

**CHARACTERIZATION OF MACRO- AND MICRO-INVERTEBRATES AND
ASSESSMENT OF WATER QUALITY IN DAMS AND RIVERS OF QWAQWA**

By

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my original work and that I have not previously in its entirety or in part submitted it at any university for a degree. I furthermore cede copyright of the dissertation in favour of the University of the Free State.

Lisemelo Francina Motholo

Signature:

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DEDICATION

This work is dedicated to my two little daughters , Tekano Cheryl Mtholo and Lielelo “Lilly” Carol Mtholo for allowing me to sacrifice their family time as they had always hoped for the best. To my husband, Mr Khateane Gideon Mtholo, “the cornerstone of all my successes”, he had always been there for me with an unlimited support.

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GLOSSARY

Summary of terms adapted from Maseti, (2005) citing Barbour *et al*, (1999)

Benthic Macroinvertebrates: are organisms large enough to be seen with a naked eye and are without a backbone such as insects (in their larval or nymph stage), crayfish, clams, snails and worms most of which live part of their cycles attached to submerged rocks, logs and submerged aquatic vegetation (Rosenberg & Resh, 1993).

Biological monitoring: The use of a biological entity as a detector and its response as a measure to determine environmental conditions, usually done through biological surveys and toxicity tests.

Biological assessment : An evaluation of the biological conditions of waterbody that uses biological surveys and other direct measurements of the resident biota in surface waters.

Biological indicators: Communities, whether plant or animal, with a narrow range of ecological tolerance that may be selected for emphasis and monitored because their presence and relative abundance serve as barometer of ecological conditions.

Biological integrity: The ability of an ecosystem to support and maintain a balanced and adaptive community of organisms having species diversity, composition and functional organization comparable to that of the natural habitats of the region (Karr & Dudley, 1981).

- Biota:** Plants and animals and other living resources of the water body (Gerritsen *et al.*, 1998).
- Biotope:** A portion of a habitat characterised by uniformity in climate and distribution of biotic and abiotic components or an area of uniform environmental conditions providing a living place for specific assemblage of plants and animals.
- Contaminants:** Materials of any kind that are polluting a source of water. These could include both biological and chemical substances in water source like rivers, sampled rivers, ponds, lakes, seas, oceans, or reservoirs used for drinking and bathing by humans. (<http://www.wisegeek.com/>).
- Ecosystem:** The interactions of the biological community and abiotic environment (Jamil, 2001).
- Environment:** The physical and biological aspects of a specific area including all living things, soil, air and water (Jamil, 2001).
- Habitat:** A place where physical and biological elements of an ecosystem provide a suitable environment including food, cover and space needed for plant and animal livelihood (Gerritsen *et al.*, 1998).
- Macroinvertebrates:** Animals that can be seen with a naked eye and are without a backbone; which can be retained by a mesh size >200µm inhabiting the bottom substrata (Rosenberg & Resh, 1993).
- Pollution:** An undesirable change in the physico-chemical or biological status characteristics of air, water and land that

may have negative impacts on living species (Jamil, 2001).

Potable water: Potable water is water which is fit for consumption by humans and other animals. It is also called drinking water, in a reference to its intended use.

Substrate: A layer underneath, or the surface where an organism grows.

Vegetation: The assemblages of plant species and the ground cover they provide. It is a general term, without specific reference to particular taxa, life forms, structure, spatial extent, or any other specific botanical or geographic characteristics.

ACRONYMS AND ABBREVIATIONS

ANOVA:	Analysis of variance
ASPT:	Average score per taxon
AWWA:	American water works association
BOD:	Biochemical oxygen demand
CFU:	Colony – forming units
COD:	Chemical oxygen demand
DO:	Dissolved oxygen
DWAF:	Department of water affairs and forestry
EC:	Electrical conductivity
EPA:	Environmental protection agency
EU:	European union
FAII:	Fish assemblage integrity index
GI:	Geomorphology index
GSM:	Gravel sand and mud
HI:	Hydrological index
HII:	Habitat integrity index
IHAS:	Integrated habitat assessment system

IUCN :	International union for conservation of nature
MAP:	Maluti – A – Phofung municipality
MID:	Minimum infectious dose
NDPES:	National discharge pollution elimination system
QA/QC:	Quality assessment / quality control
RHP:	River health program
RHS:	River habitat (quality) survey
RVI:	Riparian vegetation index
SANS:	South African national standards
SASS:	South African scoring system
SASS5:	South African scoring system version 5.0
SOP:	Standard operating procedure
TDS:	Total dissolved solids
TN:	Total nitrogen
TP:	Total phosphorus
TNTC:	Too numerous to count
TSS:	Total suspended solids
UNICEF:	United Nations international children's emergency fund

US-EPA: United States environmental protection agency

WQI: Water quality index

WSDOH: Washington state department of health

ABSTRACT

This study was aimed at assessing water quality status and documentation of waterborne invertebrate organisms in freshwaters (rivers and dams) of Qwaqwa area of Maluti-A-Phofung municipality. Water samples were collected seasonally from rivers and dams to test water quality parameter levels and the variability of the South African scoring system (SASS) bioassessment method. Variable parameters were assessed using the multiparameter equipment and spectrophotometer for water quality and nutrient assessment respectively in all sampled rivers, namely; Metsimatsho, Namahadi, Khoptjwane, Kollatshwene and Elands as well as in two dams namely; Metsimatsho and Fikapatso. The mean electrical conductivity (EC) levels were the highest in Kollatshwene (0.268 $\mu\text{S}/\text{cm}$); Elands (0.231 $\mu\text{S}/\text{cm}$) and Khoptjwane (0.214 $\mu\text{S}/\text{cm}$) rivers between February and June 2014. The EC levels varied widely across the rivers ranging from 0.017 to 0.298 $\mu\text{S}/\text{cm}$ in spring. Therefore there were no significant differences ($p > 0.05$) of the EC levels between the rivers. The EC levels of Fikapatso dam have been higher than those of Metsimatsho dam throughout the sampling period. The pH levels between the rivers were slightly significantly different ($p \leq 0.06$). There were no significant differences ($p > 0.05$) on the temperature levels between the rivers. The salinity and TDS levels between the rivers were significantly different ($p < 0.001$).

The highest winter temperature level was observed in Namahadi river (9.07 °C) with the lowest winter temperature observed in Kollatshwene river (6.22°C) which is the highest elevated sampled river (1675m). Meanwhile, with Fikapatso Dam (8.13 °C) compared to Metsimatsho dam (9.07 °C) is the opposite since temperature levels were relatively higher than those of most rivers. The pH average levels were high in the Elands river (7.57) than in Kollatshwene river (7.12) being the least of them all. On the other hand, pH average levels were high in Metsimatsho dam (7.42) than Fikapatso dam (7.25). The highest salinity levels between rivers and dams were observed in Metsimatsho river (0.223 mg/l) as compared to 0.01 mg/l in Metsimatsho dam. Lastly, the TDS levels were high in rivers ranging from 21 mg/l (Metsimatsho river) to 297 mg/l of Elands river; than that of dams ranging from 13 to 60 mg/l in both dams.

Of the various macroinvertebrate diversity indices, the South African scoring system version 5 (SASS5) index and the average score per taxon (ASPT) were the most consistently used among all biotopes. On the other hand, of the biotopes examined the Gravel/Sand/Mud (GSM) and vegetation combination is the most variable with respect to the SASS score and number of taxa encountered. The comparative SASS scores among the sampled sites were the highest in Metsimatsho Dam (SASS score = 125; No. of Taxa = 20 and the ASPT = 6.3) followed by Elands River (SASS score = 118; Number of Taxa = 21 and ASPT= 4.8); Metsimatsho River (SASS score = 117; No. of Taxa = 17 and ASPT = 6.8); and the least was Kollatshwene River (SASS score = 64; No. of Taxa = 14 and ASPT = 4.8). The lowest ASPT score of 4.8 was observed in both Kollatshwene and Elands rivers and was indicative of poor ecological category, hence poor water quality.

Aquatic insects were identified to family level. The most occurring taxa was Baetidae with 102/298 (34.22%) and was the found in all sites; followed by Corixidae with 36/298 (12.08%) and the least being Ceratopogonidae 18/298 (6.04%). Occurrence of macroinvertebrates in water sources depends on their sensitivity to water pollution, while microinvertebrates (total and faecal coliforms) are tolerant to water pollution. Of many sampled sites, Kollatshwene, Elands and Khoptjwane rivers had the highest percentage occurrence of tolerant families (71.4%; 61.9% & 60.0% respectively). The coliform bacteria such as *Escherichia coli*, *Aeromonas hydrophila*, *Acinetobacter baumannii*, *Serratia marcescens*, *Vibrio fluvialis*, *Pseudomonas putida*, *Enterobacter cloacae*, and *Burkholderia cepacia* were also identified from Qwaqwa waters. Organisms like fish and frogs (*Ameitia* spp) as well as ,crabs (*Potamonantes* spp) occurred in water sources with good water quality mainly in Fikapatso dam since they are highly sensitive to high levels of water pollution.

All studied parameters classify the Qwaqwa river water quality status to be poor as compared to the potable water from Fikapatso and Metsimatsho dams.

Keywords: Bio-indicators; biotopes; coliform bacteria; *Escherichia coli*; faecal contamination; macro-invertebrates; micro-invertebrates; protozoan parasites; Qwaqwa freshwaters; SASS5; water quality of dams and rivers.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Preamble

South Africa's freshwater resources include rivers, man-made lakes, dams, wetlands and aquifers (Oberholster & Ashton, 2008). Water resources in this country are scarce and extremely limited in global terms (DWA, 1997) due to country's climatic variations from desert to semi-desert in the west to sub-humid along the coastal area. The country is relatively dry with an average annual rainfall of about 464 mm compared to a world average of about 860 mm. While the Western Cape gets most of its rainfall in winter, the rest of the country is generally a summer-rainfall region (www.southafrica.info/travel/advice/climate). South Africa is the 30th driest country in the world and has less water per person than countries widely considered being much drier, such as Namibia and Botswana.

By 2005, more than 95% of the country's freshwater resources had already been allocated (Oberholster, 2010) to the growing population for household use and industrial works in South Africa. The quality of water in these resources has also declined due to increased pollution caused by industrialization, urbanisation, afforestation, mining, agriculture and power generation (Ashton *et al.*, 2008). Exacerbating factors are South Africa's outdated and inadequate water treatment and sewage treatment plant infrastructure and unskilled operators (Rietveld *et al.*, 2009; Snyman *et al.*, 2006).

South Africa assigns the monitoring and assessment of quality of water resources as being critically important for determination of their fitness for use for various water use sectors (Statistics South Africa, 2005). Water quality assessment provides the baseline information on water safety, UNICEF, (2010). Consumption of water containing pathogens or toxic chemicals may result in poor hygiene that poses a serious risk to human and animal health. Therefore, because water quality in any

source can change with time and other factors, continuous monitoring is basically essential (UNICEF, 2010). Bioassessment or biomonitoring techniques are used to determine responses of aquatic biota against water changes in aquatic ecosystems. Both aquatic vertebrates and invertebrates can be used as indicators of water quality.

Macroinvertebrates have been widely used as indicator organisms of environmental health (Rosenberg & Resh, 1993). Some are benthic and inhabit bottom substrates like sediments, logs, debris and macrophytes of freshwater for at least part of their life cycle and are retained by mesh sizes > 200 to 500µm (Rosenberg & Resh, 1993). Benthic macroinvertebrates are organisms large enough to be seen with a naked eye. They lack a backbone and the examples are insects (in their larval or nymphal stage), crayfish, clams, snails and worms most of which live part of their life cycles attached to submerged rocks, logs and submerged aquatic vegetation, (Rosenberg & Resh, 1993). They are widely distributed within diverse communities which constitute a broad range of trophic levels and pollution tolerances (Rosenberg & Resh, 1993; Metcalfe-Smith, 1994; Barbour *et al.*, 1999) cited by Maseti (2005). Understanding the habitat preferences of aquatic macroinvertebrates can aid in the preservation and monitoring of aquatic habitats. Their large taxonomic diversity has allowed aquatic macroinvertebrates to adapt to a wide variety of environmental conditions and factors (Demars *et al.*, 2012).

According to Farrell-Poe (2000), microinvertebrates also known as microorganisms are ever present in both terrestrial and aquatic ecosystems with most types being beneficial, functioning as agents for organic and synthetic chemical decomposition. They act as food sources for larger animals, and are essential components of nitrogen cycle and other biogeochemical cycles. A small fraction of microorganisms can be harmful if taken into the body; they could cause diseases or even death. These microorganisms are called pathogens. They occur in all environmental systems like water, soil, blood and other potential media with some contamination or pollution (Farrell-Poe, 2000). Waterborne pathogens particularly coliform bacteria and protozoan parasites such as *Cryptosporidium*, *Entamoeba* and *Giardia* species are of primary concern in areas of drinking water supplies (Fricker *et al.*, 1997).

Cyst and oocysts of *Giardia* and *Cryptosporidium* species are exceptionally resistant to water treatment (Casemore, 1991; Jakubowski *et al.*, 1991; Bellamy *et al.*, 1993); and they generally occur in low numbers in the environment and are not readily detectable. However, their minimal infections may be as low as a single cyst or oocyst making them difficult to detect in water constituting a high health risk (Regli *et al.*, 1991; Bellamy *et al.*, 1993).

On the other hand, surface water contamination includes microbial, inorganic, organic and radioactive contaminants. Microbial contaminants are bacteria, virus and protozoa (Aull, 2005) some of which are found in faecally contaminated freshwater bodies. Water pollution caused by fecal contamination is a serious problem due to the potential for contracting diseases from pathogens. Frequently, concentrations of pathogens from fecal contamination are small, and the number of different possible pathogens is large. As a result, it is not practical to test for pathogens in every water sample collected. Instead, the presence of pathogens is determined with indirect evidence by testing for an "indicator organism" such as coliform bacteria (EPA - USA, 2001). Coliforms come from the same sources as pathogenic organisms like bacteria. Coliforms are relatively easy to identify, are usually present in larger numbers than more dangerous pathogens, and respond to the environment, wastewater treatment, and water treatment as other pathogens such as bacteria and protozoan parasites. As a result, testing for coliform bacteria can be a reasonable indication of whether other pathogenic bacteria are present (EPA - USA, 2001). Microorganisms such as *Escherichia coli* (*E. coli*) and faecal coliforms are used to indicate the presence or absence of pathogens, rather than measuring individual pathogens (Aull, 2005).

As the custodian of South African's water resources, the policy of the Department of Water Affairs and Forestry (DWAF) is to ensure that aquatic ecosystems are healthy, and are utilized on a sustainable basis (DWAF, 2007). As a result, DWAF initiated the River Health Programme (RHP) in 1994. This programme is designed to develop a capacity and information based system that enables the report on the ecological state of rivers, in an objective and scientific manner.

1.2 Invertebrate inhabitants of water systems

The aquatic invertebrate communities of perennial pans of Mpumalanga (South Africa) are probably the most prominent feature in these ecosystem (Ferreira *et al.*, 2006). In arid regions, temporary water communities consist largely of crustacean orders collectively known as phyllopods. Some taxa that were commonly sampled in those temporary environments included Anostraca, Notostraca, Conchostraca, Cladocera, Ostracoda, Corixidae, Notonectidae, Dytiscidae and Hydrophilidae (Hutchinson *et al.*, 1932; Rzo'ska, 1961; Weir, 1969; Williams, 1985; Seaman, *et al.*, 1991; Meintjies, 1996). However, even though there is a general lack of literature on the invertebrate communities of perennial pans, those invertebrates were identified as possible indicators of ecological integrity for wetlands (Bird & Day, 2009).

The use of macroinvertebrates in aquatic ecosystems as indicators, including the community structure and species composition, began over a century ago (Haase & Nolte, 2008) and is now well established in many countries over the world (Dickens & Graham 2002; Hawkins, 2006; Ollis *et al.*, 2006).

1.3 Freshwater benthic invertebrates

Freshwater benthic invertebrates are small animals ranging from microscopic to several centimeters (Umar *et al.*, 2013) and are without backbones. Benthic invertebrates can be abundant in freshwater and are used as indicators of changes in water quality; they also form an important part of aquatic food webs (Umar *et al.*, 2013). Their ability to respond to temperature and pH changes in freshwater conditions can be used for bio-monitoring water quality and can provide useful information on cultural and anthropogenic disturbances (Umar *et al.*, 2013). Their abundance in water surfaces presupposes the aquatic infections by different waterborne pathogens including the enteric protozoan parasites.

In most studies invertebrates have been identified to family level and occasionally genus; and most identification had to be made using texts and keys that deal largely with European and American faunas than African freshwater invertebrates (Umar *et*

al., 2013). Many aquatic insects' larvae have not been associated with adults, and consequently their accurate identification to species is not possible (Boulton *et al.*, 2008; Pearson & Boyero, 2009; Solomon *et al.*, 2009).

A majority of fish species are freshwater pollution bio-indicators which can carry heavy infection of parasites and pathogens, which cause deterioration in the food value of other fish and may even result in their mortality. Besides, there are a number of 'helminth parasites' which are transmitted to men only through fish (Hassan, 2008). Parasitic helminths (trematodes, cestodes and nematodes) may have direct and indirect life cycles. Direct life cycle involve a single host with, sexual reproduction occurring on or in the host and eggs or larvae usually leaving the host and spending some time free-living in the environment before infecting the same species of host as the one from which it was discharged, without the obligatory involvement of any other host species (Hassan, 2008).

The basic principle behind the study of macro- and microinvertebrates and fish as pollution bio-indicators is that some are sensitive to pollution than others, that is, if a particular stream site is inhabited by pollution tolerant organisms, the pollution problem is evident and pollution – sensitive organisms may not be found at that stream site (Farrell-Poe, 2000).

1.4. Water pollution and pathology of protozoan parasites

Encysted water-borne parasites such as *Giardia* and *Cryptosporidium* spp. have presented challenges to water suppliers world-wide many decades ago (Mackintosh *et al.*, 2000). *Giardia* and *Cryptosporidium* are two protozoan parasites that occur in aquatic environment throughout the world. They have been found in most surface waters where their prevalence is related to the level of faecal pollution (Hansen & Ongerth, 1991; LeChevallier *et al.*, 1991). They are implicated in a number of outbreak and sporadic disease patterns and are endemic in livestock, domestic and feral animals.

These tiny microbes are difficult to inactivate with conventional disinfectants. Furthermore, they are difficult to remove with conventional treatment and filtration as a result is responsible for reported diarrhoeal instances, (Mackintosh *et al.*, 2000). Cryptosporidiosis, the most life threatening infection of children ≤ 5 years old, elderly and other suppressed immune systems including those who have recently had organ transplants and individuals receiving chemotherapy has a variety of hosts and hardy nature that makes vast transmission potential of parasites for widespread distribution (Mackintosh *et al.*, 2000).

1.5 Water pollution and pathology of bacterial pathogens

Drinking water supplies of poor sanitary quality has been linked to several illnesses of human populations because of a number of types of bacterial pathogens that can be present in human and / or animal wastes (faeces) depending on the source of contamination affecting the water supply,(Rahman *et al.*, 2014). The illnesses most commonly present as gastrointestinal – related symptoms such as diarrhoea and nausea. Monitoring of a broad indicator of faecal contamination such as *Escherichia coli* in drinking water systems may indicate that the source has been infected, thus, water in such systems should be deemed unsafe to drink (Maheux *et al.*, 2009).

1.5.1 Diseases from waterborne pathogens

Infectious diseases caused by pathogenic bacteria, viruses and protozoan parasites are among the most common and widespread health risk of drinking water. People are introduced to these microorganisms through contaminated drinking water, water drops, aerosols and washing or bathing (Lenntech, 2014).

Some waterborne pathogenic microorganisms spread by water and can cause severe, life-threatening diseases. Examples are typhoid fever, cholera, hepatitis A or E, shigellosis, cryptosporidiosis, giardiasis, toxoplasmosis campylobacteriosis, amebiasis, salmonellosis and legionellosis. Diarrhoea is often the main symptom of these illnesses. People with low resistance, mainly elderly people and young children, are vulnerable to these diseases as well (Lenntech, 2014).

1.6 Water quality evaluation and monitoring

1.6.1 Water quality evaluation

The term “water quality” describes physical, chemical and biological and aesthetic characteristics of water to determine its suitability for a variety of uses and for the protection of the aquatic ecosystems’ health and integrity (Maseti, 2005). For instance, these characteristics include dissolved oxygen, pH, nutrients and temperature (Farrell-Poe, 2000). Suitability of water sustains various uses of agricultural, domestic, and industrial processes. This suitability could be influenced by the concentrations of toxic substances for the drinking water uses or restrictions on temperature or pH ranges for water supporting invertebrate communities (DWAF, 2007).

The composition of surface and groundwater is dependent on natural factors of geological, biological, topographical, meteorological and hydrological nature in the drainage basin and varies with seasonal differences in runoff volumes, weather conditions and water levels (DWAF, 2009). Human intervention also poses significant effects on water quality; and some of these effects are the result of hydrological changes such as building of sampled rivers, draining of wetlands and diversion of water flow (DWAF, 2009).

The most crucial effects of human intervention are the polluting activities such as the discharge of untreated or partially treated domestic; industrial, urban and other wastewaters into the water sources; and the spreading of chemicals on agricultural lands. Eutrophication, faecal pollution or diffuse pollution may also give rise to a number of water quality problems (DWAF, 2009).

Oberholster & Ashton, (2008) reiterated that the quality of the South African water resources had declined due to pollution aggravated by the booming population growth. Water quality status of freshwaters of Qwaqwa area of Maluti-A-Phofung (MAP) municipality is not known. The major aim of this study is to document information about water quality status of this area of Qwaqwa freshwaters. Water sources of Qwaqwa comprise dams (Fikapatso and Metsimatsho) and rivers (Metsimatsho, Namahadi, Kollatshwene, Khoptjwane and Sekoto). Dams of Qwaqwa provide potable (drinking) water for humans while rivers are for waste discharge of

MAP water treatment plants; and this is the same water that supports animal drinking and irrigation purposes for crop farming. Although MAP Water conducts regular assessment of Qwaqwa water quality based on limited sampling from these two dams, there is a need for a neutral body to do water quality assessment using the biology of rivers to guide environmental management (Karr, 1998) in all water resources of this area for documentation in the public domain.

1.7 Statement of the problem

Water quality parameters such as pH, dissolved oxygen, turbidity electrical conductivity and salinity are important indicators of aquatic ecosystem health and can provide a measure of damage attributed to human activity. Significant deviation of these parameters from “natural” levels can result in ecosystem degradation; and may impact environment qualities and beneficial uses (Goudey, 2003). Aquatic ecosystems are defined as the abiotic (physical and chemical) and biotic (biological) components, habitats and ecological processes contained within lotic and lentic systems. The definition of aquatic ecosystems includes three primary abiotic and biotic components, namely, sediments (bottom or suspended), water and the riparian zone. Terrestrial biota, other than human dependent on aquatic ecosystems for survival are included in this definition (DWAF, 1996).

To date, the assessment of microbial quality of drinking water is based exclusively on culture techniques. Since these methods do not allow for the detection of specific water pathogens, most pathogens in drinking water are faecal borne (Rosenberg, 1998) found in human and animal wastes. The coliform bacteria which are always present in the digestive systems of humans and animals, are commonly used as pollution bioindicators. For example, *Escherichia coli* O157 may be present even when fecal coliform measurements show negative.

Furthermore, viruses and most protozoan parasites, such as *Giardia* and *Cryptosporidium*, are resistant to chlorination and filtration, which usually kill coliform bacteria (Rosenberg, 1998). As a result, coliform bacteria cannot be accurate indicators in such cases, particularly in chlorinated waters. Basically, indicator method usually requires cultivation on nutrient media which makes it impossible to

obtain reliable results in less than one day, by the time the results are available, pathogens might have spread wide in the water distribution system. There is clearly a need to find new approaches to monitor the microbiological quality of water.

Macroinvertebrates within the same system may be residents for several months to multiple years, depending on the lifespan of the particular organism and reflect the chronic effects of pollutants, and yet short enough to respond to relatively acute changes in water quality. Unlike fish, these populations tend to be relatively immobile, as a result they are continuously exposed to the constituents of the surface water they inhabit (Xu *et al.*, 2014). Certain fish species may disappear as a result of change in water quality, not because the fish species cannot tolerate the change in water quality; but because the organisms that are the primary food source might be eliminated by the particular change in water quality (DWAF, 1996).

Waterborne parasite infections are considered a re-emerging threat featuring in most studies carried out in developed countries in the epidemiology of cryptosporidiosis, giardiasis, amebiasis (Bakir *et al.*, 2003) and toxoplasmosis. Comparatively, there is less data documented on the occurrence of these infections in other areas (Bakir *et al.*, 2003), especially in developing countries. Infectious, water-related diseases are a major cause of morbidity and mortality world-wide. Newly recognized pathogens and new strains of established pathogens are being discovered to present an additional challenge to both the water and public health sectors (WHO, 2004). About 35 pathogens were discovered between 1972 and 1999 while many more have re-emerged as pathogens that may be transmitted by water.

Human infections caused by both free-living (associated with recreational water) and enteric protozoa (in drinking water) have been associated with several waterborne outbreaks in North America and other countries (Schuster *et al.*, 2005; Karanis *et al.*, 2007). Enteric protozoa that are associated with waterborne diseases in Canada are *Cryptosporidium* and *Giardia* spp. characterised with some strains that are highly pathogenic, and can be highly resistant to chemical disinfection (Rossignol, 2009), particularly in chlorinated water. Other enteric protozoa of human health concern are *Toxoplasma gondii*, *Cyclospora cayentanensis* and *Entamoeba histolytica* (Dixon *et al.*, 2013).

In the South African context, a study was conducted in KwaZulu-Natal, Pietermaritzburg to investigate the occurrence of *Cryptosporidium* and *Giardia* species in catchment areas and wastewater works (Jarmey-Swan, 2001). *Cryptosporidium* was detected in 3% while *Giardia* was detected in 23% of analysed samples; where high number of oocysts were detected in rivers following increased rainfalls. *Cryptosporidium* and *Giardia* species were also detected in the treated effluent and sludge samples from wastewater works indicating that neither the activated sludge process nor anaerobic digestion are able to inactivate these organisms (Jarmey-Swan, 2001).

There is dearth of information on the water quality of Qwaqwa freshwaters (dams and rivers). Furthermore, the macro- and micro-invertebrate composition of Qwaqwa freshwaters is not known. As a result the current study has been formulated to document this environmental information as it is of importance to both animal and human health.

1.8 Objectives of the study

1.8.1 General objective

The aim of this study was to conduct evaluation of macro- and microinvertebrates and seasonal assessment of water quality in sampled rivers and dams of Qwaqwa freshwaters of Maluti-A-Phofung Municipality.

1.8.2 Specific objectives

- To assess water quality of Qwaqwa freshwaters (rivers and dams) on seasonal basis.
- To document macroinvertebrates occurring in Qwaqwa freshwaters (sampled rivers and dams) using the South African scoring system (SASS) method.
- To document microinvertebrates and water-borne pathogens in Qwaqwa freshwaters (sampled rivers and dams).

CHAPTER 2

WATER QUALITY ASSESSMENT OF QWAQWA DAMS AND RIVERS

1 INTRODUCTION

According to Farrell-Poe, (2000) water quality is the term that is used to describe the chemical, physical and biological characteristics of water, generally in the terms of sustainability for a designated use. Many water quality studies conducted in South Africa (Oberholster & Ashton, 2008; Ashton, 2009; DWAF, 1996; DWAF, 2009) indicated that poor maintenance of wastewater and sewage treatment infrastructure contributes to the pollution of water resources upon which rural communities depend for all their domestic and other purposes. As a result of pollution, human health and the environment are directly affected.

In South Africa, water quality standards are tinted by human activities especially in the metropolitan areas that are located on the watersheds of river catchments. These rivers have a burden of providing water supplies and transporting waste (faecal) material to the dams located downstream of urban and metropolitan areas during the recent decade (Oberholster & Ashton, 2008).

Agricultural activities also affect water quality. Studies facilitated on salinity on irrigated agriculture indicate that irrigation has led to the deterioration of water sources (Van Rensburg *et al.*, 2011). Eutrophication in rivers has led to widespread incidents about fatal cyanobacterial poisoning in South African reservoirs (Oberholster & Ashton, 2008). However, to date, these poisoning involved deaths of livestock, domestic animals, wildlife with no human fatalities recorded in South Africa (DWAF, 2004).

Water quality assessment is important in the overall assessment of ecological status of aquatic ecosystem. Water quality data gathering is therefore vital in assessment

and monitoring of river health as water quality changes may affect the overall health of the river (Maseti, 2005).

The process of sampling water and analyzing water conditions and its characteristics is called water quality monitoring (Farrell-Poe, 2000). “Common water parameters such as pH, nutrients, oxygen and temperature can be biological, physical and chemical in nature” (Table 2.1), contented Farrell-Poe, (2000).

Table 2.1: Water quality parameters (USDA-CSREES Volunteer Monitoring 2003), Farrell-Poe, (2000).

Physical/Chemical Parameters	
Temperature	Dissolved oxygen (DO)
Alkalinity	pH
Salinity	Phosphorus
Nitrogen	Hardness
Flow/Water level	Chloride
Turbidity or Secchi transparency	Metals
Suspended sediment or Total suspended solids	Organic chemicals
Dissolved Solids (TDS)	Biochemical oxygen demand (BOD)
Electrical Conductivity	Color
Total suspended solids (TSS)	Radiochemicals
Biological Indicators	
Macroinvertebrates	Aquatic Wildlife
Bacteria	Chlorophyll
Phytoplankton	Fish
Shellfish	Exotic/Invasive species
Aquatic or terrestrial vegetation	Algae

Water quality assessment / quality control (QA/QC) strategies are required if biomonitoring results are intended for policy use. On the other hand, investigators should be able to design the QA/QC procedures that are best suited to their needs and available resources (Mandaville, 2002).

Although the Water Quality Index (WQI) has been developed to assess river health worldwide, it is not widely used in South Africa and is not currently used in river health program (RHP) monitoring (Maseti, 2005). For the RHP, water quality data are obtained from DWA gauging weirs. Physical water quality variables like conductivity, temperature, dissolved oxygen and pH are measured on site on each sampling station (Maseti, 2005). Additionally, biological assessment can provide information on the species composition at a site under consideration (EPA - Ireland, 2012). Biological information may support modification of the default species sensitivity distribution to better reflect the expected community composition at the site, for example, if there is naturally occurring warm body of water, coldwater fish species could be replaced by resident warmwater fish species in the species sensitivity distribution from which a site-specific criterion is calculated, (EPA - Ireland, 2012).

The current water quality status information in South Africa by Oberholster (2010) claims that main factors contributing to the deterioration of water quality are salinisation, eutrophication, disease-causing micro-organisms and acidification. Water quality has also declined due to increased pollution caused by industry, urbanisation, afforestation, mining, agriculture and power generation (Ashton *et al.*, 2008). Exacerbating factors include outdated and inadequate water treatment and sewage treatment plant infrastructure and unskilled operators (Rietveld *et al.*, 2009; Snyman *et al.*, 2006).

These problems are due to health-threatening microorganisms, numerous persistent and toxic metals and organic compounds. Contamination of groundwater by toxic and persistent compounds can cause irreversible pollution, influencing water users long after the original release to the environment has ceased (Oberholster, 2010). Changes in water quality conditions can have detrimental effects on aquatic biota and therefore affect their ability to provide natural cleansing activities in aquatic ecosystems such as breaking down of organic matter (Maseti, 2005).

2.2. Indicator organisms

An indicator organism is an organism used to measure environmental conditions like potential faecal contamination. Therefore the presence of coliform bacteria like *E. coli* in surface waters is a common indicator of faecal contamination (Noble *et al.*, 2003). Indicator organisms create a basis for alternative way of classifying each individual pathogen in a water sample by simply enumerating indicator organism's colonies when cultured in water. According to Noble *et al.*, (2003), indicator organisms should be present in high numbers in faecal matter to be easy to detect when the pathogen is present and vice versa. Faecal indicators are used to develop water quality criteria to support designated uses, such as primary contact recreation and drinking water supply; assess the degree of pathogen removal by treatment processes or to detect contamination of distribution systems, (EPA - USA, 2001).

2.2.1. Water quality indicators

Water quality of a stream/ river can be determined in several ways and can be compared relatively between several water sources; or can be measured absolutely in consideration of biological, chemical and physical factors or parameters that can indicate the quality of water (EPA - Ireland, 2001). Communities of fish, aquatic invertebrates and riparian vegetation are the primary indicators of ecological integrity used in the RHP, with a number of biotic indicators (including geomorphology and habitat integrity) used to characterise a site (Dallas, 2000a) and aid interpretation of the biological results (Roux *et al.*, 1999; Mangold, 2001).

2.2.1.1 Biological indicators

The use of biological surveys and other direct measurements of living systems within the watershed is one way of determining the status of water's living systems through biological assessment (bioassessment) (Fowler *et al.*, 2000).

Macroinvertebrates are valuable indicators of health of aquatic environments because they are typically found at the bottom of a stream or lakes hence “benthic” since they do not move over larger distances (Fowler *et al.*, 2000), (more details on Chapter 3).

Since different families of macroinvertebrates react differently to environmental stress like pollution, sediment loading and habitat changes, quantification of the diversity of macroinvertebrates at a study site may indicate the status of the environmental conditions of that water body

Basic microbiological tests should also be done to cover coliform groups (faecal and thermo-tolerant coliforms) since they can be affected by physical (water temperature, colour, taste and turbidity) and chemical parameters such as pH, disinfectant residuals and dissolved oxygen since these may affect both growth and reproduction of fish as an aquatic animal.

2.2.1.2 Chemical indicators

Since humans and aquatic animals depend on water with pH levels near neutral point, bioassessments are more useful when combined with chemical and habitat assessments, (EPA - Ireland, 2001). The pH is the crucial water quality indicator as it measures the concentration of hydrogen ions, which then allows for the inference of the strength of the acid and/or base. In addition, the concentration of the dissolved solutes such as nitrates, phosphates, ammonia, fluoride and iron as well as metals and other organic compounds may also affect the quality of water.

2.2.1.3 Physical indicators

Turbidity and temperature are the major physical indicators of water quality. Measuring turbidity will give or estimate the volume of suspended and colloidal matter present in the water body at a particular time (EPA - Ireland, 2012). Suspended and colloidal matter (microscopic particles that remain suspended in water and diffract light) can be anything that is suspended in water column ranging from sand, silt, clay, plankton, industrial wastes, sewage, lead and asbestos to

bacteria and viruses which occur naturally while some are produced by human activities, (EPA- Ireland 2001) such as agricultural operations.

Since cloudy water absorbs more of the sun's energy than clear water, high turbidity leads to high temperatures which can severely affect aquatic organisms especially those which have adapted to survive within narrow temperature ranges (EPA-Ireland 2001).

Aquatic organisms are particularly susceptible to the effects of increased sediments and turbidity. Many fish species need clear water water to spot their prey; while macroinvertebrate and fish eggs and larvae require oxygen - rich water circulating through the clean gravel beds to survive (Rashleigh, 2009). Sediments and other dissolved and / or suspended substances also decrease light penetration and inhibits aquatic plant photosynthesis.

2.2.2 Biotic indices and rapid bioassessment

Biological community data can be summarised and presented as simple, numeric or categorised indices (Ollis, *et al.*, 2005). These indices allow the results of ecological assessment to be communicated in a way that is understandable to the general public (Hawkes, 1979). Three basic types of indices can be generated as diversity indices, comparison (similarity or dissimilarity) indices and biotic indices (Johnson *et al.*, 1992) and of these indices, biotic indices are the most widely used.

