SELECTED HEAVY METALS CONCENTRATIONS ON SELECTED SAMPLES OF NABOOM SPRUIT, TOBIAS SPRUIT AND THE NYL FLOODPLAIN, SOUTH AFRICA

by

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THESIS

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE
in

ZOOLOGY

in the

FACULTY OF HEALTH, SCIENCE AND AGRICULTURE

at the

UNIVERSITY OF LIMPOPO

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MAY 2008

# ACKNOWLEDGEMENTS

Very special thanks to:

- Dr. W. Vlok and Prof. V. Wepener for their continuous participation and support.
- Staff at both Department of Biodiversity: University of Limpopo (UL) and Department of Zoology: University of Johannesburg (UJ).
- The UL and UJ for the use of the facilities and financial support.
- Dr. R. Greenfield (UJ), Ms. M.C. Shai and Mr. M.S. Chavalala (both UL) for their active participation and encouragements.
- My parents Morobane and Ramuji for all unconditional love and support.
- My siblings Moshidi, Galagale, Mmamogoane and Mokgoshing for always encouraging me to hold through thick and thin.
- My daughter Lesedi for being a source inspiration.
- All my friends who were part of my life most importantly Mmaserebane, Basani and Mmaphori.
- The Department of Tourism, Environment and Conservation in the Northern Cape, Institute of Natural Resources and the Department of Water Affairs and Forestry for the support and resources used.
- Above all, God for making all these possible, it is through His grace that this happened.

DECLARATION

I declare that this thesis hereby submitted to the University of Limpopo for the Master degree in Zoology has not previously submitted by me for a degree at any other University, that it is my own work in design and execution and that all material contained herein has been duly acknowledged.

Signed

ABSTRACT

The National Water Act (Act No. 36 of 1998) requires effective use of our water resources for protection of the environment and provision of safe water for current and future generations. Previous studies in the study area have shown that there are certain heavy metals occurring at elevated concentrations. These metals are cadmium, lead, zinc, copper and chromium. Metals tend to increase in concentrations in organisms at higher trophic levels through a process called bioaccumulation and this was assessed during the study. There is very little data on the tributaries of this system because the streams flow intermittently. Less attention has in the past been given to these smaller streams yet they contribute a great deal to larger rivers of interest. This study focused on Naboom Spruit and Tobias Spruit, both tributaries of the Mogalakena River.

The study was to determine the levels of certain selected metals within the system and to prove the hypothesis that wetlands act as pollutant filters. The study was also undertaken to further assess levels of those metals which have been recorded to be occurring at high concentration in certain areas within the Mogalakwena River system. A supplementary fish exposure laboratory experiment to determine the rate of uptake of cadmium and zinc by Clarias gariepinus in vitro under controlled conditions was also undertaken.

Both the high and low flow surveys were conducted where physico-chemical parameters were recorded on sites and water, fish, aquatic macro-invertebrates, sediment and aquatic plants were collected. These samples were analysed for cadmium, lead, zinc, copper and chromium content using methods described in chapter 3 of the study. The laboratory fish exposure experiment followed the analytical protocol described in Chapter 7 of this document.

Results obtained were statistically analysed and recorded and of the five metals studied in this project, the mean concentrations in all samples at the studied reaches of the sub-catchment ranked: Zinc>Copper>Chromium>Lead>Cadmium. The ranking for samples is generally: sediment>invertebrates>fish>plants. The hypothesis that sediment tend to have increased free and compound heavy metals was clearly depicted *in this study. There was increase in metal concentrations in sediment samples also increased on sites where there was less or no flow as a result of lower turbulence. In general, metal contents between sites ranked: Driefontein>Mine>Tobias>Bergland>Sacchariasboom. Metal concentrations were low at Bergland, increasing downstream and after the vlei area decreasing to as little as in the headwater at Sacchariasboom. It was then deduced that the vlei area plays its important role as a filter for pollutants in the natural system as the Sacchariasboom site is downstream of a wetland area after confluence of the Tobias Spruit with the Nylsvley.*

All concentrations of the five metals studied were higher in the other fish species as compared to values measured in C. gariepinus. These seem to relate perfectly to the low concentration of all the metals in Sacchariasboom site, where the C. gariepinus analysed for metals were collected.

It is often difficult to determine in the natural environment, which factor/s in the system affects concentrations of other metals and substance, and which one causes a problem, because of multiple occurrences. Laboratory tests such as metals exposure to biota under set conditions was used in this study to determine synergistic and or additive effects of all components in a natural system. Under conditions of the experiment performed in this study, zinc showed to have higher affinity and or was taken up much quicker by C. gariepinus than cadmium, both in pure metal (zinc or cadmium alone) and combination of metals (zinc/cadmium solution), under similar conditions. Zinc has therefore proved to have higher binding affinity to proteins of the fish than cadmium under the set condition in this experiment.

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CHAPTER 1

1. INTRODUCTION

1.1. State of freshwater ecosystems

Several, if not all, South African rivers are currently subjected to some major forms of alteration and deterioration. Generally, this modification can be equated to water quality problems and water abstraction. However, other aspects such as catchment management and physical alteration of aquatic habitats often play a major role (Kleynhans, 1996). There are only a few rivers in South Africa that have not been over-exploited, degraded, polluted or regulated by many impoundments. Many others were perennial, but now flow only seasonally or intermittently (Davies *et al.*, 1993). South Africa's freshwater environment is affected by three conditions; firstly, climate, which is characterised by inadequate and unequal rainfall and high evaporation rates. The second factor is the rapid human population growth. The last factor is the needs for economic development and the desire to meet the needs of a developing country (Walmsley *et al.*, 2001). These socio-economic activities increase water use and lead to a greater water demand and in a way increases pollution of our natural resources.

This emphasises the need for continuous and urgent effective water management with respect to both water quantity and quality. An abundance of water is of no importance if its quality makes it unfit for consumption or utilisation by users, or if the quality is such that the maintenance of natural riverine ecosystem is not ensured (Dallas and Day, 1993). The Department of Water Affairs and Forestry (1990) refers to the five water users as domestic, industrial, agriculture, recreation and the environment.

Of all the natural resources, a river is more susceptible to pollution as it is a confined, uni-directional system that drains the landscape. Activities in the catchment are reflected by changes in the aquatic ecosystem and alterations or perturbation, occurring in any reach of the river has effect on the down stream. The extent of the longitudinal and temporal effects of pollution is dependent on the ability of the riverine biota to deal with the particular pollutants entering the river. In turn, the ability of the riverine biota to purify water in polluted rivers depends on the extent and type of pollutants entering the river. Highly concentrated pollutants entering the river system may inhibit or destroy the natural self-cleaning ability of the river (Dallas and Day, 1993).

1.2. Bioaccumulation

Bioaccumulation is defined as the net accumulation of a chemical by an organism as a result of uptake from all environmental sources, i.e., both food and ambient water (Burkhard *et al.*, 2003). Bioaccumulation includes bioconcentration process, which are the net accumulation of a chemical by an aquatic organism as the result of uptake from ambient water, through gill membranes or other external body surfaces. Bioaccumulation also includes biomagnification processes that results in greater concentrations of a chemical in tissues of predators than in their prey, primarily through the mechanism of dietary accumulation. For aquatic organisms, bioaccumulation can be expressed on either a water or sediment bases (Burkhard *et al.*, 2003). No organic life can develop without the involvement of metal ions (Maartens, 1994). Metal ions play a very important role in living organisms and their deficiency results in impairment of biological functions. However, when present in excessive amounts, essential metals may become toxic. Bioaccumulation occurs in which wastes are concentrated in tissue of organism. It is an important mechanism by which wastes enter food chains (Manahan, 2001). In order for a chemical to exert a toxic effect, it must move from the ambient environment into the organism. For any compound to enter an organism, regardless of the route of entry, it must cross cell membranes (Connell, 1997). The chemical nature of the aqueous environment and characteristics of the metals primarily determine the availability and subsequent toxicity of heavy metals to aquatic biota. Most investigations of trace metal dynamics and their bioavailability in freshwaters indicates that toxicity of those metals depends on the alkalinity of the water (Villarreal-Treviño *et al.*, 1986).

Levels of pollutants in the tissue of organisms are widely used to indicate the degree of contamination of the waters in which they are living. This is particularly true when the pollutants are present only intermittently or in very low concentrations, making chemical analysis of the water difficult (Abel, 1989). Exposure of an organism to the chemicals in the environment occurs through air, water, soil and or food. Upon entry into the organism via stomach, gills, lungs, etc., chemicals are distributed through circulatory fluids into tissues and remain in an inactive form, which can be circulated again within the body (Manahan, 2001). Some chemicals forms original compounds which induce toxicant action in the body while others are biotransformed compounds which are excreted through urine, faeces, in lungs and gills (Manahan, 2001). Bioaccumulation of metals in aquatic organisms and the toxicity of metals may be affected by the following factors (Maartens, 1994):

- Form of the metal, whether inorganic, organic, particulate or soluble.
- The presence of other metals resulting in additive, synergistic or antagonistic actions.
- Physico-chemical factors such as pH, temperature, dissolved oxygen and total dissolved solids.
- Biological factors such as physiological behaviour stage in life cycle, life history, species specific, contamination by food, starvation and adaptation to metals.

1.3. Tolerance limits and their effects

Over evolutionary time, succeeding generations of organisms of each species have become adapted to certain concentrations of each water quality variable. The concentrations vary from species to species. The ranges that a particular species can survive are known as tolerance ranges and the upper and lower values are known as the tolerance limits (Dallas and Day, 1993). Within the tolerance limits, the optimal range is that concentration of the substance to which organisms are most ideally adapted and which affect growth, fecundity and other measures of the organism's health status. Organisms of a given species can survive values within this range, but as the upper or lower tolerance limits are reached, more and more abnormalities become evident. The first signs are usually behavioural, such as avoidance of non-optimal conditions by affected fish. Beyond behavioural changes, physiological stress like changes in respiratory, metabolic and excretory rates may become apparent. All of these changes may be accompanied by a decrease in egg numbers and sperm production resulting in a poor fecundity rate. As tolerance limits are reached, organisms become more susceptible to parasites and pathogens. Very often adults seem unaffected by these by sub-optimal conditions, although breeding success may be greatly reduced if eggs cannot hatch or larvae cannot grow. Juvenile stages are frequently far more sensitive than adults (Dallas and Day, 1993). If such chronic effects persist for some time or occur frequently, they can lead to the eventual death of individuals and disappearance of sensitive species from aquatic ecosystems. This can have considerable negative consequences for the health of aquatic ecosystems, since all components of the aquatic ecosystems are interdependent. The level of a constituent at which there is expected to be significant probability of measurable chronic effects up to 5% of the species in the aquatic system is known as the Chronic Effect Value (DWAF, 1996).

1.4. Metals in a natural environment

Under normal circumstances metals, which are mainly essential, such as copper and zinc, may become pollutants when present in excess by exhibiting toxic effects on organisms (van Vuren *et al.*, 1999). The effects of different metals present in water range from beneficial (calcium, zinc), through troublesome, to being dangerously toxic in certain cases (lead, mercury). Some metals may be either beneficial or toxic, depending on their concentrations and its physiological behaviour (Galvin, 1996). The distribution of metals on the earth is not uniform and living organisms have adapted to these conditions during their evolutionary development. Human intervention has resulted in redistribution of metals (van Vuren *et al.*, 1999), most commonly, localised areas of high concentrations of metals are found, not surprisingly, closely situated to areas of high industrial activity.

1.5. Metal behaviour

Metals exist in water at equilibrium between free metal ions, bound to inorganic complexes and bound to organic and inorganic matter. Heavy metals vary widely in their physical, chemical and biological properties. They share the common property of forming stable coordination complexes with a variety of elements (Nisha and Pandey, 1984). All heavy metals are potentially harmful to most organisms at some level of exposure and accumulation. Some heavy metals are toxic to most organisms even at the lowest concentrations and are rarely beneficial to life. With growing industrial activities, their concentrations in aquatic ecosystems are continuously increasing. Hence, in addition to their qualitative effects, it is essential to estimate their impact on living systems (Nisha and Pandey, 1984).

Unlike organic pollutants, heavy metals are not usually eliminated from the aquatic system by natural biodegradation processes such as microbial action. Instead they accumulate in the sediments where they may be released by various processes such as the availability of binding substances in the water (Forstner and Wittman, 1979). This brings about fluctuations of metal concentrations in surface water and the metals undergo a global eco-biological cycle in which natural water is the main pathway of pollution (van Vuren *et al.*, 1994). Toxicity and speciation of metals is strongly influenced by the chemical composition of the water (Calow, 1994), which is in turn influenced by physical parameters (Maartens, 1994).

1.5.1. Cadmium

Cadmium is a metal element, which is highly toxic to marine and freshwater aquatic life (DWAF, 1996). It is highly toxic to wildlife and is cancer-causing and teratogenic and potential mutation-causing, with severe sublethal and lethal effects at low concentrations (Eisler, 1985). Crustaceans appear to be more sensitive to cadmium than fish and molluscs (Sadiq, 1992). Cadmium is present in the earth's crust at an average concentration of 0.2 mg/kg, generally in association with zinc, lead and copper sulphide ore bodies. It is insoluble in water though many of its organic and inorganic salts are highly soluble and exists largely as the free divalent ion across the acidic pH range 7 to 4, assuming that it is not complexed by dissolved organic material (Spry and Wiener, 1990). Cadmium is affected mainly by water hardness and the presence of ligands and co-existing metal cations (Galvin, 1996). Cadmium, which is widely used and extremely toxic in relatively low dosages, is one of the principle heavy metals responsible for causing kidney damage, renal disorder, high blood pressure, bone fracture and destruction of red blood cells (Drash, 1993). The main anthropogenic pathway through which cadmium enters water bodies is via wastes from industrial processes such as electroplating, plastic manufacturing, metallurgical processes and industries of pigments and cadmium/nickel batteries (Cheremisinoff, 1995).

The presence of other metals may result in either synergistic or antagonistic interactions. The presence of cadmium and mercury may result in reduced toxic effects of both metals while the interaction of copper and cadmium leads to a fivefold increase in the toxicity of each metal (DWAF, 1996).

1.5.2. Chromium

Chromium is a relatively scarce metal, and its occurrence and quantity in aquatic ecosystems are usually very low (DWAF, 1996). Chromium however has a wide range of adverse effects in aquatic organisms. In benthic invertebrates there has been observed reduced fecundity and survival, growth inhibition and abnormal movement patterns (USEPA, 1980). Fish are reported to show reduced growth, chromosomal aberrations, reduced disease resistance and morphological changes. It is found in the mineral chromate, a trivalent state. The main sources of chromium to the environment are industrial effluents (Galvin, 1996). For example, chromium from wastes with chromic acid, used in the automobile industry and also processes involving leather tanning (de Lange, 1994). Toxicity of this metal is affected by water hardness and pH (DWAF, 1996) and available data indicated that acute toxicity decreases as water hardness and pH increase.

1.5.3. Copper

Copper is one of the most widely used metals (DWAF, 1996) and occurs in nature either in the elemental form or in several compounds such as oxides, complex sulphates and carbonates (Galvin, 1996). It is a common metallic element in the rocks and minerals of the earth's crust and this form is insoluble in water, but many copper salts are highly soluble as cupric or cuprous ions (DWAF, 1996). It is a micronutrient and an essential component of enzymes involved in redox reactions and is rapidly accumulated by plants and animals where it is toxic at low concentration in an aqueous medium (DWAF, 1996). Copper is strongly adsorbs to organic matter, carbonates and clay, which reduces its bioavailability. It is highly toxic in aquatic environments and has effects in fish, invertebrates and amphibians (USEPA, 1993; Horne and Dunson, 1995).

Although copper is toxic to living organisms, it is one of several heavy metals that are essential to life. Its toxicity can be avoided in three ways:

- By developing an active process for eliminating any excess copper ingested in the diet.
- By reducing the thermodynamic activity of copper ions virtually to zero by utilising the metal only as prosthetic element tightly bound to specific copper proteins, and
- By an interaction between zinc and copper (Scheinberg and Sternlieb, 1989).

Copper is only toxic to man and animals when one of these mechanisms is defective, either because of genetic or acquired causes.

1.5.4. Lead

Lead is physiologically non-essential, potentially hazardous to most forms of life and it is considered toxic and relatively accessible to aquatic organisms (Galvin, 1996). Fish exposed to high levels of lead exhibit a wide-range of effects including muscular and neurological degeneration and destruction, growth inhibition, mortality, reproductive problems and paralysis (Eisler, 1988). Factors such as water pH, water hardness, organic materials and other metals influence toxicity of metals. Lead partitions primarily to sediments, but becomes more bioavailable at low pH, hardness and organic matter content (among other factors). At a constant pH, solubility decreases with increasing alkalinity. Soluble lead is removed from solution by association with sediments and suspended particulates of inorganic and organic materials, such as hydrous oxides, clays and humic acids, respectively (DWAF, 1996). The combustion of oil and gasoline account for more than 50% of anthropogenic lead emissions and therefore atmospheric fallout is usually the most important source of lead in freshwater systems (Seymore *et al.*, 1995).

1.5.5. Zinc

Zinc is a metallic element and an essential micronutrient for life. It is present in several amino acids and involved in alcohol and protein metabolism. Zinc forms the active site in various metalloenzyme components (Galvin, 1996). It occurs frequently in ores and rocks in nature, being associated with iron, copper, cadmium and lead (DWAF, 1996). The toxicity of zinc is reduced in hard waters and in animals it is a metabolic antagonist of cadmium and synergistically toxic with cyanide. Copper increases the zinc toxicity in soft, but not in hard water, and zinc toxicity increases at lower oxygen concentrations (DWAF, 1996). In many types of aquatic plants and animals, growth, survival and reproduction can all be adversely affected by elevated zinc levels (Eisler, 1993). In aquatic systems, zinc tends to be partitioned in to sediment and less frequently dissolved as hydrated zinc ions and organic and inorganic complexes (MacDonald, 1993).

1.6. Determinants of the state of a river ecosystem

The state of a river ecosystem is determined by the following factors: geomorphologic characteristics, hydrological and hydraulic regimes, chemical and physical water quality and the biological nature of the in-stream and riparian habitats (Davies and Day, 1998). In this study the physical and chemical properties of water and biological components i.e. invertebrates, fish and vegetation were assessed

1.6.1. Physical and chemical qualities of water

The physical and chemical form of metals in the aquatic environment is controlled by environmental variables such as pH, redox potential, salinity, alkalinity and hardness (Maartens, 1994). It is therefore necessary to assess various water quality parameters when testing for metals in an aquatic environment.

1.6.1.1. Dissolved oxygen

The concentration of dissolved oxygen is probably one of the most important abiotic determinants of the survival of most aquatic organisms (Davies and Day, 1998). Oxygen enters water bodies largely via diffusion from the air at the water surface (Giller and Malmqvist, 1998). However, oxygen solubility in water is negatively correlated with water temperature. Dissolved oxygen in water depends on the relative rates of photosynthesis and respiration. These are influenced by aeration from the atmosphere, air pressure and salinity. Oxygen levels are naturally low where organic matter accumulates, because aerobic decomposer micro-consuming requires (and so consume) oxygen (Davies and Day, 1998). The presence of macrophyte vegetation can also affect oxygen levels. Oxygen is a by-product of photosynthesis and excessive plant growth (especially during summer) can lead to supersaturation during the day (Giller and Malmqvist, 1998). However, concentrations can decrease significantly during the night due to respiration and carbon dioxide levels can increase. Levels also vary with current speed and turbulence; small fast flowing, turbulent, unpolluted streams are usually saturated with oxygen, whereas pools and stagnant water bodies, especially with a high organic load of dead leaves, can have relatively low concentrations of dissolved oxygen (Giller and Malmqvist, 1998). The amount of ground water entering streams can also influence oxygen levels, as ground waters usually have low levels, and similarly, any stream impoundments will tend to reduce oxygen and increase carbon dioxide levels (Giller and Malmqvist, 1998). In addition to these more local variations, as a general rule, oxygen levels decrease downstream, as the upper reaches tend to be more turbulent, cooler and have a greater surface area to volume ratio for diffusion from the atmosphere (Giller and Malmqvist, 1998).

Change in oxygen levels can have a number of effects on biota (Giller and Malmqvist, 1998). A decrease in oxygen level increases an oxygen demand and may result on physiological stress on a living organism. Different species have different respiratory abilities and oxygen requirements. These differences may in turn contribute to species distributions (Giller and Malmqvist, 1998).

