ASSESSING THE EFFECTS OF WATER AND SEDIMENT QUALITY ON AQUATIC MACRO-INVERTEBRATE DIVERSITY IN THE STEELPOORT RIVER, OLIFANTS RIVER SYSTEM, LIMPOPO PROVINCE

by

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DECLARATION

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

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Ms KS Matlou   Date

ABSTRACT

In this study data was collected to establish if the water and sediment quality of the Steelpoort River has any effects on macro-invertebrate species. The Steelpoort River is a tributary of the Olifants River system but not much data about the water quality is available. The Steelpoort River is a perennial river (Ashton *et al.* 2001) situated west of Burgersfort and from there it flows in a north- easterly direction and converges with the Olifants River in the Drakensberg near Kromellenboog. High silt levels in the river, increases the risk of flooding and leads to the smothering of in-stream habitats resulting in loss of some invertebrate and fish species.

Sampling of the water, sediment and macroinvertebrates was conducted quarterly at the Steelpoort River at five sites. One site was above the impoundment (De Hoop Dam) and the rest were below the impoundment. A handheld YSI 556™ Multi Probe System (MPS instrument and a Mettler Toledo SevenGo™ conductivity meter were used to measure the physico-chemical characteristics at the sites. The macroinvertebrates were sampled using the SASS 5 bio-assessment protocol (Goodyear & McNeill 1999, Dickens & Graham 2002). Macro-invertebrate samples were collected using a 400 mm x 400 mm SASS net with a 250 µm mesh size. The substrate was disturbed for a period of two minutes to free macro-invertebrates from the substrate. The SASS score for each site was evaluated in the field for three of the five samples. The macro-invertebrate samples were preserved in 70% ethanol and sorted to family level in the University of Limpopo's Biodiversity laboratory.

For the majority of the physico-chemical parameters the most elevated were detected downstream and lowest concentrations were detected upstream. Analysis of variance (ANOVA) indicated that there were no significant differences for electrical conductivity, salinity, and TDS, between the sites ($p>0.05$). There were however significant differences in the temperature, dissolved oxygen, turbidity, and pH concentrations ($p<0.05$). The majority of the metals and metalloids in the water (Fe, Mn, V, Co, Zn, Ba, and Cu) were highest at Tiershoek (Site1) and lowest at Steelpoort (Site 4) and Burgersfort Bridge (Site 5).The mean metal concentrations

collected showed elevated levels for chromium, copper and zinc, which were above the CCME sediment guidelines.

The most abundant macroinvertebrates were from the family Ephemeroptera which are pollution sensitive and good indicators of pollution in aquatic ecosystems. When using the interpretation of the SASS 5 results from Chutter 1995, the mean Average Score Per Taxa (ASPT) scores show that the water quality for Tiershoek (Site 1) and Polopark (Site 3) is natural but the habitat diversity has been reduced. For De Hoop (Site 2) and Steelpoort (Site 4), the mean ASPT score shows that there is some deterioration in the water quality. Finally, for Burgersfort Bridge (Site 5), the mean ASPT score indicates that there is major deterioration in the water quality.

Primer statistical analysis indicated that the average dissimilarity between sites showed that the highest dissimilarity was between Tiershoek (Site 1) and Burgersfort Bridge (Site 5) which was conclusive with the results from the macro-invertebrate count and SASS, indicating that there is diverse difference from upstream to downstream.
CCA indicated positive correlations between nutrients detected at Steelpoort with Athericidae, Tabanidae, Gomphidae, Baetidae, Elmidae, Planaria, Psephenidae, and Libellulidae which was indicated that water chemistry characteristics exert influence on macroinvertebrates. The results showed that there is degradation of the water quality in river from upstream to downstream with more pollution tolerant species being abundant at Steelpoort (Site 4) and Burgersfort Bridge (Site 5). The most favourable season was autumn and the least summer/spring and this may be attributed to the life cycle of the macro-invertebrate communities.

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DEDICATION

Naledi Maboke Lesley

To my intelligent, handsome, beautiful, funny, talented and insanely lovable son. You are the holder of the key to my heart. All of this is for you my baby, you can be and do whatever your heart desires. Nothing is beyond your reach, nothing is impossible. This is only the beginning 'my son'. **I love you.**

Naledi Maboke Lesley

Go morwa waka o bohlale, o mo botse, wa go thabisa mama, wa go ba le ditalente tse fapanego, o a rategago kudu kudu. O swere senotlelo sa pelo yaka. Tse kamoka ke direla wena nana, o ka dira ebila o kaba se sengwe le sengwe pelo ya gago e se nyakago. Ga gona selo se o ka se sefihlelego. A ke mathoma "morwa waka".

Ke ya go rata.

CHAPTER 1

"Throughout the world water is recognised as the most fundamental and essential of all natural resources and it is clear that neither social and economic development, nor environmental diversity, can be sustained without water. Unfortunately water supplies continue to dwindle because of resource depletion and pollution."

(Ashton et al. 2001)

1.1 INTRODUCTION

Freshwater ecosystems provide humankind with drinking water, harvestable plants and animals, routes of travel and transport, waste removal, and renewable energy (Schwellnus *et al.* 1962, Dudgeon *et al.* 2006). High concentrations of biodiversity are found in rivers, lakes, and coastal wetlands, even though freshwater habitats constitute a small fraction of the Earth's water and only 0.8% of the Earth's surface area (Sorensen 1991). When these freshwater ecosystems are disturbed they deteriorate faster than terrestrial systems because they are more vulnerable (Sorensen 1991). An estimated 9.5% of known animal species (including a third of the vertebrate species) are represented in the *circa* 125,000 species of fresh water animals that have been described (Carpenter *et al.* 2002, Dudgeon *et al.* 2006). Dudgeon *et al.* (2006) predicts that biodiversity in freshwater is declining at a far greater rate than in terrestrial ecosystems, considering the trends in human demand for fresh water.

In arid regions, unsustainable abstraction and pollution are among the greatest threats to freshwater resources (Schwellnus *et al.* 1962, Van Den Berg *et al.* 1998, Allan 2004, Dudgeon *et al.* 2006, Strayer & Dudgeon 2010). In these regions impoundments provide a consistent supply of freshwater for domestic, industrial and agricultural demands. South Africa is a semi-arid country with low, irregular and seasonal rainfall. As a result, South Africa has a broad range of aquatic ecosystems adapted to different water quality regimes and flow patterns (Dwaf 1996f). Pollution from industry, urbanization, afforestation, mining, agriculture and power generation has led to a decline in water quality resources and almost all of the South Africa's freshwater resources have been allocated (Ashton 2007). Furthermore, the construction of impoundments also has multiple impacts on rivers which include flow reduction, altered flow regimes and changes in species diversity, composition and richness (Demirak *et al.* 2006).

The uneven distribution of water (in both quantity and quality) in South Africa has led to the need to protect aquatic ecosystems, for the maintenance of biodiversity and to sustain freshwater resources (ICMM 2010). South Africa's freshwater resources, including rivers, man-made lakes and groundwater, are under increasing stress from the growing population and an expanding economy (Ballance *et al.* 2001). Having most of its primary urban areas located on the watersheds of river catchments makes South Africa unique amongst southern Hemisphere countries. The dual burden of providing water supplies and transporting waste material is exerted on the rivers draining away from these watersheds – most of which enter downstream water storage impoundments. The impoundments have become progressively more enriched during recent decades, because most South African impoundments are located downstream of urban and metropolitan areas (Oberholster & Ashton 2008) meaning the entire runoff load ends up in the impoundments.

Habitat loss and degradation, species invasion, overharvesting, secondary extinctions, chemical and organic pollution, and global climate change have been recognised as the primary threats to biodiversity in running waters, most of which act synergistically in accelerating aquatic species demise and the deterioration of aquatic ecosystems (Van Den Berg *et al.* 1998, Dudgeon *et al.* 2006, Strayer & Dudgeon 2010). A range of interacting factors may bring about habitat degradation. This may involve direct effects on the aquatic ecosystem or indirect impacts that lead to alterations within the drainage basin.

The Olifants River System has been described as degraded and is contaminated with metal and chemicals. This is the consequences of the large number of agricultural, industrial and mining activities in the catchment (Ballance *et al.* 2001). The various land and water use activities that take place in the upper Olifants River System are of strategic importance to South Africa, e.g. mines, agriculture, power generation etc. These particular actions depend a great deal on a variety of goods and services that they acquire from the aquatic ecosystem in the area (Dabrowski & De Klerk 2013). The mining activities that do take place consist mainly of coal mining in the upper reaches of the catchment (Ashton *et al.* 2001). The middle and lower reaches consist of intensive agricultural activities but some mining activities are present on tributaries of the Olifants in the lower and middle sections (Grobler *et al.* 1994, Jezierska & Witeska 2006, De Villiers & Mkwelo 2009, Ashton 2010).

The Steelpoort River is a tributary of the Olifants River and the catchment has experienced major changes in the last ten years – the completion of the De Hoop Dam, increased mining activity, and increased human population. The water quality in this river is degraded by irrigation schemes (Opaluwa *et al.* 2012) and runoff from mines (Ballance *et al.* 2001). The area around the Steelpoort River is also affected by overgrazing and dryland cultivation, leading to erosion and high silt levels in the river (Ashton *et al.* 2001). The Steelpoort River needs to be studied because the above mentioned factors are causing an alteration of the aquatic ecosystem which in turn could be impacting on aquatic biodiversity e.g. macro-invertebrate diversity. Research is urgently required to assess the current state of the Steelpoort River and to propose measures to prevent further degradation of the river ecosystem.

1.2 LITERATURE REVIEW

The Steelpoort River has been poorly studied and not much data is available apart from the data of the River Health (Ballance *et al.* 2001, Opaluwa *et al.* 2012) which does not account for aquatic macro-invertebrates. The physical, chemical and biological integrity of rivers may be altered by human disturbances to river waters through point and non-point source and diffuse pollution that can impact aquatic organisms (Tafangenyasha & Dzinomwa 2005). The levels of physical variables and concentrations of chemical constituents that should not be exceeded in a particular reach of a river are summarized in water quality guidelines as stipulated by the National Water Act (Malan & Day 2004).

1.2.1 WATER AND SEDIMENT QUALITY

Aquatic ecosystems and their biota are affected by a number of water quality parameters such as turbidity, suspended solids, temperature, pH, salinity, concentration of dissolved ions, nutrients, oxygen, biocides and trace metals (Davies & Day 1998, Dallas & Day 2004). Season and precipitation amounts are other factors that can affect nutrient concentration in river systems since they may influence concentration and dilution of nutrients affecting its biological status (Larocque & Rasmussen 1998). The need to conduct environmental effects monitoring is critical in winter months because it is conceivable that nutrients loading in the river system during winter months may have far reaching effects on the biological status of the aquatic systems and water quality (Larocque & Rasmussen 1998). Therefore, South Africa's modification of river flow regimes and changing land use or land cover patterns, coupled with climatic conditions, the discharge of treated and untreated sewage effluents and excessive nutrient loads in return flows from agriculture, have resulted in large-scale changes to aquatic ecosystems that have resulted in the eutrophication of rivers and water storage impoundments (Oberholster & Ashton 2008, Oberholster *et al.* 2011). Rivers have become an efficient medium for transportation of pollutants from source to sink and all waste and nutrients generated on landscapes in catchment systems is mainly water (Larocque & Rasmussen 1998). The shared understanding of eutrophication in rivers remains extremely limited, despite the large amount of work that has been carried out on eutrophication in South African water supply impoundments and lakes (Oberholster & Ashton 2008).

Turbidity (containing high concentrations of suspended silts and clays) in most South African river systems is due to catchment mismanagement, erosion, siltation, unstable riverbeds and loss of in-stream fauna that feed on planktonic algae (Oberholster & Ashton 2008). Fluctuating water levels in impoundments can sometimes cause turbidity to impoundment discharge and eroded shores (Mcallister *et al.* 2001). A key indicator for survival of aquatic organisms and of organic enrichment in streams is dissolved oxygen concentration (Larocque & Rasmussen 1998). Due to metabolic requirements for oxygen to degrade organic matter, dissolved oxygen concentrations in the river water can dwindle during the winter months. Frequently in flowing streams there is a constant attrition of organic and inorganic materials to downstream areas (Tafangenyasha & Dzinomwa 2005).

Pollution-free stream waters and sediment are important in order to sustain a healthy stream flora and fauna, but due to urban surface run-off and other anthropogenic factors which make it hard to maintain an immaculate aquatic environment by impairing water quality and leave a legacy of pollution in the sediment (Beasley & Kneale 2002). Thus, the invasive transformations of riparian zones and watersheds change inputs of water, nutrients, organic matter, and sediments in lakes and rivers (Strayer & Dudgeon 2010). Therefore, in order to add causative information to water quality assessment, the addition of toxicity tests to the assessment of water quality makes especially sense for streams affected by metals, pesticides and other organic xenobiotics (Gerhardt *et al.* 2004).

1.2.2 GENERAL THREATS TO FRESHWATER ECOSYSTEMS

When it comes to freshwater biota, a bias exists relating to geography, habitat types and taxonomy; most populations and habitats in some regions have not been monitored at all (Dudgeon *et al.* 2006). Vital in influencing the biological diversity of streams is the heterogeneity of the habitat and it is linked to the larger systems and surroundings (Allan 2004). Flow alterations which can restrict access to floodplains and limit long distance migrations, may be, due to homogeneity in river channels (Dudgeon 2009). With regard to changes in particular practices or the environment itself, changes in stream ecosystems are difficult to predict in response to changes in land use because the relationship would change over time. For example, upcoming climate change may negate reduced stream temperatures through revegetation of the riparian zone, as a result eliminating one of the pathways by which agricultural land use impacts stream biota (Allan 2004).

Habitat loss and degradation, species invasion, overharvesting, secondary extinctions, chemical and organic pollution, global climate change, over abstraction and impoundment construction seem to be the major causes for concern (Van Den Berg *et al.* 1998, Dudgeon *et al.* 2006, Strayer & Dudgeon 2010, Carpenter *et al.* 2011). Freshwater ecosystems have been modified abiotically and biotically (Sorensen 1991, Dudgeon *et al.* 2006, Strayer & Dudgeon 2010) and human infrastructure now captures more than 50% of accessible fresh water runoff (Rashed 2001); whilst impoundments retain 25% of the global sediment load (Rondeau *et al.* 2000). The amount of land converted for human uses and the arrangement of riparian habitats can be useful indicators of the status of riverine ecosystems because landscape patterns influence both biotic and abiotic properties of surface waters and riparian areas (Gergel *et al.* 2002).

Habitat fragmentation and loss are foremost threats to biodiversity because socio economic and political constraints minimize the amount of land that can be established as protected areas to conserve habitats (Polasky *et al.* 2005). These factors are threatening the survival of aquatic biota and aquatic ecosystems as a whole. It is expected that even if no further degradation of habitats occurred, many populations and species are probably no longer viable over a long term and will ultimately disappear due to the degradation that river ecosystems have encountered because of human's population growth, human water use, climate change, human-made nitrogenous fertilizers invasion, number of impoundments, hydrologic alteration and overexploitation (Strayer & Dudgeon 2010). Ways in which agricultural land use may degrade rivers is through an increase in nonpoint inputs of pollutants, impact on riparian and stream channel habitat, and altered flows (Allan 2004). Restoration of a river back to its original state is not likely because new species exists and cannot be eradicated and we are also experiencing a period of climate change. All that can be done is to rehabilitate in some ways the 'natural' functioning of rivers, reduce pollution and build new aquatic landscapes that are more satisfying (Lévêque & Balian 2005).

1.2.3 CHEMICAL AND ORGANIC POLLUTION

The lithology of the catchment, atmospheric inputs, climatic conditions and anthropogenic inputs are several major influences reflected from the surface water chemistry of a river (Bricker & Jones 1995). Many industrial processes such as mining produce waste products that contain hazardous chemicals, and these are sometimes discharged directly into sewers, rivers or wetlands (Oberholster & Ashton 2008). Diffuse urban and/or agricultural runoff over large areas during storm events in addition to concentrations of nutrients (Nitrogen and Phosphorus compounds) and organic carbon increase as a result of anthropogenic inputs, particularly from sewage treatment plants (Davies & Day 1998, Bellos *et al.* 2004). Nutrients carried by rivers influence the biotic activities and may serve as key indicators of shifting conditions in their catchments (Olowu *et al.* 2010). In the majority of natural ecosystems, nutrients (especially N and P) play a major role in limiting primary productivity. Agricultural fertilizers are the main sources of inorganic nutrients which are not used by plants and are washed into streams and rivers with the domestic sewage and industrial wastes (Bellos *et al.* 2004).. The consequences of the massive input of nutrients are extremely dangerous because they lead to eutrophication which affects aquatic biota (Davies & Day 1998, Farombi *et al.* 2007).

Surface mining methods such as strip mining, show changes in topography and surface drainage and also increase the potential for soil erosion, subsidence, long-term compaction and reduced agricultural capacity. Disruption of the natural groundwater regime occurs and this may pollute both ground and surface water. Another effect that has been noticed is the changes in topsoil characteristics which increase the potential for acidity and salt content, changes in vegetation cover, development of nutrient deficiencies, surface desiccation and all these have the potential for production of atmospheric dust and other pollutants (Ashton *et al.* 2001). Acid mine drainage (AMD) is one of the most recognized consequences of mining to freshwaters worldwide and is commonly linked with coal, pyritic S, Cu, Pb, Zn and Ag mining operations (Hogsden & Harding 2011, 2012). Streams affected by AMD typically have low pH, high concentration of dissolved metals, and substrata coated with metal hydroxide precipitate (Hogsden & Harding 2011, 2012).

1.2.4 BIOACCUMULATION

Bioaccumulation of metals in organisms depends on the size, gender and season (Nussey *et al.* 1999). An increase in the bioaccumulation of metals in tissues of aquatic organisms may be caused by the increase in the bio-available metals in the water which are typically present at low concentrations in natural freshwater ecosystems (Du Preez *et al.* 1997). The temporal change in bioaccumulation provides data about the trend of bioaccumulation, which will in turn be used to recognize stability, improvement or deterioration in the organisms (Nakayama *et al.* 2010). Physico-chemical parameters which affect metal bioavailability play a vital role in the bioaccumulation and toxicity of metals in aquatic organisms (Robinson & Avenant-Oldewage 1997).

For many years humans have been utilizing metals (Järup 2003). Metals are stable and persistent environmental contaminants of both fresh and marine waters and their sediments (Yousafzai & Shakoori 2008). A wide range of pathways such as air, surface water and soil have all been responsible for the emission of heavy metals to the environment (Järup 2003). Human beings and other living organisms require metals such as Zn, Cu, Mn, Fe and Se for their wellbeing and growth. However, these metals exert toxic effects when at higher levels than they are needed. Exposure to lead, cadmium, mercury and arsenic are considered the main threats to human health because they are not essential for metabolic activities (Spehar *et al.* 1982). An increase in the uptake rate of metals into the body can be increased by the local bioavailability of heavy metals either dissolved or in the diet (Rainbow 2002). The degree of metal pollution in various aquatic ecosystems can be measured by the amount of metal accumulation in the organisms found in the aquatic system. These organisms can accumulate metals to levels greater than those necessary for physiological functioning (Coetzee *et al.* 2002, Deforest *et al.* 2007).

Metals are potentially toxic because of their ability to bind to proteins and other molecules and disturbing them from functioning in their normal metabolic rate (Rainbow 2002). Cadmium, mercury and lead have no required minimum concentration and are considered as non-essential metals and need to detoxified and excreted immediately in organisms such as macro-invertebrates (Rainbow 2002, Newman B. & Watling 2007). Whether or not the metals will be used for vital metabolic purpose, be excreted, stored in the body or even bind to the 'wrong' biomolecule and exerts a toxic effect is determined by the physiology of the organism (Coetzee *et al.* 2002, Rainbow 2002). The threshold concentration that can be tolerated can be exceeded if the maximum combined rate of excretion and detoxification of metabolically available metal is surpassed, the organism will suffer toxic effects because of the body concentration of metabolically available metal (Rainbow 2002). During periods of high flow, temporal variation in bioaccumulation may occur and generally show increased levels of bioaccumulation. This phenomenon ascribes to influence of sediment-bound metals which are brought into contact with aquatic organisms at a higher intensity during such periods (Kotzè *et al.* 1999). The overall environmental "load" of metal toxins has dramatically increased to the point that society is dependent on industrialization for proper functioning (Spehar *et al.* 1980).

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Biological systems thrive in fairly narrow physical and chemical limits even though they consist of a varying diversity of habitats and organisms. Metals play a vital role in these systems but there are vigilantly set boundaries of tolerable ranges. The temporal changes in bioaccumulation will give data about the trend of bioaccumulation, which will in turn be used to recognize stability, improvement or deterioration. Protection against the consumption of contaminated food is achieved by monitoring of concentration levels of food and other organisms (Du Preez *et al.* 1997, Rainbow 2002). Hence it is imperative to monitor the bioaccumulation in an aquatic system because of the deleterious effects of metals in aquatic ecosystems (Lee *et al.* 2000). This will give an indication of the temporal and spatial extent of metal accumulation, meanwhile assessing the potential impact on human health and organism health (Kotzè *et al.* 1999).

According to du Preez *et al*. (1997) "the monitoring of metal bioaccumulation is important because it serves the following purposes; (1) gauging the extent of bioaccumulation both temporal and spatial (2) assessing organisms health and (3) assessing fitness for human consumption." Unknown areas with high concentrations can be identified through spatial monitoring of bioaccumulation, while on the other hand for known discharges it will provide some data concerning the area being affected (Du Preez *et al.* 1997).

1.2.5 IMPACT OF IMPOUNDMENTS ON RIVERINE ECOSYSTEMS

Impoundments represent obstacles to longitudinal interactions along fluvial systems and the construction of impoundments brings about physical, chemical and biological changes to natural ecosystems (Demirak *et al.* 2006, Boyd 2010). Geomorphological adjustments of rivers below impoundments are very important, even though they may appear less obvious (Ligon *et al.* 1995). Geomorphic responses that occur like incision or aggradation, change in channel patterns, the streambed becoming coarser or finer, channel widening or narrowing, increased or decreased lateral migration of channels, loss of riparian vegetation, riparian encroachment in active channels and bank collapse depend on the type of river and type of impoundment (Hare *et al.* 1994, Stanford *et al.* 1996). The distinctiveness of the impact of an impoundment not only depends on the impoundment structure and its operations but also on the local sediment supplies, geomorphic constraints, climate, and key attributes of the local biota (Stanford *et al.* 1996, Boyd 2010).

As soon as an impoundment is active it will act like a natural lake and start to trap sediment. The sedimentation will gradually change the character of the impoundment storage and basin substrate (Boyd 2010). They endure severe morphological changes such as channelization, bank protection and aggregated mining (Hare *et al.* 1994). Habitat degradation and reduction in storage capacity of an impoundment or below the impoundment can be caused by sedimentation (Brix *et al.* 2011). Encroachment of riparian vegetation into the stream channel or filling of pools with sediments is some of the deleterious morphological changes that could occur below an impoundment (Hare *et al.* 1994). The length of the river affected by an impoundment is largely determined by the frequency of tributary confluences below the impoundment and the relative magnitude of the tributary stream (Boyd 2010).

The hydrological effects of the impoundment become less important the greater the distance downstream (Ligon *et al.* 1995, McCartney *et al.* 2000). Extensive periods of dissolved oxygen exhaustion can occur as a product of decomposition of newly submerged vegetation in many impoundments. By disturbing the movement of organisms, changing flow regimes and transforming habitats impoundments have a substantial impact on aquatic biodiversity (Vörösmarty *et al.* 2010). For the evaluation of impoundment impacts on downstream aquatic habitats the temperature of the water is a significant quality factor because it influences many important physical, chemical and biological processes (McCartney *et al.* 2000).

1.2.6 AQUATIC MACRO-INVERTEBRATES AS BIO-INDICATORS

Bio-monitoring has been marked by significant progress because of increased worldwide concern over environmental degradation unlike in the past were it lagged behind its progenitors, limnology and stream ecology (Rosenberg 1992). Until the past few decades very little was known about freshwater invertebrates in all taxonomic groups and regions even though mayflies (Ephemeroptera) have attracted naturalist since the seventeenth century (Allan & Flecker 1993). Aquatic macro-invertebrates respond quickly to localised water quality conditions in a river even though their existence also depends largely on how diverse a habitat is. The relative abundances of freshwater macro-invertebrate species has been used to make inferences about pollution loads because they vary in sensitivity to organic pollution (Azrina *et al.* 2006).

Their life-cycle durations make them good indicators of short to medium term impacts on water quality and habitat. Regardless of whether they are important or not all aquatic invertebrates take up and accumulate heavy metals, and consequent body concentrations if heavy metals show a huge variation across metals and invertebrate taxa (Rainbow 2002). For example, invertebrate groups such as Tubifucid worms and certain species of Chironomid midge larvae survive under deoxygenated conditions verging on anoxia, whereas other taxa such as larvae of Plecoptera and Ephemerotera are pollution intolerant (Hodkinson & Jackson 2005). Ranging from a single animal to the total invertebrate community, the former group can show alterations in the environment through their responses at different levels of organization (Hodkinson & Jackson 2005). To evaluate the real risk an in-depth analysis of the response of different taxa that live directly in contact with polluted wastes is vital (Nahmani & Rossi 2003).

Pollution in sediments has influence on the development of aquatic macro-invertebrates, the lowest members of the food chain, leading to changes of the entire ecological structure (Beasley & Kneale 2002). Some indicator species can be used to define tolerance ranges of the fauna for water quality conditions in different parts of a river system (Palmer *et al.* 1996). The presence or absence of sensitive or tolerant groups within communities makes them brilliant bio-indicators. (Beasley & Kneale 2002, ICMM 2010). The indication of pollution through benthic macro-invertebrate bioindicators could provide data over a longer period and therefore would be more cost-efficient.

The most frequently described macro-invertebrate distribution patterns are those of taxonomic composition, with the term `community structure' frequently being synonymous with taxonomic composition (Palmer *et al.* 1996). A functional classification is the alternative to a coarser taxonomic classification system, and there have been attempts to associate functional feeding groups (FFG) distribution to water quality variables (Faith 1990). The association is likely to be indirect as the types of food available will partly determine the physicochemical variables (Palmer *et al.* 1996). Even though invertebrate diversity generally exceeds vertebrate diversity at any locale, just like in terrestrial systems the knowledge of the number of species living in rivers of a region is more complete for vertebrates than invertebrates (Allan & Flecker 1993, Dudgeon *et al.* 2006).

Aquatic macro-invertebrates are used for the bio-assessment in the SASS 5 methodology (Dickens & Graham 2002a, Chon *et al.* 2012). The method is a qualitative, multi-biotope, rapid and field-based method and requires the identification of aquatic macro-invertebrates, mostly to family level (Chon *et al.* 2012). Aquatic macroinvertebrates are valuable organisms for bio-assessment mainly because they are visible to the naked eye, easy to identify, have rapid life cycle and usually have sedentary habitats (Dickens & Graham 2002a).

1.3 AIMS AND OBJECTIVES

1.3.1 AIM

The aim of the study was to investigate the state of the Steelpoort River and the impact of water and sediment quality and the construction of De Hoop Dam on aquatic macro-invertebrates.

1.3.2 OBJECTIVES

The Objectives of the study were:

i. To assess the water and sediment quality in the Steelpoort River at five sites,
ii. To determine the concentrations of metals (e.g. Pb, Sb, As, Cr, Al, Fe, V) in the water and sediment of the Steelpoort River, and
iii. To assess the impact of the quality of water and sediment on the aquatic macro-invertebrate assemblages in the Steelpoort River.

1.4 SIGNIFICANCE OF PROPOSED RESEARCH

Aquatic macro-invertebrate diversity in the Steelpoort River has not been studied adequately especially following the major changes in the catchment e.g. construction of the large impoundment, increase in mining and human population, and not much is known about the conditions of water and sediment quality. Hence it is imperative that studies be done on the river to help in conservation and taxonomy of aquatic macro-invertebrates.

1.5 STUDY AREA

The Olifants River catchment covers about 54 570 km^2 and is subdivided into 9 secondary catchments. The total average annual runoff is about 2400 million cubic metres per year (Ballance *et al.* 2001). The Olifants River and some of its tributaries, notably the Klein Olifants River, Elands River, Wilge River and Bronkhorstspruit, rise in the Highveld grasslands. The Olifants River meanders past the foot of the Strydpoort Mountains and through the Drakensberg, descending over the escarpment. The river has been described as degraded and is contaminated with metal and other chemicals. This is the consequences of the large number of agricultural, industrial and mining activities in the catchment (Ballance *et al.* 2001). The upper reaches of the river catchment are characterised mainly by coal mining, agricultural and conservation activities. Over-grazing and highly erodable soils result in such severe erosion, in parts of the middle section that, after heavy rains the river has a red-brown colour from all the suspended sedimentsThe middle and lower reaches consist of intensive agricultural activities (Jezierska & Witeska 2006, De Villiers & Mkwelo 2009, Ashton 2010). Other rivers such as Steelpoort and Selati rivers are major tributaries of the Olifants River.

The Steelpoort River is a perennial river (Ashton *et al.* 2001) situated west of Burgersfort and from there it flows in a north- easterly direction and converges with the Olifants River in the Drakensberg near Kromellenboog (Ballance *et al.* 2001). It covers an area of 7,139 km^2, which is about 13% of the Olifants River basin (Figure 1). The Steelpoort River is in a fair to unacceptable ecological state; overgrazing, and dryland cultivation occur throughout the area surrounding the river. High silt levels in the river, increase the risk of flooding and leads to the smothering of in-stream habitats resulting in loss of some invertebrate and fish species. Runoff from mines and other activities lowers the water quality in the Steelpoort River (Ballance *et al.* 2001).

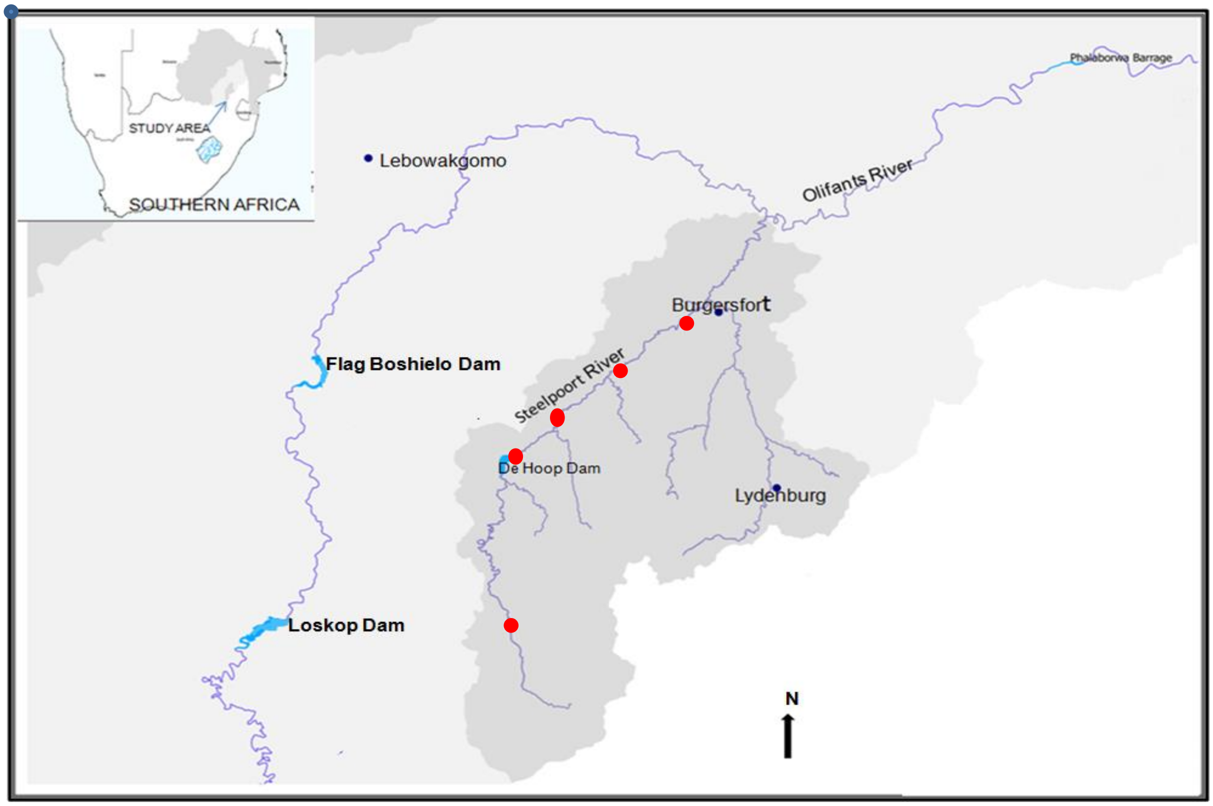

FIGURE 1. 1**:** Map showing the position of the Steelpoort River in the Olifants River catchment.

Site 1

This site is situated; about a kilometre away from the R555 road (S'25.101 E' 29.84705); it is secluded and not close to any villages or industrial area. The site is above the De Hoop Dam and is the reference site. It is below a dirt road heading to a place called Tiershoek. The river is made up of clay and silt with medium sized boulders embedded in the bed road along the river bank. The vegetation along the river bank consists of reeds and *Acacia* trees (Figure 1.2).

Site 2

The next site (most impressive of all the five sites) is; just below the De Hoop Dam a short distance from the R555 (S' 24.9530 E' 29.96141). The water here is clear and surrounded by a close canopy of *Acacia* trees. The river substrate is made up of fine gravel on the edge with small to medium boulder moving to the centre of the river.

Besides the construction of the dam the area is relatively undisturbed by anthropogenic activities (Figure 1.3).

Site 3

The site is situated; underneath a small bridge alongside the R555 (S’ 24.8939 E’ 30.01717) and is about 30 km from Steelpoort close to a commercial centre and it directly passes through a village. The water is used by the community for drinking, washing clothes, bathing, car wash, recreation activities such as fishing and hanging out. There are small scale maize farms just near the river. There are Ferrochrome mines and smelters near this site. The river bank is made of fine gravel (covered with black dust particles from pollution by the nearby smelters) and boulders in the centre of the river. The vegetation consists of reeds along the river bank with some scattered *Acacia* trees (Figure 1.4).

Site 4

Site 4 is situated; along R555 road below a small bridge about 10 km from Steelpoort (S’ 24.718 E’ 30.20092). The surrounding area has changed since the beginning of the survey in 2014. A residential area with subsidized houses has been developed just a few hundred metres from the river and a lot of construction is still taking place in the area. The river bed is made up of a combination of clay on the river bank, fine gravel towards the river and big boulders in the middle of the river. The vegetation is made up of big *Acacia* trees immersed in the water and reeds along the river bank. This area is accessible and the community uses it for laundry to a minimal extant. Cattle have also been observed drinking water from the river in most of our field surveys (Figure 1.5).

Site 5

The site is situated; near the R37 on the road to Burgersfort. It is about a kilometre from Burgersfort Mall (S’ 24.6591 E’ 30.30202). This site is just below a bridge and is easily accessible. The site contains a lot of algae (eutrophication). The river substrate is made up of clay with small rocks and consists of reeds along the river bed (Figure 1.6).

Figure 1. 2: Representation of the reference site, Tiershoek (Site 1) in the Steelpoort River.

FIGURE 1. 3: Representation of de Hoop (Site 2) below the de Hoop dam.

Figure 1.4: Representation of Polopark Site (Site 3) which is situated near the R555.

