Impact of anthropogenic pollution on selected biota in Loskop Dam

By

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Dedication

To my families and friends

Declaration

I declare that *Impact of anthropogenic pollution on selected biota in Loskop Dam* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Judy Lai

2013

Signed…………………………

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Abbreviations

Al	**A**luminium
AMD	**A**cid **M**ine **D**rainage
ANZECC	**A**ustralian and **N**ew **Z**ealand **E**nvironmental **C**onservation **C**ouncil
ASPT	**A**verage **S**core **p**er **T**axon
Ca	Calcium
$CaCO_3$	Calcium carbonate
Cd	Cadmium
cells. l^{-1}	cells per litre
cm	centimeter
CSIR	**C**ouncil for **S**cientific and **I**ndustrial **R**esearch
DOM	**D**issolved **O**rganic **M**atter
D	Berger and Parker Dominance index
DWAF	**D**epartment of **W**ater **A**ffairs and **F**orestry
E	Evenness
EC	**E**lectrical **C**onductivity
EPT	**E**phemeroptera-**P**lecoptera-**T**richoptera
et al	**et al**ibi
FC	**C**ollector-**f**ilterers
Fe	Iron
GC	**C**ollector-**g**atherers
H′	Shannon diversity index
ha	hectare
HAB	**H**armful **A**lgal **B**looms
HNO_2	Nitrous acid
IHAS	**I**nvertebrate **H**abitat **A**ssessment **S**ystem
kV	kilovolt
m	meter
Mg	Magnesium
mg/l	milli**g**ram per **l**itre
ml	milli**l**itre
Mn	Manganese
mS/m	milli**s**iemense per meter
N	Nitrogen
N	the total number of individuals collected at each site
Na	Sodium
N_{max}	the number of individuals of the most abundant species present in each sample
NH^3	Ammonia

NH_4^+	Ammonium
nm	nanometer
NO_2^-	Nitrite
NO_3	Nitrate
N:P	nitrogen:phosphorus ratio
N:Si	nitrogen:silicon ratio
P	phosphorus
ρ_i	proportion of species relative to the total number of species
PO_4	Phosphate
PR	Predators
P:Si	phosphorus:silicon ratio
SASS	**S**outh **A**frican **S**coring **S**ystem
SC	Scrapers
SEM	**S**canning **E**lectron **M**icroscope
SH	Shredders
Si	Silicon
SIC	**S**tones **I**n **C**urrent
SOOC	**S**tones **O**ut **O**f **C**urrent
SO_4	Sulphate
TN:TP	Total Nitrogen:Total Phosphorus
µg/l	**M**icrongram per **l**itre
µl	**M**icron**l**itre
µm	micronmeter
µS/cm	**m**icronsiemense per **c**ent**m**etre
TDS	**T**otal **D**issolved **S**alts
TWQR	**T**arget **W**ater **Q**uality **R**ange
WRC	**W**ater **R**esearch **C**ommission
Zn	Zinc

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Chapter 1

Introduction

South Africa is a water constrained country, with low rainfall volumes and water distributed unevenly geographically as well as social economically (DWAF 2004). With restricted water resources, increased water pollution due to increase urbanization, agricultural and industrial activities, inappropriate management and control of water resources and quality have exacerbated the already alarming situation (Oberholster et al. 2008).

Culture eutrophication and Acid Mine Drainage (AMD) are two eminent problems affecting water ecosystem health in South Africa (DWAF 2004; Oberholster et al. 2008). Acid Mine Drainage, due to improper management of abandoned mines can result in exposure of sulphur-rich minerals with oxygen and water, create oxidation of sulphur-rich mine wastes, generating AMD which can pollute the surface and ground water (Akcil and Koldas 2006). AMD is characterized with low pH values, which facilitates the dissolution and mobilization of metals from various sources, like for instance the exposed minerals in the mines, rocks and also from the soil (Wood 1985). This elevates the level of metals within a water system and increases bioavailability to the aquatic organisms (Stokes et al. 1985; Spry and Wiener 1991). However the bioavailability of metals can be related to interactions with other environmental factors involved such as the pH, temperature and dissolved organic carbon (DOC) (Wren and Stephenson 1991; Schubauer-Berigan et al. 1993; Playle 1998).

Culture eutrophication on the other hand occurs when an aquatic system is over-enriched with nutrients composed of organic and inorganic nitrogen and phosphorus (Smith 2003). Culture eutrophication can results in nuisance bloom of phytoplankton (Skei et al. 2000; Smith 2003; Oberholster et al. 2009). These harmful algal blooms (HAB) can have deleterious consequences for both the aquatic ecosystem and human health if aquatic life consumed this eutrophic water (Maso and Garces 2006). These algal blooms decrease water clarity, increase organic sedimentation, anoxia and decreasing diversity of phytoplankton assemblage (Proulx et al. 1996; Skei et al. 2000; Trainer et al. 2010). The algal blooms may lead to dominance and blooming of one species of phytoplankton (Oberholster et al. 2010). This structural change in composition of phytoplankton alters the physico-chemical characteristics of water and in turn affects the structure of the food web of a particular ecosystem (Abrantes et al. 2006). The HAB which produces bio-toxin can carry out its negative effects even when it occurs in low cellular concentration. The toxin can bioaccumulated, biotransferred and biomagnified through the food chains (Wang and Wu 2009; Brand et al. 2010). Cyanobacteria (blue-green algae) commonly occurred under eutrophic condition and produce cyanotoxins (hepto- , neuro-, and cytotoxins, irritants and gastrointestinal toxins) that poses great health risk to animals and human (Codd et al. 2005; Brand et al. 2010).

Loskop Dam which form part of the upper Olifant's River catchment receives water from both the Olifant and Wilge Rivers. The primary purpose of the dam was to provide for the irrigation needs of farmers in the Olifants, Moses and Elands River Valleys but is also used for recreation, since the dam is now part of the Loskop Nature Reserve of 25000 ha. Besides its role in providing water for domestic, industrial and irrigation purposes, it is also a fresh water angling hot spot and tourist attraction (Driescher 2007).

However, increased mining operation in the upper Olifant River catchment had lead to growth in mining, urban and industrial water requirement, putting more pressure on the existing reservoirs' in the upper Olifants catchment (DWAF 2004). The water quality in Loskop Dam has gradually deteriorated over time due to substantial discharge of acid mine water from the Witbank Coal field and return flows from sewage treatment plants (DWAF 2006). A study done by Driescher (2007) on Loskop Dam highlighted that the water quality was characterized with high total dissolved salts (TDS) with nitrate (NO_3) and sulphate (SO_4) being the major component of TDS. The heavy metals in the water column of Loskop Dam such as Cu, Cr, Ni, Zn, Cd, indicated that dam water was indeed contaminated by point and non-point source pollution (Driescher 2007). The degraded water quality is reflected by mass mortalities of fish that occurred during the time period of 2003-2008, and a sharp decline of the crocodile population due to pansteatitis, which was possibly due to intake of rancid fish fat after a fish die-off (Oberholster et al. 2010). Another impact on Loskop Dam is untreated or partly treated sewage from the upper catchment.

In my MSc thesis, I have showed that the water quality in Loskop Dam is indeed under threat of both eutrophication and AMD. I have also showed that besides chemical and physical analysis of the water, it is also very important to incorporate biotic indicators (phytoplankton and macroinvertebrate community) in a study. In this study, the phytoplankton and macroinvertebrate were used as a biomonitoring indicator by analyzing their composition and relate their response to the physical and chemical water results during the sampling period of 6 months (May 2009 to October 2009).

The study period was selected due to the previous incidents of massive fish die-offs of 14 tons occurred during September in 2006 and June to August in 2007 (Driescher 2007). For the phytoplankton study, five sampling sites were selected and are representing three different zones of Loskop Dam. These are: riverine zone (site 1 and 2, representing high flow and rapid water flushing rate); transitional zone (site 3, representing the reduced flow and flushing rate) and lacustrine zone (sites 4 and 5, representing lowest flows and water flushing rates) (Oberholster 2010). These sampling sites were chosen for physicochemical parameters and phytoplankton community analysis. Macroinvertebrate assemblage was used to determine water quality in the inflow of Loskop Dam. Two sampling sites were chosen for physico-chemical and macroinvertebrate analysis. These sites were selected in relationship to previous sampling sites from the 1968 study conducted by Kruger (1968) and Mulder (1968).

In Chapter 3, two sampling sites were chosen for macroinvertebrate assemblages. Sampling site 1 was selected in the riverine zone of Lake Loskop and sampling site 2 was an undisturbed inflowing mountain stream and act as the reference site for this study, due to its good water quality (Oberholster et al. 2012). These two sampling sites were sampled for a period of six months (May

2009 to October 2009).

Macroinvertebrate community is a reliable bio-indicator in the study of water quality over a period of time. Their diversity and composition were calculated and analyzed in conjunction with the physicochemical parameters of the sampling sites for water quality assessment. South African Scoring System 5 (SASS5) index and Shannon Diversity Index (H') were used together with the South Africa Invertebrate Habitat Assessment System (IHAS) and Ephemeroptera-Plecoptera-Trichoptera (EPT) family richness in order to gain a complete picture of the biotic status of each sampling site.

Macroinvertebrate community impacted by AMD and culture eutrophication are generally characterized with low diversity, evenness, and a community shift from sensitive to pollution-tolerant species (Harrison 1958; Hunken and Mutz 2007). In a study done by Oberholster et al. (2008) on Rietvlei Nature Reserve, a river wetland area in South Africa, showed that there was a major decrease of species diversity downstream of the point source of nutrient enrichment by the Hartbeesfontein Sewage Purification Works. The severely impacted site was dominated by tolerant family groups to organic pollution such as oligochaetes and chironomids (Oberholster et al. 2008).

The objective of Chapter 3 was determine the relationship of physical/chemical water variables and macroinvertebrates community structure between different selected sites during the 2009 study and data generated from the same sites in the 1968 study.

In Chapter 4, five sampling sites were chosen for physico-chemical and phytoplankton analysis over a period of six months (May 2009 to October 2009). The composition, diversity and evenness of the phytoplankton community in the water column was analyzed and calculated. In the study, phytoplankton was used as bioindicator in relationship with physical/chemical parameters. The autecology of each dominant phytoplankton species was determined in order to assess the biotic integrity of the sampling sites.

The objectives of the study were firstly to determine the phytoplankton assemblage in Loskop Dam over a period of 6 months, which include the same time period when previous incidents of massive fish die-offs of 14 tons occurred during September in 2006 and June to August in 2007, as well as crocodile mortality during the same period in 2009 to 2010 ; secondly to link the physical and chemical water quality parameters to the distribution and autecology of different phytoplankton communities at each sampling site.

References:

Abrantes N, Antunes SC, Pereira MJ, Gonçalves F (2006) Seasonal succession of cladocerans and phytoplankton and their interactions in a shallow eutrophic lake (Lake Vela, Portugal). International Journal of Ecology 29: 54-64

Akcil A, Koldas S (2006) Acid Mine Drainage (AMD): causes, treatment and case studies Journal of Cleaner Production 14: 1139 -1145

Brand LE, Pablo J, Compton A, Hammerschlag N, Mash DC (2010) Cyanobacterial blooms and the occurrence of the neurotoxin, beta-N-methylamino-L-alanine (BMAA), in South Florida aquatic food webs. Harmful Algae 9: 620-635

Codd GA, Morrison LF, Metcalf JS (2005) Cyanobacterial toxins: risk management for health protection. Toxicology and Applied Pharmacology 203: 264-272

Driescher AC (2007) A water resource quality study of Loskop dam and the upper catchment of the Olifants River University of the Free State, pp 1-150

DWAF (2004) Olifants River Water Management Area Internal Strategic Perspective. Department of Water Affairs and Forestry 1

DWAF (2006) The Olifants River System Pilot Study. Department of Water Affairs and Forestry Appendix D

Harrison AD (1958) The effects of sulfuric acid pollution on the biology of streams in the Transvaal. South Africa. International Association of Theoretical and Applied Limnology 13: 603-610

Hunken A, Mutz M (2007) On the ecology of the filter-feeding Neureclipsis bimaculata (Trichoptera, Polycentropodidae) in an acid and iron rich post-mining stream. Hydrobiologia 592: 135–150

Kruger EJ (1968) The seasonal variation in the quality and quantity of the net-zooplankton in Loskop Dam, Transvaal. Research project from the Department of Animal Science and Microbiology. Transvaal, South Africa

Maso M, Garces E (2006) Harmful microalgae blooms (HAB); problematic and conditions that induce them. Marine Pollution Bulletin 53: 620-630

Mulder PFS (1968) 'n Kwalitatiewe en kwantitatiewe ondersoek na die bentos en epifauna in Loskopdam. MSc Thesis, Departement Dierkunde, Potchefstroomse Universiteit vir CHO, Potchefstroom, pp1-36

Oberholster PJ, Botha AM, Ashton PJ (2009) The influence of a toxic cyanobacterial bloom and water hydrology on algal populations and macroinvertebrate abundance in the upper littoral zone of Lake Krugersdrift, South Africa. Ecotoxicology 18: 34-46

Oberholster PJ, Botha AM, Cloete TE (2008) Biological and chemical evaluation of sewage water pollution in the Rietvlei nature reserve wetland area, South Africa. Environmental Pollution 156: 184-192

Oberholster PJ, Myburgh JG, Ashton PJ, Botha AM (2010) Responses of phytoplankton upon exposure to a mixture of acid mine drainage and high levels of nutrient pollution in Lake Loskop, South Africa. Ecotoxicology and Environmental Safety 73: 326-335

Oberholster PJ, Myburgh JG, Ashton PJ, Coetzee JJ, Botha AM (2012) Bioaccumulation of aluminium and iron in the food chain of Lake Loskop, South Africa. Ecotoxicology and Environmental Safety 75: 134-141

Playle RC (1998) Modelling metal interactions at fish gills. The Science of the Total Environment 219: 147-163

Proulx M, Pick FR, Mazumder A, Hamilton PB, Lean DRS (1996) Experimental evidence for interactive impacts of human activities on lake algal species richness. Oikos 76: 191-195

Schubauer-Berigan MK, Dierkes JR, Monson PD, Ankley GT (1993) pH-Dependent toxicity of Cd, Cu, Ni, Pb and Zn to *Ceriodaphnia dubia*, *Pimephales promelas*, *Hyalella azteca* and *Lumbriculus variegatus*. Environmental Toxicology and Chemistry 12: 1261-1266

Skei J, Larsson P, Rosenberg R, Jonsson P, Olsson M, Broman D (2000) Eutrophication and Contaminants in Aquatic Ecosystems. Ambio 29: 184-194

Smith VH (2003) Eutrophication of fresh water and coastal marine ecosystems: a global problem. Environmental Science and Pollution Research International 10: 126-139

Spry DJ, Wiener JG (1991) Metal Bioavailability and Toxicity to Fish in Low-Alkalinity Lakes: A Critical Review. Environmental Pollution 71: 243-204

Stokes PM, Bailey RC, Groulx GR (1985) Effects of Acidification on Metal Availability to Aquatic Biota, with Special Reference to Filamentous Algae. Environmental Health Perspectives 63: 79-87

Trainer VL, Pitcher GC, Reguera B, Smayda TJ (2010) The distribution and impacts of harmful algal bloom species in eastern boundary upwelling systems. Progress in Oceanography 85: 33-52

Wang J, Wu J (2009) Occurrence and potential risks of harmful algal blooms in the East China Sea. Science of the Total Environment 407: 4012-4021

Wood JM (1985) Effects of Acidification on the Mobility of Metals and Metalloids: An Overview. Environmental Health Perspectives 63: 115-119

Wren CD, Stephenson GL (1991) The effect of acidification on the accumulation and toxicity of metals to fresh water invertebrates. Environmental Pollution 71: 205-241

Chapter 2

Literature Review

Introduction

2.1 Current water conditions in South Africa

Africa is the world's second-driest continent, just after Australia (United Nations Environment Programme 2010). The continent is experiencing unevenly distributed water resources with inconsistent and low rainfall (with an average yearly rainfall around 500 mm, compared with the world average of 860 mm, with some 21% of the country receiving less than 200 mm/y) and erratic climate (Pietersen and Beekman 2006; Barradas 2011). South Africa is one of the countries that are currently facing water insecurity as it is under the restriction in water availability due to geographical factors (as mentioned above) and also due to its full exploitation of existing fresh water resources (Pietersen and Beekman 2006). Adding to the already limited water resource, the water of the country is also contaminated by industrial and agricultural activities, turning available water unusable without extensive treatment (DWAF 2004). This concerning situation is also exacerbated by poor city planning, poor infrastructure management, insufficient Water Services Authorities (WSA) institutional capacity (staffing, funding, expertise and education), inadequate water and sanitation management, a lack of resources, and competition for available fresh water between industrial, municipal, agricultural and tourism sectors, and often between upstream and downstream users (Barradas 2011). Therefore the future of human's right in water usage for all citizens in the country may be threatened as more clean and safe water is demanded (demand expected to rise by 52% within the next 30 years) from the rapid growing population (current population projections by Statistics South Africa estimate that South Africa's population will grow to 53-million people by 2025) and economic development (Barradas 2011). If the government and the people do not recognize its water limit and implementing better water management, the livelihood, health and economic growth of a country would be hampered (Oberholster and Ashton 2008).

In response to the deteriorated quality of water, DWA has initiated the Blue Drop and Green Drop certification scheme, which helps to monitor and regulate drinking water quality and manage wastewater to reach the requirement of the regulation program respectively (Barradas 2011). In 2011, the Blue Drop Scheme assessment showed that Gauteng and Western Cape have the highest scores (95.1% and 94.1% respectively), indicating good tap water quality in these areas as compared to other areas such as the Eastern Cape, Mpumalanga and North West (Barradas 2011). In the Green Drop assessment, it was found that the bulk of Free State municipalities did not meet the requirement of the regulation program (Barradas 2011). This implies that millions of litres of untreated or poorly treated sewage were being discharged into rivers and streams each day, which affects usable water availability. Although the municipalities have sound policies aiming to tackle water issues in the country, they are not yet implementing these policies at a practical level (Barradas 2011). This not only put national water supply system in jeopardy, but also hinders the economic growth of South Africa (Oberholster and Ashton 2008).

Acid mine Drainage (AMD) is another issues causing great ecological problem due to improper management of abandoned mines. The water of abandoned mines, after contact with oxygen, can create oxidation of sulphur-rich mine wastes, generating AMD which pollute surface and ground water.

So, unless the perceptions about water use and reuse change, South Africa will still face water challenges in the near future. And it is important to put attention on preventing water pollution and efficient water use, in parallel with how to treat polluted water. This will require concerted effort from all people living in South Africa.

2.2 Culture eutrophication and Acid Mine drainage

South Africa is a water constrained country, with water distributed unevenly geographically as well as social economically (DWAF 2004). With restricted water resources, increased water pollution due to increase urbanization, agricultural and industrial activities, inappropriate management and control of water resources and quality have exacerbated the already alarming situation (Oberholster et al. 2008). Culture eutrophication and AMD are two eminent problems affecting ecosystem health in South Africa (DWAF 2004; Oberholster and Ashton 2008).

2.2.1 Eutrophication

2.2.1.1 Culture eutrophication and formation of toxic algal blooms

Culture eutrophication in both fresh and coastal water system is a global and imminent problem and is often associated with nuisance bloom of phytoplankton (Skei et al. 2000; Smith 2003; Oberholster et al. 2009). These harmful algal blooms (HAB) can result in deleterious consequences for both the aquatic system and human health if affected water or aquatic life consumed raw water (Maso and Garces 2006). HABs are a natural phenomenon, but anthropogenic activities due to agricultural, urbanization, damming and industrial development, can exacerbate cultural eutrophication (Smith 2003; Maso and Garces 2006). Eutrophication occurs when an aquatic system is over-enriched with nutrients composed of organic and inorganic nitrogen and phosphorus (Smith 2003). Each different phytoplankton family has its own biotic and abiotic condition requirements for its optimal growth. However fundamental elements can support the survival of some of the algal blooms commonly occurring in fresh water system such as *Microcystis* spp. blooms (Martins et al. 2008; Oberholster et al. 2010). While in marine environment, dinoflagellate such as *Karenia brevis* can cause toxic red tide conditions (Vargo et al. 2008).

The harmful effect of HABs can follow two pathways. Firstly, algal bio-toxins have harmful effect on aquatic life where it can be bioaccumulated, biotransferred and biomagnified through the food chains (Wang and Wu 2009; Brand et al. 2010). Cyanobacteria (blue-green algae) commonly occur in eutrophic condition, producing cyanotoxins (hepto-, neuro-, and cytotoxins, irritants and gastrointestinal toxins) that poses great health risk to animals and humans (Codd et al. 2005; Brand et al. 2010) (Table 2.1). Secondly, species producing bio-toxins respond differently to nutrient enrichment (Smayda 2008). For some species, growth and toxicity are stimulated by nutrient enrichment while for others, nutrient enrichment enhance their growth, but nutrient depletion can also stimulate toxin production (Smayda 2008) (Table 2.1). Environmental factors affecting bio-toxin production may be more complex than it seems. In previous studies it was found that in the case of microcystin production, the maximal levels of toxin correlated non-linearly but strongly within a range of total nitrogen (TN) concentration of 1.5 – 4 mg l^{-1} (Dickens and Graham 2002).

Table 2.1 Cyanobacterial toxins of the most dominant species in South Africa, and their functions and mechanisms of action. (Falconer 1998; Sivonen and Jones 1999; Oberholster and Ashton 2008; Codd et al. 2010).

Toxin type	Primary target organ in mammals	Cyanobacteria Taxon	Mechanism of toxicity
Hepatotoxins			
Microcystins	Liver	*Microcystis*, *Oscillatoria*, *Nostoc*, *Anabaena*	Inhibition of protein phosphatase activity, haemorrhaging of the liver
Nodularins	Liver	*Nodularia*	Inhibition of protein phosphatase activity, haemorrhaging of the liver
Cytotoxins			
Cylindrospermopsins	Liver, kidney, spleen, intestine, heart, thymus	*Cylindrospermopsis*	Inhibition of protein synthesis
Neurotoxins			
Anatoxin-a	Nerve synapse	*Anabaena*, *Oscillatoria*	Blocking of post-synaptic depolarization
Anatoxin-a(s)	Nerve synapse	*Anabaena*	Blocking of acetylcholinesterase
Saxitoxins	Nerve axons	*Anabaena*	Blocking of sodium channels
Dermatotoxins			
Aplysiatoxins	Skin	*Oscillatoria*	Protein kinase C activators, inflammatory activity
Irritant Toxins			
Lipopolysaccharides	Any exposed tissue	All	Potential irritant and allergen

However, non-toxic phytoplankton bloom formation can negatively impact the aquatic ecosystem by high biomass increases in short period of time (Maso and Garces 2006). These algal blooms often results in decrease in water clarity, increase in organic sedimentation, anoxia and decreasing diversity of phytoplankton assemblage (Proulx et al. 1996; Skei et al. 2000; Trainer et al. 2010). In a case study done by Sabater et al. (2000) on the River Oria (Northern Spain) showed that the cause of pollution was due to land use within some of the rivers' tributaries receiving urban sewage outflows. In their study the water was characterized with high nutrient concentration favourable for development of high algal biomass. Consequently, high algal biomass did lead to hypoxia causing huge mortality of fish, and allowing certain fish species such as Cyprinids that are more tolerant to hypoxia condition to dominate (Sabater et al. 2000) (Figure 2.1). The decline of dissolved oxygen can also promote production of reduced compounds, such as H_2S, which is highly toxic to aquatic animals at relatively low concentration by affecting the nervous system (Camargo et al. 2005).

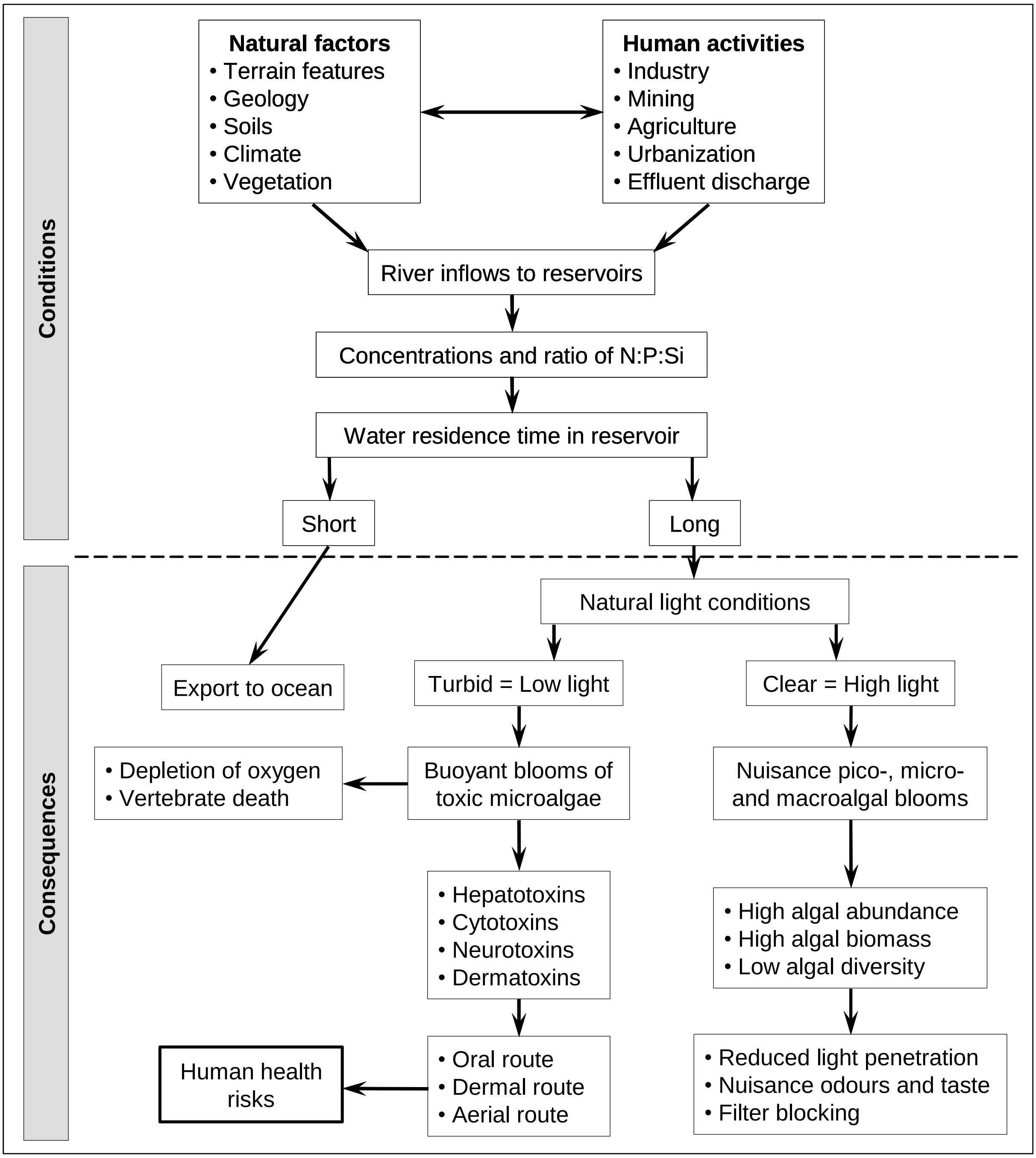

Figure 2.1: Overview of the sequence of interacting factors and the potential consequences of nutrient enrichment of fresh water in a man-made impoundment (Oberholster and Ashton 2008)

Although eutrophication is a natural ageing process of a water system, it can be exacerbated by human activities (Khan and Ansari 2005). Sewage disposal, particularly in high rainfall season, elevates the nutrient contents and enhance microbial growth to further consume the dissolved oxygen (DO) (Joubert 2008).

Few previous studies showed that eutrophication of a water system also results in reduced diversity and evenness of macroinvertebrate composition (Camargo et al. 2005; Henriques-de-Oliveira et al. 2007; Oberholster et al. 2008). In eutrophic conditions, high concentrations of nutrients promote growth of fast-growing phytoplankton and macroalgae, which may leads to dominance and blooming of one species of phytoplankton (Oberholster et al. 2010). This structural change in composition of phytoplankton alters the physico-chemical characteristics of water and in turn limited the food diversity for the macroinvertebrates in the food web. The latter reduces the richness and diversity of the community structure, and increase the abundance of organic and low-oxygen tolerant species (Hargeby et al. 1994; Oberholster et al. 2009).

2.2.1.2 Acid mine drainage (AMD) and its impacts on water resources

AMD is a mining legacy that poses a threat to the ecosystem (Dsa et al. 2008; Limpitlaw et al. 2005; Sa´nchez Espan˜a et al. 2005). AMD resulted from the decanting of water from abandoned mines where the exposed sulfide minerals (Maso and Garces 2006) made contacts with water and oxygen. This contact initiates the oxidation of sulfide minerals, producing sulfuric acid at a certain rate (Van Rensburg 2003). The rate of acid generation is determined by various factors like for example the surface area of exposed metal sulfide, oxygen content, pH, and the bacterial activity of *Acidothiobacillus ferroxidans*, *Thiobacillus ferroxidans* and *Leprosprillum ferroxidans* that directly and indirectly catalyzes the reaction involved (Akcil and Koldas 2006; Edwards et al. 2000; Schrenk et al. 1998). The reactions of acid generation are illustrated below using pyrite (FeS_2) as an example, which is by far the greatest contributor in acid mine drainage. The first reaction is the oxidation of the sulfide minerals into dissolved iron, sulfate and hydrogen:

$$2FeS_2(s) + 7O_2(g) + 2H_2O(l) = 2Fe^{2+}(aq) + 4SO_4^{2-}(aq) + 4H^+(aq)$$

When the surrounding environment is sufficiently oxidizing (depending on O_2, pH and bacterial activity), much of the ferrous iron will be oxidized to Ferric iron (Fe(III))

$$4Fe^{2+}(aq) + O_2(g) + 4H^+(aq) = 4Fe^{3+}(aq) + 2H_2O(l)$$

Ferric iron can further oxidize more pyrite into ferrous iron when in contact:

$$FeS_2(s) + 14Fe^{3+}(aq) + 8H_2O(l) = 15Fe^{2+}(aq) + 2SO_4^{2-}(aq) + 16H^+(aq)$$

This reaction generates more acid. The dissolution of pyrite by ferric iron (Fe^{3+}), together with the oxidation of the Fe^{2+} constitutes a cycle of dissolution of pyrite. At low pH (between 2.3 and 3.5) Fe^{3+} precipitates as $Fe(OH)_3$ and jarosite, leaving little Fe^{3+} in solution while simultaneously lowering pH:

$$Fe^{3+}(aq)+3H_2O(l) \rightarrow Fe(OH)_3\ (solid)+3H^+(aq)$$

Fe(OH)3 precipitates and is identifiable as the deposit of amorphous, yellow, orange, or red deposit on stream bottoms ("yellow boy") (Akcil and Koldas 2006).

When AMD-generating reactions are initiated, it is hard to stop. The impacts of AMD can last for a long time and are multifaceted (Gray 1997). AMD affect the ecosystem negatively by reducing the biodiversity, disrupting the food chain and polluting surface and ground water, which human lives depend heavily upon (Gray 1998). For plants e.g. crops, it was found that Al with Al^{3+} occurring in low pH waters, retards the growth of the crop root thereby reduces the crop yields (Delhaize and Ryan 1995).

