The Accumulation of Heavy metals by Aquatic Plants

By

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Declaration of Candidate

I, Saroja Maharaj, declare that unless indicated in this dissertation is my own work and that it has not been submitted for a degree at another Technikon or Institution.

S Maharaj
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ABSTRACT

The pollution of water bodies by heavy metals is a serious threat to humanity. The technique known as phytoremediation is used to clean up these polluted water bodies. The accumulation of heavy metals by aquatic plants is a safer, cheaper and friendlier manner of cleaning the environment. The aquatic plants studied in this project are *A.sessilis, P.stratiotes, R.steudelii* and *T.capensis*. The accumulation of heavy metals in aquatic plants growing in waste water treatment ponds was investigated. The water, sludge and plants were collected from five maturation ponds at the Northern Waste Water Treatment Works, Sea Cow Lake, Durban. The samples were analysed for Zn, Mn, Cr, Ni, Pb and Cu using ICP-MS.

In general it was found that the concentrations of the targeted metals were much lower in the water (0.002 to 0.109 mg/l) compared to sediment/sludge (44 to 1543mg/kg dry wt) and plants (0.4 to 2246 mg/kg dry wt). These results show that water released into the river from the final maturation pond has metal concentrations well below the maximum limits set by international environmental control bodies. It also shows that sediments act as good sinks for metals and that plants do uptake metals to a significant extent. Of the four plants investigated it was found that *A.sessilis* (leaves, roots and stems) and *A.sessilis* (roots and stems) are relatively good collectors of Mn and Cu respectively. These findings are described in the thesis. The concentration of heavy metals in the stems, leaves and roots of the three plants were compared to ascertain if there were differences in the ability of the plant at different parts of the plant to bioaccumulate the six heavy metals studied.

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CHAPTER 1

INTRODUCTION

1.1 Pollution

The generating of waste, containing heavy metals, is a serious threat to humanity and the planet itself. Some of the oldest cases of environmental pollution in the world are due to heavy metal use, such as Cu, Hg and Pb, mining, smelting and utilisation by ancient civilisations such as the Romans and the Phoenicians (Alloway, 1994).

The damage caused by pollution is serious, affecting human health by harmful chemical substances that are unleashed into the air, water and soil; these eventually make their way into the food chain. It also impacts negatively on the aesthetic quality of the environment through dust, noise, smoke, chemical fumes and the dumping of waste materials.

Pollution from point sources is often discharges that are known, for example discharges of effluent from sewage treatment works, whereas diffuse pollution enters watercourses from run off and land drainage. The effect of pollution may be either acute or chronic. According to Mason (2002) acute toxicity occurs rapidly and is rarely reversible whereas chronic toxicity develops after long exposure and may ultimately cause death.

Heavy metals such as Pb and Hg are commonly associated with pollution and toxicity problems, with sudden elevations in the heavy metal concentration being fatal, for example methyl mercury is highly poisonous and affects the central nervous system. Heavy metal pollution became widely recognised with the Minamata disaster in Japan, 1932. Seafood was the major diet for the local community. In 1956, the first case of nervous disease in humans was recorded where a young girl suffered from speech disturbances, delirium and experienced difficulties in walking. Mercury had accumulated in fish and after consumption of fish from the Minamata Bay by humans the mercury had accumulated in humans and hence the illness of Minamata disease. By 1988 there were 2209 confirmed cases of Minamata disease leading to 730 fatalities (Mason, 2002).

One would imagine that human beings had learnt from the Minamata disaster and mercury poisoning would never happen again. How wrong the world was, as 55 years later another disaster with mercury poisoning occurred at Thor Chemicals in South Africa. Thor Chemicals moved from England to South Africa in 1988. This plant was the only large scale mercury reclamation process in the world and became the target for many international companies facing the dilemma of what to do with waste mercury. In 1992 three workers were found to be suffering from long-term mercury exposure. One of the workers had died within months, the other was in coma and the third could not walk or talk, (http://www.umich.edu/~snre492/Jones/thorchem.htm).

A recent example of water pollution by heavy metals occurred at the Donana National Park, Spain, reported in (Mason, 2002). A mineral mine that was holding a reservoir of toxic waste burst on the 24 April 1998, releasing two million cubic meters of metal rich sludge and 4 million cubic metres of acid water into the river. In 18 days following the spill 26 tons of dead fish was removed and in the weeks to follow two hundred birds were found dead. It was postulated that the area is likely to suffer from chronic effects of pollution due to heavy metals that are now in the food chain.

1.2 Waste disposal

The disposal of waste is the cause of most pollution. Langmore (1998) defines waste as an undesirable or superfluous by - product, emission, residue or remainder of any process or activity, any matter, gaseous, liquid or solid or any combination thereof. Waste materials that are released into the atmosphere, dumped on land, or discharged into streams, rivers or sea pollute the environment (Figure 1.1).

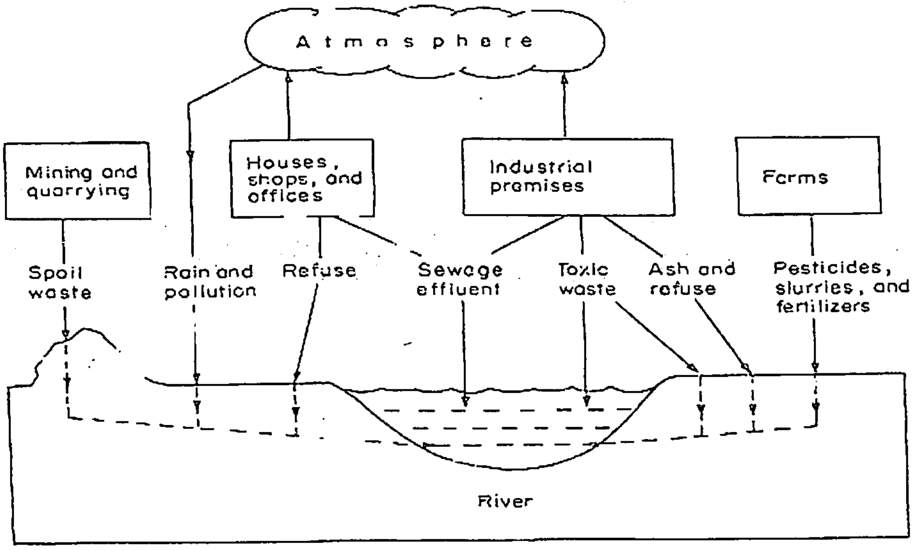

Figure 1.1) Sources and disposal of waste material into the environment (Dix, 1981)

Waste can be broadly classified into solids or liquids, or a mixture of these physical states. Liquid waste is generally called effluent, which means a stream or liquid discharge usually produced from an industrial process or sewage works. This effluent consists of a carrier liquid containing soluble and insoluble chemical substances. Solid waste can be described; according to the particle size as rubble, ash or dust; according to their origin as spoil from mining operations or slag from industrial processes. The chemical constituents of waste can be classified as organic or inorganic substances (Dix, 1981). Waste is produced from domestic, industrial and agricultural sector.

Although waste of any kind is undesirable, it appears that waste entering our water bodies has become our foremost concern. Gaseous matter emitted into the atmosphere is an increasing source of atmospheric pollution and some material is returned and deposited on land and water bodies. The leaching out of chemical substances from the soil can be a source of water pollution and liquid waste discharged into water bodies become a further source of water pollution.

1.3 Water

1.3.1 The importance of water

Water is unquestionably the lifeblood of the Earth – no animal or plant life would exist without it (Williams, 2001). Water moves through the biosphere via the processes of evaporation, condensation, precipitation and transpiration (Figure.1.2).

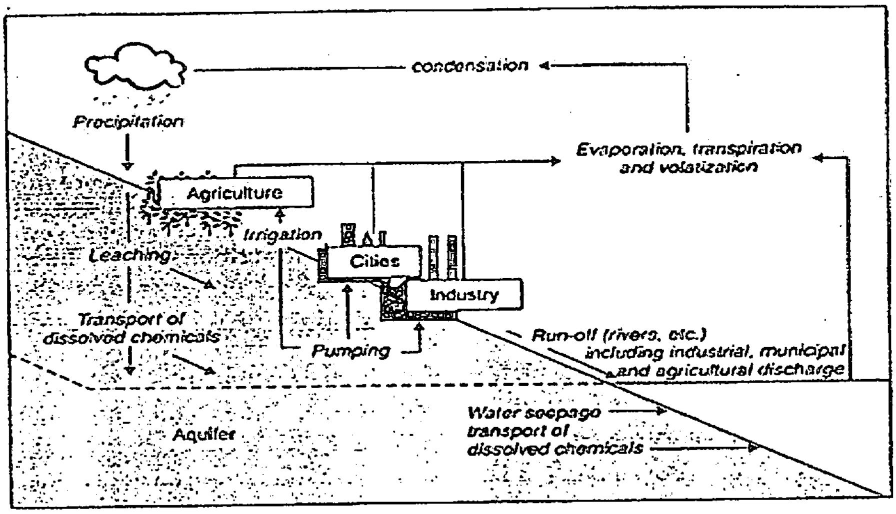

Figure 1.2) The water cycle (Williams, 2001)

Whilst it is true that the Earth's content is largely water, and that the ocean has been calculated to contain 97% of the Earth's total resource of water, the fact of the matter is that this oceanic water cannot be used for our daily domestic consumption, agriculture and industry. This means that fresh water is essential in everyday life, and being such a scarce commodity, has to be kept clean and pollution free. The scarcity of this precious commodity is revealed, when one learns that more than 20 % of the world's population do not have access to safe drinking water (Mason, 2002).

The vital role water has in maintaining life, can be demonstrated by considering the human body, which is composed of 60 to 70 % of water (approximately 40 litres). About 1,5 litres of water from the human body is lost through breathing, perspiration and faeces. A human being can only survive a week without water (Williams, 2001).

1.3.2 Water pollution

The increase in pollution of water bodies limit social and economic development and are linked closely to poverty, hunger and disease (Ashton and Seetal, 2002). In India, tubewells, from which 97% of the population obtain their drinking water, are contaminated with arsenic. The cause of the unexpected contamination is yet unknown. However, 85 million people are at risk from arsenic and one in ten will ultimately die from some form of cancer (skin, lung or bladder) (Mason, 2002). A generalised list of pollutants found in freshwater are listed in (table 1.1).

Table 1.1: Categories of pollution found in freshwater: (Mason 2002)

Acids and alkali	Anions (e.g. sulphide, sulphite, cyanide)
Detergents	Domestic sewage and farm manures
Food processing waters	Gases (e.g. chlorine and ammonia)
Heat	Metals (e.g. Cadmium, lead and Zinc)
Nutrients	Oil and oil dispersants
Organic toxic waste(e.g. formaldehyde)	Pathogens
Pesticides	Polychlorinated biphenyls
Radionuclides	

1.3.3 Wastewater

Wastewater is typically generated from domestic and industrial sources and may include urban run off. The major classes of municipal wastewater contaminants are settleable solids (sand), organic matter (Kjeldahl-nitrogen), pathogenic micro-organisms, nutrients (N and P), micro pollutants (heavy metals) and total dissolved solids. Industrial processes are the main source of heavy metal pollution of water bodies. Domestic waste also contains a substantial quantity of metals because of water that has been in prolonged contact with copper, zinc or lead pipe work or tanks. Heavy metal concentrations in domestic formulations, such as cosmetics or cleansing agents are frequently overlooked (Abel 1996).

Tap water is used for a variety of purposes, such as bathing, washing and the transport/flushing of waste. In wealthier communities, more waste is disposed by water-flushing. Domestic wastewater typically depends on water supply, climate and water availability. In industrialised countries 75% of consumed tap water ends up as sewage (Helmer and Hespanhlol, 1997).

The water demand and wastewater production in industry are sector specific. Large quantities of water are required for cooling (power plants), cleaning (textile mills), processing (paper mills) and flushing of waste. The contaminants from the wastewater may have a negative impact on the aqueous environment in which they are discharged and treatment systems are specific. The combined treatment of many contaminants reduces efficiency and increases unit treatment cost. Table (1.2) gives information on the methods of industrial wastewater treatment before discharge into sewers.

Table 1.2: Consent limits and pre treatments given to industrial wastewaters before discharge to sewers (Harrison, 1992)

Contaminant	**Consent limit(mg/l)**	**Pre treatment**
Suspended solids	400-1000	Screening and settlement
BOD(Biological oxygen demand)	500-1000	High rate bio-oxidation
Oil and grease	10	Passage through oil traps or separators
Cyanide	1-5	Chlorination or enzyme treatment for CN removal
Heavy metals	1-20	Alkaline precipitation and settlement
Acidity / alkalinity	pH 10 – 5	Neutralisation
Solvents	Substantially absent	Activated carbon treatment
Strong dyestuff colour	Low colour	Bleaching with chlorine

1.4 The removal and treatment of waste

Previously not much effort was put into the treatment of waste but this has attracted attention due to awareness of effect and drive for environmental conservation and sustainability. The treatment of solid waste and wastewater entering the environment includes physical, chemical and biological treatments.

The physical treatments available are solvent extraction, sedimentation, carbon adsorption, distillation and evaporation. Chemical treatment of waste includes neutralization of acidic or alkaline waste, ion exchange resins, incineration, pyrolysis, vitrification and solidification (Williams, 2000 and Langmore, 1998).

Biological treatment of waste is where microbes are used to destroy or reduce toxicity of a waste stream. The transformations accomplished biologically are degradation of organics, reduction of inorganic substances and complexation of heavy metals. The treatment can be divided into aerobic and anaerobic processes (Langmore, 1998). The removal of heavy metals from waste water is discussed in table (1.3) in appendix 1.

1.5 Other developments in removal of heavy metals from waste

The treatments discussed above result in transformation of metal pollutants to less toxic forms but do not totally remove the metals. The challenge to the environmental scientist is the development of methods for the complete removal of metal pollution. Therefore the use of plants to remove pollutants from the environment is being studied extensively, to clean up the environment in a cheaper, safer and friendlier manner. The cost of cleaning up large scale heavy metal pollution is enormous, with the chemicals, equipment and labour used being expensive. The technique known as phytoremediation is, however, not a complete answer to remove heavy metal pollution. Once the heavy metals are concentrated in the plants, the plants are ashed and the volume of pollution is reduced. The metals in this ash are converted to useful products.

1.6 Interest in South Africa

The shortage of water available per person and the deterioration in water quality is a challenge facing Africa's water resource managers. Most domestic and water users cannot use water without prior treatment or purification. The situation becomes precarious in poor rural areas with no access to water treatment facilities. Given the historical and political inequities of apartheid in South Africa, the socio-political content of water access and availability to the majority of the population was inadequate and inappropriate. The amount of water available per person is not uniform across Africa, some countries will have 'water abundance', whilst others will experience 'water scarcity' and 'water deficit', table (1.4) (Ashton and Seetal, 2002).

Table1.4) Three classes of water availability. Data presented as percentages (Ashton and Seetal, 2002)

Countries with water	**2000**			**2025**		
	Area	**Population**	**Water**	**Area**	**Population**	**Water**
Abundance	52.5	60.8	95.2	34.7	23.9	78.3
Scarcity	26.0	24.3	4.4	39.1	57.3	20.6
Deficit	21.5	14.9	0.4	26.2	18.8	1.1
Total African population	786 million			1.428 million		

The key to development and a good quality of life in South Africa is water.

South Africa is largely semi arid, with unpredictable drought and flood. The average rainfall country wide is less than 500mm, well below the world average of 800mm, with only 9% reaching the rivers. Clean water is important for the following reasons.

- A healthier population
- The production of food
- Sustain jobs

The rivers in South Africa are small compared to those of other countries. Ten percent of the volume of water flowing down the Zambezi River and one percent of the flow of the Congo River is carried by the Orange River. The large rivers such as the Orange and Limpopo are also shared by other countries, (Government Gazette). The degree to which populations of different Southern African countries have access to sanitation and safe supply of water are listed in table (1.5).

Table 1.5) Degree to access to safe water supplies and safe sanitation. (Ashton and Seetal, 2002)

Country	Population (millions)	Proportion urbanised (%)	Safe water (%)		Sanitation (%)	
			Urban	Rural	Urban	Rural
Angola	10.2	31	69	15	34	8
Botswana	1.6	64	100	91	91	41
D.R.C	52.0	29	37	23	23	4
Lesotho	2.1	25	65	54	53	36
Malawi	10.4	14	80	32	52	24
Mozambique	19.1	35	47	40	53	15
Namibia	1.8	37	87	42	77	32
South Africa	43.4	49	80	49	79	50
Swaziland	1.1	32	61	44	66	37
Tanzania	35.3	25	67	45	74	42
Zambia	9.6	43	64	27	75	32
Zimbabwe	11.3	43	90	69	90	42

South Africa is largely a developing country with a growing population resulting in an increase in water demand. There is also a definite need for job creation and an increase in industrial development is inevitable. This will result in an increase in waste generation and possible pollution of water bodies, as well as an increase in the cost of disposal and removal of waste. As mentioned earlier the extent of the contamination by Thor Chemicals is unknown. Reducing the demand for water and increasing the efficiency of water used should be the focus of the future.

In South Africa the environmental chemists previously concentrated on determining the levels of heavy metals in the environment. Information in South Africa on phytoremediation is limited. There is not much data available, on the remediation of water bodies polluted by heavy metals. Studies done at the ML Sultan Technikon by (Govender, 2001) determined the levels of heavy metals in air, (Paul, 2002) determined the levels of heavy metals in the sediment and water and (Msukwini, 2003) determined the levels of heavy metals in the soil. While these studies are important overall, they have been focused on determining levels of heavy metals in the environment and not the removal of heavy metals or remediation of the environment which is more important in the long run. An example of phytoremediation study done in South Africa was by Anderson *et al*, (1998), who showed how plants can be used to remove nickel from the soil. It was found that '*Berkheya codii*' has the ability to hyperaccumulate and also to tolerate nickel in the serpentine soils of the Mpumalanga province in South Africa. There is no information available in South Africa on the remediation of aquatic environments by plants.

South Africa is a country with diverse aquatic environments and abundant plant life. The study of phytoremediation on these water bodies would be extremely useful for the environmental monitoring and remediation of pollution in water bodies of South Africa.

1.7 The aim of the study

Many South Africans, rely on rivers and dams for their source of daily food, bathing, washing, drinking and irrigation, while the ocean is mainly used for recreational activities. The pollution of these water bodies would be devastating. Against this background it was deemed necessary to investigate the role of aquatic plants in removing heavy metals from waste water emanating from a sewage treatment work. The use of plants to clean up the environment has great appeal as it is a safer, cheaper and a friendlier way of removing pollutants. Hence the study of the accumulation of heavy metals by aquatic plants was the focus of this study.

1.8 The objectives of the study

1.8.1 To determine the optimal conditions for the digestion of samples to be analysed

1.8.2 To establish suitable techniques for the analysis of metals.

1.8.3 To compare results from this study with those reported in literature.

CHAPTER 2

LITERATURE REVIEW

In order to provide the context in which this project needs to be viewed, this review will touch on issues pertinent to heavy metals in the environment such as the sources of these metals, bio-indicators for metal and phytoremediation from various environmental compartments..

2.1 Heavy metals

A broad definition of heavy metals is a group of metals and metalloids with an atomic density greater than 6 g/cm^3 (Alloway, 1994). The most important heavy metals from the point of view of water pollution are zinc, copper, lead, cadmium, mercury, nickel and chromium (Abel, 1996). Knowledge of the natural concentration of the heavy metals is important because it provides a reference point for estimating the extent of pollution. Heavy metal pollution arises when the concentration of the heavy metal is high relative to the background concentration.

2.1.1 Heavy metal production

The society we live in today is predominantly industrial. The use of goods and amenities involve an increasing range of metallic products. The frequent disposal of heavy metals results in diverse types of metallic waste. Mining, smelting, refining, energy production, industrial emission, agricultural operations, sewage discharge and disposal of waste are rapidly increasing environmental pollution. The production of heavy metals has increased tremendously over years (Alloway, 1994). For example over a period of 55 years between 1930 and 1985 nickel showed the greatest increase (x35), followed by Cr (x17) and Cd (x14), whereas Hg production had not even doubled but showed a significant increase, table (2.1).

Table 2.1) Primary production of metals (10^3t/yr) (Alloway, 1994).

Bars

	Production in	
Metals	**1930**	**1985**
Cd	1.3	19
Cr	560	9940
Cu	1611	8114
Hg	3.8	6.8
Ni	22	778
Pb	1696	3077
Zn	1394	6024

2.1.2 The occurrence and use of heavy metals under investigation

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2.1.2.1 Copper

Table 2.2) Cu concentration in the environment (Cox, 1995)

Location	Concentration
Earth's Crust	50 ppm
Sea water:	
Surface	0.008 ppb
Deep	0.02 ppb
Fresh waters	1 – 10 ppb
Soils:	
Average	30 ppm
Contaminated	100 – 10 000 ppm
Human body	1 ppm

The copper metal is used in water piping, roofing material and kitchenware. Alloys of copper such as zinc (brass) and tin (bronze) are also produced. Copper sulphate is used to supplement pastures deficient in the metal and in electroplating. Cupric oxide and copper chromates are available as pigments in paints. Some of these copper compounds end up as waste products in soil and water bodies.

2.1.2.2 Zinc

Table 2.3) Zn concentration in the environment (Cox, 1995)

Location	Concentration
Earth's Crust	75 ppm
Sea water:	
Surface	0.05 ppb
Deep	0.5 ppb
Fresh waters	10 ppb
Soils:	30 – 1000 ppm
Human body	
Average	30 ppm
Blood	7 ppm

ZnO is used in the production chemicals and paints. It is also used in floor coverings, manufacture of glasses, enamels, fabrics, plastics and lubricants. Zinc oxide is a well known pharmaceutical in powders and ointments. Zinc sulphate is used as a hardener in rayon manufacturer. Zinc chloride is used in dry batteries, in the processing of cotton and as a cauterizing agent in medicine. Zinc soaps are water repellent and organozinc compounds are used as fungicides. All the stages of production and processing of zinc results in wastage which leads to the atmosphere, soil and eventually to water.

2.1.2.3 Manganese

Manganese is used in the production of alloys with aluminium, magnesium and copper. MnO_2 is used as a depolariser in dry cell batteries. In chemical reactions manganese (IV) oxide, manganese chloride and manganese stearate are used as catalysts. Manganese compounds are used as feed additives, fertilizers, pigments, dryers and wood preservative.

2.1.2.4 Nickel

Table 2.4) Ni concentration in the environment (Cox, 1995)

Location	Concentration
Earth's Crust	80 ppm
Sea water:	
Surface	0.1 ppb
Deep	0.5 ppb
Fresh waters	1ppb
Soils:	
Normal average	50 ppm
Serpentine	01 – 2 %
Human body	01 – 1ppm

More than 3000 metal alloys are made of Nickel. Ni-Cr-Fe alloys for cooking utensils and corrosion resistant equipment. Ni-Cu alloys for coinage, Ni-AL alloys for magnets and Ni-Cr alloys for heating elements. Nickel is also used in electroplating and in the manufacture of Ni-Cd batteries.

2.1.2.5 Lead

Table 2.5) Pb concentration in the environment Cox (1995)

Location	Concentration
Earth's Crust	14ppm
Ocean:	
Surface	0.02ppb
Deep	0.002ppb
Fresh waters:	
Natural	1-10ppb
polluted	20-100ppb
Mining waters	100-1000ppb
Drinking waters	50ppb
Soils:	
Normal	2-200ppb
Polluted	1-30ppm
Human body	
Average	0.5-2ppm
Blood	1-25ppm
Bone	02-10 ppm

Previously lead based pigments were used in paints. Lead is used for the production of lead acid batteries and lead chemicals are used in glassware.

2.1.2.6 Chromium

Chromium in its hexavalent form is 100 to 1000 times more toxic than the trivalent form. Chromium (III) oxide is found in pigments while chromium (III) sulphate is the most important tanning agent. Chromium compounds are used in the oil industry (as anti corrosives) and in the match industry as additive to the inflammable mixture. Residues may be found in industrial effluent.

2.1.3 Toxicity of heavy metals

Some heavy metals are required by most living organism in small but critical concentration for normal healthy growth (e.g. zinc and copper). The excess concentration of these heavy metals causes toxicity. Non essential elements are elements with no known essential biochemical function for example lead and cadmium.

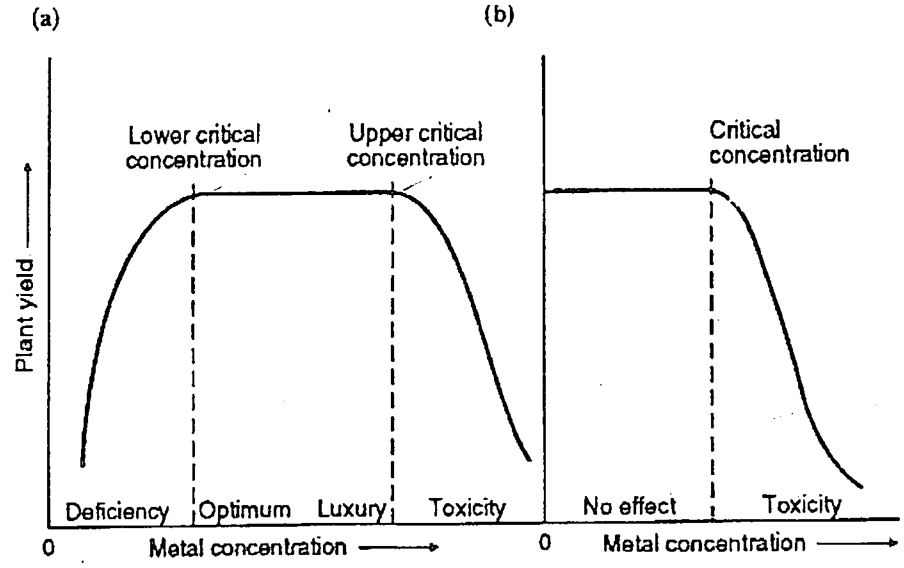

Figure 2.1) A typical dose response curve for a) micronutrients and b) non essential trace elements (Alloway, 1994)

Sudden elevations in heavy metal concentration can turn out to be fatal. The mechanisms of toxicity for the heavy metals investigated in this study are shown in the table below

Table 2.6) Toxicity of heavy metals (Athar, 1995)

Metal	Mechanism of toxicity
Cr (Chromium)	1) Carcinogenic 2) Allergic and corrosive nature 3) Damage to DNA (DNA-polymerase enzyme and free radical formation)
Cu (Copper)	1) Damage to susceptible cells including those of brain and liver. 2) Irritation of gastric mucosa 3) A consequent deficiency of ceruplasmin in serum
Pb (Lead)	1) Altered permeability of brain capillaries 2) Interferences with several enzymes involved in heame synthesis 3) Damage to the erythrocyte membrane
Mn (Manganese)	1) Inhibition of melanin synthesis in the brain. 2) Interruption of the brain catecholamine metabolism.
Ni (Nickel)	1) Carcinogenic action 2) Peroxidative degradation of membrane lipids 3) Allergenic action
Zn (Zinc)	1) Impaired immune responses

2.1.4 Heavy metals in the environment

Metals are naturally occurring substances and have been on earth since its formation. All metals that are released into the environment cannot degrade further into non-toxic product and are endangering human health and other forms of life. Heavy metals become permanent additions to the aquatic plants environment as they are not broken down i.e. conservative pollutants (Mason, 2002) (Walker *et al*, 2001).The emissions of heavy metals into the environment are the greatest source of heavy metal pollution which contaminates the aquatic ecosystems and soils through atmospheric fallout. Metallic contamination of aquatic ecosystem includes sewage and industrial discharges such as the Thor Chemicals mentioned in the introduction, domestic waste waters, base-metal mining and smelting to mention a few. Other sources of contamination include air by lead fumes from motor vehicles which invariably get into the aquatic environment through atmospheric fall out, run off and leaching

2.1.5 Method of monitoring heavy metals in the environment.

To determine concentrations of heavy metals in the environment, soil, water and air sampling and testing is conducted. However over the years studies have been extended to living organisms such as fish, earthworms and plants. These organisms gave an indication of heavy metal pollution due to their accumulation of heavy metals and their studies are found to be useful in determining the extent of pollution. According to Mader (1995), the advantages of biological monitoring are:

- Accumulation of pollutants.
- Redistribution within the organism
- Geographical distribution of the searched pollution

2.2 Bio-indication

When a wide range of different chemicals enter the aquatic environment a variety of processes occur which result in their distribution to different environmental phases, Fig.2.2. The abiotic processes which develop are the water/sediment process, the air/water process and the air/soil process (Schuurmann, 1997).

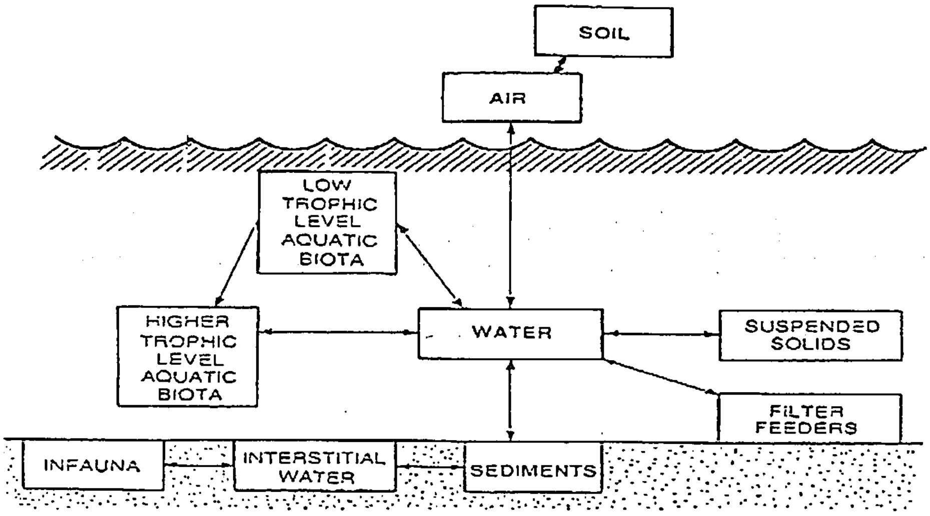

Figure 2.2) Distribution to different environmental phases (Schuurmann, 1997)

The term bioaccumulation is used when the means whereby an organism acquires a chemical is unknown. Bioconcentration is when some aquatic biota bioaccumulates compounds as a result of direct partitioning with ambient water. Biomagnification is the consumption of low trophic level aquatic biota as food by higher trophic level aquatic biota (Schuurmann, 1997).

