Bio assessment of water quality using macro-invertebrate communities in the Selati River, Lower Olifants River System

by

RASIFUDI LWENDO

DISSERTATION

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

in

ZOOLOGY

in the

FACULTY OF SCIENCE AND AGRICULTURE
(School of Molecular and Life Sciences)

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR: Prof. A Addo-Bediako
Co-SUPERVISOR: Dr A Swemmer

2019

TABLE OF CONTENTS

Chapter 3:

Macro-invertebrate distribution and diversity

Chapter 4

General discussion, conclusion and recommendations

DECLARATION

I declare that this dissertation hereby submitted for the degree of Master of Science in the Department of Zoology, Faculty of Science at University of Limpopo is original and has not been submitted by me for a degree at any other institution. This is my own work and that all other materials contained herein have been fully acknowledged.

……………………………………………… ……………………

L Rasifudi Date

Acknowledgement

I want to give a special thanks and appreciation to the following people and organisations:

Our Lord for granting me the privilege, opportunity, ability, patience and strength to start and finish this dissertation.

The Department of Biodiversity @ University of Limpopo for opportunity to do this MSc with them.

My supervisor Professor A Addo- Bediako for the support and assistance with the writing of this thesis and guidance

My co- supervisors Dr A Swemmer for assistance and guidance.

NRF and Vlir for their financial support.

Dr S Marr and Dr K Bal for helping me with statistical analysis.

Modibe Raphahlelo for his assistance with Canonical Correspondence Analysis.

I would like to thank my parents (**Mukhondo Muthuphei Esther & Rasifudi Ailwei**) and the rest of my family for their patience, encouragement, support and wisdom throughout this whole thesis.

Mukhondo Thinawanga, Rasifudi Takadzani & Khakhu Mukhethwa for thier support, encouragement, for pushing me to persevere and reminding why I am doing this.

A very special thanks to my daughter **Lwendolwashu** for always understanding when daddy had to go school and to field trips, for keeping me motivated, for inspiring me to be my best.

And once more, a really warm heart-felt thank you to the above mentioned this would have not been possible without you.

DEDICATION

Lwendolwashu Rasifudi

To my intelligent, beautiful, Pretty, funny, talented and insanely lovable daughter. You hold the key to my heart, and all this is for you my baby. You can be and do whatever your heart desires. Nothing is beyond your reach, nothing is impossible.

This is only the beginning my daughter; **I love you**

Abstract

Many South African rivers, including the Ga-Selati River have been deteriorating for the past few decades, due to an increase in mining, industrial, agricultural and domestic activities. Around mid-January 2014, the Ga-Selati River was contaminated by mine spills from a nearby phosphate mine, which polluted the river and killed many fish species. This river is a primary source of water for many activities (e.g. mining, industrial, agricultural and domestic activities), and as a result, large numbers of reservoirs, farm dams, and inter-basin transfer schemes have been constructed to increase the reliability of water supply along this river. Contamination of surface waters by agricultural pesticides and fertilizers, as well as by industrial metals, is a cause of increasing public concern.

The Ga-Selati River is a major tributary for the Olifants River, among other tributaries (Steelpoort River and Blyde River) and it plays a significant role in the Kruger National Park and other private game reserves in the catchment. This River is also known to supply water of very low quality into the main stem of the Olifants River. The Olifants River System has been described as degraded and is contaminated with metal and chemicals, and is considered to be one of the most threatened river systems in South Africa. The aim of the study was to investigate the ecological state of the Ga-Selati River and the impact of water and sediment quality on the aquatic invertebrate communities, and to propose measures to prevent further degradation of the river ecosystem by human disturbance. The main objectives were to: (i) establish the current physico-chemical composition of the river water and sediment along the entire length of the Ga-Selati River, (ii) to determine if poor water quality at the lower end of the river is due to pollution inputs in the lower reaches, or the result of cumulative pollution inputs along the entire length of the river, (iii) Assess the impact of water and sediment quality on the aquatic macro-invertebrate assemblages in the river.

The concentrations of pH, and DO were high at all sites. If there was any sort of pollution in the river, especially downstream by the mining sites, we expected these two variables to be lower. The water variables such as EC, TDS and salinity showed a gradual increase from upstream to downstream. There were also elevated levels of certain metals, such as Mg, Na, Ti, B, Sr, K and Ca showing a pollution gradient. The

high concentrations of metals in water samples indicate that the Ga-Selati River is heavily impacted downstream by anthropogenic activities such as illegal dumping/littering at site 6 and mining activities at site 7 to site 9. Some of the metal concentration (Na, Mg, K and Ca) in the river were found to be extremely high compared to other rivers in the region. Metal concentrations in sediment samples were very high compared to water samples River. The nutrient concentrations at the Ga-Selati River were high but did not show a pollution gradient.

The macro-invertebrate assemblages in the Ga-Selati River were rich in Ephemeroptera, Diptera and Trichoptera. Site 1 and site 2 accounted for most of the sensitive families, reflecting good water quality at these two sites, while site 9, a downstream site recorded the highest number of tolerant families.. The variations in the macro-invertebrate distribution were shown by the differences in the water quality at the various sites by the CCA plot. The effects of main pollution factors such as, EC, TDS, turbidity and nutrients were correlated with the distribution of tolerant taxa.

LIST OF TABLES

LIST OF FIGURES

Appendices

Chapter 1: Introduction

1.1 Introduction

Freshwater ecosystems cover only a small portion of the earth's surface (Downing *et al.* 2006) but provide very important ecosystem services and are essential for freshwater biota, many terrestrial animals and most human settlements (Allan & Flecker 1993, Davies & Day 1998). They support a high diversity of organisms such as fish, invertebrates, plants and algae (Malmqvist & Rundle 2002). Over the past century, the quality of surface water has deteriorated in many systems worldwide (Mattikalli & Richards 1996) due to growing human pressures (Jun *et al*. 2016). Factors such as habitat loss and degradation, species invasion, overharvesting, and chemical and organic pollution have been identified as threats to freshwater biodiversity, and a combination of these factors often leads to a loss of species (Turpie 2004, Dudgeon *et al.* 2006, Strayer & Dudgeon 2010, Vörösmarty *et al*. 2010).

South Africa has a broad range of aquatic ecosystems adapted to different water quality regimes and flow patterns (Department of Water Affairs and Forestry (DWAF) 1996, Goetsch & Palmer 1997). Many South African rivers have been deteriorating over the past decades, due to an increase in mining, industrial, agricultural and domestic activities releases into freshwater bodies (Ashton & Dabrowski 2011, Jooste *et al.* 2015). The Selati River, a tributary of the Olifants River, is one of the rivers currently experiencing a large amount of anthropogenic pressure (Gerber *et al*. 2015). This river is a primary source of water for many activities (e.g. agricultural, mining and domestic activities), and as a result, large numbers of reservoirs, farm dams, and canals have been constructed to increase the reliability of water supply along this river. Acid mine drainage seeping from abandoned mines and smouldering mine dumps in the Upper Olifants River is resulting in the acidification of streams and the mobilisation of metals from the sediment (McCarthy, 2011, Netshitungulwana and Yibas, 2012).Contamination of surface waters by agricultural pesticides and fertilizers, as well as by industrial metals, is a cause of increasing public concern (Gerenfes Denussa 2017, Javed & Usmani 2017).

The effects of anthropogenic activities on water and sediment quality of rivers can be determined using biological monitoring methods. Biological monitoring uses living organisms to assess ecological degradation at an environment (Bredenhand 2005). It is one of the most widely used tools to assess water quality, since it provides details about the previous and present conditions in the water. Organisms present in the river have survived chemical conditions which the river has been exposed to in the past (Resh *et al.* 1996, Davies & Day 1998) and therefore can be used to determine previous and present disturbance/alteration in the river. A bio-monitoring method for aquatic macro-invertebrates is commonly used to assess water quality of rivers throughout South Africa (Matlou *et al.* 2017, Niba & Sakwe 2018). Macro-invertebrates are widespread and sensitive to environmental changes and they are widely used for assessment of freshwater resources (Jun *et al*. 2016), and have been presented as the most reliable of all the bio-indicators used (Bredenhand & Samways 2009) and are currently used as a warning system for detecting water pollution by industry and water management agencies.

1.2 Literature review

1.2.1 South African Rivers and their pollution

The water quality of almost all of South Africa's river systems is considered to be declining progressively due to an increase in urbanisation and industrialisation (Ashton 2010). Pollution from industry, urbanization, mining, and agriculture has led to a decline in water quality in almost all of the South Africa's rivers (Ashton 2007). River systems are the primary source of water for these activities and supply more than 85% of all the water that is used in South Africa (Ashton 2007).

The Olifants River System is considered to be one of the most degraded river systems in South Africa. The state of the Olifants River reflects the cumulative effects of slightly more than a century of ecosystem stress created by anthropogenic activities in its catchment (Oberholster *et al.* 2010). It is contaminated with metal and chemicals (Kotzè *et al.* 1999, Ballance *et al*. 2001, Jooste *et al.* 2014). This is the consequence of the large number of agricultural, industrial and mining activities in the catchment (Ballance *et al.* 2001). Pollution of the Olifants River has led to a decline in the numbers of Nile crocodiles, which are keystone species for the Olifants River (Botha *et al.* 2011), and it seems like an on-going process since no solution has been found to improve their survival in these waters (Huchzermeyer *et al.* 2017)

The Ga-Selati River provides a perennial supply of water of very low quality into the main stem of the Olifants River just before it enters the Kruger National Park (Seymore *et al.* 1994, Heath *et al.* 2010). The river originates in the Wolkberg area southeast of Tzaneen at an elevation of 840 m above sea level. From there it flows for about 140 km before converging with the Olifants River near Phalaborwa at 297 m above sea level. There are two main tributaries in its upper reaches: the Ngwabitsi and Mulati rivers (Ashton *et al.* 2001). The Ga-Selati river is heavily utilised for irrigated agriculture (Chapman 2006). A significant portion of the excessive nutrients in the Ga-Selati River is associated with agricultural activities. The Ga-Selati River supports important economic activities in the region (irrigated agriculture and ecotourism), but there is considerable abstraction, as well as pollution inputs from human settlements and mining activities. Thus, the river is subjected to many anthropogenic activities and water demand exceeds the available water supply.

The Ga-Selati River has an annual flow of only about 9 million m^3/year in the upper catchment and much of this is used to irrigate agricultural fields (Chapman 2006). Alien invasive plants, abstraction for irrigation, and wasteful irrigation technologies have been found to be the main causes of reduced stream flow in the upper catchment (King 2008). Below the upper catchment, the river is partially impounded by a series of 10 small weirs over a distance of about 20 km. These weirs mark the points where irrigation water is abstracted for large-scale commercial irrigation farms (Ashton *et al.* 2001). According to Chapman (2006), the water quality declines where villages and farmlands occur adjacent to the Ga-Selati River. Abstraction in the upper catchment affects downstream water users and limits the capacity of the river to dilute pollution inputs from large mining operations in its lower reaches.

The Ga-Selati River is notorious for poor water quality due to mining activities at the Phalaborwa mines along its banks (Dos Santos & Avenant-Oldewage 2016). The town of Phalaborwa with its associated mines and industries is the largest and most important water user in the Olifants River sub-catchment. Pollution from mining industries has a major impact on the water quality of the lower Ga-Selati River.

Phalaborwa Copper Mine, FOSKOR (a phosphate mine) and Bosveld (a fertilizer factory) are located close the river, and discharge their effluents into three tailing dams located near the river channel. Contaminants from these tailings seep into groundwater and eventually into the river, while overflows occasionally result in direct pollution inputs (Wise & Musango 2006). Around mid-January 2014, the Ga-Selati River, was contaminated by overflow from a nearby phosphate mine, which polluted the river and killed many fish species. Mine spills contain acidic waters which can alter the pH of the water body and toxic metals such as zinc (Zn), lead (Pb), mercury (Hg) and arsenic (As) (Olıas *et al.* 2004), which can have a major impact on aquatic life (Kossoff *et al.* 2014). Thus excessive pollution in the Ga-Selati River could lead to the death of large terrestrial mammals in the Selati Game Reserve and Kruger National Park, which would negatively affect tourism (Saayman *et al.* 2009). The Ga-Selati River has a significant negative impact on the water quality, macro-invertebrates and fish of the Olifants River within the Kruger National Park (Marr *et al.* 2017).

1.2.2 Water quality/pollution

Many different natural and artificial factors determine the physical and chemical properties of river water quality (Xue *et al.* 2015). The term water quality is used to describe the condition of water including its chemical, physical and biological characteristics, usually with respect to its suitability for a particular purpose i.e. agricultural, domestic, industrial, and recreational or fishing (Ashton & Dabrowski 2011, Cullis *et al.* 2018). Water quality is influenced by substances that are either dissolved or suspended in the water (Dallas & Day 2004). Aquatic ecosystems and their biota are affected by a number of physico-chemical water quality factors such as turbidity, suspended solids, temperature, pH, salinity, and the concentrations of dissolved ions, nutrients, oxygen, biocides and trace metals (Dallas & Day 2004, Cullis *et al.* 2018). Human activities affect both quality and quantity of water in aquatic ecosystems (Ekeanyanwu *et al.* 2015). This in turn affects the biota which lives in rivers, and this is often a result of sub-lethal pollution.

Water pollution is a serious problem in our rivers and it is often comes from multiple sources, such as accidental spillage of chemical wastes, discharge of industrial or sewerage effluents, agricultural drainage, and domestic wastewater (Holt 2000, Mendis *et al.* 2015, Şener *et al.* 2017). Pollution may cause water to become unsuitable for human use. There are two main types of water pollution; non-point and point source pollution. Point source pollution occurs when the pollutants are released directly into the aquatic ecosystems (Dallas & Day 2004). Non-point are those which do not enter the aquatic ecosystem directly from a source. Instead pollutants enter the water either in run-off from urban, industrial and mining areas (Davies & Day 1998, Heath & Claassen 1999). Non-point pollution is difficult to control because of the way it is discharged (Dallas & Day 2004). Agricultural land use may degrade rivers through an increase in non-point inputs of pollutants (Slaughter & Mantel 2017). The high pollutant levels in Ga-selati River could be attributed to the various riparian activities leading to increased fluxes of both point and non-point pollution for example the mining activities (Mnisi 2018).

In the aquatic environment, metals are a potentially lethal form of pollution. Metals are dangerous because they cannot be destroyed by biological degradation, they accumulate with ease in the environment, and they have adverse effects to the aquatic ecosystem. Metals occur in solution, in or on suspended particles, and in sediments, from which they are taken up by different types of organisms and transferred throughout the food webs (Adams *et al.* 2011). Associated increases of waterborne metals are of concern as they may be absorbed in excessive quantities by aquatic biota like fish and macro-invertebrates, subsequently affecting their survival and reproduction (Dabrowski 2012). The toxicity of metals to aquatic organisms strongly depends on exposure scenario and exposure time. Aquatic invertebrates take up and accumulate trace metals whether they are required for growth or not, resulting in potentially toxic effects (Adams *et al.* 2011).

The primary sources of anthropogenic metal contamination in many streams are from drainage associated with mining activities, and industrial areas (Zeitoun & Mehana 2014). Freshwater organisms absorb pollutants from their environment and food (Chen *et al*. 2000). While some metals play an important role in aquatic ecosystems, their concentrations should not exceed or fall below tolerable ranges (Van Vuren *et al.* 1999). The ionic form is usually the form that causes mortalities, as opposed to complex metal compounds (Heath & Claassen 1999). Aquatic species have tolerance limits within which they may be able to survive, and increased metal concentrations may therefore lead to reduced ecosystem functioning, reduction in diversity, loss of key species and a change in the physical position of a community of an aquatic ecosystem (Dallas & Day 2004).

Sediment is an important part of the aquatic environment, and contributes to the overall quality of the river system. It adsorbs most trace elements that are in contaminates that are discharged into aquatic systems (Zhang *et al.* 2014). Thus, in most aquatic systems, the concentrations of metals in sediment are far greater than the concentrations dissolved in the water column (Harding 2005, Islam *et al.* 2014). Furthermore, pathogens, nutrients, and organic chemicals are adsorbed onto both inorganic and organic materials that eventually settle in depositional areas (Burton 2002). Sediment is known to capture hydrophobic chemical pollutants which then enter the water slowly as they are gradually released (Islam *et al.* 2014). A good sediment quality is therefore important to maintain a healthy aquatic ecosystem, and protection of aquatic life.

Water quality can also be affected by the amount of nutrients in the water. Agricultural fertilizers are the main sources of inorganic nutrient inputs into rivers, as fertilizer which is not taken up by plants often seeps into streams and rivers. Domestic sewage and industrial wastes are other important sources of nutrient inputs. In the majority of natural ecosystems, nutrients (especially N and P) play a major role in limiting primary productivity. Excessive loading of nutrients and toxins from land use changes and point sources have led to eutrophication of many freshwater ecosystems, sometimes to the point that they can no longer support natural biotic communities (Smith 2003, Smol 2008).

1.2.3 Macro-invertebrates as bio-indicators

Biological monitoring is considered more effective than physico-chemical techniques for monitoring water quality (Warren 1971, Resh & Rosenberg 1993, Herbst & Silldorff 2006, Chessman *et al.* 2007, Gebler *et al.* 2014). Chemical surveys only identify a fraction of environmental pollutants, often those included in 'priority lists (Colin *et al*. 2016). The use of bio-indicators enables assessing the overall effects of measured and unmeasured pollutants interacting with natural and other anthropogenic stressors in the complexity of natural systems (Birk *et al*. 2012) The composition of aquatic communities can indicate overall catchment conditions (Fausch *et al.* 1990), and is widely used to monitor biological integrity and overall aquatic ecosystem health (Beatty & Morgan 2010). The success of aquatic biological monitoring depends on the ability to identify and sample indicator species whose presence or absence reflects stresses in the aquatic environment (Resh & Rosenburg 1993, Davies & Day 1998). The use of bio-indicators has been traditionally used in studies of bio-monitoring, including environmental risk assessment (Friberg *et al*. 2011). The most frequently used aquatic organisms in river bio-monitoring are macro-invertebrates (Dabrowski *et al.* 2015), fish and periphyton (Li *et al.* 2010).

Aquatic organisms serve as continuous monitors of water quality because organisms present in the water have survived sporadic disturbance and pollution which the river has been exposed to in the past (Resh *et al.* 1996, Davies & Day 1998, Stein *et al.* 2008). Many aquatic macro-invertebrates spend more than half their lifetime in water (Chapman 1998), and if exposed to any type of disturbance or pollution during the early stages of development (i.e. larval or pupal stage of its life cycle), then changes in community structure will occur (Relyea *et al.* 2000).

Aquatic macro-invertebrates are valuable organisms for bio-assessment because they are ubiquitous (Resh *et al.* 1996, Roy *et al.* 2001), visible to the naked eye, easy to identify, have rapid life cycles and usually have sedentary habitats (Dickens & Graham 2002). Their life-cycle duration makes them good indicators of short to medium term impacts on water quality and their habitat (Thirion 2007). A healthy water body will contain an abundance of invertebrate taxa that are not tolerant to environmental stress, such as the mayflies, stoneflies and caddisflies (Bouchard

2004). The absence of these groups is considered an indication of pollution (Beasley & Kneale 2002).

Aquatic Macro-invertebrates are widely used as bio-indicators in many countries (Dickens & Graham 2002, Chon *et al.* 2012, Uherek & Pinto Gouveia 2014). Bio-assessments are easy to use and inexpensive, and can provide a measure of water quality variation over a time (Chutter 1994, Dickens & Graham 2002, Du *et al.* 2017). Different macro-invertebrates families have different tolerance scores (from 1-15) based on sensitivity to poor water quality (Dallas & Fowler 2000). The higher the tolerance scores the greater the invertebrate taxa's sensitivity to pollution.

The abundance of macro-invertebrate taxa is also affected by availability of the aquatic habitat it is adapted to (e.g. stones in current, marginal vegetation, sandy pools), its historical distribution of families (i.e. it must be geographically possible for a taxa to become established in a particular system) and local biotic factors (the ocurrence of parasites, predators, food supply, etc) (Davies & Day 1998, Dallas & Day 2004). While every species has its own tolerance range, species within the same family, or even order, tend to have a similar range of tolerance to environmental disturbance. However, the higher the diversity of sensitive macro-invertebrates, the more likely the water they are living in is of good quality and unaffected by pollution (Chakravorty *et al.* 2014, Xu *et al.* 2014).

1.2.4. Aim and objectives

The aim of the study was to correlate the occurrence and abundance of macro-invertebrates families from a selected biotope to the water and sediment quality of the Ga-Selati River.

The objectives of this study were to:

i. Establish the current physico-chemical composition of the river water and sediment along the entire length of the Ga-Selati River.
ii. To determine the concentrations of metals in water and sediment along the river.
iii. Assess the impact of water and sediment quality on the aquatic macro-invertebrate assemblages in the river.

1.3 Significance of proposed research

The Ga-Selati River is highly impacted by human disturbances such as agricultural activities; sewage effluent and runoff from mines, and these factors are likely to have altered aquatic ecosystem and macro-invertebrate communities within the river. However, aquatic invertebrate diversity in the river has not been studied recently, despite a number of severe impacts in recent years, including a major spill of acidic waste water into the lower reaches. It is imperative that the ecological state of the river is assessed, to determine the degree of degradation that has occurred over recent decades, and to implement a better management policies in the catchement.

1.4 References

ADAMS, W.J., BLUST, R., BORGMANN, U., BRIX, K.V., DEFOREST, D.K., GREEN, A.S., MEYER, J.S., MCGEER, J.C., PAQUIN, P.R., RAINBOW, P.S. & WOOD, C.M. 2011. Utility of tissue residues for predicting effects of metals on aquatic organisms. *Integrated Environmental Assessment and Management* **7**: 75-98.

ALLAN, J.D. & FLECKER, A.S. 1993. Biodiversity conservation in running waters. *BioScience* **43**: 32-43.

ALLAN, J.D. 2004. Landscapes and riverscapes: The influence of land use on stream ecosystems. *Annu. Rev. Ecol. Evol. Syst.* **35**: 257-284.

ASHTON, P.J., LOVE, D., MAHACHI, H. & DIRKS, P. 2001. An overview of the impact of mining and mineral processing operations on water resources and water quality in the Zambezi, Limpopo and Olifants Catchments in Southern Africa. *Contract Report to the Mining, Minerals and Sustainable Development (Southern Africa) Project, by CSIR-Environmentek, Pretoria and Geology Department, University of Zimbabwe-Harare. Report No. ENV-PC* **42.**

ASHTON, P.J. 2007. Riverine biodiversity conservation in South Africa: current situation and future prospects. *Aquatic Conservation: Marine and Freshwater Ecosystems* **17**: 441-445.

ASHTON, P.J. 2010. The demise of the Nile crocodile (*Crocodylus niloticus*) as a keystone species for aquatic ecosystem conservation in South Africa: The case of the Olifants River. *Aquatic Conservation: Marine and Freshwater Ecosystems* **20**: 489-493.

ASHTON, P.J & DABROWSKI, J.M. 2011. An overview of water quality and the causes of poor water quality in the Olifants River Catchment. WRC Project No. K8/887. *Water Research Commission, Pretoria*. 189.

BALLANCE, A., HILL, L., ROUX, D., SILBERBAUER, M. & STRYDOM, W. 2001. State of the rivers report: Crocodile, Sabie-Sand and Olifants river systems. *Resource Quality Services, DWAF, Pretoria, South Africa (www. csir. co. za/rhp)*.

BEASLEY, G. & KNEALE, P. 2002. Reviewing the impact of metals and PAHs on macro-invertebrates in urban watercourses. *Progress in Physical Geography* **26**: 236-270.

BEATTY, S. & MORGAN, D. 2010. Teleosts, agnathans and macroinvertebrates as bioindicators of ecological health in a south-western Australian river. *Journal of the Royal Society of Western Australia* **93**: 65-79.

BOTHA, H., VAN HOVEN, W. & GUILETTE, L.J. 2011. The decline of the Nile crocodile population in Loskop Dam, Olifants River, South Africa. *Water SA* **37**: 103-108.

BOUCHARD, W. 2004. Guide to aquatic macro invertebrates of the Upper Midwest Water Resources Center. *University of Minnesota*: 159-183.

BREDENHAND, E. 2005. *Evalaution of macroinvertebrates as bioindicators of water quality and the assessment of the impact of the Kleinplaas dam on the Eerste River*. MSc. Thesis, University of Stellenbosch, South Africa.

BREDENHAND, E. & SAMWAYS, M.J. 2009. Impact of a dam on benthic macro-invertebrates in a small river in a biodiversity hotspot: Cape Floristic Region, South Africa. *Journal of Insect Conservation* **13**: 297–307.

BURTON, G.A. 2002. Sediment quality criteria in use around the world. *The Japanese Society of Limnology* **3**: 65-75.

CHAKRAVORTY, P.P., SINHA, M. & CHAKRABORTY, S. 2014. Impact of industrial effluent on water quality and benthic macro invertebrate diversity in fresh water ponds in Midnapore district of west Bengal, India. *India. J. Entomology and Zoology Studies* **2**: 93-101.

CHAPMAN, R.F. 1998. *The insects: structure and function*. Cambridge university press.pp 929

CHAPMAN, A. 2006. Hydrology and land use in the Ga-Selati catchment. CSIR, Pretoria, South Africa and International Institute for Environment and Development, London, UK. pp 28

CHEN, C.Y., STEMBERGER, R.S., KLAUE, B., BLUM, J.D., PICKHARDT, P.C. & FOLT, C.L. 2000. Accumulation of heavy metals in food web components across a gradient of lakes. *Limnology and Oceanography* **45**: 1525-1536.

CHESSMAN, B., WILLIAMS, S. & BESLEY, C. 2007. Bioassessment of streams with macroinvertebrates: effect of sampled habitat and taxonomic resolution. *Journal of the North American Benthological Society* **26**: 546-565.

CHON, H.S., OHANDJA, D.G. & VOULVOULIS, N. 2012. The role of sediments as a source of metals in river catchments. *Chemosphere* **88**: 1250-1256.

CHUTTER, F.M. 1994. The rapid biological assessment of streams and rivers water quality by means of macro-invertebrate community in South Africa. Progress Report to the water research commission, South Africa, pp. 7-11

CULLIS, J.D., ROSSOUW, N., DU TOIT, G., PETRIE, D., WOLFAARDT, G., CLERCQ, W.D. & HORN, A. 2018. Economic risks due to declining water quality in the Breede River catchment. *Water SA* **44**: 464-473.

DABROWSKI, J. 2012. *Water quality, metal bioaccumulation and parasite communities of Oreochromis mossambicus in Loskop Dam, Mpumalanga, South Africa*. MSc dissertation, University of Pretoria, Pretoria.

DABROWSKI, J.M., DABROWSKI, J., HILL, L., MACMILLAN, P. & OBERHOLSTER, P.J. 2015. Fate, transport and effects of pollutants originating from acid mine drainage in the Olifants River, South Africa. *River Research and Applications* **31**: 1354-1364.

DALLAS, H.F. & DAY, J.A. 2004. *The effect of water quality variables on aquatic ecosystems:* Water Research Commission, Pretoria, South Africa. WRC Report No. TT224/04. pp 222

DALLAS, H.F & FOWLER, J. 2000. *Delineation for river types of Mpumalanga, South Africa: the establishment of a spatial framework for the selection of reference sites*. National Biomonitoring Programme for Riverine Ecosystems: Report Series No 9. Institute for Water quality Studies, Department of Water Affairs and Forestry, Pretoria.

DAVIES, B.R. & DAY, J.A. 1998. *Vanishing waters*. University of Cape Town Press, South Africa. pp 487.

.

DICKENS, C.W. & GRAHAM, P. 2002. The South African Scoring System (SASS) version 5 rapid bioassessment method for rivers. *African Journal of Aquatic Science* **27**: 1-10.

DOS SANTOS, Q.M. & AVENANT-OLDEWAGE, A. 2016. The description of a new diplozoid species, *Paradiplozoon krugerense* n. sp., from *Labeo rosae* Steindachner, 1894 and *Labeo congoro* Peters, 1852 in the Kruger National Park, South Africa with notes on the effect of water quality on its infection variables. *Hydrobiologia* **777**: 225-241.

DOWNING, J., PRAIRIE, Y., COLE, J., DUARTE, C., TRANVIK, L., STRIEGL, R., MCDOWELL, W., KORTELAINEN, P., CARACO, N. & MELACK, J. 2006. The global abundance and size distribution of lakes, ponds, and impoundments. *Limnology and Oceanography* **51**: 2388-2397.

DU, L.N., JIANG, Y.-E., CHEN, X.Y., YANG, J.X. & ALDRIDGE, D. 2017. A family-level macroinvertebrate biotic index for ecological assessment of lakes in Yunnan, China. *Water resources* **44**: 864-874.

DUDGEON, D., ARTHINGTON, A.H., GESSNER, M.O., KAWABATA, Z.I., KNOWLER, D.J., LÉVÊQUE, C., NAIMAN, R.J., PRIEUR-RICHARD, A.H., SOTO, D., STIASSNY, M.L.J. & SULLIVAN, C.A. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* **81**: 163-182.

DWAF (Department of Water Affairs and Forestry). 1996. South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems, Second Edition. Pretoria, South Africa.

EKEANYANWU, R., NWOKEDI, C. & NOAH, U. 2015. Monitoring of metals in Tilapia nilotica tissues, bottom sediments and water from Nworie River and Oguta Lake in Imo State, Nigeria. *African Journal of Environmental Science and Technology* **9**: 682-690.

FAUSCH, K.D., J. LYONS, J.R. KARR, & ANGERMEIER P.L. 1990. Fish communities as indicators of environmental degradation. In Biological indicators of stress in fish. American Fisheries **8**: 123-144.

FRIBERG, N., BONADA, N., BRADLEY, D.C., DUNBAR, M.J., EDWARDS, F.K., GREY, J., HAYES, R.B., HILDREW, A.G., LAMOUROUX, N. & TRIMMER, M. 2011. Biomonitoring of human impacts in freshwater ecosystems: the good, the bad and the ugly. in *Advances in ecological research*. Elsevier, pp. 1-68.

GEBLER, D., KAYZER, D., SZOSZKIEWICZ, K. & BUDKA, A. 2014. Artificial neural network modelling of macrophyte indices based on physico-chemical characteristics of water. *Hydrobiologia* **737**: 215-224.

GERBER, R., WEPENER, V. & SMIT, N. 2015. Application of multivariate statistics and toxicity indices to evaluate the water quality suitability for fish of three rivers in the Kruger National Park, South Africa. *African journal of aquatic science* **40**: 247-259.

GERENFES DENUSSA, D. 2017. *Levels Of Selected Heavy Metals In Water And Fish Samples From Abaya And Chamo Rift Valley Lakes*. PhD dissertation, Haramaya University.

GOETSCH, P.A. & PALMER, C. 1997. Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. *Archives of Environmental Contamination and Toxicology* **32**: 32-41.

HARDING, J.S. 2005. Impacts of metals and mining on stream communities. in MOORE, T.A., BLACK, A., CENTENO, J.A., HARDING, J.S. & TRUMM, D.A. (eds), *Metal contaminants in New Zealand.* resolutionz press, Christchurch, NZ, pp. 343-357.

HEATH, R.G.M. & CLAASSEN, M.C. 1999. An Overview of the Pesticide and Metal Levels Present in Populations of the Larger Indigenous Fish Species of Selected South African Rivers. Report to the Water Research Commission, Pretoria. WRC Report No. 428/1/99.pp 318.

HEATH, R., COLEMAN, T. & ENGELBRECHT, J. 2010. *Ecology of the Olifants River: A WRC-funded study investigated the history of water and environmental quality of the Olifants River*. Water Research Commission, Pretoria, South Africa. WRC Report No.TT452/10

HERBST, D.B. & SILLDORFF, E.L. 2006. Comparison of the performance of different bioassessment methods: similar evaluations of biotic integrity from separate programs and procedures. *Journal of the North American Benthological Society* **25**: 513-530.

HOLT, M. S. 2000. Sources of chemical contaminants and routes into the freshwater environment. *Food and chemical toxicology* **38**: S21-S27.