1.6.1.2. Temperature

Temperature plays an important role in the solubility of gasses such as H_2, N_2, CO_2, and O_2. It has an influence on the rate of chemical reactions in water (DWAF, 1996). Anthropogenic activities such as the release of warmed water used in the cooling towers of power station have an influence on temperatures of the water (DWAF, 1996). Temperature is further affected by turbidity of the water and an increase in total suspended solids (TSS) can lead to a decrease in water temperature, because heat is reflected from the surface (DWAF, 1996). Clear water absorbs and looses heat faster than turbid water resulting in thermal instability in clear waters. The degree of shading by riparian vegetation also influences water temperature (Giller and Malmqvist, 1998). Temperature changes are influenced by impoundments and dams especially if thermal stratification occurs in the reservoirs upstream of the barrier (Giller and Malmqvist, 1998).

Temperature may limit the area where species can live and they are generally adapted to certain temperature regimes (Giller and Malmqvist, 1998). Temperature changes can have a major impact on fish life. One example is the low temperature discharges from impoundments that trigger spawning (van Vuren *et al.*, 1994). The effect of temperature on biota may be indirect through its influence on metabolic rates and oxygen concentration. Most components in the life history of insects, such as egg development, larval growth rats, time for emergence, adult size and fecundity, are affected significantly by temperature (Giller and Malmqvist, 1998).

The effects of temperature on toxicity are complex. Seasonal changes in water temperature could alter lethality of copper to fish (van Vuren *et al.*, 1994). In their experiment, *Oreochromis mossambicus* was more susceptible to copper at a higher temperature. The fish has a relatively higher metabolic rate at 29±°C and therefore more water and copper passed through the gills. The higher water volume passing across the gills resulted in a greater sensitivity to copper at the higher temperature, as more water containing copper was transported to the blood (van Vuren *et al.*, 1994). Elevated temperatures do not always increase toxicity of substances. The toxicity of some substances is increased and that of others decreased by an increase in temperature (van Vuren *et al.*, 1994). Temperature also influences the rate of metabolic processes, including the uptake, metabolism and excretion of toxicants. Increased temperature will increase the oxygen requirements of aquatic organisms, while decreasing the solubility of oxygen in water (van Vuren *et al.*, 1994).

1.6.1.3. pH

pH is a measure of hydrogen ion activity and is influenced by geological and atmospheric conditions (DWAF, 1996). In natural water, pH is regulated by the carbonate-bicarbonate cycle consisting of carbonic acid ($H_2CO_3^-$), carbon dioxide (CO_2), carbonate ions (CO_3^-) and bicarbonate (HCO_3^-); and influenced by hydrophytes (photosynthesis) (Train, 1979). The pH plays an important role in the physio-chemical and biological processes of natural waters. Depending on pH values, cations and anions are released or bonded while toxicity of certain elements increases or decreases (Polling, 1999). Generally a low pH increases the solubility of most metals, thereby increasing the toxicity of those metals in the system (Sentongo, 1998).

Acidification of waters can influence interactions between the metal and the organism in at least two ways: the decrease in pH may affect metal speciation in solution, or it may affect biological sensitivity at the level of the cell surface (van Vuren *et al.*, 1999). Generally, pollution affects stream community structure mostly by reducing species diversity.

1.6.1.4. Conductivity and Total Dissolved Solids

Conductivity is a measure of a water body to conduct electrical current (DWAF, 1996) and therefore a measure of the number of ions (charged particles) in a solution (Davies and Day, 1998). The total amount of all material dissolved in a water sample is commonly measured as Total Dissolved Solids (TDS), as conductivity or as salinity (Davies and Day, 1998). Conductivity and TDS are usually closely correlated for a particular type of water. The ions that form the bulk of TDS are the sodium, potassium, calcium and magnesium cations and the chloride, sulphate, bicarbornate and carbonate anions. These are collectively known as major ions and they are needed to some extend by biota (Davies and Day, 1998).

1.6.2.1. Water

Water is a scarce commodity in South Africa and is vital for sustaining life in both terrestrial and aquatic organisms (Greenfield, 2001). Water forms the integral part of an aquatic environment and it must be of good quality for its users. Davies and Day (1998) define water quality as the value or usefulness of water, determined by the combined effects of its physical attributes and chemical constituents, and varying from user to user. Physical attributes and chemical constituents of water are influenced by climate, geomorphology, geology and soils, as well as the biota living in a given area (Davies and Day, 1998).

1.6.2.2. Sediment

By definition, sediments consist of particles of different sizes, shapes and chemical composition that have been transported by water or air from the site of their origin in a terrestrial environment and have been deposited in water bodies. They may also be formed by simple precipitation reactions (Wasswa, 1997).

As a general rule, sediments acts as natural adsorbents, binding a wide range of organic and inorganic compounds. Various sediment textures have their own binding affinity and will show selective binding of contaminating chemicals (Wasswa, 1997). Some contaminants are not readily soluble, but become rapidly fixed to particular matter in the receiving water body. This applies in particular to the metals chromium, mercury and lead (Maartens, 1994). Even close to the point of input, metal levels in the water may rapidly return to their normal level, making detection difficult. The determination of metal levels in sediments can therefore play a major role in the detection of pollution sources in the aquatic environment (Maartens, 1994). Metals immobilised into bottom sediments serve as a potential source of these pollutants to the water body and as such, sediments tend to have higher metal concentrations.

In natural systems there are three primary routes by which toxic materials bound to sediment may end up in the biota (Wasswa, 1997). The first route is by contact between a target organism and the sediment through the integument. The second route is by ingestion of sediment by the target organisms and absorption of the pollutant through the digestive system into the general circulation of the host organism. The third route is by desorption of the pollutant from the sediment to the water column and uptake of the target organism via the waters. In each case, the pollutant is active only if released from the sediment and passed to the target organisms through some biological barriers.

Under certain circumstances, metals may be released from the sediment back into the water body by changes in environmental conditions such as pH, redox potential or the presence of organic chelators and thus posing a thread to the water users. The recycling of materials, such as metals, in flowing natural waters is probably much less than in static fresh waters. Pollutants associated with sediments may be settled, resuspended during higher flows and resettled once more down stream (van Vuren *et al.*, 1994). Sediments are composed of several individual layers, each of which corresponds to a distinct condition of water flow. Layers with coarse material represent bed load depositions during strong currents, while layers with fine material consist mainly of suspended load deposited during low flows (van Vuren *et al.*, 1994). Metals found in sediments present no direct danger as long as they are bound to the sediment. Furthermore, organisms in close proximity to the sediments may accumulate metals bound on the sediments and the organisms may form part of food of other organisms in the water body.

In the aquatic environment, sediments serve as a trap to remove pollutants from the water and at the same time as a source of pollutants. In nutrient rich water, sediment may help prevent eutrophication, but it may also serve as a medium to promote the growth of aquatic plants. In either event, sediment must be considered a major factor in determining the quality of surface water (Wasswa, 1997).

1.6.2.3. Macro-invertebrates

Macro-invertebrates are those animals without a backbone (Davies and Day, 1998), and are big enough to be seen with the naked eye. In fresh waters, this group of animals occur in all habitats with specific adaptations. Macro-invertebrates, like any other water users, are subjected to the effects of pollution. According to van Vuren *et al.* (1999), elimination of non-tolerant species in a stream is often accompanied by:

- Increase in stream productivity of benthic invertebrates due to lack of predation and competition,
- Changes and simplifications in food chains,
- In the case of organic pollution a seemingly inexhaustible source of food for the remaining tolerant species.

Macro-invertebrate assemblages can be influenced by the adjacent land use and riparian vegetation, partly associated with the nature of the litter inputs, but also partly associated with the influence on water chemistry (Giller and Malmqvist, 1998).

A variety of invertebrate organisms (e.g. snails, crabs, worms, insect larvae, mussels, beetles) require specific habitat types and conditions for at least part of their life cycles. Changes in the structure of aquatic invertebrate communities are a sign of changes in the overall river conditions (Anon, 2000). As most invertebrate species are short-lived and remain in one area during their aquatic life phase, they are particularly good indicators of localised conditions in a river over a short term (Anon, 2000).

1.6.2.4. Fish

In broader terms, a fish is a cold-blooded animal, living in water, breathing by means of gills and having fins for stability and movement (Skelton, 2001). Freshwater fish distribution is more distinct and restricted because of barriers that often occur in the freshwater aquatic ecosystems. Distribution within the systems is governed by physical barriers, physico-chemical tolerance, ecological barriers, biological and behavioural factors (Skelton, 2001).

Many fish species are among the top consumers of trophic pyramids in aquatic ecosystems. They are therefore endangered by diet-borne pollutants through trophic uptake (Maartens, 1994). Fish lives depend entirely on water and any alteration in the physical and/or chemical state of the water affects their lives. The uptake and retention of chemicals in fish may take place via absorption through gills and skin and through ingestion of contaminated food and drinking water (van Vuren *et al.*, 1994). The extent of bioaccumulation of a specific element can be influenced by factors related to the organism itself such as species, its physiological conditions, growth, age, sex, the pollutant present, interactions and the physical or chemical status of the environment (van Vuren *et al.*, 1994).

Fish, being relatively long-lived and highly mobile, are good indicators of long-term influences on a river reach and the general habitat conditions within the reach. The numbers of species of fish that occur in a specific reach, as well as factors such as different size classes and the presence of parasites on the fish can be used as indicators of river health (Anon, 2000).

1.6.2.5. Marginal and emergent vegetation

Marginal vegetation is that vegetation, for example grasses, reeds and sedges, on the water's edge while emergent vegetation are those aquatic vegetation consisting of plants that live in the stream channel and that may partly or fully submerged (Gerber and Gabriel, 2002). The riparian zone of a river, stream or any water body is the land adjacent to that body of water that is, at least periodically, influenced by flooding. It is characterised by distinct vegetation and soil (Mitsch and Gosselink, 1993). The extent of the relationships between the stream and; marginal and in stream vegetation is related to stream order. Available incident light reaching the stream autotrophs is negatively related to canopy development. In tropical moist forests, the canopy reduces irradiance at the forest floor (and stream surface) to 1% of the value at the top of the canopy whereas large rivers are wide enough to be largely not shaded (Giller and Malmqvist, 1998). Marginal water vegetation includes the rooted, larger aquatic plants (macrophytes) found near the banks or rooted in shallower substrates (Giller and Malmqvist, 1998).

The riparian canopy also influences the amount and nature of particulate organic matter (litter) inputs to streams and rivers, which form a very important and often predominant energy base for food webs. Plants also influence water chemistry in a number of ways. All vegetation has some capacity to intercept chemical ions from the atmosphere onto the leaf surfaces (Giller and Malmqvist, 1998). These concentrated ions are subsequently washed off the vegetation by rain and reach the soil, where they are modified and ultimately reach streams and rivers. Evapotranspiration on the riparian and catchment vegetation also influences water yield from the catchments to streams and rivers and hence the discharge levels. Lastly, riparian vegetation can influence the physical channel morphology of the stream and even the structure of drainage patterns through, for example, bank stabilisation by roots and formation of partial or complete large woody debris dams (Giller and Malmqvist, 1998).

Heavy metals cannot be biologically degraded and are capable of accumulating in the environment, resulting in major toxicity on inhabitants (van Vuren *et al.*, 1999) and plants are not exceptions. The riparian zone is the critical component in the linkage between stream ecosystems and their catchment and plays important ecological and geomorphological roles (Uys *et al.*, 1994). These include the direct shading effects of riparian vegetation, providing habitats for life, and subsequent limiting influence on the in-stream primary production, supports aquatic food webs and also play a role in stabilising river banks (Uys *et al.*, 1994). Changes in the structure and function of riparian vegetation commonly result from changes in the flow regime of a river, exploitation for firewood, or use of the riparian zone for grazing or ploughing (Allanson, 1995).

1.7. Nyl River, its floodplain and tributaries.

Nyl River, its floodplain and tributaries form an important abiotic component by supporting the diversity of life and anthropogenic activities, such as mining, irrigation schemes and cattle and game farms in and around the catchment. These activities have been proved in other systems to induce some form of pollution in waterways. This study was an attempt to assess the status of selected metals in the selected streams. The selected metals were based on the study done on the system in the past (Greenfield, 2001). Nylsvley, being part of the Nyl River catchment, was designated a Ramsar site under sections1a, 1d, 2a, 2b, 2c, 2d, 3b and 3c of the Ramsar criteria (Anon, 2002) and in order to successfully conserve and improve the status of this wetland, a thorough knowledge of its current conditions is important. Tobias Spruit and Naboom Spruit, on which this study was done, are tributaries of the Nyl River. These tributaries are, like most South African rivers, subjected to major alterations and exposure to pollutants. It is very important to understand the extent of pollution in the system in order to successfully manage the wetlands on the area.

According to the National Environmental Management Act (Act No. 107 of 1998), *every user has the right to an environment that is not harmful to their health or well being; and to have an environment protected for the benefit of present and future generations through reasonable legislative and other measures that prevent pollution and ecologically sustainable development and use of natural resources while promoting justifiable economic and social development* (DEAT, 1998). For the successful management of a natural system, a good knowledge of its basic functioning and operation is necessary. It is absolutely essential to regularly monitor river systems that may be affected by some kind of pollution (van Vuren *et al.*, 1994). This study will provide a baseline for future studies in the Tobias Spruit, Naboom Spruit and the Nyl floodplain and help in monitoring, conservation and management of the system.

1.8. Aim and objectives of the study

The main aim of this project was to assess the bioaccumulation of selected metals in the abiotic and biotic components of selected sites in the Nylsvley system and determine *invitro* rate of uptake of selected metals by the African Sharptooth catfish, *Clarias gariepinus*.

Objectives:

- To collect samples during both high and low flows at the selected sites in the year 2002.
- To measure physical parameters of water during sampling.
- To determine concentrations of selected metals, cadmium, chromium, copper, lead and zinc on the following samples; aquatic macro-invertebrates, fish, plants, sediments and water.

- To determine the uptake rate of cadmium, zinc and cadmium-zinc combination by the African sharptooth catfish (*C. gariepinus*).

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CHAPTER 2

2. STUDY AREA

2.1. Introduction

2.1.1. Waterberg Biosphere Reserve

The Waterberg Biosphere Reserve is found in the Mogalakwena Municipality of the Waterberg district in the Limpopo Province, South Africa. The Waterberg Biosphere Reserve comprises of the core area (103 571 hectares), buffer zone (150 000 hectares) and the transition area (150 000 hectares) (DEAT, 2002). The present zonation will be re-evaluated periodically, due to the possibility of expansion. These changes could affect the present transitional and buffer zones, but the core areas will only be affected indirectly.

2.1.2. Tobias Spruit, Naboom Spruit and the Nyl Floodpain

The Tobias Spruit is a seasonal stream originating on the farm Rietfontein to the north west of Naboomspruit and is joined by another spruit, Naboom Spruit, which also originates on Rietfontein farm area. Further downstream, in the Riet Vally area, it flows into the Tobias Spruit. The Spruit joins the Nyl River about 18 km North East of Naboomspruit. This important drainage system flows into the Nyl Pans (north of Mokopane), where the river changes name to become the Mogalakwena River (Limpopo River System - Figure 2.1).

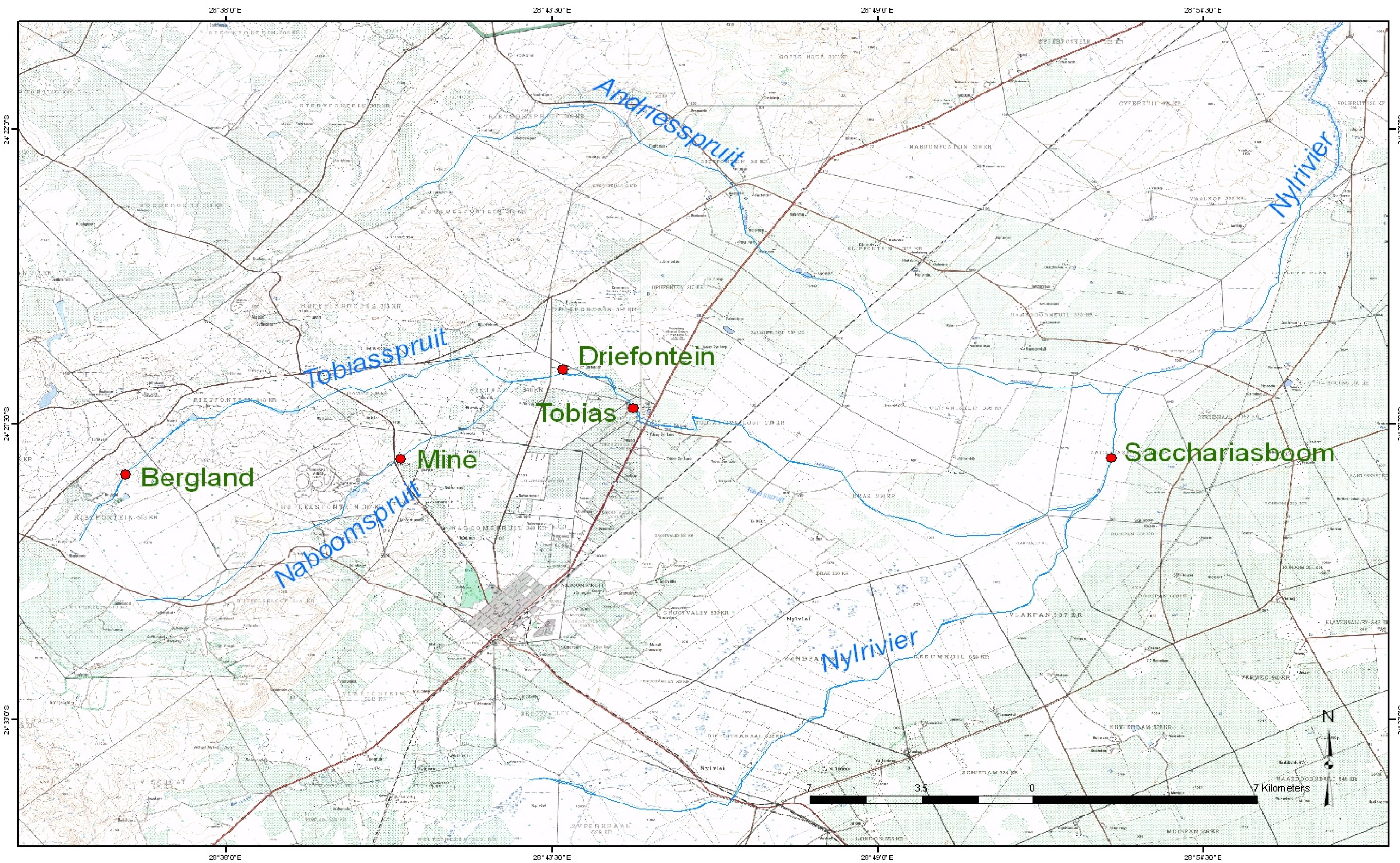

Figure 2.1 A map of the study area with sampling sites marked with red dots (●).

2.1.3. Socio-economic and environmental analysis

One of the key problems facing the Mogalakwena Municipality is the poverty situation as a result of the increased unemployment rate. The economy of the Municipality consists of several sectors including farming, mining, manufacturing, utilities, construction, trade, transport, business services, social services, private households, etc. (DEAT, 2002).

Potential environmental risks within the Mogalakwena Municipality are:

- Veld and forest fires;
- Chemical spillages;
- Drought and other natural disasters;
- Informal settlement;
- Soil erosion;
- Inadequate solid waste disposal systems;
- Urban sprawl;
- Degradation of the natural environment due to gathering of firewood;
- Sub-standard monitoring of factory effluent; and
- Inadequate sanitation systems.

Each of the above has its unique set of causes, precautions and remedies. It is the responsibility of the local authority to ensure that a balance is sustained between development and environment (DEAT, 2002). This will help in protecting a number of environmental sensitive areas existing in the Municipality.

2.1.4. Topography

South Africa is diverse in climate, geomorphology, geology and soils, and aquatic biotas. This variation results in different regions exhibiting quite considerable differences in water quality even when unaffected by human activity (Dallas and Day, 1993).

The Waterberg is unique (Figure 2.2), due to its geological formations (predominantly sandstone) (Anon 2002 a) and represents a bio-physical region found nowhere else in

South Africa (DEAT, 2002). The sandstone is mainly medium grained but it contains a few beds of fine-grained sandstone (Anon, 2002 a).

The geology of the area can be divided in three physiographical units; Kranskop Hills and surrounding drift; Piedmont areas and Alluvial plains including active floodplains, abandoned floodplains, and their uplands (Anon, 2002 b). Alluvial plains form the dominant unit along Nyl River floodplain.

Kransberg Hills form a series of isolated outcrops of bedrock in the southern part of the basin. The north-eastern alignment of these outcrops coincides with the trend of a bedrock ridge that is largely by drift and alluvium (van Wyk, undated). The Peidmont areas form a transitional zone between flats and mountainous areas of Waterberg and Strydpoort foothills. They consist largely of alluvial fans, which have been bisected by hill torrents and small intermittent streams. There is no clear boundary between Peidimont areas and Alluvial plains (van Wyk, undated). Alluvial plains dominate physiographical unit along Nyl River flood plain. It consists of sand, gravel silt and clay and it is heterogeneous, depending on their mode of deposition with limited horizontal and vertical continuity.