2.2.3 Water quality parameters

Water quality parameters are chemical, biological and physical attributes upon which assessment is based. It is measured by several factors such as the concentration of dissolved oxygen, bacterial levels, the amount of salts (salinity), or the amount of material suspended in water (turbidity). In some aquatic bodies, the concentration of microscopic algae and quantities of pesticides, herbicides, heavy metals and other contaminants may also be measured (www.freedrinkingwater.com).

2.2.3.1 Chemical parameters

2.2.3.1 (a) Total dissolved solids (TDS)

The term total dissolved solids (TDS) refers to solids that are dissolved in water (usually mineral salts). The TDS and the electrical conductivity are directly proportional (Lenntech, 2014). The more salts are dissolved in water; the higher is the value of the electric conductivity. The majority of solids which remain in water after a sand filter are dissolved ions. Sodium chloride for example is found in water as Na^+ and Cl^- . The TDS includes ionized and non-ionized matter but is reflected in conductivity, thus where the TDS are high, water may be “saline” and the applicable parameter is “salinity” (EPA – Ireland, 2001). The water temperature affects the electric conductivity so that its value increases from 2 up to 3% per 1°C of water temperature. Measuring TDS is a way to estimate water quality for irrigation and drinking (EPA - Ireland, 2001).

The TDS is the measure in milligrams per liter (mg/L) of the amount of dissolved materials in water. Ions such as potassium, sodium, chloride, carbonate, sulphate, calcium, and magnesium all contribute to the dissolved solids in the water (EPA - Ireland, 2001).

2.2.3.1 (b) Salinity

Salinity is an indicative measure of the total concentration of cations that include sodium, calcium, magnesium, and potassium (Na^+ , Ca^{2+} , Mg^{2+} , K^+), and anions that include sulphate, carbonate, bicarbonate, and chloride (i.e. SO_4^{2-} , CO_3^{2-} , HCO_3^{2-} , Cl^-) in solution (ANZECC/ARMCANZ, 2000). Salinity may also be expressed as TDS or Total Soluble Salts (TSS), which refer to the residual weight of salts after drying and filtration (Dunlop & McGregor, 2005).

Soluble salts occur naturally in aquatic ecosystems and are a vital component of the normal functioning of freshwater biota. They are ubiquitous in soils and are a remnant of geological history. Salts are also an integral part of the biochemistry of life in terrestrial and aquatic environments though for many freshwater aquatic animals exposure to high concentrations of salt can have toxic effects. Similarly, a

lack of salt can also act as a toxicant in saline and estuarine environments for freshwater species (Dunlop & McGregor, 2005).

2.2.3.1 (c) Dissolved oxygen (DO)

Dissolved oxygen (DO) is a measurement of the amount of oxygen gas dissolved in water, and available for use by plant and aquatic spp (Aull, 2005). Oxygen gas naturally mixes with water through surface interactions, for example, fast moving water typically have higher DO due to mixing with air when it comes in contact with debris such as rocks and logs (Vigil, 2003); and can be depleted by the demand from organic decomposition and use from plant and animal respiration (Aull, 2005); therefore DO relates with the concept of biochemical oxygen demand (BOD) as a measure of the use of dissolved oxygen by life forms, particularly during decomposition of organic matter (EPA - Ireland, 2001).

Aquatic populations exposed to low dissolved oxygen concentrations may be more susceptible to adverse effects of other stresses such as diseases or effects of toxic substances, hence different species of fish need different amounts of DO to thrive (Aull, 2005).

Dissolved oxygen measurements can be expressed as a concentration, milligrams per liter (mg/L), or as percent saturation to indicate the amount of oxygen the water holds compared to what it could absorb at that temperature (Addy & Green, 1997), EPA – Ireland, (2001). Turbulence interactions with air and photosynthesis can replenish DO in water; while cold water holds more DO than warm water.

2.2.3.1 (d) Nutrients

As indicated by EPA - Ireln, (2001) nutrients are chemical elements that are essential to plant and animal life and growth where nitrogen and phosphorus are two main nutrients that are important in aqautic life. Nutrients in the aquatic system are measured in milligrams per liter (mg/L). Commonly used nutrient parameters include nitrates, ammonia, orthophosphate, and total phosphorus (EPA - Ireland, 2001).

When nutrients are at high levels (concentrations), they are considered as contaminants and almost always have an impact on water pollution (www.ecy.wa.gov). Nutrient loading can result in increased algae growth. In stream segments where conditions are right, algae take the form of an attached growth – called periphyton (www.ecy.wa.gov) on rocks, logs, and other substrates grow as long green filaments or masses of algae growing in streams making rocks slippery. Excessive growths of attached algae can cause low DO, unsightly conditions, odors, and poor habitat conditions for aquatic organisms (www.ecy.wa.gov).

2.2.3.2 Physical parameters

2.2.3.2 (a) pH

The concept pH is defined as the negative logarithm of the hydrogen ion concentration of any media and thus measures whether the media is acid or alkaline. The pH scale ranges from 0 (strong acid) to 14 (strong alkaline). The range of natural pH in freshwater extends from around 4.5 for acid, to 10.0 in waters where there is intense photosynthetic activity by algae (EPA-Ireland, 2001). The range of pH suitable for fisheries is considered to be 5.0 - 9.0, though 6.5 – 8.5 is preferable (EPA – Ireland, 2001),

$$\text{pH} = -\log [\text{H}^+]$$

Several factors can be affected by the pH of water; including biological availability and solubility of elements in water (Aull, 2005). Growth and reproduction of freshwater aquatic species of fish are found to be thriving within a pH range stipulated above, however they may be thriving slightly outside this range (6.5 – 8.5) as well (Wilber, 1969), (Figure 2.1).

In waters with low dissolved solids, which consequently have a low buffering capacity (i.e. low internal resistance to pH change), changes in pH induced by external causes may be quite dramatic (EPA – Ireland, 2001). Extremes of pH can affect the palatability of water but the corrosive effect on distribution systems is a more crucial problem, EPA – Ireland, (2001). The pH can also affect the solubility and toxicity of chemicals and heavy metals in the water. Low pH results when

atmospheric oxygen reacts with water in contact with sulphides such as pyrite which reacts and forms acid; which then dissolves naturally occurring metals resulting in acid mine drainage and elevated concentrations of metals in water (Farrell-Poe, 2000).

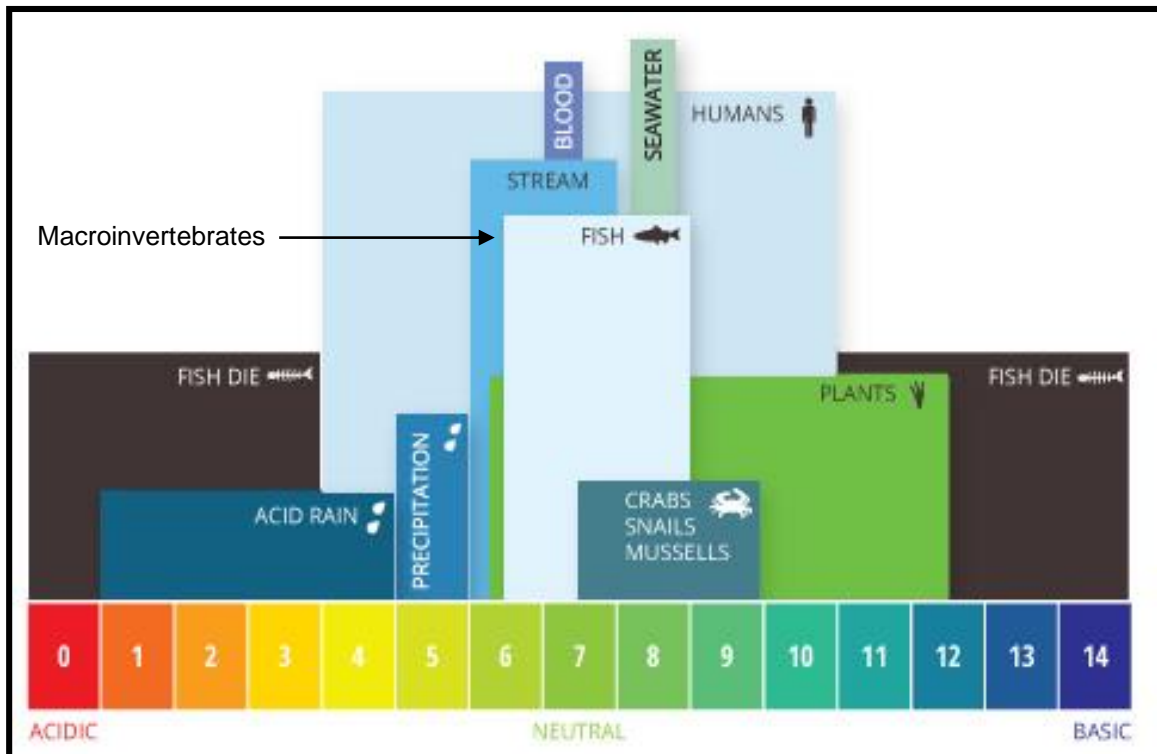


Figure 2.1: Aquatic pH levels. The optimum pH levels for fish are from 6.5 to 9.0. Outside of optimum ranges, organisms can become stressed or die. <http://www.fondriest.com/environmental-measurement/parameters/water-quality/ph>.

2.2.3.2 (b) Turbidity

Turbidity is the measure of clarity or the ability of light to pass through water. It measures the amount of particulate matter and dissolved color that is suspended in water (EPA - Ireland, 2001). Turbidity in water arises from the presence of very finely divided solids which are not filterable by routine methods (EPA-Ireland, 2001). The particles forming turbidity may also interfere with the treatability of waters; and the former could be caused by sewage matter in water posing a risk of having pathogenic pathogens shielded by turbidity particles and hence escape the action of disinfection (EPA-Ireland, 2001). On the other hand, high turbidity can cause increased temperatures because suspended particles absorb more heat; and consequently make it difficult for fish to a prey.

2.2.3.2 (c) Electrical conductivity

The conductivity of water is an expression of its ability to conduct an electrical current as this property is related to the ionic content of the sample which in turn expresses a function of the dissolved (ionisable) solids concentration (EPA - Ireland, 2001; APHA, 1998). In itself, conductivity is a property measured or reported in microsiemens per centimeter (mS/cm) as:

$$\text{Conductivity } (\mu\text{S/cm}) \times 2/3 = \text{Total Dissolved Solids (mg/L)}$$

This is a measure often used to estimate the amount of total dissolved solids / salts in the stream, rather than measuring each dissolved constituent separately (EPA - Ireland, 2001). Even though conductivity is a property of little interest to analysts, it is an invaluable indicator of the range into which hardness and alkalinity values are likely to fall (EPA - Ireland, 2001).

2.2.3.2 (d) Temperature

Water temperature is a crucial aspect of the aquatic habitat since it affects nearly all other water quality parameters; aquatic organisms are adapted to certain temperature ranges, thus, as the upper and lower limits of the range are approached, the organisms become more susceptible to diseases (EPA - Ireland, 2001). Also, fish that spend extra energy searching for cool areas might be at a disadvantage when competing for food.

The effect of temperature, especially changes in temperature on living organisms can be critical where biochemical reactions are concerned, as in the uptake of oxygen by bacteria and a rise of 10°C in temperature leads to an approximate doubling of the rate of reaction (EPA – Ireland, 2001). High temperatures also decrease gas solubility and respiration rates (Aull, 2005; citing Tchobanoglous, 1985). Warmer waters have lower dissolved oxygen solubility than cooler waters; hence low DO levels negatively affect plant and aquatic species within the water and change the character of a water body (Wilber, 1969; APHA, 1998; Kailasam & Sivakami, 2004).

According to EPA - Ireland, (2001), daily and seasonal temperature fluctuations affect an organism's metabolism, growth and reproduction. The emergence of many aquatic insects is influenced by warmer water temperatures for their early development; hence aquatic organisms' life cycles are adapted to water temperatures. Furthermore, EPA - Ireland, (2001) also indicated that stream temperature is regulated by solar energy, the surface area of stream, shade, the volume of water moving through the stream and several other factors.

2.2.3.2 (e) Stream Flow

Stream flow or discharge is the volume of water discharged or moving through a stream at any given time and is often expressed in cubic feet per second (cfs) or sometimes as gallons per minute (gpm), EPA - USA, (2001). The stream discharge can vary on a daily, weekly, monthly, or seasonal basis on response to precipitation, snowmelt, dry periods and water withdrawals (EPA - USA, 2001).

As water flows over rocks, a protective boundary layer forms at about 1-4 mm thick where the velocity of the current falls dramatically. A flattened body shape allows the insects to live in this boundary layer and not be carried away by the current (Mitchell & William, 1996). Since stream flow affects water chemistry, thus water quality measurements should always be interpreted in relation to stream flow (EPA - USA, 2001).

2.2.4 Water quality biomonitoring

Biological monitoring is the study of biological organisms and their responses to determine environmental conditions (EPA - Ireland, 2012). This involves collecting, processing and analyzing aquatic organisms (mainly macroinvertebrates) to determine health of the biological community in the river/stream, EPA - Ireland, (2012). For the purpose of assessing water quality, sampling is focused on benthic macroinvertebrates, those organisms that live at the stream bottom (Hadlev, 2014).

Through water quality monitoring, communities can assess the health of their streams and rivers over time. Once baseline data on the health of a stream is

collected, subsequent monitoring can help identify when and where pollution incidents occur (Hadlev, 2014). Water quality can be assessed using chemical sampling or biological sampling.

2.2.5 Water pollution control and surveillance in South Africa

Prevention of waterborne infections by blocking transmission via water cannot be avoided, and constitutes a challenge that humans have to face in the developing and developed world (Graczyk *et al.*, 1997). The importance of ensuring high quality of water supply is greater compared to the growing population of immune-compromised and immune-suppressed people worldwide while waterborne infections are predominantly related to poverty and low sanitation (Pozio, 2003).

For the purpose of protection and maintaining aquatic ecosystems, prevention rather than mitigation of effects of poor water quality need great emphasis (DAWF, 1996). The National government of South Africa is developing a policy for waste discharges and disposals and requires that social, environmental and economic impacts be considered. The precautionary approach with the contaminant waste reduction and minimization strategies forms the cornerstone of water quality management within the department of water affairs and forestry (DWAF, 1996).

The department of water affairs and forestry in collaboration with other organizations of the state have developed a policy on the delineation of wetlands and riparian zones to create buffer zones in which development will not be allowed (DWAF, 1996).

Protocols to determine ecological and basic human needs reserve have been developed for South Africa's water resources; and these reserves have been determined for a large number of water resources. Other protocols to determine the ecological requirements of rivers, dams and estuaries have been updated and are being used for license application requests and planning purposes (DWAF, 1996).

2.2.6 National pollution discharge elimination system

In accordance with the Title IV of the Clean Water Act, a system for permitting wastewater discharges was created and called the National Pollution discharge Elimination System (NPDES). This system required all facilities that discharged pollutants from point sources to obtain a permit that regulated technological requirements and quantitative limits on the water discharged (Aull, 2005).

Since its origination in the United State of America, this permitting system had significantly reduced the amount of point source pollution entering surface waters, preventing billions of pounds of pollution from entering surface waters every year and two thirds of the nation's surface water were safe for fishing and swimming (U.S.EPA, 2004; 1998).

In South Africa, larger urban areas along the coast have collecting systems in place for municipal wastewater although problems are often encountered in older areas where deterioration of infrastructure results in regular spillage and seepage. The supply of effective collecting systems in the rapidly expanding informal settlements in coastal urban areas is also difficult (DWAF, 2004).

In smaller coastal communities along the South African coast, collecting systems are often not supplied and non-sewered systems such as septic tanks and french drains are typically used for the treatment of domestic wastewater (DWAF, (2004). Where collecting systems are installed in smaller coastal communities, there is large seasonal fluctuation in the service population (i.e. service population during the holiday season usually increase markedly compared with the numbers in off-season). A concern with non-sewered systems used in these communities, usually situated next to sensitive areas such as estuaries, is the potential impact that spillage or seepage from these systems could have on the aquatic ecosystem and other users (e.g. recreation) of the water resource (DWAF, (2004).

According to DWAF, (2004) the risk of impact on water resources often increases markedly with the increase in number and density of non-sewered systems in a

particular area. Currently all municipal wastewater discharges to the offshore marine environment (i.e. marine outfalls) receives preliminary treatment (i.e. coarse screens and fine screens) only.

There are numerous municipal wastewater discharges to the surf zone and estuaries along the South African coast. Treatment varies from secondary to tertiary treatment to meet General Standards (Government Notice No. 991 – 18 May 1984), with a few exceptions receiving only pre-treatment (DWAF, 2004).

There is no information in the public domain about the quality of freshwaters of Qwaqwa area of the Maluti-A-Phofung (MAP) municipality. The MAP Water which is an entity of the municipality conducts regular monitoring of their potable water quality. However, there is a need for the government independent and neutral institution to conduct a research on the quality of Qwaqwa freshwaters. This kind of study will further confirm whether MAP Water is doing enough work or more is needed for improvement.

The main aim of this study was to assess water quality and aquatic ecosystem health seasonally in order to determine the levels of pollution which could affect fish, aquatic invertebrates, livestock and human health in selected sampled rivers and dams of Qwaqwa.

2.3 MATERIALS AND METHODS

2.3.1 Study area: Rivers and dams

The study was conducted on both the lotic (rivers) and lentic (dams) systems of Qwaqwa freshwaters of Maluti - A - Phofung municipality. All selected rivers and dams form the catchment for Qwaqwa drinking water systems. The underlying reason for seasonal assessment of these rivers is for their perennial flow, locality and their accessibility. Selected sites were Metsimatsho (S 28° 34' 42.3" ; E 028° 51' 51.0"), Namahadi (S 28° 34' 21.9" ; E 028° 51' 28.2"), Khoptjwane (S28° 32.1' 41.8"; E 028° 49' 10.7"), Kollatshwene (S 28° 31' 17.9"; E 028° 47' 02.8") and Elands rivers

(S 28° 29' 49.9"; E 028° 49' 40.6"), as well as Metsimatsho (S 28° 35'19.2"; E 028°56' 16.7") and Fikapatso dams (S 28° 40' 04.5"; E 028° 51' 16.2"). Figure 2.2 shows the location of the selected rivers and dams on the Maluti - A - Phofung Municipality Map.

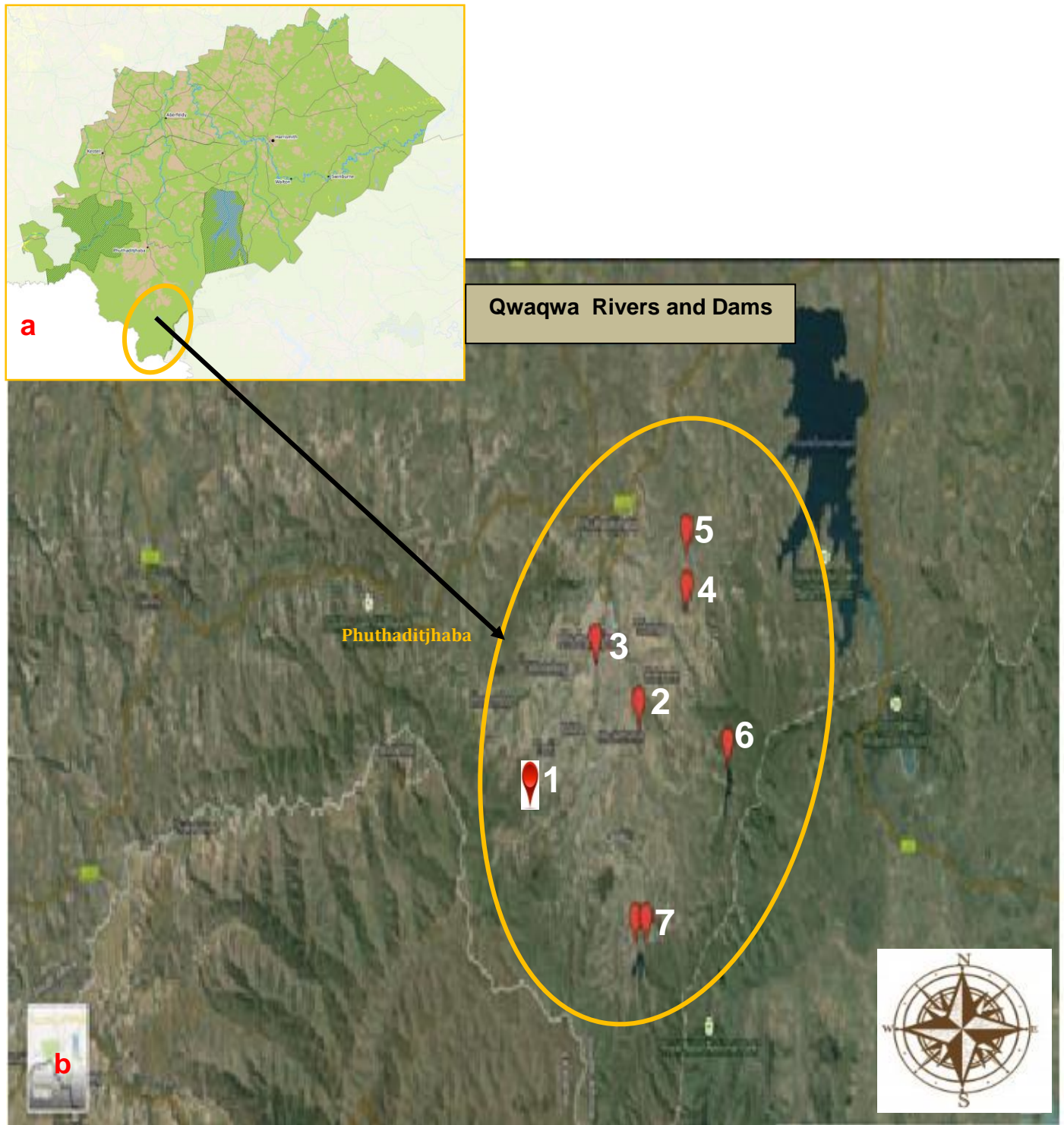


Figure 2.2: (a) Maluti-A-Phofung (MAP) terrestrial biodiversity Map (www.bgis.sanbi.org); (b) selected study sites as demarcated on Phuthaditjhaba area marking studied Rivers and dams (Google earth maps .com). Site1 = Metsimatsho River; site 2 = Namahadi River; Site 3 = Khoptjwane river; Site 4 = Kollatshwene River; Site 5 = Elands River; Site 6 = Metsimatsho Dam & Site7 = Fikapatso Dam.

2.3.2 Water sample collection

Samples were collected from different local untreated water resources like rivers and conservation dams of Qwaqwa area. Sterile polyethylene bottles were used for seasonal sampling. Habitat ecology was evaluated upon sample collection. At least 80% of water was collected from the outskirts of Phuthaditjhaba (Kollatshwene, Namahali, Metsimatsho and Kgopjwane rivers); while 20% was from the metropolitan area (Elands river). Three (3) repetitions were made on respective biotopes of vegetation, stones and gravel, sand and mud (GSM) at every collection sites. The rest of water samples were obtained from dams (Fikapatso and Metsimatsho dams) in the Qwaqwa area.

2.3 3 Water quality assessment

Sampling was conducted as indicated in section 2.3.2 above. *In situ* measurements of the following parameters: (1) dissolved oxygen (DO) saturation (%); (2) dissolved oxygen concentration (mg/l); (3) temperature (°C); (4) pH; (5) salinity (mg/l); (6) total dissolved solids (TDS) (mg/l) and (7) electrical conductivity (EC) ($\mu\text{S}/\text{cm}$) were taken using HANNA HI 9828 multiparameter instrument, (HANNA instruments Inc., Romania). Due to the technical problems encountered with the used multiparameter instrument, DO values could not be detected. However, temperature, pH, salinity, TDS and EC were analysed with regard to the relationship or correlation between sampled rivers (Metsmatsho, Namahadi, Khoptjwane, Kollatshwene and Elands rivers) and dams (Metsimatsho and Fikapatso dams) based on seasonality.

2.4 STATISTICAL ANALYSIS

Data analyses were performed by site and by season to determine any relationship from the study sites. Statistical correlation, T-test and ANOVA analyses were also performed using the STATA 11 program in order to better understand the relationships and differences of the water quality parameters (Aull, 2005).

2.5 RESULTS

2.5.1 Water quality parameter levels: comparison from autumn to summer

The results of this study address the questions of the interrelationship between individual parameters and their significance on water quality; and also lay a basis for comparison between rivers and dams. Therefore, results obtained from all tested parameters were presented with further explanations below:

Electrical conductivity levels (EC).

Electrical conductivity levels had been the lowest in July ranging from 0.0029 $\mu\text{S}/\text{cm}$ to 0.0077 $\mu\text{S}/\text{cm}$ in sampled rivers. The highest EC level was observed in Elands river from autumn to winter after which it dropped drastically in July. While Khoptjwane EC levels increased in spring from 0.22 $\mu\text{S}/\text{cm}$ to 0.98 $\mu\text{S}/\text{cm}$. On the other hand, the EC levels of Fikapatso dam had increased dramatically in spring from 0.019 $\mu\text{S}/\text{cm}$ to 0.31 $\mu\text{S}/\text{cm}$; leaving Metsimatsho dam's EC as low as 0.0015 $\mu\text{S}/\text{cm}$ to 0.034 $\mu\text{S}/\text{cm}$ in spring (Figure 2.3).

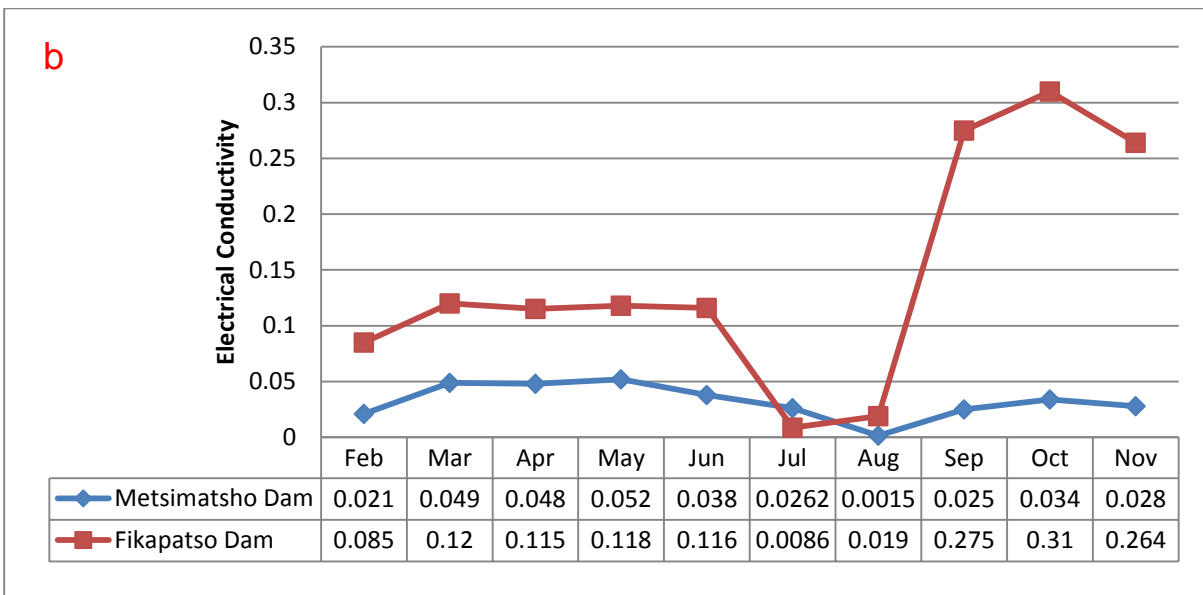
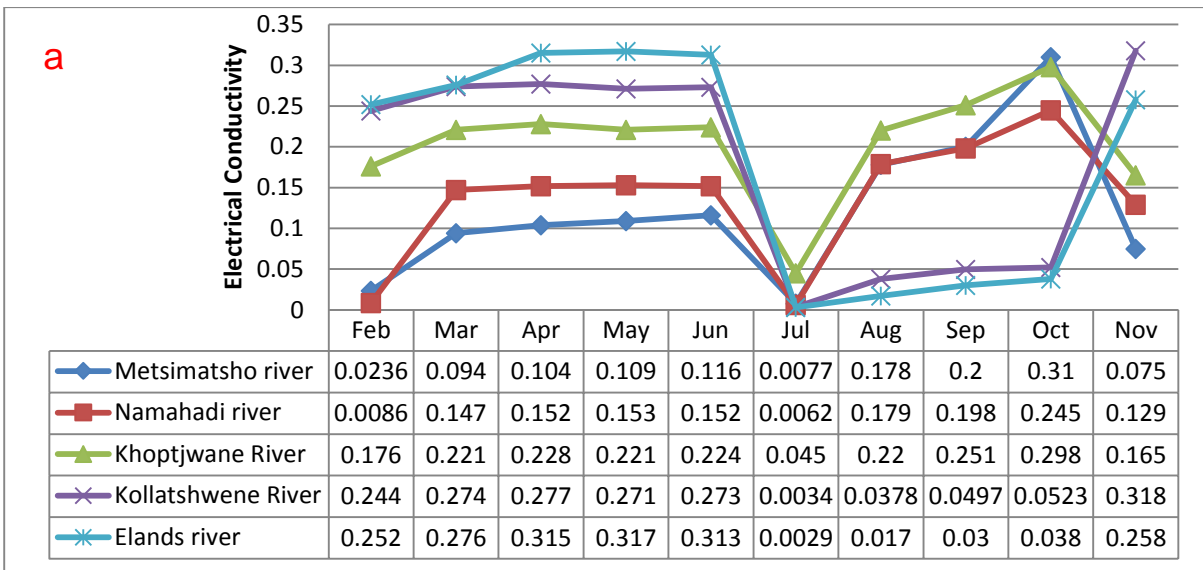


Figure 2.3: Comparison of the electrical conductivity seasonal results between sampled rivers and dams of Qwaqwa. **A** – Rivers and **B** - Dams

The pH levels

Figure 2.4 below compares the pH levels of sampled rivers and dams. The pH levels in both water systems ranged from 6.78 (Namahadi River) to 8.15 (Metsimatsho dam) in July. Elands river had been maintaining the highest pH values in autumn until June (7.83 to 7.96) where the trend dropped down in spring. The pH levels of both Fikapatso and Metsimatsho dams increased up to summer.

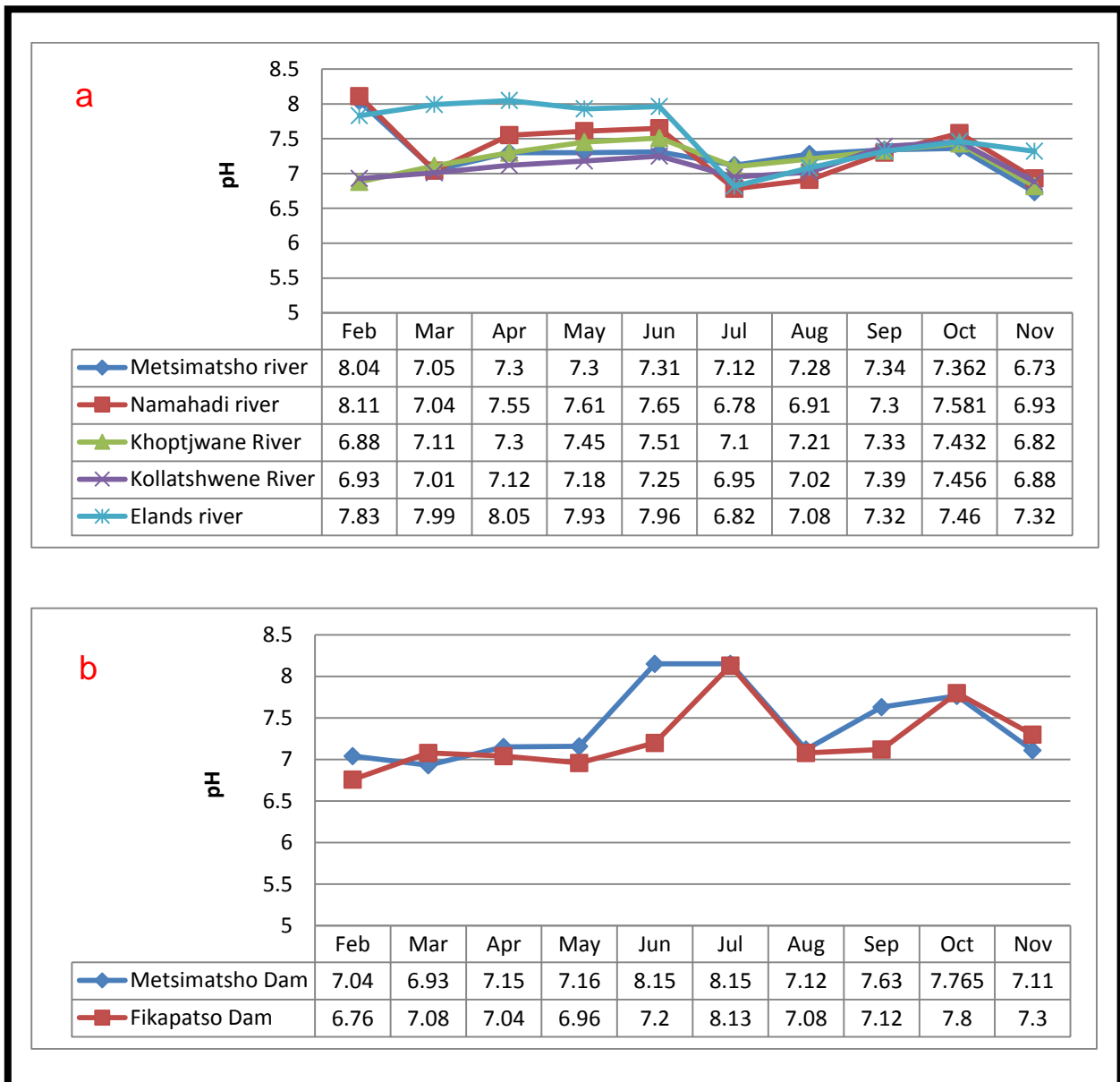


Figure 2.4: Comparison of the pH seasonal results between sampled rivers and dams of Qwaqwa. **A** – Rivers and **B** - Dams

Temperature levels

The constant decrease of temperature levels influenced by seasonal variations was observed in this study. Major temperature decrease was observed in winter particularly in July (winter) in all the sampled sites (both rivers and dams). The temperature levels of the Qwaqwa freshwater ranged from 22.26°C in July to 6.22°C in November for rivers; and 9.07 °C –to 21.06°C in dams (Figure 2.5).