Species of fauna and flora are utilised for the bioindication of environmental changes. Environmental indicators are used for the observation of long term development in an ecosystem. A study done in Ireland attempted to predict radioceasium levels in vegetation and herbivores using bioindicators (McGee *et al*, 1993). It was found that the sole use of plant material for bioindicating meant faster sampling and easier analysis than when soils are analysed in conjunction with plant samples.

Bioindicators can be of optical, chemical or physical –biochemical nature. The reduced growth of plant species and the decolouration of leaves are examples of optical indicators. Chemical indicators show the accumulation of substance under consideration. Physical-biochemical indicators show derangement of physiological functions and reduced enzymatic activity.

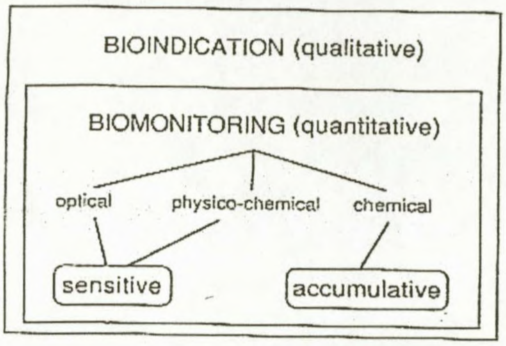

Fig 2.3) Classification of bioindication, (Harrison, 1992)

2.2.1 Sensitive biomonitors

Sensitive biomonitors are used in aquatic environments to provide early warning systems. Ecological survey uses an indicator species to make a comparison between an affected and controlled area. Ecological surveys also indicate the health of a water body exposed to pollutant loadings. Toxicity investigations can be used for the prediction of environmental effects of waste, for the comparison of toxicants on animals or for the regulation of effluent discharge. Sensitive biomonitors are also used for air quality control. In a study done to evaluate air pollution on agricultural crops, radish was used (Jones *et al*, 1993). It is mentioned that the favourable characteristics of plant bioindicators are; a short growing cycle and the ability to harvest the plant after a short time interval, a structure which enables relatively easy separation into shoot and root components , a leaf form which permits non destructive physiological measurements such as photosynthesis and transpiration. Bioindicators are termed 'biological systems which show a quantifiable response to a perturbation of their environment'.

2.2.2 Accumulation biomonitors

The bioaccumulation of heavy metals has been studied most extensively and the use of plants as indicator species for heavy metal pollution has received particular attention (Ray *et al*, 1976). An investigation involved the use of two vascular plants and one blue algae. The usefulness of the three species to qualitatively reflect the long term water quality was clearly established. In a later study on the aquatic plant *Equisetum Arvense*, it was found that seasonal variations should be taken into consideration when using the aquatic plant as a biological indicator (Ray *et al*, 1979).

In a study on the bioindication of heavy metals, the evergreen plants were collected from a polluted site in (Wroclaw) Poland and two reference sites presumed to be unpolluted; one in Poland and the other in the Netherlands (Samecka-Cymerman *et al*, 1999). The purpose of the study was to assess whether the shrubs can be used in the bioindication of heavy metals in large city environments. The shrubs are a major plant type found in urban areas with a high degree of pollution. The plants seem to be more resistant to a polluted environment. The majority of plants from the polluted site contained microelements except for Zn in amounts much higher than the plants from the background areas. It was found that one of the species examined '*Ilex*' seemed to be the best monitor for soil polluted with mercury.

Studies suggest that macrophytes have the ability to integrate and monitor variations in the concentrations of the elements in water thus they have been widely used as bio-indicators (Guilizzoni, 1991). Outridge and Noller (1987), also suggest that vascular macrophytes can be used as biomonitors because they have a longer life cycle, a higher degree of tolerance to most elements and a large amount of tissue per individual. It has been suggested that for plants to be good biological indicators they should be representative of the area, be widely distributed, abundant and easy to collect, have a high tolerance for heavy metals and be reasonably easy to identify (StiCyr *et al*, 1994). The availability of heavy metals to plants depends on the chemical form in which these elements are present (Schierup *et al*, 1981); as ions or inorganic compounds in solution, as readily exchangeable ions or organic exchange complexes, in complex or chelates with organic material, as precipitated inorganic material, incorporated in solid biological material and in the crystalline structure.

2.3 Phytoremediation

Green plants are the miners of the earth's crust, being the major accumulator of inorganic nutrients upon which other life forms are directly or indirectly dependent (Baker, 1983). Plants that are used to clean up sites contaminated with metals are known as 'hyperaccumulators'. These plants can collect hundreds or thousands of times more metal in their aboveground parts than 'normal' plants (Mc Grath, 1998). Harvesting waste is exactly how some contaminated land can be cleaned up. The use of plants to suck up toxic metals from the environment is both cheap and environmentally friendly. The various technologies of cleaning hazardous waste i.e. acid leaching, electro-osmosis are extremely expensive. Bioremediation technologies have the advantage of lower cost and minimum site disruption.

Phytoremediation has recently been defined as the use of plants to extract, sequester and/or detoxify pollutants (Meagher, 2000). Plants are used to clean up polluted soil, water or air. Due to several natural biophysical and biochemical processes, pollutants can be remediated in plants through:

- Adsorption where the growth media which is the nutrient solutions or soils is the main source of trace element for plants. Absorption usually operates at very low concentrations in solutions and depends largely on the concentration of the solution. The rate depends strongly on the pH, the intensity varies with plant species and stage development and may be selective for a particular ion (Pendias, 1989). The movement of trace elements among tissues vary greatly depending on the plant organ, its age and the element involved. The reduction and coordination of arsenic in Indian Mustard (*Brassica juncae*) was studied (Pickering, 2000). The majority of arsenic was found to be accumulated in the roots and a very low level of arsenic was detected in the stem and leaves. The uptake of arsenic was found to be inhibited by phosphate.

Table 2.7) The effect of phosphate on arsenic uptake in Indian mustard seedlings.

Sample	**Control**	**Addition of phosphate**
	umol/g fresh biomass	
Root	43.8 ± 1.6	12.3 ± 2.7
Shoot	1.1 ± 0.1	0.5 ± 0.1

The concentration of arsenic in the leaves was only a small fraction of that absorbed in the roots. However, the addition of the chelating agent dimercaptosuccinate (DMS) to the growth medium dramatically alters the distribution in the leaves and allows the arsenic to become distributed throughout the plant (Table 2.8). This process will be essential for the development of an efficient phytoremediation strategy for this element.

Table 2.8) The effect of DMS on the uptake of arsenic by Indian Mustard seedlings.

Sample	**Control**	**DMS**
	umol/g fresh biomass	
Root	1346 ± 326	777 ± 20
Stem	204 ± 71	680 ± 245
Leaves	122 ± 20	640 ± 265

- Transport and translocation. The processes involved in the transport of ions within the plant tissue and organs are: movement in xylem, movement in the phloem, storage, accumulation and immobilization. The cation translocation in the plants is most importantly controlled by chelating ligands. Other factors such as pH, the oxidation-reduction state, competing cations, hydrolysis and the formation of insoluble salts govern metal mobility within the plants tissues. The accumulation and distribution patterns of trace elements vary considerably for each element, kind of plant and growth season. Catsiski *et al* (1993) studied Cu, Cr and Ni levels in two seagrasses from three sampling areas. The distribution and translocation of the metals among the tissues were discussed. It was found that the roots presented higher accumulation of metals in relation to rhizomes due to greater surface area per unit of weight of roots. Cu was also less abundant in the adult leaves due possibly by the existence of more binding sites in older plants.

- Hyperaccumulation is usually defined as concentration of metal ion concentration >0,1-1% dry weight of plant.
- Transformation is where toxic elements are transformed into harmless forms. Most elements exist in a variety of oxidation states that vary in their toxicity (Meagher, 2000). As mentioned in 2.1.2.6, Cr $^{6+}$ is more toxic than Cr^{3+}.

From the recent works of Garbisu (2001) the following categories of phytoremediation emerge:

- Phytoextraction – contaminants from the soil are removed by plants
- Phytofiltration – pollutants from water and aqueous waste streams are adsorbed or absorbed by plant roots (rhizofiltration) or seedlings (blastofiltration)
- Phytostabilisation – Plants are used to reduce the bioavailability of pollutants in the environment
- Phytovolatilisation – Volatile pollutants that are extracted by the plants are volatilised from the plants foliage
- Phytodegradation – Plants and associated microorganism are used to degrade organic pollutants and remediate soils contaminated with organics.
- Phytomining – A technique that involves growing the hyperaccumulators, harvesting the biomass and burning it to give the commercial product. A study (Anderson and Brooks, 1998) showed the accumulation of gold in *Brassica juncae.* The highest value of 57 ug gold per g dry weight was obtained.

2.3.1 The use of Aquatic plants for phytoremediation

Freshwater vascular plants play a vital role in the ecology of many freshwater ecosystems as they are a major component of the diet of many invertebrates, fish, birds and mammals. These plants consist of a xylem, phloem and a well defined root structure. According to Outridge *et al*(1991), there are six types of plants,

- rooted emergent - the plants are rooted in the bottom of the sediment with the leaves and flowers out of the water
- free floating emergent - not rooted to the bottom of the sediment
- rooted submergent plants - leaves and flowers underwater
- free floating submergent
- rooted floating - the leaves float on the water surface with flowers and fruit either above or below water
- free floating leaved plant

The uptake of metals depends on the chemical form in which it is present in the system Fig2.4 and the factors affecting the metal accumulation by aquatic plants are listed in table (2.9).

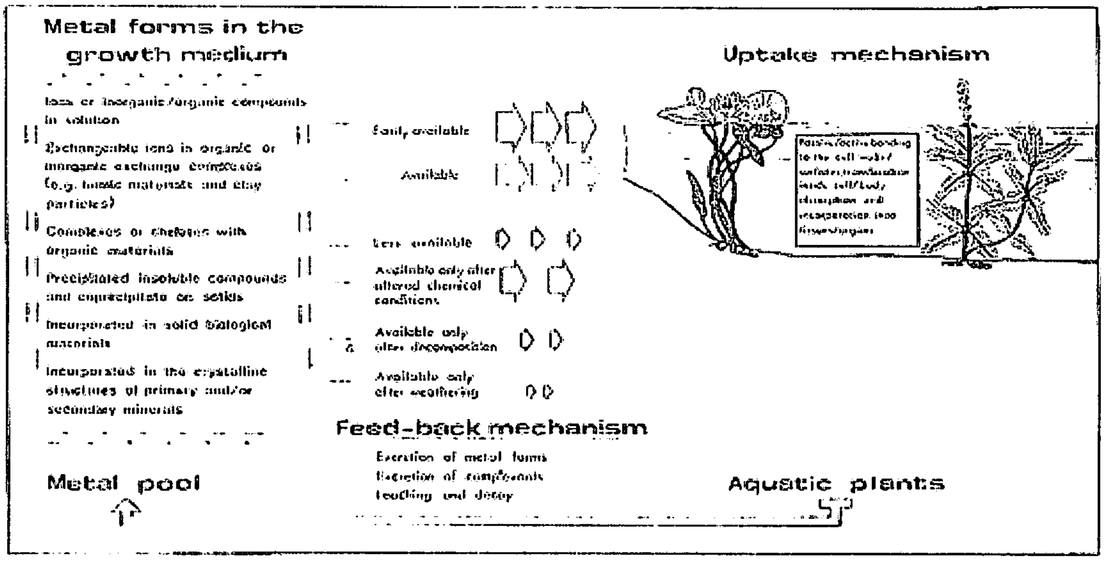

Figure 2.4) Metal cycling between aquatic medium and submerged macrophytes, Guilizzoni (1991)

Table2.9) Factors affecting accumulation (Wang, 1997)

Biological factors	Nonbiological factors	
	Environmental factors	Chemical factors
Species	Temperature	Concentration
Age	Light	Duration of exposure
Generation	Season	Disturbance or mixing
Translocation	pH	Competing ions
Route of uptake	Salinity	Complexing agents
Mode of uptake	Plant nutrients	

Some of the factors tabulated above are discussed in detail in section 2.4.

2.3.2 Investigations on phytoremediation

The use of plants to clean up effluent dates back to 1975. In this study Wolverton *et al* studied the use of two aquatic plants (*Eichornia crassipes* and *Alternanthera philoxerides*) for the final filtration of sewage. The plants were used for the study because of the high bio-mass and the ease of harvesting of the free-floating plants. Mature plants were used for the experiment. Fresh influent and effluent samples from the wastewater lagoon at Bay St. Loius were used. The plants were placed in 4,5 litre of sewage water contained in 5 litre glass cylinder. The plant roots were digested with nitric acid and hydrogen peroxide. The heavy metal concentration in the plants and effluent samples were analysed using AAS. Mercury was analysed using flameless AAS. The results obtained for the analysis of the initial effluent, digested roots of *Eichornia crassipes* and digested roots of *Alternanthera philoxerides* are tabulated below.

Table 2.10) Heavy metal concentration (ppm) in initial effluent, digested roots of *Eichornia crassipes* and digested roots of *Alternanthera philoxerides*.

	Initial effluent waste water	**Digested roots of** *Eichornia crassipes*	**Digested roots of** *Alternanthera philoxerides*
Pb	<0.008	0.063	0.036
Cd	<0.001	<0.001	<0.001
Cu	<0.01	<0.01	0.16
Ag	<0.02	<0.02	<0.02
Ni	<0.05	<0.06	<0.05
Zn	<0.08	0.58	0.84
Hg	<0.001	<0.001	<0.001
Sr	<0.01	<0.01	<0.01
Co	<0.007	<0.007	<0.007

Wolvertons findings were that the two aquatic plants studied demonstrated their ability to remove nutrients and pollutants from domestic sewage waste.

In the past 10 to 20 years phytoremediation has been studied extensively and due to the versatility of this technique, phytoremediation is viewed as a viable method for determination of extent of pollution in the environment and it can also be used to identify plant species which can accumulate heavy metals. Studies are done globally for example in Brasil, Japan, India, Argentina, Germany, France, America and Poland. The studies done determine the extent of pollution in the environment. The studies also help identify plant species which can accumulate heavy metals.

2.3.2.1 Soil/ Terrestrial

The uptake of zinc from soil and air was studied (Lagerwerff, 1971). Soil samples were collected form seven meters and 200 meters from a heavily travelled road and analysed for zinc. Radish was sown in cardboard containers filled with soil. The experiment was done in an open field 200 meters from traffic and in a growth chamber located in a windowless room. It was found that an increase in metal content in the plant increased by only a slight fraction to the increase in the soil metal content. An increase in soil pH from 5.9 to 7.2 resulted in a decrease in metal content of the radish. The zinc concentration in the roots was significantly higher in the radish grown outside than the radish grown inside.

A study (Anderson *et al*, 1998), investigated the uptake of nickel in '*Berkheya codii*'. It was found that this plant has the ability to hyperaccumulate and also to tolerate nickel in the serpentine soils of the Mpumalanga province in South Africa. It was also found that calcium chloride and lanthanum chloride significantly increased the nickel uptake in the leaves.

Muller *et al* (2002), studied the strategies of heavy metal uptake by three plant species growing near a metal smelter in northern France. The plants studied were two metallophyte (grow only on metal contaminated or naturally metal rich soil) species (*Armeria maritima ssp. halleri* and *Cardaminopsis halleri*). The other plant (*Agrostis tenuis*) is a psedometallophyte i.e. grow on both contaminated and non contaminated soil. Their aim was to investigate the metal accumulation capacity of the plants and to assess the metal – tolerance strategies adopted by each species with respect to Zn, Pb, Cd, and Cu.

The plants were separated into roots, stems and leaves. Plants were oven dried at 85° C for 15h, and then ground, before analysis. Soil samples were dried at 105° C, and then ground, before analysis. The samples were digested with hydrofluoric acid, perchloric acid and hydrochloric acid. The heavy metal concentrations were determined by either flame or graphite furnace atomic absorption spectrophotometry. Zn concentration was measured in the leaves of *C Halleri* and found to be 21500mg/kg. This value was 2 or 3 times higher than in the stems and roots. The Zn concentration in *Armeria* was 6900mg/kg, 4790mg/kg and 1280mg/kg in roots, stems and leaves respectively. *Agrostis* had twice the concentration of Zn (2320mg/kg) in the roots than in the leaves and stems. Cd concentration trends were similar to that of Zn. *C Halleri* had the highest concentration in leaves (281mg Cd/kg) whereas *Armeria* and *Agrostis* had highest concentration in roots (49 and 37 mg/kg) respectively.

Lead and copper showed similar trends which differed from those of Zn and Cd. *Armeri* had the highest, Pb and Cu concentration in the roots (1760 and 141 mg/kg respectively). The values of Pb and Cu were ten times higher than in the stem and 88 times and 18 times higher in the leaves respectively. *Agros* had the highest Pb and Cu concentration in roots 471mg/kg and 65mg/kg respectively. The leaves had 67 mg/kg Pb and 17mg/kg Cu whereas the stems had 50mg/kg Pb and 12,5mg/kg Cu. *C Halleri* had the highest Pb concentration in the roots whereas the Cu concentration in the different tissues was identical. The metal content was also determined in the brown and green leaves of *Armeri Halleri*. The findings were that the concentration of all metals in the brown leaves was higher than in the green leaves suggesting the presence of detoxification mechanism by leaf fall.

2.3.2.2 Wetlands and paddy fields

A study done by Gaur *et al*(1994) showed the effects of seven heavy metals on two vascular plants. The plants were collected from a paddy field in Kohima, India. Plants were cultivated in the laboratory and AR grade salts were used for accumulation and toxicity studies. In this study it was found that the plants had a tremendous ability to remove metal ions from the solution over a wide range of concentration.

Another study was done in India by Fazeli *et al*(1998), the uptake of heavy metals in paddy crops irrigated with paper mill effluent was compared with paddy crops irrigated with natural waters which were assumed to be unpolluted.

Table 2.11) (Fazeli, 1998) Heavy metals in soil and different part of the paddy crop from polluted and unpolluted areas.

	Cu	**Zn**	**Pb**	**Ni**	**Co**	**Cd**	**Cr**
	ppm						
Soil	32	45	42	25	21	0.9	134
Soil (a)	49	52	59	39	38	2.6	320
Root	22	16	29	14	15	0.6	31
Root (a)	34	15	35	21	27	1.3	48
Leaf	9	12	7	18	11	0.8	16
Leaf (a)	13	15	12	26	21	1.8	29
Seed	18	17	4	4	2	0.1	9
Seed (a)	15	19	9	9	8	0.2	14

(a) – Polluted region

The findings of this study were that the discharge of paper mill effluent into the agricultural land had significantly altered the uptake of toxic heavy metals into the paddy crop tissues. Rice is the common diet for people in the tropical countries and the consumption of polluted rice can lead to carcinogenic and mutagenic effects.

Mays *et al*(2001), studied the composition of heavy metal accumulation in a natural wetland and a constructed wetland receiving acid mine drainage. Wetlands act as a sink for toxic metals found in acid drainage. The growing concern is the adverse effects on the food web these accumulated elements in the constructed wetlands will have. The Tennessee Valley Authority (TVA) constructed two aerobic wetlands in the southeastern USA for treatment of acid mine drainage. These two constructed wetland and a natural wetland were used in the study.

Water, plant and sediment sample were collected from the wetlands. The plants were divided into shoot/leaves and root/rhizome components and oven dried at 65° C for 96h. The metal concentrations in the plants were not significantly correlated with sediment or water metal concentrations.

A study done by Del Rio and Font (2002), investigated the uptake of heavy metal and arsenic by wild vegetation in the Guadimar river area after the toxic spill of the

Aznakollar mine. The aim of the study was to identify a plant, growing in the contaminated soil, as a metal accumulator species. A total of 99 different plant species were studied. Soil and plant samples were also collected from an uncontaminated area. The range of heavy metal found in the plants was as follows: Pb (undetectable limits to 450mg/kg), Zn (13 to 1138mg/kg), Cu (1,2 to 152mg/kg), Cd (undetectable limits to 9,7 mg/kg), As (0,8 to 120 mg/kg).

Eleven plant species were selected for their efficiency in accumulating one or various pollutants. '*Amaranthus blitoides*' with a maximum biomass in contaminated soil (2420g/plant fresh weight) was considered the most promising species to be used for the 'phytoremediation' purpose.

2.3.2.3 Rivers, lakes and streams

The heavy metal concentration in aquatic plants was also studied by Giulio *et al* (1985), in the Chesapeake Bay, USA. The plants, clam and sediment were analysed for Cd, Pb, Cu and Zn.

Another study done by Reimer *et al* (1993) was on the concentration of zinc and chromium in aquatic macrophytes in Canada. The investigation was to obtain levels of zinc and chromium in four species of aquatic macrophytes collected from the lakes in Ontario. Another part of the study was also to investigate the possible relationships in aquatic plants and water and sediment in lakes of varying contamination.

Aquatic plants have been used extensively in monitoring of heavy metal pollution. (St-Cyr *et al*, 1994) studied trace metals in submerged plants of the St. Lawrence River in Canada. The submerged vegetation were analysed for Cr, Cd, Cu, Ni, Pb and Zn. The aquatic plants were found to offer the potential as biomonitor species reflecting the contamination of the environment with trace metals.

Kasim *et al* (1997), studied the heavy metals in water, sediment and aquatic plants of the Euphrates River in Iraq. Their study was done seasonally during 1993.

Water, sediment, and aquatic plants were collected seasonally at five sites in the upper region of the investigated river. The concentrations of the heavy metals were determined in the samples using flameless AAS.

The findings were that in water a high concentration of heavy metals was found during autumn and all metal concentration were lowest during winter and spring. In the aquatic plant all studied metals accumulated in higher concentration to that of their environment, water and sediment. Zn and Mn were found at higher concentrations in all the plants studied. The ability of the plants to accumulate metals varied because of several factors such as metabolic activity, root system and age of plant, as well as the amount of metal in water and sediment.

Szsymermanowska *et al*(1999), studied the heavy metal concentration in three lakes in West Poland. The high concentration of some elements in plants relative to the surrounding water led many to believe that the plants were accumulating elements from the water and thus 'aquatic macrophytes were thus proposed as pollution monitoring organism'. Their aim was to investigate the use in biomonitoring of some aquatic macrophytes collected in three lakes which received untreated municipal waste.

Plant, sediment and water samples were analysed. The water samples were filtered and the heavy metal content was analysed using the ICPES and AAS with graphite furnace. The plant material was dried at 60° C and then pulverised. The plant material was digested with nitric acid and hydrogen peroxide. The sediment samples were digested using a similar method as the plant samples.

Plants were divided into three groups:

- to calculate a relation between concentration of elements in water and plant
- to calculate a relation between concentration of elements in bottom sediment and plant

- to calculate a relation between concentration of elements in bottom sediment, water and emergent plant.

A strong positive correlation was observed between concentration of Cr in water and plants, concentration of Cr in bottom sediment and plants were characterised by plant in all groups. The first and second group were characterised by a positive correlation between Cd in water and plants, and Cd in bottom sediment and plants. Plants from the second group accumulated Cr at a higher rate from bottom sediment than from water. The rate of accumulation of Cr in the first group was higher with an increase in chromium in water. Cd concentration in plants in the first and second group increased with a higher ratio with an increase in its concentration in bottom sediment, than when accumulated from water. The positive correlation for Cr and Cd indicate that the plant species examined may be very useful for monitoring pollution caused by these elements.

The biomass production, Pb and Mn content in water and bottom sediment gave a negative correlation. The increased concentrations of heavy metals in plants seem to reflect the increased concentration of these metals in the environment. The aquatic macrophytes examined may be useful for monitoring Cr, Cd, Ni and Zn pollution.

Hozhima *et al*(2001) studied the uptake of heavy metals by aquatic plants in the vicinity of the ore mining and processing industries. The aquatic plants studied were *Typha Latifolia, Scirpus sylvaticus* and *Equisetum fluviatile*. The work was done at two ore refining factories in Russia (Salair and Komsomolak). The plant *Phragmites australis* was also investigated at Komsomolsk.

The plants collected at the ponds were separated into aboveground and belowground parts. Analysis was also done on the water and sediment. The plant material was digested with an acid mixture (1:4 v/v conc. H_2SO_4:HNO_3). The concentration of the elements was determined by atomic absorption spectrometry (AAS) using air –

acetylene flame with deuterium background correction and flameless AAS with Zeeman background correction.

In Salair the concentration of Pb, Zn and Cd in *Equisetum* exceeded that in the control area by a factor of ten and more. The Cu concentration was found to be twice as high. The concentration for Pb, Zn and Cu in *Typha* differed for each particular lake. The concentration of Pb varied from 7,5 to 2400ug/g dry weight. The Cu and Zn concentrations ranged from 33 to 4300 ug/g which exceeded the concentration in the control area by a factor of ten. Cd also exceeded by a factor of 100 ranging from 1, 0 to 19 ug/g dry weight. The accumulation of heavy metals was found to be in the roots, indicating a high content of mobile species in sediments or the existence of protective barriers, preventing the toxicant from the roots into the aboveground plants parts. In Komsmolsk the findings were that the habitat contained high amounts of As and Sb. It was concluded that the As and Sb concentrations in *Typha* and *Scirpus* were equal to or higher than in the sediment, The As and Sb are 0, 15 and 1, 5 ug/g fresh *Typha* respectively.

Samecka-Cymerman *et al* (2001), studied the concentration of heavy metals and plant nutrients in water, sediment and aquatic macrophytes of anthropogenic lakes differing in stage of acidification. Three lakes with a more neutral pH (numbered 1-3) and six acidotrophic lakes (numbered 4-9) were selected for the study. The study was carried out in West Poland.

Water, bottom sediment and plant samples were collected in triplicate. The leaves were separated from the plants. The moss, leaves and sediment were dried at 60° C and then homogenised. The samples were digested with nitric acid and hydrogen peroxide. Cd, Cu and Pb were analysed using AAS with graphite furnace and the rest of the heavy metals were analysed using ICPES.

One of the plant species, *Drepanocladus aduncus* growing in the Polish lake with the lowest pH contained the highest amount of Cd, Co, Cr, and Fe. Fe:Al ratio ranged from 0.3 in *Phalaris arundinacea*(lake 6) to 116 in *Juncus bulbosas* (lake 9). High concentration of heavy metals in the plants reflected high concentration of metals in the environment. Some plant species can be used in the chemical quantification of the environment of the examined lakes.

Arribere *et al* (2002), investigated the heavy metal in the vicinity of the chlor-alkali factory in the upper Negro River ecosystem, Argentina. The factory produced chlorine and NaOH using mercury as liquid cathode and was in operation from 1951 to 1995. The effluent from this factory contained high quantities of mercury in the inorganic form. The effluent contaminated the 'Alto Valle' ecosystem along the river pathway. The 'Alto Valle is an artificial irrigation region whose economy is based on agriculture i.e. fruit production and processing. The quality of water directly affected the quality of the production. The investigation was carried out on sediment from the river, two macrophytes (*Potamogeton pectinatus* and *Myriophyllum brasiliensi*) and, the liver and muscle of a native fish.

Instrumental Neutron Activation Analysis was used to determine twenty eight trace and minor elements. Total mercury was measured using Cold vapour atomic absorption spectrophotometry. The digestions of sample for mercury analysis were carried out using nitric acid. Their findings were that both the macrophtyes and fish livers might be bioindicating the presence of heavy metals in some available form introduced in the river through the drainage channels.

Cardwell *et al* (2002) studied the metal accumulation in aquatic macrophytes from southeast Queensland Australia. It was postulated that urban streams receive metal contamination from a variety of sources i.e. sewage discharge, runoff from metal-related industry and urban stormwater runoff. The heavy metals in the aquatic system eventually become associated with particulate matter which settles and accumulates in the bottom sediment. Contaminated aquatic vegetation is a source of food for a number of herbivores leading to the bioaccumulation of metals in the higher trophic levels. Cd, Cu, Zn, and Pb were chosen for this study. Cu and Zn are required by the plants for survival and Pb and Cd are toxic metals not required by the plant.

The objective of the above study was:

- To quantify the elements studied in aquatic plants and examine a relationship between sediment metal concentration and plant metal uptake
- To identify a species accumulating significant higher amounts of the four metals examined
- To study the extent of movement of the studied metals into different structural and internal tissues of the plant.

Sediment and plant species were collected from six sites. Metal accumulation between the aquatic macrophytes is known to vary. Fifteen aquatic macrophytes were collected from the six study sites. Table 2.12 in appendix 4 describes the plants investigated.

The plant material was then separated where appropriate into roots, stems, leaves and reproductive tissue. The samples were oven dried overnight at 80° C and finely ground. The heavy metals were analysed using AAS. The mean heavy metal content for the sediment samples collected was in the descending order of Zn>Pb>Cu>Cd. Data obtained revealed that sediment metals concentration were generally lower than resident plant root concentration for all metals. Zinc was the only metal to exhibit a relatively clear pattern of increasing accumulation in aquatic macrophytes with increasing sediment metal concentration. The emergent aquatic macrophyte roots were found to accumulate greater concentration of metals than stems and leaves. The plant that accumulated the highest amount of heavy metal was the submerged species *M aquaticum.* The submerged species in this study exhibited higher metal concentration than the emergent species.

Samecka – Cymermann *et al* (2002), studied heavy metals in aquatic bryophytes from the Ore Mountains in Germany. The mountains consist of various ore deposits of Ag, Sn, Pb, Zn, Ni, Cu, Co Ba, F and W which were intensively exploited by the mining industry. The aim of their study was to assess level of water pollution by monitoring the metal accumulation in native species of the aquatic bryophytes *Platyhypnidium ripariodes, Scapania sp. and Fontinalis antipyretica* and to compare the metal accumulation with those bryophytes from unpolluted areas.

