resulting in high concentrations of zinc in the river water. High rainfall and runoff containing pesticide residue and fertilizers from farms, contribute to metal contamination in rivers (Schulz, 2001; Dabrowski et al., 2002).

3.2.8 Copper

The copper concentrations found in the water at site 1 and site 2 were higher than the TWQR of 0.3 µg/l (0.0003 mg/l) for soft water (DWAF, 1996) for all sampling occasions except for the second sampling occasion at site 1, when no copper was detected and higher for all sampling occasions at site 2 (Table 3.5 & Figure 3.4). If the TWQR for medium water hardness was considered at 0.8 µg/l (0.0008 mg/l), then all but the last sampling occasion at site 1 and the third sampling occasion at site 2 were over the limits set out by TWQR (Table 3.5 & Figure 3.4). As water hardness was not measured, no comparison can be made with this document.

All the readings for copper at site 1, except for the second sampling occasion, when no copper was detected, were above the recommended CEV guidelines of 0.53 µg/l for soft water hardness (DWAF, 1996). At site 2 all concentrations from all sampling occasions were above this threshold limit. For medium water hardness, only the first, fourth and sixth sampling occasions at site 2 had concentrations of copper over the recommended CEV levels of 1.5 µg/l (0.0015 mg/l) set out by DWAF (1996) (Table 3.5 & Figure 3.4).

The AEV concentrations of copper for soft water are set at 1.6 µg/l (0.0016 mg/l) and 4.6 µg/l (0.0046 mg/l) for medium hard water (DWAF, 1996). The results for copper concentrations at site 1 were only higher than the recommended rate for soft water at sampling occasions one and four. At site 2 for soft water, only the first and last sampling occasions were over the recommended rate of 1.6 µg/l set out by DWAF (1996) for soft water. The recommended rate of 4.6 µg/l for medium water was only surpassed at the first sampling occasion at site 1 and the last sampling occasion at site 2.

Copper concentrations in the Diep River water were found to be above the general TWQR set out by DWAF (1996) for soft water at both sites. This could partly be attributed to the use of fungicides that contain copper in the vicinity of site 1. Copper-based fungicides are used by farmers to combat various fungal diseases on crops, especially in viticulture during spring and early summer before fruit set. Often several applications are necessary to be effective (Longstroth, 2002). Many crops are produced on land adjacent to the river in the upper reaches; many are vineyards (personal observations). Copper–based fungicides are commonly used in the Western Cape to prevent fungal disease on many crops (Nel et al., 2003). It is known that high rainfall occurring during the spraying season, can increase metal contaminants in rivers, as found by Schulz (2001) who studied the impact of a heavy downpour of rain on metals in the Lourens River, Western Cape, adjacent to agricultural land.

Copper concentrations in the water of the Diep River were also compared with two other recent studies conducted on metal concentrations in the water of the Diep River by Shuping (2008) and Jackson et al. (2009). As they did not use any sites north of Malmesbury in their studies, site 1 of the present study was not compared to any of the data.

Shuping (2008) detected no copper in the water samples taken within 500 m from the present study site 2 at the September 2004 and January 2005 sampling occasions but found 0.07 ±0.16 mg/l during the November 2004 sampling occasion. This amount was over the TWQR for zinc for both soft and medium water hardness set out by DWAF, (1996) and surpasses the CEV and AEV rates of DWAF (1996). Only data from these three sampling occasions corresponded with the sampling occasions of the present study.

Jackson et al. (2009) detected copper from January 2005 to September 2005 at a sampling site close to Potsdam Wastewater Treatment Works, close to the present study site 2 on all sampling occasions. The concentrations of copper in the water surpassed the TWQR set out by DWAF (1996) for both soft and hard waters as well as for the CEV and AEV rates (DWAF, 1996). The concentrations obtained by Jackson et al. (2009) were all higher than the concentrations of copper detected in the present study however the sampling months differ from the sampling period of the present study.

These results conclude that there are generally high concentrations of copper in the water of the Diep River near the Potsdam Wastewater Treatment Works throughout the year, mostly over the TWQR set out by DWAF, (1996) for soft and medium waters.

Considering all the metal results for river water, it is concluded that the entire Diep River is generally contaminated with aluminium, iron, zinc and copper and that the present study confirms previous results obtained by Shuping (2008) and Jackson et al. (2009) for the lower reaches of the river. Site 1, although situated close to the source of the Diep River, had significantly higher concentrations of zinc in the water, compared with site 2 in the lower reaches. These unusually high concentrations of zinc could have resulted from either weathering of the underlying rocks in the riverbed (as sediment samples at site 1 were also significantly higher in zinc concentrations compared with sediment samples at site 2) (Table 4.4 & Figure 4.3) or from zinc contaminants from agricultural activities like pesticides and dairy feed. As for aluminium concentrations being high at site 1, this is most likely due to weathering of bedrock in the river or from agricultural activities relating to pesticides, (aluminium being a possible ingredient of fungicides). Iron and copper in the water at site 1 could have also emanated from agricultural activities as for zinc. At site 2 the high concentrations of metals have most likely resulted from various contaminant influences, emanating from local industries, being situated in the lower reaches. The high unseasonal rainfall experienced over the study period in November 2009 may have contributed to elevated readings due to increased contaminants in the runoff.

CHAPTER FOUR

RESULTS AND DISCUSSION: SEDIMENT

4.1 RESULTS

4.1.1 Sediment characterisation

Table 4.1 shows the percentages of the various fractions in the sediment samples at the two sampling sites. The results indicate that the two sites had very similar sediment characteristics, with mostly sand making up the bulk of the sediment. At site 2 there was only 0.2% more clay in the sediment compared with site 1. The small percentage clay in the sediment at both sites will have limited impact on attracting ions, due to the small percentage present in the sediment (clay attracts positive ions) (Smith, 1986) and as the sand, silt and clay percentages are so similar at both sites, this will make comparisons of the sites easier.

Table 4.1: Relative percentages of the various fractions in sediment samples collected from two sampling sites in the Diep River.

Sampling site	Sediment fraction	Percentage (%)
Site 1	Clay	2.2
	Silt	1.2
	Sand	96.6
Site 2	Clay	2.4
	Silt	1.3
	Sand	96.3

4.1.2 ALUMINIUM

4.1.2.1 Comparisons of aluminium concentrations between consecutive sampling occasions, per site

Site 1

There was a significant decrease in the aluminium concentrations of the sediments tested between the fourth and fifth sampling occasions ($P<0.05$). There were no other significantly different readings taken between any of the other sampling occasions ($P>0.05$) (Table 4.2 & Figure 4.1).

Site 2

There was a significant decrease in the aluminium concentrations between the first and second sampling occasion ($P<0.05$), however for the second and third, fourth and fifth and last sampling occasions, the aluminium concentrations significantly increased between sampling occasions ($P<0.05$) (Table 4.2 & Figure 4.1).

4.1.2.2 Comparisons of aluminium concentrations between sampling sites 1 and 2 on the same sampling occasion

On the second, third and fourth sampling occasions a significantly higher reading of aluminium was found in the sediment samples at site 1, compared to the same sampling occasions at site 2 ($P<0.05$). There were no other significant differences between any of the other sampling occasions. The concentrations of aluminium in the sediment at site 1 were significantly higher for the combined sampling occasions pooled data than the concentrations detected at site 2 ($P<0.05$) (Table 4.2 & Figure 4.1).

4.1.3 IRON

Iron was detected in the sediment samples taken from the Diep River at site 1 and site 2 on all sampling occasions.

4.1.3.1 Comparisons of iron concentrations between consecutive sampling occasions, per site

Site 1

The iron concentrations between the first and second sampling occasions showed a significant decrease ($P<0.05$). There was then a significant increase in iron concentrations

between the second and third sampling occasions, as well as between the third and fourth sampling occasions ($P<0.05$). The concentration of iron in the sediment then showed a significant decrease between the fourth and fifth sampling occasions ($P<0.05$). There were no significant difference in the iron concentrations between the fifth and sixth sampling occasions ($P>0.05$). Overall, iron concentrations significantly decreased from sampling occasion 1 to 6 ($P<0.05$) (Table 4.3 & Figure 4.2).

Site 2

A significant decrease in iron concentrations were found between sampling occasion one and two and between sampling occasions three and four ($P<0.05$). The opposite was found between sampling occasions two and three, four and five and five and six, where concentrations of iron reflected significant increases ($P<0.05$). The concentrations of iron in the sediment at site 2 showed a significant decrease between the first and last sampling occasions ($P<0.05$) (Table 4.3 & Figure 4.2).

4.1.3.2 Comparisons of iron concentrations between sampling sites 1 and 2 on the same sampling occasion

There were significantly higher concentrations of iron in the sediment samples at site 1 on the second, third and fourth sampling occasions compared to the same sampling occasions at site 2 ($P<0.05$). The other corresponding sampling occasions between the two sites showed no significant differences in readings ($P>0.05$). The pooled data at site 1 had a significantly higher concentration of iron in the sediment than the pooled data samples from site 2 ($P<0.05$) (Table 4.3 & Figure 4.2).

4.1.4 ZINC

Zinc concentrations were detected in the sediment samples taken from the Diep River at site 1 and site 2 on all sampling occasions.

4.1.4.1 Comparisons of zinc concentrations between consecutive sampling occasions, per site

Site 1

The concentrations of zinc in the sediment at site 1 were significantly higher at the fourth sampling occasion compared to the fifth sampling occasion ($P<0.05$). No other consecutive sampling occasions showed any significant differences between the zinc concentrations ($P>0.05$) (Table 4.4 & Figure 4.3).

Site 2

There were no significant differences in the zinc concentrations of the sediment samples taken on the six consecutive sampling occasions ($P>0.05$) (Table 4.4 & Figure 4.3).

4.1.4.2 Comparisons of zinc concentrations between sampling sites 1 and 2 on the same sampling occasion

The only sampling occasion that showed a significant difference in concentrations of zinc was on the fourth sampling occasion, where at site 1 the zinc was significantly higher than that of site 2 ($P<0.05$). There were no other significant differences in the other sampling occasions per site however, overall when data were pooled, there were significantly higher concentrations of zinc detected in the sediment at site 1 than at site 2 ($P<0.05$) (Table 4.4 & Figure 4.3).

4.1.5. COPPER

Concentrations of copper were only detected at site 1 in the sediment and not at site 2.

4.1.5.1 Comparisons of copper concentrations between consecutive sampling occasions, per site

Site 1

The concentrations of copper significantly decreased between the fourth and fifth sampling occasion ($P<0.05$), while there were no significant differences between any of the other consecutive sampling occasions ($P>0.05$) (Table 4.5 & Figure 4.4).

Site 2

There were no traces of copper detected in the sediment at site 2.

4.1.5.2 Comparisons of copper concentrations between sampling sites 1 and 2 on the same sampling occasion

The concentrations of copper on the fourth sampling occasion were significantly higher at site 1 than at the same sampling occasion at site 2 ($P<0.05$). There were no further significant differences in the concentrations of copper between the sites per sampling occasion ($P>0.05$), however when data were pooled, there was a significantly higher concentration of copper in the sediment at site 1 compared to site 2 ($P<0.05$) (Table 4.5 & Figure 4.4).

Table 4.2: Mean (±SD) aluminium concentrations (mg/kg), measured in sediment from two Diep River sampling sites, per sampling occasion. Sample size: per sampling occasion: n = 6.

Sampling occasion	Site 1	Site2
1	4275.123± 220.586	1127.646 ± 185.698
2	3484.331 ± 229.962¤	#489.789 ± 80.189
3	4466.613 ± 280.216¤	701.486 ± 43.678
4	5924.437 ± 791.034¤	#585.843 ± 68.511
5	#1338.912 ± 248.942	#973.119 ± 110.620
6	1428.539 ± 100.965	#1196.893 ± 73.916
Pooled data over entire sampling period (n=30)	3486.33 ± 1809.61¤	845.80 ± 294.64

= Significant difference from the proceeding sampling occasion per site.
¤ = Significant differences between sites.

Table 4.3: Mean (±SD) iron concentrations (mg/kg), measured in sediment from two Diep River sampling sites, per sampling occasion. Sample size: per sampling occasion: n = 6.

Sampling occasion	Site 1	Site2
1	11222.649 ± 691.187~	1696.154 ± 110.107~
2	#9487.910 ± 592.317¤	#825.896 ± 61.010
3	#11249.772 ± 456.682¤	#1180.017 ± 38.750
4	#14424.288 ± 583.756¤	953.120 ± 109.459
5	#4069.111 ± 543.649	#1320.344 ± 84.786
6	4248.488 ± 323.229~	#1460.764 ± 68.982~
Pooled data over entire sampling period (n=30)	9117.04 ± 4158.63¤	1239.38 ± 322.69

= Significant differences from the proceeding sampling occasion per site.
¤ = Significant differences between sites.
~ = Significant differences between the first sampling occasion and the last sampling occasion.

Table 4.4: Mean (±SD) zinc concentrations (mg/kg), measured in sediment from two Diep River sampling sites, per sampling occasion. Sample size: per sampling occasion: n = 6.

Sampling occasion	Site 1	Site2
1	13.038 ± 0.819	1.892 ± 0.679
2	13.284 ± 1.499	1.775 ± 1.312
3	15.716 ± 3.483	2.014 ± 1.412
4	18.348 ± 3.218¤	1.946 ± 0.947
5	#5.891 ± 1.403	1.025 ± 0.549
6	7.424 ± 2.011	1.181 ± 0.275
Pooled data over entire sampling period (n=30)	12.284± 4.788¤	1.639 ± 0.425

= Significant differences from the proceeding sampling occasion per site.
¤ = Significant differences between sites.

Table 4.5: Mean (±SD) copper concentrations (mg/kg), measured in sediment from two Diep River sampling sites, per sampling occasion. Sample size: per sampling occasion n = 6.

Sampling occasion	Site 1	Site2
1	6.044 ± 0.603	0.000 ± 0.000
2	5.168 ± 0.219	0.000 ± 0.000
3	6.240 ± 0.645	0.000 ± 0.000
4	7.650 ± 1.666¤	0.000 ± 0.000
5	#2.296 ± 0.424	0.000 ± 0.000
6	2.701 ± 0.387	0.000 ± 0.000
Pooled data over entire sampling period (n=30)	5.017± 2.111¤	0.000± 0.000

= Significant differences from the proceeding sampling occasion per site.
¤ = Significant differences between sites.

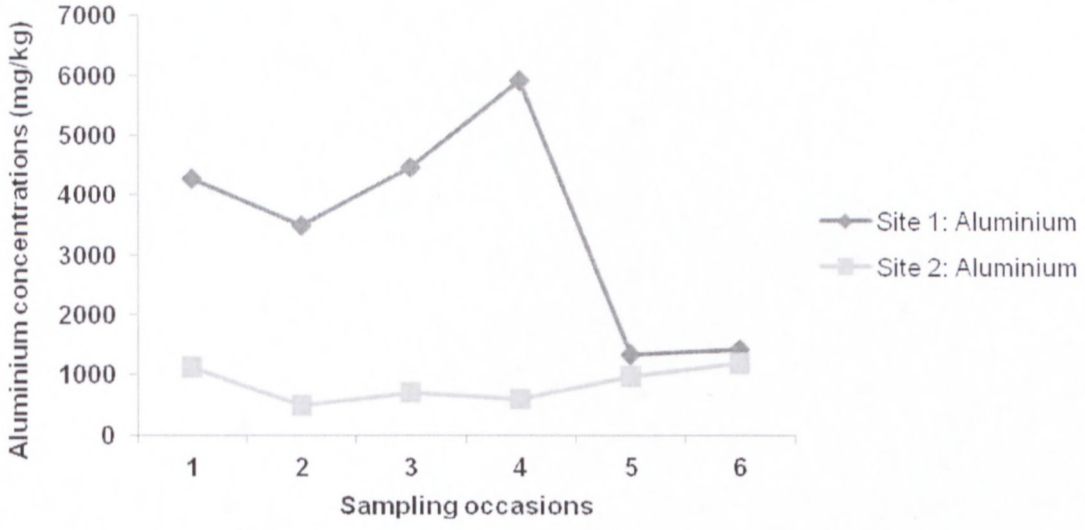

Figure 4.1: Mean aluminium concentrations (mg/kg), measured in sediment from two Diep River sampling sites, per sampling occasion.

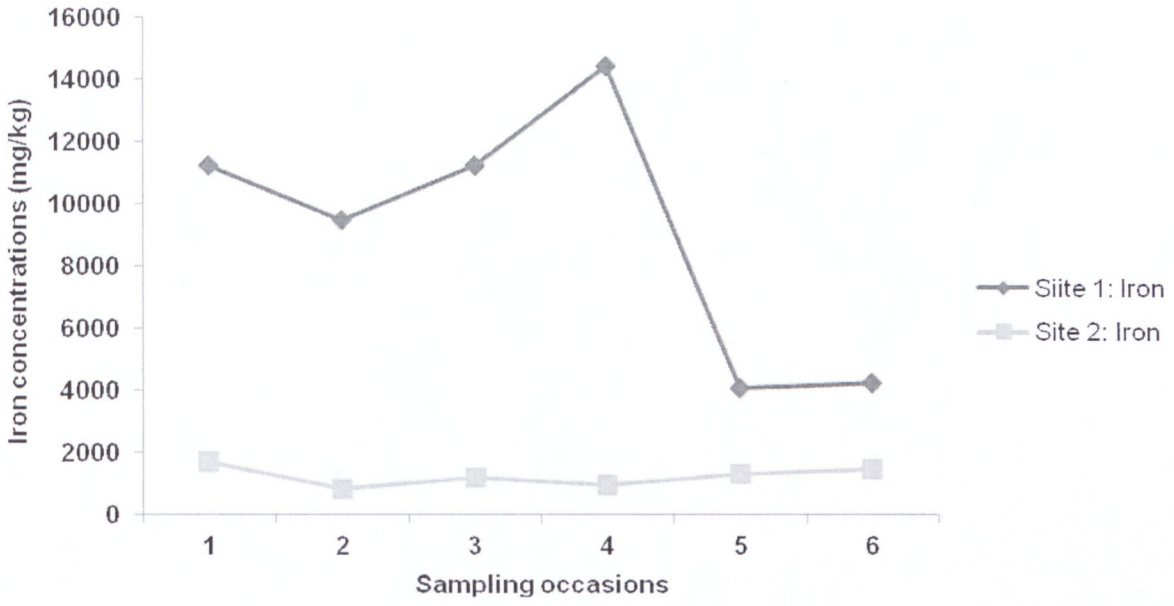

Figure 4.2: Mean iron concentrations (mg/kg), measured in sediment from two Diep River sampling sites, per sampling occasion.

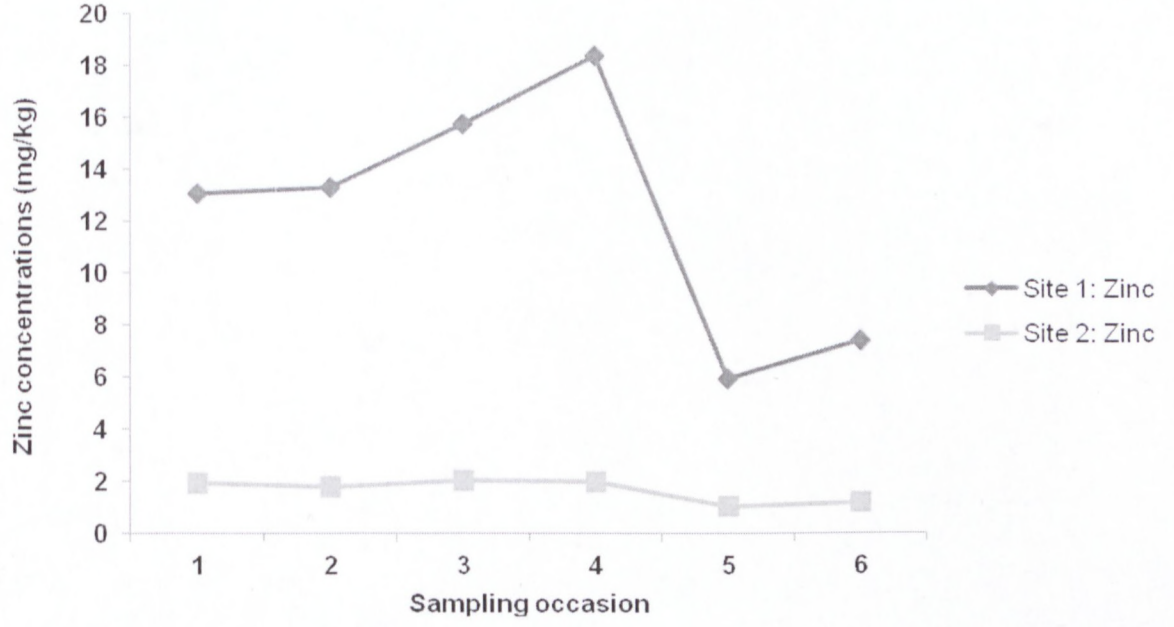

Figure 4.3: Mean zinc concentrations (mg/kg), measured in sediment from two Diep River sampling sites, per sampling occasion.

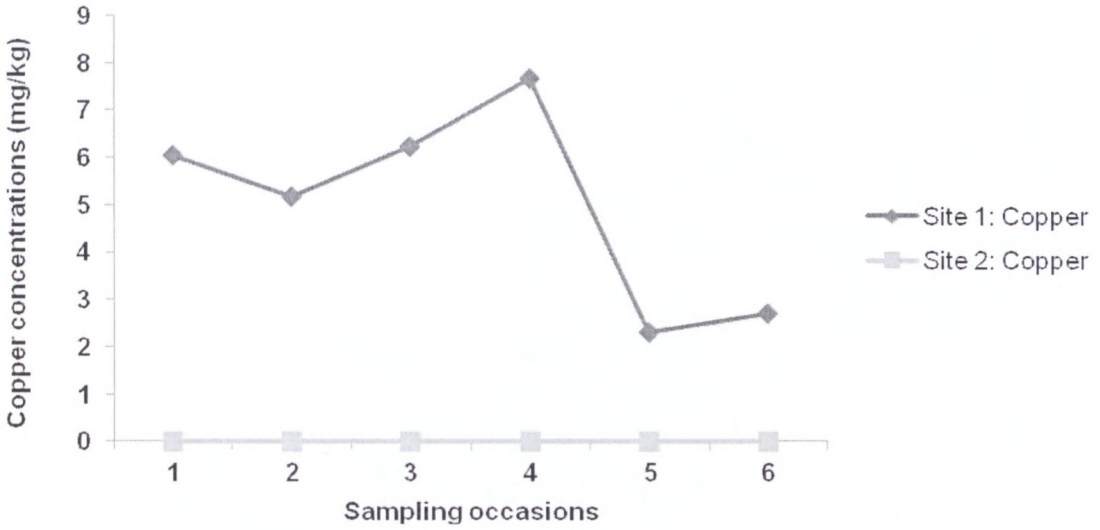

Figure 4.4: Mean copper concentrations (mg/kg), measured in sediment from two Diep River sampling sites, per sampling occasion.

4.2 DISCUSSION

Sediments are considered sinks, resulting in metals accumulating in them at high concentrations (Prasad, 2004). Contaminated sediments are soils, minerals, organic matter or sand that collects at the bottom of a water body that may contain substances that could negatively impact aquatic food chains and the surface water quality. They may be the consequence of discharges, run off from land, be deposited from the atmosphere, erode from river banks, or form from underwater breakdown or build-up of minerals. Sediment contamination contributes to the bioaccumulation of potentially harmful substances in the aquatic environment (ANZECC, 1992).

Sediment samples were sent to Bemlab, an accredited SANAS laboratory for sediment classification to establish the percentages of sand, silt and clay in the sediment at each site. The results of the sediment classification showed that the sediment at both sampling sites were predominantly sandy (96.6% sand at site 1 and 96.3% sand at site 2). The silt contents of the sediment were 1.2% at site 1 and 1.3% at site 2, respectively. The clay content of the sediment was 2.2% clay and 2.4% clay, respectively (Table 4.1). Clay dominated sediments influence the adsorption of cations, anions and pesticides and is a source of nutrients to plants (Moss, 1988). As the clay content of the sediment was very

low, one can conclude that the sediment did not play a major role in holding metals which are common in clay dominated sediment. Minerals in the sand and silt fractions of the soil have little effect on the chemical properties of soil (Wild, 1993). At site 1 there were noticeable rocky outcrops that protruded at intervals in the sandy riverbed which could have contributed to metals in the sediment due to weathering by the friction of moving water over the rocks. Organic material also plays a role in cation exchange (Moss, 1988). Sediment containing organic matter is usually darker in colour, resulting from the decomposition of the organic material. The site 2 sediment was much darker in colour, (dark grey), while the site 1 sediment appeared lighter brown/orange in colour, indicating that site 2 had more organic matter present. Biological life including fish, insects and plants were evident at site 2 which also contribute to the organic matter present in the sediment (personal observation).

As there are no sediment quality guidelines for rivers in South Africa, results were compared with the Canadian Sediment Quality Guidelines (CSQG). Out of the four metals that were studied, only copper and zinc are documented in these guidelines (CCME, 2001). There are no official sediment guidelines available for aluminium or iron.

4.2.1 Aluminium

No quality guidelines are available for aluminium concentrations pertaining to freshwater sediments. Aluminium concentrations were compared with two recent studies conducted on metals in the Diep River by Shuping (2008) and Jackson (2009), respectively.

It is expected that sites containing clay may have traces of aluminium present due to the nature of clay soils combining with positive charged aluminium ions (Moss, 1988). Even a very small amount of clay could influence the concentrations to some extent, however the sediment from both sites contained similar clay fractions (Table 4.1), yet the aluminium concentrations differed significantly (Table 4.2 & Figure 4.1), indicating that there is another aluminium source. The sediment at site 1 contained significantly higher concentrations of aluminium in the sediment compared with the sediment at site 2. The reason for the high aluminium concentrations could result from the rocky outcrops in the riverbed that are weathered by the constant flow of water over them over time, as aluminium is the third most abundant metal in the earth's crust. This may have resulted in high concentrations of aluminium in the sediment as at site 2 the water velocity is much slower and no rocky outcrops were visible.

Aluminium is also soluble at alkaline pH levels (although biologically unavailable) (DWAF, 1996). The pH levels at site 1 were high (>8) (Table 3.1) and the water flowed constantly during the study period. As the concentrations of aluminium were not constant during the study period, with a spike in aluminium concentrations at sampling occasion four and decreasing significantly at the next sampling occasion, this pattern is typical of a possible localised aluminium contaminant entering the river, rather than the weathering of bedrock being the source of aluminium. The only source of aluminium in this area could have emanated from agricultural activities on the sloped gradient on either side of the river. Farming activities may have used aluminium containing pesticides or fungicides to prevent crop diseases (Table 3.6), as there are vineyards, olive groves and citrus farms in the vicinity. The high rainfall during the study period could have increased the concentrations due to increased runoff into the river containing traces of aluminium (Table 2.1 & Figure 2.5).

At site 2 the aluminium concentrations in the sediment seemed to be more constant (Table 4.2 & Figure 4.1), however four of the six sampling occasions experienced significant differences in concentrations compared with consecutive sampling occasions indicating possible point source pollutants entering the river that caused fluctuations in the aluminium concentrations (Table 4.2 & Figure 4.1). These fluctuations could be attributed to industrial runoff from surrounding industries close to site 2 and the unseasonal rain over the study period that could have contributed to contaminated runoff (Table 2.1 & Figure 2.5).

As there have been no other metal studies in the sediments of the Diep River, north of Malmesbury, concentrations of aluminium were not compared with results obtained from any other studies for site 1. Site 1 had significantly higher concentrations of aluminium in the sediments compared with site 2 which follows the same trend for aluminium in water (Table 3.2 & Table 4.2). Shuping (2008), sampled sediment within 500 m of site 2 (present study) and found 1454.55 ±140.48 mg/kg, 17694.22 ±462.07 mg/kg and 15450.22 ±614.49 mg/kg respectively of aluminium in the sediment over the same time period (but conducted at two-monthly intervals) in 2004. These concentrations of aluminium were far higher than the concentrations of aluminium sampled in the present study (Table 4.2 & Figure 4.1). Jackson et al. (2009) detected aluminium concentrations only slightly higher than those recorded for the present study in the Diep River sediment at the site closest to the present study's site 2, at Potsdam Wastewater Treatment Works.

The reason for these higher values could be due to the dredging that occurs in the vicinity of site 2 (personal observation) or the different rainfall patterns experienced at the time of the sampling or through filtering by aquatic macrophytes. Dredging is required due to the prolific growth of reeds in the river that tends to block water flow.

4.2.2 Iron

Iron is the most useful of all metals as its alloy is steel, which is a highly versatile metal, used in the manufacturing of cars, food containers, cargo ships and paper staples (DWAF, 1996). Due to its abundant usage, it is expected that iron would constitute a large proportion of metal pollution in the environment.

Cation exchange capacity varies in sediments and soils depending on the percentage of clay present (Smith, 1986) and as the two sites have very similar clay compositions in the sediments, any differences in the iron contents will most likely not be due to clay content but rather due to other influences as both sites have just over 2% clay in the sediment (Table 4.1).

No quality guidelines are available for iron in sediments. Iron concentrations were compared with two other metal studies conducted in the Diep River on sediment. Shuping (2008) sampled sediment within 500 m of site 2 (present study) and found 1872.60 ±248.82 mg/kg, 28116.77 ±429.49 mg/kg and 13925.03 ±396.56 mg/kg respectively of iron in the sediment over the same time period (conducted at two monthly intervals) in 2004. The first sample compared well with that of the present study (Table 4.3 & Figure 4.2) but the other iron concentrations in the previous study were much higher than that detected in the present study. Jackson et al. (2009) detected the highest mean concentration of iron in the Diep River sediment at the site closest to the present study site 2, at Potsdam Wastewater Treatment Works, of 106 379.5 mg/kg during March 2005. This amount far surpasses that of any iron concentration detected in the sediment in that area during the present study (Table 4.3 & Figure 4.2).

The reasons for this could be similar to those discussed for aluminium, namely the regular dredging that occurs near site 2 due to the increase in *Phragmites* reeds in the river and the reeds themselves acting as filters for metals by absorbing them (Lee & Scholz, 2007), which could have prevented iron and other metals from entering the environment at site 2. The higher rainfall experienced at the time of the sampling (Table 2.1 & Figure 2.5) could

have resulted in faster flow rates that washed metals downstream preventing them from sinking and forming part of the sediment. The time period of 5 years that has elapsed between the present study and that of the previous study could have resulted in increased plant growth to absorb metals and an increase in water flow could have influenced sediment build-up.

At site 1, the spike in iron concentrations in the sediment at sampling occasion 4, coincided with the spike in iron concentrations of the water at site 1 at sampling occasion 3 (resulting in a two week delay for the settling of sediment) (Table 3.3 & Figure 3.2). These results indicate point-source pollution at site 1 similarly experienced for aluminium. Possible sources of iron contaminants could have been from fungicides and animal feed from dairy farms (Table 3.6 & Table 2.1).

As there have been no other metal studies in the sediments of the Diep River, north of Malmesbury, concentrations of iron were not compared with results obtained from any other studies for site 1.