HUCHZERMEYER, K.D.A., WOODBORNE, S., OSTHOFF, G., HUGO, A., HOFFMAN, A., KAISER, H., STEYL, J.C.A. & MYBURGH, J.G. 2017. Pansteatitis in polluted Olifants River impoundments: nutritional perspectives on fish in a eutrophic lake, Lake Loskop, South Africa. *Journal of fish diseases* **40**: 1665-1680.

ISLAM, M.S., HAN, S., AHMED, M.K. & MASUNAGA, S. 2014. Assessment of trace metal contamination in water and sediment of some rivers in Bangladesh. *Journal of Water and Environment Technology* **12**: 109-121.

JAVED, M. & USMANI, N. 2017. An overview of the adverse effects of heavy metal contamination on fish health. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*: 1-15.

JOOSTE, A., MARR, S.M., ADDO-BEDIAKO, A. & LUUS-POWELL, W.J. 2014. Metal bioaccumulation in the fish of the Olifants River, Limpopo province, South Africa, and the associated human health risk: A case study of rednose *labeo Labeo rosae* from two impoundments. *African Journal of Aquatic Science* **39**: 271-277.

JOOSTE, A., MARR, S.M., ADDO-BEDIAKO, A. & LUUS-POWELL, W.J. 2015. Sharptooth catfish shows its metal: a case study of metal contamination at two impoundments in the Olifants River, Limpopo river system, South Africa. *Ecotoxicology and Environmental Safety* **112**: 96-104.

JUN, Y.C., KIM, N.Y., KIM, S.H., PARK, Y.S., KONG, D.S. & HWANG, S.J. 2016. Spatial distribution of benthic macroinvertebrate assemblages in relation to environmental variables in Korean nationwide streams. *Water* **8**: 27.

KING, N., BOND, N.K.W., WISE, R. & BOND, I. 2008. Fair deals for watershed services in South Africa. IIED.

KOSSOFF, D., DUBBIN, W., ALFREDSSON, M., EDWARDS, S., MACKLIN, M. & HUDSON-EDWARDS, K.A. 2014. Mine tailings dams: characteristics, failure, environmental impacts, and remediation. *Applied Geochemistry* **51**: 229-245.

KOTZÈ, P., DU PREEZ, H. & VAN VUREN, J. 1999. Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepinus*, from the Olifants River, Mpumalanga, South Africa. *Water SA.* **25**: 99-110.

LI, L., ZHENG, B. & LIU, L. 2010. Biomonitoring and bioindicators used for river ecosystems: definitions, approaches and trends. *Procedia environmental sciences* **2**: 1510-1524.

MALMQVIST, B. & RUNDLE, S. 2002. Threats to the running water ecosystems of the world. *Environmental conservation* **29**: 134-153.

MNISI, L.N. 2018. *Development of an aquatic toxicity index for macroinvertebrates.* PhD dissertation, University of Witwatersrand, South Africa.

MARR, S., MOHLALA, T. & SWEMMER, A. 2017. The ecological integrity of the lower Olifants River, Limpopo province, South Africa: 2009–2015–Part B: Tributaries of the Olifants River. *African Journal of Aquatic Science* **42**: 181-190.

MATLOU, K., ADDO-BEDIAKO, A. & JOOSTE, A. 2017. Benthic macroinvertebrate assemblage along a pollution gradient in the Steelpoort River, Olifants River System. *African Entomology* **25**: 445-453.

MATTIKALLI, N.M. & RICHARDS, K.S. 1996. Estimation of surface water quality changes in response to land use change: application of the export coefficient model using remote sensing and geographical information system. *Journal of Environmental Management* **48**: 263-282.

MCCARTHY, T.S. 2011. The impact of acid mine drainage in South Africa. *South African Journal of Science* **107**: 01-07.

MENDIS, B., NAJIM, M., KITHSIRI, H. & AZMY, S. 2015. Bioaccumulation of heavy metals in the selected commercially important edible fish species gray mullet (*Mugil cephalus*) from Negombo estuary. Journal of Environmental Professionals Sri Lanka **4**: 1–9

NETSHITUNGULWANA R. & YIBAS B., 2012. Stream sediment geochemistry of the Olifants catchment, South Africa: Implication for acid mine drainage. In: McCullough C.D., Lund M.A. and Wyse L. (eds.), Proceedings of the International Mine Water Association Symposium, International Mine Water Association, 257–264.

OBERHOLSTER, P.J., MYBURGH, J.G., ASHTON, P.J. & BOTHA, A.M. 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.

OLıAS, M., NIETO, J., SARMIENTO, A., CERÓN, J. & CÁNOVAS, C. 2004. Seasonal water quality variations in a river affected by acid mine drainage: the Odiel River (South West Spain). *Science of the total environment* **333**: 267-281.

RELYEA, C.D., MINSHALL, G.W. & DANEHY, R.J. 2000. Stream insects as bioindicators of fine sediment. *Proceedings of the Water Environment Federation.* 663-686.

RESH, H. & ROSENBURG, D.M. 1993. Introduction to freshwater bio-monitoring and benthic macro-invertebrates. Chapman & Hall, New York.

RESH, V.H., MYERS, M.J. & HANNAFORD, M.J. 1996. Macroinvertebrates as biotic indicators of environmental quality. *Methods in stream ecology* **99**: 647-667.

ROY, A., ROSEMOND, A.D., LEIGH, D.S., PAUL, M.J. & WALLACE, J.B. 2001. Effects of changing land use on macroinvertebrate integrity: identifying indicators of water quality impairment. 229-232.

SAAYMAN, M., SAAYMAN, A. & FERREIRA, M. 2009. The socio-economic impact of the Karoo National Park. *Koedoe*, **51**:1-9.

ŞENER, Ş., ŞENER, E. & DAVRAZ, A. 2017. Evaluation of water quality using water quality index (WQI) method and GIS in Aksu River (SW-Turkey). *Science of the Total Environment* **584**: 131-144.

SEYMORE, T., DU PREEZ, H., VAN VUREN, J., DEACON, A. & STRYDOM, G. 1994. Variations in selected water quality variables and metal concentrations in the sediment of the lower Olifants and Selati Rivers, South Africa. *Koedoe* **37**: 1-18.

SLAUGHTER, A. & MANTEL, S. 2017. Land cover models to predict non-point nutrient inputs for selected biomes in South Africa. *Water SA* **43**: 499-508.

SMITH, V.H. 2003. Eutrophication of freshwater and coastal marine ecosystems a global problem. *Environmental Science and Pollution Research* **10**: 126-139.

SMOL, J.P. 2008. Pollution of lakes and rivers: a paleoecological perspective. 2nd edition. Blackwell, Malden, Massachusetts.

STEIN, H., SPRINGER, M. & KOHLMANN, B. 2008. Comparison of two sampling methods for biomonitoring using aquatic macroinvertebrates in the Dos Novillos River, Costa Rica. *Ecological Engineering* **34**: 267-275.

STRAYER, D.L. & DUDGEON, D. 2010. Freshwater biodiversity conservation: recent progress and future challenges. *Journal of the North American Benthological Society* **29**: 344-358.

THIRION C. 2007. Module E: Macroinvertebrate Response Assessment Index in River EcoClassification: Manual for EcoStatus Determination (Version 2). Joint Water Research Commission and Department of Water Affairs and Forestry report. WRC Report No. TT332/08. Water Research Commission, Pretoria

TURPIE, J. 2004. Ecosystem services supplied by the Maloti-Drakensberg Bioregion. *Report to FutureWorks for the Maloti Drakensberg Tranfrontier Conservation and Development Programme*.

UHEREK, C.B. & PINTO GOUVEIA, F.B. 2014. Biological monitoring using macroinvertebrates as bioindicators of water quality of Maroaga Stream in the Maroaga Cave System, Presidente Figueiredo, Amazon, Brazil. *International Journal of Ecology:* 1-7

VAN VUREN, J.H.J., KOTZÈ, P. & DU PREEZ, H.H. 1999. Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepinus*, from the Olifants River, Mpumalanga, South Africa. *Water SA* **25**(1): 99-110.

VISSER, Z. 2010. *The use of biomarker responses to assess pesticide exposure in the Crocodile-and Olifants River systems*. Unpublished MSc Thesis, Department of Science, University of Johannesburg, Johannesburg.

VÖRÖSMARTY, C.J., MCINTYRE, P., GESSNER, M.O., DUDGEON, D., PRUSEVICH, A., GREEN, P., GLIDDEN, S., BUNN, S.E., SULLIVAN, C.A. & LIERMANN, C.R. 2010. Global threats to human water security and river biodiversity. *Nature* **467**: 555-561.

WARREN, C.E. 1971. Biology and water pollution control. W.B. Saunders Co., Philadelphia.pp 434

WISE, R.M. & MUSANGO, J.K. 2006. A framework for decision-making using a cost-effectiveness approach: a case study of the Ga-Selati River. *Council for Scientific and Industrial Research, Pretoria and IIED, London*.pp 28.

XU, M., WANG, Z., DUAN, X. & PAN, B. 2014. Effects of pollution on macroinvertebrates and water quality bio-assessment. *Hydrobiologia* **729**: 247-259

XUE, C.H., YIN, H.L. & MING, X. 2015. Development of integrated catchment and water quality model for urban rivers. *Journal of Hydrodynamics, Ser. B* **27**: 593-603.

ZEITOUN, M.M. & MEHANA, E. 2014. Impact of water pollution with heavy metals on fish health: overview and updates. *Global Veterinaria* **12**: 219-231.

ZHANG, C., YU, Z.G., ZENG, G.M., JIANG, M., YANG, Z.Z., CUI, F., ZHU, M.Y., SHEN, L.Q. & HU, L. 2014. Effects of sediment geochemical properties on heavy metal bioavailability. *Environment international* **73**: 270-281.

.

Chapter 2: Water and sediment quality

2.1: Introduction

The term water quality is used to describe the physical, chemical, biological and aesthetic properties of water that determine its fitness for a variety of uses and for the protection of the health and integrity of aquatic ecosystems or as the physical and chemical requirements needed for water to meet a particular use (DWAF 1996). However, it is not simple to classify water as good or poor quality without the knowledge of its intended use. For example, the quality of water that is required for drinking is not of the same quality as the water required for irrigation. The Department of Water Affairs is responsible for water quality in South Africa, in terms of Act 108 of 1996 of the Constitution of the Republic of South Africa. It has developed a series of South African Water Quality Guidelines (SAWQG). These guidelines are the primary source of information and decision to support and judge the water quality for human use and other water quality management purposes.

Changes in the physical, chemical and biological composition of a water body would alter the water quality of the river and its biota (Dallas & Fowler 2000). Water quality is one of the most important factors which influence aquatic ecosystem integrity, as the abundance of freshwater organisms is controlled mainly by water quality characteristics, including pH, DO, temperature, TDS, conductivity, salinity and nutrient content (Dallas and Day 1993).

The physico-chemical parameters of aquatic ecosystems go through changes over a 24-hour period, seasonally and over years (in response to climatic fluctuations). The physico-chemical refers to a physical characteristic of water, and derives from a single or combination of chemical constituent(s) (DWAF 1996). Physico-chemical parameters include temperature, pH, dissolved oxygen (DO), total dissolved solids (TDS), electrical conductivity (EC), salinity and turbidity. The Target Water Quality Range (TWQR) of the Department of Water Affairs and Forestry (DWAF) now called the Department of Water Affairs and Sanitation (DWAS) (DWAF 1996), is the range of concentrations below which no measurable, adverse effects are expected on the health of aquatic ecosystems, and which should therefore ensure their protection (DWAF 1996).

In aquatic systems, pH plays an important role in physico-chemical and biological processes. The pH value is a measure of the hydrogen ion activity in a water sample. A lower value indicates a more acidic condition and a higher value more alkaline (DWAF 1996). The pH determines the chemical activity, and thus the availability and the potential toxicity of many heavy metals and other substances (Allard & Moreau 1987, Davies & Day 1998). Metals (e.g. silver (Ag), Al, cadmium (Cd), cobalt (Co), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn)) generally have greater detrimental environmental effects at a lower pH (DWAF 1996, Dallas & Day 2004). For instance, Al is highly toxic only in very acid waters where the low pH results in the formation of the toxic aquo-Al^{3+} ion (Davies & Day 1998). Al^{3+} is more toxic to many freshwater organisms than Al^{2+} (Crowder 1991, Cardiano *et al.* 2018). Any change in pH levels is seldom lethal to aquatic organisms, but can cause other adverse effects such as reduction in ionic balance (DWAF 1996). The majority of South African fresh waters are fairly well buffered and more or less neutral, with pH ranges between 6 and 8 (Dallas & Day 2004). The preferred pH range for natural waters in South Africa is 6.5 to 9 (Davies & Day 1998).

Electrical conductivity is simply the relative amount of electricity that can be conducted by water (Dodds 2002). Conductivity occurs because of the presence of ions such as carbonate ($CO^{2-}{}_{3}$), bicarbonate (HCO_3-), chloride (Cl-), sulphate (SO_4), nitrate (NO_3-), sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) in water, all of which carry an electrical charge (DWAF 1996). The total concentration of ions in water in relation to the temperature determines the conductivity, and thus EC provides an indirect measurement of dissolved solids (Polling 1999). TDS concentration in water can be approximated from EC using the formula: TDS (mg/ℓ) = EC (mS/cm) x 6.5 (Kempster & Van Vliet 1991)

The solubility of oxygen in water varies with chemical and physical factors, and atmospheric pressure (DWAF 1996). The concentration of DO varies at different times of the day due to processes like photosynthesis, respiration and changes in temperature (when temperature increases, dissolved oxygen decreases; Dallas & Day 2004). When dissolved oxygen is low, aquatic biota is negatively affected and may die (DWAF 1996).

Water temperature is an important abiotic driver of aquatic ecosystems. Temperature plays an essential role in water by affecting the rates of chemical reactions of organisms (Dallas & Rivers-Moore 2012), and changes in water temperature have profound effect on an aquatic organism's growth, phenology, survival and distribution (Hawkins *et al.* 1997, Mccauley *et al.* 2015), and may lead to changes in the abundance, diversity and composition of aquatic communities (Dallas & Day 2004). An increase in water temperature will lower the amount of oxygen that can remain dissolved in the water body (Davies & Day 1998). Therefore, warm water can be a limiting factor to aquatic life, due to lowered oxygen levels. Factors that can alter the water temperature include discharges of heated industrial effluent, heated return flows, irrigation water, the removal of riparian vegetation cover and inter-basin water transfers (DWAF1996).

Turbidity is the loss of water transparency that results from the scattering of light by suspended materials. Turbidity provides quantitative information as to the state of water quality (Zhang *et al.* 2003). The water turbidity is generally considered to be equivalent to some measure of the concentration of suspended solids (Wu *et al.* 2014). Total suspended solids is a measure of the quantity of material in suspension in a given water body. Suspended solids include tiny particles of silt and clay, living organisms and dead particulate organic matter (Davies & Day 1998). The concentration of suspended solids increases with the discharge of sediment washed into rivers (DWAF 1996).

Nutrients are any element required by organisms for growth (Dodds 2002). Nutrients include all major inorganic nitrogen compounds (i.e. ammonia (NH_3), ammonium (NH_4+), NO_3- and nitrite (NO_2−)) and phosphorus (P). Nitrogen (N) and P are essential macro-nutrients and are required by all living organisms (DWAF 1996) and are also essential constituents of DNA and protein (Dallas and Day 2004). There has been a substantial increase in nutrient concentrations throughout the world. For example, fewer than 10% of rivers globally can be classified as pristine in terms of their NO_3- status, as defined by World Health Organisation (i.e. < 0.1 mg/l) (Malmqvist & Rundle 2002). Although nutrients are generally not toxic, they can harm aquatic organism if present in excess (Dallas and Day 2004). Nutrient enrichment is in fact one of the major threats to aquatic ecosystems, and can lead to severe pollution problems (i.e. eutrophication). Eutrophication is the enrichment nutrients in the water body, typically compounds containing N, P, or both. Eutrophication has become the primary water quality issue for many of the freshwater ecosystem. Potential effects of eutrophication, caused by excessive inputs of P and N, are decreases in water transparency, reductions in species diversity, taste, odour and drinking water treatment problems (Smith & Schindler 2009).

Many trace metals are important in plant and animal nutrition and they also play an essential role in tissue metabolism and growth (Stankovic *et al.* 2014). Heavy metals are chemical elements with five or more specific gravity. Heavy metals include Mn, Fe, Cu, Zn, Mo, Cd, Hg and Pb. Some heavy metals are required as trace elements by living organisms, while others like Cd, Pb and Hg are not (Moss 2009). Severe imbalances of these metals can cause death, whereas marginal imbalance contributes to poor health and retarded growth (Rand & Petrocelli 1985, Prashanth *et al.* 2015).

2.1.1: Background of the river

The Ga-Selati River flows through agriculture areas, game farms and eventually industrial and mining areas before joining the Olifants River near Phalaborwa town. The dominant land-uses in the Selati catchment area are game ranching; dry land agriculture and rangelands; mining; urban settlements in villages and towns; and conservation, both within public parks (specifically the Kruger National Park) and private game reserves, such as the Selati Game Reserve (Pollard & Laporte, 2015) After the inflow of the seepage and effluent from Phalaborwa into the Ga-Selati River, the concentrations of a few constituents increase. Concentrations of phosphate (P), TDS (Van Der Merwe 1992), EC, and sulphate (SO_4^-) (Heath *et al.* 2010) were found to be high at the Ga-Selati River due to the significant mining impact on the river water quality. The water quality at the Ga-Selati River is also further impaired by agricultural return flows and other effluent discharges upstream. There is a marked deterioration in water quality in the Phalaborwa Barrage, when mixed with the water from Ga-Selati River (DWAF 2005). Sewage treatment plants in Lulekani, Namakgale and Phalaborwa discharge over two million cubic metres treated effluent into the Ga-Selati River every year (Marx 1996). As a result it introduces nitrates (NO_3^-) and PO_4^{3-}, dissolved salts, NH_3 and suspended solids into the river (Van Veelen 1990). There is an increase in NO_3^- levels in the Olifants River after the confluence with the Ga-Selati River. Thus, high NO_3^- levels received from the Ga-Selati River (Heath *et al.* 2010).

2.2 Method and materials

2.2.1 Sampled Sites

Sampling was conducted seasonally at nine sites spread along the entire length of the Ga-Selati River, from headwater to the lower reaches (site1–site 3=upper reaches, site 4-site 6=middle reaches and site 7-site 9=lower reaches) (Figure 2.2.1). These sites were selected to represent the major land-use activities in the river catchment. (Table 2.2.1)

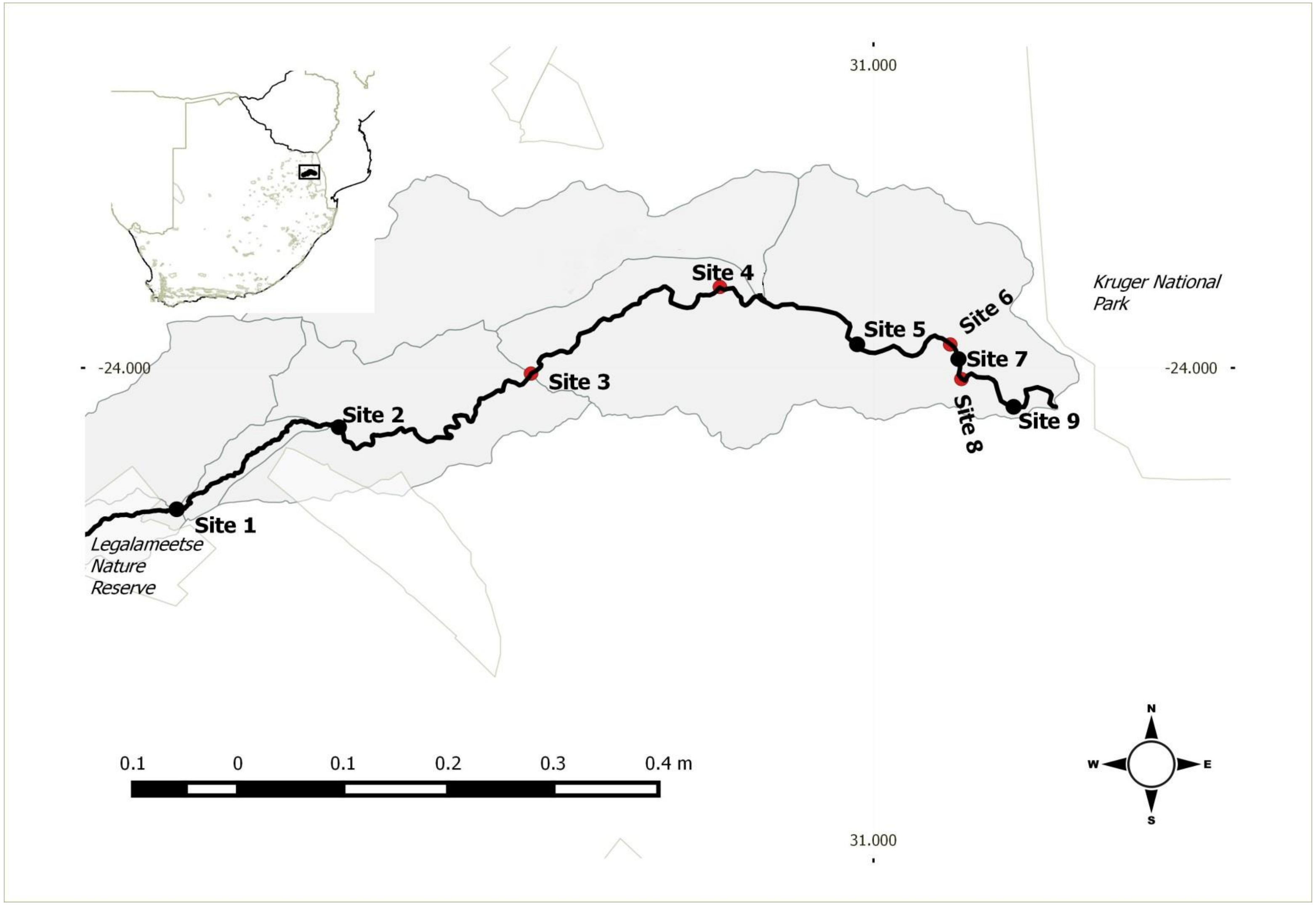


Figure 2.2.1: The Ga-Selati River showing all the sampling sites. Red dots denote sites and black dots denote sites where macro-invertebrates were sampled.

Table 2.2.1: Site numbers, coordinates and descriptions

Site number	Site name	Site coordinates	Site Description	
1	Dindinie	24°8'30.81"S 30°18'11.63"E	Near headwaters, 310 m downstream of the Legalametse Nature Reserve. Characterised by clear, fast-flowing, nutrient-poor water, with medium-sized boulders, riffles and with very little loose soil. Riparian vegetation formed a canopy over the stream with reduced light penetration. Little impact of human activities, although grazing livestock were observed.	Upper reaches
2	Harmonie	24°3'28.55"S 30°29'41.96"E	Situated 1.07 km below Harmonie Dam and is surrounded by intensive agriculture. This site is characterised by a number of disturbances such as littering, and members of the local community wash themselves and their laundry there. Fast-flowing water consisting of medium sized-boulders which form riffles, and a small section had sand and silt were observed at this site. The riparian vegetation forms a canopy around the stream.	
3	Gravelotte	24°0'19.64"S 30°40'29.25"E	Situated upstream of the Selati Game Reserve. There are game farms covering most of the catchment directly upstream of the site. The river often runs dry here during low flows (Figure 2.2.2 A & B), due to an impoundment just above it. The water here is shallow and the substrate	

			consists mainly of fine gravel. The stream is wide, and has trees along the edges of the river.	
4	Ngulube	23°55'17.13"S 30°51'13.39"E	Situated 24.2 km from Gravelotte, within Ngulube Lodge. The observed habitat here is a deep lentic pool, due to a downstream weir. The pool is surrounded by large trees.	Middle reache
5	Namakgale	23°58'36.61"S 30°59'3.95"E	Situated adjacent to Namakgale Township and the local catchment consists mostly of housing and croplands. Activities such as sand mining have altered the flow, while cropping and intensive grazing by domestic livestock have led to soil erosion and high sediment inputs into the river. The water here is shallow and the substrate consists of fine gravel.	
6	Mica	23°58'38.34"S 31° 4'26.54"E	Situated 7.7 km upstream of Phalaborwa town, but downstream of the tributaries which carry the outputs of the three sewerage plants from the town and its surrounding townships. Furthermore, the site is located adjacent to an illegal dumping site, and a large amount of litter was observed along the river channel. The surrounding vegetation is the common *Phragmites mauritianus*.	

7	Bosveld	23°59'6.89"S 31°4'44.15"E	Situated 245 m downstream of the Bosveld fertilizer factory, at the point where an overflow of highly acidic tailings water from the factory occurred in January 2014. This site also had numerous disturbances throughout the sampling campaign. Activities such as sand mining have altered the flow. The water here is shallow and the substrate consists mainly of fine gravel.	Lower reaches
8	Rail road	24°0'39.08"S 31° 5'1.39"E	Situated downstream of the Bosveld factory and about halfway along a neighbouring phosphate mine and processing plant (FOSKOR). The catchment here contains large tailings dams, and also collects run-off from an extensive industrial area. The substrate made up of clay with small rocks and consists of *Phragmites mauritianus* along the river banks.	
9	Lepelle	24°2'16.93"S 31° 7'59.64"E	Situated immediately downstream of the FOSKOR mine and industrial complex, about 750 m upstream of the confluence of the Ga-Selati River with the Olifants River. The water flow here is disturbed by a fence across the river which traps suspended leaves and logs (Figure 2.2.3). Fast-flowing, with medium-sized boulders, riffles and with fine gravel was observed. The surrounding vegetation *Phragmites mauritianus*	

Figure 2.2.2: The Ga-Selati River at site 3 (Gravelotte site) during low flow (A) and high flow (B)

Figure 2.2.3: The Ga-Selati River at site 9**.** Note the fence pole in the middle, indicating where a fence crosses the river

Sampling was conducted seasonally along the Ga-Selati River (in May, July, October 2014 and January 2015). Water and sediment samples were collected at the nine sites described in Chapter 1 (Figure 1.2.2.1).

2.2.2 Water sampling and analysis

Water samples were collected in 1000 ml acid pre-treated, polyethylene bottles, and stored at 5°C prior to analyses (Agtas *et al.* 2007). *In situ* pH, water temperature, dissolved oxygen (DO), total dissolved solids (TDS) and electrical conductivity (EC) were recorded at this depth (YSI Model 554 Datalogger Conductivity meter was used to measure TDS, EC and salinity. Turbidity was measured at Biodiversity Laboratory using Spectrophotometer. The water samples were thawed then analysed in batches with blanks using inductively coupled plasma–optical emission spectrophotometry (ICP-OES; Perkin Elmer, Optima 2100 DV) at WATERLAB, an accredited laboratory (ISO/IEC 17025:2005) in Pretoria.

2.2.3 Sediment sampling and analysis

Surface sediment samples (up to 20 cm depth) were taken at all sampling sites and were transferred to 250 ml polypropylene sampling bottles. The sediment samples were frozen immediately to prevent bacteriological and chemical activities, and were sent to a SANAS accredited laboratory in Pretoria for analysis. Metal concentrations were determined using nitric acid digestion and analysed by inductively coupled plasma - optical emission spectrometry (ICP-OES). Sediment concentrations were compared against the guideline values of Canadian Council of Ministers of the Environment (CCME 2012a).

2.2.4 Statistical analysis

The mean and standard deviation of the respective water variables and sediment were calculated. A two-way ANOVA was performed using the Statistical Package and Service Solutions (IBM SPSS version 24.0, 2016), to determine if there were significant difference between sites and seasons.

2.3 Results

2.3.1 River water

The pH values at all sites were above 7.99 (Table 2.3.1.1). The lowest pH value was 7.99 at site 1 in autumn and the highest pH was 9.3 at site 5 during spring (Figure 2.3.1.1 (A)). Statistical analysis revealed no significant difference in pH among the sites or seasons ($p > 0.05$). Electrical conductivity (EC) ranged from 146 mS/cm at site 1 to 1926.5 mS/cm at site 9. The highest mean was at site 9 (1483.5 mS/cm) and the lowest mean at site 1 (170.7 mS/cm) (Table 2.3.1.1). Site 1 had the lowest value of EC during each season except in summer where it had 198 mS/cm, which was slightly above that of site 2 which had a value of 197 mSc/m. TDS ranged from 68.6 mg/ℓ at site 1 to 839 mg/ℓ at site 9. The highest mean value was at site 9 (693.8 mg/ℓ) and the lowest at site 1 (81.6 mg/ℓ) (Table 2.3.1.1). There was a significant difference among sites for both EC and TDS ($p < 0.001$), and both increased from upstream to downstream in all seasons. However, the values dropped between site 3 and site 4 during summer (Figure 2.3.1.2 (C-D)).

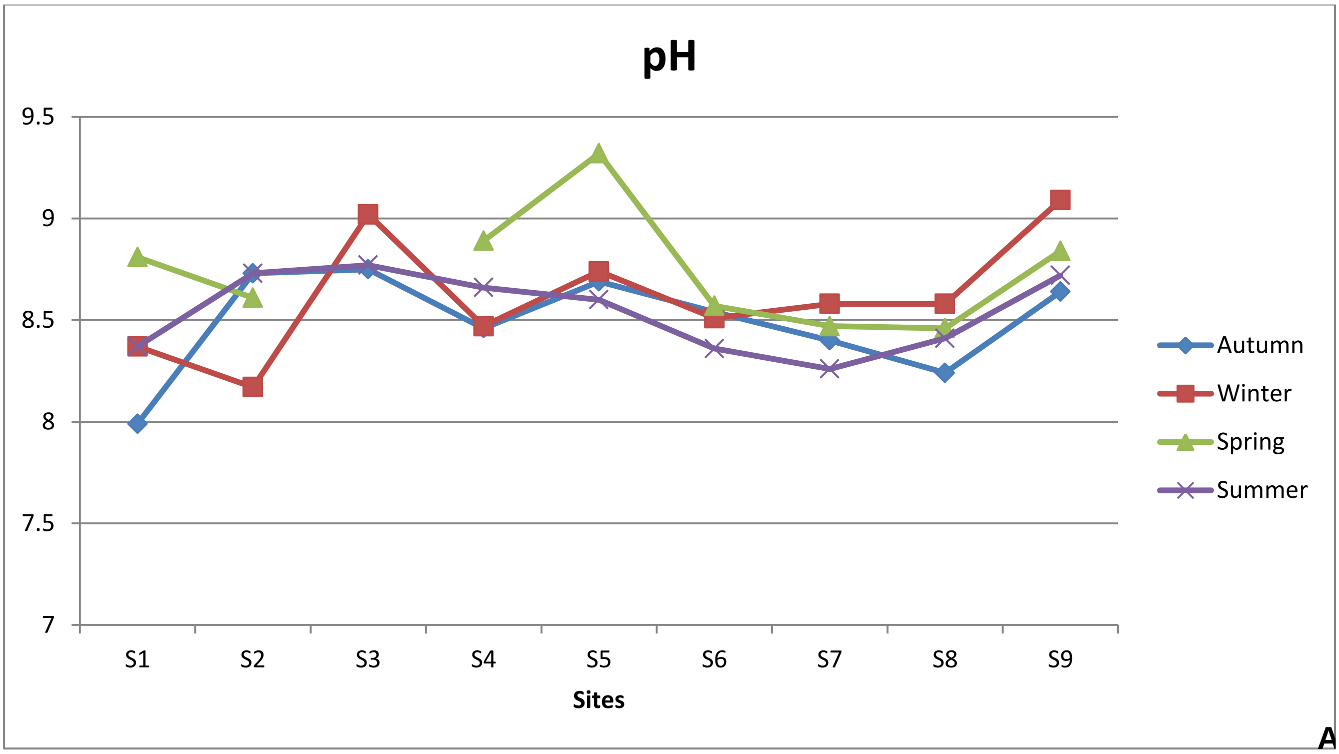
DO ranged from 6.2 mg/ℓ at site 3 during autumn to 12.3 mg/ℓ at site 8 during winter (Figure 2.3.1.1 (B)), while oxygen saturation ranged from 75.8% at site 3 during autumn to 137.1% at site 1 during winter (Figure 2.3.1.3 (E)). The highest mean DO (in both mg/ℓ and %) was at site 1 (9.9 mg/ℓ and 1197%) and lowest at site 9 (7.6 mg/ℓ and 89.7%) respectively. There was a significant difference among seasons in DO concentrations, with higher concentrations in winter. Temperature ranged from 17°C at site 6 and site 8 to 35.4°C at site 3. The highest mean was at site 3 (26.0°C) and the lowest at site 1 (20.1°C). As expected the lowest temperatures were recorded during winter with values ranging from 16.1°C at site 9 to 17.9°C at site 1 and highest during summer with values ranging from 24.2°C to 34°C (Figure 2.3.1.3 (D)).