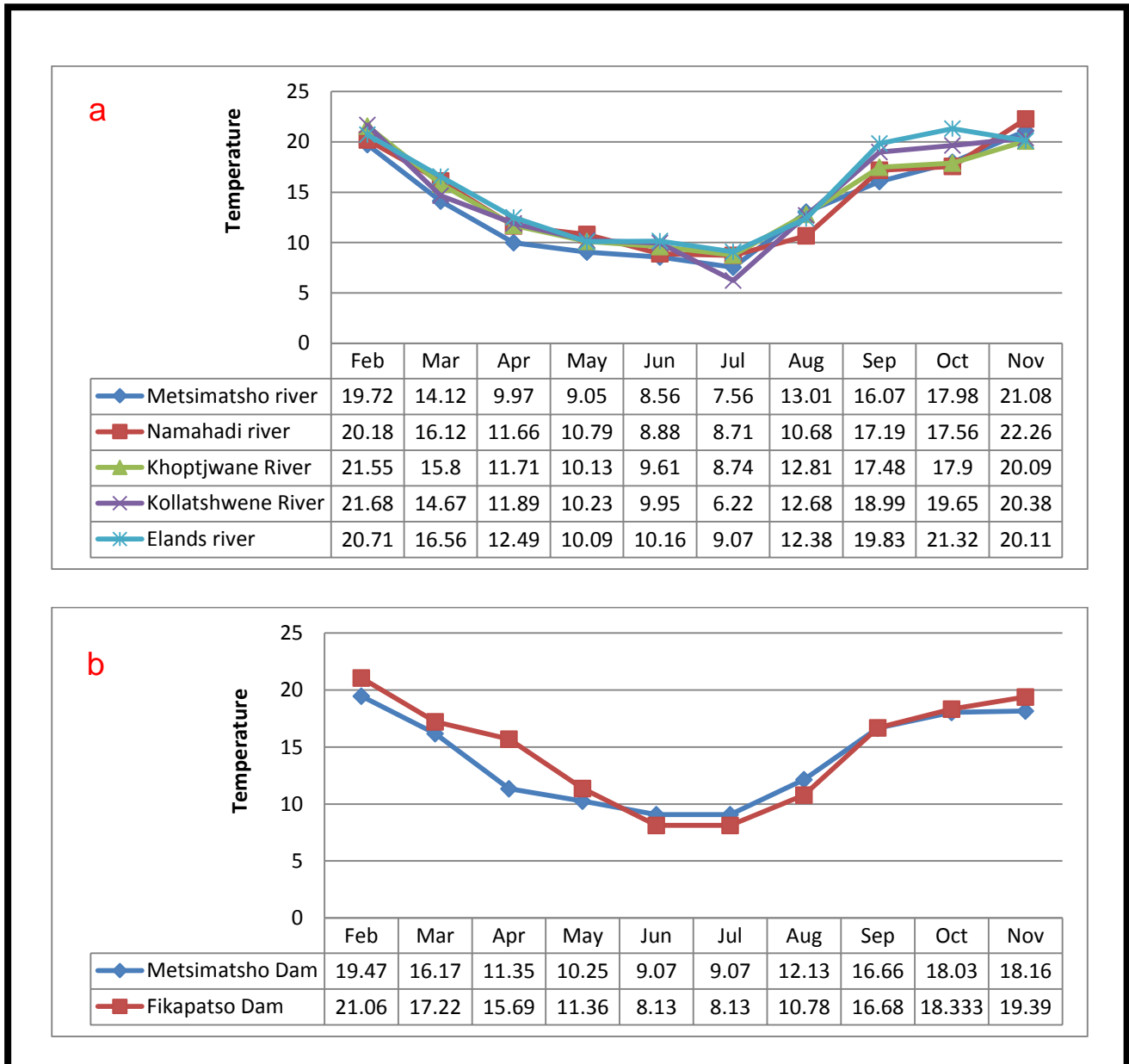


Figure 2.5: Comparison of the temperature seasonal results between sampled rivers and dams of Qwaqwa. **A** – Rivers and **B** - Dams

Salinity levels

According to (Dunlop & McGregor, 2005) salinity may also be expressed as total dissolved solids (TDS) or total soluble salts (TSS), which refers to the residual weight of salts after drying and filtration. Figure 2.6 explains residual weight of salts in water as rivers and dams' water levels and stream flow dropped with seasonality. Salinity levels in the Qwaqwa area increased further in spring when rivers in the sampled sites were almost dry and stagnant. Elands river had also maintained the highest salinity levels (0.12 mg/l in February – 0.46 mg/l October) with Metsimatsho river being the lowest (0.02 mg/l in February – 0.22 mg/l in October). In spring Namahadi river attained the lowest salinity level of 0.03 mg/l. Salinity levels of dams had been parallel since March to July with Fikapatso reading the highest values than Metsimatsho dam. The highest salinity level of 0.21 mg/l was observed in Fikapatso in October with increasing levels at Metsimatsho dam (0.09 mg/l).

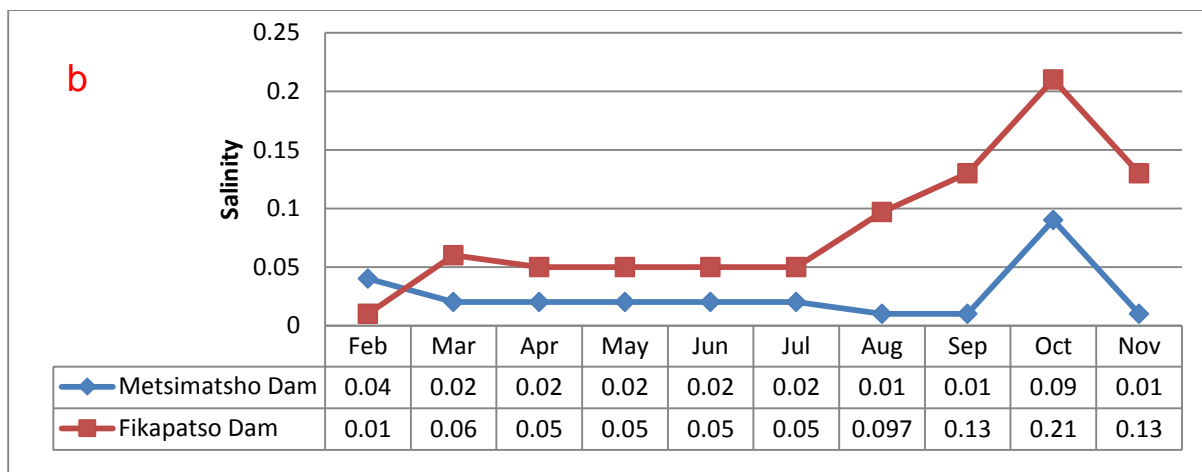
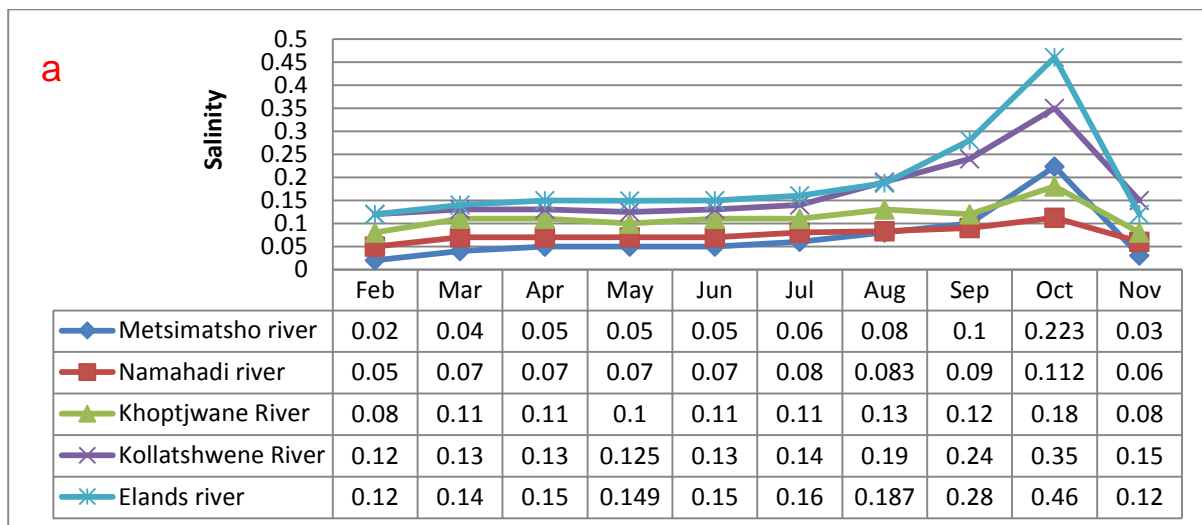


Figure 2.6: Comparison of the salinity seasonal results between sampled rivers and dams of Qwaqwa. **A** – Rivers and **B** - Dams

Total dissolved solids levels

The TDS levels of the sampled rivers (Figure 2.7) follow the same pattern of salinity levels in the same rivers. The TDS levels for all sampled rivers had been constantly increasing from February with Elands river having the highest level of 126 mg/l and Metsimatsho being the least with 21 mg/l. There was a rapid increase of TDS levels from July to October in the Elands river (198 -297 mg/l) and Kollatshwene (205 – 265 mg/l) rivers. The TDS levels in dams had been increasing in a parallel manner since February to July. A drastic decrease was observed in August and September in Fikapatso dam from 58 – 13 mg/l.

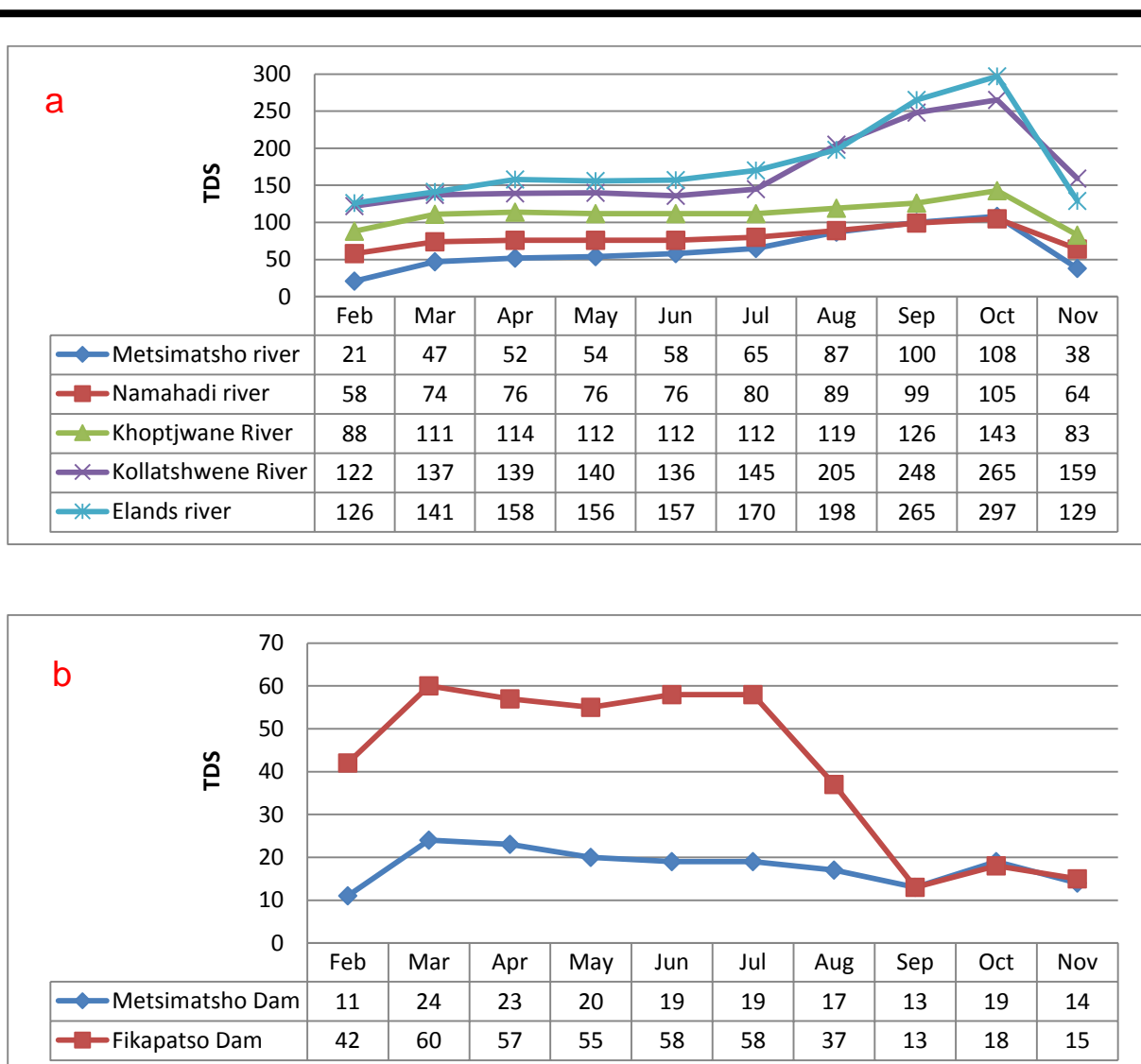


Figure 2.7: Comparison of the Total dissolved solids (TDS) seasonal results between sampled rivers and dams of Qwaqwa. **A** – Rivers and **B** - Dams

2.5.2 Relationship between parameters

Table 2.2 Correlation benchmark between parameters for rivers

Perfect (-)	Strong (-)	Moderate (-)	Weak (-)	No correlation	Weak (+)	Moderate (+)	Strong (+)	Perfect (+)
-1	-0.7 to -0.9	-0.5 to -0.6	-0.1 to -0.4	0	0.1 to 0.4	0.5 to 0.6	0.7 to 0.9	1

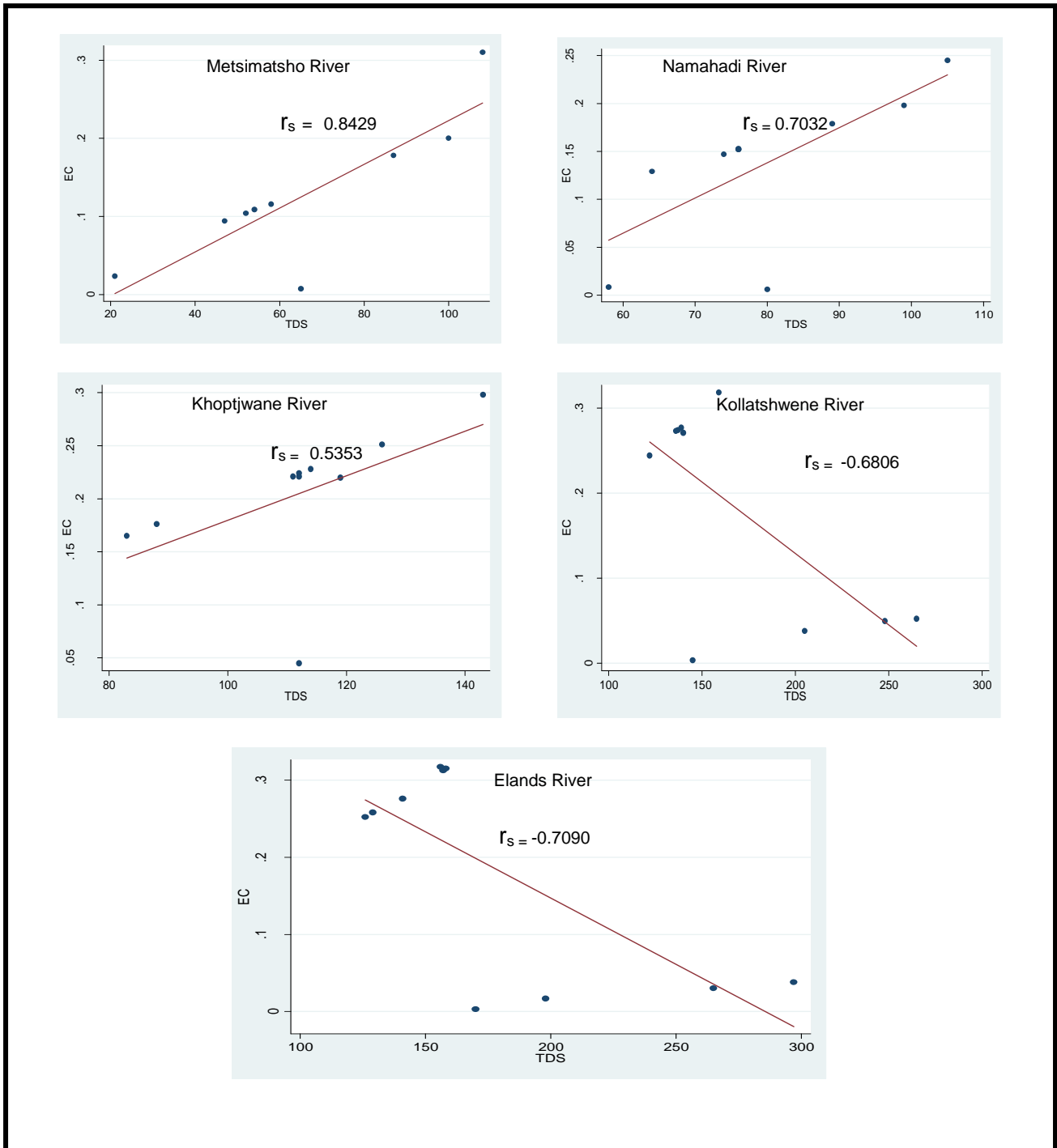


Figure 2.8: Relationship between TDS and EC for the sampled rivers of Qwaqwa.

This study was conducted within the Qwaqwa freshwaters of Maluti –A-Phofung Municipality rivers and dams in the samples size of ten units (N = 10) per study sites. Study hypotheses were:

H₀: There is no correlation if the value of r_s is obtained by chance (or by sampling error);
 H₁: A value of r_s could not be the result of the sampling error in the sample size of 10 units.

The value of r_s in these results is compared with the distribution that the calculated values may or may not exceed the critical value at $P \leq 0.05$ in order to accept or reject the null hypothesis. The calculated or observed values of Metsimatsho and Namahadi rivers exceed the critical tabulated value (0.683) at $P \leq 0.05$ (Figure 2.8). Therefore the null hypothesis is rejected with inference that there is a strong positive linear correlation ($r_s = 0.864$ and $r_s = 0.7457$ respectively) with a high significant differences between TDS and EC and negative linear correlation in Kollatshwene and Elands rivers. As for the Khoptjwane river there is a moderate linear correlation even though the calculated value is below the critical value (0.683) and infer that test was not statistically significant ($P > 0.05$). Therefore there are no significant differences ($p > 0.05$) of the EC levels between the rivers.

Furthermore, there has been a moderate correlation between TDS and EC in Metsimatsho dam with a negative strong correlation in Fikapatso dam and the results are statistically nonsignificant (Figure 2.9).

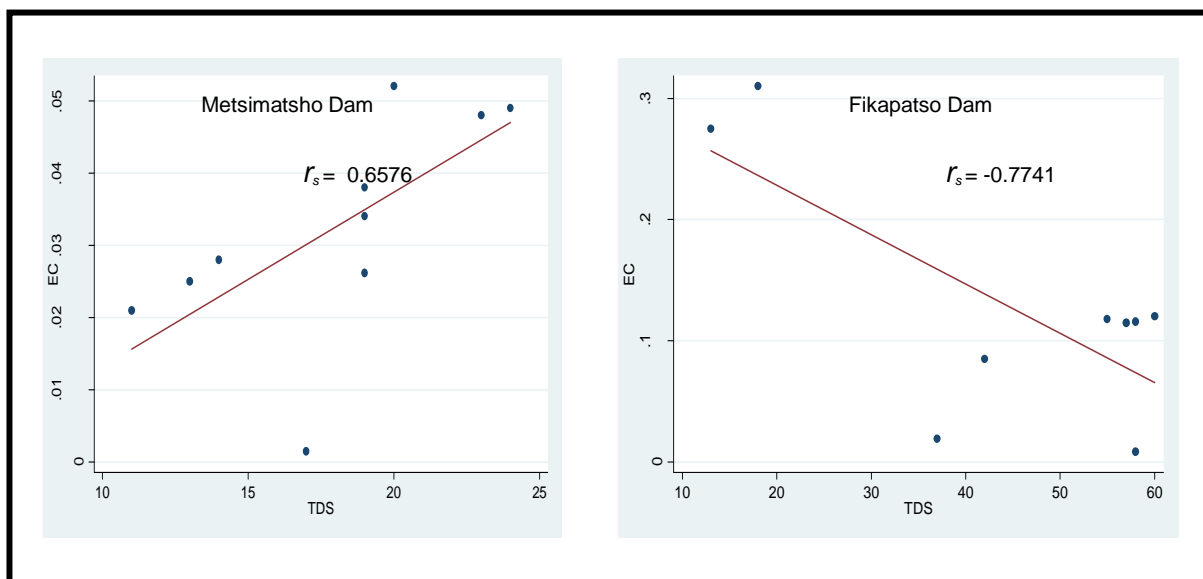


Figure 2.9: Relationship between TDS and EC for the sampled dams of Qwaqwa.

2.5.3 Relationships between salinity and electrical conductivity (EC)

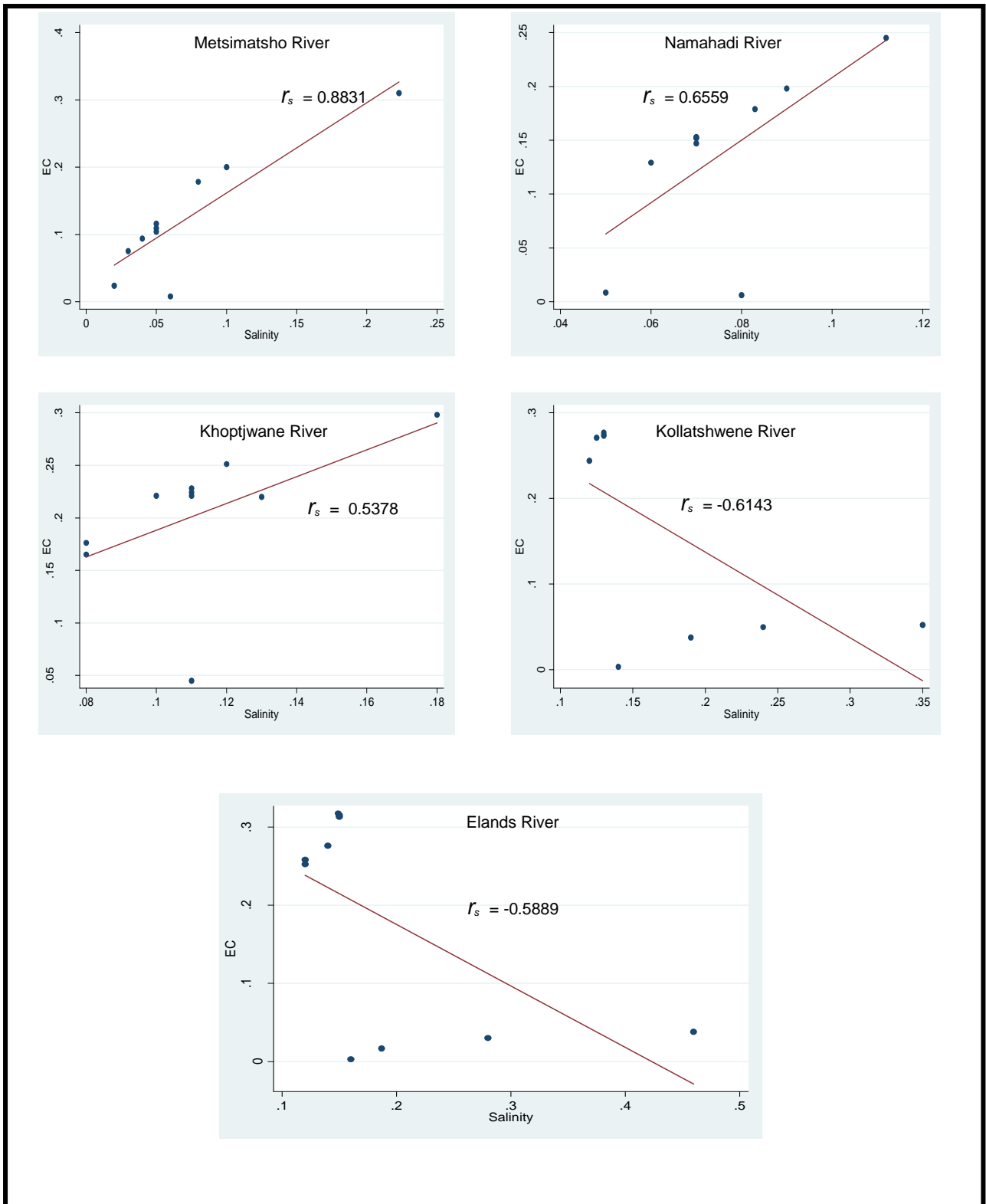


Figure 2.10: Relationship between Salinity and EC for the sampled rivers of Qwaqwa.

A strong positive linear correlation between salinity and electrical conductivity was observed at Metsimatsho river ($r_s = 0.8831$) while a medium correlation regarding the same parameters was also observed in the rest of the four sampled rivers with a negative correlation at Kollatshwene and Elands rivers. These results were indicative of significant difference ($p < 0.05$) between all the sampled rivers (Figure 2.10). Furthermore, there has been no correlation between salinity and electrical conductivity of Metsimatsho dam ($r_s = 0.0953$) compared to a strong positive correlation observed in Fikapatso dam ($r_s = 0.7788$). Therefore, a high significant difference ($p \leq 0.01$) was extrapolated from these results (Figure 2.11).

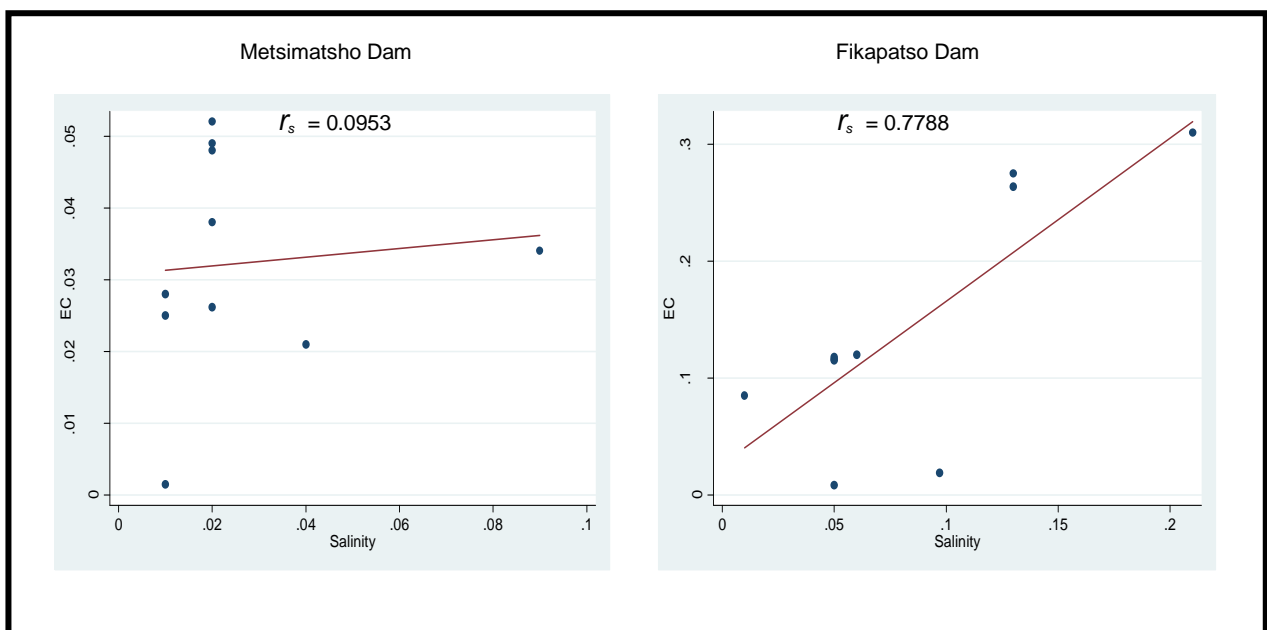


Figure 2.11: Relationship between Salinity and EC for the sampled dams of Qwaqwa.

2.5.4 Relationships between temperature and salinity

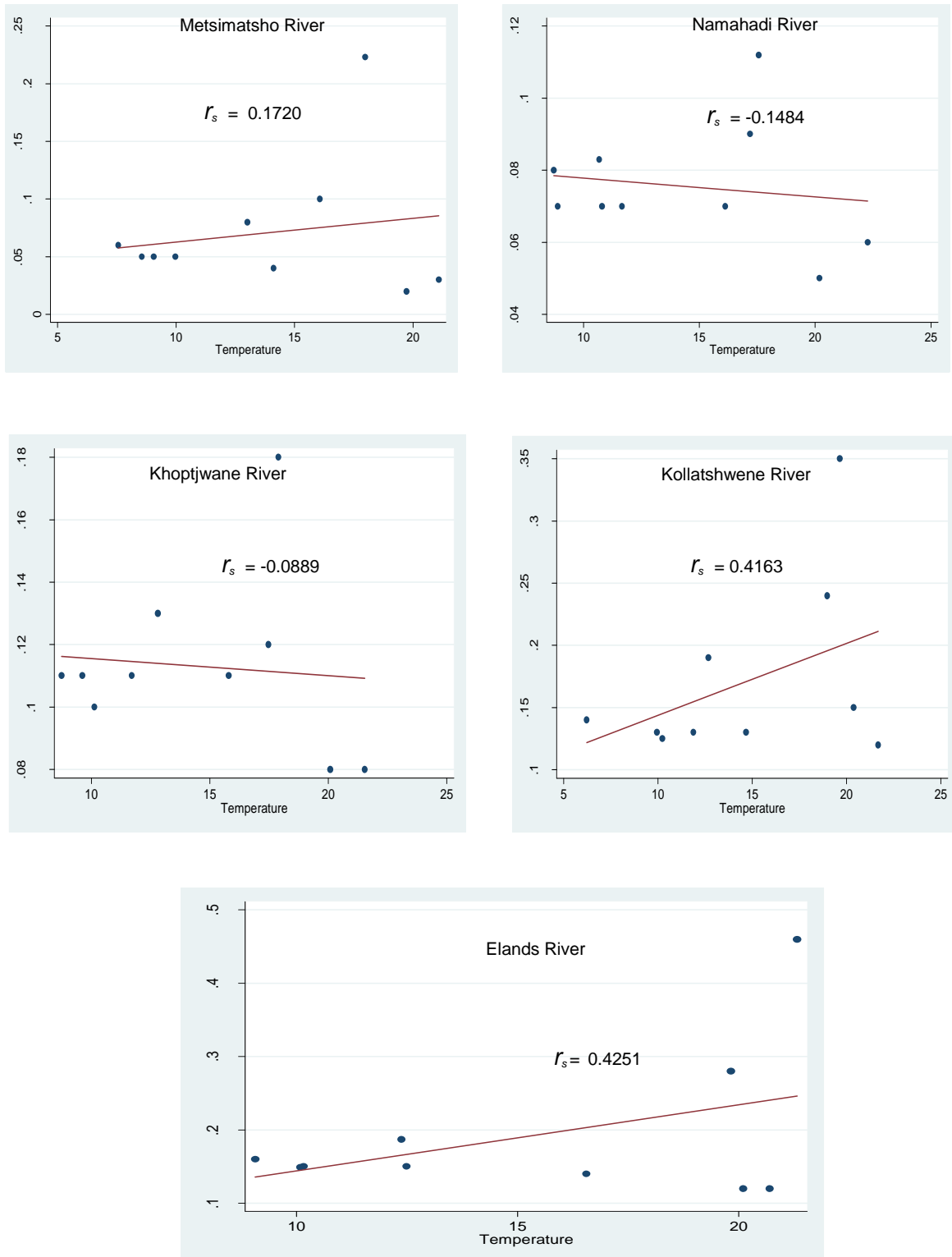


Figure 2.12: Relationship between Temperature and Salinity for the sampled rivers of Qwaqwa.

There was no correlation between temperature and salinity of Khoptjwane river ($r_s = -0.0889$) (Figure 2.12). On the other hand, a weak linear correlation was observed on the rest of the four (4) rivers with positive correlation observed in Metsimatsho ($r_s = 0.1720$); Kollatshwene river ($r_s = 0.4163$) and ($r_s = 0.4251$) Elands river and a negative correlation at Namahadi River ($r_s = -0.1484$). Therefore these rivers were statistically nonsignificant ($p > 0.05$).

Similarly, there was a weak positive correlation between salinity and temperature of Metsimatsho and Fikapatso dams (Figure 2.13) and there was no significant difference between these dams ($p > 0.05$).

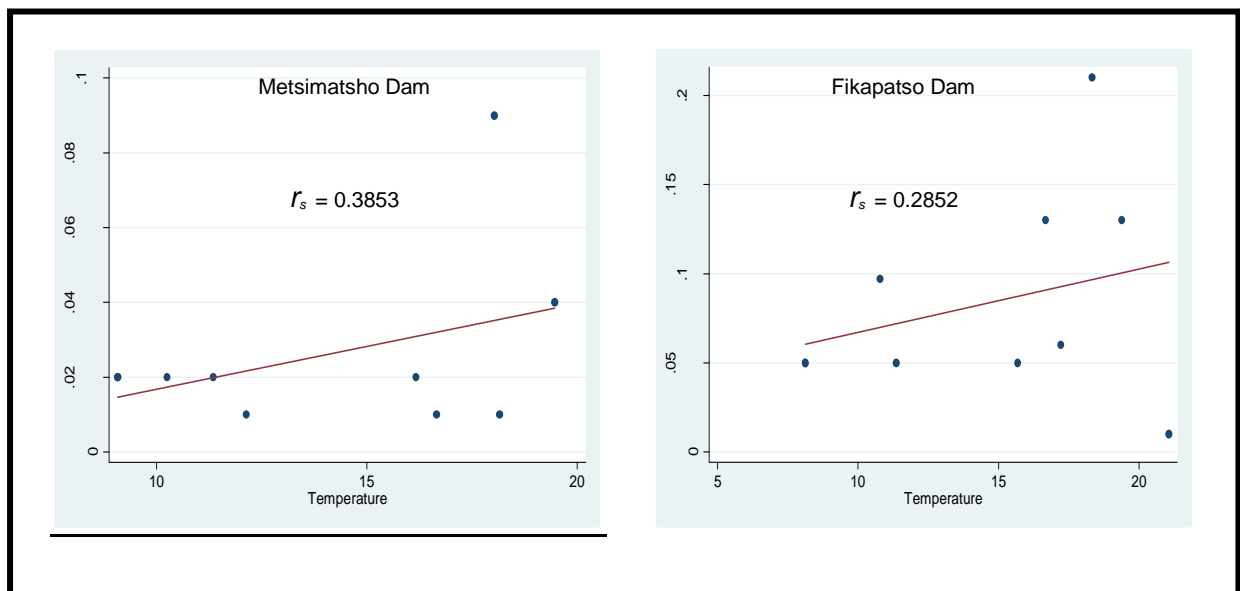


Figure 2.13: Relationship between Temperature and Salinity for the sampled dams of Qwaqwa.