Figure 1.5: Picture showing Steelpoort (Site 4) at the Steelpoort River.

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FIGURE 1.6: Picture representing Burgersfort Bridge (Site 5) at the Steelpoort River.

1.2 DISSERTATION OUTLINE

Chapter 1 is the general introduction and contains the literature review relating to all aspects covered for this dissertation including the aim and objectives, problem statement, significance of the study and the study area.

Chapter 2 describes the water and sediment quality of the Steelpoort River (both physical and chemical characteristics).

Chapter 3 is about the macro-invertebrate community structure, species richness, abundance and distribution, and how it reflects on the water quality at the Steelpoort.

Chapter 4 is about the General discussion and conclusions of the dissertation.

CHAPTER 2

WATER AND SEDIMENT QUALITY

2.1 INTRODUCTION

Aquatic systems are currently being threatened on a world-wide scale by a variety of contaminants, in addition to a multitude of imprudent water management practices and destructive land uses (Strobl & Robillard 2008). The suitability of an aquatic habitat is tied to water and sediment characteristics which the aquatic life depends on. Habitat necessities differ among species, can differ with the season, and be challenging to define (Soballe & Weiner 1998). In an aquatic ecosystem, the behaviour and impact of contaminants is complex and may involve the following processes; adsorption–desorption, precipitation–solubilization, filtration, biological uptake, excretion, and sedimentation–resuspension (Strobl & Robillard 2008).

South Africa is a semi-arid country, with high evaporation rates, and annual rainfall below the world average (Goetsch & Palmer 1997). Runoff of seasonal rainfall washes silt, organic and inorganic materials accumulated in the catchment into the water bodies. Different aquatic ecosystems with biotas are adapted to diverse water quality regimes and flow patterns and are marked by differing climatic gradients across the country (DWAF 1996d). In many rivers, there is considerable year to year variability in flow rates, and flow is strongly seasonal. Many streams and rivers may dry up completely during the dry seasons. The regulation and abstraction of water from many rivers and streams for water supply purposes is an additional problem in South Africa. Hence these regulations may lead to unnatural flow conditions, which cause stresses on aquatic ecosystems (DWAF 1996d).

The physical, chemical, biological and aesthetic properties of water that determine its fitness for a range of uses and for the protection of the health and integrity of aquatic ecosystems can be described by the term “water quality”. Water quality constituents that are either dissolved or suspended in water influence or control many of these properties (DWAF 1996d). A water quality guideline is a set of data given for a specific water quality constituent. The TWQR and water quality criteria, the CEV and the AEV collectively with the support data which includes the occurrence of the constituent in the aquatic environment, the norms used to assess its effects on water uses, and the conditions for case-, site- and region-specific modifications, make up the water quality guidelines (DWAF1996d). In the South African Water Quality Guidelines edition, the term constituent is used generically for any of the properties of water and/or the substances suspended or dissolved in it. Scientific and technical information provided for a particular water quality constituent, in the form of numerical and qualitative data that describes its potential effects on the health of aquatic ecosystems and the fitness of water for other uses are termed “water quality criteria” (DWAF 1996d).

The Target Water Quality Range (TWQR) is a management objective not a water quality criterion which has been derived from quantitative and qualitative criteria. Within this range of concentrations or levels no measurable adverse effects are expected on the health of aquatic ecosystems, and should therefore ensure their protection (DWAF 1996d). The Chronic Effect Value (CEV), is the concentration or level of a constituent where there is expected to be a noteworthy probability of measurable chronic effects to up to 5% of the species in the aquatic community. If such chronic effects continue (for some time and/or occur frequently) they can lead to the eventual demise of individuals and disappearance of susceptible species from aquatic ecosystems. For the health of aquatic ecosystems this can have considerable negative consequences, since all components of aquatic ecosystems are interdependent (DWAF1996d). The Acute Effect Value (AEV) is that concentration or level of a constituent above which there is expected to be a major probability of acute toxic effects up to 5% of the species in the aquatic community. If such acute effects persist (even a short while, or occur at too high a frequency), they can rapidly cause the demise and disappearance of susceptible species or communities from aquatic ecosystems (DWAF 1996d).

Aquatic ecosystems are defined as the abiotic (physical and chemical) and biotic components, habitats and ecological processes contained within rivers and their riparian zones, reservoirs lakes and wetlands and their fringing vegetation, for the purposes of developing water quality guidelines. Also included in this definition is terrestrial biota, other than humans dependent on aquatic ecosystems for survival. In the definition of aquatic ecosystems three primary abiotic and biotic components are included, namely, sediments (bottom or suspended), water and the riparian zone (DWAF 1996d). Based on the effects that the constituents may have on aquatic biota and the methodologies of derivation used in the criteria, the constituent-specific criteria have been divided into four categories. The constituents were chosen putting in mind the problems currently experienced in South African aquatic ecosystems. The four categories are: toxic constituents, non-toxic inorganic constituents, system variables and nutrients (DWAF 1996d).

Toxic constituents hardly ever occur in high concentrations in unimpacted systems. Their criteria are given as single numerical values related to a specific level of risk, or a value below which no adverse effect is expected. Examples of some toxic constituents are: Inorganic constituents, e.g. Al, As, Cd, Cu, F^-, Hg, Mn, NH_4^+ and Organic constituents, e.g. phenol, atrazine (DWAF 1996d). System variables control important ecosystem processes such as spawning and migration. Aquatic biotas are usually adapted to the natural seasonal cycles of changing water quality which characterize aquatic systems. Severe disruptions to the ecological and physiological functions of aquatic organisms and the ecology of the system may be caused by changes in the amplitude, frequency and duration of these cycles. Numerical ranges are given to the criteria for constituents such as temperature, pH and dissolved oxygen (DWAF 1996d). Non-toxic inorganic constituents, in that their natural concentrations depend on localised geochemical, physical and hydrological processes, they may cause toxic effects at excessive concentrations, but are generally "system characteristics". The criteria are given numerical ranges or as proportional changes from local background conditions for constituents like total dissolved solids (TDS) and total suspended solids (TSS) (DWAF 1996d). Nutrients are generally not toxic but stimulate eutrophication if present in excess. Narrative or numerical site-specific values or ranges are given criteria for constituents such as inorganic nitrogen (nitrate, nitrite, ammonium) and inorganic phosphorus (ortho-phosphates) (DWAF 1996d).

2.2 METHOD AND MATERIALS

2.2.1 WATER AND SEDIMENT SAMPLING

A YSI 556™ Multi Probe System (MPS) instrument was used to measure the water temperature, pH, electrical conductivity, redox potential and dissolved oxygen (mg/l) of the water. A Mettler Toledo SevenGo™ conductivity meter was used to measure the total dissolved solids (TDS), electrical conductivity (EC) and salinity. Acid pre-treated polypropylene sampling bottles (1000 ml) were used to collect water which was stored at 5°C immediately before sending to an accredited laboratory in Pretoria for chemical analysis. Additionally, 500 ml bottles were used to collect water for nutrient analyses at the Department of Biodiversity (UL) within two days after sampling.

Sediments were collected from all the five locations. Where necessary, large stones were moved so that the sediment beneath can be collected. Samples were stored in 250 ml polyethylene sampling bottles. All tools were rinsed with deionized water and/or acid washed before being reused for additional collections. The samples were frozen at -5°C in the field to prevent degradation and stored frozen at the Department of Biodiversity before sending to an accredited laboratory in Pretoria for chemical analysis.

2.2.2 LABORATORY ANALYSIS

Water and sediment quality analysis

Water and sediment were analysed at a SANAS accredited laboratory using an Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) for major cations and anions, nutrients and metals. The nutrients (NH_4, NO_2, NO_3 and PO_4), sulphate, turbidity, alkilinity and hardness were determined using a spectrophotometer (Merck Pharo 100 Spectroquant™) and Merck cell test kits at the University of Limpopo Biodiversity Laboratory. Metal concentrations in sediments were determined using nitric acid digestion and analysed by inductively coupled plasma - mass spectrometry (ICP-MS) and reported as mg/kg dry weight.

2.2.3 STATISTICAL ANALYSIS

The mean and standard deviation of the respective water chemistry, water sediment and metal concentrations were calculated. A One-way ANOVA was performed to determine whether the water chemistry and/or water column metal concentration varied during low and high flow, or at each site (flow levels and sites as independent variables) within the river using the aov() function in the R statistical package (R Development Core Team 2014).

2.3 RESULTS AND DISSCUSSION

2.3.1 WATER CONSTIUENTS

PHYSICO-CHEMICAL WATER QUALITY VARIABLES

Physico-chemical system variables control important ecosystem processes and aquatic animal behaviour such as emersion of invertebrates and the spawning and migration of fish (DWAF 1996d).

WATER TEMPERATURE, DISSOLVED OXYGEN AND PH

TEMPERATURE

Temperature may be defined as the state of a body that determines the transfer of heat to, or from, other bodies. It plays an essential role in water by affecting the rates of chemical reactions and therefore also the metabolic rates of organisms. In inland waters of South Africa the temperature generally ranges from 5 - 30˚C. If the organic load into a water body is high, microbial activity is accelerated by oxygen depletion which takes place at higher water temperatures. On the other hand, unnaturally low temperatures like those induced by bottom releases of dam water, may induce fish tolerance, or suppress normal activities such as, spawning. As water temperature increases, the toxicity of most substances, and the vulnerability of organisms to these substances is intensified (DWAF 1996d).

Table 2.1: The seasonal mean values of the physico-chemical water quality variables recorded at the Steelpoort River.

Water Quality Parameters	Summer		Autumn		Winter		Spring		Water quality guidelines
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Water temperature	26.1	1.5	17.7	2.2	17.1	1.7	24	2.48	Temperature should not vary more than 10% from normal (natural) value [1]
Dissolved oxygen (mg/L O_2)	8.4	1.5	8.96	0.97	11.3	1.2	9.5	1.9	≤ 5[1]
pH	8.5-9.0	0.2	7.8-8.9	0.4	8.4-9.3	0.4	9.0-9.5	0.2	Should not vary by > 5%[1]; 6.5 – 9.0[2]
Conductivity (EC) mS/m	200.3		278.5	101.5	360.1	163.9	387.9	154.5	No criteria available
TDS mg/L	91	12.8	139.5	52.6	177.2	81.1	182.2	73.2	TDS should not change by >15% from normal cycle[1]
Salinity (‰)	0.096	0.022	0.132	0.052	0.172	0.077	0.174	0.07	< 0.05% 0r < 0.5‰[1]
Turbidity NTU	22.4	7.27			42.6	12.3	38.8	12.5	120 to 180 (mg/LCaCO3) hard water[3]
Nitrate (mg/L NO_3^-N)	0.79	0.16	1.79	1.07	1.69	1.22	1.03	0.4	13[2]
Nitrite (mg/L NO_2^-N)	0.058	0.026	0.025	0.016	0.024	0.007	0.025	0.01	0.06[2]
Ammonium (mg/L NH_3^-N)	0.136	0.079	0.09	0.076	0.058	0.097	0.085	0.087	< 0.007[1]; 0.354[2]
Total Nitrogen	0.99	0.24	1.92	1.06	1.76	1.25	0.73	0.69	< 0.5 (oligotrophic); > 10 (hypertrophic)[1]
Phosphorus (mg/L P)	0.367	0.058	2.13	0.995	0.028	0.012	0.068	0.085	< 0.005 (oligotrophic); > 0.25 (hypertrophic)[1]
Calcium (mg/L)	30.6	6.9	63.6	24.9	27.8	5.1	25.9	4.1	< 200[2]
Magnesium (mg/L)	15.6	7.4	13.6	3	19.5	11	16.9	8.6	< 150[3]
Potassium (mg/L)	1.6	0.1	2.4	0.5	1.9	0.3	2	0.2	No criteria available
Sodium (mg/L)	16.6	8	12.6	3.8	41.1	27	22.8	12.8	< 200[3]

1. (DWAF 1996) - South African Water Quality guidelines: Volume 7: Aquatic Ecosystems.
2. BC-EPD (2006) – British Columbia Environmental Protection Division: Water Quality Guidelines.
3. (CCME 2012) – Canadian Council of Ministers of the Environment: Water Quality Guidelines – Aquatic Life.

For the sites, the water temperature was highest at a mean of 20.1 mg/l at Site 5 (Burgersfort Bridge) and lowest at a mean of 17.3 mg/l at Site 4 (Steelpoort) (Appendix A; Figure 2.1). The lowest reading was recorded at Site 1 (Tiershoek) during the autumn survey, with a low of 15.0˚C and the highest water temperature reading was 27.3˚C at Site 5 (beneath Burgersfort Bridge) during summer (Appendix A).The One-way ANOVA computed upon this sample show significant variance of water temperature between the different sites ($p<0.05$). In this study, for the seasons the water temperature was highest during summer at a mean of 26.1˚C and lowest during winter at a mean of 17.1˚C (Appendix A; Table 2.1). The temperature readings were always below 30˚C throughout the study.

DISSOLVED OXYGEN

For many aquatic species, dissolved oxygen is crucial and because depletion of oxygen, for example, caused by untreated sewage has highly visible effects (e.g., fish kills, macro-invertebrate decline), dissolved oxygen levels have been a key indicator of pollution for a long time (Goldman & Horne 1983). Gaseous oxygen (O_2) from the atmosphere dissolves in water and is also generated through photosynthesis by aquatic plants and phytoplankton. Oxygen is fairly soluble in water. Adequate dissolved oxygen (DO) concentrations maintenance is crucial for the survival and functioning of the aquatic biota because it is required for the respiration of all aerobic organisms. A useful measure of the health of an aquatic ecosystem may be provided by the DO concentration. Measurement of the chemical oxygen demand (COD) or the biochemical oxygen demand (BOD) are helpful for determining water quality requirements of effluents discharged into aquatic systems, in order to limit their impact, but are inappropriate for aquatic ecosystems. Sewage pollution strongly affects oxygen concentrations in the river. Under anoxic conditions (in the absence of free and bound oxygen) heavy metals such as iron and manganese can appear in solution, as ferrous (Fe) and manganous (Mn) species, and toxic sulphides (S) may also be released in the water column or in sediments (DWAF 1996d). The solubility of dissolved oxygen in water is reduced by higher temperatures, decreasing its concentration and thus its availability to aquatic organisms (Soballe and Weiner 1998).

The mean value of DO concentrations were all above 5 mg/L, which implies that the oxygen levels may not have any adverse effects on the performance and endurance of the biological communities (Table 2.1). For the sites, the highest mean value was 10.7 mg/L at Site 1 (Tiershoek) and the lowest was 8.6 mg/L at Site 3 (De Hoop) (Appendix A; Figure 2.1). The overall highest value recorded throughout all surveys at all sites was 12.66 mg/L in winter at Site 2 (De Hoop) which is right below the dam wall and the lowest was 6.85 during summer at Site 4 (Steelpoort) which is further downstream from the dam (Appendix A). The One-way ANOVA showed significant difference between sites ($p<0.05$). For the seasons, the highest mean value for DO was 11.3 mg/L during winter and the lowest was 8.4 mg/L during the summer (Appendix A; Table 2.1). The lower DO concentrations in the summer months could be attributed to the higher summer temperatures, because DO and water temperatures are inversely related. Furthermore, on numerous occasions we observed people using the river for bathing and doing laundry (e.g. Site 4), and could have increased the nutrient load, increasing the temperature and ultimately decreasing the DO.

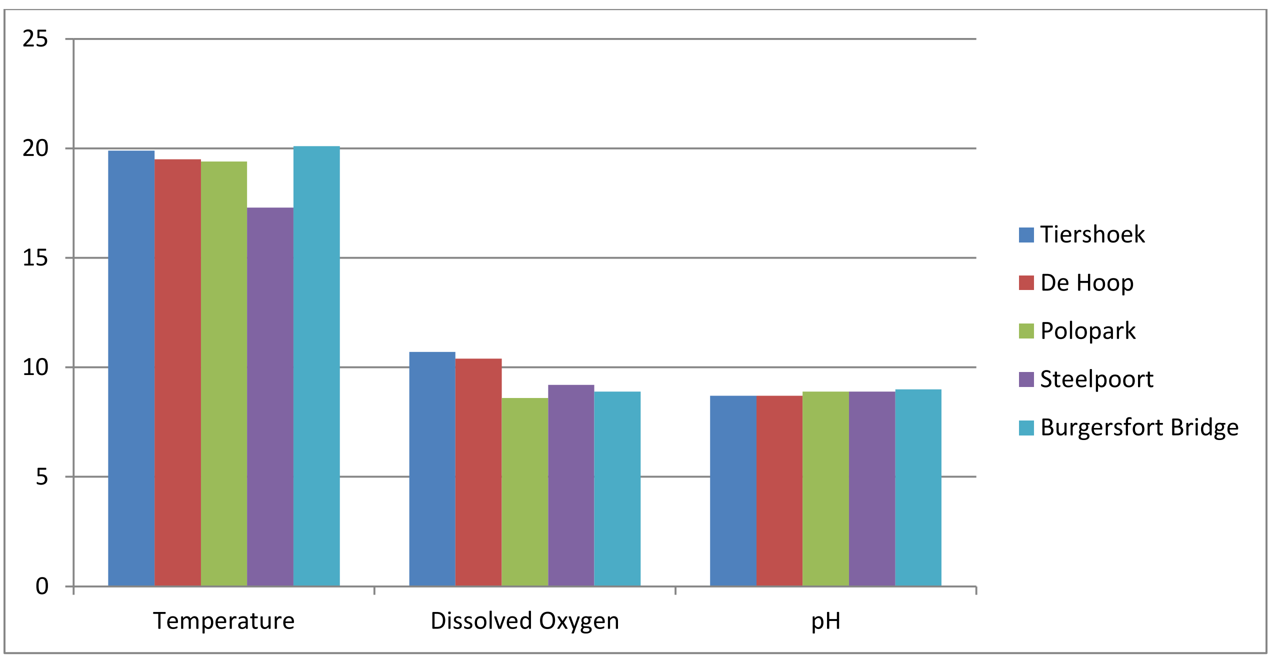

Figure 2. 1: The variations of the mean water temperature, DO and pH in the Steelpoort River.

pH

The pH value is a measure of activity of the hydrogen ion in a water sample. For surface water, pH values normally range between 4 and 11. The pH of natural waters and the relative proportions of the major ions are determined by geological and atmospheric influences. The majority of South African fresh waters are fairly well buffered and more or less neutral, with pH ranging between 6 and 8 (DWAF 1996d). In eutrophic systems, elevated pH values can be caused by increased biological activity. As a result of changing rates of photosynthesis and respiration, the pH values may fluctuate widely from below 6 to above 10 over a 24-hour period. Factors such as temperature, the concentrations of inorganic and organic ions, and biological activity can affect the pH. On the other hand, the availability and toxicity of constituents such as trace metals, non-metallic ions such as ammonium, and essential elements like selenium may be affected by the pH (DWAF 1996d, Dallas & Day 2004).

The pH readings recorded were highest at 9.5 during spring at Site 3 (Polopark) and lowest at 7.8 during autumn at Site 2 (De Hoop) (Appendix A). The One-way ANOVA showed a significant variance for pH between the sites ($p<0.05$). Thus, the pH of the water in this study was leaning more towards alkalinity. The pH can change rapidly in poorly buffered waters, which in turn may have severe effects on the aquatic biota. The rate of change of pH in aquatic systems is affected by the buffering capacity of the river. Therefore, the degree of alkalinization is vital in influencing the severity of the effects, which do not differ linearly either with pH or over time (DWAF 1996d). According to DWAF (1996), it is imperative to note that some streams are naturally more alkaline than others and their biotas are often adapted to these conditions, when assessing the potential effect of a change in pH. The high pH value at Site 3 (Polopark) could be a result of human activities at the site. It is used for laundry, car washing and bathing. Not much information is available on the effects of elevated pH (DWAF 1996d).

TURBIDITY, TDS, EC AND SALINITY

TURBIDITY

The loss of water transparency that results from the scattering of light by suspended materials is called turbidity*.* Many species of aquatic organisms have adapted to moderate turbidity. In addition, for some species, suspended particles are a source of food (or nutrients). Stream and riverine ecosystems can be degraded by excessive amounts of suspended sediment which are harmful to many aquatic organisms (Castro & Reckendorf 1995, Waters 1995). They can affect organisms that use their vision to locate prey, avoid predators, or find other members of their species to mate or care for offspring. It may also decrease the light available to algae and rooted aquatic plants (Waters 1995). The abundance of aquatic organisms in certain reaches of a river may be affected by turbidity and suspended solids (Soballe & Weiner 1998).

For the sites, the highest value was 19.3 NTU at Site 4 (Steelpoort) and the lowest was 3.3 NTU at Site 2 (De Hoop) (Appendix A; Figure 2.2).The overall lowest turbidity was recorded at Site 1 (Tiershoek) during autumn which was 1 NTU and the highest being 38 NTU during summer at Site 4 (Steelpoort) (Appendix A). The One-way ANOVA indicated a significant variance for turbidity ($p<0.05$) between sites. For the seasons, the mean turbidity value was highest during summer at 23.8 NTU and lowest during autumn at 3.8 NTU (Appendix A; Table 2.1).The high turbidity during summer is due to the rains, causing a high influx of water from upstream of Site 4 (Steelpoort) caring a lot of suspended material consisting mostly of silt, clay, and organic matter (Waters 1995). The lower turbidity value which was recorded during autumn at Site 1 (Tiershoek) (which is our reference site) is probable due to the fact that the site is in the headwater of the river so not much runoff can occur there and secondly during autumn because there was no rainfall, hence no runoff from the catchment.

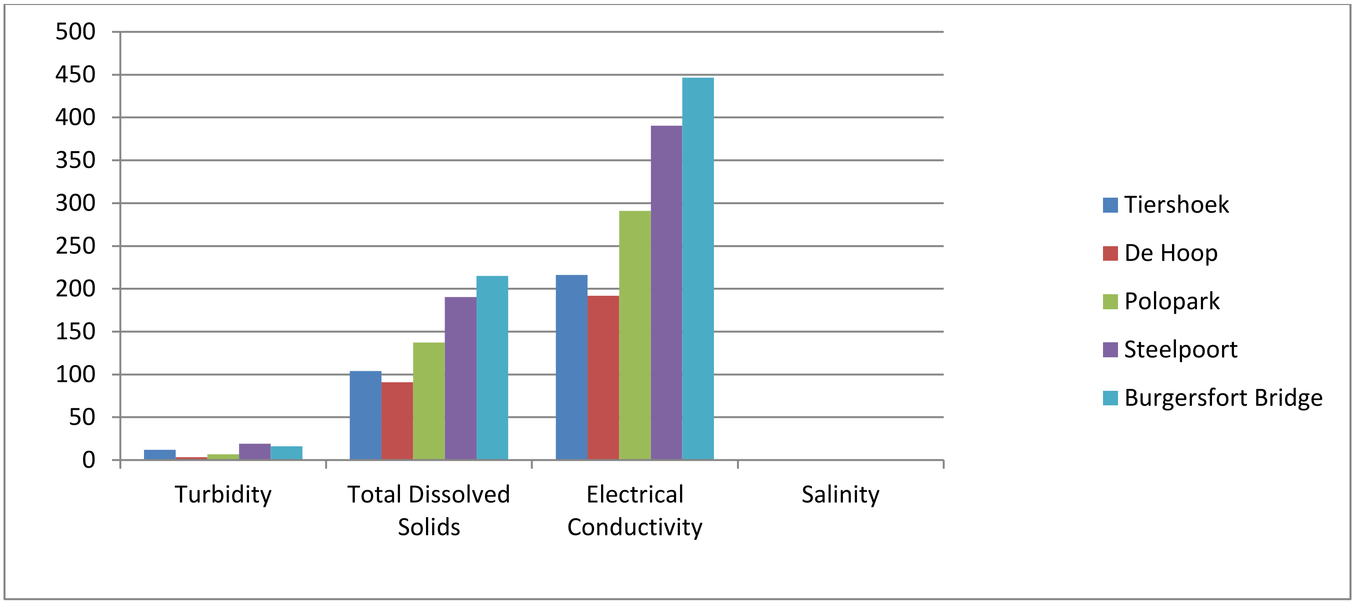

Figure 2. 2: The variation between sites for the mean turbidity, tds, ec and salinity at the Steelpoort River.

TOTAL DISSOLVED SOLIDS (TDS) AND ELECTRICAL CONDUCTIVITY (EC)

The measure of the quantity of all compounds dissolved in water is called the total dissolved solids (TDS) concentration. While the total dissolved salts (TDSalts) concentration is a measure of the amount of all dissolved compounds in water that carry an electrical charge. For the sites, the highest mean TDS value was 214.9 mg/l at Site 5 (Burgersfort Bridge) and the lowest was 90.8 mg/l at Site 2 (De Hoop) (Appendix A; Figure 2.2). Constituent inorganic salts govern the effects of the TDS. The buffering capacity of the water and the metabolism of organisms are affected by the proportional concentrations of the major ions. The lowest overall TDS values of 69.1 mg/L was recorded at Site 1 (Tiershoek) during the summer survey and the highest which was 279 mg/L was recorded at Site 5 (Burgersfort Bridge) during winter (Appendix A). The One-way ANOVA computed for this sample showed no significant variance for TDS between the sites ($p>0.05$). For the seasons, the highest mean TDS value was 182.2 mg/L during spring and the lowest was 91 mg/L during summer (Appendix A; Table 2.1). The low TDS readings in summer at Site 1 (Tiershoek) are expected since the site does not experience much anthropogenic disturbances and the water was dilute because of the rain. The high TDS values during winter at Site 5 (Burgersfort Bridge) could be due the fact that an accumulation of inorganic salts has occurred since the last wet season plus the site is further downstream so all those salts accumulated from upstream end up there.

Changes in the concentration of the total dissolved solids can affect aquatic organisms at three levels, namely: effects on microbial and ecological processes such as rates of metabolism and nutrient cycling; effects on adaptations of individual species; and effects on community structure. In systems where the organisms may not be adapted to fluctuating levels of TDS, the rate of change of the TDS concentration, and the duration of change, appears to be more important than absolute changes in the TDS concentration. Cyclical timing of the change in TDS concentration may also have significant synergistic effects with water temperature on the total community composition and functioning (Dwaf 1996d).

The measure of the ability of water to conduct an electrical current is referred to as electrical conductivity (EC) (Dwaf 2004). This ability is possible because of the presence of ions such as carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, potassium, calcium and magnesium in water, all of which carry an electrical charge (Dwaf 1996d). Therefore, the electrical conductivity (EC) is directly proportional to the TDSalts concentration of the water. EC is normally used as an approximation of the TDSalts concentration because it is much easier to measure than TDSalts. Hence, it has become common practice to use the total dissolved salts concentration, as a measure for the total dissolved solids (DWAF 1996). For the sites, the highest average EC concentration was 446.5 mS/m at Site 5 (Burgersfort Bridge) and the lowest was 191.7 mS/m at Site 2 (De Hoop) (Appendix A; Figure 2.2). The highest concentration for EC was 580 mS/m at Site 5 (Burgersfort Bridge) during spring and the lowest concentration was 149.4 mS/m at Site 1 (Tiershoek) during summer (Appendix A).Using One-way ANOVA, EC showed no significant variance between sites ($p>0.05$). For the seasons, the highest average EC concentration was 387.9 mS/m during spring and the lowest was 200.3 mS/m during summer (Appendix A; Table 2.1).

SALINITY

Salinity refers to the saltiness of water (Dallas & Day 2004) as it measures only the dissolved inorganic content. The geological characteristics, dissolution of salts from land surfaces, soil and aquifer material by the rising groundwater may be influenced in natural systems (Chapman 1996). Changes in the salt concentration of water can have adverse effects on aquatic biota, ecological and microbial processes such as rates of nutrient cycling and metabolism (Davies & Day 1998). For the sites, the highest mean value was 0.2 mg/l at Site 4 (Steelpoort) and Site 5 (Burgersfort Bridge) and the lowest was 0.1 mg/l at the rest of the sites (Appendix A; Figure 2.2). The highest value for salinity throughout the surveys and at all the sites was 0.270 mg/L at Site 5 (Burgersfort Bridge) during winter and the lowest being 0.070 mg/L at Site 1 (Tiershoek) during summer (Appendix A). Salinity showed no significant difference between sites when using One-way ANOVA ($p>0.05$). The highest mean value was 0.174 mg/L during spring and the lowest was 0.096 mg/L during summer (Appendix A; Table 2.1). Salinity is directly proportional to TDS and these salinity readings correlate exactly to the previous TDS readings. Organisms which are adapted to low-salinity habitats are mostly sensitive to changes in the TDS concentration (DWAF 1996d).

NUTRIENTS

NITRATE AND NITRITE

Nitrate (NO_{3-}) is the end product of nitrite (NO_{2-}) the inorganic intermediate, of the oxidation of nitrogen and ammonia. Nitrite and nitrate are usually measured and considered together, in view of their co-occurrence and rapid inter-conversion. Inter-conversions among the different forms of inorganic nitrogen are part of the nitrogen cycle in aquatic ecosystems. The inorganic nitrogen arising from human activities can greatly exceed "natural" sources, in highly impacted catchments. The transformation of nitrite to nitrate can occur rapidly, and vice versa, by bacterial processes. Under anaerobic conditions, nitrate is reduced to nitrite (and then to molecular nitrogen) by denitrifying bacteria. Conversely, under aerobic conditions, nitrite is oxidized to nitrate by nitrifying bacteria. Denitrification is an important process whereby nitrate is lost from aquatic systems. Water temperature, oxygen availability and pH regulate the processes of ammonification, nitrification, denitrification, and the active uptake of nitrate by algae and higher plants. Alterations to water temperature and pH influence the rates at which these processes occur and the concentration of inorganic nitrogen present in water (DWAF 1996d).

For the sites, the highest mean concentration for nitrate was 2.3 mg/l at Site 5 (Burgersfort Bridge) and 0.05 mg/l at Site 4 (Steelpoort) for nitrite. The lowest mean concentration for nitrate was 0.8 mg/l at Site 2 (De Hoop) and 0.028 mg/l at Site 3 (Polopark) for nitrite (Appendix A; Figure 2.3). The lowest concentration for nitrate was 0.42 mg/L at Site 2 (De Hoop) during winter and 0.012 mg/L at Site 2 (De Hoop) during autumn for nitrite (Appendix A). The highest concentration was 3.60 mg/L recorded at Site 5 (Burgersfort Bridge) during spring for nitrate and 0.097mg/L recorded at Site 1 (Tiershoek) during summer for nitrite (Appendix A). The One-way ANOVA showed no significant variance for nitrate ($p>0.05$) but showed a significant variance for nitrite ($p<0.05$) between sites. For the seasons, the highest mean nitrate and nitrite concentrations were 1.79 mg/L during autumn and 0.058 mg/L during summer, and their lowest concentrations were 0.79 mg/L during summer and 0.024 mg/L during winter, respectively (Appendix A; Table 2.1). The nitrate values were higher than those of nitrite because it is the more stable and is typically far more abundant in the aquatic environment (DWAF 1996d). The major sources of inorganic nitrogen which enters aquatic systems are surface runoff from the surrounding catchment area, the discharge of effluent streams containing human and animal excrement, agricultural fertilizers and organic industrial wastes (DWAF1996d). The high concentrations of nitrate during winter may have been caused by presence of faecal matter observed during the surveys and because of the lack of rain during winter, the concentrations of nitrate was increased.

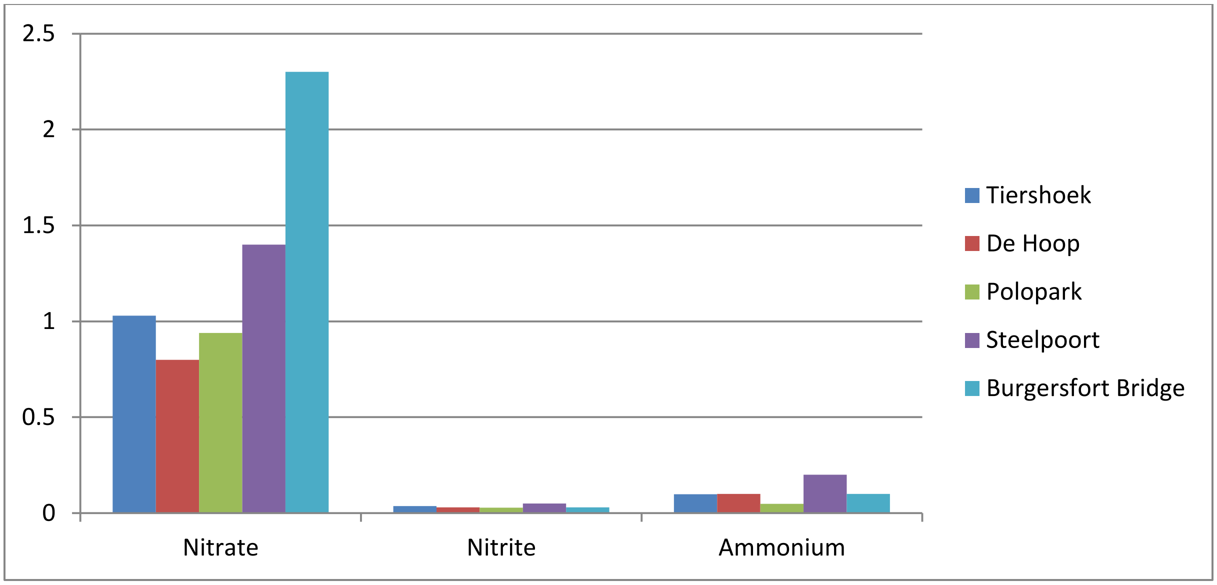

Figure 2. 3: The variation of the mean Nitrate, Nitrite, and Ammonium within sites at the Steelpoort River.

AMMONIA

Decomposition of nitrogen-containing organic matter produces ammonia, which is a vital nutrient for aquatic plants (use ammonia rapidly as a nitrogen source) but is known to be toxic to aquatic animals. It is also an energy source and nitrogen for bacteria. ammonia is converted readily to nitrite, nitrate and various organic forms of nitrogen in the presence of oxygen. Subsequently, in well-oxygenated surface water with a healthy microflora and warm temperatures, ammonia concentrations are usually low. The less toxic ionized form dominates in pH neutral or acidic water. Conversely, the relative abundance of the toxic un-ionized ammonia rises considerably with a higher pH (Soballe & Weiner 1998). In the sediment decomposing organic matter can be an important source of ammonia; and total ammonia concentrations between 1 and 10 ppm (mg/L; as nitrogen) are not uncommon in sediment pore waters (Frazier *et al.* 1996). The un-ionized fraction of high concentrations of total ammonia could have an adverse effect on burrowing organisms exposed to sediment pore water (Ankley *et al.* 1990), but the long-term impact from brief toxic episodes on the river's benthic fauna has not been evaluated adequately (DWAF 1996d).

The One-way ANOVA computed for ammonia showed no significant variance between sites ($p<0.05$). The highest ammonia concentration was 0.277 mg/L during summer at Site 1 (Tiershoek) and lowest at a concentration of 0.006 mg/L at Site 2 (De Hoop) during winter (Appendix A). The mean seasonal concentrations was highest at 0.136 mg/L during summer and lowest at 0.058 mg/L during winter (Appendix A; Table 2.1). The highest mean concentration for the sites was 0.2 mg/l at Site 4 (Steelpoort) and the lowest was 0.049 mg/l at Site 3 (Polopark) (Appendix A; Figure 2.3). When the ammonia levels in oxygenated river water are elevated, particularly during warm weathers, it suggests the existence of a nearby ammonia source, such as sewage discharge, untreated run-off, or nitrogen-enriched, organic sediments both un-ionized and ionized ammonia, relative abundance is controlled by pH and to a lesser extent water temperature (DWAF 1996d). In summer, photosynthesis by algae and aquatic plants can increase the pH of river water to 9 or greater (DWAF 1996d). The low concentrations of ammonia can be toxic, at such high pH. Looking at the pH values recorded for this survey, they are correlations between high pH and elevated ammonia levels.