Table 2.3.1.1: Water quality variables recorded in river water at the nine sampling sites in the Ga-Selati River from May 2014 to January 2015. Values are means of the four seasonal samples (± SD).

	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**	**Site 8**	**Site 9**	**Guidelines**
pH	7.99-8.81	8.17-8.73	8.75-9.02	8.46-8.89	8.46-8.89	8.36-8.57	8.26-8.56	8.24-8.58	8.64-9.09	Should not vary by > 5%[1]
EC (mS/m)	170 ±27	254 ±119	348 ±135	362 ±69	847	1017 ±330	1074 ±334	1129 ±379	1484 ±450	No guidelines
TDS (mg/ℓ)	82 ±12	118 ±57	155 ±49	169 ±26	387 ±213	472 ±139	503 ±140	514 ±157	693 ±177	No guidelines
DO (mg/ℓ)	9.9 ±1.9	9 ±1.1	7.8 ±1.8	8.3 ±1.9	8.1 ±1.8	8.6 ±2.1	9 ±2.4	8.5 ±2.7	7.6 ±0.5	No guidelines
DO (%)	120 ±20	112 ±7	95 ±20	102 ±21	94 ±14	103 ±19	106 ±3	99 ±27	90 ±3	80 % - 120 % of saturation[1]
Temperature (°C)	20.1 ±2.9	25.5 ±7.4	26 ±8.3	22.9 ±5.6	24.5 ±7.1	23.3 ±6.9	22.8 ±6	22.6 ±6.1	23.3 ±7.7	Should not vary by > 10%[1]
Turbidity (NTU)	1 ±0.8	7 ±3.1	7 ±1.5	7 ±1.3	9 ±0.6	13 ±6.5	11 ±5.2	7 ±5.1	6 ±3.6	8 (clear flow) to < 50 (turbid flow)[2]
Salinity (‰)	0.8 ±0.02	0.11 ±0.06	0.11 ±0.1	0.17 ±0.03	0.38 ±0.22	0.46 ±0.15	0.5 ±0.15	0.5 ±0.17	0.69 ±0.18	< 0.5‰[1]

1. DWAF 1996): South African Water Quality guidelines: Volume 7: Aquatic Ecosystems.

2 BC-EPD (2006): British Columbia Environmental Protection Division: Water Quality Guidelines



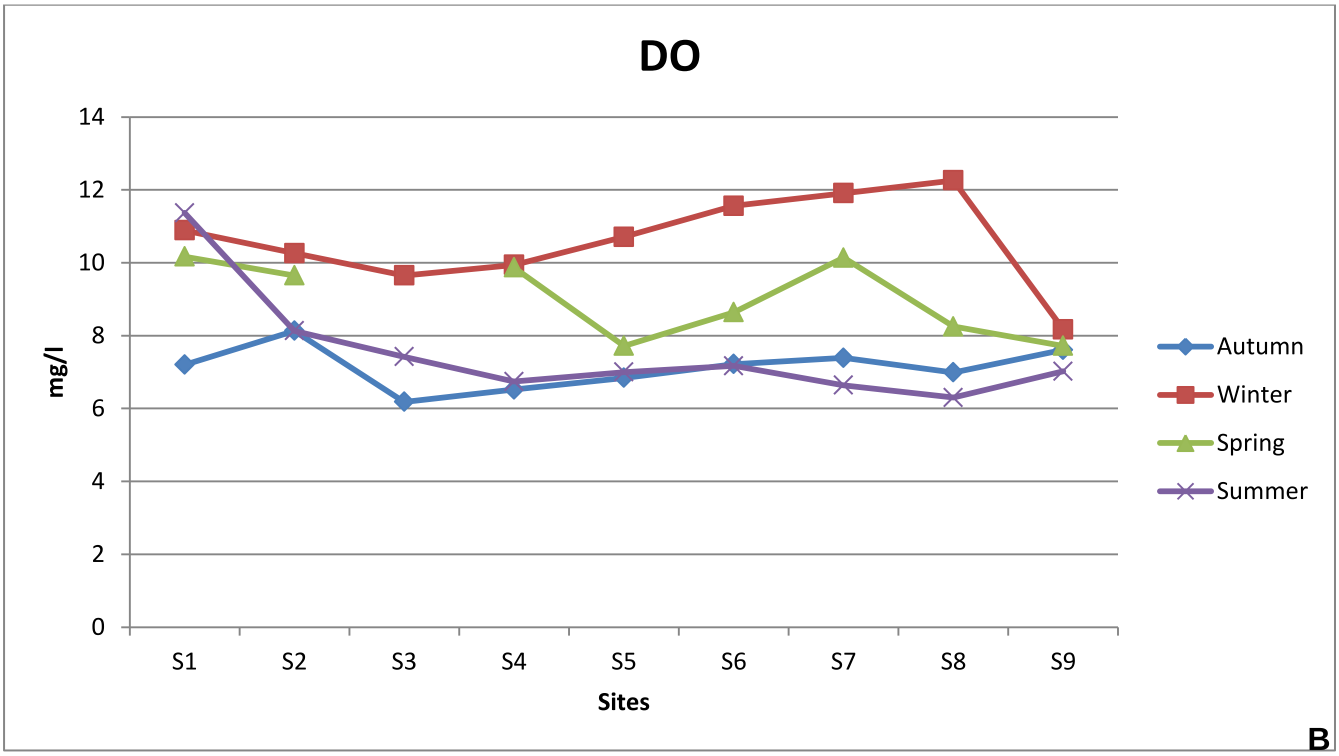
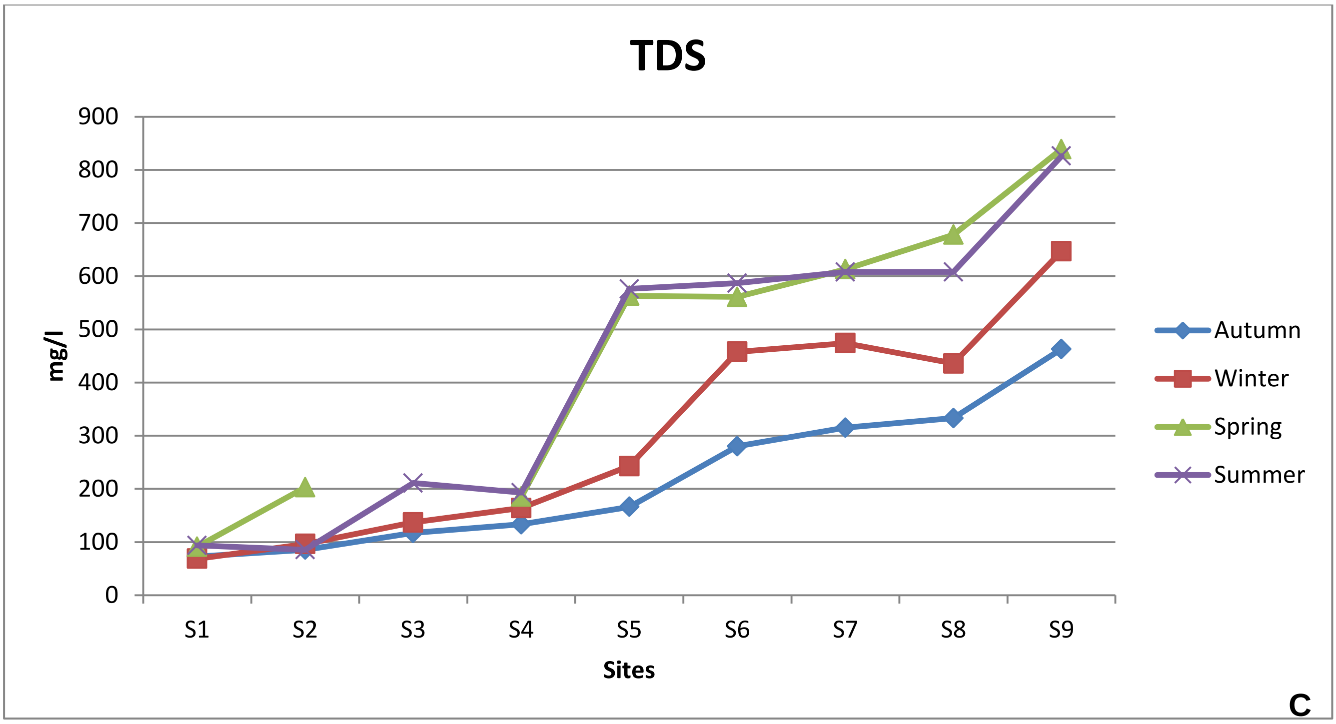


Figure 2.3.1.1 (A-B): pH & DO concentrations recorded in surface water of the Ga-Selati River from May 2014- January 2015.



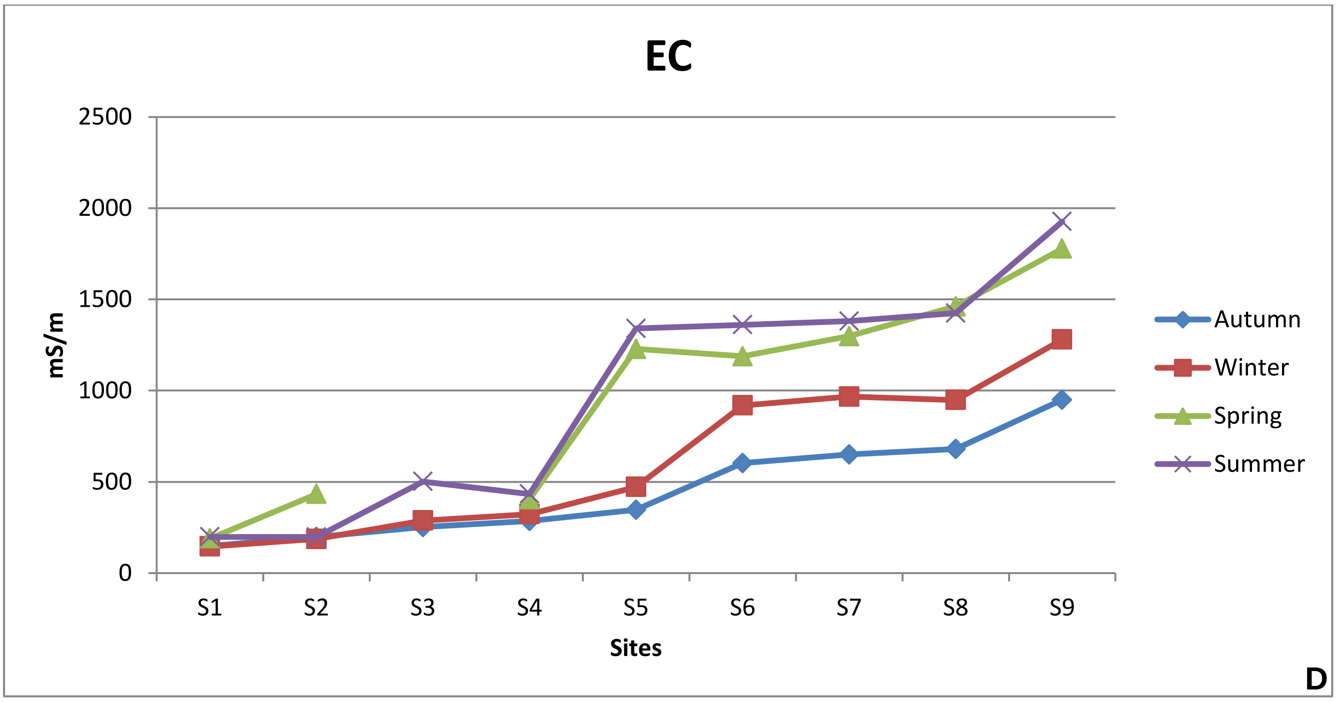
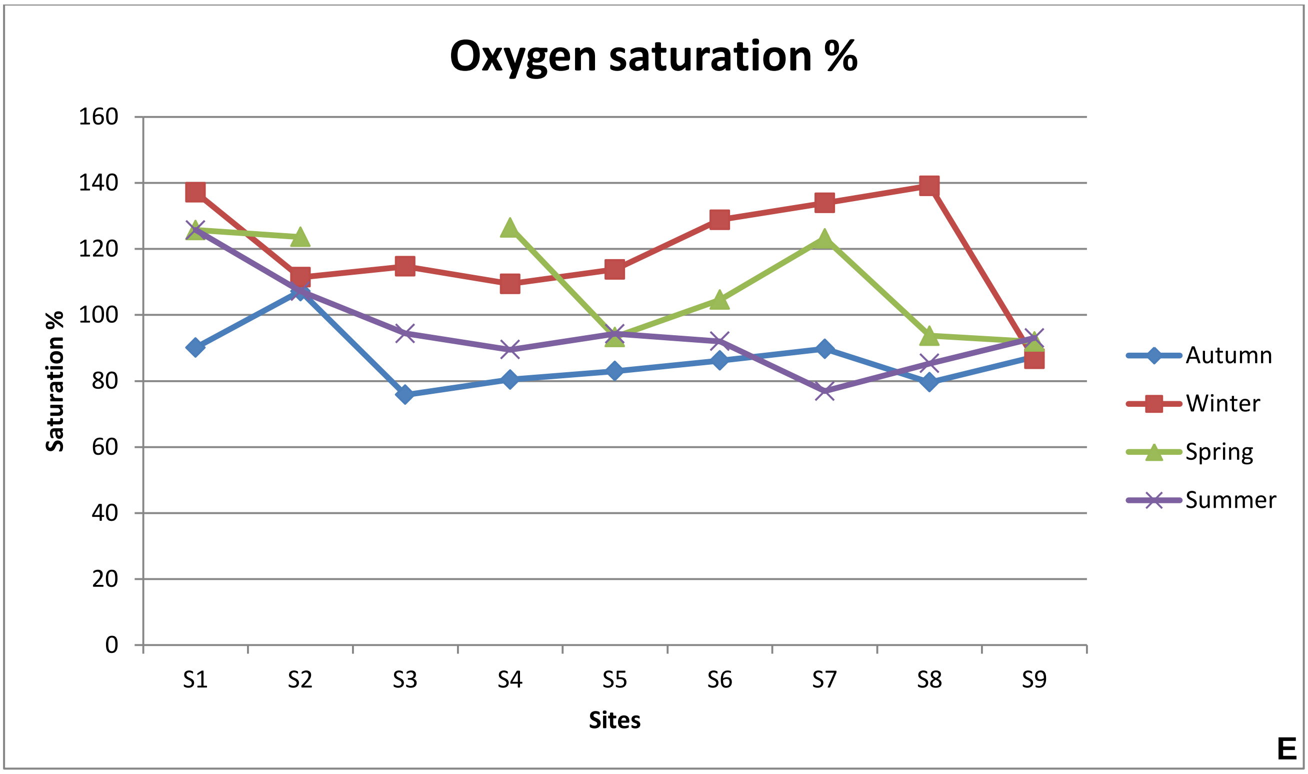


Figure 2.3.1.2 (C-D): TDS & EC concentrations recorded in the water of the Ga-Selati River from May 2014 January 2015



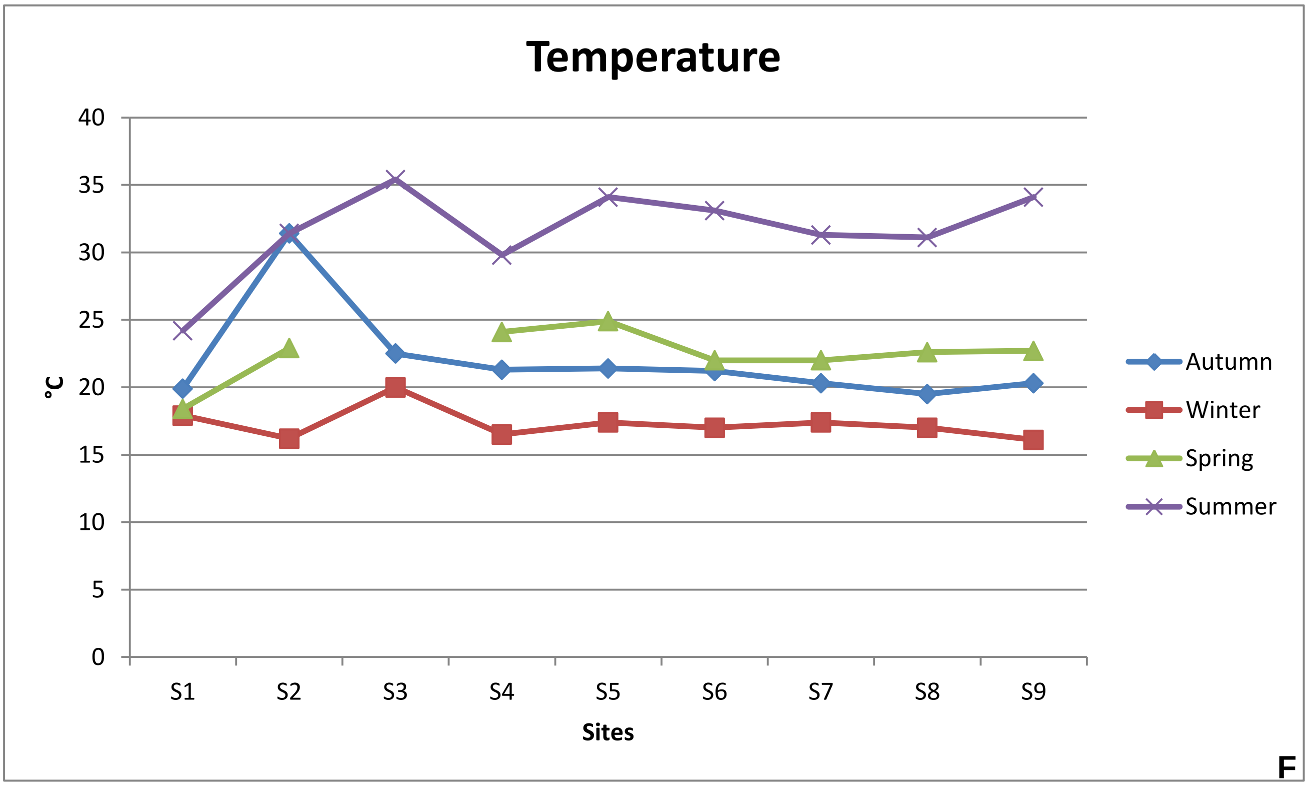
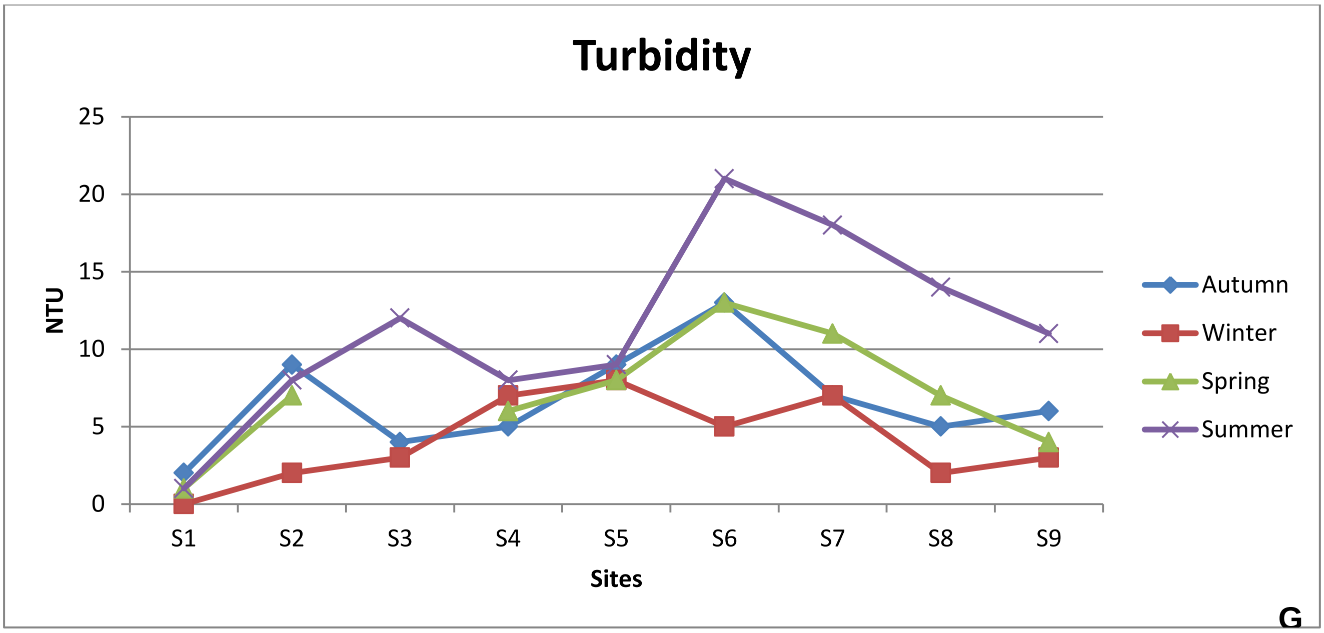


Figure 2.3.1.3 (E-F): Oxygen saturation (%) & temperature concentrations recorded in the water of the Ga-Selati River from May 2014- January 2015



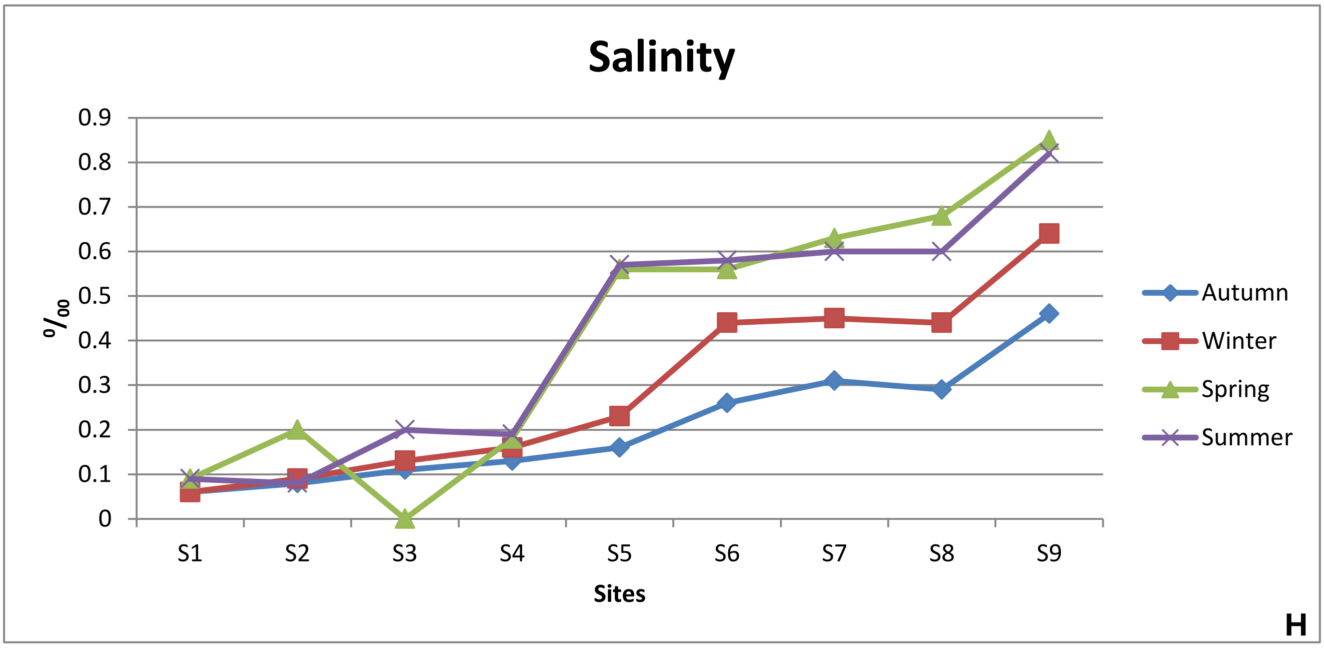


Figure 2.3.1.4 (G-H): Turbidity & salinity concentrations recorded in the water of the Ga-Selati River from May 2014- January 2015.

Turbidity ranged from 0 NTU during winter at site 1 to 21 NTU during summer at site 6 (Figure 2.3.1.4 (G)). There was no significant difference among season and sites for turbidity ($p > 0.05$). Salinity ranged from 0.06 ‰ at site 1 to 0.85 ‰ at site 9, with the lowest recorded in autumn and the highest recorded during spring (Figure 2.3.1.8). Site 1 had the lowest value recorded in each season except summer. Mean salinity across seasons increased from upstream to downstream (Table 2.3.1.1). The highest mean salinity was recorded at site 1 site 9 (0.69 ‰) and the lowest was recorded at site 1 (0.08 ‰). The salinity concentrations at site 9 were above the TWQR guidelines Statistical analysis showed no significant difference among seasons ($p = 0.194$). However, there was a significant difference among sites ($p < 0.001$).

Mean NO_3- concentrations ranged from 0.47 mg/ℓ at site 1 to 3.44 mg/ℓ at site 4 (Table 2.3.1.2). NO_2− and NH_3 concentrations were <0.01 mg/ℓ at site 1 and remained low until site 6, where there was a large increase. Mean total nitrogen (TN) concentrations ranged from 0.56 mg/ℓ at site 1 to 3.51 mg/ℓ at site 4. There was no significant difference among seasons for all the nutrients measured. Furthermore, there was no significant difference among sites for NO_3^- and P ($p = 0.36$ and $p = 0.82$) respectively. Sites 4, 6, and 7 were the only sites with NH_3 concentrations >0.010 mg/ℓ during winter. The NH_3 concentrations were >0.5 mg/ℓ during summer at site 6, site 7 and site 8 (Appendix 1 B). Mean P values ranged from 0.21 mg/ℓ at site 4 to 2.47 mg/ℓ at site 5. P values showed a significant increase during spring season downstream, with the highest value recorded at site 7 (2.21 mg/ℓ) (Appendix 1 B). The highest mean values for both TN and NO_3^- were recorded at site 4 (3.51 mg/ℓ and 3.44 mg/ℓ), while the highest mean value for both NO_2− and NH_3 were recorded at site 6 (0.2 mg/ℓ and 0.34 mg/ℓ). The highest mean value of P was recorded at site 5 (Figure 2.3.1.5 (A)). Statistical analysis showed a significant difference for NH_3 and NO_2− concentrations among the sites ($p < 0.05$).

Table 2.3.1.2: Mean (± SD) nutrients concentrations recorded in river water at the nine sampling sites from May 2014 to January 2015. (In some cases SD could not be calculated as concentrations were too low for measurement).

Nutrients (mg/ℓ)	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**	**Site 8**	**Site 9**	**Guidelines**
NO_2^-	0.03 ±0.06	0.03 ±0.03	0.05 ±0.06	0.02 ±0.01	0.03 ±0.04	0.2 ±0.14	0.11 ±0.04	0.05 ±0.02	0.05 ±0.04	No guidelines
NO_3-	0.47 ±0.31	0.55 ±0.12	1.4 ±0.6	3.44 ±6.8	1.71 ±2.3	1.51 ±0.8	1.76 ±0.8	1.68 ±0.4	2.65 ±1.1	13[2]
NH_3	0.05 ±0.05	0.04 ±0.00	0.03 ±0.02	0.06 ±0.04	0.05 ±0.03	0.34 ±0.11	0.18 ±0.1	0.14 ±0.0	0.08 ±0.03	< 0.007[1], 0.354[4]
P	0.42	0.45	0.51 ±0.3	0.21 ±0.2	2.47	0.86 ±33	1.05 ±0.7	1.15 ±0.8	0.88 ±0.7	< 0.005 (oligotrophic); > 0.25 (hypertrophic)[1]
TN	0.56 ±0.2	0.62 ±0.24	1.48 ±0.64	3.51 ±1.6	1.79 ±0.79	2.05 ±0.59	2.05 ±0.76	1.87 ±0.75	2.78 ±1.22	<0.5 (oligotrophic); >10 (hypertrophic)[1]

1. DWAF 1996): South African Water Quality guidelines: Volume 7: Aquatic Ecosystems.

2. (CCME 2012b): Canadian Council of Ministers of the Environment: Water Quality Guidelines Aquatic Life

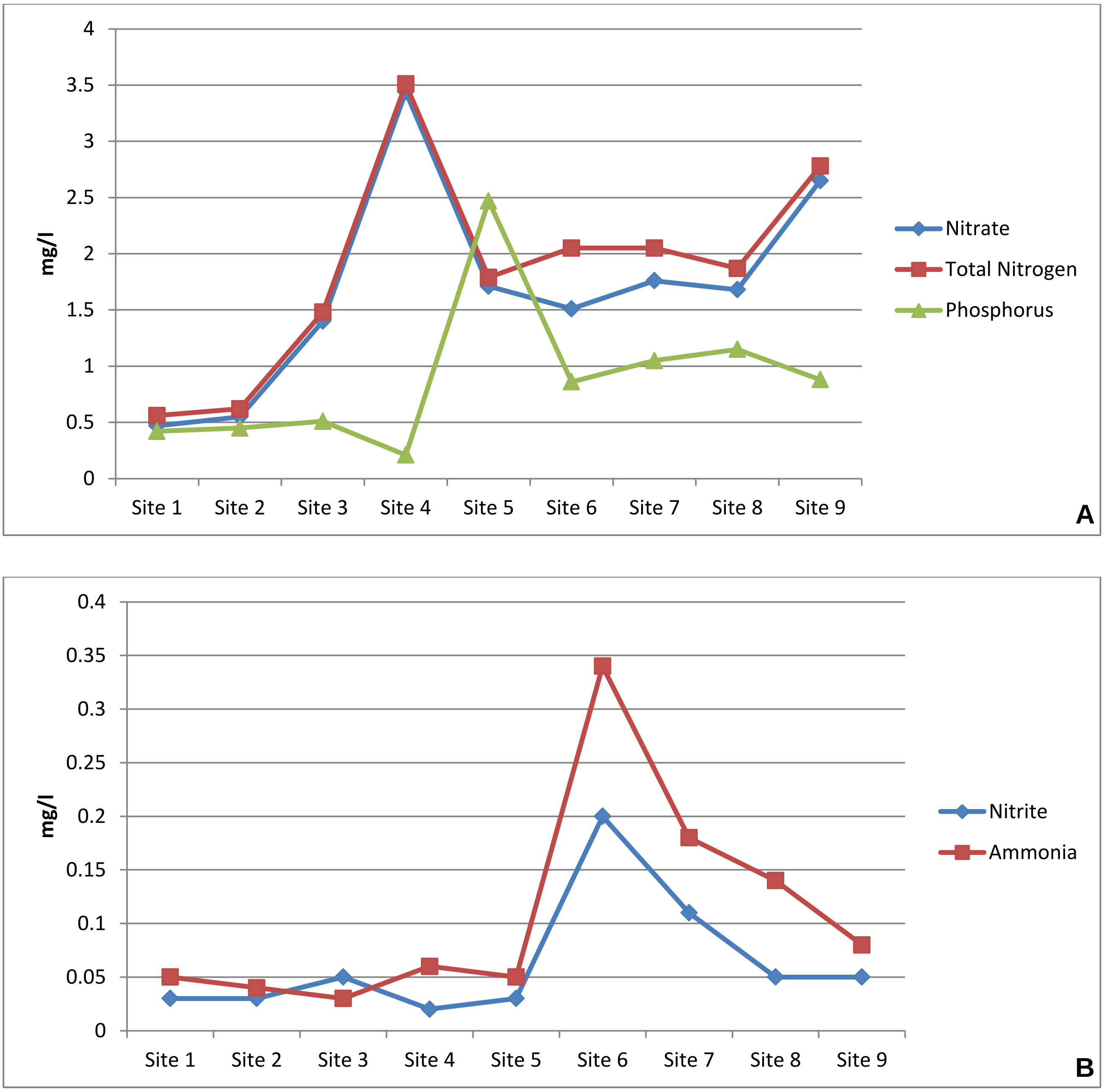


Figure 2.3.1.5 (A-B): Mean nutrient concentrations of river water across the nine sites in the Ga-Selati River.