The Waterberg system is exposed in the southern part of the Nyl River valley and is known as the Kranskop Hill, which lies at moderate depth under the floodplain (Anon, 2002b). The sampling sites as depicted in Figure 2.2 lies on the following: Waterberg, Soutpansberg, Orange River for the Bergland site, Suurberg, Drakensberg, Lebombo for both Mine and Driefontein sites; and Beaufort for Mine and Sacchariasboom sites.

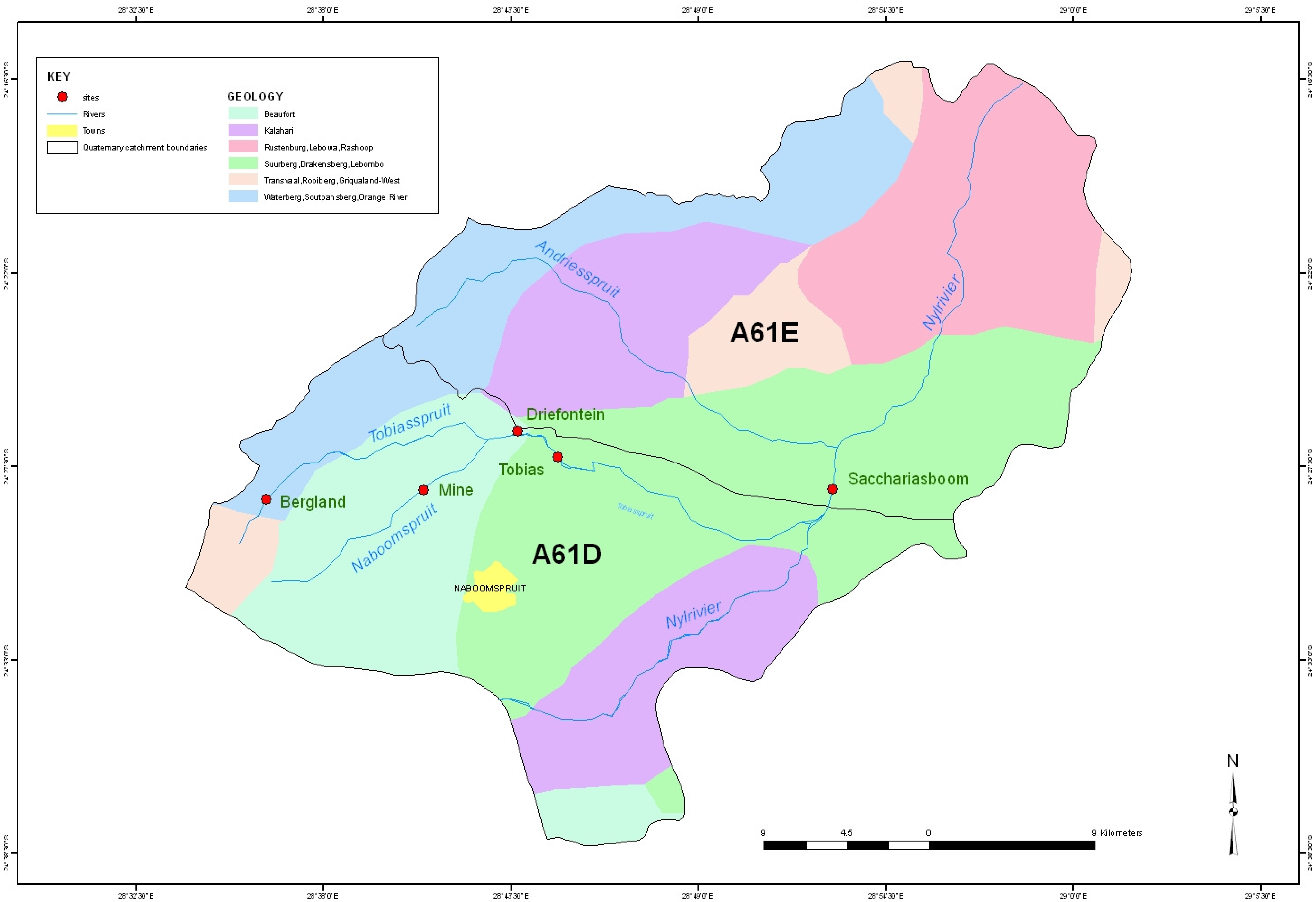

Figure 2.2 Limpopo Province geological map indicating also the study area.

Tobias Spruit, Naboom Spruit and the Nyl River form the southeastern part of the Waterberg plateau. In and along these rivers and their tributaries, the threatened wetland habitat occurs frequently. The area contains a rich diversity of species with numerous endemic forms, rare and or threatened species (DEAT, 2002).

2.2. Sampling sites

Five Sampling sites were selected for this project: Bergland, Driefontein and Tobias in the Tobias Spruit; Mine in the Naboom Spruit, a tributary of the Tobias Spruit; and Zacariasboom, which is a site in the Nyl River after its confluence with the Tobias Spruit (DWAF, 2002).

2.2.1. Bergland

Bergland is situated on the mountains on the farm Rietfontein (513KR), north west of Naboomspruit. The sampling point is situated on the headwaters of Tobias Spruit, a few hundred of meters from the origin of the Tobias Spruit (Figure 2.3).

Figure 2.3 Bergland site during high flow.

Figure 2.4 Head waters of the Tobias Spruit at Bergland site.

2.2.2. Mine

Mine site is situated in the Naboom Spruit, just below the bridge on the road from Naboomspruit to Heuningfontein on the farm Buffelsfontein (347KR), to the north east of Naboomspruit. The site is below the Buffelsfontein Flourspar Mines (van Wyk), and the sewage works of the mines residential area. Other impacts affecting the Naboom Spruit include the dam, (Figure 2.5), road, water abstraction and constructions (Figures 2.6 a, b and c), taking place during both sampling trips.

Figure 2.5 A dam on the Naboom Spruit at the Mine site.

Figure 2.6 (a) Riparian habitat and banks disturbance; and instream physical barrier at the Mine site.

Figure 2.6 (b) **Riparian habitat disturbance at the Mine site.**

Figure 2.6 (c) **Water abstraction and instream physical barrier at the Mine site.**

2.2.3. Driefontein

Driefontein site is situated on the farm Rietvalley (340KR), adjacent to Driefontein farms (317 KR), to the north east of Naboomspruit. The sampling site is just below the road bridge, (Figure 2.7), on the road from Naboomspruit to Die Oog. The influence, if any, of the Naboom Spruit on the Tobias Spruit will be detected from this point further downstream. Tobias Spruit then turns within the Driefontein farms and flow south easterly towards the Nyl River.

Figure 2.7 A down stream view of the Driefontein site.

2.2.4. Tobias

Tobias site is situated on the farm Tobias Zyn Loop (339KR) and the sampling point is just below a dam wall (Figure 2.8 a, b and c), upstream the road bridge (Figure 2.9) on the road from Naboomspruit to Potgietersrus. The Tobias Spruit at this point is flowing south easterly and later forms a confluent with the Nyl River.

Figure 2.8 (a) **A dam just above the Tobias site.**

Figure 2.8 (b) **A pool where the sample was collected during first survey at Tobias site.**

Figure 2.8 (c) **Tobias site during the second survey.**

Figure 2.9 **An upstream view of the Tobias Spruit at Tobias site.**

2.2.5. Sacchariasboom

Sacchariasboom site (Figure 2.10 a, b & c) is on the Nyl River, after the confluence of Tobias Spruit and Nyl River on the farm Sacchariasboom (354KR) where the Nyl River is flowing north easterly towards Mokopane.

Figure 2.10 (a) A farm dam at Sacchariasboom site during high flow season.

Figure 2.10 (b) A small pool with algae at Sacchariasboom site where the fish were collected during first survey.

Figure 2.10 (c) **A farm dam with freshly pumped water Sacchariasboom site during low flow survey.**

2.3. Conclusion

The physical, chemical qualities and diversities obtained from samples at Bergland site will be used as standards as the conditions at that point are considered unaffected or near natural status. All other samples and conditions further downstream will be compared with those obtained from Bergland to detect where changes, if any, starts to occur. The Mine site samples and qualities will show the influence from activities drained by Naboom Spruit into the Tobias Spruit and the Nyl Floodplain further downstream. All the information obtained on the four sites of Tobias Spruit and Naboom Spruit will be compared with the information obtained at Zacchariasboom. This will indicate the impacts of the two rivers and their tributaries have on the Nyl River and the filtering function of the wetland area, Nylsvlei.

2.4. References

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CHAPTER 3

3. MATERIALS AND METHODS

Information expressed was obtained from the Tobias Spruit, Naboom Spruit and the Nyl Floodplain. Two surveys were conducted, one during the "high flow" season and the other during a "low flow" season. Statistical Product & Service Solutions (SPSS) was used to analyse the data obtained and the mean, standard deviation and standard error were determined through summary statistics and recorded in this chapter. Logarithmic transformation of the data was done to normalize the data and then analyses of variances (ANOVA) were done to determine the differences between data sets.

3.1. Study sampling sites

Five Sampling sites were selected on this project, which are Bergland, Driefontein and Tobias are on the Tobias Spruit; Mine is on the Naboom Spruit, a tributary of the Tobias Spruit; and Zacchariasboom, which is on the Nyl River after confluence with the Tobias Spruit. Two surveys were conducted on these sites, the first one on the 30th of April 2002 and the second one from 24th – 25th of July 2002. During both surveys, water, sediments, invertebrates, fish and plant samples were collected where available, refer appendix A. Physical parameters where also collected from water at each site, where possible, during sampling, refer Table 4.1.

3.2. Cleaning of the sampling material prior to undertaking field surveys

Sampling bottles and invertebrate trays were washed in a soap bath, rinsed twice in distilled water and then filled up with a 14% sulfuric acid (H_2SO_4) solution prepared as follows:

$$240\text{ml } 98\%\ H_2SO_4 + 1760\text{ml distilled } H_2O = 2000\text{ml } 14\%\ H_2SO_4$$

Calculation:

$240\text{ml } H_2SO_4 / 1760\text{ml } H_2O \times 100\% = 13.6\%$ acid solution

After 24 hours, the acid solutions were discarded and the bottles were air dried at room temperature. New sampling plastic bags were prepared to store plants and large fish specimens.

3.3. Field sampling techniques

In the field, the prepared sampling bottles and plastic bags were used to store samples. Samples were collected as follows:

3.3.1. Plants

Emergent and fringing plants were cut into reasonable size pieces using clean stainless steel dissection instruments to prevent contamination and the plant material were rinsed in water to remove excessive material. Some of the plants were identified in the field and the remainder were identified by a specialist in the Herbarium, Department of Biodiversity at University of Limpopo (Turfloop Campus). At each site, only plants growing in the water were sampled. The selected plants were cut with clean, stainless steel dissection instruments. The plants were selected, identified and cut into smaller pieces, before it was stored in the acid prepared bags. If plants could not be identified, a sample was placed in the plant press for identification by the specialist botanist at the University Herbarium.

All the samples were labelled for future analysis and stored at about -20ºC until used in the laboratory for analysis of the material.

3.3.2. Sediment

About 500g of sediment from about top 10cm were collected in an acid-washed container at each site taken at the centre most of a sampling sites. Larger debris materials such as stones, plant pieces, etc. were removed from the samples immediately before samples were labelled and stored at about -20ºC until used in the laboratory for material analysis. One sample of sediment was collected at each site per survey.

3.3.3. Fish

Fish were collected from different biotopes (shallow and deep pools, riffles and back waters), where applicable, at each sampling site using an electrofishing technique and hand-held nets (Figure 3.1). Fish field guide (Skelton, 2001) and other relevant field fish guides were used for identification. Fish samples were placed in the acid-washed bottles and bags. Samples were labelled and stored at about -20ºC until laboratory analysis of material.

Figure 3.1 Electrofishing technique using backpack gear at Bergland site.

3.3.4. Invertebrates

Invertebrates were collected from each biotope (e.g. marginal vegetation, gravel, sand and mud), where applicable, using a SASS net and following South African Scoring System version 5 (SASS 5) techniques. SASS 5 is the biological index used for assessing aquatic invertebrate fauna based on the presence of the different aquatic invertebrate families and their perceived sensitivity to water quality (Davis and Day, 1998). White sampling trays were used for identification (Refer Appendix A) of the invertebrates using SASS 5 score sheet and the samples were then stored in the prepared containers at about -20ºC until further analyses.

3.3.5. Water

The pre-washed 500 ml sampling bottles were rinsed three times with the water at the sites before the sample was taken. The samples were stored on ice the bottles were transferred to a laboratory freezer at about -20ºC. Samples were kept at this temperature until analysis. Physical parameters of the water were measured *in situ* at each site and included: water temperature and dissolved oxygen (DO) using oxygen meter (YS15S model), conductivity, TDS and pH were measured using Aqualytic Digi Meter L21.

3.3.6. Coordinates

Coordinates of the sampling sites were taken using a hand held GPS, recorded (Table 4.1) and mapped in Figure 2.1.

3.4. Laboratory work

In the laboratory, samples were thawed at room temperature for metal analyses.

3.4.1. Plants

Samples were taken using clean stainless steel dissection instruments to cut them and then rinsed twice in distilled water. Each sample was placed in a pre-weighed clean Erlenmeyer flask and the wet mass recorded using a METTLER AE240 balance.

Samples were placed in the oven at 60^{o}C for at least 24 hours until they were completely dry. Dry samples were weighed (dry mass) and their moisture contents were calculated using the formula:

$$\frac{[(\text{wet mass} - \text{flask mass}) - (\text{dry mass} - \text{flask mass})]}{(\text{wet mass} - \text{flask mass})} * 100$$

Whereas the dry mass was calculated using the formula:

(vail weight – (dry mass sample + vail weight)

The moisture contents were recorded accordingly and the samples were ready for acid digestion.

3.4.2. Sediment

Sediment samples were placed in pre-weighed clean Erlenmeyer flasks and weighed following the same procedure as in section 3.3.1. Samples were placed in an oven at 60°C for at least 24 hours until they were completely dry. The moisture contents were calculated (as in section 3.4.1) and the samples were acid digested.

3.4.3. Fish

Fish lengths and masses were recorded and individual specimens were placed in pre-weighed clean Erlenmeyer flask. For the larger *C. gariepinus* from Sacchariasboom, the gills, liver and muscle tissue were removed and each tissue type was then prepared for acid digestion (as described in 3.3.1). Samples were placed in an oven at 60°C for at least 24 hours until they were completely dry. Moisture content was calculated using same formula as in section 3.4.1 and samples were ready for acid digestion.

3.4.4. Invertebrates

Thawed invertebrate samples, not selected according to families or biotopes sampled, were placed in pre-weighed clean Erlenmeyer flasks. Wet mass was measured and the samples were placed in an oven at 60°C for at least 24 hours until they were completely dry. Dry mass and moisture contents were calculated using similar formulae as in 3.4.1, and samples were ready for acid digestion.

All plant, sediment, fish and invertebrates samples were tightly covered with clean plastic wrap to avoid contamination and further changes in the moisture content. Samples were all dried to avoid different moisture contents, which different samples have and final concentrations of metals were expressed as metal µg/g of dry mass.

3.5. Digestion of samples

Digestion of plant, sediment, invertebrate and fish or fish tissue samples was done using the same method. Five ml perchloric acid (70% Saarchem) and 10 ml nitric acid (55% Saarchem) was added into each Erlenmeyer flasks with sample (Greenfield, 2001). The samples were left for 24 hours (cold acid digestion) in fume cardboard. After the 24 hours cold acid digestion, the samples were placed on a hotplate to complete the digestion process (hot acid digestion). The hot digestions were done at 200^{o}C for ±4 hours and or until the sample was clear. Further volumes of perchloric acid (70% Saarchem) and nitric acid (55%) were added, in the ratio of 1:2 respectively. This was done to completely digest the larger samples. The samples were then removed from the hot plate, allowed to cool down and filtered.

3.6. Filtering of samples

The filtering system, excluding air pump, was pre-washed with distilled water before and after each sample was filtered to prevent contamination. The samples were then filtered, through 0.45 µm acid resistant Millipore filter paper (Greenfield, 2001). Individual acid resistant filter paper was also used for each sample to avoid contamination. Each filtered sample was made up to a volume of 50 ml with distilled water and placed in an amber bottle and were ready for metal analysis. Metal analysis was conducted using the Atomic Absorption Spectrophotometer (AAS), Spectr AA. 10 plus model (Figure 3.2).

Figure 3.2 Atomic Absorption Spectrophotometer (AAS) Spectr AA. 10 plus.

3.7. Water samples

Thawed water samples were filtered using the same filtering system as in other samples. Only 50 ml of the filtered water was placed in a 100 ml Erlenmeyer flask and was ready for digestion.

3.7.1. Acidification

Toeach 10 ml of perchloric acid (70% Saarchem) and 20 ml of nitric acid (55% Saarchem) was added into each Erlenmeyer flask with 50 ml of filtered water. The samples were then placed on a hot plate and heated to 200^{o}C until the volume was reduced to about 25 ml. The samples were removed from the hot plate and allowed to cool down (Greenfield, 2001). The samples were made up to 50 ml with distilled water and placed in amber glass bottles and they were ready for metal analysis using AAS.

3.8. Atomic Absorption Spectrophotometry (AAS)

Following acid digestion all the samples were then analysed for the selected metal content using a Varian AAS Spectra AA-10-plus (Figure 3.2). Specific wavelengths and flame support for each of the five metals were used in calibration of the AAS as in Greenfield (2001). Standard solutions were made up for calibration using pure standards (Saarchem). The AAS reading of each sample was used to calculate the metal concentrations of each sample (µg/g) using the formula:

(AAS reading/digested mass)* Sample volume

3.9. Metal analyses

Polypropylene tubes were weighed to the fourth decimal on a Sartorius balance. Individual fish were placed in relevant marked tubes and the wet mass was recorded. The samples were then placed in an oven at 60 ^{o}C for 24 hours to dry the samples.

After 24 hours, the samples were allowed to cool at room temperature and the dry mass recorded. Initial cold acid digestion was carried out by adding 4 ml nitric acid and 400 µl hydrogen peroxide to the samples. Following cold digestion for two hours, microwave digestion of the samples was carried out in a commercial 1000 W microwave oven. The procedure was as follows:

2 minutes at 20% power
6 minutes at 30% power
6 minutes at 40% power
The fumes were released periodically in a fume cupboard from the enclosed plastic container in which the digestion was carried out. The mass of the digested material and tube were recorded after the digestion process to allow for the determination of volume lost to evaporation during this process. Samples were diluted to an approximately 10% acid concentration by adding 20 ml MilliQ water. Samples were analyzed for cadmium and zinc concentrations using the AAS.

3.10. Statistical analysis

Field work results were recorded (Chapter 4) and analyzed using Statistical Product & Service Solutions (SPSS). The mean, standard deviation and standard error were determined through summary statistics. Logarithmic transformation of the data was done to normalize the data. Analyses of variances (ANOVA) were performed to determine the differences between data sets. Levene's test for equality of variances was used to determine the significant differences between samples. The null hypothesis (H_0) states that: H_0: $V_1 = V_2$. If $P<0.05$, reject the H_0 and there is a significant difference between the means.

The laboratory experimental work on metal uptake by *C. gariepinus*, were also recorded in Chapter 4. All sets of data were tested for homoscedasticity by the log-ANOVA test for normality by the Kolmogorov-Smirnov test for goodness-of-fit. Linear and nonlinear least-squares regression and correlation were used for data fitting. One-way ANOVA was performed to detect significant differences among treatment groups, and the Dunnett's test was used to compare treatment groups with the control group. The Tukey test was used to make multiple comparisons among groups. Significance levels of tests are indicated by asterisks according to the following probability ranges (ns, not significant; *, $0.05 > P > 0.01$; **, $0.01 > P > 0.001$; ***, $P < 0.001$).

3.11. References

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CHAPTER 4

4. RESULTS

4.1 Physical parameters

Table 4.1 Physical parameters measured during sampling of the two flow regimes at all the sampling sites.