At site 2, there were four sampling occasions where iron concentrations fluctuated significantly between consecutive sampling occasions (Table 4.3). These fluctuations could have resulted from sewage, industrial activities involving iron or from algal blooms that require iron for metabolism but once the algae decompose, iron is returned to the environment by decomposition (DWAF, 1996). These fluctuations in algal blooms could have contributed to the fluctuating iron trends in the sediment as algal activity was evident at site 2 but not at site 1 (Figure 2.10). The sediment concentrations for iron at site 2 did not follow the same trends as for water, which showed greater fluctuations (Figure 3.2 & Figure 4.2) and a significant decrease over the entire study period. This may be due to the nature of water that is constantly in motion and shows a more immediate response to pollutants than sediment which comprises of layers of deposited substances over time.

4.2.3 Zinc

According to the Canadian Sediment Quality Guidelines, the recommended Interim Sediment Quality Guideline (ISQG) for zinc is 123 mg/kg with a Probable Effect Level (PEL) of 315 mg/kg (CCME, 2001).

The concentrations of zinc detected in the sediment at site 1 were well within the 123 mg/kg limit set out by the ISQGs set out by the CSQG (CCME, 2001) and at site 2 the concentrations were significantly lower than at site 1. The significantly higher zinc concentrations in the sediment at site 1 is most likely due to the influence of farming activities involving pesticide and fungicide spraying, as zinc is an ingredient of many fungicides and fertilizers, often required in viticulture (Longstroth, 2002) (Table 3.6) and can be found in food supplements of dairy cows that could enter the environment through manure (Li et al., 2005). At the time of the study in late spring and early summer, vineyards require several applications of fungicide to control diseases (Longstroth, 2002). Zinc can also be weathered from rocks within the riverbed from the constant friction of water moving over the rock surface (DWAF, 1996) and as rocks were visible in the water at site 1 and the water flowed faster at site 1 compared with site 2 , this could also have contributed to the higher zinc concentrations found at site 1. At the fourth sampling occasion at site 1, the zinc concentrations spiked significantly higher compared with the previous sampling occasion, this followed the same trend for aluminium, iron and copper at site 1 where concentrations were all significantly higher at the fourth sampling occasion compared with the fifth sampling occasions for each metal. This similarity probably indicates the use of aluminium, iron, zinc and copper in farming activities in the region of site 1 as they all increased at the same sampling occasion coinciding with heavy rainfall occurring in that region during November 2009 (Table 2.1 & Figure 2.5). The reason for site 2 having significantly less zinc than site 1 in the sediment, may possibly be due to the many *Phragmites* reeds that occur just upstream from site 2 that could have absorbed the zinc before reaching the area (Lee & Scholz, 2007). The speed of the water could have also resulted in metals not having sufficient time to settle in the sediment, as the metals were taken downstream past site 2 due to the unseasonal heavy rainfall (Table 2.1 & Figure 2.5). The zinc concentrations of pooled data in the water at site 1 were significantly higher than the pooled data for zinc in the water at site 2. Similarly, zinc concentrations for the sediment at site 1, using pooled data was significantly higher than for site 2. For pooled data, zinc in the water and sediment followed a similar pattern at both sites (Table 3.4 & Table 4.4).

Shuping (2008) measured zinc concentrations in the sediment within 500 m of site 2 (present study) during the months of September 2004, November 2004 and January 2005. The mean concentrations found in the sediment were 15.87 ±2.25 mg/kg, 290.57 ±11.91 mg/kg and 331.32 ±12.76 mg/kg, measured at two-monthly intervals. These measurements were over the CSQGs for the second and third sampling occasions in

summer and much higher than the readings obtained during the present study (Table 4.4 & Figure 4.3). As the Shuping (2008) study did not include sites north of Malmesbury, site 1 from the present study was not compared with any other data. Jackson et al. (2009) detected the highest mean concentration of zinc in the Diep River sediment at the site closest to the present study's site 2, at Potsdam Wastewater Treatment Works, of 1081.2 mg/kg during January 2005 (Jackson et al., 2009). This amount far surpasses the recommended CSQGs of 123 mg/kg for zinc (CCME, 2001) and is significantly higher than the concentrations of zinc detected in the sediment in that area during the present study (Table 4.4 & Figure 4.3). The vast differences in concentrations could be due to dredging of the river and seasonal variations in rainfall. As there have been no other metal studies pertaining to the sediments of the Diep River, north of Malmesbury, concentrations of zinc were not compared with results for site 1.

4.2.4 Copper

According to the Canadian Sediment Quality Guidelines (CCME, 2001), the recommended Interim Sediment Quality Guideline (ISQG) for copper is 35.7 mg/kg with a Probable Effect Level (PEL) of 197 mg/kg. The copper detected in the sediment samples at site 1 were well below the ISQG and PEL values set out by the CSQGs. The results for copper in the water at site 1 were opposite to that of the sediment, being over the recommended guidelines for TWQR set out by DWAF (1996), except for the second sampling occasion (Table 3.5). This could be due to the immediate contamination of water by possible fungicides used by farmers (Schulz, 2001; Dabrowski et al., 2002) in the region of site 1 and the fact that the water velocity was rapid and the water speed could have moved the copper contaminants before they could sink to the sediment. The significant decrease in copper at the fifth sampling occasion compared with the fourth could have resulted from the high rainfall experienced during November 2009 that could have washed any contaminants downstream before samples were taken and not allowing for the copper to sink to the bottom and to be adsorbed to sediment. It is suspected that the significantly higher concentrations of copper in the sediment at site 1 originated from the constant use of copper as fungicides and pesticides in the treatment of diseases on crops by farmers in the surrounding region. During summer, rainfall is normally low in the Western Cape (Table 2.2) and water flow slower, allowing for copper to sink and adsorb to the sediment particles.

At site 2 no copper was detected in the sediment samples (Table 4.5 & Figure 4.4). These results were compared with other studies done on metal contamination pertaining to the Diep River. Shuping (2008) analysed sediment for copper within 500 m of site 2, where the concentrations of copper for the months of September 2004, November 2004 and January 2005, taken at two-monthly intervals read 39.34 ±53.50 mg/kg, 108.28 ±1.60 mg/kg and 115.23 ±5.70 mg/kg, respectively. These amounts far surpassed the zero readings for copper obtained during the present study at site 2. Over the five year period, copper concentrations may have been lost due to erosion of the river banks under flood conditions or dredging of the riverbed in this region. Dredging of the river to remove excessive reeds or water hyacinth disturbs the layers of sedimentation of the river built up over time, as sediment build up can be just a few millimetres per year. Erosion of the river bank could also result from heavy earth moving machinery used to carry out operations (Moss, 1988). Dredging equipment was observed during the study period within the region of site 2 but upstream. Although no copper was detected in the sediment at site 2, the copper in the water was generally higher than the TWQR recommended by DWAF (DWAF, 1996) perhaps indicating that the copper did not have time to sink and form part of the sediment, possibly due to the higher water velocity experienced during the unseasonably high rainfall (Table 2.1 & Figure 2.5) or perhaps due to the *Phragmites* reeds upstream to rapidly absorb copper (Lee & Scholz, 2007).

A study conducted by Jackson et al. (2009), measured copper concentrations in the Diep River sediments at various sites in the Diep River. The closest site in relation to site 2 of this study would be the one at the Potsdam Wastewater Treatment Works, where Jackson et al. (2009) found that the copper concentrations fluctuated above and below the recommended guidelines of the CCME. The months of the year when this study was conducted do not correlate with the months of the present study and seasonal variations could play a significant role in differing levels of concentrations.

All four metals sampled in the sediment showed a similar pattern of concentration across the sampling period, increasing in concentration at the fourth sampling occasion (aluminium at the fifth) as indicated by Figures 4.1, 4.2, 4.3 & 4.4. The reason for this similar trend could be as a result of the heavy rainfall experienced at the time during November 2009 (Table 2.1 & Figure 2.5), coinciding with this sampling occasion, which could have added these metals as part of runoff from dairy farms and pesticide residues from vineyards near site 1 (Schulz, 2001; Dabrowski et al., 2002). Sediments collected at the fourth sampling occasion at site 1 had significantly higher copper concentrations

compared with the same sampling occasion at site 2. Generally metals have decreased in the sediment from 2004 and 2005 to 2009, however the unusually high rainfall during November 2009 may have falsely given this impression, not allowing for the contaminants to sink to the bottom of the river due to strong water currents sending the contaminants downstream.This indicates that metal concentrations in the sediment alone do not always give a conclusive indication of metal contamination in an aquatic environment. Thus, the bioaccumulation of metals in aquatic organisms such as plants plays an important role in determining the health of an environment.

CHAPTER FIVE

RESULTS AND DISCUSSION: PLANTS: METAL CONCENTRATIONS

5.1 RESULTS

5.1.1 ALUMINIUM

5.1.1.1 Metal comparison per sampling occasion, per site: Introduced Plants

5.1.1.1.1 Comparisons between consecutive sampling occasions at site 1 in the plant stems

There were significantly higher aluminium concentrations in the stems on the third sampling occasion compared to the second and on the fifth sampling occasion compared to the fourth ($P<0.05$). There was also a significant increase in aluminium concentrations between the first and last sampling occasion ($P<0.05$). There were no other significant differences in aluminium concentrations during the other consecutive sampling occasions ($P>0.05$) (Table 5.1 & Figure 5.1).

5.1.1.1.2 Comparisons between consecutive sampling occasions at site 1, in the plant leaves

There was a significant increase in aluminium concentrations in the leaves of the *Ceratophyllum sp.* from the concentrations detected in the reference plants compared to leaves on the first sampling occasion ($P <0.05$). There were no other significant differences between any of the other consecutive sampling occasions (Table 5.1 & Figure 5.1).

5.1.1.1.3 Comparisons between the stems and leaves at site 1, using pooled data

The pooled data showed the leaves of the *Ceratophyllum sp.* placed in the river at site 1 contained more aluminium in their leaves than in their stems ($P<0.05$) (Table 5.1).

5.1.1.1.4 Comparisons between consecutive sampling occasions at site 2 in the plant stems

There was a significantly higher concentration of aluminium detected in the stems on the first sampling occasion compared with the last sampling occasion at site 2 ($P<0.05$). At the second sampling occasion there were significantly higher aluminium concentrations compared to the first sampling occasion but then significantly lower concentrations in the third sampling occasion, compared to the second. At the fifth sampling occasion there were significantly lower concentrations compared to the fourth sampling occasion ($P<0.05$). There was significantly lower concentrations of aluminium in the plant stems in the last sampling occasion, compared to the first ($P<0.05$) (Table 5.1 & Figure 5.1).

5.1.1.1.5 Comparisons between consecutive sampling occasions at site 2 in the plant leaves

There were no significant differences in the aluminium concentrations in the leaves of the plants between the sampling occasions at site 2. The only significantly higher concentration of aluminium was detected between the reference plants and the first sampling occasion ($P<0.05$) (Table 5.1 & Figure 5.1).

5.1.1.1.6 Comparisons between the stems and leaves at site 2, using pooled data

The pooled data showed no significant differences in aluminium concentrations between the stems and leaves of *Ceratophyllum sp.* introduced at site 2 ($P>0.05$) (Table 5.1).

5.1.1.1.7 Comparisons between consecutive sampling occasions for the existing plants at site 2 in the plant stems

There were significantly lower concentrations of aluminium between the first and last sampling occasions in the stems of the existing plants at site 2. On the fifth sampling occasion, there was significantly lower concentrations of aluminium in the stems of the existing plants, compared to the fourth sampling occasion ($P<0.05$) (Table 5.1 & Figure 5.1).

5.1.1.1.8 Comparisons between consecutive sampling occasions for the existing plants at site 2 in the plant leaves

There was a significant decrease in aluminium concentrations in the leaves of the existing plants between the fifth and sixth sampling occasion ($P<0.05$). There were no other consecutive sampling occasions with significant differences between visits (Table 5.1 & Figure 5.1).

5.1.1.2 Metal comparisons between sites: Introduced plants

5.1.1.2.1 Comparisons between sampling sites 1 and 2 on the same sampling occasion in the plant stems

There were significantly higher concentrations of aluminium in the stem samples from site 1 on the third, fourth, fifth and sixth sampling occasions compared with the same sampling occasions at site 2 ($P<0.05$). At sampling occasion two, however, the plant stems at site 2 had significantly higher concentrations of aluminium than the plant stems at site 1 ($P<0.05$). The other corresponding sampling occasions showed no significant differences in concentrations between the two sites ($P>0.05$) (Table 5.1 & Figure 5.1).

5.1.1.2.2 Comparisons between sampling sites 1 and 2 on the same sampling occasion in the plant leaves

The only significant differences in aluminium concentrations were detected at the fifth and sixth sampling occasions between the two sites ($P<0.05$). The plant leaves at site 1 had significantly higher concentrations of aluminium than the plant leaves at site 2 for the last two sampling occasions ($P<0.05$) (Table 5.1 & Figure 5.1).

5.1.1.2.3 Comparisons between the stems at site 1 with the stems at site 2 and the leaves at site 1 with the leaves at site 2, using pooled data

The pooled data for both the stems and leaves of the plants introduced at site 1 had significantly higher concentrations of aluminium compared to the stems and leaves of the introduced plants at site 2 ($P<0.05$) (Table 5.1).

5.1.1.3 Metal comparisons at site 2: Introduced vs. existing plants

5.1.1.3.1 Aluminium concentrations between stems of the introduced plants and stems of the existing plants at site 2, per sampling occasion

The introduced plant stems at site 2 had significantly higher concentrations of aluminium in their stems at the first and last sampling occasions, compared to the aluminium concentrations detected in the stems of the existing plants ($P<0.05$). There were no other significant differences between the other sampling occasions ($P>0.05$) (Table 5.1 & Figure 5.1).

5.1.1.3.2 Aluminium concentrations between leaves of the introduced plants and leaves of the existing plants at site 2, per sampling occasion

There were significantly higher concentrations of aluminium in the leaves of the introduced plants compared to the leaves of the existing plants on the second sampling occasion at site 2 ($P<0.05$) (Table 5.1 & Figure 5.1).

5.1.1.3.3 Comparisons between the stems and leaves of the existing *Ceratophyllum sp.* and the introduced plants, using pooled data

The pooled data showed that there was a significantly higher concentration of aluminium detected in the stems of the introduced plants at site 2 compared to the stems of the existing *Ceratophyllum sp.* growing at site 2 ($P<0.05$). The leaves of the plants that were introduced to site 2 also had significantly higher concentrations of aluminium compared to the existing *Ceratophyllum sp.* ($P<0.05$) (Table 5.1).

5.1.1.3.4 Comparisons between the stems and leaves of the existing *Ceratophyllum sp.* at site 2, using pooled data

The pooled data showed significantly higher concentrations of aluminium in the leaves of the existing *Ceratophyllum sp.* compared to their stems ($P<0.05$) (Table 5.1).

5.1.2. IRON

5.1.2.1 Metal comparisons per sampling occasion, per site: Introduced Plants

5.1.2.1.1 Comparisons between consecutive sampling occasions at site 1 in the plant stems

There was a significantly lower concentration of iron in the stems on the first sampling occasion compared to the higher concentration detected in the last sampling occasion ($P<0.05$) but no significant differences in iron concentrations during the other consecutive sampling occasions ($P>0.05$) (Table 5.2 & Figure 5.2).

5.1.2.1.2 Comparisons between consecutive sampling occasions at site 1 in the plant leaves

There was a significantly lower concentration of iron in the leaves on the first sampling occasion compared to the higher concentrations detected in the sixth sampling occasion ($P<0.05$). There were no significant differences between any of the other consecutive sampling occasions ($P>0.05$) (Table 5.2 & Figure 5.2).

5.1.2.1.3 Comparisons between the stems and leaves at site 1, using pooled data

The pooled data showed that the leaves of the *Ceratophyllum sp.* introduced to the river at site 1 contained more iron in their leaves than in their stems. There was a significantly higher concentration of iron in the leaves of the plants than in the stems ($P<0.05$) (Table 5.2).

5.1.2.1.4 Comparisons between consecutive sampling occasions at site 2 in the plant stems

There were no significant differences in the iron concentrations in the stems of the *Ceratophyllum sp.* introduced to the river at site 2 between the six consecutive sampling occasions ($P>0.05$) (Table 5.2 & Figure 5.2).

5.1.2.1.5 Comparisons between consecutive sampling occasions at site 2 in the plant leaves

There were no significant differences in the iron concentrations of the leaves of the plants between the sampling occasions at site 2 ($P>0.05$) (Table 5.2 & Figure 5.2).

5.1.2.1.6 Comparisons between the stems and leaves at site 2, using pooled data

The pooled data showed that there were no significant differences in the iron concentrations between the stems and leaves of the *Ceratophyllum sp.* introduced at site 2 ($P>0.05$) (Table 5.2).

5.1.2.1.7 Comparisons between consecutive sampling occasions for the existing plants at site 2 in the plant stems

There was only a significant increase in iron concentration between the first and the last sampling occasions in the stems of the existing plants at site 2 ($P<0.05$) (Table 5.2 & Figure 5.2).

5.1.2.1.8 Comparisons between consecutive sampling occasions for the existing plants at site 2 in the plant leaves

There were no consecutive sampling occasions with significant differences between visits (Table 5.2 & Figure 5.2).

5.1.2.2 Metal comparisons between sites: Introduced plants

5.1.2.2.1 Comparisons between sampling sites 1 and 2 on the same sampling occasion in the plant stems

There were significantly higher concentrations of iron in the stem samples from site 1 on the fifth and sixth sampling occasions compared to the same sampling occasions at site 2 ($P<0.05$) (Table 5.2 & Figure 5.2).

5.1.2.2.2 Comparisons between sampling sites 1 and 2 on the same sampling occasion in the plant leaves

The only significant differences in iron concentrations in the leaves were detected at the fifth and sixth sampling occasions between the two sites. The iron concentrations in the leaves were significantly higher at site 1 at the fifth and sixth sampling occasions ($P<0.05$) (Table 5.2 & Figure 5.2).

5.1.2.2.3 Comparisons between the stems at site 1 with the stems at site 2 and the leaves at site 1 with the leaves at site 2, using pooled data

The pooled data showed that both the stems and leaves of the plants introduced at site 1 had significantly higher concentrations of iron in them than the stems and leaves of the introduced plants at site 2 ($P<0.05$) (Table 5.2).

5.1.2.3 Metal comparisons at site 2: Introduced vs. existing plants

5.1.2.3.1 Iron concentrations between stems of the introduced plants and stems of the existing plants at site 2, per sampling occasion

There were significantly higher concentrations of iron detected in the stems of the introduced plants compared to the existing plants at the second sampling occasion ($P<0.05$). There were no other significant differences between sampling occasions (Table 5.2 & Figure 5.2).

5.1.2.3.2 Iron concentrations between leaves of the introduced plants and leaves of the existing plants at site 2, to per sampling occasion

There were significantly higher concentrations of iron detected in the leaves of the introduced plants compared to the existing plants at the third sampling occasion ($P<0.05$). There were no other significant differences between sampling occasions (Table 5.2 & Figure 5.2).

5.1.2.3.3 Comparisons between the stems and leaves of the existing *Ceratophyllum sp.* and the introduced plants, using pooled data

The pooled data showed that there was a significantly higher concentration of iron detected in the stems of the introduced plants at site 2 compared to the stems of the existing *Ceratophyllum sp.* at site 2 ($P<0.05$). The leaves of the plants that were introduced to site 2 also had significantly higher concentrations of iron compared to the existing *Ceratophyllum sp.* ($P<0.05$) (Table 5.2).

5.1.2.3.4 Comparisons between the stems and leaves of the existing *Ceratophyllum sp.* at site 2, using pooled data

The pooled data showed that there was a significantly higher concentration of iron in the leaves of the *Ceratophyllum sp.* compared to the stems of the same plants sampled at site 2 ($P<0.05$) (Table 5.2).

5.1.3 ZINC

5.1.3.1 Metal comparisons per sampling occasion, per site: Introduced plants

5.1.3.1.1 Comparisons between consecutive sampling occasions at site 1 in the plant stems

There were no significant differences in zinc concentrations during the other consecutive sampling occasions ($P>0.05$) (Table 5.3 & Figure 5.3).

5.1.3.1.2 Comparisons between consecutive sampling occasions at site 1 in the plant leaves

There were no significant differences in zinc concentrations in the leaves of the plants between the sampling occasions neither on site 1 nor between the sampling occasions and the reference plants ($P>0.05$) (Table 5.3 & Figure 5.3).

5.1.3.1.3 Comparisons between the stems and leaves at site 1, using pooled data

The pooled data showed that the leaves of the *Ceratophyllum sp.* introduced at site 1 had a significantly higher concentration of zinc in them, compared to the stems ($P<0.05$) (Table 5.3).

5.1.3.1.4 Comparisons between consecutive sampling occasions at site 2 in the plant stems

There were no significant differences between the zinc concentrations in the stems of the *Ceratophyllum sp.* introduced at site 2 between the six consecutive sampling occasions ($P>0.05$). There was however a significant decrease in zinc concentration in the stems of the plants sampled on the last sampling occasion compared to the plant stems sampled on the first sampling occasion ($P<0.05$) (Table 5.3 & Figure 5.3).

5.1.3.1.5 Comparisons between consecutive sampling occasions at site 2 in the plant leaves

There were no significant differences in the zinc concentrations in the leaves of the plants at site 2, neither between the sampling occasions nor between the first visit and the reference plants ($P>0.05$). There was however, a significantly higher concentration of zinc detected in the leaves of the plants at the first sampling occasion, compared to the leaves of the plants at the last sampling occasion ($P<0.05$) (Table 5.3 & Figure 5.3).

5.1.3.1.6 Comparison between the stems and leaves at site 2, using pooled data

The pooled data showed that there were no significant differences in the zinc concentrations between the stems and leaves of the *Ceratophyllum sp.* introduced at site 2 ($P>0.05$) (Table 5.3).

5.1.3.1.7 Comparisons between consecutive sampling occasions for the existing plants at site 2 in the plant stems

The first and the last sampling occasions showed a significant increase in zinc concentrations in the plant stems of the existing plants ($P<0.05$) (Table 5.3 & Figure 5.3).

5.1.3.1.8 Comparisons between consecutive sampling occasions for the existing plants at site 2 in the plant leaves

There were significantly lower concentrations of zinc in the leaves of the reference plants compared to the first sampling occasion ($P<0.05$). There was a significant increase in zinc concentration in the existing plants leaves between the third and fourth sampling occasion ($P<0.05$) and a significant decrease between the fourth and fifth sampling occasions ($P<0.05$) (Table 5.3 & Figure 5.3).

5.1.3.2 Metal comparisons between sites: Introduced plants

5.3.2.1 Comparisons between sampling sites 1 and 2 on the same sampling occasion in the plant stems

There were no significant differences in zinc concentrations between the two sites in the plant stems during the sampling period ($P>0.05$) (Table 5.3 & Figure 5.3).

5.1.3.2.1 Comparisons between sampling sites 1 and 2 on the same sampling occasion in the plant leaves

There were no significant differences between the two sites during the sampling period ($P>0.05$) (Table 5.3 & Figure 5.3).

5.1.3.2.2 Comparisons between the stems at site 1 with the stems at site 2 and the leaves at site 1 with the leaves at site 2, using pooled data

The pooled data showed that the stems and leaves of the introduced plants at site 1 had significantly higher concentrations of zinc in their stems and leaves, compared to the stems and leaves of the introduced plants sampled at site 2 ($P<0.05$) (Table 5.3).

5.1.3.3 Metal comparisons at site 2: Introduced vs. existing plants

5.1.3.3.1 Zinc concentrations between stems of the introduced plants and stems of the existing plants at site 2, per sampling occasion

There were no significant differences in zinc concentrations in the stems of the introduced plants compared to the stems of the existing plants at any sampling occasion ($P>0.05$) (Table 5.3 & Figure 5.3).

5.1.3.3.2 Zinc concentrations between leaves of the introduced plants and leaves of the existing plants at site 2, per sampling occasion

There were significantly higher concentrations of zinc detected in the leaves of the introduced plants compared to the existing plants at the second sampling occasion ($P<0.05$). There were no other significant differences between sampling occasions (Table 5.3 & Figure 5.4).

5.1.3.3.3 Comparisons between the stems and leaves of the existing *Ceratophyllum sp.* and the introduced plants, using pooled data

The pooled data showed that there were significantly higher concentrations of zinc detected in the stems of the introduced plants at site 2, compared to the stems of the existing *Ceratophyllum sp.* growing at site 2 ($P<0.05$). The leaves of the plants that were introduced to site 2 also had significantly higher concentrations of zinc compared to the existing *Ceratophyllum sp.* ($P<0.05$) (Table 5.3).

5.1.3.3.4 Comparisons between the stems and leaves of the existing *Ceratophyllum sp.* at site 2, using pooled data

The pooled data showed that there was a significantly higher concentration of zinc in the leaves of the *Ceratophyllum sp.* compared to the stems of the same plants sampled at site 2 ($P<0.05$) (Table 5.3).

5.1.4 COPPER

5.1.4.1 Metal comparisons per sampling occasion, per site: Introduced plants

5.1.4.1.1 Comparisons between consecutive sampling occasions at site 1 in the plant stems

The concentrations of copper in the stems of the plants on the first sampling occasion was significantly higher than the stems of the reference plants ($P<0.05$). There were significantly higher concentration of copper detected at the first sampling occasion compared to that of the last (sixth) sampling occasion ($P<0.05$) (Table 5.4 & Figure 5.4).

5.1.4.1.2 Comparisons between consecutive sampling occasions at site 1 in the plant leaves

The copper concentrations in the leaves of the *Ceratophyllum sp.* were significantly lower in the reference plants, compared to the copper concentrations detected in the leaves at the first sampling occasion ($P <0.05$). There were no other significant differences between any of the consecutive sampling occasions ($P>0.05$) (Table 5.4 & Figure 5.4).

5.1.4.1.3 Comparisons between the stems and leaves at site 1, using pooled data

The pooled data showed that there were no significant differences in the copper concentrations between the stems and leaves of the introduced plants at site 1 ($P>0.05$) (Table 5.4).

5.1.4.1.4 Comparisons between consecutive sampling occasions at site 2 in the plant stems

There was a significantly higher concentration of copper detected in the stems at the first sampling occasion compared to the second sampling occasion, as well as between the first and last sampling occasions ($P<0.05$). There were no significant differences in copper concentrations between any of the other consecutive sampling occasions ($P>0.05$). There was also significantly higher copper concentration in the stems of the plants on the first sampling occasion, compared to the reference plants ($P<0.05$) (Table 5.4 & Figure 5.4).

5.1.4.1.5 Comparisons between consecutive sampling occasions at site 2 in the plant leaves

There was a significantly lower concentration of copper in the leaves of the plants at the fifth sampling occasion, compared to that of the fourth sampling occasion ($P \leq 0.05$). The copper concentrations detected in the leaves on the first sampling occasion were significantly higher than samples from the last (sixth) sampling occasion ($P \leq 0.05$). There was a significantly higher concentration of copper detected in the leaves at the first sampling occasion, compared to the reference plant leaves ($P \leq 0.05$) (Table 5.4 & Figure 5.4).

5.1.4.1.6 Comparisons between the stems and leaves at site 2, using pooled data

The pooled data showed that there were no significant differences in the copper concentrations between the stems and leaves of the introduced plants at site 2 ($P > 0.05$) (Table 5.4).

5.1.4.1.7 Comparisons between consecutive sampling occasions for the existing plants at site 2 in the plant stems

There was a significant decrease in copper concentration between the first and last sampling occasions in the stems of the existing plants ($P < 0.05$). There were no other significant differences between any of the consecutive sampling occasions ($P > 0.05$) (Table 5.4 & Figure 5.4).

5.1.4.1.8 Comparisons between consecutive sampling occasions for the existing plants at site 2 in the plant leaves

There were no significant differences between copper concentrations in the plant leaves between consecutive sampling occasions of the existing plants at site 2 ($P > 0.05$) (Table 5.4 & Figure 5.4).

5.1.4.2 Metal comparisons between sites: Introduced plants

5.1.4.2.1 Comparisons between sampling sites 1 and 2 on the same sampling occasion in the plants stems

There were significantly higher concentrations of copper in the stems from site 1 on the fifth sampling occasion, compared to the same sampling occasion at site 2 ($P<0.05$). The other corresponding sampling occasions between the two sites showed no significant differences in copper concentrations ($P>0.05$) (Table 5.4 & Figure 5.4).

5.1.4.2.2 Comparisons between sampling sites 1 and 2 on the same sampling occasion in the plant leaves

There were no significant differences in copper concentrations between the sites per sampling occasion in the plant leaves of the introduced plants ($P>0.05$) (Table 5.4 & Figure 5.4).

5.1.4.2.3 Comparisons between the stems at site 1 with the stems at site 2 and the leaves at site 1 with the leaves at site 2, using pooled data

The pooled data showed that the stems of the plants introduced at site 1 had significantly higher concentrations of copper in them compared to the stems of the plants at site 2 ($P<0.05$). The leaves of the plants introduced at site 1 also had significantly higher concentrations of copper in them compared to the leaves of the plants at site 2 ($P<0.05$) (Table 5.4).

5.1.4.3 Metal comparisons at site 2: Introduced vs. existing plants

5.1.4.3.1 Copper concentrations between stems of the introduced plants and stems of the existing plants at site 2, per sampling occasion

All the copper concentrations in the plant stems of the existing plants were significantly different to the copper concentrations in the plant stems of the introduced plants at the same sampling occasion. On all occasions the introduced plant stems showed significantly higher concentrations of copper ($P<0.05$) (Table 5.4 & Figure 5.4).

5.1.4.3.2 Copper concentrations between leaves of the introduced plants and leaves of the existing plants at site 2, per sampling occasion

There were significantly higher concentrations of copper detected in the leaves of the introduced plants compared to the existing plants at the first and second sampling occasions ($P<0.05$). There were no other significant differences between sampling occasions (Table 5.4 & Figure 5.4).

5.1.4.3.3 Comparisons between the stems and leaves of the existing *Ceratophyllum sp.* and the introduced plants, using pooled data

The pooled data showed that there were significantly higher concentrations of copper in the stems of the introduced plants at site 2 compared to the stems of the existing *Ceratophyllum sp.* at site 2 ($P<0.05$). The leaves of the plants that were introduced to site 2 also had significantly higher concentrations of copper in their leaves compared to the existing *Ceratophyllum sp.* leaves ($P<0.05$) (Table 5.4).

5.1.4.3.4 Comparisons between the stems and leaves of the existing *Ceratophyllum sp.* at site 2, using pooled data

The pooled data showed that there were significantly higher concentrations of copper in the leaves of the existing *Ceratophyllum sp.* compared to the stems of the same plants sampled at site 2 ($P<0.05$) (Table 5.4).

Table 5.1: Mean (± SD) aluminium concentrations (mg/kg), measured in *Ceratophyllum demersum* L. stems and leaves from the Diep River sampling sites 1 and 2 and existing plants at site 2, per sampling occasion. Sample size: per sampling occasion: n =6.