When highly acidic (pH 2 to pH 4) water drains into natural water systems, the physico-chemical properties of the surface and ground water are altered and consequently the biological and ecological structure of the aquatic system (Gray 1997). For example, in acidic condition (< pH 4), the carbonate and bicarbonate are converted to carbonic acid, which dissociate into carbon dioxide and water (Ashton et al. 2001). This conversion has two effects: (1) the buffering capacity of the water is reduced; (2) and it reduces photosynthesis activity for example in the case of algae which use bicarbonate as the inorganic carbon source for photosynthesis organisms (Ashton et al. 2001).

Water with a low pH facilitates the dissolution and mobilization of metals from various sources, like for instance the exposed minerals in the mines, rocks and also from the soil (Wood 1985). This elevates the level of metals within a water system and increases bioavailability to the aquatic organisms (Stokes et al. 1985; Spry and Wiener 1991). However the bioavailability of metals can be related to interactions with other environmental factors e.g. the pH, temperature, dissolved organic carbon (DOC) (Playle 1998; Schubauer-Berigan et al. 1993; Wren and Stephenson 1991). Seasonal variation also plays a major role in the level of dissolved metal contents in the water. In summer months in South Africa, with higher rainfalls, more metals are leached from tailing dams or abandoned mines into water ways and vice versa in less rainfall season during the winter months (Nussey et al. 2000).

Most studies on metal toxicities were done on fish (Henry et al. 1999; Henry et al. 2001; Kotze et al. 1999; Nussey et al. 2000; Playle 1998; Scheuhammer 1991; Spry and Wiener 1991). These studies showed that metal toxicity are species-specific when considering their feeding habits, detoxifying mechanisms, life cycle and its size (Wren and Stephenson 1991).

Macroinvertebrate community impacted by AMD generally results in increases in species abundance, reduced diversity, community shift from sensitive to more tolerant species and species loss (Harrison 1958; Hunken and Mutz 2007). Ephemeroptera-Plecoptera-Trichoptera (EPT) families are 3 families that contain species with high sensitivity towards pollutants (Wahizatul et al. 2011). According to Soucek (2001), there is a significant reduction in EPT richness and Ephemeroptera abundance in AMD receiving water, and a decrease in total taxon richness (Soucek 2001). However, water with a low pH value, does not always have negative impacts on benthic community. An earlier study done by Petrin et al. (2008) compared streams in the north of Sweden with naturally low pH's to streams in the south which were experiencing anthropogenic acidification. In the study, it was evident that invertebrate communities in southern streams exhibited reductions in density and richness. On the other hand, the northern streams with a pH as low as 4, contained diverse communities with numerous EPT taxa (Petrin et al. 2008). Petrin et al. (2008) suggested from their study that macroinvertebrates in the northern Sweden streams were adapted to lower pH values. In general, the negative influence of low pH could results from the toxicity of high concentration of dissolved metals in the water, causing mortality or physiological stress on the invertebrate fauna (Kitto 2009). Yet the response of invertebrate varies, depending on the different life stages of macroinvertebrates and how they interact with metals, metal precipitates and pH (Kitto 2009).

High level of dissolved metal can cause toxicity towards aquatic biota and can also be bioaccumulated in the food chain (Quiroz-Va´zquez et al. 2010; Oberholster et al. 2012). Acid mine drainage usually consists of high concentration of metals as metals are highly soluble in acidic water. When such drainage is released into the natural water system, the metals will precipitate as mineral suspension and can be accumulated by phytoplankton species (Bortnikova et al. 2001; Quiroz-Va´zquez et al. 2010). The phytoplankton community is able to sequester the metals from the water column which are release on the bottom sediment as they die and decompose (Smolyakov et al. 2010; Oberholster et al. 2012). Although metals in solution are bioavailable and thus exert toxic effect on the organisms, the metals in the sediment will serve as a reservoir for future pollution as resuspension occurs during dam overturn in autumn and spring (Thomas et al. 1996; DeNicol and Stapleton 2002). When the total concentration of metals exceeds the levels that the aquatic biota can process and detoxify, plankton mortality or a shift of the phytoplankton community to a more heavy-metal resistance could occur (Monteiro et al. 1995; Oberholster et al. 2012).

Sedimentation is also another major problem associated with AMD (Niyogi et al. 2002; Ritter et al. 2002). The contaminants that are transported away from the source of pollution by the water, experiences a gradual increase of pH downstream due to dilution. Metals that are in aqueous form can precipitate or are adsorb by bottom sediments in rivers or dams. Both processes may pose a devastating effect on the survival of benthic organisms as they can increase the bioavailability of metals to these organisms (Dsa et al. 2008). The metal precipitate may suspend in the water column, blocking light penetration, and thereby reducing photosynthetic activity (Ashton et al. 2001). The suspension of precipitate may also clog the gills of the fish, decrease their gas exchange rate and suffocate the fish (Ashton et al. 2001; Van Rensburg 2003).

2.3 The upper Olifants catchment

2.3.1 Upper Olifants River catchment and land use activities

The Olifants River form part of one of the South Africa's major river systems (International Water Management Institute 2008). It flows through three different provinces in the North-East of the country, through the Kruger National Park, and finally into Mozambique where it joins with Limpopo River. The upper Olifant catchment has the largest urban concentration and has the most industrialised area of the Olifants River catchment especially the towns of Middelburg and Witbank

(Fig 2.2) (Van Rensburg 2003).

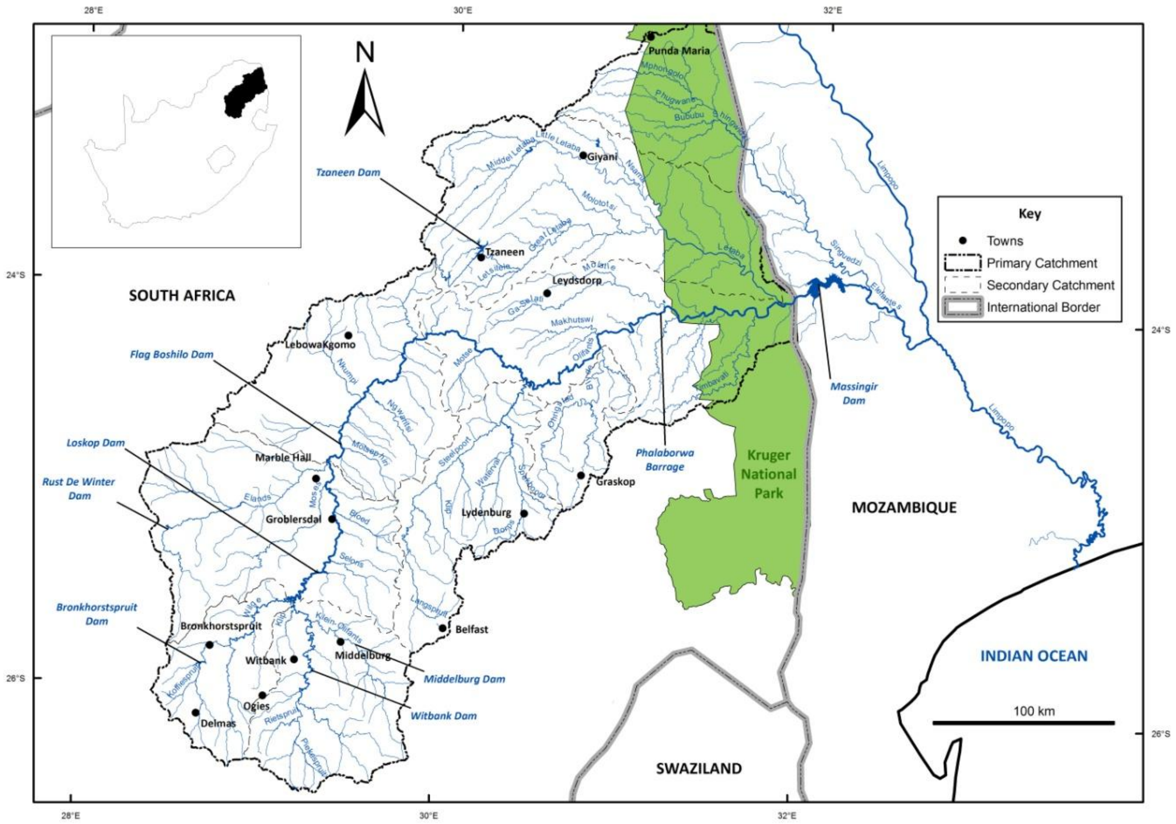

Figure 2.2: The Olifants River system which include Loskop Dam in the upper catchment

This increase in land use activities in these two areas are due to the exploitation of Witbank Coalfields which is one of the country's largest coal producing regions. The abundance of coal in this area gives way to the construction of 11 electrical-generating power stations. Consequently, the mining of the Witbank coalfield's coal also give way to the development of industrial, agriculture and mining activities in the vicinity of the towns of Witbank and Middelburg. These developments have therefore turned the highveld region into a major economic area, and indeed, the Olifants River catchment alone contribute just over 5% of the gross domestic product (GDP) of SA (Driescher 2007; Hobbs et al. 2008).

2.3.2 Impacts of land use activities from the upper Olifants catchment on Loskop Dam

Loskop Dam, 30 km long (van Vuuren 2008), with a storage capacity of 348.1 $X10^{6}$ m^{3}, was built on the farm Loskop and Vergelegen, approximately 32 km south of Groblersdal, Mpumulanga. The dam is part of the upper Olifants River catchment and receives water from the Olifant and Wilge Rivers. The dam was completed in 1938 and its wall was further rose from a height of 45 m to 54 m in 1979. Its primary purpose was to provide the irrigation needs of farmers in the Olifants, Moses and Elands River Valleys but is also used for recreation, since the dam is now part of the Loskop Nature Reserve of 25000 ha. Besides its role in providing water for domestic, industrial and irrigation purposes, it is also a hot spot for fresh water angling and a tourist attraction (Driescher 2007).

It is the biggest storage unit in the upper Olifants River catchment with 90% of its water been used for extensive irrigation by the Loskop Irrigation Board (IRB), the second largest irrigation scheme in South Africa (Joubert 2008). However, increased mining operation in the upper Olifant River catchment had lead to growth in mining, urban and industrial water requirement, putting more pressure on the existing reservoirs' in the upper catchment (DWAF 2004). The water quality in Loskop Dam has gradually deteriorated over time due to substantial discharge of AMD from the Witbank Coal field and return flows from sewage treatment plants (DWAF 2006). These contribute to the inflow into the dam with higher than normal amounts of dissolved metals, sulphates (SO_4), nutrients phosphate (PO_4) and nitrate (NO_3) (Oberholster et al. 2010). In a previous study conducted by Driescher (2007), he highlighted that the water quality was characterized with high Total Dissolved Salts (TDS) and high fluctuation between all selected sampling points. Nitrate (NO_3) and sulphate (SO_4) being the major component of TDS, together with heavy metals such as Cu, Cr, Ni, Zn, Cd, indicated that dam water was indeed contaminated by point and non-point source pollution (Driescher 2007). The degraded water quality is reflected by mass mortalities of fish that happened during the time period of 2003-2008, and a sharp decline of the crocodile population due to pansteatitis, which is due to intake of rancid fish fat after a fish die-off (Oberholster et al. 2010).

Figure 2.3: *Oreochromis mossambicus* with pansteatitis (brown spots on the body fat.)

A study conducted by Oberholster et al. (2012) on Loskop Dam showed that AMD from upstream resulted in high metal concentration in the water, and high levels of Al and Fe concentration (385.7

and 1710.0 mg kg^{-1} dry weight, respectively) were particularly found in the macroinvertebrate family of Gomphidae in comparison to other families sampled. The study indicate that the intake of certain species of phytoplankton by *O. mossambicus* could have played a role in the bioaccumulation of Al in the food chain and the possible development of pansteatitis in predators at higher trophic levels (Figure 2.3) (Oberholster et al. 2012).

In a previous study on Loskop Dam by Oberholster et al. (2010), the authors also studied the relationship between water quality and phytoplankton community structure over a study period of 6 months (Oberholster et al. 2010). The chemical and physical data from this study showed that the pollution in the dam was caused by AMD and enriched by nutrients to the point where the system has become hypertrophic. The riverine zone (inlet of the dam where Olifants River drains into) of the dam was most impacted by low pH values (pH < 6), and high total nitrogen (TN) and total phosphosrus (TP) concentrations in comparison to other sampling sites in the dam. The high nutrients values had an adverse effect on the diversity of the phytoplankton community causing the dominance of massive cyanobacterial blooms of *Microcystis aeruginosa* with complete absence of certain large zooplankton species (Figure 2.4).

Figure 2.4: Bloom of *Microcystis aeruginosa* during the 2009 study on Loskop Dam.

The only previous study on macroninvertebrate assemblages in Loskop Dam was conducted in 1968 by Mulder (1968). The structure of the benthos community was analyzed in all four seasons at a total of 19 different sites along the littoral zone of the dam. It was found that Oligochaeta and Chironomidae were the dominant groups that were found during all four season (Mulder 1968). These macroinvertebrate families have a very high tolerance level towards pollution especially to high level of nutrients in the water (Henriques-de-Oliveira et al. 2007; Martins et al. 2008). This finding also correlates well with the TN level in Loskop Dam found in a study done by Kruger (1968). In his study, the nitrogen level (4.2 mg l^{-1}) (nitrate and nitrite) was particularly high in summer and in the vicinity of the Olifants River inlet in comparison to other sampling sites (Kruger

1968). According to the criteria set by Target Water Quality Range (TWQR) (nitrogen: 2.5-10 mg l^{-1}), the nitrogen concentration (4.2 mg l^{-1}) (nitrate and nitrite) in Kruger's study suggested that the dam was classified as being eutrophic, and indicated that Olifants River definitely carries organic enrichment from upstream to the dam during the rainy season (DWAF 1996; Kruger 1968).

Eutrophication is a natural process which takes centuries to occur, but the fact that Loskop Dam only took 18 years (1938 – 1956) for its transformation from oligotrophy to eutrophy strongly suggested that such process had been catalysed and exacerbated by human-causes (Kruger 1968). Nutrients enrichment seemed to be the only stressors for Loskop Dam during the 1968 study in comparison to the present situation where the dam is under the threat of both nutrient enrichment and AMD. Although coal mining upstream of Loskop Dam in Witbank Coalfield commenced since 1895, the decanting of a number of defunct and flooded underground coal mines such as the Middelburg Colliery to the west and northwest of Witbank only started in the mid-1990's (Oberholster et al. 2012). This fact was also reflected by the physical and chemical analysis of the 1968 study where the pH remained quite neutral (pH 7-8), conductivity (100-240 μS cm^{-1}) remained low and constant, and SO_4 (<20 mg l^{-1}) level also remain fairly low in the dam in comparison with the existing studies (Kruger 1968).

2.4 Phytoplankton and macroinvertebrates as bioindicator of anthropogenic pollution

Phytoplankton is a valuable bioindicator in the water quality of an aquatic system because each species have different habitat preferences, so by analyzing the assemblage, one can determine the quality of water more accurately than with the chemical results alone (Daz-Pardo et al. 1998; Kumari et al. 2008). As different groups of phytoplankton have distinct life cycles, morphological and physiological mechanisms in adapting to the changing environment, alterations in the water quality due to human or natural cause could alter the regular succession and dynamics of phytoplankton communities within an aquatic ecosystem (Proulx et al. 1996).

Macroinvertebrate plays an important role in the aquatic biological system to balance the natural flow of energy and nutrients, therefore the condition of the benthic macroinvertebrate community reflects the stability and diversity of the larger aquatic food web (Wallace and Webster 1996; Gamito and Furtado 2009). Macroinvertebrates are valuable indicators in biological monitoring of an aquatic system, because of its visibility to the naked eye, ease of identification, rapid life cycle, and it is relatively easy to sample. Each macroinvertebrate species have specific sensitivity and tolerance towards the pollutants, therefore by analyzing the composition of macroinvertebrate community, one can assess the quality of that particular aquatic system and reveal information that is not always possible using chemical data alone (Dickens and Graham 2002; Oberholster et al. 2005; Oberholster et al. 2008). There are many factors (biological, chemical, geological and hydrological factors) in an aquatic system influencing the response of macroinvertebrate. One important factor that needs to be taken into consideration during monitoring is ‘drift’ (downstream movement of macroinvertebrate) and seasonality in invertebrate life cycle, which could obscure the extrapolation of the result of a monitoring programme (Svendsen et al. 2004; Kitto 2009)**.** These factors are also important to consider when doing assessment after rehabilitation or remediation. When a source of pollution is removed or reduced from an aquatic system, one would generally expect that the assessment result of macroinvertebrate community would re-gain its diversity and abundance of sensitive species when the chemical and physical results improved. However a study done on Spen Beck, West Yorkshire, showed that mitigating a single impact does not guarantee ecological recovery as several factors such as reduced riparian vegetation, runoffs from industrial activities and roads may also influence the re-colonisation of macroinvertebrate (Wenn 2008). Due to the complex interaction between biotic and abiotic factors in relationship with macroinvertebrates, there are very few field studies that can actually elucidate the causal relationship between a particular contaminant element to the biological response of specific macroinvertebrates (Dsa et al. 2008; Grout and Levings 2001; Jarup 2003; Nordberg et al. 1985; Spry and Wiener 1991; Soucek 2001; Soucek et al. 2002;). From these studies it is evident that the authors can only make associations between the pollutant and its impact on the benthic community (Paisley et al. 2011).

In this study, the phytoplankton and macroinvertebrate was used as a biomonitoring indicator by analyzing their composition in relationship with the physical and chemical water results during the sampling period of 6 months (May 2009 to October 2009). The study period was selected due to the previous incidents of massive fish die-offs of 14 tons occurred during September in 2006 and June to August in 2007 as well as crocodile mortality during the same period in 2003 to 2008 (Driescher

2007; Oberholster et al. 2012).

2.5 References:

Akcil A, Koldas S (2006) Acid Mine Drainage (AMD): causes, treatment and case studies. Journal of Cleaner Production 14: 1139 -1145

Ashton P, Love D, Mahachi H, Dirks P (2001) An Overview of the Impacts of Mining and Mineral Processing Operations on Water Resources and Water Quality in the Zambezi, Limopo and Olifants Catchments in Southern Africa. Mining, Minerals and Sustainable Development Report No. ENV-P-C 2001-042: 1-319

Barradas S (2011) South Africa's Water Sector 2011. Research Unit of Creamer Media (Pty) Ltd, pp 1-24

Bortnikova SB, Smolyakov BS, Sidenko NV, Kolonin GR, Bessonova EP, Androsova NV (2001) Geochemical consequences of acid mine drainage into a natural reservoir: inorganic precipitation and effects on plankton activity. Journal of Geochemical Exploration 74: 127-139

Brand LE, Pablo J, Compton A, Hammerschlag N, Mash DC (2010) Cyanobacterial blooms and the occurrence of the neurotoxin, beta-N-methylamino-L-alanine (BMAA), in South Florida aquatic food webs. Harmful Algae 9: 620-635

Camargo JA, Alonso A (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environment International 32: 831-849

Camargo JA, Alonso A, de la Puente M (2005) Eutrophication downstream from small reservoirs in mountain rivers of Central Spain. Water Research 39: 3376-3384

Codd G, Bell S, Kaya K, Ward C, Beattie K, Metcalf J (2010) Cyanobacterial toxins, exposure routes and human health. European Journal of Phycology 34: 405-415

Codd GA, Morrison LF, Metcalf JS (2005) Cyanobacterial toxins: risk management for health protection. Toxicology and Applied Pharmacology 203: 264-272

Daz-Pardo E, Vazquez G, Lopez-Lopez E (1998) The phytoplankton community as a bioindicator of health conditions of Atezca Lake, Mexico. Aquatic Ecosystem Health and Management 1: 257-266

Delhaize E, Ryan PR (1995) Aluminum Toxicity and Tolerance in Plants. Plant Physiology 107: 315-321

DeNicol DM, Stapleton MG (2002) Impact of acid mine drainage on benthic communities in streams: the relative roles of substratum vs. aqueous effects. Environmental Pollution 119: 303-315

Dickens CWS, Graham PM (2002) The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. African Journal of Aquatic Science 27: 1-10

Driescher AC (2007) A water resource quality study of Loskop dam and the upper catchment of the Olifants River University of the Free State, pp 1-150

Dsa JV, Johnson KS, Lopez D, Kanuckel C, Tumlinson J (2008) Residual Toxicity of Acid Mine Drainage-Contaminated Sediment to Stream Macroinvertebrates: Relative Contribution of Acidity vs. Metals. Water Air & Soil Pollution 194: 185-197

DWAF (1996) South African Water Quality Guidelines (Aquatic Ecosystem). Department of Water Affairs and Forestry 7

DWAF (2004) Olifants River Water Management Area Internal Strategic Perspective. Department of Water Affairs and Forestry 1

DWAF (2006) The Olifants River System Pilot Study. Department of Water Affairs and Forestry Appendix D

Edwards KJ, Bond PL, Gihring TM, Banfield JF (2000) An Archaeal Iron-Oxidizing Extreme Acidophile Important in Acid Mine Drainage. Science 287: 1796 - 1798

Falconer IR (1998) Algal toxins and human health. In the Handbook of Environmental Chemistry 5,Part C Quality and Treatment of Drinking Water II (J.Hrubee,ed.), pp 53-82

Gamito S, Furtado R (2009) Feeding diversity in macroinvertebrate communities: A contribution to estimate the ecological status in shallow waters. Ecological Indicators 9: 1009-1019

Graham JL, Jones JR, Jones SB, Downing JA, Clevenger TE (2004) Environmental factors influencing microcystin distribution and concentration in the Midwestern United States. Water Research 38: 4395-4404

Graham JL, Loftin KA, Ziegler AC, Meyer MT (2008) Cyanobacteria in lakes and reservoirs: toxin and taste-and-odor sampling guidelines. Cyanobacteria Version109

Gray NF (1997) Environmental impact and remediation of acid mine drainage:a management problem. Environmental Geology 30: 62-71

Gray NF (1998) Acid mine drainage composition and the implication for its impact on lotic systems. Water Research 32: 2122- 2134

Grout JA, Levings CD (2001) Effects of acid mine drainage from an abandoned copper mine, Britannia Mines, Howe Sound, British Columbia, Canada, on transplanted blue mussels (*Mytilus edulis*). Marine Environmental Research 51: 265-288

Hargeby A, Andersson G, Blindow I, Johansson S (1994) Trophic web structure in a shallow eutrophic lake during a dominance shift from phytoplankton to submerged macrophytes Hydrobiologia 279-280: 83-90

Harrison AD (1958) The effects of sulfuric acid pollution on the biology of streams in the Transvaal. South Africa. International Association of Theoretical and Applied Limnology 13: 603-610

Henriques-de-Oliveira C, Baptista DF, Nessimian JL (2007) Sewage input effects on the macroinvertebrate community associated to *Typha domingensis* Pers in a coastal lagoon in southeastern Brazil. Brazilian Journal of Biology 67(1): 73-80

Henry TB, Irwin ER, Grizzle JM, Brumbaugh WG, Wildhaber ML (2001) Gill lesions and death of bluegill in an acid mine drainage mixing zone. Environmental Toxicology and Chemistry 20: 1304-1311

Henry TB, Irwin ER, Grizzle JM, Wildhaber ML, Brumbaugh WG (1999) Acute Toxicity of an Acid Mine Drainage Mixing Zone to Juvenile Bluegill and Largemouth Bass. Transactions of the American Fisheries Society 128: 919-928

Hobbs PJ, Oelofse SHH, Rascher J (2008) Management of environmental impacts from coal mining in the upper Olifants River Catchment as a function of age and scale. International Journal of Water Resource Development 24: 417-432

Hunken A, Mutz M (2007) On the ecology of the filter-feeding Neureclipsis bimaculata (Trichoptera, Polycentropodidae) in an acid and iron rich post-mining stream. Hydrobiologia 592: 135–150

International Water Management Institute I (2008) Baseline Report Olifants River Basin in South Africa. A contribution to the Challenge Program Project 17 "Integrated Water Resource Management for Improved Rural Livelihoods: Managing risk, mitigating drought and improving water productivity in the water scarce Limpopo Basin", pp 1-37

Jarup L (2003) Hazards of heavy metal contamination. British Medical Bulletin 68: 167-182

Joubert A (2008) Evaluation of biomarkers for pollution in *Oreochromis Mossambicus* :a laboratory and field study. MSc Thesis, The University of Johannesburg, Department of Zoology, pp 1-117

Khan FA, Ansari AA (2005) Eutrophication: An ecological vision. The Botanical Review 71: 449-482

Kitto JAJ (2009) The application of ecological theory to the remediation of macroinvertebrate communities impacted by acid mine drainage. Master of Science in Environmental Science in the University of Canterbury, pp 1-154

Kotze P, Du Preez HH, Van Vuuren JHJ (1999) Bioaccumulation of copper and zinc in *Oreochromis massambicus* and *Clarias gariepinus*, from the Olifants River, Mpumalanga, South Africa. Water SA 25: 99-110

Kruger EJ (1968) The seasonal variation in the quality and quantity of the net-zooplankton in Loskop Dam, Transvaal. Research project from the Department of Animal Science and Microbiology. Transvaal, South Africa

Kumari P, Dhadse S, Chaudhari PR, Wate SR (2008) A biomonitoring of plankton to assess quality of water in the lakes of Nagpur City. Proceedings of Taal 2007 The 12th World Lake Conference, pp 160-164

Levings CD, Varela DE, Mehlenbacher NM, Barry KL, Piercey GE, Guo M, Harrison PJ (2005) Effect of an acid mine drainage effluent on phytoplankton biomass and primary production at Britannia Beach, Howe Sound, British Columbia. Marine Pollution Bulletin 50: 1585-1594

Limpitlaw D, Aken M, Lodewijks H, Viljoen J (2005) Post-Mining Rehabilitatiom, Land Use and Pollution at Colliers in South Africa. The South African Institute of Mining and Metallurgy Sustainable Development in the life of coal mining in South Africa, pp 1-10

Lv J, Wu H, Chen M (2011) Effects of nitrogen and phosphorus on phytoplankton composition and biomass in 15 subtropical,urban shallow lakes in Wuhan, China. Limnologica 41: 48-56

Martins R, Stephan N, Alves R (2008) Tubificidae (Annelida: Oligochaeta) as an indicator of water quality in an urban stream in southeast Brazil. Acta Limnologica Brasiliensia 20: 221-226

Maso M, Garces E (2006) Harmful microalgae blooms (HAB); problematic and conditions that induce them. Marine Pollution Bulletin 53: 620-630

Monteiro MT, Oliveira R, Vale C (1995) Metal Stress on the Plankton Communities of Sado River

(Portugal). Water Research 29: 695-701

Mulder PFS (1968) 'n Kwalitatiewe en kwantitatiewe ondersoek na die bentos en epifauna in Loskopdam. MSc Thesis, Departement Dierkunde, Potchefstroomse Universiteit vir CHO, Potchefstroom, pp1-36

Niyogi DK, Lewis WM, McKnight DM (2002) Effects of Stress from Mine Drainage on Diversity, Biomass, and Function of Primary Producers in Mountain Streams. Ecosystems 5: 554-567

Nordberg GF, Goyer RA, Clarkson TW (1985) Impact of effects of acid precipitation on toxicity of metals. Environmental Health Perspective 63: 169-180

Nussey G, van Vuren JHJ, du Preez HH (2000) Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, *Labeo umbratus* (Cyprinidae), from Witbank Dam, Mpumalanga. Water SA 26: 269-284

Oberholster PJ, Botha AM, Ashton PJ (2009) The influence of a toxic cyanobacterial bloom and water hydrology on algal populations and macroinvertebrate abundance in the upper littoral zone of Lake Krugersdrift, South Africa. Ecotoxicology 18: 34-46

Oberholster PJ, Botha AM, Cloete TE (2005) Using a battery of bioassays, benthic phytoplankton and the AUSRIVAS method to monitor long-term coal tar contaminated sediment in the Cache la Poudre River, Colorado. Water Research 39: 4913-4924

Oberholster PJ, Botha AM, Cloete TE (2008) Biological and chemical evaluation of sewage water pollution in the Rietvlei nature reserve wetland area, South Africa. Environmental Pollution 156: 184-192

Oberholster PJ, Ashton PJ (2008) An Overview of the Current Status of Water Quality and Eutrophication in South African Rivers and Reservoirs. Parliamentary Grant Deliverable State of the Nation Report, pp 1-15

Oberholster PJ, Botha AM, Myburgh JG, Ashton PJ (2010) Responses of phytoplankton upon exposure to a mixture of acid mine drainage and high levels of nutrient pollution in Lake Loskop, South Africa. Ecotoxicology and Environmental Safety 73: 326-335

Oberholster PJ, Myburgh JG, Ashton PJ, Coetzee JJ, Botha AM (2012) Bioaccumulation of aluminium and iron in the food chain of Lake Loskop, South Africa. Ecotoxicology and Environmental Safety 75: 134-141

Paisley MF, Walley WJ, Trigg DJ (2011) Identification of macroinvertebrate taxa as indicators of nutrient enrichment in rivers. Ecological Informatics 6: 399-406

Petrin Z, Englund G, Malmqvist B (2008) Contrasting effects of anthropogenic and natural acidity in streams: a meta-analysis. Proceedings of the Royal Society 275: 1143-1148

Pietersen K, Beekman H (2006) Africa Environment Outlook 2. United Nations Environment Environmental State-and-Trends: 20-Year Retrospective, pp 119-154

Playle RC (1998) Modelling metal interactions at fish gills. The Science of the Total Environment 219: 147-163

Proulx M, Pick FR, Mazumder A, Hamilton PB, Lean DRS (1996) Experimental evidence for interactive impacts of human activities on lake algal species richness. Oikos 76:191-195

Quiroz-Va´zquez P, Sigee DC, White KN (2010) Bioavailability and toxicity of aluminium in a model planktonic food chain (*Chlamydomonas–Daphnia*) at neutral pH. Limnologica 40(3): 269-277

Ritter L, Solomon K, Sibley P, Hall K, Keen P, Mattu G, Linton B (2002) Sources, pathways, and relative risks of contaminants in surface water and groundwater: a perspective prepared for the Walkerton inquiry. Journal of Toxicology and Environmental Health 65: 1-142

Sa´nchez Espan˜a J, Pamo EL, Santofimia E, Aduvire O, Reyes J, Barettino D (2005) Acid mine drainage in the Iberian Pyrite Belt (Odiel river watershed, Huelva, SW Spain): Geochemistry, mineralogy and environmental implications. Applied Geochemistry 20: 1320-1356

Sabater S, Armengol J, Comas E, Sabater F, Urrizalqui I, Urrutia I (2000) Algal biomass in a disturbed Atlantic river: water quality relationships and environmental implications. Science of the Total Environment 263: 185-195

Scheuhammer AM (1991) Acidification-related changes in the biogeochemistry and ecotoxicology of mercury, cadmium, lead and aluminium: overview. Environmental Pollution 71: 87-90

Schrenk MO, Edwards KJ, Goodman RM, Hamers RJ, Banfield JF (1998) Distribution of Thiobacillus ferrooxidans and Leptospirillum ferrooxidans: Implications for Generation of Acid Mine Drainage. Science 279: 1519-1521

Schubauer-Berigan MK, Dierkes JR, Monson PD, Ankley GT (1993) pH-Dependent toxicity of Cd, Cu, Ni, Pb and Zn to *Ceriodaphnia dubia*, *Pimephales promelas*, *Hyalella azteca* and *Lumbriculus variegatus*. Environmental Toxicology and Chemistry 12: 1261-1266

Sivonen K, Jones G (1999) Cyanobacterial toxins. In: Toxic Cyanobacteria in Water: a Guide to Public Health Significance,Monitoring and Management. I Chorus and J Bertram (Edn) The World Health Organization ISBN 0–419–23930–8. E and FN Spon, London, UK, pp 41-111

Skei J, Larsson P, Rosenberg R, Jonsson P, Olsson M, Broman D (2000) Eutrophication and Contaminants in Aquatic Ecosystems. Ambio 29: 184-194

Smayda TJ (2008) Complexity in the eutrophication–harmful algal bloom relationship, with comment on the importance of grazing. Harmful Algae 8: 140-151

Smith VH (2003a) Eutrophication of fresh water and coastal marine ecosystems: a global problem. Environmental Science and Pollution Research International 10: 126-139

Smith VH (2003b) Eutrophication of fresh water and coastal marine ecosystems: a global problem. Environmental Science and Pollution Research International 10: 126-139

Smolyakov BS, Ryzhikh AP, Romanov RE (2010) The fate of Cu, Zn, and Cd in the initial stage of water system contamination: the effect of phytoplankton activity. Journal of Hazardous Materials 184: 819-825

Soucek DJ (2001) Integrative Bioassessment of Acid Mine Drainage Impacts on the Upper Powell River Watershed, Southwestern Virginia. PhD Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, pp 1-158

Soucek DJ, Denson BC, Schmidt TS, Cherry DS, Zipper CE (2002) Impaired Acroneuria sp. (Plecoptera, Perlidae) populations associated with aluminum contamination in neutral pH surface waters. Archives of Environmental Contamination and Toxicology 42: 416-422

Spry DJ, Wiener JG (1991) Metal Bioavailability and Toxicity to Fish in Low-Alkalinity Lakes: A

Critical Review. Environmental Pollution 71: 243-204

Stokes PM, Bailey RC, Groulx GR (1985) Effects of Acidification on Metal Availability to Aquatic Biota, with Special Reference to Filamentous Algae. Environmental Health Perspectives 63: 79-87

Svendsen CR, Quinn T, Kolbe D (2004) Review of Macroinvertebrate Drift in Lotic Ecosystems. Wildlife Research Program Environmental and Safety Division Final Report-Manuscript, pp 1-92

Thomas R, Meybeck M, Beim A (1996) Water Quality Assessments - A Guide to Use of Biota, Sediments and Water in Environmental Monitoring, Chapter 7. UNESCO/WHO/UNEP Second Edition, pp 1-46

Trainer VL, Pitcher GC, Reguera B, Smayda TJ (2010) The distribution and impacts of harmful algal bloom species in eastern boundary upwelling systems. Progress in Oceanography 85: 33-52

United Nations Environment Programme (2010) World Water Atlas. Division of Early Warning Assessment United Nations Environment Programme (UNEP)

Van Rensburg RJ (2003) A Long-term Acid Mine Drainage Water Management Strategy for South Witbank Colliery, Mpumalanga. Rand Afrikaans University, Magister Scientia in Environmental Management, pp 1-68

Van Vuuren L (2008) Loskop Dam - Early Local Engineering Ingenuity. The Water Wheel, pp 18-21

Vargo GA, Heil CA, Fanning KA, Dixon LK, Neely MB, Lester K, Ault D, Murasko S, Havens J, Walsh J, Bell S (2008) Nutrient availability in support of Karenia brevis blooms on the central West Florida Shelf: What keeps Karenia blooming? Continental Shelf Research 28: 73-98

Wahizatul AA, Long SH, Ahmad A (2011) Composition and Distribution of Aquatic Insect Communities in Relation to Water Quality in Two Fresh water Streams of Hulu Terengganu, Terengganu. Journal of Sustainability Science and Management 6: 148-155

Wallace JB, Webster JR (1996) The role of macroinvertebrates in stream ecosystem function. Annual Review of Entomology 41: 115-139

Wang J, Wu J (2009) Occurrence and potential risks of harmful algal blooms in the East China Sea. Science of the Total Environment 407: 4012-4021

Wenn CL (2008) Do fresh water macroinvertebrates reflect water quality improvements following the removal of point source pollution from Spen Beck, West Yorkshire? Earth & Environment 3: 369-406

Wood JM (1985) Effects of Acidification on the Mobility of Metals and Metalloids: An Overview. Environmental Health Perspectives 63: 115-119

Wren CD, Stephenson GL (1991) The effect of acidification on the accumulation and toxicity of metals to fresh water invertebrates. Environmental Pollution 71: 205-241

Chapter 3

Habitat changes contradict historical data of macroinvertebrate assemblage in the riverine zone of Loskop Dam, South Africa.