Locality	Flow	DO mg/l	Cond mS/m	pH	T °C	TDS ppm	GPS
Bergland	High	7.06	85.7	7.88	7.28	42.9	S24°28'989''
	Low	6.89	128.4	7.44	12.9	59.6	E28°35'940''
Mine	High	3.15	272.0	7.61	18.0	137.0	S24°28'342'
	Low	4.0	322.0	7.01	10.8	161.0	E28°40'894''
Driefontein	High	9.82	417.0	8.68	24.5	209.0	S24°26'599''
	Low	.	.	.	.	.	E28°43'489''
Tobias	High	5.33	310.0	8.31	15.5	155.0	S24°27'499''
	Low	.	.	.	.	.	E28°45'092''
Sacchariasboom	High	7.24	152.2	7.28	20.2	77.1	S24°28'722''
	Low	9.45	354.0	7.28	16.5	179.0	E28°52'758''

In table 4.1 the physical parameters collected at all the sites during field work are presented. It is important to note that there was no water at Driefontein and Tobias, only on the exact point where sample was taken during first survey, during the low flow sampling survey, hence no physical parameters indicated for that period.

The highest dissolved oxygen level during high flow survey (9.82 mg/l) was recorded at Driefontein and the lowest (3.15 mg/l) was recorded at Mine. The highest dissolved oxygen level during the low flow survey (9.45 mg/l) was recorded at Sacchariasboom and the lowest (4.0 mg/l) was recorded at Mine.
The highest conductivity during the high flow survey (417.0 mS/m) was recorded at Driefontein and the lowest (85.0 mS/m) was recorded at Bergland. The highest conductivity during a low flow survey of 354.0 mS/m was recorded at Sacchariasboom and the lowest one of 128.4 mS/m was recorded at Bergland.

During the high flow survey pH of 8.68 was recorded at Driefontein and the lowest (7.28) was recorded at Sacchariasboom. The highest pH during low flow survey (7.44) at Bergland and the lowest (7.01) was recorded at Mine.

The highest water temperature during a high flow survey (24.5°C) was recorded at Driefontein and the lowest (7.28°C) was recorded at Bergland. The highest water temperature during the low flow survey (16.5°C) was recorded at Sacchariasboom and the lowest (10.8°C) was recorded at the Mine. Water temperature was highly influenced by volume and shading where smaller pools and or less water volume non-shaded areas had high water temperatures recorded.

4.2 Mean metal concentrations of field samples

Table 4.2 Mean metal concentrations in water samples during both high and low flow regimes.

Metal	Site									
	Bergland		Mine		Driefontein		Tobias		Sacchariasboom	
	Flow									
	High	Low	High	Low	High	Low	High	Low	High	Low
Cadmium (mg/l)	0.01	0.01*	0.01	0.01*	0.01	-	0.02	0.01*	0.01	0.01*
Lead (mg/l)	0.09	0.25*	0.09	0.25*	0.17	-	0.14	0.25*	0.07	0.25*
Zinc (mg/l)	0.10	0.12	0.09	0.12	0.09	-	0.12	0.13	0.09	0.21
Copper (mg/l)	0.02	0.09	0.01	0.07	0.01	-	0.01	0.09	0.01	0.09
Chromium (mg/l)	0.18	0.18	0.16	0.18	0.17	-	0.16	0.16	0.04	0.22

* = Below detection level (BDL). Level of detection (LoD) for cadmium is 0.01mg/l and for lead is 0.5 mg/l. This value is divided by 2 to give the BDL values.

It is important to note that there was no water at Driefontein site during low flow survey and also that the water sample taken at Tobias was from a nearby pool just below a dam wall in a river reach of approximately 400 m away from where a sample was taken during high flow survey as there was no water on the same spot where a sample was taken during high flow survey. Chromium had the highest concentrations at all the sites compared to other metals except at Sacchariasboom during a low flow survey. The highest metal concentration (0.22 mg/l) was Chromium followed by zinc (0.21 mg/l) at Sacchariasboom, during the low flow regime. Lead was high (0.17 mg/l) at only one site, Driefontein, during a high flow survey, whilst cadmium was the lowest of all the metal analysed in the study area. Both cadmium and lead were below detection level at all sites were samples were taken during the low flow survey and the level of detection values were used to calculate the metals concentrations provided. All these ate marked with an asterisk (*) on table 4.2 above.

The following five tables, Table 4.3 – 4.8, is a summary of the statistical analysis of the collected data at all sampled sites during both flows. Some sample sizes were too small to be computed statistically and they are indicated by dots (.) in the tables. Other variables were not sampled during low flow sampling at some of the sites where there was no water and or could not be sampled and their sample size or their N values are indicated as zero (0). The mean zinc concentration is generally higher than the other metals at all the sites.

Table 4.3 Mean metals concentration in sediment at all sampling sites during both surveys.

Sample	Metal	Locality	Flow								P value
			High				Low				
			Mean (µg/g)	Std. Dev.	N	Std. Error	Mean (µg/g)	Std. Dev.	N	Std. Error	
Sediment	Cadmium	Bergland	0.33	0.26	2.00	0.18	1.10	0.52	2.00	0.37	.
		Mine	0.18	0.00	1.00	0.00	0.28	0.00	1.00	0.00	.
		Driefontein	0.49	0.00	1.00	0.00	0.45	0.00	1.00	0.00	.
		Tobias	0.21	0.00	1.00	0.00	1.21	0.00	1.00	0.00	.
		Sacchariasboom	0.28	0.28	2.00	0.20	0.00	0.00	0.00	0.00	.
Sediment	Lead	Bergland	12.49	7.84	2.00	5.55	21.37	12.87	2.00	9.10	.
		Mine	9.39	0.00	1.00	0.00	4.87	0.00	1.00	0.00	.
		Driefontein	9.61	0.00	1.00	0.00	16.07	0.00	1.00	0.00	.
		Tobias	8.18	0.00	1.00	0.00	21.92	0.00	1.00	0.00	.
		Sacchariasboom	6.02	3.89	2.00	2.75	0.00	0.00	0.00	0.00	.
Sediment	Zinc	Bergland	10.42	6.40	2.00	4.53	20.35	14.73	2.00	10.42	0.51
		Mine	0.18	0.00	1.00	0.00	2.17	0.00	1.00	0.00	.
		Driefontein	11.08	0.00	1.00	0.00	17.41	0.00	1.00	0.00	.
		Tobias	11.37	0.00	1.00	0.00	27.07	0.00	1.00	0.00	.
		Sacchariasboom	7.69	4.44	2.00	3.14	0.00	0.00	0.00	0.00	.
Sediment	Copper	Bergland	4.16	1.40	2.00	0.99	23.42	19.62	2.00	13.88	.
		Mine	6.50	0.00	1.00	0.00	3.68	0.00	1.00	0.00	.
		Driefontein	13.30	0.00	1.00	0.00	15.66	0.00	1.00	0.00	.
		Tobias	10.12	0.00	1.00	0.00	25.98	0.00	1.00	0.00	.
		Sacchariasboom	7.22	4.48	2.00	3.17	0.00	0.00	0.00	0.00	.
Sediment	Chromium	Bergland	12.18	9.75	2.00	6.89	25.43	14.19	2.00	10.04	.
		Mine	8.66	0.00	1.00	0.00	2.12	0.00	1.00	0.00	.
		Driefontein	45.57	0.00	1.00	0.00	12.89	0.00	1.00	0.00	.

		Tobias	26.07	0.00	1.00	0.00	24.47	0.00	1.00	0.00	.
		Sacchariasboom	10.11	0.45	2.00	0.32	0.00	0.00	0.00	0.00	.

N = 1 on all sites except at Bergland and Sacchariasboom where n = 2. The highest mean metal concentration (45.57 µg/g) was chromium at Driefontein obtained during the high flow survey. The lowest mean metal concentration (0.18 µg/g) was zinc at Mine obtained during the high flow survey. The P value could not be plotted due to sample sizes on all metals except on zinc at Bergland where there was no significant difference between zinc concentrations in sediments of the two surveys.

Sediment samples collected during the low flow survey had higher mean metal concentrations than those collected during the high flow survey except for copper and chromium in sediment sample.

Table 4.4 Mean metals concentration in invertebrates at all sampling sites during both surveys.

Sample	Metal	Locality	Flow								P value
			High				Low				
			Mean (µg/g)	Std. Dev.	N	Std. Error	Mean (µg/g)	Std. Dev.	N	Std. Error	
Invertebrates	Cadmium	Bergland	2.6	2.5	12.0	0.7	1.4	1.8	4.0	0.9	0.4
		Mine	3.4	0.0	1.0	0.0	0.0	0.0	0.0	0.0	.
		Driefontein	2.3	2.0	3.0	1.1	0.0	0.0	0.0	0.0	.
		Tobias	3.6	3.2	3.0	1.8	0.0	0.0	0.0	0.0	.
		Sacchariasboom	0.1	0.2	3.0	0.1	0.0	0.0	0.0	0.0	.
Invertebrates	Lead	Bergland	5.4	4.0	12.0	1.2	1.9	1.8	4.0	0.9	0.1
		Mine	27.6	.	1.0	0.0	0.0	0.0	0.0	0.0	.
		Driefontein	0.7	3.0	0.0	0.0	0.0	0.0	.	0.0	.
		Tobias	6.5	0.8	3.0	0.5	0.0	0.0	0.0	0.0	.
		Sacchariasboom	1.6	1.4	3.0	0.8	0.0	0.0	0.0	0.0	.
Invertebrates	Zinc	Bergland	7.5	44.8	12.0	12.9	76.9	27.6	4.0	13.8	0.7
		Mine	48.3	0.0	1.0	0.0	0.0	0.0	0.0	0.0	.
		Driefontein	82.5	111.6	3.0	64.5	0.0	0.0	0.0	0.0	.
		Tobias	213.5	219.7	3.0	126.8	0.0	0.0	0.0	0.0	.
		Sacchariasboom	85.4	87.9	3.0	50.7	0.0	0.0	0.0	0.0	.
Invertebrates	Copper	Bergland	38.5	24.7	12.0	7.1	29.1	5.7	4.0	2.9	0.3
		Mine	6.9	0.0	1.0	0.0	0.0	0.0	0.0	0.0	.
		Driefontein	27.8	31.0	3.0	17.9	0.0	0.0	0.0	0.0	.
		Tobias	35.2	33.5	3.0	19.4	0.0	0.0	0.0	0.0	.
		Sacchariasboom	14.1	13.4	3.0	7.7	0.0	0.0	0.0	0.0	.
Invertebrates	Chromium	Bergland	52.4	47.9	12.0	13.8	14.3	12.5	4.0	6.3	0.0
		Mine	44.8	0.0	1.0	0.0	0.0	0.0	0.0	0.0	.
		Driefontein	28.9	36.0	3.0	20.8	0.0	0.0	0.0	0.0	.
		Tobias	5.2	7.2	3.0	4.1	0.0	0.0	0.0	0.0	.
		Sacchariasboom	2.1	2.9	3.0	1.7	0.0	0.0	0.0	0.0	.

N = 1 only at Mine site during high flow survey and no samples at all sites except Bergland during the low flow survey where n = 4. The highest mean metal concentration (213.5 µg/g) was zinc on invertebrates obtained at Sacchariasboom during the high flow survey.

Table 4.5 Mean metals concentration in plants at all sampling sites during both surveys.

Sample	Metal	Locality	Flow								P value
			High				Low				
			Mean (µg/g)	Std. Dev.	N	Std. Error	Mean (µg/g)	Std. Dev.	N	Std. Error	
Plants	Cadmium	Bergland	0.22	0.20	20.00	0.05	0.26	0.23	18.00	0.06	0.51
		Mine	0.38	0.05	4.00	0.02	0.76	0.40	5.00	0.18	0.10
		Driefontein	0.39	0.28	8.00	0.10	0.85	0.86	7.00	0.32	0.17
		Tobias	0.38	0.23	5.00	0.10	0.15	0.07	6.00	0.03	0.09
		Sacchariasboom	0.07	0.10	11.00	0.03	0.00	0.00	0.00	0.00	.
Plants	Lead	Bergland	2.28	2.11	20.00	0.47	0.68	0.49	17.00	0.12	0.00
		Mine	2.49	0.25	4.00	0.13	0.95	0.43	5.00	0.19	0.00
		Driefontein	1.65	1.02	8.00	0.36	1.84	2.63	7.00	0.99	0.86
		Tobias	3.65	2.94	5.00	1.32	1.70	1.89	6.00	0.77	0.22
		Sacchariasboom	1.03	1.01	11.00	0.31	0.00	0.00	0.00	0.00	.
Plants	Zinc	Bergland	4.07	4.07	20.00	0.91	9.52	6.98	18.00	1.65	0.01
		Mine	8.11	6.64	4.00	3.32	21.18	8.75	5.00	3.91	0.04
		Driefontein	5.07	1.69	8.00	0.60	30.05	26.23	7.00	9.91	0.05
		Tobias	7.00	3.29	5.00	1.47	17.30	11.36	6.00	4.64	0.08
		Sacchariasboom	5.05	3.95	11.00	1.19	0.00	0.00	0.00	0.00	.
Plants	Copper	Bergland	1.16	1.15	20.00	0.26	3.99	2.83	18.00	0.67	0.00
		Mine	3.58	3.73	4.00	1.86	14.70	3.29	5.00	1.47	0.03
		Driefontein	1.48	0.79	8.00	0.28	19.78	19.87	7.00	7.51	0.05
		Tobias	2.39	2.81	5.00	1.26	7.53	5.09	6.00	2.08	0.08
		Sacchariasboom	2.07	1.94	11.00	0.58	0.00	0.00	0.00	0.00	.
Plants	Chromium	Bergland	3.96	2.94	20.00	0.66	7.69	6.35	18.00	1.50	0.03
		Mine	3.51	0.87	4.00	0.43	22.27	9.72	5.00	4.35	0.01
		Driefontein	3.76	2.47	8.00	0.87	32.67	34.19	7.00	12.92	0.09

		Tobias	7.08	7.39	5.00	3.30	6.37	3.15	6.00	1.28	0.84
		Sacchariasboom	2.68	2.08	11.00	0.63	0.00	0.00	0.00	0.00	.

N = 0 only at Sacchariasboom during low flow survey and all sites have n values ranging from 5 to 20 during high flow survey. The highest mean = 32.67 µg/g of chromium at Driefontein during the low flow survey and the lowest mean = 0.07 µg/g was cadmium at Sacchariasboom during the high flow survey. There was a significant difference between high and low flow surveys in lead, zinc, copper and chromium at Bergland, zinc at Driefontein and lead, zinc, copper and chromium at the Mine site.

Plants samples collected during the low flow survey had higher mean metal concentrations than plant samples collected during the high flow survey except for lead. Cadmium has the least concentration in plants sample than all the metals analysed.

Table 4.6 Mean metals concentration in fish at all sampling sites during both surveys.

Sample	Metal	Locality	Flow								P value
			High				Low				
			Mean (µg/g)	Std. Dev.	N	Std. Error	Mean (µg/g)	Std. Dev.	N	Std. Error	
Fish	Cadmium	Bergland	0.44	0.26	26.00	0.05	0.83	0.33	15.00	0.08	0.00
		Mine	2.55	1.62	11.00	0.49	0.00	0.00	0.00	0.00	.
		Driefontein	1.42	1.53	15.00	0.40	0.00	0.00	0.00	0.00	.
		Tobias	0.89	0.00	1.00	0.00	2.94	0.49	6.00	0.20	.
		Sachariasboom	0.32	0.48	28.00	0.09	0.00	0.00	0.00	0.00	.
Fish	Lead	Bergland	3.58	1.50	26.00	0.29	0.73	0.35	15.00	0.09	0.00
		Mine	4.28	1.83	.	0.00	0.00	0.00	0.00	0.00	.
		Driefontein	4.27	1.82	15.00	0.47	0.00	0.00	0.00	0.00	.
		Tobias	6.19	0.00	1.00	0.00	1.69	0.27	6.00	0.11	.
		Sachariasboom	1.44	2.12	28.00	0.40	0.00	0.00	0.00	0.00	.
Fish	Zinc	Bergland	54.10	22.60	26.00	4.43	64.37	25.96	15.00	6.70	0.19
		Mine	89.48	46.30	11.00	13.96	0.00	0.00	0.00	0.00	.
		Driefontein	85.17	48.28	15.00	12.46	0.00	0.00	0.00	0.00	.
		Tobias	46.02	0.00	1.00	0.00	89.96	11.54	6.00	4.71	.
		Sachariasboom	26.86	28.92	28.00	5.47	0.00	0.00	0.00	0.00	.
Fish	Copper	Bergland	5.30	3.96	26.00	0.78	4.39	1.22	15.00	0.32	0.29
		Mine	29.10	23.56	11.00	7.10	0.00	0.00	0.00	0.00	.
		Driefontein	17.02	13.26	15.00	3.42	0.00	0.00	0.00	0.00	.
		Tobias	2.21	0.00	1.00	0.00	3.74	3.74	6.00	1.53	.
		Sachariasboom	3.65	5.84	28.00	1.10	0.00	0.00	0.00	0.00	.
Fish	Chromium	Bergland	9.87	7.31	26.00	1.43	7.62	3.58	15.00	0.92	0.27
		Mine	75.12	59.45	11.00	17.92	0.00	0.00	0.00	0.00	.
		Driefontein	22.56	18.90	15.00	4.88	0.00	0.00	0.00	0.00	.
		Tobias	12.39	0.00	1.00	0.00	14.78	3.05	6.00	1.25	.

		Sacchariasboom	2.25	2.40	28.00	0.45	0.00	0.00	0.00	0.00	.

N = 1 only at Tobias and ranges from 11 to 28 on all other sites during high flow survey. Fish were only collected from Bergland with n = 15 during low flow survey and n = 0 at all other sites during the same survey. The highest mean metal concentration = 89.96 µg/g was on zinc at Tobias during the low flow survey and the lowest, 0.32 µg/g of cadmium at Sacchariasboom during high flow scenario. There were significant differences between high and low flow surveys only on cadmium and lead at Bergland for fish.

Table 4.7 Group statistics of the samples at Sacchariasboom during both flow regimes combined.

Metal	Fish	Mean (µg/g)	Std. Dev.	N
Cd	P. phi	0.4545	0.2787	21
	T. spa	1.6931	1.6557	18
	C. gar	0.2155	0.0057	2
	B. pal	2.3862	1.336	14
	B. tri	0.5882	.	1
Pb	P. phi	3.5592	1.6765	21
	T. spa	3.9905	1.0369	18
	C. gar	1.5350	0.37052	2
	B. pal	3.6531	2.2841	14
	B. tri	6.4706	.	1
Zn	P. phi	62.6986	19.1161	21
	T. spa	71.1878	43.3197	18
	C. gar	11.0517	1.4128	2
	B. pal	101.3143	40.0082	14
	B. tri	51.7647	.	1
Cu	P. phi	5.9095	3.6179	21
	T. spa	22.5421	20.7641	18
	C. gar	1.1480	0.3199	2
	B. pal	14.7341	15.1789	14
	B. tri	2.9412	.	1
Cr	P. phi	9.7973	4.9217	21
	T. spa	49.7174	56.5131	18
	C. gar	3.2945	0.8722	2
	B. pal	25.3832	17.5880	14
	B. tri	17.6471	.	1

Zinc is the most abundant metal of all metals analysed while cadmium is the least occurring metal in fish species analyzed for metals.

Table 4.8 Group statistics of the combined fish species samples from all localities during high flow regime.

Fish Tissue		Cd (µg/g)	Pb (µg/g)	Zn (µg/g)	Cu (µg/g)	Cr (µg/g)
Gill	Mean	0.1172	2.1100	22.6098	3.5263	1.2354
	N	7	7	7	7	7
	Std. Dev.	0.1563	1.4804	16.6264	3.1653	0.9626
Liver	Mean	0.0598	2.7143	47.7884	9.8803	1.6719
	N	7	7	7	7	7
	Std. Dev.	0.1026	3.5240	46.9791	8.7212	1.7140
Muscle	Mean	0.0431	0.5637	3.5567	0.5332	0.3212
	N	7	7	7	7	7
	Std. Dev.	0.0552	0.2160	1.4776	0.3922	0.4069
Total	Mean	0.0734	1.7960	24.6516	4.6466	1.0762
	N	21	21	21	21	21
	Std. Dev.	0.1116	2.2931	33.0191	6.4667	1.2416

All the statistical tables above indicate numbers which were computed by the SPSS program.