There was a significant difference in Ca, Mg, K, Na, B, and Sr concentrations among sites ($p < 0.001$). There was no significant difference among seasons for all the metals except for Fe, Al and Ti ($p < 0.001$)). Mean values for Mg, Na and K were at their lowest concentrations at site 1 (10 mg/ℓ, 3 mg/ℓ, and 1.2 mg/ℓ) and were highest at site 9 (59 mg/ℓ, 111 mg/ℓ, and 19 mg/ℓ) respectively (Figure 2.3.1.6 (A-E)). Mean Ca concentrations ranged from 7 mg/ℓ to 57 mg/ℓ, increasing from site 1 to site 9. (Table 2.3.1.3, Figure 2.3.1.6 (A-E)). The lowest and highest concentrations of Ca were all recorded during winter (Appendix 1 C).

Na concentrations recorded in this study ranged from 3 mg/ℓ at site 1 to 121 mg/ℓ at site 9 (Appendix 1 C). The highest K concentration was recorded in summer (25 mg/ℓ) (Appendix 1 C). K concentrations were <1.0 mg/ℓ at site 1 and site 2 during autumn and winter and it never exceeded 1.4 mg/ℓ throughout the survey at these two sites. Mean Al concentrations were comparable at all sites except for site 5 and site 7, which were > 0.2 mg/ℓ (Table 2.3.1.3). Al concentrations were <0.010 mg/ℓ at site 1 and site 4. The highest titanium (Ti) value (0.6 mg/ℓ) was recorded at site 8 while it was <0.05 mg/ℓ at the rest of the sites. Barium (Ba), B and Zn were comparable at all the sites. Mn concentrations were comparable at all sites except for site 6. Site 1 had the highest iron concentration but the lowest concentration of the other metals (Figure 2.3.1.6 (A-E)).

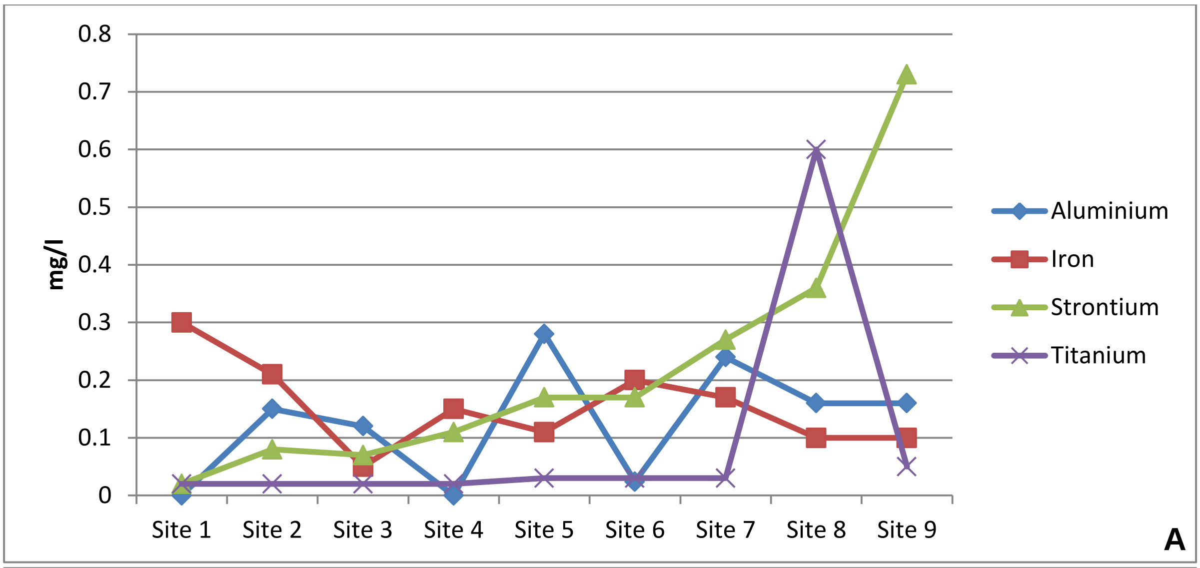
Table 2.3.1.3: Mean (± SD) values of metals recorded in river water at the nine sampling sites from May 2014 to January 2015. (Values with no SD had only one replicate due to concentrations being below detection level).

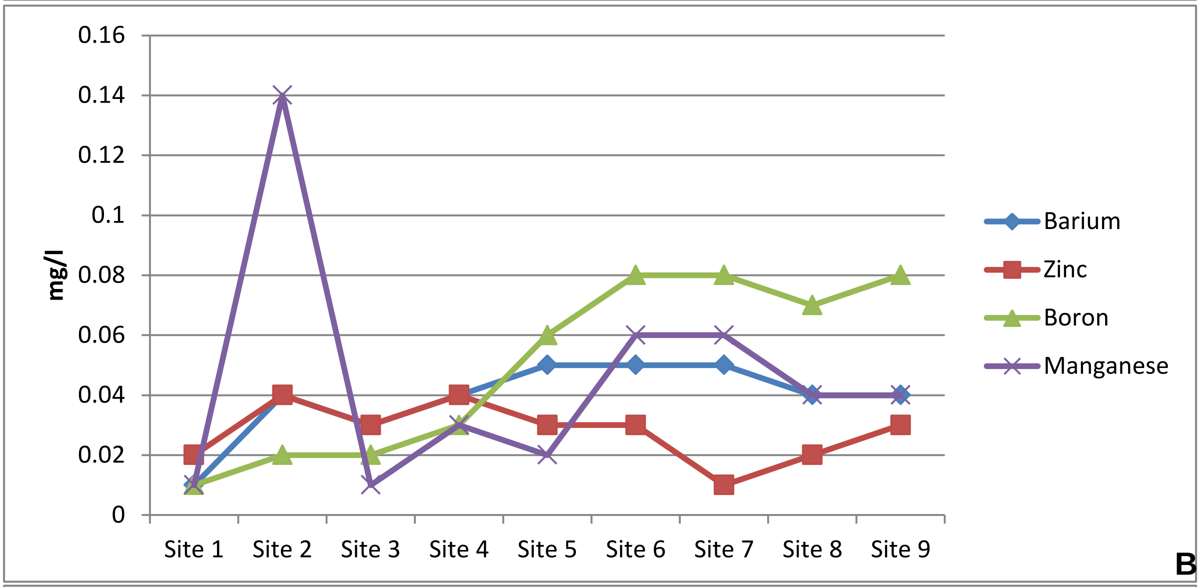
Metals in (mg/ℓ)	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**	**Site 8**	**Site 9**	**Guidelines**
Al	<0.010	0.15	0.12	<0.010	0.28	0.02	0.24	0.16	0.16	0.01[1]
Ba	0.01 ±0.00	0.04 ±0.03	0.03 ±0.02	0.04 ±0.01	0.05 ±0.03	0.05 ±0.01	0.05 ±0.00	0.04 ±0.01	0.04 ±0.01	0.7[3]
Boron (B)	0.01	0.02 ±0.02	0.02 ±0.01	0.03 ±0.02	0.06 ±0.04	0.08 ±0.02	0.08 ±0.01	0.07 ±0.02	0.08 ±0.02	No guidelines
Ca	16 ±3.4	18 ±9.4	16 ±6.5	20 ±4.1	17 ±6.8	30 ±4.2	33 ±8	37 ±7	41 ±17	< 200[4]
Fe	0.3 ±0.01	0.21 ±0.19	0.05 ±0.034	0.15 ±0.11	0.11 ±0.13	0.2 ±0.11	0.17 ±0.10	0.1 ±0.1	0.1 ±0.05	0.3[2], 1[3]
Mg	10 ±1.73	13 ±6.7	12 ±1.5	17 ±2.0	26 ±15	28 ±4.6	31 ±8.3	30 ±8	59 ±9	< 150[2], < 1.3[4]
Mn	0.01	0.14 ±0.09	0.01	0.03 ±0.02	0.02 ±0.00	0.06 ±0.02	0.06 ±0.03	0.04 ±0.02	0.04 ±0.03	0.18[1]

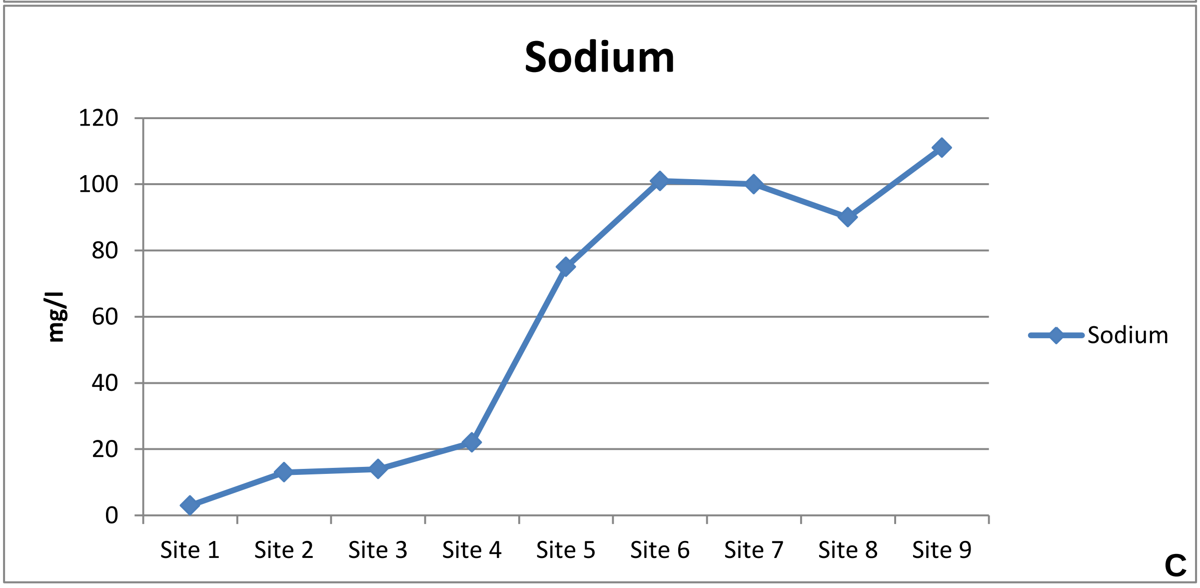
K	1.2 ±0.14	1.3 ±0.14	1.6 ±1.7	2.1 ±0.7	5 ±3	6.8 ±3.1	6.3 ±2.04	5.8 ±3.3	19 ±5	No guidelines
Na	3 ±0.5	13 ±8.1	14 ±6.4	22 ±3	75 ±64	101 ±24	100 ±19	90 ±31	111 ±13	< 200[2]
Strontium (Sr)	0.02 ±0.01	0.08 ±0.05	0.07 ±0.04	0.11 ±0.03	0.17 ±0.1	0.17 ±0.11	0.27 ±0.08	0.36 ±0.22	0.73 ±0.15	4.0[3]
Ti	0.02 ±0.00	0.02 ±0.01	0.02 ±0.00	0.02 ±0.01	0.03 ±0.00	0.03 ±0.011	0.03 ±0.01	0.61 ±0.22	0.05 ±0.02	No guidelines
Zn	0.02 ±0.01	0.04 ±0.01	0.03	0.04 ±0.02	0.03 ±0.02	0.03 ±0.02	0.01 ±0.01	0.02 ±0.01	0.03 ±0.02	0.002[1], 0.12[3]

DWAF 1996): South African Water Quality guidelines: Volume 7: Aquatic Ecosystems.

1. (CCME 2012b): Canadian Council of Ministers of the Environment: Water Quality Guidelines – Aquatic Life
2. (USEPA 2012): United States Environmental Protection Agency: Water Quality Guidelines – Aquatic Life
3. BC-EPD (2006): British Columbia Environmental Protection Division: Water Quality Guidelines


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
mg/l
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Site 7
Site 8
Site 9
Aluminium
Iron
Strontium
Titanium
A


0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
mg/l
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Site 7
Site 8
Site 9
Barium
Zinc
Boron
Manganese
B


Sodium
120
100
80
60
40
20
0
mg/l
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Site 7
Site 8
Site 9
Sodium
C

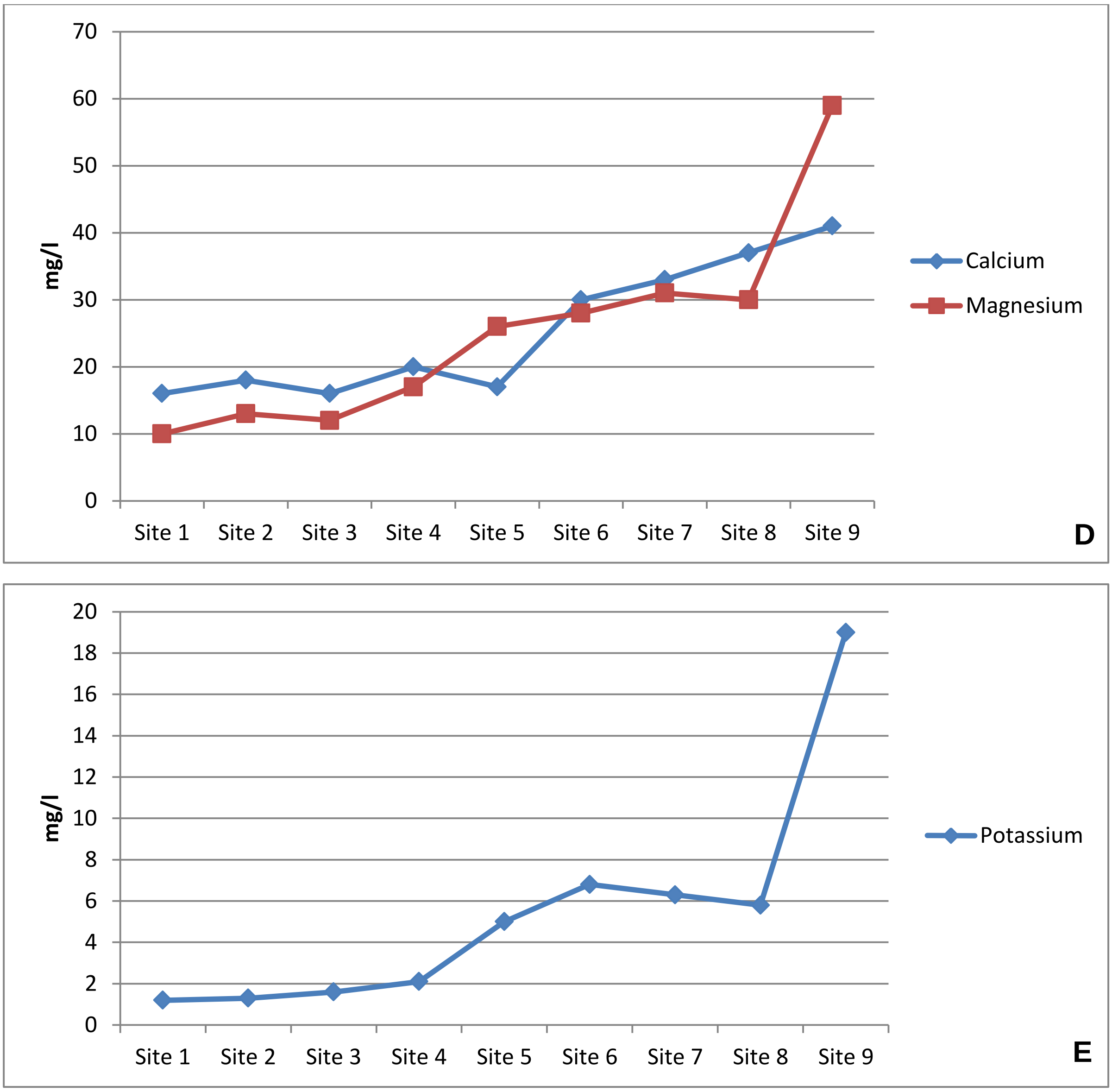


Figure 2.3.1.6 (A-E): Mean metal concentrations in river water for the nine sites in Ga-Selati River.

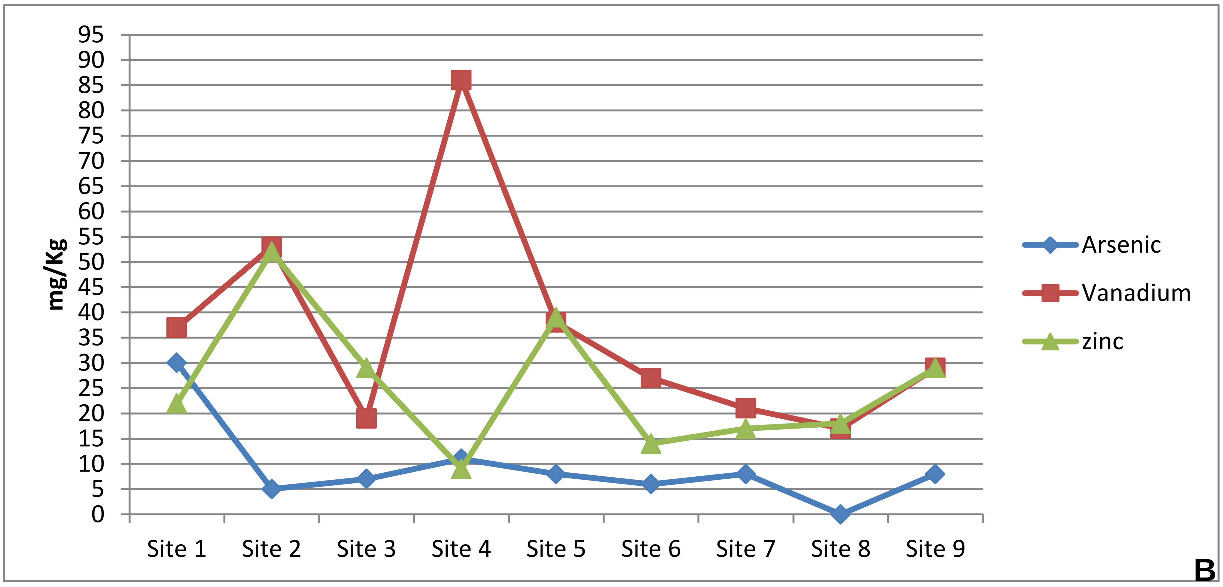
2.3.2: Sediments

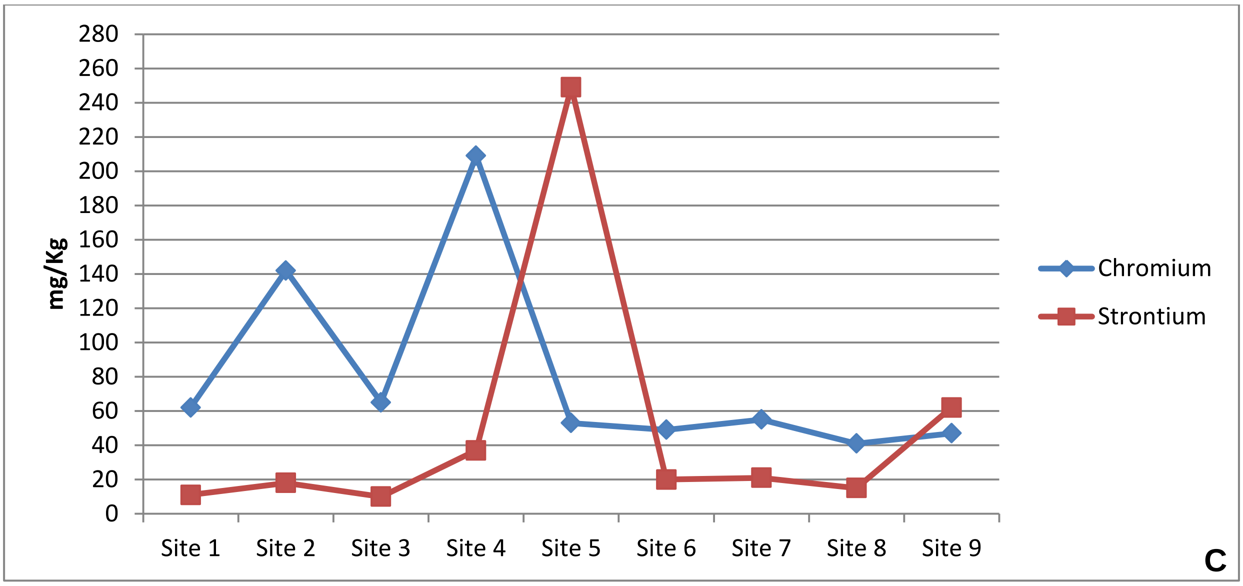
Metal concentrations analysed from the sediment samples are shown in Table 2.3.2.1. As and Zn concentrations were highest at site 1 and site 2 respectively. Mn, K, Cr, vanadium (V), Al, Fe, Ti and Mg had their highest concentrations at site 4 in the middle reaches (Figure 2.3.2.1 (A-E)). Ca, Sr and Ni had their highest concentrations at site 5 in the middle reaches. Na concentrations were highest at site 7 in the lower reaches. The lowest mean concentrations of Fe, K, Sr, Ti, Mg and Mn were recorded at site 3. Statistical analysis showed no significant difference among seasons for all the metals. However, there was a significant difference among sites for Al, Cr, Fe, Sr, Ti, Mn and V ($p < 0.05$).

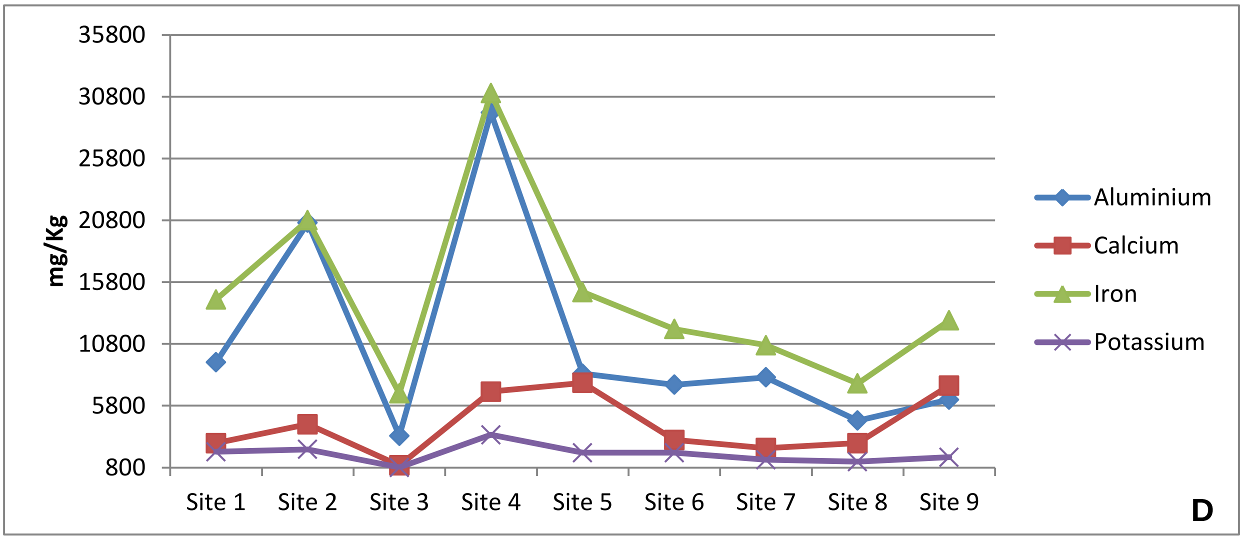
Table 2.3.2.1: Mean (± SD) metals concentrations from sediments at the nine sampling sites from May 2014 to January 2015. (Values with no SD were recorded only once).

Metals (mg/kg)	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**	**Site 7**	**Site 8**	**Site 9**	**(CCME 2012a) Sediment Quality Guidelines (mg/kg)**
Al	9326 ±6341	20600 ±27936	3368 ±743	29500 ±14724	8395 ±5586	7500 ±3305	8100 ±2559	4600 ±516	8557 ±2075	No guidelines
As	30	5 ±5	6 ±1	11 ±9	8	6	8		8 ±5	5.9
Ca	2770 ±1174	4305 ±3430	978 ±504	6948 ±2829	7659 ±6964	3032 ±1960	2376 ±641	2779 ±1619	2478 ±2628	No guidelines
Chromium (Cr)	62 ±54	142 ±86	65 ±9	209 ±80	53 ±24	49 ±19	55 ±14	41 ±7	57 ±13	37.3
Fe	14400 ±9944	20800 ±16580	6800 ±1178	31100 ±11890	15000 ±6544	12000 ±4283	10700 ±1645	7600 ±730	7469 ±5575	No guidelines
K	2078 ±1352	2273 ±2414	802 ±354	3449 ±1436	2005 ±495	2000 ±460	1436 ±322	1269 ±221	1637 ±285	No guidelines
Mg	2141 ±1739	2067 ±1602	618 ±112	5164 ±2559	4091 ±2645	1693 ±1548	1672 ±392	927 ±133	2928 ±1821	No guidelines
Mn	255 ±160	321 ±129	94 ±28	601 ±246	271 ±194	231 ±121	200 ±92	112 ±23	209 ±56	No guidelines
Na	525 ±585	861 ±399	911 ±326	983 ±301	1058 ±369	1035 ±194	1055 ±476	1031 ±171	861 ±62	No guidelines
Nickel (Ni)	106 ±73	123 ±97	72 ±59	217 ±152	744 ±947	125 ±97	102 ±73	119 ±37	88 ±81	No guidelines
Sr	11 ±10	18 ±5	10 ±2	37 ±7	249 ±40	20 ±8	21 ±165	15 ±3	62 ±25	No guidelines
Ti	736 ±544	1620 ±1416	302 ±137	3324 ±664	1691 ±807	1322 ±646	939 ±165	874 ±153	1077 ±404	No guidelines
V	37 ±19	53 ±57	19 ±1	87 ±32	38 ±17	27 ±9	21 ±7	17 ±5	29 ±10	No guidelines
Zn	22 ±11	52	29	39 ±16	39 ±14	14 ±9	17 ±6	18 ±10	29 ±16	123


800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
mg/Kg
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Site 7
Site 8
Site 9
Nickel
Manganese
A


95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
mg/Kg
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Site 7
Site 8
Site 9
Arsenic
Vanadium
zinc
B





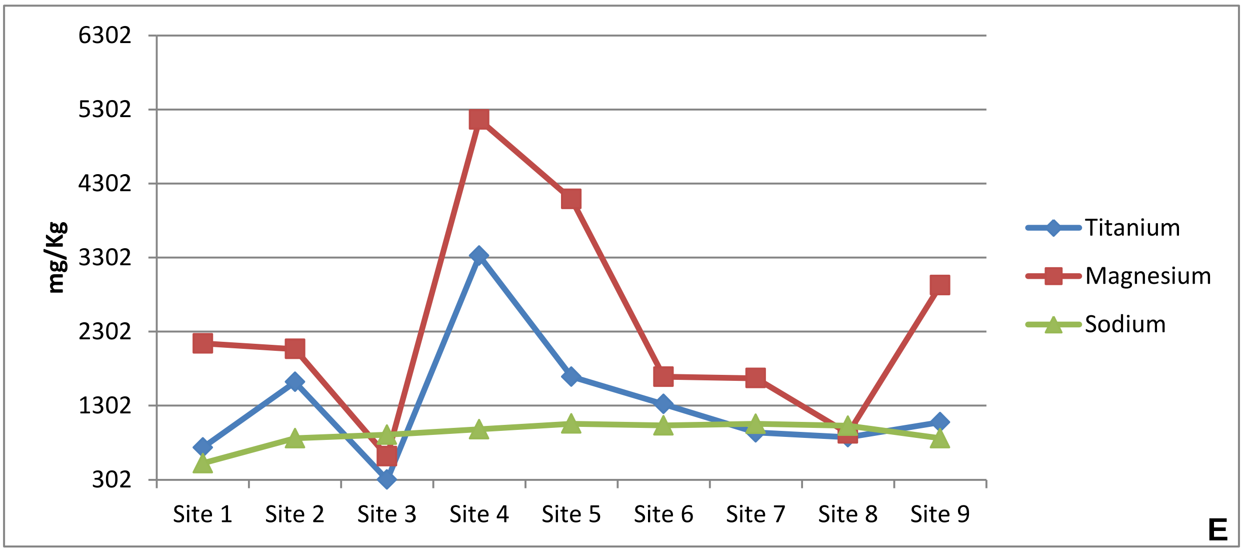


Figure 2.3.2.1 (A-E): Mean metal concentrations from sediment samples at nine sampling sites from May 2014 to January 2015.

2.4: Discussion

The water variables such as EC, TDS and salinity showed a gradual increase from upstream to downstream. There were also elevated levels of certain metals, such as Mg, Na, Ti, B, Sr, K and Ca showing a pollution gradient. This indicates inputs of salts and metals along the river, especially lower down in the catchment where there is mining activity. Salts accumulate as water moves downstream because salts are continuously being added through natural and anthropogenic sources. This high concentration of the above metals may be due to anthropogenic activities at this section of the Ga-Selati River. Anthropogenic activities are known to contribute significantly to the total aquatic burden of toxic metals in rivers (Gupta & Singh 2011). The concentration of these metals may increase due to effluent from industries (Mhatre 1991), agricultural activities (Heath & Claassen 1999) and from waste water from mining and industries (Moss 2009). The Ga-Selati River has been implicated as a possible source of metal pollution to the Olifants River, since it drains the extensive mining areas around Phalaborwa (Grobler *et al.* 1994). There is an increase awareness of the potential hazards that exist due to the contamination of freshwater ecosystems by toxic metals associated with the mining industries (Du Preez *et al.* 2003).

The Na and K concentrations showed a similar pattern (Figure 2.3.1.6 (C & E)) Na together with K plays an important role in the ionic and osmotic water balance in living organisms, and they also contribute to the TDS in the water (Kotzè 2012). High Na and K is therefore likely to be responsible for much of the increase in TDS levels from site 5 to site 9. Elevated concentrations of sodium are usually from industrial waste, especially processes that give rise to brines (DWAF 1996). The other reason for this (especially around site 5 and site 6) might be due to different geological groups that are found in the Ga-Selati River catchment (i.e. the basement complex) (Van Vuren *et al.* 1994). The weathered granite of the Basement Complex is eroded easily and may contain high concentrations of sodium and calcium (Van Vuren *et al*. 1994).

Potassium is often utilised as fertilizer, as it is an important macro-nutrient for plant and animals (Dallas and Day 2004), and fertilizer is produced by one of the industries within Phalaborwa Industrial Complex (Aken 2013). This might be the reason for elevated concentrations of K downstream. Zn mean concentrations were above the Total Water Quality Range (TWQR) (0.002 mg/l). High Zn concentrations in the Ga-Selati River could be from weathering, industrial discharges, sewage effluent and runoff. Surprisingly, Fe mean concentration was highest at site 1 and lowest at site 8 and site 9. This might be from the weathering of sulphide ores, leading to leaching of Fe from sandstones and metamorphic rocks found around site 1 (DWAF 1996). Mn mean concentrations were slightly high at site 2, but were within the TWQR (0.18 mg/l) at all sites. The higher concentrations at site 2 might be due to agricultural activities at this site, since Mn is used as a micro-nutrient fertilizer additive.

The water at site 1 was relatively clear since it had a mean concentration of 1 NTU. Site 2 was located relatively high up in the catchment but downstream of significant agricultural activity, and had a mean turbidity concentration that was similar to those recorded at sites further downstream in the Ga-Selati River. Turbidity increased further as the river flowed through the sites where there are human settlements (i.e. site 4, site 5 and site 6), but declined in the mining areas. The high turbidity values at site 3, site 4 and site 5 is probably due to overgrazing and trampling of the river banks. The decline in turbidity from site 6 to 9 might be due to the fact that there is still a lot of natural vegetation along the river and much less grazing pressure below site 6. The lowest turbidity value at the Ga-Selati River at each site was recorded during winter and the highest during summer. The lower turbidity values during winter were possibly due to lack of rainfall which can increase turbidity from the surface runoff. Turbid waters carry with them a high load of dissolved and suspended materials, and a high concentration of ions leading to high water conductivity (Kasangaki *et al.* 2008). This was evident from the progressive increase in concentration of EC from upstream to downstream.