TOTAL NITROGEN

For the sites, the highest mean concentration was 2.4 mg/l at Site 5 (Burgersfort Bridge) and the lowest was 0.78 mg/l at Site 3 (Polopark) (Appendix A; Figure 2.4). Total nitrogen was calculated to be at the highest concentration of 3.66 mg/l at Site 5 (Burgersfort Bridge) during autumn and lowest concentration of 0.05 mg/l at Site 1 (Tiershoek) during spring (Appendix A). The One-way ANOVA results for Total Nitrogen showed no significant difference between sites ($p<0.05$). For the seasons, the lowest mean concentration (0.73 mg/l) was recorded during spring while the highest at 1.92 mg/l was recorded for autumn (Appendix A; Table 2.1). The total nitrogen values were indicative of oligotrophic conditions in the Steelpoort River (DWAF 1996d).

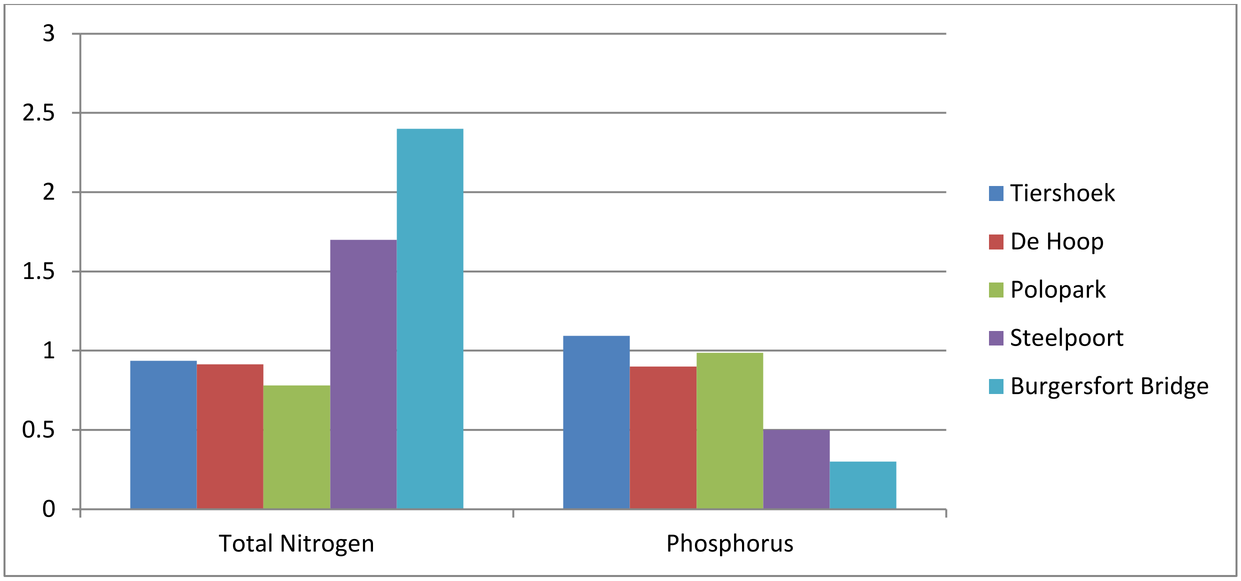

Figure 2. 4: The variations of the mean concentrations of Total Nitrogen and Phosphorus between sites in the Steelpoort River.

PHOSPHORUS

Phosphorus is seldom present in high concentrations in unimpacted surface waters because it is actively taken up by plants. Phosphorus may be present in waters as dissolved and particulate species, and can occur in numerous organic and inorganic forms. The phosphorus element does not occur in the natural environment. Found in natural waters are orthophosphates, polyphosphates, metaphosphates, pyrophosphates and organically bound phosphates. Of these, the only forms of soluble inorganic phosphorus directly utilizable by aquatic biota are orthophosphate species, H_2PO_4 and HPO_4^{-2}. In water, the forms of phosphorus are always changing because of processes of decomposition and synthesis between organically bound forms and oxidised inorganic forms. The exchange of phosphorus between sedimentary and aqueous compartments influences the phosphorus cycle. Factors such as mineral-water equilibria, water pH values, sorption processes, oxygen-dependent redox interactions, and the activities of living organisms are affected by various physical, chemical and biological modifying. Phosphorus is accumulated by a variety of living organisms, and is an essential macronutrient. It has a major role in the storage and use of energy and in cells in the building of nucleic acids. Point-source discharges such as domestic and industrial effluents, and from diffuse sources (non-point sources) in which the phosphorus load is generated by surface and subsurface drainage may result in elevated levels of phosphorus. The amount of inorganic phosphorus can be regulated by several chemical bonding processes which is bonded to iron, aluminium, calcium or organic polyphenols and adsorbed onto suspended particulate material. Under conditions of high flow and under anoxic conditions from both sediments and water adsorbed phosphorus may be released from the sediments. The pH of the water influences, the form of phosphorus in natural surface waters and the equilibrium of the different forms. Phosphorus was highest at an average of 1.09 mg/l at Site 1 (Tiershoek) and lowest at an average of 0.3 mg/l at Site 5 (Burgersfort Bridge) (Appendix A, Figure 2. 4). The highest recording was 2.98 mg/l at Site 1 (Tiershoek) during autumn and the lowest was 0 mg/l at all the sites except Site 4 (Steelpoort) mostly during winter and spring (Appendix A). For phosphorus, One-way ANOVA showed significant variance between sites ($p<0.05$). For the seasons, phosphorus was highest at an average of 2.13 mg/l during autumn and lowest at an average of 0.028 mg/l during winter (Appendix A, Table 2.1).

CATIONS

CALCIUM

Calcium is considered one of the most essential elements for living organisms because it is typically found as part of the structural material in teeth, shells, bones and exoskeletons (Dalesman & Lukowiak 2010). In most instances calcium irons are major cations in inland waters, where hard water has a high calcium concentration and soft waters have low calcium concentration (DWAF 1996d). For the sites, the highest mean concentration of Ca was 50.2 mg/l at Site 4 (Steelpoort) while the lowest was 26.3 mg/l at Site 1 (Tiershoek) (Appendix A; Figure 2.5). During autumn Ca had the highest value of 102 mg/l at Site 4 (Steelpoort) and the lowest during spring (19.8 mg/l) at Site 2 (De Hoop), which is below the dam wall (Appendix A). The results for One-way ANOVA of Calcium showed a significant difference between sites ($p<0.05$). For the seasons, the highest mean concentration for Ca was 63.6 mg/l during autumn and lowest mean concentration was 25.9 mg/l during spring (Appendix A, Table 2.1). All readings taken were below the BC-EPD guideline for calcium, since there is no DWAF guideline for calcium. Not much is known about the actual effects of changes in its concentration on aquatic biota despite the fact that it is a vital element. However, calcium is a divalent salt which can commonly be associated with water hardness (DWAF 1996e).

MAGNESIUM

Magnesium is a divalent salt and has been recognized as one of the most common sources for water hardness. It is a crucial element found in chlorophyll, in a variety of enzymes and is involved in processes such as, muscle contraction and the transmission of nerve impulses (Dallas & Day 2004). For the sites, the highest mean concentration for Mg was 26.8 mg/l at Site 5 (Burgersfort Bridge) while the lowest was 9.3 mg/l at Site 2 (De Hoop) (Appendix A; Figure 2.5). During this survey, the concentrations of magnesium recorded ranged from 8.3 mg/l at Site 2 (De Hoop) during spring to 36.5 mg/l at Site 5 (Burgersfort Bridge) during winter (Appendix A). The One-way ANOVA showed no significant variance between sites ($p>0.05$) for magnesium. For the seasons, the highest mean concentration was recorded as 19.5 mg/l during winter with the lowest being 13.6 mg/l during autumn (Appendix A; Table 2.1). In freshwaters, the range of magnesium is usually between 4 and 10 mg/l (DWAF 1996d), this implies that in the Steelpoort River the magnesium concentration during all the seasons were higher than the normal ranges. Even though magnesium was found at elevated levels it is unlikely to act as a toxin, but very little is known about its effect on aquatic organisms (DWAF 1996d, Dallas & Day 2004).

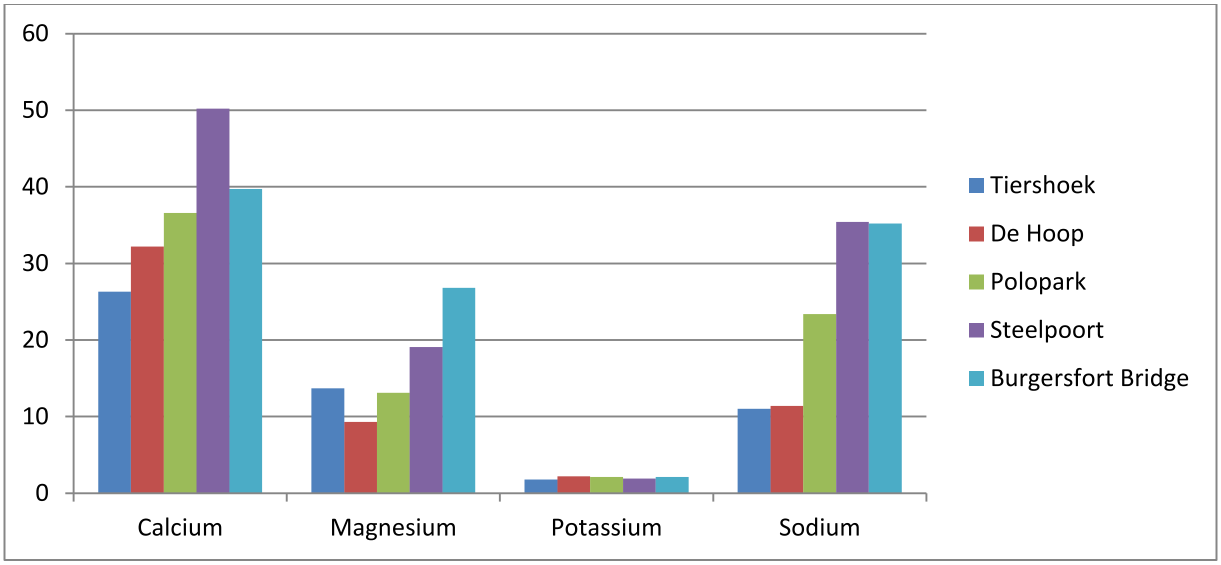

Figure 2. 5: The variation of the mean concentations of cations Calcium, Magneseium, Potassium and Sodium between sites in the Steelpoort River.

POTASSIUM

For the sites, the mean concentrations of potassium range from 1.8 mg/l at Site 1 (Tiershoek) to 2.2 mg/l at Site 2 (De Hoop) (Appendix A; Figure 2.5). Potassium was found to be at its least elevated concentration (< 1.0 mg/l) at Site 1 (Tiershoek) during summer and at its most elevated concentration (3 mg/l) at Site 5 (Burgersfort Bridge) during autumn (Appendix A). The One-way ANOVA computed for potassium showed significant variance between sites ($p<0.05$). For the seasons, the mean concentrations of potassium range from 1.6 mg/l during summer to 2.4 mg/l during autumn (Appendix A; Table 2.1). The toxic effects of potassium are not known because they are not available in SAWQG (DWAF 1996b). Potassium generally ranges from 2 to 5 mg/l in freshwater so in this study it was below the range during all seasons except winter. Potassium is responsible for ionic balance in all organisms, muscle contraction and the transmission of nerve impulses in animals. Potassium may operate as a limiting nutrient for animal communities and plants because it typically occurs in much lower concentrations than sodium, therefore it can occasionally act as a nutrient and the lack of it may limit plant growth (Hellgren *et al.* 2006).

SODIUM

Sodium is a major cation in many South African inland waters and is found everywhere in aquatic ecosystems (DWAF 1996b). Like potassium, it is responsible for ionic and osmotic water balance in all organisms, the transmission of nerve impulses and muscle contraction in animals (DWAF 1996c). For the sites, the highest mean concentration for sodium was 35.4 mg/l at Site 4 (Steelpoort) and the lowest was 11 mg/l at Site 1 (Tiershoek) (Appendix A; Figure 2.5). Sodium concentrations ranged from 8 mg/l at Site 2 (De Hoop) during summer and Site 1 (Tiershoek) during autumn to 69.4 mg/l at Site 5 (Burgersfort Bridge) during winter (Appendix A). The One-way ANOVA for sodium showed a significant variance between sites ($p<0.05$). For the seasons, the mean sodium concentration was highest at 41.1 mg/l during winter and lowest at 12.6 mg/l during autumn (Appendix A; Table 2.1). Sodium is most likely the least toxic cation and is relevant in the aquatic ecosystem because it is a major contributor to TDS (DWAF 1996d). There is no SAWQG available for sodium but CCME Water Quality Guidelines for Sodium is 200 mg/l (Ccme 2012a). All the Sodium values for this study were below the CCME value.

2.3.2 WATER QUALITY RESULTS

Table 2.2: Seasonal variations of the mean concentrations of metals and metalloids in the water column at the Steelpoort River.

Metals	Summer		Autumn		Winter		Spring		Water quality guidelines
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Aluminium	0.269	0.186	85	32	0.017	0.005	0.0005	0.001	0.001[1]; 0.1[3]
Iron	0.413	0.22	289	167	0.076	0.058	0.06	0.072	Fe vary < 10% background conc[1]; 0.3[3]
Titanium	-	-	41	26	0.022	0.005	0.036	0.007	No criteria
Barium	0.03	0.002	0	0	0.033	0.027	0.005	0.006	0.74
Manganese	0.068	0.038	3	1	0.007	0.005	0.003	0.004	0.18[1]; < 1.3[2]
Nickel	-	-			0.002	0.001	-	-	< 0.47[4]
Vanadium	-	-	2	1	0.015	0.018	0.006	0.001	No criteria
Chromium	-	-			0.001	0.001	-	-	Cr III: 0.012[1]; 0.0089[3]
Strontium	0.111	0.024	0	0	0.176	0.041	0.02	0.027	4.0[4]
Zinc	-	-	0	0	0.004	0.001	0.0014	0.002	0.002[1]; 0.04 - 0.115[2]; 0.03[3]; <0.12[4]

1. (DWAF 1996) - South African Water Quality guidelines: Volume 7: Aquatic Ecosystems.
2. BC-EPD (2006) – British Columbia Environmental Protection Division: Water Quality Guidelines
3. (CCME 2012) – Canadian Council of Ministers of the Environment: Water Quality Guidelines – Aquatic Life
4. (USEPA 2012) – United States Environmental Protection Agency: Water Quality Guidelines – Aquatic Life

ALUMINIUM

Aluminium is the third most abundant metal in the earth's crust. It is widely distributed and occurs naturally in soil, water, and air (Stahl *et al.* 2011). It is never found as the free metal in nature and is a very reactive element (Wauer *et al.* 2004). Most commonly it is found combined with other elements such as oxygen, silicon, and fluorine and these chemical compounds are commonly found in soil, rocks (especially igneous rocks), minerals (e.g., sapphires, rubies, turquoise), and clays. For the sites, the highest mean concentration for Al was 34.1 mg/l at Site 4 (Steelpoort) and the lowest concentration was 19.7 mg/l at Site 5 (Burgersfort Bridge) (Appendix A; Figure 2.6). Aluminium concentration was highest at 136 mg/l during autumn at Site 4 (Steelpoort) and the lowest concentration was recorded at 0.01 mg/l during spring at Site 2 (De Hoop) (Appendix A). The One-way ANOVA showed no significant variance between sites ($p>0.05$). For the seasons, the mean Al concentration was highest at 85.4 mg/l during autumn and lowest at 0.1 mg/l during spring (Appendix A; Table 2.2). The mean concentrations for Al were above the TWQRs for aquatic ecosystems (DWAF 1996a, CCME 2011). To a large extent most aluminium-containing compounds do not dissolve in water unless the water is acidic or very alkaline (ATSDR 2008a) and in this instance the water was a bit alkaline with pH ranging between 7.8 and 9.5.

IRON

Iron is often a major constituent of soils (especially clays), and is the fourth most abundant element by weight in the earth's crust (BC-EPD 2008). It is an absolute requirement for all forms of life. Its significance is particularly notable in biogeochemical processes because of its unique ability to serve as both an electron donor and acceptor and thus can play a vital role in metabolic processes of many organisms. In water, the solubility of iron varies with compounds and temperature (as well as pH and other physical factors). In freshwater systems, of primary biological significance are iron (III) hydroxide ($Fe(OH)^3$) with very low solubility, and in contrast, iron (II) hydroxide $Fe(OH)^2$, being relatively more soluble. The lowest mean concentration for the sites was 25.5 mg/l at Site 5 (Burgersfort Bridge) and the highest was 119.4 mg/l at Site 1 (Tiershoek) (Appendix A; Figure 2.6). Iron (mean) was above the recommended TWQRs during summer and autumn (CCME 2012b). The lowest overall value for Fe was 0.05 mg/l at Site 2 (De Hoop) during winter and highest at Site 1 (Tiershoek) during autumn at a value of 447 mg/l (Appendix A). The One-way ANOVA computed on Fe showed no significant variance between sites ($p>0.05$). Iron levels were detectable during all the seasons, the lowest mean value of 0.08 mg/l was taken during winter and the highest mean value of 289 mg/l was taken during autumn (Appendix A; Table 2.2). Furthermore, in the British Columbian Guidelines (BC-EPD 2008), for the protection of freshwater aquatic life, 0.35 mg/l is acceptable for dissolved iron and 1 mg/l for total iron.

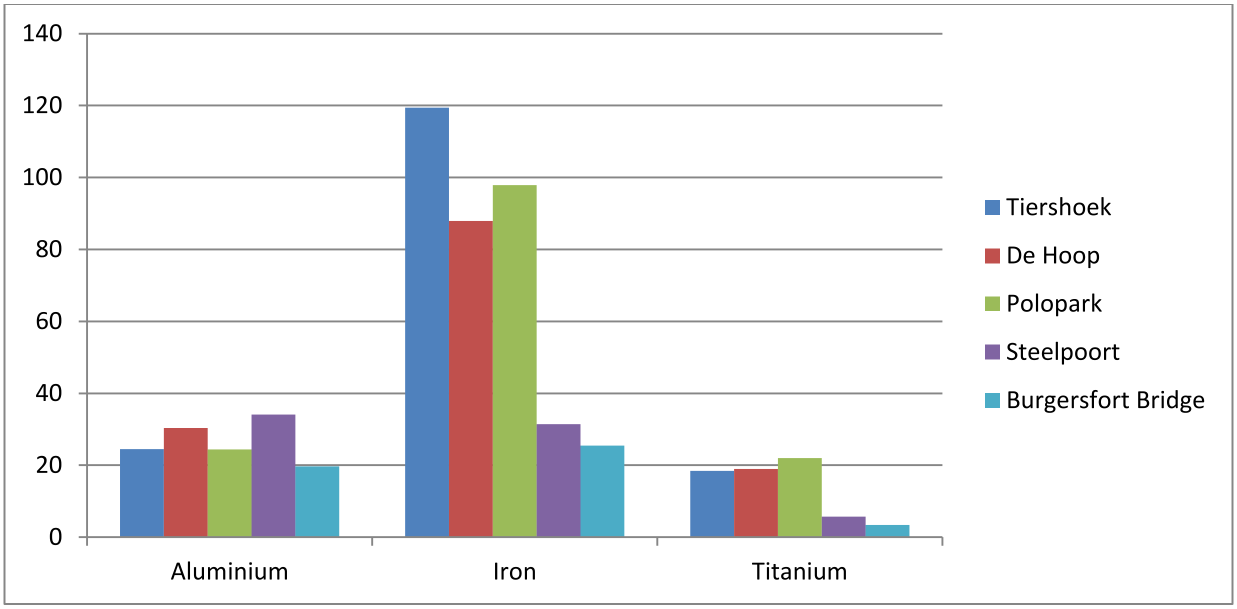

Figure 2. 6: The variation of the mean Aluminium, Iron and Titanium concentration between sites in the Steelpoort River.

TITANIUM

The highest mean concentration for the sites was 22 mg/l at Site 3 (Polopark) and the lowest mean concentration was 3.36 mg/l at Site 5 (Burgersfort Bridge) (Appendix A; Figure 2.6). The overall highest concentration was 66 mg/l at Site 3 (Polopark) during autumn while the lowest was 0.015 mg/l at Site 2 (De Hoop) during winter (Appendix A). The One-way ANOVA for Ti showed no significant variance between sites ($p>0.05$). For the seasons, the highest mean Ti was recorded at 41 mg/l during autumn while the lowest mean was recorded at 0.02 mg/l during winter (Appendix A, Table 2.3).

MANGANESE

Manganese was detected during three seasons (summer, winter and autumn). The lowest mean concentration for sites was 0.393 mg/l at Site 4 (Steelpoort) and the highest was 1.226 mg/l at Site 1 (Tiershoek) (Appendix A; Figure 2.7). The highest Mn concentration (3.6 mg/l) was recorded during autumn at Site 1 (Tiershoek) while the lowest concentration (0.002 mg/l) was taken at Site 4 (Steelpoort) and Site 5 (Burgersfort Bridge) during winter (Appendix A). The One-way ANOVA analysis showed no significant variance between sites ($p>0.05$) for Mn. For the seasons, the highest mean concentration was observed during autumn at 2.6 mg/l while the lowest mean concentration was observed during winter at 0.01 mg/l (Appendix A; Table 2.2). During autumn Mn was above the TWQR at a concentration of 2.6 mg/l ((DWAF 1996a, BC-EPD 2008). Manganese is found in many types of rocks and soil and is a naturally occurring substance. It is found combined with other substances such as oxygen, sulphur, and chlorine. Pure manganese is a silver-coloured metal; yet, it does not occur in the environment as a pure metal (Howe *et al.* 2004).

VANADIUM

Vanadium is a natural element in the earth which is white to grey in colour and is often found as crystals. It is usually combined with other elements such as oxygen, sodium, sulphur, or chloride, in the environment. Trace levels of vanadium are detectable in most living organisms because it is ubiquitous in the biosphere. No criteria are available for the concentrations of vanadium, so the readings were not compared to any of the guidelines used. The highest mean concentration for the sites was 1.256 mg/l at Site 1 (Tiershoek) and the lowest concentration was 0.182 mg/l at Site 5 (Burgersfort Bridge) (Appendix A; Figure 2.7). For the seasons, the lowest mean concentration was 0.02 mg/l recorded during the winter survey while the highest mean concentration was 1.7 mg/l recorded during the autumn survey (Appendix A; Table 2.2). The One-way ANOVA showed no significant variance between site ($p>0.05$). The lowest V concentration was 0.0018 mg/l at Site 3 (Polopark) during winter while highest concentration was 2.51 mg/l at Site 1 (Tiershoek) during autumn (Appendix A).

COBALT

Elemental cobalt is a hard, silvery grey metal which is a naturally-occurring element that has properties similar to those of iron and nickel with an atomic number of 27 (ATSDR 2004a). Small amounts of cobalt are naturally found in most plants, animals, rocks, soil and water. Nonetheless, cobalt is regularly found in the environment combined with other elements such as oxygen, sulphur, and arsenic. Cobalt is even found in dissolved or ionic form, in water, usually in small amounts (WHO 2006). Vitamin B12 or cyanocobalamin is a biochemically key cobalt compound and is essential for good health in animals and humans (ATSDR 2004a). When comparing all the sites, Site 1

(Tiershoek) had the highest mean concentration of 0.121 mg/l and Site 5 (Burgersfort Bridge) had the lowest mean concentration of 0.017 mg/l (Appendix; Figure 2.7). The highest Co concentration was 0.241 mg/l at Site 1 (Tiershoek) during autumn while the lowest was 0 mg/l all throughout winter (Appendix A).The One-way ANOVA computed for Co showed no significant variance ($p>0.05$) between sites. For Co, autumn was the only season it was detected (Appendix A; Table 2.2).

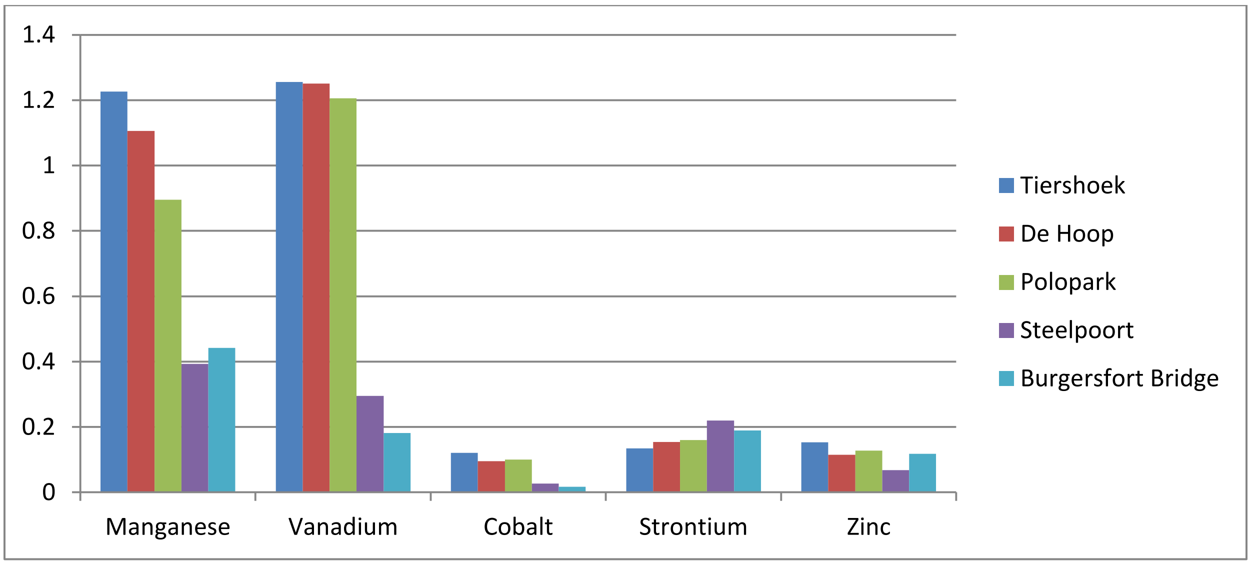

Figure 2. 7: The variation between sites of the mean Manganese, Vanadium, Cobalt, Strontium and Zinc concentrations in the Steelpoort River.

STRONTIUM

Strontium has physical and chemical properties similar to those of calcium and is a soft, silvery metal which is a fairly common alkaline earth metal (ATSDR 2004c). It is present in small quantities in most plant tissues although it has not been shown to be vital for their growth & development. Strontium is a significant freshwater quality ion which contributes to water "hardness", in localities where it is elevated. The lowest mean concentration for the sites was 0.135 mg/l at Site 1 (Tiershoek) and the highest concentration was 0.220 mg/l at Site 4 (Steelpoort) (Appendix A; Figure 2.7). Strontium was highest during autumn at Site 4 (Steelpoort) with a value of 0.4 mg/l and lowest during winter at Site 2 (De Hoop) during summer at a value of 0.085 mg/l (Appendix A).The One-way ANOVA showed no significant variance between sites ($p>0.05$) for Sr. The mean highest concentration was 0.3 mg/l during autumn and lowest at 0.11 mg/l during summer (Appendix A; Table 2.2).

ZINC

Zinc is a metallic element, an essential micronutrient for all organisms as it forms the active site in various metalloenzymes. Two zinc oxidation states occur in aquatic ecosystems, namely as a metal, and as zinc(II) (DWAF 1996d). Zinc (II) ion is toxic at relatively low concentrations to fish and other aquatic organisms. A variety of interactions affect the toxicity of zinc in aquatic ecosystems (Eisler 1993). The site with highest mean concentration of 0.153 mg/l was at Site 1 (Tiershoek) and the lowest mean concentration of 0.068 mg/l was at Site 4 (Steelpoort) (Appendix A; Figure 2.7). The lowest Zn concentration of 0.002 mg/l was taken at Site 3 (Polopark) during winter while the highest concentration of 0.425 mg/l was taken at Site 1 (Tiershoek) during autumn (Appendix A). The One-way ANOVA showed no significant variance ($p>0.05$) between sites. For seasonal variation Zn was above detection level during all seasons except summer. For the seasons, the highest mean concentration was 0.317 mg/l during autumn and the lowest was 0.001 mg/l during spring (Appendix A; Table 2.2).

BARIUM

The lowest mean concentration for the sites was 0.057 mg/l at Site 5 (Burgersfort Bridge) and the highest mean concentration was 0.082 mg/l at Site 1 (Tiershoek) (Appendix A; Figure 2.8). The highest Ba concentration was recorded as 0.186 mg/l at Site 2 (De Hoop) during autumn and the lowest concentration as 0.018 mg/l at Site 2 (De Hoop) during winter (Appendix A). When using One-way ANOVA no significant variance ($p>0.05$) was found between sites for Ba. For the seasons, the highest mean Ba concentration (0.2 mg/l) was recorded during autumn, the lowest being 0.03 mg/l during winter (Appendix A; Table 2.2). The Ba recordings were all below the TWQRs, hence they are no calls for concern when it comes to this metal. It is occasionally found naturally in drinking water and food. The amount usually found in drinking water is small because certain barium compounds (barium sulphate and barium carbonate) do not mix well with water and can persist for a long time in the environment. The form of barium released determines the length of time that barium will last in air, land, water, or sediments following release of barium into these media (ATSDR 2007a).

CHROMIUM

For the sites, the lowest mean Cr concentration was 0.069 mg/l at Site 1 (Tiershoek) and the highest mean concentration was 0.147 mg/l at Site 4 (Steelpoort) (Appendix A; Figure 2.8). The One-way ANOVA computed for Cr showed no significant variance between sites ($p>0.05$). Chromium was only above detection level during autumn and winter (Appendix A). For the seasons, the highest mean concentration was 0.23 mg/l during autumn and the lowest was 0.001 mg/l during winter (Appendix A; Table 2.2). The toxicity of chromium to aquatic biota is notably influenced by abiotic variables such as hardness, temperature, pH, and salinity of water; and biological factors such as species, life stage, and potential differences in sensitivities of local populations (Eisler 1986, Dallas & Day 2004). Hydrolysis and precipitation are the most imperative processes that determine the outcome and effects of chromium, whereas adsorption and bioaccumulation are relatively minor, in both freshwater and marine environments (Irwin *et al.* 1997b). Cr^{+6} is the dominant dissolved stable Cr species under oxygenated conditions in aquatic systems. The hexavalent form exists as a component of a complex anion that varies with pH and may take the form of chromate (CrO_4^{-2}), hydrochromate ($HCrO_4^{-1}$), or dichromate ($Cr_2O_7^{-2}$). These ionic Cr^{+6} forms are mobile in the aquatic environment because they are highly soluble in water (Eisler 1986).

BORON

Boron is found in the earth's crust and is a widely occurring element in minerals. Through the natural weathering of soil and rocks it can be released into air, water or soil. Boron cannot be destroyed in the environment instead it can only change its form or become attached or separated from particles in soil, sediment, and water. It is extensively circulated in ground and surface water. The average concentration is about 0.1 mg/L in surface water. Minute amounts of boron can be released into the environment from glass manufacturing plants, coal-burning power plants, copper smelters and agricultural fertilizer and pesticide usage (ATSDR 2010). The lowest mean for the sites was 0.003 mg/l at Site 1 (Tiershoek) while the highest mean was 0.022 mg/l at Site 4 (Steelpoort) and Site 5 (Burgersfort Bridge) (Appendix A; Figure 2.8). The lowest concentration was 0 mg/l at Site 1 (Tiershoek) during winter while the highest was 0.03 mg/l at Site 4 (Steelpoort) during spring (Appendix A). One-way ANOVA showed no significant variance between sites ($p>0.05$). Boron was only detectable during winter and spring. For the seasons, the highest mean was 0.008 mg/l during spring and the lowest was 0.007 mg/l during winter (Appendix A; Table 2.2). It is a crucial trace element for the development and growth of higher plants, even though the range between insufficiency and excess is normally narrow, varying with the plant; boron is not required in fungi and animals (Eisler 1990).

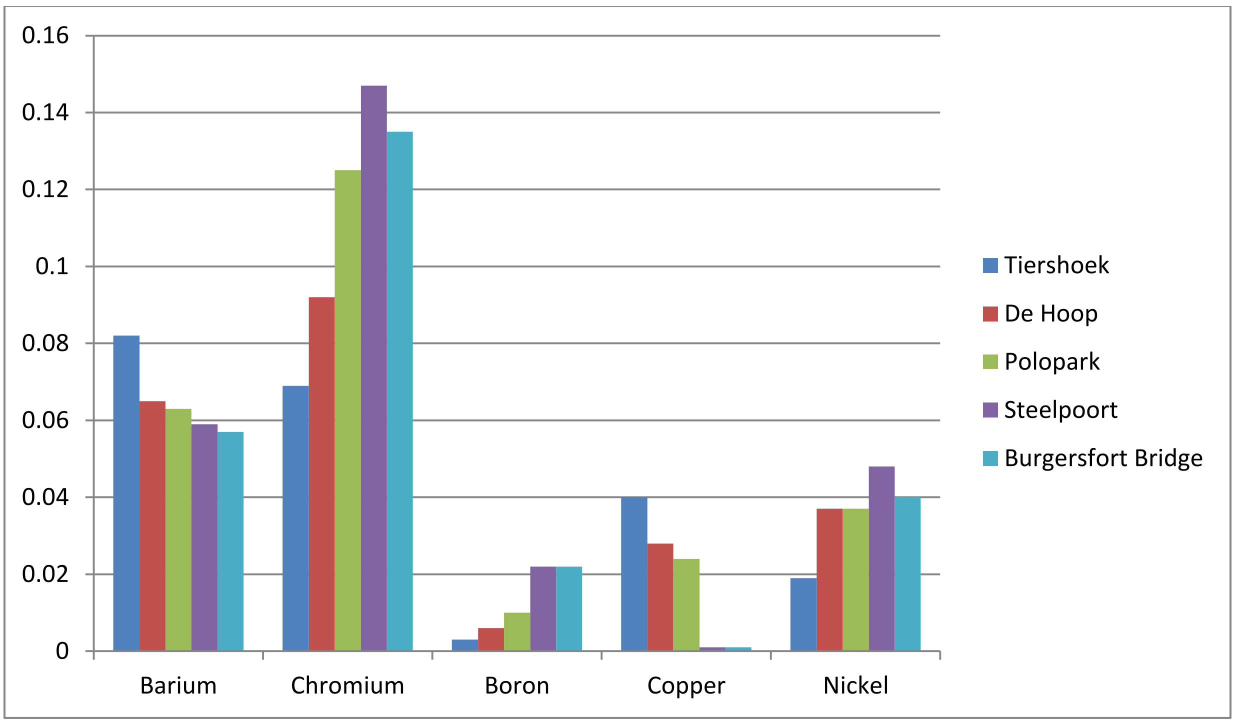

Figure 2. 8: The variation between sites in the Steelpoort of the mean Barium, Chromium, Boron, Copper and Nickel concentrations.