Water and aquatic bryophytes were collected in triplicate from the streams in Erzgebirge. The water samples were filtered and analysed for the heavy metals on the ICPES and Cu, Cr and Pb were analysed on the AAS with graphite furnace. The bryophytes were washed thoroughly and dried at 40° C and then homogenised. The plant material was digested in duplicate using nitric acid and hydrogen peroxide.

Platyhpnidium riparioides exceeded the background values, (Cd 195mg/kg), Cu 33mg/kg), Zn (22500mg/kg), Pb (595 mg/kg) and Co (140mg/kg). An increase in heavy metals in the plants seems to reflect an increase in heavy metal in the environment as indicated by the significant positive correlation obtained for Al, Ba, Ni, Zn, Sr and Cd in water and plants. It was found that Cu in bryophytes depends on the joint influence of Ni and Cd in water, Pb depends on the joint influence of Zn, Mg and K in water and Zn depends on the joint influence of Mn, V, Zn, Mg and K in water. Elements present in the environment influence element concentration in the plant.

In this study it was found that the aquatic bryophytes could be used in the evaluation of exposure of the environment to contaminants. The investigation also indicated that, the remnants of high pollutant loadings released in the 19th century are still present in the Erzebrige environment.

Klumpp *et al* (2002), studied the variation of nutrient and metal concentration in aquatic macrophytes along the Rio Cachoeira in Bahia (Brasil). Cocoa is an important crop in Bahia , however, due to climatic conditions the tree is susceptible to fungal diseases. Cuprous fungicide is used to control the fungal diseases. Rio Cachoeira is a river that crosses the cocoa plantation. The degradation of this river represents a possible health risk to riverine population which makes use of the river for fishing, food resource for cattle, washing of clothes, bathing and watering of garden-grown vegetables.

In this study two aquatic macrophytes (*Eichornia crassipes* and *Pistia stratiotes*) were investigated. *Eichornia* was chosen for the study because of its abundance and large biomass produced. The plants were collected along the river course to investigate possible Cu, Cr, and Al contamination of the river ecosystem. The study area extended upper 50 km of the river and sampling was done at six sites along the river course. *E. crassipes* was separated into tops and roots. The plants were dried at 70° C and ground for analysis. The plant material was digested with nitric acid /perchloric acid (5:1). The Cu, Cr and Al concentration was determined using atomic absorption spectrometry.

Pistia showed high concentration of Cu at the Itabuna (downstream from the city) and in the tributary water. Cu in macrophtyes and river water were not as high as expected. The Cu concentration in roots was higher than in tops – low uptake and translocation of Cu in plants. They conclude that their results demonstrated that there is an input of metals going into the river from agricultural (Cu), industrial (Cr) and urban sources (Al, Cu).

2.3.2.4 Sea

The concentration of metals in a variety of seaweeds from the Vostok Bay, Sea of Japan, (Saenko *et al*, 1975) was carried out. In this survey it was found that several plants were specific in the type of metals they accumulate, Mn being one of the metals studied. It was found that seaweeds of the order *Ceramialos* concentrated manganese to a high degree while *Chondrus yendoi* from another order concentrated manganese to a lesser degree.

The lead concentration in *Zostera marina* was studied, (Lyngby *et al*, 1983) in Denmark. In this study the highest lead concentration in below and above ground parts was found in the city of Aalborg (the largest city in the area). It was also found that eelgrass can be used as an indicator organism of trace metal contamination and bioavailability in coastal areas.

A study was done in Indonesia, (Nienhuis, 1986), where the background levels of heavy metals in nine tropical seagrass species were investigated. Heavy metal pollution from a variety of sources increasingly threatens the tropical marine environment and background data from unspoiled areas is needed to act as a reference for samples from polluted areas. The nine seagrass species were collected from pristine coastal localities in the Flores Sea, Indonesia. The leaves, shoots etc were analysed and the results are shown in the table 2.13 listed in appendix 3.The results obtained in this study can be used for background data from pristine tropical coastal areas. However, it was found that more data over the years are needed.

Study on copper uptake in plants was done by Carter *et al* (1992), where the seagrass '*Zostera muelleri*' was investigated as a sentinel accumulator for copper. The seagrass was supplied with a range of copper concentrations, ranging from those found naturally to levels indicative of severe pollution. It was found that the leaves were the major site of accumulation for copper.

The determination of manganese in '*P. oceanica*' was done by Catsiki *et al* (1992). This study was done to obtain background levels of heavy metals in the East Mediterranean. It was found that Mn accumulated more in the leaves of '*P. oceanica*'.

A study done in the NW Mediterranean Romeo *et al* (1995), showed the accumulation of Zinc in the scales of seagrass '*Posidonia oceanica*'. The study was done at two areas, Cote d Azur (new and old pleasure boat harbours) and Corsica (a protected area in a natural park). It was found that the zinc concentration in scales from Corsica were lower than in Cote d Azur. A study was also done in north western Mediterranean, Warnau *et al* (1995). In this study heavy metals was determined in the seagrass, *Posidonia oceanica* from three different sites subject to different pollution conditions. It was found that two of the meadows although subject to different pollution status showed very similar patterns of metal levels.

The above discussion is pertinent to the present investigation in the following areas:

- the plants investigated
- the area studied
- instrumentation used for analysis
- sampling and digestion of samples.
- The identification of a heavy metal accumulator species
- To determine a correlation between the plants, water and sediment
- Plants were also used to give an indication of the contamination of heavy metal concentration in the vicinity.

2.4 Factors affecting the accumulation of metals

The processes by which heavy metals are removed in a wetland are physical, chemical, biological and biochemical. The processes occur in the water, biota, substratum and suspended solids. The difficulty associated, with what actually occurs or which reaction takes place is due to processes such as sedimentation, complexation and cation exchange being dependent on each other, thus making the process of heavy metal removal very complex (Matagi *et al*, 1998).The knowledge of the interaction between trace metals, water, sediment and aquatic plants is unfortunately incomplete. This lack of information makes it difficult to offer a comprehensive discussion of all the factors affecting uptake of metals by aquatic plants (Guilizzoni, 1991).

2.4.1 Sedimentation and flocculation

The role of organic matter connects the behaviour of heavy metals in an aquatic ecosystem. A large number of functional groups (e.g. humic and fulvic acids) have an impact on how dissolved organic matter bonds with heavy metals. In an aquatic system when metals are bound to organic and inorganic compounds they settle in the sediment (Guilizzoni, 1991).The transport of heavy metals may be from the water to sediment or biota or suspended solids and vice versa. Particles that are denser than water would settle out in calmer waters. Lighter particles settle after floc formation. The clay and organic material particles have electronic charge aggregate to form flocs, which adsorb the heavy metals. Flocculation is enhanced with increasing pH, turbulence, concentration of suspended matter, ionic strength and high algal growth. Small particles have a greater surface area than large particles and therefore flocs more easily with greater adsorption potential (Matagi *et al*, 1998).

In Kassim's (1997) study the suspended particles had much higher concentration in all the metals studied, most of the particles consisted of planktonic organisms which are well known in their ability in metal accumulation by a factor of 10^6 or even more. The high river current also allows for only a small part of the suspended particles to settle down and thus results in lower heavy metal concentration in the sediment than that found in suspended particles. The high current causes the particle size of the suspended solids to decrease thus giving more chance for metal adsorption in the suspended solids. Di Guilio (1985) suggests that a measure of particle size could be used to normalise sediment samples for variability in metal concentration.

2.4.2 Adsorption

When the organic molecules which bind the heavy metals decompose, or when sediment conditions change, the speciation of heavy metals may change. The properties of heavy metals that affect adsorption are valence, radius, degree of hydration, and co-ordination with oxygen. Other factors affecting adsorption are redox status, pH, complex forming ligands, concentration and properties of other metals and the presence of soluble ligands. Pb and Cu tend to be adsorbed more strongly than Zn, making zinc more labile and bioavailable. Plants translocate oxygen from the shoots to the roots, which leak the oxygen in the reduced environment. The oxidise conditions promote precipitation of oxyhydroxide of Fe^{3+} and Mn^{2+} which act as absorption sites for other heavy metals present in the water (Matagi *et al*, 1998).

Romeo (1995) suggested that the high concentration of lead is due to the adsorption of lead released from sediments onto the cellulose cell wall of the '*Posidonia oceanica*' scales. Catsiki (1993) mentions that greater surface area per unit of weight of roots increases the adsorption capacity of heavy metals.

2.4.3 Co - precipitation

Cu, Mn, Ni and Zn are co-precipitated in Fe oxides whereas Ni, Pb, and Zn are co-precipitated with Mn oxide. Fe^{3+} has a large surface area and acts as a scavenger for cations (heavy metals) and anions such as AsO_4^{3-}. The oxidation of FeS_2 causes acidification of wetland soils which causes, the heavy metals to go back into solution. The dissolution of Mn and Fe oxides by reducing conditions is likely to cause an increase in heavy metal concentration (Matagi *et al*, 1998). Romeo (1995) mentions that low Zn content can be due to diffusion from the nearby sediment and co-precipitation with iron may have also taken place in the oldest scales of *P. oceanica.*

Ahumada *et al*(1999) studied the sequential extraction of heavy metals in soils irrigated with wastewater. The purpose of their study was to characterise the chemical forms of Cr, Ni, Zn, Pb, and Cu in soils that have been irrigated with wastewaters of domestic and industrial origin for more than five decades. One area was cultivated with lettuce and the other with celery. The pH, organic carbon, and cation exchange (CEC) was determined. Mn, Fe and Al free oxides were extracted and total metal determination was done using AAS. The extraction procedures used are listed in the table below.

Table 2.14 : Sequential extraction of heavy metals in soil, Ahumada *et al*(1999)

Forms	**Extractants**	**Equilibrium**
Exchangeable	1M $MgCl_2$	1 hour
Carbonate occluded	1M NaOAc	5 hours
Mn-oxide occluded	NH_2OH-HCl + 0.01M HNO_3	30 minutes
Organically bound	$K_4P_2O_7$	24 hours
Fe oxide occluded	NH_2OH-HCl + 25% (v/v) HOAc	4 hours to 96 deg C

Their findings were that Cr had the lowest percent of extraction indicating association with silicates. The Cr extracted was associated mainly with iron oxides and to a smaller extent with organic matter. Cu bound to iron oxide is predominant in soil cultivated with celery while in soil cultivated with lettuce the metal associated with organic matter prevails. Ni associated with oxides is prevailing. Pb is associated with iron oxides and organic matter. Zn associated with iron oxide and organic matter prevails in soil cultivated with lettuce while Zn associated with iron oxide prevails in the soil cultivated with celery.

It was found that Cu in the roots is four to five times higher than in the shoots. Cr content found in celery was higher than in lettuce with the highest concentration found in the roots. In celery the lead is distributed equally in the roots and shoots, while in lettuce twice the amount is found in the radicular part than in the aerial part. Most of the zinc is concentrated in radicular part of the plant. The relation between the metal content in the roots and shoots of the plant and the different metallic forms was determined through linear correlation (Table 2.15).

Table 2.15: Linear correlation between heavy metal found in roots and shoots of celery and lettuce and the same metals found in the different forms in soil, Ahumada *et al*(1999)

	Celery		Lettuce	
Element	**Chemical form**	**R (Correlation Coefficients)**	**Chemical form**	**R (Correlation Coefficients)**
	Roots		**Roots**	
Cu	Ox Fe	0.68	Exchangeable	0.50
	Organic matter	0.54	Carbonate	0.68
			Ox Mn	0.57
Zn	Organic matter	0.79	Exchangeable	0.63
			Carbonate	0.71
			Ox Mn	0.69
			Ox Fe	0.64
Cr	-	-	Organic matter	0.53
Ni	-	-	Organic matter	0.78
Pb	-	-	Carbonate	0.70
			Ox Mn	0.91
			Ox Fe	0.87
	Shoots		-	-
Cu	Organic matter	0.61	-	-
	Ox Fe	0.76		
Cr	Ox Fe	0.67	-	-
Zn	Organic matter		-	-

It was found that in lettuce Cu and Zn were found in exchangeable form, Cu and Pb as carbonate, Pb and Zn associated with Mn oxides, Cr and Ni complexed with organic matter and Pb and Zn bound to Fe oxides.

2.4.4 Precipitation

The insoluble heavy metal precipitate that forms, limits the bioavailability of heavy metals to the aquatic ecosystem. The solubility product Ksp of the metal specie, pH and concentration of metal ion influences precipitation.

$MX_2(s) \rightarrow \quad M^{2+}(aq) \quad + \quad 2X^{-}(aq)$

$Ksp = (M^{2+})(X^{-})^2$

At equilibrium the rate of removal of metal ions as precipitates equals the rate of dissolution from precipitates. When the product of the concentration of cations and anions exceeds Ksp, precipitation occurs. Carbonates, hydroxides and sulphides of heavy metals are precipitated under reducing conditions (Matagi *et al*, 1998).

2.4.5 Cation- Anion exchange

Due to the negative charge on sediment colloid, cation exchange occurs. This results in the exchange of hydrogen ions with the metal. Anion exchange is the attraction of anions to the positive charge on sediment colloid. The replacing power of the anion or cation will depend on its valence, diameter in hydrated form and concentration of other ions present. Ions with a larger hydrated radius have a lower replacing power than ions with smaller radii (Matagi *et al*, 1998).

2.4.6 pH

A pH of 3.7 to 4.5, favours the dissolution of carbonates and hydroxide minerals, thus freeing the associated metal (Guilizzoni, 1991). Lakes that had the lowest pH contained the highest concentration of heavy metals in the water and sediment with the lowest pH had the highest concentration of heavy metals (Samecka – Cymermann, 2001). Reimer (1993) mentions that in areas of high sulphur content the unbuffered lakes became more acidic increasing the solubility of Zn. Langewerf (1971) found that an increase in soil pH from 5.9 to 7.2 resulted in a decrease in metal content of the radish (Table 5.2). This could be attributed to a possible decrease in solubility and /or mobility of the metals because of the change in matrix.

Table 2.16: Percent of total amount of metals in radish attributed to aerial contamination, Langewerf (1971).

	pH 5.9		pH 7.2	
	Tops	**Roots**	**Tops**	**Roots**
Soil				
Pb (L)	48	*	40	*
(M)	45	*	39	*
(H)	39	*	29	*
Cd (L)	48	*	49	*
(M)	45	*	39	*
(H)	41	*	33	*
Zn (L)	50	42	47	43
(M)	43	37	45	36
(H))	33	32	41	31

* Negligible

Scheirup and Larsen (1981) mention that generally a low pH, high Eh and low CEC values favour the existence of easily available heavy metal forms.

2.4.7 Seasonal variations

Seasonal changes also affect metal uptake. As discussed in chapter 2, Ray *et al* (1979) studied the seasonal variation in *Equisetum Arvensis.* In order to draw effective conclusion, seasonal variations should be taken into consideration. There is also evidence of this for Mn, Cu and Zn in *Typha latifolia.* It was found that large amounts of Zn, were translocated from the roots and rhizome to leaf and shoot growth in spring. The movement was reversed in autumn. The seasonal variation was affected by light and temperature. The bioavailabilities of trace elements are not accumulated at a constant rate all year round (Outridge and Noller, 1987). Lyngby and Brix (1982) found that the highest heavy metal in *Zostera Marina* was recorded when the growth had ceased and a distinct decline was observed at the beginning of the growth season. St-Cyr (1994) reports that an increase in trace metal concentration could be attributed to an increase in biomass during the growing season. Maximum concentration noted when growth had ceased.

2.4.8 Chelating agents

The uptake of heavy metals is also influenced by organic chelates. Different synthethic and natural organic chelates varied in the uptake of heavy metals. It is mentioned by Gauer (1994) that natural waters contain several complexing agents such as (humic acid and fulvic acid) which can chelate metals thus reducing the concentration of free metal ions. Deram (2000) studied the effect of EDTA on two growth mediums. There was a dramatic increase in copper and nickel concentration in '*Arrhenatherum elatius*' when EDTA was added to a growth medium of copper ore. Cu increased from 200 to 7500 mg/kg dry weight and nickel increased from 8 to 1276 mg/kg dry weight. The addition of EDTA to the mine base metal tailing resulted in an increase in Pb concentration in *A.Elatius* from <100 mg/kg dry weight to about 25000 mg/kg dry weight. It was also noted that raising the pH of the growth medium rendered Pb less mobile.

2.4.9 Redox potential

A negative potential is exhibited at an anoxic-water interface releasing heavy metals for example Cu and Zn. The competition between dissolved cations and adsorbed heavy metal ions partially decreases the heavy metal concentration (Guilizzoni, 1991).

2.4.10 Water hardness

The toxicity of heavy metals is reduced in high carbonate waters due to inorganic metal carbonate being less toxic to other metal species (Guilizzoni, 1991).

2.4.11 Salinity, light and microbial activity

The competition between dissolved cations and adsorbed heavy metals ions partially decreases heavy metal concentration. Light enhances the toxicity of metals and microbial activity enhances the release of metals (Guilizzoni, 1991). As mentioned by Brix (1983), several investigations have indicated that factors such as temperature, salinity and seasonal variation have a significant influence on the concentration of heavy metals.

2.4.12 Presence of other ions

The uptake of trace element by various species was influenced by the presence of other ions. For example there was a decline in uptake of some metals at high concentration of Ca and Mg in water. The rate of uptake of heavy metals is also influenced by the atomic weight of the ion. High molecular weight ions (Hg and Pb) were concentrated by *Elodea nuttalii* more effectively than lower atomic weight ions for example Cd and Ni, (Guilizzoni, 1991).

2.4.12 Ferric oxide 'plaque'

The change in metal bioavailibity in sediment by aquatic plants is due to the species oxidising their roots zones through diffusion of oxygen from roots or secretion of oxidising compounds resulting in the formation of a ferric oxide 'plaque' on roots. The plant is protected against phytotoxins such as reduced Mn and Fe. The plaque will critically affect the metal uptake, for example the Zn entry in *Aster trifolium* was restricted only up to a point were the plaque became saturated with Zn. Above the threshold point the plaque may act as a concentrated source of metals for the root (Outridge and Noller, 1987). Cardwell (2002) mentions that some metal found in the root tissue samples may be associated with iron plaque, a co-precipitate of iron oxyhydroxides which can co-precipitate other metals such as zinc and copper.

2.4.14 Age of plant species

The comparison of heavy metal uptake in macrophytes is often difficult because of differences in sampling time (age of the plant) and tissue analysis (growing tips, whole plant). Metal data therefore cannot be extrapolated from one species to another or even within the same species. Nienhuis (1986) mentions that the age of leaves and greater surface area per unit weight in roots compared to rhizomes results in a larger absorption capacity. St – Cyr (1994) mentions that the collection of species of differing age and the sampling of plants at slightly different stages in their growth cycle can cause within site variability in heavy metal concentration. Plants with longer stems were also noted to have a lower overall trace metal concentration in their aboveground parts than do rosette type plants. High surface to volume ratios are known to favour metal uptake from water

CHAPTER 3

EXPERIMENTAL

3.1 Sampling location

The area chosen for investigation was the Northern Waste Water Treatment Works at Sea Cow Lake. The Northern works serves as a catchment area which is mainly domestic in character. However, leachates from the Bisasar Road landfill site is also pumped into the treatment works. The works is in the order of 10 000 ha encompassing the Durban North coastal area.

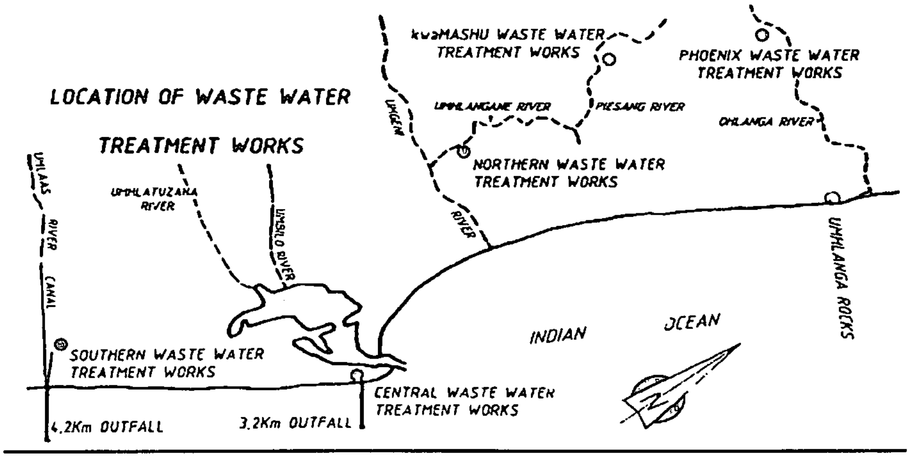

Figure 3.1) Location of waste water treatment works in Durban. (Durban Metro)

The sampling location comprises inter alia, a sewage treatment plant and a series of sewage maturation ponds. These different sectors will be discussed below. The Northern Wastewater Works, Durban, was chosen as the area to be used for this study because of it's:

- Aquatic environment
- Serves a large Durban population with the outlet to rivers and eventually the sea.

- Possible heavy metal pollution (industrial and domestic effluent, as well as leachates from Bisasar landfill sites)
- The availability of aquatic plants.
- Easy access to area

3.1.1 Sewage treatment

The complete treatment of the sewage is combination of mechanical, chemical and biological processes. The processes that occur at the Northern Water Works are described briefly below, see figure (3.2) in appendix 2 for a schematic diagram of the process.

3.1.1.1 Primary treatment

The waste water is passed through 'screens' that remove large particles example paper and plastic which are often referred to as rags. The rags are loaded onto a transport container which is removed when full and emptied at a landfill site at Shongweni. Before being loaded onto a transport container the rags are squeezed dried to reduce spillages and transport cost. The waste water then passes into a 'degritter' where the sand and grit is removed. The sand and grit that is removed is also sent to the landfill site. The waste water then goes into a 'primary settling tank' where the biodegradable solids 'primary sludge' can settle to the bottom of the tank and the suspended and dissolved matter flows over the top of the tank. Oil on the surface is also removed.

3.1.1.2 Secondary treatment

The 'primary effluent' undergoes a second treatment called an 'activated sludge' process where air is introduced into the system by mechanical aerators and the bacteria purify the liquid in the presence of oxygen. Biological treatment is where microbes are used to destroy or reduce toxicity of a waste stream. The transformations accomplished biologically are:

- degradation of organics e.g. phenols

- the reduction of inorganic substances e.g. nitrates
- complexation of heavy metals e.g. nickel.

The treatment can be divided into aerobic and anaerobic processes. Aerobic decomposition is the breakdown of organic waste in the presence of oxygen. This treatment is increasingly being used in the treatment of leachates from landfill site. After aeration the mixed liquor is passed into secondary settling tanks. The sludge containing the bacteria is separated from the 'secondary effluent'. Some of the sludge is returned to the activated sludge process to aid in the bacterial process.

3.1.1.3 Tertiary treatment

3.1.1.3.1 Maturation ponds

The 'secondary effluent' is passed into the first maturation pond and then flows through the other four maturation ponds which are set out in series (figure 3.5 in appendix 5). The maturation ponds are used for further purification of the effluent before discharge into the rivers. In countries with limited water supply the re-use of effluent is necessary and thus further purification may be required. Maturation ponds reduce bacteriological count, ammonia, nitrates and phosphates thus purifying the effluent and making it more suitable for discharge.

The importance of algae in pond operation needs to be considered, particularly in summer when they are abundant. The green pigment in algae fulfills the following functions:

- The oxygen supplied from photosynthesis prevents the pond from turning anaerobic too quickly.
- The plant material absorbs nitrogen hence decreasing the nitrogen content in the water.
- Due to a rise in pH, it is indirectly responsible for precipitating some of the phosphates from the water.

Temperature regulates the presence of algae in the ponds and at low temperature the removal of nitrogen and phosphates will decrease (The institute of water pollution control ,1973).

Figure 3.3) One of the Maturation ponds at Northern Waste Water Works.

The advantages of pond systems are:

- low construction and operational cost
- excellent final effluent quality
- effectiveness in hot and cold climates
- attractiveness for birdlife.

However the disadvantages as mentioned by Outridge and Noller (1991) are:

- monospecific cultures are susceptible to pathogenic infections which results in destruction of plants and loss of treatment efficiency
- the ponds require continual harvesting of plant biomass
- the disposal of plant biomass that contains high concentrations of toxic elements.

3.1.1.3.2 Chlorination

The water leaving pond 5 is chlorinated. The chlorine is added as a gas and the main purpose is to disinfect the water. The water leaving pond 5 is discharged into the

Umhlange River, then to the Umgeni River and finally into the Indian ocean at Blue Lagoon or sold to industry for re - use.

Figure 3.4) Outlet at pond 5

3.2 Sampling Collection

The plants, sludge and water samples were collected from the outlet of each of the five ponds at the Northern Water Works. The plants were collected in plastic bags. The water and sludge were collected in plastic containers. A flow diagram of the layout of the ponds is shown in figure 3.5 in appendix 5.

3.2.1 Sampling of plants

The four plants under investigation are the *Typha, Rumex, Pistia* and *Alternanthera.* These plants gave us a range of aquatic plants, i.e. free floating to submerged species. The plants were abundant and easily available in the study area. All the information on the description of the plants was obtained from Aston (1973), Cook (1974) and Leistner (2000).

3.2.1.1 *Typha capensis (Rohrb.)N.E.Br*

Typha a member of the family *Typhaceae,* is found in clumps or dense stands of erect, robust plants in ditches, swamps, lakes and watercourses, in fresh (sometime brackish) waters to about 1.5m deep. The plant (figure3.6 in appendix 6) consists of branched rhizomes with the shoots ending in stiff, erect leaf-bearing stems. The clum*s* are rigid, 1-3m high and 0.3-2cm in diameters. The *leaves* are mostly linear, erect with the open sheath closely enveloping the clum. The leaves of the plant arise close to the base of the clum and appear to arise from the rhizome itself. The blades are several cm to >1m long x 0.2-3 cm wide, tough and often spongy within with the male spikes above the female spikes. The clums are erect, simple and ending with dense, cylindrical flower-spikes with the male flowers zoned towards the upper portion and separated from the female flowers in the lower portion. The *flowers* are unisexual, cylindrical, velvety brownish.

3.2.1.2 *Rumex steudeli Hoschst. ex A.Rich.*

Rumex a member of the family *Polgonacea* is an herbaceous, emergent, rooted perennial (figure 3.7 in appendix 7). The *stems* are 1,5m long, hollow and swollen. The *stems* are about 15mm diameter between nodes. The *leaves* alternate and are erect. The blades are 30 cm long x 4,5 cm wide. The petiole about 9cm long and the upper leaves decreasing in size. The *flowers* are unisexual with the leaves absent or reduced on the flowering portion of the stem. The male and female flowers are both 2,5 – 4 mm long. The species is found in freshwater swamps, stream edges, rooted on mud or in water several cm deep

3.2.1.3 *Pistia stratoites L.*

Pistia a member of the family *Araceae* is a free floating plant that grows in a very wide variety of aquatic habitats (figure 3.8 in appendix 8). The overlapping leaves arises from a very short stem (from nearly absent to 2,5cm long) and several long hanging roots (up to approximately 45cm long). The *leaves* are pale green, 3-15cm long and up to 8cm wide. The leaves form a rosette, are green and are roughly egg shaped with the broadest part above. The flowers are small and hidden among the leaf bases having between 2 to 8 male flowers and a solitary female flower. The fruit is an ellipsoid, many seeded, berry with the seeds themselves being hard, subovoid and truncated at both ends.

3.2.1.4 *Alternanthera sessilis (L.) DC.*

Alternanthera a member of the family *Amaranthaceae* is an annual or perennial herb sometimes becoming woody at the base, being prostrate or erect to floating or scrambling, with leaves being at opposite sides (figure 3.9 in appendix 9). The stems are erect or floating (often swollen when floating), often rooted at internodes. The flowers are small each subtended by 2 membranous bracteoles. The flower arrangements are found in heads or short spikes with the flowers themselves being solitary, bisexual, producing lenticular seeds. Stamens (2-4) are usually alternating with short pseudostaminodes, anthers of 2-locular. The perianth segments are glabrous or hairy with the outer not terminating in a subulate tip.

The plants that were collected from each of the five ponds were *Pistia* and *Alternanthera*. The plants that were collected from the 'old sludge site' were *Rumex*, *Typha* and *Alternanthera*. In the laboratory the plants were rinsed thoroughly with tap water and separated. In *Alternanthera* the leaves were separated from the roots and stems. *Rumex* was separated into leaves, stems and roots. *Typha* was separated into aboveground and belowground parts. *Pistia* was not separated. The different portions of the plant were placed in brown paper bags. The paper bags were placed in the 60 deg C oven for two days. Once dried the plants were crushed in a mill and kept in labelled plastic vials.

3.2.2 Sampling of sludge

Sludge samples were collected from each of the five ponds, an 'old sludge site' (disposal site for sludge over the years) and a 'new sludge site' (a storage facility for sludge when there are problems with the belt press. When the problems are resolved the sludge is then processed in the belt press). The sludge samples were placed in porcelain dishes and oven dried at 60 deg C for two days. Once dried, the sludge sample was sieved through a 500 micron sieve to remove larger particles. The finer particles were crushed in a mill and kept in labelled vials.

3.2.3 Sampling of water

Water samples were collected from each of the five ponds. A water sample was also taken after chlorination. The water samples were kept frozen until analysis.

3.3 Materials and reagents

All glassware was soaked in 10 % nitric acid prior to use. Only A grade glassware was used for the analysis.

3.3.1 Reagents:

3.3.1.1) Nitric Acid - Reidel de Haen (70%)

3.3.1.2) Perchloric acid - ACE (70%)

3.3.1.3) HCl - BDH (32%)

3.3.1.4) 1000 ppm standards of Pb, Cr, Mn, Zn, Ni and Cu made up in 1N HNO_3 - Saarchem

3.4 Digestion

The digestion of each plant, soil and water sample was done in triplicate. A certified soil sample was also digested in triplicate. The acids used for the digestion were concentrated nitric acid, perchloric acid and hydrochloric acid. A blank digestion was also done using the acids and the level of heavy metals in the blank was subtracted from the sample reading.