2.5.5 Relationships between temperature and total dissolved solids (TDS)

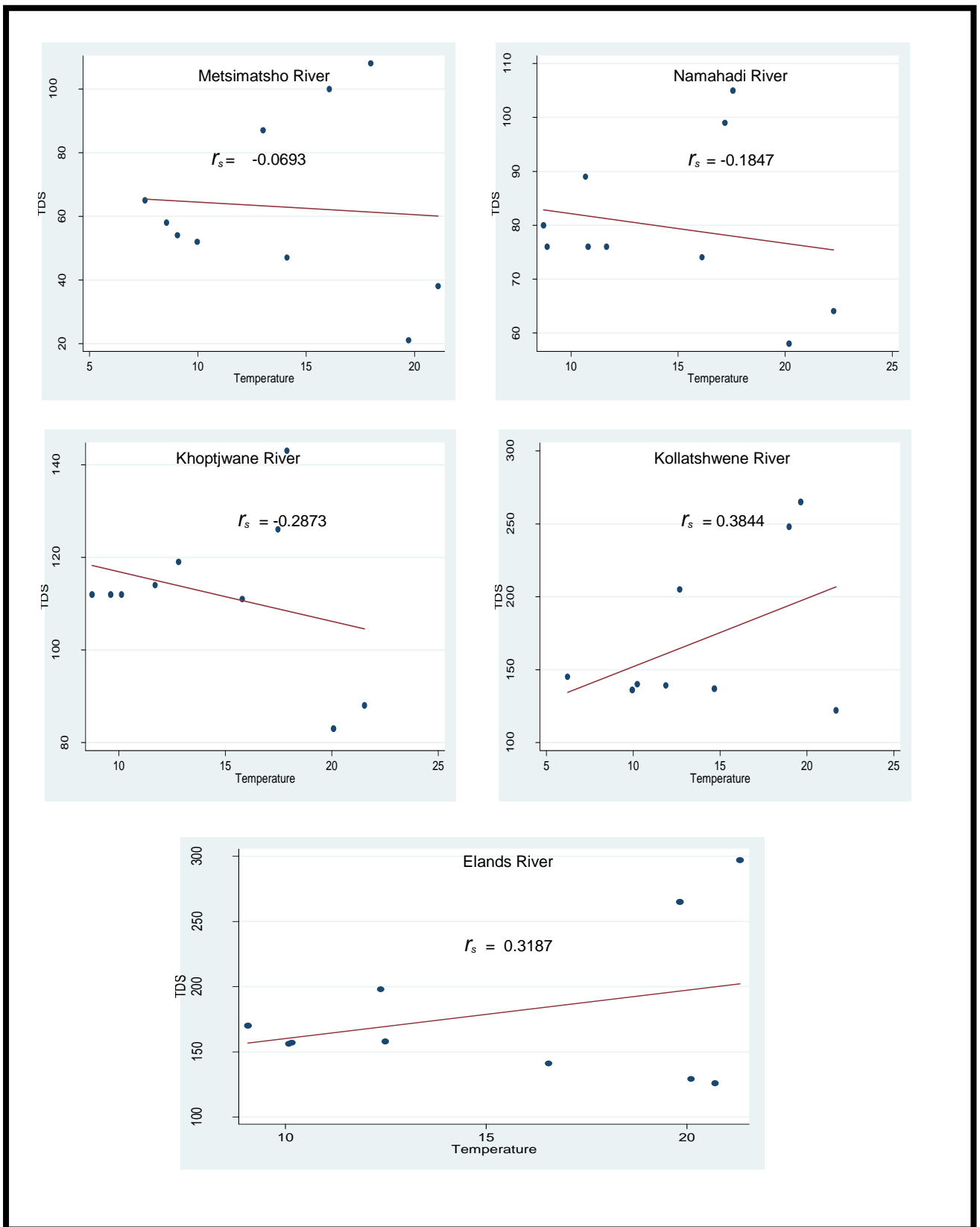


Figure 2.14: Relationship between Temperature and TDS for the sampled rivers of Qwaqwa.

The analysis results indicate negative linear correlation between the temperature and TDS of Metsimatsho ($r_s = -0.0693$); Namahadi ($r_s = -0.1847$) and Khoptjwane ($r_s = -0.2873$) rivers. In addition there was a weak correlation in these rivers (Figure 2.14). On the other hand, a weak positive correlation was computed between the temperature and TDS of Kollatshwene and Elands rivers and therefore there was no significant difference ($p > 0.05$) between all the sampled rivers.

A medium negative correlation between temperature and TDS of Metsimatsho ($r_s = -0.5202$) and Fikapatso ($r_s = -0.5406$) dams was extrapolated and there was no significant difference ($p > 0.05$) between these two dams (Figure 2.15).

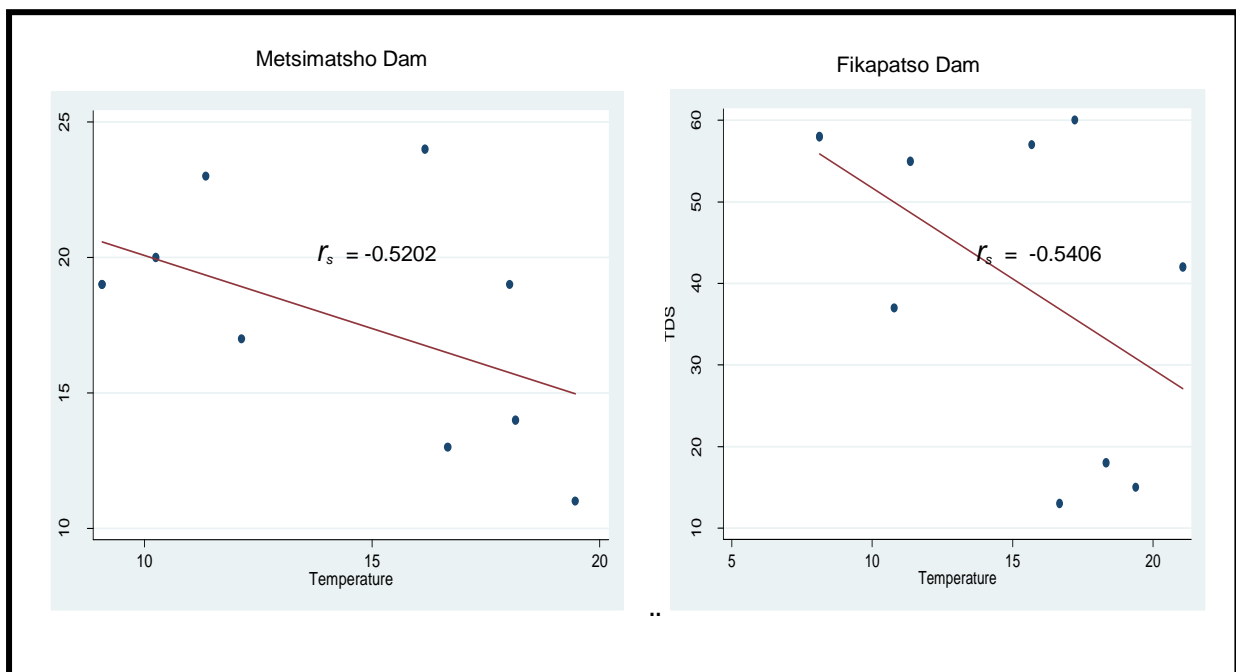


Figure 2.15: Relationship between Temperature and TDS for the sampled dams of Qwaqwa.

2.5.6 Relationships between temperature and pH

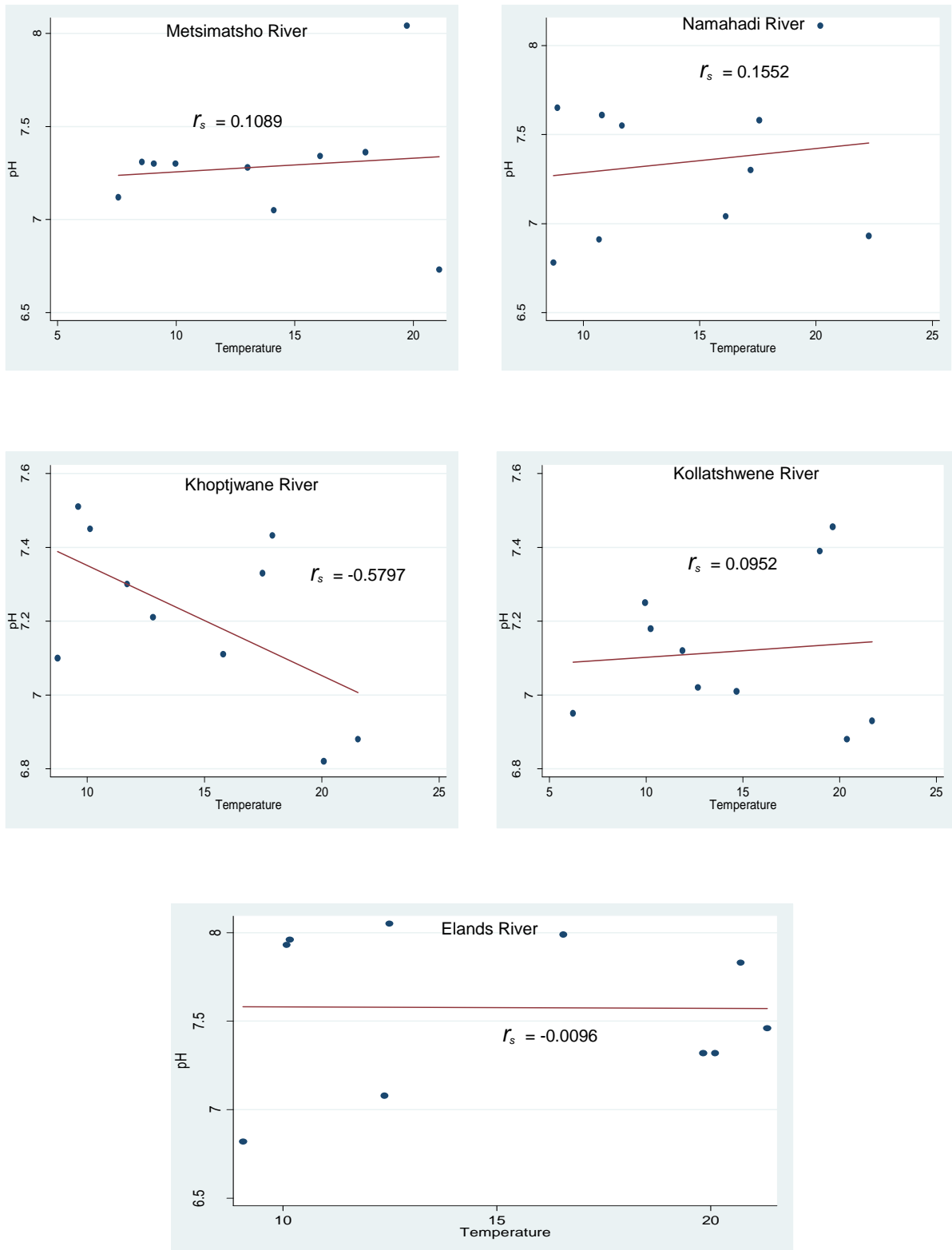


Figure 2.16: Relationship between Temperature and pH for the sampled rivers of Qwaqwa.

Khoptjwane river had a moderate negative correlation between temperature and the pH ($r_s = -0.5797$); a weak positive linear correlation in both Metsimatsho and Namahadi rivers ((Figure 2.16); no correlation between temperature and pH of Kollatshwene and Elands Rivers ($r_s = 0.0952$) and ($r_s = -0.0096$) respectively and therefore there was a significant difference between rivers ($p < 0.05$).

There was no significant difference ($p > 0.05$) between temperature and pH of Metsimatsho and Fikapatso dams (Figure 2.17) with a weak linear correlation of these parameters. In addition, there was a negative correlation between temperature and the pH of these dams ($r_s = -0.4378$ and $r_s = -0.3207$, respectively).

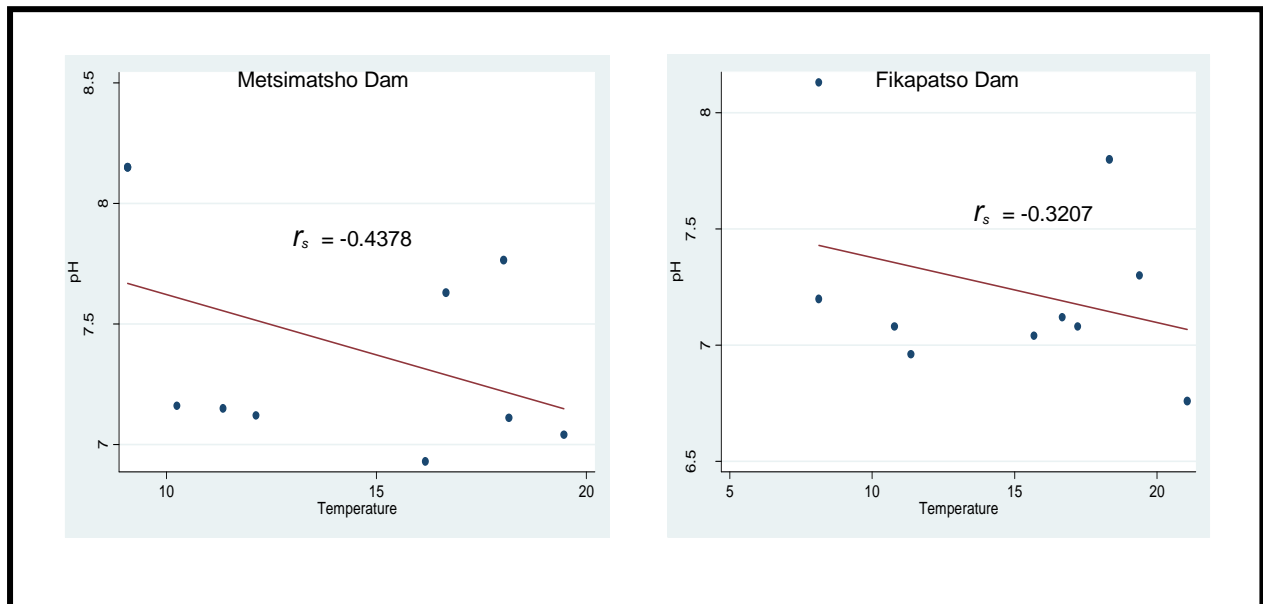


Figure 2.17: Relationship between Temperature and pH for the sampled dams of Qwaqwa.

2.5.7 Relationships between temperature and EC

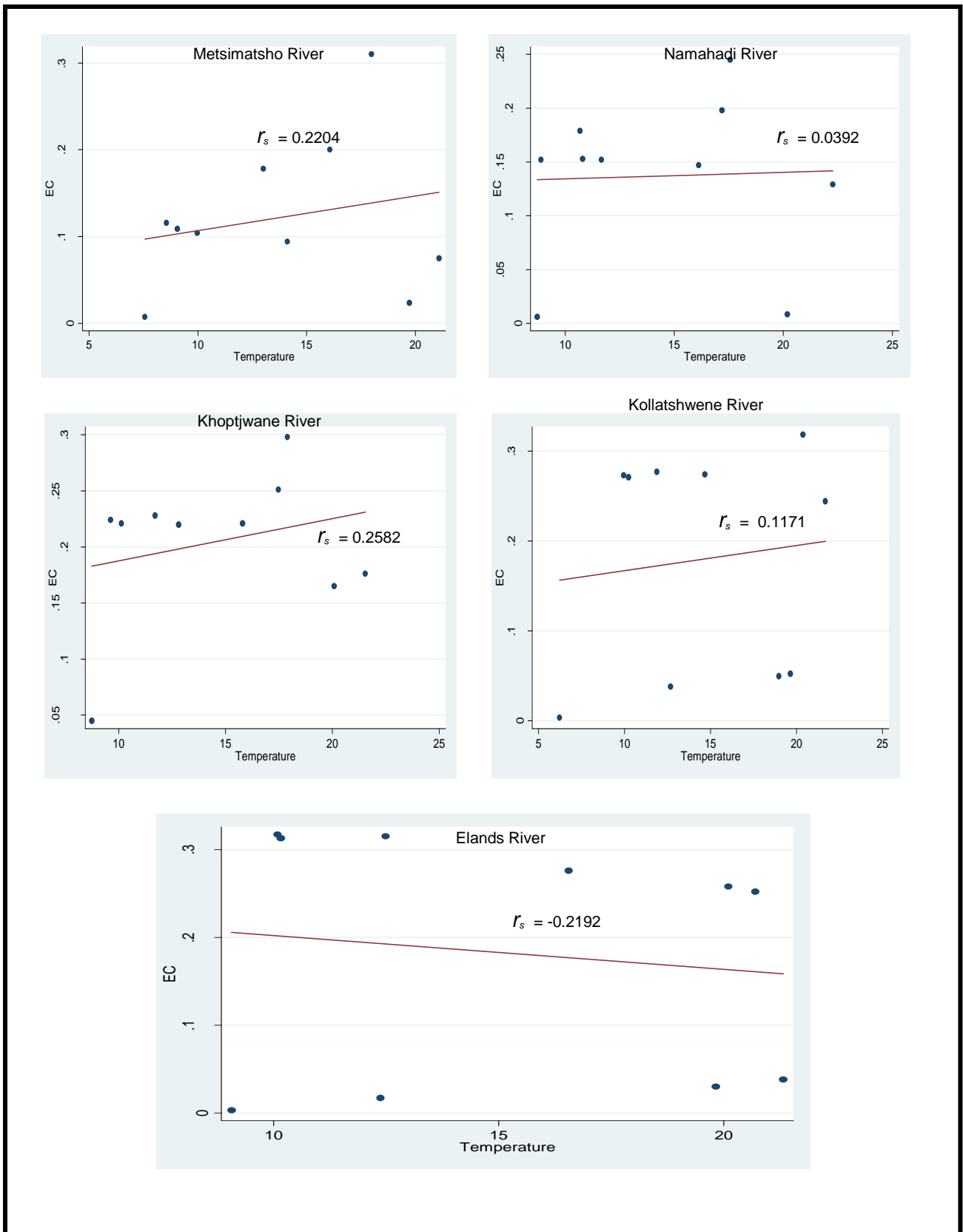


Figure 2.18: Relationship between Temperature and EC for the sampled rivers of Qwaqwa.

There has been a weak linear correlation between temperature and electrical conductivity of all sampled rivers (Figure 2.18) and indicated a significant difference ($p < 0.05$) between rivers. A positive linear correlation in the four (4) rivers and negative correlation in Elands River ($r_s = -0.2192$).

A negative correlation between temperature and electrical conductivity of Metsimatsho dam ($r_s = -0.1989$) with a moderate linear correlation ($r_s = 0.5859$) in Fikapatso dam (Figure 2.19).

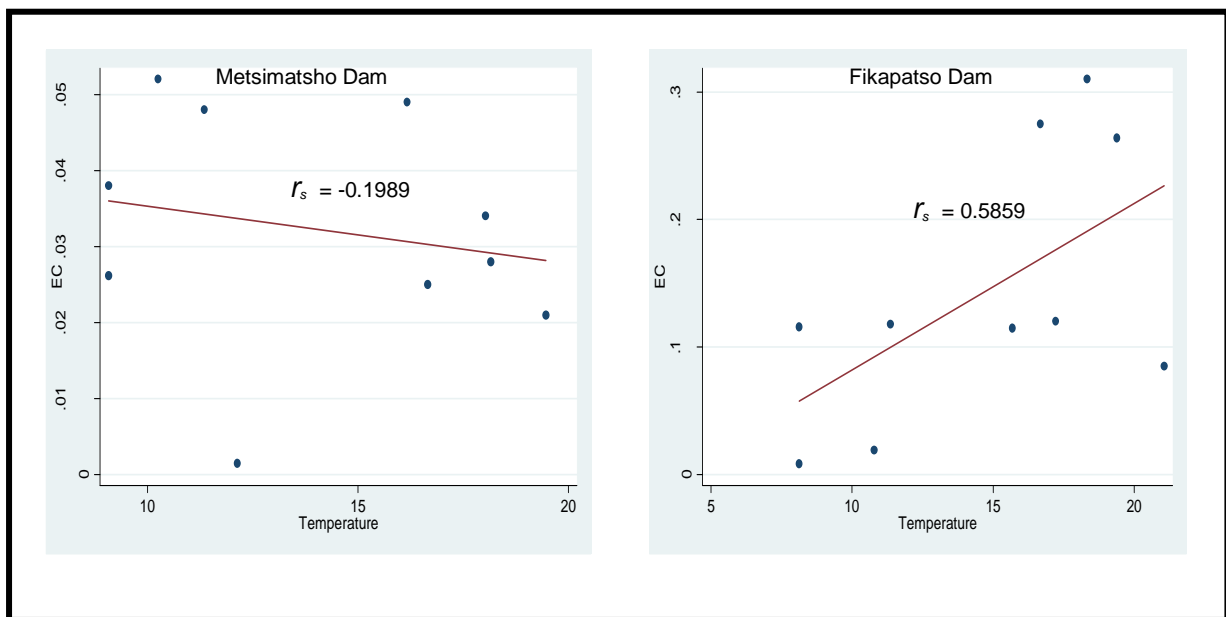


Figure 2.19: Relationship between Temperature and EC for the sampled dams of Qwaqwa.

A representation of Water quality parameter measurements for the different study sites are summarised on table 2.3. The values presented on this table are mean measurements for the sampling seasons and were derived from the data presented on the figures 2.3 to 2.7 for the sampled rivers and dams of Qwaqwa freshwaters.

Table 2.3: Representation of water quality mean measurements from different study sites within Qwaqwa freshwaters.

Sample Sites	pH				DO (mg/l)				Temp. (°C)				Salinity (mg/l)				TDS (mg/l)				EC (mS/cm)			
	A	W	S	S	A	W	S	S	A	W	S	S	A	W	S	S	A	W	S	S	A	W	S	S
Site 1	7.46	7.24	7.33	6.73	-	-	-	-	14.60	8.39	15.69	21.08	0.53	0.13	0.04	0.03	40	59	98.33	38	0.07	0.11	0.23	0.08
Site 2	7.57	7.35	7.26	6.93	-	-	-	-	15.99	9.46	15.14	22.26	0.07	0.09	0.06	0.06	69.33	77.33	89	64	0.10	0.10	0.21	0.13
Site 3	7.09	7.35	7.32	6.82	-	-	-	-	16.35	9.49	16.06	20.09	0.11	0.14	0.10	0.08	104.3	112	129.3	83	0.20	0.16	0.26	0.17
Site 4	7.02	7.13	7.29	6.88	-	-	-	-	16.08	8.8	17.11	20.38	0.13	0.26	0.13	0.15	132.7	140.3	239.3	159	0.27	0.18	0.05	0.33
Site 5	7.96	7.57	7.29	7.32	-	-	-	-	15.59	9.77	17.84	20.11	0.15	0.31	0.14	0.12	141.7	161	253.3	129	0.28	0.21	0.03	0.26
Site 6	7.04	7.82	7.51	7.11	-	-	-	-	15.66	9.46	15.61	18.16	0.02	0.04	0.03	0.01	19.33	19.33	16.33	14	0.04	0.04	0.02	0.03
Site 7	6.96	7.43	7.33	7.30	-	-	-	-	17.99	9.21	15.26	19.39	0.15	0.15	0.04	0.13	53	57	22.67	15	0.11	0.08	0.20	0.26

Site1 = Metsimatsho River; site 2 = Namahadi River; Site 3 = Khoptjwane river; Site 4 = Kollatshwene River; Site 5 = Elands River; Site 6 = Fikapatso Dam & Site7 = Metsimatsho Dam; (-) = No measurement.

2.5.8 NUTRIENT ANALYSIS

Nutrient analysis in 25 milligrams per liter (mg/l) of water in this study was performed to determine the concentration of dissolved salts (salinisation). Table 2.3 presents summary salinisation sources for the sampled water resources of Qwaqwa constituting nitrates (NO_3^-), phosphates (PO_4^-), ammonium compounds (NH_4^+) and free chlorine (Cl) with accompanying TSS and COD values. The concentration of salts (NO_3^- , PO_4^- and NH_4^+) was measured in rivers not in potable water from dams. The concentration levels of ammonia ranged between 0.20 mg/l and 0.65 mg/l. Relatively, the concentration of nitrogen ranged from 0.10 mg/l in Khoptjwane river to 1.80 mg/l in the Elands river. The lowest phosphorus concentration (0.10 mg/l) was observed in Kollatshwene river with the highest level of 0.83 mg/l.

Table 2.4: Analysis of nutrient levels detected from the sampled rivers and dams.

	National standard ranges	MAP Internal standards	Observed measurements (mg/l)						
			Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
NH₃	<6	<4	0.40	0.05	0.20	0.50	0.65	*	*
NO₃	<15	<9	1.40	1.50	0.10	0.50	1.80	*	*
PO₄	<10	<6	0.57	0.20	0.23	0.10	0.83	*	*
TSS	<25	<15	30	9	8	3	40	4	14
Cl	Optional	0.15 – 0.25	**	**	**	**	**	**	**
COD	≤ 75	≤ 50	30	29	09	17	11	40	15
pH	5.5 – 9.5	6.5 – 8.7	7.4	7.7	7.9	7.8	8.2	8.1	6.9

Site1 = Metsimatsho River; site 2 = Namahadi River; Site 3 = Khoptjwane river; Site 4 = Kollatshwene River; Site 5 = Elands River; Site 6 = Fikapatso Dam & Site7 = Metsimatsho Dam.

*Not measured in potable water from the sampled dams

**Optional. No apparatus to measure chlorine at MAP Water

2.6 DISCUSSION

Measuring the physical, chemical and biological characteristics of surface water provides crucial information for identification, addressing and tackling water quality problems (Palaniappan *et al.*, 2010). By providing baseline data, trends over time and comparison between different water bodies, these data may help to determine the water quality impacts of industrial, agricultural and other human activities; quantify the effectiveness of policies and management plans; develop water management models; prioritize where management effort should be concentrated; and communicate to key stakeholders about pollution, human health concerns and degraded ecosystems (Palaniappan *et al.*, 2010).

Water quality status through the *in situ* assessment of the three rivers, Khoptjwane, Kollatshwene and Elands advocates seasonal decline in water quality. This is due to constant increase of total dissolved solids, salinity and electrical conductivity values which subjects water bodies to eutrophication particularly if concentration of phosphates and nitrates increase (Oberholster & Ashton, 2008). Water quality parameter results shown on table 2.3 are meant to assist in the interpretation of direct influence of water quality on aquatic life forms (Aull, 2005).

The results indicate high solubility of elements or salts in water particularly in spring resulting in high concentrations of salts. This could be subsequent to low stream / river flow and erratic rainfall distributions during autumn and spring. Electrical conductivity (EC) also increased with increasing salt concentration especially when temperature increased in spring. However a deviation of a declined electrical conductivity in Kollatshwene and Elands rivers in spring was due to unknown factors. This phenomenon fails to make sense especially when salinity (Figure.2.6) and TDS values (Figure 2.7) in these rivers are the highest among other sampled sites. The descriptive analysis of water quality data indicates that there is a strong positive linear correlation (0.84; 0.99) between TDS and EC.

Electrical conductivity (EC) is related to the ionic content of the sample that expresses a function of the dissolved (ionisable) solid concentration (EPA - Ireland, 2001; APHA., 1998) and is a measure of determining the total dissolved solids/salts (TDS).

The EC levels between Metsimatsho and Fikapatso dams were significantly different ($p < 0.05$). The mean EC level of Fikapatso dam was significantly higher than the mean EC level of Metsimatsho dam. However, there are no significant differences ($p > 0.05$) of the EC levels between the rivers. It could be argued that EC levels were elevated by concentration of the water TDS of these rivers including nitrates and ammonium compounds. These were the rivers with much of faecal contamination due to the dense township settlements in their proximity whose dumping areas are habitually either close to the rivers or directly into rivers. In winter, levels of EC had regressed due to the lower temperature levels (ranging from 6.22°C in rivers and 9.07°C in dams) since EC activity increases 3 times when the temperature increases by 1°C. Now, this describes high levels of EC during autumn and towards the end of spring (Figure 2.3). Factors that had kept EC of Kollatshwene and Elands rivers too low in spring following opposite trends with other rivers are not yet known. In dams, EC of Fikapatso dam was relatively higher than that of Metsimatsho dam both in autumn and spring and could be for the chemical concentration of Metsimatsho dam than that of Fikapatso dam, other than that it is indicative of lower concentration of total dissolved solids, hence there was relatively low faecal contamination than in rivers.

The pH levels of both sampled rivers and dams of Qwaqwa freshwaters ranged around neutral point (7) even though Namahadi river was slightly acidic (6.78) while Metsimatsho dam was slightly alkaline (8.15) in July. The pH levels between Metsimatsho and Fikapatso dams were not significantly different ($p > 0.05$). The mean pH level of Metsimatsho dam was higher than the mean pH level of Fikapatso, whereas the pH levels between the rivers are slightly significantly different ($p \leq 0.06$). The temperature levels between Metsimatsho and Fikapatso dams were not significantly different ($p > 0.05$). The mean temperature level of Fikapatso dam was higher than the mean temperature level of Metsimatsho dam, but not significant; meanwhile there were no significant differences ($p > 0.05$) of the temperature levels between the rivers.

The salinity levels between Metsimatsho and Fikapatso dams were significantly different ($p < 0.05$). The mean salinity level of Fikapatso dam was significantly higher than the mean salinity level of Metsimatsho dam. On the other hand salinity levels

between the rivers are highly significantly different ($p < 0.001$). Salinity levels of rivers were higher than that of dams because sampled rivers of Qwaqwa receive the effluent discharge from MAP Water, therefore there is more of chemical and faecal contamination in these rivers. This makes waters from these rivers not suitable for human consumption than the potable water provided by sampled dams. The difference between salinity levels between rivers could be on levels of chemical or salts concentration.

The TDS levels between Metsimatsho and Fikapatso dams were highly significantly different ($p < 0.001$). The mean TDS level of Fikapatso dam was significantly higher (41.3 mg/l) than 17.9 mg/l mean TDS level of Metsimatsho dam. The mean TDS levels (179.7 mg/l) between rivers are highly significantly different ($p < 0.001$) from those of dams ranging from 21 mg/l to 297 mg/l. The TDS also presupposes the total soluble salts (TSS) of sampled waters of which the latter also complies with the acceptable standards (Table 2.3). However, the deviant levels of TSS from Metsimatsho and Elands Rivers (30 mg/l and 40mg/l respectively) were higher than the standard ranges. The COD and pH of the sampled waters constitute acceptable water parameter levels.

The reason for Fikapatso dam to have high levels of the chemical parameters like salts (salinity and total dissolved salts) could be dependent on water levels where sampling was done. Water samples collected from the seepage outlet of the dam with low volumes of water, therefore there are possibilities of high concentration of salts in this area than in the entire dam.

Nutrient levels of NO_3^- , PO_4^- and NH_4^+ observed from the sampled waters (Table 2.3 and Appendix C – Table 1C) were at minimal levels that comply with MAP Water standards and anticipate low chances of eutrophication. In contrast, Metsimatsho and Elands rivers fail the MAP water standards with high TSS levels (30 mg/l and 40 mg/l, respectively) which could have been influenced by high faecal contamination particularly in the Elands river. Furthermore, high TSS levels of Metsimatsho river were also due to unknown factors. Salinity results indicated minimal levels of the ammonia, nitrogen and phosphorus since the observed figures (Table 2.3) comply with both the National and MAP internal nutrient standards; even though relatively

higher concentration levels of ammonia, nitrogen and phosphorus were observed in the Elands river.

In general, there was no significant difference between rivers and dams of Qwaqwa in terms of water temperatures even though these two dams are at higher elevation than the sampled rivers. The biota found in all the sampled sites (dams and rivers) might have adapted to low temperatures even though the number of identified taxa had been relatively lower in winter for the sampled sites, with Metsimatsho dam experiencing the lowest number of taxa (Figure 3.10).

CHAPTER 3

CHARACTERIZATION OF WATERBORNE MACROINVERTEBRATES IN DAMS AND RIVERS OF QWAQWA

3.1 INTRODUCTION

Macroinvertebrates are well known to have different sensitivities to pollution and habitat transformation and are therefore very useful indicators of pollution. Different taxa of macroinvertebrates also exhibit differing tolerances to individual water quality variables (Dallas & Day, 1993), therefore water of suitable quality is essential to maintain healthy populations of aquatic organisms (Malan & Day, 2003). As a result, sensitivity classification can be undertaken using the South African scoring system version 5 (SASS5) sensitivity scores.

The SASS5 sensitivity scores as assigned by Dickens & Graham, (2001) were derived from the tolerance levels of aquatic macroinvertebrates to pollution (Gerber & Gabriel, 2002). The tolerance score ranging from 1 - 5 denotes high tolerance to pollution; 6 – 10 denotes moderate tolerance whereas 11 -15 denotes high sensitivity to pollution.

Understanding the habitat preferences of aquatic macroinvertebrates can aid in the preservation and monitoring of aquatic habitats. Their large taxonomic diversity has allowed aquatic macroinvertebrates to adapt to a wide variety of environmental conditions and factors (Demars *et al.*, 2012).

The type of river habitat being studied can be used to predict the traits of macroinvertebrates within them, as well as the taxonomic composition present (Demars *et al.*, 2012). Factors such as pH, elevation, water depth, and substrate type affect how different organisms adapt to their aquatic habitats. For example, in sand and silt substrate, deposit feeders like crabs (Davey, 2000) are responsible for detritus, whereas it is done by filter feeders such as *Tricopteran* larvae (Wallace *et*

al., 1977) within water bodies containing high amounts of submerged vegetation (Demars *et al.*, 2012).

In a study conducted by Wolmarans *et al.*, (2014) in the Olifants River within the seven selected study sites, it was concluded in that study that water quality of the Olifants River was of poor state of health indicated by a small number of highly sensitive taxa (7 out of 95 taxa).

Aquatic macroinvertebrates were also sampled to determine the nature of the aquatic habitat at Wilge River (a tributary of the Olifants River) and a total of 40 taxa were sampled in that study. According to the Gerber & Gabriel, (2002), a low number of pollutant sensitive Ephemeropterans (>2spp Baetidae, Heptagenidae and Leptophlebiidae) were found to be indicators of poor water quality. On the other hand a cumulative investigation on impacts of dams on macroinvertebrates indicated that opportunistic taxa that are pollution tolerant have increased in numbers both in the Western Cape and Mpumalanga (Mantel, 2010) also indicating poor water quality. Furthermore, studies on the perennial endorheic reed pans on the Mpumalanga Highveld, South Africa also showed that macroinvertebrates could reflect various changes along with seasonal and anthropogenic impacts on water quality. These impacts were found to have caused the disappearance of sensitive taxa and promoted the increase of pollution tolerant macroinvertebrate taxa (De Klerk & Wepener, 2013).

On the above mentioned studies undertaken in South Africa, research results indicate that macroinvertebrates can be used to assess water quality and the subsequent river health. In this regard they are water quality bioindicators. The type of river habitat studied can be used to predict the traits of macroinvertebrates found in them, as well as the taxonomic composition present (Demars *et al.*, 2012). Factors such as pH, elevation, water depth, and substrate type indicate how different organisms adapt to their aquatic habitats. For example, in sand and silt substrates, deposit feeders like crabs (Davey, 2000) are responsible for detritus, whereas it is done by filter feeders such as *Tricopteran* larvae (Wallace *et al.*, 1977) particularly in water bodies that contain high amounts of submerged vegetation (Demars *et al.*, 2012).

The biological evaluation of water quality is linked to the number of pollution tolerant organisms compared to a number of pollution intolerant ones, Voshell, (2002b). Voshell, (2002b) further contented that if a survey of the stream yielded a higher proportion of pollution tolerant macroinvertebrates and there are no sensitive ones that could be the indication of poor water or habitat quality index. A favourable water quality index would be characterised by finding sensitive organisms as well as tolerant organisms. The macroinvertebrate assessment form is one sample of how water quality may be assessed in the stream using occurring macroinvertebrates (Voshell, 2002a).