COPPER

Copper is one of the world's most widely used metals. It is regarded as potentially hazardous by the USEPA, even though it occurs naturally in most waters. Copper occurs in four oxidation states, specifically, 0, I, II and III (US-EPA, 2000). Copper is commonly found as an impurity in mineral ores, and is a common metallic element in the rocks and minerals of the earth's crust. Copper is an essential component of enzymes involved in redox reactions, is a micronutrient and is rapidly accumulated by plants and animals. It can be toxic even at low concentrations in water and is known to cause brain damage in mammals (ATSDR 2004b). The highest mean Cu concentration for the sites was 0.040 mg/l at Site 1 (Tiershoek) and the lowest mean concentration was 0.001 mg/l at Site 4 (Steelpoort) and Site 5 (Burgersfort Bridge) (Appendix A; Figure 2.8). The overall highest concentration was 0.079 mg/l during autumn and the lowest was 0.001 mg/l at all the sites during winter (Appendix A). The One-way ANOVA for Cu showed no significant variance between sites ($p>0.05$). Cu was only detectable during two seasons, winter and autumn. For the seasons, the highest mean concentration was 0.06 mg/l during autumn and the lowest was 0.001 mg/l during winter (Appendix A; Table 2.2).

NICKEL

Nickel is a hard, silvery metal greatly used in industrial purposes which is also abundant in the earth's crust (Irwin *et al.* 1997f). Mixtures called alloys can be formed from nickel because it has properties that make it very desirable for combining with other metals. Iron, copper, chromium, and zinc are some of the metals that nickel is alloyed with. In this study, nickel was only detectable during winter and autumn. It was below the TWQRs in both seasons (US-EPA, 2012a). The highest mean Ni concentration for the sites was 0.048 mg/l at Site 4 (Steelpoort) and the lowest mean concentration was 0.019 mg/l at Site 1 (Tiershoek) (Appendix A; Figure 2.8). The highest concentration of 0.094 mg/l was recorded at Site 4 (Steelpoort) during autumn while lowest concentration of 0.002 mg/l was recorded at all the sites except Site 1 (Tiershoek) during winter (Appendix A). The One-way ANOVA computed for Ni showed no significant variance between sites ($p>0.05$). For the seasons, the highest mean Ni concentration was 0.07 mg/l during autumn and the lowest was 0.002 mg/l during winter (Appendix A; Table 2.2).

2.3.3 SEDIMENT RESULTS

Table 2.3: Seasonal variations for the mean concentrations of metals and metalloids in the sediment at the Steelpoort River.

Metals	Summer		Autumn		Winter		Spring		Water quality guidelines
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Aluminium	41789.4	26813.2	39120	21577	34160	12846	33520	6115	No guidelines
Iron	163323	137094	131920	117222	115600	66883	121200	48073	No guidelines
Titanium	32765	24456.3	12560	9354	16400	10225	13760	5296	No guidelines
Manganese	798.5	460.7	1061	608	1045	455	973	361	No guidelines
Vanadium	682.7	602.1	731	931	694.2	406	544	272	No guidelines
Cobalt	43	30.64	43	34.92	57.2	37.56	40.2	19	No guidelines
Strontium	93.3	57.4	90	48.64	100.8	31.46	87.2	19	No guidelines
Zinc	117.2	85.4	106	92	127	36.93	113.2	54	123mg/kg
Barium	62.3	15.87	60	21.45	66.4	8.08	30.74	27	No guidelines
Chromium	99.8	34.37	117	65.59	90.6	25.48	112.8	50	37.3mg/kg
Boron	9	21.42	-	-	-	-	24.5	4.95	No guidelines
Copper	44.4	26.56	32	20.66	24.3	6.81	38.6	25	35.7 mg/kg
Nickel	1158.1	941.8	828	684	28.2	8.76	1095.4	436	No guidelines

(CCME 2012) - Canadian Council of Ministers of the Environment: Water Quality Guideline- Aquatic life.

ALUMINIUM

No guidelines for sediment are available for aluminium. The lowest mean value for the sites was 25452.6 mg/kg at Site 1 (Tiershoek) and the highest mean value was 60163.0 mg/kg at Site 4 (Steelpoort) (Appendix A; Figure 2.9). The overall highest Al value was 84252.1 mg/kg recorded at Site 4 (Steelpoort) during summer and the lowest value of 19600 mg/kg was recorded at Site 1 (Tiershoek) during winter (Appendix A). Using the One-way ANOVA, no significant variance ($p>0.05$) was detected between sites. For the seasons, the highest mean of Al was 41789.4 mg/kg and recorded during summer and the lowest was 33520 mg/kg during spring (Appendix A; Table 2.3). The concentrations of Al in the sediment at such high levels during summer can be attributed to the fact that it is the third most abundant element in the earth's crust (DWAF 1996d). Nonetheless, Al is among the most toxic trace metals that are physiologically non-essential. Aluminium compounds are used in several diverse and significant industrial applications such as alums (aluminium sulphate) in water-treatment and alumina in abrasives and furnace linings. The mining and processing of aluminium ores or the production of aluminium metal, alloys, and compounds can cause high levels in the environment (Rosseland *et al.* 1990), this could be the reason behind the elevated aluminium level. Also, coal-fired power plants and incinerators release small amounts of aluminium into the environment.

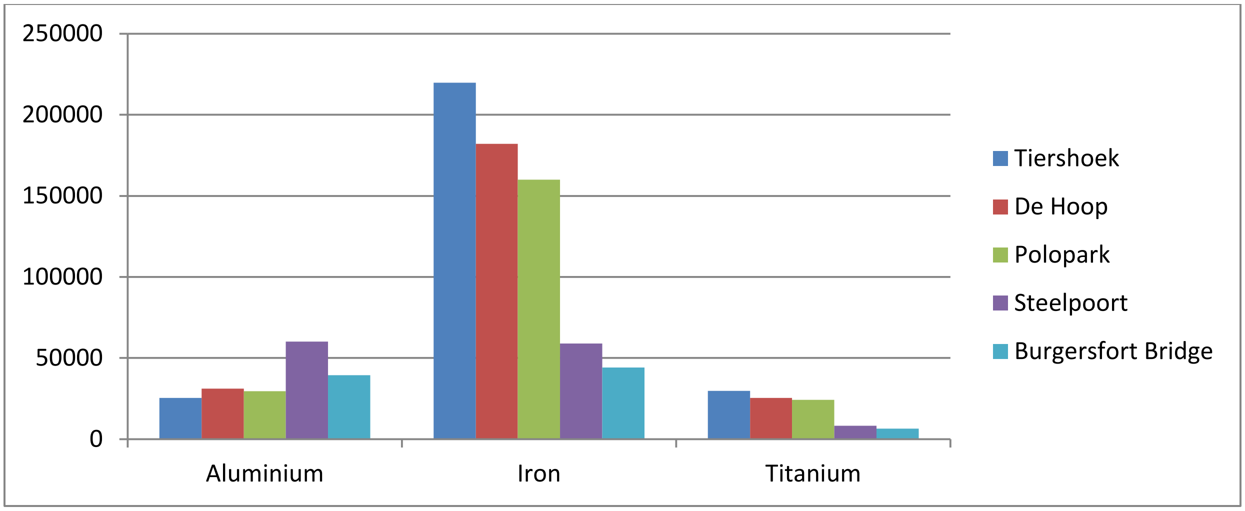

Figure 2.9: The mean variations of Aluminium, Iron and Titanium concentrations in the Sediment at the Steelpoort River.

IRON

The Fe concentrations in the sediment were very elevated as compared to the other metals. The highest mean concentration for the sites was 219719.7 mg/kg at Site 1 (Tiershoek) and the lowest concentration was 44255.1 mg/kg at Site 5 (Burgersfort Bridge) (Appendix A; Figure 2.9). The highest overall Fe concentration was 313639.9 mg/kg at Site 3 (Polopark) in summer and the lowest overall Fe concentration was 27200 mg/kg at Site 4 (Steelpoort) during autumn (Appendix A). The One-way ANOVA for Fe showed no significant variance between sites ($p>0.05$). The highest mean concentration was 163323.4 mg/kg during summer, the lowest mean concentration value being 115600 mg/kg in winter (Appendix A; Table 2.3). The extremely elevated iron levels may be attributed to the Ferrochome mines in the area. In surface water, the anthropogenic sources of iron are often related to mining activities. Iron pyrites (FeS^2) are exposed to weathering and bacterial action by mining which is common in coal seams.

Ferrous iron exists only between pH 4.0 and 5.0 in low oxygen conditions and is generally chemically unstable in water (BC-EPD 2008). The relationship between the relative proportions of the bioavailable and bioactive ferrous (Fe^{2+}) (II) iron and the almost insoluble ferric Fe^{3+} iron vary with a wide range of factors including pH, dissolved oxygen, dissolved and total organic carbon (DOC/TOC) ratio, colour, humic and other organic acids, exposure to sunlight and chloride concentration (WHO 2003). Ferrous iron oxidizes to its ferric (Fe^{3+}) forms (e.g. ferric hydroxide, $Fe(OH)_3$) as pH and the partial pressure of oxygen (pO_2) increase. It is insoluble in these forms and may precipitate from solution, sometimes producing a thick sludge on the bottom of streams (BC-EPD- 2008).

TITANIUM

For the sites, the lowest mean value was 6561.2 mg/kg at Site 5 (Burgersfort Bridge) and highest mean concentration was 29742.6 mg/kg at Site 1 (Tiershoek) (Appendix A, Table 2.4). The highest concentration value (59370.2 mg/kg) was recorded at Site 1 (Tiershoek) during summer and the lowest concentration (4000 mg/kg) was recorded at Site 4 (Steelpoort) during autumn (Appendix A). The One-way ANOVA showed no significant variance between sites ($p>0.05$). For the seasons, titanium was highest during summer at a mean concentration of 32765 mg/kg and lowest during autumn at mean concentration of 12560 mg/kg (Appendix A; Table 2.3).

ANTIMONY

Antimony is a silvery white metal of medium hardness that breaks easily. It is found in the earth's crust in small amounts. Antimony ores are mined and then either combined with oxygen to form antimony oxide or changed into antimony metal. In order to make it stronger, other metals such as lead and zinc are mixed with a little antimony to form mixtures of metals called alloys. Its entry into the environment is through mining and processing of its ores and in the production of antimony metals, alloys, antimony oxide, and combinations of antimony with other substances (ATSDR 1992). For the sites, antimony was highest with a mean of 13.5 mg/kg at Site 2 (De Hoop) and was lowest with a mean of 1.5 mg/kg at Site 5 (Burgersfort Bridge) (Appendix A; Figure 2.10). The highest recording was 26 mg/kg during winter at Site 1 (Tiershoek) and Site 2 (De Hoop) while the lowest was 0.7 mg/kg during summer at Site 2 (De Hoop) (Appendix A). The One-way ANOVA computed for Sb showed no significant variance between sites ($p>0.05$). Antimony was only detectable during summer and winter. For the seasons, antimony was highest at 21.5 mg/kg during winter and lowest at 2.0 mg/kg during summer (Appendix A; Table 2.3).

Antimony is found at such low levels in the environment to an extent that it is sometimes difficult to measure it. Antimony that is dissolved in rivers and lakes is at very low concentration, usually less than 5 parts of antimony in 1 billion parts of water (ppb). There is no evidence that supports the accumulation of antimony in fish and other aquatic animals. Antimony is transported into streams and waterways in runoff either due to natural weathering or disturbed soil because it is a natural constituent of soil (ATSDR 1992).

BARIUM

Barium is a silvery-white metal that takes on a silver-yellow colour when exposed to air. In nature it occurs in many different forms of compounds (ATSDR 2007a). These compounds do not burn well and are solids, existing as powders or crystals. The Barium concentrations ranged from 15mg/kg at Site 5 (Burgersfort Bridge) to 96 mg/kg at Site 3 (Polopark) (Appendix A). The lowest mean value was 40.9 mg/kg at Site 5 (Burgersfort Bridge) and the highest mean value was 66.3 mg/kg at Site 1 (Tiershoek) (Appendix A; Figure 2.10). One-way ANOVA for Ba showed no significant variance ($p>0.05$) between sites. For the seasons, a high mean Ba concentration value of 66.4 mg/kg was recorded during winter and a lower concentration value of 30.7 mg/kg during spring (Appendix A, Table 2.3). Barium sulphate and barium carbonate, are the two forms of barium often found in nature as underground ore deposits (Choudhury & Cary 2001).

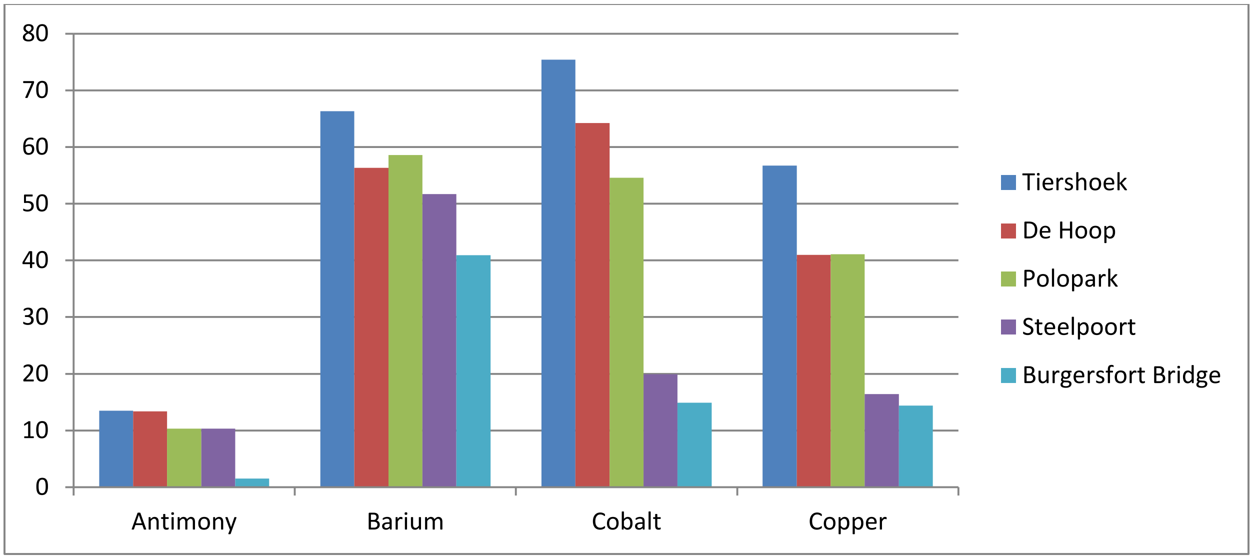

Figure 2.10: The mean variation of Antimony, Barium, Cobalt and Copper concentrations in the sediment at the Steelpoort River.

COBALT

Concentrations of Cobalt were only detected in the sediment and not the water samples and has no sediment guidelines. For the sites, the highest mean concentration for Co was 75.4 mg/kg at Site 1 (Tiershoek) and the lowest mean concentration was 14.9 mg/kg at Site 5 (Burgersfort Bridge) (Appendix A; Figure 2.10). The highest concentration value of 96 mg/kg was recorded at Site 1 (Tiershoek) during winter while the lowest concentration value (10 mg/kg) was recorded at Site 4 (Steelpoort) during autumn (Appendix A). One-way ANOVA showed a significant variance between sites ($p<0.05$). For the seasons, the lowest mean concentration (40.2 mg/kg) was recorded during spring and the highest mean concentration (57.2 mg/kg) was recorded during winter (Appendix A; Table 2.3). The Natural sources and human activities may both provide entry for cobalt into the environment. It may get into surface water from runoff and leaching when rainwater washes through soil and rock containing cobalt and may additionally enter air and water, and settle on land from windblown dust, seawater spray, volcanic eruptions, and forest fires (ATSDR 2004a, WHO 2006). In the environment cobalt cannot be destroyed but it can only change its form or become attached or separated from particles. Cobalt released into water may remain in the water column in ionic form or stick to particles in the water column, or to the sediment at the bottom of the body of water into which it was released. Many factors such as the chemistry of the water and sediment at a site as well as the cobalt concentration and water flow will determine the specific fate of the cobalt (ATSDR 2004a).

COPPER

The highest mean concentration of the sites was 56.7 mg/kg at Site 1 (Tiershoek) and the lowest mean concentration was 14.4 mg/kg at Site 5 (Burgersfort Bridge) (Appendix A; Figure 2.10). The Cu concentrations detected in the sediment were highest with a value of 76.7 mg/kg at Site 1 (Tiershoek) during summer and lowest with a value of <10 mg/kg at Site 4 (Steelpoort) and 5 (Burgersfort Bridge) during winter (Appendix A). The One-way ANOVA results for Cu showed no significant variance ($p>0.05$) between sites. For the seasons, the highest mean value of 44.4 mg/kg was recorded during summer and the lowest mean value of 24.3 mg/kg being recorded during winter (Appendix A; Table 2.3). According to the CCME sediment quality guidelines, Cu levels were above the standard level of 35.7 mg/l during summer and spring. At alkaline pH copper is easily adsorbed and precipitated in sediments which was the general pH in this study (DWAF 1996d). In natural waters only less than 1% of total copper exists in the free ionic form. Most of the soluble copper is present as complexes of cupric carbonate at pH levels and inorganic carbon concentrations characteristic of natural fresh waters. Therefore, adsorption and precipitation are essential in determining the abiotic fate of copper in the aquatic environment (Eisler 1998a). Copper toxicity is reliant on local water quality conditions. For example copper toxicity increases: when water hardness and dissolved oxygen decrease; and when it is combined with other metals (Irwin *et al.* 1997d). The toxicity decreases with an increase in alkalinity and in the presence of zinc, molybdenum, sulphate, calcium or magnesium (DWAF 1996d).

CADMIUM

Cadmium is a somewhat rare, soft, silver-white, transition element metal closely related to zinc. Only two oxidation states are possible (0 and +2) in nature, and the zero or metallic state is rare. Cadmium has no recognized necessary biological function (Eisler 1985, ATSDR 2008b). Though, it has been detected in more than 1000 species of aquatic and terrestrial flora and fauna. Entry of cadmium into the environment is via three routes: refining and use of cadmium, copper and nickel smelting, and fuel combustion (Irwin *et al.* 1997a). Cadmium was recorded only during summer with a mean concentration of 2 mg/kg (Appendix A, Table 2.3). The highest concentration value recorded was 4.7 mg/kg at Site 3 (Polopark) and the lowest was 0 mg/kg at Site 2 (De Hoop) just below the dam wall (Appendix A). The mean Cd concentration recorded was above the CCME sediment quality level (0.6 mg/kg) (CCME 2012b).

It has been documented that contamination of the environment by cadmium is especially severe in the localities of smelters and urban industrialized areas (Eisler 1985). Non-ferrous metal mining and refining, manufacture and application of phosphate fertilizers, fossil fuel combustion, waste incineration and disposal emit cadmium into soil, water and air. The high Cd concentration at Site 3 could be due to the mining activities close by the site. Accumulation of cadmium can be observed in aquatic organisms and agricultural crops. The form in which cadmium exists is as the hydrated ion or as ionic complexes with other inorganic or organic substances. Wherein soluble forms migrate in water and insoluble forms of are immobile and will deposit and absorb to sediments (ATSDR 2008b).

LEAD

Elemental lead is a bluish-grey, soft metal. In hard, basic waters metallic lead is sparingly soluble to 30 ug/l, and up to 500 ug/l in soft, acidic waters. Four lead stable isotopes occur in the environment: Pb-204 (1.5%), Pb- 206 (23.6%), Pb-207 (22.6%), and Pb-208 (52.3%) (Irwin *et al.* 1997e). Lead has an average abundance in the earth's crust of 16 mg/kg and is a comparatively rare metal. It is a key component of more than 200 recognized minerals and only three are adequately abundant to form mineral deposits, which are; galena (PbS), angelesite ($PbSO_4$), and cerusite ($PbCO_3$). Lead is everywhere and is a distinctive trace constituent in rocks, soils, water, plants, animals, and air (Eisler 1988). The Pb concentrations were only detectable during summer and spring with the highest concentration of 8.25 mg/kg at Site 1 (Tiershoek) in spring and the lowest concentration of 2.6 mg/kg at Site 2 (De Hoop) during summer (Appendix A). The lowest mean concentration for the sites was 3.3 mg/kg at Site 5 (Burgersfort

Bridge) and the highest mean concentration was 5.6 mg/kg at Site 1 (Tiershoek) (Appendix A; Figure 2.11). The One-way ANOVA showed no significant variance between sites for Pb ($p>0.05$). The mean concentration was higher in spring (6 mg/kg) than in summer (1.6 mg/kg) (Appendix A; Table 2.3). All the concentrations were below the sediment quality guidelines (CCME 2012b).

The wastes of Lead mining activities have severely reduced or eliminated populations of fish and aquatic invertebrates, either indirectly through toxicity to prey species or directly through lethal toxicity (Eisler 1988). In most of its chemical forms Lead is toxic and can be incorporated into the body by inhalation, ingestion, dermal absorption, and placental transfer to the foetus. Effects of lead are modified significantly by various biological and abiotic variables because it is to all phyla of aquatic biota (ATSDR 2007b).

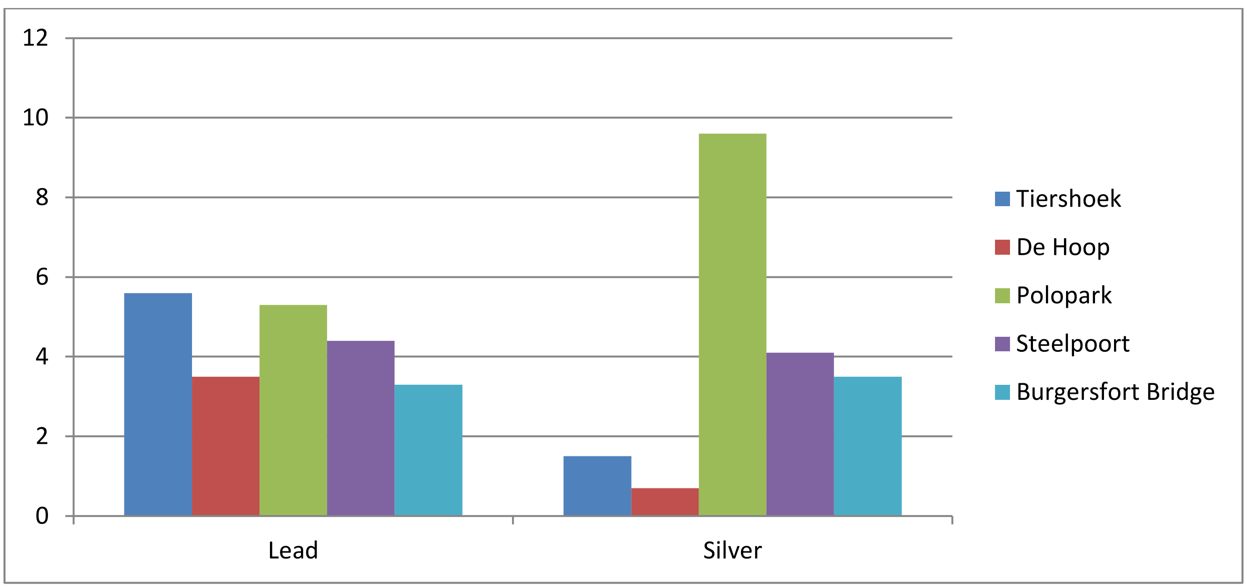

Figure 2. 11: The mean variations of Lead and Silver concentrations in the sediment at the Steelpoort River.

SILVER

Silver occurs naturally in pure form or in ores and is a white, ductile metal. It has the highest thermal and electrical conductivity of all metals. There are some compounds of silver that are extremely photosensitive but are stable in water and air except for tarnishing readily when exposed to sulphur compounds. For many organisms silver is a normal trace constituent. In natural environments, silver is intimately associated with other metal sulphides such as lead, copper, iron and gold, or occurs primarily in the form of sulphide, for example, it readily forms compounds with antimony, arsenic, selenium, and tellurium (Irwin 1997). The lowest mean concentration for the sites was 0.7 mg/kg at Site 2 (De Hoop) and the Highest mean was 9.6 mg/kg at Site 3 (Polopark) (Appendix A; Figure 2.11). It was highest at a concentration of 9.6 mg/kg during summer at Site 3 (Polopark) and lowest at 0.7 mg/kg also during summer at Site 2 (De Hoop) (Appendix A). The One-way ANOVA for Ag showed no significant variance ($p>0.05$) between sites. Silver was only detectable during two seasons (summer and spring). The mean concentration was higher during spring (5.39 mg/kg) than in summer (3.5 mg/kg) (Appendix A; Table 2.3).

There are no man-made sources of silver because it is an element. Silver cannot be broken down, but can change its form by combining with other substances. Large amounts of silver are released into the environment through the natural wearing down of silver-bearing rocks and soil by wind and rain. It is a stable metal and remains in the environment in one form or another until it is taken again by people (ATSDR 1990).

MANGANESE

Manganese is necessary for good health and is a trace element. Manganese is found naturally in most foods and may be added to food or made available in nutritional supplements. It could also be added as an additive in gasoline to improve the octane rating of the gas (Howe *et al.* 2004, ATSDR 2008d). Manganese is a normal element of air, water, soil, and food. After release from the manufacture, additional manganese can be found in air, soil, and water. Manganese cannot break down in the environment, as with other elements. It can simply alter its form or become attached or separated from particles. In water, most of the manganese usually attaches to particles in the water or settle into the sediment (ATSDR 2008d). The lowest mean concentration was 480.0 mg/kg at Site 5 (Burgersfort Bridge) and the highest mean concentration was 1419.0 mg/kg at Site 1 (Tiershoek) (Appendix A; Figure 2.12). The highest concentration for Mn was recorded at Site 1 (Tiershoek) during autumn with a value of 1678 mg/kg while the lowest concentration was recorded at Site 2 (De Hoop) during spring at a value of 133 mg/kg (Appendix A).The One-way ANOVA computed for Mn showed no significant variance between sites ($p>0.05$). For the seasons, Mn had the highest mean concentration value of 1061 mg/kg during autumn and lowest mean concentration value of 733 mg/kg during spring (Appendix A; Table 2.3).

NICKEL

The highest mean concentration for the sites was 1240.5 mg/kg at Site 1 (Tiershoek) and the lowest mean was 291.1 mg/kg at Site 5 (Burgersfort Bridge) (Appendix A; Figure 2.12). The highest overall Ni concentration (2202.2 mg/kg) was recorded at Site 4 (Steelpoort) during summer while lowest concentration (14 mg/kg) was recorded at Site 1 (Tiershoek) during winter (Appendix A). The One-way ANOVA showed no significant variance between sites ($p>0.05$). The seasonal lowest mean concentration (28.2 mg/kg) was recorded during winter while the highest concentration (1158.1 mg/kg) during summer (Appendix A; Table 2.3). Nickel compounds can be formed through the combination of nickel with other substances such as chlorine, sulphur, and oxygen. Most of these compounds dissolve rather easily in water and have a characteristic green colour (ATSDR 2005a). Mining, smelting, refining, alloy processing, scrap metal reprocessing, fossil fuel combustion, and waste incineration are some of the human activities that contribute to nickel loadings in aquatic and terrestrial ecosystems. Nickel and its salts, chemical and physical forms strongly influence bioavailability and toxicity (WHO 2006). There are three natural sources of entry for nickel into surface waters: as particulate matter in rainwater, through the dissolution of primary bedrock materials, and from secondary soil phases. In fresh water and marine water the fate of nickel is affected by the pH, pE, ionic strength, type and concentration of ligands, and the availability of solid surfaces for adsorption (Eisler 1998b).

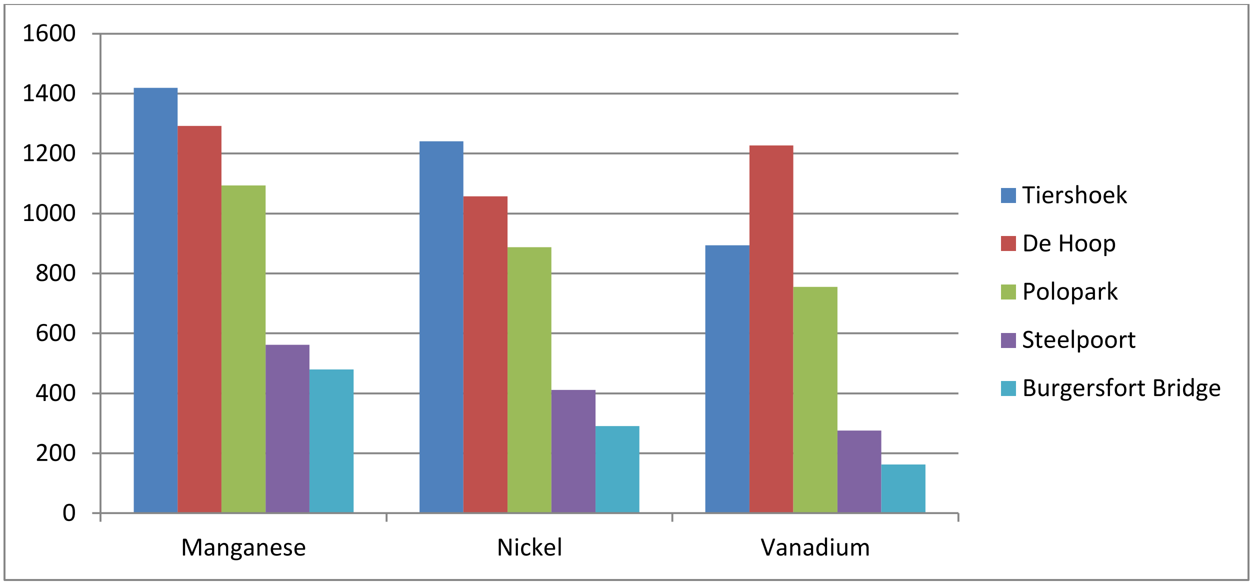

Figure 2. 12: The mean variation of Manganese, Nickel and Vanadium concentrations In the sediment at the Steelpoort River.

VANADIUM

The lowest mean concentration of V was 162.3 mg/kg at Site 5 (Burgersfort Bridge) and the highest was 1227.2 mg/kg at Site 2 (De Hoop) (Appendix A; Figure 2.12). The highest value recorded for V was 2297 mg/kg at Site 2 (De Hoop) during the autumn survey, while the lowest value recorded was 76.8 at Site 4 (Steelpoort) during the summer survey (Appendix A). Using One-way ANOVA for V a significant variance ($p<0.05$) was found between sites. For the seasons, the highest mean concentration recorded for V was 731.2 mg/kg during autumn while the lowest mean concentration recorded was 544 mg/kg during spring (Appendix A; Table 2.3). In the environment, small amounts of vanadium tend to stimulate plants but large amounts are toxic. Vanadium is considered to be one of the 14 most noxious heavy metals. It stimulates photosynthesis in higher plants, is an oxygen carrying metal in some invertebrates and is essential for the growth of fungi and algae. There is much debate on the function of vanadium in the metabolism in higher plants and organisms, although vanadium is recognized as an essential element for certain species of algae (ATSDR 2009).

CHROMIUM

In crystalline form, Chromium is a steel-gray, lustrous, hard metal. Naturally only four Cr isotopes occur —Cr-50 (4.3%), -52 (83.8%), -53 (9.6%), and -54 (2.4%)—and seven are human-made (Eisler 1986, Irwin *et al.* 1997c, b, ATSDR 2008c). Elemental Cr is not usually found pure in nature, but is very stable. It is most frequently found in the environment in the trivalent (+3) and hexavalent (+6) oxidation states, but can exist in oxidation states ranging from -2 to +6 (Irwin *et al.* 1997b). The relation between concentrations of total Cr in a given environment and biological effects on the organisms living there is not well understood (Eisler 1986). For the sites, the highest mean concentration was 133.0 mg/kg at Site 5 (Burgersfort Bridge) and the lowest mean concentration was 65.1 mg/kg at Site 1 (Tiershoek) (Appendix A; Figure 2.13). The lowest concentration of 27 mg/kg was recorded at Site 5 (Burgersfort Bridge) during spring and the highest concentration was also recorded during spring at a concentration of 153 mg/kg at Site 4 (Steelpoort) (Appendix A). One-way ANOVA was performed on Cr and no significant variance was found between sites ($p>0.05$). For the seasons, the highest mean concentration of Cr in sediment was recorded as 117.2 mg/kg during autumn while the lowest concentration was recorded in spring as 82.6 mg/kg (Appendix A; Table 2.3). During all the four seasons the Cr concentrations were all above the sediment quality guidelines (CCME 2012b).

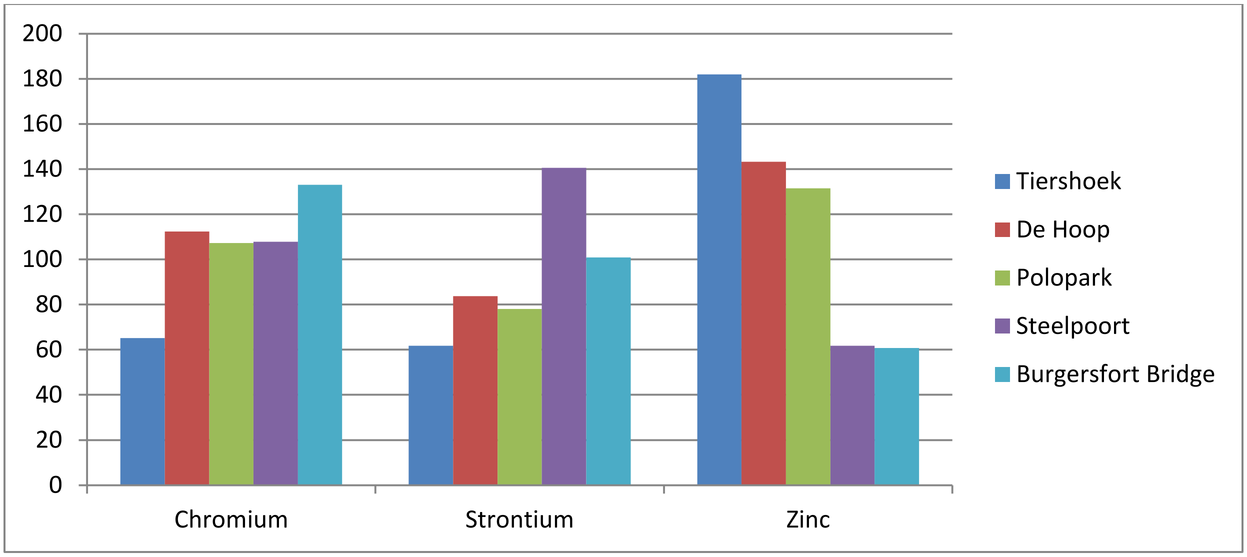

Figure 2. 13: The mean variations for Chromium, Strontium and Zinc concentrations in the sediment at the Steelpoort River.

STRONTIUM

High concentrations of strontium are occasionally a marker for pollution from cattle feedlots. When comparing the United States Environmental Protection Agency (US-EPA 2012a) TWQRs with our values, they are within range and cannot cause any specific effect. Many strontium compounds are hazardous to fish and wildlife, even though pure strontium does not appear to be very toxic (Irwin *et al.* 1997g). The highest mean concentration for the sites was 140.5 mg/kg at Site 4 (Steelpoort) and the lowest mean concentration was 61.7 mg/kg at Site 1 (Tiershoek) (Appendix A; Figure 2.13). The lowest Sr concentration was recorded at Site 1 (Tiershoek) during summer at a value of 45.7 mg/kg with the highest Sr concentration recorded at Site 4 (Steelpoort) again during summer at a value of 183.9 mg/kg (Appendix A). One-way ANOVA showed significant variance between sites ($p<0.05$) for Sr. Strontium had the lowest seasonal mean concentration of 87.2 mg/kg during spring and the highest mean concentration of 100.8 mg/kg during winter (Appendix A; Table 2.3).