3.4.1 Digestion of plant material

A sample of 1g was weighed in a 100ml beaker. Two glass beads were added to each beaker. Nitric acid (15 ml) was added and the sample was digested at room temperature for ± 12 hours. The beakers were placed in a sand bath and digested for 1 hour at 150°C. The beakers were cooled to room temperature and Perchloric acid (10 ml) was added to each beaker. Digestion at 235°C for two hours was carried out. The beakers were cooled to room temperature and HCl (5ml) was added. A further digestion at 150°C for 20 minutes was carried out. The samples were cooled and transferred to a 25ml volumetric flask. The flasks were made up to mark with double deionised water. The flasks were shaken thoroughly and the contents filtered through a 0, 45 micron filter into a volumetric flask.

3.4.2 Digestion of the soil material

A sample of 1g was weighed in a 100ml beaker. Two glass beads were added to each beaker. Nitric acid (15 ml) was added and the sample was digested at room temperature for ± 12 hours. The beakers were placed in a sand bath and digested for 1 hour at 150°C. The beakers were cooled to room temperature and Perchloric acid (10ml) was added to each beaker. Digestion at 200°C for two hours was carried out. The beakers were cooled to room temperature and HCl (5ml) was added. A further digestion at 150°C for 20 minutes was carried out. The samples were cooled and transferred to a 50ml volumetric flask. The flasks were made up to mark with double deionised water. The flasks were shaken thoroughly and allowed to stand overnight.

3.4.3 Preparation of water sample

A 95.0 ml water sample was acidified with 5.0 ml of concentrated nitric acid. The sample was then filtered through a 0,45 micron filter into a volumetric flask.

3.5 Instrumentation

The identification and quantification of heavy metals require analytical techniques that are rapid and sensitive. The accurate and precise concentration of trace elements can be determined because of the improvements and developments of new analytical techniques. The greatest advances are the use of computers for the control of various instrument functions, data manipulation and interferences corrections (Pierzynski, 1998). The following parameters should be considered when choosing an analytical technique:

- detection limit
- precision
- concentration range
- time and cost
- automation
- operating skills
- interferences
- difficulties with contamination.

The instrumentation used for the preliminary work were the ICP-AES (Inductively Coupled Plasma – Atomic Emission Spectroscopy), AAS (Atomic Absorption Spectroscopy) and GF-AAS (Graphite Furnace Atomic Absorption Spectroscopy). The detection limits for the analytical techniques that were used for the preliminary work are listed in the table below.

Table 3.1) Detection limit (ug/l) (Vandecasteele and Block, 1993)

Element	Flame AAS	ICP AES	GF-AAS
Ni	4	4	0.1
Cu	1	0.02	0.9
Cr	2	0.01	2
Pb	10	0.05	20
Zn	0.8	0.01	1
Mn	1	0.01	0.04

Due to the number of elements per sample and the large amount of samples, the routine analysis of the samples was done by Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS) conducted at the University of Natal (UND), Durban, South Africa. The instrument is located at the Geology Department. Some of the samples were also done on ICP- AES at the ML Sultan Technikon (MLS). The detection limit for ICP-MS is noted in appendix 10. Results obtained for the ICP-AES and ICP-MS are shown in appendix A and appendix B respectively.

According to Ivanova (2001), ICP – MS permits the direct determination of more than 30 elements in plants. According to Date and Gray (1989) the ICP-MS provides the following features which makes it an attractive technique for environmental trace – element studies:

- Multielement capability is excellent for the determination of a large number of elemental concentrations over the range of ug/g to sub ng/g.
- The detection limits in aqueous solution is generally below 0,1 ng/ml, desirable for evaluating water quality in fresh, groundwater and sea water samples.
- The lower detection limits for ICP-MS enables smaller sample sizes to be analysed, this avoids preconcentration steps which introduces contamination or analyte loss.
- Rapid, simultaneous analysis.
- Due to the direct determination of many heavy metals it provides an excellent facility for undertaking pollution studies.
- A large range of sample introduction techniques for example conventional pneumatic, ultrasonic and high solid nebulisers, flow injection, electrothermal volatilisation.
- The ability to determine isotope ratios.

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3.5.1 ICP – AES

The plasma is the source that is used to atomise the sample. The ICP consist of a quartz tube that has argon gas flowing through. Surrounding the quartz tube is water cooled induction coil that is connected to a high frequency generator (4-50) MHz. The flowing argon is ionised by a spark from the Tesla coil. The magnetic field that has developed induces an electron and an ion current that flows in a closed circular path. The support gas is heated to high temperatures and sustain the ionisation necessary for achieving a stable plasma.(Gas kinetic temperature - 5000 – 6000 K; Ionisation temperature - 7500 K; Excitation temperature- 6500 – 7000 K; Electron temperature - 10 000K) (Vandecasteele and Block, 1993).

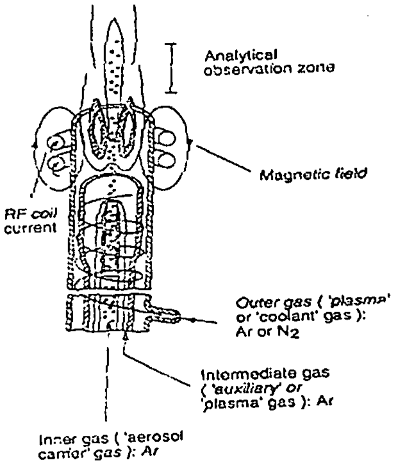

Figure 3.13) Inductively coupled plasma. (Vandecasteele and Block, 1993)

The reaction that occurs in the plasma when an aqueous sample containing MX is introduced are as follows.

H_2O (l) - H_20 (g)

$2H_20$ - $2H_2 + O_2$

MX = M + X

M + O = MO

MO - M + O

M - $M n^+ + e'$

The advantages of ICP-AES are:

- complete solute vaporisation
- low matrix effects
- low ionisation interferences
- simultaneous multi element analysis.

For efficient vaporisation and dissociation of aerosol high gas temperatures, small particle size and high residence time is required. The most common introduction of liquid sample in ICP is the pneumatic nebulization. The sample is introduced to the nebuliser by force feed i.e. peristaltic pump or a venture effect aspiration. A small portion of the sample is nebulised and the remainder flows through to waste. Matrix and chemical interferences are less in ICP AES than in flame AES due to, inert atmosphere, high plasma temperature and long residence time of analyte in the source.

3.6 Calibration

The quantitative analytical tool that is central to both ICP and AAS is the calibration curve (Chasteen , 1993). The calibration curve is a means of determining how an instrument response to different concentrations of analyte. The calibration is usually represented by an x-y plot of analyte concentration versus instrument respond. The standards that are used to calibrate response must have a concentration that is known as exactly as possible. The calibrations are performed on the same day that the samples are analysed to correct for day to day drift of instrument response. Even if the response from the instrument to the amount of analyte is almost completely linear - not all results fall on the line. Linear regression is used to find the best straight line. Samples with responses falling outside the linear range have to be diluted before being analysed. The concentration of sample is determined by measuring the instrument responses to the sample and referring to the calibration curve. An example of a calibration curve is illustrated below.

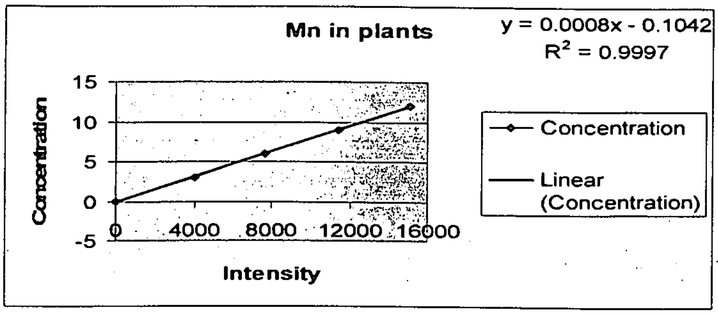

Figure 3.14) Calibration curve for Manganese.

3.7 Quality control

A quality control check sample (known concentration) for each element under investigation was run with the samples. The limitation of this study was the availability of a certified plant reference material. However, a certified sediment reference material (CRM), sample (SARM No.51) was used as a check. According to Stoeppler (1992) a CRM is used to:

- Demonstrate the accuracy of results obtained in the laboratory
- Monitor the performance of the method
- Demonstrate equivalence between methods
- Detect errors in the application of standardised methods
- Calibrate equipment which requires a calibrant similar to the matrix.

The values obtained for the certified reference material is tabulated below.

Table 3.2: Certified reference values

Element	Actual value (mg/kg)	ICP-AES (mg/kg)
Cr	509	313
Mn	1626	1524
Ni	200	212
Cu	268	268
Zn	0.22%	0.21%
Pb	0.52%	0.51%

As mentioned earlier Ahumuda *et al*(1999), chromium is extracted the least indicating association with silicates. The chromium values reported could be lower than the true value.

3.8 Typical analysis by ICP AES

The appropriate standards were prepared in 10 % HNO_3. The instrument was switched on for at least half hour prior to use. The wavelength for each element (Table 3.2) was set on the instrument. The argon gas was opened and the plasma was lit. Deionised water was aspirated to flush the tubes of any contamination. The highest standard was aspirated to optimise the photomultiplier tube. Once the instrument was optimised the blank and standards were aspirated and a calibration curve obtained. The samples were then run on the instrument and values obtained.

Table 3.3: Wavelengths used for each element

Element	**Wavelength**
Cu	324.754
Cr	205.552
Zn	257.610
Mn	213.856
Pb	220.353
Ni	221.647

3.9 Statistical analysis

The metal concentration in water is reported as mg/l. The metal concentration in the plants and sludge are reported as mg/kg dry wt and the means of three replicates were done. Any outliers were discarded and the better of two results were reported. The mean, standard deviations and relative standard deviations with a confidence level of 95% were calculated using statistical function available in the spreadsheet package Microsoft excel 97.

CHAPTER 4

RESULTS

To ascertain the distribution of heavy metals among water, plants and sediment of a pond, the concentrations of heavy metals in these phases were determined. Furthermore the plants were separated into roots, stems and leaves for purposes of analysis. The values obtained are discussed, using bar-graphs as an aid in detecting any trends in variation of concentrations of the metals in the plants, sludge and water.

4.1 Heavy metal concentration in water

The concentration (mg/l) of heavy metal in water that was sampled from the five ponds is shown in table 4.1. The results obtained for the chlorinated water is also listed in the same table.

Table 4.1 : Heavy metal concentration (mg/l) in Water

	Pb	Cu	Ni	Cr	Zn	Mn
Pond 1	0.006	0.006	0.003	0.003	0.051	0.043
Pond 2	0.016	0.004	0.005	0.004	0.109	0.082
Pond 3	0.012	0.004	0.005	0.004	0.08	0.067
Pond 4	0.005	0.003	0.002	0.002	0.031	0.034
Pond 5	0.005	0.003	0.005	0.002	0.062	0.068
Chlorinated	0.011	0.006	0.004	0.003	0.064	0.068

It can be seen from the results in table 4.1 that the highest concentration was obtained for Zn (0,109 mg/l), and the lowest concentration was obtained for Ni and Cr (0,002 mg/l). The concentrations of Mn and Zn were higher than those of the other heavy metals. The concentration of heavy metals in the chlorinated water was comparable to the concentrations of these metals in the 5 ponds.

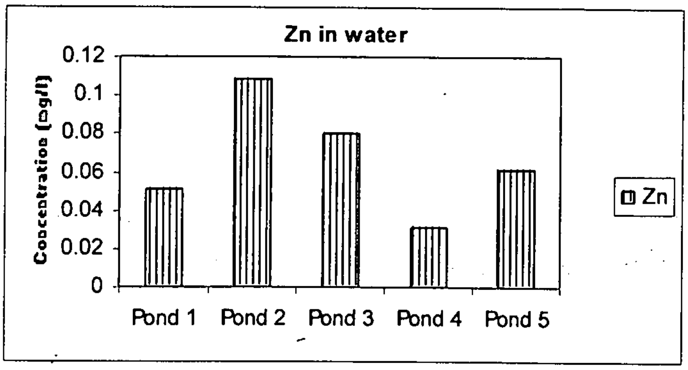

Figure 4.1) Zn concentration (mg/l) in water

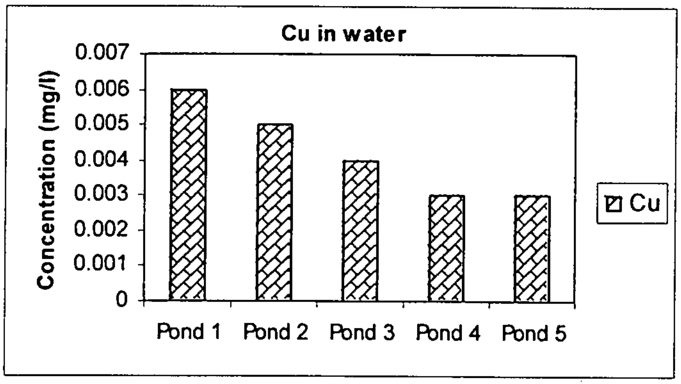

Figure 4.2) Cu concentration (mg/l) in water

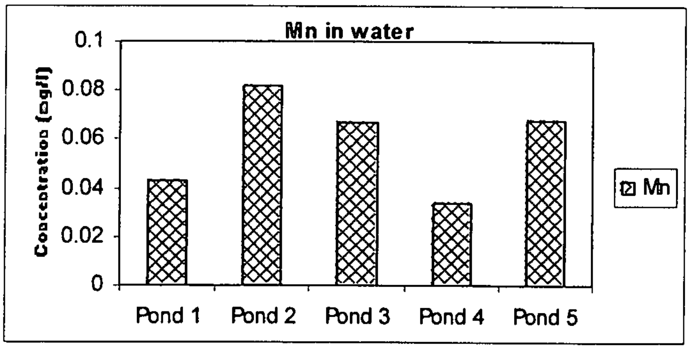

Figure 4.3) Mn concentration (mg/l) in water

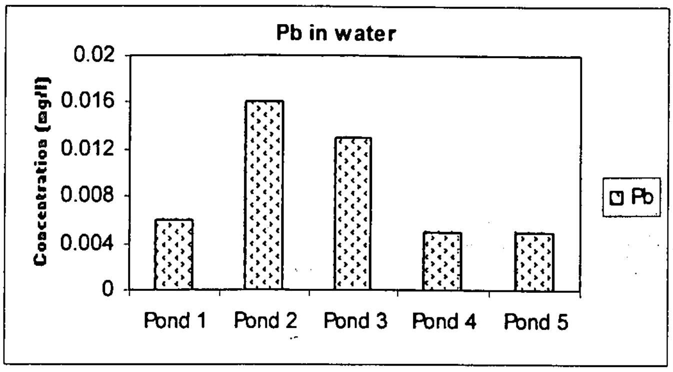

Figure 4.4) Pb concentration (mg/l) in water

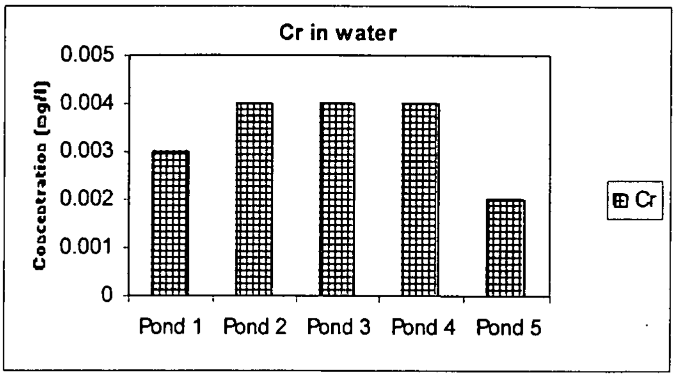

Figure 4.5) Cr concentration (mg/l) in water

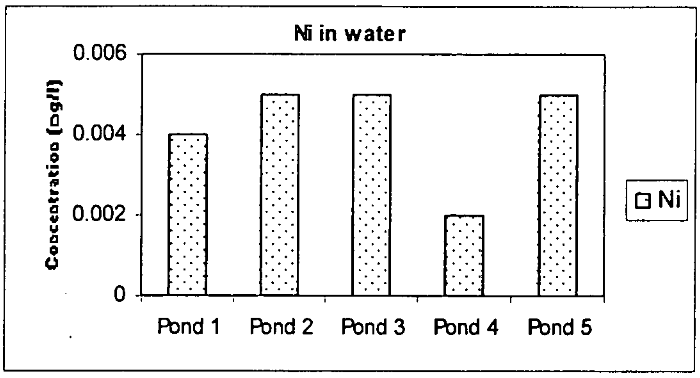

Figure 4.6) Ni concentration (mg/l) in water

The trend for Cu is an ideal trend since it is expected that in the water phase the concentration of heavy metal would decrease from pond1 to pond 5. However the rest of the heavy metals under investigation do not show this trend and this can be attributed to the interplay of factors such as sedimentation, co-precipitation,

precipitation and pH which are discussed in detail in section 2.4. The trend for Cu is the anticipated one on the basis that metals would partition between the water and sediment phase. The assumption used here is supported by the observation that the concentration of metals is much higher in the sediment/sludge compared to the water phase.

4.2 Heavy metal concentration in sludge

Table 4.2 lists the heavy metal concentration (mg/kg dry wt) in sludge sampled from the five ponds. The results obtained for the 'old sludge site' and 'new sludge site' are also listed in the table.

Table 4.2 : Heavy metal concentration (mg/kg) dry weight in sludge

	Pb	Cu	Ni	Cr	Zn	Mn
Pond 1	86	120	60	132	458	1543
Pond 2	44	65	149	115	311	699
Pond 3	45	90	217	109	332	1085
Pond 4	43	58	130	80	395	517
Pond 5	65	62	118	84	338	602
Old sludge	72	315	108	203	769	1221
New sludge	112	340	142	395	1367	474

From the results obtained in table 4.1 it is seen that the highest concentration of heavy metals was obtained for Mn (1543mg/kg dry wt) from pond 1. The lowest concentration of heavy metals was obtained for Pb (43mg/kg dry wt) from pond 4. The concentration of Mn, Cr and Zn in sludge from pond 1 is almost double to that obtained in pond 5. The lowest concentration of Ni was found to be in pond 1, which was contrary to the general trend that all the other heavy metals had their highest concentration in pond 1. This could be attributed to environmental factors which are discussed in section 2.4. The variation in the concentration of the six metals in the five ponds is shown in figures 4.7 to 4.12 below.

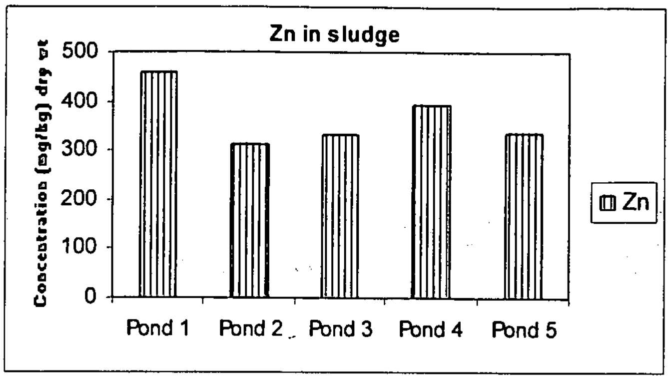

Figure 4.7) Zn concentration (mg/kg) in sludge

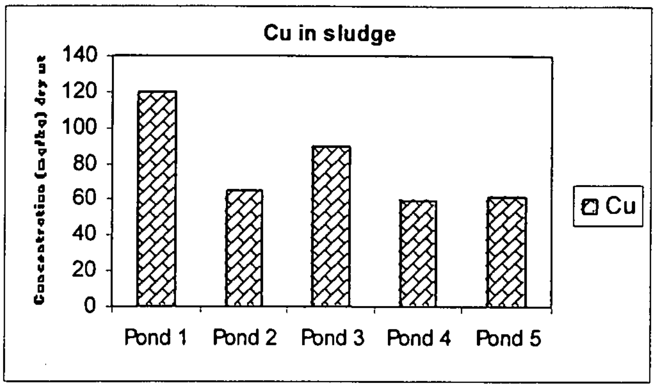

Figure 4.8) Cu concentration (mg/kg) in sludge

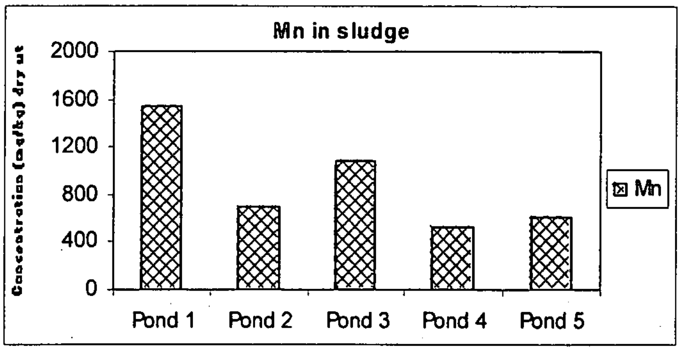

Figure 4.9) Mn concentration (mg/kg) in sludge

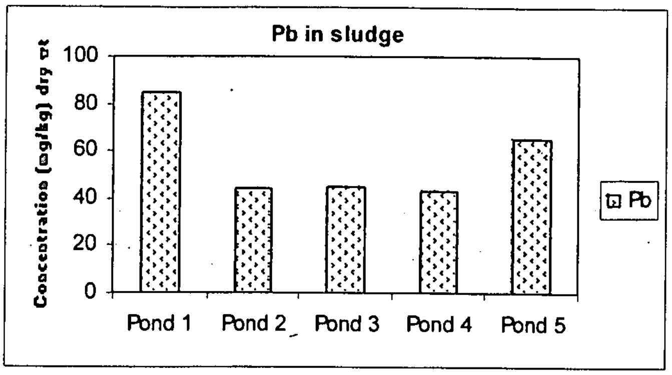

Figure 4.10) Pb concentration (mg/kg) in sludge

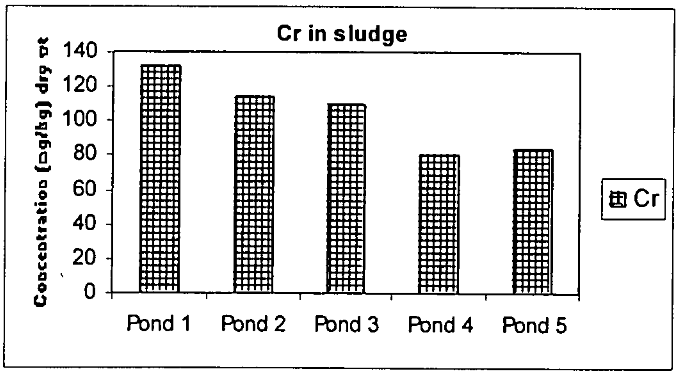

Figure 4.11) Cr concentration (mg/kg) in sludge

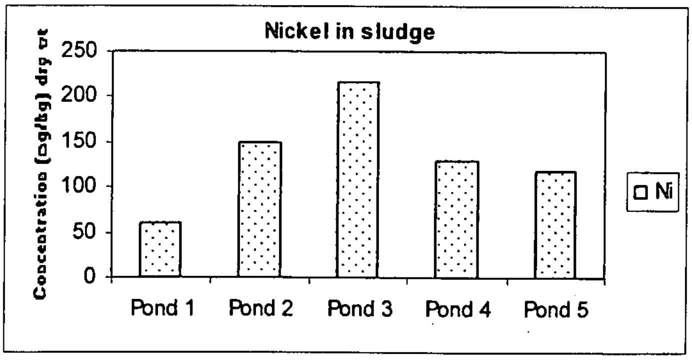

Figure 4.12) Ni concentration (mg/kg) in sludge

It can be seen from the bar-graphs above that the trend for Cu and Mn are similar. The trend for Cr is close to anticipated where by the concentration of metal decreases from pond1 to pond 5. However for the rest of the heavy metals under investigation there are no clear trends.

4.3 Heavy metal concentration in plants from the ponds.

43.1 Heavy metal concentration in *A.sessilis* leaves

The heavy metal concentrations (mg/kg dry wt) in *A.sessilis* leaves that were sampled from the five ponds are shown in table 4.3.

Table 4.3) Heavy metal concentration (mg/kg) dry weight in *A.sessilis* leaves

	Pb	Cu	Ni	Cr	Zn	Mn
Pond 1	12	22	7	2	303	1060
Pond 2	5	21	9	7	142	358
Pond 3	5	19	5	2	309	922
Pond 4	3	27	8	3	169	215
Pond 5	9	19	7	4	139	492

The metal with the highest concentration in the *A.sessilis* leaves was found to be Mn (1060mg/kg dry wt) from pond 1, and the metal with the lowest concentration was found to be Cr (2mg/kg dry wt) from pond 1 and 3. The concentration of Mn and Zn from Pond 1 is almost double to that found in pond 5. This may show accumulation of Zn and Mn depending on the concentration of these heavy metals in the sludge and water. A similar trend has been observed for Mn in sludge (see figure 4.9). The results indicate that *A.sessilis* leaves are relatively good bio accumulators of Mn. The results given in table 4.3 are depicted as bar-graphs in figure 4.13 to 4.18.

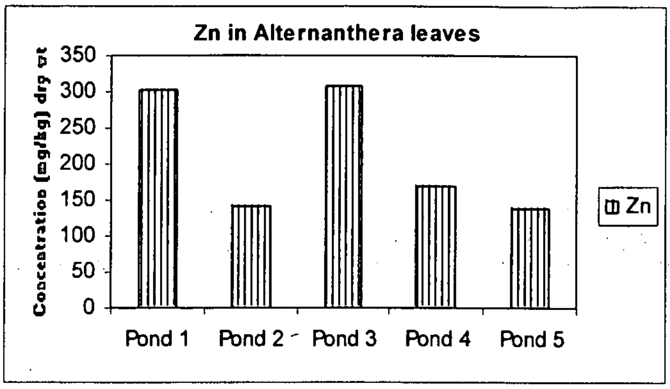

Figure 4.13) Zn concentration (mg/kg) in *A.sessilis* leaves

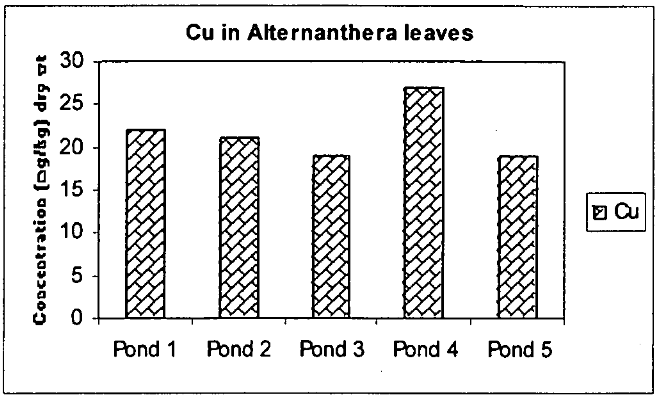

Figure 4.14) Cu concentration (mg/kg) in *A.sessilis* leaves.

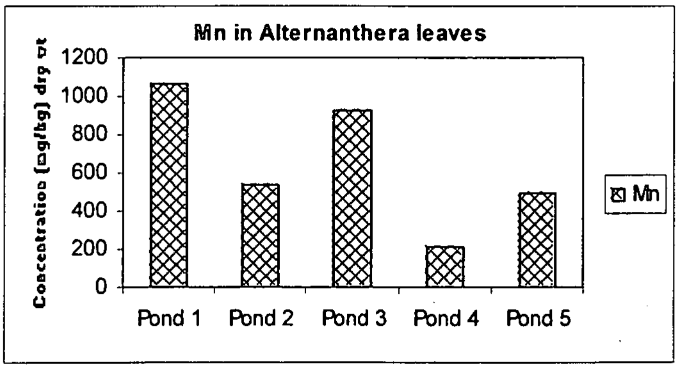

Figure 4.15) Mn concentration (mg/kg) in *A.sessilis* leaves.

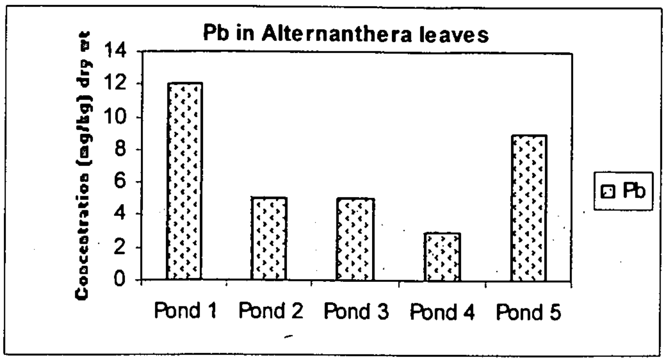

Figure 4.16) Pb concentration (mg/kg) in *A.sessilis* leaves

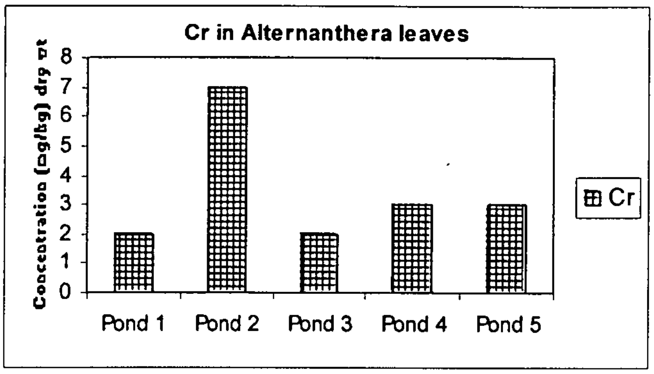

Figure 4.17) Cr concentration (mg/kg) in *A.sessilis* leaves

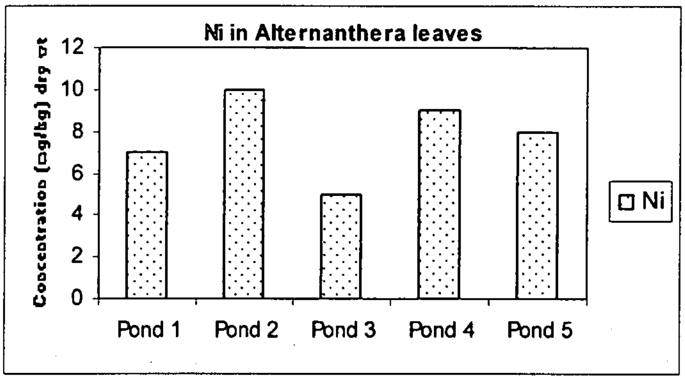

Figure 4.18) Ni concentration (mg/kg) in *A.sessilis* leaves

Figures 4.13 to 4.18 indicate that the trends for Zn and Mn are roughly similar. Variability in heavy metal concentration in the ponds could be attributed to pH, iron oxide plaque and age of specie which is discussed in detail in section 2.4.