The DO concentrations were higher upstream. High values of oxygen in both mg/ℓ and (%) occurred during winter, when water temperatures were lower compared with the other seasons. This is due to the fact that cold water holds more oxygen than warm water (Bartram & Ballance 1996). The low DO concentration at site 3 might be due to high temperature recorded at the site. Another factor might be due to the water impoundment just above the site. There are at least 17 unplanned impoundments along the Ga-Selati River (King *et al.* 2008). Water impoundment can affect physical and chemical factors such as stream substrate, DO and water temperature (Waters 1995, Hayes 1999). Ward & Stanford (1987) stated that deep release dams often draw water from anoxic hypolimnion causing DO reductions downstream. This statement can be supported by the fact that, the DO concentrations at site 2, site 3 and site 5 were lower compared to the other sites. All these sites were located downstream of an impoundment. The DO values recorded throughout the study were within TWQR (80-120%) range. During the sampling campaign, the highest temperatures were recorded at site 3. This might be due to the lack of riparian vegetation to provide shade, thus water is exposed to direct solar radiation, which leads to high temperatures. The mean temperature was low at site 1, which was probably due to trees alongside the riverbank forming a canopy reducing light penetration.

The nutrient concentrations at the Ga-Selati River did not show a pollution gradient, thus there are different factors contributing to high nutrient concentrations at each site. NO_3^- and TN concentrations were highest at site 4. This may be due to fertilizers used on the gardens at Ngulube Lodge which is adjacent to the river. The main source of N in residential catchments is fertilizer application to lawns and gardens (Poor & Mcdonnell 2007). N levels can also be greatly enhanced by sewage effluent, agricultural fertilizers and organic industrial waste products.

TN concentrations for various trophic levels as suggested by DWAF (1996) are as follows: <0.5 mg/ℓ =oligotrophic conditions, 0.5-2.5 mg/ℓ = mesotrophic conditions, 2.5-10 mg/ℓ = eutrophic conditions, and >10 mg/ℓ = hypertrophic conditions. Site 1 therefore represented an oligothrophic condition, site 4 (3.51 mg/ℓ) and site 9 (2.78 mg/ℓ) indicated a degree of eutrophication while the rest of the sites had mesotrophic conditions. Algal blooms were visually observed at site 4, 5, 6 and 9. Excessive algal growth due to nutrient enrichment results in utilisation of available dissolved CO_2 which reduces the carbonic acid content of the water, thus increasing pH levels (Oberholster *et al.* 2012). However, pH concentrations were comparable at all the sites. Excessive algal growth is known to hyper-saturate dissolved oxygen concentrations due to the release of oxygen produced by photosynthesis (DWAF 1996, Mainstone & Parr 2002, Hilton *et al.* 2006). However, DO concentrations were lower at sites with algae compared to the sites which had no algae. The algae might be playing a significant role at the Ga-Selati River, by increasing the pH of the water, especially at site 9.

P is delivered to the river system from a range of sources, varying in its bioavailability from source to source. Elevated levels of P may result from point-source discharges such as domestic and industrial effluents and from diffuse sources (non-point sources) in which the P load is generated by surface and subsurface drainage. Non-point sources include atmospheric precipitation, urban runoff, and drainage from agricultural land, in particular from land on which fertilizers have been applied (DWAF 1996). High concentrations of phosphorous occur in waters that receive sewage, leaching or runoff from cultivated land (Palmer *et al.* 2004). The mean P concentration was high at site 5. The high concentration of P at site 5 might be coming from surface run-off attached to soil particles, from livestock excreta, since this site is subjected to cropping and intensive grazing by domestic livestock which has led to soil erosion and high sediment inputs into the river, or from sewerage, since there are many houses close to the river system in this area, and they all have pit latrine toilets. P is considered to be the principal nutrient controlling the degree of eutrophication in aquatic ecosystems and is an essential macro-nutrient, and is accumulated by a variety of living organisms (DWAF 1996). P concentrations for various trophic levels as suggested by DWAF (1996) are as follows: < 0.005 mg/ℓ =oligotrophic conditions, 0.005-0.025 mg/ℓ = mesotrophic conditions, 0.025-0.25 mg/ℓ = eutrophic conditions, and >0.25 mg/ℓ = hypertrophic conditions. The P concentrations were all above 0.21 mg/ ℓ in the Ga-Selati River.

The mean NH_3 concentrations were high at site 6 to site 8. At pH values greater than 8, NH_4^+ ions are converted to the highly toxic unionised NH_3 (Seymore *et al.* 1994, DWAF 1996). According to Dallas & Day (2004), increases in pH can result from certain alkaline effluents from industries, as well as from anthropogenic eutrophication when excessive primary production leads to depletion of CO_2 from water in the presence of sunlight. Low pH values were expected at site 7, site 8, and site 9, since these sites are adjacent to the mining industries; however the pH values were basic throughout the sampling periods. Surprisingly, the highest pH value was recorded at site 9 during winter. NH_3 concentrations are usually low in well-oxygenated surface water with a healthy micro-flora and warm temperatures

(Soballe and Wiener 1998). This is further supported by the high DO values recorded at all sites. High concentrations of NH_3 at site 6 are the results of sewage input.

Increased sediment load in the Ga-Selati River has changed the channel morphology. Sediment input can cause habitat reduction, and can introduce pollutants like pesticides, metals and nutrients which are adsorbed to the sediment (Ahmad *et al.* 2010), thus it can impact stream communities direct or indirect. Metals such as As, Cd, Cr, Cu and Hg frequently accumulate in aquatic sediments (Harding 2005). Metal concentrations in sediment samples were very high compared to water samples in the Ga-Selati River. Metal concentrations in sediments can exceed water concentrations by 3 to 5 orders of magnitude (Bryan & Langston 1992). This is due to the fact that these metals bind to organic or inorganic particles that eventually settle to the bottom of our streams and once these contaminants are bound to a particle surface or adsorb into its interior matrix, they become less likely to be bio-transformed and desorption is usually very slow; therefore, adsorbed contaminants will reside for long periods in the sediment (Burton 2002). Thus, sediment provides binding sites for metals, thus eliminating pollutants from the water body and reducing the toxicity of the metal or making it unavailable to aquatic organisms (Salomons *et al*. 1987, Grobler *et al.* 1994). These bounded metals can be released back into the water if there are changes in pH, water hardness, salinity and temperature (Van Vuren *et al.* 1994).

There are currently no sediment quality guidelines in South Africa, so the Canadian sediment quality guidelines were used. As concentrations at the Ga-Selati River were above sediment quality guidelines (CCME 2012a) at all sites except for site 2 and site 8. The higher concentration at site 1 might be coming from rock weathering since site 1 is located upstream next to the mountains. This metal can accumulate in water from natural sources (Kaltreider *et al.* 2001) and certain geological formations contain high levels of As that can easily leach into freshwater ecosystems (Klaue and Blum 1999). As is a naturally-occurring element (Irwin *et al.* 1997) and is fairly water-soluble and higher concentrations are found in aquatic environment than in most areas of land (Edmonds and Francesconi 1993). Cr concentrations were above sediment quality guidelines at all sites and Zn concentrations were within sediment quality guidelines (CCME 2012a) at all sites. High concentrations of metals in sediment were expected at the mining sites downstream (site 7, site 8, and site 9);

however Na was the only metal which had a higher sediment concentration at these sites. High metal concentration where expected at the sites around the mining area. Mining create a potential source of metal pollution in the aquatic environment (Dabrowski *et al.* 2015). However, higher sediment concentration of Al, Cr, Fe, Ni, K, Ti, V, and Mn were found at site 4. This might be due to the water impoundment at this site. Anthropogenic sources of Fe are often related to mining activities; however the concentration of Fe was lower at the mining sites compared to upstream sites (site 1 and site 2) and this could be due to geology rather than pollution.

In conclusion, the high concentrations of metals in water samples indicate that the Ga-Selati River is heavily impacted downstream by anthropogenic activities such as illegal dumping/littering at site 6 and mining activities at site 7 to site 9. The high concentrations of metals were recorded at downstream sites (site 6 to site 9) of the river with an exception of Fe which was high at site 1. Some of the metal concentration (Na, Mg, K and Ca) in the river were found to be extremely high compared to other rivers in the region (Table 2.4.1), while Zn, Mn, Fe, and Cr concentrations were comparable (du Preez and Steyn 1992, Seymore *et al*. 1994, Gerber *et al*. 2015). Generally, the Ga-Selati River has been found to be most polluted and was in a critically modified condition, compared to other rivers (Klaserie, Steelpoort, and Blyde) in the Olifants River System (Marr *et al.* 2017) and that of Steelpoort River (Matlou et al. 2017). These high concentrations in the Ga-Selati River might be due to agriculture and mining activities around the Selati catchment. The high concentrations of metals in sediment in the middle reaches are due to impoundments and slow current. NO_3-, and P concentrations were high in the middle reaches (site 4 and site 5) of the river due to application of fertilizers at site 4 and livestock excreta and sewage input at site 5. The water variables in the Ga-Selati River did not show that there is pollution input in the lower reaches of the river. The mean concentrations of pH, and DO were high at all sites. If there was any sort of pollution in the river, especially downstream by the mining sites, we expected these two variables to be lower. These shows that the poor water quality in the Ga-Selati River is mainly due to different land uses and anthropogenic activities along the entire length of the river. The middle and lower Ga-Selati River flows through populated areas with various anthropogenic activities, making it vulnerable to different forms of pollution. The river has been systemically impaired by increasing human activities in its catchment, resulting in contamination by acidification, metals, industrial and agricultural chemicals, organic pollutants and domestic waste, and as a result significantly impacting the water quality of the river.

Table 2.4.1: Metal concentrations from the literature and current study, at selected sites in the Olifants, Balule, Mamba, Letaba and Luvuvhu River. NS = not sampled

			Dissolved metal concentrations (mg/l)											
	Source	Site & sample date	Cd	Cr	Ni	Pb	Fe	Cu	Mn	Zn	K	Ca	Mg	Na
	du preez & Steyn (1992)	Balule, October 1990	BD	<0.010	0.2 ±0.1	0.4 ± 0.1	2.3 ± 0.6	0.1 ± 0.04	0.05 ± 0.02	1.1 ± 0.6	NS	NS	NS	NS
	Seymore *et al*. (1994)	Whole river, October 1991	NS	0.01	0.02	0.2	0.44	0.03	0.04	0.1	0.02	0.04	0.1	0.1
	Gerber *et al*. 2015	Olifants River all Sites, 2009-2011	0.01 ± 0.001	0.004 ± 0.001	0.001 ± 0.00	0.004 ± 0.001	0.04 ± 0.006	0.001 ± 0.00	0.004 ± 0.001	0.002 ± 0.001	0.007+- 0.001	0.02 ± 0.002	0.03 ± 0.01	0.1 ± 0.03
	Gerber *et al*. 2015	Letaba River, all sites 2000-2011	0.01 ± 0.003	0.01 ± 0.002	0.002 ± 0.0003	0.01 ± 0.003	0.05 ± 0.01	0.001 ± 0.0002	0.01 ± 0.001	0.01 ± 0.01	0.01+- 0.01	0.02 ± 0.01	0.03 ± 0.02	0.2 ± 0.13
	Gerber *et al*. 2015	Luvuvhu River all sites 2009-2011	0.01 ± 0.002	0.003 ± 0.001	0.001 ± 0.00	0.01 ± 0.001	0.04 ± 0.004	0.001 ± 0.0001	0.003 ± 0.0002	0.002 ± 0.001	0.003 ± 0.001	0.01 ± 0.002	0.01 ± 0.003	0.03 ± 0.01
Site 1	This study	Ga-Selati River 2014-2015	NS	<0.010	NS	<0.010	0.03	NS	0.01	0.02	1.2	16.25	9.5	3.25
Site 2	This study	Ga-Selati River 2014-2015	NS	<0.010	NS	<0.010	0.19	NS	0.14	0.04	1.3	18	13.25	13
Site 5	This study	Ga-Selati River 2014-2015	NS	<0.010	NS	<0.010	0.11	NS	0.02	0.03	5.03	17.25	25.5	75
Site 7	This study	Ga-Selati River 2014-2015	NS	<0.010	NS	<0.010	0.17	NS	0.06	0.01	6.25	33.25	31.25	99.5
Site 9	This study	Ga-Selati River 2014-2015	NS	<0.010	NS	<0.010	0.09	NS	0.04	0.03	19.2	40.5	59.25	110.5

2.5 Reference

AGTAS, S., GEY, H. & GUL, S. 2007. Concentrations of heavy metals in water and chub, Leuciscus cephalus (Linn.) from the river Yildiz, Turkey. *Journal of environmental biology* **28**: 845.

AKEN, W.R. 2013. *An assessment of the effects of water quality on the ichthyofauna of the Ga-Selati river, Limpopo, South Africa*. MSc Dissertation, University of Johannesburg

ALLARD, M. & MOREAU, G. 1987. Effects of experimental acidification on a lotic macroinvertebrate community. *Hydrobiologia* **144**: 37-49.

BARTRAM, J. & BALLANCE, R. 1996. *Water quality monitoring: a practical guide to the design and implementation of freshwater quality studies and monitoring programmes*. CRC Press.

BRITISH COLUMBIAN ENVIRONMENTAL PROTECTION DIVISION (BC-EPD). 2006. Water quality – A compendium of working water quality guidelines for British Columbia. In: Government of British Columbia Environmental Protection Division. http://www.env.gov.bc.ca/wat/wq/BCguidelines/working.html. Accessed March 2017

BRYAN, G. & LANGSTON, W. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. *Environmental pollution* **76**: 89-131.

BURTON, G.A. 2002. Sediment quality criteria in use around the world. *Limnology* **3**: 65-75.

CARDIANO, P., CHILLE, D., FOTI, C. & GIUFFRE, O. 2018. Effect of the ionic strength and temperature on the arsenic, Fe^{3+} and Al^{3+} interactions in aqueous solution. *Fluid Phase Equilibria* **458**: 9-15

CCME. 2012a. Canadian water quality guidelines for the protection of aquatic life and sediment quality guidelines for the protection of aquatic life. in*.* Canadian Council of Ministers of the Environment.

CCME. 2012b. Canadian water quality guidelines for the protection of aquatic life: nutrients, Cl, F, Al, As, B, Cd, Cr, Cu, Fe, Pb and Zn. in*.* Canadian Council of Ministers of the Environment.

CROWDER, A. 1991. Acidification, metals and macrophytes. *Environmental Pollution* **71**: 171-203.

DABROWSKI, J.M., DABROWSKI, J., HILL, L., MACMILLAN, P. & OBERHOLSTER, P.J. 2015. Fate, transport and effects of pollutants originating from acid mine drainage in the Olifants River, South Africa. *River Research and Applications* **31**: 1354-1364.

DALLAS, H.F. & DAY, J.A. 1993. The effect of water quality variables on riverine ecosystems: a review. Water Research Commission Report No. TT 61/93. Water Research Commission, Pretoria.

DALLAS, H.F. & DAY, J.A. 2004. *The effect of water quality variables on aquatic ecosystems: a review*. Water Research Commission Pretoria.

DALLAS, H.F & FOWLER, J. 2000. Delineation of river types for rivers of Mpumalanga, South Africa: establishing a spatial framework for selection of reference sites.

DALLAS, H.F. & RIVERS-MOORE, N.A. 2012. Critical thermal maxima of aquatic macroinvertebrates: towards identifying bioindicators of thermal alteration. *Hydrobiologia* **679**: 61-76.

DAVIES, B.R. & DAY, J.A. 1998. *Vanishing waters*. University of Cape Town Press, South Africa pp 487.

DODDS, W.K. 2002. *Freshwater ecology: concepts and environmental applications*. Academic press.

DU PREEZ, H., HEATH, R., SANDHAM, L. & GENTHE, B. 2003. Methodology for the assessment of human health risks associated with the consumption of chemical contaminated freshwater fish in South Africa. *Water SA.* **29**: 69-90.

DU PREEZ, H. & STEYN, G. 1992. A preliminary investigation of the concentration of selected metals in the tissues and organs of the tigerfish (*Hydrocynus vittatus*) from the Olifants River, Kruger National Park, South Africa. *Water SA.* **18**: 131-136.

DWAF (Department of Water Affairs and Forestry). 1996. South African Water Quality Guidelines: Volume 7: Aquatic Ecosystems, Second Edition. Pretoria, South Africa.

DWAF (Department of Water Affairs and Forestry). 2005. *Olifants River Water Resources Development Project. Environmental Impact Assessment*. Final Environmental Impact Report No 90. Department of Water Affairs and Forestry, Pretoria, South Africa.

EDMONDS, J.S. & FRANCESCONI, K.A. 1993. Arsenic in seafoods: human health aspects and regulations. *Marine Pollution Bulletin* **26**: 665-674.

GERBER, R., WEPENER, V. & SMIT, N. 2015. Application of multivariate statistics and toxicity indices to evaluate the water quality suitability for fish of three rivers in the Kruger National Park, South Africa. *African journal of aquatic science* **40**: 247-259.

GROBLER, D., KEMPSTER, P. & VAN DER MERWE, L. 1994. A note on the occurrence of metals in the Olifants River, Eastern Transvaal, South Africa. *Water SA* **20**: 195-204.

GUPTA, S.K. & SINGH, J. 2011. Evaluation of mollusc as sensitive indicator of heavy metal pollution in aquatic system: a review. IIOAB journal **2**: 49-57.

HARDING, J.S. 2005. Impacts of metals and mining on stream communities. Moore, TA, Black, A., Centeno, JA, Harding, JS and Trumm, DA (Eds.): 343-357.

HAWKINS, C.P., HOGUE, J.N., DECKER, L.M. & FEMINELLA, J.W. 1997. Channel morphology, water temperature, and assemblage structure of stream insects. *Journal of the North American Benthological Society*: 728-749.

HAYES, D. 1999. *Issues affecting fish habitat in the Great Lakes basin*. Michigan State University Press: East Lansing, MI. pp. 209-237

HEATH, R.G.M. & CLAASSEN, M.C. 1999. An Overview of the Pesticide and Metal Levels Present in Populations of the Larger Indigenous Fish Species of Selected South African Rivers. Report to the Water Research Commission, Pretoria. WRC Report No. 428/1/99. pp. 318

HEATH, R., COLEMAN, T. & ENGELBRECHT, J. 2010. Water quality overview and literature review of the ecology of the Olifants River. *WRC Report No. TT452* **10**.

HILTON, J., O'HARE, M., BOWES, M.J. & JONES, J.I. 2006. How green is my river? A new paradigm of eutrophication in rivers. *Science of the Total Environment* **365**: 66-83.

IBM Corp 2016. IBM SPSS statistics for windows, version 24.0 Armonk NY: IBM corp.

IRWIN, R.J., VAN MOUWERIK, M., STEVENS, L., DUBLER SEESE, M. & BASHAM, W. 1997. Arsenic. in IRWIN, R.J., VAN MOUWERIK, M., STEVENS, L., DUBLER SEESE, M. & BASHAM, W. (eds), *Environmental Contaminants Encyclopedia.* Water Resources Divisions, Water Operations Branch, Fort Collins, Colorado. pp. 2007.

KALTREIDER, R.C., DAVIS, A.M., LARIVIERE, J.P. & HAMILTON, J.W. 2001. Arsenic alters the function of the glucocorticoid receptor as a transcription factor. *Environmental Health Perspectives* **109**: 245-251.

KASANGAKI, A., CHAPMAN, L.J. & BALIRWA, J. 2008. Land use and the ecology of benthic macroinvertebrate assemblages of high-altitude rainforest streams in Uganda. *Freshwater Biology* **53**: 681-697.

KEMPSTER, P. & VAN VLIET, H. 1991. Water Quality Fitness for Domestic Water use: Draft Internal Report. *Hydrological Research Institute: Pretoria*.

KING, N., BOND, N.K.W., WISE, R. & BOND, I. 2008. *Fair deals for watershed services in South Africa*. Natural Resource Issues No. 12. IIED, London.

KLAUE, B. & BLUM, J.D. 1999. Trace analyses of arsenic in drinking water by inductively coupled plasma mass spectrometry: high resolution versus hydride generation. *Analytical Chemistry* **71**: 1408-1414.

KOTZÈ, P.J. 2012. *Aspects of water quality, metal contamination of sediment and fish in the Olifants River, Mpumalanga*. PhD dissertation, University of Johannesburg

MAINSTONE, C.P. & PARR, W. 2002. Phosphorus in rivers—ecology and management. *Science of the Total Environment* **282**: 25-47.

MALMQVIST, B. & RUNDLE, S. 2002. Threats to the running water ecosystems of the world. *Environmental conservation* **29**: 134-153.

MARR, S., MOHLALA, T. & SWEMMER, A. 2017. The ecological integrity of the lower Olifants River, Limpopo province, South Africa: 2009–2015–Part B: Tributaries of the Olifants River. *African Journal of Aquatic Science* **42**: 181-190.

MARX, H.M. 1996. *Evaluation of the Health Assessment Index with reference to metal bioaccumulation in Clarias gariepinus and aspects of the biology of the parasite Lamproglena clariae*. MSc Dissertation, Rand Afrikaans University, Johannesburg 249 pp.

MATLOU, K., ADDO-BEDIAKO, A. & JOOSTE, A. 2017. Benthic macroinvertebrate assemblage along a pollution gradient in the Steelpoort River, Olifants River System. *African Entomology* **25**: 445-453.

MCCAULEY, S.J., HAMMOND, J.I., FRANCES, D.N. & MABRY, K.E. 2015. Effects of experimental warming on survival, phenology, and morphology of an aquatic insect (O donata). *Ecological entomology* **40**: 211-220.

MHATRE, G. 1991. Bioindicators and biomonitoring of heavy metals. *Journal of Environmental Biology* **12**: 201-209.

MOSS, B.R. 2009. *Ecology of fresh waters: man and medium, past to future*. John Wiley & Sons.

OBERHOLSTER, P.J., ASHTON, P.J., BOTHA, A.M., DABROWSKI, J., DABROWSKI, J.M., DE KLERK, A.R., DE KLERK, L.P., GENTHE, B., HILL, L., LE ROUX, W., SCHACHTSCHNEIDER, K., L, S., SOMERSET, V. & WALTERS., C. 2012. *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*. Report to the Olifants River Forum. August 2012, CSIR report number: CSIR/NRE/WR/ER/2012/00051/B. CSIR Natural Resources and Environment, Pretoria 423 pp.

PALMER, C.G., R.S. BEROLD & W.J. MULLER. 2004. Environmental water quality in water resources management. WRC Report No. TT 217/04, Water Research Commission, Pretoria, South Africa.

POLLARD, S., & LAPORTE, A. 2015. *Living in Phalaborwa: a collaborative view of the system through the eyes of people*. Association for Water and Rural Development (AWARD), Hoedspruit, Limpopo, South Africa.

POLLING, L. 1999. *Ecological aspects of the Ga-Selati River, Olifants River system, Northenm province, Republic of South Africa.* PhD dissertation, University of the North, Peietersburg, South Africa: 312 pp.

POOR, C.J. & MCDONNELL, J.J. 2007. The effects of land use on stream nitrate dynamics. *Journal of Hydrology* **332**: 54-68.

PRASHANTH, L., KATTAPAGARI, K.K., CHITTURI, R.T., BADDAM, V.R.R. & PRASAD, L.K. 2015. A review on role of essential trace elements in health and disease. *Journal of dr. ntr university of health sciences* **4**: 75.

RAND, G.M. & PETROCELLI, S.R. 1985. Fundamentals of aquatic toxicology: methods and applications. in, FMC Corp., Princeton, NJ.

RELYEA, C.D., MINSHALL, G.W. & DANEHY, R.J. 2000. Stream insects as bioindicators of fine sediment. Proceedings of the Water Environment Federation 2000: 663-686

SALOMONS, W., DE ROOIJ, N., KERDIJK, H. & BRIL, J. 1987. Sediments as a source for contaminants? *Hydrobiologia* **149**: 13-30.

SEYMORE, T., DU PREEZ, H., VAN VUREN, J., DEACON, A. & STRYDOM, G. 1994. Variations in selected water quality variables and metal concentrations in the sediment of the lower Olifants and Selati Rivers, South Africa. *Koedoe* **37**: 1-18.

SMITH, V.H. & SCHINDLER, D.W. 2009. Eutrophication science: where do we go from here? *Trends in Ecology & Evolution* **24**: 201-207.

SOBALLE, D. & WEINER, J. 1998. Water and sediment quality. *USGS (ed.), Ecological Status and Trends of the Upper Mississippi River System*.

STANKOVIC, S., KALABA, P. & STANKOVIC, A.R. 2014. Biota as toxic metal indicators. *Environmental Chemistry Letters* **12**: 63-84.

VAN DER MERWE, M. 1992. Aspects of heavy metal concentration in the Olifants River, Kruger National Park and the effect of copper on the haematology of *Clarias gariepinus* (Clariidae). in*,* University of Johannesburg.

VAN VEELEN, M. 1990. *Kruger National Park-Assessment of current water quality status*.

VAN VUREN, J.H.J., DU PREEZ, H.H. & DEACON, A.R. 1994. *Effect of Pullants [ie Pollutants] on the Physiology of Fish in the Olifants River (Eastern Transvaal)*. Water Research Commission of South Africa.

WARD, J.V. & STANFORD, J.A. 1987. The ecology of regulated streams: past accomplishments and directions for future research. in *Regulated streams.* Springer, pp. 391-409.

WATERS, T.F. 1995. *Sediment in streams: sources, biological effects, and control*. American Fisheries Society.

WU, J.L., HO, C.R., HUANG, C.C., SRIVASTAV, A., TZENG, J.H. & LIN, Y.T. 2014. Hyperspectral sensing for turbid water quality monitoring in freshwater rivers: empirical relationship between reflectance and turbidity and total solids. *Sensors* **14**: 22670-22688.

ZHANG, Y., PULLIAINEN, J.T., KOPONEN, S.S. & HALLIKAINEN, M.T. 2003. Water quality retrievals from combined Landsat TM data and ERS-2 SAR data in the Gulf of Finland. *IEEE Transactions on Geoscience and Remote Sensing* **41**: 622-629.

Chapter 3: Macro-invertebrate distribution and diversity

3.1. Introduction

Aquatic macro-invertebrates have been traditionally used in the bio-monitoring of stream and river ecosystems for various environmental stresses, such as organic pollution, metals, hydromorphological degradation, nutrient enrichment, acidification and other stressors (Li *et al.* 2010). Water of suitable quality is essential to maintain healthy populations of aquatic organisms (Malan and Day, 2003). Each family has different environmental requirements and responds to changes in environmental factors (Mwedzi *et al*., 2016, Niedrist & Füreder, 2016). However, some species can tolerate a broad range of conditions, while others are very sensitive to their environmental conditions. Macro-invertebrate communities should occur at a site in the absence of any environmental stress in any type of stream (Dickens *et al.* 2018), often sensitive species decrease in abundance while tolerant species increase in abundance (Neumann & Dudgeon 2002). According to Markert *et al.* (1999) a bio-indicator is "an organism (or part of an organism or a community of organisms) that contains information on the quality of the environment (or a part of the environment)". Macro-invertebrates can therefore serve as good bio-indicators, providing an integrated measure of the quality of the aquatic environment (Dalu *et al.* 2017).

Land use has been found to be a strong predictor of biological habitat integrity (Allan *et al.* 1997). As the demand for land for urban and agricultural uses increases, habitats degradation increases, and therefore biodiversity is increasingly under threat (Turpie *et al.* 2008). Land use activities change physical, chemical, and biological structures and functioning of aquatic ecosystems, and in turn reduce benthic macro-invertebrate density and diversity (Penrose *et al.* 1980, Lenat & Crawford 1994, Kennen 1999, Thorpe & Lloyd 1999, Verschuren *et al.* 1999, Külköylüoğlu 2004).

Rivers flowing through human-impacted sites generally differ in water quality and macro-invertebrate composition from relatively undisturbed sites (Kasangaki *et al.* 2006). Physical habitat quantity and quality are important, since they can determine the structure and composition of biotic communities (Poff & Allan 1995, Ebrahimnezhad & Harper 1997, Sweeney & Newbold 2014, Battin *et al.* 2016). Aquatic organisms directly respond to aspects of the environment and are highly affected by habitat change/reduction (Relyea *et al*. 2000). In this chapter, aquatic macro-invertebrates were sampled at five different sites in the Ga-Selati River in order to access the state of water quality and compare with chemical measures of water quality.

3.2 Method and materials

3.2.1 Aquatic macro-invertebrate sampling

Aquatic macro-invertebrates were sampled at five of the nine sites in the Ga-Selati River where water and sediment chemistry was sampled (see chapter 2). The biotope types of each site are given in Table 1.4.1. These sites were selected due to the fact that they were more accessibility than the other sites for macro-invertebrates sampling. Five samples were collected during each season (in autumn (May 2014), winter (July 2014), spring (October 2014) and summer (January 2015. Macro-invertebrates sampling was terminated after the first sampling campaign at site 7, since the macro-invertebrate biotopes were compromised by a spill of acidic waste-water and intensive sand mining, and were not included in the results. Macro-invertebrate samples were collected by kick sampling the substrate using a 30 cm by 30 cm SASS net with a 1 mm mesh size. The stones in current with riffles were disturbed for a period of five minutes to free macro-invertebrates from the substrate. The macro-invertebrate samples were stored in 70% ethanol immediately after being sampled to prevent predacious invertebrates to prey on other invertebrates. Collected samples were carefully separated from the debris and stored in 70% alcohol.

3.2.2 Data analysis

The macro-invertebrates were sorted, identified to family level and counted in the University of Limpopo’s Biodiversity laboratory. The identification was done using a field guide manual with illustrations (Gerber & Gabriel 2002), with the aid of a stereomicroscope (Leica EZ4) and magnifying glass. For the classification of sensitivity the SASS5 sensitivity scores for individual taxa were used (Dickens and Graham, 2002): tolerant (score 1-5), moderately sensitive (6-10), and highly sensitive to pollution (11-15). The mean aquatic macro-invertebrate family abundance was calculated for each site from the four seasonal values. A two-way ANOVA on the Statistical Package and Service Solutions (IBM SPSS version 24) was used to determine whether there were any significant differences in macro-invertebrates abundance between the sites and seasons.

Multivariate analyses of family abundances were conducted using Primer E (Clarke & Warwick 2001). A similarity matrix was constructed using Bray Curtis similarity to compare the multivariate differences in communities between the sites and seasons. A non-metric multidimensional scaling (NMDS) plot was prepared from the similarity matrix to display graphical differences in invertebrate communities between sites and seasons. NMDS is an ordination method that preserves the rank-ordered distances between sample points in ordination space and for our purposes provided a useful approach for visualizing changes in faunal similarity over time. NMDS uses an iterative approach that rearranges samples in the ordination space to minimize a measure of disagreement (referred to as stress) between the compositional dissimilarities and the distance between the points. In two-dimensional NMDS ordinations, stress values <0.1 correspond to a good ordination with no real prospect of a misleading interpretation (Clarke & Warwick, 2001). Permutational multivariate analysis of variance (PERMANOVA) was also used to determine whether there were statistical differences between the sites or sampling seasons. Canonical correspondence analysis (CCA) was used to explore the relationship between macro-invertebrates and water quality parameters. CCA visualises a pattern of community variation and the main features of the distributions of species along the environmental variables. CCA can be used both for detecting species-environment relations, and for investigating specific questions about the response of species to environmental variables (Salmon *et al.* 2014).

3.3 Results

3.3.1 Macro-invertebrate community composition

During the study period (May 2014–January 2015), a total of 40 220 individual macro-invertebrates were sampled and identified. Macro-invertebrates varied in abundance and diversity across the sites. Site 9 had the highest number of individuals (21 519), followed by site 2 with 9 136 individuals (Table 3.3.1.1). Upstream sites (site 1 and site 2) had the highest number of families (35 and 39 respectively). Site 1 and site 9 had a highest number of orders (10 each), followed by site 2 and site 5 with nine orders (Figure 3.3.1.1.). The order Plecoptera was only present at upstream sites 1 and 2, contributing 0.1% and 0.4% respectively (Figure 3.3.1.1). Ephemeroptera, Trichoptera, Odonata, and Diptera were the only orders present at all the sites.

Table 3.3.1.1: Numbers of macro-invertebrates recorded per family and order at the four sites along Ga-Selati River during the year 2014-2015. A = autumn, W = winter, Sp = spring, S = summer.