Abstract

In the current study of Loskop Dam the water quality shows that the dam receives acid mine drainage (AMD) and nutrients from upstream abundant coalmines, sewage works, and agriculture activities during the winter and spring season of 2009. It was evident from the study that the water at sampling site 1 (near the inlet of Loskop Dam) was of a poorer quality than site 2, the reference site. The water quality at site 1 was characterized by a maximum, sulphate concentration of 199 mg l^{-1} while the average electrical conductivity was 544 µS cm^{-1}. On the other hand the maximum sulphate concentration at the reference site was 5 mg l^{-1}, while conductivity was 18.33 µS cm^{-1}. During 2009 spring, site 1 had a higher macroinvertebrate diversity than the reference site with only a few highly sensitive families (Perlidae, Heptageniidae, Chlorocyphidae and Psepheniidae) despite increasing sulphate, conductivity and TDS levels. Scraper and shredder that is usually an indicator of a good habitat quality were also present at site 1 during spring. In contrast to site 1, the reference site had no sensitive taxa present during both seasons; and also the community was composed only of predators, collector gatherers/filters. Low habitat heterogeneity such as no aquatic macrophytes and less riparian vegetation may account for the low diversity and unbalanced in functional feeding group composition at the reference site. Consequently it was evident from this study that water chemistry alone was not a reliable indicator to determine macroinvertebrate assemblage and that the habitat plays a major role in this system. The macroinvertebrate communities at the selected sites in present study in comparison with some sites in the study conducted in Loskop Dam in 1968 also indicate that the habitat plays a major role in macroinvertebrate assemblage. The number of taxa

from the same period in 1968 was comparatively lower and was dominated by more tolerant taxa such as Chironomidae and Oligochaeta. The comparison showed that there was a significant shift of macroinvertebrate community from more tolerant group in 1968 to more sensitive groups in the present study. This phenomenon can possible be related to the development of reed bed in the riverine zone over a period of 42 years at site 1 that act as good habitat and providing shelters for macroinvertenbrates.

3.1 Introduction

Generally, a macroinvertebrate community impacted by AMD and organic pollution have a reduced diversity, an increase in species abundance, a community shift from sensitive to more tolerant species, or species loss, (Harrison 1958; Hunken and Mutz 2007). In a study conducted by Soucek (2001), he reported a significant reduction in Ephemeroptera-Plecoptera-Trichoptera (EPT) families richness and abundance, as well as a decrease in total taxon at sites receiving AMD water. According to Soucek (2001) the sites impacted by mining activity had higher water column and sediment toxicity due to high water column metal concentration and high Iron (III) hydroxide precipitate deposition respectively. He found that the precipitation of Al at a neutral pH occurring downstream from mining activities with AMD source causes acute toxicity to macroinvertebrates. The phenomenon was probably the cause of reduced benthic macroinvertebrate diversity. The product of oxidation of pyrite from coal mining, Fe(III) hydroxide precipitates, can also smothers the benthic habitat and thus inhibits the periphyton productivity that serves as an important primary food source for aquatic food webs (Soucek 2001).

Sewage inflow in rivers on the other hand has similar effects in altering the community assemblage in a coastal lagoon in South East Brazil (Henriques-de-Oliveira et al. 2007). According to a study by Henriques-de-Oliveira et al. (2007), macroinvertebrate community composition shifts may occurs from highly sensitive macroinvertebrate families (Trichoptera and Ephemeroptera) due to organic pollution to those ones that are more tolerant (Oligochaeta 41%, Chironomidae 40%). Although, in the latter study the impacted site had the highest abundance of macroinvertebrates, yet the greatest diversity was reported in un-impacted site (Henriques-de-Oliveira et al. 2007). A study conducted by Oberholster et al. (2008) on Rietvlei nature reserve, a river wetland area in South Africa, also showed that there was a major decrease of species diversity downstream of the point source of nutrient enrichment by the Hartbeesfontein Sewage Purification Works. The severely impacted site was dominated by tolerant family groups such as Oligochaetes and Chironomids (Oberholster et al. 2008). Often studies only point out the relationship between the observed changes in biotic indices and the level of pollutants. There are very few studies that can actually determine the causal relationship between a particular contaminant to the biological response of the organisms (Dsa et al. 2008; Grout and Levings 2001; Jarup 2003; Nordberg et al. 1985; Spry and Wiener 1991; Soucek 2001; Soucek et al. 2002). Therefore, it is difficult to elucidate data due to the complex interaction between biological, chemical, and physical factors that are involved in the system during a particular period of sampling. These factors can interact greatly altering the level of toxicities of the contaminants, its bioavailability and also the sensitivities of the organisms towards these contaminants (Wren and Stephenson 1991). The natural habitat may also play an important role and may also reduce the biodiversity as observed in this study (Wang et al. 2008). The objectives of the study was (1) to compare historical macroinvertebrate data of 1968 with the same sampling period in 2009 at two selected sites (2) to determine if any shifts in macroinvertebrate assemblage did occurred over the period of 42 year.

3.2 Materials and Methods

3.2.1 Study area

Loskop Dam (25°25'S; 29°2'25'E) is situated in the Mpumalanga Province of South Africa. The surrounding geology of the area is mainly composed of volcanic granite, namely Rooiberg felcite, and the southern border is composed of sediments including large pebbles of quartzite held together by a sandy matrix. The impoundment is fed by both the Olifants and Wilge Rivers, and has an area of 2427 ha and a volume of 374.3 m^3 at full supply capacity. In the upper Olifants River catchment, AMD, sewage pollution and extensive agriculture are the main sources of anthropogenic stressors on the aquatic environment (Driescher 2008). The catchment area of Loskop Dam is 11464 km^2 (Oberholster et al. 2010) . The lake water is utilized mainly for irrigation supply and drinking water for the towns of Groblersdal and Marble Hall, downstream of the dam wall. It also forms part of the 25000 ha Loskop nature reserve which is managed by the Mpumalanga Tourism and Parks Agency (MTPA). Over the past fifteen years, isolated incidents of fish mortality have been recorded at different times in Loskop Dam. These incidents have become more frequent during the past five years (2003-2008) during the winter and spring months (Driescher 2008), and have coincided with Nile crocodile (*Crocodylus niloticus*) mortalities (Oberholster 2011; Oberholster et al. 2010, 2012).

Two sampling sites were selected for the determination of macroinvertebrate distribution during the winter and spring months of 2009 in comparison with the same period in the 1968 study. This time frame was selected due to previous crocodile and fish mortalities of 14 tons during October 2007

(Driescher 2008; Oberholster et al. 2012). The sites were selected in relationship to previous sampling sites from the 1968 study conducted by Kruger (1968) and Mulder (1968). Sampling site 1 was selected in the riverine zone of Loskop Dam while sampling site 2 (undisturbed inflowing mountain stream) act as the reference site for this study as in the case of the 1968 study. This site was selected on the bases of its good water quality. The biotope of sampling site 1 was made up of boulders and sand, while sampling site 2 consists of bedrock. Sampling site 1 was dominated by dense reedbeds of the common water reeds *Phragmites communis* and the bulrush *Typha australis*, while macrophytes were totally absent at site 2 (Figure 3.1).

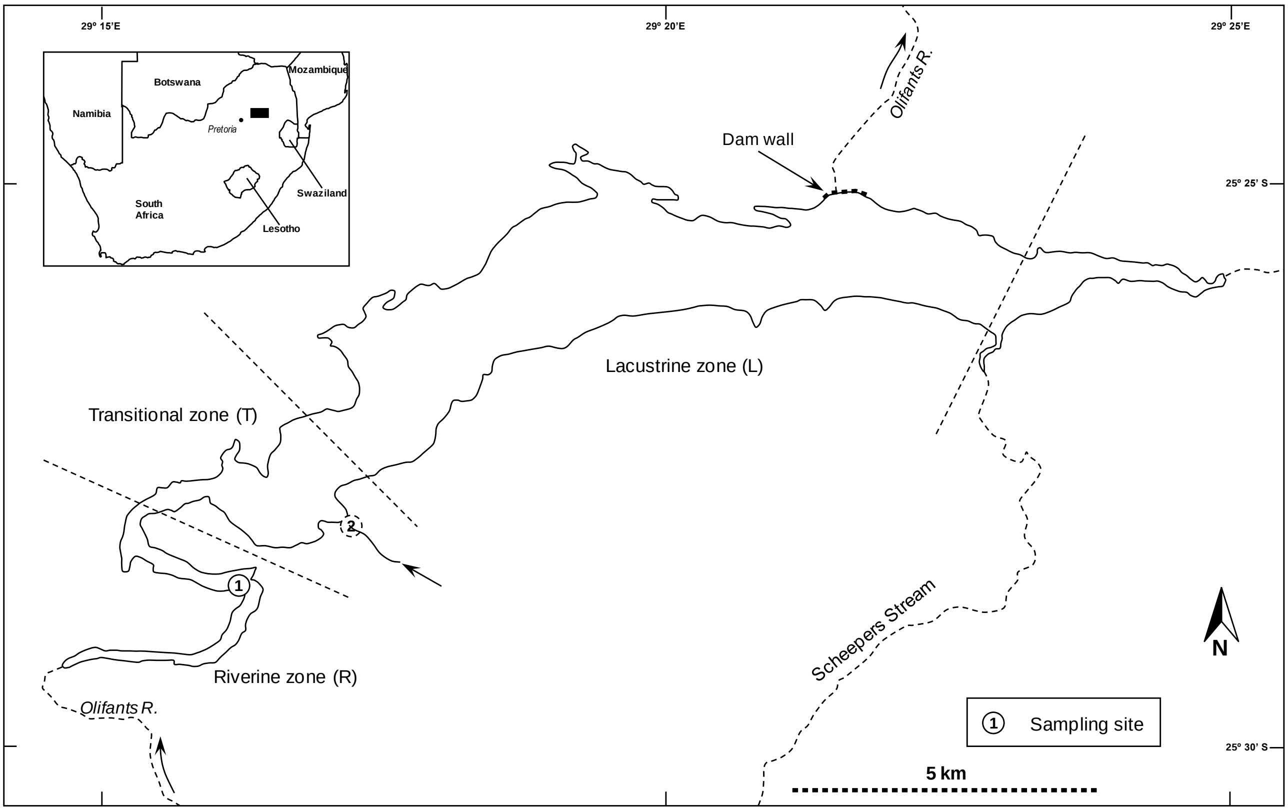

Figure 3.1: A map showing sampling site (1) inflow of the Olifant River and site (2) the reference site, Fontein sonder eind.

3.2.2 Macroinvertebrate sampling

Macroinvertebrates were collected from the surface sediments at sampling sites 1 and 2 with a SASS net (25 cm diameter; 50 µm mesh) (Oberholster et al. 2009). The macroinvertebrates were collected from sand, rocks, sediments, stones in current (SIC), stones out of current (SOOC), and marginal vegetation (Oberholster et al. 2008), according to the protocol as detailed in the South African Scoring System (SASS) Version 5, Rapid bioassessment method for rivers (Dickens and Graham 2002). The organisms collected were identified to family level as listed on the SASS5 scoring sheet. The sum of the SASS5 scores represents the index of the river health, while the average score per taxon (ASPT) is devided by the SASS5 score and by the number of sampled taxa (Dickens and Graham 2002). The abundance of organisms within each family was estimated as: 1 = 1; A = 2-10; B = 10-100; C = 100-1000; D = > 1000 , while macroinvertebrate diversity was calculated using the Shannon's diversity index (Shannon and Weaver 1949):

$$H = -(\sum(\rho_i \ \ln \rho_i)) \qquad (1)$$

where (H) is Shannon's diversity index, the proportion of species (i) relative to the total number of species (ρ_i) present in the aquatic ecosystem are calculated. The product of ($\rho_i \ln \rho_i$) for each family in the aquatic ecosystem is summed, and multiplied by -1 to give H. This index was used in conjunction with the SASS5 index. With the aid of the SASS5 scoring system, the presence and absence of certain families could be determined at the different sampling sites.

The diversity and evenness of the macroinvertebrate functional feeding groups were also used as indicators, as they play a vital role in assimilating and transferring energy in the aquatic food web. The feeding groups are classified based on how the invertebrates acquire food resources and particle size fraction consume (Cummins 1974). Therefore spatial and temporal variation in food source can shape the distribution and composition of aquatic macroinvertebrates (Wallace and Anderson 1995). This classification system includes the following functional feeding groups: shredders (SH); scrappers (SC); collector-gatherers (GC); collector-filterers (FC) and predators (PR). The South Africa Invertebrate Habitat Assessment System (IHAS) (McMillan 1998) and Ephemeroptera-Plecoptera-Trichoptera (EPT) families richness (Soucek 2001) were also included in this study. Because EPT families consist of pollutant-intolerant species, their richness was found to be potential bioindicators for a cleanliness of an ecosystem (Wahizatul et al. 2011). The IHAS is a numerical evaluation of the habitat condition at a particular sampling site and at the time of sampling. This assessment is important to provide a complete picture of biotic status in conjunction with SASS5, due to the fact that differences in habitat may also results in certain families being absent from a particular site, even if the water quality was good as observed in this study. However, it must be taken in account that these indices were not yet developed and employed in the 1968 study. The only information available on the habitat condition of sampling site 1 was that small patches of the common water reed *Phragmites australis* and the bulrush *Typha communis* was observed in the 1968 study. In the current study in 2009 these small patches of common reeds had formed dense reed bed at sampling site 1.

3.2.3 Physical and chemical analysis

Dissolved O_2, temperature, pH and conductivity were measured *in situ* at the surface of the water column using a Hach sension^{TM}156 Portable Multiparameter (Coveland, USA) according to Oberholster et al. (2010). Surface water (the top 5 cm) samples were taken during the June and October 2009 field trips at both sites with a surface grab sampler. These surface water samples were filtered in the field (using 0.45μm Whatman GF/filters), stored in pre-rinsed 500 ml polyethylene bottles and preserved with sulfuric acid (pH 2.0) for analysis of dissolved nutrients. The samples were transported to the laboratory in a dark container. All analysis was carried out according to standard methods (American Public Health Association, APHA 1992).

3.2.4 Rainfall Collection

The rainfall data was collected using a standardized rain gauge at the weather station at the Loskop Dam Nature Reserve Offices. The rainfall data was used to determine the increase in flow regime.

3.2.5 Statistical analysis

Differences in water temperature, conductivity and macroinvertebrate functional feeding groups of sampling sites 1 and 2 during the present study, were compared using one way ANOVA. Correlations among the above variables were calculated using the Spearman correlation test. In all cases comparisons that shows a p value < 0.05 were considered significant. Statistical analysis was conducted with Origin software (version 7.0.0).

3.3 Results

3.3.1 Macroinvertebrate distribution of the 1968 and 2009 studies

During the winter sampling in June 2009, a total of 17 macroinvertebrate families were identified at site 1 in the riverine zone of Loskop Dam. This site had a higher EPT richness yet it was dominated by the family Simuliidae (order Diptera) which accounts for 70% of the population, and is known to have low level of tolerance to contaminants (Thirion 2007). Other less dominant families included Baetidae, Coenagrionidae (order Odonata) and Hydropsychidae (order Trichoptera). Site 2 had a lower diversity with 14 macroinvertebrate families sampled. Site 2 was dominated by families such as Baetidae (order Ephemeroptera), Gomphidae (order Odonata) and Chironomidae (order Diptera), all with relatively high tolerance to contaminants. The number of sensitive families was low in comparison with site 1, with only Philopotamidae (order Trichoptera) observed. Both the diversity (H' = 1.18) and evenness (E = 0.416) of the macroinvertebrate community at site 1 were relatively lower than that of site 2 (H' = 1.86, E = 0.707). However more sensitive taxa were encountered at site 1, such as Baetidae and Perlidae (Plecoptera). There was no significant difference in ASPT value between the sites (site 1 = 5.6, site 2= 5.2), and both are assessed as border line good/bad water quality values with some deterioration (Tamatamah et al. 2007).

In October 2009 (spring) a total of 21 macroinvertebrate families were collected at sampling site 1. The site had a SASS score of 133 and ASPT score of 6.3, indicating a good water quality with high habitat diversity. The good habitat was reflected by a high IHAS score of 72%. This site was also characterized by clear water, good flow with some filamentous algae. Compared to the winter sampling, the spring sample at site 1 had a higher Shannon's diversity index (*H'* = 2.06) yet slightly lower evenness (E = 0.677). The dominant family was Veliidae, and four highly sensitive families (Perlidae, Heptageniidae, Chlorocyphidae and Psepheniidae) were also present. The presence of Leptophlebidae shows that there was relatively high oxygen content at site 1 (Magoba 2005). In October 2009 a total of 15 macroinvertebrate families were collected at site 2. This sampling site had a SASS score of 80 and ASPT sore of 5.3, indicating a "border line" water quality with moderate habitat diversity. The site was characterized with slow water flows (< 10 cm s^{-1}) and a habitat consisting mainly of sand and bedrock. Compared to the winter sampling, the spring sampling had a higher family diversity and evenness at sampling site 2. The dominant families observed were Gomphidae (order Odonata), Corixidae (order Hemiptera) and Gyrinidae (order Coleoptera). No family with high sensitivity (QV>10) scores were recorded at this site during spring. The evenness of families at site 2 (E = 0.73) was higher than that of site 1 (E = 0.677). However, site 1 (*H'* = 2.0615) had a higher Shannon's diversity index than site 2 (*H'* = 1.988) during this sampling period.

At sampling site 2, the make-up of functional feeding groups of the macroinvertebrate community in winter and spring did differ substantially. It comprised predominantly of predators (winter: 44.55%, spring: 91.44%) with the rest made up by collector-gatherer and filterers (Figure 3.2). However, great differences were observed between winter and spring sampling at sampling site 1.

In June, the community was primarily made up of predators, with the rest of the macroinvertebrate families consist of collector-gatherers and filterer-feeders **(**Figure 3.2). The increase in average surface water temperature from 16℃ in June to 21℃ in October correlated positively (r = 0.94; $p < 0.05$) with the increases of predators at both sites. A vast difference was observed in both the diversity of the macroinvertebrate families and functional feeding groups during the October 2009 sampling **(**Figure 3.2, Table 3.1**).** The community had a more balanced structure in terms of the functional feeding groups which include scrapers/shredders, collector gatherers, filterers and predators (Table 3.1). According to the 1968 study, macroinvertebrate family numbers seemed to be lower in comparison with the 2009 study, and were dominated by the order and families of Oligochaeta, Chironomidae and Caenidae at both sampling sites 1 and 2 during winter and spring sampling period (Table 3.1). The dominant functional feeding groups that were found in the littoral zone at site 1 in the 1968 study, were collector-gatherers (Mulder 1968).

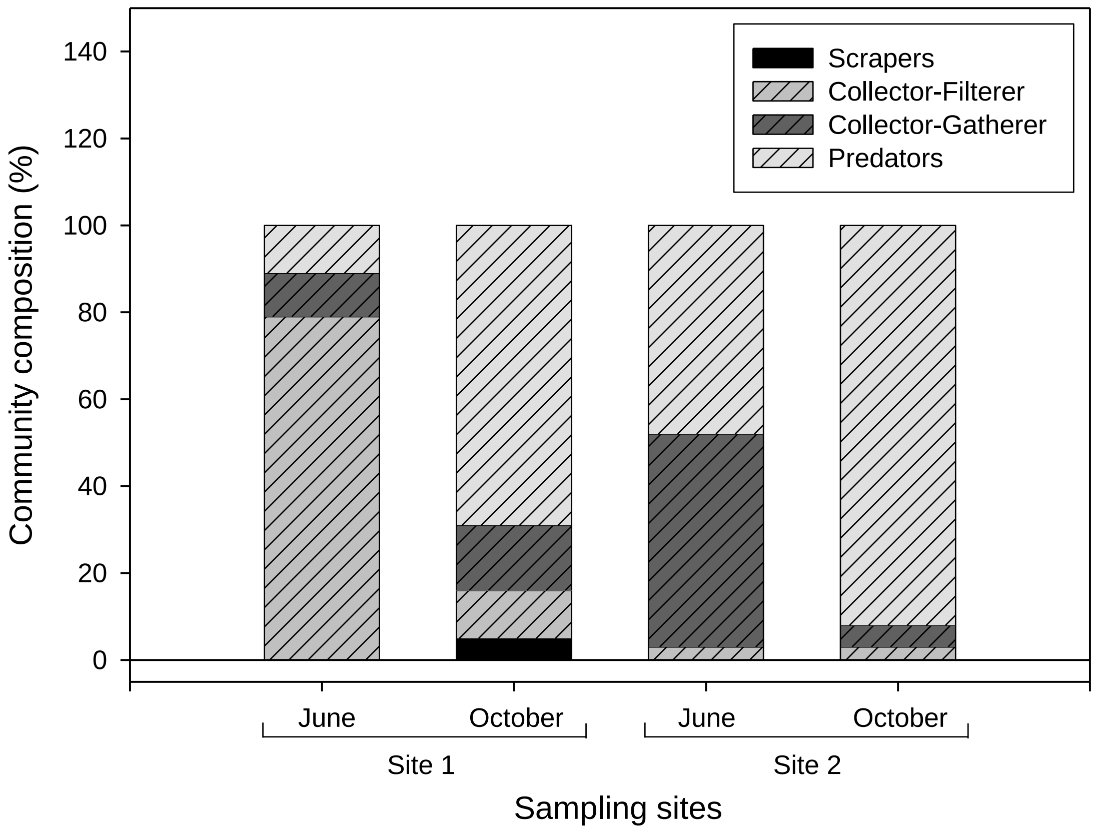

Figure 3.2: The average abundance (%) of macroinvertebrate functional-feeding groups at site 1 and 2 (reference site) during June (winter) and October (spring) 2009 (n=2) at Loskop Dam.

Table 3.1 Composition differences of macroinvertebrate assemblage from the 2009 and 1968 studies at Loskop Dam. (W) 2009 winter; (S) 2009 spring; (WS) 1968 winter and spring

W	Site 1				Site 2			
Year 2009 June winter	**Family**		**Estimated number of organisms**	**Functional feeding group**	**Family**		**Estimated number of organisms**	**Functional feeding group**
	Annelida	Hirudinea	A	predator	**Ephemeroptera**	Baetidae	B	collector-gatherer
	Crustacea	Potamonautidae	1	collector-gatherer	**Odonata**	Coenagrionidae	A	Predator
	Plecoptera	Perlidae	1	predator		Gomphidae	B	Predator
	Ephemeroptera	Baetidae > 2 sp	B	collector-gatherer		Libellulidae	A	Predator
	Odonata	Coenagrionidae	B	predator	**Hemiptera**	Belostomatidae	A	Predator
		Aeshnidae	A	predator		Gerridae	A	Predator
		Gomphidae	A	predator		Naucoridae	A	Predator
	Hemiptera	Belostomatidae	A	predator		Veliidae	A	Predator
		Gerridae	A	predator	**Trichoptera**	Hydropsychidae	A	collector-filterer
		Veliidae	A	predator		Philopotamidae	1	collector-filterer
	Trichoptera	Hydropsychidae	B	collector-filterer	**Coleoptera**	Dytiscidae/Noteridae	1	Predator
	Cased caddis:	Leptoceridae	1	collector/predator		Gyrinidae	1	Predator
	Coleoptera	Dytiscidae/Noteridae	A	predator	**Diptera**	Ceratopogonidae	1	Predator
		Gyrinidae	1	predator		Chironomidae	B	collector/predator/gatherer
		Hydrophilidae	A	predator				
	Diptera	Chironomidae	A	collector/predator/gatherer				
		Simuliidae	C	collector/filterer				

Estimate abundances: 1=1, A= 2-10, B= 10-100, C= 100-1000, D> 1000

Table 3.1 continued

S	Site 1				Site 2			
Year 2009 October Spring	**Porifera**		A		**Hydracarina**		1	Parasite
	Annelida	Oligochaeta	B	collector/gatherer	**Ephemeroptera**	Baetidae	A	collector-gatherer
	Crustacea	Potamonautidae	1	collector-gatherer	**Odonata**	Coenagrionidae	A	Predator
		Atyidae	B			Corduliidae	A	Predator
	Plecoptera	Perlidae	B	Predator		Gomphidae	B	Predator
	Ephemeroptera	Baetidae 1sp	B	collector-gatherer		Libellulidae	1	Predator
		Heptageniidae	B	Scraper	**Hemiptera**	Corixidae	B	Predator
		Leptophlebiidae	B	collector-gatherer		Hydrometridae	A	Predator
	Odonata	Chlorocyphidae	A			Naucoridae	1	Predator
		Coenagrionidae	B	Predator		Nepidae	A	Predator
		Corduliidae	1	Predator	**Trichoptera**	Hydropsychidae	A	collector-filterer
		Gomphidae	B	Predator		Leptoceridae	A	collector/predator
	Hemiptera	Belostomidae	B		**Coleoptera**	Dytiscidae	A	Predator
		Nepidae	1	Predator		Gyrinidae	B	Predator
		Veliidae	C	Predator		Hydrophilidae	A	Predator
	Trichoptera	Hydropsychidae	B	collector-filterer				
	Coleoptera	Elmidae	1	scraper/shredder				
		Psephenidae	1	Scraper				
	Diptera	Simuliidae	A	collector/filterer				
		Tabanidae	A	Predator				
	Pelecypoda	Corbiculidae	B	collector/filterer				

Estimate abundances: 1=1, A= 2-10, B= 10-100, C= 100-1000, D> 1000

Table 3.1 continued

WS	Site 1				Site 2			
Year 1968 Winter	**Family**		**Estimated number of organisms**	**Functional feeding group**	**Family**		**Estimated number of organisms**	**Functional feeding group**
	Annelida	Oligochaeta	B	collector/gatherer	**Annelida**	Oligochaeta	C	collector/gatherer
	Diptera	Culicidae	A	collector/gatherer/filterer	**Diptera**	Culicidae	A	collector/gatherer/filterer
		Chironomidae	B	collector/predator/gatherer		Ceratopogonidae	A	Predator
	Ephemeroptera	Caenidae	B	collector/gatherer		Chironomidae	C	collector/predator/gatherer
	Hemiptera	Corixidae	A	Predator	**Ephemeroptera**	Caenidae	C	collector/gatherer
		Belostomatidae	A	predator	**Trichoptera**	Ecnomidae	A	
	Odonata	Coenagrionidae	A	predator		Leptoceridae	A	collector/predator
					Hemiptera	Notonectidae	A	Predator
					Odonata	Coenagrionidae	A	Predator
Year 1968 Spring	**Annelida**	Oligochaeta	A	collector/gatherer	**Annelida**	Oligochaeta	A	collector/gatherer
	Crustacea	Ostracoda	A		**Crustacea**	Ostracoda	A	
	Ephemeroptera	Caenidae	A	collector/gatherer	**Ephemeroptera**	Caenidae	A	collector/gatherer
	Diptera	Chironomidae	A	collector/predator/gatherer		Baetidae	B	collector/gatherer
	Hemiptera	Notonectidae	A	Predator	**Diptera**	Chironomidae	C	collector/predator/gatherer
		Belostomatidae	A	Predator	**Hemiptera**	Corixidae	B	Predator
						Notonectidae	A	Predator
						Belostomatidae	A	Predator
					Trichoptera	Ecnomidae	A	
					Odonata	Coenagrionidae	A	Predator

Estimate abundances: 1=1, A= 2-10, B= 10-100, C= 100-1000, D> 1000

3.3.2 Physical and chemical data of the 1968 study in comparison to the 2009 study

In June 2009, sampling site 1 was characterised by average nitrate nitrogen (N) value of 1,500 µg l^{-1} and a sulphate (SO_4) value of 135 µg l^{-1}. A decrease in average N value (240 µg l^{-1}) at this sampling site was observed in October 2009. However the latter was still significantly above the TWQR guideline standard of <0.2 µg l^{-1} for South Africa. The sulphate value at site 1 on the other hand increased in October to 199 mg l^{-1}, but was still below TWQR guideline standard for South Africa (0-200 mg l^{-1}). Conductivity (June: 323 µScm^{-1}, October: 544 µS cm^{-1}) and TDS (June: 202 µg l^{-1}, October: 270 µg l^{-1}) increased throughout the study period. The much lower average conductivity value in June 2009 correlated positively (r = 0.83; p < 0.05) with the high percentage of collector filterers (Table 3.2). An average chloride level (22 mg l^{-1}) at site 1 was also much higher than the TWQR guideline standard (0.2 µg l^{-1}), especially in October. Site 2, in both winter and spring, was low in nitrate nitrogen (<0.2 mg l^{-1}), orthophosphate (<0.2 mg l^{-1}) and sulphate (<5 mg l^{-1}) concentrations in comparison to site 1. In contrast to site 1, site 2 for both seasons had very low conductivity (June: 18.33 µS cm^{-1}, October: 16 µS cm^{-1}) and TDS (June: 10.4 µg l^{-1}, October: 7.2 µg l^{-1}) (Figure 3.3). The pH values for both sites in both seasons ranged from neutral to alkaline, with site 2 having a higher pH (8.38~8.7) in comparison to site 1 (7.6 ~ 8.09). However the pH values at both sites increased slightly in October.