3.2. Aquatic invertebrate insects of freshwater systems

Aquatic macroinvertebrates form a rather large group of organisms, which includes representatives from multiple phyla, such as arthropods, annelids, and molluscs. The aquatic arthropods, when considering freshwater species, are comprised of multiple orders of insects, spiders, and crustaceans. Insects constitute the most numerous arthropods, and orders such as *Odonata* (dragonflies and damselflies), *Trichoptera* (caddisflies), *Plecoptera* (stoneflies), *Coleoptera* (beetles), *Diptera* (flies), *Ephemeroptera* (mayflies) and *Hemiptera* (water bugs) are common inhabitants of freshwater bodies (Richardson, 2008). Annelids (worms and leeches), though less represented, are also present in freshwater ecosystems. Molluscs are represented in freshwater ecosystems mainly by the gastropod class. Most aquatic gastropods are snails (Kershner & Lodge 1990) and are found near the substrate or on supporting structures like rocks.

3.2.1 Importance of freshwater benthic invertebrates

According to Ronsenberg & Resh, (1993) benthic macroinvertebrates are common inhabitants of lakes and streams where they move energy through food webs. However, benthic macroinvertebrates can be difficult to work with unless the proper study design is used since (a) quantitative sampling is difficult because of their contagious distribution large sample numbers; (b) their distribution an abundance are

affected by large number of natural factors accounting for changes in biodiversity; and (c) some groups of benthic macroinvertebrates are taxonomically difficult, although new improved keys are developed (Ronsenberg & Resh, 1993). Examples of new taxonomic keys is standard operating procedure (SOP) which is designed to be used as a reference by biologists who analyze aquatic macroinvertebrate samples from Missouri (Sarver, 2005). Its purpose is to establish consistent levels of taxonomic resolution among agency, academic and other biologists. The information in this SOP has been established by researching current taxonomic literature. It should assist an experienced aquatic biologist to identify organisms from aquatic surveys to a consistent and reliable level. The criteria used to set the level of taxonomy beyond the genus level are the systematic treatment of the genus by a professional taxonomist and the availability of a published key (Sarver, 2005).

The collection of benthic macroinvertebrates from lakes and streams is usually a straightforward procedure using standard equipment even though the removal of organisms from background material can be tedious and time-consuming (Ronsenberg & Resh, 1993). Furthermore, their identification to species level requires substantial training and skill while in the contrary; data analysis procedures are standard and can be performed by anyone trained in elementary statistics (Ronsenberg & Resh, 1993).

The occurrence and distribution of benthic macroinvertebrates along streambeds are influenced by a variety of environmental factors. Environmental conditions from single-rock microhabitats to upland catchments directly affect assemblage organizations and the important stream trophic function which those assemblages provide (Covich *et al.*, 1999, Wallace & Webster 1996). At large spatial scales, benthic macroinvertebrates can show distinct assemblage patterns due to particular physiographic region (Brussock *et al.*, 1985), zones created longitudinally along stream systems that are defined by energy supply and channel morphology (Vannote *et al.*, 1980), or differences in geomorphic context within complex watershed landscapes (Montgomery, 1999).

The distribution and abundance of aquatic organisms in a benthic community involve many factors; some of which are major physical factors that determine what species are found in particular areas (Voshell, 2002a), namely:

- Water temperature
- Volume and velocity of water flow (discharge)
- Substrate
- Energy relationships

3.2.2 Adaptations of organisms for Aquatic habitats

Most insects land on water trapped by the water surface tension while tiny ones may be drown inside a water droplet. Aquatic insects cope in water bodies by having waterproofed skin so that large amounts of fresh water do not diffuse into the body (Mitchell & William, 1996). Many are covered with water repellent waxy layer; with hairy or waxy legs which repel water so that they do not get trapped by water surface tension. Many of them are strong swimmers or crawlers and can also fly as nymphs, larvae or adults at differing degree (Mitchell & William, 1996).

Some in the Hemipteran order: Corixidae (water boatmen) are the only aquatic beetles that can take off from water – without having to crawl out of the water first (Mitchell & William, 1996). The following characteristics assist aquatic organisms to adapt to their habitat:

Breathing underwater:

According to Mitchell & William, (1996), water is much heavier than air and there is much more oxygen in air than in the water; therefore an insect needs to process a lot of water to get sufficient amount of oxygen. That is probably one reason why only aquatic insect larvae develop gills to absorb oxygen from the water, while adult insects continue to breathe air instead of developing gills.

Other aquatic adaptations include “ripple effect”, “double vision” and “oars”:

Ripple effect:

Most aquatic insects are sensitive to water ripples to detect predators or prey and some may even create their own ripples on the water surface and process the returning “echoes” to detect prey (Mitchell & William, 1996). Some example including *Coleoptera*: Gyrinidae (whirligig beetle) also create ripples to find mates and communicate with each other.

Double vision:

The whirligig beetle has eyes that are divided horizontally to see both under and above water (Mitchell & William, 1996).

Oars:

Many aquatic insects paddle underwater with oar-like legs as they are long, flattened, hairy and fringed to increase the surface area, and bend in on the return stroke to reduce water resistance (Mitchell & William, 1996). Examples include water scavenger beetle (*Coleoptera*: Hydrophilidae) and water boatmen (Corixidae) (Figure 3.1).



Figure 3.1: Images of aquatic insects that adapt to their environment by using the oars.

3.2.3 Classification of aquatic insects

Each of the Classes of arthropods, including the insects, are split into a number of smaller groups, which reflect progressively more detailed structural similarities between the group members. These smaller groups follow a strict hierarchy and the major class divisions in descending order of size are called Subclass, Order, Suborder, Family, Subfamily and Genus (Kendall, 2010). A genus is the smallest group of any real importance in the naming of individual species, although in some classifications generic groups may be further split into Subgenera (Kendall, 2010). The scientific name of a species includes, first, the genus to which it belongs and, second, its specific name, e.g. the European Violet Ground Beetle is called *Carabus violaceus*, meaning the species *violaceus* in the genus *Carabus* (by convention, generic and specific names are always printed in italics; the generic name spelt with a capital letter and the specific name with a small letter). The full classification of this insect by Kendall, (2010) is as follows:

PHYLUM:	Arthropoda	arthropod	
CLASS:	Insecta	insect	
SUBCLASS:	Pterygota	winged	insect
ORDER:	Coleoptera	beetle	
SUBORDER:	Adephaga	carnivorous	beetle
FAMILY:	Carabidae	ground	beetle
SUBFAMILY:	Carabinae	-	
GENUS:	<i>Carabus</i>	-	
SPECIES:	<i>Carabus violaceus</i> L.	violet ground beetle	

3.2.4 Life cycles for aquatic insects

Life cycles for aquatic insects may be very short or too long. For example; mosquito has a life cycle of two weeks, while some hellgrammites take 4-5 years to complete their life cycle (Edelstein, 2013)

In temperate streams insects undergo three types of life cycles, namely, slow season, fast season and non-seasonal life cycles (Edelstein, 2013)

Slow season life cycle:

This may occur in cooler streams where insects grow during the fall and winter while feeding on leaf detritus; pupae and adults emerge from late winter to early summer (e.g mayflies, stoneflies and caddisflies).

Fast season life cycle:

Growth of immature is fast after a long egg or larval diapauses, that is, they may stay in the egg stage from August to March; the larval stage from March to May and become adults in June or July, this includes some caddisflies

Non-seasonal life cycles

Several stages and / or sizes are present in all seasons and these life cycles are common in hellgrammites.

Insects either undergo complete or incomplete metamorphosis. Four stage life cycle in complete metamorphosis and three stages in incomplete metamorphosis (Figures 3.2 & 3.3).

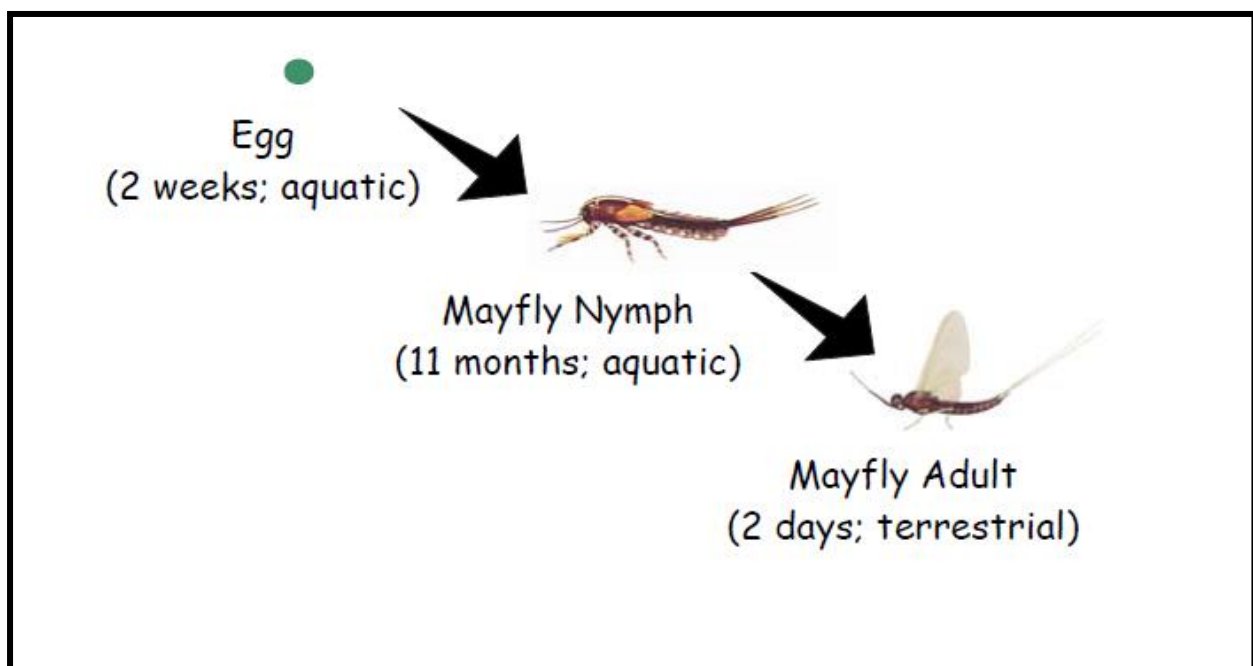


Figure 3.2: Incomplete metamorphosis of a Mayfly. Adapted from Maryland State Envirothon, (2013).

In incomplete metamorphosis female insects lay eggs covered by the egg case that protect and hold them together (Figure 3.3). Eggs hatch into nymphs that look like the small adults but without wings. Nymphs undergo ecdysis (shedding out of the hard outer casing called chitin) for several times, i.e. 4 – 8 times in most insects. This phenomenon is also called molting of the exoskeleton and facilitates growth. When these insects reach the adult stage, they stop molting but develop wings and start lay eggs as well.

Most aquatic insects go through complete metamorphosis where a female insect lays eggs. Eggs hatch into larvae that do not resemble the adult insect as they usually have worm-like shape and most have legs in the larval stage (Figure 3.3). Larvae also shed their skin several times as grow slightly larger (Edelstein, 2013). Larvae pupate (pupa) making cocoons around themselves without feeding but their bodies develop into adults and develop wings, legs and other parts including internal organs (Edelstein, 2013). This change may take from 4 days to many months. As the pupa changes into an adult after a period of time, it breaks out of the cocoon and emerges out as an adult moth.

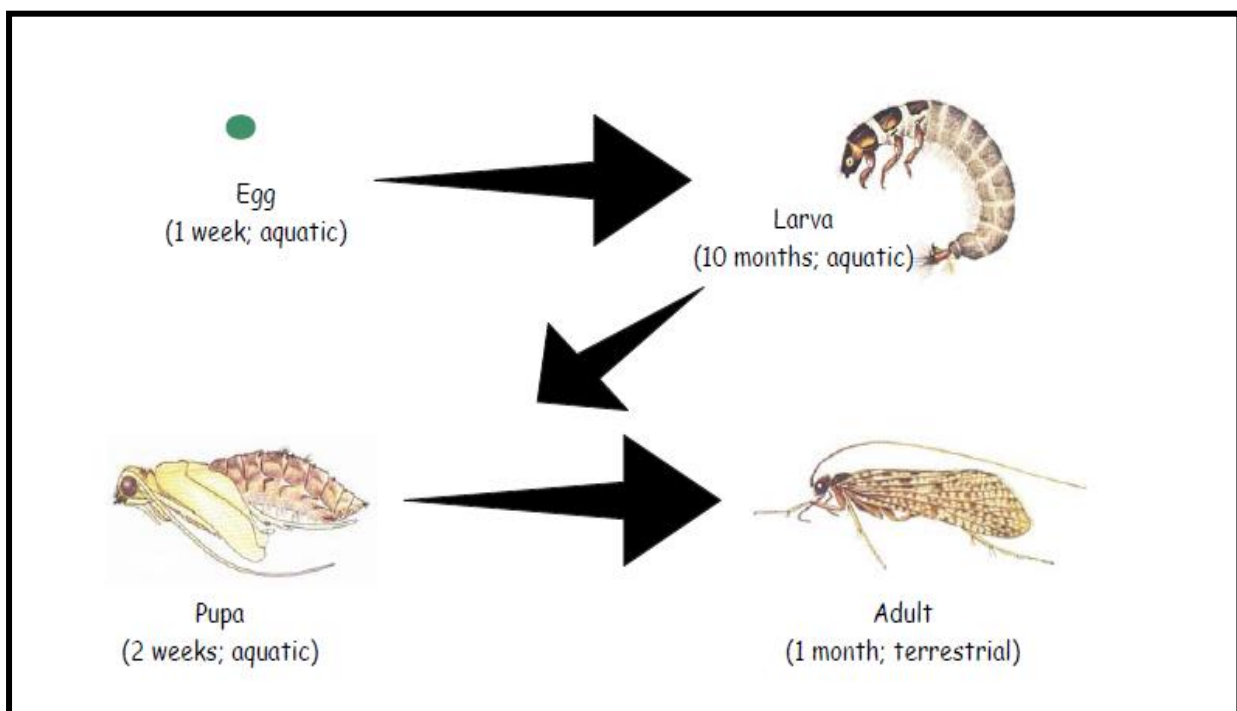


Figure 3.3: Complete metamorphosis of a Caddisfly. Adapted from Maryland State Envirothon, (2013).

3.2.5 Habitat complexity

Habitat is made more complex by *organic matter* constituted by large woody debris, root wads and leaf packs/debris jams; *channel morphology* (geologic formations, forces of erosion, slope of the stream and pools, riffles, runs, glides); and *landscape influences* including slope of the land adjacent to stream; land-use near the stream and low extent of upstream environmental impacts (Cushman *et al*, Undated).

In South Africa, the most currently used method of invertebrate habitat assessment is the integrated habitat assessment system (IHAS, version 2), developed by McMillan, (1998) even though Dallas, (2000b), Dickens & Graham, (2002) argued that IHAS has not, to date been tested and validated scientifically.

3.2.5.1 River habitat quality Survey (RHS)

RHS is a system for assessing the character and quality of rivers based on their physical structure. It has four distinct components: (i) a standard method for field survey; (ii) a computer database, for entering results from survey sites and comparing them with information from other sites throughout the UK and the Isle of Man; (iii) a suite of methods for assessing habitat quality; and (iv) a method for describing the extent of artificial channel modification (Raven *et al.*, 1998).

Habitat quality is determined according to the occurrence and diversity of habitat features of known value for wildlife, and is derived by comparing observed features at a site with those recorded at sites from rivers of similar character (Figure 3.4). Habitat features associated with high quality are generally to be found at sites in a predominantly unmodified physical state.

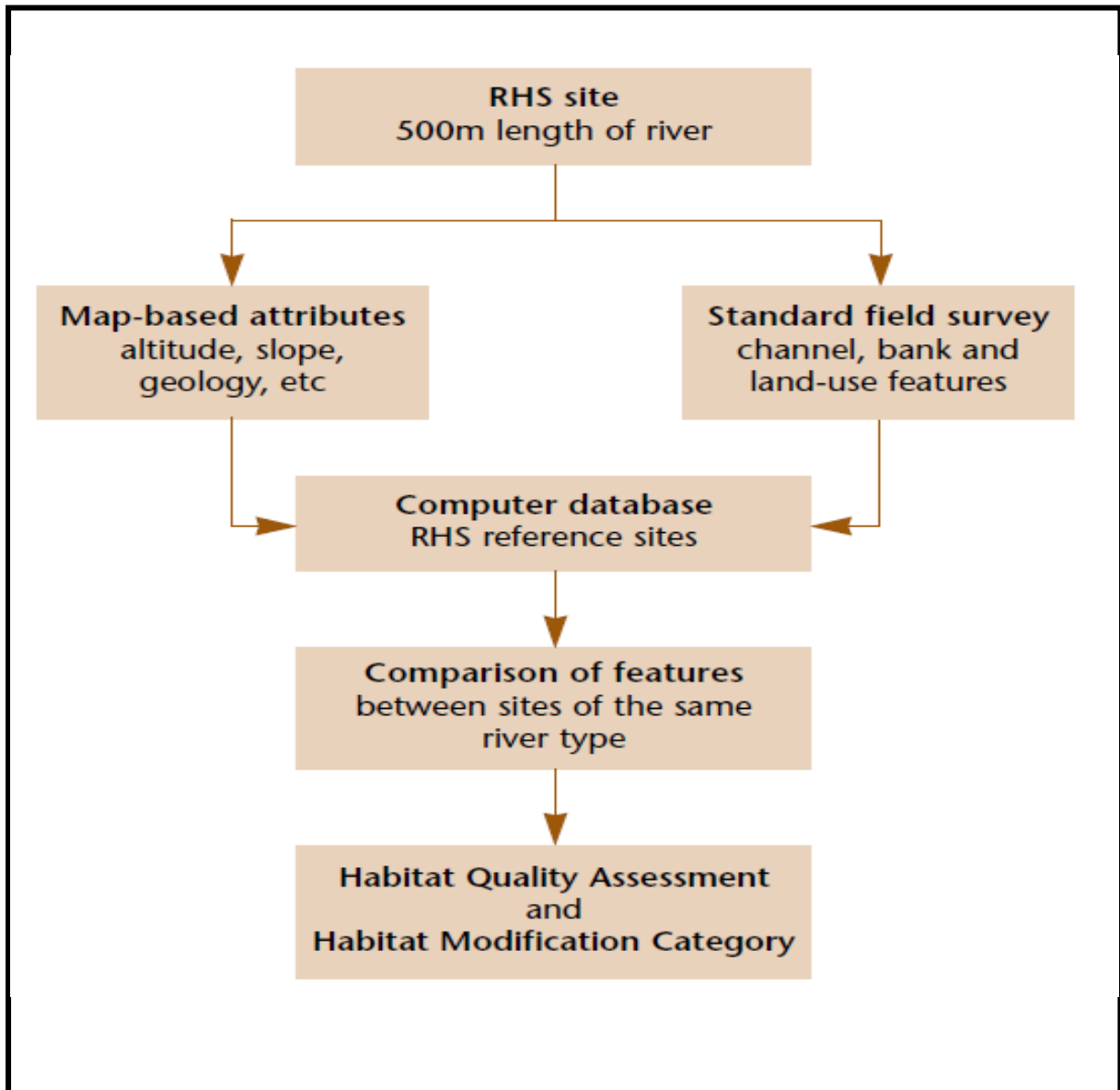


Figure 3.4: An introduction to how RHS works. Adapted from Raven *et al.*, (1998).

3.2.5.2 River health program (RHP)

According to Roux, (1997); Roux *et al.*, (1999) cited by Maseti, (2005), the formal design and implementation of RHP was initiated in 1994 for the purpose of serving as the source of information for the overall ecological status of South African river systems; and to support their management. The major objective of the RHP is to ensure that all reports provide scientifically and managerially relevant information for the national aquatic ecosystem management. This program uses in-stream and riparian biological information (fish, invertebrates and riparian vegetation) to characterize the response of the aquatic environment disturbances; and also to

determine the ecological health of the rivers using the prevailing biota (macroinvertebrates, fish and vegetation) and abiotic components such as water quality and geomorphology. This activity is known as “Bio-monitoring” and is the technique commonly used in water resource management, worldwide.

The health of the biota inhabiting the river ecosystems provides a direct and integrated measure of the health of the river as a whole (Roux *et al.*, 1999) and to support and maintain a balanced integrated and adaptive community of organisms; to have species diversity, composition and functional organization comparable to that of natural habitats (Karr & Dudley, 1981).

The RHP uses both biological and physical indicators where the former provides a framework for the interpretation of biological data. Biological indicators used in this programme include aquatic invertebrates (South African scoring system - SASS), (Chutter, 1998); fish assemblages (Fish Assemblage Integrity Index - FAII), (Kleynhans, 1999); and riparian vegetation (Riparian Vegetation Index - RVI), (Kemper, 2001). Respective physical indicators are also used in the RHP programme as habitat (Habitat Integrity Index – HII), (Kleynhans, 1999); geomorphology (Geomorphology Index – GI), (Rowntree and Ziervogel, 1999); water quality (water Quality Index - WQI), (Moore, 1990); river flow (Hydrological Index – HI), (Hughes & Smakhtin, 1996).

3.2.5 Riparian vegetation

According to Kemper, (2001) riparian vegetation is the vegetation found in close proximity of the river. Therefore riparian zone is that area located next to a river, influenced by river processes such as flooding and alluvial deposition; and characterised by vegetation adapted to mesic conditions and occasional inundation (Kotze *et al.*, 1997; Maseti, 2005).

Functional riparian vegetation stabilizes river channels, attenuate floods, maintain water temperature and quality; intercept and deposit nutrients as well as sediments (Kemper, 2001). Changes in the stream/river flow, vegetation removal, grazing,

construction, erosion and alien vegetation invasion within the riparian zone alter the structural and functional characteristics of the riparian vegetation, thereby affecting the health of the river (Maseti, 2005).

For that notion, the riparian vegetation index was developed for the use in RHP to assess and monitor the degree of the riparian vegetation modification. This index was tested in Mpumalanga (Crocodile River) and was to be adapted for the use in other provinces (Kemper, 2001).

3.2.7 River zones, biotopes and substrates

Water systems (resources) are generally classified as a general river ecosystem; river biotopes and river substrate particle sizes (Gerber & Gabriel, 2002). Water resources specifically rivers can have different geographical regions due to some factors like: geology, geomorphology, climate, soils and human activities in the catchment (Gerber & Gabriel, 2002). River ecosystem is further broken into river zones as described by Dallas & Day, (1993), namely;

- The headwater zones (mountain stream)
- The middle zone, and
- The lower zone (Figure 3.5)

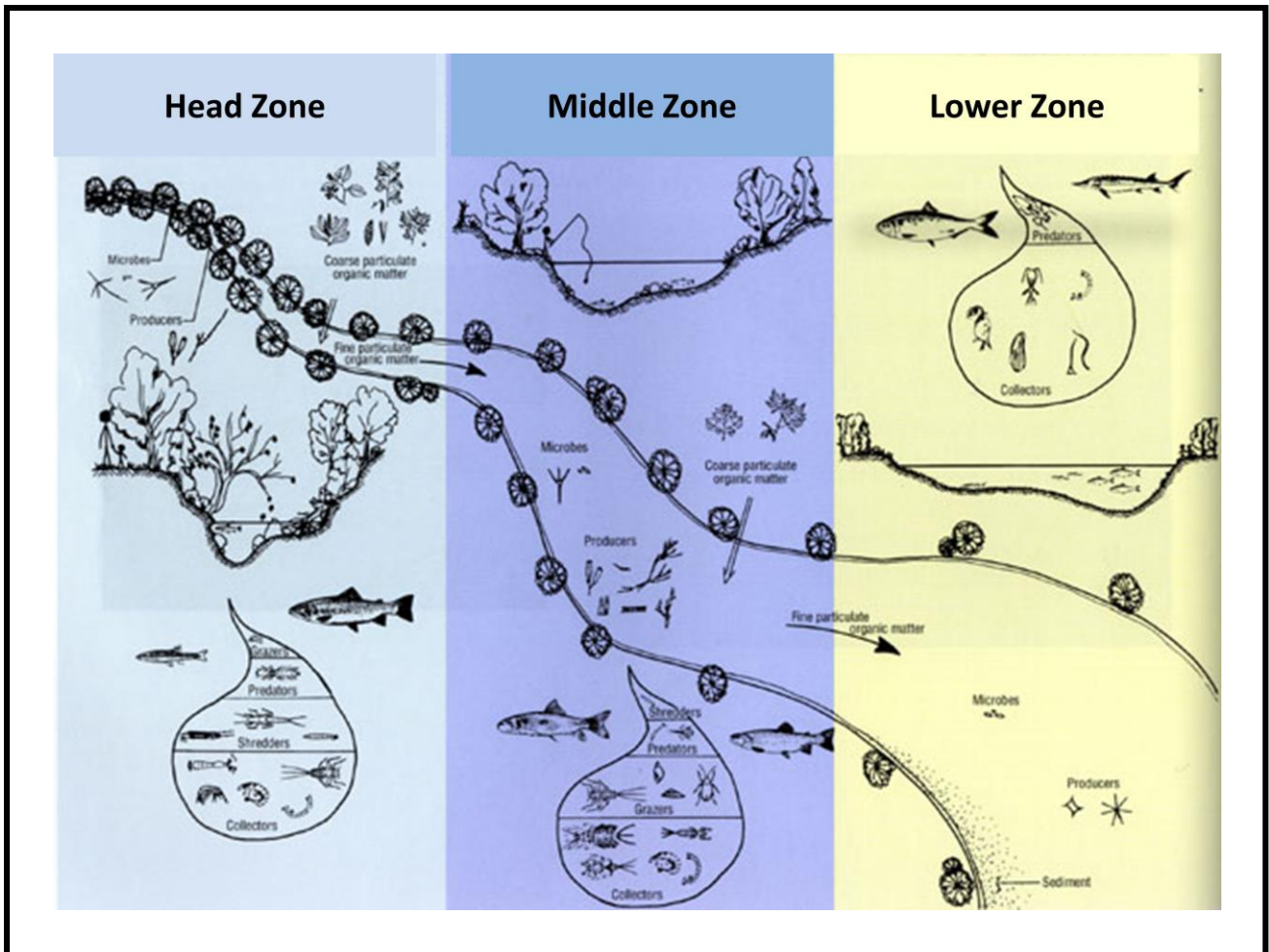


Figure 3.5: Diagrammatic representation of a general river ecosystem based on the River Continuum Concept of Vannote, Minshall, Cummins, Sedell and Cushing (1980); <http://www.trincoll.edu/orgs/Sample/ProjectOverview/RiverEcosystems.htm>.

The stream bed composition is one of the most important physical factors controlling the structure of invertebrate community (Mackay & Eastburn, 1990) and the stream bed can be described by biotopes (Figure 3.6). A biotope in a river ecosystem refers to the environment of a community of closely associated organisms (Gerber & Gabriel, 2002). Biotopes are categorised into SASS and specific groups (Ollis *et al.*, 2005).

SASS biotopes:

- Stones
- Vegetation
- GSM

Specific biotopes:

- Riffles and runs
- Pools
- Boulders
- Algae



Figure 3.6: Representation of different river biotopes as follows: a) Riffles and runs; b); pool and bedrock; c) marginal and aquatic vegetation and d) algae. Adapted from Gerber & Gabriel, (2002).

The material that constitutes the bottom of a river is called substrate which often characterise the different zones and biotopes found in a river. For instance, headwater zone of a typical river would have a substrate composed of boulders and bedrock that gradually changes to cobbles and pebbles that are collectively called stones. As these stones flow in the middle zones they eventually become sandy and silty in the lower zones and are presented in different particle sizes (Table 3.1) (Dickens & Graham, 2001).

Table 3.1: Representation of river substrate with their different particle sizes (Gerber & Gabriel, 2002).

Category	Particle size range
Silt	<0.06 mm
Sand	0.06 – 2 mm
Gravel	2 -20 mm
Stones	2 -30 mm
Boulders	>30 mm
Bedrock	Slab of rock

3.2.8 Macroinvertebrates and SASS5

As cited by Maseti (2005), benthic macroinvertebrates are those organisms that inhabit the bottom substrates like sediments, logs, debris and macrophytes of freshwater habitats for most parts of their life cycles and can be retained by mesh sizes > 200 – 500 µm (Rosenberg & Resh, 1993).

Macroinvertebrates play an important role in the ecosystem of which they are a part (Voshell, 2002a). They also serve as food for fish, amphibians, and water birds; and are also involved in the breakdown of organic matter and nutrients. Macroinvertebrates from freshwater bodies are used to assess the health of a stream/river; and also serve as indicators of water quality for the following reasons:

- They are easy to collect.
- Many live in water for many years.
- They are sensitive (intolerant) to pollution, habitat changes and severe natural events; while others may be tolerant.
- They are generally sessile, i.e. they cannot escape pollution like fish and birds do.

Macroinvertebrates are also widely distributed with diverse communities which constitute a broad range of trophic levels and pollution tolerances (Rosenberg & Resh, 1993; Metcalfe-Smith, 1994; Barbour *et al.*, 1999).

The South African scoring system version 5 (SASS 5) is a rapid biomonitoring index which uses macroinvertebrate families as indicators (Dickens & Graham, 2002); and has been tested and widely used in South Africa as a tool for assessing water quality and river health (Dallas, 1997; Vos *et al.*, 2002).

A modified method of Dallas, (2007) has been used to generate biological bands for SASS 5 score and average score per taxon (ASPT) values and analysis was based on Dallas, (2007) method. This method uses natural variation in SASS 5 scores and ASPT at reference sites to determine the percentiles and band widths. Preliminary biological bands have been generated where data permits and were analysed using analysis of variance (ANOVA) to determine if the differences in SASS 5 score and ASPT were significant (Dallas, 2007).

3.2.8.1 Interpretation of SASS5 and ASPT

The SASS method produces three different and complimentary scores, SASS Score, Number of taxa and average score per taxon (ASPT). While the reserve procedure relies on ASPT, as this is the least variable of the scores (Dallas, 2000a; Dickens & Graham, 2002) and also provides the most reliable measure of a Natural Class or band A (Table 3.1), the other two scores can be used to aid interpretation. Interpretation is based on the premise that if either SASS5 score or ASPT is above the band value it will fall in band; for example, a site would fall in the biological band

A or natural (defined as SASS5 score > 150 or ASPT > 8.0) if the site had a SASS5 score of 160 and an SPT of 7.2 (Dallas, 2007).

For example, in “clean” rivers, ASPT gives more reliable results, while in “polluted” rivers, SASS Score may be more reliable (Chutter, 1998). There are also exceptional cases where, in polluted rivers, the ASPT score can be unreasonably high. In these cases the SASS Score will indicate the presence of pollution. Table 3.2 below indicates the biological bands and ecological categories that interpret SASS5 data (Dallas, 2007).

Table 3.2: The default benchmark category boundaries for the biotic index (SASS) (Dallas, 2000; Dickens & Graham, 2002).

Biological Band / Ecological category	Class Boundary	Description	Range of ASPT Scores
A	Natural	Unmodified natural	7
B	Good	Natural with few modifications	6 - 6.9
C	Fair	Moderately modified	5 - 5.9
D	Poor	Largely modified	<5

Qwaqwa freshwater macroinvertebrate status is also not known. Expected outcome of this study is to document the abundance or occurrence of macroinvertebrate taxa particularly pollution specific taxa; and also to document pollution bioindicators of Qwaqwa freshwaters.

This study was aimed at evaluating benthic macroinvertebrates in the aquatic habitats and also determine the river health using macroinvertebrates as bioindicators of water pollution and water quality biomonitoring with the aid of the South African scoring system (SASS5).

3.3 MATERIALS AND METHODS

3.3.1 Study Area: Rivers and dams

The study was conducted on both the lotic (rivers) and lentic (dams) freshwater systems of Qwaqwa area of Maluti-A-Phofung (MAP) municipality. All selected rivers and dams form the catchment of MAP water resource for Qwaqwa drinking water systems. The underlying reason for seasonal assessment of rivers is for their perennial flow, locality and their accessibility. Selected study sites were Metsimatsho (S 28° 34' 42.3" ; E 028° 51' 51.0"), Namahadi (S 28° 34' 21.9" ; E 028° 51' 28.2"), Khoptjwane (S 28° 32.1' 41.8"; E 028° 49' 10.7"), Kollatshwene (S 28° 31' 17.9"; E 028° 47' 02.8") and Elands rivers (S 28° 29' 49.9"; E 028° 49' 40.6"), as well as Metsimatsho (S 28° 35'19.2"; E 028°56' 16.7") and Fikapatso dams (S 28° 40' 04.5"; E 028° 51' 16.2").

3.3.2 River habitat survey

Habitat evaluation was conducted prior to water sample collection as suggested by Raven *et al.*, (1998) where the environmental, climatic and social factors were assessed at the river ecosystem. A standard method for field survey and assessment was based on the geographical aspects of the habitat such as coordinates, elevation and habitat description. Other physical factors like dominant type of aquatic and riparian vegetation, specific river zone, river depth and width, water flow condition, temperature and the human impact on the habitat quality were also assessed. Habitat quality was determined according to the occurrence and diversity of habitat features as compared to observed features from rivers of the similar features.

Riparian vegetation was sampled from each site and individual plant species were identified by a plant science researcher (Shezi, T.A) and Dr E.Sieben, a senior lecturer in the Department of Plant Science, University of the Free State, Qwaqwa Campus herbarium.

3.3.3 Water sample collection

Water samples were collected from different local untreated water resources like sampled rivers and conservation dams in the Qwaqwa area. Samples were collected from autumn (February) to summer (November). Freshwater habitat ecology was evaluated upon sample collection. At least 80% of river water will be collected from the outskirts of Phuthaditjhaba in Qwaqwa (Kgopjwane, Kollatshwene, Namahali and Metsimatsho rivers); and 20% from the metropolitan area (Elands river) with 3 repetitions per collection site.

3.3.3.1 Replication

During the seasonal survey, replicate samples were taken on different selected biotopes, namely; stone, hard bed rock, vegetation and GSM. SASS scores were taken; number of taxa determined and finally calculated the ASPT for each biotope in each sampled site.

3.3.4 Aquatic invertebrate diversity assessment

Aquatic invertebrate communities were sampled from a variety of biotopes in each water source. These biotopes include stones; gravel, sand and mud (GSM) and aquatic vegetation. Aquatic Invertebrate samples were collected by the use of an aquatic net (500 µm netting mesh size), with a number of sweeps depending on the extent of a particular biotope (Ferreira *et al.*, 2006). Benthic macroinvertebrate communities were sampled and the content of each grab was emptied into the pan (50 cm x 40 cm x 5 cm) filled with half contents of water. Suspended debris was decanted; while the remainder of the sample was transferred into the polyethylene bottles (Ferreira *et al.*, 2006) for further identification of macroinvertebrates in the laboratory.

3.3.4.1 Diversity indices

Diversity indices Shannon Wiener and Simpson Yule were computed to determine the diversity of taxa found in all sampled sites. The Shannon Wiener index was used as:

$$H = -\text{SUM} [(pi) * \ln (pi)] \quad E=H/H_{\max}$$

Where, SUM = Summation; pi = Number of species / total number of samples; S = Number of species or species richness; H_{max} = Maximum diversity possible; E = Evenness = H/H_{max} (www.easycalculation.com/statistics).

On other the Simpson Yule index was calculated using the following formula as prescribed by Usher (1983):

$$D = \sum_{i=1}^s pi - 2$$

Since D is the probability that two individuals selected randomly belong to the same species (Usher, 1983); where:

\sum = Summation; pi = Number of species / total number of samples; S = Number of species, then;

$$D' = 1 - D$$

Therefore, D' = 1 - D is the probability that the two individuals are of different species. As a community becomes more diverse, then the probability that two individuals are of the same species decreases, hence D approaches 0 and D' approaches 1 (Usher, 1983) and the greater the diversity.