ZINC

For the sites, Zn had the lowest mean concentration of 60.8 mg/kg at Site 5 (Burgersfort Bridge) and the highest mean concentration of 182.0 mg/kg at Site 1 (Tiershoek)

(Appendix A; Figure 2.13). The lowest value recorded for Zn was 24 mg/kg at Site 4 (Steelpoort) during autumn and the highest value recorded was 226 mg/kg at Site 2 (De Hoop) again during autumn (Appendix A). The One-way ANOVA showed no significant variance ($p>0.05$) between sites. Zinc was above the recommended sediment quality guideline only during winter (127 mg/kg) (CCME 2012b). The lowest seasonal mean concentration recorded for Zn was 106 mg/kg during autumn and the highest mean concentration recorded was 127 mg/kg during winter (Appendix A; Table 2.3). In hard waters the toxicity of Zn is reduced. Zinc is found in rocks and ores and is readily refined into a pure stable metal. Through both natural processes such as weathering and erosion, and through industrial activity, it can enter aquatic ecosystems (Eisler 1993, Irwin *et al.* 1997h). Zinc is a metabolic antagonist of cadmium and its toxicity is reduced in animals (ATSDR 2005b). The bio-availability and toxicity of zinc is affected by the adsorption of zinc by hydrous metal oxides, clay minerals and organic materials which are important processes in aquatic ecosystems (Irwin *et al.* 1997h). The prerequisite for trace elements often varies considerably between species, but the optimal concentration range is generally narrow. Marginal imbalances contribute to reduced fitness, whereas severe imbalances can cause death (DWAF 1996d).

2.4 CONCLUSION

For the majority of the physico-chemical parameters the most elevated were detected downstream either at Steelpoort (Site 4) or Burgersfort Bridge (Site 5) and lowest concentrations were detected at De Hoop (Site 2). This indicates that the physico-chemical parameters increased from upstream to downstream. Since each parameter/constituent has an effect which is either beneficial or detrimental to aquatic biota, it is usually hard to determine the magnitude of the combined effect of the physico-chemical parameters (Dallas & Day 2004). Analysis of variance (ANOVA) indicated that there were no significant differences for electrical conductivity, salinity, and TDS, between the sites ($p>0.05$). There were however significant differences in the temperature, dissolved oxygen, turbidity, and pH concentrations ($p<0.05$). The overall water temperature was normal with temperatures ranging from 15 ˚C – 27 ˚C. Dissolved oxygen was above the TWQR during all seasons. The pH recorded ranged from 7.8 to 9.5 (intermediate to alkaline). The lowest pH levels recorded in autumn then winter and the highest in spring then summer. Therefore, the reason behind the high pH levels in spring and summer could be due to turnover because more biological activities occur in the catchment (Davies & Day 1998). The EC, TDS, salinity and turbidity recorded were all within the TWQR, even though we observed very high increases in the turbidity during summer, this may be due to annual precipitation which drags soil from the river bank into the stream causing it to become more turbid.

The nutrients, nitrite and phosphorus indicated significant variance between sites ($p<0.05$). The values of nitrate and nitrite (inorganic nitrogen) were all very low indicative of oligotrophic conditions during all the seasons. The ammonium mean levels were all above the TWQR for DWAF and BC-EPD values. Hypertrophic conditions can be observed during all the seasons with the total nitrogen levels. Elevated nutrients concentration, when related to the total nitrogen may result in the increase/abundance of aquatic and/or algae (DWAF 1996d). Phosphorus concentrations were high, indicative of hypertrophic conditions. Total nitrogen and phosphorus both at high concentrations may lead to eutrophication (DWAF 1996d). The cations were all below the guideline values (BC-EPD 2008, CCME 2012b, US-EPA 2012a). Analysis of variance (ANOVA) indicated that all the cations except for Mg showed significant variance between sites ($p<0.05$).

The majority of the metals and metalloids in the water were below detection level and the once that were detectable had low concentrations. For the sites, the majority of the metals and metalloids (Fe, Mn, V, Co, Zn, Ba, and Cu) were highest at Tiershoek (Site1) and lowest at Steelpoort (Site 4) and Burgersfort Bridge (Site 5). The remainder of the metals and metalloids (Sr, Cr, B, and Ni) were highest at Steelpoort (Site 4) and lowest at Tiershoek (Site 1). Aluminium and Titanium were the exception because the former was highest (mean) at Steelpoort (Site 4) and the latter was highest at Polopark (Site 3) but they were both lowest (mean) at Burgersfort Bridge (Site 5). The general trend showed more elevated levels upstream as compared to downstream implying that most of the metals and metalloids are concentrated in the headwaters of the river.

Analysis of variance (ANOVA) indicated that there were no significant differences for all the water metals and metalloids between sites ($p>0.05$). However, the levels that deviated from the norm may have been influenced by a number of factors such as; the geology or the bedrock, the physico-chemical characteristics, pollution, and/ or a combination of all these factors (Du Preez & Steyn 1992, Davies *et al.* 1993, DWAF 1996d, BC-EPD 2008, Whitehead *et al.* 2009, Ccme 2012a, US-EPA 2012b). For the seasons, autumn seemed to have the highest concentrations for most of the metals detected which correlates with literature because generally most accumulation happens in the warmer months of the year.

Metal concentrations were higher in the sediment than in the water. For the sites, the metals and metalloids Fe, Ti, Ba, Co, Cu, Pb, Mn, Ni, Sb, V, and Zn were highest at Tiershoek (Site 1) and lowest at Burgersfort Bridge (Site 5). Al, Sr, and Cr were highest at Steelpoort (Site 4) or Burgersfort Bridge (Site 5) and lowest at Tiershoek (Site 1). Ag was the only one highest at Polopark (Site 3) and lowest at De Hoop (Site 2). The trend in the sediment also indicated a concentration gradient from upstream to downstream for the majority of the metals and metalloids. For the sediment metals and metalloids, there were only significant differences in the Sr, Co, Cu, and V concentrations between the sites ($p<0.05$). The mean metal concentrations collected showed elevated levels for chromium, copper and zinc, which were above the CCME sediment guidelines. The rest of metals and metalloid could not be stated because no guidelines exist for them except for lead which was below its guideline of 35 mg/kg (CCME 2012b). Nonetheless, the metals detected were mostly elevated during summer and lower in the spring beside lead (Pb). The typically elevated levels are not unusual because sediment serves as sink for metal contamination in aquatic ecosystems (Chapman *et al.* 1999). Hence, metal concentrations linked with sediment greatly surpass the concentrations dissolved in water, in most aquatic systems (Chapman *et al.* 1998, Ikem *et al.* 2003, Chon *et al.* 2012, Ciparis *et al.* 2012).

CHAPTER 3

MACRO-INVERTEBRATE BIOMONITORING

3.2 INTRODUCTION

The use of biological variables to assess the environment is known as biomonitoring. The main task in biomonitoring is to look for the ideal indicator (or bioindicator) whose presence, abundance, and/or behavior reflects a stressor's result on biota. An indicator may be used for biomonitoring at several ranks of organization, ranging from suborganismal (i.e. gene, cell, tissue) and organismal to population, community, and even ecosystem levels (Bonada *et al.* 2006a). Biological monitoring (biomonitoring) indicates and assesses ecological degradation, transformation, improvement or other effects, resulting from a localized event or variable by using the living components of the studied environment. Results from biological monitoring can be obtained rapidly and are cost effective. Biological monitoring examines organisms whose exposure to pollutants is continuous, hence it is advantageous. Therefore, species present in riverine ecosystems show both the present and past history of the water quality in the river, permitting detection of disturbances that might otherwise be missed (Eekhout *et al.* 1996). Benthic macroinvertebrates are widespread and sensitive to environmental changes and is the reason they are widely used for assessment of freshwater resources (Resh 1995), and have been presented as the most reliable of all the bioindicators used (Rosenberg & Resh 1993, Bredenhand & Samways 2009).

For the bio-assessment of the integrity of aquatic systems numerous methods have been developed. Most are based on the attributes of whole assemblages of organisms such as fish, algae or invertebrates while some of these are based on one or other aspect of a single species. Some important tools to support decision making within river management are biological monitoring and assessment methods. For assessment of its quality, the biotic component of an aquatic ecosystem may indeed be considered as an 'integrating-information-yielding unit' (De Pauw *et al.* 2006). There has been little consistency in methods of sampling, sample processing, and data interpretation among studies, even though aquatic macroinvertebrates are used throughout the world in stream bioassessments (Carter & Resh 2001, Bonada *et al.* 2006b, Herbst & Silldorff

2006, Chessman *et al.* 2007). Furthermore, a variety of quantitative and qualitative collection techniques may be used in the collection of benthic macroinvertebrates. Quantitative techniques often consume large amounts of time and money but are thought to be more reliable and more amenable to statistical analysis. Also, large portion of the aquatic community cannot be sampled using quantitative sampling because they are usually habitat specific (Lenat 1988).

Many macroinvertebrates species are diagnostic of specific water quality and certain kinds of habitats (Mackie 1998). Their abundance, occurrence and activities expose something about the state of the ecosystem in which they are found, and whether processes are functioning according to expectations within normal bounds (Kevan 1999, Bredenhand & Samways 2009). In literature, biological water quality assessment methods of aquatic invertebrates are often referred to as benthic macroinvertebrates or macrozoobenthos because the majority of them have a benthic life and inhabit the bottom substrates (sediments, debris, logs, macrophytes, filamentous algae, etc.) (Rosenberg & Resh 1993). The community present in a given situation reflects its environment because the individual species have different habitat preferences. It is logical to detect pollution by monitoring the aquatic ecosystem since the effect of stream pollution is to alter the aquatic environment and its biological community (Hilsenhoff 1977, Stark 1993). For detecting the diversity of environmental perturbations associated with human activities, no single group of organisms is always best suited. There is a need to monitor the status of different taxonomic groups, if, the maintenance of ecosystem integrity is the aim of river management (De La Rey *et al.* 2004). Change in relative abundances, followed by the disappearance of certain species has been linked to human disturbances of the environment. Among the first to be affected are those having biotope preferences making them more susceptible to specific types of disturbance (Clark & Samways 1996). To assess pollution and other anthropogenic disturbances of rivers and streams often requires that each taxon be allocated a number indicating its level of sensitivity in the construction of biotic indices that use macroinvertebrates. Depending on the nature of the particular disturbance, individual taxa may vary quite widely in sensitivity which may cause problems in constructing such indices. One possible solution to this problem is to create a suite of indices, each gathered using sensitivity numbers targeted to a particular impact (Chessman & Mcevoy 1997).

Macroinvertebrates are an important component of riverine ecosystems and are normally abundant (Hilsenhoff 1977, Quinn & Hickey 1990), and in freshwater assessment they are easily the most commonly used group of freshwater organisms (Rosenberg & Resh 1993, Stark 1993). Historically, in the study of running water ecosystems, invertebrates have received considerable attention; in particular relationships between environmental variables have been the subject of numerous investigations and macro-invertebrate community structure. Also, benthic macro-invertebrate responses to inorganic or organic pollutants have been used to develop biotic indices and they are considered one of the best biological indicators of water quality (Duran 2006). For environmental monitoring and assessment of aquatic systems, community structure or species composition of benthic invertebrates has frequently been used. Three common approaches have been taken: the 'saprobic' approach, which is effective in measuring impacts from sewage effluents and requires detailed knowledge of taxonomy; diversity indices, which ignore information provided by important species and tend to lose information and does not require detailed knowledge of species requirements; and biotic indices, which merges both approaches (Reynoldson & Metcalfe-Smith 1992).

Macro-invertebrate community structure may be affected by particular habitat characteristics such as current velocity, water depth, and substratum type. Hence, macroinvertebrate-based biomonitoring or pollution assessment is intended to monitor water quality through its influence on community composition (Stark 1993). The most frequently described macro-invertebrate distribution patterns are those of taxonomic composition, with the term `community structure' frequently being synonymous with taxonomic composition (Palmer *et al.* 1996).

Biomonitoring has only as recently as 1996 become a routine tool in the management of South Africa’s inland waters even though other methods have been available for many years (De La Rey *et al.* 2004). At this time, the backbone of the National River Health

Programme is SASS (South African Scoring System), a macro-invertebrate index established by Chutter (1998). This system has undergone a number of refinements to suit all conditions; the latest of these modifications is SASS 5 (Dickens & Graham 2002b).

Macroinvertebrates have the advantage to be easy to collect and identify, and are indicative of the changing water qualities as they can be confined for most part of their life to one locality on the river bed. Therefore, instead of chemical samples of the water taken at one time they act as continuous monitors of the water flowing over them. Throughout the whole river system, macroinvertebrates are ubiquitous and abundant in the crenal and rhitral as well as the potamal part (Illies 1961). They occupy a very significant niche in the operation of the river continuum food web (Vannote *et al.* 1980,Giller & Malmqvist 1998, De Pauw *et al.* 2006). They are ever-present in rivers and often display greater taxonomic and trophic variety than fish. Plafkin *et al.* (1989) suggest that fish reflect conditions over broader spatial areas because of their relative mobility and longevity while macroinvertebrates are more indicative of local habitat conditions (Lammert & Allan 1999). In this chapter macro-invertebrate species richness and abundance would be used to determine how impacted the Steelpoort River is, by looking at seasonal differences from upstream to downstream.

3.2 METHODS AND MATERIALS

3.2.1 AQUATIC MACRO-INVERTEBRATE SAMPLING

Aquatic macro-invertebrates were collected at the five sites of the Steelpoort River. The invertebrates were sampled using the SASS 5 bio-assessment protocol (Goodyear & Mcneill 1999, Dickens & Graham 2002b). Macro-invertebrate samples were collected using a 400 mm x 400 mm SASS net with a 250 µm mesh size. The substrate was disturbed for a period of two minutes to free macro-invertebrates from the substrate. The SASS score for each site was evaluated in the field for three of five samples. The macro-invertebrate samples were preserved in 70% ethanol and sorted to family level.

3.2.2 LABORATORY ANALYSIS

MACRO-INVERTEBRATE ANALYSIS

The samples were sorted by removing the macroinertebrates from the debris (sediment, plants, algae etc.) in the Biodiversty Laboratory, University of Limpopo. The macroinvertebrates were then sorted and identified to the family level and counted. The ASPT and the SASS Score were calculated to determine the state of the water quality in the Steelpoort River. SASS is a scoring system based on macroinvertebrates, whereby each macro-invertebrate taxon is allocated a sensitivity/tolerance score according to the water quality conditions it is known to tolerate (Dallas 1995, Dallas 1997). Data interpretation is based on two calculated values, namely SASS Score, which is the sum of the sensitivity/tolerance scores for taxa present at a site, and average score per taxon (ASPT), which is the SASS Score, divided by the number of taxa.

3.2.3 STATISTICAL ANALYSIS

SIMPER analysis was performed to determine the main factors contributing to the differences found during the different seasons and sites for aquatic macoinvertebrate using the vegan package for R (Oksanen *et al.* 2013). Canonial correspondence analysis (CCA) was used to determine the relationship between the water quality parameters and the macroinvertebrates and the sediment metals and the macroinvertebrates (CANOCO version 5). Canonical correspondence analysis (CCA) is a direct gradient analysis used to examine the relationships between the measured variables and the distribution of communities. The data was $\log_{(x+1)}$ transformed to stabilize the variance and the statistical package CANOCO 5 was used (Ter Braak & Smilauer 2012).

3.3 RESULTS

3.3.1 OVERALL TAXON DIVERSITY AND RICHNESS OF BENTHIC MACROINVERTEBRATES

The total number of macroinvertebrates individuals that were collected from the Steelpoort River throughout the whole sampling period (February to October, 2014) was

21720, which were classified into 13 orders (Ephemeroptera, Trichoptera, Coleoptera, Hemiptera, Odonata, Diptera, Turbellaria, Plecoptera, Megaloptera, Crustacea, Porifera, Annelida, Mollusca), and 55 families (Figure 3.1). Diptera was the most diverse order with 10 families, followed by Ephemeroptera, Trichoptera and Odonata with 8 families each, Coleoptera with 7 families, Mollusca with 4 families, Hemiptera with 3 families, then Crustacea with 2 families, and finally Turbellaria, Plecoptera, Megaloptera, Porifera and Annelida with 1 family each (Figure 3.1).

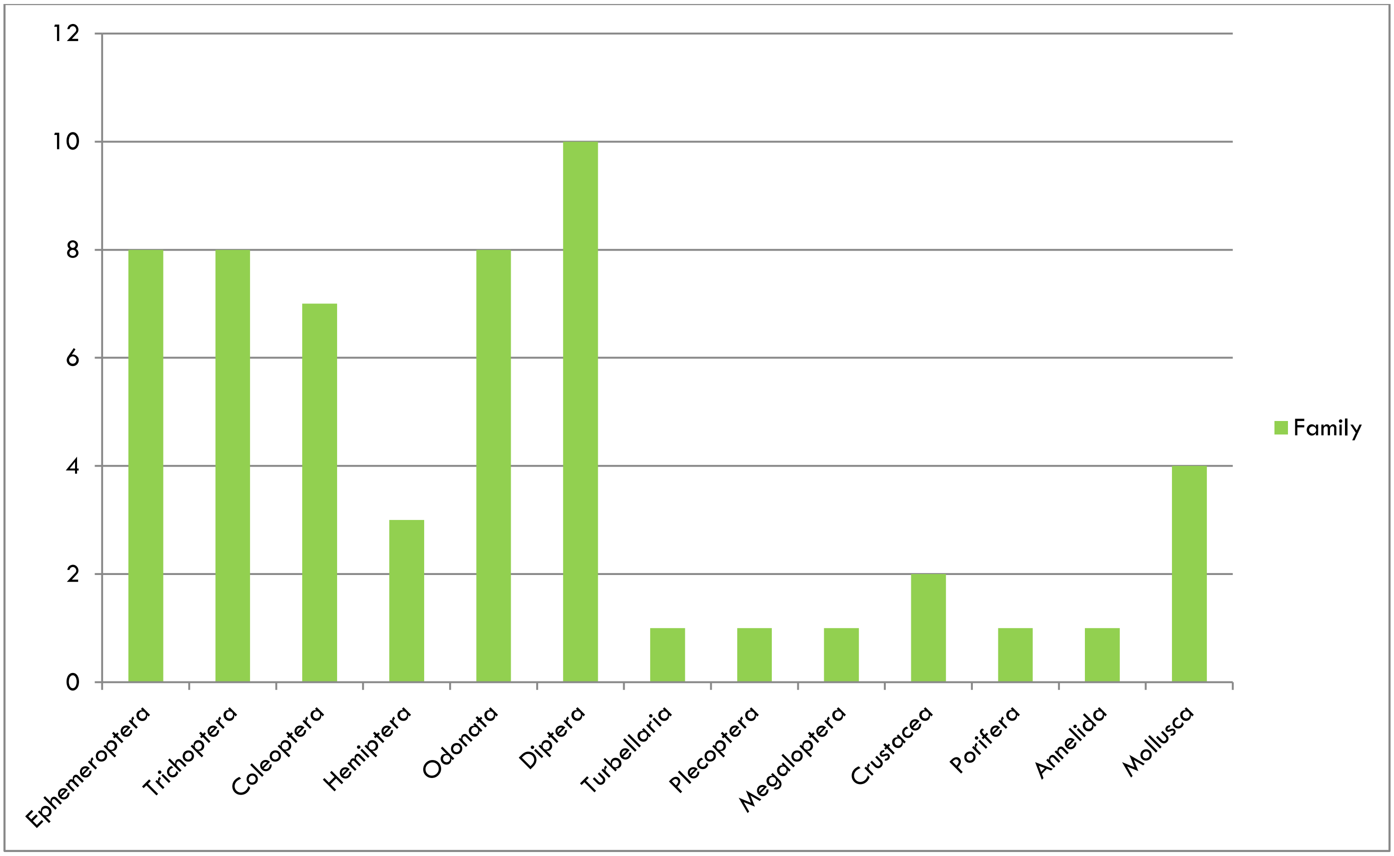

Figure 3.1: Representation of the overall number of macroinvertebrates taxa (Family) in the Steelpoort River.

Ephemeroptera had the highest number of individuals collected from all the five sites, 11332 (constituting 52.2% of the total number of individuals found). Diptera had the second highest number with 5577 individuals contributing 25.7%. Trichoptera followed with 3718 individuals contributing 17.1% of the total number of individuals. The order with least amount of individuals was Porifera with 1 individual collected throughout the surveys, contributing merely 0.005% to the total number of specimens collected.

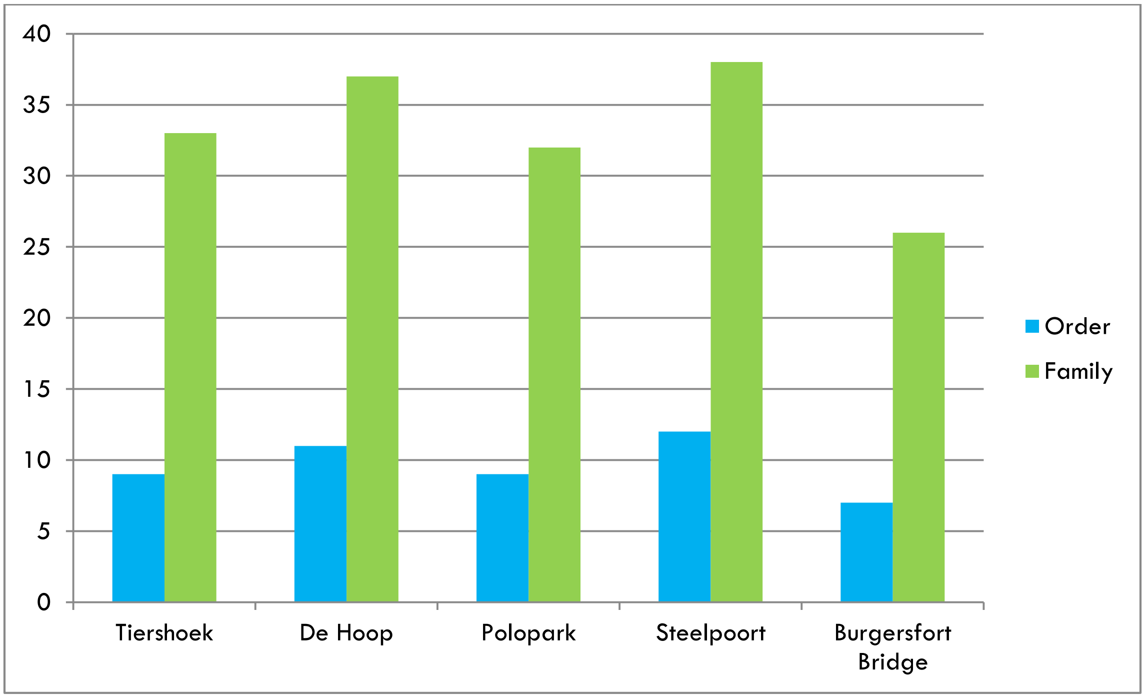

Figure 3.2: Representation of the number of macroinvertebrates taxa (Order and Family) between sites in the Steelpoort River.

From Figure 3.2 it was observed that, Tiershoek (Site 1) was represented by 9 orders and 33 families. De Hoop (Site 2) was represented by 11 orders and 37 families. Polopark (Site 3) was represented by 9 orders and 32 families. Steelpoort (Site 4) was represented by 12 orders and 38 families while Burgersfort Bridge (Site 5) was represented by 7 orders and 26 families. Steelpoort (Site 4) was represented by the highest number of orders and families of all the five sites and Burgersfort Bridge (Site 5) had least representation of orders and families (Figure 3.2). Therefore the sequence for the site with highest and the least number of orders and families from high to low was; Steelpoort>De Hoop>Tiershoek>Polopark>Burgersfort Bridge.

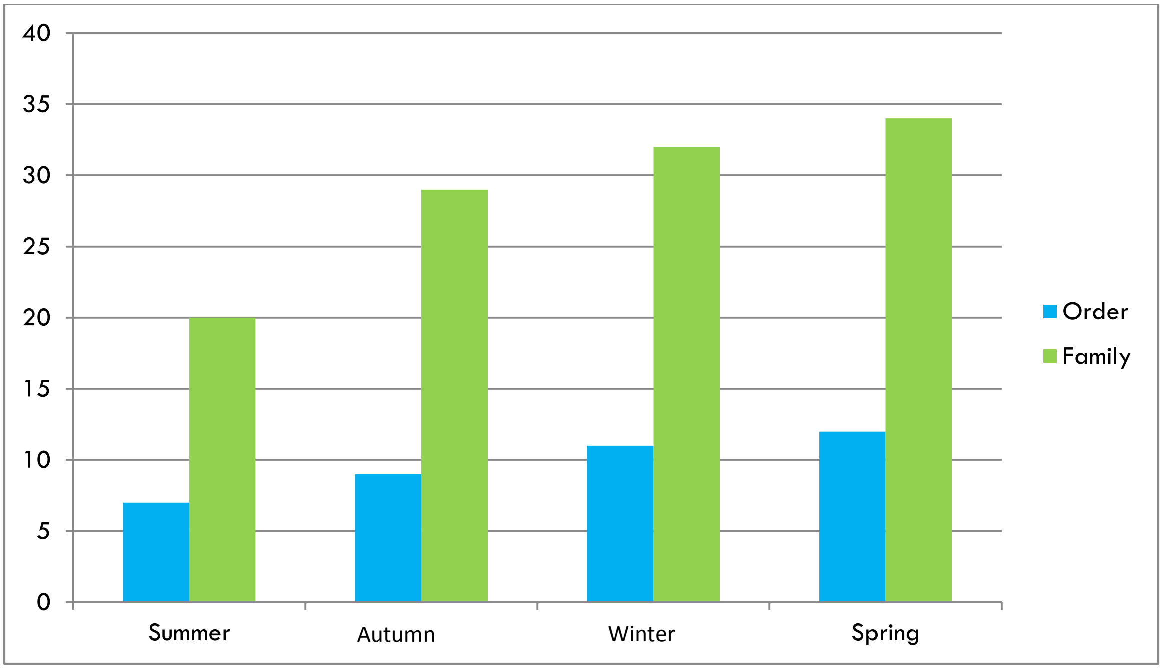

Figure 3.3: The number of macro-invertebrate taxa (Order and Family) between seasons in the Steelpoort River.

When we compare the seasons, summer had 7 orders and 20 families. Autumn had 11 orders and 32 families. Winter only contributed 9 orders and 29 families while spring had 12 orders and 32 families. The sequence of the season with most orders and families to the least is spring>autumn>winter>summer, in that order (Figure 3.3). Ephemeroptera, Trichoptera, Coleoptera, Hemiptera, Odonata, Diptera and Crustacea orders were present during all the seasons.

A comparison of the percentage and total number of individuals collected at the Steelpoort River throughout the sites shows that Tiershoek (Site 1) had 8638 individuals contributing 40%. De Hoop (Site 2) had 3935 individuals contributing 18%. Polopark (Site 3) had 2479 individuals contributing 11%. Steelpoort (Site 4) had 4810 individuals contributing 22% while Burgersfort Bridge (Site 5) had 1858 individuals contributing 9% (Table 3.1). Tiershoek (Site 1) had the highest number of individuals while Burgersfort Bridge (Site 5) had the lowest. The sequence of the sites from high to low number of individuals was: Tiershoek>Steelpoort>De Hoop>Polopark>Burgersfort Bridge. In all the sites Ephemeroptera, Trichoptera and Diptera were the most contributing orders.

Table 3.1: Total number of macroinvertebrates in each order collected at all the sites in the Steelpoort River.

	Tiershoek		De Hoop		Polopark		Steelpoort		Burgersfort bridge	
Orders	Total	%	Total	%	Total	%	Total	%	Total	%
Ephemeroptera	6488	75.1	2092	53.2	1160	46.8	793	16.5	798	43
Trichoptera	534	6.2	632	16.1	375	15.1	1991	41.4	186	10
Coleoptera	128	1.5	118	3	74	3	136	2.8	6	0.3
Hemiptera	0	0	8	0.02	2	0.08	23	0.5	10	0.5
Odonata	12	0.1	111	2.8	51	2.1	197	4.1	87	4.7
Diptera	1457	16.9	934	23.7	775	31.3	1644	34.2	767	41.3
Turberallia	6	0.07	18	0.5	27	1.1	12	0.3	0	0
Plecoptera	10	0.1	0	0	0	0	2	0.04	0	0
Megaloptera	2	0.02	0	0	0	0	5	0.1	0	0
Crustacea	1	0.01	9	0.2	2	0.08	4	0.08	0	0
Porifera	0	0	1	0.03	0	0	0	0	0	0
Annelida	0	0	4	0.1	13	0.5	1	0.02	4	0.2
Mollusca	0	0	8	0.2	0	0	2	0.04	0	0

In terms of seasons, for summer only 3169 individuals were collected contributing only 15%. Winter had 8936 individuals collected which contributed 41%. Autumn had 5873 individuals contributing 27% while spring had 3742 individuals contributing 17%. From high to low the sequence goes; winter>autumn>spring>summer. Similar to the sites, the families that contributed most during all the seasons were Ephemeroptera, Trichoptera and Diptera.

Table 3.2: Total abundance of each order during the four seasons in the Steelpoort River.

	Summer		**Autumn**		**Winter**		**Spring**	
Orders	Total	%	Total	%	Total	%	Total	%
Ephemeroptera	1265	39.9	1301	22.2	6320	70.7	2445	65.3
Tricoptera	1213	38.2	2179	37.1	252	2.8	74	1.9
Coleoptera	137	4.3	57	1	80	0.9	188	5
Hemiptera	15	0.5	1	0.02	17	0.1	10	0.3
Odonata	108	3.4	196	3.3	99	1.1	55	1.5
Diptera	429	13.5	2099	35.7	2102	23.5	947	25.3
Turberallia	0	0	30	0.5	32	0.4	1	0.03
Plecoptera	0	0	2	0.03	6	0.07	4	0.1
Megaloptera	0	0	0	0	7	0.08	0	0
Crustacea	2	0.06	8	0.1	2	0.02	4	0.1
Porifera	0	0	0	0	0	0	1	0.03
Annelida	0	0	0	0	19	0.2	3	0.08
Mollusca	0	0	0	0	0	0	10	0.3

3.3.2 BRAY-CURTIS MEASURE ACROSS SITE AND SEASON SAMPLING UNIT

The MDS plots represent a SIMPER analysis of the average similarity (AS) and average dissimilarity (AD) of the macro-invertebrate species collected during the four sampling seasons at the five different sites.

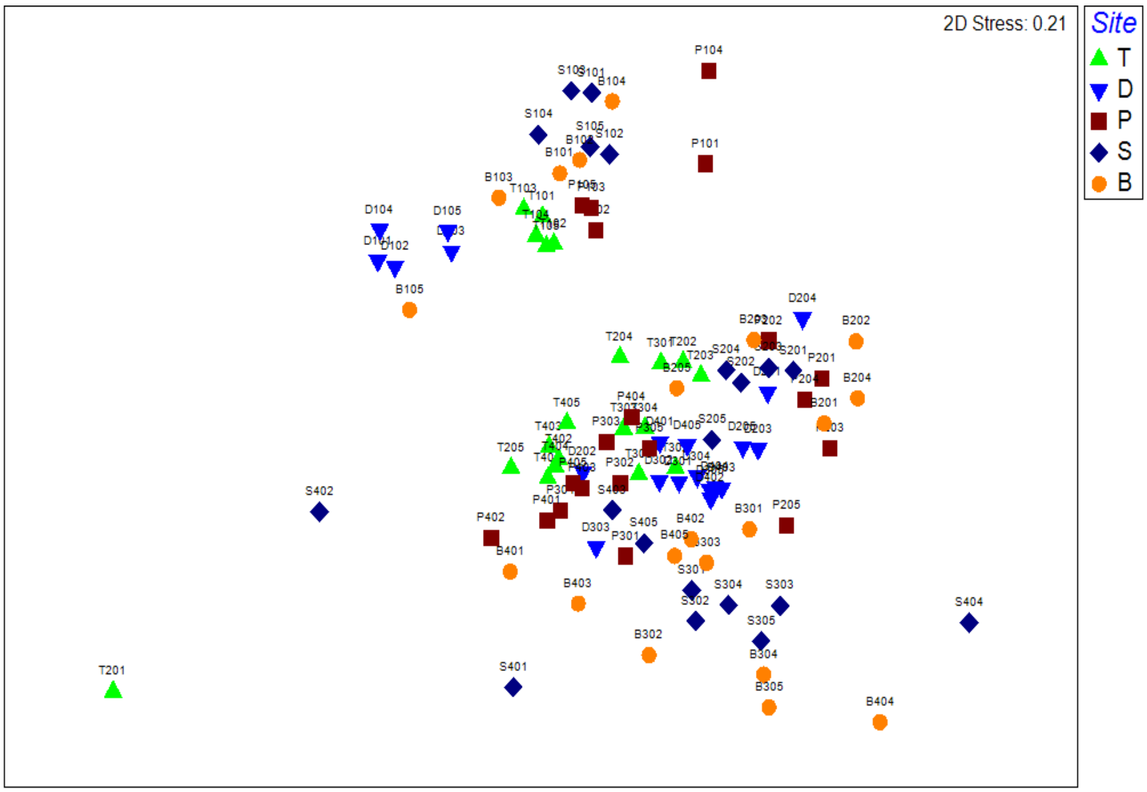

Figure 3.4: MDS plot representing the differences between sites during the four seasons in the Steelpoort River (T = Tiershoek, D = De Hoop, P = Polopark, S = Steelpoort and B = Burgersfort Bridge). .

A SIMPER analysis was performed to determine the differences between the same sites during different seasons. At Tiershoek (Site 1) the SIMPER analysis found 46.17% average similarity between the families Caenidae (24.88%), Leptophlebiidae (24.20%), Chironmidae (14.94%), Baetidae 3sp (5.81%), Elmidae larvae (5.54%), Hydropsychidae 1sp (5.34%), Tabanidae (4.61%), Baetidae 1sp (4.48%) and Baetidae 2sp (1.83%). Caenidae, Leptophlebidae, and Chironmidae contributed most to the average similarity at Tiersheok (Site 1) during the four seasons.

At De Hoop (Site 2), the SIMPER analysis found 45.89% between the families Caenidae (32.93%), Chironmidae 13.06%), Tabanidae (11.89%), Baetidae 1sp (11.27%), Elmidae (5.85%), Hydropsychidae 2sp (4.16%), Simulidae (3.76%), Gomphidae (3.74%), Leptoceridae (1.98%) and Ceratopogonidae (1.77%) during the four seasons. The greatest average similarity at De Hoop (Site 2) was generated mostly by the families Caenidae, Chironmidae, Tabanidae and Baetidae 1sp as they contributed more than 10% to the similarity.

Polopark (Site 3) had an average similarity of 41.80% according to the SIMPER analysis between all the four seasons. Chironmidae (21.20%), Baetidae 1sp (17.72%), Caenidae (13.56%), Leptophlebiidae (12.59%), Tabanidae (11.32%), Elmidae larvae (6.76%), Hydropsychidae 1sp (6.26%) and Gomphidae (3.67%) were the families that contributed to the similarity between the site during all the four seasons. The families mostly responsible for similarity were Chironmidae, Baetidae 1sp, Caenidae, Leptophlebiidae and Tabanidae.