The chemical data generated in June 2009 were compared with the maximum value recorded in winter at the littoral zone of Loskop Dam by Kruger (1968) as well as the data by Mulder (1968).

However, Mulder (1968) did not record the chemical data of the winter of 1968. However, the physical and chemical data for spring was compared with Mulder's study in 1968 (Table 3.2). The most noticeable difference shown in the table is higher levels of calcium, magnesium, sodium, sulphate, conductivity and TDS in the present study. Particularly for sulphate, the increase was almost ten-fold in comparison to the 1968 study. The conductivity increased almost in two-fold while the nitrate/nitrite (N) level of the 1968 study was eight times higher than in the current study during spring time.

Table 3.2 Comparison of physical and chemical data of Loskop Dam between 2009 and 1968 studies

		2009				1968	
Parameter	Units	June (Winter)		October (Spring)		Winter	Spring
		Site 1	reference site	Site 1	reference site	maximum recorded of littoral zone	inlet site (2)
Alkalinity	mg/ℓ CaCO3	40	4.3	54	11	56	61
Aluminium	mg/ℓ Aℓ	0.07	0.16	0.09	0.19	No data	No data
Ammonia nitrogen	mg/ℓ N	0.1	0.1	0.1	0.1	No data	No data
Cadmium	mg/ℓ Cd	0.01	0.01	0.01	0.01	No data	No data
Calcium	mg/ℓ Ca	35	0.63	42	0.41	13.6	21
Chloride	mg/ℓ Cℓ	5	5	22	5	No data	No data
Fluoride	mg/ℓ F	0.2	0.2	0.2	0.2	No data	No data
Iron	mg/ℓ Fe	0.06	0.3	0.06	0.3	No data	No data
Magnesium	mg/ℓ Mg	18	0.65	24	0.33	6.2	7.25
Manganese	mg/ℓ Mn	0.05	0.05	0.05	0.05	No data	No data
Nitrate + Nitrite (N)	mg/ℓ N	1.5	0.2	0.24	0.2	0.95	1.47
ortho Phosphate	mg/ℓ P	0.2	0.2	0.2	0.2	(Total P) 3.2	(Total P)2.15
Potassium	mg/ℓ K	3	0.5	7	0.5	8.4	3.65
Silicon	mg/ℓ Si	1.9	3.2	1.8	3.8	No data	No data
Sodium	mg/ℓ Na	25	1	34	0.8	5.3	20.3
Sulphate	mg/ℓ SO4	135	5	199	5	18	9.5
Zinc	mg/ℓ Zn	0.06	0.06	0.06	0.06	No data	No data
pH		7.6	8.38	8.08	8.7	7.5	7.5
Conductivity	(μS/cm)	323	18.33	544	16	135	205
Temp	(℃)	13.3	13.9	24.4	24.5	13.2	25.6
D.O	(mg/L)	6.56	7.77			7.2	8.22
TDS	(μg/L)	202	10.4	270	7.2	193	155.5

Average of site 1 throughout sampling period (2009) : 0.93 average of nitrate throughout 4 season (1968): 0.87

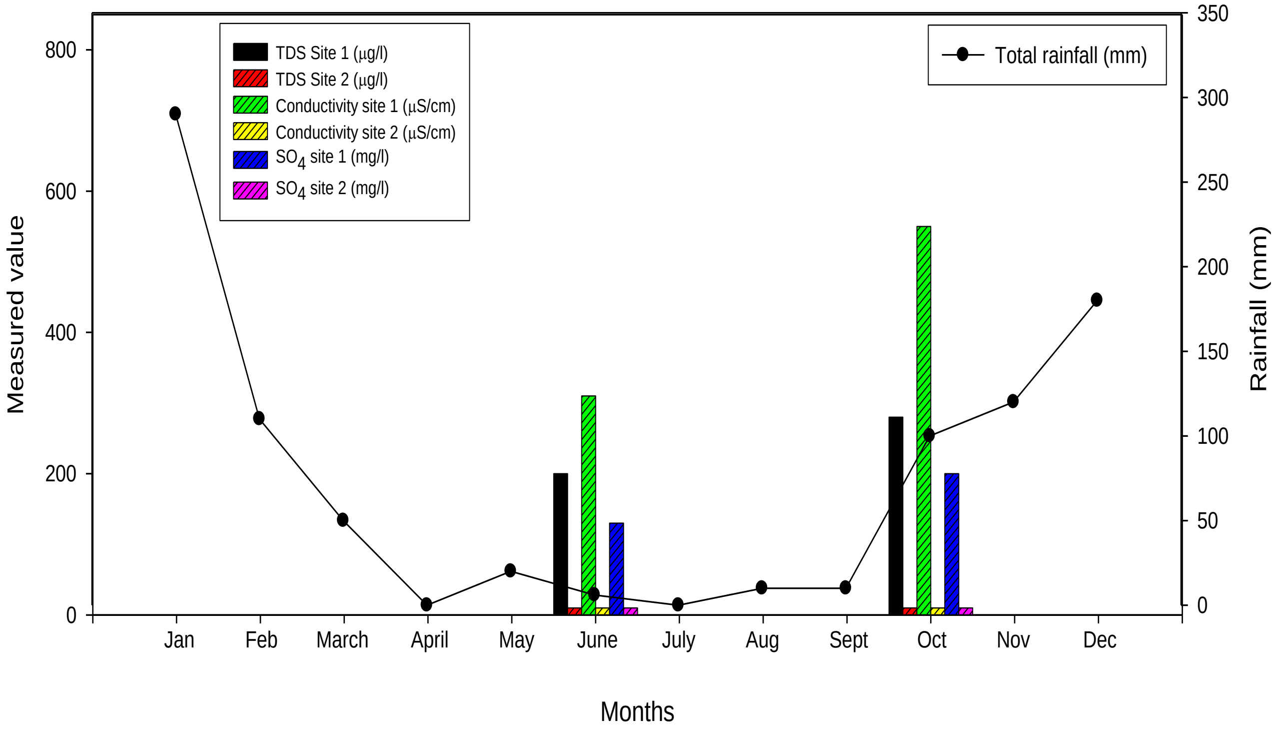

Figure 3.3: Total monthly rainfall in 2009 (data supplied by Ecologist Jannie Coetzee, MTPA, Lake Loskop) and trend of conductivity, TDS, and SO_4 during winter (June) and spring (October) sampling period.

3.4 Discussion

3.4.1 Macroinvertebrate distribution and habitat heterogeneity

The higher number of macroinvertebrate taxa in the present study, when compared to data from the 1968 study, as well as the shift from more tolerant groups in1968 to more sensitive groups in the present study can possibly be related to habitat changes at sampling site 1. According to Kruger (1968) low numbers of the common water reed *Phragmites communis* and the bulrush *Typha australis* could be found at the riverine zone of the lake during 1968. However, forty two years later in 2009 these common reeds had formed dense reed bed at sampling site 1. These reed beds can act as ‘filters’ and absorbed phosphates and nitrogen out of the water column and may be the reason for the low values of ortho-phosphate measured at this site (Oberholster et al. 2008). The reed bed also acts as good habitat providing oxygen through their roots for macroinvertenbrates. The available habitat also plays a major role in reproduction, foraging for food, shelter, and escape from predators (Olomukoro and Nduh Tochukwu 2006).

The reference site in winter (June 2009) had a higher Shannon diversity index and evenness in comparison to site 1. This observation was expected since higher diversity should occur at the un-impacted site, characterized by having a low TDS, conductivity, SO_4 and higher pH (Last 2001). However, site 2 had a less diversity than site 1, and only one sensitive taxa (Philopotamidae) was collected. This may be due to the different biotope of site 2 that contains only bedrock, whereas site 1 contains cobbles, boulders and macrophytes. It is generally known that higher diversity of invertebrates can be found at biotopes with boulders, cobbles and macrophytes, than that with only bedrock (Wang et al. 2008). The macroinvertebrate family Plecoptera that generally contains sensitive species was not found at this site. This could be related to the slower flow regime (<10 cm s^{-1}) observed at sampling site 2, since Plecoptera prefer cool, clean streams with high dissolved oxygen, a condition that often occurs in riffles (Last 2001). Analysis of the macroinvertebrates feeding groups at sampling site 2 shows that predators seem to have a higher family diversity than other groups. Due to the absence of scrapers and shredders at sampling site 2, it seems that this site has an imbalanced macroinvertebrate community structure. This could possibly be related to the lower habitat heterogeneity at this site. This site also had no aquatic macrophytes present, like in the case of site 1. It is known that aquatic macrophytes do increase physical heterogeneity and create a more diverse habitat for benthos (Magoba 2005; Buckup et al. 2007). The dominance of the family Simuliidae at sampling site 1 may be linked with the standing stocks of vascular plants which provide an attachment surface for this species (Oberholster et al. 2008). The abundance of Simuliidae and Hydropsychidae are indicators of organic pollution and may be the reason for their dominance at sampling site 1 (Last 2001; Buckup et al. 2007). Both these families belong to filter-feeding groups feeding on fine particles including algae (Fuller and O'Reilly 2005; Resh et al. 2007). Their dominance at sampling site 1 suggests there was an increase in planktonic and periphyton algae, as a result of higher nitrogen and phosphate concentrations during the winter months with lower river flows (Coimbra et al. 1996).

Spring samples at both sites showed an increase in diversity, evenness, ASPT and richness. The spring sample at sampling site 2 was 7 points higher in terms of the SASS score. The increase in macroinvertebrate diversity was possibly due to an increase in temperature during the spring time, as most macroinvertebrates were in a larval stage during the winter months (Boyero et al. 2005). The higher abundance of predators in October at both sampling sites can possibly be linked to the number of prey as water temperature increases. In terms of the make-up of the functional feeding groups, spring and winter samples showed that the community consisted of predators, collector-gatherers and collector-filterers, with a total absence of scrapers and shredders (Figure

3.2). This was quite similar to the feeding groups that were also found in the littoral zone at site 1 in the 1968 study, with collector-gatherers reported as the dominant group (Mulder 1968). The absence of scrapers and shredders at sampling site 2 during the spring sampling could possibly be due to low habitat heterogeneity.

Higher diversity and presence of highly sensitive taxa at sampling site 1 during the spring sampling can be attributed to the increase in the amount of rainfall during the rainy season. The increase in the inflow of drainage into the inlet of the dam creates riffles that favour the survival of sensitive taxa and more habitat diversity supporting establishment of a diverse community. The good habitat quality at sampling site 1 in spring was reflected by the IHAS assessment which showed that this site was characterised by good flows (Eddy and Feck 1998).

The macroinvertebrate family numbers seemed to be lower in 1968 in comparison with the 2009 study, and were dominated by Oligochaeta, Chironomidae and Caenidae. These order and families are generally good indicators of high nutrient input and low oxygen content in the water (Dura 2005) (Table 3.1). Oligochaeta was particularly high in winter time ($n > 400$) in 1968, indicating there may have been a higher nutrient inflow during that sampling period. Similarly, a study done on Rietvlei Nature Reserve wetland area, South Africa showed a similar trend where the impacted sites were dominated by the macroinvertebrate families Oligochaeta, Nematodes, Syrphidae, and had low diversity and eveness (Oberholster et al. 2008). The family Chironomidae generally has a very short generation time, which enables them to quickly colonize an area where pollution occurred (Martins et al. 2008). This advantage help reduced competition and predation (Eddy and Feck 1998). Both Oligochaeta (n = 442 in winter) and Chironomidae (n = 156 in spring) have high tolerance to pollutants and they normally occur in abundance, particularly in areas with high organic input (Martins et al. 2008). Oligochaeta feeds on fine organic matter and can colonize quickly in absence of predators, and particularly when there is high level of organic pollution resulting in a reduction of dissolved oxygen (Eddy and Feck 1998). Their dominance reflected that the water quality in Loskop Dam during the 1968 samples was primarily degraded by enrichment of organic substances coming from the upper Olifants River catchment during the rainy season (Oberholster et al. 2010). The present study showed that Oligochaeta, Caenidae and Chironomidae were either absent or in low abundance at both sites. Instead, families with higher sensitivity (> 7), such as Perlidae, Leptophlebiidae, Atyidae, Aeshnidae, Heptageniidae, Chlorocyphidae, Elmidae, Psephnidae, Philopotamidae, Corduliidae, and Hydracarina were found during the current study. Therefore by comparing the families between the two studies, it is apparent that there was a shift in macroinvertebrate community make-up from tolerant families during the 1968 study to more sensitive families in 2009.

Another difference between the macroinvertebrate communities were that the community of the current study has a more evenly distribution of uni-, semi-, and multivoltinism than the 1968 study. Voltinism is a measure of the life cycle of taxa. Univoltine taxa are families that require a year to complete a single life cycle; semivoltine taxa require several years to complete a single life cycle; while multivoltine taxa has several life cycles during a single year (Eddy and Feck 1998). In the 1968 study, multivoltine taxa such as Oligochaeta and Chironomidae dominated the community, which possibly indicated a seasonal or intermittent period of poor water quality as these taxa can re-colonize after exposure of water to pollutants faster than uni/semivoltine taxa (Eddy and Feck 1998). They are thus more competitive than univoltine or semi-voltine taxa which require a more stable aquatic system to be able to survive throughout a year or longer periods of time (Eddy and Feck 1998). In contrast to the previous study in 1968, the voltinism of taxa in the current study seem to be more evenly distributed at sampling site 1. Besides multi-voltine taxa (Simuliidae, Belostomatidae, Hydrometridae), there was a greater number of univoltine taxa (Coenagrionidae, Hydropsychidae, Dytiscidae, Gyrinidae, Heptaganiidae, Leptophlebiidae, Tabanidae) and semi-voltine taxa (Elmidae, Gomphidae, Psephnidae) at sampling site 1 during the 2009 sampling (Wallace and Anderson 1995).

3.4.2 Physical and chemical analyses

Sulphate, TDS, and conductivity levels were much higher at sampling site 1 than at site 2 during the 2009 study. These three values increased throughout the 2009 study period, showing that there was a constant inflow of AMD into Loskop Dam from the upstream mining area’s or due to evaporation in winter leaving water more concentrated with salts during the spring. The above three values were particularly high in October, which could be the consequence of decanting coal mines upstream after the increase in rainfall during spring. If there was a constant input of AMD, then one would also expect a low pH and particularly high level of dissolved metals in the water column. However from the chemical and physical analyses, it showed water column conditions of a neutral to alkaline pH (6.84 to 9.39), but with high concentrations of Al, Fe and other metal ions. These metals were usually the chemical indicators for the extent of the impact made by AMD, yet they are easily modified by other physical, biological and chemical factors as it move downstream (DWAF 1996; Soucek 2001; Ritter et al. 2002). The solubility of Al is highly pH-dependent where it is most soluble and toxic at low pH (< 4.0), least soluble in neutral pH (6.5-7.5) in the absence of complexing agents, and soluble but biologically unavailable under alkaline conditions (pH > 7.5) (DWAF 1996).

Therefore the high concentration of Al detected at site 1 may be due to its solubility under alkaline conditions. Fe concentration appears to be lower at sampling site 1 than site 2. One factor that affects the level of Fe concentration in the water column is the hardness ($CaCO_3$ mg l^{-1}) of water where soft water (< 60 mg l^{-1} $CaCO_3$) containing low concentration of bicarbonate usually contains higher concentration of Fe (DWAF 1996). This may explain the higher Fe level found at sampling site 2, whereas the lower Fe concentrations at site 1 may be due to harder water. A possible reason for the higher level of metal concentrations detected in the water column during June could also be due to AMD discharge that dilutes as it moves further downstream from the point of entry in the aquatic system. The pH is then lowered and dissolved metals will form colloids that precipitate as metal sulphates and hydroxides (Last 2001). The precipitate can leave the water column and settle on the bottom sediment, particularly at low discharge periods (winter). Sedimentation of metals and pollutants are particularly destructive to macroinvertebrate in terms of diversity, richness and population density, because their whole life cycle are spent on the substrate (Gray 1997). As colonization and abundance of macroinverebrate species are dependent on particle size and spaces between substrate, sedimentation and coating of substrate can thus decrease heterogeneity, making it unstable and unfit for benthic organisms to live on (Quigley 1981; Gray 1996; Jackson et al. 2000; Soucek 2001; Nedeau et al. 2003; Stoddard et al. 2006).

Total nitrate (N) and phosphate (P) are indicators for level of organic nutrient in a water system. Out of all the phosphate species, ortho-phosphate is the only form of phosphate that can be assimilated by autotrophs. Phosphate is usually the limiting factor for phytoplankton production in a fresh water system. This is because of the quick turn-over rate of ortho-phosphate and also the efficient trapping of P-input by biological assimilation and deposition in the sediment (Correll 1998). Therefore this may explain the low level of ortho-phosphate throughout the study.

The relatively high nitrate level at sampling site 1 in June may be indicative of sewage input or runoffs from agricultural activities upstream of Loskop Dam. By comparing data obtained during the 1968 study with data from the present study, there was an increase in dissolved metal ions in the present study (i.e. Ca^{2+}, Mg^{2+}, Na^{+}). Other metals such as iron, manganese, aluminum and cadmium were not measured during the 1968 study, and could not be compared. Despite the huge increases in the level of sulphate, TDS and conductivity as compared to the 1968 study, it was still within the TWQR standard for aquatic ecosystems (DWAF 1996).

3.5 Conclusion

It is evident from the this study that the shift from more pollution tolerant macroinvertebrate groups in1968 to more sensitive groups in the present study can be related to the habitat changes over a period of 42 years. Furthermore, it seems that the formation of dense reed bed at sampling site 1 did increase the habitat heterogeneity which over shadowed the poorer water quality measured in the 2009 study in comparison with the 1968 study. The study also indicated site 2, (undisturbed inflowing mountain stream) that act as the reference site for this study as in the case of the 1968 was not suitable for reference conditions due to its lack of habitat heterogeneity.

3.6 References

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF) (1992) Standard Methods for the Examination of Water and Wastewater. (19th edition). APHA, AWWA, and WPCF, Washington, D.C., USA

Boyero L, Valladolid M, Arauzo M (2005) Dynamics of Invertebrate Benthic Communities and Drift in a Regulated River of Central Spain. International Review of Hydrobiology 90: 392-411

Buckup L, Bueno AAP, Bond-Buckup G, Casagrande M, Majolo F (2007) The benthic macroinvertebrate fauna of highland streams in southern Brazil: composition, diversity and structure. Revista Brasileira de Zoologia 24: 294–301

Coimbra CN, Graca MA, Cortes RM (1996) The effects of a basic effluent on macroinvertebrate community structure in a temporary Mediterranean river. Environmental Pollution 94: 301-307

Correll DL (1998) The Role of Phosphorus in the Eutrophication of Receiving Waters: A Review. Journal of Environmental Quality 27: 261-266

Cummins WK (1974) Structure and Function of Stream Ecosystems. American Institute of Biological Sciences 24:631-641

Dickens CWS, Graham PM (2002) The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. African Journal of Aquatic Science 27: 1-10

Driescher AC (2008) A water quality study of Loskop Dam and the upper catchment of the Olifants River. Unpublished M.Sc. Thesis. University of the Free State, Bloemfontein, South Africa, pp 1-150

Dsa JV, Johnson KS, Lopez D, Kanuckel C, Tumlinson J (2008) Residual Toxicity of Acid Mine Drainage-Contaminated Sediment to Stream Macroinvertebrates: Relative Contribution of Acidity vs. Metals. Water Air & Soil Pollution 194: 185-197

Dura JO (2005) Response of the benthic macroinvertebrate community to a point source in La Tordera Stream (Catalonia, Ne Spain). Universitat de Girona

DWAF (Department of Water Affairs and Forestry) (1996) South African Water Quality Guidelines (first edition). Volume 7: Aquatic Ecosystem, Pretoria, South Africa

Eddy T, Feck J (1998) Beneficial use Reconnaissance Monitoring and Assessment Report. Wyoming Department of Environmental Quality Water Quality Division: 1-25

Fouche PSO, Vlok W (2010) Water quality impacts on instream biota of the Shingwedzi River, South Africa. African Journal of Aquatic Science 35: 1-11

Fuller M, O'Reilly C (2005) Characterization and comparison of benthic macroinvertebrate communities in small streams of forested and deforested catchments bordering Lake Tanganyika. Nyanza Projects Publications. Paleoclimatology-Limnogeology Team Report.

Gray NF (1996) A substrate classification index for the visual assessment of the impact of acid mine drainage in lotic systems. Water Research 30(6): 1551-1554

Gray NF (1997) Environmental impact and remediation of acid mine drainage: a management problem. Environmental Geology 30: 62-71

Grout JA, Levings CD (2001) Effects of acid mine drainage from an abandoned copper mine, Britannia Mines, Howe Sound, British Columbia, Canada, on transplanted blue mussels (*Mytilus edulis*). Marine Environmental Research 51: 265-288

Harrison AD (1958) The effects of sulfuric acid pollution on the biology of streams in the Transvaal. South Africa. International Association of Theoretical and Applied Limnology 13: 603-610

Henriques-de-Oliveira C, Baptista DF, Nessimian JL (2007) Sewage input effects on the macroinvertebrate community associated to *Typha domingensis* Pers in a coastal lagoon in southeastern Brazil. Brazilian Journal of Biology 67(1): 73-80

Hunken A, Mutz M (2007) On the ecology of the filter-feeding *Neureclipsis bimaculata* (Trichoptera, Polycentropodidae) in an acid and iron rich post-mining stream. Hydrobiologia 592: 135–150

Jackson LE, KurtZ JC, Fisher WS (2000) Evaluation Guidelines for Ecological Indicators. US Environmental Protection Agency, Office of Research and Development, Research Triangle Park, NC.EPA/620/R-99/005, pp 1-109

Jarup L (2003) Hazards of heavy metal contamination. British Medical Bulletin 68: 167-182

Joubert A (2008) Evaluation of biomarkers for pollution in *Oreochromis Mossambicus*: a laboratory and field study. MSc Thesis, The University of Johannesburg, Department of Zoology, pp 1-117

Kruger EJ (1968) The seasonal variation in the quality and quantity of the net-zooplankton in Loskop Dam, Transvaal. Research project from the Department of Animal Science and Microbiology. Transvaal, South Africa, pp 1-59

Last LJ (2001) Impact of specific Acid Mine Drainage contaminants on macroinvertebrate communities in southeastern Ohio streams. MSc Thesis, College of Arts and Sciences of Ohio University, Ohio, pp 1-97

Magoba RNN (2005) Effects of invasion and clearing of alien riparian vegetation on benthic macroinvertebrate and adult Odonata assemblages in Soutpansberg River. MSc Thesis, Conservation Ecology and Entomology, University of Stellenbosch, pp 1-127

Martins R, Stephan N, Alves R (2008) Tubificidae (Annelida: Oligochaeta) as an indicator of water quality in an urban stream in southeast Brazil. Acta Limnologica Brasiliensia 20: 221-226

McMillan PH (1998) An Integrated Habitat Assessment System for the Rapid Biological Assessment of Rivers and Streams (An Internal STEP report), number ENV-P-I 98088 for the Water Resources Management Programme, CSIR, Pretoria, South Africa

Mulder PFS (1968) 'n Kwalitatiewe en kwantitatiewe ondersoek na die bentos en epifauna in Loskopdam. M.Sc. Thesis, Departement Dierkunde, Potchefstroomse Universiteit vir CHO, Potchefstroom, pp 1-36

Nedeau EJ, Merritt RW, Kaufman MG (2003) The effect of an industrial effluent on an urban stream benthic community: water quality v.s habitat quality. Environmental Pollution 123: 1-13

Nordberg GF, Goyer RA, Clarkson TW (1985) Impact of effects of acid precipitation on toxicity of metals. Environmental Health Perspective 63: 169-180

Oberholster PJ (2011) Using epilithic filamentous green algae communities as indicators of water quality in the headwaters of three South African river systems during high and medium flow periods. In: Zooplankton and phytoplankton. Kattel G (ed). Chapter 5, ISBN

978-1-61324-508-8, Nova Science Publishers, Inc. USA, pp 121-125

Oberholster PJ, Botha AM, Ashton PJ (2009) The influence of a toxic cyanobacterial bloom and water hydrology on algal populations and macroinvertebrate abundance in the upper littoral zone of Lake Krugersdrift, South Africa. Ecotoxicology 18: 34-46

Oberholster PJ, Botha AM, Cloete TE (2008) Biological and chemical evaluation of sewage water pollution in the Rietvlei nature reserve wetland area, South Africa. Environmental Pollution 156: 184-192

Oberholster PJ, Botha AM, Myburgh JG, Ashton PJ (2010) Responses of phytoplankton upon exposure to a mixture of acid mine drainage and high levels of nutrient pollution in Lake Loskop, South Africa. Ecotoxicology and Environmental Safety 73: 326-335

Oberholster PJ, Myburgh JG, Ashton PJ, Coetzee JJ, Botha AM (2012) Bioaccumulation of aluminium and iron in the food chain of Lake Loskop, South Africa. Ecotoxicology and Environmental Safety 75: 134-141

Olomukoro JO, Nduh Tochukwu OC (2006) Macrobenthic colonization of artificial substrates II: weeds and plant stems. Asian Journal of Plant Science 5: 990-995

Olomukoro JO, Okologume U (2008) Macroinvertebrate colonization of artificial substrates in a Nigerian river I: gravel and leaf litter. African Journal of Aquatic Science 33: 143-148

Quigley MA (1981) Fresh water macroinvertebrates. Water Pollution Control Federation 53: 1015-1027

Resh V, Betts E, Groff M, Cover M (2007) Bioindicators of Strawberry Creek. Department of Integrative Biology, University of California-Berkeley, pp 1-14

Ritter L, Solomon K, Sibley P, Hall K, Keen P, Mattu G, Linton B (2002) Sources, pathways, and relative risks of contaminants in surface water and groundwater: a perspective prepared for the Walkerton inquiry. Journal of Toxicology and Environmental Health 65: 1-142

Shannon CE, Weaver W (1949) The mathematical theory of communications. University of Illinois Press, Urbana

Soucek DJ (2001) Integrative Bioassessment of Acid Mine Drainage Impacts on the Upper Powell River Watershed, Southwestern Virginia. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, pp 1-158

Soucek DJ, Denson BC, Schmidt TS, Cherry DS, Zipper CE (2002) Impaired Acroneuria sp. (Plecoptera, Perlidae) populations associated with aluminum contamination in neutral pH surface waters. Archives of Environmental Contamination and Toxicology 42: 416-422

Spry DJ, Wiener JG (1991) Metal Bioavailability and Toxicity to Fish in Low-Alkalinity Lakes: A Critical Review. Environmental Pollution 71: 243-204

Stoddard JL, Herlihy AT, Hill BH, Hughes RM, Kaufmann PR, Klemm DJ, Lazorchak JM, McCormick FH, Peck DV, Paulsen SG, Olsen AR, Larsen DP, Van Sickle J, Whittier TR (2006) Mid-Atlantic Integrated Assessment (MAIA) State of the Flowing Water Report. EPA/620/R-06/001 (NTIS PB2007-107909), US Environmental Protection Agency, Office of Research and Development,Washington DC, pp 1-85

Tamatamah R, D Wasonga, Muthike J (2007) Mara River EFA Study, ANNEX 6.1 Starter

Document for BBM Workshop

Thirion C (2007) Macroinvertebrate Response Assessment Index in River Eco-Classification: Manual for EcoStatus Determination (version 2). Department of Water Affairs & Forestry, Resource Quality Services. MODULE E: Macroinvertebrate Response Assessment Index (MIRAI)

Wallace JB, Anderson NH (1995) Habitat, Life History and Behavioral Adaptation of Aquatic Insects. An Introduction to the Aquatic Insects of North America .Editor: Merrit, RW, Cummins, KW. Third Edition, Kendall/Hunt Publishing Company, Iowa, pp. 41-73

Wang Z-Y, Lee JHW, Cheng D, Duan X (2008) Benthic invertebrates investigation in the East River and habitat restoration strategies Journal of Hydro-environment Research 2: 19-27

Wren CD, Stephenson GL (1991) The effect of acidification on the accumulation and toxicity of metals to fresh water invertebrates. Environmental Pollution 71: 205-241

Chapter 4

Assessing the impact of water quality on phytoplankton assemblages at Loskop Dam

Abstract

The phytoplankton assemblages were studied and associated with the physical and chemical parameters of the water quality in Loskop Dam in order to evaluate the impacts of acid mine drainage and eutrophication. The study suggested that the water quality of the dam was deteriorated by algal blooms and high concentration of dissolved salts due to Acid Mine Drainage (AMD) inflow from the upper catchment. The values of TDS, Sulphate, conductivity and nitrate were particularly high at the riverine zone (sites 1 and 2) indicating that these sites were receiving AMD and untreated/partly treated sewage from waste water plants upstream of Loskop Dam. The autecology of the phytoplankton species (*Microcystis* sp., *Ceratium* sp., *Cyclotella* sp.) found at all sampling sites suggested that the species were tolerant to high electrolyte and nutrient-rich environment. Yet, the transitional (site 3) and lacustrine zone (sites 4 and 5) had a lower diversity (site 3: H'= 1.23; site 4: H' = 1.25; site 5: H' = 1.56) as compared to the riverine sites (site 1: H' = 2.075; site 2: H' = 1.93). The lower diversity at site 3 could be due to the massive bloom of *Ceratium hirundinella* occurred during the onset of spring (September and October), where temperature (20℃ – 30℃) was in favour for the species. *Microcystis* bloom was particularly prominent around the transitional zone and less at the riverine and lacustrine zone, but they were less frequent than the occurrence of *C.hirundinella* bloom during the winter-spring period. This could result from the inhibitory effects of *C.hirundinella* on the *Microcystis* spp. due to the allelopathic interaction between these two species.