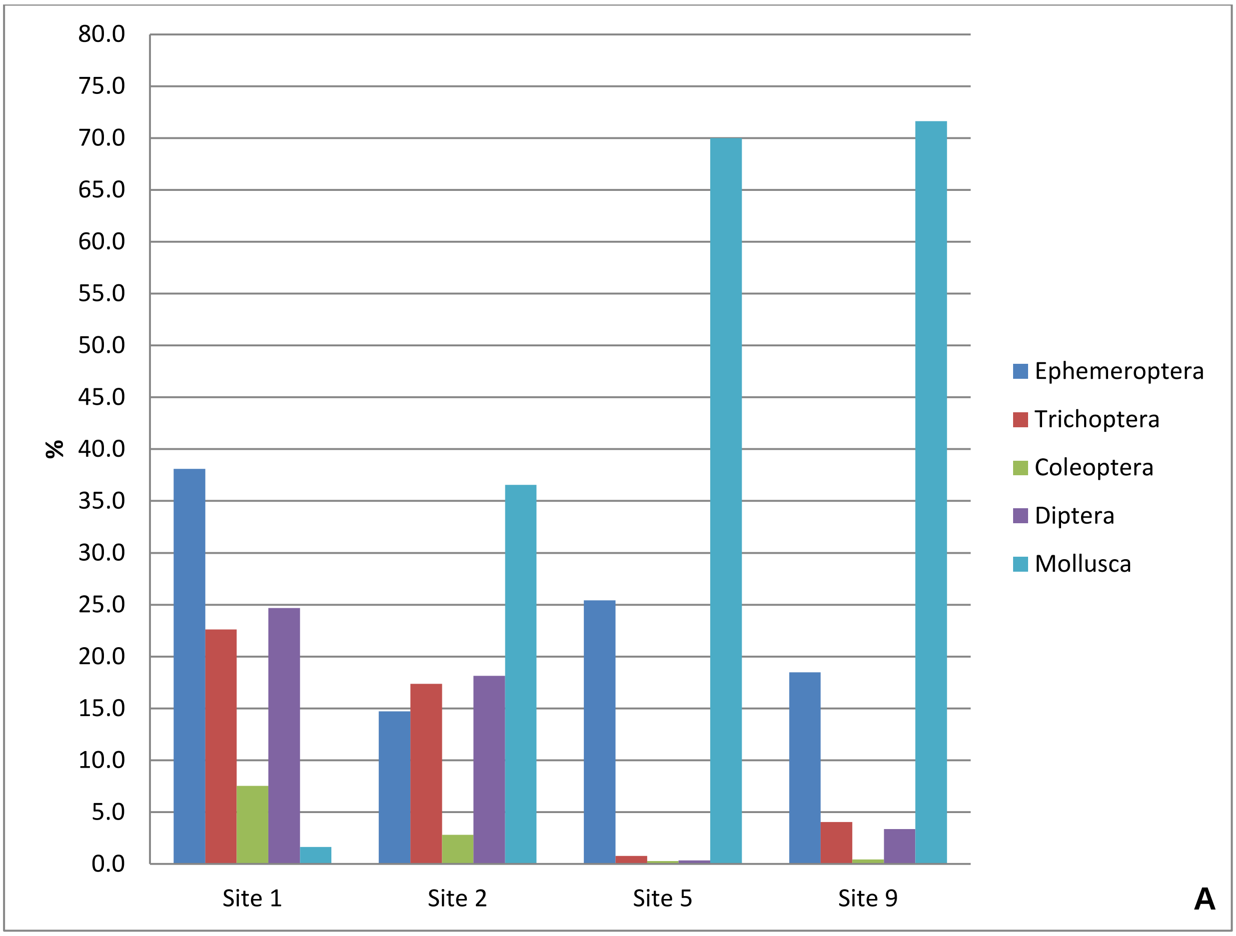
Order	Family	Site 1				Site 2				Site 5				Site 9			
		A	W	Sp	S	A	W	Sp	S	A	W	Sp	S	A	W	Sp	S
Ephemeroptera	Baetidae	177	29	115	17	94	22	47	7	68	17	0	1	630	507	2	1
	Caenidae	42	214	443	157	19	56	54	12	7	144	98	30	111	1552	856	24
	Polymitarcyidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Heptageniidae	6	1	2	2	316	203	0	0	0	3	0	0	0	0	0	0
	Telogonodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Oligoneuridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Prosopistomatidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Leptophlebiidae	49	52	32	17	203	296	0	8	362	549	0	0	107	185	2	0
	Tricorythidae	165	57	140	16	6	0	0	11	0	0	0	0	4	0	0	0
Trichoptera	Hydropsychidae	466	380	70	27	479	514	0	100	30	0	0	0	248	374	29	6
	Polycentropodidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Philopotamidae	31	33	10	0	323	118	0	3	0	0	2	0	8	6	0	0
	Ecomidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Psychomyiidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Petrothrincidae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Leptoceridae	2	1	0	0	0	0	0	4	0	0	0	0	0	0	0	0
	Hydroptilidae	0	1	0	0	0	0	0	0	0	0	1	0	2	174	0	0
	Pisuliidae	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Coleoptera	Dytiscidae	0	0	0	0	1	10	0	0	0	0	0	7	0	1	3	0
	Gyrinidae	2	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0
	Hydraenidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Elmidae	28	24	101	45	75	44	40	84	0	1	2	4	17	22	36	9
	Helodidae	0	1	4	0	0	0	0	1	0	0	0	0	0	0	8	0
	Psephenidae	53	18	32	33	0	0	0	0	0	0	0	0	0	0	0	0
	Hydrophilidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hemiptera	Naucoridae	0	0	0	0	1	0	0	0	0	0	0	0	0	1	5	0
	Notonectidae	0	0	0	0	0	0	6	0	0	0	3	1	0	0	0	0
	Belostomatidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Gerridae	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
	Hydrometridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Corixidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Veliidae	0	0	0	0	0	1	0	0	1	0	0	0	34	0	0	0
	Nepidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Pleidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Odonata	Libellulidae	34	16	2	0	218	180	131	35	31	3	43	11	71	101	36	1
	Corduliidae	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
	Aeshnidae	11	0	3	9	0	1	0	0	0	0	0	0	0	0	0	0
	Gomphidae	14	6	10	22	21	11	39	0	8	1	1	0	133	20	1	0
	Calopterygidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Chlorocyphidae	20	43	6	6	65	63	1	1	0	0	0	0	0	0	0	1
	Platycnemididae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	Coenagrionidae	0	0	0	0	1	4	15	1	1	40	0	0	3	11	1	0
	Chlorolestidae	0	0	0	0	0	1	4	0	3	9	0	0	0	0	0	0
	Lestidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diptera	Athericidae	246	80	14	13	44	37	93	0	0	0	0	0	0	1	0	0
	Culicidae	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Tabanidae	10	8	4	23	56	44	9	25	0	1	0	1	13	5	14	2
	Psychodidae	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	Dixidae	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Chirinomidae	134	483	25	24	283	260	3	2	2	2	11	1	53	376	4	0
	Ceratopogonidae	2	11	3	2	0	0	0	0	0	0	0	0	2	1	0	0
	Muscidae	0	0	0	0	0	1	0	0	0	0	0	0	2	70	57	2
	Ephydridae	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	Syrphidae	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	Tipulidae	6	2	2	7	0	0	0	0	0	0	0	0	0	0	0	0
	Simulidae	16	1	1	0	173	634	0	3	0	0	0	0	14	106	0	0
Turbellaria	Planaria	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plecoptera	Perlidae	1	0	4	0	17	22	0	2	0	0	0	0	0	0	0	0
Lepidoptera	Pyralidae	6	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Megaloptera	Corydalidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crustacea	Potamonautidae	2	1	10	8	0	0	0	0	1	1	0	0	0	0	1	5

	Palaemonidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Amphipoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Atyidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Annelida	Hirudinea	0	1	5	0	8	59	22	0	0	0	1	0	0	4	3	0
	Oligochaeta	5	0	0	0	7	3	11	0	0	0	0	0	2	0	2	0
Mollusca	Physidae	0	0	0	0	0	0	0	3	0	0	0	0	3	0	13	0
	Lymnaeidae	0	0	0	0	0	0	6	12	0	0	0	0	0	1	0	0
	Planorbidae	11	9	1	13	1	0	68	31	0	0	0	1	0	0	0	0
	Ancylidae	0	0	20	20	0	0	0	0	0	0	0	0	0	0	0	0
	Thiaridae	0	0	0	0	12	0	34	200	126	6	178	2888	700	25	123	10728
	Unionidae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	Sphaeriidae	0	0	0	0	1	0	0	0	92	13	3	0	5	0	0	0
	Corbiculidae	0	0	0	0	285	55	23	2630	183	1	1	29	644	294	63	2832
Total number of individuals		1540	1476	1065	461	2711	2642	608	3175	915	791	345	2974	2808	3840	1260	13611

Site 1 was dominated by the order Ephemeroptera, followed by Diptera. The same number of families within the Ephemeroptera were recorded throughout the study at site 1 (Table 3.3.1.1), indicating a less variation in water quality throughout the year at this site. The family Athericidae was a major contributor in the Diptera order at site 1. The order, Ephemeroptera had less families compared to Diptera, but had more individuals. Site 5 and 9 had a high number of Mollusca, with this order contributing more than 70% to the total number of individuals recorded.



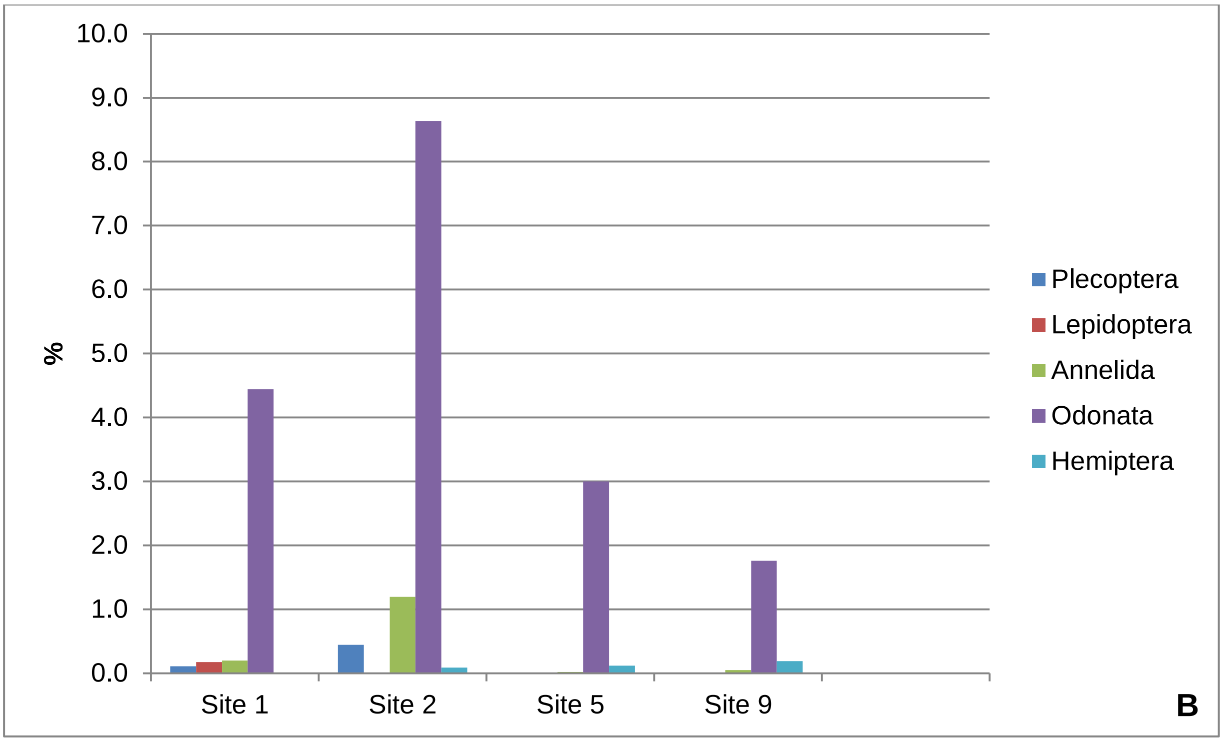


Figure 3.3.1.1: The relative abundance of the most common (A) and least common (B) macro-invertebrate orders, averaged across the four sample sites (May 2014 to January 2015).

The relative abundance of Coleoptera was higher at the upstream sites (7.5% at site 1 and 2.8% at site 2), but lower at the downstream sites, contributing only 0.3% at site 5, and 0.4% at site 9. Figure 3.3.1.2 A & B show seasonal influence on the number of individuals in each order. Seasonal variation was particularly large for Mollusca, as a result of the extremely high abundance of Thiaridae at site 9 during summer. The orders Plecoptera, Ephemeroptera and Annelida were more abundant in winter than summer, while Mollusca and Crustacea were highest during summer.

Site 1 had the highest number of families and site 5 had the lowest families recorded compared to other sites (Figure 3.3.1.3). Site 1 had the highest number of families during spring and site 2 had the highest number of families in autumn. The results showed that the numbers of families at site 9 were decreasing after each sampling (however the number of families were equal during autumn and winter). The lowest number of families recorded at site 9 was during summer.

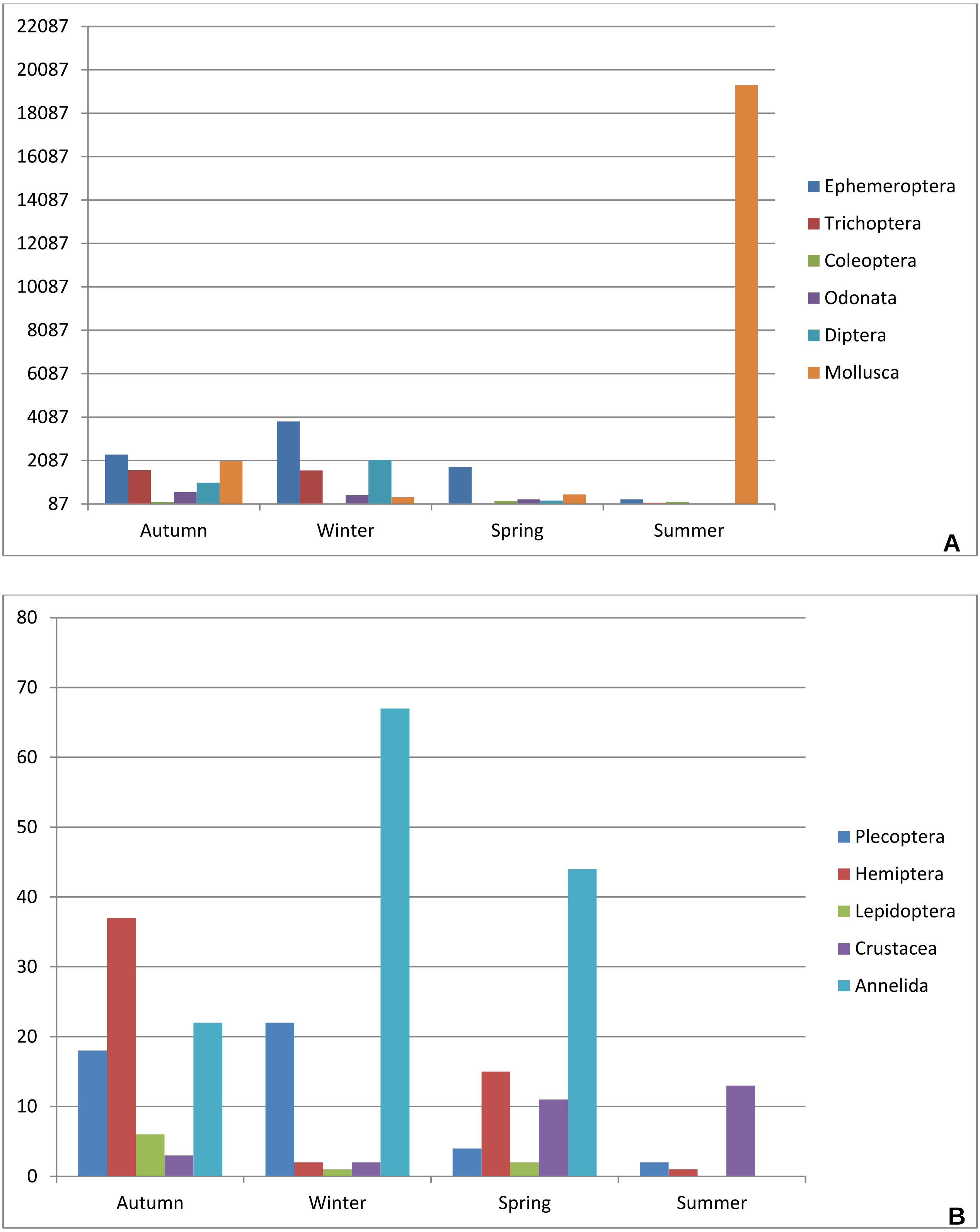


Figure 3.3.1.2: Seasonal variation in the abundances of macro-invertebrate orders at the four sites sampled along the Ga-Selati River (May 2014 to January 2015). Bars show total individuals sampled at all sites **A** shows the dominant macro-invertebrates orders, **B** shows less common orders.

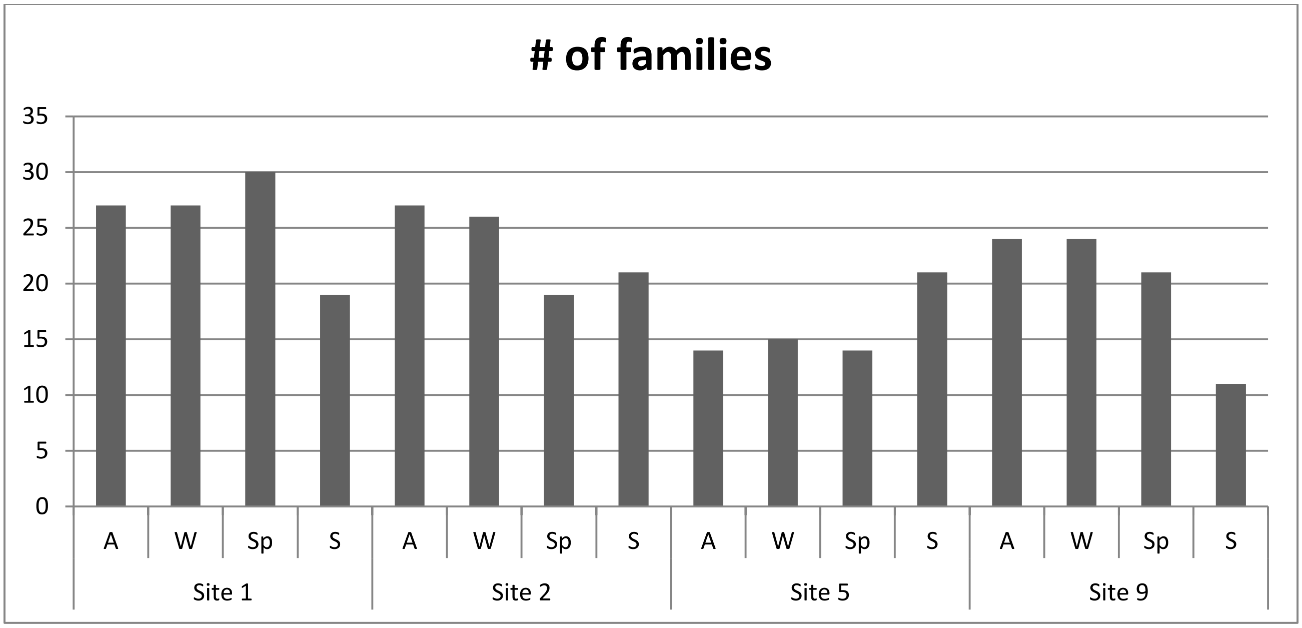


Figure 3.3.1.3: Seasonal variation of the abundances of macro-invertebrate families (May 2014 to January 2015).

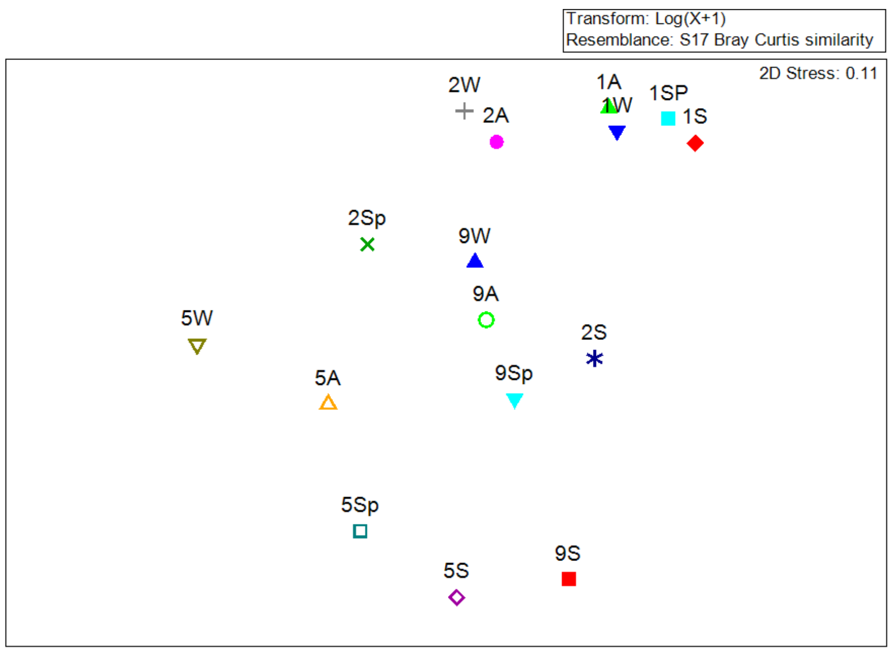


Figure 3.3.1.4: NMDS plot indicating similarities and dissimilarities of macro-invertebrate families between sites in the Ga-Selati River. The stress value was 0.1 and each point represents a site number and different seasons (A = autumn, W = winter, Sp = spring, S = summer).

The NMDS revealed clear clustering of sites, with the least variation for site 1. SIMPER analysis gave an average of 67.48% dissimilarity between sites 1 and site 2 (Appendix 3 A-F) The dissimilarity between site 1 and the rest of the sites was mainly due to the absence of Corbiculidae and Thiaridae and the presence of Perlidae. Site 1 had the highest average abundance of Hydropsychidae 3.17%. There was 79.82% average dissimilarity between sites 1 and site 5. This dissimilarity was due to 3.24% average abundance contributed by Thiaridae at site 5. Site 1 had 2.37% average abundance of Tricorythidae, 2.27% average abundance of Athericidae, while there were no Tricorythidae and Athericide recorded at site 5. There was 67.48 % dissimilarity between site 1 and site 2; this was mainly due by the absence of Corbiculidae at site 1 and low abundance of Hydropsychidae at site 2. There was 75% average dissimilarity between site 1 and site 9. This significant dissimilarity was due to absence of Corbiculidae and Thiaridae at site 1; whereas at site 9 Corbiculidae had 3.99% average abundance and Thiaridae had 4.17% average abundance. The average dissimilarity between site 2 and site 9 was 63.15%, and was mainly due to the high abundance of Thiaridae and Caenidae at site 9. The dissimilarity between site 2 and site 5 was 73.74%; this was due to the absence of Athericidae and Simulidae at site 5. Dissimilarity between site 5 and site 9 was 64.34%.

The CCA results showed that there was a significant difference among macro-invertebrate families and water variables ($p = 0.0020$, $F = 2.51$). The sum of all eigenvalues was 0.582 (Table 3.3.1.2). The CCA showed that the families Baetidae, Gomphidae, Leptophlebiidae, Caenidae and Sphaeriidae are negatively correlated to salinity, P and the nutrients (Figure 3.3.1.7). Iron and dissolved oxygen were positively correlated to families such as chironomidae, Hydropsychidae, Tricorythidae, Psephenidae and Athericidae. Zinc was positively correlated to the families Tabanidae, philopotamidae, Elmidae, Planorbidae and Chlorocyphidae. The pH, TDS, EC, salinity and temperature were positively correlated to Hydroptilidae, Muscidae, Corbiculidae, Simulidae and Thiaridae.

Table 3.3.1.2: Eigenvalues of the correlation matrix of the species-environment relationship. Between macro-invertebrates and water parameters.

Macro-invertebrates and water parameters	Axis 1	Axis 2	Axis 3	Axis 4	Total Inertia
Eigenvalues	0.285	0.114	0.099	0.084	0.582
Species-environment correlations	1.000	1.000	1.000	1.000	
Cumulative percentage variance *of species data	48.9	68.5	85.6	100.0	
*of species-environment relation	48.9	68.5	85.6	100.0	
Sum of all eigenvalues					0.582
Sum of all canonical eigenvalues					0.582

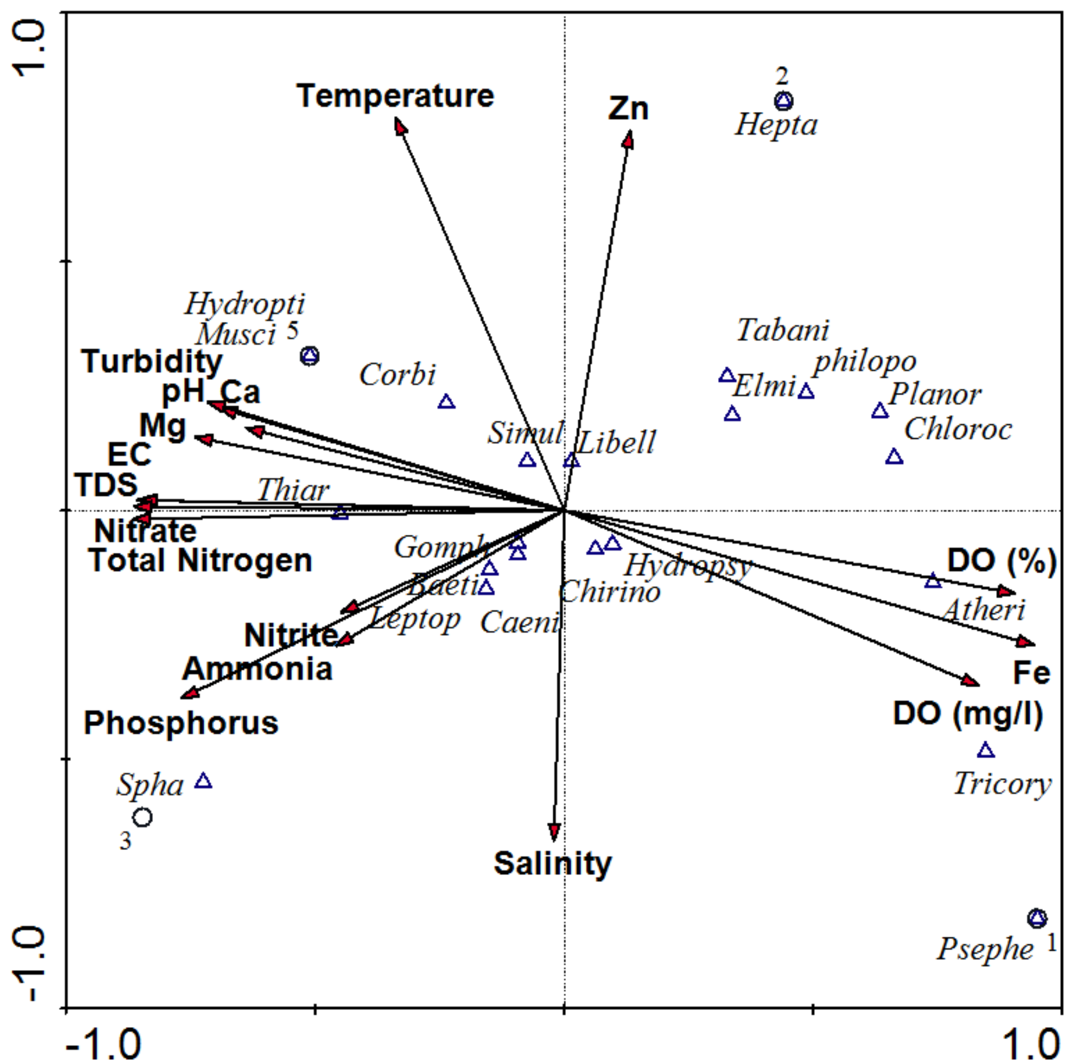


Figure 3.3.1.7: CCA plot of the relationship between water quality parameters and macro-invertebrate families, for the four sites sampled in the Ga-Selati River. Dots show sites and triangles show families. 1 = *site 1,* 2 = *site 2,* 3 = *site 5, &* 5 = *site 9.* Atheri = *Athericidae,* Baeti = *Baetidae,* Caeni = *Caenidae,* Chirino = *Chirinomidae,* Chloroc = *Chlorocyphidae,* Corbi = *Corbiculidae,* Elmi = *Elmidae,* Gomph = *Gomphidae,* Hepta = *Heptageniidae,* Hydrophil = *Hydrophilidae,* Hydropsy = *Hydropsychidae,* Hydropti = *Hydroptilidae,* Leptop = *Leptophlebiidae,* Libell = *Libellulidae,* Musci = *Muscidae*, Philopo = *Philopotamidae*, Planor = *Planorbidae*, Psephe = *Psephenidae* Simul = *Simulidae* Spha = *Sphaeriidae,* Tabani = *Tabanidae,* Tricory = *Tricorythidae,* Thiar = *Thiaridae.*

3.4 Discussion

The upstream sites (site 1 and site 2); which showed relatively good water quality in chapter 2 had the highest abundance of families. High macro-invertebrate family diversity reflects good/fair water quality conditions and low diversity expresses low water quality (Lenat & Crawford 1994, Kartikasari 2013, Kidd *et al.* 2014). Declines in water quality, would result in the loss of sensitive macro-invertebrate taxa (Mcclain *et al.* 2014). Even though site 1 and site 2 had the highest abundance of families, there was a difference in the types of families present at these two sites. Site 2 had 15 different families (Appendix 2 B) which were not present at site 1. While, site 1 had nine different families (Appendix 2 A) which were not present at site 2, an indication of different environmental conditions. Changes in diversity could be attributable to the difference across sites (i.e. habitat diversity and land use activities) (Table 1.4.1).

Different taxa of macro-invertebrates differ in their physical habitat requirements (Dallas & Day, 1993). The variation in macro-invertebrate composition at different sites is therefore a combination of water chemistry and physical conditions (Khoza *et al.* 2012, Wolmarans *et al.* 2014). Physical conditions include habitat availability, flow rate and seasonal variation. Site 1 was characterised by clear and low nutrient-concentrations (see chapter 2), with many medium-sized boulders and riffles. The riparian vegetation formed a canopy around the stream which reduced light penetration. This habitat may be more favourable to the more sensitive macro-invertebrate families and may therefore partly explain the greater abundance of these taxa at this site.

Distribution and diversity of macro-invertebrates in a river system can be influenced by anthropogenic activities (Edegbene & Arimoro 2012, Zajac *et al.* 2013). The low family richness at site 5 is more likely due to high level of anthropogenic disturbances at the site. Kasangaki *et al* (2006) found that richness and total invertebrate abundance were lower at high disturbance sites than at the minimally disturbed sites. The sand mining activities at site 5 are increasing sediment loads in the stream, and this is affecting the flow regime of the river. Different macro-invertebrate respond differently to flow variability (Monk *et al.* 2018) and flow reduction has been found to reduce macro-invertebrate abundance (Mcintosh *et al*. 2002, Dewson *et al*. 2007). Land use change has also been found to be one of the main drivers of change in stream flow (Mwangi *et al.* 2016). This might be the cause of low macro-invertebrate abundance in this site. The fact that site 5 showed the lowest mean family richness throughout the study could also be linked to poor habitat diversity, as this site was characterised by high sedimentation. This site lacked habitat diversity (no stones in current or riffle and less vegetation); it consisted mainly of gravel.