	Site 1		Site 2			
	Introduced plants		Introduced plants		Existing plants	
Sampling occasion	Stems	Leaves	Stems	Leaves	Stems	Leaves
Reference plants	319.52±134.15	211.77±54.61*	319.52±134.15	211.77±54.61*	319.52±134.15	211.77±54.61
1	~1004.57±460.40¤	1330.04±454.88	~964.00±211.34	1419.31±512.53	~310.72±113.95•	499.08±189.54
2	1400.17±228.02¤	1866.32±545.32	#1791.97±251.87	2323.29±607.38	223.95±101.28	349.08±149.18•
3	#2175.94±513.15¤	2809.41±848.25	#315.30±49.07	480.28±178.28	303.98±161.71	628.58±202.77¤
4	1651.62±387.56¤	2181.71±322.91	436.68±172.40	456.39±242.51	290.27±78.97	601.09±73.02
5	#3589.45±822.69¤	4522.61±932.62¤	#203.79±108.65	328.99±110.50	#79.90±33.85	139.59±51.27
6	~2762.09±797.73¤	3501.80±1232.44¤	~237.53±128.74	378.34±287.13	~60.05.09±28.86•	#161.77±86.81
Pooled data for entire study period (n=36)	2097.31±954.29♥¤	2701.98±1167.66♥¤	658.21±621.14♥	897.77±808.28♥	241.76 ± 96.82♥♦	396.53 ± 214.29♥♦

= Significant differences between consecutive sampling occasions (from preceding sampling occasion).
¤ = Significant differences between sites (indicated in column of site 1).
* = Significant differences between the reference plants and the first sampling occasion.
~ = Significant differences between the first sampling occasion and the last sampling occasion.
• = Significant differences at site 2: existing plants versus introduced plants per sampling occasion (indicated in column of existing plants).
♦ = Significant differences at site 2: existing plants versus introduced plants: pooled data (indicated in column of existing plants).
♥ = Significant differences between stems and leaves per plant group: pooled data.

Table 5.2 : Mean (± SD) iron concentrations (mg/kg), measured in *Ceratophyllum demersum* L. stems and leaves from the Diep River sampling sites 1 and 2 and existing plants at site 2, per sampling occasion. Sample size: per sampling ocassion n =6.

	Site 1		Site 2			
	Introduced plants		Introduced plants		Existing plants	
Sampling occasion	Stems	Leaves	Stems	Leaves	Stems	Leaves
Reference plants	484.00±144.54	213.99±70.99	484.00±144.54	213.99±70.99	484.00±144.54	213.99±70.99
1	~1294.62±1226.80	~2148.32±1156.88	896.29±290.43	1575.68±781.32	~286.15±162.16	509.50±281.18
2	5491.60±1226.80	6855.22±1781.25	2172.80±309.18	3940.09±1015.90	199.99±116.45•	265.58±141.51
3	6542.83±2426.75	10073.83±3814.00	2296.05±580.84	3669.36±1053.81	507.13±277.64	684.03±282.33•
4	6887.38±1075.07	9179.52±726.28	2220.51±535.70	2261.14±1245.55	562.73±210.56	795.26±160.25
5	8138.99±1631.55¤	13980.56±3067.75¤	751.06±464.08	1520.71±735.31	992.39±376.93	1413.73±512.46
6	~9841.11±4107.48¤	~11386.74±3540.24¤	231.07±127.35	824.93±400.50	~728.90±393.32	1201.74±663.55
Pooled data for entire study period (n=36)	6366.09 ± 2898.50♥¤	8937.37 ± 4079.46♥¤	1427.96 ± 906.63♦♥	2298.65 ± 1254.94♦♥	546.22 ± 290.43♦♥	811.64 ± 429.12♦♥

¤ = Significant differences between sites (indicated in column of site 1).
~ = Significant differences between the first sampling occasion and the last sampling occasion.
• = Significant differences at site 2: existing plants versus introduced plants per sampling occasion (indicated in column of existing plants).
♦ = Significant differences at site 2: existing plants versus introduced plants: pooled data.
♥ = Significant differences between stems and leaves per plant group: pooled data.

Table 5.3: Mean (± SD) zinc concentrations (mg/kg), measured in *Ceratophyllum demersum* L. stems and leaves from the Diep River sampling sites 1 and 2 and existing plants at site 2, per sampling occasion. Sample size: per sampling occasion: n =6.

	Site 1		Site 2			
	Introduced plants		Introduced plants		Existing plants	
Sampling occasion	Stems	Leaves	Stems	Leaves	Stems	Leaves
Reference plants	101.12±23.39	134.54±21.81	101.12±23.39	134.54±21.81	101.12±23.39	134.54±21.81*
1	170.05±90.25	255.14±167.60	~193.68±37.33	~433.03±153.20	~58.55±24.93	62.84±24.18•
2	185.60±87.49	230.09±114.00	136.99±32.20	246.13±44.83	37.99±12.81	30.53±5.78
3	170.63±23.07	262.72±84.21	95.42±24.14	120.03±41.36	45.88±27.41	56.05±35.78
4	177.18±35.94	293.48±62.85	132.90±45.93	120.61±45.50	50.39±23.22	#105.31±27.97
5	149.97±23.98	219.56±40.96	56.22±30.07	84.50±49.46	32.52±10.84	#46.58±10.31
6	112.31±47.70	163.36±54.89	~36.84±10.87	~40.34±11.47	~15.87±10.68	47.55±18.87
Pooled data for entire study period (n=36)	160.96 ± 26.59¤♥	237.39 ± 44.62¤♥	108.68 ± 57.80♦	174.11 ± 144.18♦	40.2 ± 15.02♦♥	58.14 ± 25.53♦♥

¤ = Significant differences between sites (indicated in column of site 1).
* = Significant differences between the reference plants and the first sampling occasion.
~ = Significant differences between the first sampling occasion and the last sampling occasion.
• = Significant differences at site 2: existing plants versus introduced plants per sampling occasion (indicated in column of existing plants).
♦ = Significant differences at site 2: existing plants versus introduced plants: pooled data.
♥ = Significant differences between stems and leaves per plant group: pooled data.

Table 5.4 : Mean (± SD) copper concentrations (mg/kg), measured in *Ceratophyllum demersum* L. stems and leaves from the Diep River sampling sites 1 and 2 and existing plants at site 2, per sampling occasion. Sample size: per sampling occasion: n =6.

	Site 1		Site 2			
	Introduced plants		Introduced plants		Existing plants	
Sampling occasion	Stems	Leaves	Stems	Leaves	Stems	Leaves
Reference plants	4.15±3.85*	4.90±0.57*	4.15±3.85*	4.90±0.57*	4.15±3.85	4.90±0.57
1	17.23±2.24	14.86±2.32	~17.52±2.01	~15.42±1.74	~4.27±2.56•	5.22±2.57•
2	16.15±3.24	13.23±1.20	#11.13±1.42	12.16±2.39	1.11±1.09•	3.10±0.77•
3	13.16±1.49	13.49±3.86	11.29±3.04	8.66±2.58	3.72±2.04•	5.35±1.40
4	15.18±3.89	18.68±3.88	14.80±2.94	10.77±3.04	2.80±1.69•	5.26±0.75
5	12.77±5.43¤	13.24±1.79	5.92±1.93	#6.67±2.75	0.75±1.19•	5.47±3.42
6	9.29±3.49	12.35±2.12	~6.04±1.93	~4.80±1.70	~0.03±0.07•	4.32±1.78
Pooled data for entire study period (n=36)	13.96 ± 2.86¤	14.31 ± 2.29¤	11.12 ± 4.63♦	9.75 ± 3.85♦	2.11 ± 1.73♦♥	4.79 ± 0.92♦♥

¤ = Significant differences between sites (indicated in column of site 1).
* = Significant differences between the reference plants and the first sampling occasion.
~ = Significant differences between the first sampling occasion and the last sampling occasion.
• = Significant differences at site 2: existing plants versus introduced plants per sampling occasion (indicated in column of existing plants).
♦ = Significant differences at site 2: existing plants versus introduced plants: pooled data.
♥ = Significant differences between stems and leaves per plant group: pooled data.

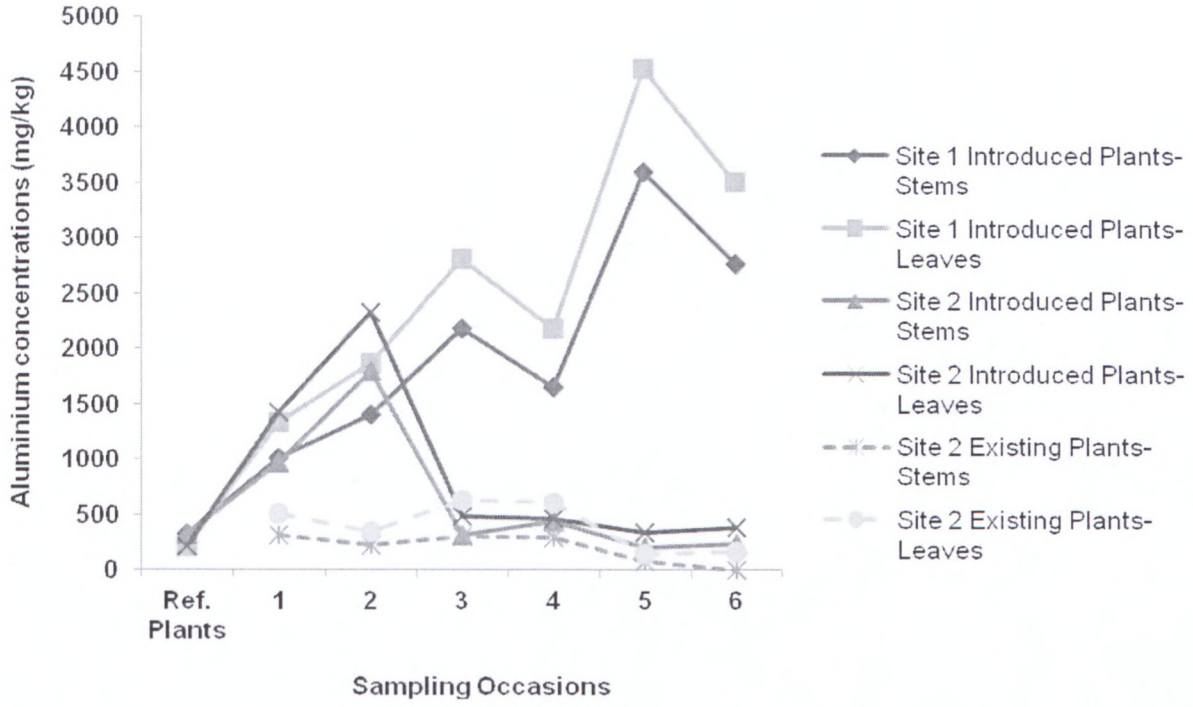

Figure 5.1: Mean aluminium concentrations (mg/kg), measured in *Ceratophyllum demersum* L. stems and leaves from the Diep River sampling sites 1 and 2 and existing plants at site 2, per sampling occasion.

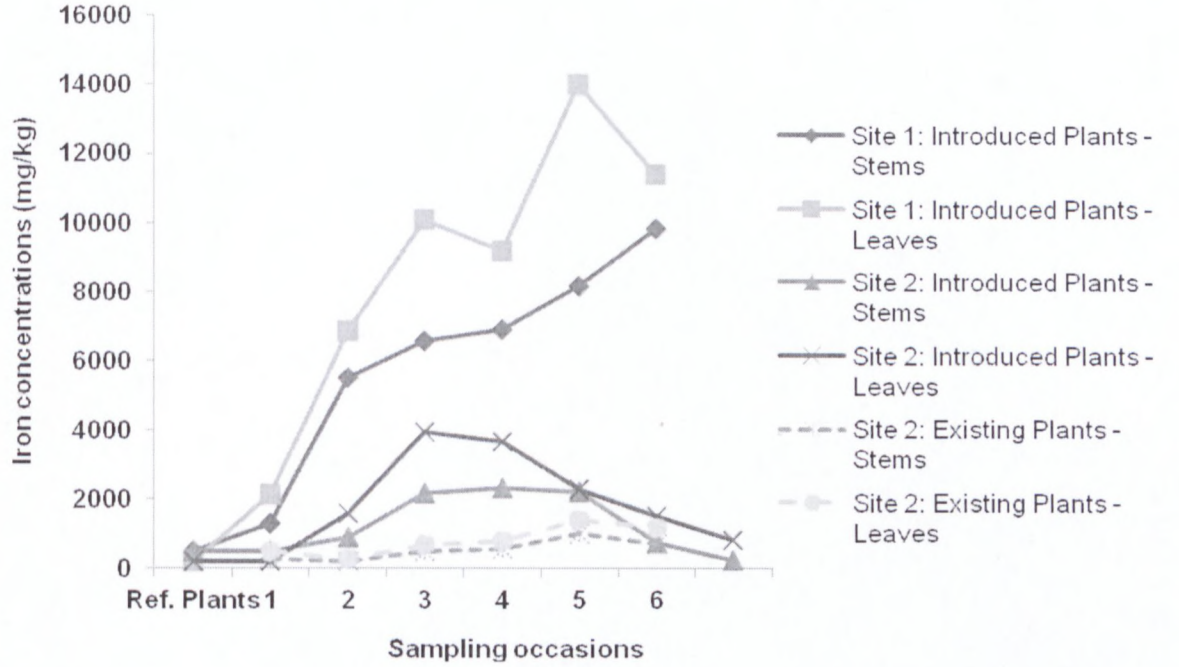

Figure 5.2 : Mean iron concentrations (mg/kg), measured in *Ceratophyllum demersum* L. stems and leaves from the Diep River sampling sites 1 and 2 and existing plants at site 2, per sampling occasion.

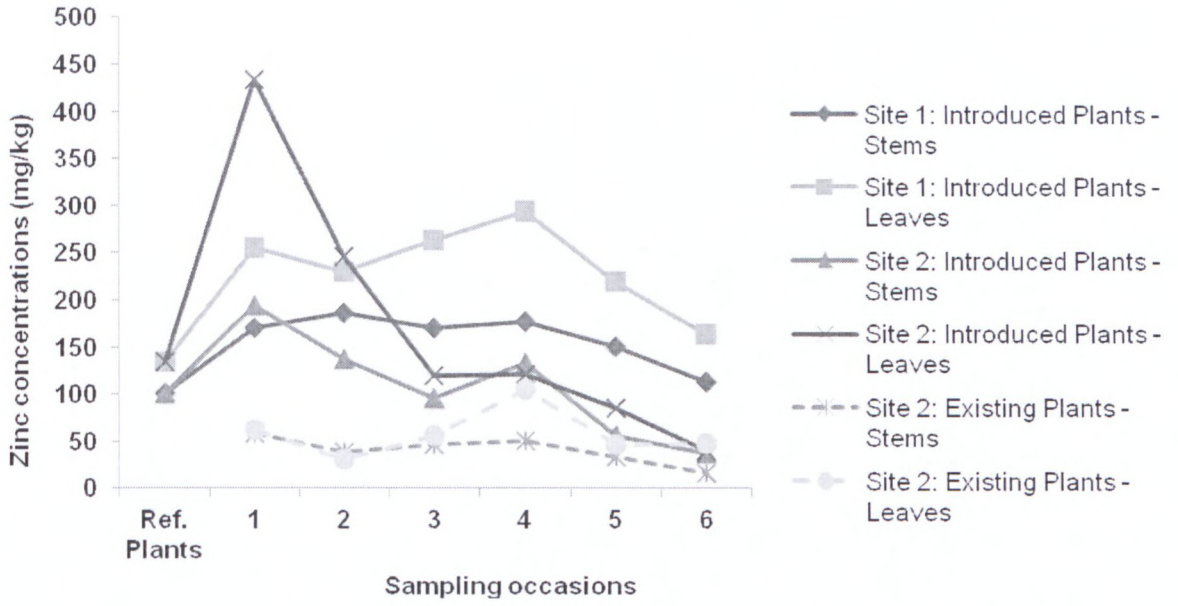

Figure 5.3: Mean zinc concentrations (mg/kg), measured in *Ceratophyllum demersum* L. stems and leaves from the Diep River sampling sites 1 and 2 and existing plants at site 2, per sampling occasion.

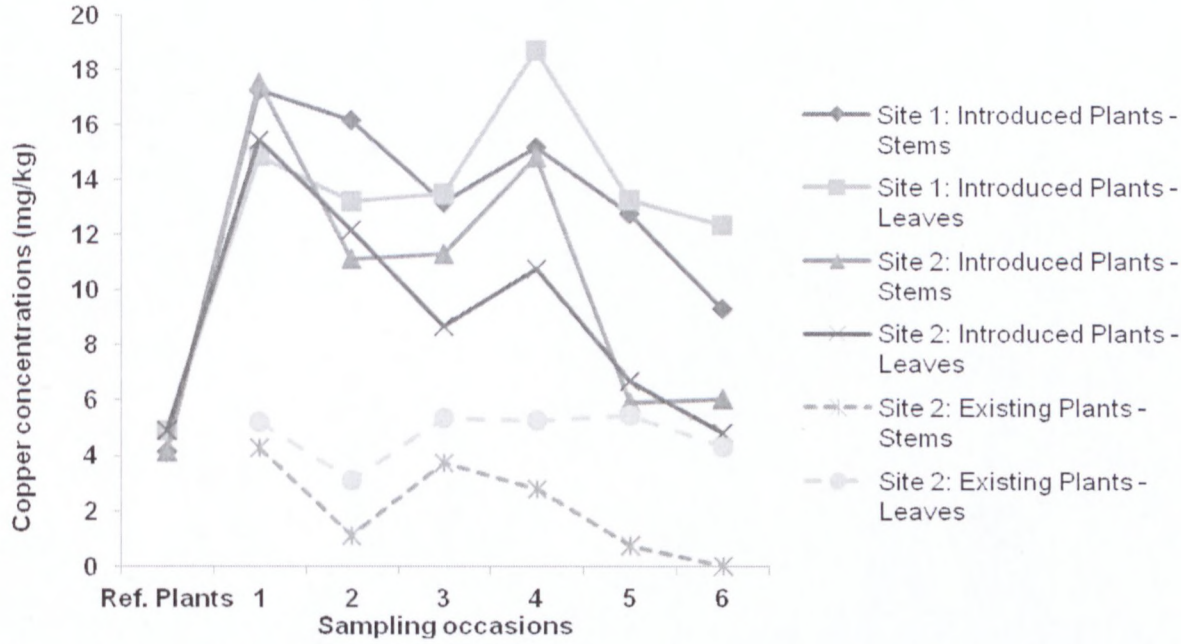

Figure 5.4: Mean copper concentrations (mg/kg), measured in *Ceratophyllum demersum* L. stems and leaves from the Diep River sampling sites 1 and 2 and existing plants at site 2, per sampling occasion.

5.2 DISCUSSION

5.2.1 Metals in the introduced *Ceratophyllum demersum* L.

Metals were bioaccumulated by the introduced *C. demersum* at both sites. The plants at site 1 bioaccumulated metals (aluminium, iron, zinc and copper) to a greater extent than the plants at site 2 and at a more rapid rate, in just two weeks (Table 5.7). Rapid biosorption of heavy metals by certain aquatic macrophytes is well documented (Schneider & Rubio, 1999; Kamal et al., 2004; Maine et al., 2004; Peng et al., 2008). In the latter study it was documented that it took just two hours for the two *Potamogeton* species to absorb the metals studied. Veglio & Beolchini (1997), suggested that the initial rapid absorption by plants and the slower more linear phase of absorption which usually followed, was due to the initial fast metal binding process (biosorption), followed by the slower transport of the metals across the plasma membrane into the cytoplasm (bioaccumulation). Salisbury & Ross (1985) describe the initial process of ion adsorption by the cell membrane as being fast and then the movement of the ions into the cytoplasm, across the membranes as being at a more constant rate. The results of the present study of the introduced *C. demersum*, concur with the findings of Veglio & Beolchini (1997). The introduced plants were genetically not adapted to limit metal uptake, due to their relatively short term exposure to the waters. Plants need to be exposed for a long period of time to an external stimulus before they show signs of genetically modifying themselves to overcome any setbacks to their metabolism by that external stimulus, as shown by Dickinson et al. (1991).

The concentrations of aluminium and iron detected in the site 1 plants were much higher than the concentrations detected in the water but not as high as the sediment samples. For zinc and copper however, the introduced plants at site 1 contained higher concentrations of these metals compared with concentrations detected in the water as well as the sediment (Table 5.7). At site 2, all the introduced plants contained higher concentrations of metals than the water and sediment of their immediate environment.

Research into bioaccumulation of metals by aquatic plants has been well documented for various metals (Samecka-Cymerman & Kempers, 2001; Aksoy et al., 2005; Kara, 2005; Marques et al., 2007; Deng et al., 2008; Peng et al., 2008; Hu et al., 2010). In most of

these studies it was found that the roots of the aquatic plants were able to bioaccumulate metals more readily than that of the leaves.

Certain metals within the sediment or water, depending on the mode of uptake by the plant, can influence the concentration of metals the plant is able to absorb (Robinson et al., 2003). There are many variables involved regarding the uptake and storage of metals within plants, especially that of the bioavailability of metals to plants. Biologically available metals are those that occur in a form that are assimilable by living organisms (bioavailable), as metals occur in various forms and are not all bioavailable to plants (Wright & Welbourn, 2002).

Research involving submerged macrophytes as bioaccumulators of metals within their tissues was conducted by for example Cardwell et al. (2002); Duman et al. (2006); Fritioff & Greger (2006); Deng et al. (2008) and Peng et al. (2008). Babovic et al. (2010) found that *C. demersum* accumulated the highest amount of zinc, copper and iron in its tissues compared to other macrophytes used in the study of a fishpond in Serbia. In a study conducted by Rashed (2002) of the three aquatic plants from the Nile River that were studied, *C. demersum* was found to accumulate most of the metals that were tested and was considered to be an excellent biomonitor of metal pollution.

Studies have revealed that plant species differ in the concentration of metals that they accumulate and differ according to where the accumulated metals are stored (Göthberg et al., 2002; Giordani et al., 2005; Intawongse & Dean, 2006). Not only the plant organ but also the age of the plant organ can influence the amount of metal that is stored, as discussed by Robinson et al. (2003) in a study of nickel in *Berkheya coddii* leaves and stems. Older leaves accumulated more nickel while older stems accumulated less nickel than new growth. This study differed from previous studies as nickel accumulated in the cuticle of the upper epidermis significantly more than the rest of the leaf. Previous studies of other hyperaccumulator plant species, found that nickel accumulated in vacuoles and epidermal cells. Krämer et al. (2000) found that nickel was found mostly in the vacuoles of *Thlaspi goesingense* and Küpper et al. (2001) found that nickel was compartmentalised in the vacuoles of *T. goesingense*, *Alyssum bertolonii* and *A. lesbiacum* respectively.

The present results obtained for aluminium, iron, zinc and copper all follow similar trends where generally more metals were bioaccumulated in the leaves of the plants rather than in the stems, at all sampling occasions and at both sites (except for copper at site 2 where the stems bioaccumulated more copper than the leaves). These results are most likely due to the external morphology of the plant, where leaves also have a far greater surface area which allows for greater absorption of surrounding metals (Figure 2.6). Leaves have more stomata, where the uptake of metals occurs, through ectodesmata situated in the epidermal cell walls (Franke, 1961). Certain leaves possess trichomes and may secrete excess metals from these structures (Robinson et al., 1996).

Metals in plants are generally found to be greater/higher in the organs directly in contact with the source of metals, so in rootless aquatic submerged plants, the leaves are more exposed to the water source of metal contaminants than the stems (Jarvis et al., 1976). Although various studies have shown that different plants vary in the quantities of metals stored and in which parts of the plants they are stored (Leavitt et al., 1979; Cardwell et al., 2002; Windham et al., 2003; Intawongse & Dean, 2006), leaves are more temporary than stems, as leaves are able to senesce thus reducing the metal burden on the plant (Windham et al., 2003). By storing metals in leaves, it is possible that the leaves may become unpalatable to potential predators and thus the plant exhibits a mechanism of self preservation (Boyd & Martens, 1994).

The concentrations of aluminium, iron and zinc (pooled data) were all found to be significantly higher in the plants at site 1 compared with the plants at site 2, for both stems and leaves, indicating a more polluted environment at site 1 and that the metals are possibly more bioavailable at site 1 (Tables 5.1-5.4).

Hewitt and Smith (1975) gave the average quantity of mineral elements a normal foliage plant requires for healthy growth (Table 5.5). There was no information available relating to aluminium, probably as aluminium is not an essential element and is toxic to plants (Wright & Welbourn, 2002). Although all plants differ and have various mineral requirements for optimum growth, according to this table, both the introduced plants at site 1 and site 2 bioaccumulated considerably higher concentrations of iron and zinc in their leaves than what are required for optimal growth. The site 1 plants had concentrations of copper just under the highest recommended copper concentration for plants (Table 5.5). The site 2 plants were within the suggested guidelines as illustrated by Table 5.5.

Although these figures may not be completely accurate for *C. demersum,* this information gives an indication as to whether the metal concentrations are above what are considered normal concentrations.

Table 5.5: Typical concentrations (ppm) of mineral elements in the foliage of normal plants (Hewitt & Smith, 1975) and mean (± SD) results for pooled data of *Ceratophyllum demersum* L. leaves from the Diep River.

Element	ppm. in dry matter (Hewitt and Smith, 1975).	Reference plants	Site 1 Introduced Plants	Site 2 Introduced Plants	Site 2 Existing plants
Aluminium	N/A	N/A	N/A	N/A	N/A
Iron	50-300	213.99±70.99	8936.8±4079.46	2298±1254.94	811.64±429.12
Zinc	15-75	134.54±21.81	237.39±44.62	174.11±144.18	58.14±25.53
Copper	5-15	4.90±0.57	14.31±2.29	9.75±4.63	4.79±0.92

A popular nutrient solution widely used to grow plants hydroponically is Hoagland's solution, developed by Hoagland and Snyder in 1933. It consists of various macro and micronutrients considered to be ideal for optimum plant growth (Hoagland & Arnon, 1950). Table 5.6 compares the concentrations of the elements studied with those of Hoaglands solution to show how much more the plants accumulated in their leaves compared with the nutrient requirements of plants in general. The Hoagland solution quantities of elements were far lower than the concentrations detected in the plant samples of this study.

Table 5.6: Comparison of mean (±SD) concentration of metals obtained in the leaves of *Ceratophyllum demersum* L. (pooled data) with Hoagland's nutrient solution, measured in ppm (Hoagland & Arnon, 1950).

Element	Hoagland's nutrient solution (ppm)	Reference plants	Site 1 Plants	Site 2 Plants	Existing plants
Aluminium	N/A	N/A	N/A	N/A	N/A
Iron	1-5	213.99±70.99	8936.8±4079.46	2298±1254.94	811.64±429.12
Zinc	0.05	134.54±21.81	237.39±44.62	174.11±144.18	58.14±25.53
Copper	0.02	4.90±0.57	14.31±2.29	9.75±4.63	4.79±0.92

5.2.2 Introduced site 2 plants and existing site 2 plants

With all four metals sampled, in all instances, the introduced plants bioaccumulated significantly higher concentrations of metals in their leaves and stems compared with the existing plants growing at the same site. As the introduced plants did not originate from a site with excess metals (Refer to Table 5.5), these plants initially had low levels of ions in their cells, allowing them to absorb ions at a much faster rate than the plants growing in the high metal environment (Lee, 1982). In all cases, the leaves accumulated more metals than the stems but this is most likely due to the leaves having a larger surface area exposed to the surrounding waters to absorb metals.

It seems that the existing plants are able to limit the quantities of aluminium, iron, copper and zinc that they bioaccumulate by selective adaptations relating to the survival in a polluted environment over the years and adapting to their contaminated environment. Plants are either able to exclude potentially phytotoxic metals and resist their uptake or once the metal has entered the plant cells, have a high tolerance level of that specific metal (Baker, 1987). Dickinson et al. (1991) discussed how metal tolerant plants may result from tolerant mutants, through natural selection over time and that these tolerant plants are actually induced by the toxic substances. Plants with higher tolerance to excess metals are favoured by natural selection in polluted environments as they survive the changes in the environment better. Gene expression in environmentally stressed plants, over time, can be altered, affecting plant proteins, by either inducing new proteins or repressing others. This allows the plant to make biochemical and structural changes within to handle metal induced stress (Sachs & Ho, 1986). Long-lived plants may also produce genetic mutations that are able to produce rogue shoots. As long-lived plants have millions of meristems present and each meristem is able to produce shoots, shoots that are better suited to the environment will be better adapted to the stress of excess metals (Gill, 1986). Studies over the decades, of heavy metal tolerance by plants, especially grasses, have illustrated the power of selection and how plants are selected according to their tolerant genotypes (Prat, 1934; Broker, 1963; Urquhart, 1971; Bradshaw, 1976).

In many cases once a metal has entered a plant in excess quantities, plants are able to store unwanted substances within their vacuoles in the cytosol, where they will have least effect on plant metabolism. There are various ways that a plant avoids metal build up within its organs but each plant species has specific mechanisms in place to reduce the

uptake of excess metals into the cytosol (Hall, 2002). The method that the existing *C. demersum* at site 2 have implemented to overcome phytotoxicity of the metals is not known, but the results indicate that they probably limit metal toxicity.

There is evidence that plants living in an environment of high metal concentrations have certain metabolic inhibitors present that are able to slow down the rate of diffusion once more than sufficient concentrations have been acquired for general metabolism (Kochian & Lucas, 1982; Glass, 1983). Plants that are able to overcome the phytotoxic effects of excess metal accumulation and are able to survive contaminated aquatic environments are valuable resources in phytoremediation of metal polluted water bodies (Azadpour & Mathews, 1996). Prasad (2004) indicated that there are presently 400 plant species that are considered to be hyperaccumulators of heavy metals and that these plants are able to accumulate 100 -1000 times of a certain metal normally accumulated by plants. Prasad (2003) listed *C. demersum* as a 'metal accumulator' and included it in a list of plants used for phytoremediation. The present study confirms that *C. demersum* is an aluminium, iron, zinc and copper accumulator in the Diep River. The possibility of using this species in phytoremediation of the Diep River needs further investigation.

The present study has recognized that by introducing *C. demersum*, a well documented aquatic plant in its ability to accumulate metals (Gupta & Chandra, 1996; Zurayk et al., 2001; Demirezen & Aksoy, 2006; Babovic et al., 2010), to a known metal contaminated environment (Shuping, 2008), such a plant, if it is not from a similar polluted environment, will rapidly accumulate metals in its organs, as shown by the present study. These results concur with Sandermann (1992) who stated that plants are like 'green livers' able to bioaccumaulate high levels of excess environmental chemicals.

Table 5.7: Comparisons of pooled data for aluminium, iron, zinc and copper in the reference plants, introduced plants, existing plants, sediment and water samples from the two sampling sites in the Diep River over the six sampling occasions.
Sample size: Pooled data: n = 36.

	Aluminium	Iron	Zinc	Copper
Reference Plants	211.77±54.61	213.99±70.99	134.54±21.81	4.90±0.57
Introduced Plants at site 1	2701.5±1167.66	8936.8±4079.46	237.39±44.62	14.31±2.29
Introduced Plants at site 2	897.3±808.28	2298±1254.94	174.11±144.18	9.75±4.63
Existing Plants at site 2	396.53±214.29	811.64±429.12	58.14±25.53	4.79±0.92
Sediment at site 1	3486.33±1809.61	9117.04±4158.63	12.28±4.79	5.02±2.11
Sediment at site 2	845.1±294.64	1239±322.67	1.64±0.43	0±0
Water at site 1	0.53±0.36	0.71±0.59	0.03±0.02	0.0024±0.0031
Water at site 2	0.27±0.1	0.91±0.49	0.02±0.04	0.0046±0.0080

Plant concentrations based on leaf samples measured in mg/L.
Sediment samples measured in mg/kg.
Water samples measured in mg/L.