4.1 Introduction

Phytoplankton, like macroinvertebrate is a useful bio-indicators for evaluating the water quality of a system due to their quick population response to many environmental stressors (Domingues et al. 2008). They usually compensate the insufficiency of chemical analysis alone, which is only a point in time and space (Zhou et al. 2008). It is a known fact that the rate of nutrient loading in an aquatic system is directly proportional to the algal biomass (Smith et al. 1999). However it does not necessarily favour harmful species, as the occurrence of some of these species do not depend only on high nutrient concentration (Heisler et al. 2008). In fact, occurrence and types of algal blooms involves complex interaction of a variety of factors such as the composition of the loaded nutrients, bioavailability, physiological factors of the species, chemical and physical factors, hydrographic and topographical conditions (Maso and Garces 2006; Heisler et al. 2008). N (NH_4^{+}, NH^3, NO_2^{-}, HNO_2, NO_3) and P (PO_4) serves as the primary macronutrient for phytoplankton, therefore both compounds are the limiting factors for growth (Smith et al. 1999). TN:TP ratio is thus an indicator pointing out the potential limiting nutrients within a system (Dzialowski et al. 2005). P is usually the limiting factor in South Africa fresh water system (Oberholster et al. 2009). By looking at the TN:TP ratio, the relative biomass of species in a community can be predicted. At high N:P ratio (20~50:1), Chlorophyta (green algae) growth is favored and dominated the aquatic system in dams; whereas in low N:P ratio (5~10:1), the community structure is shifted favoring dominance of nitrogen-fixing cyanophyta (blue-green algae) (Bulgakov and Levich 1992). This shift may be due to N limitation in the water which allows certain blue-green algae (e.g. *Anabaena* spp.) to fix N from the atmosphere, thus gain an advantage over other taxa in N-limited water system (Camargo et al. 2005; Havens et al. 2003;Van Ginkel 2002).

However this relationship or the TN:TP ratio rule seems less applicable in a eutrophic/hypertrophic system (Nuccio et al. 2003). In these aquatic systems, the emergence and the dominant blooms of cyanobacteria seem to be more associated with an increase in the absolute concentration of P, and this low TN:TP ratio is not the cause, but rather a result of the bloom (Flynn 2010). One other important element, but to a lesser extent than N and P, is silicon (Si). This nutrient is particularly essential for the growth of diatoms as it is a key element in the building of their cell wall (Martin-Jézéquel et al. 2000; Oberholster et al. 2010). Diatoms are a large group of algae contributing 20-25% of the global net marine primary production, forming an important food source for zooplankton and providing Dissolved Organic Matter (DOM) for growth of bacterial population (Pete et al. 2010). Under condition with sufficient P supply, high concentration of Si is able to stimulate growth of diatoms (Hecky and Kilham 1988). Si is a nutrient which has a relatively low internal recycling rate, thus its supply mainly relies on external sources (Wu and Chou 2003). High input of domestic wastes, which usually contains relatively low Si concentration, contains high P: Si or N: Si nutrient ratio, favouring the population growth of larger algae like cyanobacteria in water bodies (Hecky and Kilham 1988; Watson et al. 1997). This event is not only controlled by bottom-up (nutrient) factors, but also by top-down (grazing by zooplankton) factors (Hashioka and Yamanaka 2007). In such situation, cyanobacterial cells are large in size and inedible, therefore experiences less grazing pressure by zooplankton than other smaller size algae (Watson et al. 1997; Muylaert et al. 2006). Therefore one cannot use the nutrient loading as the only reference for the prediction of algal blooms. A further understanding of the critical features and mechanisms underlying population dynamics of algal blooms are required in order to improve monitoring and to build models for predicting the occurrence, movement, toxicity and environmental effect of Harmful Algal Blooms (HAB) (Zingonea and Enevoldsenb 2000).

The succession and the structure of the phytoplankton community within a system are influenced by the distinct climatic cycles in a region, with physical, chemical and biotic factors playing an important role in controlling the sequence of its seasonal succession (Ashton 1985; Arhonditsis et al. 2004). As different groups of phytoplankton have distinct life cycles, morphological and physiological mechanisms in adapting to the changing environment, alterations in the water quality due to human or natural causes could alter the regular succession and dynamics of phytoplankton communities within an aquatic ecosystem (Proulx et al. 1996). This makes phytoplankton a suitable tool for biomonitoring an aquatic system. Since phytoplankton is the primary producer fuelling the food web, community changes can have a significant health and community impact on the secondary and tertiary trophic levels (Abrantes et al. 2006). Additionally, phytoplankton population dynamics also affect the physical and chemical water quality such as the turbidity, oxygen concentration and the total productivity of the aquatic system (Los and Wijsman 2007).

The objectives of the study was to (i) determine the phytoplankton assemblage in Loskop Dam over a period of 6 months, which include the same time period when fish and crocodile die off occurred in 2007 and (ii) to link the physical and chemical water quality parameters to the distribution and autecology of different phytoplankton communities at each sampling site.

4.2 Materials and Method

4.2.1 Study Area

Five sampling sites were selected and are representing three different zones of Loskop Dam (Figure 4.1). These are: riverine zone (sites 1 and 2, representing high flow and rapid water flushing rate); transitional zone (site 3, representing the reduced flow and flushing rate) and lacustrine zone (site 4 and 5, representing lowest flows and water flushing rates). The study period last for 6 months and was conducted from early autumn (May) to early spring (October). The study period was selected due to the previous incidents of massive fish die-offs of 14 tons occurred during September in 2006 and June to August in 2007 (Driescher 2007). This period is also the overturn of the dam causing bottom sediment to resuspend into the water column due to wind movement and changes in temperature. Since no reference site was available, we compared different sites to see longitudinal changes in the dam from the inflow to the dam wall.

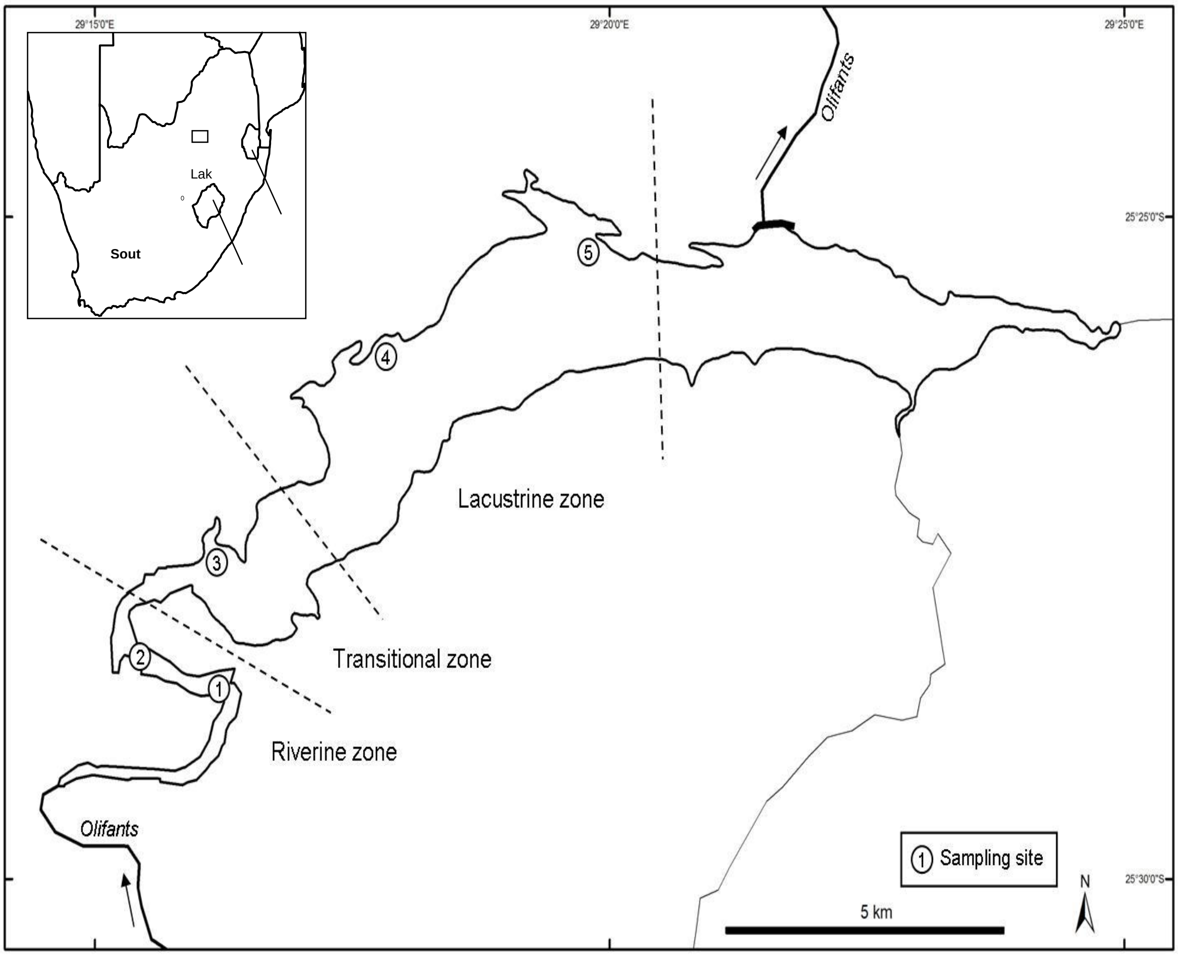

Figure 4.1: Map of Loskop Dam showing different zones and sampling sites.

4.2.2 Physical and Chemical analysis

Dissolved O_2, temperature, pH and conductivity were measured *in situ* at the surface of water column using a Hach sension^{TM}156 Portable Multiparameter (Coveland, USA) according to Oberholster et al. (2009). Transparency was measured with a 20 cm diameter, black and white quadrant Secchi disk (Oberholster et al. 2008). Surface water (first 2 m) of the euphotic zone was sampled with 0.5 meter interval using a 6 litre Van Dorn Sampler from June to October 2009 at 5 sampling sites. The water samples of each depth at each sampling site was pooled together to form one integrated water sample for each site. Two litre sub-sample of the 12 litre integrated water sample at each site collected was taken for phytoplankton and chlorophyll analysis. Water samples for chemical analysis were filtered through Whatman (0.45µm) GF/filters. The filtered water was preserved in pre-rinsed polyethylene bottles with sulfuric acid (pH 2.0) for analysis of dissolved nutrients. All analysis was done according to standard method of the American Public Health Association (APHA 1992). Concentration of total nitrogen (TN) and total phosphorus (TP) were determined by persulfate digestion. Nitrogen concentration was determined on an autolyzer by the cadmium reduction method. Soluble reactive phosphate was determined by the ascorbic acid method. Values of TN, ammonia (NH_3), silicon (Si), calcium (Ca), magnesium (Mg), and TP were compared between 5 sampling sites.

4.2.3 Phytoplankton sampling

100 ml of the two litre sampled for phytoplankton and chlorophyll ***a*** were preserved in the field by addition of acidic Lugol's solution to a final concentration of 0.7%, followed after one hour by the addition of buffered formaldehyde to a final concentration of 2.5% for phytoplankton analyses.

All phytoplankton identifications were made with a compound microscope at 1250 x magnification (Van Vuuren et al. 2006; Taylor et al. 2007). Strip counts were made until at least 100 individuals of each of the dominant phytoplankton species had been counted. All counts were based on the numbers of cells observed and the individual species were grouped into major algal groups (Lund et al. 1958; Willen 1991). The relative abundance of phytoplankton taxa at each sampling site was categorised according to Hörnström (1999): 1 = ≤ 250, 2 = 251-1000, 3 = 1001-5000, 4 = 5001-25 000 cells l^{-1}.

4.2.4 Preparation for Scanning Electron Microscopy

Scanning Electron Microscopy was used to identify phytoplankton specimen that was difficult to identify under the light microscope (e.g. centric diatoms). The phytoplankton material was filtered through 2.0µm polyester membrane filter placed inside a Swinnex 13 Millipore, and the material was filtered by pushing air through the filter with a syringe. The filter membrane was examined under a light microscope to ensure that there was adequate material for examination. The membrane was then mounted on an aluminum microscope stub with carbon tape and coated with gold palladium, and examined with an FEI QUANTA ESEM 2000 at a voltage between 10 and 15 kV.

4.2.5 Chlorophyll *a* extraction and analysis

One litre of the 2 litre sub-sample of each site was filtered through a 45 µm filter with a hand filter in the field for chlorophyll analysis. The filters (triplicate at each site) were stored in a 10 ml glass tube, and were transferred from the field to the laboratory in a dark cool box. 10 ml of 80% acetone were added to the glass tube containing Whatman 45µm filter with the filter containing phytoplankton. The solution in the tube was vortexed till the algae was completely removed from the filters. The vortexed solution was then closed with foil and placed in the dark for 24 hours. After

24 hours, 1 ml of the solution was used for analysis of chlorophyll *a* and *b* content spectrophotometrically at 664 nm, and 647 nm respectively (Porra et al. 1989).

4.2.6 Diversity and Dominance Calculation

Phytoplankton diversity was calculated using the Shannon's diversity index (Shannon and Weaver 1949):

$$H = -(\sum(\rho_i \ \ln \rho_i)) \qquad (1)$$

where (H) is Shannon's diversity index, the proportion of species (i) relative to the total number of species (ρ_i) present in the aquatic ecosystem are calculated. The product of (ρ_i ln ρ_i) for each species in the aquatic ecosystem is summed, and multiplied by -1 to give H. The Berger-Parker dominance index (Berger and Parker 1970) was used to measure the evenness or dominance of specimens at each site.

$$D = N_{max}/N$$

Where N_{max} = the number of individuals of the most abundant species present in each sample, and N = the total number of individuals collected at each site (Oberholster et al. 2009).

4.2.7 Autecological classification of algal taxa collection in Loskop Dam.

The autecology, or physiological requirements and tolerance of algal species to nutrients (nitrogen and phosphorus concentrations), organic enrichment, dissolved oxygen, major ions (such as Ca, chloride, iron and sulfate), temperature, or pH, was used to classify phytoplankton of Loskop Dam qualitatively from the literature (Porter 2008). It is known that the autecological characterization of environmental conditions based on taxonomic composition should reflect the physical and chemical changes caused by humans (Stevenson and Smol 2003).

4.2.8 Rainfall Collection

The rainfall data was collected using a standardized rain gauge at the weather station at the Loskop Dam Nature Reserve Offices.

4.3 Results

4.3.1 Chemical and Physical Values

High rainfall occurs during spring – summer period. From the graph (Figure 4.2), sharp increase in TDS and conductivity was in correlation with increase in rainfall during early spring (August – September). All sampling sites showed an increasing trend in sulphate, conductivity and TDS from May to October, with highest value occurring in October (Figures 4.2, 4.3, 4.4). The average sulphate, conductivity and TDS values were higher at the riverine zones (sites 1 and 2) relative to transitional and lacustrine zones (sites 3, 5 and 6). Despite high TDS and conductivity occurring at these sampling sites, the values are still within Target Water Quality Range (TWQR) standard (Domestic Use, TDS: 0 - 450 mg l^{-1}; Conductivity: 0 – 70 mS m^{-1}) set by Department of Water Affairs and Forestry (DWAF), where within this range, the water quality is maintained (DWAF 1996b). The sulphate concentration (Figure 4.5) for sites 1, 2, 3, 4 and 5 exceeds the river water sulphate level (100 mg l^{-1}) for aquatic ecosystem health proposed by Canada (Ministry of Environment 2000) but was below the South Africa Water Quality Guideline value of 400 mg l^{-1} for domestic water supplies (DWAF 1996a). From the graphs (Figure 4.2, 4.3 and 4.4), it was evident that sulphate and TDS seemed to increase at the same time. This was particularly evident at the riverine (sites 1 and 2) and transitional zone (site 3) (Figure 4.4).

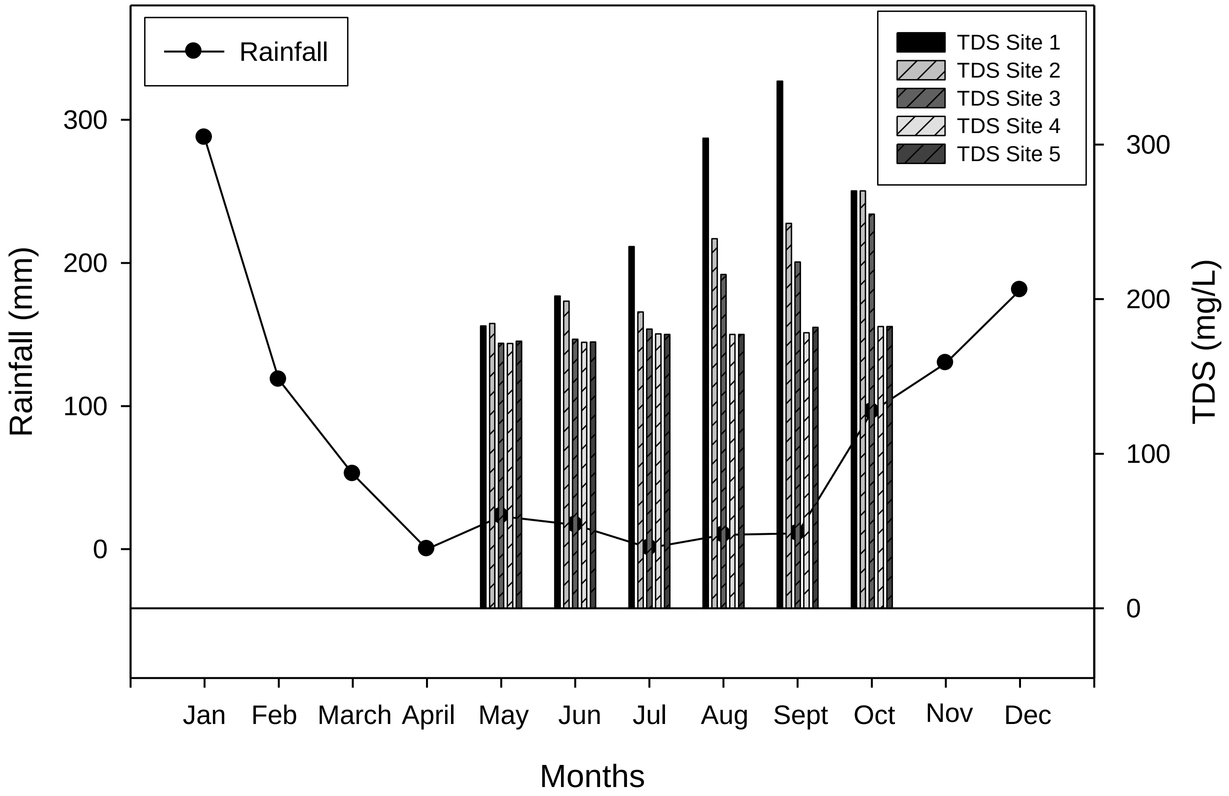

Figure 4.2: Total monthly rainfall in 2009 (data supplied by Jannie Coetzee, Loskop Office) and trends of TDS at the different sampling sites in Loskop Dam during the sampling period.

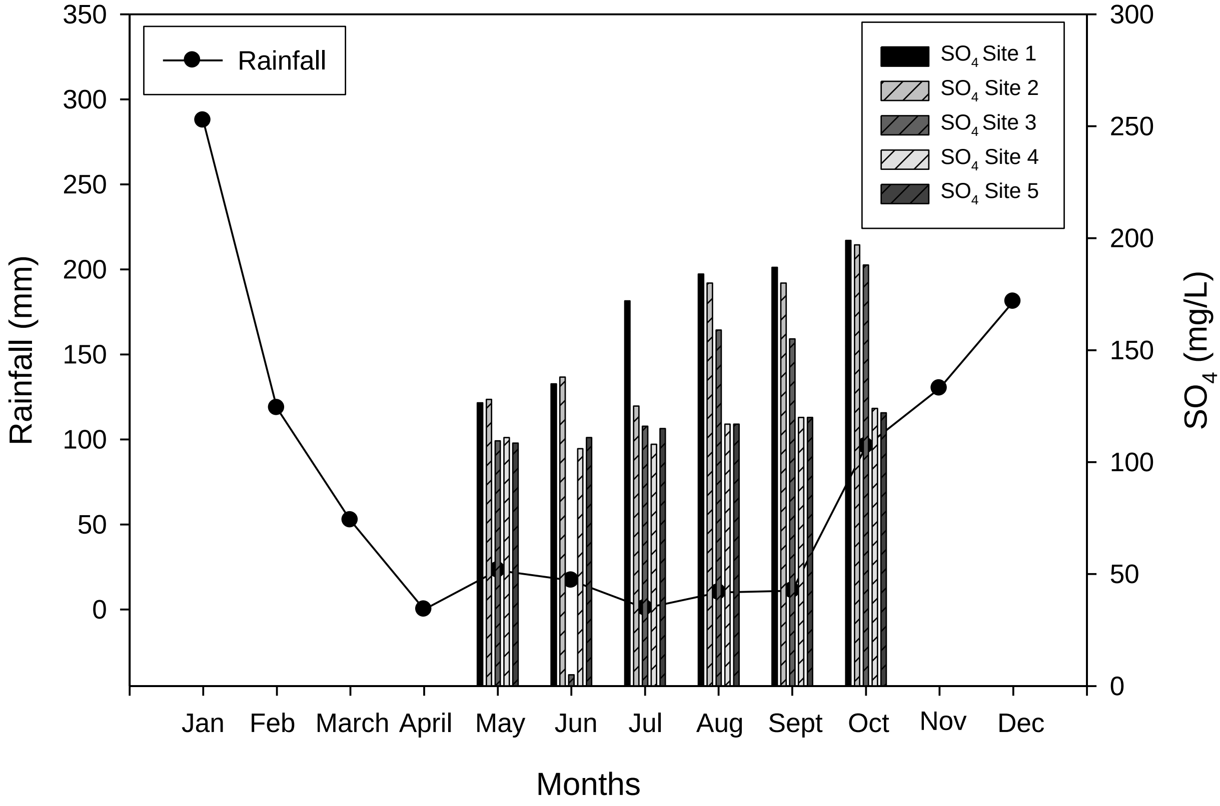

Figure 4.3: Total monthly rainfall in 2009 (data supplied by Jannie Coetzee, Loskop Office) and trends of SO_4 at the different sampling sites in Loskop Dam during the sampling period.

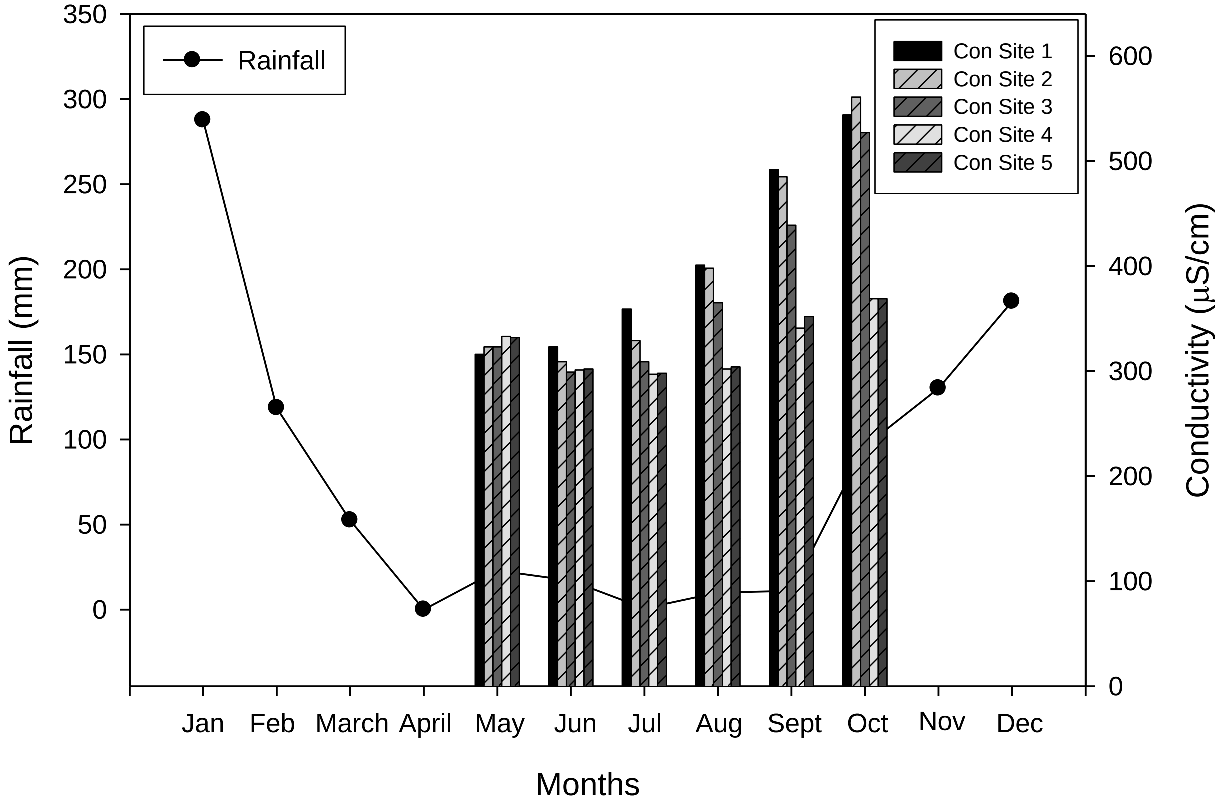

Figure 4.4: Total monthly rainfall in 2009 (data supplied by Jannie Coetzee, Loskop Office) and trends of conductivity at the different sampling sites in Loskop Dam during the sampling period.

The average concentration of aluminum (Al) (<0.09 mg l^{-1}) was above TWQR standard (0.01 mg l^{-1}) yet constant throughout the sampling sites, but maximum average concentration occurred at site 1 (May = 110 ug l^{-1}) and 2 (June= 290 ug l^{-1}) (Figure 4.6). Iron (Fe) average concentration most of the time were generally below the detection limit (<0.06 mg l^{-1}). However it was still above the ANZECC (Australian and New Zealand Environmental Conservation Council) standard on water quality for aquatic ecosystem (0.01 mg l^{-1}) (ANZECC 2000) (Figure 4.7). The maximum value did occur during June at site 2 (0.14 mg l^{-1}). ANZECC standard for aquatic water quality was used as a guideline for Fe because it specify the levels at which the concentration of Fe is harmful or safe for the aquatic environment.

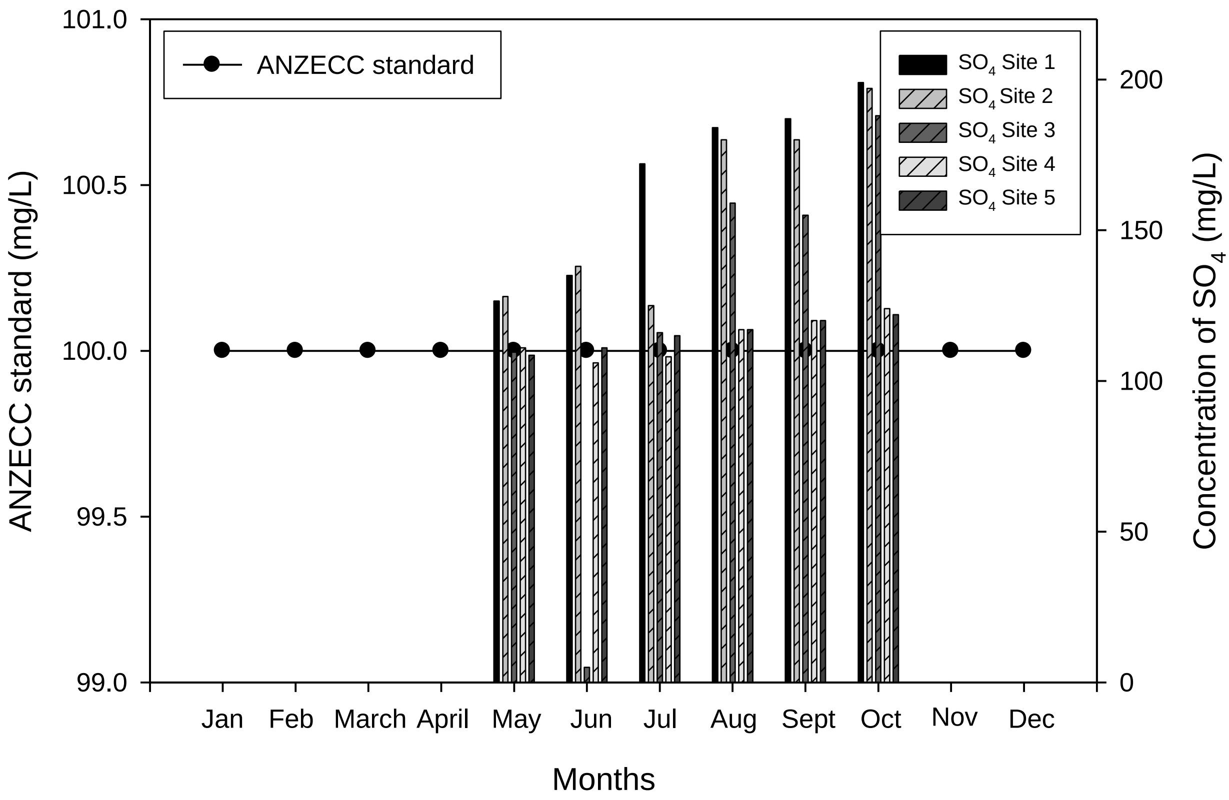

Figure 4.5: Graph comparing sulphate level with ANZECC (Australian and New Zealand Environmental Conservation Council) quality guideline for aquatic ecosystem throughout the sampling period.

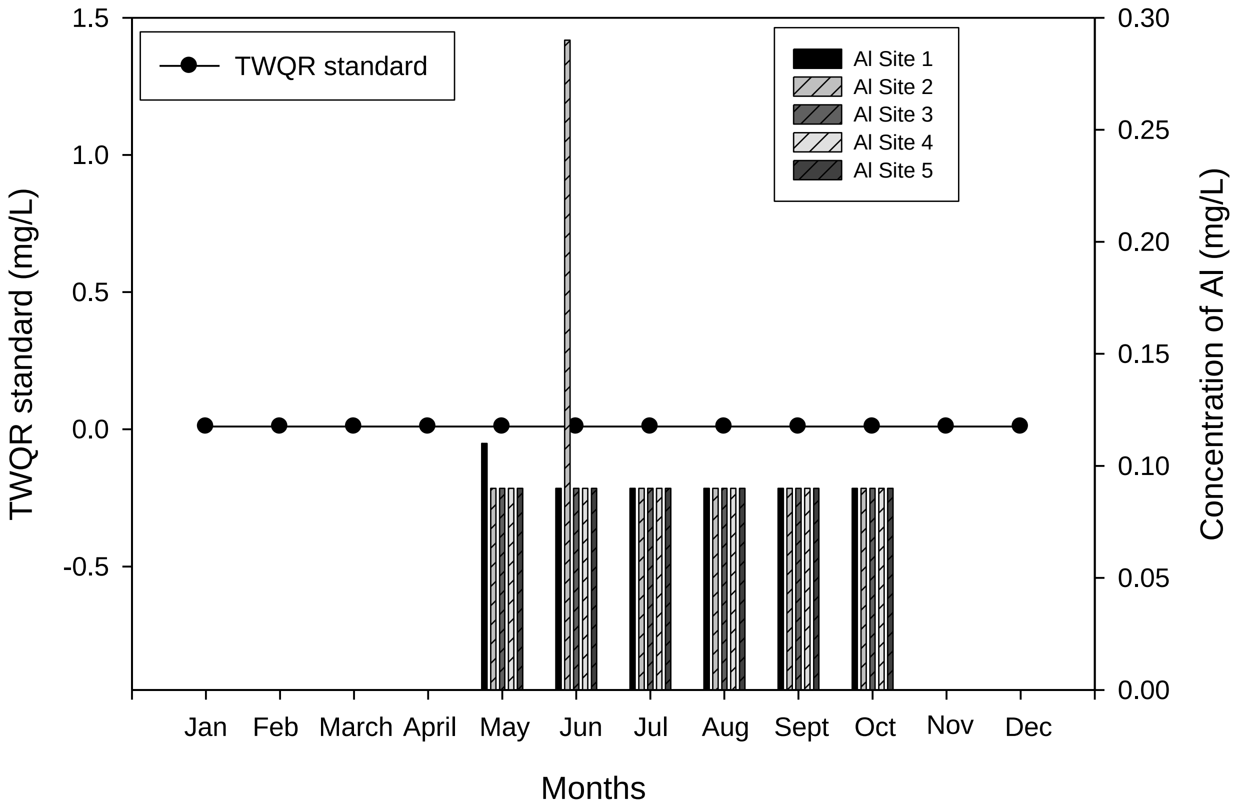

Figure 4.6: Graph comparing Al level with TWQR quality guideline for aquatic ecosystem throughout the sampling period.