At Steelpoort (Site 4) the SIMPER analysis found an average similarity of 35.10% including the families Chironmidae (12.20%), Leptophlebiidae (10.35%), Simulidae (10.23%), Tabanidae (7.70%), Hydrotilidae (6.50%), Gomphidae (5.69%), Caenidae (5.02%), Baetidae 3sp (4.09%), Elmidae (3.06%), Psychodidae (2.69%), Elmidae (2.03%) and Ceratopogonidae (1.96%). The greatest average similarity between the sites during the four seasons was generated by the families Chironmidae, Leptophlebiidae, Simulidae and Baetidae 1sp.

The SIMPER analysis found an average similarity of 37.84% at Burgersfort Bridge (Site 5) between the families Chironmidae (18.61%), Caenidae (17.74%), Simulidae (17.15%), Baetidae 1sp (15.63%), Hydropsychidae 1sp (6.75%), Hydrotilidae (5.75%), Leptophlebiidae (4.01%), Tabanidae (2.46%) and Gomphidae (2.25%). Chironmidae, Caenidae, Simulidae and Baetidae 1sp made the most contribution to the similarity at Burgersfort Bridge (Site 5) during all the seasons.

The SIMPER analysis found that the average dissimilarity (AD) was highest between Tiershoek (Site 1) and Burgersfort Bridge (Site 5) at 69.84% with the families contributing to the AD represented by Leptophlebiidae (11.40%), Chironmidae (8.58%), Caenidae (8.28%), Baetidae 1sp (6.77%), Hydropsychidae 1sp (6.55%), Simulidae (6.34%), Baetidae 3sp (6.17%), Baetidae 2sp (4.31%), Elmidae larvae (4.10%), Tabanidae (3.57%), Hydroptilidae (3.22%), Baetidae 4sp (2.82%), Ceratopogonidae (2.80%), Hydropsychidae 2sp (2.29%), Corduliidae (2.01%), Gomphidae (1.97%), Psychodidae (1.94%), Helodidae (1.91%), Leptoceridae (1.75%), Tipulidae (1.73%) and Libellulidae (1.62%). The family that contributed greatly to the AD was Leptophlebiidae between Tiershoek (Site 1) and Burgersfort Bridge (Site 5) during the four seasons.

The lowest AD was observed between Tiershoek (Site 1) and Polopark (Site 3) at 61.10%. The families that contributed to the AD were Leptophlebiidae (10.02%), Caenidae (9.06%), Chironmidae (8.98%), Baetidae 1sp (8.05%), Hydropsychidae 1sp (7.64%), Baetidae 3sp (7.55%), Tabanidae (4.78%), Baetidae 2sp (4.74%), Elmidae larvae (3.91%), Ceratopogonidae (3.68%), Simulidae (3.52%), Hydropsychidae 2sp (3.24%), Psychodidae (2.98%), Gomphidae (2.87%), Helodidae (2.43%), Tipulidae (1.96%), Planaria (1,68%), Hydroptilidae (1.44%), Leptoceridae (1.37%) and Elmidae (1.30%). Leptophlebiidae was the family that contributed greatly to the lowest AD between the two sites during the four seasons.

A SIMPER analysis was performed to determine the differences between the same seasons at different sites. From the macroinvertebrates collected during summer (Survey 1), SIMPER analysis showed there was 50.82% AS between all the sites, which was the highest average similarity of all the four visits. The species that contributed most of the AS were from the families Caenidae (20.44%), Hydropsychidae 1sp (18.53%), Baetidae 1sp (13.15%), Psychodidae (10.93%), Leptophlebiidae (9.85%), Ceratopogonidae (9.18%), Corduliidae (3.92%), Helodidae (3.80%) and Elmidae larvae (3.22%), in descending order.

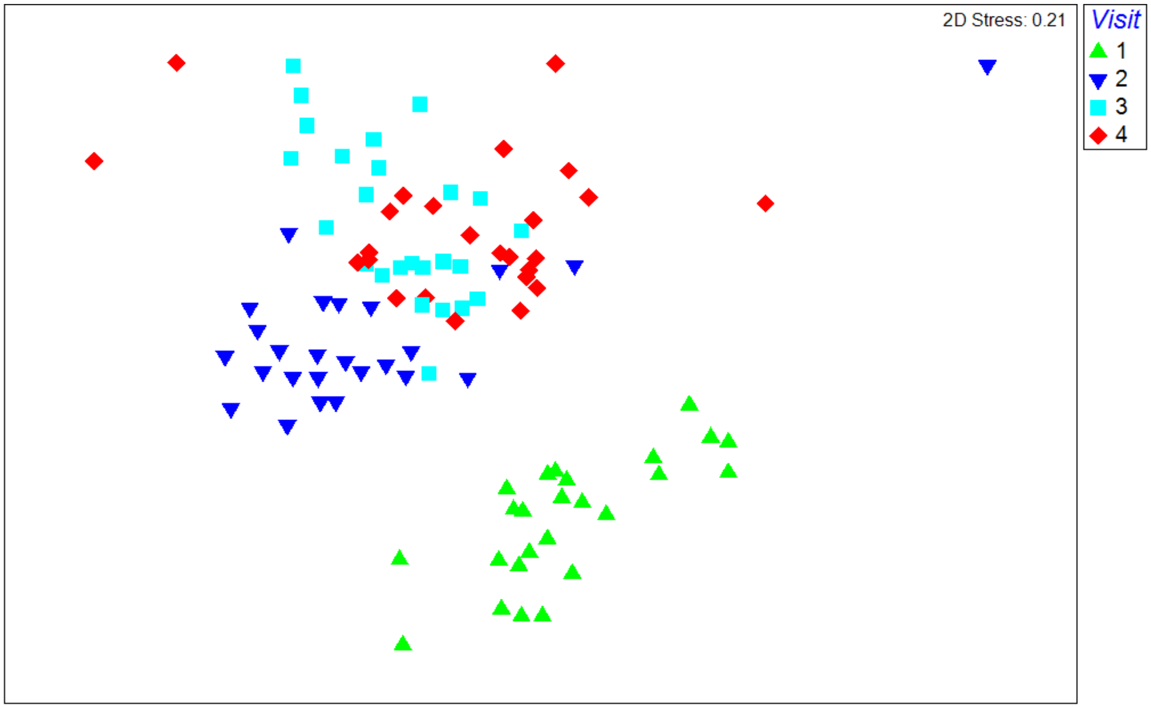

Figure 3. 5: MDS plot representing the differences between seasons at the five sites in the Steelpoort River (Visit 1 = summer, 2 = autumn, 3 = winter, 4 = spring).

For autumn SIMPER analysis found 49.19% average similarity between the five sites. The families responsible for the AS included Chironmidae (25.85%), Tabanidae (12.37%), Caenidae (11.66%), Simulidae (9.44%), Gomphidae (6.00%), Leptophlebiidae (5.93%), Baetidae 1sp (5.62%) and Baetidae 4sp (428%).

A SIMPER analysis found 48.33% AS for winter, the similarities are due to contributions from the families Chironmidae (21.69%), Hydropsychidae 1sp (11.42%), Baetidae 1sp (11.06%), Tabanidae (11.01%), Simulidae (9.73%), Hydropsychidae 2sp (8.63%), Caenidae (6.25%), Gomphidae (6.07%) and Leptophlebiidae (4.52%).

A SIMPER analysis for spring (Survey 4) found 43.44% AS between the five sites and showed that the families contributing were Chironmidae (27.77%), Caenidae (25.42%), Tabanidae (9.68%), Baetidae 1sp (9.64%), Elmidae larvae (7.23%), Leptophlebiidae (6.25%) and Simulidae (5.16%).

The highest AD from SIMPER analysis was observed between summer and winter at 79.09%. Chironmidae (10.94%), Hydropsychidae 1sp (7.99%), Simulidae (6.11%),Tabanidae (5.85%), Baetidae 3sp (5.32%), Leptophlebiidae (5.22%), Baetidae 1sp (5.11%), Caenidae (5.00%), Baetidae 4sp (4.73%), Ceratopogonidae (4. 66%), Psychodidae (4.51%), Gomphidae (3.35%), Baetidae 2sp (2.92%), Leptoceridae (2.78%), Corduliidae (2.72%), Hydroptilidae (2.71%), Helodidae (2.66%), Elmidae larvae (2.58%), Athericidae (1.51%), Chlorolestidae (1.20%), Hydropsychidae 2sp (1.20%) and Planaria (1.11%). Chironmidae was the family that contributed the most towards such a high dissimilarity.

The lowest AD of 57.63% was observed between winter and spring, as can clearly be seen on the MDS plot (they are grouped closer to each other). The families that contributed were Leptophlebiidae (8.30%), Baetidae 1sp (7.91%), Caenidae (7.86%), Simulidae (7.51%), Baetidae 3sp (7.36%), Baetidae 4sp (6.74%), Chironmidae (6.00%), Tabanidae (5.09%), Elmidae larvae (4.77%), Hydroptilidae (4.38%), Gomphidae (4.23%), Baetidae 2sp (4.11%), Leptoceridae (3.83%), Athericidae (2.24%), Muscidae (1.99%), Naucoridae (1.88%), Elmidae (1.80%), Planaria (1.63%), Oligochaeta (1.46%) and Chlorocyphidae (1.43%). None of the families contributed above 10% to the AD.

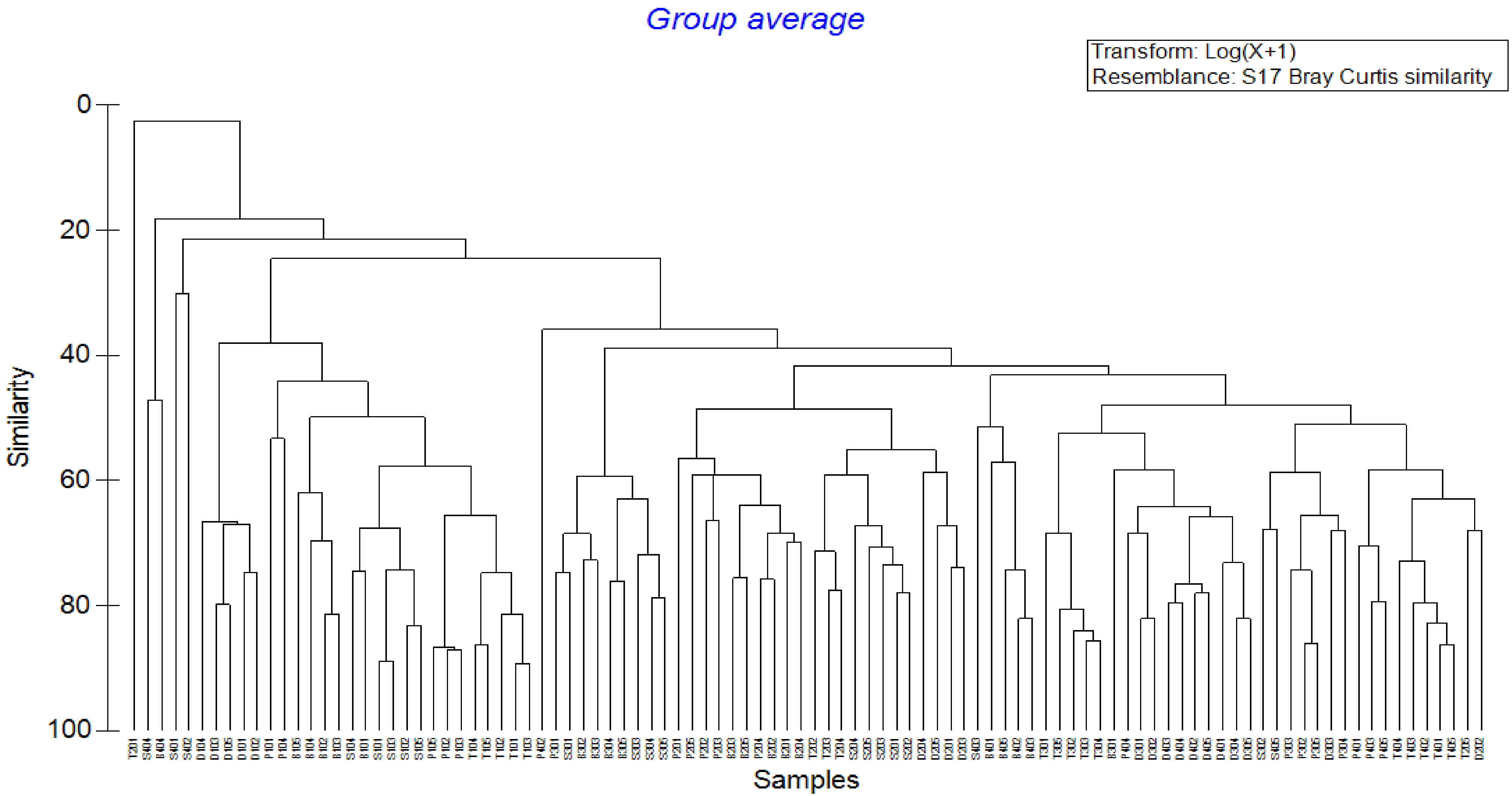

Figure 3.6: Euclidian distance matrix during the four seasons at the five sites in the Steelpoort River.

3.3.3 SOUTH AFRICAN SCORING SYSTEM 5TH EDITION (SASS 5)

The backbone of the National River Health Programme is SASS (South African Scoring System), a macro-invertebrate index established by Chutter (1998). This system has undergone a number of refinements to suit all conditions; the latest of these modifications is SASS 5 (Dickens & Graham 2002b).

Table 3.3: SASS score, number of taxa and ASPT values of macro-invertebrate families obtained at each sampling site of the Steelpoort River.

	Tiershoek				De Hoop				Polopark				Steelpoort				Burgersfort Bridge			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
SASS Score	61	77	62	47	54	49	56	73	22	61	72	46	48	63	68	22	54	31	47	32
No of Taxa	9	13	12	8	8	10	9	15	4	10	12	7	9	11	14	5	9	7	8	6
ASPT	7	6	5	6	7	5	6	5	6	6	6	7	5	6	5	4	6	4	6	5

When comparing the mean SASS score amongst the sites, it was observed that Tiershoek (Site 1) had a mean score of 61.75 which ranged from 47 – 77. De Hoop (Site 2) had a mean score of 58 which ranged from 49 – 73. Polopark (Site 3) had a mean score of 50.25 which ranged from 22 – 72. Steelpoort (Site 4) had a mean score of 50.25 which ranged from 22 – 68 while Burgersfort Bridge (Site 5) had a mean score of 41 which ranged from 31 – 54. The sequence for the average SASS score from high to low was; Tiershoek > De Hoop > Polopark and Steelpoort > Burgersfort Bridge (Table 3.3: Figure 3.7).

For the average No of Taxa, Tiershoek (Site 1) had an average of 10.5 which ranged from 8 – 13. De Hoop (Site 2) had an average of 10.5 which ranged from 8 – 15. Polopark (Site 3) had an average of 8.25 which ranged from 4 – 12. Steelpoort (Site 4) had an average of 9.75 which ranged from 5 – 14 while Burgersfort Bridge (Site 5) had an average of 7.5 which ranged from 6 – 9. The site with the highest average No of Taxa were Tiershoek and De Hoop followed by Steelpoort then Polopark and then Burgersfort Bridge (Table 3.3: Figure 3.7).

When comparing the mean ASPT, Tiershoek (Site 1) had an average of 6 which ranged from 5 – 7. De Hoop (Site 2) had an average of 5.75 which ranged from 5 – 7. Polopark (Site 3) had an average of 6.25 which ranged from 6 – 7. Steelpoort (Site 4) had an average of 5 which ranged from 4 – 6 while Burgersfort Bridge (Site 5) had an average of 5.25 which ranged from 4 – 6. In an order from high to low the mean ASPT for the sites was; Polopark > Tiershoek > De Hoop > Burgersfort Bridge > Steelpoort (Table 3.3: Figure 3.7).

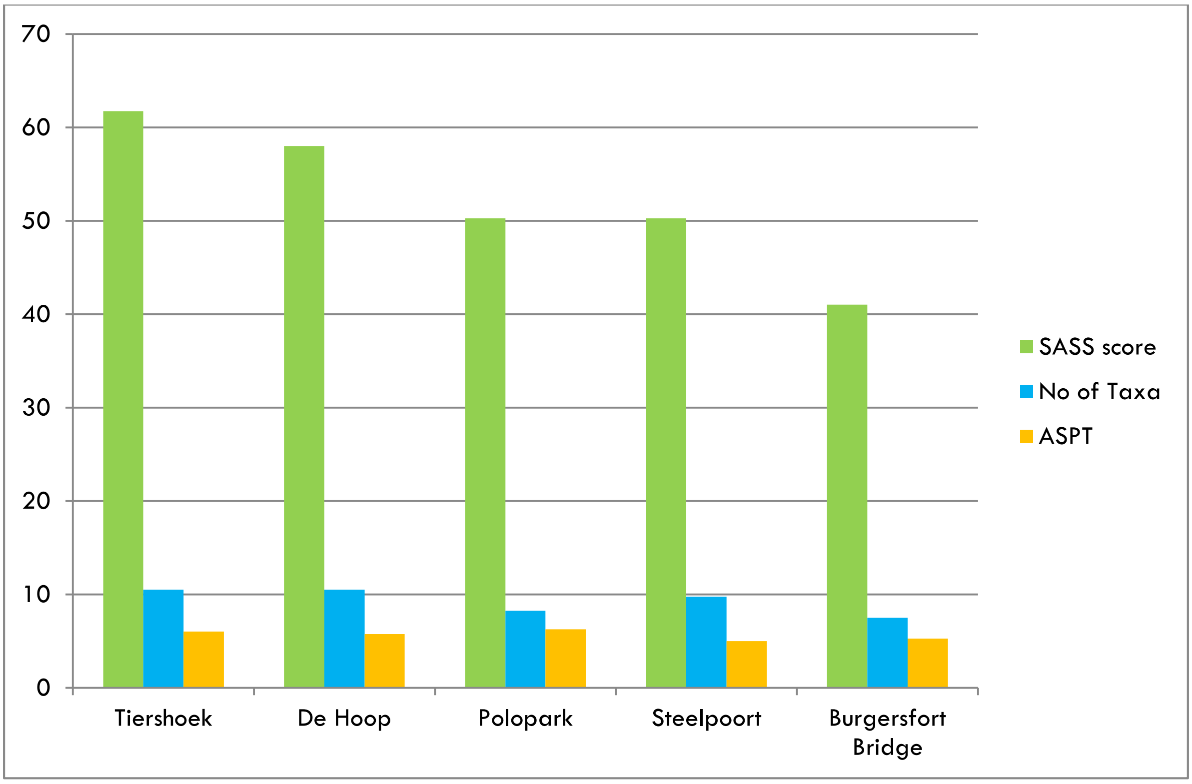

Figure 3. 7: The Mean SASS Scores, ASPT and No of Taxa for macroinvertebrates collected at each site in the Steelpoort River.

Table 3.4: SASS score, number of taxa and ASPT values of macro-invertebrate families obtained during each sampling season at the Steelpoort River.

	Summer					Autumn					Winter					Spring				
	Tiershoek	De hoop	Polopark	Steelpoort	Burgersfort Bridge	Tiershoek	De hoop	Polopark	Steelpoort	Burgersfort Bridge	Tiershoek	De hoop	Polopark	Steelpoort	Burgersfort Bridge	Tiershoek	De hoop	Polopark	Steelpoort	Burgersfort Bridge
SASS Score	61	54	22	48	54	77	49	61	63	31	62	56	72	68	47	47	73	46	22	40
No of Taxa	9	8	4	9	9	13	10	10	11	7	12	9	12	14	8	8	15	7	5	6
ASPT	7	7	6	5	6	6	5	6	6	4	5	6	6	5	6	6	5	7	4	7

When comparing the mean SASS score of the different seasons, summer had an average score of 47.8 ranging from 22 – 61. Autumn had an average score of 56.2 ranging from 31 – 77. Winter had an average score of 61 ranging from 47 – 72 while spring had an average score of 45.6 ranging from 22 – 73. The season with the highest average SASS score was winter followed by autumn then summer and finally spring (Table 3.4: Figure 3.8). The high SASS score during winter may be attributed the high No of Taxa that were collected during that sampling period.

For the mean No of Taxa during the different seasons, summer had a mean of 7.8 ranging from 4 – 9. Autumn had a mean of 17 ranging from 7 – 13. Winter had a mean of 11 ranging from 8 – 14 while spring had a mean of 8.2 ranging from 5 – 15. The sequence for the average No of Taxa from high to low was; autumn > winter > spring > summer (Table 3.4: Figure 3.8).

When comparing the average ASPT, summer had a mean of 5.8 ranging from 5 – 7. Autumn had a mean of 5.4 ranging from 4 – 6. Winter had a mean of 5.6 ranging from 5 – 6 while spring had a mean of 5.8 ranging from 4 – 7. In an order from high to low the mean ASPT for the season was; summer and spring > winter > autumn (Table 3.4: Figure 3.8).

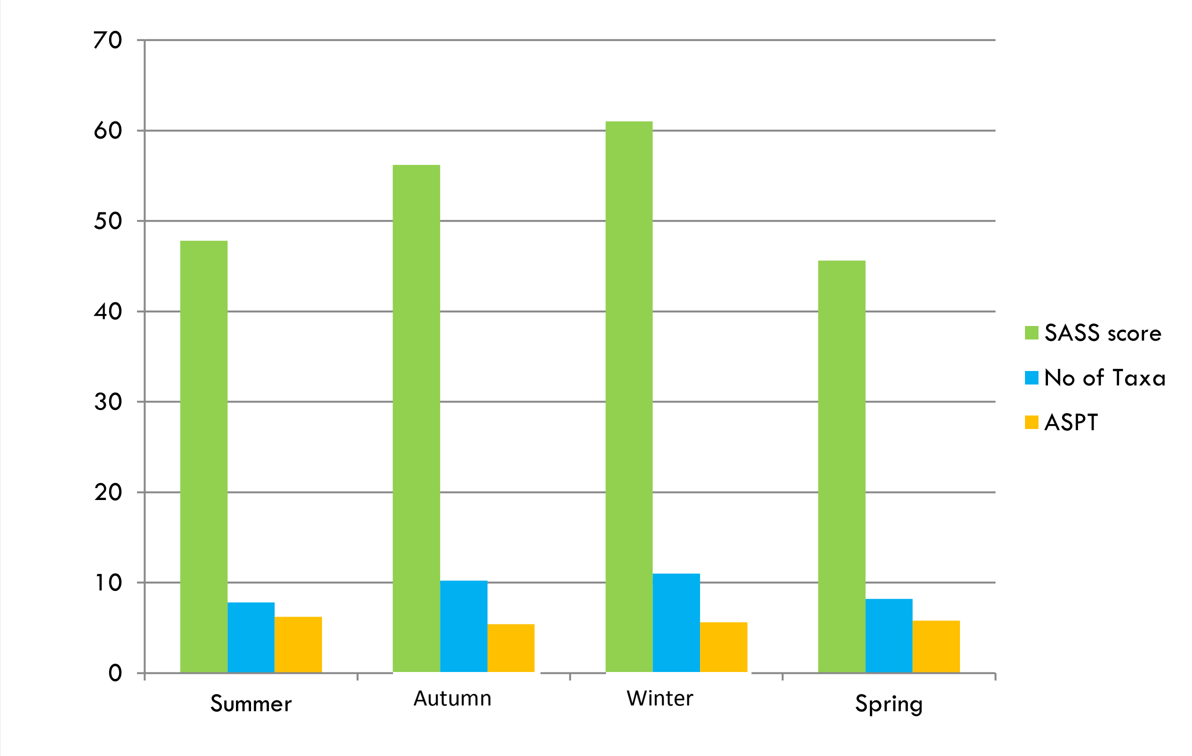

Figure 3.8: The mean SASS Scores, ASPT And No of Taxa for macroinvertebrates collected in the Steelpoort River.

Table 3.5: Interpretation of SASS results (from Chutter 1995)

SASS score	ASPT (Average Score per Taxon)	Interpretation
>100	>6	Water quality natural, habitat diversity high
<100	>6	Water quality natural, habitat diversity reduced
>100	<6	Borderline between water quality natural and some deterioration interpretation should be based on the extent by which the SASS exceeds 100 and the ASPT<6
50-100	<6	Some deterioration in water quality
<50	Variable	Major deterioration in water quality

3.3.4 CANONICAL CORRESPONDENCE ANALYSIS

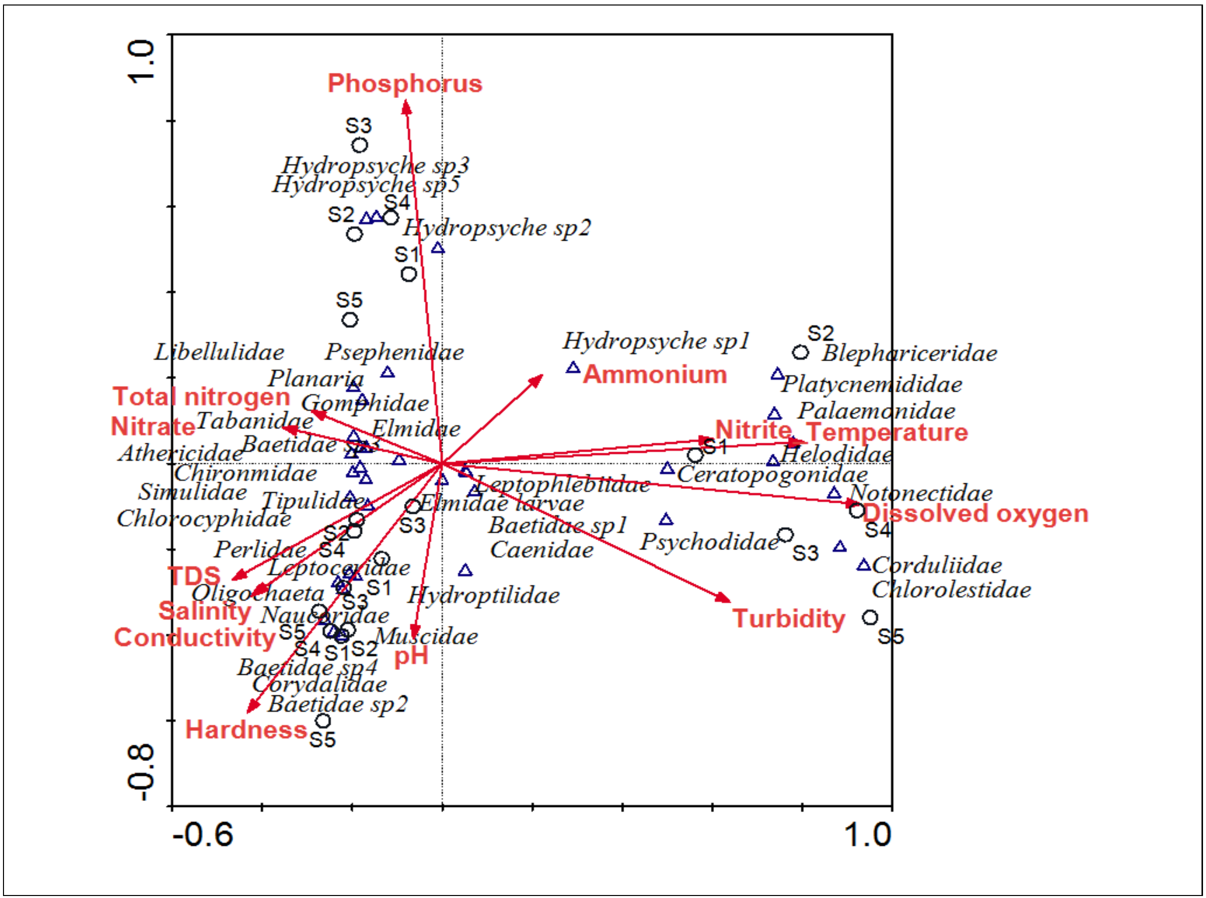

Figure 3.9: CCA plot of the relationship between water quality parameters and macroinvertebrates.

The community structure of benthic macroinvertebrates was well correlated to environmental factors in this study, DO, phosphorous temperature, turbidity and hardness were the most important predictors of benthic macro-invertebrate assemblage (Figure 3.9). The taxa-environment factor correlations (*r*) for factor 1 and factor 2 were 0.99 and 0.98 (Table 3.6). Most aquatic insects were distributed at the low phosphorous concentration and turbidity but higher DO.

The effects of pollution factors, total nitrogen and nitrate were correlated with the distribution of Athericidae, Tabanidae, Gomphidae, Baetidae, Elmidae, Planaria, Psephenidae, and Libellulidae. The conditions for total nitrogen and nitrate prevailed at S5 (Burgersfort Bridge). Salinity, conductivity, hardness, TDS and pH were correlated with Chironmidae, Simulidae, Tipulidae, Chlorocyphidae, Perlidae, Oligochaeta, Leptoceridae, Naucoridae, Baetidae, Corydalidae, Elmidae larvae, Hydroptilidae and Muscidae. The cumulative variation explained by the first two axes of the taxa–environment relationship in the CCA was 81.5%. This implies that the measured environmental variables were sufficient in explaining much of the variance in the benthic macro-invertebrate assemblage.

Table 3.6: The result of canonical correlation analysis between environmental factors and macroinvertebrates taxa of the Steelpoort River

Axes	1	2	3	4	Total Inertia
Eigenvalues	0.414	0.204	0.171	0.16	1.629
Taxa-environment correlations	0.995	0.976	0.954	0.931	
Cumulative percentage variance					
*of taxa data	25.4	38	48.5	58.3	
*of taxa-environment relation	32.7	48.8	62.3	74.8	
Sum of all eigenvalues					1.629
Sum of all canonical eigenvalues					1.268

Canonical correspondence analysis was used to determine the relationship between macroinvertebrates and sediment metals and metalloids. The distribution of taxa and factors loading on the plan of factor 1 and factor 2 is shown in Figure 3.10. The distribution of macroinvertebrates in the river was restricted mainly by iron and copper as the first factor. The taxa-environment factor correlations (*r*) for factors 1 and 2 were 0.93 and 0.99 respectively (Table 3.7). Results of the CCA show that Manganese, Cobalt, were the most important predictors of benthic macro-invertebrate assemblage structure. CCA axis 1 and axis 2 explained 29% and 16.5% of the total variation respectively. The effect of the pollution factors were mainly independent on the taxa distribution (Figure 3.7). The effect of the pollution factors such as iron, copper and titanium were more independent than other metals.

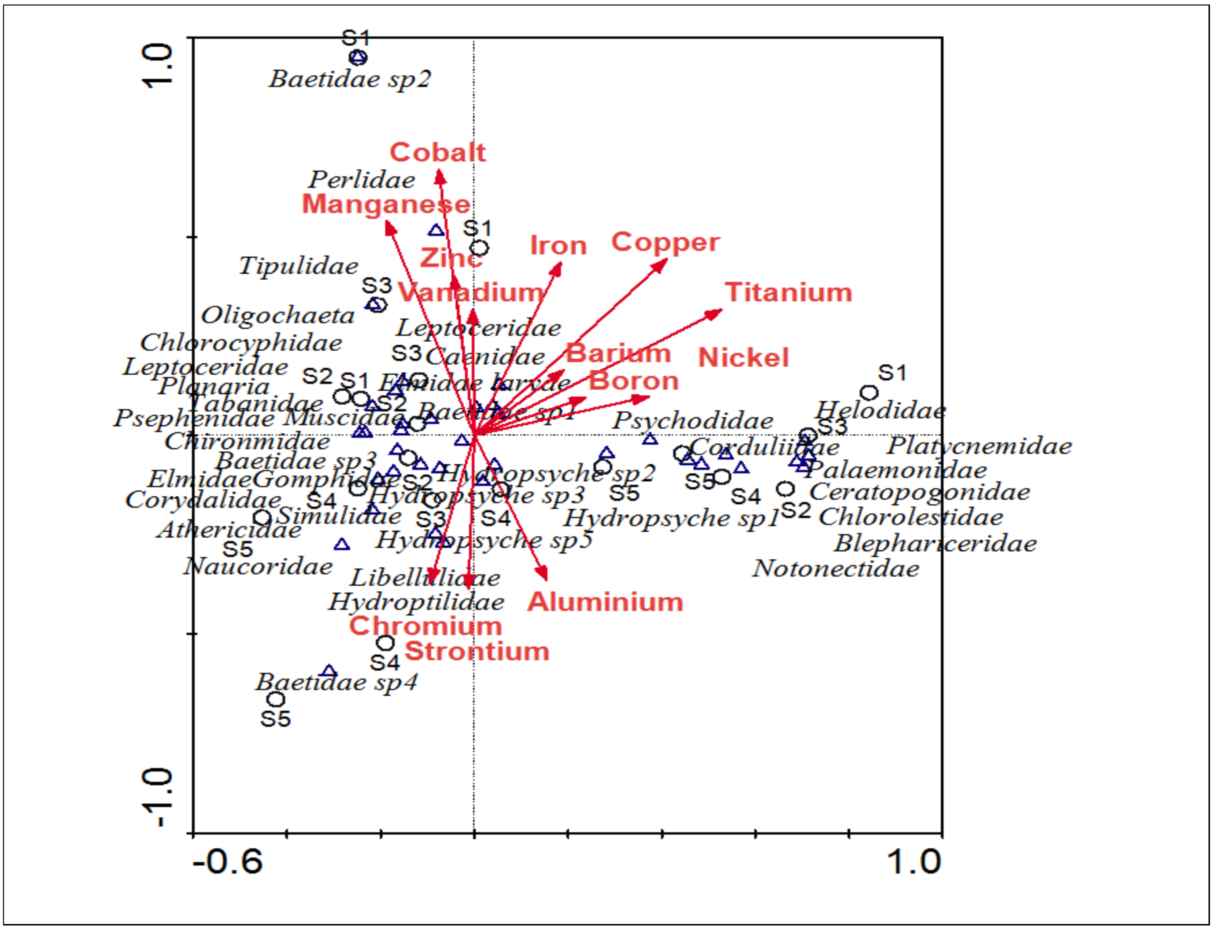

Figure 3.10: CCA plot of the relationship between macroinvertebrates and sediment bioaccumulation in the Steelpoort River.

Table 3.7: The result of canonical correlation analysis between environmental factors and macroinvertebrates taxa of the Steelpoort River

Axes	1	2	3	4	Total Inertia
Eigenvalues	1.1 0.349	1.2 0.198	1.3 0.162	1.4 0.138	1.5 1.629
Species-environment correlations	1.6 0.93	1.7 0.992	1.8 0.931	1.9 0.866	1.10
Cumulative percentage variance *of species data	1.11 21.4	1.12 33.5	1.13 43.5	1.14 52	1.15
*of species-environment relation	1.16 29	1.17 45.5	1.18 58.9	1.19 70.4	1.20
Sum of all eigenvalues					1.21 1.629
Sum of all canonical eigenvalues	1.22	1.23	1.24	1.25	1.26 1.202

3.4 DISCUSSION AND CONCLUSION

Freshwater organisms serve as continuous monitors of water quality and can detect sporadic disturbances and pollutants that enter as pulses, which is one of the most important advantages in using them for biomonitoring. Generally, the disturbance will take place during at least one stage (egg, larva, pupa and adult) of the invertebrate's life cycle. Changes will be detected in community structure when sampled at a later time, if one of the stages is susceptible to a particular disturbance (Relyea *et al.* 2000). Due to their limited ability to migrate, macroinvertebrates (especially aquatic insects) are widely used as bioindicators and are highly susceptible to environmental impacts (Oertli *et al.* 2008).