4.3.2 Heavy metal concentration in *A.sessilis* stems and roots

Table 4.4 lists the heavy metal concentration (mg/kg dry wt) in *A.sessilis* roots and stems sampled from the five ponds.

Table 4.4) Heavy metal concentration (mg/kg) dry weight in *A.sessilis* roots and stems

	Pb	Cu	Ni	Cr	Zn	Mn
Pond 1	1.7	77	7	3	251	709
Pond 2	0.6	15	5	2	112	230
Pond 3	2	29	6	2	273	490
Pond 4	0.8	9	10	2	211	164
Pond 5	0.9	12	3	0.5	127	299

The metal with the highest concentration in *A.sessilis* roots and stems was found to be Mn (709 mg/kg dry wt), the lowest concentration was found for Cr (0,5 mg/kg dry wt). The concentration of Mn and Pb in the roots and stems from pond 1 is almost double to that found in pond 5. The concentration of Cr and Cu (pond1) is almost six times higher than that found in pond 5. This may show accumulation of Mn, Pb, Cr and Cu depending on the concentration of these heavy metals in the sludge and water. These results show that the stems and roots of *A.sessilis* are relatively good bio accumulators of Cu and Mn. The variations of the concentrations of the individual metals with the ponds are shown as bar-graphs in figures 4.19 to 4.23.

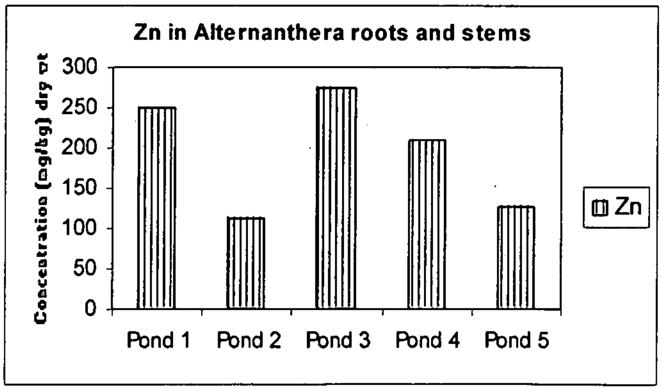

Figure 4.19) Zn concentration (mg/kg) in *A.sessilis* roots and stems

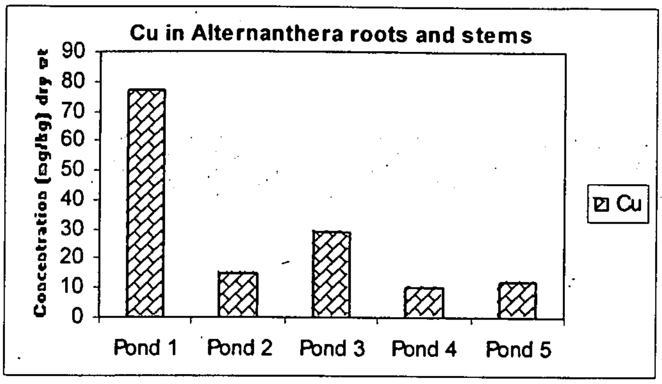

Figure 4.20) Cu concentration (mg/kg) in *A.sessilis* roots and stems

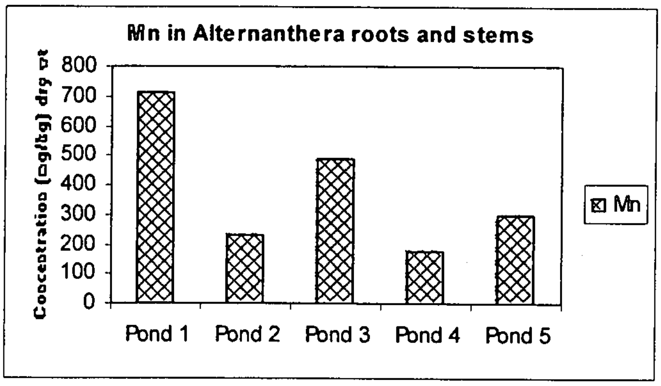

Figure 4.21) Mn concentration (mg/kg) in *A.sessilis* roots and stems

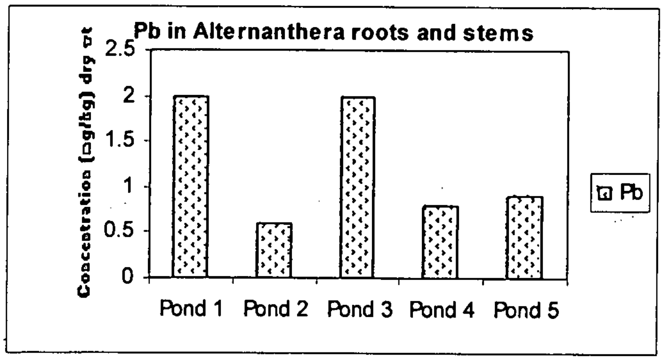

Figure 4.22) Pb concentration (mg/kg) in *A.sessilis* roots and stems

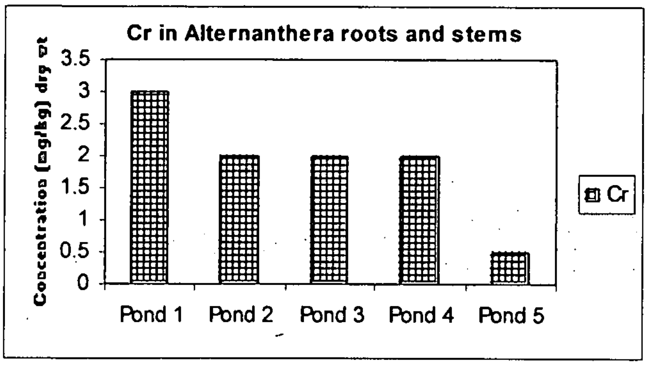

Figure 4.23) Cr concentration (mg/kg) in *A.sessilis* roots and stems

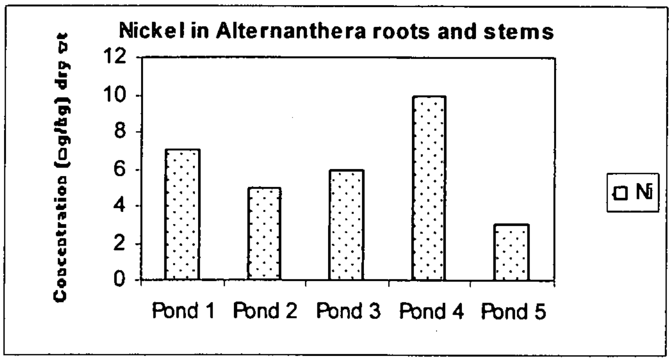

Figure 4.24) Ni concentration (mg/kg) in *A.sessilis* roots and stems

As shown in figures 4.20, 4.21 and 4.22 the variations in the concentrations of Cu, Mn and Pb are roughly similar.

4.3.3 Heavy metal concentration in *P.stratiotes*

Table 4.5 lists the heavy metal concentration (mg/kg dry wt) in *P.stratiotes* sampled from the five ponds.

Table 4.5) Heavy metal concentration (mg/kg) dry weight in *P.stratiotes*

	Pb	Cu	Ni	Cr	Zn	Mn
Pond 1	9	101	24	10	669	2246
Pond 2	4	36	16	6	566	747
Pond 3	4	37	15	3	505	739
Pond 4	3	30	23	3	489	608
Pond 5	4	35	17	3	613	1035

The metal with the highest concentration in *P.stratiotes* was found to be Mn (2246 mg/kg dry wt) and the one with the lowest concentration was found to be Cr and Pb (3 mg/kg dry wt). All the heavy metals studied had their highest concentrations in pond 1. The concentration of Cr and Cu in *P.stratiotes* from pond1 is almost three times to that found in pond 5. The concentration of Zn and Mn was higher in pond 5 than in pond 4. The Mn concentration in *P.stratiotes* from pond 1 is almost double to that found in pond 5. The variations in the concentrations of each metal in the 5 ponds is shown in bar-graphs i.e. figures 4.25 to 4.29.

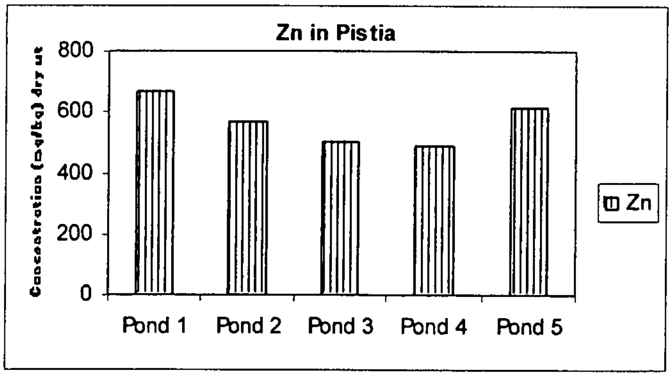

Figure 4.25) Zn concentration (mg/kg) in *P.stratiotes*

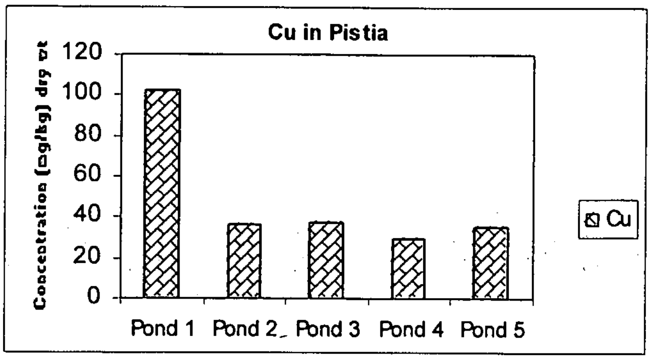

Figure 4.26) Cu concentration (mg/kg) in *P.stratiotes*

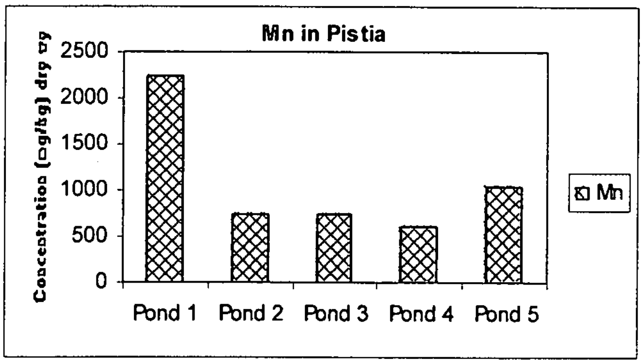

Figure 4.27) Mn concentration (mg/kg) in *P.stratiotes*

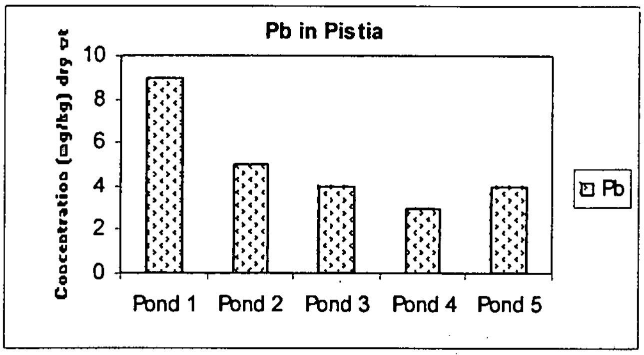

Figure 4.28) Pb concentration (mg/kg) in *P.stratiotes*

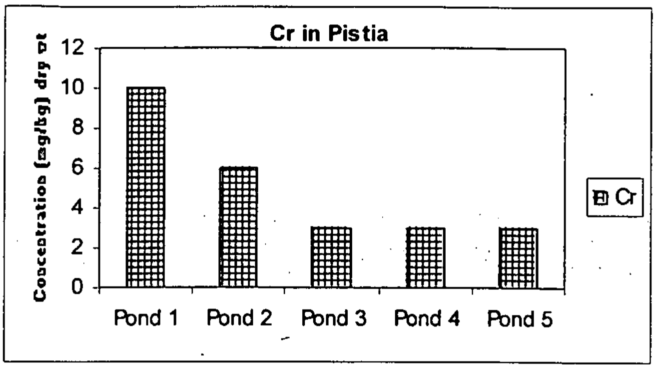

Figure 4.29) Cr concentration (mg/kg) in *P.stratiotes*

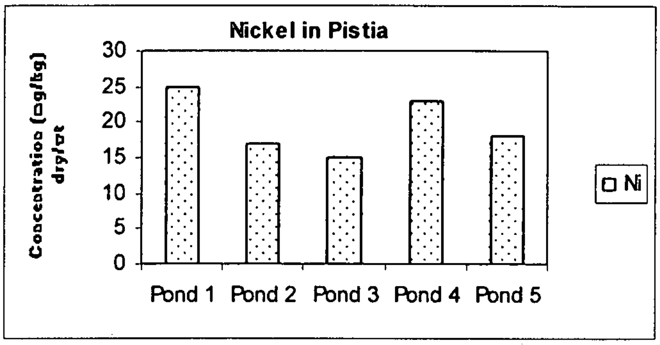

Figure 4.30) Ni concentration (mg/kg) in *P.stratiotes*

A similar trend for Cu was obtained in water. The trend for Cr is close to what was expected, namely a steady decrease from pond1 to pond 5. However the rest of the heavy metals under investigation do not show this trend and this can be attributed by the same factors as discussed for water.

4.4 Heavy metal concentration in plants from the old sludge.

4.4.1 Heavy metal concentration in *R.steudelii*

Table 4.6 lists the heavy metal concentration (mg/kg dry wt) in *R.steudelii* sampled from the 'old sludge site'.

Table 4.6) Heavy metal concentration (mg/kg) dry weight in *R.steudelii*

	Pb	Cu	Ni	Cr	Zn	Mn
Leaves	1.1	21	6	4	204	111
Stems	0.8	19	4	1.5	193	59
Roots	3	33	6	4	282	80

The metal with the highest concentration in *R.steudelii* was found to be Zn (282 mg/kg dry wt), which was found in the roots. The heavy metal with the lowest concentration in *R.steudelii* was found to be Pb (0,8 mg/kg dry wt), which was found in the stems. The variations of the concentrations of the 6 metals in leaves, roots and stems are shown as bar-graphs in figures 4.31 to 4.36.

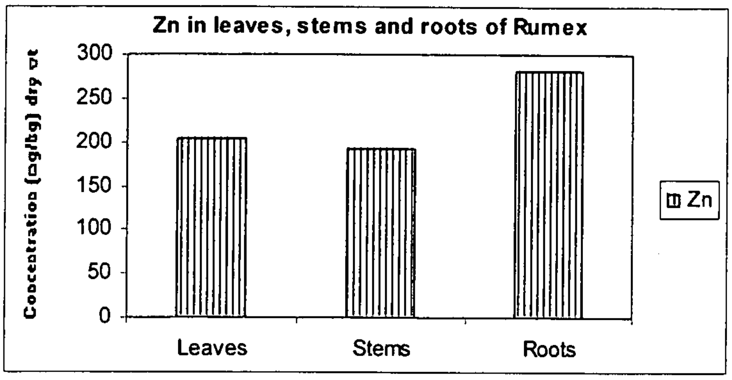

Figure 4.31) Zn concentration (mg/kg) in roots, stems and roots of *R.steudelii*

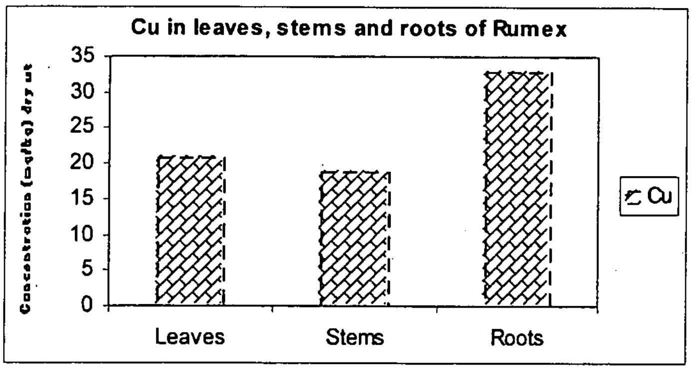

Figure 4.32) Cu concentration (mg/kg) in leaves, stems and roots of *R.steudelii*

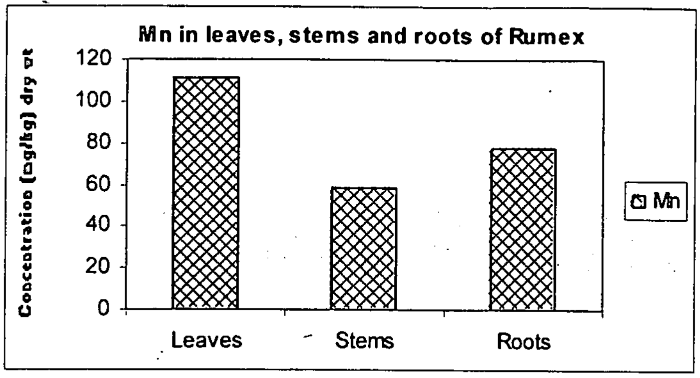

Figure 4.33) Mn concentration (mg/kg) in leaves, stems and roots of *R.steudelii*

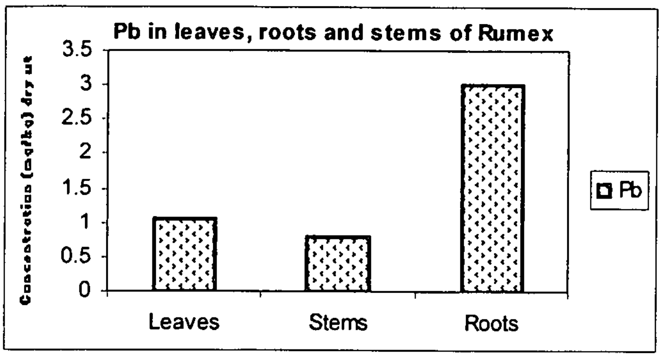

Figure 4.34) Pb concentration (mg/kg) in leaves, stems and roots of *R.steudelii*

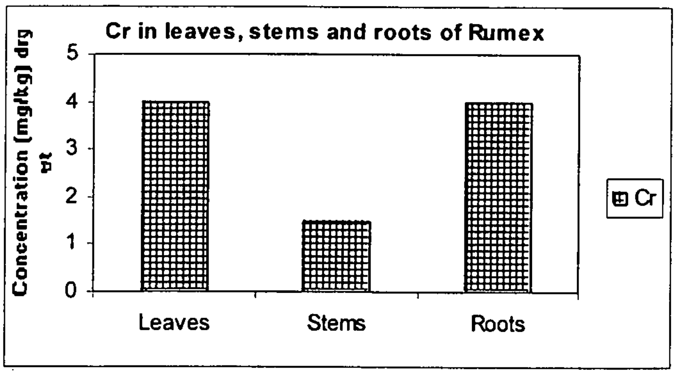

Figure 4.35) Cr concentration (mg/kg) in leaves, stems and roots of *R.steudelii*

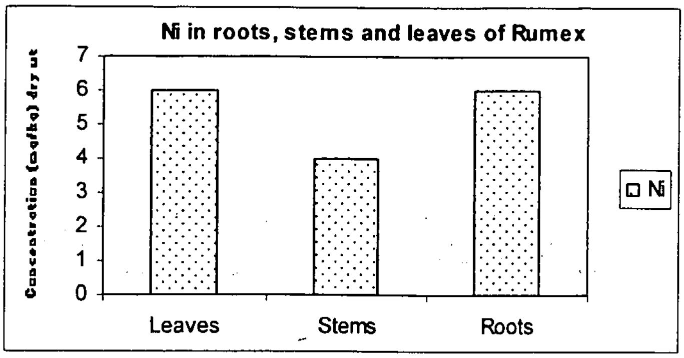

Figure 4.36) Ni concentration (mg/kg) in leaves, stems and roots of *R.steudelii*

As shown in figures 4.31, 4.32 and 4.34 the pattern of variations is roughly similar. The pattern for variation is similar for Ni and Cr, in roots and leaves. However a high concentration of Mn occurred in the leaves as shown in figure 4.33.

4.4.2 Heavy metal concentration in *T.capensis*

Table 4.7 lists the heavy metal concentration (mg/kg dry wt) in *T.capensis* sampled from the 'old sludge site'.

Table 4.7) Heavy metal concentration (mg/kg) dry weight in *T.capensis*

	Pb	Cu	Ni	Cr	Zn	Mn
Aboveground	0.5	13	3	1.3	92	665
Belowground	0.4	28	6	0.6	377	366

The metal with the highest concentration in *T.capensis* was found to be Mn (665mg/kg dry wt) in the aboveground part of the plant. The metal with the lowest concentration in *T.capensis* was found to be Pb (0,4 mg/kg dry wt) in the belowground part of the plant. The belowground part of *T.capensis* had a higher concentration of Cu, Zn and Ni compared to the aboveground part, whereas the aboveground part had a higher concentration of Mn and Cr than the belowground part. The concentrations of Pb obtained in the aboveground and belowground parts were similar. The variations of the 6 metals in the aboveground and belowground parts are shown in bar-graphs in figures 4.37 to 4.39.

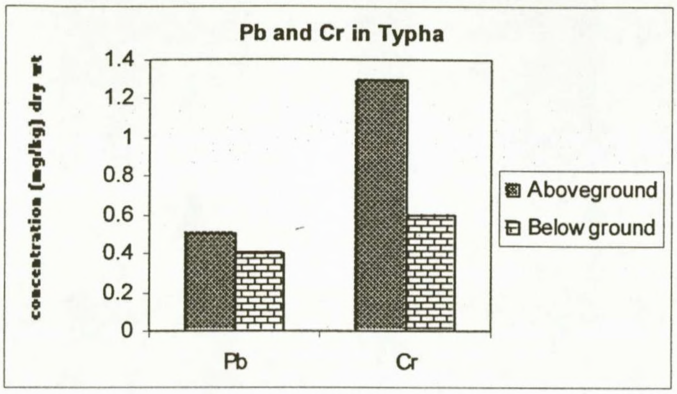

Figure 4.37) Pb and Cr concentration (mg/kg) in aboveground and belowground parts of *T.capensis*

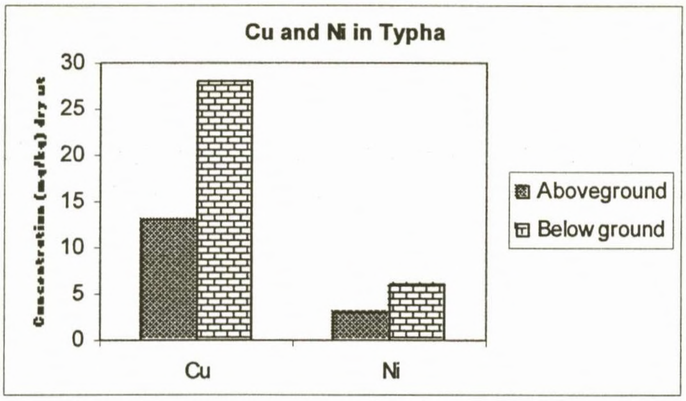

Figure 4.38) Ni and Cu concentration (mg/kg) in aboveground and belowground parts of *T.capensis*

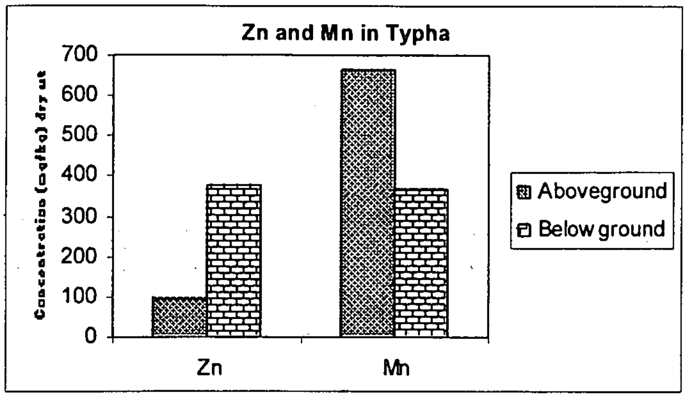

Figure 4.39) Zn and Mn concentration (mg/kg) in aboveground and belowground parts of *T.capensis*

4.4.3 Heavy metal concentration in *A.sessilis*

Table 4.8 lists the heavy metal concentration (mg/kg dry wt) in *A.sessilis* sampled from the 'old sludge site'.

Table 4.8) Heavy metal concentration in *A.sessilis* (mg/kg) dry weight from the old sludge area

	Pb	Cu	Ni	Cr	Zn	Mn
Leaves	4	29	7	5	547	263
Roots/stems	0.8	90	4	0.6	578	131

Except for Zn and Cu all the other metals had a higher concentration in leaves than the roots and stems. The highest concentration was obtained for Mn (263 mg/kg dry wt) and the lowest concentration was obtained for Cr (0,6 mg/kg dry wt).

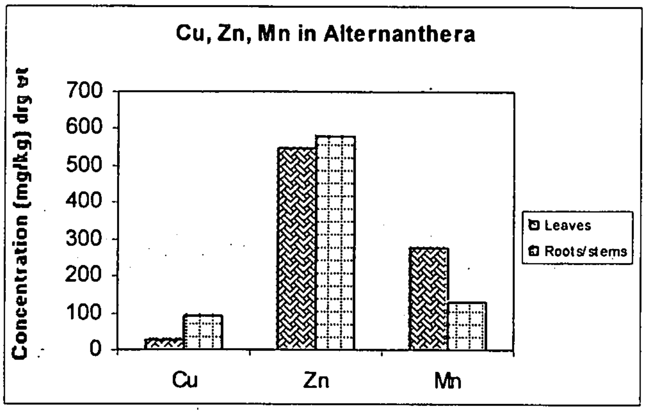

Figure 4.40) Cu, Zn, Mn (mg/kg) in *A.sessilis*

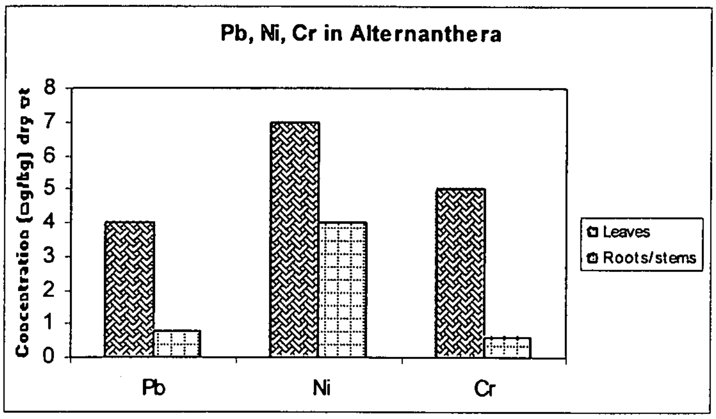

Figure 4.41) Pb, Ni, Cr (mg/kg) in *A. sessilis*

4.5 The distribution of heavy metals in the aquatic environment

Table 4.9) Manganese concentration.

	A.sessilis leaves (mg/kg)	*A.sessilis* stems/roots (mg/kg)	*P.stratiotes* (mg/kg)	Sludge (mg/kg)	Water (mg/l)
Pond 1	1060	709	2246	1543	0.043
Pond 2	358	230	747	699	0.082
Pond 3	922	490	739	1085	0.067
Pond 4	215	164	608	517	0.034
Pond 5	492	299	1035	602	0.068

The Mn concentration was the highest in *P.stratiotes* (2246 mg/kg dry wt). The *A.sessilis* leaves concentrated Mn more than the roots and stems. *A.sessilis* leaves and root system show a similar trend to the sludge. It appears that *A.sessilis* is an accumulator of Mn.

Table 4.10) Zinc concentration

	A.sessilis **leaves (mg/kg)**	*A.sessilis* **stems/roots (mg/kg)**	*P.stratiotes* **(mg/kg)**	**Sludge (mg/kg)**	**Water (mg/l)**
Pond 1	303	251	669	458	0.051
Pond 2	142	112	566	311	0.109
Pond 3	309	273	505	332	0.08
Pond 4	169	211	489	395	0.031
Pond 5	139	127	613	338	0.062

The highest value obtained for Zn was in *P.stratiotes* (669 mg/kg dry wt). Generally the *A.sessilis* leaves concentrated Zn more than the roots and stems

Table 4.11) Copper concentration

	A.sessilis **leaves (mg/kg)**	*A.sessilis* **stems/roots (mg/kg)**	*P.stratiotes* **(mg/kg)**	**Sludge (mg/kg)**	**Water (mg/l)**
Pond 1	22	77	101	120	0.006
Pond 2	21	15	36	65	0.004
Pond 3	19	29	37	90	0.004
Pond 4	27	9	30	58	0.003
Pond 5	19	12	35	62	0.003

The highest value was obtained for sludge (120mg/kg dry wt). The Cu in *A.sessilis* varied from leaves to root/stem system. The *A.sessilis* root/stem showed a similar trend to that found in sludge, possible accumulation of Cu in *A.sessilis* roots/stems.

Table 4.12) Nickel concentration

	A.sessilis leaves (mg/kg)	*A.sessilis* stems/roots (mg/kg)	*P.stratiotes* (mg/kg)	Sludge (mg/kg)	Water (mg/l)
Pond 1	7	7	24	60	0.003
Pond 2	9	5	16	149	0.005
Pond 3	5	6	15	217	0.005
Pond 4	8	10	23	130	0.002
Pond 5	7	3	17	118	0.005

The highest value was obtained for sludge (217 mg/kg dry wt). The concentration of Ni varied from pond to pond.