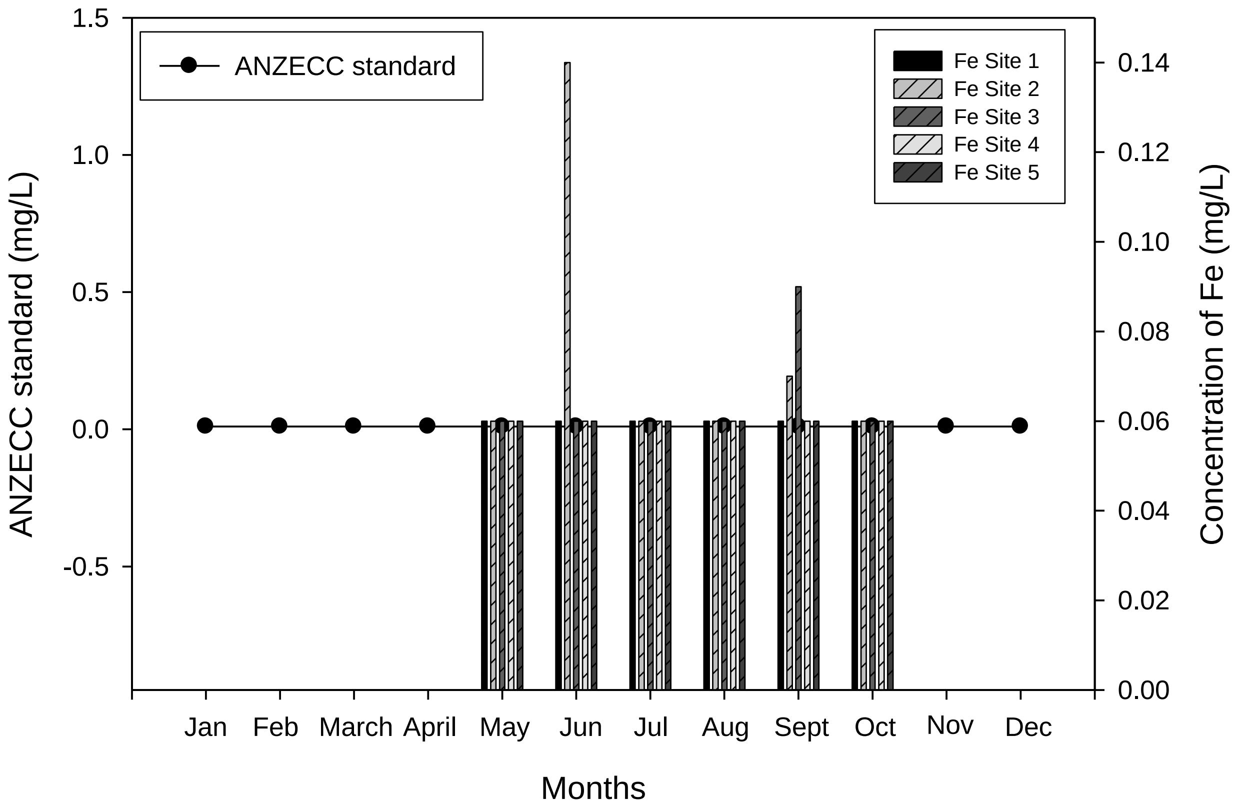

Figure 4.7: Graph comparing Fe level with ANZECC (Australian and New Zealand Environmental Conservation Council) quality guideline for aquatic ecosystem throughout the sampling period.

Other metals such as manganese (Mn), cadmium (Cd) and zinc (Zn) did remain below detecting limit throughout the study. Nitrate level was higher at the riverine zone relative to sites 3, 4 and 5 (Table 4.1). At site 1, the average nitrate concentration during May (1.9 mg l^{-1}), June (1.5 mg l^{-1}), August (0.91 mg l^{-1}) and September (0.62 mg l^{-1}) indicated that the dam's trophic status was mesotrophic according to TWQR standard (0.5 mg l^{-1} – 2.5 mg l^{-1}). For site 2, the average nitrate concentration was high occurred during May (1.9 mg l^{-1}), June (1.5 mg l^{-1}) and August (0.96 mg l^{-1}). At site 3, average nitrate concentration reached maximum only during August (0.66 mg l^{-1}) (Table

4.1). Orthophosphate level was below detection limit at all sites throughout the sampling period (<0.2 mg l^{-1}). The average silicon (Si) concentration was higher at sites 4 and 5 in the lacustrine zone relative to transitional and riverine zone of the dam (Table 4.1). The pH level was the lowest during July for all sites in comparison the other sampling period (Table 4.1, Figure 4.8). The average pH in the riverine zone (site1 = 7.87, site 2 = 7.86) was found to be lower than the other sampling sites (site 3 = 8.2, site 4 = 8.16, site 5 = 8.26). The average water temperature ranged between 14.98℃ to 20.1℃ from May to August, while the average water temperature increased during September and October (21.76℃ – 24.32℃) (Table 4.1). Secchi depth average ranged between 0.71 m – 2.27 m with site 3 having the lowest average secchi depth (0.33 m to 1.62 m) in comparison with the other sites. The highest secchi depth were measured at site 4 (2.27 m) and 5 (2.11 m).

Table 4.1 Physical and chemical water quality characteristics during the sampling periods (May – October 2009) at Loskop Dam.

Parameter	Units	Site 1 Riverine zone						Site 2 Riverine zone						Site 3 Transitional zone					
		May	June	July	August	Sept	Oct	May	June	July	Aug	Sept	Oct	May	June	July	Aug	Sept	Oct
Aluminium (Al)	$mg.l^{-1}$	0.11	0.09	0.09	0.09	0.09	0.09	0.09	0.29	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Nitrogen (NH_3)	$mg.l^{-1}$	0.01	0.1	0.1	0.2	0.1	0.1	0.01	0.1	1.1	0.2	0.1	0.1	0.01	0.38	1.3	0.38	0.1	0.21
Iron (Fe)	$mg.l^{-1}$	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.14	0.06	0.06	0.07	0.06	0.06	0.06	0.06	0.06	0.09	0.06
Calcium (Ca)	$mg.l^{-1}$	29	35	38	40	34	42	29	34	30	36	33	41	25	28	27	32	31	39
Nitrogen (NO_3)	$mg.l^{-1}$	1.9	1.5	0.2	0.91	0.62	0.24	1.9	1.5	0.27	0.96	0.44	0.2	0.2	0.2	0.2	0.66	0.25	0.2
Phosphorus (PO_4)	$mg.l^{-1}$	0.2	0.2	0.2	0.2	0.2	0.2	<0.2	0.2	0.2	0.2	0.2	0.2	<0.2	0.2	0.2	0.2	0.2	0.2
Silicon (Si)	$mg.l^{-1}$	3.6	1.9	0.4	0.57	0.42	1.8	3.3	2.1	2.8	0.56	0.39	1.6	3.5	3.8	3.3	1.2	1.4	1.5
Sulphate (SO_4)	$mg.l^{-1}$	126.5	135	172	184	187	199	128	138	125	180	180	197	109.5	5	116	159	155	188
pH		9.39	7.6	6.84	7.1	8.2	8.09	8.12	7.65	6.87	7.74	8.55	8.2	8.99	7.61	6.72	7.59	9.08	9.2
TDS	$\mu g.l^{-1}$	182.6	202	234	245	256	270	184.2	198.7	191.7	239	249	270	171.5	174.1	180.6	216	224	255
Temperature	℃	16.7	13.3	11.9	14.4	21.6	24.4	17.3	14.5	16.4	15.2	22.1	25.4	20.4	16.2	16.1	15.6	22.3	25.1
Conductivity	$\mu S.cm^{-1}$	316	323	359	401	492	544	324	309	329	398	485	561	323	299	309	365	439	527
Secchi Depth	m	1	0.9	1.2	2.25	1.6	1.4	1	0.61	0.7	1.83	1.12	0.8	0.39	0.42	0.6	1.62	0.91	0.33

Parameter	Units	Site 4 Lacustrine zone						Site 5 Lacustrine zone					
		May	June	July	Aug	Sept	Oct	May	June	July	Aug	Sept	Oct
Aluminium (Al)	$mg.l^{-1}$	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Nitrogen (NH_3)	$mg.l^{-1}$	0.12	0.97	0.1	0.2	0.1	0.1	0.01	0.1	0.1	0.2	0.1	0.1
Iron (Fe)	$mg.l^{-1}$	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Calcium (Ca)	$mg.l^{-1}$	25	27	26	25	23	28	25	27	26	25	24	27
Nitrogen (NO_3)	$mg.l^{-1}$	0.01	0.31	0.24	0.2	0.2	0.2	0.2	0.26	0.22	0.2	0.2	0.2
Phosphorus (PO_4)	$mg.l^{-1}$	<0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Silicon (Si)	$mg.l^{-1}$	3.9	4	3.7	3.3	2.6	1.6	3.9	4	3.7	3.2	2.4	1.5
Sulphate (SO_4)	$mg.l^{-1}$	111	106	108	117	120	124	108.5	111	115	117	120	122
pH		8.47	7.38	6.95	7.92	9	9.23	8.71	7.4	7.15	7.81	9.15	9.35
TDS	$\mu g.l^{-1}$	171.3	172.1	177.5	177.1	178.3	182.2	172.8	172.3	177.2	177.1	181.8	182.2
Temperature	℃	24.8	17	15.1	16.2	21.1	23.2	21.3	17.1	15.4	16.2	21.7	23.5
Conductivity	$\mu S.cm^{-1}$	333	301	297	302	341	369	332	302	298	304	352	369
Secchi Depth	m	1.6	2.4	1.25	3.46	2.4	2.5	1.8	2.3	1.55	2.63	1.86	2.5

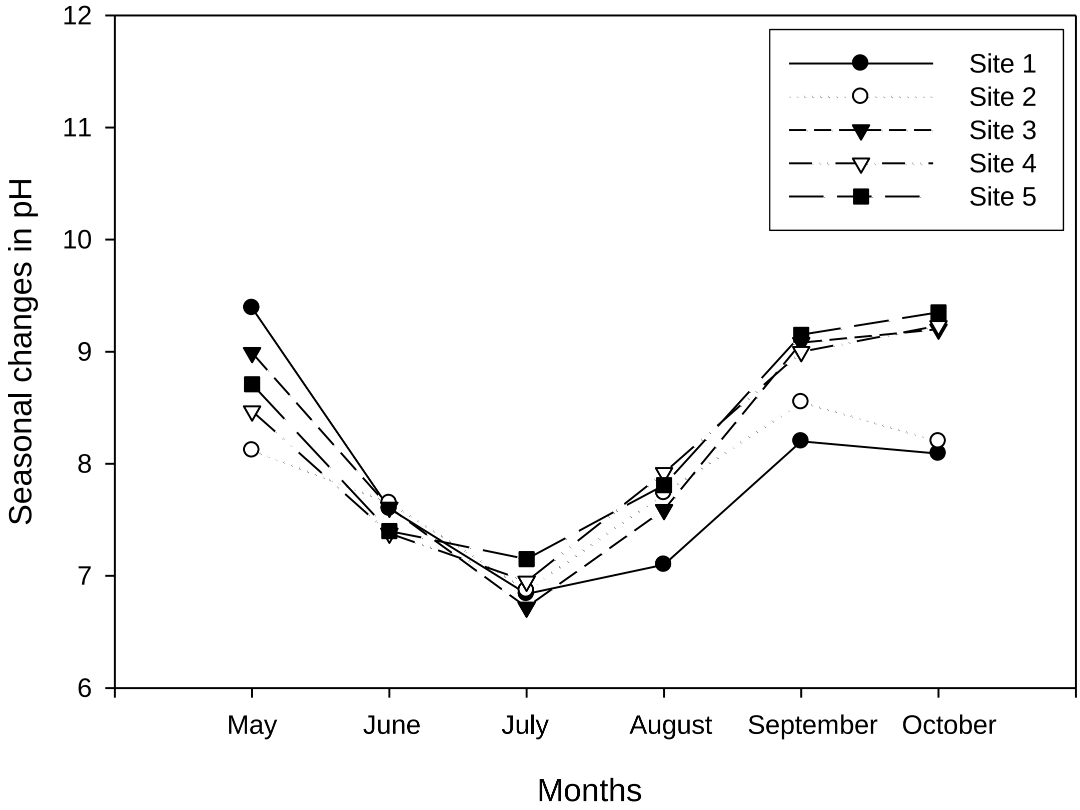

Figure 4.8: Temporal fluctuation of pH in the water column during the sampling period in Loskop Dam.

4.3.2 Phytoplankton

The phytoplankton community at site 1 had a fairly high average diversity (*H'*=2.08) when compared to the other sites (2, 3, 4 and 5). Site 1 was generally dominated by Bacillariophyceae species (Berger and Parker dominance: 0.9 – 1) that are tolerant to eutrophic and high electrolyte condition (Table 2). The diversity at site 1 was the highest between June (*H'*=2.73) and September (*H'*=2.39), and fell sharply in October (*H'*=1.2). At site 2, the phytoplankton community was also characterized with eutrophic and high electrolyte tolerant species, such as *Cyclotella meneghiniana*

(Berger and Parker dominance: 0.22 - 0.53), *Microcystis aeruginosa* (Berger and Parker dominance: 0.1 – 0.45) and *Ceratium hirundinella* (Berger and Parker dominance: 0.22-0.45). The following species occurred at site 2, *M. aeruginosa* and *M. flos-aqua*, the Dinophyceae *C. hirundinella* and the Chlorophyceae *Scenesdes quadricauda* (Berger and Parker dominance: 0.3). The diversity at site 2 (*H'*=2.44) was the highest in May, but decreased in June, increased again and remained high from July to October in comparison to May. The average Shannon diversity index of this site was the second highest (*H'*=1.93) in comparison to all other sites. At site 3, the average Shannon diversity index (*H'*= 1.23) was the lowest in comparison to all other sites. In May, June, September and October, a mono-species bloom of *C. hirundinella* (5001-25000 cells ml^{-1}) occurred at site 3. For other sampling period (July and August) at site 3, *C. hirundinella* were also present, but at lower numbers (251-1000 cells ml^{-1}) amongst other eutrophic tolerant species such as *C. meneghiana*, *M. aeruginosa* and *M. flos-aqua*.

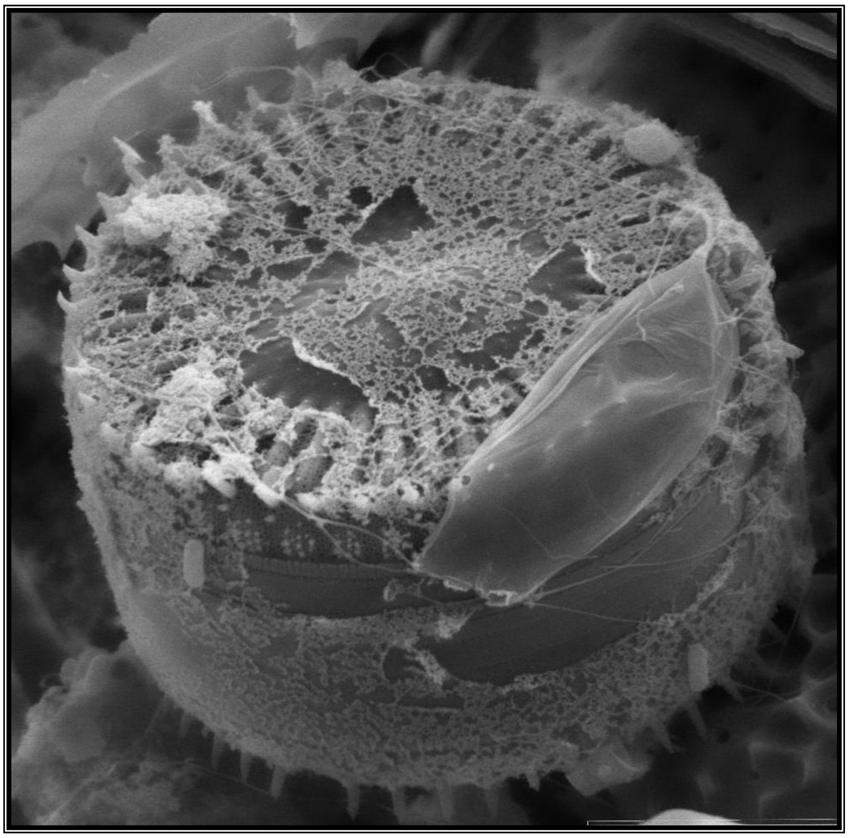

Figure 4.9: SEM micrograph of the centric diatom *C. meneghiniana* occurring in high number particularly at sites 1, 2 and 3 of Loskop Dam.

C. hirundinella at site 3 dominated particularly in May and October in comparison to other months (Berger and Parker Index, 0.98 and 0.81 respectively). Low average phytoplankton diversity (*H'* = 1.25) was observed at site 3 during May in comparison to other months when each algal group was dominated by one species. *Fragilaria crotonensis* and *C. hirundinella* occurred frequently in abundance (5001-25000 cells ml^{-1}) at site 4 especially from July to October. Species observed at this site range from meso- to eutrophic tolerants, according to their autecology (Table 4.2). At site 5, the Shannon diversity index (June, *H'*: 1.1, July, *H'*: 1.68, August, *H'*:2.18, September, *H'*: 1.83, October, *H'*:1.35) increased, reaching its peak in August and dropped again in September and

October. *Fragilaria crotonensis* (Berger and Parker Index: July: 0.38, August: 0.24, September: 0.27, October: 0.31) and *C. hirundinella* (Berger and Parker index: July: 0.38, August: 0.25, September: 0.32, October: 0.31) were dominant species at this site from July to October. Species favouring eutrophic, standing water and high conductivity were found particularly at this site (Table 4.2) (Carty 2002; Taylor et al. 2007). The autecological characteristics of selected species sampled in the Loskop Dam from May 2009 to October 2009 indicate that the dam water was eutrophic with high electrolyte concentration (Table 4.2). The shift in phytoplankton composition at each site for each month is shown in Figures 4.12 – 4.17. Throughout the sampling period, diatom was ubiquitous with pennated diatoms as the prominent specimens, indicating that the water was stable with low turbidity due to low water discharge in the low rainfall season. Dinophyta was also a prominent species at Loskop Dam, particularly at sites 2, 3, 4 and 5 during the month of May, June, September and October. The total absence of Dinophytes at site 1 was possibly an indication of unstable water column due to higher water mixing at the riverine zone where Olifants River flows into the dam. Cyanophyta was not so prominent in this study except at riverine (site 2) and transitional zone (site 3) in June due to the fact that these species are usually late summer species.

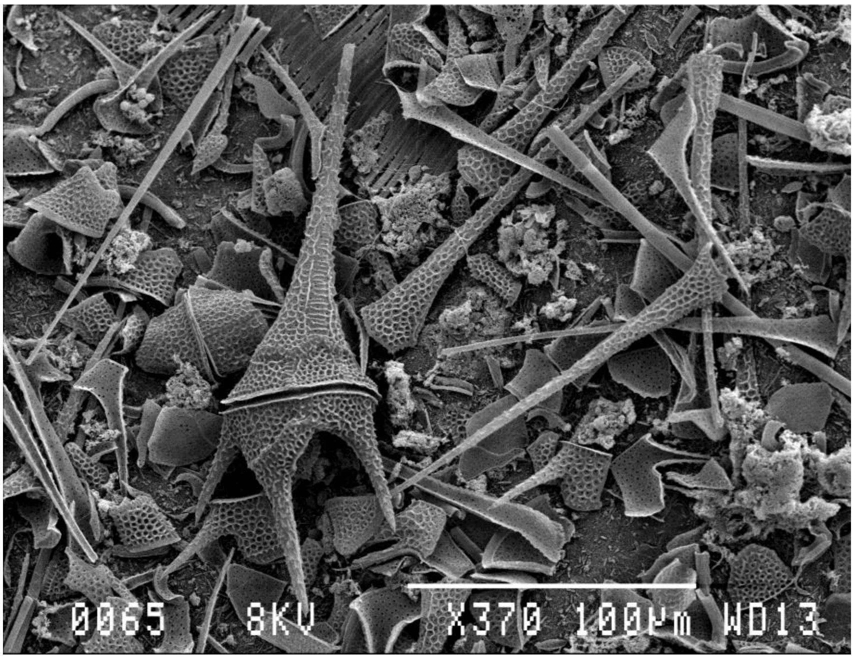

Figure 4.10: SEM micrograph of *C. hirundinella* specimens that dominate site 3 of Loskop Dam.

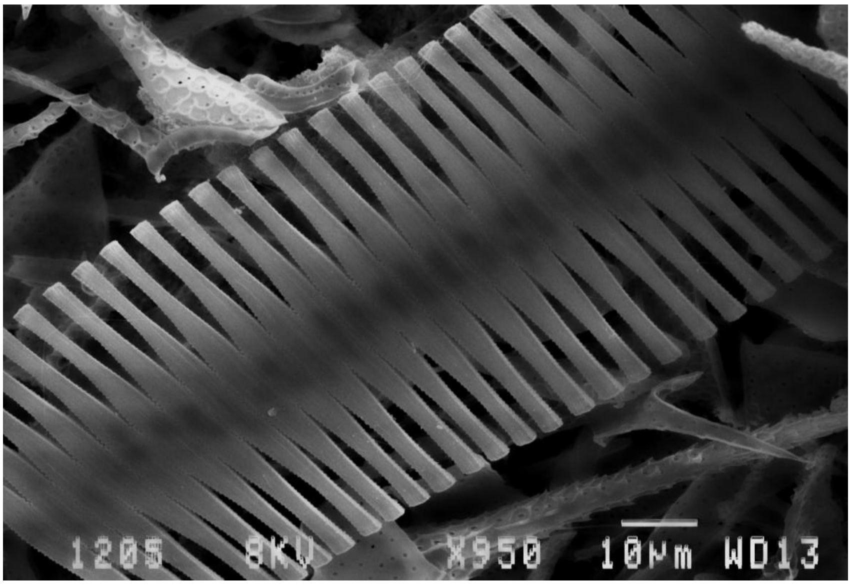

Figure 4.11: SEM micrograph of *F. crotonensis* specimens that was the dominant diatom during the sampling period at Loskop Dam.

Table 4.2 Metrics that are based on autecological characteristics of dominant phytoplankton species sampled in the Loskop Dam from May 2009 to October 2009 at 5 sampling sites.

Species	Site	Autecology	References
Cyclotella meneghiniana	1,2,3,4,5	Indicator of electrolyte rich eutrophic water	Taylor et al. (2007) Reynolds et al. (2002)
Microcystis aeruginosa	2,3,4,5	Absent at high levels of metal pollution, and low pH. Indicator of eutrophic water with medium to high orthophosphate concentrations.	Reynolds et al. 2002 Oberholster et al. (2005)
Microcystis flos aquae	2,3,4,5	Present in slightly eutrophic water with medium to high orthophosphate concentrations.	Chaudhary and Meena (2007)
Navicula gregalis	1,2,4,5	Indicators of eutrophic and high electrolyte condition.	Taylor et al. (2007)
Navicula libonensis	1,2,4,5	Indicators of eutrophic and electrolyte-rich condition.	Taylor et al. (2007)
Fragilaria ulna	1,2,3,4,5	Mesotophic to eutrophic, alkaline water	Taylor et al. (2007)
Fragilaria crotonensis	1,2,3,4,5	Oligotrophic to weakly eutrophic, slightly alkaline fresh water with moderate electrolyte level.	Reynold (2002), Taylor et al. (2007)
Ceratium hirundinella	1,2,3,4,5	Present in high ionic and eutrophic water with medium orthophosphate concentrations..	Reynold (2002)
Scenedesmus quadricauda	1,2,3	Indicators of shallow, mixed, highly enriched system	Padisák (2009)
Diatom vulgaris	1,2,3,5	Indicator of meso- to eutrophic condition, with average conductivity (100 μScm^{-1} to 500 μScm^{-1})	Walsh and Wepener (2009)

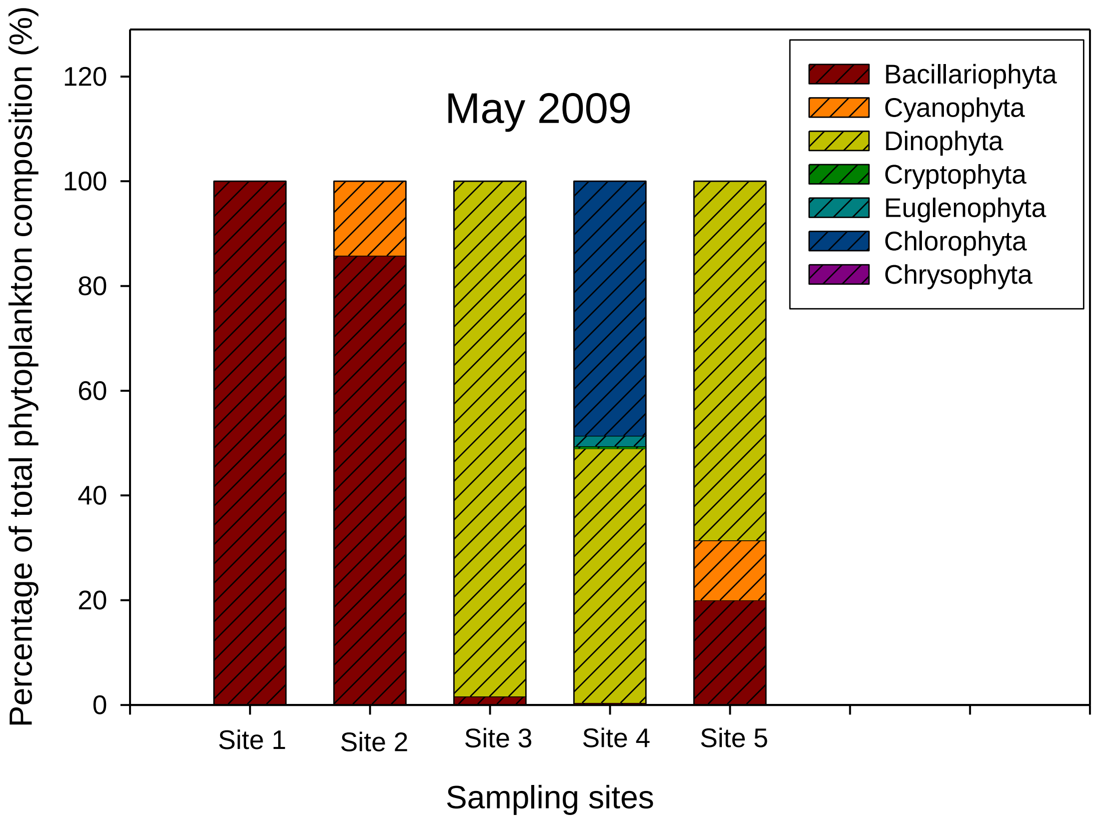

Figure 4.12: Phytoplankton species composition in Loskop Dam in May.

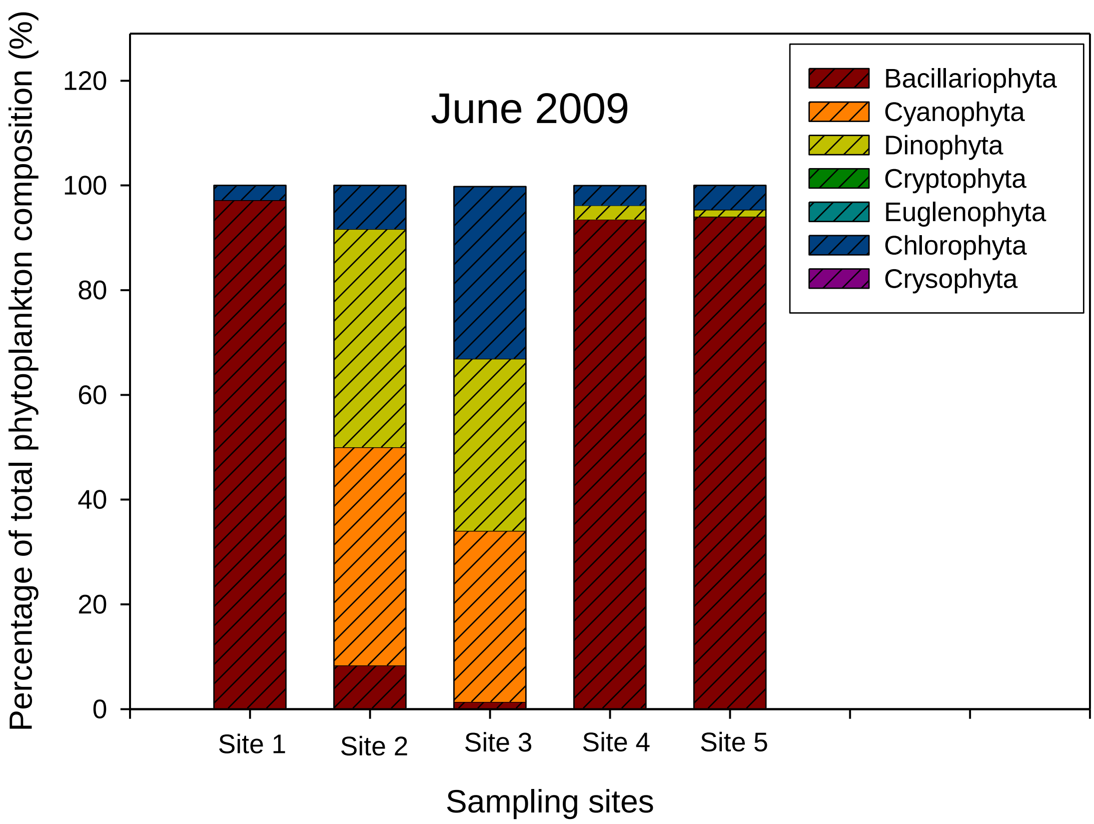

Figure 4.13: Phytoplankton species composition in Loskop Dam in June.