5.2.3 Aluminium

Aluminium is generally toxic to plants when it is in a form that is bioavailable, as it has no biological function in plants (Wright & Welbourn, 2002). The plants at site 1 accumulated significantly higher concentrations of aluminium in their leaves than in their stems (pooled data). The reasons why leaves are good storage places for excess metals has already been discussed in section 5.5. *C. demersum* has the ability to quickly accumulate aluminium from its environment. In just two weeks significantly higher concentrations of aluminium had been measured in the plant samples at the first sampling occasion at site 1 and site 2, compared to the reference plants (Table 5.1 & Figure 5.1). The sediment at site 1 contained high concentrations of aluminium, slightly higher than concentrations found in the plants (Table 4.2), but as the plants are not in direct contact with the sediment, they do

give a better indication of metals in the aquatic environment compared with the much lower concentrations of metals detected in the water. The introduced plants at site 2 were able to accumulate aluminium in their leaves to higher concentrations than in the water and sediment, indicating possible biomonotoring capabilities for aluminium. Using plants as biomonitors of metal pollution can contribute additional information regarding metals in the environment and environmental quality (Madejón et al, 2006; Mertens et al., 2006).

In a study conducted by Goulet et al. (2005) on four different aquatic plant species, the highest concentrations of aluminium were detected in the roots of *Typha latifolia* (a rooted aquatic plant species) compared with floating-leaved species. They also established that the distribution of aluminium varied among various plant organs from species to species. In another similar study, Gallon et al. (2004) studied aluminium accumulation in five different aquatic plants and found that *Myriophyllum exalbescens* (a submerged macrophyte) accumulated more aluminium than any other plants sampled (among them the rooted aquatic, *Typha latipholia*) and the aluminium was mostly accumulated in the leaves.

In total, the introduced plants at site 1 accumulated a significantly higher amount of aluminium in their stems and leaves compared with the introduced plants at site 2 (Table 5.1). Results after a two-week period in the river showed that the plants introduced at both sites showed high concentrations of aluminium in both their stems and leaves. After just 4 weeks of being exposed to their new environment in the river, the plants at site 1 showed significantly higher concentrations of aluminium in their stems and leaves compared with the reference plants. The stems at site 1 bioaccumulated 3.1 times the concentration of aluminium detected in the reference plant stems, while the leaves accumulated 6.3 times. At site 1 the aluminium concentrations in the leaves and stems increased reasonably steadily over the study period, decreasing slightly at sampling occasion four and then spiking significantly in concentration at sampling occasion 5 (Table 5.1). This trend shown by the aluminium concentrations in the introduced plants at site 1 did not correspond with that of the water concentrations for aluminium, where there was an initial spike in concentrations at sampling occasion two and then decreasing over the study period (Table 3.2). The concentrations of aluminium in the plants did however correspond with the sediment trends, significantly spiking in concentration at the fifth sampling occasion (Table 4.2 & Figure 4.1). It is possible, due to the high rainfall that

turbulence of the sediment occurred, causing an increase in aluminium concentration in the water, which was then rapidly moved downstream due to the increased water velocity.

At site 2 the stems bioaccumulated 3 times the amount of aluminium than the reference plant stems. The leaves of the plants at site 2 showed 6.7 times more aluminium than the reference plant leaves. These results indicate that *C demersum* L. is able to bioaccumulate aluminium especially in its leaves (Table 5.1 & Figure 5.1). The plants at site 2 seemed to regulate the aluminium concentrations better than the plants at site 1, as after the initial high body loads in plants at both sites, the concentrations steadily decreased at site 2 over the sampling period, while the plants at site 1 had a generally higher aluminium concentration throughout the study period except for the final sampling occasion (Table 5.1). This trend in metal uptake of initial rapid biosorption and then slower transport of the metal through the plasma membrane into the cytoplasm of the cells is described by Veglio & Beolchini (1997), Vannela & Verma (2006) and Poljsak et al. (2011). Bioavailability of aluminium may have also differed at the two sites, causing the differences in aluminium concentrations detected in the plants. The significantly higher aluminium concentrations in the plants at site 1 are also most likely related to the higher water and sediment readings obtained at site 1 compared with site 2 (Tables 3.2 & Table 4.2). The concentrations of aluminium detected in the introduced plant stems at site 2 followed a similar pattern to the aluminium concentrations in the water at site 2, where the initial sampling occasions had a significantly higher aluminium reading compared with the final sampling occasion where the readings were significantly lower.

The introduced *C. demersum* stems bioaccumulated aluminium, following a similar pattern to the concentration of aluminium in the water at site 2, thus indicating the possibility of *C. demersum* of being a potential biomonitoring species for aluminium contaminants in the Diep River. According to Madejón et al. (2006) and Mertens et al. (2006) plants play an important role in the monitoring of metal pollution in environments and if they can provide quantitative information that reflects the concentrations of metals in the environment they can be used as biomonitors (Wright & Welbourn, 2002). The leaves of the plants at site 2 also followed a similar trend to that of the aluminium detected in the water samples over the sampling period, where the initial aluminium concentrations were high but then gradually decreased over the sampling period in a similar pattern but for the leaves this was not considered significantly different.

Aluminium concentrations were compared between *C. demersum* introduced to the lower Diep River for a twelve week period and *Ceratophyllum* of the same species that were already growing in the river. Compared with the existing plants at site 2, the introduced plants accumulated significantly higher concentrations of aluminium in both their stems and leaves compared with the existing plants. The existing plants accumulated a significantly higher concentration of aluminium in their leaves compared to their stems. This could possibly be attributed to the new introduced plants not being adapted to the polluted environment and thus were more vulnerable to absorbing aluminium than the existing plants (Table 5.1 & Figure 5.1). Prasad (2004) describes how studies using Al tolerant plants and Al sensitive plants indicate that Al tolerant plants are able to secrete substances such as malic acid, citric acid or oxalic acid as a preventative mechanism to prevent the uptake of aluminium by the roots. In an experiment conducted by Zheng et al. (1998) it was established that aluminium resistance was displayed by *Triticum aestivum* (wheat) plants. These plants continuously secreted organic acids at high levels, which could be attributed to aluminium resistance. Ma et al. (1997) concluded that the secretion of citric acid by *Cassia tora* L. was a mechanism of aluminium resistance. As most studies of aluminium resistance in plants have been conducted on plant roots (Ma et al., 1997; Zheng et al., 1998; Jansen et al., 2002; Poschenrieder, et al., 2008) and *C. demersum* is rootless, it may be more likely that aluminium tolerance in the existing plants at site 2 could be attributed to the many other ways that certain metal tolerant plants display resistant traits. Extracellular ways that plants are known to resist aluminium are by the adhesion of aluminium to the cell wall (Cuenca et al., 1991) or by the selectively permeable plasma membrane (Jansen et al., 2002). Internal mechanisms that could have been displayed by the existing *Ceratophyllum sp.* are the formation of aluminium chelates once the aluminium has entered the cytoplasm, vacuole storage, and the synthesis of aluminium tolerant proteins or perhaps increased enzyme activity (Jansen et al., 2002).

The results obtained for aluminium indicate that the stems of the existing plants were able to regulate the aluminium concentrations, as they showed a more even distribution of concentrations over the sampling period and the amounts detected in the stems were fairly stable (Table 5.1 & Figure 5.1). The leaves of the existing plants did not display the same rate of stability and regulation as the stems but this could be due either to a detoxification mechanism of the plant to accumulate aluminium in the leaves rather than the stems or that it takes longer for the metal to get to the stem via the leaves. The introduced plants initially acquired high dosages of aluminium, however they displayed a

rapid response after the first two sampling occasions, in decreasing the concentrations of the metal quite substantially.The stems of the introduced plants displayed the most significant differences between sites per sampling occasion, with all sampling occasions except the first one being significantly different and in most cases lower concentrations were achieved at site 2, except for the second sampling occasion, compared to site 1 (Table 5.1).

The existing plants at site 2 accumulated significantly more aluminium in their leaves compared with their stems over the entire study period (Table 5.1). They also accumulated significantly lower concentrations of aluminium than the introduced plants at the same site for both the stems and leaves (pooled data), indicating perhaps a genetically tolerant genotype that has developed over time (as discussed previously).

5.2.4 Iron

Iron is essential in plants for various metabolic processes in the plant, such as chlorophyll development, energy transfer, respiration, nitrogen fixation and forms part of proteins and enzymes (Salisbury & Ross, 1985). It is unknown what concentration of iron was in the plants before they were sampled, however according to Table 5.5, a normal foliage plant has 50-300 ppm iron present in their leaves (Hewitt and Smith, 1975). The reference plants had 313.99 ±70.99 ppm present in their leaves before they were placed in the river. There are also many factors that affect the uptake of iron such as pH, the amount of organic matter present, the amounts of nitrogen and bicarbonate ions, balances between iron and certain other elements like zinc, manganese, potassium and molybdenum, and thus in some cases iron deficiency can affect many plants (Salisbury & Ross, 1985). Although iron is the fourth most abundant metal in the earth's crust, it is the availability of iron that limits its uptake by plants. It is important for a plant to obtain sufficient amounts of iron for metabolic processes but also not to absorb excessive quantities, as excessive iron can lead to phytotoxicity (Guerinot & Yi, 1994).

At site 1 the iron concentrations in the sediment were just over the concentrations detected in the introduced plant leaves, however the concentrations of iron detected in the plants were much higher than the iron concentrations of the water. At site 1 the iron concentrations in the plant stems and leaves steadily increased after each consecutive sampling occasion. Compared with the reference plants, the stems at site 1 accumulated

2.7 times the amount of iron on the first sampling occasion (over a two week period) and 20 times the concentrations initially shown in the reference plants. The leaves accumulated 10 times the amount of iron of the reference plants at the first sampling occasion and at the last sampling occasion just over 53 times their initial concentrations over the 12 week study period. This is probably due to the leaves having more stomata present than the stems, where uptake of metals in the leaves occurs through ectodesmata situated in the epidermal cell walls (Franke, 1961). These results indicate that the introduced *C. demersum* were able to relatively rapidly bioaccumulate high concentrations of iron from the water. It is presumed that the leaves, having the larger surface area and being more exposed to the iron containing water would accumulate more iron than the stems (Cardwell et al., 2002; He & Yongfeng, 2009; Shuping et al., 2010).

At site 2, the introduced plants bioaccumulated more iron in their leaves compared with their stems but over the last two sampling occasions the concentration of iron began to decrease, perhaps due to being in an unavailable state or due to less iron in the water (Table 5.2 & Figure 5.2). The stems at site 2 accumulated 1.9 times the amount of iron at the first sampling occasion, compared with the reference plants, and the leaves 7.4 times. At the last sampling occasion the iron concentrations in the stems were only twice the concentration initially detected in the reference plants and just under four times in the leaves. These results possibly relate to the decrease in iron concentrations in the water over the last two sampling occasions, as shown by Figure 3.2. High levels of phosphorus in the water often associated with effluent from Wastewater Treatment Works (Smith, 1986), could have attributed to the reduced iron concentrations, as high levels of phosphorus can be responsible for iron deficiencies (Landner & Reuther, 2004). High levels of phosphorus in this region of the river, resulting from Potsdam Wastewater Treatment Works, could have resulted in lower iron concentrations, as negatively charged phosphate ions could combine with positively charged iron molecules that may then be unavailable to plants (Smith, 1986). The loss of membrane integrity in metal stressed plants, could have allowed for the loss of iron from the plant cells (Quartacci et al., 2001; Souza-Santos et al., 2001; Tripathi et al., 2003; Kumar & Prasad, 2004; Sinha et al., 2005).

In total the pooled data for leaves and stems at site 1 had significantly higher concentrations of iron compared with the leaves and stems at site 2 (Table 5.2). These results could possibly reflect the concentrations of iron in the immediate environment,

depending on the bioavailability of the iron to the plants. As the introduced plants at both sites had the same origin, it can be said that iron at site 1 was more bioavailable than the iron at site 2. The site 1 introduced plants were able to bioaccumulate more iron than the site 2 introduced plants. At site 1, the increased concentrations of iron in the river could have resulted from fertilizer contamination from surrounding farms, as iron is a micronutrient required by plants for healthy growth (Salisbury & Ross, 1985). Iron is also a supplement in dairy feed (Li et al., 2005), possibly used by dairy farmers in the vicinity of site 1. It is also sold as part of a biological soil fungicide (Natural Industries, 1992-2011), which could have been introduced, as runoff from surrounding farms (Table 3.6). In alkaline soils iron is often not available to plants and chelated iron is required to supplement plant growth (Salisbury & Ross, 1985). Sediment samples taken at site 1 contained more iron than sediment samples taken at site 2 which could indicate that iron occurs more abundantly in the underlying rock structures at site 1 (Table 4.3) or iron has built up in the sediment from fertilizers introduced by farmers to their crops over time. However, as there was very little difference in the clay content at both sites in the sediment at both sites (only 2.2 and 2.4% respectively) it is most likely that the iron originated mostly from the surrounding environment and not from the bedrock.

At site 1 and site 2 the iron in the water was significantly higher at the first sampling occasions compared with the last sampling occasions. The sediment results followed the same trend with initial samples having a significantly higher concentration of iron compared with the final sampling occasions for both sites (Table 4.3). This trend was opposite in the site 1 plants where the stems and leaves of the plants at site 1 had significantly lower readings of iron at the initial sampling occasion, compared with the final sampling occasion. The concentration of iron steadily increased over time at site 1 in the plants' stems and leaves which indicates the ability of the plants to bioaccumulate iron in both their stems and leaves and probably the increase in iron bioavailability over the study period. After initial high iron concentrations, the introduced plants at site 2 reduced iron uptake, possibly due to iron being less bioavailable for uptake due to changes in speciation of iron within the water (Smith, 1986).

Iron concentrations were compared between *C. demersum* introduced to an aquatic environment for a twelve week period and compared with *Ceratophyllum sp.* of the same species that were already growing in the same aquatic environment. Compared with the existing plants at site 2, the introduced plants accumulated significantly higher

concentrations of iron in both their stems and leaves compared with the existing plants. The existing plants accumulated a significantly higher concentration of iron in their leaves compared to their stems. Although various studies have shown that different plants vary in the quantities of metals stored and in which parts of the plants they are stored (Leavitt et al., 1979; Cardwell et al., 2002; Windham et al., 2003; Intawongse & Dean, 2006), leaves are considered to be more temporary than stems. Leaves are able to senesce and are consumed by predators, thus reducing the metal burden on the plant and possibly being a form of detoxification (Windham et al., 2003).

The lower iron concentrations in the existing plants growing at site 2 compared with the introduced plants at that site, could be due to the introduced plants not being adapted to the site over the short period that the plants were exposed to that environment and thus resistant to metal uptake than the existing plants (Table 5.2 & Figure 5.2). The existing plants could have adapted over time and developed a regulatory control over iron uptake, as shown by studies conducted on *Arabidopsis thaliana* that indicate complex regulatory control mechanisms of iron (Connolly et al., 2002; Yuan et al., 2005). The introduced plants seemed to have a more stable iron uptake after the fourth sampling occasion, while the stems of the existing plants had a more stable iron concentration throughout the 12 week study period. This may indicate the importance of the plant to regulate iron uptake and store it in the stems over time, as stems are the more permanent organ of the plant. As leaves could be consumed by organisms and are able to be detached from the plant body more easily than the stems (Windham et al., 2003), plants may move excess metals to the leaves for storage. Iron deficiency and the problem of iron availability is well known (Guerinot & Yi, 1994) and by storing iron in stems, this may be a more permanent storage mechanism used by plants to combat possible iron deficiency, as iron is an important micronutrient in plants (Salisbury & Ross, 1985).

The site 2 introduced plants bioaccumulated more iron to greater concentrations than were found in both the sediment and water, and the iron concentrations followed similar trends to that of the water and sediment. *C. demersum* has shown the potential of being a possible future biomonitor for iron in this location of the river but further investigations are required due to the many factors assosciated with successful biomonitoring (Madejón et al., 2006; Mertens et al., 2006).

5.2.5 Zinc

Zinc is required in plants to produce auxins, regulates sugars and activates enzymes, forms starch, influences seed and stalk maturation, is involved in the formation of chlorophyll and carbohydrates and assists plants in withstanding low temperatures (Salisbury & Ross, 1985). Zinc availability is affected by pH and balances between itself and certain elements like phosphorus, copper, manganese, magnesium and arsenic. Organic matter, nitrogen stress and soil saturation also influence zinc availability (Landner & Reuther, 2004).

As the zinc concentrations detected in all the introduced plants, as well as the existing plants, were far higher than the concentrations of zinc detected in the water and sediment at the relevant sites, it can be said that the introduced and existing plants definitely bioaccumulated zinc (Table 5.7).

The stems of the introduced plants at site 1 on the first sampling occasion were able to bioaccumulate 1.6 times the concentration of zinc detected in the reference plant stems but at the sixth sampling occasion this amount had been reduced to just over 1% more than the original concentrations. The leaves accumulated 1.9 times the concentration of zinc at the first sampling occasion and also reduced the concentrations to just over 1% of the original concentrations at the last sampling occasion (Table 5.3 & Figure 5.3). Zinc accumulated significantly more in the leaves compared with the stems of the introduced plants at site 1. Plants tend to store excess metals in their leaves and they also have a larger surface area than the stems for possible metal uptake, as previously discussed in section 5.5).

The introduced plants at site 1 initially accumulated zinc but maintained a steady pattern of zinc uptake over the study period, with a final decrease in zinc concentrations, as the final concentrations of zinc were well below the concentrations of the initial sampling occasion, comparable to the zinc concentrations detected in the reference plants. The pattern that the site 1 introduced plants showed, indicated an initial rapid bioaccumulation but a possible strong regulation thereafter, much more regulated than for aluminium and to a lesser extent iron. There is also a possibility that the bioavailability of zinc decreased over the study period. Zinc concentrations in plants (for pooled data) were higher than the

concentrations in the immediate environment (water and sediment) for all the plants studied (Table 5.7).

The plants at site 2 bioaccumulated zinc in both their stems and leaves, at far higher concentrations than that of the water and sediment (Table 5.7). The plant stems at site 2 bioaccumulated 1.9 times the amount of zinc compared with the reference plants on the first sampling occasion but then finally after the 12 week study period 2.8 times less zinc was detected in the plants from the last sampling occasion, indicating a decline in zinc concentrations over the study period compared with the initial reference plants. The leaves accumulated 3.2 times the zinc concentrations of the reference plants on the first sampling occasion but at the final sampling occasion, the leaves had 3.4 times less zinc compared with the reference plants (Table 5.3 & Figure 5.3). This initial accumulation of zinc by the plant could be related to the need of the plant to absorb zinc as a micronutrient to facilitate normal metabolic processes (Salisbury & Ross, 1985). The plants may have had a slight zinc deficiency originally. The gradual decline of zinc from the third sampling occasion to below the initial concentrations of the reference plants at the final sampling occasion, could possibly be attributed to the high amounts of phosphates in the water that are often associated with effluent from Waste Water Treatment Works (Smith, 1986), as high levels of phosphates are responsible for zinc deficiencies. Zinc is positively charged and is able to bind with negatively charged phosphates to form insoluble compounds not readily bioavailable for plants (Landner & Reuther, 2004). Possible high levels of phosphates in this region of the river, resulting from Potsdam Wastewater Treatment Works, could have resulted in lower zinc concentrations in the plants. Loss of membrane integrity in the plants, related to metal toxicity, could have also contributed to the loss of zinc from the plant cells (Quartacci et al., 2001; Souza-Santos et al., 2001; Tripathi et al., 2003; Kumar & Prasad, 2004; Sinha et al., 2005).

Concentrations for zinc were significantly higher for the stems and leaves of the introduced plants at site 1 when data was pooled, compared with the concentrations of zinc for the stems and leaves of the introduced plants at site 2 (Table 5.3). This could be due to the variation of metal bioavailability at the two different sites.

Zinc was only detected in the water at site 2 during the third and fourth sampling occasions (Table 3.4). Zinc concentrations were also very low in the sediment (Table 4.4) while in the plants, zinc concentrations were over 100 times more than that found in the

water and sediment (Table 5.3). This indicates the prevalence of zinc in the environment but it is not always reflected in water or sediment samples and illustrates the importance of plants as indicators of metal pollution.

Zinc concentrations were compared between *C. demersum* introduced to the Diep River over a twelve week period and those that were already growing in the same aquatic environment. Compared with the existing plants at site 2, the introduced plants accumulated significantly higher concentrations of zinc in both their stems and leaves. The reasons for this will be similar to that for the other metals, as the introduced plants have not had time to adapt to the polluted environment, like the existing plants and do not display genetic adaptations to metal tolerance (Dickinson et al., 1991).

The existing plants accumulated a significantly more zinc in their leaves compared to their stems (previously discussed concerning the other metals). The existing plants had a steadier pattern of zinc uptake compared with the introduced plants, following a similar pattern to that of copper (Tables 5.3 & 5.4). At the fourth sampling occasion, the existing plants bioaccumulated the most zinc over the study period but quickly decreased the concentrations in the successive two sampling occasions (Table 5.3 & Figure 5.3). This spike in zinc concentrations at the fourth sampling occasion followed the increase in both the water and sediment zinc concentrations on the third sampling occasion, indicating a delayed but positive response to the bioaccumulation of zinc by the existing plants at site 2. These results show the initial rapid bioaccumulation of zinc by the existing *C. demersum* and then the steadier trend of uptake shown by the plants, as suggested by Kochian & Lucus (1982) and Glass (1983) for plants growing in an environment of high metal nutrients.

5.2.6 Copper

Copper plays a role in photosynthesis and respiration. It is involved with the production of proteins, forms lignin in plant cell walls and is important in carbohydrate and protein metabolism. Copper is probably the most immobile of the micronutrients and various factors affect the availability of copper to the plant. These include pH, organic matter, lack of oxygen, lack of nitrogen and balances between copper and other elements like zinc, nitrogen and phosphorus (Salisbury & Ross, 1985).

The introduced plants at site 1 bioaccumulated copper in both their stems and leaves at higher levels than the water and sediment concentrations (Tables 3.5, 4.5 & 5.4). At site 1 the concentrations of copper in the plant stems were all significantly higher than the reference plants, except for the last sampling occasion. Initially the stems of the *C. demersum* at site 1 bioaccumulated 4.2 times the concentration of copper at the first sampling occasion (after two weeks) compared with the reference plants but then a more steady copper uptake was seen, until the last sampling occasion where copper was reduced to 2.2 times more than the reference plants. Only in the last sampling occasion was this lower amount significantly less than that of the previous sampling occasion (Table 5.4 & Figure 5.4). The fourth sampling occasion saw the highest copper concentrations in the plant stems and leaves which corresponded precisely to the water and sediment copper concentrations, also showing an increase on the fourth sampling occasion and the highest concentrations attained over the study period (Tables 3.5, 4.5 & 5.4). This was the trend experienced for the other metals, adding to the probability that the runoff from the unseasonal rainfall and increased bioavailability could have had an impact on the plant metal results. Iron, zinc and copper and to some extent aluminium are used in viticulture which predominates agricultural activities in the vicinity of site 1 (Table 3.6).

The leaves of the *Ceratophyllum sp.* at site 1 bioaccumulated 3 times the concentrations of copper than the reference plants after two weeks but this declined slightly to 2,5 times at the final sampling occasion (after 12 weeks). Copper concentrations in the stems of the plants at site 1 showed a significant higher concentration on their first sampling occasion compared with their last sampling occasion. Copper concentrations in the plants at site 1 did not show any significant differences between the stem and leaf concentrations (Table 5.4), while for aluminium, iron and zinc the leaves showed significant increases in concentrations compared with the stems for pooled data.

As the introduced *Ceratophyllum sp.* follow the environmental trends for copper at site 1, *C. demersum* can possibly play an important role in future active biomonitoring studies in the upper reaches of the Diep River for copper contaminants, especially as the region has much agricultural activities that seem to contribute to the contamination of the Diep River, as shown by the results for metals in this study.

At site 2 the introduced plants were able to bioaccumulate 4.2 times the concentrations of copper in their stems after just two weeks but after 12 weeks this was reduced to 2.5

times of that of the reference plants. The leaves of the introduced plants at site 2 bioaccumulated 3 times the concentrations of copper in their leaves compared with the reference plants but after 12 weeks this amount decreased by a fraction less than the reference plants (Table 5.4). These data indicate that *C. demersum* is able to bioaccumulate copper from its environment in the Diep River. The results obtained for copper show that the stems of the plants accumulated more copper than the plant leaves on the initial sampling occasion, which was different to the aluminium, iron and zinc studied, where the leaves bioaccumulated metals to a greater extent (Table 5.4).

The plant stems and leaves of the introduced plants at site 2 had significantly higher concentrations of copper on the first sampling occasion compared with the last sampling occasion (Table 5.4). At site 2 zinc followed the same pattern with zinc concentrations significantly higher in the stems and leaves on the initial sampling occasions compared with the final sampling occasions (Table 5.3). Although no copper was detected in the sediment samples at site 2 (Table 4.5) and trace amounts of copper was detected in the water (Table 3.5), the introduced plants and to a lesser extent, the existing *C. demersum* were able to bioaccumulate copper in their stems and leaves. Compared to the reference plants, after the first sampling occasions, significant increases in both the stems and leaves were detected in the introduced plants. This confirms the importance of aquatic plants as indicators of metal pollution and the potential of *C.demersum* L. as an active and passive biomonitoring species in the lower parts of the Diep River (Table 5.4).

Copper concentrations were compared between *C. demersum* introduced to an aquatic environment for a twelve week period and the same species already growing in the same aquatic environment. The introduced plants accumulated significantly higher concentrations of copper in both their stems and leaves. The existing plants at site 2 accumulated a significantly higher concentration of copper in their leaves compared with their stems. The existing plants displayed a more stable copper uptake in their stems over the sampling period, perhaps indicating that the stems, being a more permanent organ of the plant, were able to maintain the copper concentrations more successfully, compared with the leaves, as leaves could be shed in the event of phytotoxicity or stand the chance of being consumed by organisms (Boyd & Martens, 1994). Being in an environment where copper is more likely to be phytotoxic to the plant than a deficiency, it is probably a survival mechanism of the plant to store less copper in the stems than the leaves. To avoid copper deficiency and phytotoxicity from excess copper, plants are able to control

the absorption of certain metals in an environment of excess metal concentrations (Kochian & Lucas, 1982; Glass, 1983; Cohu, 2009).

Although concentrations of copper decreased after the fourth sampling occasion, overall the introduced plants bioaccumulated in most cases triple the amount of copper than the existing plants (Table 5.4 & Figure 5.4). It is possible that lower ion concentrations in the introduced plants, could have facilitated rapid ion uptake (Lee, 1982). The new introduced plants were not adapted to the site and polluted environment and thus were more vulnerable to taking up copper than the existing plants. Dickinson et al. (1991) discusses many examples of how plants survive polluted environments and how plants adapt genetically to their environment by natural selection over time. This is probably why the existing plants displayed a more stable, consistent pattern of copper uptake over the study period, not only for copper but also for the other metals studied.

Filter-feeding mussels have been found to be ideal biomonitoring species for metal contaminants in estuaries, as often water does not contain very high metal concentrations when measured and thus does not reflect the metal concentrations in that particular environment, but may contain sufficient concentrations of metals that are of toxicological importance. Mussels act as bioaccumulators of metals and by measuring metal concentrations in their tissues, a more accurate account of metal pollution of a particular environment can be deduced (Connell, et al., 1999). Similarly, plants living in an aquatic environment can provide valuable information regarding the state of metal pollution by measuring responses at the level of the individual organism, ie. biomonitoring.

As aquatic plants actually need certain metals as micronutrients for nutrition they will absorb metals like iron, copper and zinc as part of their normal growth requirements and in a similar way, like mussels, are able to provide important information regarding metal concentrations in a specific aquatic system. By biomonitoring metals in plants, a relationship between the metal concentrations in the environment and the response they elicit in the living organisms can be obtained which can then be used in environmental protection and management (Connell, et al., 1999).

By measuring selective metal accumulation in *Ceratophhyllum demersum* L plants, both by passive and active biomonitoring procedures, these results not only show how *C. demersum* are able to adapt to metal contaminated environments but indicate how

introduced plants that have not been affected by metal pollutants, rapidly biaccumulate relatively large concentrations of metals within a short period of time. These results for metal bioaccumulation may be of relevance in any future biomarker studies conducted in the Diep River, as it has been shown that rapid bioaccumulation in organisms, may lead to rapid toxic responses within the body. These responses can then be used as biomarkers and early warning signals of metal induced stress (Snyman et al., 2000).

CHAPTER SIX

RESULTS AND DISCUSSION: PLANTS: MEMBRANE INTEGRITY

6.1 RESULTS

For this chapter, the term "subtracted amounts" always refers to EC reading/nutrient concentration after 24 hour period – EC reading/nutrient concentration before 24 hour period.

6.1.1 Electrical Conductivity (EC)

For consecutive sampling occasions at site 1 and site 2, EC readings of deionised water prior to placement of plants in the water were subtracted from the EC readings after a 24 hour period. Only these subtracted amounts were used for statistical analyses.

6.1.1.1 Comparison of subtracted amounts between consecutive sampling occasions at site 1

The subtracted amounts calculated for the plants from site 1 were significantly higher for the fifth sampling occasion compared to the fourth ($P<0.05$) (Table 6.1). No other significant differences were found between any other consecutive sampling occasions at site 1. There was also no significant difference between the subtracted amounts for the reference plants and the plants from the first sampling occasion ($P>0.05$).

6.1.1.2 Comparison of subtracted amounts between consecutive sampling occasions at site 2: Introduced plants

The subtracted amounts calculated for the introduced plants from site 2 were significantly higher for the first sampling occasion compared to the reference plants ($P<0.05$). Plants from the first sampling occasion lost solutes to the water, whereas the reference plants gained solutes from the water. The subtracted amounts calculated for the plants from site 2 were significantly lower for the second sampling occasion compared to the first sampling occasion but significantly higher for the fourth sampling occasion compared to the third ($P<0.05$). No other significant differences were found between any other consecutive sampling occasions at site 2 (Table 6.1).

6.1.1.3 Comparison of Electrical Conductivity of deionised water, between consecutive sampling occasions at site 2: Existing plants

The subtracted amounts calculated for the existing plants from site 2 were significantly higher for the first sampling occasion compared to the reference plants and for the third sampling occasion, compared to the second and for the sixth sampling occasion compared with the fifth ($P<0.05$). Plants from the first sampling occasion lost solutes to the water, whereas the reference plants gained solutes from the water. No other significant differences were found between any other consecutive sampling occasions ($P>0.05$) (Table 6.1).

6.1.1.4 Comparison of subtracted amounts between introduced and existing plants at site 2, using pooled data

The subtracted amounts, calculated using pooled data, were significantly higher for the introduced plants at site 2, compared to the existing plants at site 2 ($P<0.05$) (Table 6.1).

6.1.1.5 Comparison of subtracted amounts between sites 1 and 2, using pooled data

The subtracted amounts, calculated using pooled data, were significantly higher for the introduced plants at site 2, compared to the introduced plants at site 1 ($P<0.05$) (Table 6.1).