Table 4.13) Chromium concentration

	A.sessilis leaves (mg/kg)	*A.sessilis* stems/roots (mg/kg)	*P.stratiotes* (mg/kg)	Sludge (mg/kg)	Water (mg/l)
Pond 1	2	3	10	132	0.003
Pond 2	7	2	6	115	0.004
Pond 3	2	2	3	109	0.004
Pond 4	3	2	3	80	0.002
Pond 5	4	0.5	3	84	0.002

The highest value was obtained in the sludge (132mg/kg dry wt).

Table 4.14) Lead concentration

	A.sessilis leaves (mg/kg)	*A.sessilis* stems/roots (mg/kg)	*P.stratiotes* (mg/kg)	Sludge (mg/kg)	Water (mg/l)
Pond 1	12	1.7	9	86	0.006
Pond 2	5	0.6	4	44	0.016
Pond 3	5	2	4	45	0.012
Pond 4	3	0.8	3	43	0.005
Pond 5	9	0.9	4	65	0.005

The highest value for Pb was found in the sludge (86mg/kg dry wt). This is the only element for which *A.sessilis* leaves had a higher concentration than *P.stratiotes.*

CHAPTER 5

DISCUSSION and CONCLUSION

To provide a backdrop for the discussion of the results obtained in this study a brief discussion of sewage treatment is provided in section 5.1.

5.1 Sewage treatment

Sewage contains inorganic and organic wastes that pollute water bodies rendering the water unfit for human use. The polluted water bodies can cause widespread diseases such as typhoid and cholera. Due to the shortage of water as discussed in section 1.6, the pollution of these water bodies must be prevented. The treatment of sewage is carried out for inter alia, the following reasons:

- To render it suitable for discharge into rivers or other water bodies.
- To render it fit for re-use in agriculture and industry

The South African National Water Act No.36 of 1998 is to ensure that the nation's water resources are protected, used, developed, conserved and controlled. The by-law also states that an owner shall at his/her own cost take the necessary steps, acceptable to the authorised delegate, to prevent the entry of substances which may be a danger to health or adversely affect the potability of water into the water supply system. The by-law also states that no trade effluent shall be accepted for discharge into the sewage disposal system, unless the effluent does not contain concentrations of substances in excess of those listed in the by-law. Table (5.1) lists the limits for the concentrations of the relevant heavy metals set by the by-law.

Table 5.1: Heavy metal limit in trade effluent (Sewage disposal by-law)

General quality limits	Large sewage works (>25 M/d)	Small sewage works (< 25 M/d)	Units (mg/l)
Copper	50	5	mg/l
Nickel	50	5	mg/l
Zinc	50	5	mg/l
Manganese	50	5	mg/l
Lead	20	5	mg/l
Chromium	20	5	mg/l

5.2 Heavy metal concentration in water

As shown in figures 4.1 to 4.6 there is no discernible trend in the concentrations of most of the heavy metals studied. The exception is copper for which there is a decrease from Pond 1 to Pond 5. The variations of heavy metal concentration (see Table 4.1) in the water from the ponds could be attributed to pH and sedimentation. Several factors discussed section 2.4 including a high pH will cause the metals to precipitate and a decrease in heavy metal concentration in the water phase would result.

An important factor that could impact on the concentrations of metals is the frequency of removal of free floating plant species from the ponds. However, it was not possible to ascertain what species were removed and when the species were removed from the ponds. This was so because no records were kept by the Northern Treatment Works regarding the dates of clearing the ponds of plants. It was therefore, not possible to factor in this aspect to account for the variations observed. Furthermore, the age of the free floating plants was also not known. Since mature plants would have accumulated a higher concentration of metals than young plants, the differences in ages of the plants sampled may also have contributed to the variations in metal concentrations that were recorded. Another aspect that should be considered in accounting for the variations in metal concentrations in water, is the competition for heavy metal accumulation between *Eichornia crassipes* and *P. stratiotes* for example. Since algae also accumulate heavy metals and if each of these species are removed at different times from the ponds there would be changes of heavy metal concentration in the water.

5.3 Heavy metal concentration in sludge

The results obtained (see figures 4.7 to 4.12) show variations in the concentration of heavy metals in the sludge and this could be attributed to factors similar to those discussed above for water. Factors such as pH, sedimentation, removal and age of submerged plants and sampling would cause variations in heavy metal concentration. As reported by Guilio and Scanlon (1985) heavy metal concentration is affected by organic content of the sludge. These researchers found a significant correlation between metal concentration and organic content. Another important factor which could account for the variations is the frequency of sediment removal from the ponds. As was the case for the removal of plants no records were kept of the dredging dates by the Northern Treatment Works

5.4 Heavy metal concentration in the plants investigated

The variations in heavy metal concentration in the plants obtained from different ponds could be attributed to factors discussed above such as pH, sedimentation and ferric oxide plaque. As discussed under section 5.2, the differences in ages of the plants sampled could account for part of the variations observed. Mature plants could have been sampled from one pond and young plants or a mixture of mature and young plants could have been collected from another pond.

Some data on concentrations of the heavy metals, before pertinent to this study, in freshwater plants from uncontaminated environments and polluted ecosystems obtained from Outridge and Noller (1987) are listed in the Table 5.2.

Table 5.2) Summary of trace metal concentrations (ug/g dry wt) in freshwater plants from uncontaminated environments and polluted ecosystems (Outridge and Noller, 1987).

	Uncontaminated environments		Polluted ecosystems
Element	**Minimum**	**Maximum**	**Maximum**
Cr	0.12	45	65
Cu	0.14	55	190
Mn	34	6,880	8,370
Ni	0.85	23	290
Pb	0.30	35	1,200
Zn	11	250	7,030

The values obtained for Mn, Cr, Pb, and Ni in the present study fall within the values obtained for the uncontaminated environment as listed in the above table. The Zn concentrations obtained for *P. stratiotes* (Table 4.10) were higher than the maximum value obtained for the uncontaminated environment. The value for Cu obtained for *A. sessiles* stems/roots and *P. stratiotes* in Pond 1 was higher than the maximum value obtained for the uncontaminated environment. It should be noted in Outridge and Noller's review the type of aquatic plant (submerged, emergent or floating) was not considered.

5.4.1 *P. stratiotes*

From the results obtained (Table 4.5), lead is the only element for which *A. sessiles* leaves had a higher concentration than *P. stratiotes.* As reported by Outridge and Noller (1987), free floating species had less lead than any other life forms and submerged species. St-Cyr and Campbell (1994) found that the concentration of heavy metals in plants having long stems (*Potamogeton richardsonii*) was lower, overall, in their aboveground parts than do rosette type plants (*Vallisneria americana*). In contrast to the other trace metals, it was found that lead concentrated more in *P. richardsonii* than in *V. americana.* It was found that greater surface to volume ratio of *P. richardsonii* relative to *V. americana* influenced the accumulation of lead more than the other heavy metals. In this study it was found that *A. sessiles* leaves had a higher concentration of Pb than *P. stratiotes* which is a rosette type plant.

Klupp *et al* (2002) studied *P.stratiotes* to determine metal contamination in a freshwater ecosystem (see section 2.3.2.3 for details). The values obtained for the concentration of heavy metals are listed in the table below. The values are reported for five sites along a river course.

Table 5.3) Mean values for Cu and Cr in *P.stratiotes* from freshwater ecosystem (Klumpp, 2002)

	Itape	**Ferrades**	**Itabuna**	**Tributary**	**Ponto di Pitu**	**Vila Cachoeira**
Cu	7.57	6.12	10.81	12.69	5.15	7.20
Cr	2.36	1.86	2.21	3.14	1.57	1.43

The values obtained for Cu and Cr in the present study is much higher than these values. Cu is 10 times higher whereas Cr is 3 times higher. This could be attributed to samples being collected from different aquatic systems where the heavy metal loading into the environment is varying. The Cu concentration in *Pistia* (Table 5.4) was found to be higher than that for Cr, the same trend is observed in the present study (Table 4.5).

5.4.2 A. sessilis

From the results shown in Tables 4.3 and 4.4, it is seen that the Pb concentration in the leaves is higher than that in the stems. This could be a mechanism used by the plant wants remove lead. The death or decay of leaves could be means by which a plant gets rid of excess Pb. A similar trend was observed for the Mn concentration in *A. sessilis* and the Mn concentration in the sludge. *A. sessilis* appears to be a bioaccumulator of Mn (1060 mg/kg dry wt in the leaves and 709 mg/kg dry wt in the roots/stems) (figures 4.15, 4.19 and 4.9).

Del Rio and Font (2002) found that *Amaranthus blitiodes,* belonging to the family Amaranthaceae (see section 2.3) is the most promising species for the bioremediation of the contaminated area. This plant belongs to the same family as *A. sessilis* which was chosen for the present study. The concentration of the heavy metals in the plant species in the contaminated and uncontaminated area is listed below (table 5.4).

Table 5.4) Concentration (mg/kg dry wt) in *Amaranthus blitiodes* from contaminated and uncontaminated areas.

	Contaminated sites	**Uncontaminated site**
Pb	40	7.6
Zn	192	47
Cu	26	12

The values in the contaminated site are average values obtained from the four sites (Soberbina, Vicario, P241 and Quema). The Pb value in the contaminated site tabulated above is higher than the values obtained in *A. sessilis* (Table 4.3 and 4.4) in the present study. The mean value of Pb in the sediment in the contaminated site was found to be 466 mg/kg dry wt which is much higher than the values obtained for lead in the sludge (Table 4.2) in the present study, therefore greater accumulation of lead occurred in *Amaranthus blitiodes* in the contaminated site. It was found that *Amaranthus blitiodes* was efficient in the accumulation of heavy metals.

5.4.3 *R. steudelii*

A review by Outridge and Noller (1987) showed that the leaves or stems of many species had a higher concentration of Mn than the roots, (see Table 5.5). It was found that *R. steudelii* in the present study (Table 4.6) had higher concentration of Mn in the leaves compared to the other heavy metal under investigation. In another study by Catsiki and Bei (1992) it was found that Mn was higher in the leaves than in the other parts of the plant.

Table 5.5) Element distribution among different plant parts from uncontaminated ecosystems.

Element	Cr	Cu	Mn	Ni	Pb	Zn
Leaves	2	1	6	2	0	2
Stems	2	1	2	1	1	2
Roots	7	10	5	6	5	6
No of species examined	12	15	15	12	9	11

Cardwell *et al* (2002) studied *Rumex crispus* to determine metal contamination in urban streams, (see section 2.3). The values obtained for the concentration of heavy metals are listed in Table (5.6). The mean values (ug/g dry wt) are reported for one site.

Table 5.6) Mean values for Cu, Pb and Zn (ug/g dry wt) in *Rumex* from urban streams (Cardwell *et al*, 2002)

	Cu	Pb	Zn
Roots	111	432	621
Leaves	12	14	124

The heavy metal concentration obtained for the roots are higher than the values obtained for roots in the present study. Cardwell's values for Zn and Cu in the leaves are higher than the values recorded in the present study. However, Zn, Pb and Cu were found to accumulate to a greater extent in the roots than in the leaves, a similar trend was observed in the present study.

5.4.4 *T.capensis*

The concentrations of heavy metals in *Typha* reported by Outridge and Noller (1987), for unpolluted and polluted environments are shown in Table 5.7.

Table 5.7) Element concentration (ug/g dry wt) in *Typha latifolia* (Outridge and Noller 1987).

Element	**Source**	**Uncontaminated**	**Polluted**
Cr	2	1.2	-
	8	-	4.0
	9	-	1.3
Cu	12	7.0	-
	19	-	5.4
Mn	3	330	-
	11	-	430
Ni	6	<6.0	-
	8	-	3.6
Zn	8	22	-
	13	-	8.6
	14	-	2.8

The Zn, Mn and Cu values (Table 4.7) obtained for *T. capensis* in the present study are higher than the values tabulated above.

Hozhina *et al*(2001) studied *Typha latifolia* in the uptake of heavy metals in the vicinity of the ore mining and processing industries (see section 2.3 for details).The values obtained by these researchers for the concentration of heavy metals are listed in the Table 5.8. The values (ug/g dry wt) are reported from two sites.

Table 5.8) Concentration for Cu, Pb and Zn (ug/g dry wt) in *Typha* (Hozhina *et al*, 2001)

Salair collection ponds (Russia)		**Pb**	**Cu**	**Zn**	**Mn**
Control area	Aboveground	23	4.7	51	-
	Belowground	20	20	31	-
Dyukov ravine (Well)	Aboveground	46	28	340	-
	Belowground	100	150	4300	-
Dyukov ravine (Southern lake)	Aboveground	820	99	40	-
	Belowground	2400	580	450	-
Dyukov ravine (Northern lake)	Aboveground	7.5	7.0	33	-
	Belowground	600	39	430	-
Settling	Aboveground	48	34	400	-
	Belowground	46	130	1400	-
Salagaev ravine	Aboveground	18	19	210	-
	Belowground	80	81	510	-
Komsomolsk collection pond	Aboveground	-	-	-	630
	Belowground	-	-	-	300

It is interesting to note that the values listed above show a similar trend to the values obtained in the present study (Table 4.7). Mn is accumulated to a greater extent in aboveground parts whereas Zn and Cu have greater accumulation in the belowground parts. The heavy metal accumulation in the roots was attributed to a high content of mobile species in the sediment or to a protective barrier which prevents the heavy metals from penetrating from the roots into the aboveground part of the plant. However it is also likely that the well developed subterranean rhizome could serve as a storage site for heavy metals.

As reported by Kassim (1997) aquatic plants accumulate varying amounts of metals due to factors such as concentration of metal in sediment and water as well as metabolic activity of a given plant, the root system and the age. Reimer (1993) also points out that Zn and Cr concentrations in aquatic macrophytes may be influenced by lake location, plant species and the water and sediment chemistry. To the best of my knowledge the tolerable levels of the heavy metals in the plants studied is unknown. When the plants are cultivated in controlled conditions the tolerable levels of heavy metals can be determined.

5.5 Conclusion and Recommendations

The water quality from the final pond is acceptable for release into rivers or for re-use by industry. It also confirms that the ponds are serving the purpose for which they were made, namely, to allow metals to settle in the sediment which act as metal sinks.

However it is a concern that the ponds are dredged periodically and the sludge is deposited adjacent to the ponds. The concern is connected with the possible leaching of the metals back into the ponds with regard to the uptake of metals in the plants.

The present study has shown that the plants that occur in the ponds are serving a very valuable function in removing metals from the ponds. Of the four plants investigated it was found that *A.Sessilis* for the accumulation of Mn and *A.Sessilis* root/stem for the accumulation of Cu from the sludge was the most effective.

In view of our findings it is recommended that aquatic macrophytes existing in the ponds be maintained as a manageable population such that it does not adversely affect the system in terms of biological oxygen demand (BOD) and chemical oxygen demand (COD). Furthermore it is noted that the plants selected from the ponds have also been the subject of bioaccumulation studies in other parts of the world as shown by our literature survey. This confirms that these plants be maintained in the systems of ponds.

Future work can be the cultivating of the plants in controlled conditions to monitor the heavy metal uptake. An improvement suggestion is to lower the digestion temperature for the plants and soil whilst increasing the digestion time, as organometallic compounds can volatilise and the metal concentrations obtained can be lower than the true value.

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APPENDIX 1

Table 1.3) Physical and chemical treatment technologies (Langmore, 1998)

	Description of process	Examples of application
	Physical Treatment	
Ion exchange	An organic resin is used to exchange ions with those dissolved in waste water. The resin have cationic (positive) or anionic (negative) groups.	High quality water is produced by deionisation but the recovery of toxic materials such as mercury and Cr (VI) find wide use.
Reverse osmosis	Pressure is applied to one side of a semi permeable membrane (allows passage of certain components of a solution), to concentrate the dissolved components on that side.	The removal of heavy metals such as Ni (II) or Cr (VI) has found wide use.
	Chemical Treatment	
Electrolysis	When a current is passed through a solution, the positive ions (cations) go to the cathode where they are reduced and negative ions (anions) go to the anode where they are oxidised.	Copper and nickel are selectively removed from waste solutions.
Precipitation	Precipitating agents such as lime and alkali metal sulphides are used to precipitate some or all of the substance in a solution.	Heavy metals as their insoluble hydroxides or sulphides are removed from solution.
Reduction	Reducing agents such as ferrous sulphate and sodium sulphite are used to remove one or more electrons from a molecule.	Ferrous sulphate reduces Cr (VI) to Cr (III), followed by a precipitation of the Chromium (III) hydroxide.

APPENDIX 2

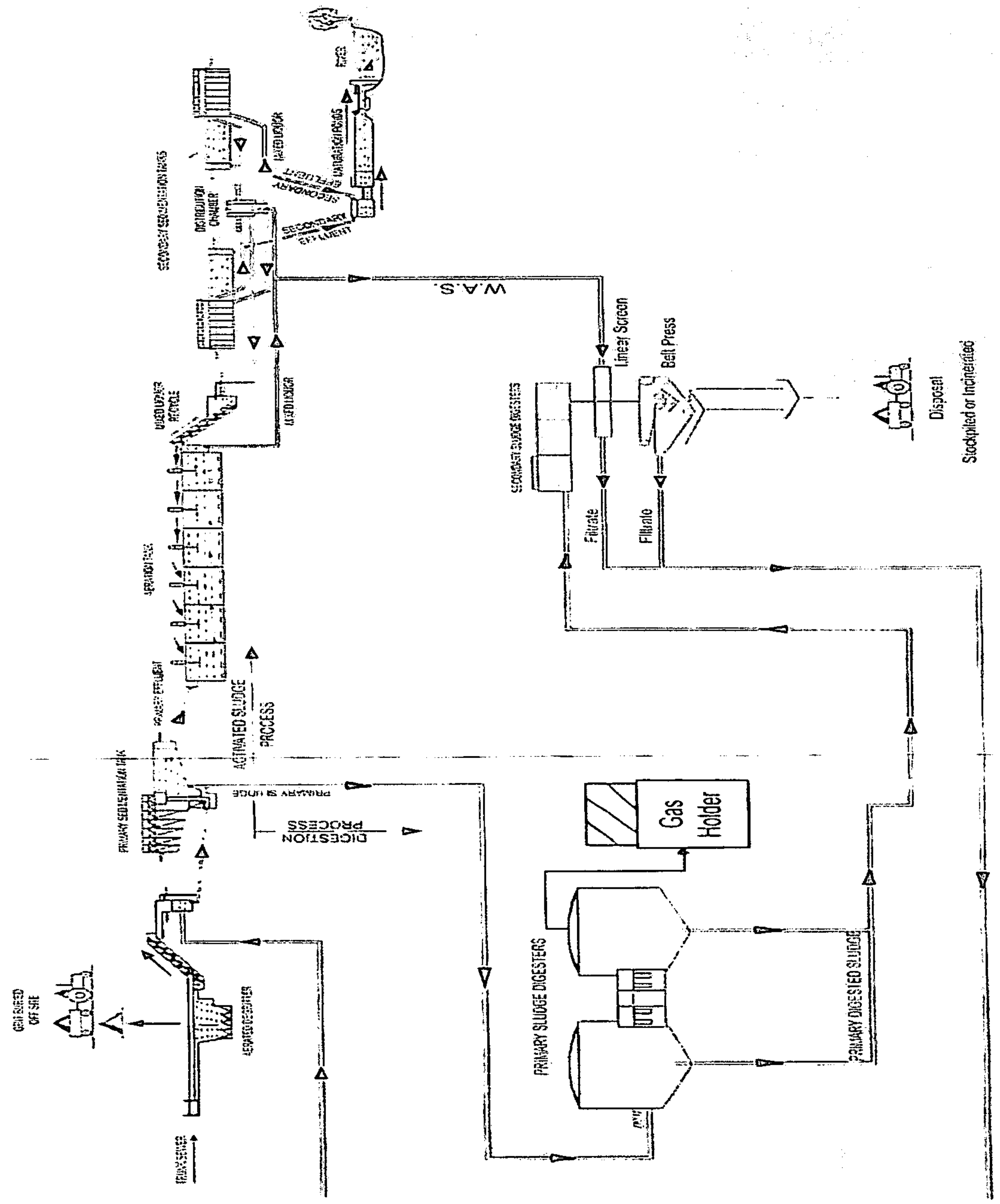


Fig 3.2) Schematic flow diagram of Northern Wastewater Treatment Works. (Durban Metro).

APPENDIX 3

Table 2.13: Heavy metal concentration in seagrass species from the Flores Sea (Nienhuis, 1986).

		Cd	Cu	Pb	Zn
		Ug/g dry wt			
Cymodocae rotundata	**leaves**	0.49	6.6	2.0	21
	Shoots	0.40	4.5	1.8	22
	Rhizome and root	0.22	4.5	2.5	27
Cymodocae serrulata	**leaves**	0.68	5.8	2.1	23
	Shoots	0.27	3.1	1.4	21
	Rhizome and root	0.17	3.1	1.4	20
Thalassia hemprichii	**leaves**	0.57	7.0	2.8	23
	Shoots	0.42	5.0	2.3	18
	Rhizome and root	0.24	2.9	3.0	14
Syringodium isoetifolium	**leaves**	0.16	3.9	2.6	15
	Shoots	0.21	4.1	2.8	23
	Rhizome and root	0.16	6.8	6.1	19
Enhalus acoroides	**leaves**	0.36	4.4	1.7	16
	Shoots	0.16	3.7	3.7	14
	Rhizome and root	0.12	2.6	1.1	19
Halodule uniervis	**leaves**	0.60	5.6	2.6	24
	Shoots	0.24	3.4	2.0	16
	Rhizome and root	0.25	2.7	2.5	13
Halodule pinifolia	**Entire plant**	0.36	6.7	5.4	27
Thalassodendron ciliatum	**leaves**	1.54	6.0	2.5	20
	Shoots	0.98	3.3	1.3	6
	Rhizome and root	0.23	3.4	1.6	6
Halophila ovalis	**Entire plant**	0.41	17.3	3.9	63

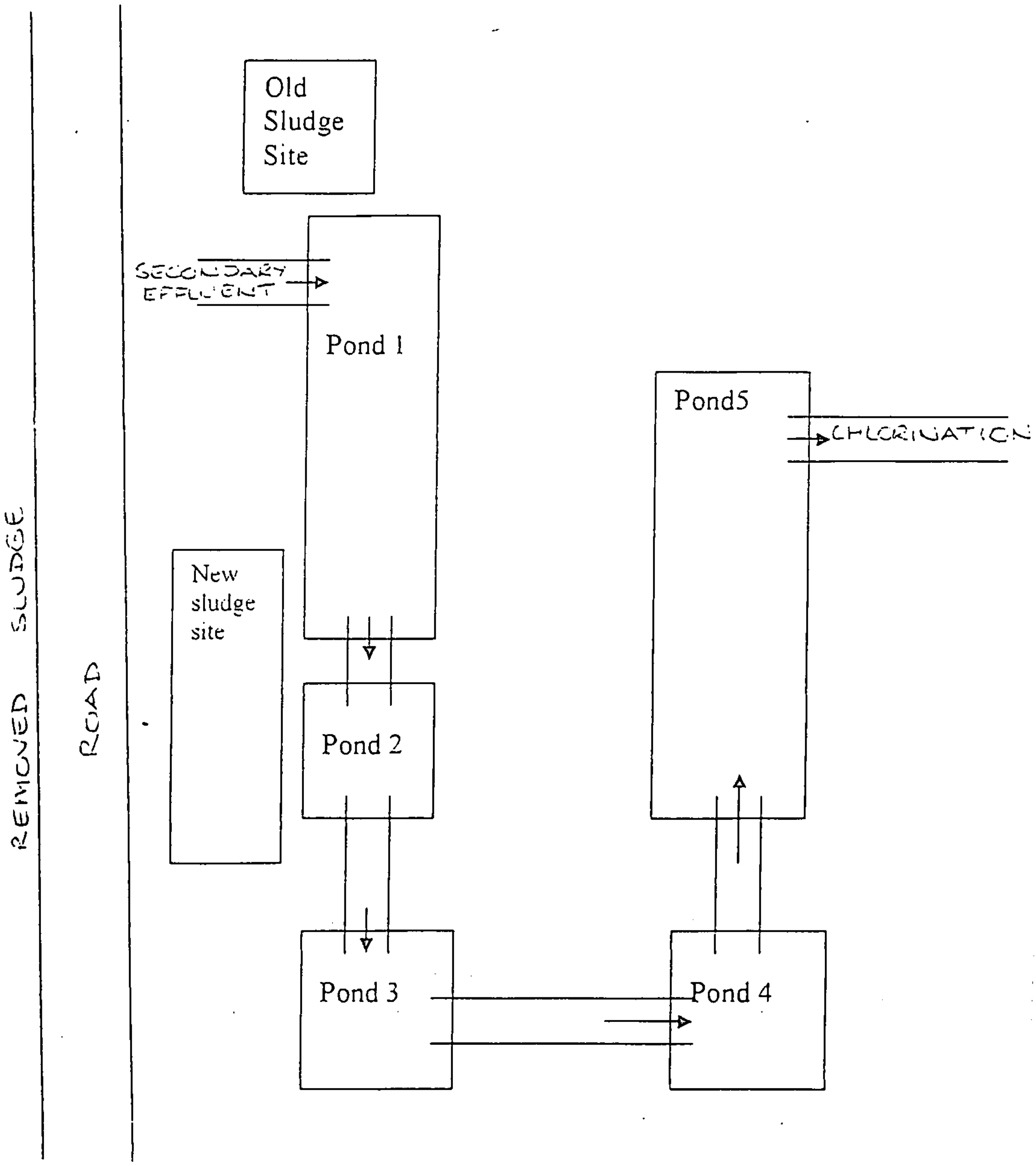
APPENDIX 4

Table 2.12) Description of macrophytes species collected

Species	Plant type	Site
Colocasia esculenta (Taro, Elephants Ear)	Emergent plant found in hydric soils (native to Asiatic region).	4
Cyperus eragrostis (Umbrella sedge)	Emergent tufted perennial sedge to 1m tall (American)	3, 4
Eleocharis equisitina (Spike rush)	Erect rhizomatous perennial to 1,5m tall (native)	6
Myriophyllum aquaticum (Parrots feather or thread of life)	Submerged and sometimes partly emergent feathery species (South American)	4
Nymphaea violacea (Waterlily)	Emergent species, attached to substrates with floating leaves and prominent flowers (native)	6
Nymphoides germinate (Marshwort)	Emergent perennial with floating leaves and small white hairy flowers (native)	5
Persicaria attenuatum	Knotweeds, Smartweeds	3
Persicaria orientalis		1, 6
Persicaria subsessilis		3, 5
Persicaria lapathifolium (Willow smartweed)	Emergent erect, branched annual found in marshy areas and creek banks (native)	1
Potamogeton javanicus	Rhizomatous perennial, mostly submerged with a few small floating leaves (native)	6
Rumex crispus (Curled dock)	Emergent, erect broad leafed perennial with cylindrical stems to 1.5m tall (native)	
Schoenoplectus validus (River culbrush, River bulrush)	Emergent erect perennial with cylindrical stems to 3m tall (native)	5
Typha domingensis (Cumbungi)	Emergent erect perennial, extensive rhizome system, flat leaves, to 4m tall (Native, potential weed)	3,4,5