Diptera was the most diverse order with 10 families (Figure 3.1) but Ephemeroptera was the richest with 11332 individuals collected (Table 3.1). Steelpoort (Site 4) had the highest number of orders and families while Burgersfort Bridge (Site 5) had the least (Figure 3.2). Steelpoort (Site 4) having the most orders and families could be attributed to the nature of the site even though it is further downstream. That section of the river could be described as having multiple habitat types. It consisted of a section of fine gravel on the one end of the river bank, a muddy section on the opposite side, a rocky section near the middle of the river, boulder in the middle of the river and vegetation on banks of the river. These different habitats provide different niches for the macroinvertebrates found, hence the high numbers of orders and families. Burgersfort Bridge (Site 5) was visibly in a deteriorating state because of the sewage, cow excrement, and upstream pollution and the river bed was primarily made up of muddy silt embedded with boulders, hence the low number of orders and families. For the seasons we observed that spring had the highest number of orders and families while summer had the least (Figure 3.3). This may have been due to the water quality during summer.

Ephemeroptera larvae are commonly used as bioindicators in many monitoring programmes because they are recognized worldwide for their sensitivity to oxygen depletion in running waters (Menetrey *et al.* 2008). Considering the sites at the Steelpoort River, it was observed that Tiershoek (Site 1) had the highest number of individuals collected at this site throughout all the seasons. Ephemeroptera was the richest family contributing 75.1% of all the individuals collected (Table 3.1). These high numbers were because there were usually more than two species of Baetidae present during the surveys. This indicates that the water quality of the Steelpoort River at Tiershoek (Site 1) was relatively good (Dickens & Graham 2002b). Burgersfort Bridge (Site 5) had the lowest number of individuals collected throughout the study (Table 3.1). Ephemeroptera still contributed the most at this site but with fewer numbers and one Baetidae species during every season which is a clear indication of poor/deteriorating water quality (Dickens & Graham 2002b).

In terms of seasons, winter had the highest number of individuals collected and Ephemeroptera still had the highest number of individuals collected contributing 70.1% of the total from all the sites (Table 3.2). These results show that winter was the season with the best water quality conditions for even sensitive macro-invertebrates to thrive while summer was the least suitable season having the lowest number of individuals collected (Table 3.2) and the least number of orders and families (Figure 3.3) (Day *et al.* 2001). The poor water quality in summer could have come from run-off from the various anthropogenic activities especially mining and agriculture in the catchment. The poor water quality resulting from pollution can lead to lethal or sub lethal deterioration of aquatic organisms. The type of pollutant and its concentration determine the toxic effect (Gupta & Singh 2011).

The SIMPER analysis indicated the average similarity/dissimilarity of the sites and seasons. From the sites it was observed that they are less than 50% (Figure 3.5) similar to each other during all the four seasons. This means that every season produces slightly different macroinvertebrates (even though there were similar families encountered in all seasons). The variations in the macro-invertebrate populations could be due to the differences in seasonal water quality. Therefore, the number of individuals for each family would fluctuate depending on their adaptation to the prevailing seasonal conditions in the river. The SIMPER analysis for seasons showed that summer (Survey 1) had an AS of 51% (Figure 3.6) between the sites meaning that during summer all the five sites had macro-invertebrate families that were similar. The rest of the seasons were all below 50% leading to the conclusion that the species encountered differed slightly from season to season.

The dissimilarity between sites showed that the highest dissimilarity was between Tiershoek (Site 1) and Burgersfort Bridge (Site 5) with an AD of about 70% which is conclusive with the results from the macro-invertebrate count and SASS, indicating that there is a diverse difference from upstream to downstream. The highest AD of 79% between the seasons was between summer and winter which also coincides with SASS and macro-invertebrate counts. Thus, the most favourable season for most macroinvertebrates was winter and the least favourable was summer. The presence of mayflies, which is considered as ‘‘keystone’’ species, is believed to be an important environmental indicator of oligotrophic to mesotrophic (i.e. low to moderately productive) conditions in running waters (Barbour *et al.* 1999, Bauernfeind & Moog 2000). A high sensitivity has been demonstrated in both observational and experimental studies of mayfly taxa to oxygen depletion, acidification, and various contaminants including metals, ammonia and other chemicals (Hickey & Clements 1998, Menetrey *et al.* 2008). Ephemeroptera were observed to contribute a lot to similarity between the sites and the seasons (e.g. MDS plots through SIMPER analysis).

There are two general approaches to assess the pollutants and their toxic effects at different levels from species to community level of any ecosystem which are “active and passive monitoring”. In passive monitoring, at the level of individual’s accumulation of toxic substances in specimen, in organs and tissues indicative of pollution in the environment can be traced, while at the level of populations degradation of the ecosystem, elimination of sensitive species and reduction of biodiversity can be revealed as adverse consequences of pollution. In active monitoring the behavioral patterns of specimen, the response of artificial or modified populations, specific function of organs like movement, feeding, respiration, reproduction and the neural regulation, as well as cellular and subcellular events are studied under the effect of toxic substances (Gupta & Singh 2011). In this study passive monitoring was done whereby population degradation, elimination of sensitive species and reduction of biodiversity was revealed using the SASS 5 protocol and macro-invertebrate count (analysed using the Primer software).

Macroinvertebrates can point out changes in the environment ranging from the individual animal to the total invertebrate community through their responses at different levels of organization (Hodkinson & Jackson 2005). Different macro-invertebrate taxa tolerate organic pollution to a lesser or greater extent and that their differing responses can be used to indicate water quality conditions this has been recognized by monitoring methods. For example, in rivers, macro-invertebrate groups such as tubificid worms and particular species of chironomid midge larvae, survived under deoxygenated conditions verging on anoxia, whereas other taxa, such as larvae of Plecoptera and Ephemeroptera have been shown to be pollution intolerant (Rosenberg & Resh 1993, Reyers *et al.* 2000, Hodkinson & Jackson 2005).

From the results obtained by using the SASS 5 bio-assessment protocol it was observed that the mean SASS scores and the No of Taxa decrease from Tiershoek (Site 1) moving downstream to Burgersfort Bridge (Site 5) (Figure 3.7). A closer observation of the results reveals that the highest SASS score of 77 was recorded at Tiershoek (Site 1) during autumn while the lowest SASS score of 22 was recorded at Polopark (Site 3) during summer and Steelpoort (Site 4) during spring (Table 3.3: Table 3.4). The highest No of Taxa was 14 at Steelpoort (Site 4) during winter and lowest (4 taxa) at Polopark (Site 3) during summer. This implies that Tiershoek (Site 1) had more pollution sensitive families as compared to the other sites because it had a high mean No of Taxa the highest SASS score. Due to the presence of family Helodidae, Perlidae and more than two species of Baetidae and Hydropsychidae which are pollution sensitive with high SASS score of 12 Tiershoek (Site 1). Therefore, Tiershoek (Site 1) is in better water quality condition than the rest of sites, solely based on the SASS score. This indicates that there is a gradual decline in the water quality condition of the river from upstream (above the dam wall) to downstream because of the reduction in the No of Taxa and absence of more sensitive macro-invertebrate families downstream. The lowest mean SASS score was recorded at Burgersfort Bridge (Site 5) with a score of 41 ranging from 31 – 54. The mean No of Taxa was also lowest at Burgersfort Bridge (Site 5) with an average of 7.5 taxa, ranging from 6 – 9 (Table 3.3: Figure 3.7). The low mean SASS score at Burgersfort Bridge (Site 5) was due to the lack of many pollution sensitive species. The only sensitive species found was from the family Athericidae with a SASS score of 10 but the rest had SASS scores below 9. Therefore, this reach of the river was mostly made up of species which are more tolerable of pollution. During autumn the only pollution sensitive species were from the family Baetidae (>2sp) with a SASS score of 12 and Athericidae with a SASS score of 10. These two families could be the reason behind this season’s high SASS score, implying that during autumn the

Steelpoort River was less polluted as compared to the other seasons. On the contrary, the high No of Taxa could have also contributed to the higher SASS score. Spring had the lowest mean SASS score of 45.6 ranging from 22 – 73 (Table 3.4: Figure 3.8). This is because spring had one pollution sensitive species from the family Chlorocyphidae with a SASS score of 10 and the majority of species found during this season had a SASS score below 6. Therefore, spring had poor water quality conditions for macroinvertebrates. The season with the lowest mean No of Taxa was summer with 7.8 ranging from 4 – 9.

The ASPT mean recorded amongst the sites was highest at De Hoop (Site 3) with a value of 6.25 from a range of 6 – 7 and the lowest mean ASPT was 5 at Steelpoort (Site 4) ranging from 4 – 6. When using the interpretation of the SASS 5 results from Chutter 1995, the mean ASPT scores show that the water quality for Tiershoek (Site 1) and Polopark (Site 3) is natural but the habitat diversity has been reduced. For De Hoop (Site 2) and Steelpoort (Site 4), the mean ASPT score shows that there is some deterioration in the water quality. Finally, for Burgersfort Bridge (Site 5), the mean ASPT score indicates that there is major deterioration in the water quality (Table 3.3: Table 3.5: Figure 3.7).

Seasonally, the interpretation for mean ASPT score indicates that the water quality during summer and spring shows major deterioration as compared to that of autumn and winter. The lowest mean ASPT of 5.4 was recorded in autumn (ranged from 4 – 6) and the highest mean ASPT of 6.2 was recorded in summer (ranged from 5 – 7). Even though summer had the highest mean ASPT, the condition at the Steelpoort River was still poor due to the mean SASS score which is below 50 and it had the lowest mean No of taxa. Similar results were observed for spring (Table 3.4: Table 3.5: Figure 3.8).

For river health assessment obtaining the ASPT score is a more repeatable and consistent measure for a given reach of a river and/or for a particular period (Dickens & Graham 2002b). The ASPT shows that Tiershoek (Site 1) during autumn had natural water quality with reduced habitat diversity while Polopark (Site 3) during summer and Steelpoort (Site 4) during spring the water quality had deteriorated (Table 3.3: Table 3.4). Furthermore, emphasizing that the Steelpoort River water quality is worsening from upstream to downstream. The seasonal variations indicate that the water quality in the Steelpoort River were more favourable during autumn, followed by winter, then summer and finally spring (Figure 3.8).

Canonial correspondence axes 1 and 2, indicated high negative loading of DO, phosphorous temperature, turbidity and hardness. These parameters were associated with perlidae, oligochaeta, leptoceridae, naucoridae, baetidae sp 2 and baetidae sp 4, corydalidae, elmidae larvae, hydroptilidae and muscidae. The above species range from sensitive to tolerant species (SSAS 5). Perlidae is highly pollution sensitive species while oligochaete is highly pollution tolerant (Dickens and Graham 2002). Perlidae prevailed at S2 (below the dam) while oligochaete prevailed at S4 (further downstream). This is another indication that the condition in the Steelpoort River deteriorates from upstream to downstream. Though Site 4 (Steelpoort) had the highest number of orders and families and the second highest number of individuals collected, this has more to do with composition of the site itself and less to do with the physico-chemical parameters because it was the most versatile of all the sites in terms of habitat heterogeneity. The salinity, conductivity, hardness and TDS for this study were all below the TWQR with exception of pH which was elevated at all the site during the spring survey. At a pH above about 8, non-metallic ions are gradually converted to the highly toxic form e.g. un-ionized ammonia (NH^{3+}) (Dallas & Day 2004). Therefore, the species present in the Steelpoort River are representative of fair conditions.

For the sediment and macroinvertebrates, CCA axes 1 and 2, showed negative loadings of chromium and strontium which were associated with the following macroinvertebrates baetidae sp 4, hydroptilidae, libellulidae and hydropsyche sp 5. According to SASS 5 these species are pollution sensitive and they prevailed at S4. The presence of pollution sensitive macroinvertebrates such as those of the Ephemeropteran family, is an indication that water is suitable for sustaining pollution sensitive aquatic organisms including fish (Seanego and Moyo 2013). Strontium is a significant freshwater quality ion which contributes to water "hardness", in localities where it is elevated (ATSDR 2004). On the other hand, the toxicity of chromium to aquatic biota is notably influenced by abiotic variables such as hardness, temperature, pH, and salinity of water; and biological factors such as species, life stage, and potential differences in sensitivities of local populations (Eisler 1986, Dallas & Day 2004).. The rest of the metals and metalloids showed positive correlation on axes 1 and 2. They were associated with perlidae, caenidae, baetidae sp 1, leptoceridae, helodidae, psychodidae and hydropsyche sp 1. Of these, perlidae and helodidae were highly pollution tolerant, baetidae sp 1 and hydropsyche sp 1 were pollution sensitive while leptoceridae and caenidae were moderately pollution tolerant. The presence of all these macroinvertebrates implies that the conditions in the Steelpoort River are not uniform (some parts are more polluted then others).

In conclusion, the results showed that there is degradation of the water quality in river from upstream to downstream with more pollution tolerant species being abundant at Steelpoort (Site 4) and Burgersfort Bridge (Site 5). The most favourable season was autumn/winter and the least summer/spring and this may be attributed to the life cycle of the macro-invertebrate communities. Regardless, macroinvertebrates with the earliest response to the pollutants enabling them to indicate the presence and predict the consequences of undesirable anthropogenic effects are indicative of a good bioindicator (Gupta & Singh 2011).

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

4.2 PHYSICO-CHEMICAL VARIABLES

There were no significant differences in electrical conductivity, salinity, and TDS, among the sites ($p>0.05$). There were however, significant differences in the temperature, dissolved oxygen, and turbidity ($p<0.05$). The overall water temperature was normal with temperatures ranging from 15 ˚C – 27 ˚C with the highest records being taken in summer and the lowest in autumn, for the seasons, the site with lowest temperature was Tiershoek (Site 1) and the highest was Burgersfort Bridge (Site 5). Implying the water temperature increased as we moved further downstream. The solubility of dissolved oxygen in water may be reduced by higher temperatures, decreasing its concentration and thus its availability to aquatic organisms. Oxygen depletion may be further accelerated by greater microbial activity at the higher temperature, if the organic loading is high (Dallas & Day 2004). Dissolved oxygen was above the TWQR during all seasons, therefore, temperature at Steelpoort River were within the natural limits. The dissolved oxygen concentration in water may increase or decrease. The decrease in dissolved oxygen in aquatic ecosystems is usually observed and may have adverse effects on many aquatic organisms (e.g. micro-organisms, invertebrates and fish), for their normal functioning. Several other factors may affect the concentration of dissolved oxygen in water. The rate at which oxygen is dissolved in water may be modified by atmospheric pressure and salinity. The concentration of dissolved oxygen in the water is also affected by respiration by animals and plants, photosynthesis by plants, and aerobic decomposition of organic matter by micro-organisms (Dallas & Day 2004).

The pH recorded ranged from 7.8 to 9.5 (intermediate to alkaline). In alkaline conditions, metals such as aluminium occur as unavailable hydrated hydroxides (Campbell & Tessier 1987). Changes in pH may affect non-metallic ions such as ammonium. However, at a pH above 8, they are gradually converted to the highly toxic un-ionized ammonia (Dallas & Day 2004). The lowest pH levels were recorded in autumn then winter and the highest in spring then summer. The reason behind the high pH levels in spring and summer could be due to turnover because more biological activities occur in the catchment (Davies & Day 1998). The pH values in this study correlate to those previously recorded by the WRC whereby the pH was neutral to alkaline (Dallas 2009).

The EC, TDS, salinity and turbidity recorded were all within the TWQR, even though we observed very high increases in the turbidity during summer. Increased turbidity often results in a reduction in water temperature as more heat is reflected from the surface and less absorbed by the water therefore temperature-sensitive species may also be affected (Dallas & Day 2004). However, in the Steelpoort River it did not result in the reduction of water temperature. The elevated turbidity levels during summer may have resulted from the annual precipitation which drags soil from the river bank and upstream into the stream causing it to become more turbid. Previous studies have also indicated that the Steelpoort River has a high concentration of salts which results from urban and agricultural runoff (Dallas 2009).

The nutrients, nitrate and ammonium showed no significant variance between sites ($p>0.05$) while nitrite and phosphorus indicated significant variance between sites ($p<0.05$). The values of nitrate and nitrite (inorganic nitrogen) were all very low, indicative of oligotrophic conditions during all the seasons. The mean ammonium levels were all above the TWQR for DWAF and BC-EPD values. Ammonium ions dominate at low to medium pH values, but as pH increases ammonia is formed (Schubauer-Berigan *et al.* 1995), the latter being toxic to aquatic organisms. Therefore at low pH a high concentration of ammonium ions in water is not toxic, but if the pH is raised toxicity will develop (Dallas & Day 2004). These elevated ammonium levels can be attributed to dung from large herds of cattle seen around the river during sampling. Total nitrogen was too high throughout and the water is said to be in hypertrophic condition. Elevated nutrients concentration, when related to the total nitrogen may result in the increase/abundance of aquatic algae (DWAF 1996d). According to previous studies the Steelpoort River contributes nitrate to the overall load of the Olifants River as a result of the agricultural activities and small urban areas in the lower reaches (Dallas 2009). Phosphorus concentrations were high during autumn, indicative of hypertrophic conditions. Sediments act as a sink for P entering the stream at high concentrations from point sources during low-flow periods. Adsorbed P may be released from the sediments under high flow and/or anoxic conditions (Webster *et al*. 2001). High concentrations of total nitrogen and phosphorus may lead to eutrophication (DWAF 1996d).

The cations were all below the guideline values (BC-EPD 2008, CCME 2012b, US-EPA 2012a). The Statistical analysis indicated that all the cations except for Mg showed significant variance between sites ($p<0.05$). The Transvaal and the Drakensberg are made up of igneous rocks which usually contain sufficient calcium and magnesium that water flowing over or through them picks up measurable quantities of these elements, and of nutrients such as phosphates, nitrates and silicates. Hence, water affected by igneous rocks has a pH higher than 7 because it is usually dominated by calcium and/or magnesium cations and bicarbonate anions. Waters containing these variables are said to be "rock dominated" (Gibbs 1970). These conditions prevailed at Steelpoort River, hence, it could be said that the geology of the area had an impact on the conditions in the River.

For the majority of the physico-chemical parameters, the most elevated were detected downstream either at Steelpoort (Site 4) or Burgersfort Bridge (Site 5) and lowest concentrations were detected at De Hoop (Site 2, below the dam). All bedload sediment and all or part of the suspended load (depending upon the reservoir capacity relative to inflow) (Brune 1953) is deposited in the quiet water of the reservoir (reducing reservoir capacity) and upstream of the reservoir in reaches influenced by backwater, upstream of the dam. The water released from the dam possesses the energy to move sediment, but has little or no sediment load, downstream of the dam. This clear water released from the dam is often referred to as hungry water, because the excess energy is usually depleted on erosion of the channel bed and banks for some years following dam construction. The resulting effect is incision (downcutting of the bed) and coarsening of the bed material until equilibrium is reached and the material cannot be moved by the flows. Thus, the De Hoop Dam may be responsible for the low concentrations of materials/substances at Site 2. This indicates that the physico-chemical parameters increased from upstream to downstream. Since each parameter/constituent has an effect which is either beneficial or detrimental to aquatic biota, it is usually hard to determine the magnitude of the combined effect of the physico-chemical parameters (Dallas & Day 2004).

4.2 METALS IN THE WATER

Metals and metalloids that were detected in the Steelpoort River were the following; aluminium, boron, barium, cobalt, chromium, copper, iron, manganese, nickel, strontium, titanium, vanadium and zinc. The majority of the metals and metalloids in the water were all below detection level and the once that were detectable had low concentrations. Although, metals like aluminium, iron and manganese were detected in elevated levels, sometimes exceeding the ecologically acceptable standards. In a study by Polling (1995), similar results for manganese and iron were observed. The high Iron concentration recorded is expected because the Steelpoort River is surrounded by ferrochrome mines (Ballance *et al.* 2001). Autumn seemed to have the highest concentrations for most of the metals detected which correlates with literature because generally most accumulation happens in the warmer months of the year. However, there were no significant differences between the sites.

The majority of the metals and metalloids (Fe, Mn, V, Co, Zn, Ba, and Cu) were highest at Tiershoek (Site1) and lowest at Steelpoort (Site 4) and Burgersfort Bridge (Site 5). The general trend showed more elevated levels upstream as compared to downstream implying that most of the metals and metalloids are concentrated near the headwaters of the river. However, the levels that deviated from the norm may have been influenced by a number of factors such as; the geology or the bedrock, the physico-chemical characteristics, pollution, and/ or a combination of all these factors (Du Preez & Steyn 1992, Davies *et al.* 1993, DWAF 1996d, BC-EPD 2008, Whitehead *et al.* 2009, CCME 2012a, US-EPA 2012b).

4.3 METALS IN THE SEDIMENT

In decreasing order the metalsand metalloids detected in the sediment at the Steelpoort River were as follows; Fe>Al>Ti>Mn>Ni>V>Zn>Cr>Sr>Ba>Co>Cu>Pb>Sb>Ag. Metal concentrations were higher in the sediment than in the water. Antimony and silver were not detected in the water but were detected in sediment at low concentrations. For the sediment metals and metalloids, there were only significant differences in the Sr, Co, Cu, and V concentrations between the sites ($p<0.05$) and the rest indicated no significant differences ($p>0.05$). The mean metal concentrations collected showed elevated levels for chromium, copper and zinc, which were above the CCME sediment guidelines. The rest of metals and metalloid could not be stated because no guidelines exist for them except for lead which was below its guideline of 35 mg/kg (CCME 2012b). Titanium had elevated concentrations but there are no available guidelines. Nonetheless, the metals detected were mostly elevated during summer and lower in the spring with the exception of lead (Pb).

For the sites, the majority of the metals and metalloids Fe, Ti, Ba, Co, Cu, Pb, Mn, Ni, Sb, V, and Zn were highest at Tiershoek (Site 1) and lowest at Burgersfort Bridge (Site 5). The trend in the sediment also indicated a concentration gradient from upstream to downstream for the majority of the metals and metalloids. The anomaly with Ag, Al, Sr, and Cr may be attributed to a number of factors like the activities taking place in the surrounding catchment. The typically elevated levels in the sediment are not unusual because sediment serves as sink for metal contamination in aquatic ecosystems (Chapman *et al.* 1999). Sediment accumulates contaminants and pollutes the ecosystems that are associated with it. Hence, metal concentrations linked with sediment greatly surpass the concentrations dissolved in water, in most aquatic systems (Chapman *et al.* 1998, Ikem *et al.* 2003, Chon *et al.* 2012, Ciparis *et al.* 2012). The high concentrations of metals in sediment are an indication of the chronic nature of metal pollution.

4.4 MACROINVERTEBRATES

MACRO-INVERTEBRATE SPECIES ABUNDANCE AND DIVERSITY

Freshwater organisms serve as continuous monitors of water quality and can detect sporadic disturbances and pollutants that enter as pulses, which is one of the most important advantages in using them for biomonitoring. Generally, the disturbance will take place during at least one stage (egg, larva, pupa, adult) of the invertebrate's life cycle. Changes will be detected in community structure when sampled at a later time, if one of the stages is susceptible to a particular disturbance (Relyea *et al.* 2000). Due to their limited ability to migrate, macroinvertebrates (especially aquatic insects) are widely used as bioindicators and are highly susceptible to environmental impacts (Oertli *et al.* 2008).

Diptera was the most diverse order while Ephemeroptera was the richest. Steelpoort (Site 4) had the highest number of orders and families while Burgersfort Bridge (Site

5) had the least. Steelpoort (Site 4) having the most orders and families could be attributed to the conditions at the site. It has a multiple habitat types which provide different niches for the macroinvertebrates found, hence the high number of orders and families. Burgersfort Bridge (Site 5) was visibly in a deteriorating state because of the sewage, cow excrement, and pollution from upstream, hence the low number of orders and families. The density of benthic organisms may be decreased by an increase in the turbidity levels which have been shown to increase drift (i.e. the rate at which organisms move by floating downstream) (Ryder 1989 cited by Ryan 1991). Turbidity at this site was relatively high hence might have contributed to the low macro-invertebrate numbers at this site.

Ephemeroptera larvae are commonly used as bioindicators in many monitoring programs because they are recognized worldwide for their sensitivity to oxygen depletion in running waters (Menetrey *et al.* 2008). Considering the sites at the Steelpoort River, it was observed that Tiershoek (Site 1) had the highest number of individuals collected at this site throughout all the seasons. Ephemeroptera was the richest family of all the individuals collected. These high numbers were because there was usually more than two species of Baetidae present during the surveys. Therefore, this indicates that the water quality of the Steelpoort River at Tiershoek (Site 1) was relatively good (Dickens & Graham 2002b). Burgersfort Bridge (Site 5) had the lowest number of individuals collected throughout the study. Ephemeroptera still contributed the most at this site but with fewer numbers and one Baetidae species during every season which is a clear indication of poor/deteriorating water quality (Dickens & Graham 2002b).

Seasonal variations indicate that winter had the highest number of individuals collected and Ephemeroptera still had the highest number of individuals collected of the total from all the sites. These results show that winter was the season with the best water quality conditions for even sensitive macro-invertebrates to thrive while summer was the least suitable having the lowest number of individuals collected and the least number of orders and families (Day *et al.* 2001). The poor water quality in summer could have come from run-off from the various anthropogenic activities especially mining in the catchment. The poor water quality resulting from pollution can lead to lethal or sub lethal deterioration of aquatic organisms.

BRAY-CURTIS MEASURE ACROSS SITES AND SEASON SAMPLING UNIT

The SIMPER analysis (Primer software) indicated the average similarity/dissimilarity of the sites and seasons. From the sites it was observed that they are less than 50% similar to each other during all the four seasons. This means that every season produces slightly different macroinvertebrates. The variations in the macro-invertebrate populations could be due to the differences in seasonal water quality. Therefore, the number of individuals for each family would fluctuate depending on their adaptation to the prevailing seasonal conditions in the river. The SIMPER analysis for seasons showed that summer (Visit 1) had an AS of 51% between the sites meaning that during summer all the five sites had macro-invertebrate families that were at least 50% similar. The rest of the seasons were all below 50% leading to the conclusion that the species encountered differed gradually from season to season.

The dissimilarity between sites showed that the highest dissimilarity was between Tiershoek (Site 1) and Burgersfort Bridge (Site 5) which was conclusive with the results from the macro-invertebrate count and SASS, indicating that there is diverse difference from upstream to downstream. The highest AD of 79% between the seasons was between summer and winter which also agrees with SASS and macro-invertebrate counts. Thus, the most favourable season for most macroinvertebrates was autumn and the least favourable was summer.

SOUTH AFRICAN SCORING SYSTEM 5TH EDITION (SASS 5)

Macroinvertebrates can point out changes in the environment ranging from the individual animal to the total invertebrate community through their responses at different levels of organization (Hodkinson & Jackson 2005). Different macro-invertebrate taxa tolerate organic pollution to a lesser or greater extent and that their differing responses can be used to indicate water quality conditions. For example, in rivers, macro-invertebrate groups such as tubificid worms and particular species of chironomid midge larvae, survived under deoxygenated conditions verging on anoxia, whereas other taxa, such as larvae of Plecoptera and Ephemeroptera were shown to be pollution intolerant (Rosenberg & Resh 1993, Reyers *et al.* 2000, Hodkinson & Jackson 2005).

From the results obtained by using the SASS 5 bio-assessment protocol it was observed that the mean SASS scores and the No of Taxa decrease from Tiershoek (Site 1) moving downstream to Burgersfort Bridge (Site 5).The high SASS score at Site 1 was due to the presence of sensitive families; Helodidae, Perlidae and more than two species of Baetidae and Hydropsychidae. Therefore, Tiershoek (Site 1) is in better water quality condition than the rest of the sites. This indicates that there is a gradual decline in the water quality condition of the river from upstream (above the dam wall) to downstream because of the reduction in the No of Taxa and absence of more sensitive macro-invertebrate families downstream. The lower mean SASS score at Burgersfort Bridge (Site 5) was due to the lack of many pollution sensitive species. The season with the lowest mean No of Taxa was summer.

The mean ASPT scores show that the water quality for Tiershoek (Site 1) and Polopark (Site 3) is natural (Chutter 1995 interpretation of SASS results) but the habitat diversity has been reduced. For De Hoop (Site 2) and Steelpoort (Site 4), the mean ASPT score shows that there is some deterioration in the water quality. Finally, for Burgersfort Bridge (Site 5), the mean ASPT score indicates that there is major deterioration in the water quality. Seasonally, the interpretation for mean ASPT scores indicate that the water quality during summer and spring show major deterioration as compared to that of autumn and winter. The lowest mean ASPT was recorded in autumn and the highest mean ASPT was recorded in summer. Even though summer had the highest mean ASPT, the condition at the Steelpoort River was still poor due to the mean SASS score which is below 50 and it had the lowest mean No of taxa. For river health assessment obtaining the ASPT score is a more repeatable and consistent measure for a given reach of a river and/or for a particular period (Dickens & Graham 2002b). The ASPT shows that Tiershoek (Site 1) during autumn had good water quality with reduced habitat diversity while Polopark (Site 3) during summer and Steelpoort (Site 4) during spring the water quality had deteriorated (Table 3.3: Table 3.4). Furthermore, emphasizing that the Steelpoort River water quality is worsening from upstream to downstream. The seasonal variations indicate that the water quality in the Steelpoort River were more favourable during autumn, followed by winter, then summer and finally spring.

Canonial correspondence analysis indicated that the conditions of the Steelpoort River are deteriorating. The nutrients; nitrate, nitrite, ammonium, phosphorus and total nitrogen showed positive correlation to Hydropsyche sp 2,3 and 5, Libellulidae, Psephenidae, Planaria, Gomphidae, Elmidae, Tabanidae, Baetidae sp 3, Athericedia, Blephariceridae, Platycnemidae, Palaemonidae and Helodidae. According to SASS 5 the majority of these species are pollution sensitive except for Libellulidae, Planaria, Tabanidae and Baetidae sp 3 (Dickens & Graham 2005). However, these species were only found in very low numbers which implies that the nutrients may be having a negative impact on the community structure in the Steelpoort River. Even though many pollution sensitive families were collected from the Steelpoort pollution tolerant dominated. This is an indication that the conditions in the Steelpoort are being altered by pollutants throughout the whole river system because we observed the % of Ephemeropterans being highest from upstream to downstream.

In the sediment, the high presence of many pollution tolerant species such as Oligochaeta, Muscidae, Psychodidae, Tipulidae, Tabanidae, Baetidae sp (etc), further demonstrates the poor state of the Steelpoort River even though they were recorded in lower numbers. Thus, the result from the species diversity, primer and SASS there seems to be a gradual deterioration from upstream to downstream of the Steelpoort River.

4.5 CONCLUSION

The physico-chemical variables demonstrated that the overall effects of changes in the magnitude of more than one variable may be greater or lesser than the effect of each in isolation because each water quality variable has an effect on aquatic organisms (beneficial or detrimental) (Dallas & Day 2004). The general trend in the Steelpoort River demonstrated that the river is deteriorating from upstream to downstream. Each site was unique with respect to its composition of constituents (physico-chemical parameters, heavy metals and macroinvertebrates). The impact of the dam was noticeable because the sites below the dam demonstrated effects stated in literature. Dams and diversions are constructed and operated for a wide variety of purposes including flood and/or debris control; residential, commercial, and agricultural water supply; and hydropower production. All dams trap sediment to some degree and most alter the flood peaks and seasonal distribution of flows, thereby profoundly changing the character and functioning of rivers, regardless of their purpose (Kondolf *et al.* 2006). During low-flow conditions damming or abstraction may result in increased evaporation and consequently in increased concentrations of chemical constituents in natural waters (Dallas & Day 2004). This was evident for some of the chemical constituents which decreased during the high-flow conditions in the Steelpoort River.

4.6 RECOMMENDATIONS FOR FUTURE STUDIES

Not many studies have been conducted on the Steelpoort River catchment. Bio-monitoring of this river should be continued to increase the database. The sediment and water quality studies should be conducted more frequently to monitor the impact of the mines around that catchment (Steelpoort River) to safeguard from the deleterious effect that may occur. Such monitoring will aid in early warning systems from pollution and habitat alterations which will ensure that preventative measures be taken in good time to maintain the integrity of the Steelpoort River. Furthermore, database will help the future mitigations strategies for the whole Olifants River System.

Further diversity studies should be done to build a database on the species that are present. These results should be incorporated in the database of the Olifants River in the Limpopo Province. The control and monitoring of the contamination of pollutants in the Steelpoort River is vital.

The objectives of this study were to; 1) to assess the water and sediment quality in the Steelpoort River at five sites, 2) to determine the concentrations of metals in the water and sediment of the Steelpoort River, and 3) to assess the impact of the quality of water and sediment on the aquatic macro-invertebrate assemblages in the Steelpoort River. All these objectives have been fulfilled. Moving forward from here would be the continuous monitoring of this river system to prevent further deterioration in the water quality. The majority of the major ions, metals and metalloids in this study have not been properly evaluated at the Steelpoort River; hence it is imperative that continuous monitoring be conducted by re-sampling at various sites upstream and downstream at least twice annually.

In previous studies the water quality problems in these sub-areas of the middle-Olifants were salinity, eutrophication, toxicity and sediment. The salinity and eutrophication problems were due to the irrigation return flows and sewage treatment plant discharges. The toxicity problems have been related to the use of pesticides and herbicides in the irrigation schemes. The sediment is related to poor agricultural practise due to overgrazing in the rural areas. The extent of the toxicity problem needs to be determined through a monitoring program. The current irrigation return flow volumes and qualities need to be quantified and the changes that will occur if irrigation practises are changed in the future (DWAF 2004).

Further neglect to this tributary of the Olifants River may lead to more deterioration of the water quality and even extinction species that have not been document and may offer key ecological significance. The Olifants River has several large dams which serve as traps for sediment, nutrients, toxins, and heavy metals. These regions have been the epicentre for most of the recent mortalities in aquatic organisms. Therefore, in future studies should also focus on the accumulation of sediments and the interactive effects on benthic organisms, remobilisation of elements and microbiological activity near sediment-water interface (Dallas 2009). The metals that were recorded at elevated quantities in the sediment should continue being monitored and their effects on macroinvertebrates evaluated. Field biomonitoring in collaboration with government stakeholders should be implemented to determine the sources of pollution into the Steelpoort River and the impact it may have on the biological components of the river and the aquatic ecosystem as a whole. Future studies should also include functional feeding groups and bioaccumulation of macroinvertebrates as key stone species to present a more detailed account of the state of the river. Other aspects should include surveys of fish and other organisms to create a larger database, and the use of aquatic organisms in dams as sentinels of water quality problems. Eutrophication, algal blooms, changes in functional algal groups and its relation to the potential changes in the food chain and mortalities in aquatic species also require investigation (Dallas 2009). The Steelpoort River requires further assessment to clearly identify the condition of the river and document the impact of the activities taking place in the catchment.

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APPENDICES

APPENDIX A: WATER AND SEDIMENT QUALITY

Table A 1. 1: The seasonal water quality parameters at the Steelpoort River.