Key:

N = number

Std. Dev. = standard deviation

Std. Error = standard error

Cd = cadmium

Pb = lead

Zn = zinc

Cu = copper

Cr = chromium

(.) = Sample could not be computed

AB = There is a significant difference between high and low flow on the same site

P. phi = *Pseudocranilabrus philander*

T. spa = *Tilapia sparrmanii*

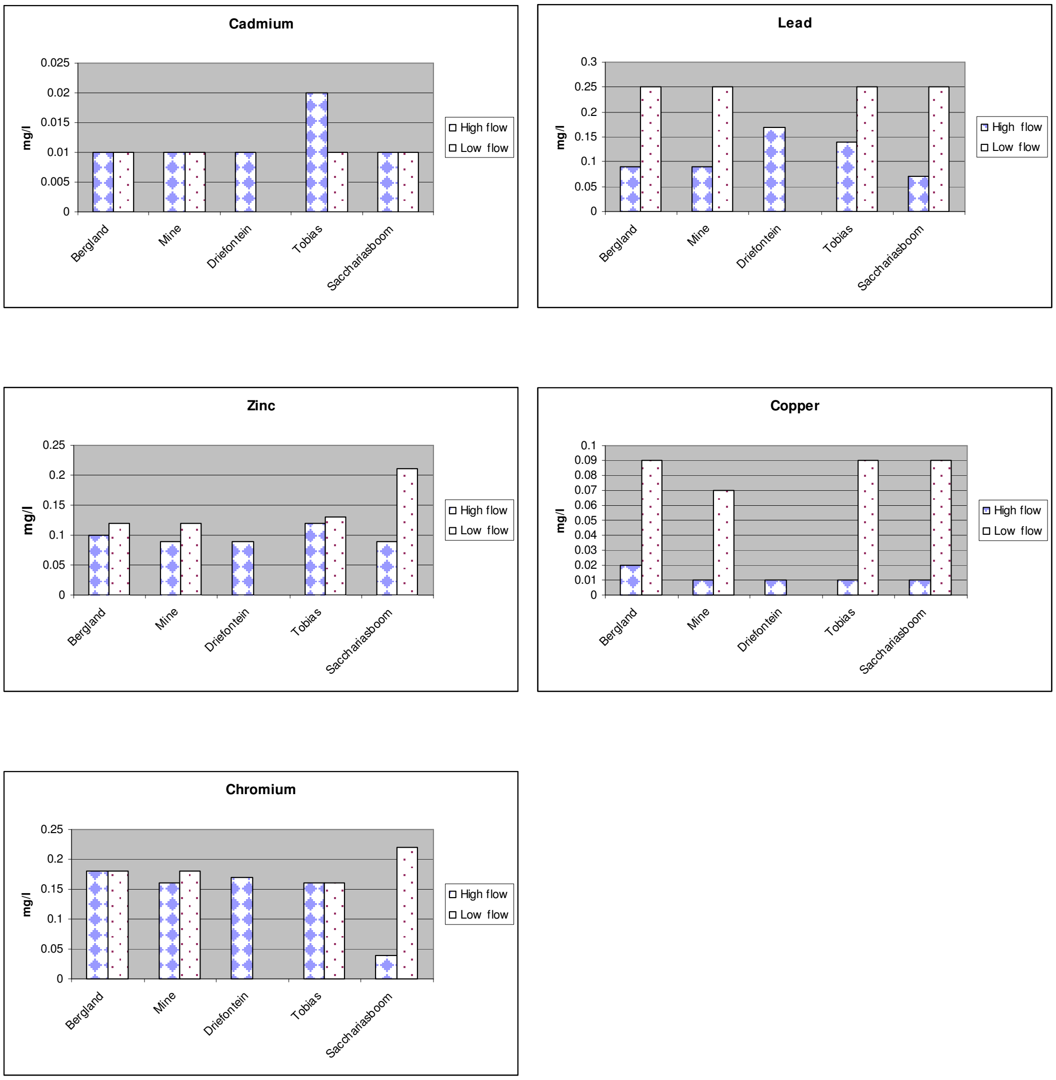
C. gar = *Clarias gariepinus*

B. pal = *Barbus paludinosus*

B. tri = *Barbus trimaculatus*

Zinc has the highest mean concentration in fish than all the other metals analysed while cadmium has the least mean metal concentration in plants than all other samples.
Generally, the mean metal concentrations are higher in the low flow survey than in the high flow survey. Only chromium in plants and sediment, lead in plants and fish; and cadmium in plants sample were higher in high flow than low in flow surveys.

4.4 Water samples



Figures 4.1 Metal concentrations in water samples from the selected sites in the Nyl River catchment during both high and low flow conditions.

The cadmium concentration during the high flow survey in the water samples ranking showed Tobias>Bergland=Mine=Driefontein=Sacchariasboom and was below detection limit during the low flow scenario where water was sampled.

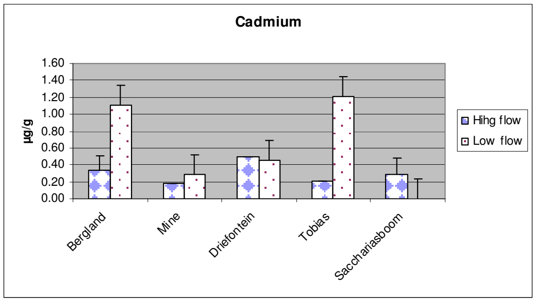
The lead concentration during the high flow survey in the water samples was ranked as follows: Driefontein>Tobias>Bergland=Mine>Sacchariasboom. The lead concentration was below detection limit by the spectrophotometer during the low flow scenario where water samples were collected.

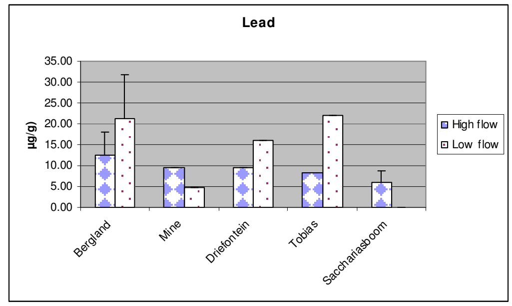
The zinc concentration during the high flow survey in the water samples ranking showed Driefontein>Tobias>Bergland>Mine=Sacchariasboom and was Sacchariasboom>Tobias>Bergland=Mine during the low flow scenario.

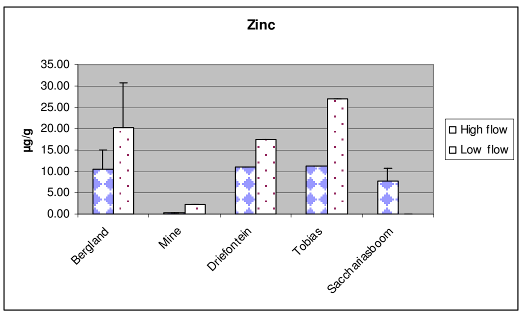
The copper concentration during the high flow survey in the water samples was ranked as follows: Bergland>Mine=Driefontein=Tobias=Sacchariasboom and was Bergland=Tobias=Sacchariasboom>Mine during the low flow survey.

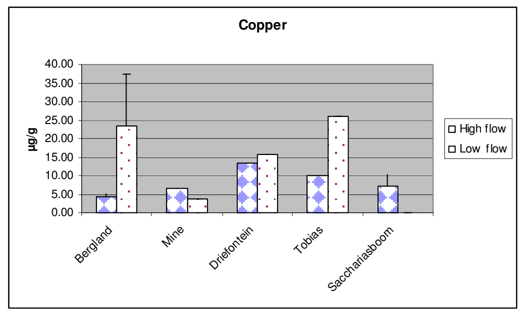
The copper concentration during the high flow survey in the water samples was ranked as follows: Bergland>Driefontein>Mine=Tobias>Sacchariasboom and was Sacchariasboom>Bergland=Mine>Tobias=Sacchariasboom during the low flow survey.

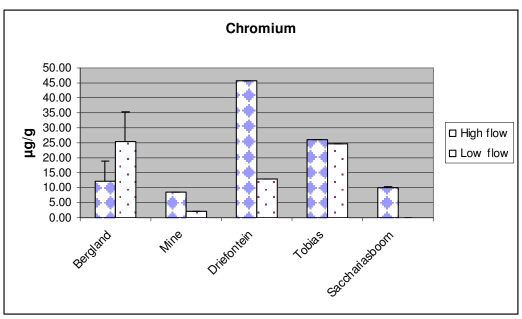
4.5 Sediment samples











Figures 4.2 Metal concentrations in sediment samples from selected sites in the Nyl River catchment during both high and low flow conditions.

The mean cadmium concentration during high flow in sediment are Driefontein>Bergland>Sacchariasboom>Tobias>Mine and the low flow scenario showed the following ranking: Tobias>Bergland>Driefontein>Mine.

The mean lead concentration during high flow in sediment are Bergland>Driefontein>Mine>Tobias>Sacchariasboom and the low flow scenario showed the following ranking: Tobias>Bergland>Driefontein>Mine.

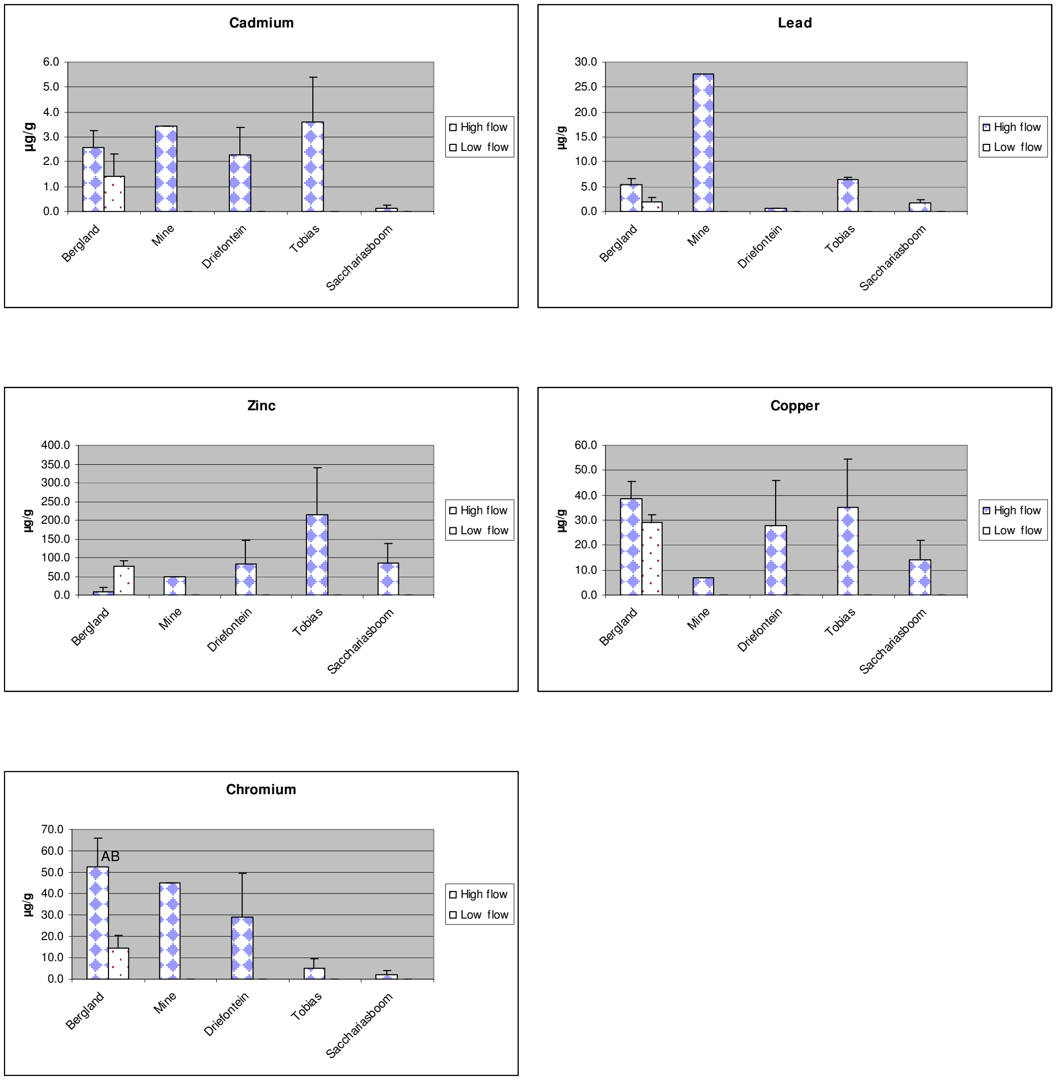
The mean zinc concentration during high flow in sediment are Tobias>Driefontein>Bergland>Sacchariasboom>Mine and the low flow scenario showed the following ranking: Tobias>Bergland>Driefontein>Mine.

The mean copper concentration during high flow in sediment are Driefontein>Tobias>Sacchariasboom>Mine>Bergland and the low flow scenario showed the following ranking: Tobias>Bergland>Driefontein>Mine.

The mean chromium concentration during high flow in sediment are Driefontein>Tobias>Bergland>Sacchariasboom>Mine and the low flow scenario showed the following ranking: Bergland>Tobias>Driefontein>Mine.

There was no sediment sample taken during the low flow survey at Sacchariasboom. Generally, the low flow surveys have higher mean cadmium concentration than the high flow surveys which is mainly accounted for by the dilution factor and more so, settling on sediment as there is less tumbling as a result of less water flow during low flow. The mine site has generally the lowest mean metal concentration compared to all other sites. This is because Mine site is immediately below a dam which is a sediment trap. This therefore helps in trapping excess pollutants and thus reducing the cumulative metal effect on all downstream users. Bergland site being at the head waters and in a protected private land was expected to have less mean metal concentration especially in sediment where metals are expected to be mostly available in water column as a result of increased tumbling, but the results shows the opposite. This may therefore prove that the selected metals are naturally available in the catchment and are not necessarily mainly induced by human activities. The sample size is small, only two samples at most, and could not be computed for determination of significant differences.

4.6 Invertebrates samples



Figures 4.3 Metal concentrations in invertebrates samples from selected sites in the Nyl River catchment during both high and low flow conditions.

Ranking of the invertebrates sample for the mean cadmium concentration during the high flow in invertebrates are Tobias>Mine>Bergland>Driefontein>Sacchariasboom. Only Bergland sample size is enough to compare variances between invertebrate samples of both flow regimes and there is no significant difference between both flow regimes in invertebrates for cadmium concentration.

Ranking of the invertebrates sample for the mean lead concentration during high flow in invertebrates are Mine>Tobias>Bergland>Sacchariasboom>Driefontein. Significant difference is only determined for the Bergland where enough sample size was collected and there is no significant difference between both flow regimes in invertebrates for lead concentration.

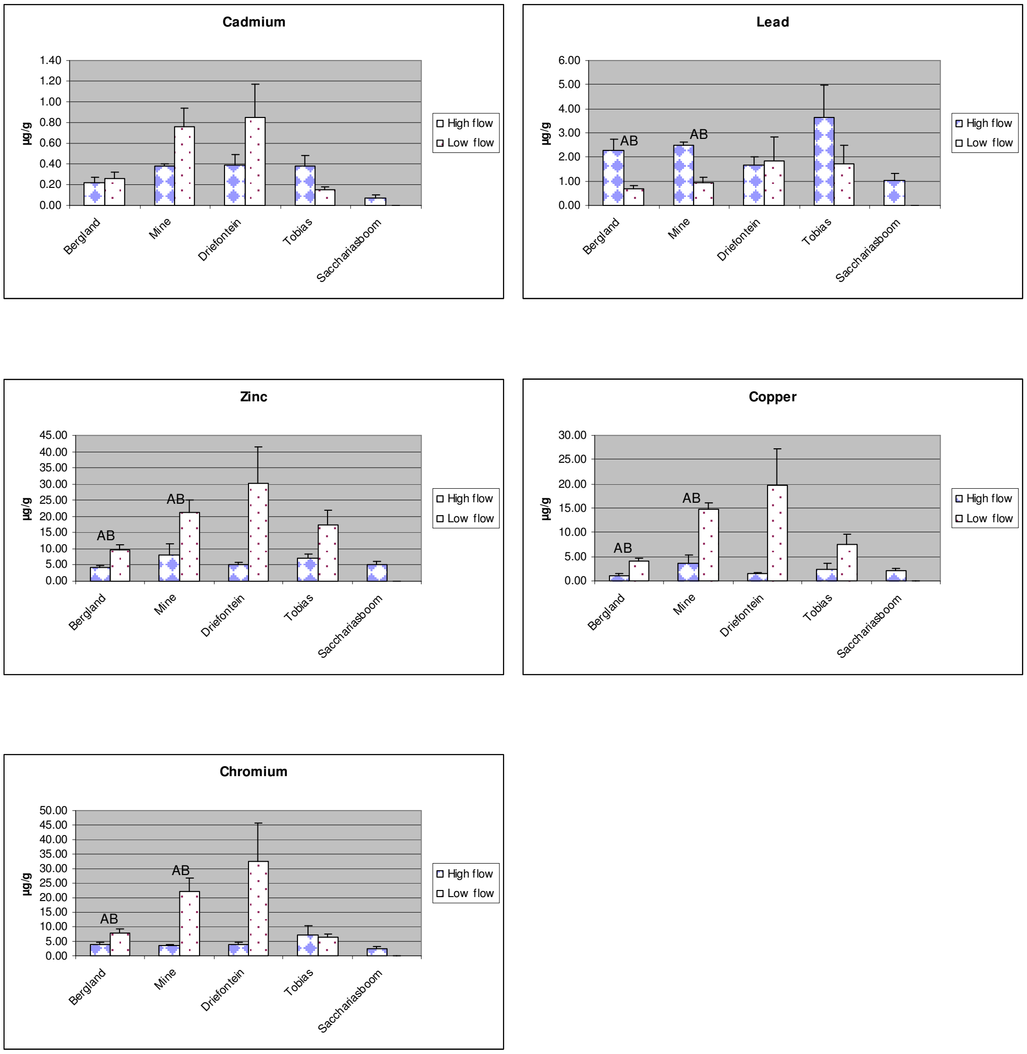
Ranking of the invertebrates sample for the mean zinc concentration during high flow in invertebrate samples are Tobias>Sacchariasboom>Driefontein>Mine>Bergland. Significant difference is only determined at Bergland where enough sample size was collected and there is no significant difference between both flow regimes in invertebrates for zinc concentration.

Ranking of the invertebrates sample for the mean copper concentration during high flow in invertebrate samples are Bergland>Tobias>Driefontein>Sacchariasboom>Mine. Significant difference was only determined at Bergland where enough sample size was collected and there is no significant difference between both flow regimes in invertebrates for copper concentration.

Ranking of the invertebrates sample for the mean chromium concentration during high flow in invertebrate samples are Bergland>Mine>Driefontein>Tobias>Sacchariasboom. Significant difference was only determined at Bergland where enough sample size was collected and there is a significant difference between high and low flow regimes in invertebrates for chromium concentration where the P value = 0.0310.

The only significant differences between both survey is in chromium with the P value = 0.0310. This is indicated by AB on the chart and the comparison is only between flows of each site and not amongst different sites.

4.7. Plants samples



Figures 4.4 Metal concentrations in plants samples from selected sites in the Nyl River catchment during both high and low flow conditions.

The mean cadmium concentration during high flow in plants samples ranking showed Driefontein>Mine>Tobias>Bergland>Sacchariasboom and the low flow survey showed the following ranking: Driefontein>Mine>Bergland>Tobias. There was no plants sample at Scchariasboom during the low flow regime and significant differences could not be computed.

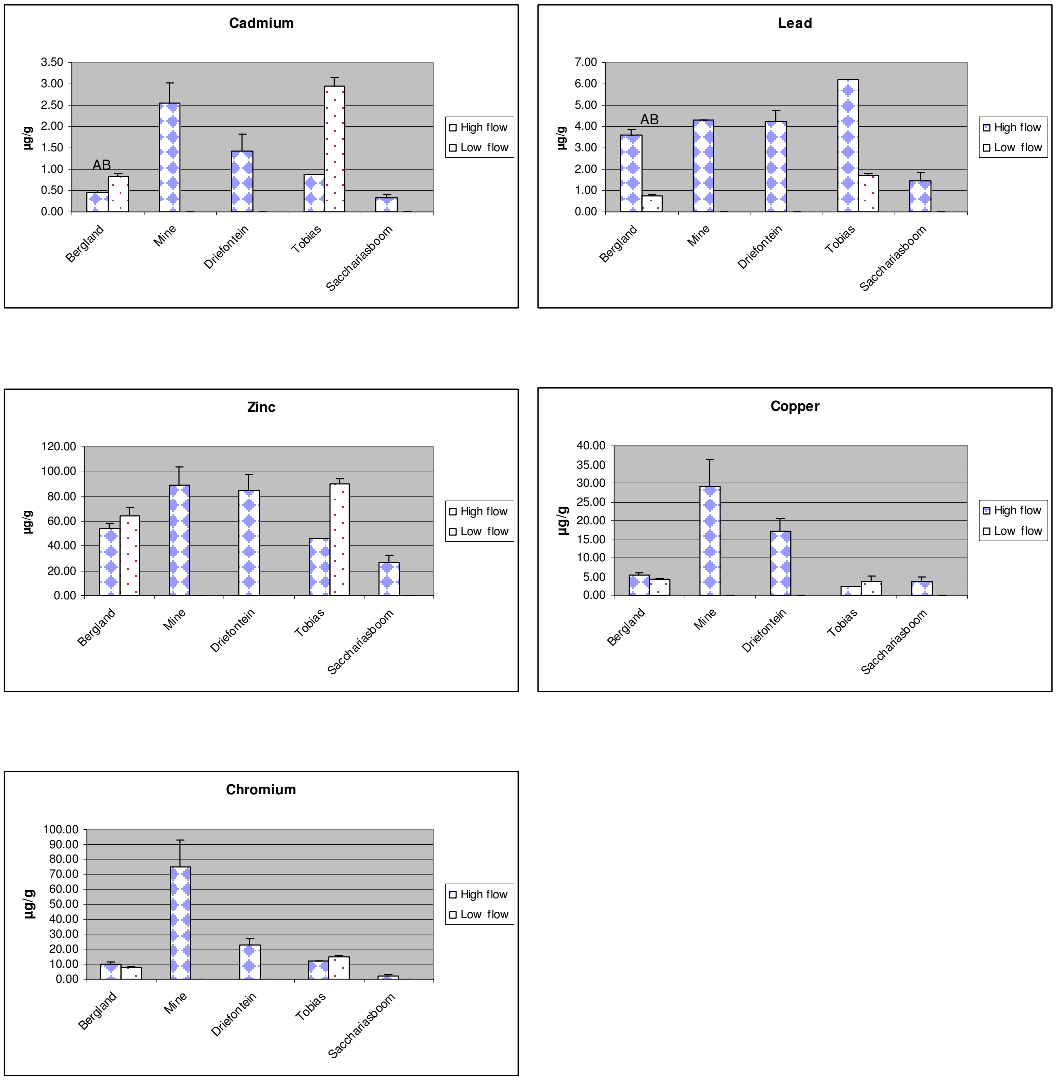
The mean lead concentration during high flow in plants samples ranking showed Tobias>Mine>Bergland>Driefontein>Sacchariasboom and the low flow scenario showed the following ranking: Driefontein>Tobias>Mine>Bergland. There is a significant difference at Bergland and Mine in mean lead concentration between both flow regimes with the P values of 0.0030 and 0.000 respectfully. This is indicated by AB on the chart and the comparison is only between flows of each site and not amongst different sites. There was no plants sample at Schhariasboom during the low flow regime and significant differences could not be computed.