APPENDIX 5

Figure 3.5) Flow diagram of the maturation ponds



APPENDIX 6

Figure 3.6) *T.capensis*

APPENDIX 7

Figure 3.7) *R.steudelii*

APPENDIX 8

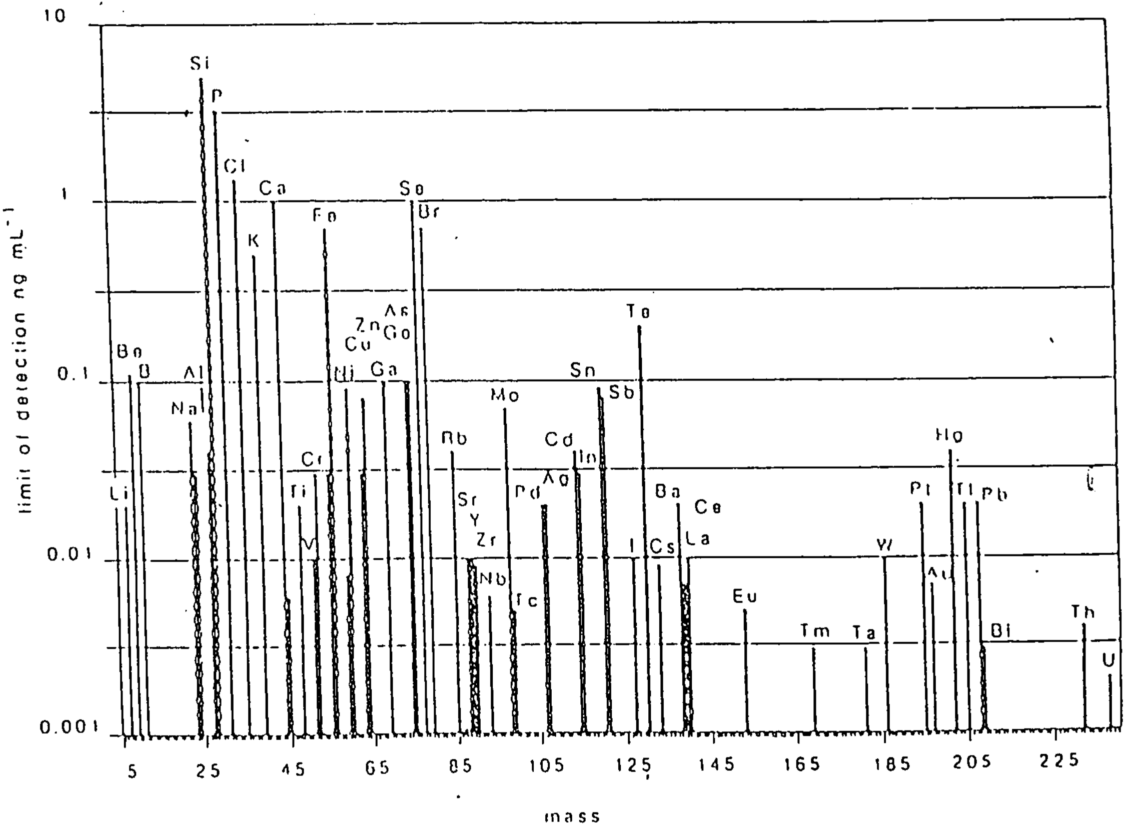
Figure 3.8) *P.stratiotes*

APPENDIX 9

Figure 3.9) *A.sessilis*

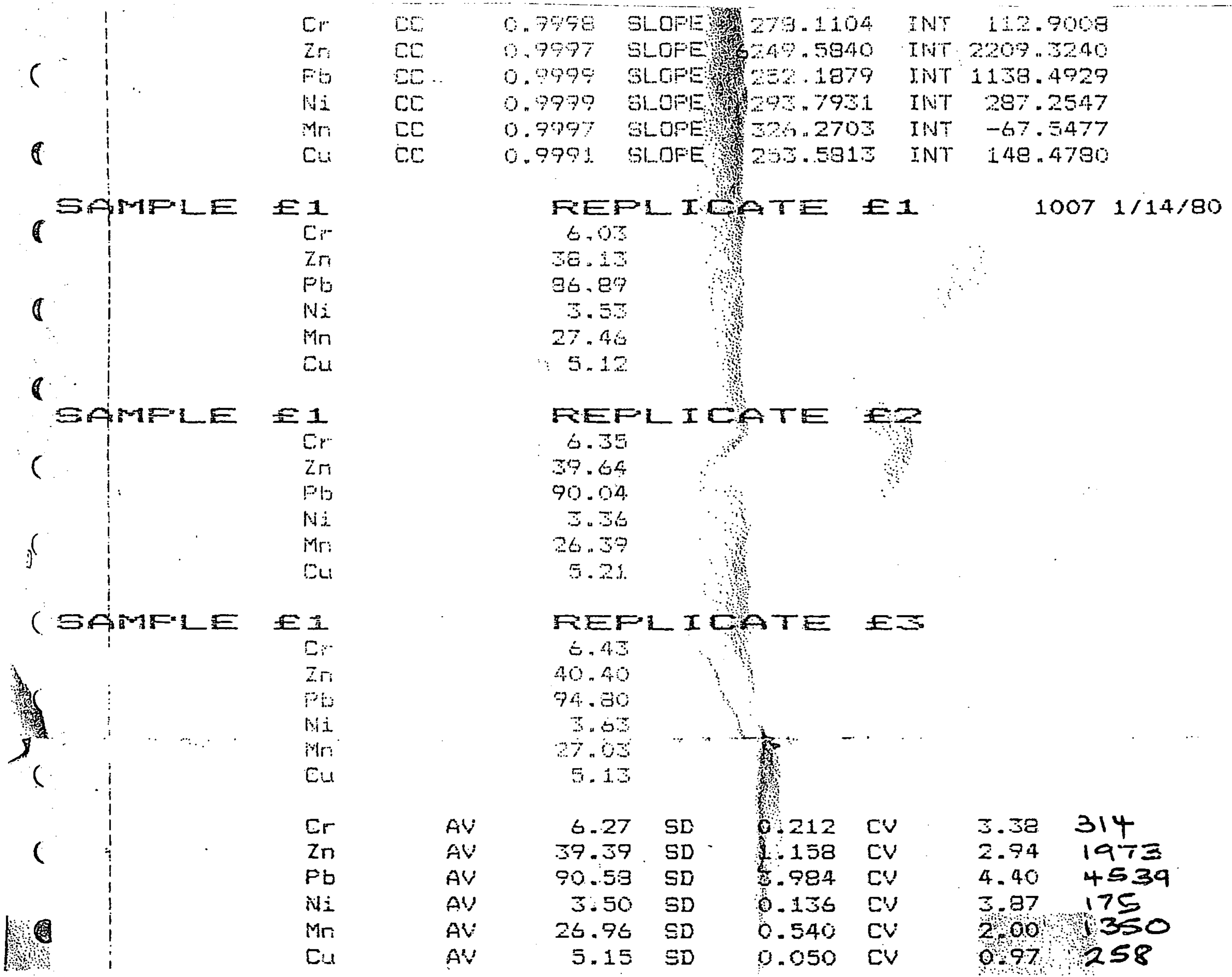
APPENDIX 10

Figure 3.10) Detection limit in ICP-MS (Date and Gray, 1989)



APPENDIX A

A set of results obtained for ICP-AES



```
         Cr      CC      0.9998   SLOPE  278.1104   INT   112.9008
         Zn      CC      0.9997   SLOPE 6249.5840   INT  2209.3240
         Pb      CC      0.9999   SLOPE  252.1879   INT  1138.4929
         Ni      CC      0.9999   SLOPE  293.7931   INT   287.2547
         Mn      CC      0.9997   SLOPE  326.2703   INT   -67.5477
         Cu      CC      0.9991   SLOPE  253.5813   INT   148.4780

SAMPLE £1                 REPLICATE £1          1007 1/14/80
         Cr                 6.03
         Zn                38.13
         Pb                86.89
         Ni                 3.53
         Mn                27.46
         Cu                 5.12

SAMPLE £1                 REPLICATE £2
         Cr                 6.35
         Zn                39.64
         Pb                90.04
         Ni                 3.36
         Mn                26.39
         Cu                 5.21

SAMPLE £1                 REPLICATE £3
         Cr                 6.43
         Zn                40.40
         Pb                94.80
         Ni                 3.63
         Mn                27.03
         Cu                 5.13

         Cr        AV       6.27   SD   0.212   CV      3.38   314
         Zn        AV      39.39   SD   1.158   CV      2.94   1973
         Pb        AV      90.58   SD   3.984   CV      4.40   4539
         Ni        AV       3.50   SD   0.136   CV      3.87   175
         Mn        AV      26.96   SD   0.540   CV      2.00   1350
         Cu        AV       5.15   SD   0.050   CV      0.97   258
```

APPENDIX B

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water.

Quantitative Analysis - Summary Report

Sample ID: S43
Sample Date/Time: Tuesday, January 07, 2003 13:23:40
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	641101	4674	9.444	ppm
Mn	55	4564928	4105	41.3	ppm
Ni	58	299541	-188	7.307	ppm
Cu	63	282809	395	7.573	ppm
Zn	64	903102	3598	58.914	ppm
Pb	208	27767162	2187	121.224	ppm

Sample ID: QC
Sample Date/Time: Tuesday, January 07, 2003 13:28:21
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	748840	4674	1.017	ppm
Mn	55	1184709	4105	0.981	ppm
Ni	58	434626	-188	1.011	ppm
Cu	63	387630	395	0.978	ppm
Zn	64	167393	3598	0.991	ppm
Pb	208	2232129	2187	0.888	ppm

Sample ID: BL 1
Sample Date/Time: Tuesday, January 07, 2003 13:25:37
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	263645	4674	0.431	ppm
Mn	55	46269	4105	0.041	ppm
Ni	58	63821	-188	0.17	ppm
Cu	63	7337	395	0.02	ppm
Zn	64	15300	3598	0.053	ppm
Pb	208	294609	2187	0.128	ppm

Sample ID: S1
Sample Date/Time: Tuesday, January 07, 2003 13:27:48
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	6204	4674	0.003	ppm
Mn	55	79071	4105	0.072	ppm
Ni	58	1477	-188	0.004	ppm
Cu	63	2075	395	0.005	ppm
Zn	64	14457	3598	0.077	ppm
Pb	208	21524	2187	0.01	ppm

Sample ID: S2
Sample Date/Time: Tuesday, January 07, 2003 13:31:56
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	5793	4674	0.002	ppm
Mn	55	71734	4105	0.063	ppm
Ni	58	1129	-188	0.003	ppm
Cu	63	1723	395	0.004	ppm
Zn	64	11082	3598	0.051	ppm
Pb	208	12907	2187	0.005	ppm

Sample ID: S3
Sample Date/Time: Tuesday, January 07, 2003 13:36:04
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	6772	4674	0.004	ppm
Mn	55	114730	4105	0.108	ppm
Ni	58	2161	-188	0.006	ppm
Cu	63	2983	395	0.008	ppm
Zn	64	16424	3598	0.092	ppm
Pb	208	24743	2187	0.012	ppm

Sample ID: S4
Sample Date/Time: Tuesday, January 07, 2003 13:40:14
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	6104	4674	0.002	ppm
Mn	55	48574	4105	0.041	ppm
Ni	58	790	-188	0.002	ppm
Cu	63	1409	395	0.003	ppm
Zn	64	10225	3598	0.044	ppm
Pb	208	13169	2187	0.005	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: S5
Sample Date/Time: Tuesday, January 07, 2003 13:44:24
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	8672	4674	0.006	ppm
Mn	55	70322	4105	0.062	ppm
Ni	58	1789	-188	0.005	ppm
Cu	63	1934	395	0.004	ppm
Zn	64	13074	3598	0.065	ppm
Pb	208	50254	2187	0.023	ppm

Sample ID: S6
Sample Date/Time: Tuesday, January 07, 2003 13:48:34
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	6025	4674	0.002	ppm
Mn	55	38993	4105	0.031	ppm
Ni	58	689	-188	0.002	ppm
Cu	63	1242	395	0.002	ppm
Zn	64	7620	3598	0.026	ppm
Pb	208	13126	2187	0.005	ppm

Sample ID: S7
Sample Date/Time: Tuesday, January 07, 2003 13:52:45
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	5657	4674	0.001	ppm
Mn	55	45665	4105	0.037	ppm
Ni	58	794	-188	0.002	ppm
Cu	63	1171	395	0.002	ppm
Zn	64	6938	3598	0.021	ppm
Pb	208	9346	2187	0.003	ppm

Sample ID: S8
Sample Date/Time: Tuesday, January 07, 2003 13:56:56
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	5882	4674	0.002	ppm
Mn	55	82566	4105	0.074	ppm
Ni	58	1735	-188	0.005	ppm
Cu	63	1766	395	0.004	ppm
Zn	64	11610	3598	0.055	ppm
Pb	208	13976	2187	0.006	ppm

Sample ID: S9
Sample Date/Time: Tuesday, January 07, 2003 14:01:08
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	7024	4674	0.004	ppm
Mn	55	71854	4105	0.062	ppm
Ni	58	1427	-188	0.004	ppm
Cu	63	1485	395	0.003	ppm
Zn	64	13924	3598	0.068	ppm
Pb	208	31387	2187	0.014	ppm

Sample ID: S10
Sample Date/Time: Tuesday, January 07, 2003 14:05:20
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	6939	4674	0.004	ppm
Mn	55	76473	4105	0.068	ppm
Ni	58	1853	-188	0.005	ppm
Cu	63	1739	395	0.004	ppm
Zn	64	14475	3598	0.075	ppm
Pb	208	27287	2187	0.012	ppm

Sample ID: S44
Sample Date/Time: Tuesday, January 07, 2003 14:26:02
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	556910	4951	7.536	ppm
Mn	55	4231363	4293	34.989	ppm
Ni	58	329190	-368	7.335	ppm
Cu	63	278054	309	6.805	ppm
Zn	64	868497	3733	51.644	ppm
Pb	208	25659918	2301	106.415	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: BL 2
Sample Date/Time: Tuesday, January 07, 2003 14:28:38
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	312171	4951	0.448	ppm
Mn	55	60468	4293	0.043	ppm
Ni	58	81791	-368	0.19	ppm
Cu	63	14225	309	0.035	ppm
Zn	64	16634	3733	0.05	ppm
Pb	208	81961	2301	0.024	ppm

Sample ID: S11
Sample Date/Time: Tuesday, January 07, 2003 14:30:16
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	6994	4951	0.004	ppm
Mn	55	101327	4293	0.088	ppm
Ni	58	2149	-368	0.006	ppm
Cu	63	2017	309	0.005	ppm
Zn	64	22590	3733	0.125	ppm
Pb	208	24397	2301	0.01	ppm

Sample ID: S12
Sample Date/Time: Tuesday, January 07, 2003 14:34:30
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	7664	4951	0.004	ppm
Mn	55	78126	4293	0.065	ppm
Ni	58	1571	-368	0.005	ppm
Cu	63	1558	309	0.003	ppm
Zn	64	16763	3733	0.084	ppm
Pb	208	33885	2301	0.014	ppm

Sample ID: S13
Sample Date/Time: Tuesday, January 07, 2003 14:38:43
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	7851	4951	0.005	ppm
Mn	55	98409	4293	0.085	ppm
Ni	58	1908	-368	0.005	ppm
Cu	63	2131	309	0.005	ppm
Zn	64	21426	3733	0.116	ppm
Pb	208	36748	2301	0.016	ppm

Sample ID: S14
Sample Date/Time: Tuesday, January 07, 2003 14:42:54
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	6761	4951	0.003	ppm
Mn	55	91381	4293	0.078	ppm
Ni	58	1695	-368	0.005	ppm
Cu	63	1869	309	0.004	ppm
Zn	64	19346	3733	0.102	ppm
Pb	208	16183	2301	0.006	ppm

Sample ID: S15
Sample Date/Time: Tuesday, January 07, 2003 14:47:01
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	8022	4951	0.005	ppm
Mn	55	72864	4293	0.06	ppm
Ni	58	1314	-368	0.004	ppm
Cu	63	1720	309	0.004	ppm
Zn	64	17321	3733	0.086	ppm
Pb	208	39588	2301	0.016	ppm

Sample ID: S16
Sample Date/Time: Tuesday, January 07, 2003 14:51:08
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	6893	4951	0.003	ppm
Mn	55	53610	4293	0.042	ppm
Ni	58	1084	-368	0.003	ppm
Cu	63	2978	309	0.007	ppm
Zn	64	12573	3733	0.055	ppm
Pb	208	20277	2301	0.008	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID:	S17	Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample Date/Time:	Tuesday, January 07, 2003 14:55:16	Cr	52	6538	4951	0.003	ppm
Number of Replicates:	2	Mn	55	68610	4293	0.056	ppm
Aliquot Volume (mL):	1	Ni	58	1376	-368	0.004	ppm
Diluted To Volume (mL):	10	Cu	63	2793	309	0.006	ppm
		Zn	64	15995	3733	0.078	ppm
		Pb	208	15915	2301	0.006	ppm

Sample ID:	S18	Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample Date/Time:	Tuesday, January 07, 2003 14:59:25	Cr	52	6211	4951	0.002	ppm
Number of Replicates:	2	Mn	55	55010	4293	0.043	ppm
Aliquot Volume (mL):	1	Ni	58	790	-368	0.003	ppm
Diluted To Volume (mL):	10	Cu	63	2127	309	0.005	ppm
		Zn	64	11211	3733	0.046	ppm
		Pb	208	13533	2301	0.005	ppm

Sample ID:	S19	Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample Date/Time:	Tuesday, January 07, 2003 15:03:33	Cr	52	65356	4951	0.094	ppm
Number of Replicates:	2	Mn	55	28597	4293	0.023	ppm
Aliquot Volume (mL):	1	Ni	58	2350	-368	0.007	ppm
Diluted To Volume (mL):	10	Cu	63	2550	309	0.006	ppm
		Zn	64	7365	3733	0.027	ppm
		Pb	208	105275	2301	0.047	ppm

Sample ID:	S20	Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample Date/Time:	Tuesday, January 07, 2003 15:07:43	Cr	52	69320	4951	0.101	ppm
Number of Replicates:	2	Mn	55	27538	4293	0.022	ppm
Aliquot Volume (mL):	1	Ni	58	2205	-368	0.006	ppm
Diluted To Volume (mL):	10	Cu	63	4012	309	0.01	ppm
		Zn	64	7200	3733	0.026	ppm
		Pb	208	274549	2301	0.127	ppm

Sample ID:	S45	Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample Date/Time:	Tuesday, January 07, 2003 15:28:22	Cr	52	618032	5792	8.266	ppm
Number of Replicates:	2	Mn	55	4156538	8022	34.1	ppm
Aliquot Volume (mL):	0.1	Ni	58	343658	-312	7.484	ppm
Diluted To Volume (mL):	10	Cu	63	277109	326	6.587	ppm
		Zn	64	867476	3929	50.737	ppm
		Pb	208	25695484	8007	96.083	ppm

Sample ID:	S21	Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample Date/Time:	Tuesday, January 07, 2003 15:32:32	Cr	52	42377	5792	0.057	ppm
Number of Replicates:	2	Mn	55	26597	8022	0.018	ppm
Aliquot Volume (mL):	1	Ni	58	2069	-312	0.006	ppm
Diluted To Volume (mL):	10	Cu	63	3164	326	0.008	ppm
		Zn	64	11505	3929	0.054	ppm
		Pb	208	180751	8007	0.079	ppm

Sample ID:	S22	Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample Date/Time:	Tuesday, January 07, 2003 15:36:44	Cr	52	2222853	5792	3.82	ppm
Number of Replicates:	2	Mn	55	23540545	8022	24.677	ppm
Aliquot Volume (mL):	1	Ni	58	856014	-312	2.376	ppm
Diluted To Volume (mL):	10	Cu	63	2117536	326	6.429	ppm
		Zn	64	2127161	3929	15.921	ppm
		Pb	208	3580262	8007	1.56	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

		Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample ID:	S23						
Sample Date/Time:	Tuesday, January 07, 2003 15:40:55	Cr	52	2338791	5792	4.173	ppm
Number of Replicates:	2	Mn	55	22467835	8022	24.444	ppm
Aliquot Volume (mL):	1	Ni	58	727938	-312	2.097	ppm
Diluted To Volume (mL):	10	Cu	63	1990379	326	6.272	ppm
		Zn	64	1946730	3929	15.121	ppm
		Pb	208	3371719	8007	1.519	ppm

		Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample ID:	S24						
Sample Date/Time:	Tuesday, January 07, 2003 15:45:07	Cr	52	2289466	5792	4.105	ppm
Number of Replicates:	2	Mn	55	22200520	8022	24.274	ppm
Aliquot Volume (mL):	1	Ni	58	711658	-312	2.06	ppm
Diluted To Volume (mL):	10	Cu	63	1962794	326	6.216	ppm
		Zn	64	1933475	3929	15.093	ppm
		Pb	208	3368162	8007	1.522	ppm

		Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample ID:	S25						
Sample Date/Time:	Tuesday, January 07, 2003 15:49:20	Cr	52	3465394	5792	6.399	ppm
Number of Replicates:	2	Mn	55	8448269	8022	9.504	ppm
Aliquot Volume (mL):	1	Ni	58	928737	-312	2.767	ppm
Diluted To Volume (mL):	10	Cu	63	2088640	326	6.808	ppm
		Zn	64	3407860	3929	27.399	ppm
		Pb	208	6173532	8007	2.305	ppm

		Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample ID:	S26						
Sample Date/Time:	Tuesday, January 07, 2003 15:53:33	Cr	52	4273089	5792	7.933	ppm
Number of Replicates:	2	Mn	55	8374215	8022	9.469	ppm
Aliquot Volume (mL):	1	Ni	58	957920	-312	2.869	ppm
Diluted To Volume (mL):	10	Cu	63	2052730	326	6.725	ppm
		Zn	64	3386649	3929	27.372	ppm
		Pb	208	6188820	8007	2.29	ppm

		Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample ID:	S27						
Sample Date/Time:	Tuesday, January 07, 2003 15:57:46	Cr	52	4243929	5792	8.003	ppm
Number of Replicates:	2	Mn	55	8146522	8022	9.356	ppm
Aliquot Volume (mL):	1	Ni	58	951579	-312	2.894	ppm
Diluted To Volume (mL):	10	Cu	63	2076333	326	6.909	ppm
		Zn	64	3335116	3929	27.376	ppm
		Pb	208	6241262	8007	2.359	ppm

		Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample ID:	S28						
Sample Date/Time:	Tuesday, January 07, 2003 16:02:00	Cr	52	1532191	5792	2.739	ppm
Number of Replicates:	2	Mn	55	27620980	8022	30.142	ppm
Aliquot Volume (mL):	1	Ni	58	381832	-312	1.104	ppm
Diluted To Volume (mL):	10	Cu	63	716977	326	2.265	ppm
		Zn	64	1140441	3929	8.876	ppm
		Pb	208	3532454	8007	1.559	ppm

		Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Sample ID:	S29						
Sample Date/Time:	Tuesday, January 07, 2003 16:06:10	Cr	52	1491676	5792	2.735	ppm
Number of Replicates:	2	Mn	55	28451813	8022	31.85	ppm
Aliquot Volume (mL):	1	Ni	58	427280	-312	1.266	ppm
Diluted To Volume (mL):	10	Cu	63	781172	326	2.532	ppm
		Zn	64	1253031	3929	10.006	ppm
		Pb	208	3865590	8007	1.737	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: S30
Sample Date/Time: Tuesday, January 07, 2003 16:10:17
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	1261047	5792	2.274	ppm
Mn	55	26056971	8022	28.698	ppm
Ni	58	443133	-312	1.293	ppm
Cu	63	753244	326	2.402	ppm
Zn	64	1215384	3929	9.548	ppm
Pb	208	4235306	8007	1.883	ppm

Sample ID: S43
Sample Date/Time: Thursday, January 09, 2003 11:52:05
Number of Replicates: 2
Aliquot Volume (mL): 0.01
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	61705	5683	6.246	ppm
Mn	55	435996	7617	29.039	ppm
Ni	58	40001	-282	7.151	ppm
Cu	63	28979	373	5.536	ppm
Zn	64	97822	7287	43.222	ppm
Pb	208	2496601	1889	79.478	ppm

Sample ID: QC
Sample Date/Time: Thursday, January 09, 2003 11:56:13
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	697014	5683	0.897	ppm
Mn	55	1101918	7617	0.864	ppm
Ni	58	422168	-282	0.873	ppm
Cu	63	387528	373	0.872	ppm
Zn	64	160922	7287	0.859	ppm
Pb	208	2080643	1889	0.628	ppm

Sample ID: BL 1
Sample Date/Time: Thursday, January 09, 2003 11:58:37
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	263645	5683	0.432	ppm
Mn	55	46269	7617	0.042	ppm
Ni	58	63821	-282	0.17	ppm
Cu	63	7337	373	0.02	ppm
Zn	64	15300	7287	0.051	ppm
Pb	208	294609	1889	0.13	ppm

Sample ID: S31
Sample Date/Time: Thursday, January 09, 2003 12:00:51
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	116055	5683	1.312	ppm
Mn	55	1982520	7617	14.241	ppm
Ni	58	164789	-282	3.117	ppm
Cu	63	65944	373	1.35	ppm
Zn	64	136406	7287	6.576	ppm
Pb	208	309689	1889	1.01	ppm

Sample ID: S32
Sample Date/Time: Thursday, January 09, 2003 12:04:59
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	216280	5683	2.644	ppm
Mn	55	3408823	7617	25.927	ppm
Ni	58	251614	-282	5.029	ppm
Cu	63	110890	373	2.406	ppm
Zn	64	216606	7287	11.271	ppm
Pb	208	503775	1889	1.716	ppm

Sample ID: S33
Sample Date/Time: Thursday, January 09, 2003 12:09:07
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	134371	5683	1.575	ppm
Mn	55	1874968	7617	13.857	ppm
Ni	58	147270	-282	2.868	ppm
Cu	63	60448	373	1.273	ppm
Zn	64	120535	7287	5.948	ppm
Pb	208	283577	1889	0.936	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: S34
Sample Date/Time: Thursday, January 09, 2003 12:13:17
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	191290	5683	2.257	ppm
Mn	55	2713157	7617	19.977	ppm
Ni	58	175610	-282	3.401	ppm
Cu	63	71046	373	1.49	ppm
Zn	64	123415	7287	6.068	ppm
Pb	208	229206	1889	0.751	ppm

Sample ID: S35
Sample Date/Time: Thursday, January 09, 2003 12:17:27
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	191214	5683	2.296	ppm
Mn	55	3894238	7617	29.197	ppm
Ni	58	232482	-282	4.579	ppm
Cu	63	99625	373	2.129	ppm
Zn	64	175355	7287	8.924	ppm
Pb	208	315841	1889	1.059	ppm

Sample ID: S36
Sample Date/Time: Thursday, January 09, 2003 12:21:38
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	185574	5683	2.228	ppm
Mn	55	3112921	7617	23.349	ppm
Ni	58	204568	-282	4.034	ppm
Cu	63	81410	373	1.74	ppm
Zn	64	142931	7287	7.217	ppm
Pb	208	261705	1889	0.87	ppm

Sample ID: S37
Sample Date/Time: Thursday, January 09, 2003 12:25:50
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	144580	5683	1.71	ppm
Mn	55	1633922	7617	12.145	ppm
Ni	58	123076	-282	2.412	ppm
Cu	63	59111	373	1.253	ppm
Zn	64	136364	7287	6.819	ppm
Pb	208	429709	1889	1.422	ppm

Sample ID: S38
Sample Date/Time: Thursday, January 09, 2003 12:30:01
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	219103	5683	2.695	ppm
Mn	55	2303215	7617	17.603	ppm
Ni	58	164375	-282	3.305	ppm
Cu	63	87556	373	1.909	ppm
Zn	64	197144	7287	10.288	ppm
Pb	208	671946	1889	2.239	ppm

Sample ID: S39
Sample Date/Time: Thursday, January 09, 2003 12:34:12
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	150053	5683	1.803	ppm
Mn	55	1590057	7617	11.982	ppm
Ni	58	117480	-282	2.334	ppm
Cu	63	57254	373	1.23	ppm
Zn	64	133418	7287	6.762	ppm
Pb	208	407056	1889	1.349	ppm

Sample ID: S40
Sample Date/Time: Thursday, January 09, 2003 12:38:25
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	91331	5683	1.066	ppm
Mn	55	1431030	7617	10.709	ppm
Ni	58	130138	-282	2.568	ppm
Cu	63	55061	373	1.175	ppm
Zn	64	167979	7287	8.543	ppm
Pb	208	268734	1889	0.9	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: S41
Sample Date/Time: Thursday, January 09, 2003 12:59:06
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	133113	5457	1.512	ppm
Mn	55	1410446	10751	10.109	ppm
Ni	58	132509	-233	2.6	ppm
Cu	63	55057	394	1.173	ppm
Zn	64	156168	6712	7.882	ppm
Pb	208	314555	2365	1.169	ppm

Sample ID: S42
Sample Date/Time: Thursday, January 09, 2003 13:03:20
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	161443	5457	1.845	ppm
Mn	55	1438189	10751	10.296	ppm
Ni	58	135627	-233	2.658	ppm
Cu	63	55603	394	1.183	ppm
Zn	64	158370	6712	7.987	ppm
Pb	208	267669	2365	0.983	ppm

Sample ID: S46
Sample Date/Time: Thursday, January 09, 2003 13:07:34
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	390116	5457	0.586	ppm
Mn	55	21546460	10751	19.942	ppm
Ni	58	206538	-233	0.519	ppm
Cu	63	298878	394	0.821	ppm
Zn	64	826010	6712	5.545	ppm
Pb	208	1017846	2365	0.408	ppm

Sample ID: S47
Sample Date/Time: Thursday, January 09, 2003 13:11:44
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	253852	5457	0.358	ppm
Mn	55	21403102	10751	18.717	ppm
Ni	58	191615	-233	0.455	ppm
Cu	63	299954	394	0.779	ppm
Zn	64	885629	6712	5.618	ppm
Pb	208	1180338	2365	0.47	ppm

Sample ID: S48
Sample Date/Time: Thursday, January 09, 2003 13:15:51
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	361196	5457	0.546	ppm
Mn	55	22022885	10751	20.534	ppm
Ni	58	150149	-233	0.38	ppm
Cu	63	270868	394	0.75	ppm
Zn	64	835357	6712	5.649	ppm
Pb	208	1062653	2365	0.437	ppm

Sample ID: S49
Sample Date/Time: Thursday, January 09, 2003 13:19:59
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	54304	5457	0.555	ppm
Mn	55	6292404	10751	43.605	ppm
Ni	58	23144	-233	0.44	ppm
Cu	63	45190	394	0.924	ppm
Zn	64	254202	6712	12.529	ppm
Pb	208	140899	2365	0.513	ppm

Sample ID: S50
Sample Date/Time: Thursday, January 09, 2003 13:24:06
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	78217	5457	0.833	ppm
Mn	55	9412187	10751	65.724	ppm
Ni	58	33409	-233	0.638	ppm
Cu	63	65623	394	1.355	ppm
Zn	64	386124	6712	19.347	ppm
Pb	208	145944	2365	0.542	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID:	S51
Sample Date/Time:	Thursday, January 09, 2003 13:28:15
Number of Replicates:	2
Aliquot Volume (mL):	0.1
Diluted To Volume (mL):	10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	49337	5457	0.487	ppm
Mn	55	6144458	10751	41.716	ppm
Ni	58	22271	-233	0.415	ppm
Cu	63	45085	394	0.903	ppm
Zn	64	246685	6712	11.896	ppm
Pb	208	85927	2365	0.307	ppm

Sample ID:	S52
Sample Date/Time:	Thursday, January 09, 2003 13:32:24
Number of Replicates:	2
Aliquot Volume (mL):	1
Diluted To Volume (mL):	10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	310646	5457	0.465	ppm
Mn	55	7091123	10751	6.563	ppm
Ni	58	213057	-233	0.536	ppm
Cu	63	145446	394	0.4	ppm
Zn	64	1136261	6712	7.646	ppm
Pb	208	248176	2365	0.1	ppm

Sample ID:	S53
Sample Date/Time:	Thursday, January 09, 2003 13:36:33
Number of Replicates:	2
Aliquot Volume (mL):	1
Diluted To Volume (mL):	10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	313517	5457	0.493	ppm
Mn	55	6903537	10751	6.7	ppm
Ni	58	194751	-233	0.514	ppm
Cu	63	137817	394	0.397	ppm
Zn	64	1164214	6712	8.218	ppm
Pb	208	336273	2365	0.14	ppm