Water Quality Parameters	Summer					Autumn					Winter					Spring				
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5
Water temperature	23.6	26.4	26.9	26.2	27.3	15	19.9	20	16.5	17.2	17.1	17.2	15.4	15.8	19.8	23.8	14.3	15.3	10.5	16.2
Dissolved oxygen (mg/L O_2)	10.8	8.75	7.57	6.85	7.85	10.24	7.97	8.08	8.96	9.54	12.53	12.66	11.1	10.12	10.21	9.04	12.04	7.5	10.91	8.06
Dissolved oxygen (%)	116.3	95.4	89.4	91.4	90	114.2	96.4	97.6	94.38	106.7	146.1	145.5	121.6	113.5	120.9	118.2	126.3	100.7	113.2	101.7
pH	8.53	8.96	8.83	8.68	8.73	8.63	7.84	8.92	8.74	8.84	8.37	8.7	8.7	9	9.3	9.3	9.2	9.5	9	9.3
Conductivity (EC) mS/m	149.4	204	214.2	217.7	226.3	189.4	177.7	260.9	356.8	407.7	236.9	183.3	326	482	572	289.3	201.9	363	505	580
TDS mg/L	69.1	91.6	94.5	98.4	101.4	96.8	86	127.9	178.8	208	113.7	89.9	162.5	241	279	136.7	95.7	164.4	243	271
Salinity (‰)	0.07	0.09	0.09	0.1	0.13	0.09	0.08	0.12	0.17	0.2	0.11	0.09	0.16	0.23	0.27	0.13	0.09	0.16	0.23	0.26
Turbidity NTU	24	4	16	38	37	1.0	2	3	8	5	9	4	4	7	8	14	3	4	24	15
Nitrate (mg/L NO_3^-N)	0.97	0.54	0.8	0.87	0.78	1.0	1.71	0.92	1.72	3.58	1.12	0.5	1.1	2.1	3.6	<0.5	0.6	<0.5	1.1	1.4
Nitrite (mg/L NO_2^-N)	0.097	0.052	0.046	0.067	0.028	0.013	0.012	0.015	0.048	0.037	0.019	0.02	0.03	0.03	0.03	0.02	<0.010	0.02	0.04	0.03
Ammonium (mg/L NH_3^-N)	0.277	0.102	0.106	0.1	0.097	0.072	0.077	0.036	0.223	0.043	0.012	0.006	0.01	0.203	<0.010	0.032	0.058	0.043	0.24	0.052
Total Nitrogen	1.344	0.694	0.952	1.037	0.905	1.202	1.799	0.971	1.991	3.66	1.151	0.501	1.138	2.372	3.63	0.047	0.658	0.061	1.376	1.484
Phosphorus (mg/L P)	0.30	< 0.5	< 0.5	0.4	0.4	2.982	2.607	2.959	1.209	0.916	0.0	0.00	0.00	0.10	0.00	<0.010	0.00	0.00	0.2	0.00
Calcium (mg/L)	20	29	38	35	31	33	59	57	102	67	25.9	20.8	26.8	33.1	32.4	26.4	19.8	24.5	30.5	28.3
Magnesium (mg/L)	11	9	12	19	27	18	10	14	12	14	13	9.7	14.1	24.3	36.5	12.6	8.3	12.4	21.2	29.8
Potassium (mg/L)	<1.0	1.7	1.5	1.5	1.6	1.8	2.3	2.852	2.127	3	1.7	2.4	1.9	1.7	1.8	1.9	2.3	2	2.1	1.9
Sodium (mg/L)	9	8	17	24	25	8	17	11	16	11	15	12.3	42.9	65.8	69.4	12	8.2	22.6	35.8	35.2

Table A 1. 2: The seasonal metal and metalloids constituents at the Steelpoort River.

Metals and Metalloids	Summer					Autumn					Winter					Spring				
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5
Alumium	<0.100	0.143	0.106	0.315	0.513	49	91	73	136	78	0.025	0.011	0.015	0.018	0.017	<0.100	<0.100	<0.100	0.112	0.113
Boron	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025	0.000	0.001	0.003	0.014	0.017	0.007	0.011	0.018	0.030	0.028
Barium	0.031	0.029	0.026	0.031	0.031	0.175	0.186	0.180	0.152	0.138	0.080	0.018	0.021	0.022	0.023	0.040	0.026	0.026	0.033	0.035
Cobalt	<0.025	<0.025	<0.025	<0.025	<0.025	0.241	0.19	0.199	0.053	0.033	0.000	0.000	0.000	0.000	0.000	<0.010	<0.010	<0.010	<0.010	<0.010
Chromium	<0.025	<0.025	<0.025	<0.025	<0.025	0.138	0.184	0.251	0.292	0.268	0.000	0.000	0.000	0.001	0.002	<0.010	<0.010	<0.010	<0.010	<0.010
Copper	<0.025	<0.025	<0.025	<0.025	<0.025	0.079	0.056	0.048	<0.025	<0.025	0.001	0.001	0.001	0.001	0.001	<0.010	<0.010	<0.010	<0.010	<0.010
Iron	0.169	0.601	0.24	0.381	0.674	477	351	391	125	101	0.178	0.045	0.065	0.049	0.045	0.182	0.054	0.098	0.226	0.196
Manganese	<0.025	0.122	0.054	0.062	0.033	3.64	3.18	3.49	1.48	1.29	0.006	0.015	0.009	0.002	0.002	0.033	<0.025	0.026	0.027	<0.025
Nickel	<0.025	<0.025	<0.025	<0.025	<0.025	0.034	0.072	0.072	0.094	0.078	0.003	0.002	0.002	0.002	0.002	<0.010	<0.010	<0.010	<0.010	<0.010
Strontium	0.094	0.085	0.104	0.129	0.141	0.145	0.299	0.228	0.353	0.236	0.152	0.124	0.171	0.219	0.216	0.150	0.108	0.137	0.180	0.164
Titanium	<0.025	<0.025	<0.025	<0.025	<0.025	55	57	66	17	10	0.022	0.015	0.020	0.028	0.027	0.036	0.025	0.034	0.042	0.041
Vanadium	<0.025	<0.025	<0.025	<0.025	<0.025	2.51	2.5	2.41	0.799	0.464	0.003	0.002	0.002	0.037	0.033	<0.010	<0.010	<0.010	0.050	0.049
Zinc	<0.025	<0.025	<0.025	<0.025	<0.025	0.425	0.309	0.355	0.172	0.324	0.005	0.005	0.002	0.004	0.004	0.030	0.030	0.027	0.027	0.026

Table A 1. 3: The seasonal sediment metal and metalliods concentrations in the Steelpoort River.

Metals and Metalloids	Summer					Autumn					Winter					Spring				
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5
Aluminium	22210.3	31194.0	19808.6	84252.1	51481.9	21600	25200	39600	75600	33600	19600	36400	29200	54400	31200	38400	31600	30000	26400	41200
Antimony	0.9	0.7	4.5	2.5	1.5	<4.00	<4.00	<4.00	<4.00	<4.00	26	26	16	18	<4.00	<4.00	<4.00	<4.00	<4.00	<4.00
Barium	62.2	65.3	48.3	87.1	48.6	61	44	96	52	45	70	74	72	61	55	72	42	18	6.72	15
Cobalt	69.7	37.8	79.5	13.6	14.4	73	87	28	10	16	96	76	80	21	13	63	56	31	35	16
Chromium	81.5	66.3	137.9	77.3	136.0	58	217	116	59	136	55	74	100	117	107	66	92	75	178	153
Copper	76.7	52.8	58.5	17.8	16.1	43	38	57	7.49	14	32	22	19	<10	<10	75	51	30	24	13
Iron	297278.7	139736.4	313639.9	25741.5	40220.4	220400	290800	80000	27200	41200	190800	140400	156400	50000	40400	170400	157600	89600	133200	55200
Lead	2.9	2.6	6.5	4.4	3.3	<4.00	<4.00	5	<4.00	<4.00	<8.00	<8.00	<8.00	<8.00	<8.00	8.25	4.3	4.61	<4.00	<4.00
Manganese	1225.0	906.7	1231.0	294.0	336.0	1678	1658	1050	375	542	1455	1271	1394	591	514	1318	1333	700	986	528
Nickel	2065.0	998.7	2202.2	210.4	314.3	1351	1751	511	208	317	14	29	29	38	31	1532	1448	806	1189	502
Strontium	45.7	76.6	47.8	183.9	112.5	55	51	96	171	79	58	120	91	141	94	88	87	77	66	118
Silver	1.5	0.7	9.6	4.1	1.6	<4.00	<4.00	<4.00	<4.00	<4.00	<10	<10	<10	<10	<10	<4.00	<4.00	<4.00	<4.00	5
Titanium	59370.2	36360.4	52747.4	5102.4	10244.6	20000	24800	9200	4000	4800	22000	22800	26400	6800	4000	17600	18000	8800	17200	7200
Vanadium	1137.4	665.8	1423.1	76.8	110.3	884	2297	225	106	144	1002	1000	963	320	186	553	946	410	602	209
Zinc	194.1	90.1	220.8	36.9	44.0	182	226	64	24	34	170	124	142	69	130	182	133	99	117	35

APPENDIX B: MACROINVERTEBRATES BIOMONITORING

Table B 1. 1: The seasonal macro-invertebrate count in the Steelpoort River.

Taxa	Summer																								
	Tiershoek					De Hoop					Polapark					Steelpoort					Burgersfort bridge				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Baetidae 1sp	1	0	0	9	18	0	6	12	0	13	22	29	52	10	32	11	6	5	0	9	2	2	1	6	2
Baetidae 2sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae 3sp	17	0	22	79	84	0	0	0	0	0	22	0	0	0	15	0	0	0	0	0	0	0	0	0	0
Baetidae 4sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae 5sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
caenidae	7	6	11	32	25	46	94	52	21	66	4	31	40		25		12		4	3	5	17	21	8	10
telogonodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
leptophlebiidae	10	14	15	41	57	0	0	0	0	0	0	14	21	2	12	11	1	9	7	3	6	4	7	0	4
Hydropsychidae1	34	25	38	42	61	0	0	0	0	0	17	43	72	12	105	221	147	59	118	98	11	10	5	3	0
Hydropsychidae2	0	0	0	0	0	34	29	2	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
philopotamidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydroptilidae	0	2	0	4	1	2	3	0	1	0	0	0	0	0	0	1	0	2	2	1	1	0	2	0	1
Dytiscidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gyrinidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydraenidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae larvae	10	3	6	0	6	1	0	3	6	0	2	2	0	0	1	0	2	0	1	2	1	0	0	3	0
Helodidae	1	11	2	15	17	9	14	1	5	1	0	2	7	0	3	0	0	0	0	0	0	0	1	0	1

TABLE B 1.1: CONTINUES

Psephenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Naucoridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notonectidae	0	0	0	0	0	0	1	0	3	1	0	0	0	0	1	1	0	3	1	0	0	1	1	2	0
Corixidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Libellulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corduliidae	0	0	1	2	2	0	0	7	0	0	0	0	0	0	0	16	8	12	3	7	2	7	13	5	1
Aeshnidae	0	1	0	0	3	7	0	0	0	0	6	0	0	0	0	5	0	0	0	0	0	0	0	0	0
Gomphidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorocyphidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Platycnemididae	0	0	0	0	1	3	2	2	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Coenagrionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorolestidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	1	0	3	2	1	1	1	4
Athericidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blephariceridae	0	0	0	0	1	0	3	4	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psychodidae	3	3	2	4	2	5	0	5	8	3	0	15	18	0	17	16	11	18	9	22	4	1	0	2	1
Dixidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chirinomidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceratopogonidae	12	24	19	13	11	11	39	16	12	38	0	0	2	10	14	11	0	10	0	5	0	0	0	0	0
Muscidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tipulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corydalidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamonautidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palaemonidae	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Porifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oligochaeta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Physidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE B 1.1: CONTINUES

Lymnaeidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planorbidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaeriidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Taxa	Autumn																								
	Tiershoek					De Hoop					Polapark					Steelpoort					Burgersfort bridge				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Baetidae 1sp	48	182	212	69	142	0	0	0	0	0	7	0	2	3	0	0	19	3	28	0	0	5	0	0	0
Baetidae 2sp	2	8	4	3	14	6	212	52	23	48	0	54	82	0	0	0	0	0	73	0	0	0	0	0	0
Baetidae 3sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae 4sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae 5sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
caenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
telogonodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
leptophlebiidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae1	0	39	32	36	0	241	0	2	19	0	13	7	6	24	0	48	0	44	0	0	0	0	0	3	6
Hydropsychidae2	0	6	55	12	15	6	8	43	81	79	0	13	18	10	0	18		173	21	7	0	10	9	12	9
Hydropsychidae3	0	13	0	19	60	8	2	7	13	0	8	1	17	4	0	82	8	3	0	6	0	7	0	12	0
Hydropsychidae4	0	15	148	224	88	93	2	3	0	10	43	0	5	1	0	2	0	30	8	3	3	8	0	3	0
Hydropsychidae5	0	0	0	0	0	0	0	0	0	7	0	0	26	0	8	4	6	7	0	27	0	0	0	0	0
Hydropsychidae6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0
philopotamidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Hydroptilidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dytiscidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gyrinidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydraenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae	0	2	1	0	0	0	0	0	0	0	0	1	0	0	1	0	4	0	10	1	2	0	0	0	0
Elmidae larvae	0	0	2	0	0	2	0	0	19	0	1	0	1	2	1	0	0	0	5	0	0	0	0	0	0

TABLE B 1.1: CONTINUES

Helodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psephenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Naucoridae	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Notonectidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corixidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Libellulidae	0	0	0	0	0	0	1	0	9	3	0	6	0	9	0	3	4	4	12	7	3	1	3	4	4
Corduliidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aeshnidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gomphidae	0	0	0	0	0	16	5	14	0	5	2	2	2	4	1	6	4	6	19	19	1	3	1	10	3
Chlorocyphidae	0	0	0	0	0	6	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Platycnemididae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coenagrionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorolestidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	0	1	0	0	0	1	0	0	1	6	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
Blephariceridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	0	2	5	5	1	50	5	44	11	51	8	7	5	9	19	7	14	35	44	9	3	0	2	0	2
Psychodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dixidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chirinomidae	0	74	149	236	255	28	16	27	10	17	8	15	24	14	62	31	25	29	101	22	23	5	5	51	17
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
Muscidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Tipulidae	0	4	3	1	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Simulidae	0	9	4	10	0	6	0	11	4	7	1	0	2	20	17	44	83	28	151	22	62	8	7	6	4
Perlidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corydalidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamonautidae	0	1	0	0	0	0	2	0	1	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Palaemonidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Porifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oligochaeta	0	0	0	0	0	5	1	1	3	5	3	0	0	0	0	0	0	6	1	0	0	0	0	0	0

TABLE B 1.1: CONTINUES

Physidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymnaeidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planorbidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaeriidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Taxa	Winter																								
	Tiershoek					De Hoop					Polapark					Steelpoort					Burgersfort bridge				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Baetidae 1sp	47	194	469	29	23	25	16	0	7	19	0	0	3	0	4	0	0	6	2	1	38	0	3	0	0
Baetidae 2sp	226	582	241	468	798	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae 3sp	0	0	0	0	0	0	0	0	184	211	2	132	66	48	135	0	50	0	0	0	3	2	0	0	0
Baetidae 4sp	0	0	0	0	0	0	0	58	0	0	78	0	0	0	0	24	0	89	43	52	8	142	76	25	15
Baetidae 5sp	51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
caenidae	48	121	79	47	189	45	43	47	35	38	7	9	10	20	7	2	2	0	0	0	14	3	10	0	0
telogonodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
leptophlebiidae	64	240	139	143	151	0	0	0	0	0	10	34	22	8	24	4	1	0	1	1	0	3	4	0	0
Hydropsychidae1	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae4	0	0	0	0	0	5	5	15	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
philopotamidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	0	8	0	0	4	2	3	0	5	0	0	0	0	2	0	2	0	0	3	0	0	0	2	0	0
Hydroptilidae	3	0	0	0	0	0	0	0	0	0	3	0	1	1	0	1	25	17	3	4	0	9	18	5	2
Dytiscidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gyrinidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydraenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elmidae	0	0	0	0	3	0	0	0	0	0	0	0	0	1	0	0	1	5	0	5	0	0	0	0	1

TABLE B 1.1: CONTINUES

Elmidae larvae	7	1	1	2	3	2	0	1	4	11	3	11	1	2	4	1	0	8	1	0	0	0	0	0	0
Helodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psephenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Naucoridae	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	6	2	3	0	0	0	0	1
Notonectidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corixidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Libellulidae	0	0	0	0	0	0	0	0	0	0	3	0	0	5	0	0	0	9	0	1	0	2	5	2	0
Corduliidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aeshnidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Gomphidae	0	1	0	1	0	2	2	1	7	2	4	8	8	2	7	6	3	1	2	2	2	3	5	1	1
Chlorocyphidae	0	0	0	0	1	1	1	0	0	4	0	3	0	0	1	0	0	0	0	0	1	0	0	0	0
Platycnemididae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coenagrionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chlorolestidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	6	5	8	3	5
Blephariceridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	6	12	9	7	8	6	6	13	25	13	11	12	8	2	15	10	5	5	4	5	1	1	7	4	0
Psychodidae	1	0	0	2	1	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Dixidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chirinomidae	71	28	26	23	22	33	26	32	7	8	124	85	14	16	72	115	46	39	29	16	49	88	103	32	48
Ceratopogonidae	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3	6	3	0	0	0	0	0
Muscidae	0	0	0	1	3	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	3	2	0	2	0
Tipulidae	1	1	1	1	7	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Simulidae	23	12	3	4	5	2	0	0	12	21	1	3	0	0	1	8	86	336	6	66	3	9	24	26	24
Perlidae	1	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corydalidae	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	1	0	0	0	0	0
Potamonautidae	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palaemonidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Porifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE B 1.1: CONTINUES

Oligochaeta	0	0	0	0	0	1	0	1	0	0	0	0	1	10	0	1	0	0	0	0	4	0	0	0	0
Physidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymnaeidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planorbidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaeriidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0
Taxa	Spring																								
	Tiershoek					De Hoop					Polapark					Steelpoort					Burgersfort Bridge				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Baetidae 1sp	0	121	0	1	2	31	152	178	136	96	0	2	2	0	1	3	1	0	1	0	73	43	0	89	0
Baetidae 2sp	0	0	0	0	0	0	0	44	0	0	0	0	0	51	0	0	0	0	0	0	0	0	0	0	0
Baetidae 3sp	0	6	0	5	0	35	0	0	0	0	3	0	0	0	0	2	4	0	0	0	0	0	0	0	0
Baetidae 4sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae 5sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
caenidae	111	100	52	187	82	34	20	16	49	21	5	3	12	26	7	0	0	0	0	0	0	0	0	0	0
telogonodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
leptophlebiidae	212	235	44	0	121	0	0	0	0	0	3	1	4	11	4	1	0	0	0	0	0	0	0	0	0
Hydropsychidae1	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae2	0	0	0	0	6	0	0	0	0	0	7	3	0	0	0	0	0	0	0	0	0	0	1	0	0
Hydropsychidae3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	3	0	0	0	0	0	0	0	0
Hydropsychidae4	0	0	0	0	0	30	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Hydropsychidae6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
philopotamidae	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	0	0	0	0	0	0	2	2	1	0	0	0	0	1	0	0	0	0	0	1	0	3	2	0	0
Hydroptilidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2
Dytiscidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gyrinidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydraenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE B 1.1: CONTINUES

Elmidae	1	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	1	4	0	4	0	1	0	0	0
Elmidae larvae	2	7	2	16	2	3	11	12	5	8	3	0	0	18	3	0	4	15	0	60	0	0	0	0	0
Helodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psephenidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Naucoridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	1	0	0	0	3	2
Notonectidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corixidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Libellulidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
Corduliidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aeshnidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0
Gomphidae	1	0	1	0	0	13	2	3	1	1	1	0	0	0	0	0	0	9	1	15	0	0	0	0	0
Chlorocyphidae	0	0	0	0	0	0	2	0	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Platycnemididae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coenagrionidae	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Chlorolestidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Athericidae	0	0	0	0	0	0	2	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blephariceridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tabanidae	0	2	1	3	2	12	21	15	21	12	2	1	6	3	3	10	0	2	0	8	2	0	1	0	0
Psychodidae	0	0	1	0	1	0	0	1	2	3	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
Dixidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chirinomidae	52	41	78	88	59	14	24	21	58	18	13	12	22	11	44	1	0	3	4	27	8	7	0	0	10
Ceratopogonidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muscidae	0	0	0	0	0	2	2	5	3	5	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Tipulidae	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Simulidae	0	1	0	0	0	2	0	25	14	2	0	0	0	2	0	0	1	7	0	34	0	18	19	0	0
Perlidae	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corydalidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Potamonautidae	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Palaemonidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE B 1.1: CONTINUES

Porifera	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oligochaeta	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Physidae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymnaeidae	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planorbidae	0	0	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaeriidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0

Table B 1. 2: The presence and absence of macro-invertebrate families at each site in the Steelpoort River.

	Site 1 Tiershoek			
	Feb 2014	May 2014	Aug 2014	Oct 2014
Taxon				
Emphemeroptera				
Baetidae	**X**	**X**	**x**	**X**
Caenidae	**X**	**X**	**x**	**X**
Polymitarcyidae				
Heptageniidae				
Telogonodidae				
Oligoneuridae				
Prosopistomatidae				
Leptophlebiidae	**X**	**X**	**x**	**X**
Tricorythidae				
Trichoptera				
Hydropsychedae	**X**	**X**	**x**	
Macrostemum capense				
Cheumatopsyche afra type				
Amphisyche scottae				
Cheumatopsyche thomasseti type				
Polymorphanisus bipunctatus				
Aethaloptera maxima				
Polycentropodidae				
Philopotamidae				
Ecomidae				
Psychomyiidae				
Paracnomina				
Cassed caddisflies				
Petrothrincidae				
Leptoceridae			**x**	**X**
Hydroptilidae	**X**	**X**	**x**	
Barbarochthonidae				
Pisuliidae				
Sericostomatidae				
Glossosomatidae				
Coleoptera				
Dytiscidae				
Dytiscidae larvae				
Gyrinidae			**x**	
Gyrinidae larva				
Hydraenidae	**X**			
Elmidae		**X**	**x**	**X**
Elmidae larvae	**X**	**X**	**x**	**X**
Helodidae	**X**			
Psephenidae		**X**		**X**
Hydrophilidae				
Hemiptera				
Naucoridae				
Notonectidae				
Belostomatidae				
Gerridae				
Hydrometridae				
Corixidae				
Veliidae				
Nepidae				

Pleidae				
Odonata				
Libellulidae				
Corduliidae	**X**			
Aeshnidae				
Gomphidae	**X**		**x**	**x**
Zygoptera				
Calopterygidae				
Chlorocyphidae			**x**	
Platycnemididae	**X**			
Coenagrionidae				
Chlorolestidae				
Lestidae				
Diptera				
Athericidae		**X**		
Blephariceridae	**X**			
Culicidae				
Tabanidae		**X**	**x**	**x**
Psychodidae	**X**		**x**	**x**
Dixidae	**X**			
Chironmidae		**X**	**x**	**x**
Ceratopogonidae	**X**		**x**	
Muscidae larva			**x**	
Muscidae pupa				
Ephydridae				
Syrphidae				
Tipulidae		**X**	**x**	**x**
Simulidae		**X**	**x**	**x**
Turbellaria				
Planaria			**x**	**x**
Plecoptera				
Perlidae			**x**	**x**
Notonemouridae				
Lepidoptera				
Pyralidae				
Hyracarina				
Hydrachnellae				
Megaloptera				
Corydalidae			**x**	
Crustacea				
Potamonautidae		**X**		
Palaemonidae				
Amphipoda				
Atyidae				
Porifera				
Porifera				
Annelida				
Hirudinea				
Oligochaeta				
Mollusca				
Physidae				
Lymnaeidae				
Planorbidae				
Ancylidae				
Sphaeriidae				

Appendix B: Macro-invertebrate families collected at Steelpoort River throughout the sampling period (May 2014 to January 2015) at site 1. "**X**" denotes family presence.

TABLE B 1.2: CONTINUES

	Site 2 De Hoop			
	Feb 2014	May 2014	Aug 2014	Oct 2014
Taxon				
Emphemeroptera				
Baetidae	X	x	x	x
Caenidae	X	x	x	x
Polymitarcyidae				
Heptageniidae				
Telogonodidae				
Oligoneuridae				
Prosopistomatidae				
Leptophlebiidae		x	x	
Tricorythidae				
Trichoptera				
Hydropsychedae	X	x		
Macrostemum capense				
Cheumatopsyche afra type				
Amphisyche scottae				
Cheumatopsyche thomasseti type				
Polymorphanisus bipunctatus				
Aethaloptera maxima				
Polycentropodidae				
Philopotamidae				x
Ecomidae				
Psychomyiidae				
Paracnomina				
Cassed caddisflies				
Petrothrincidae				
Leptoceridae		x	x	x
Hydroptilidae	X			
Barbarochthonidae				
Pisuliidae				
Sericostomatidae				
Glossosomatidae				
Coleoptera				
Dytiscidae				
Dytiscidae larvae				
Gyrinidae				
Gyrinidae larva				
Hydraenidae				
Elmidae				
Elmidae larvae	x	x	x	x
Helodidae	x			
Psephenidae				
Hydrophilidae				
Hemiptera				
Naucoridae		x	x	
Notonectidae	x			
Belostomatidae				
Gerridae				
Hydrometridae				
Corixidae				
Veliidae				
Nepidae				

Pleidae				
Odonata				
Libellulidae		x		
Corduliidae				
Aeshnidae				
Gomphidae		x	x	x
Zygoptera				
Calopterygidae				
Chlorocyphidae		x	x	x
Platycnemididae	x			
Coenagrionidae				
Chlorolestidae				
Lestidae				
Diptera				
Athericidae		x	x	x
Blephariceridae	x			
Culicidae				
Tabanidae		x	x	x
Psychodidae	x		x	x
Dixidae				
Chironmidae		x	x	x
Ceratopogonidae	x			
Muscidae larva				
Muscidae pupa			x	x
Ephydridae				
Syrphidae				
Tipulidae		x		
Simulidae		x	x	x
Turbellaria				
Planaria		x	x	
Plecoptera				
Perlidae				
Notonemouridae				
Lepidoptera				
Pyralidae				
Hyracarina				
Hydrachnellae				
Megaloptera				
Corydalidae				
Crustacea				
Potamonautidae		x	x	x
Palaemonidae	x			
Amphipoda				
Atyidae				
Porifera				
Porifera				x
Annelida				
Hirudinea				
Oligochaeta			x	x
Mollusca				
Physidae				x
Lymnaeidae				x
Planorbidae				x
Ancylidae				
Sphaeriidae				

Appendix B: Macro-invertebrate families collected at Steelpoort River throughout the sampling period (May 2014 to January 2015) at site 1. "**X**" denotes family presence.

TABLE B 1.2: CONTINUES

	Site 3 Polopark			
	Feb 2014	May 2014	Aug 2014	Oct 2014
Taxon				
Emphemeroptera				
Baetidae	x	x	x	x
Caenidae	x	x	x	x
Polymitarcyidae				
Heptageniidae				
Telogonodidae				
Oligoneuridae				
Prosopistomatidae				
Leptophlebiidae	x	x	x	x
Tricorythidae				
Trichoptera				
Hydropsychedae	x	x		
Macrostemum capense				
Cheumatopsyche afra type				
Amphisyche scottae				
Cheumatopsyche thomasseti type				
Polymorphanisus bipunctatus				
Aethaloptera maxima				
Polycentropodidae				
Philopotamidae				
Ecomidae				
Psychomyiidae				
Paracnomina				
Cassed caddisflies				
Petrothrincidae				
Leptoceridae			x	x
Hydroptilidae			x	
Barbarochthonidae				
Pisuliidae				
Sericostomatidae				
Glossosomatidae				
Coleoptera				
Dytiscidae				
Dytiscidae larvae				
Gyrinidae				
Gyrinidae larva				
Hydraenidae				
Elmidae		x	x	x
Elmidae larvae	x	x	x	x
Helodidae	x			
Psephenidae				
Hydrophilidae				
Hemiptera				
Naucoridae				
Notonectidae	x			
Belostomatidae				
Gerridae				
Hydrometridae				
Corixidae			x	
Veliidae				
Nepidae				

Pleidae				
Odonata				
Libellulidae		x		
Corduliidae				
Aeshnidae			x	
Gomphidae		x	x	x
Zygoptera				
Calopterygidae				
Chlorocyphidae		x	x	
Platycnemididae	x			
Coenagrionidae				x
Chlorolestidae				
Lestidae				
Diptera				
Athericidae			x	
Blephariceridae				
Culicidae				
Tabanidae		x	x	x
Psychodidae	x		x	x
Dixidae				
Chironmidae		x	x	x
Ceratopogonidae	x			
Muscidae larva				
Muscidae pupa				
Ephydridae				
Syrphidae				
Tipulidae		x		
Simulidae		x	x	x
Turbellaria				
Planaria		x	x	
Plecoptera				
Perlidae				
Notonemouridae				
Lepidoptera				
Pyralidae				
Hyracarina				
Hydrachnellae				
Megaloptera				
Corydalidae				
Crustacea				
Potamonautidae			x	
Palaemonidae	x			
Amphipoda				
Atyidae				
Porifera				
Porifera				
Annelida				
Hirudinea				
Oligochaeta			x	x
Mollusca				
Physidae				
Lymnaeidae				
Planorbidae				
Ancylidae				
Sphaeriidae				

Appendix B: Macro-invertebrate families collected at Steelpoort River throughout the sampling period (May 2014 to January 2015) at site 1. "**X**" denotes family presence.

TABLE B 1.2: CONTINUES

	Site 4 Steelpoort			
	Feb 2014	May 2014	Aug 2014	Oct 2014
Taxon				
Emphemeroptera				
Baetidae	**x**	**x**	**x**	**x**
Caenidae	**x**	**x**	**x**	**x**
Polymitarcyidae				
Heptageniidae				
Telogonodidae				**x**
Oligoneuridae				
Prosopistomatidae				
Leptophlebiidae	**x**	**x**	**x**	**x**
Tricorythidae				
Trichoptera				
Hydropsychedae	**x**	**x**		
Macrostemum capense				
Cheumatopsyche afra type				
Amphisyche scottae				
Cheumatopsyche thomasseti type				
Polymorphanisus bipunctatus				
Aethaloptera maxima				
Polycentropodidae				
Philopotamidae				
Ecomidae				
Psychomyiidae				
Paracnomina				
Cassed caddisflies				
Petrothrincidae				
Leptoceridae		**x**	**x**	**x**
Hydroptilidae	**x**		**x**	**x**
Barbarochthonidae				
Pisuliidae				
Sericostomatidae				
Glossosomatidae				
Coleoptera				
Dytiscidae				**x**
Dytiscidae larvae				
Gyrinidae				
Gyrinidae larva				
Hydraenidae				
Elmidae		**x**	**x**	**x**
Elmidae larvae	**x**	**x**	**x**	**x**
Helodidae				
Psephenidae				**x**
Hydrophilidae				
Hemiptera				
Naucoridae			**x**	**x**
Notonectidae	**x**			
Belostomatidae				
Gerridae				
Hydrometridae				
Corixidae				
Veliidae				
Nepidae				

Pleidae				
Odonata				
Libellulidae		**x**	**x**	**x**
Corduliidae	**x**			
Aeshnidae				**x**
Gomphidae		**x**	**x**	**x**
Zygoptera				
Calopterygidae				
Chlorocyphidae				
Platycnemididae				
Coenagrionidae		**x**		
Chlorolestidae	**x**			
Lestidae				
Diptera				
Athericidae		**x**		
Blephariceridae				
Culicidae				
Tabanidae		**x**	**x**	**x**
Psychodidae	**x**		**x**	**x**
Dixidae				
Chironmidae		**x**	**x**	**x**
Ceratopogonidae	**x**	**x**	**x**	
Muscidae larva		**x**	**x**	
Muscidae pupa				
Ephydridae				
Syrphidae				
Tipulidae			**x**	**x**
Simulidae		**x**	**x**	**x**
Turbellaria				
Planaria				
Plecoptera				
Perlidae		**x**		
Notonemouridae				
Lepidoptera				
Pyralidae				
Hyracarina				
Hydrachnellae				
Megaloptera				
Corydalidae			**x**	
Crustacea				
Potamonautidae		**x**		**x**
Palaemonidae				
Amphipoda				
Atyidae				
Porifera				
Porifera				
Annelida				
Hirudinea				
Oligochaeta			**x**	
Mollusca				
Physidae				
Lymnaeidae				
Planorbidae				
Ancylidae				
Sphaeriidae				**x**

Appendix B: Macro-invertebrate families collected at Steelpoort River throughout the sampling period (May 2014 to January 2015) at site 1. “**X**” denotes family presence.

TABLE B 1.2: CONTINUES

	Site 5 Burgersfort Bridge			
	Feb 2014	May 2014	Aug 2014	Oct 2014
Taxon				
Emphemeroptera				
Baetidae	x	x	x	x
Caenidae	x	x	x	x
Polymitarcyidae				
Heptageniidae				
Telogonodidae				
Oligoneuridae				
Prosopistomatidae				
Leptophlebiidae	x	x	x	
Tricorythidae				
Trichoptera				
Hydropsychedae	x	x		
Macrostemum capense				
Cheumatopsyche afra type				
Amphisyche scottae				
Cheumatopsyche thomasseti type				
Polymorphanisus bipunctatus				
Aethaloptera maxima				
Polycentropodidae				
Philopotamidae				
Ecomidae				
Psychomyiidae				
Paracnomina				
Cassed caddisflies				
Petrothrincidae				
Leptoceridae			x	x
Hydroptilidae	x		x	x
Barbarochthonidae				
Pisuliidae				
Sericostomatidae				
Glossosomatidae				
Coleoptera				
Dytiscidae				
Dytiscidae larvae				
Gyrinidae				
Gyrinidae larva				
Hydraenidae				
Elmidae		x	x	
Elmidae larvae	x			
Helodidae	x			
Psephenidae				
Hydrophilidae				
Hemiptera				
Naucoridae			x	x
Notonectidae	x			
Belostomatidae				
Gerridae				
Hydrometridae				
Corixidae				
Veliidae				

Nepidae				
Pleidae				
Odonata				
Libellulidae		x	x	
Corduliidae	x			
Aeshnidae				
Gomphidae		x	x	
Zygoptera				
Calopterygidae				
Chlorocyphidae			x	
Platycnemididae				
Coenagrionidae				
Chlorolestidae	x			
Lestidae				
Diptera				
Athericidae			x	
Blephariceridae				
Culicidae				
Tabanidae		x	x	x
Psychodidae	x			
Dixidae				
Chironmidae		x	x	x
Ceratopogonidae				
Muscidae larva				
Muscidae pupa			x	x
Ephydridae				
Syrphidae				
Tipulidae			x	
Simulidae		x	x	x
Turbellaria				
Planaria				
Plecoptera				
Perlidae				
Notonemouridae				
Lepidoptera				
Pyralidae				
Hyracarina				
Hydrachnellae				
Megaloptera				
Corydalidae				
Crustacea				
Potamonautidae				
Palaemonidae				
Amphipoda				
Atyidae				
Porifera				
Porifera				
Annelida				
Hirudinea				
Oligochaeta			x	
Mollusca				
Physidae				
Planorbidae				
Ancylidae				
Sphaeriidae				

Appendix B: Macro-invertebrate families collected at Steelpoort River throughout the sampling period (May 2014 to January 2015) at site 1. "**X**" denotes family presence.