The mean zinc concentration during high flow in plants samples ranking showed Mine>Tobias>Driefontein>Sacchariasboom>Bergland and the low flow scenario showed the following ranking: Driefontein>Mine>Tobias>Bergland. There is a significant difference in the mean zinc concentrations between both high and low flow scenarios at Bergland, Mine and Driefontein with the P values of 0.0070, 0.0430 and 0.045 respectfully. This is indicated by AB on the chart and the comparison is only between flows of each site and not amongst different sites. There was no plants sample at Sacchariasboom during the low flow regime and significant differences could not be computed.

The mean copper concentration during high flow in plants samples ranking showed Mine>Tobias>Sacchariasboom>Driefontein>Bergland and the low flow scenario showed the following ranking: Driefontein>Mine>Tobias>Bergland. There is a significant difference in the mean copper concentrations between both flow regimes at Bergland and Mine with P values of 0.0010 and 0.0290 respectfully. This is indicated by AB on the chart and the comparison is only between flows of each site and not amongst different sites. There was no plants sample at Sacchariasboom during the low flow regime and significant differences could not be computed.

The mean chromium concentration during high flow in the plants samples ranking showed Tobias>Bergland>Driefontein>Mine>Sacchariasboom and the low flow scenario showed the following ranking: Driefontein>Mine>Bergland>Tobias. There is a significant difference in the mean chromium concentrations between both flow regimes at Bergland and Mine with P values of 0.0320 and 0.0120 respectfully. This is indicated by AB on the chart and the comparison is only between flows of each site and not amongst different sites. There was no plants sample at Sacchariasboom during the low flow regime and significant differences could not be computed.

4.8. Fish samples



Figures 4.5 Metal concentrations in fish samples from selected sites in the Nyl River catchment during both high and low flow conditions.

Ranking of the fish sample for the mean cadmium concentration during high flow in fish are Mine>Driefontein>Tobias>Bergland>Sacchariasboom compared to the low flow scenario where Tobias>Bergland. There is a significant difference in the mean cadmium concentrations between both flow regimes at Bergland only with the P value of 0.0000. This is indicated by AB on the chart and the comparison is only between flows of each site and not amongst different sites. The sample sizes from all other sites were small and could not be computed for statistical purposes.

Ranking of the fish sample for the mean lead concentration during high flow in fish samples are Tobias>Mine>Driefontein>Bergland>Sacchariasboom compared to the low flow scenario where Tobias>Bergland. There is a significant difference in the mean lead concentrations between both flow regimes at Bergland only with the P value of 0.0000. This is indicated by AB on the chart and the comparison is only between flows of each site and not amongst different sites. The sample sizes from all other sites were small and could not be computed for statistical purposes.

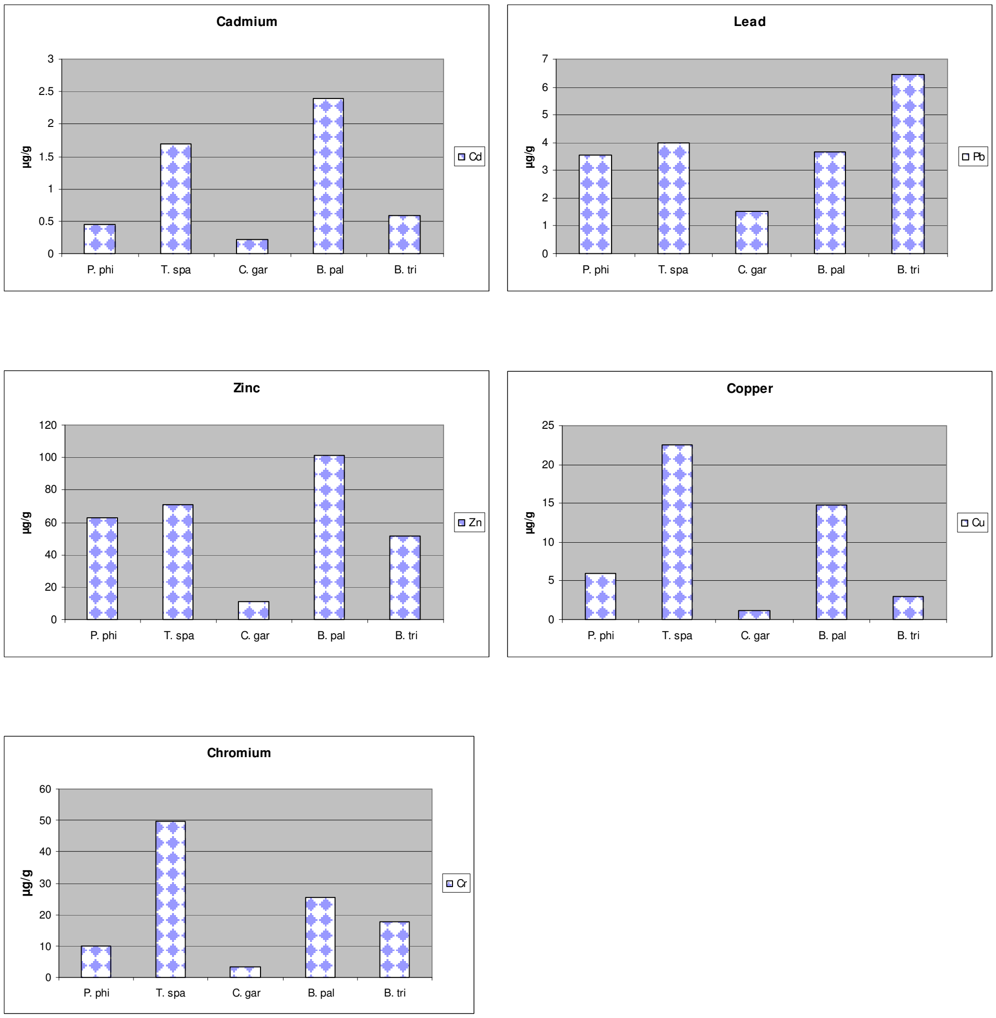
Ranking of the fish sample for the mean zinc concentration during high flow in the fish samples are Mine>Driefontein>Bergland>Tobias>Sacchariasboom compared to the low flow scenario where Tobias>Bergland. There is no significant difference in the mean zinc concentrations between both flow regimes at Bergland. The sample sizes from all other sites were small and could not be computed for statistical purposes.

Ranking of the fish sample for the mean copper concentration during high flow in the fish samples are Mine>Driefontein>Bergland>Sacchariasboom>Tobias compared to the low flow scenario where Bergland>Tobias. There is no significant difference in the mean copper concentrations between both flow regimes at Bergland. The sample sizes from all other sites were small and could not be computed for statistical purposes.

Ranking of the fish sample for the mean chromium concentration during high flow in fish samples are Mine>Driefontein>Tobias>Bergland>Sacchariasboom compared to the low flow scenario where Tobias>Bergland. There is no significant difference in the mean chromium concentrations between both flow regimes at Bergland. The sample sizes from all other sites were small and could not be computed for statistical purposes.

There was no fish sample at Mine, Driefontein and Sacchariasboom during low flow. The mine site was highly turbid as a result of pipeline construction across the river. The water in Sacchariasboom was just pumped into the farm dam and was turbid with no connection to both upstream and downstream pools.

4.9. Fish species samples



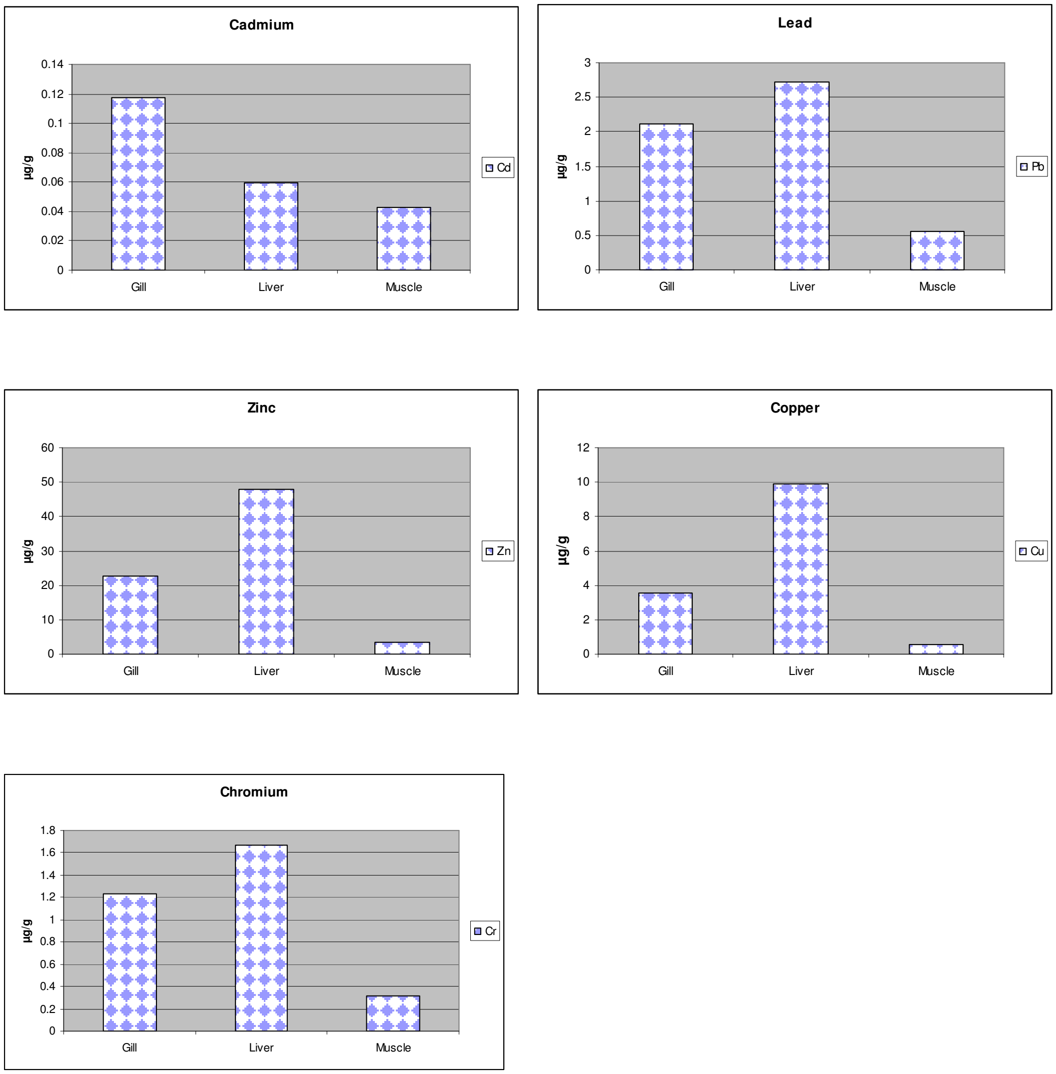
Figures 4.8 **Metal concentrations in different fish species from the Nyl River catchment collected only during the high flow regime.**

Ranking the samples for the mean cadmium concentration are *Barbus trimaculatus>Tilapia sparrmanii>Barbus paludinosus>Pseudocranilabrus philander>Clarias gariepinus.*

Ranking the samples for the mean lead concentration are *Barbus trimaculatus>Tilapia sparrmanii> Pseudocranilabrus philander>Barbus paludinosus>Clarias gariepinus.*

Ranking the samples for the mean zinc concentration are *Barbus paludinosus*> *Tilapia sparrmanii*> *Pseudocranilabrus philander*>*Barbus trimaculatus*>*Clarias gariepinus.*
Ranking the samples for the mean copper concentration are *Tilapia sparrmanii*>*Barbus paludinosus*> *Pseudocranilabrus philander*>*Barbus trimaculatus*>*Clarias gariepinus.*
Ranking the samples for the mean chromium concentration are *Tilapia sparrmanii*>*Barbus paludinosus*>*Barbus trimaculatus*>*Pseudocranilabrus philander*>*Clarias gariepinus.*

4.10. Fish tissues samples



Figures 4.7 The mean metal concentrations and standard deviations of each metal in selected tissues of *C. gariepinus*.

The mean concentrations of four metals *viz*, lead, zinc, copper and chromium ranked liver>gill>muscle and gill>liver>muscle for cadmium only. The liver shows higher mean concentration of all the metals studied except for cadmium and muscles the least metal concentrations of all metals studied.

CHAPTER 5

5. DISCUSSION AND CONCLUSIONS

5.1 Natural Environment

In the aquatic environment, metals and other pollutants accumulate in the ecosystems and concentrations normally will increase down stream through additive effect. The longitudinal changes in physical and chemical characteristics impose significant consequential changes on ecosystem processes (Giller and Malmqvist, 1998). Highly concentrated pollutants entering the river system may inhibit or destroy the natural self-cleaning ability of the river (Dallas and Day, 1993).

Many fish species are considered as top consumers in aquatic ecosystems (Dallinger *et al*., 1987). As a result, pollutants discharged in the aquatic environment are likely to accumulate in fish and represent a potential risk not only in those fish but also to piscivorous birds (van Eeden and Schoonbee, 1993); and mammals including human being (Grimanis *et al*., 1978). This study was conducted to determine the levels of selected metal concentrations in selected samples in the study area and assess the phenomenon of the pollutants filtering effect of Nyl Floodplain, in order to address the safety of fish consumption by humans from the system.

De Wet *et al.* (1990) indicated that different metals tend to accumulate at different rates between the sediment and *Azolla spp*. These plants have demonstrated the ability to absorb metals more effectively than it is stored in the sediment (de Wet *et al.,* 1990). In metals uptake, several factors such as the presence of a variety of different metals in the system can influence the absorption of one another.

Generally, there is a direct relation of metal concentrations between the water sample and other samples at a given site. This study also shows that chromium has higher concentrations followed by zinc, while lead and copper seem to be occurring at approximately equivalent concentrations, and cadmium occurs at the least concentration compared to all other metals studied. Bervoets and Blust (2003) found a direct correlation of metal concentrations in fish organs and levels in water and sediment. In this study, there was however local differences in metal concentrations between different samples and sampling sites as discussed in detail below. The variability is often associated with various physicochemical properties of the water during sampling (Nussey *et al.*, 2000). Different metals can be accumulated by the different samples under similar conditions. For example, the water fern, *Azolla filiculoides* Lam. will accumulate the pollutant at a different level to the actual concentrations in the aquatic environments, e.g. in the bottom sediment (de Wet *et al.*, 1990).

5.1.1. Pooled samples

Individuals of each sample at a given site were pooled into one metal analysis to compare each sample of one site with a similar sample of the other different sites. Samples were pooled to determine the general trends in metal concentrations between high and low flow surveys of each metal. Generally, samples collected during the low flow survey had a higher mean concentration compared to those collected during the high flow survey. The higher mean metal concentration during the low flow survey can be related to the over saturation in the small volume of water. Water levels were generally lower during the low flow survey compared to the high flow survey. It is however known that some metals have antagonistic effects on others, e.g. copper on cadmium on the uptake by *Spirodela polyrhiza* duckweed (Saadi *et al.*, 2002). High mean metal concentration may be associated with competition of metals to the binding side of substrates. Those metals with higher affinity to certain substrates will have higher chances of being absorbed and or taken up.

In general, the mean zinc concentration was high in all samples throughout the study area than all other metals. Zinc is an essential element needed for the proper functioning of animal bodies and is homeostatically controlled in fish. It also play a role in healing process of body tissues and is required for physiological processes such as hormone metabolism, immune responses and stabilisation of membranes (Marx and Avenant-Oldewage, 1998) and thus present at elevated levels on invertebrates and fish.

5.1.2. Bergland

Bergland is situated in the protected headwaters of the Tobiasspruit and metal concentrations at this site are lower than those for the sites downstream (Tables 4.3 to

4.7). Generally, all metals were higher in fish and invertebrates during the high flow survey compared to values for the low flow survey. Most pollutants are normally transported by stormwater and are associated with suspended sediments and the sediments are retained into the beds of rivers and infiltrated basins (Mermillod-Blondin *et al.*, 2004). Metals tend to settle more on the sediment during low flows as there is little tumbling, if any, which affects such settling during high flow survey. Sediments at this site showed low levels of metal contamination due to lower impact at the site which is in a protected area. The flow was fast at Bergland due to the steep landscape which resulted in less settling of metal and other particles in the sediment. Fish and invertebrates have accumulated higher mean metal concentrations compared to sediment and plants. This is attributed to turbulence associated with high flow which makes particles to be more available on the water column and in contact with mobile organisms. The low flow survey indicates the opposite with metal concentrations being high in both plants and sediment due to less tumbling resulting in particles settling on substrates.

5.1.3. Mine

Generally, all metals are present in lower concentrations in the sediment and plants samples from the low flow survey than the high flow survey for same reasons as on section 5.1.2. No invertebrates and fish were collected at this site during low flow survey. Mine site was the only sampling site in Naboom Spruit, a tributary of Tobias Spruit and metal concentrations at this site were generally compared to concentrations at Driefontein which is downstream of the confluence of the Naboom Spruit with Tobias Spruit. There were several signs of negative impacts (Figures 2.5 and 2.6 a, b & c) on aquatic life at this site, including plastics waste, cans, habitat destruction as a result of pipeline construction crossing the stream, water abstraction and a layer of oil on the top surface of the water column.

Low counts and or absence of aquatic macro-invertebrates at the site are associated with elevated TDS. Aquatic macro-invertebrates are depending on vision for most of their activities and are not as mobile as fish. High turbidity was due to the bank disturbance on the site as a result of the pipeline construction across the river at the sampling site (Figures 2.6a and 2.6b) during the low flow survey and rusted pipes and oils on the pipes used for abstraction of water from the dam (Figure 2.5). Also associated with high turbidity were high bank erosions as a result of free roaming cattle drinking water at this site and dust produced by locomotives at the Flourspar Mine just upstream. The site is also very deep just below the dam wall and there was no water release from the dam resulting on less pollutants flushing and dilution.

There is no downstream additive effect of metal concentration because during both sampling surveys, there was no flow connection between the two sites. Water resources are shared resources and the National Water Act (Act No. 36 of 1998) makes provision for equitable use of water resources by all users and continued fragmentation of the catchment have a negative effect on the downstream users. There are several farm dams and weirs in the Tobias Spruit cutting off completely the flow of water, especially during low flow scenarios. These barriers are not only reducing downstream flow requirements but also trap lots of particles. The trapped particles, if toxic, may become lethal to aquatic life down stream during very high flows as they are tumbled and released at high concentrations and become available to organisms and other users down stream.