6.1.2 SODIUM

For consecutive sampling occasions at site 1 and site 2, sodium readings of deionised water prior to placement of plants in the water were subtracted from the sodium readings after a 24 hour period. Only these subtracted amounts were used for statistical analyses.

6.1.2.1 Comparison of subtracted amounts, between consecutive sampling occasions at site 1

The subtracted amounts calculated for the plants from site 1 were significantly lower for the first sampling occasion compared to the reference plants ($P<0.05$). No other

significant differences were found between any other consecutive sampling occasions ($P>0.05$) (Table 6.2).

6.1.2.2 Comparison of subtracted amounts between consecutive sampling occasions at site 2: Introduced plants

The subtracted amounts calculated for the introduced plants from site 2 were significantly lower for the first sampling occasion compared to the reference plants ($P<0.05$). There were significant differences between the subtracted amounts calculated for plants from the first and last sampling occasions. Plants from the first sampling occasion lost sodium to the water, whereas plants from the last sampling occasion gained sodium from the water. The subtracted amounts calculated for the introduced plants from site 2 were significantly lower for the last sampling occasion compared to the first and significantly higher for the fourth sampling occasion, compared to the third, while the sixth sampling occasion was significantly lower compared to the fifth ($P<0.05$). No other significant differences were found between any other consecutive sampling occasions ($P>0.05$) (Table 6.2).

6.1.2.3 Comparison of subtracted amounts between consecutive sampling occasions at site 2: Existing plants

There was no significant differences between the subtracted amounts calculated for the existing plants from the first sampling occasion, compared to the reference plants ($P>0.05$). The subtracted amounts calculated for the existing plants from site 2 were significantly higher for the last sampling occasion compared to the first, and for the sixth sampling occasion compared to the fith ($P<0.05$). No other significant differences were found between any other consecutive sampling occasions (Table 6.2).

6.1.2.4 Comparison of subtracted amounts between introduced and existing plants at site 2, using pooled data

The subtracted amounts, calculated using pooled data, were not significantly different between the existing plants and introduced plants at site 2 ($P>0.05$) (Table 6.2).

6.1.2.5 Comparison of subtracted amounts between sites 1 and 2, using pooled data

The subtracted amounts, calculated using pooled data, were not significantly different between the introduced plants at site 1 and the introduced plants at site 2 ($P>0.05$) (Table 6.2).

6.1.3 CALCIUM

For consecutive sampling occasions at site 1 and site 2, calcium readings of deionised water prior to placement of plants in the water were subtracted from the calcium readings after a 24 hour period. Only these subtracted amounts were used for statistical analyses.

6.1.3.1 Comparison of subtracted amounts, between consecutive sampling occasions at site 1

The subtracted amounts calculated for the plants from site 1 did not differ significantly between the reference plants and the first sampling occasion ($P>0.05$). There were also no significant differences between any consecutive sampling occasion between the plants at site 1 ($P>0.05$) (Table 6.3).

6.1.3.2 Comparison of subtracted amounts, between consecutive sampling occasions at site 2: Introduced plants

The subtracted amounts calculated for the introduced plants from site 2 were significantly lower for the first sampling occasion compared to the reference plants ($P<0.05$). The subtracted amounts calculated for the introduced plants from site 2 were significantly lower for the second sampling occasion (where calcium was gained by the plants) compared to the first sampling occasion (where calcium was lost to the water) and significantly higher for the fourth sampling occasion (at zero), compared to the third (where calcium was gained to the plants), No other significant differences were found between any other consecutive sampling occasions ($P>0.05$) (Table 6.3).

6.1.3.3 Comparison of subtracted amounts between consecutive sampling occasions at site 2: Existing plants

The subtracted amounts calculated for the existing plants from site 2 were significantly higher for the first sampling occasion compared to the reference plants ($P<0.05$). The subtracted amounts calculated for the existing plants from site 2 were significantly higher for the last sampling occasion compared to the fifth ($P<0.05$). On the fifth sampling occasion, plants gained calcium from the water while on the sixth sampling occasion, calcium was lost to the water. No other significant differences were found between any other consecutive sampling occasions (Table 6.3).

6.1.3.4 Comparison of subtracted amounts between introduced and existing plants at site 2, using pooled data

The subtracted amounts, calculated using pooled data, were significantly lower for the introduced plants at site 2, compared to the existing plants at site 2. Existing plants lost calcium to the water, whereas introduced plants gained calcium from the water ($P>0.05$) (Table 6.3).

6.1.3.5 Comparison of subtracted amounts between sites 1 and 2, using pooled data

The subtracted amounts, calculated using pooled data, were not significantly different between the introduced plants at site 1 and the introduced plants at site 2 ($P>0.05$) (Table 6.3).

6.1.4 POTASSIUM

For consecutive sampling occasions at site 1 and site 2, potassium readings of deionised water prior to placement of plants in the water were subtracted from the potassium readings after a 24 hour period. Only these subtracted amounts were used for statistical analyses.

6.1.4.1 Comparison of subtracted amounts between consecutive sampling occasions at site 1

The subtracted amounts calculated for the plants from site 1 did not differ significantly between the reference plants and the first sampling occasion ($P>0.05$). There were also no significant differences between any consecutive sampling occasions between the plants at site 1 ($P>0.05$) (Table 6.4).

6.1.4.2 Comparison of subtracted amounts between consecutive sampling occasions at site 2: Introduced plants

The subtracted amounts calculated for the plants from site 2 did not differ significantly between the reference plants and the first sampling occasion ($P>0.05$). The subtracted amounts calculated for the introduced plants from site 2 were significantly lower for the last sampling occasion compared to the first and significantly lower for the second compared to the first, as well as the fifth compared with the fourth sampling occasion. However, on the fourth sampling occasion the subtracted amounts were significantly higher than the third sampling occasion ($P<0.05$). No other significant differences were found between any other consecutive sampling occasions ($P>0.05$) (Table 6.4).

6.1.4.3 Comparison of subtracted amounts between consecutive sampling occasions at site 2: Existing plants

The subtracted amounts calculated for the existing plants from site 2 were significantly lower for the first sampling occasion compared to the reference plants ($P<0.05$). There were no other significant differences. The subtracted amounts calculated for the existing plants from site 2 were significantly higher for the last sampling occasion compared to the first and the sixth sampling occasion compared to the fifth ($P<0.05$). No other significant differences were found between any other consecutive sampling occasions ($P>0.05$) (Table 6.4).

6.1.4.4 Comparison of subtracted amounts between introduced and existing plants at site 2, using pooled data

The subtracted amounts , calculated using pooled data, were significantly higher for the introduced plants at site 2, compared to the existing plants at site 2 ($P<0.05$) (Table 6.4).

6.1.4.5 Comparison of subtracted amounts between sites 1 and 2, using pooled data

The subtracted amounts, calculated using pooled data, were not significantly different between the introduced plants at site 1 and the introduced plants at site 2 ($P>0.05$) (Table 6.4).

6.1.5 MAGNESIUM

For consecutive sampling occasions at site 1 and site 2, magnesium readings of deionised water prior to placement of plants in the water were subtracted from the magnesium readings after a 24 hour period. Only these subtracted amounts were used for statistical analyses.

6.1.5.1 Comparison of subtracted amounts between consecutive sampling occasions at site 1

The subtracted amounts calculated for the plants from site 1 did not differ significantly between the reference plants and the first sampling occasion ($P>0.05$). There were also no significant differences between any consecutive sampling occasion between the plants at site 1 ($P>0.05$) (Table 6.5).

6.1.5.2 Comparison of subtracted amounts between consecutive sampling occasions at site 2: Introduced plants

The subtracted amounts calculated for the plants from site 2 did not differ significantly between the reference plants and the first sampling occasion ($P>0.05$). The subtracted amounts calculated for the introduced plants from site 2 were significantly higher for the

fourth and fifth sampling occasions compared to the preceeding sampling occasions. However, on the sixth sampling occasion the subtracted amounts were significantly lower than the fifth sampling occasion ($P<0.05$) (Table 6.5).

6.1.5.3 Comparison of subtracted amounts between consecutive sampling occasions at site 2: Existing plants

The subtracted amounts calculated for the existing plants from site 2 did not differ significantly between the reference plants and the first sampling occasion ($P>0.05$). There were also no significant differences between any consecutive sampling occasions ($P>0.05$) (Table 6.5).

6.1.5.4 Comparison of subtracted amounts between introduced and existing plants at site 2, using pooled data

The subtracted amounts, calculated using pooled data, were significantly higher for the introduced plants at site 2, compared to the existing plants at site 2 ($P<0.05$). The introduced plants at site 2 lost magnesium to the water, whereas the existing plants gained magnesium from the water (Table 6.5).

6.1.5.5 Comparison of subtracted amounts between sites 1 and 2, using pooled data

The subtracted amounts, calculated using pooled data, were significantly higher for the introduced plants at site 2, compared to the introduced plants at site 1 ($P<0.05$). The introduced plants at site 1 gained magnesium from the water, whereas the introduced plants at site 2 lost magnesium to the water (Table 6.5).

Table 6.1: Mean (±SD) Electrical Conductivity (EC) of deionised water before and after containing *Ceratophyllum demersum* L. for 24 hours, measured in mS/cm.
Sample sizes: Per sampling occasions: n=4-6.
Pooled data: n=29-35.

Sampling occasions	Site 1			Site 2					
				Introduced plants			Existing plants		
	Before	After	EC (after) minus EC (before)	Before	After	EC (after) minus EC (before)	Before	After	EC (after) minus EC (before)
Reference plants	17.9	15.88 ± 3.56	-2.02 ± 3.56	17.9	15.88 ± 3.56	-2.02 ± 3.56*	17.9	15.88 ± 3.56	-2.02 ± 3.56*
1	17.3	23.23 ± 5.08	7.12 ± 4.65	17.3	31.32 ± 3.04	14.02 ± 3.04	16.6	20.2 ± 3.94	3.63 ± 3.98
2	16.6	25.32 ± 3.16	9.62 ± 3.16	43	46.65 ± 3.91	# 3.65 ± 3.91	19.1	19.75 ± 4.92	0.65 ± 4.92
3	19.1	29.13 ± 1.86	10.03 ± 1.86	49	57.82 ± 7.42	8.82 ± 7.42	21.6	23.02 ± 2.08	# 5.62 ± 2.08
4	21.6	25.24 ± 7.20	3.64 ± 7.20	49.3	80.72 ± 12.39	# 31.42 ± 12.39	18.7	22.45 ± 6.16	3.75 ± 6.16
5	18.7	30.88 ± 2.19	#12.18 ± 2.19	50.6	76.53 ± 14.80	25.93 ± 14.80	49.7	47.18 ± 7.84	-2.52 ± 7.84
6	49.7	56.15 ± 24.66	6.45 ± 24.66	68.4	98.12 ± 18.77	29.72 ± 18.78	49.3	57.45 ± 2.39	# 8.15 ± 2.39
Pooled data of subtracted amounts	3.96 ± 1.26¤ (n=29)			4.47 ± 3.95 (n=35)			3.84 ± 2.36 ♦ (n=30)		

= Significant differences between consecutive sampling occasions (from preceding sampling occasion).
¤ = Significant differences between sites (indicated in column of site 1).
* = Significant differences between the reference plants and the first sampling occasion.
♦ = Significant differences at site 2: existing plants versus introduced plants: pooled data (indicated in column of existing plants).

Table 6.2: Mean (±SD) sodium (Na) concentrations (mg/L), measured in the deionised water before and after containing *Ceratophyllum demersum* L. for 24 hours.
Sample sizes: Per sampling occasions: n=4-6.
Pooled data: n=29-32.

	Site 1			Site 2					
	Introduced plants			Introduced plants			Existing plants		
Sampling occasion	**Before**	**After**	**Na (after) minus Na (before)**	**Before**	**After**	**Na (after) minus Na (before)**	**Before**	**After**	**Na (after) minus Na (before)**
Reference plants	0 ± 0 (n=4)	4.06 ± 0.99 (n = 24)	4.06 ± 0.99* (n = 24)	0 ± 0 (n=4)	4.06 ± 0.99 (n = 24)	4.06 ± 0.99* (n = 24)	0 ± 0 (n=4)	4.06 ± 0.99 (n = 24)	4.06 ± 0.99 (n = 24)
1	0.47 ± 0.53	2.99 ± 0.58	2.44 ± 0.29	0.47 ± 0.53	2.98 ± 0.68	~2.52 ± 0.39	0.74 ± 1.06	3.46 ± 1.13	~2.64 ± 1.91
2	0.74 ± 1.06	4.22 ± 1.54	3.56 ± 2.33	3.62 ± 0.28	6.67 ± 0.45	3.18 ± 0.62	1.03 ± 0.62	3.63 ± 1.19	2.86 ± 0.73
3	1.03 ± 0.62	4.49 ± 3.50	3.37 ± 3.97	4.34 ± 0.32	8.16 ± 1.74	3.15 ± 0.43	0.90 ± 0.84	4.14 ± 1.55	3.27 ± 1.07
4	0.90 ± 0.84	4.71 ± 2.23	4.34 ± 2.14	0 ± 0	9.27 ± 2.02	#0.08 ± 1.90	1.00 ± 0.61	3.26 ± 1.21	2.69 ± 0.67
5	1.00 ± 0.61	6.73 ± 6.70	6.20 ± 7.10	0.36 ± 0.81	8.98 ± 1.02	8.35 ± 1.06	4.34 ± 0.32	7.24 ± 1.60	2.94 ± 2.05
6	4.34 ± 0.32	6.80 ± 3.00	3.86 ± 0.53	1.28 ± 2.64	0.70 ± 1.25	~#-0.44 ± 1.42	0 ± 0	8.66 ± 1.13	~#8.63 ± 1.26
Pooled data for entire study period	3.96 ± 1.26¤ (n=32)			4.47 ± 3.95 (n=29)			3.84 ± 2.36♦ (n=30)		

= Significant differences between consecutive sampling occasions (from preceding sampling occasion).
¤ = Significant differences between sites (indicated in column of site 1).
* = Significant differences between the reference plants and the first sampling occasion.
~ = Significant differences between the first sampling occasion and the last sampling occasion.
♦ = Significant differences at site 2: existing plants versus introduced plants: pooled data (indicated in column of existing plants).

Table 6.3: Mean (±SD) calcium (Ca) concentrations (mg/L), measured in the deionised water before and after containing *Ceratophyllum demersum* L. for 24 hours.
Sample sizes: Per sampling occasions: n=4-6.
Pooled data: n=27-30.

	Site 1			Site 2					
				Introduced plants			Existing plants		
Sampling occasion	**Before**	**After**	**Ca (after) minus Ca (before)**	**Before**	**After**	**Ca (after) minus Ca (before)**	**Before**	**After**	**Ca (after) minus Ca (before)**
Reference plants	5.30 ± 11.86 (n=5)	1.35 ± 17.30 (n=24)	1.57 ± 19.9	5.30 ± 11.86 (n=5)	1.35 ± 17.30 (n=24)	1.57 ± 19.9*	5.30 ± 11.86 (n=5)	1.35 ± 17.30 (n=24)	1.57 ± 19.9*
1	3.27 ± 1.24	3.41± 2.07	-0.22 ± 2.24	3.27 ± 1.24	3.70 ± 2.98	1.17 ± 1.55	2.22 ± 1.30	7.80 ± 16.78	6.86 ± 18.77
2	2.22 ± 1.30	1.23 ± 1.70	-0.34 ± 2.43	4.37 ± 0.74	0.03 ± 0.08	#- 4.34 ± 0.67	2.66 ± 1.63	1.98 ± 1.86	-1.26 ± 2.54
3	2.66 ± 1.63	3.55 ± 8.69	1.60 ± 10.29	4.93 ± 0.47	2.52 ± 5.87	- 4.80 ± 0.41	2.26 ± 0.79	1.74 ± 1.23	-0.51 ± 1.59
4	2.26 ± 0.79	0.31 ± 0.54	-2.11 ± 0.27	0.73 ± 1.80	- 0.55 ± 1.35	#0 ± 0	2.62 ±1.62	2.30 ± 4.83	0.94 ± 6.16
5	2.62 ± 1.62	0 ± 0	-2.62 ± 1.62	1.68 ± 1.99	0.11 ± 0.19	- 1.58 ± 2.03	4.93 ± 0.47	2.11 ± 1.85	-2.78 ± 1.71
6	4.93 ± 0.47	0.76 ± 1.51	-4.16 ± 1.73	2.96 ± 1.21	1.11 ± 0.97	- 1.63 ± 1.42	0.73 ± 1.80	4.39 ± 3.87	#5.15 ± 3.79
Pooled data for entire study period		-1.31 ± 3.60 (n=27)		-1.86 ± 0.77 (n=30)			1.40 ± 6.59 ♦ (n=30)		

= Significant differences between consecutive sampling occasions (from preceding sampling occasion).
* = Significant differences between the reference plants and the first sampling occasion.
♦ = Significant differences at site 2: existing plants versus introduced plants: pooled data (indicated in column of existing plants.

Table 6.4: Mean (±SD) potassium (K) concentrations (mg/L), measured in the deionised water before and after containing *Ceratophyllum demersum* L. for 24 hours.
Sample sizes: Per sampling occasions: n=4-6.
Pooled data: n=27-29.

	Site 1			Site 2					
				Introduced plants			Existing plants		
Sampling occasion	**Before**	**After**	**K (after) minus K (before)**	**Before**	**After**	**K (after) minus K (before)**	**Before**	**After**	**K (after) minus K (before)**
Reference plants	0 ± 0 (n=5)	2.90 ± 0.92 (n = 24)	2.90 ± 0.92 (n = 24)	0 ± 0 (n=5)	2.90 ± 0.92 (n = 24)	2.90 ± 0.92 (n = 24)	0 ± 0 (n=5)	2.90 ± 0.92 (n = 24)	2.90 ± 0.92* (n = 24)
1	0.06 ± 0.07	1.81 ± 0.97	1.90 ± 1.01	0.06 ± 0.07	2.01 ± 0.59	~2.09 ± 0.49	0.21 ± 0.13	0.14 ± 0.29	~-0.05± 0.31
2	0.21 ± 0.13	1.68 ± 1.10	1.60 ± 1.21	0.31 ± 0.12	0.62 ± 0.52	#0.21 ± 0.50	0.09 ± 0.09	0.09 ± 0.16	0.02 ± 0.22
3	0.09 ± 0.09	0.46 ± 0.75	0.11 ± 0.37	0.52 ± 0.07	2.17 ± 1.44	1.08 ± 0.45	0.47 ± 0.27	0.19 ± 0.33	-0.31 ± 0.48
4	0.47 ± 0.27	1.06 ± 0.84	0.64 ± 0.82	0 ± 0	3.52 ± 1.12	#3.96 ± 1.13	0.09 ±0.12	0.57 ± 0.47	0.59 ± 0.53
5	0.09 ± 0.12	1.75 ± 0.95	1.78 ± 1.01	0 ± 0	1.53 ± 1.05	#1.21 ± 0.78	0.52 ± 0.0 7	1.07 ± 0.80	0.32 ± 0.42
6	0.52 ± 0.07	2.70 ± 1.84	2.86 ± 1.18	0 ± 0	0.20 ± 0.24	~0.24 ± 0.25	0 ± 0	1.63 ± 0.98	~#1.59 ± 1.09
Pooled data for entire study period		1.40 ± 0.31 (n=27)			1.47 ± 0.31 (n=29)			0.36 ± 0.31♦ (n=29)	

= Significant differences between consecutive sampling occasions (from preceding sampling occasion).
* = Significant differences between the reference plants and the first sampling occasion.
♦ = Significant differences at site 2: existing plants versus introduced plants (indicated in column of existing plants).

Table 6.5: Mean (±SD) magnesium (Mg) concentrations (mg/L), measured in the deionised water before and after containing *Ceratophyllum demersum* L. for 24 hours.
Sample sizes: Per sampling occasions: n=4-6.
Pooled data: n=27-31.

	Site 1			Site 2					
				Introduced plants			Existing plants		
Sampling occasion	**Before**	**After**	**Mg (after) minus Mg (before)**	**Before**	**After**	**Mg (after) minus Mg (before)**	**Before**	**After**	**Mg (after) minus Mg (before)**
Reference plants	0 ± 0 (n=5)	0.31 ± 0.19 (n = 25)	0.31 ± 0.19 (n = 25)	0 ± 0 (n=5)	0.31 ± 0.19 (n = 25)	0.31 ± 0.19 (n = 25)	0 ± 0 (n=5)	0.31 ± 0.19 (n = 25)	0.31 ± 0.19 (n = 25)
1	0.13 ± 0.05	0.14 ± 0.08	0 ± 0.10	0.13 ± 0.05	0.10 ± 0.08	0 ± 0.08	0.18 ± 0.07	0.09 ± 0.12	-0.08 ± 0.16
2	0.18 ± 0.07	0.05 ± 0.11	-0.14 ± 0.13	0.31 ± 0.06	0.15 ± 0.06	-0.16 ± 0.08	0.20 ± 0.08	0.40 ± 0.63	-0.18 ± 0.08
3	0.20 ± 0.08	0.40 ± 0.63	0.32 ± 0.72	0.33 ± 0.04	0.48 ± 0.24	0.08 ± 0.14	0.20 ± 0.05	0.06 ± 0.07	-0.16 ± 0.08
4	0.20 ± 0.05	0.06 ± 0.09	-0.14 ± 0.06	0 ± 0	0.55 ± 0.11	#0.55 ± 0.19	0.25 ± 0.10	0.05 ± 0.06	-0.19 ± 0.08
5	0.25 ± 0.10	0.06 ± 0.12	-0.18 ± 0.17	0 ± 0	1.32 ± 0.40	#1.32 ± 0.40	0.33 ± 0.04	0.35 ± 0.09	0.02 ± 0.10
6	0.33 ± 0.04	0.25 ± 0.16	-0.06 ± 0.16	0.14 ± 0.32	0.30 ± 0.35	#0.22 ± 0.30	0 ± 0.05	0.51 ± 0.26	0.54 ± 0.28
Pooled data for entire study period		-0.03 ± 0.25¤ (n=27)			0.33 ± 0.13 (n=31)			-0.01 ± 0.08♦ (n=31)	

= Significant differences between consecutive sampling occasions (from preceding sampling occasion).
¤ = Significant differences between sites (indicated in column of site 1).
♦ = Significant differences at site 2: existing plants versus introduced plants (indicated in column of existing plants).

6.2 DISCUSSION

6.2.1 Electrical Conductivity (EC)

Electrical conductivity is the ability of water to conduct an electrical current, which depends on the dissolved mineral content of the water. The more solutes in a solution, the higher the EC reading will be (DWAF, 1996). Measuring solute leakage from plant tissue has widely been used to obtain information regarding membrane permeability of plants relating to various environmental stresses (Gupta, 1977; Palta et al., 1977; Leopold et al., 1981; McKersie et al., 1982; Whitlow et al., 1992). When a plant endures environmental stress and is submerged into a deionised water solution, depending on the state of the cell membrane, certain substances, including ions, will leak from the plant cells into the surrounding solution (McKersie et al., 1982; De Vos et al., 1991; Sinha et al., 1997; Quartacci et al., 2001) and by measuring the amount of solutes in that solution one can determine the degree of leakage over time or compare different plant samples from various sites or sampling occasions (Hall, 2002) as performed in the present study. By osmotic pressure, solutes in a plant cell will pass from an area of lower concentration to an area of higher concentration, via a membrane in order to gain equilibrium (Mauseth, 2003)

In the present study, only the data for the leaked solutes were analysed. The difference in the solutes, after the reference plants (taken from the fishpond) had been left in the water for a 24 hour period was negative (Table 6.1). This indicates that the plants absorbed solutes from the water, implying that the cell membrane integrity had not been lost, as normal osmotic pressure had been maintained. There was however, no significant difference in the EC results between the reference plants and the first sampling occasion at site 1. At site 1, the difference in EC readings, for the subtracted amounts of solutes in the water, showed no definite pattern but peaking at sampling occasion 5 and being significantly higher than the preceding sampling occasion (Table 6.1). This increase in solute loss at site 1 can be linked to the unseasonally high rainfall experienced of 107.9 mm during the month of November 2009 at the time of the present study (Tables 2.1 & 2.2 & Figure 2.5): Increased rainfall results in increased runoff (Schulz, 2001; Dabrowski et al., 2002). A fairly steep gradient (Figure 2.4) and farming activities, prevalent in the area, are likely reasons for an increase in contaminants in the river due to runoff. With increased

metals in the river, aquatic plants can bioaccumulate excess metals that could become toxic to them, resulting in cell membrane leakage, all contributing to the increase in EC.

At the fifth sampling occasion, solute loss was significantly higher than the solute loss of the reference plants and highest of all sampling occasions. The high solute loss could possibly be a response to excess metals in the plants, as established from this study in chapter 5, resulting in loss of membrane integrity and an increase in solute loss. The high solute loss at sampling occasion 5 corresponds with the highest iron reading in the leaves of the introduced plants at site 1, at sampling occasion 5 (which was significantly higher than the fourth sampling occasion) (Table 5.2 & Figure 5.2). It also similarly corresponds with the fifth sampling occasion for the leaves of the introduced plants at site 1, where aluminium was significantly higher than the aluminium concentrations in the fourth sampling occasion and highest over the study period (Table 5.1 & Figure 5.1). These high concentrations of iron and aluminium could have been the reason for the particularly high solute loss at the fifth sampling occasion, indicating loss of cell membrane integrity, as both excess iron and aluminium concentrations are known to cause this condition in plants (Gallego et al., 1996; Quartacci et al., 2001; Souza-Santos et al., 2001). There were no other significant differences in solute loss or gain by the plants at site 1 between the consecutive sampling occasions.

There was significantly more solute loss by the introduced plants from site 2 or rather significantly greater solute uptake by the reference plants compared to the plants from the first sampling occasion. The reference plants actually gained solutes from the water, compared to the plants from the first sampling occasion. At the first sampling occasion, solutes were lost to the water by the introduced plants at site 2 (Table 6.1). There was significantly lower solute loss to the water by plants at the second sampling occasion compared to the first sampling occasion and then a dramatic increase in solute loss at sampling occasion 4, which was significantly higher than the third sampling occasion (Table 6.1). Solute loss of introduced plants from site 2 were significantly higher (using pooled data) than the plants from site 1. This could be attributed to a more severe loss of membrane integrity compared with the introduced plants at site 1. At site 2, the salinity of the river water was much higher than at site 1 (Table 3.1), thus the introduced plants at site 2 could have absorbed more salts from the river water and naturally these salts would diffuse into the surrounding deionised water to obtain equilibrium. An increased saline

environment could also affect membrane integrity by lipid peroxidation, as documented by Upadhyay and Panda (2005) and thus result in a higher solute loss, as established by the present study. In the study by Kumar and Prasad (2004), as cadmium concentrations were experimentally increased, the electrical conductivity readings of the water in which *C. demersum* were placed, increased, while the solutes decreased in the solution containing the reference plants. Although no cadmium was detected in the water of the Diep River in the vicinity of site 2, in a previous study (Shuping, 2008) it is possible that cadmium or any other potentially harmful metal or organic chemical was present at the time of the study which could have contributed to the effect on cell membrane integrity.

Certain metals (eg. copper) negatively affect cell membrane integrity, resulting in an increase in electrical conductivity of water (de Vos et al., 1989; Devi & Prasad, 1998; Kumar & Prasad, 2004). In the present study copper was detected in the water and plant samples taken at both sites and often in significantly high quantities (Table 3.5 & Figure 3.4). Copper concentrations for water were over the Target Water Quality Range set out by DWAF (1996). In the plant samples at both sites copper was detected in the stems and leaves of the plant samples (Table 5.7) while copper was only detected in the sediment at site 1 and not at site 2 (Table 4.5 & Figure 4.4). Even though copper was not detected in the sediment samples at site 2, copper was found in the plant samples. As the water had high concentrations of copper, one can deduce that the copper concentrations in the plants could have caused oxidative stress in the plants, resulting in lipid peroxidation and leakage of ions, as proved by Devi and Prasad (1998) when they studied copper toxicity in *C. demersum*.

There was significantly more solute loss by the reference plants, compared to the plants from the first sampling occasion for the existing plants at site 2. The reference plants actually gained solutes from the water, compared to the existing plants from the first sampling occasion that lost solutes to the deionised water. There were significant increases in solute loss from the existing plants at sampling occasions three and six, compared to their preceeding sampling occasions. These significant increases in solute loss coincide with the significant increase in aluminium in the sediment and increased concentrations of aluminium measured in the leaves of the existing plants on the same sampling occasion (Tables 4.2 & 5.1). There is a possibility that the increase in aluminium in the environment contributed to solute loss at the last sampling occasion, as aluminium,

as well as other metals in excess, have direct effects on most physiological functions in plants (Barceló & Poschenrieder, 1990). The solute loss, as shown by the pooled data was significantly lower for the existing plants at site 2, compared to the introduced plants from the same site (Table 6.1). This indicates that the introduced plants had a decrease in membrane integrity, losing more solutes into the surrounding deionised water compared with the existing plants. At sampling occasion five, when the introduced plants at both sites showed the highest solute loss, the existing plants at the same sampling occasion, actually absorbed ions from the solution. This could indicate that possibly metals have adhered to membranes, causing slower transportation of ions across them or for transportation to cease completely. This adhesion of metals to the membranes could result in less solute loss from the plants, and causing the entire process of nutrient transport to be compromised (Delhaize & Ryan, 1995).

6.2.2 Sodium

In plant cells, osmosis occurs through selectively permeable membranes, allowing solutes to diffuse from an area of high solute concentration, to areas of lower solute concentrations. Sodium ions occur in very low concentrations intracellularly compared with the external environment (Salisbury & Ross, 1985). Sodium is required by plants in minute quantities and is especially important in the maintenance of pH within plant cells and for the control of osmotic pressure of extracellular fluids (Smith, 1986). As sodium is a monovalent ion it is most likely to move more freely (like potassium) compared with divalent ions like magnesium and calcium (DWAF, 1996). Excess metals cause loss of cell membrane integrity and disrupt the functioning of membranes as documented by many authors (Sinha et al., 1997; Quartacci et al., 2000; Souza-Santos et al., 2001; Kumar & Prasad, 2004; Sinha et al., 2005; Panda, 2007). This would affect the ability of the plant to regulate elements successfully to maintain optimum growth (Reichman, 2002). By doing experiments to establish if certain ions are lost from the plant under laboratory conditions, these results can assist in establishing if heavy metals could have resulted in negatively affecting the plants situated in the Diep River or whether there are any trends between the introduced plants and the existing plants regarding membrane integrity and whether the results indicate a possible metal tolerance by the existing plants.

The first experiment was to establish if any sodium had leaked through the plant cell membranes of *C. demersum* after being submerged in deionised water for 24 hours. The results showed that sodium moved from the reference plant samples, from the introduced plant samples taken at both site 1 and site 2 and the existing plants at site 2 into the water (Table 6.2). The amounts of leaked sodium from the introduced plants at site 1 and 2 were significantly lower at the first sampling occasion, compared to the reference plants. The existing plants had the least loss of sodium overall, followed by the introduced plants at site 1 and then the introduced plants at site 2 (Table 6.2).