3.3.5 Aquatic macroinvertebrate assessment indices

3.3.5.1 South African Scoring System version 5 (SASS5)

Macroinvertebrates from each biotope rinsed into the SASS tray half filled with water. Invertebrates obtained from each biotope were recorded on the SASS score sheet indicating their abundance. The SASS scoring system allocates each taxon (usually a family) of invertebrates from the South African rivers a score ranging from 1 – 15. The score of 1 is for taxa (families) that are most tolerant to water pollution; and 15 for those that are most sensitive to water pollutants (Chutter, 1998).

The prevalence of aquatic benthic macroinvertebrates was determined through field water sampling and evaluation (identification) using the aquatic invertebrate's field guide and the South African scoring system (SASS5) version 5 as well as the water invertebrate pollution sensitivity scale (Gerber & Gabriel, 2002). Present invertebrates were identified to family level and used as bio-indicators of water pollution and recorded in every seasonal water collection to determine SASS scores. To complete the SASS exercise the scores for all the taxa were added together as total score. The average score per taxon (ASPT) was calculated by dividing the total score by number of taxa. All these three scores (SASS, ASPT and number of taxa/families) were used to interpret the status of the river assessed (Chutter, 1998).

In the laboratory, aquatic macroinvertebrates and few aquatic invertebrates were identified using a dissection microscope (Nikon C – LEDS, China) to identify taxa difficult to differentiate mainly at nymphal and larval stages. Some aquatic insects that were difficult to differentiate were preserved in 70% ethanol and were finally identified to the family level by Dr Emile Bredenhand (UFS – Zoology & Entomology, Qwaqwa Campus). A South African aquatic invertebrate guide (Gerber & Gabriel, 2002) was used for aquatic macroinvertebrate identification. Identified macroinvertebrate taxa were counted in order to calculate the average species per taxon (ASPT) (Ferreira *et al.*, 2006).

3.4 STATISTICAL ANALYSIS

The SASS5 score and ASPT recorded for each biotope group such as stones, vegetation and GSM during autumn, winter, spring and summer were analysed separately as indicated by Ollis *et al.*, (2005). Comparisons were made by analyzing SASS5 and ASPT values against the median values for each biotope as calculated by Dallas, (2000a & b). The ASPT values below 5 were indicative of poor category or Biological Band D - E while ASPT above 7 indicate a natural habitat category or Biological band A of water quality. The stata 11 analysis programme was used to create similarity dendrograms, scatter plots and the descriptive analyses of macroinvertebrate data.

Diversity indices like the Shannon Wiener and Simpson Yule indices were used to determine diversity between rivers and dams of the Qwaqwa area. The use of ANOVA and the T-test was instrumental to determine the significant differences among rivers and between rivers and dams of Qwaqwa area.

3.5 RESULTS

3.5.1 Habitat evaluation

The habitat description for the sampled sites give better details on the geographical features of Qwaqwa freshwater sources. Sampled dams of the Qwaqwa area, Metsimatsho and Fikapatso are lying on the mountains of this area than the sampled rivers. Metsimatsho dam is the most highest above the sea level (1882 m) followed by Fikapatso Dam (1798 m). As compared to the sampled rivers; these two dams underlie a bedrock with steady clear waters (Table 3.3). Similarly some rivers on the head zone had relatively higher elevation like Kollatshwene (1675 m) and Metsimatsho (1658 m) rivers underlie bedrock with a bit of turbid waters with moderate to faster flow of the stream. Most of the middle zone rivers are dominated by stone substrate (Elands river); and sand gravel and mud (GSM) observed in Namahadi and Khoptjwane rivers) with high turbid water in the Elands river. The most dominant vegetation species of all sampled rivers are sedges (*Cyperus* spp) and lavender (*Lavandula* spp); with *Salix* and *Poplars* spp of exotic trees. along the Elands river (Table 3.3). On the other hand, dams are dominated by alien invasive species, *Acacia mearnsii*. The topography of Qwaqwa area comprises steep slopes of Drakensberg and Maloti mountain range (DWAF, 2008) with evident runoff during heavy rains. This influences high turbidity of water in rivers during high rainfall seasons.

Table 3.3: Habitat assessment giving details on the geographical features and description of the sampling site.

Sampling site	River Zone	Coordinates	Elevation	Habitat Description	Dominant Vegetation
Metsimatsho River	Head Zone	S 28°34'42.4" E 028°51'51.0"	1658 m	Bed rock substrate, turbid water, fast to slow water flow (slow in winter) and animal faecal deposition.	<i>Cyperus</i> spp (Sedges), <i>Lavandula</i> spp (Lavender), <i>Juncus oxycarpus</i> , <i>Eragrostis planuculnis</i>
Namahadi River	Middle Zone	S 28°34'21.7" E 028°51' 28.2"	1647 m	Stones, Gravel sand and gravel substrate with little mud, clear water, moderate flow, and minimal faecal deposition and by humans and animals.	<i>Cyperus</i> spp (Sedges), <i>Lavandula</i> spp (Lavender)
Kgoptjane River	Middle Zone	S 28°32' 41.8" E 028°49'10.7"	1660 m	Stone, gravel, sand and mud, a bit of turbid water, moderate flow, disposal of nappies (faecal contamination).	<i>Cyperus</i> spp (Sedges), <i>Salix babylonica</i>
Kollatshwene River	Head Zone	S 28°31.17.8" E 028°47'01.1"	1675 m	Bed rock substrate, bit of turbid water, moderate to fast flow, faecally contaminated by disposal of nappies.	<i>Cyperus</i> spp (Sedges), <i>Lavandula</i> spp (Lavender), <i>Berula erecta</i> , <i>Vernonia hatalonsis</i>
Elands River	Middle Zone	S 28°29'49.9" E 028°49'39.8"	1641 m	Stones, moderate flow, water highly turbid, faecally contaminated by disposal of nappies	<i>Salix babylonica</i> , <i>Populus canescens</i> , <i>Robinia pseudoacacia</i>
Metsimatsho Dam	N/A	S 28°35'26.0" E 028°56'11.2"	1882 m	Bed rock; Clear water	<i>Helichrysum</i> sp. , <i>Juncus</i> sp., <i>Acacia mearnsii</i>
Fikapatso Dam	N/A	S 28°40'04.8" E 028°51'16.5"	1798 m	Bed rock; Clear water	<i>Acacia mearnsii</i> , <i>Hypericum perforatum</i> , <i>Robinia pseudoacacia</i> .

3.5.2 Macroinvertebrate evaluation and SASS5

Table 3.4 presents the SASS score results and the average score per taxon (ASPT) for all the sampled sites (rivers). Figure 3.7 shows the SASS scores for the sampled sites adapted from the information presented on table 3.4. The results indicate a significant difference between the SASS score and the ASPT of the sampled sites ($p < 0.05$). Furthermore, a number of taxa per season and the sampled sites is presented on figure 3.8 with Khoptjwane river presenting the highest number of taxa in autumn and winter while Metsimatsho river had the highest number of taxa in spring. Table 3.5 presents comparison of macroinvertebrate taxa abundance of all sampled sites (both sampled rivers and dams); Baetidae taxa dominated all the sampled rivers (102) followed by Corixidae (36) and Pleiidae (27) with Ceratopogonidae being the least (18) most of which are tolerant to water pollution with reference to table 3.6.

Table 3.4: SASS5 score, number of taxa and ASPT for the sampled sites of Qwaqwa area for the combined seasons.

Sampled site	SASS Score	Number of Taxa	Average score per taxon (ASPT)
Metsimatsho River	117	17	6.8
Namahadi River	101	12	7.6
Khoptjwane River	94	15	6.7
Kollatshwene River	64	14	4.8
Elands River	118	21	4.8
Metsimatsho Dam	125	20	6.3
Fikapatso Dam	104	15	6.9

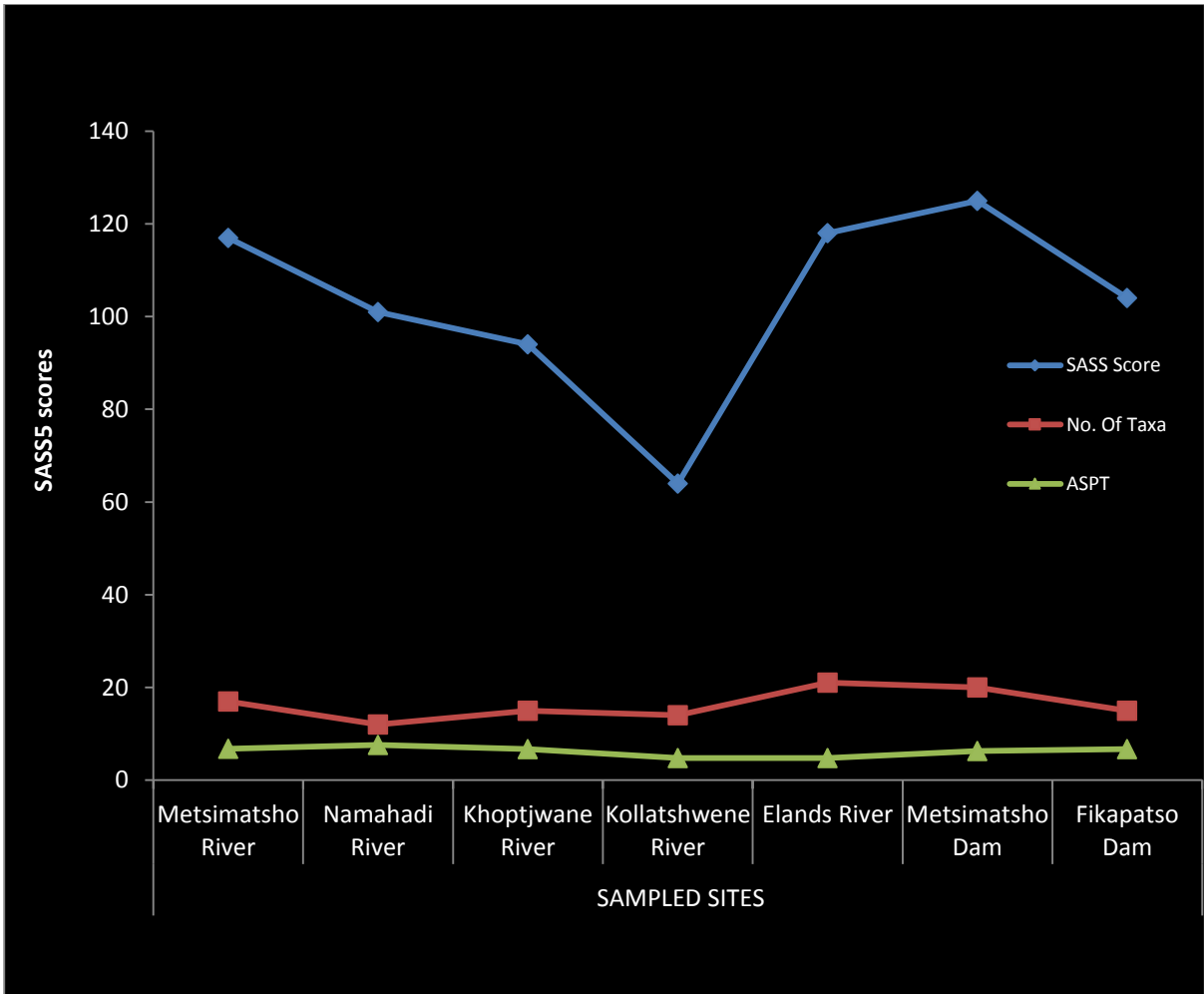


Figure 3.7: Representation of SASS5 scores across both lotic (rivers) and lentic (dams) systems of Qwaqwa area

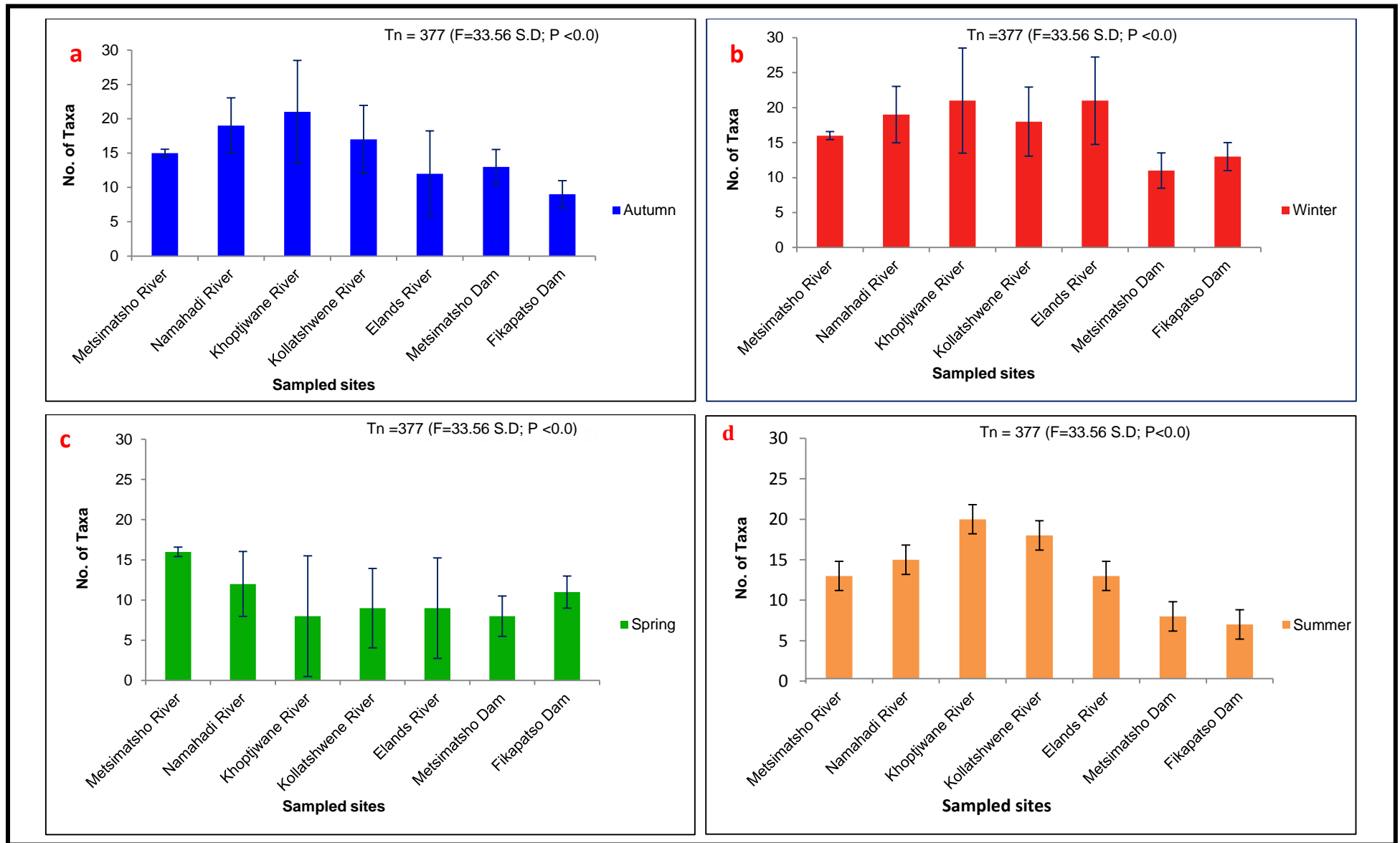


Figure 3.8: Comparison of the number of taxa across sampled sites of Qwaqwa are during different sampling seasons (a. Autumn; b. Winter; c. Spring and d. Summer).

Table 3.5: Representation of the most abundant taxa, their numbers and percentage occurrence at each sampled study sites (N=36).

FAMILY	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Total/ Taxa
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	N
Hydrachnellae	4	17.39	3	13.04	3	13.04	2	8.69	2	8.69	7	30.43	2	8.69	23
Baetidae	24	23.53	10	9.80	27	26.47	21	20.59	6	5.88	11	10.78	3	2.94	102
Teloganodidae	6	23.08	5	19.23	3	11.54	3	11.54	2	7.69	3	11.54	4	15.38	26
Coenagrionidae	4	16.67	5	20.83	3	12.5	2	8.33	3	12.5	3	12.5	4	16.67	24
Corixidae	4	11.11	2	5.56	7	19.44	6	16.67	3	8.33	10	27.78	4	11.11	36
Pleidae	3	11.11	4	14.81	4	14.81	8	29.63	3	11.11	3	11.11	2	7.41	27
Ecnomidae	3	15.79	2	10.53	2	10.53	5	26.32	2	10.53	2	10.53	3	15.79	19
Gyrinidae	4	17.39	3	13.04	8	34.78	3	13.04	2	8.69	-	0	3	13.04	23
Ceratopogonidae	2	11.11	2	11.11	2	11.11	3	16.67	3	16.67	3	16.67	3	16.67	18
Total per site	54		36		59		53		26		42		28		298

Site1 = Metsimatsho River; site 2 = Namahadi River; Site 3 = Khoptjwane river; Site 4 = Kollatshwene River; Site 5 = Elands River; Site 6 = Fikapatso Dam & Site7 = Metsimatsho Dam; (-) = Family not available.

Table 3.6: Representation of total number of sampled families and percentage occurrence of tolerant, moderately sensitive and highly sensitive macroinvertebrate families from different sampled sites.

Sampling site	Total no. of families / sampling site	% of total no. of families	Number of tolerant families	Percentage occurrence	Number of moderately sensitive families	Percentage occurrence	Number of highly sensitive families	Percentage occurrence
Site 1	17	47.2	9	52.9	4	23.5	4	23.5
Site 2	12	19.1	3	25.0	5	41.7	4	33.3
Site 3	15	41.7	9	60.0	3	20.0	3	20.0
Site 4	14	38.9	10	71.4	5	35.7	0	0
Site 5	21	58.3	13	61.9	4	19.0	2	9.52
Site 6	20	55.6	11	55.0	6	30.0	3	15.0
Site 7	15	41.7	8	53.3	4	26.7	3	20.0

Site1 = Metsimatsho River; site 2 = Namahadi River; Site 3 = Khoptjwane river; Site 4 = Kollatshwene River; Site 5 = Elands River; Site 6 = Fikapatso Dam & Site7 = Metsimatsho Dam.

3.5.3 Diversity Indices

3.5.3.1 Shannon Wiener diversity index for macroinvertebrate taxa

Given a very large sample size, with more than 5 taxa, the Shannon Wiener index value (H) can range between 0 to ~4.6 using the natural log (ln). A value near 0 would indicate that every taxon in the sample is the same. A value near 4.6 would indicate that the number of individuals are evenly distributed between all the taxa.

Table 3.7 shows that there is no Shannon index that is 2 or above. They are all close to 1 and below 1. This implies that everything in the sample (per respective community and season) was the same. For better interpretation of these results, Shannon Wiener Index (H) was converted to ENS = exp (H). Therefore a community with Shannon Wiener index of H had an equivalent diversity as a community with equally-common species of exp (H). For example, for the autumn season of Metsimatsho river, the Shannon Index = 0.673 and its corresponding ENS = 1.96 ≈ 2 implies that a community with Shannon Index of 0.673 has an equivalent diversity as a community with 2 equally common species.

Table 3.7: The Shannon Wiener diversity index interpretation of results for the macroinvertebrates seasonal analysis for the sampled rivers.

Season	Metsimatsho (ENS)*	Namahadi (ENS)	Khoptjwane (ENS)	Kollatshwene (ENS)	Elands (ENS)
Autumn	0.673 (1.96)	1.0795 (2.94)	1.052 (2.87)	1.073 (2.93)	0.679 (1.97)
Winter	0.693 (2)	1.0959 (2.99)	1.091 (2.98)	1.0986 (3)	0.692 (1.20)
Spring	0.685 (1.98)	1.0928 (2.80)	1.082 (2.95)	1.1 986(3)	0.687 (1.99)
Summer	0.685 (1.98)	1.0925 (2.79)	1.08 (2.94)	1.0999(3)	0.598 (1.73)
Combined seasons	0.684 (1.98)	1.090 (2.98)	1.076 (2.94)	1.1173(3.0)	0.664(1.95)

* Effective Number of Species (ENS) = exp (Shannon Index)

Equitability or evenness

$E_H = H / \ln S$, where H is Shannon Wiener's index and S is the total number of species in a community. Equitability assumes a value between 0 and 1 with 1 being complete evenness. The E_H values in table 3.8 were very close to one, this implies that the taxa within each community and for a particular season are even. All the evenness values of this study for the combined seasons range between 0.98 and 1.0, this advocates the complete evenness of the sampled taxa.

Table 3.8: Shannon's Equitability representation of macroinvertebrate taxa for the sampled rivers of Qwaqwa.

Season	Metsimatsho (ENS)	Namahadi (ENS)	Khoptjwane (ENS)	Kollatshwene (ENS)	Elands (ENS)
Autumn	0.97	0.98	0.96	0.98	0.98
Winter	1.0	1.0	0.99	1.0	1.0
Spring	0.99	0.94	0.99	1.0	0.99
Summer	1.0	0.99	1.0	1.0	0.99
Combined- seasons	0.99	0.98	0.99	1.0	0.99

3.5.3.2 Simpson Yule's Indices of diversity for macroinvertebrate taxa

Simpson Yule's Index (D) measures the probability that two individuals randomly selected from a sample will belong to the same species or taxa (or some category other than species). It is a value between 0 and 1 where 0 represents infinite diversity and 1 represents no diversity. That is, the bigger the value of D , the lower the diversity.

Simpson's Index of Diversity (1 – D)

The value of this index also ranges between 0 and 1, but now, the greater the value, the greater the sample diversity. This makes more sense. In this case, the index represents the probability that two individuals randomly selected from a sample will belong to different species or taxa.

Table 3.9 presents Simpson Yule's diversity indices results and their interrelationship. For example, the value D is finally reduced from one (1 – D) to determine diversity of the sampled taxa within the Qwaqwa freshwaters.

Table 3.9: Representation of Simpson index (D) and [Simpson's Index of Diversity \(1 – D\)](#) for macroinvertebrate taxa of Qwaqwa freshwaters.

Season	Metsimatsho	Namahadi	Khoptjwane	Kollatshwene	Elands
Autumn	0.51 (0.49)	0.35 (0.65)	0.37 (0.63)	0.35 (0.65)	0.52 (0.48)
Winter	0.50 (0.50)	0.34 (0.66)	0.34 (0.66)	0.33 (0.67)	0.50 (0.50)
Spring	0.51 (0.49)	0.39 (0.61)	0.35 (0.65)	0.34 (0.66)	0.50 (0.50)
Summer	0.52 (0.49)	0.42 (0.58)	0.37 (0.63)	0.35 (0.65)	0.51 (0.49)
Combined seasons	0.51 (0.49)	0.37 (0.63)	0.35 (0.65)	0.34 (0.66)	0.50 (0.50)

*Colour codes:

- Black = Simpson index (D)
- Blue = Simpson diversity index (1-D)

3.5.3.3 Similarity analysis of rivers based on averages of seasons for the respective biotopes

For the vegetation biotope, Metsimatsho river was similar to Khoptjwane in terms of the average SASS scores and ASPT values; Namahadi was similar to Kollatshwene and all these four rivers were dissimilar Elands (Table 3.10 & Appendix E – 1, 2 & 3).

Regarding the GSM biotope, there was no similarity between Metsimatsho river and other four rivers (Namahadi, Khoptjwane, Kollatshwene and Elands rivers). On the other hand Namahadi was similar to Khoptjwane; while Kollatshwene was similar to Elands river.

Lastly, the average SASS scores for the stone biotope indicated that Namahadi, Khoptjwane and Elands were similar but were neither similar to Metsimatsho river nor Kollatshwene river.

Table 3.10: Averages of the three seasons per river for three respective biotopes.

River	Biotope	SASS score	Taxa	ASPT
Metsimatsho	Vegetation	51.67	7.67	6.74
Namahadi	Vegetation	35.67	5.3	6.73
Khoptjwane	Vegetation	50	7	7.14
Kollatshwene	Vegetation	33.3	5	6.66
Elands	Vegetation	0	0	0
Metsimatsho	GSM	0	0	0
Namahadi	GSM	36	5.67	6.35
Khoptjwane	GSM	37.67	4.67	8.07
Kollatshwene	GSM	26	4.33	6.00
Elands	GSM	28.33	6.33	4.48
Metsimatsho	Stones	51.67	8	6.46
Namahadi	Stones	36.33	5.67	11.17
Khoptjwane	Stones	33.33	5	6.67
Kollatshwene	Stones	27.67	5.33	5.19
Elands	Stones	38.67	7.67	5.04

3.6 DISCUSSION

The habitat was evaluated in both rivers and dams of Qwaqwa area prior to water sample collection considering factors like riparian vegetation, altitude, water flow and water depth. Riparian vegetation surrounding rivers was dominated by *Cyperus* and *Lavandula* species. Invasive alien plant species were dominant around Metsimatsho and Fikapatso dams. According to Mucina & Rutherford (2006), alien plant invasions are generally localised around these two dams, but can be severe. Some important problem species that is found in this vegetation type include: *Acacia dealbata*, *A. mearnsii*, *Hypericum perforatum*, *Pinus patula*, *Populus canescens*, *Pyracantha angustifolia*, *P. crenulata*, *Robinia pseudo-acacia*, *Rubus cuneifolius* and *Salix fragilis*. *Acacia mearnsii* which is declared an invader plant species was observed at both dams (DWAF, 2008). However, riparian vegetation acts as a natural sponge, soaking up water as it runs off the land, and slowly releasing that water back into the stream; or contributes shade, food and shelter for aquatic organisms (<http://extension.usu.edu/waterquality/htm>).

As altitude increases, atmospheric pressure decreases causing lower oxygen partial pressure; low metabolic rates in highland fish and reduced dissolved oxygen to a point where it becomes detrimental to aquatic life. Aquatic invertebrates may have a general trend of smaller body sizes and lower species richness at higher altitude due to lower oxygen partial pressure (Juarez *et al.*, 2011).

The SASS5 biotope groups sampled at Metsimatsho, Namahadi, Khoptjwane, Kollatshwene and Elands rivers were assessed using the SASS5 scoring sheet (Appendix B) and the SASS scores for autumn, winter, spring and summer were determined (Appendix D – Tables 1D, 2D, 3D & 4D). The number of taxa was also counted in order to calculate the ASPT of each replicate biotope as outlined in tables in Appendix D (excluding dams since biotopes were not sampled in dams). A list of macroinvertebrate taxa collected from different biotope groups is provided for each river in appendix B (also including Metsimatsho and Fikapatso dams).

Similarity measures among sampled rivers for the vegetation, GSM and stones biotopes indicated the relatedness between these sampling sites (Appendix E – 1, 2

& 3). Based on SASS scores per individual river biotope, there was a similarity of scores (51.67) between the vegetation and stone biotopes at Metsimatsho river. The average SASS score of 36 was obtained from the three biotopes (Vegetation, GSM and stones) at Namahadi river. This indicated that there was a similarity in terms of the number macroinvertebrate taxa found in this river. At Kollatshwene river, the vegetation and stones biotopes obtained the SASS scores of 26 and 27.67 respectively; and this indicated that the number of macroinvertebrate taxa found from these two river biotopes were similar (Table 3.10). These results indicate general dissimilarity of SASS scores between biotopes of the sampled sites.

River health was evaluated using the SASS5 and ASPT indices. This excluded the dams since these indices were first developed for the river health programme (RHP) in South Africa. The ASPT for these entire rivers is varied and could be based on the seasonality, locality of individual rivers, their geographic factors as well as anthropogenic influences.

Based on these aspects, Elands river has scored the lowest ASPTs in all seasons (Figure 3.11) and would advocate an intense faecal contamination influenced by the residential activities since it is located in the metropolitan area where sewer systems discharge; also with surrounding communities dumping diapers in the water sources. According to the SASS5 system the lower the ASPT the poorer the water quality.

Table 3.6 shows a representation of total number of families sampled and percentage occurrence of tolerant, moderately sensitive and highly sensitive families from different sampled sites. Of all the sampled sites, Kollatshwene, Elands and Khoptjwane rivers have the highest percentage occurrence (71.4%; 61.9% & 60.0% respectively) of tolerant macroinvertebrate families. In addition; the river health among these mentioned sites seems to be highly degraded due to the prevailing water pollution. This is symbolized by the low respective percentage occurrence (0%; 9.52% & 15%) of highly sensitive macroinvertebrate families at Kollatshwene, Elands and Khoptjwane rivers. Furthermore, Elands river constituted three (3) families of highly sensitive SASS status and these include Oligochaeta, Culicidae and Syrphidae, all with the SASS value "1"; whereas Kollatshwene river has Oligochaeta and Culicidae. According to the sensitivity scale by Dickens & Graham,

(2001), Culicidae, Oligochaeta and Syrphidae are bioindicators of deteriorated water quality. Therefore there is a significant difference ($p < 0.05$) between the pollution tolerance of macroinvertebrate taxa of the sampled rivers. It can be concluded that water quality status at sampled rivers and dams may be of different pollution levels since Qwaqwa rivers comprises wastewater with MAP water sewer discharge effluent, whereas dams provide potable water for the Qwaqwa communities (DAWF, 2008).

The results presented in Table 3.9 indicate that Simpson diversity values for the sampled rivers range between 0 and 1; and therefore indicate an infinite diversity among the sampled macroinvertebrate taxa. The blue figures are the Simpson diversity index indicators ($D' = 1 - D$) and the values for the combined seasons per sample site (river) are indicative of a greater diversity as they all range from 0.50 to 1.00. According to the sensitivity scales by Dickens & Graham, (2001), Culicidae, Oligochaeta and Syrphidae are bioindicators (Appendix B) for a deteriorated water quality. Therefore there is a significant difference ($p < 0.05$) between the sampled rivers. It could be concluded that water quality status at sampled rivers and dams may be different hence rivers at Qwaqwa area contain wastewater with discharged materials from MAP water treatment plants; whereas dams supply potable water suitable for drinking purposes for the Qwaqwa communities.

CHAPTER 4

DETECTION OF WATERBORNE MICROINVERTEBRATES OCCURRING IN DAMS AND RIVERS OF QWAQWA

4.1 INTRODUCTION

According to Zamxaka *et al.*, (2004), South Africa still lacks a constant supply of potable water in the communities affecting more than 7 Million people (almost 17% of the population) who do not have access to potable water supply, particularly in the Eastern Cape Province (DWAF, 1996). Nearly 80% of the South African population rely on surface water as the main source of water (Venter, 2001). This relatively high percentage of the population that is without proper water supply services indicates that many of the people still utilize untreated surface water for domestic purposes and rely on State intervention for improved water quality supply. Pegram *et al.*, (1998) showed that a substantial number (about 43 000) of South Africans die every year from diarrhoeal diseases. Most of the pathogens are distributed world-wide, but outbreaks of some diseases such as cholera, shigellosis, and typhoid tend to be regional (Grabow *et al.*, 1994) being caused by most prevalent water-borne pathogens.

Results of the study conducted in 75 dairy farms of Mangaung area in the Free State indicated that at least nine farm boreholes were found to contain high levels of nitrates and *Escherichia coli* (*E. coli*) beyond the recommended standards (Esterhuizen *et al.*, 2012). According to Oliver *et al.*, (2009) faecally- driven pathogens like *E. coli* can impact water quality and human health especially when water is consumed prior treatment. Other bacterial pathogens like *Salmonella tryphimurium* and *Vibrio cholera* were also reported to be significantly higher at Baviaanspoort wastewater in the Gauteng Province of South Africa (Dungeni *et al.*, 2010). On the other hand giardiasis and cryptosporidiosis are common infections of domestic and wild animals, which shed large number of cysts and oocyst into the environment (WHO, 2004). A study conducted in Kwa-Zulu Natal on the prevalence

of diarrhoeal diseases caused by water-borne pathogens indicated that *Cryptosporidium* and *Giardia* occurrence is endemic with the prevalence of 39.3% or even higher than recorded in this province in 3- to 4 - years age group (Jarmey-Swan *et al.*, 2001).

The microbial content of water represents one of the primary determinants of fitness for use. Human settlements, inadequate sanitation and waste water removal practices, storm water wash-off and sewage spills are the major sources of deteriorating microbiological water quality in South Africa. The spread of cryptosporidiosis, dysentery, cholera and typhoid are the exemplary diseases found in waters contaminated with faecal mater (Momba *et al.*, 2004) in Alice (Eastern Cape). Consequently, after HIV / AIDS and low birth weight, diarrhoea was found to be the third highest cause of death among children under five (5) years of age and represented 10% of all deaths in this age in South Africa (Bradshaw *et al.*, 2003).

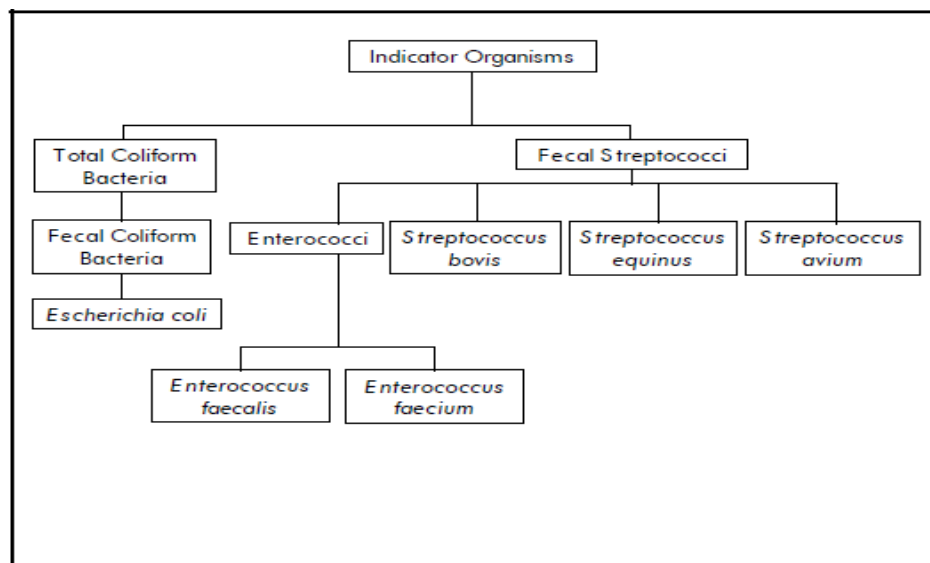


Figure 4.1: Indicator organism relationship. Adapted from EPA - USA, (2001).

Figure 4.1 clarifies the relationship among indicator organisms as they feature in the aquatic ecosystems. Aquatic systems with faecal contamination comprise both the total coliform bacteria and faecal streptococci bacteria. Both bacterial groups occur naturally in the intestinal tract of warm –blooded mammals

4.2 Coliform bacteria

4.2.1 Total and faecal coliforms

The term “total coliforms” includes several genera of gram-negative, facultative anaerobic, non-spore-forming, rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 37°C, some of which occur naturally in the intestinal tract of animals and humans (Rosinger & Tanner, 2014), as well as others that occur naturally in soil and in fresh or marine waters and could be pathogenic to variety of specific hosts. Total coliform bacteria are common in the environment (soil or vegetation) (Figure 4.2) and are generally harmless. If a laboratory detects only total coliform bacteria in drinking water, the source is probably environmental and fecal contamination is unlikely (EPA – USA, 2001). However, if environmental contamination can enter the system, pathogens could get in too.