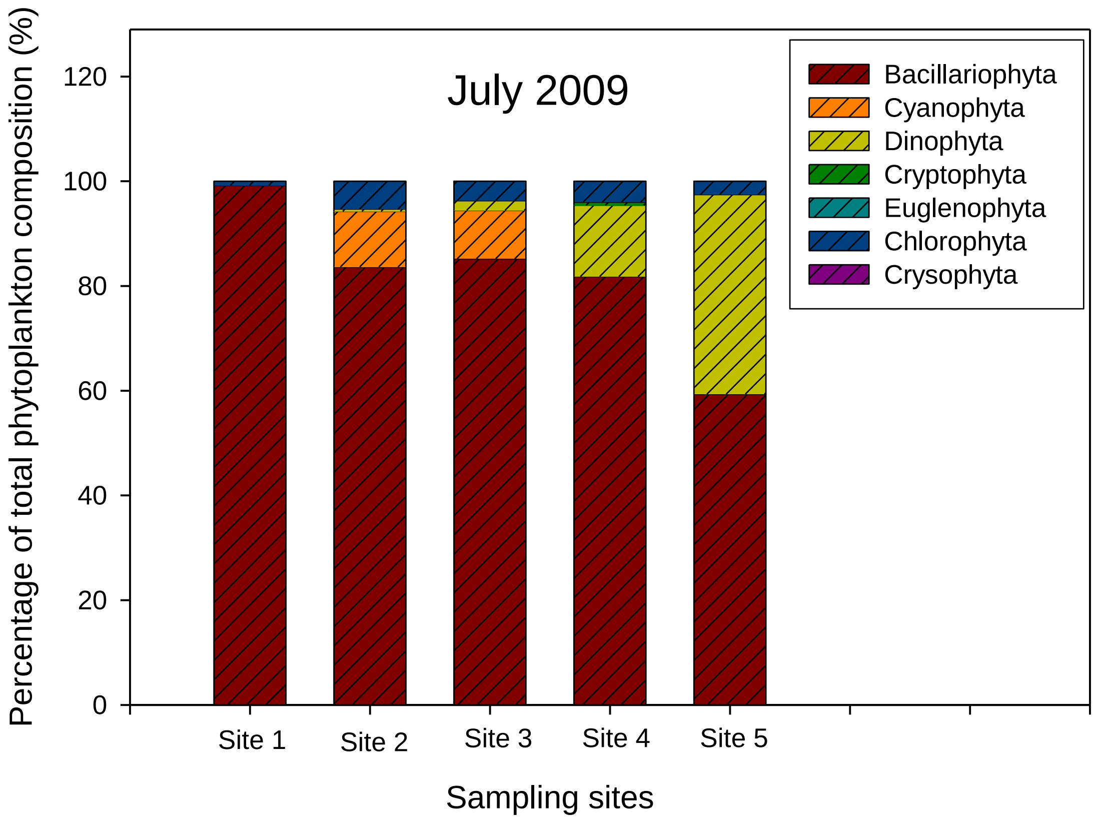

Figure 4.14: Phytoplankton species composition in Loskop Dam in July.

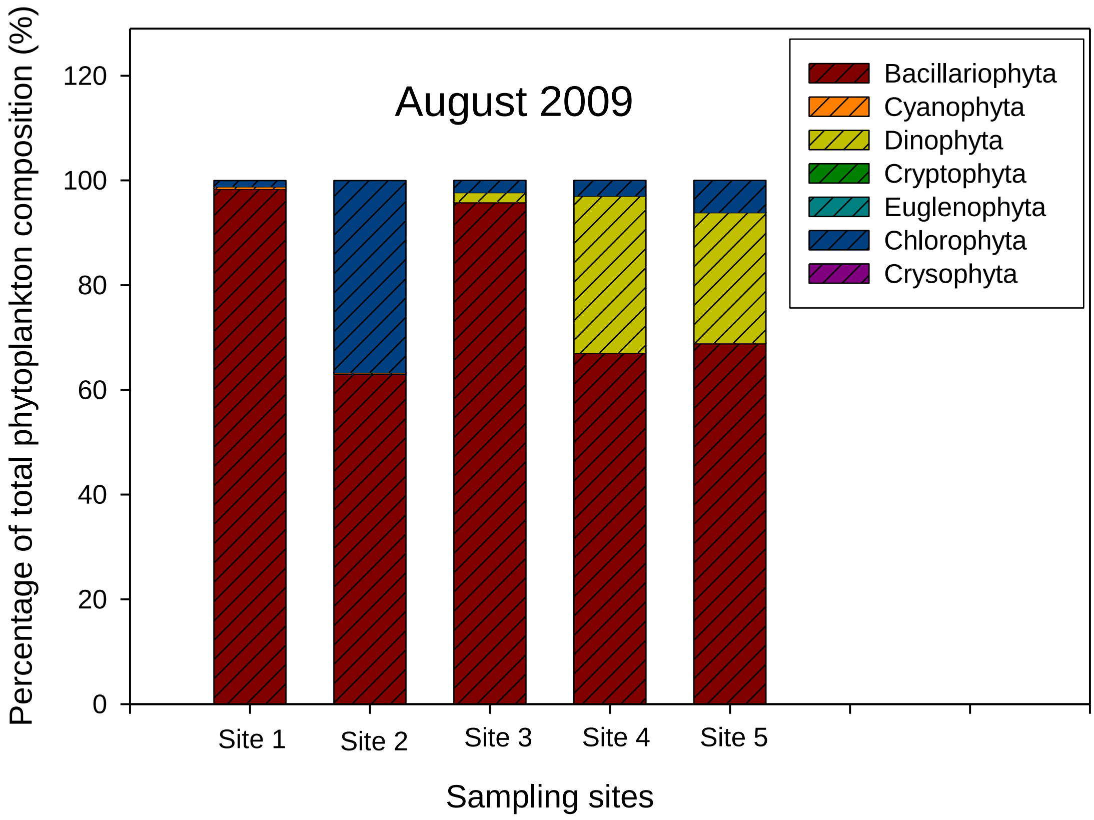

Figure 4.15: Phytoplankton species composition in Loskop Dam in August.

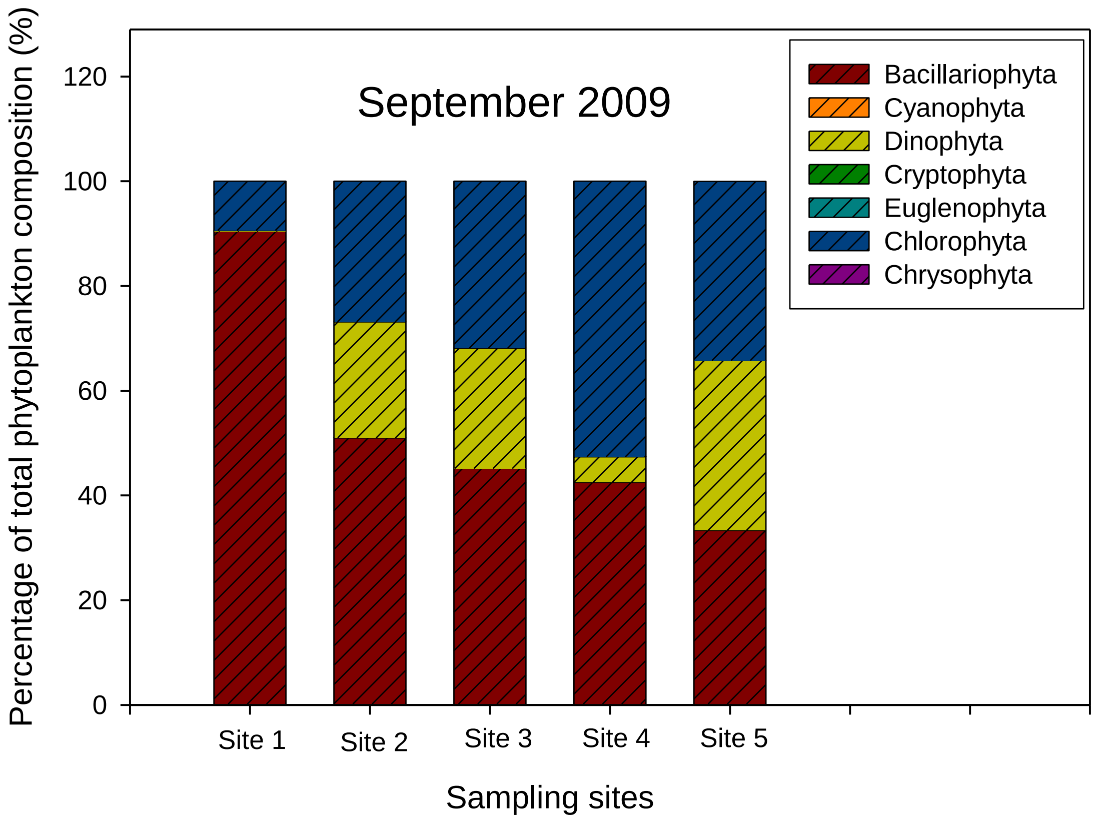

Figure 4.16: Phytoplankton species composition in Loskop Dam in September.

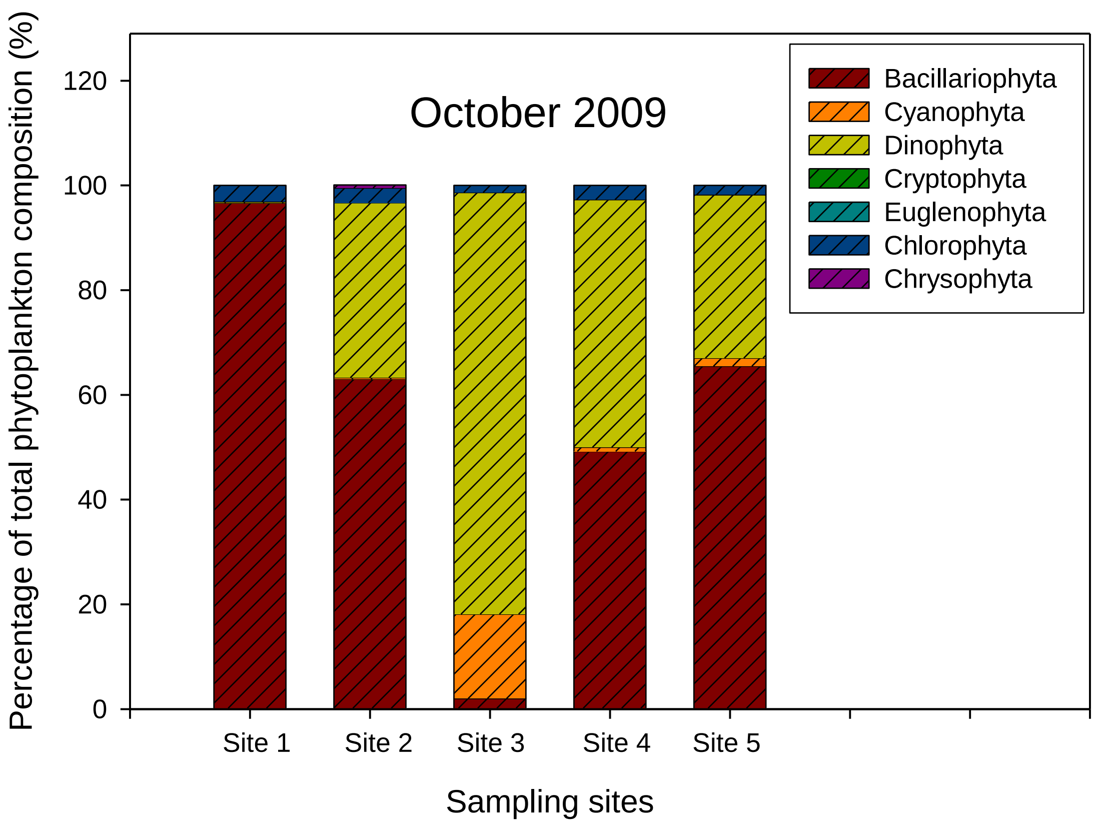

Figure 4.17: Phytoplankton species composition in Loskop Dam in October.

4.4 Discussion

4.4.1 Chemical and Physical data analysis

The high sulphate, conductivity and TDS levels showed that the dam water was most likely receiving AMD from the intensive mining, industrial activities upstream of the dam. Noticeably at the inlet, where the drainage flows into the dam. The average sulphate, conductivity and TDS levels were detected to be higher at the riverine zone (sites 1 and 2) in comparison to the other sites. High sulphate content in the system may exacerbate the extent of eutrophication, as sulphate has high preference in binding to Fe, which in turn mobilizes the phosphate (Oberholster et al. 2011). The high levels of TDS and conductivity at sampling sites 1 and 2 were most likely contributed by the metals which become highly soluble in acidic condition that occurred at AMD site upstream of Loskop Dam. High level of TDS was also detected in a previous study done by Driescher (2008) on Loskop Dam. In the latter study, he observed high fluctuation of TDS level in the dam over a period of 2006 to 2007. This result indicated that pollution occurred in pulses and may also be the cause of sporadic incidents of fish mortalities in Loskop Dam. In previous study by Oberholster et al. (2010) on Loskop Dam, Al and Fe concentration, as indicators of AMD, were much higher than the present study. In his study, Al and Fe reached a maximum of 1.56 mg l^{-1} and 1.2 mg l^{-1} respectively at the inlet of the dam. In the present study, Al and Fe only reached a maximum of 0.11 mg l^{-1} and 0.29 mg l^{-1} at the riverine zone sites. This great difference in the metal concentration can also be related to pulses of pollution. The study done by Oberholster et al. (2010) was conducted during the high rainfall season (February, March), so extra seepage of acid water from the abandoned coal mines did possibly coincide with elevated Al concentrations. This study was conducted during the low rainfall season, together with higher average pH and much lower dissolved metal concentration. The dominant anion at Loskop dam was sulphate, a characteristic of water contaminated by AMD (Villiers and Mkwelo 2009). The effect of AMD could also be shown by the lower average pH level in the riverine zone (site 1 = 7.87, site 2 = 7.86) as compared to other sites (site 3 = 8.2, site 4 = 8.16, site 5 = 8.26). pH was the lowest during July at all sites, possibly indicating inflow of the mine drainage from upstream. The high pH during September and October could result from the increase in rates of CO_2 consumption of algal photosynthesis as water temperature increase in spring (Oberholster et al. 2011). The uptake and assimilation of CO_2 drives the CO_2/bicarbonate/carbonate equilibrium towards the more alkaline carbonate end of the equilibrium (Oberholster et al. 2011). High concentration of Al, Fe and low pH are typical indicators of AMD, therefore one would expect a high concentration of dissolved metal and low pH in the receiving water body (Akcil and Koldas 2006). The Al concentration was fairly constant and above TWQR standard, at the sampling points except for May at site 1 and June at site 2. Although the solubility of Al is highly pH dependant, it is also influenced by other physical, biological and chemical factors (DWAF 1996b).

From the high level of nitrate (0.2 mg l^{-1} – 1.9 mg l^{-1}) found especially at the riverine zone of Loskop Dam, it was obvious that the dam is currently contaminated by untreated waters from the sewage works of municipality upstream. During the sampling trip, dead fish were found floating on the water or lying on the bank of the dam, one possible reason could be high nitrate level detected during May, June and August at sites 1 and 2. According to Australian and New Zealand Environmental Conservation Council (ANZECC) guidelines on water quality for aquatic ecosystem (ANZECC 2000), nitrate level above 0.17 mg l^{-1} are harmful with a potential to cause fish mortalities in high concentration. Phosphate level (<0.2 mg l^{-1}) was low during the study as this might be due to quick turn-over rate of ortho-phosphate and also the efficient trapping of P-input by biological assimilation and deposition in the sediment (Correll 1998). The water column turbidity (as measured by Secchi depth) at site 3 was particularly high in May (0.39 m) and October (0.33 m), and both of these periods were dominated by *C. hirudinella* and *M. flos-aquae*. Thus the low Secchi depths measured at these sites were possibly related to phytoplankton and not to suspended solids.

4.4.2 Phytoplankton

Phytoplankton is often used as a bioindicator in the water quality of an aquatic system because each species have different habitat preferences, so by analyzing the assemblage, one can deduce the quality of water more accurately than with the chemical results alone (Daz-Pardo et al. 1998; Kumari et al. 2008). They are used as bioindicators also due to their rapid response to environmental changes, fast turnovers, and they are also easy to collect in large numbers (US Environmental Protection Agency 2011). Algae are particularly valuable in environmental assessment because they serve as an indicator of environmental conditions based on the variable environmental sensitivities and tolerances of individual taxa and species. Algae’s species-specific sensitivity to environmental conditions and their high diversity in habitats provide the potential for more accurate and precise assessment of chemical, physical and biological conditions. Algae occur in all aquatic environments, thus one could compare the taxonomic composition and diversity of algal assemblages to environmental change among reference (healthy) and polluted ecosystems. Through comparison, one could assess the ecological health of habitats and deduce probable environmental causes of ecological impairment (Stevenson and Smol 2003).

The result of the composition of phytoplankton community in Loskop Dam indicated that the dam is indeed in an eutrophic state, which could not be detected solely from the phosphate concentration. The low phosphate level could be due to that the available phosphate were absorbed and consumed by the phytoplankton to support their growth. The phytoplankton assemblage at all sites and throughout the sampling trip are composed primarily by species that are tolerant to eutrophic condition and high electrolyte contents. Experimental evidence of nutrient requirements has been reported for certain phytoplankton species to determine their autecology in laboratory cultures and has been used in this study (Tilman and David 1977; Tilman and David 1982; Tilman et al. 1982; Porter 2008). The water at sites 1 and 2 throughout the sampling period shows that the water was characterized with low light penetration caused by diatom and *Microcystis*. Sites 1 and 2 are at the riverine zone where it receives surface runoffs from the upper Olifants River carrying pollutants from the abandoned mine sites and sewage work upstream. *C. hirundinella* seems to be a prominent dinophyta species found in Loskop Dam throughout the study, particularly at sites 2, 3, 5 and 6.

They are commonly found in SA man-made impoundments and its blooming usually indicates eutrophic conditions (high PO_4 and NO_3 level) within a water system (van Ginkel et al. 2001). But in this study, the phosphate concentration was low, as compared to previous study on Loskop Dam (0.7 mg l^{-1}) (Oberholster et al. 2009). A similar result was also found in the study done on a mesotrophic lake in Poland, where there was also a negative correlation between dinophyte biomass and phosphate concentration (Solis 2005). The maximum increase of *C. hirundinella* cells occurred around September and October, which was found to be in correspondence with the period of *C. hirundinella* bloom found in Hartbeespoort Dam (van Ginkel et al. 2001). The water temperature raised and ranges from 20℃ to 30℃. This range seems to be the most favourable temperature for massive growth of *C. hirundinella*, even though their optimum growth temperature were predicted to be between 5℃ to 30℃ (van Ginkel et al. 2001). *C. hirudinella* thrives the best in warm and stratified water and it has the following adaptive features that make them a better competitor than others in obtaining resources for growth (Reynolds et al. 2002) namely: the large size (mean length = 180 μm) of *C. hirudinella* makes it relatively ungrazed by most of the zooplankton, thus its blooming can alter the community structure of zooplankton. Its ability to migrate into different water layers, thus in a stratified water condition, *C. hirundinella* can migrate to the bottom of dam to obtain nutrients, and upward during the day time to expose itself to sunlight for photosynthesis (Reynolds et al. 2002). Furthermore, this species has the ability of assimilating both organic and inorganic phosphorus which give the species and advantage over other algal species, as inorganic orthophosphate (PO_4) is the only phosphorus that are biologically available to most of phytoplankton species (van Ginkel et al. 2001).

The occurrence of *Microcystis* bloom was particularly prominent around the transitional zone and less at the riverine and lacustrine zone, but they were less frequent than the occurrence of *C. hirundinella* bloom during the winter-spring period. This could be due to that *Microcystis* bloom is highly seasonal, usually occurs in late summer period when temperature is usually warm (above 17 ℃). However, according to Graham et al (2008), their dominance may also occur any time of the year. The co-occurrence of *C. hirundinella*, may possibly have an inhibitory effect on *Microcystis* during our sampling period, since allelopathic interaction between these species if *C. hirundinella* average cell number exceeds 1000 cells.ml^{-1} has previously been reported by Oberholster et al. (2010).

4. 5 Conclusion

In general this study shows that the water quality in Loskop Dam is deteriorated due to occurrence of algal blooms and high concentration of dissolved salts due to AMD inflow from the upper catchment. Algal diversity is particularly poor at the transitional zone and was dominated mainly by *C. hirundinella* and *M. aeruginosa*. Although the other sampling sites had higher phytoplankton diversity, the phytoplankton assemblage at these sites was composed mainly by species that were tolerant to eutrophic and high electrolyte conditions. The loss in diversity of phytoplankton may suggest that the system is losing its integrity and ecosystem functioning, due to progressive eutrophication.

4.6 References:

Abrantes N, Antunes SC, Pereira MJ, Gonçalves F (2006) Seasonal succession of cladocerans and phytoplankton and their interactions in a shallow eutrophic lake (LakeVela, Portugal). International Journal of Ecology 29: 54-64

Akcil A, Koldas S (2006) Acid Mine Drainage (AMD): causes, treatment and case studies Journal of Cleaner Production 14: 1139 -1145

ANZECC (2000) Australian and New Zealand Guidelines for Fresh water and Marine Water Quality Volume 1: The Guidelines. Australian and New Zealand Environment and Conservation Council

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF) (1992) Standard Methods for the Examination of Water and Wastewater. (19^{th} edition). APHA, AWWA, and WPCF, Washington, D.C., USA

Arhonditsis GB, Winder M, Brett MT, Schindler DE (2004) Patterns and mechanisms of phytoplankton variability in Lake Washington (USA). Water Research 38:4013-4027

Ashton P, Love D, Mahachi H, Dirks P (2001) An Overview of the Impacts of Mining and Mineral Processing Operations on Water Resources and Water Quality in the Zambezi, Limopo and Olifants Catchments in Southern Africa. Mining, Minerals and Sustainable Development Report No. ENV-P-C 2001-042: 1-319

Ashton PJ (1985) Seasonality in Southern Hemisphere fresh water phytoplankton assemblages. Hydrobiologia 125: 179-190

Barradas S (2011) South Africa's Water Sector 2011. Research Unit of Creamer Media (Pty) Ltd, pp 1-24

Bell FG, Hälbich TFJ, Bullock SET (2002) The effects of acid mine drainage from an old mine in the Witbank Coalfield, South Africa. Quarterly Journal of Engineering Geology and Hydrogeology 35: 265–278

Berger WH, Parker FL (1970) Diversity of planktonic *Foraminifera* in deep sea sediments. Science168:1345–1347

Bortnikova SB, Smolyakov BS, Sidenko NV, Kolonin GR, Bessonova EP, Androsova NV (2001) Geochemical consequences of acid mine drainage into a natural reservoir: inorganic precipitation and effects on plankton activity. Journal of Geochemical Exploration 74: 127-139

Boyero L, Valladolid M, Arauzo M (2005) Dynamics of Invertebrate Benthic Communities and Drift in a Regulated River of Central Spain. International Review of Hydrobiology 90: 392-411

Brand LE, Pablo J, Compton A, Hammerschlag N, Mash DC (2010) Cyanobacterial blooms and the occurrence of the neurotoxin, beta-N-methylamino-L-alanine (BMAA), in South Florida aquatic food webs. Harmful Algae 9: 620-635

Buckup L, Bueno AAP, Bond-Buckup G, Casagrande M, Majolo F (2007) The benthic macroinvertebrate fauna of highland streams in southern Brazil: composition, diversity and structure. Revista Brasileira de Zoologia 24: 294–301

Bulgakov NG, Levich AP (1992) Environmental Biogenic Elements and the Phytoplankton: The Nitrogen-to-Phosphorus Ratio as an Independant Regulating Factor. Russian Journal of Aquatic Ecology 2: 149-159

Camargo JA, Alonso A, de la Puente M (2005) Eutrophication downstream from small reservoirs in mountain rivers of Central Spain. Water Research 39: 3376-3384

Carty S (2002) Fresh water Algae of North America : Ecology and Classification Elsevier Science & Technology Books Chapter 20: 685-714

Chaudhury BL, Meena L (2007) A environmental hazard – a case study of toxic bloom of microcystis (anacystis) spp. in Udaipur lakes, Rajasthan (India). Journal of Herbal Medicine and Toxicology 1(1): 55–59

Codd G, Bell S, Kaya K, Ward C, Beattie K, Metcalf J (2010) Cyanobacterial toxins, exposure routes and human health. European Journal of Phycology 34:405-415

Codd GA, Morrison LF, Metcalf JS (2005) Cyanobacterial toxins: risk management for health protection. Toxicology and Applied Pharmacology 203: 264-272

Coimbra CN, Graca MA, Cortes RM (1996) The effects of a basic effluent on macroinvertebrate community structure in a temporary Mediterranean river. Environmental Pollution 94: 301-307

Correll DL (1998).The Role of Phosphorus in the Eutrophication of Receiving Waters: A Review. Journal of Environmental Quality 27: 261-266

Cummins WK (1974) Structure and Function of Stream Ecosystems. American Institute of Biological Sciences 24: 631-641

Daz-Pardo E, Vazquez G, Lopez-Lopez E (1998) The phytoplankton community as a bioindicator of health conditions of Atezca Lake, Mexico. Aquatic Ecosystem Health and Management 1: 257-266

Delhaize E, Ryan PR (1995) Aluminum Toxicity and Tolerance in Plants. Plant Physiology 107: 315-321

DeNicol DM, Stapleton MG (2002) Impact of acid mine drainage on benthic communities in streams: the relative roles of substratum vs. aqueous effects. Environmental Pollution 119: 303-315

Dickens CWS, Graham PM (2002) The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. African Journal of Aquatic Science 27: 1-10

Domingues RB, Barbosa A, Galvão H (2008) Constraints on the use of phytoplankton as a biological quality element within the Water Framework Directive in Portuguese waters. Marine Pollution Bulletin 56: 1389-1395

Driescher AC (2007) A water resource quality study of Loskop dam and the upper catchment of the Olifants River. MSc Thesis in Environmental Management, University of Freestate, ZA, pp 1-150

Dsa JV, Johnson KS, Lopez D, Kanuckel C, Tumlinson J (2008) Residual Toxicity of Acid Mine

Drainage-Contaminated Sediment to Stream Macroinvertebrates: Relative Contribution of Acidity vs. Metals. Water Air & Soil Pollution 194: 185-197

Dura JO (2005) Response of the benthic macroinvertebrate community to a point source in La Tordera Stream (Catalonia, Ne Spain). Universitat de Girona

DWAF (1996a) South African Water Quality Guidelines. Department of Water Affairs and Forestry Volume 1: Domestic Use

DWAF (1996b) South African Water Quality Guidelines (Aquatic Ecosystem). Department of Water Affairs and Forestry 7

DWAF (2004) Olifants Water Management Area: Internal Strategic Perspective. National Water Resource Planning DWAF Report No P WMA 04/000/00/0304

DWAF (2006) The Olifants River System Pilot Study. Department of Water Affairs and Forestry Appendix D

Dzialowski AR, Wang SH, Lim NC, Spotts WW, Huggins DG (2005) Nutrient limitation of phytoplankton growth in central plains reservoirs, USA. Journal of Plankton Research 27: 587-596

Eddy T, Feck J (1998) Beneficial use Reconnaissance Monitoring and Assessment Report. Wyoming Department of Environmental Quality Water Quality Division, pp 1-25

Edwards KJ, Bond PL, Gihring TM, Banfield JF (2000) An Archaeal Iron-Oxidizing Extreme Acidophile Important in Acid Mine Drainage. Science 287: 1796 - 1798

Falconer IR (1998) Algal toxins and human health. In the Handbook of Environmental Chemistry 5,Part C Quality and Treatment of Drinking Water II (J.Hrubee,ed.), pp 53-82

Flynn KJ (2010) Do external resource ratios matter? Implications for modelling eutrophication events and controlling harmful algal blooms. Journal of Marine Systems 83: 170-180

Fuller M, O'Reilly C (2005) Characterization and comparison of benthic macroinvertebrate communities in small streams of forested and deforested catchments bordering Lake Tanganyika. Nyanza Projects Publications.Paleoclimatology-Limnogeology, pp 1-6

Gamito S, Furtado R (2009) Feeding diversity in macroinvertebrate communities: A contribution to estimate the ecological status in shallow waters. Ecological Indicators 9: 1009-1019

Graham JL, Loftin KA, Ziegler AC, Meyer MT (2008) Cyanobacteria in lakes and reservoirs: toxin and taste-and-odor sampling guidelines. Cyanobacteria Version109

Gray NF (1996) A substrate classification index for the visual assessment of the impact of acid mine drainage in lotic systems. Water Research 30(6): 1551-1554

Gray NF (1997) Environmental impact and remediation of acid mine drainage:a management problem. Environmental Geology 30: 62-71

Gray NF (1998) Acid mine drainage composition and the implication for its impact on lotic systems. Water Research 32: 2122 - 2134

Grout JA, Levings CD (2001) Effects of acid mine drainage from an abandoned copper mine, Britannia Mines, Howe Sound, British Columbia, Canada, on transplanted blue mussels (Mytilus edulis). Marine Environment Research 51: 265-288

Hargeby A, Andersson G, Blindow I, Johansson S (1994) Trophic web structure in a shallow eutrophic lake during a dominance shift from phytoplankton to submerged macrophytes Hydrobiologia 279-280: 83-90

Harrison AD (1958) The effects of sulfuric acid pollution on the biology of streams in the Transvaal. South Africa. International Association of Theoretical and Applied Limnology 13: 603-610

Hashioka T, Yamanaka Y (2007) Seasonal and regional variations of phytoplankton groups by top–down and bottom–up controls obtained by a 3D ecosystem model. Ecological Modelling 202: 68-80

Havens KE, James RT, East TL, Smith VH (2003) N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. Environmental Pollution 122: 379-390

Hecky RE, Kilham P (1988) Nutrient limitation of phytoplankton in fresh water and marine environments: A review of recent evidence on the effects of enrichment. American Society of Limnology and Oceanography 33: 796-822

Heisler J, Glibert PM, Burkholder JM, Anderson DM, Cochlan W, Dennison WC, Dortch Q, Gobler CJ, Heil CA, Humphries E, Lewitus A, Magnien R, Marshall HG, Sellner K, Stockwell DA, Stoecker DK, Suddleson M (2008) Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae 8: 3-13

Henriques-de-Oliveira C, Baptista DF, Nessimian JL (2007) Sewage input effects on the macroinvertebrate community associated to *Typha domingensis* Pers in a coastal lagoon in southeastern Brazil. Brazilian Journal of Biology 67(1): 73-80

Henry TB, Irwin ER, Grizzle JM, Brumbaugh WG, Wildhaber ML (2001) Gill lesions and death of bluegill in an acid mine drainage mixing zone. Environmental Toxicological Chemistry 20: 1304-1311

Henry TB, Irwin ER, Grizzle JM, Wildhaber ML, Brumbaugh WG (1999) Acute Toxicity of an Acid Mine Drainage Mixing Zone to Juvenile Bluegill and Largemouth Bass. Transactions of the American Fisheries Society 128: 919-928

Hobbs PJ, Oelofse SHH, Rascher J (2008) Management of environmental impacts from coal mining in the upper Olifants River Catchment as a function of age and scale. International Journal of Water Resource Development 24: 417-432

Hörnström E (1999) Long-term phytoplankton changes in acid and limed lakes in SW Sweden. Hydrobiology 394: 93–102

Hunken A, Mutz M (2007) On the ecology of the filter-feeding *Neureclipsis bimaculata* (Trichoptera, Polycentropodidae) in an acid and iron rich post-mining stream. Hydrobiologia 592: 135–150

International Water Management Institute I (2008) Baseline Report Olifants River Basin in South Africa. A contribution to the Challenge Program Project 17 “Integrated Water Resource Management for Improved Rural Livelihoods: Managing risk, mitigating drought and improving water productivity in the water scarce Limpopo Basin”, pp 1-37

Jackson LE, KurtZ JC, Fisher WS (2000) Evaluation Guidelines for Ecological Indicators. US Environmental Protection Agency, Office of Research and Development, Research Triangle

Park, NC.EPA/620/R-99/005, pp 1-109

Jarup L (2003) Hazards of heavy metal contamination. British Medical Bulletin 68: 167-182

Joubert A (2008) Evaluation of biomarkers for pollution in *Oreochromis Mossambicus*: a laboratory and field study. The University of Johannesburg, Department of Zoology, pp 1-117

Khan FA, Ansari AA (2005) Eutrophication: An ecological vision. The Botanical Review 71: 449-482

Kitto JAJ (2009) The application of ecological theory to the remediation of macroinvertebrate communities impacted by acid mine drainage. Master of Science in Environmental Science in the University of Canterbury, pp 1-154

Kotze P, Du Preez HH, Van Vuren JHJ (1999) Bioaccumulation of copper and zinc in *Oreochromis Mossambicus* and *Clarias gariepinus*, from the Olifants River, Mpumalanga, South Africa. Water SA 25: 99-110

Kruger EJ (1968) The seasonal variation in the quality and quantity of the net-zooplankton in Loskop Dam, Transvaal. Research project from the Department of Animal Science and Microbiology.Transvaal, South Africa, pp 1-60

Kumari P, Dhadse S, Chaudhari PR, Wate SR (2008) A biomonitoring of plankton to assess quality of water in the lakes of Nagpur City. Proceedings of Taal 2007 The 12th World Lake Conference, pp 160-164

Last LJ (2001) Impact of specific Acid Mine Drainage contaminants on macroinvertebrate communities in southeastern Ohio streams. MSc Thesis, College of Arts and Sciences of Ohio University, Ohio, pp1-97

Limpitlaw D, Aken M, Lodewijks H, Viljoen J (2005) Post-Mining, Rehabilitatiom, Land Use and Pollution at Colliers in South Africa, pp 1-10

The South African Institute of Mining and Metallurgy Sustainable Development in the life of coal mining in South Africa, pp 1-10

Los FJ, Wijsman JWM (2007) Application of a validated primary production model (BLOOM) as a screening tool for marine, coastal and transitional waters Journal of Marine Systems 64: 201-215

Lund JWG, Kipling C, Le Cren EO (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11**:**143-170.