As mentioned, the existing plants displayed the least sodium loss, (except for the final sampling occasion) which could mean that membrane integrity was mainly maintained due to genetic adaptations to their environment over time (Lambers et al., 2008; Munns & Tester, 2008). An example of a plant displaying genetic adaptations is that of *Arabidopsis thaliana,* which has a high tolerance rate for saline conditions and plants growing in different regions differ in salt tolerance. Baxter et al. (2010) found genetic evidence supporting local adaptations of various *Arabidopsis thaliana* plants and that plants growing near the coast had more of a certain sodium transporter allele, than plants growing in less saline conditions.

The existing plants displayed a level of adaptability to their environment and had a more stable loss of sodium compared with the introduced plants at both sites (except for the final sampling occasion). There was a significant increase in sodium loss to the deionised water from the existing plants at the last sampling occasion, compared to the first sampling occasion. As site 2, which had higher water salinity, due to its proximity to the ocean, the introduced plants had more sodium leakage from their cells than the plants from site 1. This could be due to the increased salinity of the water at site 2 and the stress associated with increased salinity on the plant that negatively impacts general plant metabolism (Grattan & Grieve, 1999; Upadhyay & Panda, 2005).

At site 2, for the introduced plants, at the last sampling occasion, a negative difference between readings for sodium was obtained, unlike any of the other occasions. This means that the plants absorbed sodium, possibly indicating a nutrient deficiency from being in the new saline environment, as salinity affects nutrient uptake (Grattan & Grieve, 1999). The amounts of leaked sodium from the introduced plants at site 2 were significantly lower at

the last sampling occasion compared to the first. Plants actually gained sodium during the last sampling occasion, unlike the preceeding 5 sampling occasions, when sodium was lost to the deionised water. There was no significant sodium loss or gain by the introduced plants at site 1 and 2 or between the site 2 introduced plants and existing plants (using pooled data) (Table 6.2).

As the EC results indicated significant differences between the pooled data of site 1 and the introduced plants at site 2, as well as the pooled data between site 2 and the existing plants at site 2 (Table 6.1). The results obtained for the EC readings, indicate that the sodium in the deionised water experiments did not contribute to the differences in the EC readings.

6.2.3 Calcium

Calcium is involved with controlling activities of enzymes and forms a component of the middle lamella between cells and is responsible with the movement of substances across cell membranes. It also plays a role in nitrate uptake and starch metabolism (Salisbury & Ross, 1985). Unlike sodium, calcium is a divalent ion. Most of the results for the introduced plants, for leaked calcium, were negative, except for the fourth sampling occasion at site 1, indicating that the plants absorbed the calcium from the surrounding water or released the calcium into the water only to reabsorb it (Table 6.3). The reference plants followed the same trend by having less calcium in the water. The introduced plants at site 2, displayed similar trends, with all but the first sampling occasion having solute loss from the plants to the deionised water (Table 6.3). The amounts of leaked calcium from the introduced plants at site 2 were significantly lower at the first sampling occasion compared to the reference but significantly higher from the existing plants at the same site at the first sampling occasion compared to the reference plants (Table 6.3).

The existing plants at site 2 seemed to lose the most calcium to the water, as the pooled data reflect, while the pooled data for the site 1 and site 2 introduced plants showed negative readings, indicating uptake of calcium by the plants. These results may indicate that the introduced plants had the need to absorb calcium as a required macronutrient due to a possible calcium deficiency, or due to the very high salt content of the water that reduces calcium availability and reduces its mobility in the plant (Grattan & Grieve, 1999).

High aluminium concentrations in solution have been known to decrease calcium uptake, as documented by Horbowicz et al. (2011). In the presence of aluminium, the uptake and translocation of calcium is hindered and plants become deficient in calcium (Huang et al., 1992; Rengel, 1992). At site 1, aluminium was abundant in the water, sediment and particularly the plant samples (Tables 3.2, 4.2 & 5.1) which could have led to the onset of calcium deficiency in the introduced plants. This was revealed by the mostly negative readings obtained from the subtracted amounts calculated indicating uptake by plants of calcium. The Diep River was found to have concentrations of aluminium in the water, over the recommended Target Water Quality Range set out by DWAF (1996) (Table 3.2), indicating that possibly the occurance of aluminium in solution (released by the plant) may have resulted in the plant unable to reabsorb the calcium.

Perhaps, the existing plants at site 2 had accumulated more calcium over time to compensate for the effects of the saline environment and had more calcium in its tissues, compared with the introduced plants, thus contributing to the higher calcium loss from the existing plants (Table 6.3). Another possible reason for the increased calcium in the plants could be due to the calcium/sodium ratio that is known to occur in plants as discussed by Lahaye and Epstein (1971), when sodium concentrations were found to be low in bean plants when calcium levels were high. Another theory could be that the divalent ions of calcium are more prone to attach themselves to membrane surfaces and are thus not as easily lost as monovalent potassium and sodium (Salisbury & Ross, 1985).

6.2.4 Potassium

Potassium is one of the easiest mineral elements to be lost from plant leaves, and by osmosis it will move through plant membranes from a region of higher solutes to a region of lower solutes (Meyer et al., 1973). Potassium is generally found in larger quantities than the micronutrients, as it is classified as a macronutrient and is immediately available and soluble. Like sodium, potassium is a monovalent ion and more mobile (DWAF, 1996). It is used in the manufacture of amino acids, for osmotic balance and functions as an enzyme activator in the cells (Salisbury & Ross, 1985). Potassium leakage from plant cells is a consequence of the alteration of plasma membrane permeability and is frequently used by

researchers to assess possible damage to cell membranes (de Vos et al., 1989; Sinha et al., 1997).

The difference between potassium concentrations in the water samples before and after site 1 plants were placed, was a positive value, indicating that potassium had leaked from the plant cells (containing more solutes) into the surrounding water (that had less solutes). The reference plant samples showed even greater potassium losses after the 24 hour period, indicating possible higher potassium solutes in the plant. At site 1 and site 2, all subtracted amounts for potassium were positive, indicating the loss of potassium from the plant cells into the surrounding water (Table 6.4).

For the plants from site 1, the degree of potassium loss did not vary significantly over the experimental period. However at site 2, there were significantly lower potassium concentrations in the deionised water from the introduced plants at the last sampling occasion compared to the first. The consecutive sampling for the introduced plants at site 2, showed three significant differences of differing values, with no definite trend (Table 6.4).

The existing site 2 plants showed the least potassium loss over the entire study period (Table 6.4). This may indicate that initially, the concentration of potassium in the existing plants was far less than the potassium of the introduced plants, as this could be expected in a more saline environment (salt reduces potassium uptake in plants) (Grattan & Grieve, 1999). Also, with the high aluminium concentrations detected in the water samples (Table 3.2), aluminium may have played a negative role in potassium uptake by the existing plants over time (Horbowicz et al., 2011). The existing plants could either have been unhealthy plants with potassium deficiency or suffered loss of membrane integrity resulting in little potassium being lost due to excess metals bonding to negatively charged pectic material on the membrane surfaces, associated with excess metal absorption in plants, especially that of a trivalent such as aluminium (Delhaize & Ryan, 1995; Prasad, 2004).

The existing plants at site 2 significantly gained potassium at the first sampling occasion compared to the reference plants. The plants then significantly lost potassium at the last sampling occasion compared to the first. These results showed that the existing plants at

site 2 finally lost potassium to the water, from initially taking up potassium from the water. On two sampling occasions (the first and third sampling occasions) the plants absorbed a small amount of potassium. These varied results could indicate problems relating to general cell metabolism due to oxidative stress and lipid peroxidation indicative of excess heavy metals that alter the structures of cell membranes and modify their transport activities (Delhaize & Ryan, 1995; Sinha, 1997). The findings of the present study agree with the findings of De Vos et al. (1989) and Sinha et al. (1997) regarding potassium leakage from plant cells under excess metal stress.

6.2.5 Magnesium

Magnesium has many important functions in the plant cell. It forms the central atom of the chlorophyll molecule and activates enzymes. It is also a carrier of phosphorus in the plant and is involved with starch translocation and sugar synthesis, it forms fats and oils, increases the utilization of iron and controls nutrient uptake. Calcium and magnesium ions have two positive charges compared with sodium and potassium only having one (Salisbury & Ross, 1985). This generally makes the calcium and magnesium ions adhere better to negatively charged pectin that occurs naturally in the primary cell walls of plants (Jarvis, 1982) which hinders the movement of calcium and magnesium from the cells into the surrounding water.

The amounts of leaked magnesium did not differ significantly between the reference plants and the first sampling occasion for both groups of introduced plants or for the existing plants. The amounts of leaked magnesium also did not differ significantly between the consecutive six sampling occasions at site 1 or for those of the existing plants at site 2. At site 2, the loss of magnesium was significantly higher at the fourth and fifth sampling occasions which coensides with the high rainfall during these weeks (Figure 2.5). It is possible that the increase in contaminants via runoff into the river could have resulted in an increase in metals in the water which could damage cell membranes in the plants (Sinha et al., 1997; Quartacci et al., 2001), causing higher losses of magnesium ions (Souza-Santos et al., 2001; Kumar & Prasad, 2004; Panda 2007).

The introduced plants at site 1 generally absorbed magnesium ions while the introduced plants growing at site 2 lost magnesium ions to the water after the 24 hour period. The

existing plants at site 2 showed different results to the introduced plants at the same site, as magnesium ions were mostly absorbed (Table 6.5). This could mean that the existing plants over time have become magnesium deficient and have absorbed the magnesium ions or the latter may have attached themselves onto the pectin of the cell walls in a similar manner as calcium, as the results for calcium and magnesium show similar trends.

Pooled data for the introduced plants at site 2 show that they lost more magnesium to the water than the existing plants. At site 2, the introduced plants lost significantly more magnesium to the water than the plants at site 1. These results indicate that the introduced plants at site 2 lost magnesium ions while the introduced plants at site 1 absorbed magnesium. All plants based at site 2 had higher magnesium losses than the plants introduced at site 1, based on the results of pooled data relating to the magnesium losses and gains, however only the introduced plants at site 2 actually absorbed magnesium compared to the other plants losing magnesium to the water (Table 6.5).

In conclusion, after exposure to contaminants in the Diep River to contaminants, *C. demersum* displayed various results in the laboratory experiment which was used to determine cell membrane integrity. The reference plants (not exposed to the Diep River) all leaked sodium, potassium, calcium and magnesium into the water during 24 hours. The responses of the plants in the Diep River varied. For sodium and potassium, the ions leaked out of the plants. But for calcium and magnesium, most results indicated that the plants actually absorbed ions from the water during the 24 hour period. If the responses of the plants are compared with the reference plants, only sodium and potassium mostly lost ions to the water, probably due to their monovalent nature. The most solute loss (as indicated by the subtracted amounts calculated from the EC readings) was experienced by the site 2 introduced plants, followed by the introduced plants at site 1. The reference plants absorbed solutes in contrast to all the sampled plants in the Diep River (Table 6.1), indicating the loss of cell membrane integrity of all the *Ceratophyllum sp.* sampled from the Diep River. These results can be attributed to the generally high levels of contaminants found in the Diep River and the plants themselves during the course of the study.

Recommendations for further study would be to investigate whether the existing *C. demersum* are physically different from plants originating from a known cleaner environment. It is known that plants adapted to saline conditions may have salt glands in

the form of modified trichomes or bladders (modified epidermal cells) to excrete excess salt (Munns & Tester, 2008). Other studies could be conducted under laboratory conditions, where controlled amounts of metals are added to the water over time to compare tolerance of plants growing in a polluted environment and plants harvested from a relatively non-polluted environment to test their tolerance levels, similar to that of Kumar and Prasad (2004).

CHAPTER SEVEN
RESULTS AND DISCUSSION: PLANTS: CHLOROPHYLL

7.1 RESULTS

7.1.1 Comparison of chlorophyll concentrations in *Ceratophyllum demersum* L. between consecutive sampling occasions per site in the plant leaves

7.1.1.1 Chlorophyll a

Site 1

There was a significantly higher concentration of chlorophyll a detected in the leaves of *C. demersum* from the sixth sampling occasion compared to the fifth sampling occasion and in the third sampling occasion, compared to the fourth ($P<0.05$). No other significant differences were detected (Table 7.1).

Site 2

Introduced plants

There were significantly higher concentrations of chlorophyll a detected in the leaves from the third and sixth sampling occasions, compared to respective preceding visits. There was also a significantly higher concentration of chlorophyll a at the sixth sampling occasion compared to the first ($P<0.05$) (Table 7.1).

Existing plants

There was a significantly higher concentration of chlorophyll a in the leaves of the existing plants at the fifth sampling occasion compared to the preceding visit, as well as between the first and last sampling occasions when chlorophyll a was significantly higher on the last sampling occasion ($P<0.05$). No other significant differences were detected ($P>0.05$) (Table 7.1).

7.1.1.2 Chlorophyll b

Site 1

There was a significantly lower concentration of chlorophyll b detected in the leaves of the plants at site 1 on the fourth sampling occasion compared to that of the third ($P< 0.05$). There was a significant increase in chlorophyll b concentrations from the fourth to the fifth sampling occasion ($P<0.05$) (Table 7.1).

Site 2

Introduced plants

There were significantly higher concentrations of chlorophyll b in the leaves of the plants at the third sampling occasion compared to the second, and the sixth sampling occasion compared to the fifth ($P<0.05$). There was also a significantly higher concentration of chlorophyll b at the sixth sampling occasion compared to the first ($P<0.05$) (Table 7.1).

Existing plants

There was a significantly higher concentration of chlorophyll b in the leaves of the existing plants at site 2 at the second and fifth sampling occasions compared to their preceding visits, while at the fourth sampling occasion the chlorophyll b detected in the leaves was significantly lower than in the preceding sampling occasion ($P<0.05$). There was a significantly higher chlorophyll b concentration detected at the last sampling occasion compared to the first sampling occasion ($P<0.05$) (Table 7.1).

7.1.1.3 Total chlorophyll content

Site 1

At the fourth sampling occasion there was a significantly lower concentration of total chlorophyll contents compared to the preceding visit, while at the fifth sampling occasion the total chlorophyll contents was significantly higher compared to the fourth visit ($P<0.05$) (Table 7.1).

Site 2

Introduced plants

There were significantly higher concentrations of total chlorophyll contents in the leaves of the plants detected at the third and sixth sampling occasions compared to their preceding visits ($P<0.05$). There was also significantly higher total chlorophyll reading detected at the sixth sampling occasion compared to the first sampling occasion $P\leq0.05$) (Table 7.1).

Existing plants

There were significantly higher concentrations of total chlorophyll contents in the leaves of the existing plants detected at the second and fifth sampling occasions compared to the preceding visits ($P<0.05$). There was a significant decrease from the third to the fourth sampling occasion ($P<0.05$). There was also significantly higher total chlorophyll reading detected at the sixth sampling occasion compared to the first sampling occasion $P\leq0.05$) (Table 7.1).

7.1.2 Comparison of chlorophyll concentrations between and within sites, per sampling occasion in the plant leaves

7.1.2.1 Chlorophyll a

There were significantly higher concentrations of chlorophyll a in the leaves of the introduced plants at the fourth, fifth and sixth sampling occasions at site 2 compared to site 1 ($P<0.05$). At the first, third, fourth, fifth and sixth sampling occasions, the chlorophyll a contents was significantly lower in the existing plant leaves compared to the corresponding sampling occasion of the introduced plants at the same site ($P<0.05$) (Table 7.1).

7.1.2.2 Chlorophyll b

There were significantly higher concentrations of chlorophyll b in the leaves of the plants at the sampling occasion at site 1 on the second sampling occasion, while on the fourth and sixth sampling occasions there were significantly lower chlorophyll b concentrations at

site 1 compared to the corresponding visits at site 2 ($P<0.05$). All but the second sampling occasion for the existing plants produced significantly lower chlorophyll b concentrations in the leaf samples compared to the introduced plants at site 2 ($P<0.05$) (Table 7.1).

7.1.2.3 Total chlorophyll content

There were no significant differences between sites on corresponding sampling occasions ($P>0.05$). The site 2 introduced plants had a significantly higher total chlorophyll contents than the existing plants at site 2 on all but the second sampling occasion ($P<0.05$) (Table 7.1).

7.1.3 Comparison between and within sites, using pooled data

7.1.3.1 Chlorophyll a

There was a significantly higher concentration of chlorophyll a in the introduced plants at site 2 compared to the introduced plants at site 1 as well as with the existing plants at site 2 over the entire sampling period ($P<0.05$) (Table 7.1).

7.1.3.2 Chlorophyll b

There was a significantly higher concentration of chlorophyll b in the introduced plants at site 2 compared to the introduced plants at site 1 as well as with the existing plants at site 2 over the entire sampling period ($P<0.05$) Table 7.1.

7.1.3.3 Total chlorophyll content

There was a significantly higher concentration of total chlorophyll content in the introduced plants at site 2 compared to the introduced plants at site 1 as well as with the existing plants at site 2 over the entire sampling period ($P<0.05$) (Table 7.1).

Table 7.1: Mean (±SD) chlorophyll concentrations (mg/L), measured in *Ceratophyllum demersum* L. leaves at the different sites in the Diep River, per sampling occasion, per chlorophyll group.
Sampling sizes: Per sampling occasion: n = 6.
Pooled data: n = 36.

	Site 1			Site 2					
				Introduced plants			Existing plants		
Sampling occasion	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**
Reference plants	6.98±1.06	2.22±0.89	9.21±1.62	6.98±1.06	2.22±0.89	9.21±1.62	6.98±1.06	2.22±0.89	9.21±1.62
1	7.83±2.43	2.85±0.99	10.68±3.05	~7.03±1.41	~2.00±0.41	~9.03±1.81	~3.50±1.09«	~1.04±0.32«	~4.54±1.41«
2	7.25±1.18	2.50±0.36¤	9.47±1.68	6.63±1.16	1.97±0.38¤	8.59±1.54	4.97±1.40	#1.76±0.48	#6.73±1.86
3	7.65±1.65	2.35±0.51	10.01±2.13	#9.26±1.22¤	#3.12±0.58	#12.38±1.75	4.82±0.50«	1.93±0.78«	6.75±1.05«
4	#5.04±1.61¤	#1.39±0.54¤	#6.42±2.15¤	10.00±1.25*¤	3.22±0.53¤	13.22±1.76	3.94±0.84«	#1.05±0.31«	#4.99±1.15«
5	3.93±0.84¤	#2.58±0.32	#10.74±1.21	8.70±1.10¤	3.06±0.44	11.76±1.40	#6.67±1.46«	#1.95±0.45«	#8.62±1.91«
6	#7.52±0.69¤	2.38±0.28¤	9.90±0.90¤	~#12.68±1.98¤	~#4.48±0.77¤	~#17.16±2.74	~6.69±1.19«	~1.99±0.41«	~8.94±1.42«
Pooled data for entire study period	6.5±2.07¤	2.34±0.26¤	9.54±0.77¤	9.05±2.41	2.77±0.13	12.02±0.47	5.10±0.36♦	1.62±0.17♦	6.76±0.36♦

= Significant differences between consecutive sampling occasions (from preceding sampling occasion).
¤ = Significant differences between sites (introduced plants).
~ = Significant differences between the first sampling occasion and the last sampling occasion.
« = Significant differences at site 2: introduced plants versus existing plants (per sampling occasion).
♦ = Significant differences at site 2: existing plants versus introduced plants (pooled data).

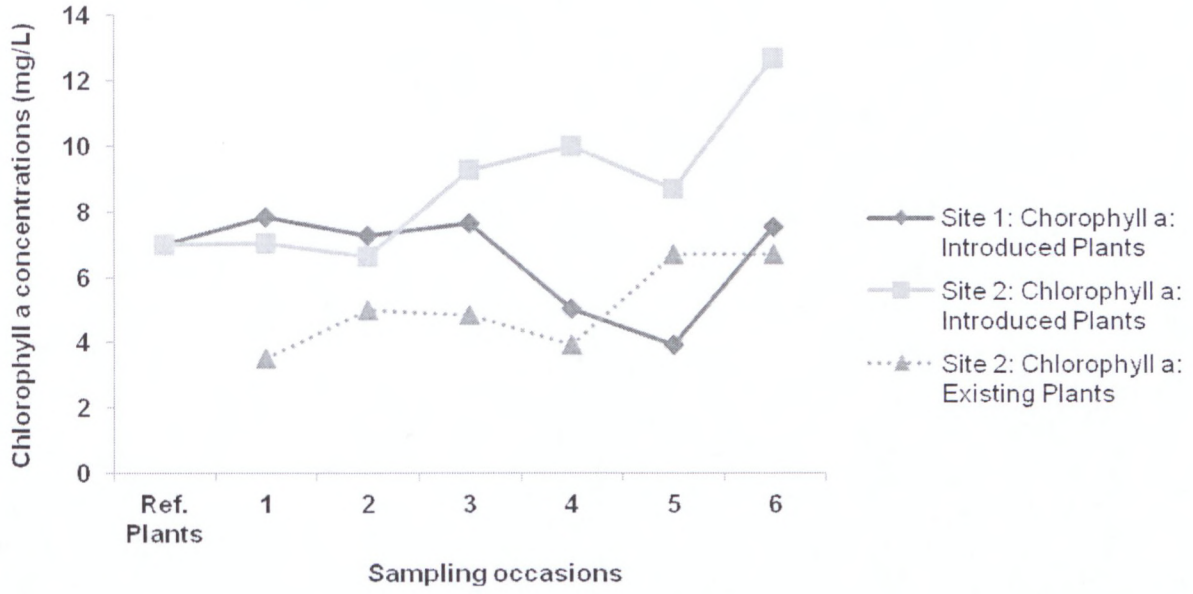

Figure 7.1: Mean chlorophyll a concentrations (mg/L), measured in *Ceratophyllum demersum* L. leaves at the different sites in the Diep River, per sampling occasion.

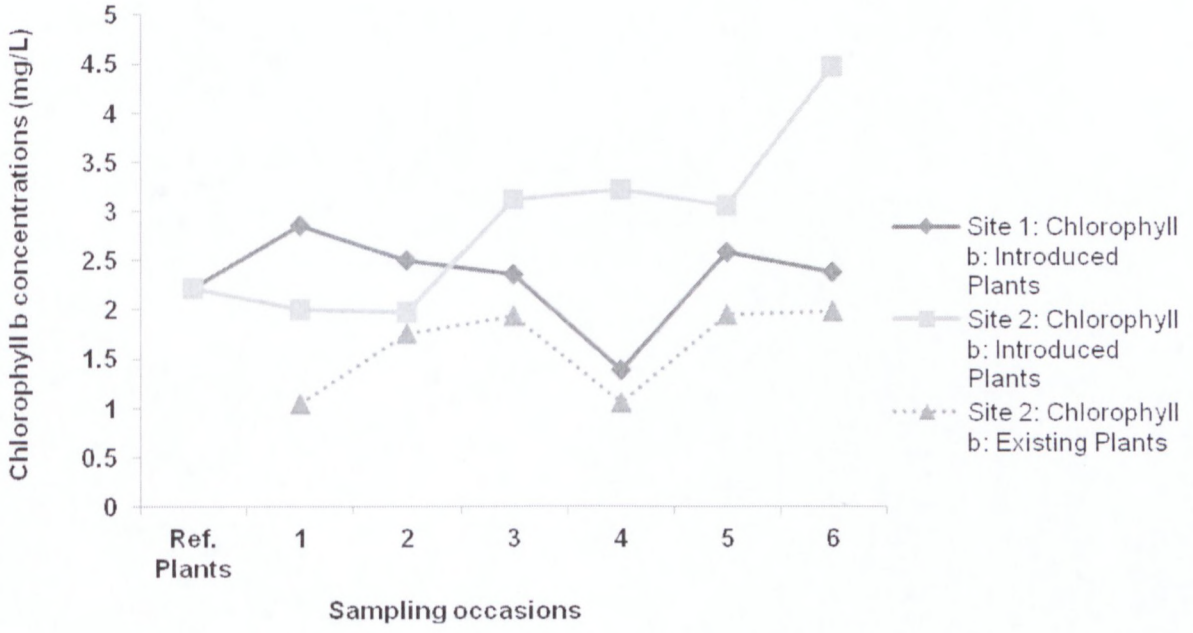

Figure 7.2 Mean chlorophyll b concentrations (mg/L), measured in *Ceratophyllum demersum* L. leaves at the different sites in the Diep River, per sampling occasion.

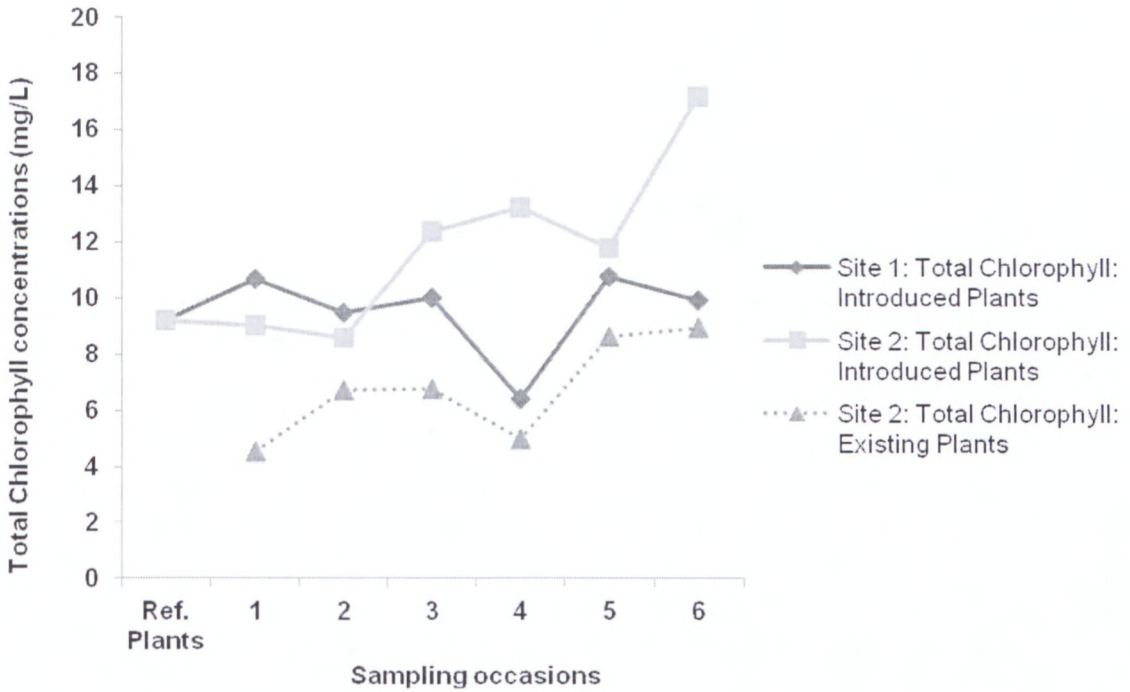

Figure 7.3: Mean total chlorophyll concentrations (mg/L), measured in *Ceratophyllum demersum* L. leaves at the different sites in the Diep River, per sampling occasion.

7.2 DISCUSSION

Metals in excess concentrations are known to affect photosynthesis and chlorophyll production in a negative way, disadvantaging the plant (Küpper et al., 1996; Küpper at al., 1998; Mukherjee et al., 2004; Myśliwa-Kurdziel & Strzatka, 2002; Shakya et al., 2008). Plasma membranes in plant cells consist of 50% lipids and the plasma membrane is the first structure that comes into contact with metals from the environment. Any excess metal ions will change the structure of the membrane lipids which will in turn disrupt normal cellular functions in the plant. In chloroplasts, the thylakoid membranes are also affected by heavy metals, changing their structures, lipid content and thus affecting their functioning, resulting in peroxidation of the chloroplast membranes (Devi & Prasad, 2004). The results obtained for copper in the present study indicate the opposite effect to findings by Baszyński et al. (1988). A decrease in copper in the plant leaves at the fifth sampling

occasion, (compared with the fourth), coincided with a decreased chlorophyll a concentration in both site 1 and site 2 introduced plants, while the existing plants at site 2 showed an increase in chlorophyll a. For the site 2 introduced plants, this trend was also followed for total chlorophyll content and to a lesser extent chlorophyll b, where the introduced plants had a decrease in total chlorophyll content at the fifth sampling occasion, which coincided with a decrease in copper concentrations in the leaves, while the existing plants at the same site experienced increased total chlorophyll production (Figures 5.4, 7.1, 7.2 & 7.3). Chlorophyll and copper data for site 2 introduced plants at the fifth sampling occasion, did not follow the same trend as for chlorophyll b and total chlorophyll contents (Figures 5.4, 7.2 & 7.3) which could perhaps be attributed to the generally higher copper concentrations found at site 1 (Tables 5.4 & 5.7) and the different environmental conditions associated with site 1 from agricultural activities (possible copper fungicide influences).

Between the third and fourth sampling occasions when the copper concentrations in the plant leaves increased, clear differences in the chlorophyll results are seen when the site 2 introduced and existing plants are compared (Table 5.4). At site 2 when there was an increase in copper in the plant leaves (Table 5.4), chlorophyll a, chlorophyll b and total chlorophyll content increased slightly in the plant leaves of the introduced plants, while the chlorophylls all decreased in the existing plants (Figures 5.4, 7.1 7.2 & 7.3). These results indicate that possibly, increased copper concentrations in the plant leaves of introduced *Ceratophyllum sp.* could have positively influenced chlorophyll activity due to a copper deficiency prior to placement or that the introduced plants were unable to regulate copper uptake, due to their short exposure to contaminants in the river. Chlorophyll production of the existing *Ceratophyllum sp.* could have been negatively influenced by excess copper uptake due to the effect over time that excess metals have on general plant metabolim (Barceló & Poschenrieder, 1990) (Table 5.5).

Copper plays an important role in chlorophyll production but excess copper inhibits chlorophyll production by altering cell membrane properties and affecting the enzymes that assist with chlorophyll production (Shakya et al., 2008). Excess metals, especially

copper, can influence the thylakoid membranes of the chloroplasts both structurally and functionally eg. copper ions in wheat (Quartacci et al., 2000), runner beans (Maksymiec et al., 1994) and spinach (Maksymiec et al., 1992). Copper has been found to decrease the percentage of chloroplastic membranes (Murata et al., 1990). Granum stacking in the chloroplast depends on glycolipids, so any modification in the membrane would negatively impact photosynthetic efficiency. Photosynthesis II depends completely on monogalactosyldiglycerols (MGDG) levels and a decrease in lipids would cause a decrease in photosynthetic activity (Murata et al., 1990). Maksymiec et al. (1994) showed that the composition of lipids is crucial in maintaining thylakoid function and that thylakoid membranes are not able to function optimally when excess metal ions were present.

Reduced chlorophyll content due to excess of certain metals is well documented in particular for zinc and copper in various plant species, as well as for cadmium, lead, nickel and mercury. These metals were found to inhibit the biosynthesis of photosynthetic pigments that resulted in decreased chlorophyll content (Myśliwa-Kurdziel & Strzatka, 2002). Excess copper and zinc ions are able to substitute magnesium ions in the chlorophyll molecule, resulting in a breakdown of photosynthesis (Küpper et al., 1996, 1998). Copper affected chlorophyll biosynthesis more strongly than cadmium in *Lemna trisulca* (Prasad et al., 2001). At concentrations of 25 and 50 µM (3.18 mg), plants tested had a 50% decrease in chlorophyll contents, compared with untreated plants. For the present study, the introduced plants had much higher concentrations of copper present in their leaves (compared to the previous study) but high chlorophyll concentrations. The existing plants at site 2 had 4.79 ±0.92 mg copper in their leaves and showed lower chlorophyll concentrations (pooled data) (Table 5.4 & 7.1). The introduced plants therefore did not follow the trend of the the *Lemna* plants studied by Prasad et al. (2001). As the introduced plants had only inhabited the water for 12 weeks, it is unknown what consequences such high concentrations of copper had on plant metabolism and further studies incorporating longer exposure periods to copper are recommended.