Faecal coliforms (FC) (subset of total coliforms – Figure 4.2) in water samples include several species of coliform bacteria and are found in the intestines and faeces of warm-blooded animals (Jawetz *et al*, 1987). Faecal coliforms can grow at an elevated temperature of 44.5°C (AWWA, 1999), and is an indication that pathogens found from the intestines of warm-blooded mammals may be present (Aull, 2005). They include a number of genera and species that have common biochemical and morphological attributes found in the *Escherichia coli* (Figure 4.2). On the other hand, faecal streptococci group (FS) includes five species namely *Enterococcus faecalis*, *E. faecium*, *Streptococcus bovis*, *S.equinus*, and *S. avium*, (EPA - USA, 2001). The first two species are enterococci bacteria, a subset of faecal streptococci group which were formerly classified as part of the group D *Streptococcus* system.

The *E. coli* are a subgroup of the fecal coliform group (Figure 4.2). Most *E. coli* bacteria are harmless and exist in the intestines of people and warm-blooded animals. However, some strains can cause illnesses, for example, a specific strain of *E. coli* bacteria known as *E. coli* O157:H7 (Figure 4.2a) causes most of those outbreaks. When a drinking water sample is reported as “*E. coli* present,” it does not mean that O157:H7 is present. The presence of *E. coli* in a drinking water sample

usually indicates recent fecal contamination that presupposes a greater risk that other pathogens like *Shigella* and *Salmonella* species are also present (Figure 4.2b). Boiling or disinfecting contaminated drinking water destroys all forms of *E. coli*, including O157:H7 (WSDOH, 2011).

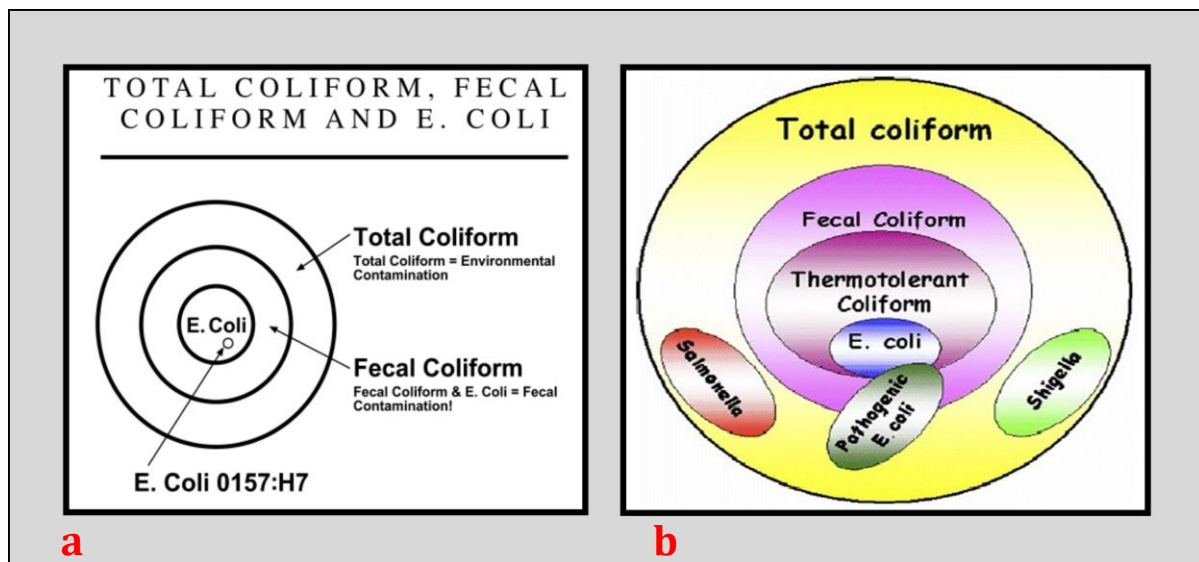


Figure 4.2: Relationship between (a) coliform bacteria and *Escherichia coli*; (b) *E.coli* indicates the presence of pathogens. Adapted from WSDOH, (2011). www.aqua-elite.com.

Faecal and total coliform enumeration for water treatment facilities must have lower than five percent of the samples positive in a month (U.S EPA, 2004a). The presence of *E. coli* (a subset of faecal coliforms) in a water sample also indicates faecal contamination since it is one of the ubiquitous coliform members of the intestinal microflora of warm-blooded animals (Jawetz *et al.*, 1987).

4.2.2 Faecal streptococci bacteria

According to Davies – Colley *et al.*, 1994, there has been a resurging interest in the enterococcus group of indicators. Enterococci a subgroup of the faecal streptococci [FS] group are round coccoid bacteria that live in the intestinal tract. Different strains of faecal streptococci (*Streptococcus faecalis* and *Streptococcus faecium*) form part of the enterococci family (EPA - USA, 2001), now species transferred to *Enterococcus faecalis* and *E. faecium* are human – specific than other streptococci found in the intestinal tract of other warm-blooded animals such as cats, dogs, cattle,

horses and sheep. Gastrointestinal illnesses are better predicted in human and animals by enterococci than by faecal coliform bacteria since the die-off rate of faecal coliforms is greater than the enterococci bacteria.

4.2.3 Microbial contaminants and pathogen types

Rather than measuring individual indicator organisms such as *E.coli* and faecal coliforms as indicators of harmful bacteria (Aull, 2005), other pathogens include protozoan parasites like amoebas, *Giardia*, *Cyanobacteria*, *Cryptosporidium* and *Toxoplasma species*. These and many other organisms can be pathogenic to humans, livestock and other mammals consuming water.

Most commonly identified pathogens associated with waterborne diseases are grouped into the three general categories of bacteria, protozoa and viruses (EPA - USA, 2001).

4.2.3.1 Bacteria

Bacteria are unicellular organisms without a definite nucleus (Chapra, 1997). They contain single strand of DNA and typically reproduce through binary fission during which a single cell divides to form two identical daughter cells. When conditions are favourable such as the right temperature and nutrients are available, some bacteria like *E. coli* can divide every 20 minutes. This means that in just 7 hours one bacterium can generate 2,097,152 bacteria. After one more hour the number of bacteria will have risen to a colossal 16,777,216. That's why we can quickly become ill when pathogenic microbes invade our bodies (Chapra, 1997). Faecal wastes from warm-blooded animals and humans are sources of many types of bacteria found in water bodies, including coliform groups and *E. coli*, *Streptococcus*, *Lactobacillus*, *Staphylococcus* and *Clostridia* (EPA - USA, 2001). However, not all bacteria are pathogen since others are only water contaminants. Table 4.1 indicates list of pathogenic bacteria of concern to water quality and their associated disease.

Table 4.1: Pathogenic bacteria and their associated diseases. Adapted from EPA - USA, (2001)

Bacteria	Disease	Symptoms
<i>Enteropathogenic Salmonella typhi</i>	Typhoid fever	High fever, diarrhoea, ulceration of the small intestine
<i>Salmonella</i>	Salmonellosis	Diarrhoea, dehydration
<i>Shigella</i>	Shigellosis	Bacillary dysentery
<i>Vibrio cholera</i>	Cholera	Extremely heavy diarrhoea, dehydration
<i>Yersinia enterocolitica</i>	Yersinosis	Diarrhoea

Bacteria populations fluctuate in response to the stream flow, temperature, energy sources and disturbances of streambed, time of the year and time of the day (EPA - USA, 2001). They can survive for long periods on land and in stream sediments.

4.2.3.1 (a) Structure of bacteria

Bacteria are classified into 5 groups according to their basic shapes: spherical (cocci), rod (bacilli), spiral (spirilla), comma (vibrios) or corkscrew (spirochaetes). They can exist as single cells, in pairs, chains or clusters. Figures 4.3(a) and (b) indicate the different shapes and the general structure of the bacterial cell, respectively.

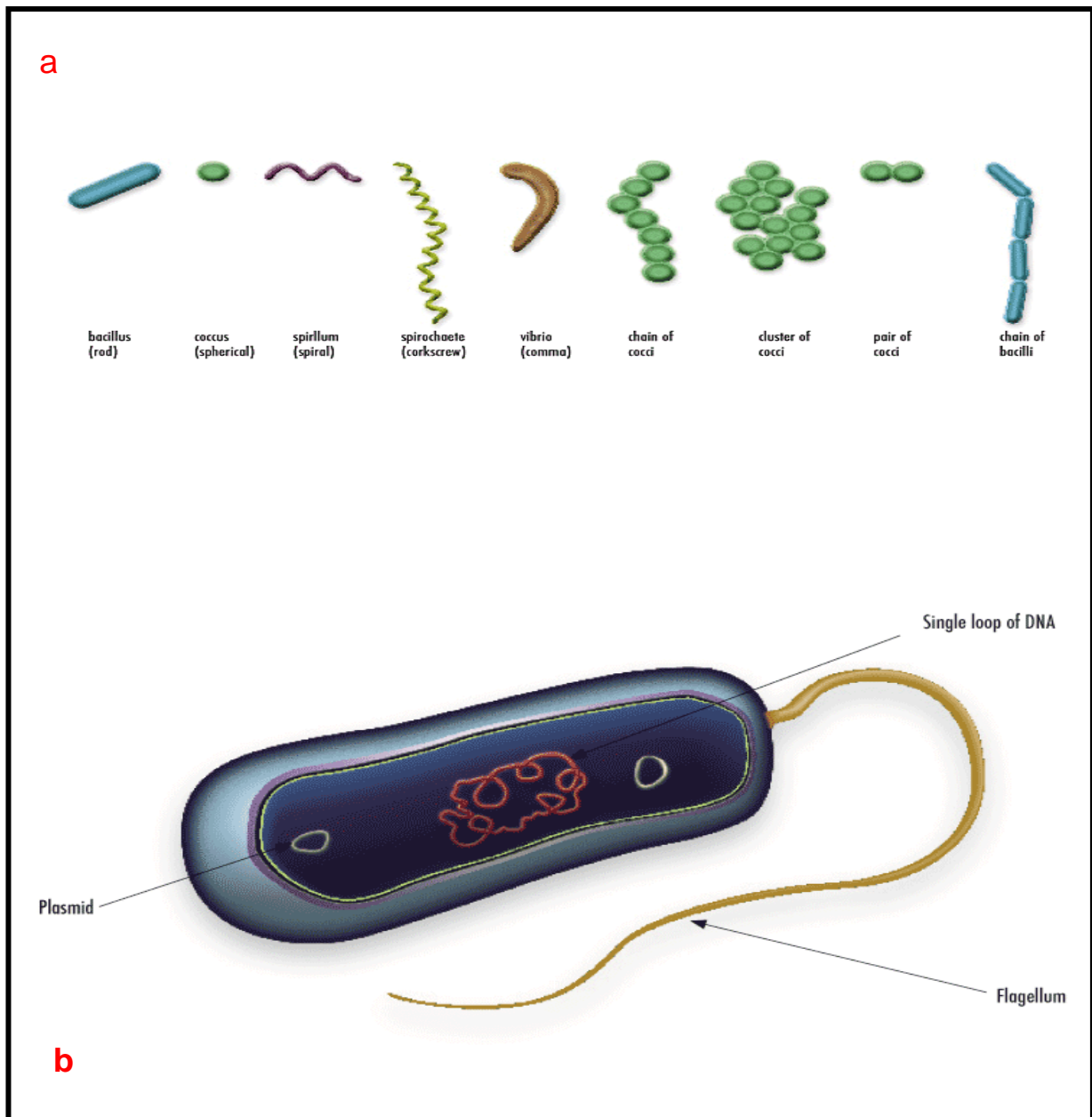


Figure 4.3: (a) Identification of different bacterial shapes; and (b) indicates the typical structure of the bacterial cell. Adapted from Microbiology Online, (2014).

There has been a resurgence of interest in the enterococcus group as indicators (Davies-Colley *et al.*, 1994). Enterococci (a subgroup of the faecal streptococci [FS] group) are round coccoid bacteria that live in the intestinal tract (EPA - USA, 2001). *Streptococcus faecalis* and *S. faecium* (part of the enterococci family) are brought to be more human-specific than other streptococci, but can be found in intestinal tracts of other warm-blooded animals such as cats, dogs, cattle, horses and sheep, (EPA - USA, 2001). The risk of swimmers of contracting gastro-intestinal illness seems to be

predicted better by enterococci than by faecal coliform since the die-off rate of faecal coliforms is much greater than the enterococci die-off rate (EPA - USA, 2001).

4.2.3.1(b) The structural differences in the bacterial cell wall

Danish scientist Hans Christian Gram devised a method to differentiate two types of bacteria based on the structural differences in their cell walls (Figure 4.4). In his test, bacteria that retain the crystal violet dye do so because of a thick layer of peptidoglycan and are called Gram-positive bacteria. In contrast, Gram-negative bacteria do not retain the violet dye and are coloured red or pink. Compared with gram-positive bacteria, gram-negative bacteria are more resistant against antibodies because of their impenetrable cell wall. These bacteria have a wide variety of applications ranging from medical treatment to industrial use and Swiss cheese production, http://www.diffen.com/difference/Gram-negative_Bacteria_vs_Gram-positive_Bacteria.

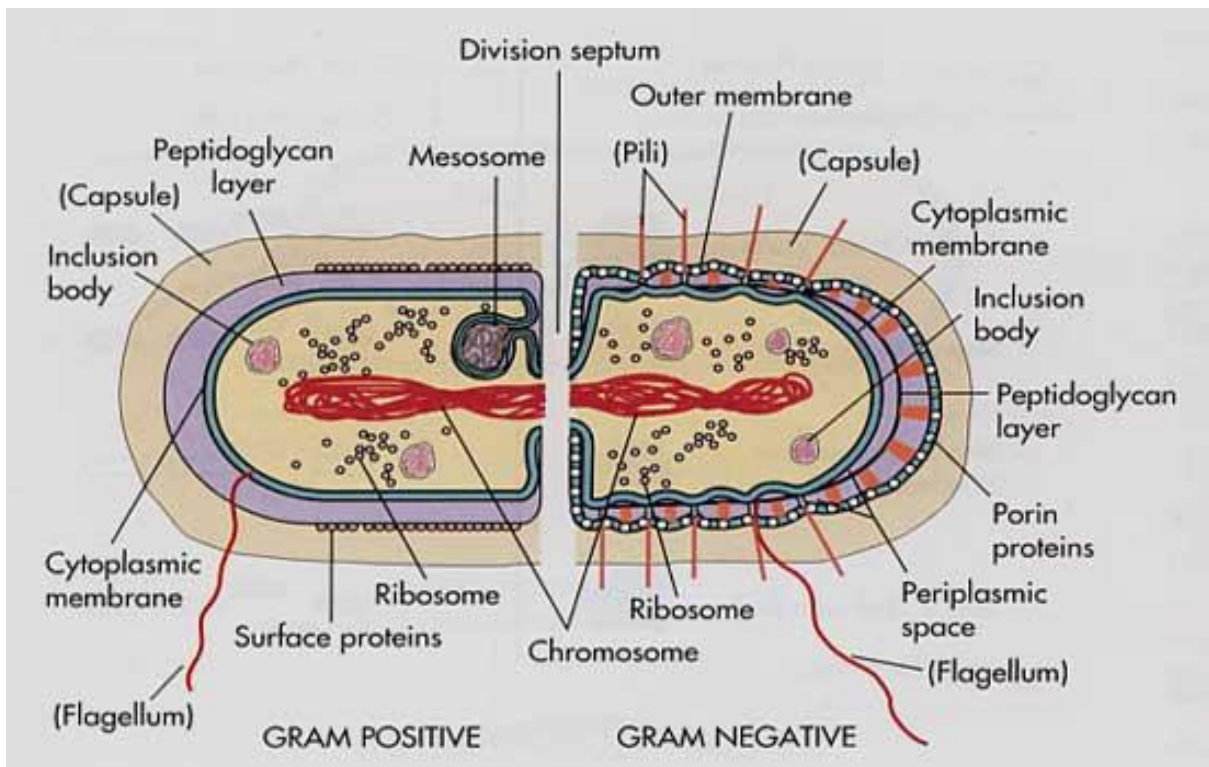


Figure 4.4: The structural differences of the bacterial cell wall.

http://www.diffen.com/difference/Gram-negative_Bacteria_vs_Gram-positive_Bacteria

4.2.3.2. Protozoan parasites

According to EPA - USA, (2001), protozoans are unicellular organisms that reproduce by binary fission and occur primarily in the aquatic environment. Pathogenic protozoans constitute almost 30% of known species of protozoans (Mitchell *et al.*, 1988). Pathogenic protozoa exist in the environment as cysts that hatch, grow and multiply after ingestion. Encystations of protozoans facilitates their survival, and protection from harsh conditions such as high temperature and salinity (EPA - USA, 2001). Two major waterborne protozoan parasites of great concern from the faecal contamination as waterborne pathogens are *Cryptosporidium* spp. and *Giardia lamblia* (EPA - USA, 2001). Some waterborne protozoan parasites from faecal sources posing threads to human health are *Entamoeba histolytica* (Figure 4.5) and *Toxoplasma gondii* in animals.

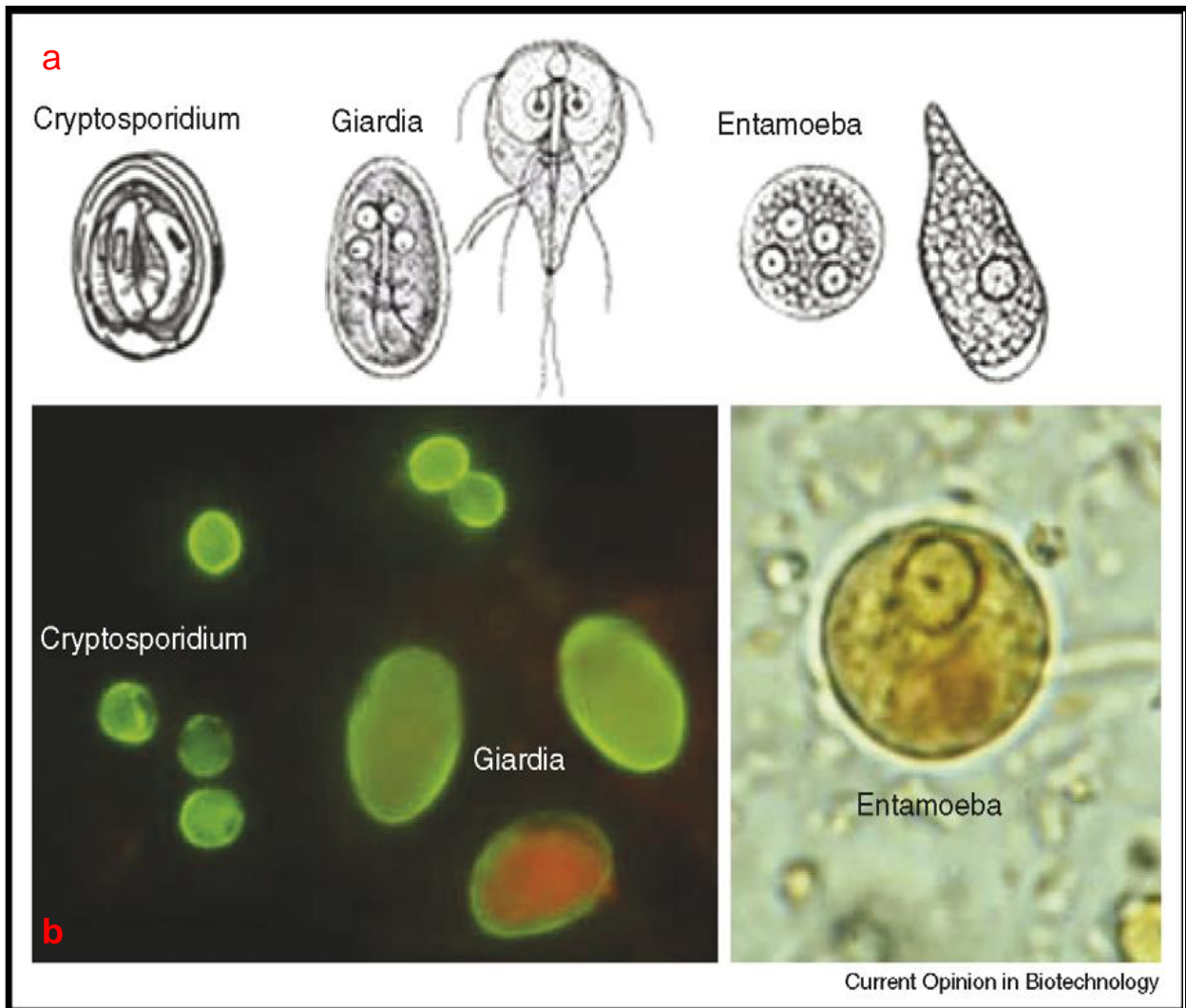


Figure 4.5: Representations of: **(a)** *Cryptosporidium* oocysts, *Giardia* cysts and *Entamoeba* cysts; **(b)** immunofluorescence images of *Cryptosporidium* oocysts, *Giardia* cysts and *Entamoeba* cysts. Adapted from Maha *et al*, (2008).

The microinvertebrate study of Qwaqwa freshwaters was conducted to fill the information gap in the public domain since there is no documented report about the microbial status of Qwaqwa freshwaters. Although the MAP Water regularly monitors *E.coli* from potable water (from dams only) there is a need to document the general coliform bacterial information in both dams and rivers of Qwaqwa area. Therefore it is important to have a research study conducted by an independent neutral institution to compare with the work done by the MAP Water.

This study was aimed at conducting microbial water quality assessment in order to determine the health of faecally contaminated freshwaters of Qwaqwa area.

4.3 MATERIALS AND METHODS

4.3.1 Study area: Rivers and dams

The study was conducted on both the lotic (rivers) and lentic (dams) freshwater systems of Qwaqwa area of Maluti-A-Phofung (MAP) municipality. All selected rivers and dams form the catchment of MAP water resource for Qwaqwa drinking water systems. The underlying reason for microbial assessment of these rivers and dams is for their perennial flow, locality and their accessibility. Selected sites were Metsimatsho (S 28° 34' 42.3" ; E 028° 51' 51.0"), Namahadi (S 28° 34' 21.9" ; E 028° 51' 28.2"), Khoptjwane (S28° 32.1' 41.8"; E 028° 49' 10.7"), Kollatshwene (S 28° 31' 17.9"; E 028° 47' 02.8") and Elands rivers (S 28° 29' 49.9"; E 028° 49' 40.6"), as well as Metsimatsho (S 28° 35'19.2"; E 028°56' 16.7") and Fikapatso dams (S 28° 40' 04.5"; E 028° 51' 16.2").

4.3.2. Sample collection

Water samples were collected from different locally untreated water resources like conservation sampled rivers and dams in Qwaqwa. Samples were collected using sterile polyethylene bottles, in each and every month starting from February to November 2014. At least 80% of river water was collected from the outskirts of Phuthaditjhaba, Qwaqwa (Kollatshwene, Namahali, Metsimatsho and Kgopjwane Rivers); and 20% from the metropolitan area (Elands river). Water samples were also collected from Metsimatsho and Fikapatso dams as the sources of potable water supply for Qwaqwa communities. Water was collected in 1liter capacity polyethylene plastic bottles and transported to the laboratory for assessment of microinvertebrate occurrence.

4.3.3 Microscopy

Stained slides prepared from bacterial cultures were observed under the light microscope (Nikon Eclipse E 100, China). Slides were observed at magnification of 40X under oil immersion. Both gram positive and negative bacteria were observed under the microscope and identified by their morphological characteristics (shape) and the gram stain colour.

4.3.3.1 Preparations of bacterial cultures

Overnight cultures

Overnight cultures were prepared as described by Maniatis *et al.*, (1982). Using the YT agar gel in the agar plates, water sample of 10µl was inoculated on the agar. Single colonies from streaked plates were picked and inoculated into the broth. The loop used for inoculation was flamed and briefly cooled before touching the colony, and the culture was grown overnight at 37°C with vigorous shaking in the incubator.

Bacterial inoculums were picked from a frozen culture by scratching the sterile loop across the surface of the culture of the streaked plate. Colonies were visible after 12-24 hours growth at 37°C. Plates were inverted in the incubator to prevent dripping on the colonies from condensation (Maniatis *et al.*, 1982).

4.3.3.2 Preparation of YT Agar gel

The YT agar gel was prepared as described by Sambrook *et al.*, (1989).

For making 1 L 2x liquid medium, 46 g of agar powder was suspended in 900 ml distilled water, the pH was adjusted to 7.0 with approximately 0.2 ml of 5 N NaOH. Amounts of 16 g of tryptone; 10 g of yeast extract and 5 g of NaCl were weighed and added to fill up to a final volume of 1 L with deionized water and the mixture was sterilize by autoclaving. The mixture was cooled to 45°C prior to dispensing into sterile petri dishes.

4.3.3.3 Preparation of permanent bacterial slides

Sterile technique was used to prepare bacterial slides. An inoculating needle or sterile loop was used to take a sample of a single bacterial colony from the agar and mix it into the broth and place on to the clean slide. The mixture was left to dry by heat fixing as the slide was passed through the flame a few times until a white film appeared.

a) Staining of slides

Gram staining was done to make slides of bacteria by first preparing the gram stains. Main reagents for gram staining are crystal violet, gram iodine and safranin. Crystal violet was prepared by dissolving the crystal violet in 20 ml of 95% ethanol and this solution was added to 80 ml of 1% ammonium oxalate solution and was left to stand for 24 hours and filtered thereafter. Other reagents were readily prepared to be used for gram staining.

b) Gram staining conditions

The slide was flooded with crystal violet for 20 seconds; washed with distilled water for 2 seconds; the slide was washed with the gram iodine for 1 minute and was decolorized by tilting the slide while it was rinsed drop by drop with 95% ethanol for 10 – 20 seconds until ethanol runs clear. The slide again washed with distilled water for 2 seconds, after which it was flooded with safranin for 20 seconds followed by another wash with distilled water finally blotted dry before the slide could be observed under the microscope.

4.3.3.4 Identification of gram negative bacteria

The use of API 20E test strip was as per protocol described from the bioMerieux, Inc. (<http://delrio.dcccd.edu/jreynolds/microbiology>).

A large colony (2-3mm diameter) of the pure culture bacterium was inoculated into the 0.85% NaCl solution, making sure that the suspension was homogenous and without clumps of floating bacteria. A McFarland barium sulfate standard #3 was used to quantitate the suspension and to produce a standard inoculum size. The API strip was inoculated by holding the strip at a slight angle up from the table top inoculating the bacterial suspension into each cupule (well) with the sterile pipette. By capillary action, the fluid was drawn into the cupule slowly squeezing the bulb of the pipette eliminating any bubbles forming in the wells. Each well was filled up to the neck. The test wells of CIT, VP and GEL were filled up to the top of the well with boxes around their names. Other test wells, namely; LCD, ODC, ADH, H₂S and URE were also filled up to the top with sterile mineral oil.

Strips were incubated in the incubation chambers with small indented wells in water that filled the indentations at the bottom of the chambers. Strips were placed in the bottoms making sure that water does not slop onto the API strip. The top of the incubation chamber was placed over the chamber bottom and labelled accordingly. Strips were incubated in their chambers at 37°C for 18 – 24 hours.

For interpretation of the reaction, proper reagents were added. At least 1 drop of Kovac's was added to the IND and was read within a few minutes; 1 drop of Barritt's A and B was added to VP and the reaction took up to 10 minutes and lastly 1 drop of FeCl₃ was added to TDA. Readings for these tests were as described by the provided chart. Results were recorded on the diagram handed out in the laboratory and therefore the oxidase test reaction which was negative was added as the last test result. The result sheet was used to input the reactions in to the computer for the API 20E website database provided the identified potential organisms.

4.4 RESULTS

4.4.1 *In vivo* detection of faecal coliform and *Escherichia coli*

The percentage occurrence of coliform bacteria is presented on Table 4.2. The interpretation is that all sampled sites (rivers and dams) are indicative of both gram positive and gram negative at varying percentages with highest gram negative occurrence in Kollatshwene river (90%), followed 80% occurrence in Namahadi, Khoptjwane and Elands rivers; while Metsimatsho River (70%) and Metsimatsho dam (60%) were the least populated with this category of coliform bacteria. There has been a balance of 50% between gram positive and – negative coliforms in Fikapatso dam.

Table 4.2: Summary of coliform types and their percentage occurrence

Sampled Site	Coliform Gram Status	Morphological features (Shape)	Occurrence (%)
Metsimatsho River	Gram negative	Rod,	70
	Gram Positive	Spherical	30
Namahadi River	Gram negative	Rod,	80
	Gram Positive	spherical (Clustered)	20
Khoptjwane River	Gram negative	All Spherical	80
	Gram Positive	(clustered)	20
Kollatshwene River	Gram negative	Rod (Chain),	90
	Gram Positive	spherical	10
Elands River	Gram negative	Rod,	80
	Gram Positive	spherical	20
Metsimatsho Dam	Gram negative	Long rod chains	60
	Gram Positive	Spherical (clustered)	40
Fikapatso Dam	Gram negative	Clustered rods	50
	Gram Positive	Clustered rods	50

Table 4.3 presents faecal coliform analysis results of all sampled sites. Smaller traces of faecal coliform were observed in Metsimatsho (14 cfu/100 ml) and Namahadi (12 cfu/100 ml) rivers with none (0 cfu/100 ml) at Fikapatso dam. However, for the rest of sampled rivers and Metsimatsho dam, coliform bacterial population was too numerous to be counted (TNTC). On the other hand, *E.coli* population of all sampled sites was also TNTC.

Table 4.3: Analysis of faecal coliforms and *Escherichia coli* (*E. coli*) detected from water samples from the sampled rivers and dams of Qwaqwa.

Water sample Id	Faecal coliforms (cfu /100ml)	<i>Escherichia coli</i> (cfu /100ml)
Metsimatsho River	14	TNTC
Namahadi River	12	TNTC
Khoptjwane River	*TNTC	TNTC
Kollatshwene River	TNTC	TNTC
Elands River	TNTC	TNTC
Metsimatsho Dam	23	TNTC
Fikapatso Dam	0	0

*TNTC = Too numerous to count

4.4.2 Identification and characterization of bacterial species

A number of coliform bacteria species was identified from the culture plates using the API E20 protocol. Of many sampled, table 4.4 indicates the species of bacteria and coliform bacteria identified. The coliform bacteria such as *Escherichia coli*, *Aeromonas hydrophila*, *Acinetobacter baumannii*, *Serratia marcescens*, *Vibrio fluvialis*, *Pseudomonas putida*, *Enterobacter cloacae*, and *Burkholderia cepacia* were isolated and identified to species level by color detection and colony size on MacConkey agar plates. The difference between Gram positive and Gram-negative bacteria lies in the ability of the cell wall of the organism to retain the crystal violet. Gram-negative bacteria do not retain crystal violet dye in the Gram staining protocol. Gram-positive bacteria retain the crystal violet dye when washed in a decolorizing solution. All isolated strains were characterised by their morphological feature of shape; Gram-negative species were rod shaped and therefore retained the safranin color.

Table 4.4: Representation of coliform bacteria species identified from the agar plates sourced from different sampled sites of Qwaqwa freshwaters.

Source	Identified species	Gram status
Elands river	<i>Aeromonas hydrophila</i>	Gram negative
Fikapatso dam	<i>Acinetobacter baumannii</i>	Gram negative
Fikapatso dam	<i>Serratia marcescens</i>	Gram negative
Namahadi and Khoptjwane rivers	<i>Vibrio fluvialis</i>	Gram negative
Kollatshwene river	<i>Pseudomonas putida</i>	Gram negative
Khoptjwane river	<i>Enterobacter cloacae</i>	Gram negative
Metsimatsho river	<i>Burkholderia cepacia</i>	Gram negative
Metsimatsho dam	None*	None

*No growth on the agar culture plates ever.

4.5 DISCUSSION

The results based on microinvertebrate occurrence indicate a poor water quality status among all the sampled rivers. The presence of coliform bacteria both gram negative and gram positive bacteria advocates a composition of faecal coliform as well as faecal streptococcus both of which are bioindicators of faecally contaminated water bodies Aull, (2005).

The ratio between faecal coliform (FC) and faecal streptococcus (FS) counts in water was used in several studies to determine the origin of faecal pollution (Arango & Long, 1998; Sinton *et al.*, 1998; and Gilpin *et al.*, 2002). This ratio method was highly applicable in this study as it is based on the fact that FS are more abundant in animal faeces than in human faeces, while FC are abundant in human faeces than in animal faeces (Sinton *et al.*, 1998). According to this method, the results of this study are indicative of human faecal pollution since in every sampled site gram negative faecal coliform is the most abundant with the percentage occurrence range between 70% and 90%. This test stipulates that a FC: FS ratio greater than 4 is indicative of human faeces and a FC: FS ratio of less than 7 indicates animal faecal pollution (Sinton *et al.*, 1998). Similarly, the results of this study advocates human faecal contamination in rivers and animal faecal contamination in dams hence Metsimatsho rivers had the FC: FS ratio of 70%: 30% i.e. 7: 3; Namahadi river with the ratio of

8: 2; Khoptjwane river with 8: 2; Kollatshwene river with 9 : 1 while Elands river had 8: 2 as well. Ratios of 6: 4 and 5: 5 were obtained in Metsimatsho and Fikapatso dams respectively.

All sampled sites were assessed for the bacterial infection from 100 ml volume of water to determine the faecal coliform units (cfu / 100 ml) particularly for detection of *E. coli*. Fikapatso Dam was free from faecal coliform and *E. coli* contamination. This information could also be extrapolated that there was no bacteria in the sampled 100 ml of water; or water collection and water handling method could have been inadequate since there has been an indication of faecal contamination presented on Table 4.2. Other species of coliform bacteria than *E.coli* were identified as shown in table 4.4.

Aeromonas hydrophila

Aeromonas are commonly isolated from a variety of aquatic environments, including freshwater, estuarine, brackish, and salt waters. Its presence does not indicate the water has been polluted (www.water.epa.gov) since it could also be isolated from a variety of foods, including red meats (beef, pork, lamb), poultry, produce, fish, and shellfish. Its presence in drinking water causes gastroenteritis and some diarrheal infections especially to young children under 3 years of age ([www.who.int/water sanitation health](http://www.who.int/water_sanitation_health)).

Acinetobacter baumannii

Acinetobacter species have been involved in hospital outbreaks and are common inhabitants of some water sources. *Acinetobacter* spp. (especially *Acinetobacter Baumannii*) have recently emerged as pathogens of considerable interest, particularly as the causative agents of nosocomial pneumonia or of complications in immunocompromised patients (Lee *et al.*, 2010). It is one of the pathogens that inhabit faecally contaminated waters. However, in more recent times, *A. baumannii* infections involving the central nervous system, skin and soft tissue, and bone have emerged as highly problematic for certain institutions since it has a strong mechanism to resist all antibiotics (Peleg *et al.*, 2008).

Serratia marcescens

Serratia marcescens is a faecal coliform bacterium, the standard human faecal coliform bacteria causing white pox and killing the coral. A DNA analysis makes this one hundred percent certain. It is the same species of faecal coliform bacteria found in humans and animals and human and animal sewage (Davison, 2002). Its presence symbolizes infection risks particularly to human beings due to water pollution.

Enterobacter cloacae

The genus *Enterobacter* is Gram-negative, facultative anaerobic, rod-shaped, non-spore-forming bacteria; a member of the coliform group of bacteria. It does not belong to the faecal coliforms (or thermotolerant coliforms) group of bacteria, unlike *E. coli*, because it is incapable of growth at 44.5°C in the presence of bile salts (Tan *et al.*, 2014). Its presence in water indicates a risk of infection and water quality status will be a threat to human health.