5.1.4. Driefontein

All metal concentrations were higher during the low flow survey compared to the high flow survey in the plants and the sediment samples. Only limited samples were collected at Driefontein, where there was only a small pool which was not connected to any flowing water during sampling. Fish, invertebrates and water samples were not collected during the low flow survey as the site was dry during the low flow survey. Generally, the mean metal concentrations were higher in plants samples during the low flow survey than high flow survey. The sediment samples had higher mean metal concentrations in low flow survey except for cadmium and chromium with chromium much higher than all metals at this site except zinc in invertebrates and fish. Much higher metal concentrations at this site may be attributed to the isolated small pool sampled with no dilution factor.

5.1.5. Tobias

There were no significant differences in all samples at this site between high and low flows. Only plants and sediment were sampled during both flow regimes at this site.

Cadmium, lead and chromium mean concentrations were lower in plants during the low flow compared to the high flow survey, while zinc and copper mean concentrations were higher for the low flow survey.

Generally, metal mean concentrations were higher in sediment during the low flow compared to the high flow survey. Again, this is because metals tend to settle well when there is reduced turbulence in the water.

5.1.6. Sacchariasboom

Generally, all the metals in all samples are less concentrated at Sacchariasboom than at all other sites. This again indicates the filtering factor of the Nylsvley, on the Nyl River.

There was no similar pattern followed of mean metal concentrations in the fish species, except that *C. gariepinus* had the lowest mean metal concentrations. *C. gariepinus* is not as active as all other collected fish species and this phenomenon can be associated with fish activity. More active fish needs more energy, which is obtained through food and respiration. Fish take up metals mainly through food and respiration, and to a very small extend, through diffusion. Though more metal can be absorbed, fish are capable of regulating metal concentrations in their bodies.

5.1.6.1. Fish tissues

Metal uptake maybe influenced by several factors in an aquatic ecosystem, during life stage development, the species analysed and the physiological processes in the fish species studied (Kotze *et al.*, 1999). The stable element analysis of liver, muscle and bone samples from fish of different mass and age show that the concentrations in adult fish remain fairly constant (Pentreath, 1997). The *C. gariepinus* analysed in this study were approximately the same age with the average total length ranging from 16.4cm to 30cm. Differences in Zn and Cu accumulation seem to occur only in certain organs (gonads) and during certain stages of its reproductive cycles such as spawning (Kotze *et al.*, 1999). Fish during this project were not differentiated according to gender and comparison between males and females was not done. For this study, only the gills, liver and muscle tissue were selected for metal analysis because of the potential effect the elevated metal concentration can have on humans utilising this fish as source of protein.

In most cases, the gonads do not form part of the diet of humans consuming fish as food and therefore were not analysed for metal concentrations.

Figures 4.9 indicate the mean metal concentrations and standard deviations of each metal in selected tissues of *C. gariepinus* collected during the study. The liver showed a higher mean concentration for all the metals studied, followed by the gills and then muscle tissues. A similar trend was observed by Kotze *et al.* (1999).

Other studies (Avenant-Oldewage and Marx, 1994) showed that there was no consistency on which tissue of the fish accumulated high metal concentration at any given time. Contrary to this, Nussey *et al*. (2000) found that gills generally had the highest mean metal concentration of the metals analysed due to direct contact with aquatic environment and their importance as an effecter of ionic and osmotic regulation.

Based on the phenomenon that some metals accumulate in the tissues and organs of fish when increased in the system, seven *C. gariepinus* with total length ranging from 16.4cm to 30cm of both sexes, collected from Sacchariasboom were analysed for metal accumulation in gill, liver and muscle tissues. Fish used in this experiment were collected at Sacchariasboom during the high flow survey only. The results of this analysis are graphically indicated in Figure 4.9. The liver had the highest mean concentration of all the metals analysed, followed by gills and then muscles and this is consistent with results obtained by van Vuren *et al.* (1994), Marx and Avenant-Oldewage (1998), Nussey (1998), Robinson and Avenant-Oldewage, (1997) and Seymore *et al*. (1996).

In contrast, studies of selected metals accumulation on fish tissues of different fish species show that the gills had the highest metal concentration, followed by liver, muscle and skin (Nussey *et al.*, 2000; Coetzee *et al.,* 2002; Avenant-Oldewage and Marx, 2000; Kotze *et al.*, 1999; Bezuidenhout, 1990). The authors also acknowledged that the liver, in its role as a storage and detoxification organ, can accumulate high levels of metals, which was the case in this study. Muscle tissue accumulated the lowest concentrations of the metals studied in this project and since muscle is the most important tissue type consumed by humans, it will be important to conduct similar analysis on a regular basis where people consume large quantities of potentially polluted fish. Life stage and body size of a fish play a role on levels of metals accumulated in fish tissues where smaller fish tend to have higher levels of metal concentrations than larger fish (Nussey *et al.*, 2000; Coetzee *et al.*, 2002; Kotze *et al.*, 1999), a phenomenon which was not considered in this study, due to the extend of the study period.

The lower metal concentration in the gills of *C. gariepinus* can be associated with the lower respiration in the water and a capability of air breathing. The higher metal content in the liver can be a result from accumulation from the food source (Baudin, 1987) whilst the metal found in the gills were probably obtained via breathing (Kotze *et al.*, 1999). The amount of metal bioaccumulation is influenced by various environmental and biological factors, leading to differences in metal accumulation between individuals, species, seasons (Mzimela *et al.*, 2003) and sites (Kotze *et al.*, 1999). The phenomenon that different metals are accumulated differently by various tissues of fish is based on the function of each tissue type and fish activity during the time of sampling (Figure 4.9). It will be important to look at the fish size and the amount of accumulation observed when one wants to determine which size class can be consumed by humans in the system.

5.1.7. General conclusions

Generally, zinc and chromium are the most abundant metals in all samples throughout the study area, as also reported by Greenfield (2004) and cadmium being the least occurring metal of the five metals analysed. The physiological effects of all metals analysed was discussed in Chapter 1. Only sediment samples have higher mean metal concentrations of chromium, copper and zinc. Sediment has no mechanism of getting rid of excess substances and all particles tend to settle on the sediment. This may however be dangerous if the particles are toxic as they may be released during high flows. This will release the metals and the higher concentrations will be available to aquatic organisms and other users. The effects of this on the natural water resources may have serious economic and social implications. The health of humans may be compromised and there may be associated adverse impacts on the tourism industry. Information obtained from this study provide basis for fish health which is important to protect human casualties which may result from consumption of fish with elevated metal contents and drinking water without prior treatment.

Floods often provide cleansing of pollutants in river systems, but that does not occur regularly to compensate for the pressure exerted. We however need not rely on floods for reasonable ecosystem functioning which will be perfect in an unregulated environment. The Tobias Spruit and Naboom Spruit are highly regulated by several small farm dams and weirs which may act as pollutant traps as they trap lots of sediment which have shown to have higher mean metal concentrations during this study. These structures may be dangerous during high flows when water are spilling over, resulting in exposure of toxins to resident organisms at higher concentrations. Sources of metals in the study area other than natural occurrences includes effluents from agricultural activities, waste disposal sites, storm water from Modimolle, sewerage from various sources, rusted pipes on dams and use of leaded fuel and water abstraction pumps (Figures 2.5 and 2.6 c) in the area. Direct effluents discharge and diffused agricultural effluents from riparian farming areas contribute a large proportion of nutrients loads and ions on the system. Compliance monitoring of these activities and various other aspects of water resources, which is a requisite of the National Water Act (Act No. 36 of 1998), is highly recommended for sustainable management of the catchment to ensure proper ecosystem functioning.

All the metal concentrations were generally lower at Bergland which is in the headwaters of the system and showed an increase downstream unto the Tobias site. This was due to the fact that Bergland is in the headwaters and there were fewer influences around the site. There are generally fewer activities on the steep areas of land, compared to flat reaches of a river. In this case, Bergland is situated in a protected area with very little activities with potential negative impacts. The headwaters are also more influenced by the surface runoff and scouring of the fast flowing water due to gradient of the landscape removing most of the potential pollutants. Once the river reaches the flat areas, the potential for settlement of materials and pollutants increases and this will lead to a higher potential for pollutants to occur. This will then increase the potential for metal accumulation in the plants, invertebrates and fish; and settling on the sediment. Increased metal contents also pose a potential impact on other organisms higher on the trophic level such as human through subsistence fishing. The need to compensate for household and economic demands through traditional practices and socio-economic needs for a developing country put an enormous pressure on our ecosystems. Ecosystem benefits within and outside Ramsar sites have been high on the agenda by the Ramsar Convention and Chapter 6 of the National Environmental Management: Biodiversity Act (2004). Subsistence fishing in the study area is important for local communities as a source of protein rich food and as such pose potential threat to human life through bioaccumulation.

The metal concentrations are lower at Sacchariasboom, which is downstream from the wetland area. This proves the hypothesis that wetlands serves as filters for pollutants. It can further be related to the dilution factor of the volume of water from Nyl River. Wetlands are the most productive ecosystems and may provide long term socio-economic benefits if properly managed, yet they are the most sensitive ecosystems. The mean metal concentrations obtained from this study were generally falling within the target water quality ranges by DWAF (1996). During his study on the same system, Greenfield (2004) indicated that pollutant levels were acceptable in the system.

The National Water Act (Act No. 36 of 1998) makes specific provision for the Ecological Reserve, which refers to the quality and quantity of water needed to ensure that the natural environment continues to meet the demands for ecosystem goods and services. The Act also makes provision for establishment of Catchment Management Agencies (CMAs), which overlook activities in each catchment in the country to ensure that aquatic ecosystem goods and services are not compromised. This study, amongst others, may provide baseline information when catchment management objectives (CMOs) are set, which is a requirement by DWAF.

Establishment of conservation areas and or river conservancies/stewardship along the stretch of the highly regulated tributaries of Mogalakwena River will help restore ecosystem functioning of the river. The highly fragmented flows by farm weirs and dams have negative impacts on the distribution of aquatic fauna other than some macro-invertebrates taxa with aerial stages on their lifecycles. Upstream movement of fish and other invertebrates is highly affected and establishment of river conservancies along the rivers will help in proper management of these rivers. Establishment of water users associations, local authorities and CMAs as required by the National Water Act (Act

No. 36 of 1998) will be important to jointly combine efforts on resource protection and sustainable management practices.

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CHAPTER 6

6. CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

Tobias and Naboom Spruits, amongst others, Groot Nyl, Klein Nyl, Olifant Spruit, Modderloop/Rasloop, Middlefontein Spruit, Hassie-se-Water, De Wet Zyn Loop, Bad se Loop, Andries Spruit and Kootjie se Loop, are in the sub-catchment feeding the Nylsvlei, a Ramsar site. It was designated a Ramsar site under criteria: 1a, 1d, 2a, 2b, 2c, 2d, 3b and 3c. In order to keep the Ramsar status of Nylsvley, it is critically important to continually monitor both the drivers and responses in the whole catchment. This will enhance the understanding of the dynamics of the catchment as a whole. Negative changes from the sub-catchments have negative influence on the overall system such as water quality and quantity, biological integrity and overall ecosystem goods and services.

Due to its landscape, the Nyl River floodplain is dependant to some extent on surface runoff water, which has a negative impact on the system's biota (Higgins *et al.*, 1995). Storm water contains high concentrations of heavy metals, organic compounds and pathogens, and can also be an important source of nutrients (Mermillod-Blondin *et al.*, 2004). Compliance monitoring by the relevant government authorities is important to ensure discharge of acceptable levels of pollutants by others users. The types of invertebrates found in a system are dependant on the environment and their fate is determined by the heavy metals in the sediment. It is recommended that a relationship and comparison of metals in sediment and resident invertebrate species is studied and these can play a major conservation role should such invertebrate species be determined in the system. A follow up study should be considered to investigate this relationship, to help conserve and use more of those invertebrates playing a role on metal reduction on sediments. Depending on the intended use, metals can therefore be controlled by invertebrates in natural water bodies, so as to restrict metals and nutrients to the sediment or facilitate release to the water.

Control of stream supply of water for drinking by riparian farmers and communities will help in the reduction of several water quality problems. Other management issues include suppression of dust particles through spraying with water on areas where there is more vehicle traffic such as mines and roads across rivers.

This study and a few others similar in the area are aiming at protection and conservation the ecological integrity of the whole Mogalakwena catchment. Although all the metals are present at acceptable levels (Greenfield, 2001), it is very important to continuously study and monitor these metals for maintenance of the conservation status of the Nylsvley Ramsar site and for the benefit of other water users, such as industrial, agricultural, recreational and domestic users in the area. This will benefit the environment and the South African economy through eco-tourism and at the same time ascertaining certain legislative obligations and the Constitution of South Africa.

The National Aquatic Ecosystem Health Monitoring Programme (NAEHMP) commonly known as River Health Programme (RHP) in DWAF, which uses the responses which are aquatic biota to determine the extent of drivers which are physical and chemical conditions of a river reach, is one most appropriate tool to be used. It is however important to be applied by accredited individuals and information obtained should be reported accordingly on the Rivers Database. The River Database is aiming at providing a one stop information site which will help with data when required for various reasons such as reserve determinations, decisions making and others.

Quite often when development are proposed to be undertaken in many catchments, it is not always easy to quickly refer for biodiversity record to help in decision making for approval of developments. South Africa is a developing country and in order to maintain balance between ecosystem health and economic demands of the developing country, it is essential to continuously monitor biological integrities of our natural environment. Environmental awareness is currently gaining momentum in South Africa and all water users will need to work cooperatively in order to successfully implement set legislations environmental objectives and utilize resources in a way which does not substantially compromise the environment.

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CHAPTER 7

7. UPTAKE KINETICS OF CADMIUM AND ZINC BY THE AFRICAN SHARPTOOTH CATFISH (*C. GARIEPINUS*)

7.1. Toxicology

Toxicology is the quantitative study of the effects of harmful substances or stressful conditions on organisms (Laws, 1993). Toxicology is divided into three main types. The first one is *Economic toxicology* which is concerned with the deliberate use of chemicals to produce harmful effects on target organism such as bacteria, parasites and insects, followed in this exposure experiment of African sharptooth catfish to different concentrations of cadmium and zinc. The second one is *Forensic toxicology* which is concerned with the medical and legal aspect of adverse effects of harmful chemicals and stressful conditions on human. The third one is *Environmental toxicology* which is concerned with the incidental exposure of living organism/s including humans, to pollutant chemicals and unnatural environmental stresses (Laws, 1993).

The information obtained from the field samples alone will not be enough to indicate the relationship between metals themselves and also between a metal and an organism. Thus, results from the exposure experiment can supplementing those from Tobias Spruit, Naboom Spruit and Nyl River to further understand the factors contributing to the overall availability and toxicity of metal in a natural water system. It is often important to combine a field study with an experimental work, in order to determine the effects of the physical and chemical environment on toxicity of metal.

7.2. Metal kinetics

Laboratory experiments where living organisms are exposed under controlled conditions to different metals at different concentrations provide important information on lethal and sub-lethal effects of the pollutants. This information is essential for the improvement of water quality guidelines for metals and will help in cases of chemicals entering a natural system accidentally (spillage) or intentionally (dumping). The "health" status of the fish can also be accessed from such experiment (van Vuren *et al.*, 1999).

This experiment was used to determine the rate of uptake of cadmium and zinc by juvenile albino *C. gariepinus* under controlled laboratory conditions. The experiment was based on the free ion activity model (FIAM), metal bioavailability and the effects of concentration on the rate of uptake the metals by the fish. The original FIAM postulates that the biological effects of metals are best predicted by the activity of the free metal ion, rather than the concentration of total metal. Trace metal interaction with aquatic organisms generally involve the following steps (Campbell, 1995): advection or diffusion of the metal in the bulk solution to the cell membrane surface; sorption/surface complexation of the metal at binding sites on the cell membrane surface; and metal uptake (transport) through the cell membrane into the cellular environment by the involvement of specific channels, pumps or carriers.
The uptake kinetics of metals during exposure should therefore display normal Michaelis-Menten kinetics (van Ginneken *et al.* 1999) where the rate of metal uptake and or binding to the cell membrane surface reaches a point where there is no more available substrate and the complexation reaches an equilibrium state.

Channels, carriers and pumps are integral proteins that provide gateways through lipid bilayer (Vercauteren and Blust, 1999). The gateways are very selective and their selectivity is strongly dependent on the charge and the dimensions of the ion and protein. Cation-selective channels have a negative charge so that positive ions are attracted, but only species that fit into the binding sites can be transported. Generally it is found that only the free metal ion can be transported, while most other species are not translocated by these systems (Vercauteren and Blust, 1999). Metal uptake can also be related to the free metal ion rather than the total metal ion concentration. However, ions of similar size and charge, such as calcium and cadmium, may compete for the same binding sites and be taken up via the same gateways (Vercauteren and Blust, 1999).

Metal must first interact with, or traverse, the cell membrane surface to elicit a biological response. Certain metal species, however, are not able to react directly with cell surface binding sites, and thus, are considered biologically inactive. Such species include colloidal metals (Rich and Morel, 1990) and those complexed to strong organic ligands (Anderson and Morel, 1982).

It has been shown previously that the uptake of cadmium and zinc by a mussel *Mytilus edulis* can be affected by calcium channel blockers and other inhibitors (Vercauteren and Blust, 1999). The inhibitors also influenced physiological condition, but a significant correlation with the effect on metal uptake did not exist in most cases. Cadmium and zinc also inhibit each other's uptake, but the type of inhibition is not clear. The effects of the inhibitors on cadmium and zinc uptake are very different from the effects on calcium uptake, indicating that cadmium and zinc are preferentially taken up through other gateways (Vercauteren and Blust, 1999).

FIAM has more rigorous conceptual basis and its extend is capable of modelling concentration-response experiments from a wider range of water chemistry conditions (i.e. varying pH, hardness and dissolved organic matter) than the original model and, as such, potentially provides a more useful tool for evaluating metal-organism interaction (Brown and Markich, 2000).

C. gariepinus was chosen for this experiment because it has a diverse diet (Skelton, 2001) and was found to occur throughout the natural system of interest during surveys. Small fish have larger metabolic rates per unit body mass than larger fish and hence require relatively more oxygen (Collvin, 1984; Van der Merwe, 1992). Small fish meet this requirement via a higher rate of flow over the gills and by having a larger gill surface area per gram body weight than large fish. Small fish have higher expected frequency of toxic molecules passing over the gills per unit time compared to larger fish. The metals uptake rate was measured at concentrations ranging from 0.00 μM (control) to 5 μM.

Zinc is an essential micronutrient to all organisms, but may become toxic at higher concentrations (Hogstrand *et al.*, 1996). In the absence of dietary zinc, uptake through waterborne sources can become significant (Spry and Wood, 1989). The gills also take up zinc, but some of this is quickly eliminated from the body (Mohan and Choudhary, 1991). It is present in several amino acids and involved in alcohol and protein metabolism. Cadmium is very toxic to organisms even at low concentrations. It is primarily taken up through water, while zinc is mostly acquired through food (van Ginneken *et al.*, 1999).

7.3. Methods

This experiment was used to determine the rate of cadmium and zinc uptake by juvenile *C. gariepinus* under controlled laboratory conditions.

C. gariepinus was chosen for this experiment because it has a diverse diet (Skelton, 2001) and was found to occur throughout the Nylsvley sampling sites during surveys.

7.3.1. Experimental procedure and setup

7.3.1.1. Glassware preparation

Before performing the experiment, all glassware to be used were washed using a method set out by Giesy and Wiener (1977). The apparatus was pre-washed using Teepol ™ detergent to remove all solid particles and pen marks. The apparatus was then rinsed in distilled water and placed in a soap bath containing a phosphate-free 2% Contrad ™ (Merck chemicals) solution for 24 hours. After 24 hours, the apparatus was rinsed in distilled water and placed in a 1M HCl (Merck chemicals) acid bath for another 24 hours. The glassware was rinsed again in distilled water and then left to dry.

7.3.1.2. Preparation of media

Table 7.1. Mass of salts diluted in four 1L beakers and the calculated concentrations of each salt in the final prepared medium (i.e. in the 18.6L solution).

Beaker (1000ml)	Salt crystal	Mass of salt (g)	Volume of distilled water (ml)	Final concentration of salt in the medium (µM)
A	$CaCl_2$	5.88	500	37.6
B	$MgSO_4$	2.47	500	8.84
C	$NaHCO_3$	1.30	500	11.0
D	KCl	0.12	500	1.14

All the solutions were mixed completely using a magnetic stirrer for each mixture. After all the crystals were dissolved, 25 ml of each was placed in a new beaker and a further 900 ml of distilled water was added to make up to a total volume of 1000 ml. This solution was poured into a clean 20 L container and 17.6 L of deionised water was added to make up a total volume of 18.6 L. The solution was aerated for 24 hours after which it was ready for use as a medium for preparation of stock solutions and a medium in which fish were exposed. For both metals, i.e. zinc and cadmium, sublethal metal concentrations were obtained from values provided by the US Environmental Protection Agency's Ecotox database. For both zinc and cadmium, 10% of the mean LC_{50} values for fish were used as the maximum exposure concentrations (refer Table 7.1 – 7.3). Lower percentages of the mean LC_{50} of the metals were chosen to ensure that the fish exposed to the metals do not die during the exposure.