The existing plants had a much lower copper concentration in their leaves but displayed a decreased chlorophyll concentration (compared to the introduced plants that had high copper and high chlorophyll concentrations). Only the existing plants at site 2 showed a

similar trend to that of *Lemna trisulca* studied by Prasad et al. (2001). Copper concentrations of 4.79 ±0.92 mg detected in the existing plant leaves, resulted in decreased chlorophyll contents in *C. demersum* growing in the Diep River. Excess copper (Table 5.4) and zinc (Table 5.3), had similar affects on the existing *C. demersum* at site 2, resulting in decreased chlorophyll production, compared with the newly introduced plants which had higher chlorophyll production but over a shorter time period.

The age of the plant can influence the effect that the excess metal ions have on plants, as found by Skórzyńska-Polit & Baszyński (1995) where more mature leaves were negatively affected compared with younger leaves. However Malik et al. (1992), found that young wheat seedlings were negatively affected by chromium which decreased chlorophyll production and chloroplast development in very young leaves. As the younger leaves of *C. demersum* appear on the ends of the long stems and older leaves are rather sparse on the stem, it can be said that younger leaves were used in the present study and follow the trend determined by Malik et al. (1992). Copper concentrations in the plants were highest at site 1 which may have contributed to the lower chlorophyll readings found in the plants at site 1 compared with site 2 (Table 5.4).

Excess zinc, negatively affects chlorophyll production in plants (Symeonidis & Karataglis, 1992; Shakya et al., 2008). Zinc, along with some other metals, which include copper, is known to replace the central magnesium ion in the chlorophyll molecule, especially in aquatic plants. This replacement of magnesium affects the harvesting of light and results in a breakdown of photosynthesis (Küpper et al., 1996, 1998). Evidence of possible metal damage can be seen in the present study, as when zinc concentrations generally increased (although only slightly) at the fourth sampling occasion (Figure 5.3), the results for all chlorophylls at site 1 and the existing plants at site 2 decreased (Figures 7.1, 7.2 & 7.3), possibly confirming the negative effects of zinc established by the above authors. Only the site 2 chlorophyll data of the introduced plants were opposite to that of the above findings, where an increase in zinc concentration in the plant leaves at the fourth sampling occasion, resulted in an increase in chlorophyll a, chlorophyll b and total chlorophyll contents. The higher concentrations of zinc detected in the water and sediments (Tables

3.4 & 4.4), resulting in higher zinc concentrations in the plants at site 1 (Table 5.3) may have had a more detrimental effect on chlorophyll production of the introduced plants there compared with site 2. High iron concentrations in the environment could have lessened the effects of zinc toxicity in the plants, as documented by Fontes & Cox (1998), where high iron concentrations prevented most of the toxic effects of excess zinc.

Excess iron interferes with chemical processes within the plant cells that produce proteins essential for plant metabolism. In small quantities, iron is required by the plant for chlorophyll production, however excess iron can change the chlorophyll in such a way that the plant struggles to photosynthesise (Kampfenkel et al., 1995). As for all the other metals, iron concentrations at site 1 were significantly higher in the sediment (Table 4.2) and the introduced plants than at site 2 (Table 5.2), indicating a possible reason for the lower chlorophyll readings in the plants at site 1. The highest concentrations of iron in the introduced plant leaves at site 1 were found at the fifth sampling occasion (Figure 5.2) and the corresponding chlorophyll concentrations for chlorophyll a, chlorophyll b and the total chlorophyll contents in the plants at the same sampling occasion, all decreased, thus reflecting the findings of many authors that excess iron has a detrimental effect on chlorophyll production (Figures 7.1, 7.2 & 7.3) (Kim & Jung, 1993; Kampfenkel et al., 1995; Gallego et al., 1996). At site 2, when iron concentrations were highest in the introduced plant leaves, at the third sampling occasion, the chlorophyll production for all chlorophyll forms increased in concentration. For the existing plants at site 2, the most iron was found in the leaves on the fifth sampling occasion and corresponding chlorophyll data at the same sampling occasion indicated that all chlorophyll forms increased. O'Kelley (1974) reviewed studies that indicated that iron and manganese were linked with chlorophyll production and that light played an important role together with iron to stimulate chlorophyll production in certain algae, adding to the important role that metal and environmental interactions have on chlorophyll production in plants.

Although the introduced plants accumulated significantly more aluminium, iron, zinc and copper in their leaves than the existing plants, this did not affect chlorophyll production negatively. Baszyński et al. (1982) found that chlorophyll content depended on the copper tolerance of a specific plant and that excess copper actually increased the total chlorophyll

content in spinach plants that were known to be copper tolerant, while in copper sensitive plants, a decrease in chlorophyll content was found (Baszyński et al., 1988). At site 1, where the pooled data for aluminium, iron, zinc and copper were higher than the concentrations detected in the site 2 plants, for the first three sampling occasion's chlorophyll concentrations were the highest for all chorophyll data, indicating that high metal concentrations positively affected chlorophyll production (Table 5.7). After sampling occasion 3 though, the chlorophyll concentrations of the site 2 introduced plants showed higher chlorophyll readings for the proceeding three sampling occasions (Figures 7.1, 7.2, 7.3). It may be possible that the high metal concentrations detected in the plants at site 1 could have negatively affected chlorophyll production only from the fourth sampling occasion onwards, as previously discussed and that possibly the plants were beginning to show intolerance of excess metals.

The lower chlorophyll production results obtained for the site 1 introduced plants, compared with site 2, could be either metal related or light related. At site 1 the light intensity was generally lower due to the more shaded environment and leaf cover was evident on the baskets of the introduced plants on most of the sampling days. At site 1, the concentrations of aluminium were higher in the plants, sediment and water compared with site 2 (Tables 5.1, 4.2 & 3.2) and could have negatively affected chlorophyll production. Aluminium is known to displace vital ions on the cell wall and negatively affect membrane activity and it is rapidly absorbed into plant cells within minutes of being exposed (Lazof et al., 1994). Aluminium is able to displace ions and prevent the transport of nutrients within the plant, as it binds with proteins and pectic residues on the cell wall, as documented by Haug (1994) and thus affects general plant metabolism, including chlorophyll production.

It is possible that the site 2 introduced plants first needed to acclimatise to the extreme conditions of their new environment but then took advantage of possible nitrates and phosphates, that is a well known byproduct of sewage sludge (Wright & Welbourn, 2002) and most likely enters the river near this area from Potsdam (Coastal & Environmental Consulting, 2011). High levels of nitrates and phosphates , associated with increased

organic matter (Smith, 1986) at site 2 compared with site 1 (personal observation) and sewage treatment works could have affected the higher chlorophyll results at site 2, as the site 1 plants would not be affected by this, being situated upstream. There is a direct relationship between phosphates and chlorophyll concentrations; an increase in phosphates, increases chlorophyll production (Dillon & Rigler, 1974). Nitrogen, being a component of chlorophyll can stimulate chlorophyll production (Meeks, 1974) and this may have resulted in the higher levels of chlorophyll obtained in the site 2 introduced plants. The site 2 introduced plants had significantly higher levels of chlorophyll (pooled data) of the three plant groups tested (Figures 7.1, 7.2, 7.3). There was also a significant increase in all chlorophyll groups for all plants at site 2 (introduced and existing plants) for concentrations of chlorophyll between the first and last sampling occasion. These results clearly indicate that the site 2 environment promoted chlorophyll production.

The introduced plants at site 2 and the existing plants at site 2 were both exposed to the same concentrations of metals and experienced the same environmental conditions, yet their chlorophyll data differed significantly (Table 7.1). The introduced plants at site 2 had almost double the amount of chlorophyll in all forms, in their leaves compared with the existing plants (Table 7.1). The existing plants at site 2 had significantly lower chlorophyll concentrations than the introduced plants at each sampling occasion (except for the second). The existing plants may have developed ways to regulate certain metals but at the expense of other functions, like chlorophyll production (Dickinson et al., 1991). It is possible that certain metals bioaccumulated over time by the existing plants may have contributed to the loss of chlorophyll production due to metal phytotoxicity and oxidative stress (Haug, 1994; Kampfenkel et al., 1995; Gallego et al., 1996; Sinha et al., 1997; Shakya et al., 2008).

These results may indicate that time plays an important role in the lower chlorophyll readings of the existing plants and that genetic adaptations, as discussed for the tolerance of excess metals in plants, may have played an important role in the lower chlorophyll content obtained for the existing plants in this study. There was no decline in chlorophyll production over the 12 week period for any of the existing plant samples. The results

obtained for chlorophyll contents for the introduced plants and existing plants differed significantly (Table 7.1). A suggested study would be to monitor the plants over a longer period of time, to observe if the chlorophyll contents decrease further over time. As the existing plants showed no radical variations in chlorophyll concentrations over the study period, using chlorophyll readings as a biomarker for metal concentrations in the environment are not recommended without further research, particularly laboratory exposures.

As there are so many environmental factors that could influence plant growth and thus chlorophyll production, like light intensity, temperature, metal toxicity to the plants, metal interactions within the water and pH that influences metal bioavailability (Salisbury & Ross, 1985), it is difficult to speculate on the exact reasons for the chlorophyll results obtained. In conclusion one can deduce that the higher concentrations of metals (aluminium, copper, iron and zinc) detected in the introduced plants at site 1, compared with introduced plants at site 2, directly correspond with the lower chlorophyll concentrations obtained from the same plants at site 1 compared with the introduced plants at site 2. This trend in the plants, where in the presence of high concentrations of metals, chlorophyll production is negatively influenced, indicates that *C. demersum* may possibly be considered as a potential active biomonitor of metal contamination in the Diep River. It is important to note that as metals are not independent of each other in an aquatic environment and influence metabolic processes within plants, a laboratory experiment would give more conclusive results of cause and effect.

CHAPTER EIGHT

CONCLUSION

As metal pollution in rivers become more prevalent in our modern society, it is becoming necessary to regularly monitor pollution levels in rivers, particularly in the urban environment. *C. demersum*, an aquatic submerged macrophyte, commonly found in stagnant water bodies worldwide, is an ideal plant to use for monitoring metal contamination in rivers, as it is abundant and easy to grow. Measuring metal concentrations in plants is a more comprehensive approach for establishing the degree of contamination in a river, than just sampling water and sediment, as determined by this study.

Results found that aluminium, iron, zinc and copper concentrations in the Diep River at both study sites were higher than the guidelines set out by DWAF for water quality in aquatic environments. Even in the upper reaches of the river the concentrations for these metals were higher than expected, especially for aluminium and zinc, which were found to be higher in the water there, than at the known polluted site downstream. These findings indicate the presence of metal contaminants in the water in the upper reaches of the river and signify a possible association with farming activities in that region. The results from this study are in contrast to the 'State–of-Rivers Report' document from the River Health Programme, compiled by DWAF in 2003, when the upper Diep River, near its source, was considered to have its biodiversity and integrity largely intact and the envisioned future ecological state of the river in that vicinity was considered to be 'good' (River Health Programme, 2003). In just six years the quality of the upper parts of the Diep River has deterioated considerably. This indicates the need for future monitoring of water quality in the Diep River, especially in the upper reaches.

Sediment metal analysis revealed that for all metals studied, sediment in the upper reaches of the river had higher concentrations of metals than sediments sampled at the lower study site. As sediment is deposited in larger quantities downstream, it was unusual that the sediment in the upper reaches had higher concentrations of all four metals. All

sediment samples for the four metals sampled, had much higher concentrations of metals present than the water. These results are probably also related to the farming activities (for example pest control methods and dairy feeds) around site 1.

As bedrock can have an influence on the metal makeup of sediment, measuring metal concentrations in aquatic plants, as shown by *C.demersum*, has indicated the importance of metal bioaccumulation in plants as indicators of metal pollution. *C.* demersum is rootless and is therefore not directly influenced by the sediment makeup. During the study period of 12 weeks, it was found that the introduced plants bioaccumulated high concentrations of aluminium, iron, zinc and copper. These concentrations were far higher than the concentrations detected in the water and in most cases the sediment too. The introduced plants at the site in the upper reaches of the river bioaccumulated more metals than the plants at the site downstream, indicating the high degree of metal contamination in the upper parts of the river, within the farming district and probably higher metal bioavailability.

At the site in the lower reaches of the river, existing *Ceratophyllum sp.* growing naturally at the site, were also analysed for aluminium, iron, zinc and copper and compared with the introduced plants at the same site. For all metals sampled, the introduced plants had higher metal concentrations than the existing plants, possibly indicating the susceptibility of the new plants to the polluted environment and the genetic adaptation of the existing plants.

In all cases, except for copper in the introduced plants at site 2 (in the lower reaches), metal concentrations were higher in the leaves compared to the stems, even for the existing plants. Leaves have more surface area exposed to contaminants than stems and are less permanent organs, as they senesce and are food for herbivores. Metals accumulated in leaves could possibly be a survival mechanism of the plant. If metals are accumulated in high quantities within *Ceratophyllum sp.*, these metals are only temporarily removed from the water and may find their way back into the environment via the food

chain. *C. demersum* is consumed by many organisms like snails, fish and water fowl, or may decompose, contributing metals to the sediment.

It is recommended for *Ceratophyllum sp.* to be used in assisting in the control of metal pollution (phytoremediation). Plants should be introduced from especially produced clean stock, introduced to the river and then harvested after a period of time and removed from the river, thus removing metal contaminants in the process. It is unkown how long it would take for the introduced plants to adapt to the environment and what their threshold level of metal contaminants would be, before metal accumulation stabilises and possibly declines or kills the plant, this needs investigation. The removal of metal contaminated plants is essential to prevent further movement of these metals in the food chain via birds and fish who use *Ceratophyllum sp.* as food. It is clear from this study that established plants do not accumulate as much metals as new plants and have evolved mechanisms over time to adapt to their contaminated environment.

After *C. demersum* were placed in water for 24 hours, comparisons between the consecutive sampling occasions, sites and existing plants from the river could be compared with the introduced plants, concerning solute loss. The most solute loss (consisting of all possible solutes, not only the four ions tested) was experienced by the site 2 introduced plants, followed by the introduced plants at site 1. The reference plants absorbed solutes in contrast to all the sampled plants in the Diep River (Table 6.1), indicating the loss of cell membrane integrity of all the *Ceratophyllum sp.* sampled from the Diep River. The reference plants (not exposed to the Diep River) gained sodium, potassium, calcium and magnesium after 24 hours. For sodium and potassium, the ions leaked out of the Diep River plants. But for calcium and magnesium, most results indicated that the plants actually absorbed ions from the water after the 24 hour period. If the responses of the plants are compared with the reference plants, only sodium and potassium lost solutes, probably due to the monovalent nature of those ions. These results can be attributed to the generally high levels of contaminants found in the Diep River and plants themselves during the course of the study.

Copper is known to play an important role in chlorophyll production but excess copper inhibits chlorophyll production by altering cell membrane properties and affecting the enzymes that assist with chlorophyll production. In this study, at site 2, where both existing plants and introduced plants could be compared, generally when copper increased in the leaves of the plants, the chlorophylls also increased in the introduced plants but showed a decrease in the existing plants. These results could possibly indicate that the new plants benifited from the increased copper concentrations while the existing plants did not, possibly due to their copper threshold level being lower from being exposed to metals for a much longer period than the introduced plants.

In conclusion, the present study has shown that *C. demersum*, as an introduced plant to the Diep River bioaccumulates metals to high concenvcations and follows environmental metal trends to some extent. Analysing this species for metal content has also definitely provided additional information to what was gathered with water and sediment analyses alone. Therefore, the species shows potential as a possible biomonitor species for aluminium, iron, zinc and copper contamination. It is however not conclusive whether cell membrane integrity and chlorophyll contents *C. demersum* can be used as reliable biomarkers of metal exposure, but the results obtained from this study show potential. Further research is however vital for this species.

Finally, as few studies have been conducted on the Diep River and metal pollution, it is recommended that further seasonal studies be conducted in various parts of the river on various metals but especially in the upper reaches, to record levels of possible pollution throughout the year. It is also recommended that other organisms be tested, for possible use as biomonitors and models in biomarker studies, like aquatic invertebrates and other aquatic plant species. Laboratory experiments are highly recommended, as they will give a clearer indication of how metals affect plants and organisms. By applying various concentrations of metals to plants in a controlled environment, a clearer link between cause and effect can be established. It is also advisable to look at other contaminants in the Diep River, other than metals, like polychlorinated biphenyls (PCBs) or polycyclic

aromatic hydrocarbons (PAHs), pesticides and anions that can have an environmental impact on waterbodies.

REFERENCES

Adekoya, J.A., Williams, A.B. & Ayejuyo, O.O., 2006. Distribution of heavy metals in sediments of Igbede, Ojo and Ojora rivers of Lagos, Nigeria. *The Environmentalist* 26 (4): 277-280.

Aksoy, A., Demirezen, D. & Duman, F., 2005. Bioaccumulation, detection and analyses of heavy metal pollution in sultan marsh and its environment. *Water, Air and Soil Pollution* 164 (1-4): 241-255.

Alibaba, 1999-2012. Untitled. http://pesticides.en.alibaba.com [24/04/2012].

ANZECC, 1992. Australian water quality guidelines for fresh and marine waters. National Water Quality Management Strategy, Australian and New Zealand Environmental Conservation Council, Canberra.

Aravind, P. & Prasad, M.N.V., 2004. Zinc protects chloroplasts and associated photochemical functions in cadmium exposed *Ceratophyllum demersum* L., a freshwater macrophyte. *Plant Science* 166 (5): 1321-1327.

Aravind, P. & Prasad, M.N.V., 2005. Cadmium-induced toxicity reversal by zinc in *Ceratophyllum demersum* L. (a free floating aquatic macrophyte) together with exogenous supplements of amino-and organic acids. *Chemosphere* 61 (11): 1720-1733.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24 (1): 1-15.

Ash, M. & Ash, I., 2004. *Handbook of Green Chemicals*. 2nd Ed. Synapse Information Resources. New York.

Azadpour, A. & Mathews, J.E., 1996. Remediation of metal-contaminated sites using plants. *Remediation Journal* 6 (3): 1-18.

Babovic, N., Drazic, G., Djordjevic, A. & Mihailovic, N., 2010. Heavy and toxic metal accumulation in six macrophyte species from fish pond Ecka, Republic of Serbia. Preceedings of the 2010 Conference of BALWOIS, Ohrid, Republic of Macedonia, 25-29/05/ 2010.

Babu, T.S., Akhtar, T.A., Lampi, M.A., Tripuranthakam, S., Dixon, D.G. & Greenberg, B.M., 2003. Similar stress responses are elicited by copper and ultraviolet radiation in the aquatic plant *Lemna gib*ba: Implication of reactive oxygen species as common signals. *Plant and Cell Physiology* 44 (12): 1320-1329.

Baker, A.J.M., 1987. Metal tolerance. *New Phytologist* 106: 93–111.

Barceló, J & Poschenrieder, C., 1990. Plant water relations as affected by heavy metal stress: A review. *Journal of Plant Nutrition* 13 (1): 1-37.

Baszyński, T., Król, M., Krupa, Z., Ruszkowska, M., Wojcieska, U. & Wolińska, D., 1982. Photosynthetic apparatus of spinach exposed to excess copper. *International Journal of Plant Physiology* 108 (5): 385-395.

Baszyński, T., Tukendorf, A., Ruszkowska, M., Skórzyńska, E. & Maksymiec, W., 1988. Characteristics of the photosynthetic apparatus of copper non tolerant spinach exposed to excess copper. *Journal of Plant Physiology* 132: 708-713.

Baxter, I., Brazelton, J.N., Yu, D., Huang, Y.S., & Lahner, B., 2010. A coastal cline in sodium accumulation in Arabidopsis thaliana is driven by natural variation of the sodium transporter AtHKT1;1. *Plos Genetics* 6 (11): e1001193.doi:10.1371/journal.pgen.1001193.

Benedict, C.R., 1972. Net CO_2 fixation in yellow-green plants. In: Black, C.C.(Ed). *Net carbon dioxide assimilation in higher plants*. Symp. Sect. American Society Plant Physiology.

Bonide, 2012. Untitled. Htp://www.bonide.com [24/04/2012].

Boyd, R.S. & Martens, S.N., 1994. Nickel hyperaccumulated by *Thlaspi montanum* var. *montanum* is acutely toxic to an insect herbivore. *Oikos* 70: 21-25.

Bradshaw, A.D., 1976. Pollution and Evolution. In: Mansfield, T. A. (Ed). *Effects of air pollution on plants.* Cambridge University Press. London.

Broker, W., 1963: Genetisch-physiologische Untersuehungen fiber die Zinkvertr/~glidhkeit yon *Silene inflata SM. Flora* B 153: 122-156.

Brown, C. & Magoba, R. (Eds). 2009. *Rivers and wetlands of Cape Town*. South Africa: Water Research Commission.

Buczacki, S. 1995. *Best water plants*. Hamlyn. London.

Butterworth, F.M., Villalobos-Pietrini, R. & Gonsebatt, M.E., 2000. Biomonitors and biomarkers as indicators of environmental change, Vol. 2. In: Butterworth, F. M., Gunatilaka, A. & Gonsebatt, M. E. (Eds). *Biomonitors and biomarkers as indicators of environmental change 2: A handbook*. Kluwer Academic/Plenum. New York.

Cardwell, A.J., Hawker, D.W. & Greenway, M., 2002. Metal accumulation in aquatic macrophytes from southeast Queensland, Australia. *Chemosphere* 48 (7): 653-663.

Caussy, D., Gochfeld, M., Gurzau, E., Corneliu, N. & Reudel, H., 2003. Lessons from case studies of metals: investigating exposure, bioavailability, and risk. *Ecotoxicology and Environmental Safety* 56: 45-51.

CCME, 2001. Canadian sediment quality guidelines for the protection of aquatic life: Summary tables. Updated. In: Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

Chen, Z., Salem, A., Xu, Z. & Zhang, W. 2009. Ecological implications of heavy metal concentrations in the sediments of Burullus Lagoon of Nile Delta, Egypt. *Estuarine, Coastal and Shelf Science* 86: 491-498.

Coastal & Environmental Consulting, 2011. C.A.P.E. Estuaries Programme: Estuary Management Plan for the Diep River Estuary.

Cohu, C.M., 2009. Regulation of copper homeostasis in plants: A focus on chloroplastic superoxide dismutases and copper delivery mechanisms. PhD thesis, Colorado State University, U.S.A.

Connell, D., Lam, P., Richardson, B. & Wu, R., 1999. *Introduction to Ecotoxicology.* Blackwell. London.

Connolly, E.L., Fett, J.P. & Guerinot, M.L., 2002. Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. *The Plant Cell* 14: 1347-1357.

Cuenca, G., Herrera, R., & Merida, T., 1991. Distribution of aluminium in accumulator plants by X-ray microanalysis in *Richeria grandis* Vahl leaves from a cloud forest in Venezuela. *Plant Cell Environment* 14: 437-441.

Dabrowski, J.M., Peall, S.K.C., Reinecke, A.J., Liess, M. & Schulz, R., 2002. Runoff-related pesticide input into the Lourens River, South Africa: Basic data for exposure assessment and risk mitigation at the catchment scale. *Water, Air and Soil Pollution* 135: 265-283.

Delhaize, E & Ryan, P.R., 1995. Aluminium toxicity and tolerance in plants. *Plant Physiology* 107: 315-321.

Demirezen, D. & Aksoy, A., 2006. Common hydrophytes as bioindicators of iron and manganese pollutions. *Ecological Indicators* 6: 388-393.

Deng, P.Y., Shu, W.S., Lan, C.Y. & Liu, W., 2008. Metal contamination in the sediment, pondweed and snails of a stream receiving effluent from a lead/zinc mine in southern China. *Bulletin of Environmental Contamination and Toxicology* 81: 69-74.

Devi, S.R. & Prasad, M.N.V., 1998. Copper toxicity in *Ceratophyllum demersum* L. (Coontail), a free floating macrophyte: Response of antioxidant enzymes and antioxidants. *Plant Science* 138: 157-165.

Devi, S.R. & Prasad, M.N.V., 2004. Membrane lipid alterations in heavy metal exposed plants. In: Prasad, M.N.V. (Ed). *Heavy metal stress in plants from biomolecules to ecosystems.* 2nd Edition. Springer. Berlin.

De Vos C.H.R., Schat, H., Vooijs R. & Ernst, W.H.O., 1989. Copper-induced damage to the permeability barrier in roots of *Silene cucubalus*. *Journal of Physiology* 135: 164-168.

De Vos, C.H.R, Schat, H., De Waal, M.A.M., Vooijs, R. Ernst, W.H.O., 1991. Increased resistance to copper-induced damage of the root cell plasmalemma in copper tolerant *Silene cucubalus*. *Physiologia Plantarum* 82: 523-528.

Dewez, D., Geoffroy, L., Vernet, G., Popovic, R., 2005. Determination of photosynthetic and enzymatic biomarkers sensitivity used to evaluate toxic effects of copper and fludioxonil in alga *Scenedesmus obliquus*. *Aquatic Toxicology* 74: 150-159.

Dickinson, N.M., Turner, A.P. & Lepp, N.W., 1991. How do trees and other long-lived plants survive in polluted environments? *Functional Ecology* 5 (1): 5-11.

Dillon, P.J. & Rigler, F.H., 1974. The phosphorus-chlorophyll relationship in lakes. *Limnology & Oceonography* 19 (5): 767-773.

Dobson, M. & Frid, C., 1998. *Ecology of aquatic systems.* Longman. Essex.

Duman, F., Obali, O., Demirezen, D., 2006. Seasonal changes of metal accumulation and distribution in shining pondweed (*Potamogeton lucens*). *Chemosphere* 65: 2145-2151.

Dushenkov, V, Kumar, P.B.A.N., Motto, H. & Raskin, I., 1995. Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. *Environmental Science & Technology* 29: 1239-1245.

DWAF, 1996. South African Water Quality Guidelines. Volume 7: Aquatic Ecosystems. Department of Water Affairs and Forestry, 1996.

DWAF, 2002. Water Resources Management Plan in the Diep River Catchment: A situation assessment. www.dwaf.gov.za/IWQS/reports/Diep/Downloaded.

Ebbs, S. & Uchil, S., 2008. Cadmium and zinc induced chlorosis in Indian mustard (*Brassica juncea* (L.) Czern) involves preferential loss of chlorophyll b. *Photosynthetica* 46 (1): 49-55.

Ferrat, L., Pergent-Martini, C. & Roméo, M., 2003. Assessment of the use of biomarkers in aquatic plants for the evaluation of environmental quality: application to seagrasses. *Aquatic Toxicology* 65: 187-204.

Fitzgerald, E.J., Caffrey, J.M., Nesaratnam, S.T. & McLoughlin, P., 2003. Copper and lead concentrations in salt marsh plants on the Suir Estuary, Ireland. *Environmental Pollution* 123: 67-74.

Fontes, R.L.F. & Cox, F.R., 1998. Zinc toxicity in soybean grown at high iron concentrations in nutrient solution. *Journal of Plant Nutrition* 21(8): 1723-1730.

Frankart, C., Eullaffroy, P. & Vernet, G., 2002. Comparative effects of four herbicides on non-photochemical fluorescence quenching in *Lemna minor*. *Environmental and Experimental Botany* 49: 159-168.

Franke, W., 1961. Ectodesmata and foliar absorption. *American Journal of Botany* 48 (8): 683-691.

Friends of Rietvlei, 2008. Potamogeton *in Rietvlei.* http://www.friendsofrietvlei.co.za/Potamogeton.html [18/06/2008].

Fritioff, A. & Greger, M., 2006. Uptake and distribution of Zn, Cu, Cd, and Pb in an aquatic plant *Potamogeton natans. Chemosphere* 63: 220-227.

Gallego, S.M., Benavídes, M.P. & Tomaro, M.L., 1996. Effect of heavy metal ion excess on sunflower leaves: evidence for the involvement of oxidative stress. *Plant Science* 121: 151-159.

Gallon, C., Munger, C., Prémont, S. & Campbell, P.G.C., 2004. Hydroponic study of aluminium accumulation by aquatic plants: Effects of fluoride and pH. *Water, Air, and Soil Pollution* 153: 135-155.

Gaynor, A. & Gray, N.F., 2004. Trends in sediment metal concentrations in the river Avoca, south-east Ireland. *Environmental Geochemistry and Health* 26: 411-419.

Gill, D.E., 1986. Individual plants as genetic mosaics: ecological organisms versus evolutionary individuals. In: Crawley, M.J. (Ed). *Plant Ecology.* Blackwell Scientific Publications. Oxford.

Giordani, C., Cecchi, S. & Zanchi, C., 2005. Phytoremediation of soil polluted by nickel using agricultural crops. *Environmental Management* 36 (5): 675-681.

Glass, A.D.M. 1983. Regulation of ion transport. *Annual Review of Plant Physiology* 34: 311-326.

Göthberg, A., Greger, M. & Bengtsson, B., 2002. Accumulation of heavy metals in water spinach (*Ipomoea aquatica*) cultivated in the Bangkok region, Thailand. *Environmental Toxicology and Chemistry* 21 (9): 1934-1939.

Goulet, R.R., Lalonde, J.D., Munger, C., Dupuis, S., Dumont-Frenette, G., Prémont, S. & Campbell, P.G.C., 2005. Phytoremediation of effluents from aluminium smelters: A study of Al retention in mesocosms containing aquatic plants. *Water Research* 39 (11): 2291-2300.

Grasmück, N., Haury, J., Léglize, L. & Muller, S., 1995. Assessment of bio-indicator capacity of aquatic macrophytes using multivariate analysis. *Hydrobiologia* 300/301: 115-122.

Grattan, S.R. & Grieve, C.M., 1999. Salinity-mineral nutrient relations in horticultural crops. *Scientia Horticulturae* 78: 127-187.

Grindley, J.R. & Dudley, S., 1988. Estuaries of the Cape: Part II: Synopses of available information on individual systems. Report No. 28 Reitvlei/Diep CW24/25. In: Heydorn, A.E. F. and Morant, P.D. (Eds.) CSIR Research Report 427. Stellenbosch.

Gross, E.M., Erhard, D. & Iványi, E., 2003. Allelopathic activity of *Ceratophyllum demersum* L. and *Najas marina ssp. intermedia* (Wolfgang) Casper. *Hydrobiologia* 506 (1): 583-589.

Guerinot, M.L. & Yi, Y., 1994. Update on mineral nutrition, Iron: nutritious, noxious, and not readily available. *Plant Physiology* 104: 815-820.

Gümgüm, B., Ünlü, E., Tez, Z. & Gülsün, Z. 1994. Heavy metal pollution in water, sediment and fish from the Tigris River in Turkey. *Chemosphere* 29: 114-116.

Gupta, P. & Chandra, P., 1996. Response of cadmium to *Ceratophyllum demersum* L., a rootless submerged plant. *Waste Management* 16: 335-337

Gupta, R.K., 1977. A study of photosynthesis and leakage of solutes in relation to the desiccation effects in bryophytes. *Canadian Journal of Botany* 55 (9): 1186-1194.

Haberlant, G., 1914. *Physiological plant anatomy*. 3rd Edition. Macmillan. London.