The Ephemeroptera, Plecoptera and Trichoptera (EPT) group was much higher upstream, where there is less sedimentation, than downstream. The richness for the intolerant EPT consistently declined with sediment addition (Lenat 1983, Kefford *et al.* 2010, Ramezani *et al.* 2014). Increased sediment load can also affect flow regime patterns, which can eliminate macro-invertebrate communities (Jeffries & Mills 1990). Sand mining at site 5 had altered the water flow in such a way that it was resulting into a pool at this site. Mcclain *et al.* (2014) found that the diversity of macroinvertebrates were generally greater during high-flow compared to low-flow conditions

The results showed that most of the families found at site 9 are highly tolerant to pollution. Site 9 had 37 families, which is only one family less than site 1. Site 1 had 14 different families which were not present at site 9, and six of these families have a sensitivity score of 11-15 which means they have a low tolerance to pollution (Gerber & Gabriel 2002). Site 9 also had 14 different families which were not present at site 1. All these families are highly tolerant to pollution except for Naucoridae and Unionidae, which are moderately tolerant to pollution (Gerber & Gabriel 2002). The high family abundance at site 9 might be due to the presence of medium-sized boulders, riffles, fine gravel and surrounding vegetation at this site, which reflects high habitat diversity.

The family Perlidae was the only family present under the order Plecoptera. This family was only recorded at site 1 and site 2 .Perlidae is sensitive towards environmental pollutants and thus plays an important role as indicator organisms in water quality assessments. (Popijač & Sivec 2009, Elbrecht *et al.* 2015). Judging from the absence of Perlidae downstream shows pollution in the Ga-Selati River downstream. This family has also been found to decline in density in response to anthropogenic disturbance (Relyea *et al.* 2000) and pollution (Lenat, 1983). High abundance of Chironomidae was recorded at site 1 and site 2. Chironomidae were up to a thousand times higher in the area which was strongly contaminated by mine tailings (Smolders *et al.* 2003), however in this study high abundance of chironomidae were recorded at the site which reflected good/fair water quality. Another study found that Chironomidae became dominant at the expense of sensitive macro-invertebrate groups such as Ephemeroptera and Plecoptera (Kiffney & Clements 1994, Mandaville 1999). This might explain the low abundance of Perlidae at site1, where chironomidae abundance was highest. A detailed analysis of the Chironomidae community in the upper Ga-Selati River is needed to explain this anomaly.

Corbiculidae and Thiaridae were the most abundant macro-invertebrate families collected in this study and were present at all sites except site 1. Thiaridae were represented the highest numbers of all the organisms collected during the course of this study and was recovered mainly at site 5 and site 9. Thiaridae were found to be a dominant taxa in the Ga-Selati River, and one of the two species collected is an exotic invader species (Wolmarans *et al.* 2014) A plausible reason for the abundance of Thiaridae at site 9 is their high tolerance to pollution (Dickens & Graham 2002). Another factor might be the habitat preferences of this family, which includes slow current speed < 0.1 m/s; (Wolmarans *et al.* 2014), organic enrichment and the presence of aquatic vegetation (Thirion 2007). Thiaridae are known to prefer warmer climates, although can survive in water with temperatures ranging from 0 to 47°C (Miranda *et al.* 2010). This may also explain their greater abundance at the sites which had higher temperatures (sites 2, 5 and 9; see Table 2.3.1.1, Chapter 2). High number of Thiaridae was also observed at site 2 in backwaters during the sampling campaign, which are likely to be warmer than the main stream. An association between Thiaridae and temperature was also indicated by the CCA (Figure 3.3.1.7).

The order Coleoptera is a good bio-indicator for trace elements in impacted and non-impacted environments and can be used in environmental monitoring (Burghelea *et al.* 2011). The most abundant families recorded in the order Coleoptera were Elmidae and Psephenidae, both of which are considered to be moderately tolerant to pollution (Dickens & Graham 2002). The family Psephenidae was only found at site 1, while

Elmidae were only present at site 1 and site 2. The reason why they were less abundant downstream might be due to slightly low dissolved oxygen concentration. The CCA showed that the family Psephenidae is positively correlated to dissolved oxygen. Clements (1994) identified that the majority of Ephemeroptera (mayflies) and Plecoptera (stoneflies) species were sensitive to metal contamination. This can explain the abundance of these two families at site 1 and site 2 where most metal (Ca, Mn, Na, K and B) concentrations were lower. These two sites also had lower salinity, and nutrient values compared to the rest of the sites. The presence of Ephemeroptera is also believed to be an important environmental indicator of oligotrophic to mesotrophic conditions in running waters (Barbour *et al*. 1999, Bauernfeind & Moog 2000). It was found that Ephemeroptera diversity declined due to increased nutrient levels (Ngodhe *et al.* 2014). The high abundance of Ephemeroptera at site 1 might also be due to lower nutrient concentration. The nutrient results in chapter 2 showed that site 1 represented an oligothrophic condition.

The lower reaches of Ga-Selati River did not have any sensitive families due to the poor water quality, which was most likely as a result of run-off from the various anthropogenic activities in the lower catchment, particularly the mining at Phalaborwa. The River Health Programme report (RHP 2005) on the Olifants River catchment in the Limpopo province found that the lower Ga-Selati River reflected a largely impaired condition, with no sensitive families present. This was attributed to the poor water quality from agricultural and mining activities. They also found high diversity of aquatic invertebrates upstream which reflected good water quality. Thus, for a decade the poor condition at downstream of Ga-Selati River has not changed.

There was seasonal variation in water quality from sites 2 to 9, which leads to more scatter in the ordination plot. Only site 1 showed the clustering of the samples, because there are no major anthropogenic activities at this site. Furthermore, the high number of families at sites 2 and 9 may be partly due to the high diversity of habitat types (stones in current, gravel, sand, mud and aquatic vegetation). Site 2 had more stone biotopes, and this could be the reason the site had the highest percentage (48.1%) of Odonata especially in winter where there was less sediments from run-off. The order Odonata is known to prefer stony habitat (Gerber & Gabriel 2002).

The CCA showed the correlation between macro-invertebrate families and environmental variables. The high tolerant families (Muscidae, Thiaridae, Corbiculidae and Simulidae) were associated with high concentrations of TDS and EC at site 9 which had low dissolved oxygen concentrations. Polluted sites support highly tolerant taxa (Thorne *et al.* 2000). High populations of taxa that belong to the family Corbiculidae have been found in the location where the dissolved oxygen concentration was the lowest (Nguyen & De Pauw 2002). High tolerant families (Chironomidae and Hydropsychidae) and moderately tolerant families (Tricorythidae, Psephenidae and Athericidae) were associated with high dissolved oxygen at site 1. Chironomidae has been found to have significant correlation with dissolved oxygen (Thorne *et al.* 2000). Even though Chironomidae is highly tolerant to pollution, it seems to depend on dissolved oxygen. Chironomidae, mortality were observed when oxygen concentrations were below 8% saturation (Connolly *et al.* 2004). Baetidae, Caenidae and Sphaeridae were correlated with high nutrient concentrations at site 5. Baetidae is a family with several species, some of them are moderately tolerant to nutrients (Justus *et al.* 2010, Ratia *et al.* 2012, Xu *et al*. 2014). The Caenidae has been reported to be tolerant pollution (Menetrey *et al.* 2007) and this might explain their high abundance at site 5 and site 9 were EC, TDS, P, NO_3- and TN concentrations were higher.

3.5 References

ALLAN, J.D., ERICKSON, D.L. & FAY, J. 1997. The influence of catchment land use on stream integrity across multiple spatial scales. *Freshwater Biology* **37**: 149-161.

BARBOUR, M.T., GERRITSEN, J., SNYDER, B. & STRIBLING, J. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers. *USEPA, Washington*.

BATTIN, T.J., BESEMER, K., BENGTSSON, M.M., ROMANI, A.M. & PACKMANN, A.I. 2016. The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology* **14**: 251.

BAUERNFEIND, E. & MOOG, O. 2000. Mayflies (Insecta: Ephemeroptera) and the assessment of ecological integrity: a methodological approach. in *Assessing the Ecological Integrity of Running Waters.* Springer, pp. 71-83.

BURGHELEA, C.I., ZAHARESCU, D.G., HOODA, P.S. & PALANCA-SOLER, A. 2011. Predatory aquatic beetles, suitable trace elements bioindicators. *Journal of Environmental Monitoring* **13**: 1308-1315.

CLARKE, K. & WARWICK, R. 2001. An approach to statistical analysis and interpretation. *Change in Marine Communities.*

CLEMENTS, W.H. 1994. Benthic invertebrate community responses to heavy metals in the Upper Arkansas River Basin, Colorado. *Journal of the North American Benthological Society*: 30-44.

CONNOLLY, N., CROSSLAND, M. & PEARSON, R. 2004. Effect of low dissolved oxygen on survival, emergence, and drift of tropical stream macroinvertebrates. *Journal of the North American Benthological Society* **23**: 251-270.

DALLAS, H.F. & DAY, J.A. 1993. *The effect of water quality variables on riverine ecosystems: A review*. Freshwater Research Unit, University of Cape Town.

DALLAS, H. 2007. River health programme: South African Scoring System (SASS) data interpretation guidelines. *Report produced for the Department of Water Affairs and Forestry (Resource Quality Services) and the Institute of Natural Resources*.

DALU, T., WASSERMAN, R.J., TONKIN, J.D., MWEDZI, T., MAGORO, M.L. & WEYL, O.L. 2017. Water or sediment? Partitioning the role of water column and sediment chemistry as drivers of macro-invertebrate communities in an austral South African stream. *Science of the Total Environment* **607**: 317-325.

DICKENS, C.W. & GRAHAM, P. 2002. The South African Scoring System (SASS) version 5 rapid bioassessment method for rivers. *African Journal of Aquatic Science* **27**: 1-10.

DICKENS, C., COX, A., JOHNSTON, R., DAVISON, S., HENDERSON, D., MEYNELL, P. & SHINDE, V. 2018. Monitoring the health of the Greater Mekong's Rivers.

DEWSON, Z.S., JAMES, A.B. & DEATH, R.G. 2007. A review of the consequences of decreased flow for instream habitat and macroinvertebrates. *Journal of the North American Benthological Society* **26**: 401-415.

EBRAHIMNEZHAD, M. & HARPER, D.M. 1997. The biological effectiveness of artificial riffles in river rehabilitation. *Aquatic Conservation: Marine and Freshwater Ecosystems* **7**: 187-197.

EDEGBENE, A. & ARIMORO, F. 2012. Ecological Status of Owan River, Southern Nigeria Using Aquatic Insects as Bioindicators. *Journal of Aquatic Sciences* **27**: 99-111.

GERBER, A. & GABRIEL, M.J.M. 2002. Aquatic invertebrates of South African Rivers. Illustrations. Institute for Water Quality Studies. Department of Water Affairs and Forestry, Pretoria.

JEFFRIES, M. & MILLS, D. 1990. *Freshwater ecology: principles and applications*. Belhaven Press.

JUSTUS, B., PETERSEN, J.C., FEMMER, S.R., DAVIS, J.V. & WALLACE, J. 2010. A comparison of algal, macroinvertebrate, and fish assemblage indices for assessing low-level nutrient enrichment in wadeable Ozark streams. *Ecological Indicators* **10**: 627-638.

KASANGAKI, A., BABAASA, D., EFITRE, J., MCNEILAGE, A. & BITARIHO, R. 2006. Links between anthropogenic perturbations and benthic macroinvertebrate assemblages in Afromontane forest streams in Uganda. *Hydrobiologia* **563**: 231-245.

KARTIKASARI, D. 2013. Application of water quality and ecology indices of benthic macroinvertebrate to evaluate water quality of tertiary irrigation in Malang District. *Journal of Tropical Life Science* **3**: 193-201.

KENNEN, J.G. 1999. RELATION OF MACROINVERTEBRATE COMMUNITY IMPAIRMENT TO CATCHMENT CHARACTERISTICS IN NEW JERSEY STREAMS1. in*,* Wiley Online Library.

KHOZA, Z., POTGIETER, M. & VLOK, W. 2012. A preliminary survey of biotic composition of the Olifantspruit catchment, South Africa. *African Journal of Aquatic Science* 37: 201-208.

KIDD, K.R., AUST, W.M. & COPENHEAVER, C.A. 2014. Recreational stream crossing effects on sediment delivery and macroinvertebrates in southwestern Virginia, USA. *Environmental management* **54**: 505-516.

KIFFNEY, P.M. & CLEMENTS, W.H. 1994. Effects of heavy metals on a macroinvertebrate assemblage from a Rocky Mountain stream in experimental microcosms. *Journal of the North American Benthological Society*: 511-523.

KÜLKÖYLÜOĞLU, O. 2004. On the usage of ostracods (Crustacea) as bioindicator species in different aquatic habitats in the Bolu region, Turkey. *Ecological Indicators* **4**: 139-147.

LENAT, D.R. 1983. Chironomid taxa richness: natural variation and use in pollution assessment. Freshwater Invertebrate Biology: 192-198.

LENAT, D.R. & CRAWFORD, J.K. 1994. Effects of land use on water quality and aquatic biota of three North Carolina Piedmont streams. *Hydrobiologia* **294**: 185-199.

LI, L., ZHENG, B. & LIU, L. 2010. Biomonitoring and bioindicators used for river ecosystems: definitions, approaches and trends. *Procedia environmental sciences* **2**: 1510-1524.

MALAN, H., BATH, A., DAY, J. & JOUBERT, A. 2003. A simple flow-concentration modelling method for integrating water quality and water quantity in rivers. *Water SA* **29**: 305-312

MANDAVILLE, S. 1999. *Bioassessment of Freshwaters Using Benthic Macroinvertebrates: A Primer*. Soil & Water Conservation Society of Metro Halifax.

MARKERT, B., WAPPELHORST, O., WECKERT, V., HERPIN, U., SIEWERS, U., FRIESE, K. & BREULMANN, G. 1999. The use of bioindicators for monitoring the heavy-metal status of the environment. *Journal of Radioanalytical and Nuclear Chemistry* **240**: 425-429.

MCCLAIN, M.E., SUBALUSKY, A.L., ANDERSON, E.P., DESSU, S.B., MELESSE, A.M., NDOMBA, P.M., MTAMBA, J.O., TAMATAMAH, R.A. & MLIGO, C. 2014. Comparing flow regime, channel hydraulics, and biological communities to infer flow–ecology relationships in the Mara River of Kenya and Tanzania. *Hydrological Sciences Journal* **59**: 801-819

MCINTOSH, M.D., BENBOW, M.E. & BURKY, A.J. 2002. Effects of stream diversion on riffle macroinvertebrate communities in a Maui, Hawaii, stream. *River Research and Applications* **18**: 569-581.

MENETREY, N., OERTLI, B., SARTORI, M., WAGNER, A. & LACHAVANNE, J. 2007. Eutrophication: are mayflies (Ephemeroptera) good bioindicators for ponds? in *Pond Conservation in Europe.* Springer, pp. 125-135.

MIRANDA, N.A., PERISSINOTTO, R. & APPLETON, C.C. 2010. Salinity and temperature tolerance of the invasive freshwater gastropod Tarebia granifera. *South African Journal of Science* **106**: 01-07.

MWANGI, H.M., JULICH, S., PATIL, S.D., MCDONALD, M.A. & FEGER, K.H. 2016. Relative contribution of land use change and climate variability on discharge of upper Mara River, Kenya. *Journal of Hydrology: Regional Studies* **5**: 244-260.

MWEDZI, T., BERE, T. & MANGADZE, T. 2016. Macroinvertebrate assemblages in agricultural, mining, and urban tropical streams: implications for conservation and management. *Environmental science and pollution research* **23**: 11181-11192.

NEUMANN, M. & DUDGEON, D. 2002. The impact of agricultural runoff on stream benthos in Hong Kong, China. *Water Research* **36**: 3103-3109.

NGODHE, S.O., RABURU, P.O. & ACHIENG, A. 2014. The impact of water quality on species diversity and richness of macroinvertebrates in small water bodies in Lake Victoria Basin, Kenya. *Journal of Ecology and the Natural Environment* **6**: 32-41.

NGUYEN, L.T. & DE PAUW, N. 2002. The invasive Corbicula species (Bivalvia, Corbiculidae) and the sediment quality in Flanders, Belgium. *Belgian journal of zoology* **132**: 41-48.

NIEDRIST, G.H. & FÜREDER, L. 2016. Towards a definition of environmental niches in alpine streams by employing chironomid species preferences. *Hydrobiologia* **781**: 143-160.

PENROSE, D., EAGLESON, K. & LENAT, D. 1980. *Biological evaluation of water quality in North Carolina streams and rivers*. Department of Natural Resources and Community Development, Division of Environmental Management, Technical Services Branch, Biological Monitoring Group.

POFF, N.L. & ALLAN, J.D. 1995. Functional organization of stream fish assemblages in relation to hydrological variability. *Ecology* **76**: 606-627.

POPIJAČ, A. & SIVEC, I. 2009. Diversity and distribution of stoneflies in the area of Plitvice Lakes National Park and along the Mediterranean river Cetina (Croatia). *Aquatic Insects* **31**: 731-742

RAMEZANI, J., RENNEBECK, L., CLOSS, G.P. & MATTHAEI, C.D. 2014. Effects of fine sediment addition and removal on stream invertebrates and fish: a reach-scale experiment. *Freshwater Biology* **59**: 2584-2604.

RATIA, H., VUORI, K.M. & OIKARI, A. 2012. Caddis larvae (Trichoptera, Hydropsychidae) indicate delaying recovery of a watercourse polluted by pulp and paper industry. *Ecological Indicators* **15**: 217-226.

RELYEA, C.D., MINSHALL, G.W. & DANEHY, R.J. 2000. Stream insects as bioindicators of fine sediment. *Proceedings of the Water Environment Federation* **2000**: 663-686.

RIVER HEALTH PROGRAMME (RHP). 2005. A biomonitoring survey of the olifants river catchment falling within limpopo province. Field survey of 2004.

SALMON, S., PONGE, J.F., GACHET, S., DEHARVENG, L., LEFEBVRE, N. & DELABROSSE, F. 2014. Linking species, traits and habitat characteristics of Collembola at European scale. *Soil Biology and Biochemistry* **75**: 73-85.

SMOLDERS, A., LOCK, R., VAN DER VELDE, G., HOYOS, R.M. & ROELOFS, J. 2003. Effects of mining activities on heavy metal concentrations in water, sediment, and macroinvertebrates in different reaches of the Pilcomayo River, South America. *Archives of Environmental Contamination and Toxicology* **44**: 0314-0323.

SWEENEY, B.W. & NEWBOLD, J.D. 2014. Streamside forest buffer width needed to protect stream water quality, habitat, and organisms: a literature review. *JAWRA Journal of the American Water Resources Association* **50**: 560-584.

THIRION C. 2007. Module E: Macroinvertebrate Response Assessment Index in River EcoClassification: Manual for EcoStatus Determination (Version 2). Joint Water Research Commission and Department of Water Affairs and Forestry report. WRC Report No. TT332/08. Water Research Commission, Pretoria

THORNE, R.S.J., WILLIAMS, W.P. & GORDON, C. 2000. The macroinvertebrates of a polluted stream in Ghana. *Journal of Freshwater Ecology* **15**: 209-217.

THORPE, T. & LLOYD, B. 1999. The macroinvertebrate fauna of St. Lucia elucidated by canonical correspondence analysis. *Hydrobiologia* **400**: 195-203.

TURPIE, J., MARAIS, C. & BLIGNAUT, J.N. 2008. The working for water programme: Evolution of a payments for ecosystem services mechanism that addresses both poverty and ecosystem service delivery in South Africa. *Ecological economics* **65**: 788-798.

VERSCHUREN, D., TIBBY, J., LEAVITT, P.R. & ROBERTS, C.N. 1999. The environmental history of a climate-sensitive lake in the former'White Highlands' of central Kenya. *Ambio* **28**: 494-501.

WOLMARANS, C., KEMP, M., DE KOCK, K., ROETS, W., VAN RENSBURG, L. & QUINN, L. 2014. A semi-quantitative survey of macroinvertebrates at selected sites to evaluate the ecosystem health of the Olifants River. *Water SA* 40: 245-254.

WORF, D.L. 1980. *Biological monitoring for environmental effects*. DC Heath and Company.

XU, M., WANG, Z., DUAN, X. & PAN, B. 2014. Effects of pollution on macroinvertebrates and water quality bio-assessment. *Hydrobiologia* **729**: 247-259

ZAJAC, R.N., VOZARIK, J.M. & GIBBONS, B.R. 2013. Spatial and temporal patterns in macrofaunal diversity components relative to sea floor landscape structure. *PloS one* **8**: e65823.

Chapter 4: General discussion, conclusion and recommendation's

4.1 General discussion

The objectives of the study were to: (i). establish the current physico-chemical composition of the river water and sediment along the entire length of the Ga-Selati River, (ii). Determine the concentrations of metals in water and sediment along the river, & (iii). Assess the impact of water and sediment quality on the aquatic macro-invertebrate assemblages in the river. Generally, the levels of physico-chemical parameters increased from upstream to downstream in the Ga-Selati River. The highly disturbed midstream and downstream sites generally had high nutrient, turbidity, TDS and conductivity values, while these parameters were relatively low at the upstream sites while DO decreased downstream. This indicates that the some of the physico-chemical parameters increased from upstream to downstream and vice versa. Since each parameter/constituent has an effect which is either beneficial or detrimental to aquatic biota, it is usually hard to determine the magnitude of the combined effect of the physico-chemical parameters (Dallas & Day 2004).

Most of the metals in the water were within the recommended levels at all sites (DWAF 1996) (Table: 2.3.1.3). Generally, the metal concentrations showed a concentration gradient, with higher concentrations downstream and lower concentrations upstream, with the exception of Al, Fe and Mn which were higher at the upstream sites (site 1 and site 2) than the rest of the sites. It was evident that the major source of pollution of the Ga-Selati River is the combination of different anthropogenic activities along the catchment of the river. These activities are sewage works and domestic waste (at site 4, 5 and 6), mining discharges (at site 7, 8 and 9) and agricultural runoff (at site 2) (Table: 2.2.1).

The concentrations of metals in the sediment were higher than in the water. This is because sediments act as reservoirs for pollutants (Pekey 2006, Chandrasekaran *et al.* 2013). Sediment accumulates contaminants and pollutes the ecosystems that are associated with it. Hence, metal concentrations linked with sediment greatly surpass the concentrations dissolved in water, in most aquatic systems (Chon *et al.* 2012, Ciparis *et al.* 2012). High concentrations of metals in sediment samples were found in the middle reaches. High concentrations of Al, Cr, Fe, Ni, K, Ti, V and Mn were found in sediments at site 4, compared to sites further downstream where there are more anthropogenic activities. However, this deviation may have been influenced by a number of factors such as; the geology or the bedrock, the physico-chemical characteristics, pollution, in this case could be the impoundment present at this site, and/ or a combination of all these factors (BC-EPD 2006 Whitehead *et al.* 2009, CCME 2012a, US-EPA 2012).

Macro-invertebrates have limited ability to migrate, highly susceptible to environmental impacts, and are widely used as bio-indicators. The macro-invertebrate assemblages in the Ga-Selati River were rich in Ephemeroptera, Diptera and Trichoptera. Site 1 and site 2 accounted for most of the sensitive families, reflecting good water quality at these two sites, while site 9, a downstream site recorded the highest number of tolerant families. The lowest taxa richness was recorded at site 5 and this is the site which had the highest level of anthropogenic disturbances. There is intensive sand mining activity at this site and coupled with poor habitat diversity might be the cause of the low macro-invertebrate abundance in this site.

The presence of EPT at site 1, further support the fact that the water quality at this site is good (oligothrophic condition). As the members of these groups are sensitive to pollution and it is known that a reduction in DO and pH, for example, can reduce the abundance of mayflies (Wesner *et al.* 2014). The ordination plot also shows that site 1 is different from the other sites, as all the samples were grouped together, while the samples of the other sites were scattered. The SIMPER analysis showed the highest dissimilarity to be 79.82% average dissimilarity between sites 1 and site 5, followed by 75%, between site 1 and site 9, then 73.74%, between site 2 and site 5 and then 67.48% between sites 1 and site 2. The average dissimilarity between site 2 and site 9 was 63.15%. The dissimilarity between site 5 and site 9 was 64.34%. Thus, the percentage dissimilarities are all above 60%, an indication that the conditions at the sites are different and the variations could be due to the different activities near the sites.

The variations in the macro-invertebrate distribution could further be explained by the differences in the water quality at the various sites as seen on the CCA plot. The effects of main pollution factors such as, conductivity, TDS, turbidity and nutrients were correlated with the distribution of tolerant taxa. The other factor that had an impact on the macro-invertebrate distribution was the habitat characteristics at each site.

There was seasonal variation, with the highest numbers of taxa, including the sensitive taxa in winter and lowest in summer. Thus, an indication that winter was the season with the best water quality conditions while summer had poor water quality. This might be due to low temperature during winter and which also affects the oxygen levels (solubility of oxygen increases as temperature decreases). The poor water quality in summer could have come from run-off from the various anthropogenic activities especially mining in the catchment. The catchment receives rainfall during summer, warm temperatures which usually affects DO. Deterioration of water quality affects distribution and diversity of aquatic organisms.

4.2 Conclusion

In conclusion, the current physico-chemical composition of the river water and sediment at the Ga-Selati River shows that the river has low water quality, especially at the lower reaches of the river. The poor water quality at the lower reaches in the Ga-Selati River is due to cumulative pollution inputs along the entire length of the river. This is due to different land uses along the catchment of the river and this land uses contaminate the Ga-Selati River with different pollutants. The study shows that the water and sediment quality at Ga-Selati River is having a negative impact on the macro-invertebrates distribution. The negative effect of human impact in the Ga-Selati River is evident, especially water contamination and macro-invertebrates habitat disturbance. Information gathered from this study demonstrates the value of aquatic macro-invertebrates as water quality indicators of environmental impacts in rivers.

4.3 Recommendation.

The objectives of this study were to; 1) to assess the water and sediment quality in the Ga-Selati River at nine sites, 2) to determine the concentrations of metals in the water and sediment, and 3) to assess the impact of the quality of water and sediment on the aquatic macro-invertebrate assemblages in five sites of the Ga-Selati River. All these objectives have been met. From the results it is evident that the river is polluted with contaminants from different sources. The Ga-Selati River has several impoundments which serve as traps for sediment, nutrients, toxins, and heavy metals. Further neglect to this system may lead to more deterioration of the water quality and even extinction of species that have not been documented and may offer key ecological significance. Future studies should also include functional feeding groups and bioaccumulation of macro-invertebrates as key stone species to present a more detailed account of the state of the river. The Ga-Selati River requires further assessment to clearly identify the condition of the river and document the impact of the human activities taking place in the catchment. Failure to take action may lead to more deterioration of the water quality or causing irreversible losses of biological diversity.

It is therefore important to implement a continuous monitoring programme and manage the river system to prevent further deterioration in the water quality. The Lepelle water board should regulate the intensity of sand mining at the Ga-Selati River, because it is one of the main causes of aquatic biota habitat disturbance.

4.4 References

BRITISH COLUMBIAN ENVIRONMENTAL PROTECTION DIVISION (BC-EPD). 2006. Water quality – A compendium of working water quality guidelines for British Columbia. In: Government of British Columbia Environmental Protection Division. http://www.env.gov.bc.ca/wat/wq/BCguidelines/working.html. Accessed March 2017

CCME. 2012a. Canadian water quality guidelines for the protection of aquatic life and sediment quality guidelines for the protection of aquatic life. in. Canadian Council of Ministers of the Environment

CCME. 2012b. Canadian water quality guidelines for the protection of aquatic life and sediment quality guidelines for the protection of aquatic life. in. Canadian Council of Ministers of the Environment.

CHANDRASEKARAN, A., MUKESH, M., ANANTHARAMAN, P., TAMILSELVI, M., MUTHUKUMARASAMY, R., MANIVEL, T. & RAJMOHAN, R. 2013. Trace Metal Concentration in Sediments of Tamirabarani River in Relationships with Physico Chemical Characteristics. A Study Using Gis Application. *International Journal of InnovativeTechnology and Exploring Engineering (IJITEE)*.

CHON, H.S., OHANDJA, D.G. & VOULVOULIS, N. 2012. The role of sediments as a source of metals in river catchments. *Chemosphere* 88: 1250-1256.

CIPARIS, S., SCHREIBER, M.E. & VOSHELL JR, J.R. 2012. Using watershed characteristics, sediment, and tissue of resident mollusks to identify potential sources of trace elements to streams in a complex agricultural landscape. *Environmental monitoring and assessment* 184: 3109-3126.

DALLAS, H.F. & DAY, J.A. 2004. *The effect of water quality variables on aquatic ecosystems: A review*. WRC Report No. TT224/04. Water Research Commission, Pretoria, South Africa 222 pp.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF). 1996. South African Water Quality Guidelines**.**

PEKEY, H. 2006. The distribution and sources of heavy metals in Izmit Bay surface sediments affected by a polluted stream. *Marine Pollution Bulletin* 52: 1197-1208.

WESNER, J.S., KRAUS, J.M., SCHMIDT, T.S., WALTERS, D.M. & CLEMENTS, W.H. 2014. Metamorphosis enhances the effects of metal exposure on the mayfly, Centroptilum triangulifer. *Environmental science & technology* **48**: 10415-10422.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA). 2012. National recommended water quality criteria: Aquatic life. http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm. Accessed March 2017.

WHITEHEAD, P.G., WILBY, R.L., BATTARBEE, R.W., KERNAN, M. & WADE, A.J. 2009. A review of the potential impacts of climate change on surface water quality. *Hydrological Sciences Journal* 54: 101-123.