Magoba RNN (2005) Effects of invasion and clearing of alien riparian vegetation on benthic macroinvertebrate and adult Odonata assemblages in Soutpansberg River. MSc Thesis, Conservation Ecology and Entomology, University of Stellenbosch, pp 1-127

Martin-Jézéquel M, Hildebrand M, Brzezinski MA (2000) Silicon Metabolism in diatoms: implication for growth. Journal of Phycology 36: 821-840

Martins R, Stephan N, Alves R (2008) Tubificidae (Annelida: Oligochaeta) as an indicator of water quality in an urban stream in southeast Brazil. Acta Limnologica Brasiliensia 20: 221-226

Maso M, Garces E (2006) Harmful microalgae blooms (HAB); problematic and conditions that induce them. Marine Pollution Bulletin 53: 620-630

McMillan PH (1998).An Integrated Habitat Assessment System for the Rapid Biological

Assessment of Rivers and Streams. Research Project number ENV-P-I-98132. Council for Scientific and Industrial Research (CSIR), Water Resources Management Programme, South Africa.

Ministry of Environment LaP, Province of British Columbia (2000) Ambient Water Quality Guidelines for Sulphate. http://wwwenvgovbcca/wat/wq/BCguidelines/sulphate

Monteiro MT, Oliveira R, Vale C (1995) Metal Sress on the Plankton Communities of Sado River (Portugal). Water Research 29: 695-701

Mulder PFS (1968) 'n Kwalitatiewe en kwantitatiewe ondersoek na die bentos en epifauna in Loskopdam. MSc Thesis, Departement Dierkunde, Potchefstroomse Universiteit vir CHO, Potchefstroom, pp 1-36

Muylaert K, Declerck S, Van Wichelen J, De Meester L, Vyverman W (2006) An evaluation of the role of daphnids in controlling phytoplankton biomass in clear water versus turbid shallow lakes. Limnologica 36: 69-78

Nedeau EJ, Merritt RW, Kaufman MG (2003) The effect of an industrial effluent on an urban stream benthic community: water quality vs habitat quality. Environmental Pollution 123: 1-13

Niyogi DK, Lewis WM, McKnight DM (2002) Effects of Stress from Mine Drainage on Diversity, Biomass, and Function of Primary Producers in Mountain Streams. Ecosystems 5: 554-567

Nordberg GF, Goyer RA, Clarkson TW (1985) Impact of effects of acid precipitation on toxicity of metals. Environmental Health Perspective 63: 169-180

Nuccio C, Melillo C, Massi L, Innamorati M (2003) Phytoplankton abundance, community structure and diversity in the eutrophicated Orbetello lagoon (Tuscany) from 1995 to 2001. Oceanologica Acta 26: 15-25

Nussey G, van Vuren JHJ, du Preez HH (2000) Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, *Labeo umbratus* (Cyprinidae), from Witbank Dam, Mpumalanga. Water SA 26: 269-284

Oberholster PJ, Aneck-Hahn NH, Ashton PJ, Botha A-M, Brown J, Dabrowski JM, de Klerk AR, et al. (2011) Risk Assessment of Pollution in Surface Waters of the upper Olifants River System: Implications for Aquatic Ecosystem Health and the Health of Human Users of Water CSIR Natural Resources and the Environment Executive Summary Report to the Olifants River Forum. CSIR/NRE/WR/ER/2010/0025/B

Oberholster PJ, Ashton PJ (2008) An Overview of the Current Status of Water Quality and Eutrophication in South African Rivers and Reservoirs Parliamentary Grant Deliverable State of the Nation Report. Council for Scientific and Industrial Research (CSIR), Pretoria, pp 1-14

Oberholster PJ, Botha AM, Ashton PJ (2009) The influence of a toxic cyanobacterial bloom and water hydrology on algal populations and macroinvertebrate abundance in the upper littoral zone of Lake Krugersdrift, South Africa. Ecotoxicology18: 34-46

Oberholster PJ, Botha AM, Cloete TE (2005) Using a battery of bioassays, benthic phytoplankton and the AUSRIVAS method to monitor long-term coal tar contaminated sediment in the

Cache la Poudre River, Colorado. Water Research.39: 4913-4924
Oberholster PJ, Botha AM, Cloete TE (2008) Biological and chemical evaluation of sewage water pollution in the Rietvlei nature reserve wetland area, South Africa. Environmental Pollution.156: 184-192
Oberholster PJ, Botha AM, Myburgh JG, Ashton PJ (2010) Responses of phytoplankton upon exposure to a mixture of acid mine drainage and high levels of nutrient pollution in Lake Loskop, South Africa. Ecotoxicology and Environmental Safety 73: 326-335
Oberholster PJ, Myburgh JG, Ashton PJ, Coetzee JJ, Botha AM (2012) Bioaccumulation of aluminium and iron in the food chain of Lake Loskop, South Africa. Ecotoxicology and Environmental Safety 75: 134-141
Padisa´k J, Crossetti LO, Naselli-Flores L (2009) Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. Hydrobiologia 621:1-19
Paisley MF, Walley WJ, Trigg DJ (2011) Identification of macroinvertebrate taxa as indicators of nutrient enrichment in rivers. Ecological Informatics 6: 399-406
Pete R, Davidson K, Hart MC, Gutierrez T, Miller AEJ (2010) Diatom derived dissolved organic matter as a driver of bacterial productivity: The role of nutrient limitation. Journal of Experimental Marine Biology and Ecology 391: 20-26
Petrin Z, Englund G, Malmqvist B (2008) Contrasting effects of anthropogenic and natural acidity in streams: a meta-analysis. Proceedings of the Royal Society 275: 1143-1148
Pietersen K, Beekman H (2006) Africa Environment Outlook 2. United Nations Environment Environmental State-and-Trends: 20-Year Retrospective, pp 119-154
Playle RC (1998) Modelling metal interactions at fish gills. The Science of the Total Environment 219: 147-163
Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficient and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents:verification of the concentration of chlorophyll standards by atomic absorption spectrometry. Biochimica et Biophysica Acta 975: 384-394
Porter SD (2008) Algal attributes: An Autecological Classification of Algal Taxa Collected by the National Water-Quality Assessment Program. US Geological Survey Data Series 329.
Proulx M, Pick FR, Mazumder A, Hamilton PB, Lean DRS (1996) Experimental evidence for interactive impacts of human activities on lake algal species richness. Oikos 76: 191-195
Quigley MA (1981) Fresh water macroinvertebrates. Water Pollution Control Federation 53: 1015-1027
Quiroz-Va´zquez P, Sigee DC, White KN (2010) Bioavailability and toxicity of aluminium in a model planktonic food chain (*Chlamydomonas–Daphnia*) at neutral pH. Limnologica 40(3): 269-277
Resh V, Betts E, Groff M, Cover M (2007) Bioindicators of Strawberry Creek. Department of Integrative Biology, University of California-Berkeley, pp 1-14
Reynolds CS, Huszar V, Kruk C, Naselli-Flores L, Melo S (2002) Towards a functional

classification of the freshwater phytoplankton. Journal of Plankton Research 24:417-428
Ritter L, Solomon K, Sibley P, Hall K, Keen P, Mattu G, Linton B (2002) Sources, pathways, and relative risks of contaminants in surface water and groundwater: a perspective prepared for the Walkerton inquiry. Journal of Toxicology and Environmental Health 65: 1-142
Sa´nchez Espan˜a J, Pamo EL, Santofimia E, Aduvire O, Reyes J, Barettino D (2005) Acid mine drainage in the Iberian Pyrite Belt (Odiel river watershed, Huelva, SW Spain): Geochemistry, mineralogy and environmental implications. Applied Geochemistry 20: 1320-1356
Sabater S, Armengol J, Comas E, Sabater F, Urrizalqui I, Urrutia I (2000) Algal biomass in a disturbed Atlantic river: water quality relationships and environmental implications. Science of the Total Environment 263: 185-195
Scheuhammer AM (1991) Acidification-related changes in the biogeochemistry and ecotoxicology of mercury, cadmium, lead and aluminium: overview. Environmental Pollution 71: 87-90
Schrenk MO, Edwards KJ, Goodman RM, Hamers RJ, Banfield JF (1998) Distribution of Thiobacillus ferrooxidans and Leptospirillum ferrooxidans: Implications for Generation of Acid Mine Drainage. Science 279: 1519-1521
Schubauer-Berigan MK, Dierkes JR, Monson PD, Ankley GT (1993) pH-Dependent toxicity of Cd, Cu, Ni, Pb and Zn to *Ceriodaphnia dubia*, *Pimephales promelas*, *Hyalella azteca* and *Lumbriculus variegatus*. Environmental Toxicology and Chemistry 12: 1261-1266
Shannon CE, Weaver W (1949) The mathematical theory of communications. University of Illinois Press, Urbana, pp 1-144
Sivonen K, Jones G (1999) Cyanobacterial toxins. In: Toxic Cyanobacteria in Water: a Guide to Public Health Significance,Monitoring and Management. I Chorus and J Bertram (Edn) The World Health Organization ISBN 0–419–23930–8. E and FN Spon, London, UK, pp 41-111
Skei J, Larsson P, Rosenberg R, Jonsson P, Olsson M, Broman D (2000) Eutrophication and Contaminants in Aquatic Ecosystems. Ambio 29: 184-194
Smayda TJ (2008) Complexity in the eutrophication–harmful algal bloom relationship, with comment on the importance of grazing. Harmful Algae 8: 140-151
Smith VH (2003) Eutrophication of fresh water and coastal marine ecosystems: a global problem. Environmental Science and Pollution Research International 10: 126-139
Smith VH, Tilman GD, Nekola JC (1999) Eutrophication: impacts of excess nutrient inputs on fresh water, marine, and terrestrial ecosystems. Environmental Pollution 100: 179-196
Smolyakov BS, Ryzhikh AP, Romanov RE (2010) The fate of Cu, Zn, and Cd in the initial stage of water system contamination: the effect of phytoplankton activity. Journal of Hazard Materials 184: 819-825
Solis M (2005) Relationships between selected abiotic variables and phytoplankton composition in deep mesotrophic Lake ZAGŁĘBOCZE. Oceanological and Hydrobiological Studies 34: 81-96
Soucek DJ (2001) Integrative Bioassessment of Acid Mine Drainage Impacts on the Upper Powell River Watershed, Southwestern Virginia. PhD Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, pp 1-158

Soucek DJ, Denson BC, Schmidt TS, Cherry DS, Zipper CE (2002) Impaired Acroneuria sp. (Plecoptera, Perlidae) populations associated with aluminum contamination in neutral pH surface waters. Archives of Environmental Contamination and Toxicology 42: 416-422

Spry DJ, Wiener JG (1991) Metal bioavailability and toxicity to fish in low-alkalinity lakes: A critical review. Environmental Pollution 71: 243-304

Stevenson RJ, Smol JP (2003) Use of Algae in Environmental Assessments. Fresh water Algae of North America 23: 775-804

Stoddard JL, Herlihy AT, Hill BH, Hughes RM, Kaufmann PR, Klemm DJ, Lazorchak JM, McCormick FH, Peck DV, Paulsen SG, Olsen AR, Larsen DP, Van Sickle J, Whittier TR (2006) Mid-Atlantic Integrated Assessment (MAIA) State of the Flowing Waters Report. EPA/620/R-06/001 (NTIS PB2007-107909), US Environmental Protection Agency, Office of Research and Development,Washington DC , pp 1-85

Stokes PM, Bailey RC, Groulx GR (1985) Effects of Acidification on Metal Availability to Aquatic Biota, with Special Reference to Filamentous Algae. Environmental Health Perspectives 63: 79-87

Svendsen CR, Quinn T, Kolbe D (2004) Review of Macroinvertebrate Drift in Lotic Ecosystems. Wildlife Research Program Environmental and Safety Division Final Report-Manuscript, pp 1-92

Tamatamah R, Wasonga D, Muthike J (2007) Mara River EFA Study, Annex 6.1 Starter Document for BBM Workshop

Taylor JC, Harding WR, Archibald CGM (2007) An illustrated Guide to some common Diatom species from South Africa. Water Research Commission WRC Report TT 282/07

Thirion C (2007) Macroinvertebrate Response Assessment Index in River Eco Classification: Manual for EcoStatus Determination (version 2). Department of Water Affairs & Forestry, Resource Quality Services.Module E: Macroinvertebrate Response Assessment Index (MIRAI)

Thomas R, Meybeck M, Beim A (1996) Water Quality Assessments - A Guide to Use of Biota, Sediments and Water in Environmental Monitoring, Chapter 7. UNESCO/WHO/UNEP Second Edition, pp 1-46

Tilman, David (1977) Resource competition between planktonic algae - an experimental and theoretical approach. Ecology 58: 338 - 348

Tilman, David (1982) Resource competition and community structure. Princeton, NJ, Princeton University Press

Tilman, David, Kilham SS, Kilham P (1982) Phytoplankton community ecology - The role of limiting nutrients. Annual Review of Ecological Systematics 13: 349-372

Trainer VL, Pitcher GC, Reguera B, Smayda TJ (2010) The distribution and impacts of harmful algal bloom species in eastern boundary upwelling systems. Progress in Oceanography 85: 33-52

United Nations Environment Programme (2010) World Water Atlas. Division of Early Warning Assessment United Nations Environment Programme (UNEP)

US Environmental Protection Agency (2011) Biological Indicators of Watershed Health. EPA http://www.epa.gov/bioiweb1/html/periphyton.html

Van Ginkel CE, Hohls BC, Vermaak E (2001) A *Ceratium hirundinella* (O.F. Müller) bloom in Hartbeespoort Dam, South Africa. Water SA 27: 269-276

Van Ginkel CE (2002) Trophic Status Assessment Executive Summary. Department of Water Affairs and Forestry, pp 1-15

Van Rensburg RJ (2003) A Long-term Acid Mine Drainage Water Management Strategy for South Witbank Colliery, Mpumalanga. Rand Afrikaans University Magister Scientia in Environmental Management, pp 1-68

Van Vuuren S, Taylor JC, Gerber A, Van Ginkel C (2006) Easy identification of the most common freshwater algae. – North-West University and Department of Water Affairs and Forestry, Pretoria, South Africa, pp 1-200

Van Vuuren L (2008) Loskop Dam - Early Local Engineering Ingenuity. The Water Wheel, pp 18-21

Vargo GA, Heil CA, Fanning KA, Dixon LK, Neely MB, Lester K, Ault D, Murasko S, Havens J, Walsh J, Bell S (2008) Nutrient availability in support of Karenia brevis blooms on the central West Florida Shelf: What keeps Karenia blooming? Continental Shelf Research 28: 73-98

Villiers Sd, Mkwelo. ST (2009) Has monitoring failed the Olifants River, Mpumalanga? Water SA 35: 671-676

Wahizatul AA, Long SH, Ahmad A (2011) Composition and Distribution of Aquatic Insect Communities in Relation to Water Quality in Two Fresh water Streams of Hulu Terengganu, Terengganu. Journal of Sustainability Science and Management 6: 148-155

Wallace JB, Anderson NH (1995) Habitat, Life History, and Behavioural Adaptations of Aquatic Insects. An Introduction to the Aquatic Insects of North America. Editor: Merrit, RW, Cummins, KW. Third Edition, Kendall/Hunt Publishing Company, Iowe, pp 41-73

Wallace JB, Webster JR (1996) The role of macroinvertebrates in stream ecosystem function. Annual Review of Entomology 41: 115-139

Walsh G, Wepener V (2009) The influence of land use on water quality and diatom community structures in urban and agriculturally stressed rivers. Water SA 35:579-594

Wang J, Wu J (2009) Occurrence and potential risks of harmful algal blooms in the East China Sea. Science of the Total Environment 407: 4012-4021

Watson SB, McCauley E, Downing JA (1997) Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. Limnology and Oceanography 42: 487-495

Wenn CL (2008) Do fresh water macroinvertebrates reflect water quality improvements following the removal of point source pollution from Spen Beck, West Yorkshire? Earth & E-nvironment 3: 369-406

Willen E (1991) Planktonic diatoms – an ecological review. Algological Studies 62**:** 69-106

Wood JM (1985) Effects of Acidification on the Mobility of Metals and Metalloids: An Overview. Environmental Health Perspectives 63: 115-119

Wren CD, Stephenson GL (1991) The effect of acidification on the accumulation and toxicity of metals to fresh water invertebrates. Environmental Pollution71: 205-241

Wu JT, Chou TL (2003) Silicate as the limiting nutrient for phytoplankton in a subtropical eutrophic estuary of Taiwan. Estuarine Coastal and Shelf Science 58: 155-162

Zhou Q, Zhang J, Fu J, Shi J, Jiang G (2008) Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Analytica Chimica Acta 606: 135-150

Zingonea A, Enevoldsenb HO (2000) The diversity of harmful algal blooms: a challenge for science and management. Ocean & Coastal Management 43: 725-748

Summary

Data generated in this study indicated that the water quality in Loskop Dam was deteriorated due to AMD and sewage inflows from the upper Olifants catchment. The water was characterized with high TDS, conductivity, sulphate and nutrients. The surface water at the riverine zone (site 1) was found to be of much poorer water chemistry when compared to the reference site (site 2) (unimpacted). Yet at site 1, macroinvertebrate community, which serves as the bioindicator in this study, was found to have higher diversity with few sensitive species (Perlidae, Heptageniidae, Chlorocyphidae and Psepheniidae) present in comparison to site 2. The functional macroinvertibrate feeding groups namely scraper and shredders which are usually indicators of a good habitat quality, were also present at site 1 during spring 2009. At site 2, the community was composed only of predators, collector gatherers/filterers. The dense reed bed at site 1 may have act as good habitat providing oxygen and shelter against predators through their roots for macroinvertenbrates. These factors could have been the reason for higher diversity of the macroinvertebrate community and few sensitive species found when compared to the macroinvertebrate community in the 1968 study. On the other hand, low habitat heterogeneity, such as no aquatic macrophytes and less riparian vegetation may accounted for the lower diversity and unbalanced functional feeding group composition at the reference site.

When the macroinvertebrate community results of the 2009 and 1968 study were compared it was evident that there was a shift from more tolerant groups in1968 to more sensitive groups in the present study. This macroinvertebrate community shift can possibly be related to changes in the habitat of site 1. According to the 1968 study, low numbers of the common water reed *Phragmites* *communis* and the bulrush *Typha australis* were found at the riverine zone of Loskop Dam. However, forty two years later in 2009 these common water reeds had formed dense reedbeds at sampling site 1. These reed bed can act as 'filters' and absorbed phosphates and nitrogen out of the water column and may be the reason for the low values of ortho-phosphate measured at this site. Consequently it was evident from this study that water chemistry alone was not a reliable indicator to determine macroinvertebrate assemblage and that habitat plays a major role at the study sites.

During the study period (May 2009 to October 2009), the water was characterized with high TDS, sulphate, conductivity and nitrate levels, particularly at the riverine zone where the dam receives AMD and other pollutant from upstream. The phytoplankton communities at the selected sampling sites were composed by species (*Microcystis* sp., *Ceratium* sp., *Cyclotella* sp.) that were tolerant to high electrolyte and nutrient-rich environment. Yet, the transitional (site 3) and lacustrine zone (site 4 and 5) had a lower diversity (site 3: *H'*= 1.23; site 4: *H'* = 1.25; site 5: *H'* = 1.56) as compared to the riverine sites (site 1: *H'* = 2.075; site 2: *H'* = 1.93). The lower diversity at site 3 could be due to the massive bloom of *C.hirundinella* occurring during the onset of spring (September and October), where temperature (20℃ – 30℃) was in favour for the species. *Microcystis* bloom was particularly prominent around the transitional zone and less at the riverine and lacustrine zone, but they were less frequent than the occurrence of *C.hirundinella* bloom during the winter-spring period. This could result from the inhibitory effects of *Ceratium hirundinella* on the *Microcystis* due to the allelopathic interaction between these two species.

Therefore, from the study it was evident that the water quality of the dam was deteriorated due to the formation of algal blooms caused by nutrient enrichment and high concentration of dissolved salts from AMD inflows from the upper catchment.

Appendices

INVERTEBRATE HABITAT ASSESSMENT SYSTEM (IHAS)

version 2.2 *peter mac 1/2001*	River Name: Olifants River (Loskop inflow)	
	Site Name: Site 1	Date: 23/10/09

SAMPLING HABITAT

	0	1	2	3	4	5
Stones in Current (SIC)						
Total length of white water rapids (ie: bubbling water) (in metres)	none	~~0-1~~	>1-2	>2-3	>3-5	>5
Total length of submerged stones in current (run) (in metres)	none	0-2	>2-5	~~>5-10~~	>10	
Number of separate SIC *area's* kicked (not individual stones)	0	1	2-3	~~4-5~~	6+	
Average stone size's kicked (cm's)(<2 *or* >20 is '<2>20')(gravel is <2; bedrock is >20).	none	<2>20	2-10	~~11-20~~	2-20	
Amount of stone surface clear (of algae, sediment etc.) (in percent %) *	n/a	0-25	~~26-50~~	51-75	>75	
PROTOCOL: time spent actually kicking SIC's (in minutes)(gravel/bedrock = 0 min)	0	<1	>1-2	~~2~~	>2-3	>3

(* *NOTE:* up to 25% of stone is usually embedded in the stream bottom)

SIC Score: 15 (max. 20)

	0	1	2	3	4	5
Vegetation						
Length of fringing vegetation sampled (river banks) (PROTOCOL - in metres)	none	0-½	>½-1	>1-2	~~2~~	>2
Amount of aquatic vegetation/algae sampled (underwater) (in square metres)	~~none~~	0-½	>½-1	>1		
Fringing vegetation sampled in: ('still'=pool/still water only; 'run'=run only)	none		run	still		~~mix~~
Type of veg. (percent leafy veg. as opposed to stems/shoots) (aq. veg. only=49%)	none		1-25	26-50	~~51-75~~	>75

Vegetation Score: 13 (max. 15)

	0	1	2	3	4	5
Other Habitat / General						
Stones Out Of Current (SOOC) sampled: (PROTOCOL - in square metres)	~~none~~	0-½	>½-1	1	>1	
Sand sampled: (PROTOCOL – in minutes) ('under' = present, but only under stones)	none	under	0-½	>½-1	~~1~~	>1
Mud sampled: (PROTOCOL – in minutes) ('under' = present, but only under stones)	none	under	~~0-½~~	½	>½	
Gravel sampled: (PROTOCOL – in minutes) (*if all gravel, SIC stone size = '<2'*)**	~~none~~	0-½	½	>½**		
Bedrock sampled: ('all'=no SIC, sand, or gravel; *then SIC stone size ='>20'*)**	~~none~~	some			all**	
Algal presence: ('1-2m²'=algal bed; 'rocks'=on rocks; 'isol.'=isolated clumps) ***	>2m²	~~rocks~~	1-2m²	<1m²	isol.	none
Tray identification: (PROTOCOL – using time: 'corr' = correct time)		under		~~corr~~		over

(** *NOTE:* you must still fill in the SIC section)

Other Habitat Score: 10 (max. 20)

Habitat Total: 38 (max. 55)

STREAM CONDITION

	0	1	2	3	4	5
Physical						
River make up: ('pool'=pool/still/dam only; 'run' only; 'rapid' only; '2mix'=2 types etc.)	pool		run	rapid	~~2 mix~~	3 mix
Average width of stream: (metres)		>10	~~>5-10~~	<1	1-2	>2-5
Average depth of stream: (metres)	>1	1	>½-1	½	~~¼-½~~	<¼
Approximate velocity of stream: ('slow'=<½m/s; 'fast'=>1m/s) (use twig etc. to test).	still	slow	fast	med.		~~mix~~
Water colour: ('disc.'=discoloured with visible colour but still transparent)	silty	opaque		disc.		~~clear~~
Recent disturbances due to: ('constr.'=construction; 'fl/dr'=flood or drought) ***	fl/dr	fire	constr.	other		~~none~~
Bank / riparian vegetation is: ('grass'=includes reeds; 'shrubs'=includes trees)	none		grass	~~shrubs~~	mix	
Surrounding impacts: ('erosn'=erosion/shear bank; 'farm'=farmland/settlement)***.	erosn.	farm	~~trees~~	other		open
Left bank cover (rocks and vegetation): (in percent %)	0-50	51-75	~~75-95~~	>95		
Right bank cover (rocks and vegetation): (in percent %)	0-50	51-75	~~75-95~~	>95		

(*** *NOTE:* if more than one option, choos ethe lowest)

Stream Conditions Total: 34 (max. 45)

Web site: www.csir.co.za/ihas

Total IHAS Score: 72 %

Figure S1: Invertebrate Habitat Assessment System for site 1

INVERTEBRATE HABITAT ASSESSMENT SYSTEM (IHAS)

version 2.2 *peter mac 1/2001*	River Name: Tweeloopiespruit (loskop control).	
	Site Name: Site 4	Date:

SAMPLING HABITAT	0	1	2	3	4	5
Stones in Current (SIC)						
Total length of white water rapids (ie: bubbling water) (in metres)	~~none~~	0-1	>1-2	>2-3	*>3-5*	>5
Total length of submerged stones in current (run) (in metres)	none	0-2	~~>2-5~~	*>5-10*	>10	
Number of separate SIC *area's* kicked (not individual stones)	0	1	2-3	~~4-5~~	6+	
Average stone size's kicked (cm's)(<2 *or* >20 is '<2>20')(gravel is <2; bedrock is >20).	none	<2>20	2-10	~~11-20~~	*2-20*	
Amount of stone surface clear (of algae, sediment etc.) (in percent %) *........	n/a	0-25	~~26-50~~	51-75	>75	
PROTOCOL: time spent actually kicking SIC's (in minutes)(gravel/bedrock = 0 min)	0	<1	>1-2	~~2~~	>2-3	>3
(* *NOTE:* up to 25% of stone is usually embedded in the stream bottom)					*max. 20*	
SIC Score:					13	

Vegetation	0	1	2	3	4	5
Length of fringing vegetation sampled (river banks) (PROTOCOL - in metres)	none	~~0-½~~	>½-1	>1-2	*2*	>2
Amount of aquatic vegetation/algae sampled (underwater) (in square metres)	~~none~~	0-½	*>¼-1*	>1		
Fringing vegetation sampled in: ('still'=pool/still water only; 'run'=run only)	none		run	~~still~~		*mix*
Type of veg. (percent leafy veg. as opposed to stems/shoots) (aq. veg. only=49%) ..	none		~~1-25~~	26-50	*51-75*	>75
					max. 15	
Vegetation Score:					6	

Other Habitat / General	0	1	2	3	4	5
Stones Out Of Current (SOOC) sampled: (PROTOCOL - in square metres)	~~none~~	0-½	>½-1	*1*	>1	
Sand sampled: (PROTOCOL - in minutes) ('under' = present, but only under stones) .	none	under	0-½	>½-1	~~1~~	>1
Mud sampled: (PROTOCOL - in minutes) ('under' = present, but only under stones). .	none	under	~~0-½~~	*½*	>½	
Gravel sampled: (PROTOCOL - in minutes) (*if all gravel, SIC stone size = '<2'*)**....	~~none~~	0-½	*½*	>½**		
Bedrock sampled: ('all'=no SIC, sand, or gravel; *then SIC stone size ='>20'*)**	none	~~some~~			all**	
Algal presence: ('1-2m²'=algal bed; 'rocks'=on rocks; 'isol.'=isolated clumps) ***	>2m²	~~rocks~~	1-2m²	<1m²	*isol.*	none
Tray identification: (PROTOCOL - using time: 'corr' = correct time)		under		~~corr~~		over
(** *NOTE:* you must still fill in the SIC section)					*max. 20*	
Other Habitat Score:					11	
					max. 55	
Habitat Total:					30	

STREAM CONDITION	0	1	2	3	4	5
Physical						
River make up: ('pool'=pool/still/dam only; 'run' only; 'rapid' only; '2mix'=2 types etc.)	pool		run	rapid	~~2 mix~~	3 mix
Average width of stream: (metres)		>10	>5-10	<1	1-2	~~>2-5~~
Average depth of stream: (metres)	>1	1	>½-1	½	<½-¼	~~<¼~~
Approximate velocity of stream: ('slow'=<½m/s; 'fast'=>1m/s) (use twig etc. to test).	still	slow	fast	med.		~~mix~~
Water colour: ('disc.'=discoloured with visible colour but still transparent)	silty	opaque		disc.		~~clear~~
Recent disturbances due to: ('constr.'=construction; 'fl/dr'=flood or drought) *** ..	fl/dr	fire	constr.	other		~~none~~
Bank / riparian vegetation is: ('grass'=includes reeds; 'shrubs'=includes trees)	none		grass	shrubs	~~mix~~	
Surrounding impacts: ('erosn'=erosion/shear bank; 'farm'=farmland/settlement)***.	erosn.	farm	~~trees~~	other		open
Left bank cover (rocks and vegetation): (in percent %)	0-50	~~51-75~~	75-95	>95		
Right bank cover (rocks and vegetation): (in percent %)	0-50	~~51-75~~	75-95	>95		
(*** *NOTE:* if more than one option, choos ethe lowest)					*max. 45*	
Stream Conditions Total:					37	

Web site: www.csir.co.za/ihas

Total IHAS Score: 67 %

Figure S2: Invertebrate Habitat Assessment System for site 2 (reference site).