Pseudomonas putida

A member of the fluorescent group of pseudomonads, is a flagellated, gram-negative rod that is found throughout the natural environment (Thomas *et al.*, 2013). Case reports in the literature describe a wide range of conditions that have led to *P putida* bacteremia including pneumonia, catheter-related blood stream infections, acute cholecystitis and cholangitis, tonsillitis, thrombophlebitis, and skin and soft tissue infections (SSTIs). This bacterial species is also an indicator for faecal contamination.

Burkholderia cepacia

A catalase-producing, lactose-nonfermenting, Gram-negative bacteria. *Burkholderia cepacia* organisms are typically found in water and soil and can survive for prolonged periods in moist environments. The organism is usually cultured in *Burkholderia cepacia* agar (BC agar) which contains crystal violet and bile salts to inhibit the growth of gram positive cocci and Ticarcillin and Polymyxin B to inhibit the growth of other gram negative bacilli, (Lipuma, 2005). It also contains Phenol Red pH indicator which turns pink when it reacts with alkaline by-products generated by

the bacteria when it grows. The effects of *B. cepacia* on people vary widely, ranging from no symptoms at all to serious respiratory infections, especially in patients with cystic fibrosis (www.cdc.gov/HAI/organisms). It is an indicator of poor water (polluted water) quality with high risk of human infection.

CHAPTER 5

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

The basic principle behind the study of macro- and microinvertebrates as pollution bio-indicators is that some are sensitive to pollution than others. For instance, if a particular stream site is inhabited by pollution tolerant organisms, the pollution problem is evident and pollution – sensitive organisms may not be found at that stream site (Farrell-Poe, 2000) or vice versa. The South African reality is that in recent years numerous studies contained alarming findings regarding the threat to the country's water resources. Over half of the ecosystems associated with our rivers are classified as threatened (Farrell-Poe, 2000) due to the alarming rate of pollution, resulting in poor river health and low river integrity. Therefore it is expected that South Africa's freshwater resources will be depleted in 2030 and unable to meet the needs of people and industry since they have been fully allocated to the growing population in 2005 (Oberholster & Ashton, 2008).

The current study tested a number of factors that indicate the purity or quality of water found in sampled rivers and dams of Qwaqwa. Geographical and physico-chemical as well as biological aspects were all taken into consideration for determining the aquatic ecosystem and the effects of water quality on biota involved. Habitat evaluation prior to water sample collection was mandatory to determine the expected results of this study. Water sample collection was the cornerstone of this study since every research information was obtained from the collected water samples. Macroinvertebrates (Appendix B) and microinvertebrates data (Table 4.3) were obtained from water samples and were the basic indicators of the current water quality status of Qwaqwa freshwaters. Nutrient content of these waters was also tested in order to determine their values and subsequent effects to water quality and the inhabitants of the sampled rivers and dams.

The aim of this study was to assess water quality and characterise macro- and microinvertebrate inhabitants of the sampled freshwaters. Documentation of this data is valuable in that it will now give a picture of the status of Qwaqwa freshwaters.

The riparian vegetation composition along the sampled rivers is uniform, comprising exotic trees (*Salix* and *poplars* species), *Acacia* species, *Cyperus* (sedges), and *Lavandula* (lavender) species. Vegetation around rivers was dominated by *Cyperus* and *Lavandula* species. The habitat evaluation procedure used in the current study was considered to be an appropriate analytical tool to assess the proposed project (Ashley & Muse, 2008).

Results from the habitat evaluation indicate that all the sampled rivers were subject to faecal contamination by human deliberate deposition of baby nappies directly in rivers. This could be the reason for the turbid water condition in the Khoptjwane, Kollatshwene and Elands rivers especially with slow water flow; whereas Metsimatsho and Namahadi rivers ran clear waters as well as in the two sampled dams (Metsimatsho and Fikapatso) with minimal faecal contamination by animals. The surface runoff, industrialisation and urban (residential) activities were found to be major pollution effective factors in most sampled sites. Metsimatsho river is on the upper zone whereas other rivers are on the middle zone where water flow is low hence these four rivers were the most polluted rivers of Qwaqwa area. The faecal contamination status of Qwaqwa rivers describes the status of drinking water quality especially potable water from the sampled dams while rivers are used as outlets for water treatment (effluent) discharge by the MAP Water treatment plant.

Water quality information available for the major impoundment of the Qwaqwa freshwaters included measurements of temperature, pH, salinity, dissolved oxygen, total dissolved solids and electrical conductivity. Based on the results of the current study, the correlation coefficient of 0.99 was statistically significant (Taylor, 1990) and was therefore used to determine the strength between TDS and EC; hence a strong positive correlation between the TDS and EC of all the sampled rivers of Qwaqwa area particularly during the low water flow seasons like winter and spring.

However, water quality of the sampled sites varied with their degree of contamination and chemical concentrations. Measurements of water quality parameters per sampled site particularly salinity, total dissolved solids and electrical conductivity were high in the middle zone rivers like Khoptjwane, Kollatshwene and Elands. These parameters are relatively high due to high solubility of chemicals in water most of which affect quality of water.

The pH levels of all sampled rivers were constantly ranging between 6.82 (Elands river) and 8.15 (Metsimatsho dam). This could be argued that temperature influences water pH since the pH levels of pure water decreases as the temperature increases (Ozyasar, 2014). The pH average levels were observed lower in most sampled rivers (all were below 7) in November as the temperature increased; except in Elands river (7.32). High water temperatures can increase the solubility and thus toxicity of certain compounds. These elements include heavy metals such as cadmium, zinc and lead as well as compounds like ammonia (EPA - Ireland, 2012). Ammonia is known for its toxicity at high pH levels, but temperature can also influence its acute and chronic criteria concentrations; while solubility of chemical substances (nutrients) or sewage materials can also influence pH levels as they dissolve in water (EPA - Ireland, 2012). As the temperature increases or decreases, the ion concentrations will also shift, thus shifting the pH value down (Ozyasar, 2014). However, with the average pH levels of sampled rivers and dams all being close to the neutral point across the year, high pH is less common than low pH as a candidate cause because anthropogenic sources are acidic more often than basic (EPA - Ireland, 2012). This indicates that organism growth and chemical solubility were highly facilitated in all sampled sites and could also influence aquatic organism abundance or vice versa (Aull, 2005).

It could be expected to get lowest winter temperatures in Kollatshwene river (6.22°C) since it is the highest elevated river sampled (1675m), but with Fikapatso dam (8.13 °C) compared to Metsimatsho dam (9.07°C) is the opposite. In winter as air (atmospheric) temperature grows colder, surface water also gets colder (Addy & Green, 1997). The reason for this dramatic deviation was the time of the day at which readings were taken since samples from rivers were taken in the mornings of the first day; sampling from dams always followed on the second day (morning). As a

result, Kollatshwene river had always been the first to sample in the morning as well as Fikapatso dam, respectively.

The neutral pH status of the sampled sites could have favoured the rapid solubility of chemicals as rivers dried up. As a result, salinity levels of Fikapatso dam were relatively higher than that of Metsimatsho dam since water samples in Fikapatso were collected from the dam outlet rather than in Metsimatsho dam where samples were collected in high masses of water in the dam.

Total dissolved solids of the water samples varied from 21 mg/l in autumn of 2014 to 297 mg/l in spring of 2014 during the sampling periods. At high flows, the TDS values tended to be diluted by surface runoff (Figure 2.7) and for all sampled rivers there was an inverse correlation between discharge rate and TDS (Charkhabi & Sakizadeh, 2006). This explains why in these rivers, the level of TDS values in summer 2014 was lesser than the values of winter and spring seasons (Figure 2.7) particularly in Metsimatsho, Namahadi, Khoptjwane and Elands rivers. It is normal for streams with a substrate of bed rock to dissolve and accumulate ions from minerals in the rock and soil over which they flow (Johnson *et al.*, 1999). On the other hand, TDS levels may change when ions are introduced to water from salts, acids, base, hard-water minerals or soluble gases. This explains possible reasons why water bodies happen to have high TDS levels. High TDS levels in the aquatic system particularly due to dissolved salts may affects many forms of aquatic life (Johnson *et al.*, 1999).

A comparison of results from ANOVA and the t-test between sampled sites regarding the computed water quality parameters were indicative of high significant difference on TDS amongst rivers, Metsimatsho and Fikapatso dams. Sampled rivers had a high significant difference in salinity ($p < 0.001$); slight significant difference in pH ($p < 0.06$) and no significant difference of pH in dams ($p > 0.05$); no significant difference in temperature ($p > 0.05$) in rivers. There was also a significant difference in mean levels of electrical conductivity ($p < 0.05$) between the sampled rivers; no significant difference in temperature ($p > 0.05$) between Metsimatsho and Fikapatso dams; and a significant differences in salinity mean levels ($p < 0.05$) in both dams.

Therefore the significant differences of water quality parameters and their correlation clearly indicate degraded quality of water found in both rivers and dams of Qwaqwa area whose effect impinged most on the composition of aquatic macroinvertebrate taxa found in each study site.

According to Palaniappan *et al.*, (2010), water quality contamination weakens or destroys natural ecosystem that supports human and livestock health, food production and biodiversity. Studies estimated that the value of ecosystem services is double the gross national product of global economy, and the role of freshwater ecosystems in purifying water and assimilating wastes has been valued at US\$ 400 billion (Constanza *et al.*, 1997). Revenga *et al.*, (2000), reiterated that freshwater ecosystems are among the most degraded resources on the planet, and have suffered proportionately greater species and habitat losses than terrestrial or marine ecosystems.

In a study conducted by Maseti, (2005) in the Eastern Cape, there was a similar evidence of water quality impairment in rivers located downstream like Elands river with identified salinisation and nutrient pollution as variables of concern (O’Keeffe *et al.*, 1996) in Qwaqwa freshwaters as well.

It has been observed that the occurrence of several taxa in all sampled sites (five rivers and two dams) is closely related to pollution levels and environmental gradients (Johnson *et al.*, 1992). Macroinvertebrate evaluation results indicate that of all sampled families (n=298) found in Qwaqwa freshwaters comprise three groups of taxa influenced by water pollution levels, namely; tolerant taxa (SASS value ranges, 1 – 5); moderately sensitive (6 -10) and highly sensitive (11-15) with their corresponding percentage occurrence (Table 3.6). Of many sampled taxa, 71.4 % occurrence of highly tolerant taxa was found in Kollatshwene river; followed by Elands river (61.9%) and Khoptjwane river (60%); moderately sensitive taxa had the highest percentage occurrence (41.7%) in Namahadi river; followed by Kollatshwene river (35.7%) and 30% in Metsmatsho dam; while highly sensitive taxa were found Namahadi river (33.3%); 23.5% in Metsimatsho river and Khoptjwane river (20.0%). Therefore the ASPT 4.8 observed at Kollatshwene and Elands rivers indicate poor water quality. The high ASPT values of Metsimatsho river (6.8) and 7.6 of Namahadi

river declare that water quality in these rivers is relatively good given the condition that all these sampled rivers contain wastewater since the MAP Water discharges municipal effluent into them.

Among the most occurring taxa in this study, Baetidae was the highest found in all sites (34.22%) followed by Corixidae (12.08%) and the least being Ceratopogonidae (6.04). Based on the study conducted in China (Xu *et al.*, 2013), results indicated that Baetidae was a good indicator of sites that were relatively free from pollution while it seems contradictory to the results of this study where Baetidae was also dominant (20.59%) in rivers with poor water quality like Kollatshwene river (ASPT of 4.8) following its highest percentage occurrence (26.47%) in Khoptjwane river (ASPT of 6.7) being least occurring (9.8%) in the less polluted river, Namahadi based on its ASPT of 7.6 (Table 3.5). The variation between these two studies is due to unknown factors unless a variety of Baetidae species was identified as Baetidae >2 spp with the SASS value of 12 in most sampled rivers in China (Xu *et al.*, 2013) than in this study, otherwise macroinvertebrate taxa in this study was identified to the family level only.

According to the findings of this study, this variation could be consequent to the locality of individual rivers since their pollution levels are diverse. All the affected rivers had the evidence of high faecal contamination hence the disposal of diapers in river waters, or eutrophication because eutrophication management is only focussing on implementation of inappropriately high phosphates concentrations (1 mg / L as P) for all effluent discharged from sewage treatment plants to South African freshwater systems (Oberholster & Ashton, 2008).

Diversity indices employed in this study clarified the diversity of macroinvertebrate taxa within the sampled sites. The diversity results indicated that the community with Shannon Wiener index of H had an equivalent diversity as a community with equally-common species. On the other hand the Simpson Yule index results indicated that there was an infinite diversity among the sampled macroinvertebrate taxa. The variability of number of taxa among sampled rivers and dams was expected as there were natural differences in biotopes in terms of substrate, flow and other aspects (Dickens and Graham, 2002). Therefore it could be concluded that there was a slight

significant difference ($p < 0.06$) between the macroinvertebrate taxa sampled in this study.

To investigate bacterial presence, the culture plate method was used with cultures grown on selective agar media for total bacteria, total coliforms, and *Staphylococcus* spp, respectively. Species of *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *Vibrio*, *Serratia*, and *Burkholderia* were isolated and quantified from the water samples and were identified with all characterised gram negative species with rod shape morphological feature.

Faecal coliforms have long been used as indicators of pollution in water (Gearheart, 1999; Hernandez *et al.*, 1997; McMath *et al.*, 1999; Perkins & Hunter, 2000; Tyrrell *et al.*, 1995) due to the potential for introduction of pathogens and other pollutants along with these bacteria (Ricca & Cooney, 1999; Tyrrell *et al.*, 1995).

Coliform bacteria are the most common microbiological contaminants of natural waters and indicators of degraded water quality. Although most of these bacteria are not harmful and are part of the normal digestive system, some are pathogenic to humans and may cause disease such as gastroenteritis, ear infections, typhoid, dysentery, hepatitis A, and cholera (Jolley & English, 2014). If disease-causing bacteria are present, the most common symptoms are gastrointestinal upset and general flu-like symptoms such as fever, abdominal cramps, and diarrhea. Symptoms are most likely in children or elderly household members (Swistock *et al.*, 2014).

The presence of these faecal coliform bacteria in the Qwaqwa freshwaters indicate faecal contamination mainly by human faeces than animal faeces. A smaller percentage (with a minimum of 10% to 30% among rivers) of animal faecal contamination was evident in sampled rivers than in dams (table 4.3) since Metsimatsho and Fikapatso dams are geographically isolated from human settlements and situated up in the mountains of Qwaqwa area. Their faecal contamination could emanate from birds and animals in the cattle posts for subsistent farming.

A microbial study was conducted in Limpopo and Mpumalanga where results revealed that 85% and 69% of water treatment plants complied with the limits set by DWAF, (1996) in terms of faecal coliforms (0 cfu/100 ml) and total coliforms (0-10 cfu/100ml) respectively (Obi *et al.*, 2007). In that study *Salmonella* species constituted the most common isolates (26.9%), followed by *Vibrio* species (25%) while *Campylobacter* was the least found bacteria and underlined the possibilities of the outbreak of cholera epidemic in parts of the country like KwaZulu-Natal (Obi, *et al.*, 2007).

A similar water quality study was also conducted in the Western Cape and Mpumalanga using the SASS4 and the observation was that multivariate analysis found differences in invertebrate communities in rivers with high densities of small dams, foothill- cobble streams and foothill- gravel streams (Mantel *et al.*, 2010) like Kollatshwene and Metsimatsho Rivers. Both in these studies, opportunistic taxa that are tolerant to pollution because they are capable of exploiting different habitats particularly those with slower currents had increased in numbers (Mantel *et al.*, 2010), while other taxa that are sensitive to pollution and disturbance had declined in numbers. In this study family relative abundance of collected aquatic macroinvertebrate taxa across all selected lotic and lentic systems of Qwaqwa (Figure 3.8) were calculated to compare rivers in terms of pollution levels amongst all sampled sites based on SASS5 scores during all sampling seasons. Distribution of SASS5 scores are presented in figure 3.9. There is a significant difference ($P < 0.05$: $n=298$) between combined biotopes groups SASS5 scores than in separate biotope groups. Similarly, in the study conducted by Ollis, (2005) there was a significant difference ($P < 0.05$) between the SASS5 score in Mpumalanga also with a high significant difference ($P < 0.01$) in SASS scores in the Western Cape indicating a relatively large degree of scatter in their data. In this case it could be argued that some of statistically significant results obtained (at $P < 0.05$ or $P < 0.01$) are of little consequence because of this high degree of scatter in the underlying data relative to the required precision for the application under scrutiny (Ollis *et al.*, 2005).

According to the further microbial analysis of water samples using the membrane filtration, Fikapatso dam is actually not infected with faecal coliforms particularly

E.coli (Table 4.4). However, the rest of all sampled sites were indicative of high occurrence of *E.coli*, hence too numerous to count (TNTC). The difference in the occurrence of coliform bacteria in Fikapatso dam could justify the 50:50 ratio or there has never been any existence of the faecal coliform and *E.coli* in the analysed 100 ml of water hence 0 cfu / 100 ml.

According to Xu *et al.*, (2013), nitrates and phosphates are the main polluting nutrients in rivers. In the study conducted in China, total nitrogen (TN) was seriously high in most polluted rivers seconded by the total phosphorus (TP). Results of this study are complimentary to the results of the current study where nitrogen had the highest concentration (1.8 mg/l); high phosphorus concentration (0.83 mg/l) and the least concentration of ammonia (0.65 mg/l). In this study, Elands river is the most seriously polluted river. According to SASS scores of macroinvertebrate taxa of this study (Appendix B and table 3.6) macroinvertebrate tolerance levels in Elands river had the highest occurrence percentage (61.9%) of tolerant macroinvertebrate taxa. The nutrient pollution affects the taxa richness while macroinvertebrates composition changes with concentrations of nitrogen and phosphorus compounds and suspended organic matter (Camargo, 1993). With higher concentrations of these nutrients, eutrophication by accumulation of metabolic products (e.g. hydrogen sulphide in deep waters); discoloration or turbidity of water (resulting in low or poor light penetration); deterioration in the taste of water and depletion of dissolved oxygen (Oberholster & Ashton, 2008) in water subject aquatic life to suffocation due to anaerobic conditions of water and subsequently killing macroinvertebrates and fish. This explains lower number of highly sensitive taxa in the sampled sites particularly in Kollatshwene river with a zero number of highly sensitive taxa and 2 taxa in Elands river (Table 3.6).

5.2 CONCLUSIONS

Water quality parameters and biological constituents of the sampled sites are indeed indicators of water pollution. It is evident that water quality of the sampled sites was influenced by seasonality and anthropogenic pollution related activities. However, water quality of dams in Qwaqwa is relatively better than that of rivers hence it is used for human drinking and household purposes and for breeding of natural fish species like rainbow trout and carp in the Fikapatso dam (DWAF, 2007). In fact water from rivers of Qwaqwa is said to be wastewater according to MAP Water since it contains effluent discharge. Therefore its quality is poor for use in agriculture and household purposes.

The pH values in all sampled sites were around the neutral point which means biological availability and solubility of elements are highly facilitated. Therefore this indicates that water quality status in most rivers is deteriorating with seasonality as shown by other water quality parameters. At some point there was a similarity in between rivers and dams of Qwaqwa in terms of the pH. On the other hand, there was a difference between rivers and dams on the basis of EC, salinity, TDS and temperature. Major contributing factors to high pollution in these rivers includes urbanization and faecal contamination either by human and animals, therefore the prevalence or occurrence of several macroinvertebrate taxa is closely related to the pollution levels and environmental gradients (Johnson *et al.*, 1992).

The presence of highly tolerant macroinvertebrates in the sampled rivers also indicate high water pollution status. Their biological growth also depends on high nutrient values in water as well as high prevalence of waterborne microorganisms that constitute their food sources. Macroinvertebrates particularly aquatic insect are bioindicators of water pollution and their prevalence or occurrence depends entirely on the quality of water. Based on seasonality, macroinvertebrate populations may vary depending on the river/stream flow.

Occurrence of microinvertebrates like coliform bacteria that includes faecal coliforms indicate faecal contamination of Qwaqwa aquatic systems. Identified coliform

bacteria particularly *E. coli* advocates the presence of pathogenic bacteria in the sampled rivers and dams. The microbial quality of water in Qwaqwa freshwaters necessitates a regular monitoring of water treatment plants to the community. More consideration must be given to the filtration systems and training of communities on basic home water treatment methods like boiling, sedimentation and filtration, with more emphasis on boiling of drinking water hence poor water quality is subject to diarrheal disease outbreaks.

The results of this study suggest that consumption of unchlorinated drinking water could represent a notable risk to the health of communities. As such, there is a need for continuous monitoring of these water sources and to establish standards.

5.3 RECOMMENDATIONS

- It is therefore recommended that surveillance measures and good sanitary provisions be formulated by the responsible bodies or all stakeholders assigned for the monitoring of the environment to control and regulate all anthropogenic pollution induced activities that degrade the aquatic environment.
- It is recommended that taxa-specific indicators for water quality assessment are reliable and be used for effective monitoring of South African aquatic ecosystem.
- Future studies should include use of molecular techniques especially for microinvertebrate detection and identification.
- Future water quality studies should not focus on microorganisms prevalence alone, but also on the virulence of these microorganisms especially in drinking water (both in water sources and container - stored water) in the rural communities.
- Communities must be educated about the status of freshwaters and how to avoid their contamination and danger or risks of using unchlorinated water.

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APPENDIX A

The SASS Version 5 scoring sheet

SASS Version 5 Score Sheet		Taxon	S	Veg	GSM	TOT	Taxon	S	Veg	GSM	TOT	Taxon	S	Veg	GSM	TOT
Date: / /200__		PORIFERA	5				HEMIPTERA					DIPTERA				
Collector:		COELENTERATA	1				Belostomatidae*	3				Athericidae	10			
Grid Reference: WGS-84 Cape datum		TURBELLARIA	3				Corixidae*	3				Blepharoceridae	15			
S: ° ' " E: ° ' "		ANNELIDA					Gerridae*	5				Ceratopogonidae	5			
Site code:		Oligochaeta	1				Hydrometridae*	6				Chironomidae	2			
River:		Leeches	3				Naucoridae*	7				Culicidae*	1			
Site description:		CRUSTACEA					Nepidae*	3				Dixidae*	10			
Weather Condition:		Amphipoda	13				Notonectidae*	3				Empididae	6			
Temp:.....°C pH:.....		Potamonautidae*	3				Pleidae*	4				Ephyridae	3			
DO:.....mg/l Cond:.....mS/m		Atyidae	8				Velidae/M...velidae*	5				Muscidae	1			
Biotopes sampled:		Palaemonidae	10				MEGALOPTERA					Psychodidae	1			
SIC..... Time.....minutes		HYDRACARINA	8				Corydalidae	8				Simuliidae	5			
SOOC..... Time.....minutes		PLECOPTERA					Sialidae	6				Syrphidae*	1			
Average size of stones.....cm		Notonemouridae	14				TRICHOPTERA					Tabanidae	5			
Bedrock.....		Perlidae	12				Dipseudopsidae	10				Tipulidae	5			
Aquatic veg'n..... Dom. sp.....		EPHEMEROPTERA					Ecnomidae	8				GASTROPODA				
Mveg/C..... Dom. sp.....		Baetidae 1sp	4				Hydropsychidae 1 sp	4				Ancylidae	6			
Mveg/OOC..... Dom. sp.....		Baetidae 2 sp	6				Hydropsychidae 2 sp	6				Bulininae*	3			
Gravel..... Sand.....		Baetidae > 2 sp	12				Hydropsychidae > 2 sp	12				Hydrobiidae*	3			
Mud.....		Caenidae	6				Philopotamidae	10				Lymnaeidae*	3			
Hand picking/Visual observation.....		Ephemeridae	15				Polycentropodidae	12				Physidae*	3			
Flow: Low/Medium/High/Flood		Heptageniidae	13				Psychomyiidae/Xiphocent	8				Planorbinae*	3			
Turbidity: Low/Medium/High		Leptophlebiidae	9				Caseid caddis:					Thiaridae*	3			
Riparian land use:		Oligoneuridae	15				Barbarochthonidae SWC	13				Viviparidae* ST	5			
Disturbance in the river: eg. sandwinning, cattle drinking point, floods etc.		Polymitarcyidae	10				Calamoceratidae ST	11				PELECYPODA				
Observations: eg. smell and colour of water, petroleum, dead fish, etc.		Prosopistomatidae	15				Glossosomatidae SWC	11				Corbiculidae	5			
		Teloganodidae SWC	12				Hydroptilidae	6				Sphaeriidae	3			
		Tricorythidae	9				Hydrosalpingidae SWC	15				Unionidae	6			
		ODONATA					Lepidostomatidae	10				SASS Score				
		Calopterygidae ST,T	10				Leptoceridae	6				No. of Taxa				
		Chlorocyphidae	10				Petrothrincidae SWC	11				ASPT				
		Chlorolestidae	8				Pisuliidae	10				Sample collection effort exceeds method?				
		Coenagrionidae	4				Sericostomatidae SWC	13								
		Lestidae	8				COLEOPTERA									
		Platycnemidae	10				Dytiscidae*	5								
		Protoneuridae	8				Elmidae/Dryopidae*	8								
		Aeshnidae	8				Gyrinidae*	5								
		Corduliidae	8				Halplidae*	5								
		Gomphidae	6				Helodidae	12								
		Libellulidae	4				Hydraenidae*	8								
		LEPIDOPTERA					Hydrophilidae*	5								
		Pyralidae	12				Limnichidae	10								
							Psephenidae	10								
												Other biota including juveniles:				

APPENDIX B

THE SASS SCORE SHEET USED FOR THE QWAQWAG RIVERS and DAMS

FAMILIES	SAMPLED SITES						
	Metsimatsho River	Namahadi River	Khoptjwane River	Kollatshwene River	Elands River	Metsimatsho Dam	Fikapatso Dam
Planaria				3		3	3
Oligochaeta				1		1	
Hirudinea					3		
Potamonautidae	3		3			3	3
Atyidae						8	
Hydrachnellae	8	8	8		8	8	8
Perlidae	12	12					
Beatidae	12	12	12	6	12	12	12
Oligoneuridae		15			15		
Tricoyrthidae		9					
Leptophlebiidae		9	9				9
Polymitarcyidae						10	
Prosopistomatidae	15		15			15	
Teloganodidae SWC	12	12	12		12	12	12
Coenagrionidae	4	4	4		4		4
Protoneuridae	8	8			8		
Aeshnidae						8	8
Belastomatidae	3						
Corixidae	3	3	3	3	3	3	3
Gerridae				5			5
Naucoridae	7			7		7	
Notonectidae	3		3	3		3	
Pleiiidae	4		4	4	4	4	4
Ecnomidae	8	8	8	8	8	8	8
Hydropsychidae			4	6			4
Hydrophilidae					6		
Dytiscidae	5				5	5	
Gyrinidae	5		5		5		5
Ceratopogonidae	5		5	5	5	5	5

Chironomidae			2		2		2		2
Culicidae					1		1		1
Dixidae					10		10		
Simuliidae							5		
Syrphidae		1					1		
Tipulidae								5	
Thiaridae								3	
Notonemourida									14
SASS Score	117	101	94		64		118	125	104
No. Of Taxa	17	12	15		14		21	20	15
ASPT	6.8	7.6	6.7		4.8		4.8	6.3	6.9

APPENDIX C

Table 1C. Summary of average and standard deviation values of water quality parameters in rivers and dams of Qwaqwa of Maluti-A-Phofung municipality during four subsequent seasons.

Seasons	Value	EC mS/cm	pH	Temp. °C	Salinity Mg/l	TDS Mg/l	NO ₃ ⁻ Mg/l	PO ₄ ⁻ Mg/l	NH ₄ ⁺ Mg/l
Autumn	Ave.	0.186	7.42	15.92	0.093	97.6	*	*	*
	Stdev	0.02	0.176	0.352	0.004	3.435	*	*	*
Winter	Ave.	0.155	7.33	9.18	0.104	109.93	*	*	*
	Stdev	0.049	0.234	0.67	0.0008	3.004	*	*	*
Spring	Ave.	0.154	7.29	16.37	0.188	161.87	1.066	0.372	0.376
	Stdev	0.026	0.113	1.683	0.048	18.089	0.72	0.343	0.239
Summer	Ave.	**	**	**	**	**	1.054	0.4	0.343
	Stdev	**	**	**	**	**	0.746	0.271	0.270

*Analysis only started with spring samples.

**Done in November only (No sample mean and standard deviation values calculated).

APPENDIX D

Macroinvertebrate Data

Table 1D: SASS5 score, number of taxa and ASPT for each replicate sample in different biotopes of all study sites during the autumn survey.

Site	Replicate Biotope	SASS Score	Number of Taxa	ASPT
Metsimatsho River	Vegetation	56	6	9.3
	¹ GSM	-	-	-
	Stones/bed rock	48	9	5.3
Namahadi River	Vegetation	32	5	6.4
	GSM	41	8	5.1
	Stones/bed rock	38	6	6.3
Khoptjwane River	Vegetation	78	10	7.8
	GSM	42	5	8.4
	Stones/bed rock	36	6	6
Kollatshwene River	Vegetation	47	6	7.8
	GSM	28	4	7
	Stones/bed rock	35	7	5
Elands River	Vegetation	² -	-	-
	GSM	31	5	6.2
	Stones/bed rock	39	7	5.6

¹ GSM = Gravel, Sand and Mud

² (-) = Biotope not available

Table 2D: SASS5 score, number of taxa and ASPT for each replicate sample from different biotopes from all replicated site during the winter survey.

Site	Replicate Biotope	SASS Score	Number of Taxa	ASPT
Metsimatsho River	Vegetation	63	8	7.9
	³ GSM	-	-	-
	Stones/bed rock	68	8	8.5
Namahadi River	Vegetation	42	6	7
	GSM	39	7	5.5
	Stones/bed rock	39	6	6.5
Khoptjwane River	Vegetation	50	8	6.3
	GSM	48	6	8
	Stones/bed rock	40	7	5.7
Kollatshwene River	Vegetation	31	6	5.2
	GSM	29	6	4.8
	Stones/bed rock	24	6	4
Elands River	Vegetation	⁴ -	-	-
	GSM	39	10	3.9
	Stones/bed rock	60	11	5.1

³ GSM = Gravel, Sand and Mud

⁴ (-) = Biotope not available

Table 3D: SASS5 score, number of taxa and ASPT for each replicate sample from different biotopes from all replicated site during the spring survey.

Site	Replicate Biotope	SASS Score	Number of Taxa	ASPT
Metsimatsho River	Vegetation	36	9	4
	⁵ GSM	-	-	-
	Stones/bed rock	39	7	5.6
Namahadi River	Vegetation	33	5	6.6
	GSM	28	2	5.6
	Stones/bed rock	32	5	6.4
Khoptjwane River	Vegetation	22	3	7.3
	GSM	23	3	7.7
	Stones/bed rock	24	2	6
Kollatshwene River	Vegetation	22	3	7.3
	GSM	21	3	7
	Stones/bed rock	24	3	8
Elands River	Vegetation	⁶ -	-	-
	GSM	15	4	3.8
	Stones/bed rock	17	5	3.4

⁵GSM = Gravel, Sand and Mud

⁶ (-) = Biotope not available

Table 4D: SASS5 score, number of taxa and ASPT for each replicate sample in different biotopes of all study sites during the autumn survey.

Site	Replicate Biotope	SASS Score	Number of Taxa	ASPT
Metsimatsho River	Vegetation	38	6	6.3
	⁷ GSM	-	-	-
	Stones/bed rock	42	7	6.0
Namahadi River	Vegetation	28	4	6.0
	GSM	38	5	7.6
	Stones/bed rock	40	6	6.7
Khoptjwane River	Vegetation	56	8	7.0
	GSM	32	6	5.3
	Stones/bed rock	34	6	5.7
Kollatshwene River	Vegetation	38	8	4.8
	GSM	23	5	6.4
	Stones/bed rock	31	5	6.2
Elands River	Vegetation	⁸ -	-	-
	GSM	28	6	4.7
	Stones/bed rock	33	7	4.7

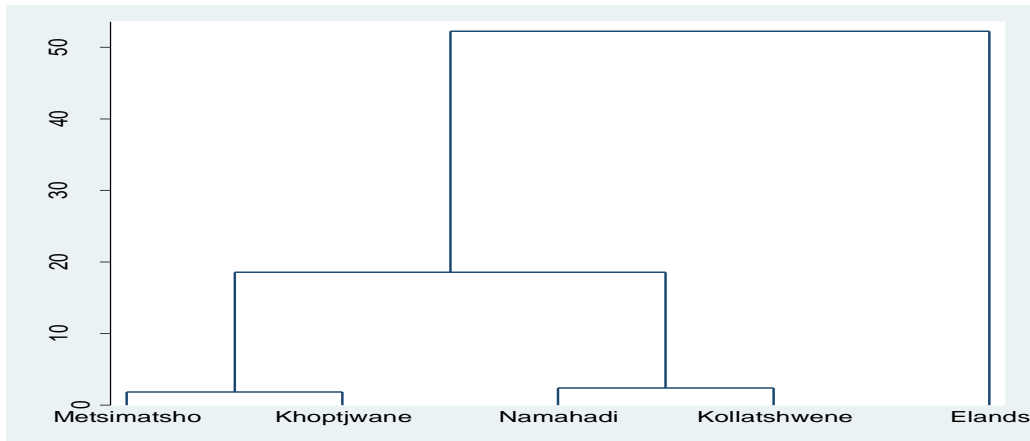
⁷ GSM = Gravel, Sand and Mud

⁸ (-) = Biotope not available

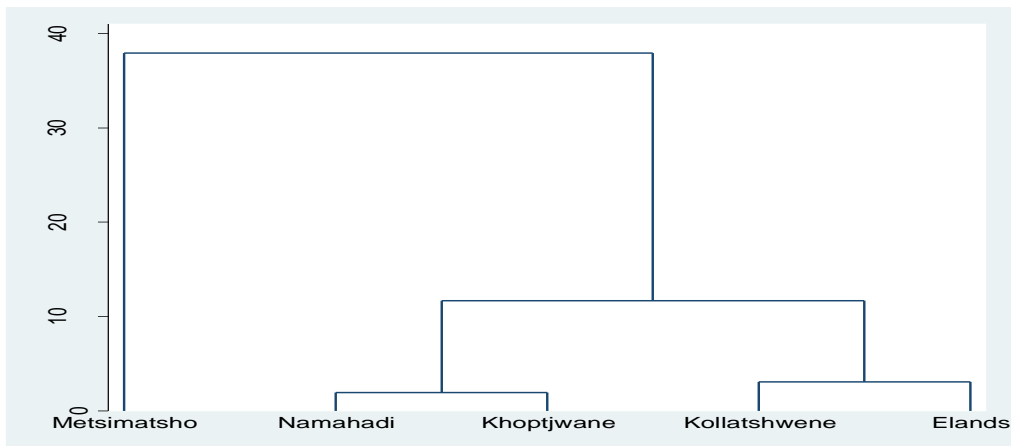
APPENDIX E:

Similarity dendrograms

1. Dendrogram of sampling sites for vegetation biotope during autumn, winter and spring based on the macroinvertebrate SASS scores and number of taxa.



2. Dendrogram of sampling sites for gravel, sand and mud biotope during autumn, winter and spring based on the macroinvertebrate SASS scores and number of taxa.



3. Dendrogram of sampling sites for stones biotope during autumn, winter and spring based on the macroinvertebrate SASS scores and number of taxa