7.3.1.3. Preparation of metal solutions

7.3.1.3.1. Preparation of zinc exposure solution

Different concentrations of the zinc exposure solutions were prepared from a 100 µM (0.01363 g $ZnCl_2$ /L) stock solution made up in medium solution. A final exposure volume of 800 ml was attained by diluting the stock solution as presented in Table 7.2.:

Table 7.2 Preparation of zinc stock solution.

Zinc (µM)	Volume (ml) of zinc stock solution	Volume (ml) of medium added
0	0	800
0.2	1.6	798.4
0.5	4	796
1	8	792
2.5	20	780
5	40	760

7.3.1.3.2. Preparation of cadmium exposure solution

Different concentrations of the cadmium stock solution were prepared from a 100 µM of 0.01833 g$CdCl_2$/L stock solution made up in medium solution. A final exposure volume of 800 ml was attained by diluting the stock solution as presented in Table 7.3:

Table 7.3 Preparation of cadmium stock solution.

Cadmium (µM)	Volume (ml) of cadmium stock solution	Volume (ml) medium
0	0	800
0.02	0.16	798.4
0.06	0.48	796
0.1	0.08	792
0.4	3.2	780
0.8	6.4	760

7.3.1.3.3. Preparation of cadmium/zinc exposure solution

Different concentrations of the cadmium/zinc stock solution were prepared from 0.01833 g$CdCl_2$/L and 0.01363 g $ZnCl_2$/L stock solutions, each 100 µM, made up in medium solution. A final exposure volume of 800 ml was attained by diluting the stock solutions as presented in Table 7.4:

Table 7.4 Preparation of cadmium/zinc stock solution.

Concentration (µM) $CdCl_2$/$ZnCl_2$	Volume (ml) $CdCl_2$/$ZnCl_2$	Volume (ml) medium
0/0	0/0	800
0.02/0.2	0.16/1.6	798.2
0.06/0.5	0.48/4	795.5
0.1/1	0.08/8	791.2
0.4/2.5	3.2/20	779.8
0.8/5	6.4/40	753.6

7.3.2. Experimental setup

The experiment was conducted in the environmental control rooms of the Department of Zoology, UJ. Juvenile *C. gariepinus* were exposed to different concentrations of cadmium, zinc and cadmium/zinc (Table 7.2, 7.3 and 7.4), for three hours. Exposures were carried out in 1000 ml acid-washed beakers, containing 800 ml of the relevant exposure solution. Four *C. gariepinus* were used per metal exposure and three for each control. Physical parameters of each exposure were measured during the time of exposure using pHScan2 Tester for pH and Cyberscan100 for conductivity, TDS and temperature.

7.4. Results

Figure 7.1 A, B, C and D indicate the rate of uptake of zinc, cadmium and zinc/cadmium mixture by juvenile *C. gariepinus* in a laboratory situation. Uptake of ionic metals is affected by several physical factors such as pH and temperature and availability of binding sites on cell membranes of organisms exposed. Table 7.5 – 7.7 indicate the physical-chemical conditions under which fish was exposed.

Table 7.5 Physical-chemical conditions under which *C. gariepinus* were exposed to different concentrations of cadmium.

Physico-chemical parameter	**0µM Cd**	**0.2 µM Cd**	**0.5 µM Cd**	**1 µM Cd**	**2.5 µMCd**	**5 µMCd**
Temperature (^{0}C)	23.8	23.0	24.3	23.5	23.6	23.3
pH	6.3	6.4	6.1	6.1	6.1	5.9
Conductivity (mS/m)	41.2	44.2	46.7	48.5	49.2	52.2
Dissolved Oxygen (%)	32.9	28.2	23.2	31.8	36.0	31.6
Total Dissolved Solids (ppm)	20.7	22.0	23.4	24.2	24.7	26.1
Number of fish	3	3	4	4	4	4

There was a general increase in conductivity in all exposure media from least cadmium concentration to the highest cadmium concentration. All other variables remained fairly constant except dissolved oxygen in 2 – Cd 2 where dissolved oxygen was very low compared to the others.

Table 7.6 Physical conditions under which *C. gariepinus* were exposed to at different concentrations of zinc.

Physico-chemical parameter	0µM Zn	0.2 µM Zn	0.5 µM Zn	1 µM Zn	2.5 µM Zn	5 µM Zn
Temperature (^{0}C)	23.2	23.8	23.5	23.8	23.7	23.7
pH	5.8	5.6	5.8	5.8	5.9	5.8
Conductivity (mS/m)	47.5	50.1	48.8	52.0	53.8	56.5
Dissolved Oxygen (%)	24.2	20.5	27.4	22.4	31.5	25.9
Total Dissolved Solids (ppm)	23.9	25.1	24.5	26.1	27.0	28.2
Number of fish	3	4	4	4	4	4

The variables remained fairly constant except for the dissolved oxygen and conductivity.

Table 7.7 Physical conditions under which *C. gariepinus* were exposed to at different concentrations of cadmium/zinc combination.

Physico-chemical parameter	0µM Cd/Zn	0.2 µM Cd/Zn	0.5 µM Cd/Zn	1 µM Cd/Zn	2.5 µM Cd/Zn	5 µM Cd/Zn
Temperature (^{0}C)	23.3	23.4	23.5	23.5	23.5	23.4
pH	5.8	5.8	5.8	5.8	5.8	5.8
Conductivity (mS/m)	51.3	55.1	50.8	57.5	54.1	62.4
Dissolved Oxygen (%)	32.7	32.0	33.3	32.0	33.4	40.2
Total Dissolved Solids (ppm)	25.7	27.5	25.5	28.7	27.1	31.2
Number of fish	2	4	4	4	4	4

The variables remained fairly constant in 0µM Cd/Zn to 2.5 µM Cd/Zn media except conductivity, dissolved oxygen and total dissolved solids only in the 5 µM Cd/Zn sample. Total dissolved solids were higher (31.2 ppm) in the 5 – Cd/Zn5 compared to the rest of the other media.

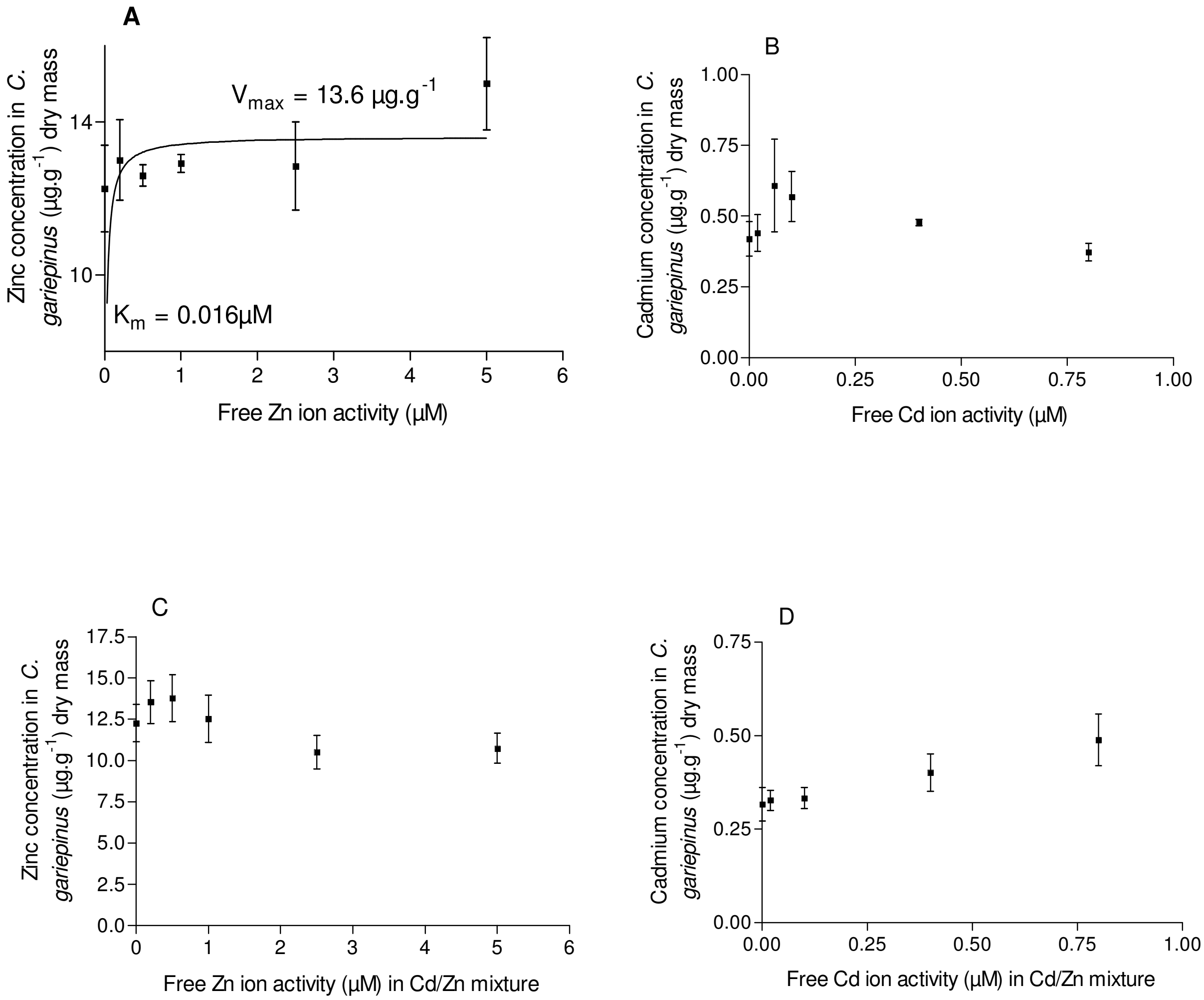

Figure 7.1 The rate of uptake of free zinc ion (A), free cadmium ion (B), free zinc ion in a cadmium/zinc mixture (C) and free cadmium in a cadmium/zinc mixture (D) by *C. gariepinus*.

The rate of uptake of zinc by *C. gariepinus* is higher (Figure 7.1a), Vmax = 13.6µg/g^{-1}, than for cadmium (Figure 7.1b), Vmax = 0.53µg/g^{-1}. Zinc uptake followed the Michaelis-Menton equation during the free zinc exposure experiment (Figure 7.1a) with the K_M value (dissociation value) = 0.016 µM. The rate of uptake of zinc by this fish in the cadmium/zinc mixture is also higher, Vmax = 12.9µg/g^{-1}, than for cadmium also in the cadmium/zinc mixture, Vmax = 0.35µg/g^{-1}, under same conditions (Figures 7.1 c and d respectively).

7.5. Discussions and conclusion

Since zinc is an essential trace element for many biological functions, fish have relatively high need for the metal and are able to regulate the element (Shears and Fletcher, 1993; Spry *et al.*, 1988). Glynn (1991) indicated in his experiment that the cadmium levels in the gills, liver and kidney increased drastically because of the cadmium exposure. On the other hand, the zinc levels in the organs were not markedly affected by a fifteen-fold increase in external zinc concentration, indicating a homeostatic control of zinc in the fish. The toxicity of cadmium is probably due to both the affinity of Cd^{2+} ion for sulphydryl groups in biological molecules (Shukla and Singhal, 1984), and to the lack of regulatory mechanism for cadmium in fish.

The Michaelis-Menton model for mediated transport of solutes across biological interfaces provides a good explanation of the observed variation in metal uptake rates (van Ginneken *et al.*, 1999). In this model the V_{max} value describes the rate of uptake, while the K_M value describes the affinity of the transporter for the metal. A lower K_M value indicates a higher affinity of the transporter for the metal. The lower, $K_M = 0.016$ µM (Figure 7.1a) indicates a higher affinity of the transporters in the fish cell membranes to zinc. Studies done on the Common Carp (*Cyprinus carpio*) by van Ginneken *et al.* (1999) found that zinc uptake is six times faster than cadmium uptake, while the affinity of the transporter for cadmium is 10 times higher than the affinity for zinc. Zinc has higher affinity to the fish proteins than cadmium as zinc is involved in dietary components than cadmium in fish. They also found that both zinc and cadmium had an inhibitory effect on each other. Cadmium increased the transporters' affinity for zinc, but it decreased the uptake rate of zinc by almost half. Thus cadmium is an inhibiter of zinc uptake. Zinc had the opposite effect on cadmium. It increased the uptake rate of cadmium ten fold. The results for cadmium in this experiment are opposite to those of van Ginneken *et al.* (1999). The rate of uptake of zinc by *C. gariepinus* in this experiment is higher (Figure 7.1a), Vmax = 13.6$\mu g/g^{-1}$, than for cadmium (Figure 7.1b), Vmax = 0.53$\mu g/g^{-1}$. The rate of uptake of zinc by the albino *C. gariepinus* in this experiment was 25.7 times higher than cadmium uptake in the free ion exposure media. Vercauteren and Blust (1999) found that the rate of uptake of zinc by *Mytilus edulis* is about 100 times faster than cadmium uptake. Both metals however have an inhibitory effect on each other as displayed by reduced Vmax values of both metals in the cadmium/zinc medium, thus both metals competing for same binding sites in the fish cell membranes. Zinc is however displays even a higher affinity, 36.8 times higher than cadmium in the cadmium/zinc mixture. Zinc has higher affinity to proteins than cadmium as zinc is involved in dietary components than cadmium in fish. Vercauteren and Blust (1999) indicated that the affinity of zinc to organic ligands is roughly 10 times higher than that of cadmium. Metal uptake by aquatic organisms is a two-phased process, which involves initial rapid adsorption or binding to the surface, followed by a slower transport into the cell interior (Crist *et al.*, 1988; Wepener *et al.*, 2001). In this experiment, time frame was not considered and all fish were exposed to different concentrations of metal and metal combinations for equal time.

This experiment proved the antagonistic effect of cadmium on zinc and vice versa at different concentrations the metals analysed under similar laboratory conditions. It also proved zinc to follow the Michaelis-Menten kinetics by the albino *C. gariepinus*. Only zinc uptake followed the Michaelis-Menten kinetics in the experiment similarly as observed by Meyer and Wepener (2001). In the same study Meyer and Wepener (2001) also observed that cadmium uptake on *Oreochromis mossambicus* followed the Michaelis-Menton equation during zinc and cadmium mixture exposure. The same case is observed for both zinc and cadmium at lower concentrations (Figure 7.1 c and d). At the lower zinc concentrations the zinc uptake followed the Michaelis-Menton equation, but not at the higher concentrations.

Other studies (Moolman *et al.* 2006), used the linear form of the Langmuir isotherm model to determine the uptake of cadmium, zinc and a mixture of both metals at five different concentrations by two freshwater gastropods species. The model in that study showed that both gastropods species have different uptake rate of zinc but not cadmium. The linear uptake of cadmium was similar in both species and thus did not yield a good fit to the model (Moolman *et al.*, 2006). There was also no clear cadmium/zinc interaction of the mixed metal exposure and both metals showed a net reduction in the uptake of the metals in the mixture similarly as observed in the albino *C. gariepinus* exposure using Michaelis-Menton equation observed in this study.

It is generally known that water quality influences availability, toxicity and chemical form of pollutants, and here under similar conditions, different metals, *viz* zinc and cadmium, are not taken up by fish at the same rate, thus proving the phenomenon that different metals are taken up differently by the same fish species (Kotze *et al*., 1999). The rate of uptake of zinc and cadmium is faster in pure metal solution or environment, than in metal combination. These indicate the negative effect of the rate of uptake of metals by the presence of another metal, which is the situation in the natural environment. This is because metals are most often competing for same binding site on proteins and other biological components, where an affinity and size of the metal on an ionic state determines the success of such binding This was clearly displayed by this experiment where Zn had higher affinity in the cadmium/zinc mixture than cadmium (Figure 7.1 c and d). It is recommended that models such as Langmuir isotherm are used in future comparison studies. This will not only determine the rate of uptake by different metals of a given species but also variations between species.

In conclusion, zinc has proved to have a higher affinity to the binding sites in the juvenile *C. gariepinus* than cadmium. Both cadmium and zinc are competing for same binding sites in the fish species. The experiment also proved that the Michaelis-Menten kinetics can be best displayed by the uptake of zinc by the fish but not cadmium.

7.6. References

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APPENDIX A

A list of fish species collected at the sampling sites during both flows.

Bergland

Aplocheilichthys johnstoni[1]

Clarias gariepinus[1]

Pseudocranilabrus philander[1 & 2]

Tilapia sparrmanii[1]

Mine

Barbus paludinosus[1]

Chaetia flaviventris[2]

Pseudocranilabrus philander[1 & 2]

Tilapia sparrmanii[1 & 2]

Driefontein

Barbus paludinosus[1]

Pseudocranilabrus philander[1]

Tilapia sparrmanii[1]

Tobias

Barbus paludinosus[1]

Barbus trimaculatus[1]

Tilapia sparrmanii[1]

Sacchariasboom

Barbus paludinosus[1&2]

Clarias gariepinus[1]

Barbus trimaculatus[2]

Pseudocranilabrus philander[2]

Tilapia sparrmanii[2]

A list of invertebrate families collected during sampling on both flows at each locality.

Bergland

Athericidae

Baetidae[1 & 2]

Belostomatidae[1 & 2]

Cased caddis[1 & 2]

Chironomidae[1 & 2]

Chlorocyphidae[1 & 2]

Chlorolestidae[2]

Corixidae[2]

Crabs[1 & 2]

Culicidae

Dytiscidae[1 & 2]

Gerridae

Gomphidae

Gyrinidae[1 & 2]

Helodidae[2]

Hydrometridae

Hydrophilidae[1 & 2]

Hydropsychidae[1 & 2]

Leptophlebiidae[2]

Nepidae

Notonectidae

Shrimps[1 & 2]

Simulidae[2]

Tabanidae[2]

Tibulidae[2]

Velidae[1 & 2]

Mine

Aeshnidae[1]

Baetidae[1]

Belostomatidae[1 & 2]

Chironomidae[1 & 2]

Chlorolestidae[1 & 2]

Corduliidae[1 & 2]

Corixidae[1 & 2]

Crabs[1]

Culicidae[1]

Dytiscidae[1 & 2]

Hydrachnellae[1]

Hydrophilidae[1]

Hydrophilidae[1]

Libellulidae[1]

Lymnaeidae[1]

Nepidae[1 & 2]

Notonectidae[1 & 2]

Oligochaet[1 & 2]

Driefontein

Cuilidae[1]

Tobias

Ccorduliidae[1]

Chironomidae[1]

Chlorolestidae[1]

Coroxidae[1]

Crabs[1]

Dytscidae[1]

Gerridae[1]

Notonectidae[1]

Veliidae[1]

Sacchariasboom

Baetidae[1]

Ceratopogonidae[1]

Chironomidae[1]

Chlorolestidae[1]

Corixidae[1 & 2]

Culicidae[2]

Dytiscidae[2]

Gerridae[1]

Hdrophilidae[2]

Hydraenidae[1]

Labellulidae[1]

Lymnaeidae[1]

Notonectidae[1 & 2]

Tibulidae[2]

List of plants collected during sampling of both surveys at the five localities.

Bergland

Cyperus sp. [2]

Ficus sp. [2]

Joncus oxycarpus[2]

Juncus lomatophlus[1]

Persicaria decipiens[1 & 2]

Poaceae[1]

Schoenoplectus sp. [1 & 2]

Tetraria sp. [1]

Mine

Cyperus distans[1]

Cyperus sp. [2]

Floscopa glomerata[1 & 2]

Miscanthus junceus[1]

Nymphaea nouchali var. caerulea[1]

Persicaria senegalensis[1]

Persicaria sp. [2]

Phragmites moratiatus. [2]

Schoenoplectus muricinux[1]

Scoenoplectus sp. [2]

Typha capensis[1 & 2]

Driefontein

Cyperus immensus[1]

Cyperus sexangularis[2]

Oryza longistaminata[1]

Phragmites moratiatus[1 & 2]

Schoenoplectus sp.[2]

Tobias

Cyperus distans[1]

Cyperus sexangularis[2]

Ludwigia sp.[1]

Marsilia sp.[2]

Oryza longistaminata[1 & 2]

Persicaria attenuata[1]

Persicaria decipiens[1]

Phragmites moratiatus[2]

Schoenoplectus sp.[2]

Saccariasboom

Cyperacea cf *Bolboschoenus sp.*[2]

Naja sp.[1]

Ottelia ulvifolia[1]

Persicaria attenuata[1]

Persicaria sp.[2]

Schoenoplectus carymbosus[1]

Schoenoplectus sp.[2]

Key

Survey:

[1] = First survey

[2] = Second survey