Sample ID:	S43
Sample Date/Time:	Thursday, January 09, 2003 13:57:12
Number of Replicates:	2
Aliquot Volume (mL):	0.01
Diluted To Volume (mL):	10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	64327	6936	5.982	ppm
Mn	55	451047	10283	28.324	ppm
Ni	58	40530	-506	7.087	ppm
Cu	63	29077	357	5.432	ppm
Zn	64	100017	7292	42.919	ppm
Pb	208	2597156	2380	93.49	ppm

Sample ID:	QC
Sample Date/Time:	Thursday, January 09, 2003 14:01:20
Number of Replicates:	2
Aliquot Volume (mL):	1
Diluted To Volume (mL):	10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. SD
Cr	52	793208	6936	0.993	0.01
Mn	55	1231258	10283	0.948	0.015
Ni	58	473945	-506	0.988	0.006
Cu	63	426334	357	0.972	0.006
Zn	64	178281	7292	0.963	0.011
Pb	208	2321049	2380	0.846	0.012

Sample ID:	BL 2
Sample Date/Time:	Thursday, January 09, 2003 14:23:38
Number of Replicates:	2
Aliquot Volume (mL):	1
Diluted To Volume (mL):	10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	312171	6936	0.45	ppm
Mn	55	60468	10283	0.043	ppm
Ni	58	81791	-506	0.17	ppm
Zn	64	16634	7292	0.04	ppm
Pb	208	81961	2380	0.025	ppm

Sample ID:	S54
Sample Date/Time:	Thursday, January 09, 2003 14:05:58
Number of Replicates:	2
Aliquot Volume (mL):	1
Diluted To Volume (mL):	10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	356518	6936	0.563	ppm
Mn	55	8066985	10283	7.923	ppm
Ni	58	239748	-506	0.634	ppm
Cu	63	153385	357	0.443	ppm
Zn	64	1230631	7292	8.694	ppm
Pb	208	275212	2380	0.11	ppm

Sample ID:	S55
Sample Date/Time:	Thursday, January 09, 2003 14:10:08
Number of Replicates:	2
Aliquot Volume (mL):	1
Diluted To Volume (mL):	10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	353523	6936	0.563	ppm
Mn	55	29081081	10283	28.842	ppm
Ni	58	160901	-506	0.429	ppm
Cu	63	1126124	357	3.286	ppm
Zn	64	1483984	7292	10.587	ppm
Pb	208	324144	2380	0.131	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: S56
Sample Date/Time: Thursday, January 09, 2003 14:14:19
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	353007	6936	0.576	ppm
Mn	55	28364073	10283	28.827	ppm
Ni	58	166454	-506	0.455	ppm
Cu	63	1030104	357	3.08	ppm
Zn	64	1426059	7292	10.425	ppm
Pb	208	357386	2380	0.146	ppm

Sample ID: S57
Sample Date/Time: Thursday, January 09, 2003 14:18:30
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	325171	6936	0.536	ppm
Mn	55	26785836	10283	27.532	ppm
Ni	58	156113	-506	0.432	ppm
Cu	63	952953	357	2.883	ppm
Zn	64	1317818	7292	9.742	ppm
Pb	208	356861	2380	0.145	ppm

Sample ID: S58
Sample Date/Time: Thursday, January 09, 2003 14:22:42
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	304344	6936	0.492	ppm
Mn	55	19920365	10283	20.098	ppm
Ni	58	148883	-506	0.404	ppm
Cu	63	409468	357	1.215	ppm
Zn	64	1523276	7292	11.059	ppm
Pb	208	335117	2380	0.137	ppm

Sample ID: S59
Sample Date/Time: Thursday, January 09, 2003 14:26:55
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	318819	6936	0.526	ppm
Mn	55	19151619	10283	19.696	ppm
Ni	58	141355	-506	0.391	ppm
Cu	63	389463	357	1.178	ppm
Zn	64	1498871	7292	11.092	ppm
Pb	208	370243	2380	0.153	ppm

Sample ID: S60
Sample Date/Time: Thursday, January 09, 2003 14:31:08
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	316933	6936	0.516	ppm
Mn	55	19224052	10283	19.514	ppm
Ni	58	148958	-506	0.407	ppm
Cu	63	385599	357	1.152	ppm
Zn	64	1505022	7292	10.993	ppm
Pb	208	339569	2380	0.138	ppm

Sample ID: S61
Sample Date/Time: Thursday, January 09, 2003 14:35:20
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	317403	6936	0.525	ppm
Mn	55	27265279	10283	28.121	ppm
Ni	58	260742	-506	0.723	ppm
Cu	63	477302	357	1.448	ppm
Zn	64	2575454	7292	19.138	ppm
Pb	208	555693	2380	0.229	ppm

Sample ID: S62
Sample Date/Time: Thursday, January 09, 2003 14:39:34
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	322926	6936	0.549	ppm
Mn	55	28236883	10283	29.875	ppm
Ni	58	258180	-506	0.734	ppm
Cu	63	479198	357	1.492	ppm
Zn	64	2615933	7292	19.941	ppm
Pb	208	595430	2380	0.247	ppm

Sample ID: S63
Sample Date/Time: Thursday, January 09, 2003 14:43:45
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	340674	6936	0.594	ppm
Mn	55	28738961	10283	31.16	ppm
Ni	58	254269	-506	0.741	ppm
Cu	63	493526	357	1.574	ppm
Zn	64	2646469	7292	20.675	ppm
Pb	208	666725	2380	0.282	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: QC
Sample Date/Time: Thursday, January 09, 2003 15:04:21
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	748840	6449	1.017	ppm
Mn	55	1184709	7958	0.981	ppm
Ni	58	434626	-445	1.014	ppm
Cu	63	387630	277	0.987	ppm
Zn	64	167393	6880	0.991	ppm
Pb	208	2232129	2044	0.881	ppm

Sample ID: S64
Sample Date/Time: Thursday, January 09, 2003 15:08:58
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	300104	6449	0.477	ppm
Mn	55	27358408	7958	26.893	ppm
Ni	58	101127	-445	0.279	ppm
Cu	63	178292	277	0.536	ppm
Zn	64	527801	6880	3.783	ppm
Pb	208	178425	2044	0.073	ppm

Sample ID: S65
Sample Date/Time: Thursday, January 09, 2003 15:13:05
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	290747	6449	0.45	ppm
Mn	55	27127525	7958	25.994	ppm
Ni	58	109993	-445	0.296	ppm
Cu	63	184387	277	0.54	ppm
Zn	64	516658	6880	3.608	ppm
Pb	208	229788	2044	0.093	ppm

Sample ID: S66
Sample Date/Time: Thursday, January 09, 2003 15:17:13
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	313487	6449	0.502	ppm
Mn	55	27826425	7958	27.539	ppm
Ni	58	107631	-445	0.299	ppm
Cu	63	168999	277	0.511	ppm
Zn	64	538797	6880	3.889	ppm
Pb	208	245608	2044	0.102	ppm

Sample ID: S67
Sample Date/Time: Thursday, January 09, 2003 15:21:21
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	338004	6449	0.597	ppm
Mn	55	4999591	7958	5.434	ppm
Ni	58	117521	-445	0.359	ppm
Cu	63	1116411	277	3.716	ppm
Zn	64	2958634	6880	23.645	ppm
Pb	208	271795	2044	0.117	ppm

Sample ID: S68
Sample Date/Time: Thursday, January 09, 2003 15:25:30
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	260037	6449	0.437	ppm
Mn	55	5186129	7958	5.392	ppm
Ni	58	101565	-445	0.297	ppm
Cu	63	1166833	277	3.716	ppm
Zn	64	3125672	6880	23.9	ppm
Pb	208	240666	2044	0.102	ppm

Sample ID: S69
Sample Date/Time: Thursday, January 09, 2003 15:29:39
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	269782	6449	0.467	ppm
Mn	55	4782919	7958	5.115	ppm
Ni	58	86527	-445	0.26	ppm
Cu	63	1055356	277	3.457	ppm
Zn	64	2851274	6880	22.423	ppm
Pb	208	175741	2044	0.076	ppm

Sample ID: S70
Sample Date/Time: Thursday, January 09, 2003 15:33:49
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	309223	6449	0.564	ppm
Mn	55	34008949	7958	38.264	ppm
Ni	58	115007	-445	0.363	ppm
Cu	63	235161	277	0.809	ppm
Zn	64	1560670	6880	12.886	ppm
Pb	208	600481	2044	0.267	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: S71
Sample Date/Time: Thursday, January 09, 2003 15:37:59
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	294361	6449	0.537	ppm
Mn	55	32486547	7958	36.565	ppm
Ni	58	107039	-445	0.338	ppm
Cu	63	220149	277	0.758	ppm
Zn	64	1481462	6880	12.235	ppm
Pb	208	601096	2044	0.27	ppm

Sample ID: S72
Sample Date/Time: Thursday, January 09, 2003 15:42:10
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Units
Cr	52	284458	6449	0.517	ppm
Mn	55	32740927	7958	36.7	ppm
Ni	58	105664	-445	0.333	ppm
Zn	64	1503927	6880	12.373	ppm
Pb	208	567694	2044	0.252	ppm

Sample ID: S43
Sample Date/Time: Monday, January 13, 2003 12:00:50
Number of Replicates: 2
Aliquot Volume (mL): 0.01
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	70988	6729	6.142	1.52	ppm
Mn	55	501777	9221	28.241	0.19	ppm
Ni	58	46669	134	7.13	4.98	ppm
Cu	63	33766	1070	5.43	1.7	ppm
Zn	64	112178	13803	40.627	0.45	ppm
Pb	208	2738243	11560	77.867	0.13	ppm

Sample ID: QC
Sample Date/Time: Monday, January 13, 2003 12:04:58
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	797377	6729	0.867	3.12	ppm
Mn	55	1263560	9221	0.829	3.64	ppm
Ni	58	482649	134	0.851	2.42	ppm
Cu	63	439254	1070	0.837	1.74	ppm
Zn	64	184641	13803	0.818	1.83	ppm
Pb	208	2203225	11560	0.694	1.1	ppm

Sample ID: BL 1
Sample Date/Time: Monday, January 13, 2003 12:09:06
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	359006	6729	0.479	0.32	ppm
Mn	55	54941	9221	0.04	1.61	ppm
Ni	58	80458	134	0.175	1.02	ppm
Cu	63	10026	1070	0.022	0.71	ppm
Zn	64	18948	13803	0.057	2.58	ppm
Pb	208	390979	11560	0.122	0.21	ppm

Sample ID: S73
Sample Date/Time: Monday, January 13, 2003 12:13:15
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	398512	6729	0.551	0.37	ppm
Mn	55	2.9E+07	9221	24.656	0.16	ppm
Ni	58	477320	134	1.074	0.3	ppm
Cu	63	513647	1070	1.25	0.09	ppm
Zn	64	3297135	13803	19.812	0.93	ppm
Pb	208	549047	11560	0.183	0.4	ppm

Sample ID: S74
Sample Date/Time: Monday, January 13, 2003 12:17:24
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	396331	6729	0.553	0.15	ppm
Mn	55	2.9E+07	9221	24.334	0.82	ppm
Ni	58	464898	134	1.057	0.86	ppm
Cu	63	489714	1070	1.204	0.9	ppm
Zn	64	3219586	13803	19.54	0.87	ppm
Pb	208	544648	11560	0.187	1.47	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: S75
Sample Date/Time: Monday, January 13, 2003 12:21:34
Number of Replicates: 2
Initial Sample Quantity (mg):
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	395039	6729	0.561	1.55	ppm
Mn	55	2.8E+07	9221	24.346	0.93	ppm
Ni	58	456810	134	1.057	0.02	ppm
Cu	63	472393	1070	1.181	0.54	ppm
Zn	64	3182219	13803	19.654	0.8	ppm
Pb	208	595054	11560	0.208	0.11	ppm

Sample ID: S76
Sample Date/Time: Monday, January 13, 2003 12:25:44
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	488260	6729	0.705	0.45	ppm
Mn	55	3.4E+07	9221	29.825	0.6	ppm
Ni	58	341420	134	0.801	1.67	ppm
Cu	63	605913	1070	1.538	0.43	ppm
Zn	64	3756272	13803	23.547	0.71	ppm
Pb	208	693679	11560	0.249	0.02	ppm

Sample ID: S77
Sample Date/Time: Monday, January 13, 2003 12:29:55
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	452725	6729	0.668	0.09	ppm
Mn	55	3.5E+07	9221	31.069	0.73	ppm
Ni	58	347733	134	0.834	1.21	ppm
Cu	63	566117	1070	1.467	1.56	ppm
Zn	64	2525679	13803	16.153	0.57	ppm
Pb	208	766582	11560	0.281	1.56	ppm

Sample ID: S78
Sample Date/Time: Monday, January 13, 2003 12:34:06
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	462367	6729	0.672	0.42	ppm
Mn	55	3.3E+07	9221	28.999	1.4	ppm
Ni	58	330480	134	0.78	0.16	ppm
Cu	63	529799	1070	1.353	0.21	ppm
Zn	64	3485924	13803	21.983	0.48	ppm
Pb	208	643392	11560	0.234	0.17	ppm

Sample ID: S79
Sample Date/Time: Monday, January 13, 2003 12:38:17
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	379154	6729	0.546	0.7	ppm
Mn	55	1E+07	9221	8.873	1.25	ppm
Ni	58	200466	134	0.47.	0.81	ppm
Cu	63	443058	1070	1.123	0.18	ppm
Zn	64	1092781	13803	6.805	0.19	ppm
Pb	208	548403	11560	0.198	0.44	ppm

Sample ID: S80
Sample Date/Time: Monday, January 13, 2003 12:42:29
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	340541	6729	0.456	1.02	ppm
Mn	55	1E+07	9221	8.394	0.34	ppm
Ni	58	211210	134	0.462	0.22	ppm
Cu	63	445471	1070	1.053	0.07	ppm
Zn	64	1167467	13803	6.78	0.69	ppm
Pb	208	556817	11560	0.192	0.39	ppm

Sample ID: S81
Sample Date/Time: Monday, January 13, 2003 12:46:42
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	357239	6729	0.505	0.21	ppm
Mn	55	1E+07	9221	8.73	1.77	ppm
Ni	58	192780	134	0.444	0.94	ppm
Cu	63	446150	1070	1.112	0.55	ppm
Zn	64	1117244	13803	6.843	0.42	ppm
Pb	208	484262	11560	0.171	0.36	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: S82
Sample Date/Time: Monday, January 13, 2003 12:50:55
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	369235	6729	0.539	0.84	ppm
Mn	55	4.1E+07	9221	36.206	0.17	ppm
Ni	58	338281	134	0.804	0.11	ppm
Cu	63	520434	1070	1.338	1.08	ppm
Zn	64	3442655	13803	21.863	0.2	ppm
Pb	208	615917	11560	0.224	1.13	ppm

Sample ID: BL 2
Sample Date/Time: Monday, January 13, 2003 13:11:38
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	312171	8150	0.438	1.14	ppm
Mn	55	60468	13121	0.046	0.75	ppm
Ni	58	81791	-76	0.19	0.44	ppm
Cu	63	14225	911	0.035	0.69	ppm
Zn	64	16634	13803	0.049	3.13	ppm
Pb	208	81961	13344	0.026	0.08	ppm

Sample ID: S83
Sample Date/Time: Monday, January 13, 2003 13:15:52
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	379066	8150	0.569	0.67	ppm
Mn	55	4.4E+07	13121	41.463	0.16	ppm
Ni	58	353996	-76	0.877	1.63	ppm
Cu	63	538908	911	1.456	0.86	ppm
Zn	64	3710291	13803	24.821	0.68	ppm
Pb	208	635091	13344	0.255	0.17	ppm

Sample ID: S84
Sample Date/Time: Monday, January 13, 2003 13:20:02
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	384605	8150	0.593	2.25	ppm
Mn	55	4.4E+07	13121	41.759	2.1	ppm
Ni	58	340376	-76	0.865	4.11	ppm
Cu	63	533678	911	1.479	2.58	ppm
Zn	64	3575372	13803	24.53	1.98	ppm
Pb	208	625365	13344	0.256	1.5	ppm

Sample ID: S85
Sample Date/Time: Monday, January 13, 2003 13:24:09
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	537096	8150	0.879	0.07	ppm
Mn	55	9E+07	13121	91.653	0.11	ppm
Ni	58	438610	-76	1.178	1.97	ppm
Cu	63	1430383	911	4.193	1.65	ppm
Zn	64	3767369	13803	27.338	0.44	ppm
Pb	208	1401564	13344	0.598	2.11	ppm

Sample ID: S86
Sample Date/Time: Monday, January 13, 2003 13:28:46
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	514313	8150	0.841	1.01	ppm
Mn	55	8.9E+07	13121	89.972	0.75	ppm
Ni	58	416144	-76	1.118	3.71	ppm
Cu	63	1386829	911	4.066	2.63	ppm
Zn	64	3700680	13803	26.858	1.21	ppm
Pb	208	982891	13344	0.422	1.93	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: S87
Sample Date/Time: Monday, January 13, 2003 13:32:55
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	488076	8150	0.817	0.59	ppm
Mn	55	8.6E+07	13121	89.604	0.7	ppm
Ni	58	407036	-76	1.12	2.94	ppm
Cu	63	1339901	911	4.022	1.15	ppm
Zn	64	3587548	13803	26.657	0.43	ppm
Pb	208	887288	13344	0.389	0.33	ppm

Sample ID: S88
Sample Date/Time: Monday, January 13, 2003 13:37:03
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	318979	8150	0.493	1.46	ppm
Mn	55	9392684	13121	9.049	1.8	ppm
Ni	58	142634	-76	0.364	1.66	ppm
Cu	63	224820	911	0.625	0.04	ppm
Zn	64	662438	13803	4.52	0.73	ppm
Pb	208	231214	13344	0.092	0.09	ppm

Sample ID: S89
Sample Date/Time: Monday, January 13, 2003 13:41:12
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	328280	8150	0.505	0.79	ppm
Mn	55	9892033	13121	9.489	1.39	ppm
Ni	58	146383	-76	0.372	0.43	ppm
Cu	63	231710	911	0.641	0.03	ppm
Zn	64	677859	13803	4.607	0.16	ppm
Pb	208	268884	13344	0.108	1	ppm

Sample ID: S90
Sample Date/Time: Monday, January 13, 2003 13:45:22
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	326764	8150	0.506	0.1	ppm
Mn	55	9892262	13121	9.547	0.81	ppm
Ni	58	150090	-76	0.384	2.03	ppm
Cu	63	241370	911	0.672	1.07	ppm
Zn	64	683463	13803	4.675	0.94	ppm
Pb	208	281331	13344	0.114	1.65	ppm

Sample ID: S91
Sample Date/Time: Monday, January 13, 2003 13:49:31
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	432150	8150	0.674	0.34	ppm
Mn	55	1.4E+07	13121	14.016	1.82	ppm
Ni	58	199065	-76	0.511	0.32	ppm
Cu	63	293753	911	0.822	0.88	ppm
Zn	64	812714	13803	5.595	1.2	ppm
Pb	208	645592	13344	0.263	0.42	ppm

Sample ID: S92
Sample Date/Time: Monday, January 13, 2003 13:53:42
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	460094	8150	0.734	0.6	ppm
Mn	55	1.5E+07	13121	14.971	0.15	ppm
Ni	58	213532	-76	0.561	3.2	ppm
Cu	63	308728	911	0.883	0.94	ppm
Zn	64	835019	13803	5.878	1.26	ppm
Pb	208	696502	13344	0.291	2.31	ppm

Sample ID: S43
Sample Date/Time: Monday, January 13, 2003 16:12:57
Number of Replicates: 2
Aliquot Volume (mL): 0.01
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	56278	7253	6.096	0.28	ppm
Mn	55	389040	11139	28.181	1.21	ppm
Ni	58	32549	-99	6.67	3.21	ppm
Cu	63	24543	789	5.401	1.31	ppm
Zn	64	83972	12161	40.427	0.06	ppm
Pb	208	2212526	9341	76.373	2.69	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: QC
Sample Date/Time: Monday, January 13, 2003 14:18:27
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	753421	7807	0.982	0.64	ppm
Mn	55	1180384	12113	0.936	0.57	ppm
Ni	58	431106	-142	0.928	1.26	ppm
Cu	63	393798	851	0.927	0.08	ppm
Zn	64	165841	12738	0.916	0.61	ppm
Pb	208	2096439	10063	0.746	0.98	ppm

Sample ID: BLANK1
Sample Date/Time: Monday, January 13, 2003 14:22:37
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	263645	7807	0.421	2.11	ppm
Mn	55	46269	12113	0.039	2.43	ppm
Ni	58	63821	-142	0.17	0.55	ppm
Cu	63	7337	851	0.02	0.29	ppm
Zn	64	15300	12738	0.055	0.1	ppm
Pb	208	294609	10063	0.125	1.33	ppm

Sample ID: S93
Sample Date/Time: Monday, January 13, 2003 14:26:47
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	413901	7807	0.711	1.09	ppm
Mn	55	1.3E+07	12113	14.083	2.12	ppm
Ni	58	174727	-142	0.497	0.42	ppm
Cu	63	288815	851	0.898	0.3	ppm
Zn	64	729337	12738	5.588	0.31	ppm
Pb	208	610057	10063	0.283	0.21	ppm

Sample ID: S94
Sample Date/Time: Monday, January 13, 2003 14:30:58
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	280561	7807	0.442	0.83	ppm
Mn	55	1.2E+07	12113	11.895	2.19	ppm
Ni	58	103648	-142	0.272	0.17	ppm
Cu	63	173376	851	0.497	0.11	ppm
Zn	64	681132	12738	4.811	0.61	ppm
Pb	208	239393	10063	0.104	1.15	ppm

Sample ID: S95
Sample Date/Time: Monday, January 13, 2003 14:35:10
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	298583	7807	0.46	0.72	ppm
Mn	55	1.3E+07	12113	12.39	0.07	ppm
Ni	58	113044	-142	0.29	3.42	ppm
Cu	63	174249	851	0.488	1.18	ppm
Zn	64	769713	12738	5.313	1.75	ppm
Pb	208	338785	10063	0.145	1.6	ppm

Sample ID: S96
Sample Date/Time: Monday, January 13, 2003 14:39:22
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	296939	7807	0.468	0.28	ppm
Mn	55	1.2E+07	12113	11.724	1.1	ppm
Ni	58	106051	-142	0.278	1.41	ppm
Cu	63	162397	851	0.465	1.01	ppm
Zn	64	699984	12738	4.939	1.24	ppm
Pb	208	297934	10063	0.128	0.62	ppm

Sample ID: S97
Sample Date/Time: Monday, January 13, 2003 14:43:35
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	301941	7807	0.472	0.58	ppm
Mn	55	1.6E+07	12113	15.004	2.11	ppm
Ni	58	158913	-142	0.413	1.36	ppm
Cu	63	419794	851	1.194	0.82	ppm
Zn	64	2091807	12738	14.746	0.57	ppm
Pb	208	239622	10063	0.102	0.43	ppm

APPENDIX B continued

Raw data (ICP-MS)of heavy metal concentration in plants, sludge and water, continued

Sample ID: S98
Sample Date/Time: Monday, January 13, 2003 14:47:48
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	261212	7807	0.377	0.28	ppm
Mn	55	1.6E+07	12113	14.3	1.8	ppm
Ni	58	173010	-142	0.417	0.02	ppm
Cu	63	339553	851	0.896	0.01	ppm
Zn	64	2362546	12738	15.454	1.7	ppm
Pb	208	213012	10063	-0.084	0.96	ppm

Sample ID: S99
Sample Date/Time: Monday, January 13, 2003 14:52:02
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	300572	7807	0.461	1.55	ppm
Mn	55	1.6E+07	12113	14.729	1.22	ppm
Ni	58	153056	-142	0.391	1.25	ppm
Cu	63	380583	851	1.063	0.62	ppm
Zn	64	2192165	12738	15.175	0.1	ppm
Pb	208	975935	10063	0.427	0.32	ppm

Sample ID: S100
Sample Date/Time: Monday, January 13, 2003 14:56:12
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	353156	7807	0.565	0.14	ppm
Mn	55	3289229	12113	3.237	0.38	ppm
Ni	58	143840	-142	0.382	1.34	ppm
Cu	63	462958	851	1.345	0.19	ppm
Zn	64	1570358	12738	11.292	0.64	ppm
Pb	208	359442	10063	0.157	0.07	ppm

Sample ID: S101
Sample Date/Time: Monday, January 13, 2003 15:00:18
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	386823	7807	0.631	1.56	ppm
Mn	55	3249260	12113	3.255	0.31	ppm
Ni	58	149656	-142	0.405	1.75	ppm
Cu	63	462787	851	1.369	0.13	ppm
Zn	64	1561771	12738	11.433	1.04	ppm
Pb	208	515699	10063	0.229	0.51	ppm

Sample ID: QC
Sample Date/Time: Monday, January 13, 2003 15:23:01
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	778386	7253	1.029	2.17	ppm
Mn	55	1214044	11139	0.982	2.46	ppm
Ni	58	442305	-99	0.992	3.11	ppm
Cu	63	398249	789	0.987	2.26	ppm
Zn	64	169691	12161	0.964	1.1	ppm
Pb	208	2212731	9341	0.829	1.67	ppm

Sample ID: BL2
Sample Date/Time: Monday, January 13, 2003 15:27:11
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	268399	7253	0.444	1.25	ppm
Mn	55	51874	11139	0.046	0.62	ppm
Ni	58	67514	-99	0.192	0.28	ppm
Cu	63	11059	789	0.033	1.58	ppm
Zn	64	13834	12161	0.05	1.99	ppm
Pb	208	65669	9341	0.027	0.46	ppm

Sample ID: S102
Sample Date/Time: Monday, January 13, 2003 15:31:22
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	360182	7253	0.618	1.32	ppm
Mn	55	3084000	11139	3.27	0.97	ppm
Ni	58	134046	-99	0.393	0.97	ppm
Cu	63	403799	789	1.306	0.35	ppm
Zn	64	1477292	12161	11.521	0.45	ppm
Pb	208	447656	9341	0.216	0.43	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: S103
Sample Date/Time: Monday, January 13, 2003 15:35:29
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	296945	7253	0.481	0.54	ppm
Mn	55	2437559	11139	2.447	0.61	ppm
Ni	58	111751	-99	0.31	1.73	ppm
Cu	63	279569	789	0.856	1.44	ppm
Zn	64	1064768	12161	7.85	0.89	ppm
Pb	208	273940	9341	0.126	1.34	ppm

Sample ID: S104
Sample Date/Time: Monday, January 13, 2003 15:39:37
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	283909	7253	0.448	0.15	ppm
Mn	55	2320818	11139	2.272	0.17	ppm
Ni	58	103564	-99	0.28	0.69	ppm
Cu	63	235836	789	0.704	0.7	ppm
Zn	64	1025119	12161	7.37	0.03	ppm
Pb	208	189649	9341	0.084	0.84	ppm

Sample ID: S105
Sample Date/Time: Monday, January 13, 2003 15:43:46
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	314266	7253	0.524	0.57	ppm
Mn	55	2449251	11139	2.526	1.25	ppm
Ni	58	113691	-99	0.324	1.73	ppm
Cu	63	260408	789	0.819	1.56	ppm
Zn	64	1062103	12161	8.046	1.39	ppm
Pb	208	217952	9341	0.101	0.79	ppm

Sample ID: S106
Sample Date/Time: Monday, January 13, 2003 15:47:55
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	397911	7253	0.669	0.96	ppm
Mn	55	1.2E+07	11139	11.98	1.41	ppm
Ni	58	159225	-99	0.456	2.47	ppm
Cu	63	389259	789	1.231	1.96	ppm
Zn	64	3189181	12161	24.385	0.72	ppm
Pb	208	502873	9341	0.237	1.99	ppm

Sample ID: S107
Sample Date/Time: Monday, January 13, 2003 15:52:05
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	377416	7253	0.587	0.09	ppm
Mn	55	1.1E+07	11139	10.844	0.33	ppm
Ni	58	162477	-99	0.432	0.31	ppm
Cu	63	401633	789	1.178	0.16	ppm
Zn	64	3234130	12161	22.933	0.05	ppm
Pb	208	576691	9341	0.255	1.13	ppm

Sample ID: S108
Sample Date/Time: Monday, January 13, 2003 15:56:15
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	293514	7253	0.438	0.54	ppm
Mn	55	1.1E+07	11139	10.345	0.96	ppm
Ni	58	156610	-99	0.4	0.22	ppm
Cu	63	411340	789	1.161	0.18	ppm
Zn	64	3099859	12161	21.154	6.29	ppm
Pb	208	445948	9341	0.191	0.62	ppm

Sample ID: S109
Sample Date/Time: Monday, January 13, 2003 16:00:25
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	352724	7253	0.598	1	ppm
Mn	55	4270433	11139	4.478	0.66	ppm
Ni	58	130114	-99	0.377	1.05	ppm
Cu	63	258218	789	0.825	0.36	ppm
Zn	64	1075525	12161	8.276	0.06	ppm
Pb	208	318421	9341	0.148	0.59	ppm

APPENDIX B continued

Raw data (ICP-MS) of heavy metal concentration in plants, sludge and water, continued

Sample ID: S110
Sample Date/Time: Monday, January 13, 2003 16:04:36
Number of Replicates: 2
Aliquot Volume (mL): 1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	359956	7253	0.604	0.28	ppm
Mn	55	4299388	11139	4.46	0.38	ppm
Ni	58	132640	-99	0.38	2.4	ppm
Cu	63	279283	789	0.883	0.38	ppm
Zn	64	1077651	12161	8.202	0.72	ppm
Pb	208	180893	9341	0.082	0.1	ppm

Sample ID: S32
Sample Date/Time: Monday, January 13, 2003 16:08:47
Number of Replicates: 2
Aliquot Volume (mL): 0.1
Diluted To Volume (mL): 10

Analyte	Mass	Peak Intens.	Blank Intens.	Concentration	Conc. RSD	Units
Cr	52	166686	7253	2.117	5.47	ppm
Mn	55	2670086	11139	21.391	3.56	ppm
Ni	58	171413	-99	3.797	0	ppm
Cu	63	81869	789	1.99	5.01	ppm
Zn	64	166055	12161	9.291	4.09	ppm
Pb	208	354281	9341	1.258	3.77	ppm