Hall, J.L., 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany* 53 (366): 1-11.

Hargrave, B.T., 1991. Ecology of deep-water zones. In: Barnes, R.S.K. & Mann, K.H. (Eds). *Fundamentals of Aquatic Ecology*. Blackwell. London.

Haug, A., 1984. Molecular aspects of aluminium toxicity; critical revue. *Plant Science* 1: 345-373.

He, W. & Yongfeng, J., 2009. Bioaccumulation of heavy metals by *Phragmites australis* cultivated in synthesized substrates. *Journal of Environmental Sciences* 21: 1409-1414.

Henderson, L. & Cilliers, C.J., 2002. *Invasive aquatic plants*. Agricultural Research Council: Cape Town.

Hesketh, J.D., 1963. Limitations to photosynthesis responsible for differences among species. *Crop Science* 3: 493-496.

Hewitt, E.J. & Smith, T.A., 1975. *Plant Mineral Nutrition*. English University Press. London.

Hill, M.K., 2010. *Understanding environmental pollution*. Cambridge University Press. Cambridge.

Hiscock, P., 2003. *Encyclopedia of aquarium plants*. Barrons. New York.

Hiscox, J.D, Israelstam, G.F., 1979. A method for extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* 57: 1332-1334.

Hoagland, D.R. & Arnon, D.I., 1950. *The water-culture method for growing plants without soil.* Circulation 347, University of Calfornia, Agricultural Experimental Station. Berkley.

Horbowicz, M., Kowalczyk, W., Grzesiuk, A. & Mitrus, J., 2011. Uptake of aluminium and basic elements, and accumulation of anthocyanins in seedlings of common buckwheat (*Fagopyrum esculentum* Moench) as a result increased level of aluminium in nutrient solution. *Ecological Chemistry and Engineerings* 18 (4) 479-488.

Hu, J., Zheng, A., Pei, D. & Shi, G., 2010. Bioaccumulation and chemical forms of cadmium, copper and lead in aquatic plants. *Brazilian Archives of Biology and Technology* 53: 235-240.

Huang, J.W., Grunes, D.L. & Kochian, L. V., 1992. Aluminium effects on the kinetics of calcium uptake into cells of the wheat root apex. *Planta* 188: 414-421.

Huxley, A., Griffiths, M. & Levy, M. (Eds), 1999. *The New Royal Horticultural Society Dictionary of Gardening*. 4th Edition. MacMillan. London.

Intawongse, M & Dean, J.R., 2006. Uptake of heavy metals by vegetable plants grown on contaminated soil and their bioavailability in the human gastrointestinal tract. *Food Additives and Contaminants* 23 (1): 36-48.

Jackson, V.A., Paulse, A.N., Odendaal, J.P. & Khan, W. 2009. Investigation into the metal contamination of the Plankenburg and Diep Rivers, Western Cape, South Africa. *Water SA* 35 (3): 289-299.

Jansen, S., Broadley, M.R., Robbrecht, E. & Smets, E., 2002. Aluminium hyperaccumulation in angiosperms: A review of its phylogenetic significance. *The Botanical Review* 68 (2): 235-269.

Järup, L., 2003. Hazards of heavy metal contamination. *British Medical Bulletin* 68: 167-182.

Jarvis, M.C., 1982. The proportion of calcium-bound pectin in plant walls. *Planta* 154: 344-346.

Jarvis, S.C., Jones, L.H.P. & Hopper, M.J., 1976. Cadmium uptake from solution by plants and its transport from roots to shoots. *Plant and Soil* 44: 179-191.

John, D.A. & Leventhal, J.S., 1995. Bioavailability of metals. In: du Bray, E. A. (Ed) *Preliminary compilation of descriptive geoenvironmental mineral deposit models.* Open-file Report 95-831. United States Geological Survey, Department of Interior. Denver, Colorado.

Kaglyan, A, Klenus, V., Kuz'Menko, M., Belyaev, V., Yu. Nabyvanets, D. & Gudkov, D., 2005. Macrophytes as bioindicators of radionuclide contamination in ecosystems of different aquatic bodies in Chernobyl exclusion zone. In: Bréchignac, F. & Desmet, G. (Eds). *Equidosimetry. Ecological standardization and equidosimetry for radioecology and environmental ecology.* Proceedings at the NATO Advanced Research Workshop of Ecological Standardization and Equidosimetry for Radioecology and Environmental Ecology. Ukraine 14-20/04/ 2002.

Kamal, M., Ghaly, A.E., Mahmoud, N. & Côte, R., 2004. Phytoaccumulation of heavy metals by aquatic plants. *Environment International* 29: 1029-1039.

Kampfenkel, K., Van Montagu, M. & Inze, D., 1995. Effects of iron excess on *Nicotiana plumbaginifolia* plants (implications to oxidative stress). *Plant Physiology* 107 (3): 725-735.

Kara, Y., 2005. Bioaccumulation of Cu, Zn and Ni from the wastewater by treated *Nastursium officinale*. *International Journal of Environmental Science and Technology* 2: 63-67.

Kara, Y. & Zeytunluoglu, A., 2007. Bioaccumulation of toxic metals (Cd and Cu) by *Groenlandia densa* (L.) Fourr. *Bulletin of Environmental Contamination Toxicology* 79: 609-612.

Keskinkan, O., Goksu, M.Z.L., Basibuyuk, M. & Forster, C.F., 2004. Heavy metal adsorption properties of a submerged aquatic plant (*Ceratophyllum demersum*). *Bioresource Technology* 92: 197-200.

Khellaf, N. & Zerdaoui, M., 2009. Phytoaccumulation of zinc by the aquatic plant, *Lemna gibba* L. *Bioresource Technology* 100: 6137-6140.

Kim, C.S. & Jung, J., 1993. The susceptibility of mung bean chloroplasts to photoinhibition is increased by an excess supply of iron to plants: A photobiological aspect of iron toxicity in plant leaves. *Photochemistry and Photobiology* 58 (1): 120-126.

Kingtai chemicals, 2012. Untitled. Htp://www.chemicalsinchina.com [24/04/2012].

Kochian, L.V. & Lucas, W.L., 1982. Potassium transport in corn roots. Resolution of kinetics into a saturable and linear component. *Plant Physiology* 70: 1723-1731.

Kochian, L.V., Piñeros, M.A. & Hoekenga, O.A., 2005. The physiology, genetics and molecular biology of plants aluminium resistance and toxicity. *Plant and Soil* 274: 175-195.

Krämer, U, Smith, R.D., Wenzel, W.W., Raskin, I. & Salt, D.E., 1997. The role of metal transport and tolerance in nicel hyperaccumulation by *Thlaspi goesingense*. *Plant Physiology* 115: 1641-1650.

Krämer, U, Pickering, I.J., Prince, R.C., Raskin, I. & Salt, D.E., 2000. Subcellular localisation and speciation of nickel in hyperaccumulator and non-accumulator *Thlaspi species*. *Plant Physiology* 122: 1343-1353.

Kucera, T, Horakova, H. & Sonska, A. 2008. Toxic metal ions in photoautotrophic organisisms. *Photosynthetica* 46 (4): 481-489.

Kumar, G.P. & Prasad, M.N.V., 2004. Cadmium Toxicity to *Ceratophyllum demersum* L.: Morphological symptoms, membrane damage, and ion leakage. *Bulletin of Environmental Contamination and Toxicology* 72: 1038-1045.

Küpper, H., Küpper, F. & Spiller, M., 1996. Environmental relevance of heavy metal-substituted chlorophylls using the example of water plants. *Journal of Experimental Botany* 47 (295): 259-266.

Küpper, H., Küpper, F. & Spiller, M., 1998. *In situ* detection of heavy metal substituted chlorophylls in water plants. *Photosynthesis Research* 58: 123-133.

Küpper, H., Lombi, E., Zhao, F.J., Wieshammer, G. & McGrath, S.P., 2001. Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goesingense*. *Journal of Experimental Botany* 52: 2291-2300.

Lahaye, P.A. & Epstein, E., 1971. Calcium and salt toleration by bean plants. *Physiologia Plantarum* 25 (2): 213-218.

Lambers, H., Chapin, F.S. & Pons,T.L., 2008. *Plant Physiological Ecology*. Springer. New York.

Landner, L & Reuther, R., 2004. *Metals in society and in the environment*. Kluwer Academic Press. Dordrecht.

Lazof, D.B., Goldsmith, J.G., Rufty, T.W. & Linton, R.W., 1994. Rapid uptake of aluminium into cells of intact soybean root tips. A micro analytical study using secondary ion mass spectroscopy. *Plant Physiology* 106: 1107-1114.

Leavitt, S.W., Dueser, R.D. & Goodell, H.G., 1979. Plant regulation of essential and non-essential heavy metals. *Journal of Applied Ecology* 16: 203-212.

Lee, B & Scholz, M. 2007. What is the role of *Phragmites australis* in experimental constructed wetland filters treating urban runoff? *Ecological Engineering* 29: 87-95.

Lee, R.B., 1982. Selectivity and kinetics of ion uptake by barley plants following nutrient deficiency. *Annals of Botany* 50: 429-449.

Leopold, A.C., Musgrave, M.E. & Williams, K.M., 1981. Solute leakage resulting from leaf desiccation. *Plant Physiology* 68: 1222-1225.

Li, T.Y. & Xiong, Z.T., 2004. Cadmium-induced colony disintergration of duckweed (*Lemna paucicostata* Hegelm.) and as biomarker of phytotoxicity. *Ecotoxicology and Environmental Safety* 59: 174-179.

Li, Y., McCrory, D.F., Powel, J.M., Saam, H. & Jackson-Smith, D.B., 2005. Heavy metal concentrations in dairy feed: a survey of heavy metal concentrations in Wisconsin dairy feeds. *Journal of Dairy Science* 88: 2911-2922.

Liu, Y., Wang, X., Zeng, G., Qu, D., Gu, J., Zhou, M. & Chai, L., 2007. Cadmium-induced oxidative stress and response of the ascorbate-glutathione cycle in *Bechmeria nivea* (L.) Gaud. *Chemosphere* 69 (1): 99-107.

Longstroth, M., 2002. *Proposed Michigan grape spray programs for 2002*. Michigan State University. http://web3.canr.msu.edu/vanburen/gspray.htm [20/09/2011].

Ma, J.F., Zheng, S.J. & Matsumoto, H., 1997. Specific secretion of citric acid induced by Al stress in Cassia tora L. *Plant and Cell Physiology* 38 (9): 1019-1025.

Madejón, P., Marañón, T., Murillo, J.M. & Robinson, B., 2006. In defense of plants as biomonitors of soil quality. *Environmental Pollution* 143: 1-3.

Maine, M.A., Suñe, N.L. & Lagger, S.C., 2004. Chromium bioaccumulation: comparison of the capacity of two floating aquatic macrophytes. *Water Research* 38: 1494-1501.

Maksymiec W., Russa, R., Urbanik-Sypniewska, T. & Baszynski, T., 1992. Changes in acyl lipid and fatty acid composition in thylakoids of copper non-tolerant spinach exposed to excess copper. *Journal of Plant Physiology* 140: 52-55.

Maksymiec W., Russa, R., Urbanik-Sypniewska, T. & Baszynski, T., 1994. Effect of excess Cu on the photosynthetic apparatus of runner bean leaves treated at two different growth stages. *Physiologia Plantarum* 91 (4): 715-721.

Malik, D., Sheoran, I.S. & Singh, R., 1992. Lipid composition of thylakoid membranes of cadmium treated wheat seedlings. *Indian Journal of Biochemistry and Biophysics* 29: 350-354.

Markert, B., Wappelhorst, O., Weckert, V., Herpin, U., Siewers, U., Friese, K. & Breulmann, G., 1999. The use of bioindicators for monitoring the heavy-metal status of the environment. *Journal of Radioanalytical and Nuclear Chemistry* 240: 425-429.

Marques, A.P.G.C., Rangel, A.O.S.S. & Castro, P.M.L., 2007. Zinc accumulation in plant species indigenous to a Portuguese polluted site: Relation with soil contamination. *Journal of Environmental Quality* 36: 646-653.

Maton, A., Hopkins, J., McLaughlin, C.W., Johnson, S., Warner, M.Q., LaHart, D. & Wright, J.D., 1993. *Human Biology and Health.* Prentice Hall. Englewood Cliffs, New Jersey.

Mauseth, D., 2003. *Botany. An introduction to plant biology.* 3rd Ed. Jones & Bartlett. Sudbury.

McKersie, B.D., Huci, P. & Beversdorf, W.D., 1982. Solute leakage from susceptible and tolerant cultivars of *Phaseolus vulgaris*. *Canadian Journal of Botany* 60 (1): 73-78.

Meck, M., Love, D. & Mapani, B., 2006. Zimbabwean mine dumps and their impacts on river water quality – a reconnaissance study. *Physics and Chemistry of the Earth* 31: 797-803.

Meeks, J.C., 1974. Chlorophylls. In Stewart, W.D.P. (Ed.) *Algal Physiology and Biochemistry.*Blackwell Scientific Publications. Oxford.

Megateli, S., Semsari, S. & Couderchet, M., 2009. Toxicity and removal of heavy metals (cadmium, copper, and zinc) by *Lemna gibba*. *Ecotoxicology and Environmental Safety* 72: 1774-1780.

Mertens, J., Luyssaert, S. & Verheyen, K., 2006. Comment on 'In defense of plants as biomonitors of soil quality'. *Environmental Pollution* 144: 715.

Meyer, B.S., Anderson, D.B., Bohning, R.H. & Fratianne, D.G., 1973. *Introduction to plant physiology*. 2nd Edition. Van Nostrand. New York.

Milan, B., Slobodanka, P., Živko, S. & Borivoj, K., 2006. Macrophytes as phytoindicators and potential phytoremediators in aquatic ecosystems. Paper presented at the 36th International Conference: International Austrian Committee Danube Research, Vienna, September 2006.

Mishra, S., Srivastava, S., Tripathi, R.D., Kumar, R., Seth, C.S. & Gupta, D.K., 2006. Lead detoxification by coontail (*Ceratophyllum demersum* L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere* 65: 1027-1039.

Moss, B., 1988. *Ecology of fresh waters, man and medium.* 2nd Edition. Blackwell. London.

Mukherjee, S., Mukherjee, S. & Bhattacharyya, P., 2004. Heavy metal levels and esterase variations between metal-exposed and unexposed duckweed *Lemna minor:* field and laboratory studies. *Environmental International* 30 (6): 811-814.

Munns, R. & Tester, M, 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59: 651-681.

Murata, N., Higashi, S.I. & Fujimura, Y., 1990. Glycerolipids in various preparations of photosystem II from spinach chloroplasts. *Biochimica et Biophysica Acta* 1019 (3): 261-268.

Myśliwa-Kurdziel, B. & Strzatka, K., 2002. Influences of metals on biosynthesis of photosynthetic pigments. In: Prasad, M.N.V., Strzatka, K. (Eds). *Physiology and biochemistry of metal toxicity and tolerance in plants.* Kluwer. Dortrecht.

Natural Industries, 1992-2011. Untitled. http://naturalindustries.com. Downloaded 24/04/2012.

Nel, A., Krause, M. & Khelawanlall, N., 2003. *A guide for the control of plant diseases.* Department of Agriculture of the Republic of South Africa. Pretoria.

Ntengwe, F.W. & Maseka, K.K., 2006. The impact of effluents containing zinc and nickel metals on stream and river water bodies: The case of Chambishi and Mwambashi streams in Zambia. *Physics and Chemistry of the Earth* 31: 814-820.

Nyquist, J. & Greger, M., 2007. Uptake of Zn, Cu, and Cd in metal loaded *Elodea canadensis*. *Environmental and Experimental Botany* 60: 219-226.

O'Kelley, J.C., 1974. Chlorophylls. In: Stewart, W.D.P. (Ed). *Algal physiology & biochemistry*. Blackwell. London.

Okonkwo, J.O., Mothiba, M., Awofolu, O.R. & Busari, O., 2005. Levels and speciation of heavy metals in some rivers in South Africa. *Bulletin of Environmental Contamination and Toxicology* 75: 1123-1130.

Pajević, S., Borišev, M., Rončević, S., Vukov, D. & Igić, R., 2008. Heavy metal accumulation of Danube river aquatic plants – indication of chemical contamination. *Central European Journal of Biology* 3: 285-294.

Palta, J.P., Levitt, J. & Stadelmann, E.J., 1977. Freezing injury in onion bulb cells. *Plant Physiology* 60: 393-397.

Panda, S.K., 2007. Chromium-mediated oxidative stress and ultrastructural changes in root cells of developing rice seedlings. *Journal of Plant Physiology* 164: 1419-1428.

Pavlikova, D., Pavlik, M., Staszkova, L., Motyka, V., Szakova, J., Tlustos, P. & Balik, J., 2008. Glutamate kinase as a potential biomarker of heavy metal stress in plants. *Ecotoxicology and Environmental Safety* 70: 223-230.

Peng, K., Luo C., Lou L., Li, X. & Shen, Z., 2008. Bioaccumulation of heavy metals by the aquatic plants *Potamogeton pectinatus* L. and *Potamogeton malaianus* Miq. and their potential use for contamination indicators and in wastewater treatment. *Science of The Total Environment* 392: 22-29.

Poh, B.L., Gevens, A., Simonne, E. & Snodgrass, C., 2009. *Estimating copper, manganese and zinc micronutrients in fungicide applications*. Horticultural Sciences Department. Florida Cooperative Extension Service, Institute of Food & Agricultural Sciences, University of Florida, 14/08/2009 http://edis.ifas.ufl.edu.

Poljsak, B., Pócsi, I. & Pesti, M., 2011. Interference of chromium with cellular functions. In: Cellular effects of heavy metals, Part 2, 59-86. Springer.com.

Poschenrieder, C., Gunsé, B., Corrales, I. & Barceló, J., 2008. A glance into aluminium toxicity and resistance in plants. *Science of the Total Environment* 400 (1-3): 356-368.

Prasad, M.N.V., Malec, P., Waloszek, A., Bojko, M. & Strzalka, K., 2001. Physiological responses of *Lemna trisulca* L. (Duckweed) to cadmium and copper bioaccumulation. *Plant Science* 161: 881-889.

Prasad, M.N.V., 2003. Phytoremediation of metal-polluted ecosystems: hype for commercialization. *Russian Journal of Plant Physiology* 50 (5): 686-700.

Prasad, M.N.V., 2004. Metallothioneins, metal binding complexes and metal sequestration in plants. In: Prasad, M.N.V. (Ed). *Heavy metal stress in plants*. 2nd Ed. Springer. Berlin.

Prasad, M.N.V., Greger M. & Smith B.N., 2001. Aquatic macrophytes. In: Prasad M.N.V. (Ed) *Metals in the environment: Analysis by biodiversity*. Marcel Dekker. New York.

Prat, H., 1934. A biometric study of the culms of cereals and grasses. *Canadian Journal of Research* 10: 563-570.

Quartacci, M.F., Pinzino, C., Sgherri, C.L.M., Vecchia, F.D. & Navari-Izzo, F., 2000. Growth in excess copper induces changes in the lipid composition and fluidity of PSII-enriched membranes in wheat. *Physiologia Plantarum* 108 (1): 87-93.

Quartacci, M.F., Cosi, E. & Navari-Izzo, F., 2001. Lipids and NADPH-dependent superoxide production in plasma membrane vesicles from roots of wheat grown under copper defiency or excess. *Journal of Experimental Botany* 52 (354): 77-84.

Ramadan, A.A., 2003. Heavy metal pollution and biomonitoring plants in lake Manzala, Egypt. *Pakistan Journal of Biological Sciences* 6 (13): 1108-1117.

Rashed, M.N., 2002. *Biomarkers as indicator for water pollution with heavy metals in rivers, seas and oceans.* http://www.wrrc.dpri.kyotou.ac.jp/~aphw/APHW2004/proceedings/OHS/56-OHS-A344/56-OHS-A344.pdf.

Reichman, S. M., 2002. *The responses of plants to metal toxicity: A review focusing on copper, manganese and zinc.* Australian Minerals and Energy Environment Foundation. Melbourne.

Rengel, Z., 1992. Role of calcium in aluminium toxicity. *New Phytology* 121: 499-513.

River Health Programme, 2003. State-of-Rivers Report: Diep, Hout Bay, Lourens and Palmiet River Systems. Department of Water Affairs and Forestry. Pretoria.

Robach, F., Thiébaut, G., Trémolières, M. & Muller, S., 1996. A reference system for continental running waters: plant communities as bioindicators of increasing eutrophication in alkaline and acidic waters in north-east France. *Hydrobiologia* 340: 67-76.

Robinson, B., Outred, H., Brooks, R. & Kirkman, J., 1995. The distribution and fate of arsenic in the Waikato River system, North Island, New Zealand. *Chemical Speciation and Bioavailability* 7(3): 89-96.

Robinson, B.H., Lombi, E., Zhao, F.J. & McGrath, P., 2003. Uptake and distribution of nickel and other metals in the hyperaccumulator *Berkheya coddii*. *New Phytologist* 158(2): 279-285.

Robinson, N.J., Wilson, J.R. & Turner, J.S., 1996. Expression of the type 2 metallothionein-like gene MT2 from *Arabidopsis thaliana* in Zn (2+)-metallothionein-deficient *Synechococcus* PCC 7942: putative role for MT2 in Zn^{2+} metabolism. *Plant Molecular Biology* 30: 1169-1179.

Robinson, P. 2003. *Plants for ponds rock and water features*. Southwater. London.

Rook, E.J.S., 2009. *Ceratophyllum demersum,* common hornwort. www.rook.org/earl/bwca/nature/aquatics/ceratophyllum [13/08/2009].

Ryan, P.R., Kinraide, T.B. & Kochian, L.V., 1994. Al – Ca interactions in aluminium rhizotoxicity. I. Inhibition of root growth is not caused by reduction of calcium uptake. *Planta* 192: 98-102.

Sachs, M.H. & Ho, T-H.D., 1986. Alteration of gene expression during environmental stress in plants. *Annual Review of Plant Physiology* 37: 363-376.

Salisbury, F.B. & Ross, C.W., 1985. *Plant Physiology*. 3rd Edition. Wadsworth. Belmont.

Salt, D.E., Blaylock, M., Kumar, N.P.B.A., Dushenkov, V., Ensley, B.D., Chet, I. & Raskin, I., 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants of outstanding interest. Nature Biotechnology 13: 468–474.

Samecka-Cymerman, A. & Kempers, A.J., 2001. Concentrations of heavy metals and plant nutrients in water, sediments and aquatic macrophytes of anthropogenic lakes (former open cut brown coal mines) differing in stage of acidification. *The Science of the Total Environment* 281: 87-98.

Samecka-Cymerman, A. & Kempers, A.J., 2004. Toxic metals in aquatic plants surviving in surface water polluted by copper mining industry. *Ecotoxicology and Environmental Safety* 59: 64-69.

Sánchez-Viveros, G., González-Mendoza, D., Alarcón, A. & Ferrera-Cerrato R., 2010. Copper effects on photosynthetic activity and membrane leakage of *Azolla filiculoides* and *A. caroliniana*. *International Journal of Agriculture and Biology* 12: 365–368.

Sandermann Jr., H., 1992. Plant metabolism of xenobiotics. *Trends in Biochemical Sciences* 17 (2): 82-84.

Sawidis, T., Chettri, M.K., Zachariadis, G.A. & Stratis, J.A., 1995. Heavy metals in aquatic plants and sediments from water systems in Macedonia, Greece. *Ecotoxicology and Environmental Safety* 32 (1): 73-80.

Schneider, I.A.H. & Rubio, J. 1999. Sorption of heavy metal ions by the nonliving biomass of freshwater macrophytes. *Environmental Science & Technology* 33: 2213-2217.

Schulz, R., 2001. Rainfall-induced sediment and pesticide input from orchards into the Lourens River, Western Cape, South Africa: importance of a single event. *Water Research* 35 (8): 1869-1876.

Shakya, K., Chettri, M.K. & Sawidis, T., 2008. Impact of heavy metals (copper, zinc, and lead) on the chlorophyll content of some mosses. *Archives of Environmental Contamination and Toxicology* 54: 412-421.

Shrestha, B.B., 2003. Metal toxicity in plants: How do metallophytes manage to grow? *Himalayan Journal of Sciences* 1: 51-54.

Shuping, L.S., 2008. *Biomonitoring of metal contamination in the lower Diep River, Milnerton, Western Cape*. M.Tech. thesis, Cape Peninsula University of Technology, Cape Town.

Shuping, L.S., Snyman, R.G., Odendaal, J.P. & Ndakidemi, P.A., 2011. Accumulation and distribution of metals in *Bolboschoenus maritimus* (Cyperaceae), from a South African River. *Water, Air and Soil Pollution* 216: 319-328.

Siebert, A., Bruns, I., Krauss, G. J., Miersch J. & Markert, B., 1996. The use of the aquatic moss *Fontinalis antipyretica* L. ex Hedw. as a bioindicator for heavy metals: 1. Fundamental investigations into heavy metal accumulation in *Fontinalis antipyretica* L. ex Hedw. The *Science of the Total Environment* 177: 137-144.

Singh, M., Ansari, A.A., Müller, G. & Singh, I.B., 1997. Heavy metals in freshly deposited sediments of Gomati River (a tributary of the Ganga River): Effects of human activities. *Environmental Geology* 29: 246-252.

Sinha, S., Gupta, M. & Chandra, P., 1997. Oxidative stress induced by iron in *Hydrilla verticillata* (l.f.) Royle: response of antioxidants. *Ecotoxicology and Environmental Safety* 38 (3): 286-291.

Sinha, S., Saxena, R., Singh, S., 2005. Chromium induced lipid peroxidation in the plants of *Pistia stratiotes* L.: role of antioxidants and antioxidant enzymes. *Chemosphere* 58: 595-604.

Skórzyńska-Polit, E. & Baszyński, T., 1995. Photochemical activity of primary leaves in cadmium stressed *Phaseolus coccineus* depends on their growth stage. *Acta Societalis Botanicorum Poloniae* 64 (3): 273-279.

Smith, R.L., 1986. *Elements of Ecology*. 2nd Edition. Harper & Row. New York.

Smol, J.P. 2002. *Pollution of lakes and rivers*. Arnold. London.

Snyman, R.G., Reinecke, S.A., Reinecke, A.J., 2000. Hemocytic lysosome responses in the snail *Helix aspersa* after exposure to the fungicide copper oxychloride. Archives of Environmental Contamination and Toxicology 69: 117 – 122.

Soares, H.M.V.M., Boaventura, R.A.R., Machado, A.A.S.C. & Esteves da Silva, J.C.G., 1999. Sediments as monitors of heavy metal contamination in the Ave river basin (Portugal): multivariate analysis of data. *Environmental Pollution* 105: 311-323.

Soares, D.C.F., de Oliveira, E.F., de Fátima Silva, G.D., Duarte, L.P., Pott, V.J. & Filho, S.A.V., 2008. *Salvinia auriculata*: Aquatic bioindicator studied by instrumental neutron activation analysis (INAA). *Applied Radiation and Isotopes* 66: 561-564.

Souza-Santos, P., Ramos, R.S., Ferreira, S.T. & Carvalho-Alves, P.C., 2001. Iron-induced oxidative damage of corn root plasma membrane H^+-ATPase. *Biochemica et Biophysica Acta (BBA)-Biomembranes* 1512 (2): 357-366.

Symeonidis, L. & Karataglis, S., 1992. The effect of lead and zinc on plant growth and chlorophyll content of *Holcus lanatus* L. *Journal of Agronomy and Crop Science* 168 (2): 108-112.

Syngenta, 2012. Untitled. Htp://www.syngenta.com [24/04/2012].

Tele Atlas, 2009. Burslem. Google Maps. http://maps.google.co.uk. [19/07/2011].

Tripathi, R.D., Rai, U.N., Vajpayee, P., Ali, B., Khan, E., Gupta, D.K., Mishra, S., Shukla, M.K. & Singh, S.N., 2003. Biochemical responses of *Potomogeton pectinatus* L. exposed to higher concentrations of zinc. *Bulletin of Environmental Contamination and Toxicology* 71: 255-262.

Upadhyay, R.K. & Panda, S.K., 2005. Salt tolerance of two aquatic macrophytes, *Pistia stratiotes* and *Salvinia molesta*. *Biologia Plantarum* 49 (1): 157-159.

Urquhart, C., 1971. Genetics of lead tolerance in *Festuca ovina*. *Heredity* 26: 19-33.

Van Gestel, C.A.M. & Van Brummelen, T.C., 1996. Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms. *Ecotoxicology* 5: 217-225.

Vannela, R. & Verma, S.K., 2006. Co2+, Cu2+, and Zn2+ Accumulation by cyanobacterium *Spirulina platensis*. *Biotechnology Progress* 22: 1282-1293.

Veglio, F. & Beolchini, F., 1997. Removal of metals by biosorption: A review. *Hydrometallurgy* 44: 301-316.

Walker, C.H., 1995. Biochemical biomarkers in ecotoxicology – some recent developments. *The Science of the Total Environment* 171: 189-195.

Walker, C.H., Hopkin, S.P., Sibly, R.M. & Peakall, D.B., 2006. *Principles of Ecotoxicology*. 3rd Ed. Taylor and Francis. London.

Water Institute of Southern Africa, 2009. The profile of the Diep River. www.ewisa.co.za [09/06/2011].

Wegener, J.W.M., van Schaik, M.J.M. & Aiking, H., 1992. Active biomonitoring of polycyclic aromatic hydrocarbons by means of mosses. *Environmental Pollution* 76: 15-18.

Wepener, V., van Vuren, J.H.J., Chatiza, F.P., Mbizi, Z., Slabbert, L. & Masola, B., 2005. Active biomonitoring in freshwater environments: early warning signals from biomarkers in assessing biological effects of diffuse sources of pollutants. *Physics and Chemistry of the Earth* 30: 751-761.

Whitlow, T.H., Bassuk, N.L., Ranney, T.G. & Reichert, D.L., 1992. An improved method for using electrolyte leakage to assess membrane competence in plant tissues. *Plant Physiology* 98: 198-205.

Wild, A., 1993. *Soils and the environment. An introduction*. Cambridge University Press. New York.

Windham, L., Weis, J.S. & Weis, P., 2003. Uptake and distribution of metals in two dominant salt marsh macrophytes *Spartina alterniflora* (cordgrass) and *Phragmites australis* (common reed). *Estuarine, Coastal & Shelf Science* 56 (1): 63-72.

Wright, D.A. & Welbourn, P., 2002. *Environmental toxicology.* Cambridge Environmental Chemistry Series 11. Cambridge University Press. Cambridge.

Yuan, Y.X., Zhang, J., Wang, D.W. & Ling. H.Q., 2005. AtbHLH29 of *Arabidopsis thaliana* is a functional ortholog of tomato FER involved in controlling iron acquisition in strategy 1 plants. *Cell Research* 15: 613-621.

Zheng, S.J., Ma, J.F. & Matsumoto, H., 1998. Continuous secretion of organic acids is related to aluminium resistance during relatively long-term exposure to aluminium stress. *Physiologia Plantarum* 103 (2): 209-214.

Zurayk, R., Sukkariyah, B. & Baalbaki, R., 2001. Common Hydrophytes as bioindicators of nickel, chromium and cadmium pollution. *Water, Air, and Soil Pollution* 127: 373-388.