Appendix 1 A: Water quality variables

Site	Season	Temp	Doxygen	Doxygen_Per	pH	Conductivity	TDS	Salinity	Turbidity
1	Autumn	19.9	7.2	90.1	7.99	149.2	72.9	0.06	2
1	Winter	17.9	10.89	137.1	8.37	146	68.6	0.06	0
1	Spring	18.4	10.17	125.7	8.81	189.5	91.3	0.09	1
1	Summer	24.2	11.37	125.7	8.37	198.2	93.7	0.9	1
2	Autumn	31.4	8.13	107.2	8.73	197.5	85.7	0.08	9
2	Winter	16.2	10.26	111.4	8.17	187.25	96.5	0.09	2
2	Spring	22.9	9.65	123.6	8.61	433.35	203	0.2	7
2	Summer	31.4	8.13	107.2	8.73	197.5	85.7	0.08	8
3	Autumn	22.5	6.18	75.8	8.75	252.2	117.1	0.11	4
3	Winter	20	9.65	114.7	9.02	288.2	137	0.13	3
3	Summer	35.4	7.42	94.4	8.77	502	211	0.2	12
4	Autumn	21.3	6.52	80.4	8.46	285.45	133.5	0.13	5
4	Winter	16.5	9.94	109.4	8.47	323.2	164.1	0.16	7
4	Spring	24.1	9.87	126.5	8.89	404.5	185.4	0.18	6
4	Summer	29.8	6.74	89.5	8.66	433.8	192.9	0.19	8
5	Autumn	21.4	6.84	83	8.69	346.35	166.2	0.16	9
5	Winter	17.4	10.71	113.8	8.74	472	243	0.23	8
5	Spring	24.9	7.72	93.3	9.32	1228.5	563	0.56	8
5	Summer	34.1	7	94.3	8.6	1340.5	576	0.57	9
6	Autumn	21.2	7.22	86.2	8.54	602.5	280	0.26	13
6	Winter	17	11.56	128.8	8.51	918.5	458	0.44	5
6	Spring	22	8.64	104.6	8.57	1187.5	561	0.56	13
6	Summer	33.1	7.17	92	8.36	1360.5	587	0.58	21
7	Autumn	20.3	7.39	89.7	8.4	649.5	315	0.31	7
7	Winter	17.4	11.91	133.9	8.58	967	474	0.45	7
7	Spring	22	10.14	123.2	8.47	1298.5	613	0.63	11
7	Summer	31.3	6.64	76.9	8.26	1380.5	608	0.6	18
8	Autumn	19.5	6.99	79.6	8.24	680	333	0.29	5
8	Winter	17	12.26	139.1	8.58	948.5	436	0.44	2
8	Spring	22.6	8.25	93.7	8.46	1461	678	0.68	7
8	Summer	31.1	6.3	85.3	8.41	1424.5	608	0.6	14
9	Autumn	20.3	7.61	87.3	8.64	949.5	463	0.46	6
9	Winter	16.1	8.17	867	9.09	1280	647	0.64	3
9	Spring	22.7	7.72	91.9	8.84	1778	839	0.85	4
9	Summer	34.1	7.02	93	8.72	1926.5	826	0.82	55

Appendix 1 B: Nutrients concentrations

Site	Season	Nitrate_NO3	Nitrite_NO2	Ammonia	Phosphorus
1	Autumn	0.13	0.01	0.025	<0.05
1	Winter	0.56	0.01	<0.010	<0.05
1	Spring	<0.5	<0.010	0.028	<0.05
1	Summer	0.72	0.108	0.11	0.426
2	Autumn	0.55	0.015	0.038	<0.05
2	Winter	0.43	0.012	<0.010	<0.05
2	Spring	<0.5	0.017	0.043	<0.05
2	Summer	0.66	0.083	0.045	0.453
3	Autumn	2	0.008	0.018	0.05
3	Winter	<0.5	0.014	<0.010	<0.05
3	Summer	1.11	0.121	0.047	0.513
4	Autumn	<0.20	0.015	0.02	<0.05
4	Winter	9.86	0.012	0.015	<0.05
4	Spring	<0.5	0.013	0.0104	0.07
4	Summer	0.25	0.022	0.102	0.346
5	Autumn	<0.20	0.01	0.02	<0.05
5	Winter	4.09	<0.010	<0.010	<0.05
5	Spring	<0.5	0.017	0.074	<0.05
5	Summer	0.84	0.089	0.05	2.47
6	Autumn	1.42	0.107	0.22	0.3
6	Winter	>10.00	0.159	0.372	67
6	Spring	2.3	0.403	0.425	1.55
6	Summer	0.81	0.123	>0.5	0.94
7	Autumn	2.17	0.106	0.242	0.38
7	Winter	1.88	0.137	0.226	1.03
7	Spring	2.4	0.058	0.083	2
7	Summer	0.59	0.133	>0.5	0.804
8	Autumn	1.22	0.03	0.141	0.45
8	Winter	1.72	0.038	<0.010	0.95
8	Spring	<0.5	0.07	0.146	2.21
8	Summer	2.1	0.045	>0.5	0.97
9	Autumn	2.41	0.038	0.05	0.49
9	Winter	1.73	0.022	<0.010	0.67
9	Spring	<0.5	0.035	0.093	1.92
9	Summer	3.8	0.104	0.101	0.453

Appendix 1 C: Metal concentrations

Site	Season	Calcium	Magnesium	Potassium	Sodium	Aluminium	Barium	Boron	Iron	Manganese	Strontium (Sr)	Titanium	Zinc
1	Autumn	15	10	<1.0	4	<0.100	<0.025	<0.025	0.033	<0.025	<0.025	<0.025	<0.025
1	Winter	12	7	<1.0	3	<0.100	0.008	0.007	0.021	<0.025	0.013	0.016	0.014
1	Spring	19	11	1.3	3	<0.100	0.01	<0.01	0.035	<0.025	0.014	0.021	0.025
1	Summer	19	10	1.1	3	<0.100	0.014	<0.010	0.028	0.012	0.025	0.016	0.029
2	Autumn	15	12	<1.0	11	0.155	0.033	<0.025	0.47	0.229	0.052	<0.025	<0.025
2	Winter	13	10	<1.0	8	<0.100	0.024	0.014	0.044	<0.025	0.053	0.016	0.048
2	Spring	32	23	1.2	25	<0.100	0.085	0.041	0.113	0.129	0.144	0.036	0.025
2	Summer	12	8	1.4	8	<0.100	0.027	0.014	0.121	0.056	0.057	0.01	0.04
3	Autumn	17	15	1.1	15	0.115	0.034	<0.025	0.097	<0.025	0.071	<0.025	<0.025
3	Winter	17	14	1.3	15	<0.100	0.029	0.017	0.029	<0.025	0.084	0.019	<0.010
3	Summer	28	17	4.1	26	<0.100	0.073	0.031	0.066	0.024	0.139	0.023	0.032
4	Autumn	19	17	1.3	20	<0.100	0.039	<0.025	0.196	0.029	0.092	<0.025	<0.025
4	Winter	20	16	1.8	20	<0.100	0.041	0.024	0.042	<0.025	0.109	0.025	<0.010
4	Spring	25	19	2.9	26	<0.100	0.054	0.052	0.283	0.056	0.148	0.027	0.023
4	Summer	15	14	2.3	20	<0.100	0.038	0.021	0.063	0.018	0.099	0.012	0.046
5	Autumn	21	18	1.6	26	0.278	0.039	0.028	0.305	<0.025	0.114	<0.025	<0.025
5	Winter	7	8	<1.0	15	<0.100	0.014	0.017	0.015	<0.025	0.058	<0.010	0.047
5	Spring	20	39	6.7	139	<0.100	0.054	0.099	0.084	0.022	0.26	0.022	0.014
5	Summer	21	37	6.8	120	<0.100	0.09	0.082	0.052	0.016	0.243	0.016	0.022
6	Autumn	28	27	3.2	70	0.24	0.047	0.066	0.31	0.054	0.183	<0.025	<0.025
6	Winter	33	29	5.4	96	<0.100	0.048	0.078	0.057	<0.025	0.026	0.037	0.041
6	Spring	34	33	10.3	114	<0.100	0.05	0.095	0.24	0.084	0.278	0.041	<0.010
6	Summer	25	22	8.1	124	<0.100	0.06	0.1	0.177	0.047	0.204	0.02	0.011
7	Autumn	30	28	3.4	72	0.23	0.048	0.069	0.301	0.059	0.204	<0.025	<0.025
7	Winter	42	39	6.2	114	<0.100	0.053	0.097	0.07	<0.025	0.364	0.045	0.02
7	Spring	37	37	8	104	<0.100	0.046	0.067	0.204	0.097	0.281	0.031	0.012
7	Summer	24	21	7.4	108	<0.100	0.051	0.084	0.116	0.034	0.209	0.018	0.011
8	Autumn	44	31	3.4	76	0.161	0.046	0.071	0.175	0.058	0.22	2.47	<0.025
8	Winter	38	23	2.6	53	<0.100	0.04	0.055	0.059	<0.025	0.292	0.041	0.027
8	Spring	36	41	8.8	119	<0.100	0.036	0.068	0.085	0.04	0.249	0.027	<0.010
8	Summer	28	25	8.4	110	<0.100	0.051	0.094	0.067	0.022	0.684	0.023	0.012
9	Autumn	21	50	12.5	91	0.162	0.055	0.081	0.151	0.074	0.556	<0.025	<0.025
9	Winter	57	66	19.9	121	<0.100	0.037	0.101	0.09	<0.025	0.893	0.066	0.018
9	Spring	52	67	19.4	115	<0.100	0.027	0.061	0.077	0.013	0.793	0.04	0.026
9	Summer	32	54	25	115	<0.100	0.052	0.09	0.043	0.024	0.684	0.029	0.05

Appendix 2 A: Macro-invertebrate families collected at Selati River throughout the sampling period (May 2014 to January 2015) at site 1. "**X**" denotes family presence.

	Site 1			
	Autumn	Winter	Spring	Summer
Taxon				
Emphemeroptera				
Baetidae	**X**	**X**	**X**	**X**
Caenidae	**X**	**X**	**X**	**X**
Polymitarcyidae				
Heptageniidae	**X**	**X**	**X**	**X**
Telogonodidae				
Oligoneuridae				
Prosopistomatidae				
Leptophlebiidae	**X**	**X**	**X**	**X**
Tricorythidae	**X**	**X**	**X**	**X**
Trichoptera				
Hydropsychidae	**X**	**X**	**X**	**X**
Polycentropodidae				
Philopotamidae	**X**	**X**		
Ecomidae				
Psychomyiidae				
Paracnomina	**X**			
Cassed caddisflies				
Petrothrincidae	**X**	**X**		
Leptoceridae	**X**			
Hydroptilidae	**X**	**X**		
Pisuliidae				**X**
Coleoptera				
Dytiscidae				
Dytiscidae larvae				
Gyrinidae	**X**		**X**	**X**
Gyrinidae larva				
Hydraenidae				
Elmidae	**X**			**X**
Elmidae larvae	**X**	**X**	**X**	**X**
Helodidae				
Psephenidae	**X**	**X**	**X**	**X**
Hydrophilidae				
Hemiptera				
Naucoridae				
Notonectidae				
Belostomatidae				
Gerridae				
Hydrometridae				
Corixidae				
Veliidae				
Nepidae				
Pleidae				
Odonata				
Libellulidae	**X**	**X**		
Corduliidae				
Aeshnidae	**X**		**X**	**X**
Gomphidae	**X**	**X**	**X**	**X**
Calopterygidae				
Chlorocyphidae	**X**	**X**	**X**	**X**

Platycnemididae				
Coenagrionidae				
Chlorolestidae				
Lestidae				
Diptera				
Athericidae	X	X	X	X
Blephariceridae				
Culicidae	X			
Tabanidae	X	X	X	X
Psychodidae				
Dixidae				
Chirinomidae	X	X	X	X
Ceratopogonidae	X	X	X	X
Muscidae larva				
Muscidae pupa				
Ephydridae				
Syrphidae				
Tipulidae	X	X	X	X
Simulidae	X			X
Turbellaria				
Planaria				
Plecoptera				
Perlidae	X			X
Notonemouridae				X
Lepidoptera				
Pyralidae	X	X		X
Hyracarina				
Hydrachnellae				
Megaloptera				
Corydalidae				
Crustacea				
Potamonautidae	X	X	X	X
Palaemonidae				
Amphipoda				
Atyidae				
Porifera				
Porifera				
Annelida				
Hirudinea		X		X
Oligochaeta	X			
MOLLUSCA				
Physidae				
Lymnaeidae				
Planorbidae	X	X	X	X
Ancylidae		X	X	X
Thiaridae				
Unionidae				
Sphaeriidae				
Corbiculidae				

Appendix 2 B: Macro-invertebrate families collected at Selati River throughout the sampling period (May 2014 to January 2015) at site 2. "X" denotes family presence.

	Site 2			
	Autumn	Winter	Spring	Summer
Taxon				
Emphemeroptera				
Baetidae	**X**	**X**	**X**	**X**
Caenidae	**X**	**X**	**X**	**X**
Polymitarcyidae				
Heptageniidae	**X**	**X**		
Telogonodidae				
Oligoneuridae				
Prosopistomatidae				
Leptophlebiidae	**X**	**X**		**X**
Tricorythidae	**X**			**X**
Trichoptera				
Hydropsychidae		**X**		
Polycentropodidae				
Philopotamidae	**X**	**X**		**X**
Ecomidae				
Psychomyiidae				
Paracnomina				
Cassed caddisflies				
Petrothrincidae				
Leptoceridae				**X**
Hydroptilidae				
Pisuliidae				
Coleoptera				
Dytiscidae				
Dytiscidae larvae	**X**	**X**		
Gyrinidae		**X**		
Gyrinidae larva				
Hydraenidae	**X**			
Elmidae	**X**	**X**		**X**
Elmidae larvae	**X**	**X**	**X**	**X**
Helodidae				
Psephenidae				
Hydrophilidae				
Hemiptera				
Naucoridae	**X**			
Notonectidae			**X**	
Belostomatidae				
Gerridae				
Hydrometridae				
Corixidae				
Veliidae		**X**		
Nepidae				
Pleidae				
Odonata				
Libellulidae	**X**	**X**	**X**	**X**
Corduliidae				
Aeshnidae				
Gomphidae	**X**	**X**	**X**	
Calopterygidae				
Chlorocyphidae	**X**	**X**	**X**	**X**
Platycnemididae				

Coenagrionidae	X		X	
Chlorolestidae			X	
Lestidae				
Diptera				
Athericidae	X	X	X	
Blephariceridae				X
Culicidae				
Tabanidae	X	X	X	X
Psychodidae				
Dixidae		X		
Chirinomidae	X			X
Ceratopogonidae				
Muscidae larva				
Muscidae pupa		X		
Ephydridae				
Syrphidae				
Tipulidae				
Simulidae	X	X		X
Turbellaria				
Planaria				
Plecoptera				
Perlidae	X	X		X
Notonemouridae				
Lepidoptera				
Pyralidae				
Hyracarina				
Hydrachnellae				
Megaloptera				
Corydalidae				
Crustacea				
Potamonautidae				
Palaemonidae				
Amphipoda				
Atyidae				
Porifera				
Porifera				
Annelida				
Hirudinea	X	X	X	
Oligochaeta	X	X	X	
Mollusca				
Physidae				
Lymnaeidae				
Planorbidae	X		X	X
Ancylidae				
Thiaridae	X		X	X
Unionidae				
Sphaeriidae	X			
Corbiculidae	X	X	X	X

Appendix 2 C: Macro-invertebrate families collected at Selati River throughout the sampling period (May 2014 to January 2015) at site 5. “X” denotes family presence.

	Site 5			
	Autumn	Winter	Spring	Summer
Taxon				
Emphemeroptera				
Baetidae	X	X		X
Caenidae	X	X	X	X
Polymitarcyidae				
Heptageniidae		X		
Telogonodidae				
Oligoneuridae				
Prosopistomatidae				
Leptophlebiidae	X	X		
Tricorythidae				
Trichoptera				
Hydropsychidae	X			
Polycentropodidae				
Philopotamidae			X	
Ecomidae				
Psychomyiidae				
Paracnomina				
Cassed caddisflies				
Petrothrincidae				
Leptoceridae				
Hydroptilidae			X	
Pisuliidae				
Coleoptera				
Dytiscidae				X
Dytiscidae larvae				
Gyrinidae				
Gyrinidae larva				
Hydraenidae				
Elmidae				
Elmidae larvae		X	X	X
Helodidae				
Psephenidae				
Hydrophilidae				
Hemiptera				
Naucoridae				
Notonectidae				X
Belostomatidae				
Gerridae			X	
Hydrometridae				
Corixidae				
Veliidae	X			
Nepidae				
Pleidae				
Odonata				
Libellulidae	X	X	X	X
Corduliidae				
Aeshnidae				

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

Appendix 2 D: Macro-invertebrate families collected at Selati River throughout the sampling period (May 2014 to January 2015) at site 7. "X" denotes family presence.

	Site 7
	Autumn
Taxon	
Emphemeroptera	
Baetidae	**X**
Caenidae	**X**
Polymitarcyidae	
Heptageniidae	
Telogonodidae	
Oligoneuridae	
Prosopistomatidae	
Leptophlebiidae	**X**
Tricorythidae	
Trichoptera	
Hydropsychidae	
Polycentropodidae	
Philopotamidae	**X**
Ecomidae	
Psychomyiidae	
Paracnomina	
Cassed caddisflies	
Petrothrincidae	
Leptoceridae	**X**
Hydroptilidae	
Pisuliidae	
Coleoptera	
Dytiscidae	
Dytiscidae larvae	
Gyrinidae	
Gyrinidae larva	
Hydraenidae	
Elmidae	
Elmidae larvae	
Helodidae	
Psephenidae	
Hydrophilidae	**X**
Hemiptera	
Naucoridae	**X**
Notonectidae	
Belostomatidae	
Gerridae	
Hydrometridae	
Corixidae	
Veliidae	**X**
Nepidae	
Pleidae	
Odonata	
Libellulidae	**X**
Corduliidae	
Aeshnidae	
Gomphidae	**X**
Calopterygidae	
Chlorocyphidae	
Platycnemididae	

Coenagrionidae	
Chlorolestidae	
Lestidae	
Diptera	
Athericidae	
Blephariceridae	
Culicidae	
Tabanidae	**X**
Psychodidae	
Dixidae	
Chirinomidae	**X**
Ceratopogonidae	**X**
Muscidae larva	**X**
Muscidae pupa	**X**
Ephydridae	
Syrphidae	
Tipulidae	
Simulidae	**X**
Turbellaria	
Planaria	
Plecoptera	
Perlidae	
Notonemouridae	
Lepidoptera	
Pyralidae	
Hyracarina	
Hydrachnellae	
Megaloptera	
Corydalidae	
Crustacea	
Potamonautidae	
Palaemonidae	
Amphipoda	
Atyidae	
Porifera	
Porifera	
Annelida	
Hirudinea	**X**
Oligochaeta	
Mollusca	
Physidae	
Lymnaeidae	
Planorbidae	
Ancylidae	
Thiaridae	**X**
Unionidae	
Sphaeriidae	**X**
Corbiculidae	**X**

Appendix 2 E: Macro-invertebrate families collected at Selati River throughout the sampling period (May 2014 to January 2015) at site 9. “X” denotes family presence.

	Site 9			
	Autumn	Winter	Spring	Summer
Taxon				
Emphemeroptera				
Baetidae	X	X	X	X
Caenidae	X	X	X	X
Polymitarcyidae				
Heptageniidae				
Telogonodidae				
Oligoneuridae				
Prosopistomatidae				
Leptophlebiidae	X	X	X	
Tricorythidae	X			
Trichoptera				
Hydropsychidae			X	
Polycentropodidae				
Philopotamidae	X	X		
Ecomidae				
Psychomyiidae		X		
Paracnomina				
Cassed caddisflies				
Petrothrincidae				
Leptoceridae				
Hydroptilidae	X	X		
Pisuliidae				
Coleoptera				
Dytiscidae			X	
Dytiscidae larvae		X		
Gyrinidae				
Gyrinidae larva				
Hydraenidae				
Elmidae	X	X	X	
Elmidae larvae	X	X	X	X
Helodidae			X	
Psephenidae				
Hydrophilidae				
Hemiptera				
Naucoridae		X	X	
Notonectidae				
Belostomatidae				
Gerridae	X			
Hydrometridae				
Corixidae				
Veliidae	X			
Nepidae				
Pleidae				
Odonata				
Libellulidae	X	X	X	X
Corduliidae				
Aeshnidae				
Gomphidae	X	X	X	
Calopterygidae				
Chlorocyphidae				X
Platycnemididae				

Coenagrionidae	X	X	X	
Chlorolestidae				
Lestidae				
Diptera				
Athericidae		X		
Blephariceridae				
Culicidae				
Tabanidae	X	X	X	X
Psychodidae				
Dixidae				
Chirinomidae	X	X	X	
Ceratopogonidae	X	X		
Muscidae larva		X		
Muscidae pupa		X	X	X
Ephydridae				
Syrphidae				
Tipulidae				
Simulidae	X	X		
Turbellaria				
Planaria				
Plecoptera				
Perlidae				
Notonemouridae				
Lepidoptera				
Pyralidae			X	
Hyracarina				
Hydrachnellae				
Megaloptera				
Corydalidae				
Crustacea				
Potamonautidae			X	X
Palaemonidae				
Amphipoda				
Atyidae				
Porifera				
Porifera				
Annelida				
Hirudinea		X	X	
Oligochaeta			X	
Mollusca				
Physidae	X		X	
Lymnaeidae		X		
Planorbidae				
Ancylidae				
Thiaridae	X	X	X	X
Unionidae	X			
Sphaeriidae	X			
Corbiculidae	X	X		X

Appendix 3 A: Average dissimilarity between Site 1 & 2

Site 1 & 2
Average dissimilarity = 67.48

Species	Site 1 Av.Abund	Site 2 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Corbiculidae	0.00	3.47	4.99	1.55	7.40	7.40
Hydropsychidae.	3.17	0.09	4.26	2.18	6.31	13.71
Libellulidae	0.91	3.14	3.19	1.80	4.72	18.43
Chirinomidae	2.30	2.04	2.92	1.30	4.33	22.76
Caenidae	3.36	1.51	2.92	1.58	4.32	27.08
Tricorythidae	2.37	0.40	2.78	1.77	4.12	35.43
Psephenidae	1.81	0.00	2.48	2.16	3.67	39.10
Simulidae	0.34	1.96	2.40	1.01	3.55	42.65
Thiaridae	0.00	1.52	2.37	1.02	3.51	46.16
Leptophlebiidae	1.97	2.03	2.36	1.83	3.50	49.66
Philopotamidae	1.12	1.69	2.26	1.40	3.35	53.01
Athericidae	2.27	1.51	2.24	1.34	3.33	56.33
Heptageniidae	0.32	1.70	2.08	1.04	3.08	62.69
Baetidae	1.04	1.43	1.82	1.36	2.70	65.40
Planorbidae	0.80	1.13	1.75	1.28	2.59	67.98
Chlorocyphidae	1.27	1.31	1.73	1.51	2.57	70.55
Tabanidae	0.88	1.69	1.60	1.58	2.38	72.93
Hirudinea	0.18	1.09	1.39	1.07	2.05	77.10
Gomphidae	1.15	1.17	1.24	1.31	1.85	78.95
Elmidae	2.13	2.38	1.14	1.20	1.68	84.22
Ancylidae	0.71	0.00	1.07	0.78	1.58	85.80
Perlidae	0.16	0.82	0.98	1.13	1.46	87.26
Aeshnidae	0.54	0.03	0.76	0.83	1.12	88.38
Potamonautidae	0.52	0.00	0.74	0.84	1.10	89.48
Oligochaeta	0.09	0.49	0.73	0.76	1.09	90.57

Appendix 3 B: Average dissimilarity between Site 1 & 5

Site 1 & 5
Average dissimilarity = 79.82

Species	Site 1 Av.Abund	Site 5 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Thiaridae	0.00	3.24	7.08	1.32	8.87	8.87
Hydropsychidae	3.17	0.05	6.45	2.48	8.08	16.95
Leptophlebiidae	1.97	2.24	4.82	2.23	6.04	22.99
Tricorythidae	2.37	0.00	4.80	2.22	6.02	29.01
Athericidae	2.27	0.00	4.60	2.11	5.77	34.78
Chirinomidae	2.30	0.37	4.30	1.33	5.39	40.17
Elmidae	2.13	0.21	4.16	2.20	5.21	45.38
Psephenidae	1.81	0.00	3.74	2.28	4.68	50.06
Caenidae	3.36	2.13	3.50	1.34	4.39	54.45
Chlorocyphidae	1.27	0.00	2.61	1.61	3.27	57.72
Baetidae	1.04	0.90	2.54	1.18	3.18	60.90
Corbiculidae	0.00	1.19	2.43	0.79	3.05	63.95
Libellulidae	0.91	1.32	2.38	1.20	2.98	66.94
Philopotamidae	1.12	0.05	2.21	1.20	2.77	69.70
Gomphidae	1.15	0.26	2.20	1.46	2.76	72.46
Tabanidae	0.88	0.07	1.85	1.18	2.31	77.24
Planorbidae	0.80	0.03	1.70	1.16	2.13	81.61
Ancylidae	0.71	0.00	1.69	0.79	2.11	83.73
Aeshnidae	0.54	0.00	1.15	0.83	1.44	85.17
Potamonautidae	0.52	0.07	1.14	0.88	1.43	86.59
Ceratopogonidae	0.48	0.00	1.02	0.83	1.27	87.87
Sphaeriidae	0.00	0.51	1.01	0.48	1.27	89.14
Tipulidae	0.46	0.00	0.93	0.81	1.17	90.30

Appendix 3 C: Average dissimilarity between Site 2 & 5

Site 2 & 5

Average dissimilarity = 73.74

Species	Site 2 Av.Abund	Site 5 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Corbiculidae	3.47	1.19	5.56	1.23	7.54	7.54
Thiaridae	1.52	3.24	4.83	1.36	6.55	14.09
Leptophlebiidae	2.03	2.24	4.32	1.16	5.86	19.95
Elmidae	2.38	0.21	4.25	2.34	5.77	25.71
Hydropsychidae	2.22	0.00	3.80	1.47	5.16	30.87
Libellulidae	3.14	1.32	3.52	1.65	4.77	35.64
Chirinomidae	2.04	0.37	3.16	1.30	4.29	39.93
Simulidae	1.96	0.00	3.07	0.93	4.16	44.09
Tabanidae	1.69	0.07	2.88	1.88	3.90	48.00
Athericidae	1.51	0.00	2.82	1.01	3.83	55.72
Caenidae	1.51	2.13	2.80	1.36	3.80	59.52
Planorbidae	1.13	0.03	2.64	0.91	3.57	63.10
Heptageniidae	1.70	0.09	2.58	0.91	3.50	66.60
Philopotamidae	1.69	0.05	2.55	0.94	3.46	70.06
Baetidae	1.43	0.90	2.52	1.26	3.42	73.48
Gomphidae	1.17	0.26	2.09	1.12	2.83	76.31
Chlorocyphidae	1.31	0.00	2.02	1.07	2.74	79.04
Hirudinea	1.09	0.03	1.94	1.06	2.63	81.67
Coenagrionidae	0.40	0.42	1.32	0.69	1.78	85.71
Perlidae	0.82	0.00	1.25	1.04	1.70	87.41
Oligochaeta	0.49	0.00	0.95	0.71	1.28	88.69
Sphaeriidae	0.03	0.51	0.94	0.49	1.27	89.96

Appendix 3 D: Average dissimilarity between Site 1 & 9

Site 1 & 9

Average dissimilarity = 75.00

Species	Site 1 Av.Abund	Site 9 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Thiaridae	0.00	4.17	7.23	1.37	9.64	9.64
Corbiculidae	0.00	3.99	6.60	1.76	8.80	18.45
Hydropsychidae	3.17	0.08	4.82	2.24	6.43	24.88
Tricorythidae	2.37	0.08	3.57	1.94	4.76	29.63
Baetidae	1.04	2.38	3.46	1.40	4.62	34.25
Athericidae	2.27	0.03	3.46	1.91	4.61	38.86
Chirinomidae	2.30	1.58	3.37	1.27	4.49	43.35
Psephenidae	1.81	0.00	2.82	2.16	3.76	47.10
Caenidae	3.36	3.67	2.67	1.48	3.55	50.66
Leptophlebiidae	1.97	1.64	2.58	1.63	3.44	57.62
Libellulidae	0.91	1.73	2.15	1.34	2.86	60.49
Muscidae	0.00	1.38	2.09	1.08	2.79	63.27
Gomphidae	1.15	0.93	1.94	1.38	2.58	65.86
Chlorocyphidae	1.27	0.03	1.93	1.53	2.58	68.44
Elmidae	2.13	1.31	1.88	1.39	2.50	70.94
Philopotamidae	1.12	0.26	1.68	1.16	2.24	73.18
Simulidae	0.34	0.90	1.36	0.84	1.81	77.03

Planorbidae	0.80	0.00	1.28	1.15	1.71	80.54
Hydroptilidae	0.03	0.96	1.27	0.64	1.70	82.24
Tabanidae	0.88	0.74	1.27	1.15	1.70	83.94
Ancylidae	0.71	0.00	1.23	0.78	1.64	85.58
Potamonautidae	0.52	0.17	0.89	0.93	1.18	86.76
Aeshnidae	0.54	0.00	0.86	0.81	1.15	89.05
Ceratopogonidae	0.48	0.10	0.78	0.88	1.04	90.09

Appendix 3 E: Average dissimilarity between Site 2 & 9

Site 2 & 9
Average dissimilarity = 63.15

Species	Site 2 Av.Abund	Site 9 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Thiaridae	1.52	4.17	4.75	1.20	7.53	7.53
Caenidae	1.51	3.67	3.46	1.47	5.48	13.01
Baetidae	1.43	2.38	3.25	1.67	5.14	18.15
Corbiculidae	3.47	3.99	3.22	1.21	5.11	23.25
Hydropsychidae	1.87	1.95	2.96	1.09	4.69	27.95
Chirinomidae	2.04	1.58	2.80	1.20	4.44	37.04
Leptophlebiidae	2.03	1.64	2.68	1.27	4.25	41.29
Simulidae	1.96	0.90	2.63	1.07	4.16	45.45
Libellulidae	3.14	1.73	2.59	1.25	4.10	49.56
Athericidae	1.51	0.03	2.15	1.02	3.41	52.97
Philopotamidae	1.69	0.26	2.08	0.96	3.29	56.26
Heptageniidae	1.70	0.00	2.05	0.86	3.24	59.50
Planorbidae	1.13	0.00	1.95	0.88	3.08	62.58
Muscidae	0.03	1.38	1.92	1.07	3.04	65.63
Elmidae	2.38	1.31	1.90	1.39	3.02	68.64
Gomphidae	1.17	0.93	1.85	1.12	2.93	71.58
Tabanidae	1.69	0.74	1.77	1.47	2.80	74.37
Chlorocyphidae	1.31	0.03	1.61	1.06	2.55	76.93
Hirudinea	1.09	0.18	1.46	1.06	2.31	79.24
Hydroptilidae	0.00	0.96	1.17	0.62	1.85	83.02
Perlidae	0.82	0.00	1.01	1.02	1.59	84.61
Coenagrionidae	0.40	0.36	0.83	0.77	1.32	87.35
Oligochaeta	0.49	0.11	0.76	0.77	1.21	88.56
Tricorythidae	0.40	0.08	0.66	0.66	1.04	90.79

Appendix 3 F: Average dissimilarity between Site 5 & 9

Average dissimilarity = 64.34

Species	Site 5 Av.Abund	Site 9 Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Corbiculidae	1.19	3.99	7.71	1.27	11.98	11.98
Thiaridae	3.24	4.17	6.90	1.11	10.72	22.71
Leptophlebiidae	2.24	1.64	5.11	1.10	7.94	30.65
Baetidae	0.90	2.38	4.73	1.29	7.35	38.00
Caenidae	2.13	3.67	4.66	1.45	7.25	45.25
Hydropsychidae	0.03	1.95	3.58	0.99	5.57	50.82
Chirinomidae	0.37	1.58	3.05	1.05	4.73	55.55
Libellulidae	1.32	1.73	2.99	1.34	4.64	60.20
Muscidae	0.00	1.38	2.95	1.07	4.59	64.79
Elmidae	0.21	1.31	2.59	1.48	4.02	68.81
Gomphidae	0.26	0.93	1.95	0.77	3.02	71.83
Hydroptilidae	0.03	0.96	1.71	0.65	2.65	74.49
Tabanidae	0.07	0.74	1.57	1.00	2.44	76.92
Simulidae	0.00	0.90	1.53	0.68	2.38	79.30
Coenagrionidae	0.42	0.36	1.36	0.66	2.11	81.41
Sphaeriidae	0.51	0.09	1.17	0.52	1.82	85.10
Physidae	0.00	0.36	0.83	0.60	1.28	86.39
Helodidae	0.00	0.23	0.61	0.54	0.95	88.42

Appendix 3 G: Macro-invertebrate SASS 5 score

Taxa	**SASS 5 Score**
Emphemeroptera	
Baetidae 1sp	4
Baetidae 2sp	6
Baetidae>2sp	12
Caenidae	6
Polymitarcyidae	10
Heptageniidae	13
Telogonodidae	9
Oligoneuridae	15
Prosopistomatidae	10
Leptophlebiidae	9
Tricorythidae	9
TRICHOPTERA	
Hydripsychidae 1sp	4

Hydropsychidae 2sp	6
Hydropsychidae >2sp	12
Polycentropodidae	12
Philopotamidae	10
Ecomidae	8
Psychomyiidae	8
Petrothrincidae	11
Leptoceridae	6
Hydroptilidae	6
Barbarochthonidae	13
Pisuliidae	10
Sericostomatidae	13
Coleoptera	
Dytiscidae	5
Gyrinidae	5
Hydraenidae	8
Elmidae	8
Helodidae	12
Psephenidae	10
Hydrophilidae	5
Hemiptera	
Naucoridae	7
Notonectidae	3
Belostomatidae	3
Gerridae	5
Hydrometridae	6
Corixidae	3
Veliidae	5
Nepidae	3
Pleidae	4
ODONATA	
Libellulidae	4

Corduliidae	8
Aeshnidae	8
Gomphidae	6
Calopterygidae	10
Chlorocyphidae	10
Platycnemididae	10
Coenagrionidae	4
Lestidae	8
DIPtera	
Athericidae	10
Blephariceridae	15
Culicidae	1
Tabanidae	5
Psychodidae	1
Dixidae	10
Chirinomidae	2
Ceratopogonidae	5
Muscidae larva	1
Muscidae pupa	1
Ephydridae	3
Syrphidae	1
Tipulidae	5
Simulidae	5
Turbellaria	
Planaria	3
Plecoptera	
Perlidae	12
Notonemouridae	14
LEPIDOPTERA	
Pyralidae	12
MEGALOPTERA	
Corydalidae	8

CRUSTACEA	
Potamonautidae	3
Palaemonidae	10
Amphipoda	13
Atyidae	8
PORIFERA	
Porifera	5
ANNELIDA	
Hirudinea	3
Oligochaeta	1
MOLLUSCA	
Physidae	3
Lymnaeidae	3
Planorbidae	3
Ancylidae	6
Thiaridae	3
Unionidae	6
Sphaeriidae	3
Corbiculidae	5