THE USE OF WATER QUALITY, AQUATIC SPECIES COMPOSITION AND AQUATIC HABITAT CONDITIONS TO ASSESS THE RIVER HEALTH CONDITION OF THE NZHELELE RIVER, LIMPOPO PROVINCE, SOUTH AFRICA

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DEDICATION

This thesis is dedicated to my late father Moloko Johannes Mokgoebo

DECLARATION

I, **Matjutla John Mokgoebo**, hereby declare that the thesis, which I hereby submit for the degree of Doctor of Philosophy at the University of South Africa, is my own work and has not previously been submitted by me for a degree at this or any other institution.

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STUDENT SIGNATURE DATE

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Abstract

Health condition of a river is a necessity for the sustainability of aquatic ecosystems. River health status of the Nzhelele River was assessed through the use of water quality, macroinvertebrate taxa composition and aquatic habitat conditions. The study was conducted along the Nzhelele River in Limpopo Province of South Africa where the river transcends six tribal villages. The objectives were to assess water quality conditions in order to determine the magnitude of pollution impact, to correlate species diversity and water quality parameters, to measure the size of degraded areas with respect to species richness and to develop a model for managing river health condition of the Nzhelele River. Data were collected monthly between February and December 2016 (early autumn to mid-summer). Macroinvertebrates were sampled where water samples were collected to ensure that the relationship between water quality and macroinvertebrates was adequately correlated. Water quality parameters that were analysed were pH, stream temperature, river velocity, conductivity, Dissolved Oxygen, Total Dissolved Solids, nitrates, nitrites and chlorine. Assessment of habitat conditions was done through the assessment of habitat and riparian zone integrity. One-way ANOVA was used to determine if there were significant differences between the six sampling areas in terms of water quality and aquatic species composition. Principal Correspondence Analysis (PCA) was used to correlate water quality and macroinvertebrate data.

The results indicated that water quality parameters significantly differed among the six sampling sites and that also explained the variations in diversity of macroinvertebrates that were sampled from the six sampling sites. Pollution tolerant organisms constituted a total of 46.7% and the remaining 53.3% represented pollution sensitive organisms. PCA results showed positive and negative correlations between macroinvertebrates and water quality parameters to indicate variations in the levels of pollution along the Nzhelele River. Habitat integrity results indicated that the Musekwa sampling site was the most degraded and had lower species diversity. The Ratio of Ephemeroptera, Plecoptera and Trichoptera (EPT) and Chironomidae Abundances should be reviewed to read as Ratio of EPT and Chironomidae-Thiaridae Abundances in order to strengthen the study of the relationship between pollution tolerant and pollution sensitive organisms.

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CHAPTER ONE: INTRODUCTION

1.1 Background to the problem

Rivers and their adjacent lands serve as habitats for a variety of organisms. These organisms tend to survive in a particular state of a river. The organisms suffer when the state of a river is altered naturally or through anthropogenic activities. Assessing and understanding the impacts of human activities on aquatic ecosystems has long been a focus of ecologists, water resource managers and fisheries scientists (Kwak and Freeman, 2010). According to Kwak and Freeman (2010), ecological integrity is the synopsis of chemical, physical and biological integrity. Dlamini (2009) has observed that the consequences of anthropogenic water-use activities and land-use management are becoming noticeable on the environment. According to Van Ael *et al*. (2011), pressure on the biological community of an aquatic environment is a result of pollution, habitat deterioration, spatial isolation, and the spreading of invasive species. Aquatic ecosystem biodiversity requires specific chemical and physical environmental conditions for survival. Physical habitat and water quality are strongly related to aquatic species composition and distribution, and as such, become a measure of river health status (DEAT, 2007). River health conditions and the sustainability of aquatic ecosystems are a good measure of the presence and concentration of a variety of pollutants, as well as the degraded or altered environments (DWAF, 1997). Roux (2000) defined ecosystem health as the ability of an ecosystem to support and maintain a balanced, integrated and adaptive community of organisms having a diversity of species, composition and functional organisation comparable to that of the natural habitats of the region.

Moiseenko *et al*. (2008) stated that many groups of organisms can be used as indicators of environmental and ecological change, even though fish are good indicators of aquatic environmental change and ecosystem health. Gyedu-Ababio and van Wyk (2004) have similarly argued that the overall ecological integrity of a river is reflected by biological communities through the integration of the effects of different stressors over time. Flautt (2007) argued that the main focus should particularly be on Ephemeroptera (E), Tricoptera (T), and Plecoptera (P) orders because of their sensitivity to stream conditions and viability to act as stream health indicators. Yeom and Adams (2007) noted that a class of environmental health indicators that has grown rapidly in use over the past two decades is multimetric indices.

Angermeier and Davideanu (2004) noted that these indices integrate information on several attributes of a biotic community into a number that is scaled to reflect ecological health. An example of an integrative index at the organism level is the health assessment index (HAI), which is a quantitative methodology that allows statistical comparisons of fish health among data sets and evaluates the general health status of individuals within a population (Kleynhans et al., 2008).

Miserendino *et al*. (2011) asserted that all over the world the ecological integrity of river systems is being endangered by land use changes. These changes are brought about by urbanisation, agriculture, pasture conversion, deforestation, and the replacement of native species by exotic ones with commercial value. This represents a real threat to biodiversity and conservation of lotic ecosystems (Miserendino *et al*., 2012). More often, habitat valuation processes are mostly used to inform decisions about which lands to conserve because ecological risk assessment has been criticised for ignoring habitat range limitations of a site as well as spatial patterns in habitat quality (Efroymson *et al*., 2008).

The quality of the environmental conditions is also a determining factor in macroinvertebrate assemblages in any river. Yamada *et al*. (2014) have indicated that faunal communities inhabiting plant leaves and rhizomes (macro-and-microinvertebrates), the quantity and quality of environmental variations as well as the dynamics of the spatial distribution patterns of the vegetation determine the dynamics of species diversity, functional diversity and composition of the faunal communities. Miserendino *et al*. (2012) have also indicated that extensive damage on riparian and aquatic habitats can adversely affect fish and macroinvertebrate communities. Suspended particulate matter (SPM) has been found to have a range of detrimental effects on water resource (Bilotta *et al*., 2012).

Varnosfaderany *et al*. (2010) stated that the use of macroinvertebrate communities as bioindicators for assessment of water quality has more advantages than those based on diatoms, fish, riparian and aquatic vegetation. This is because freshwater macroinvertebrate species vary in their sensitivity to organic pollution (Czerniawska-Kusza, 2005). Habitat alteration has been seen as a factor contributing to biodiversity loss at the global scale (Almeida *et al.*, 2013) such as loss of adaptive capacity to natural environmental fluctuation causing changes in community structure and function, resulting from suboptimal and more homogenous abiotic environments

(Perez-Quintero, 2007). Almeida *et al.* (2013) stated that near-bed hydraulic variables are the key factors to analyse spatial distribution patterns of macrobenthos.

According to Giordani *et al.* (2009), several indicators are available for assessing trophic status and quality aquatic ecosystems and their evolution under different anthropogenic pressures and inherent threats. Xu *et al*. (1999) argued that more environmental managers consider the protection of ecosystem health as a new goal of environmental management. Aquatic macroinvertebrates are some of the most important organism groups selected to evaluate the integrity of biological communities the ecological status assessment process (Alvarez-Cabria, 2010).

However, there are various models that have been used to estimate the rate of pollution in water bodies, the rate of aquatic environmental deterioration and the resident time of pollutants in rivers and streams. Many of these models have helped in the management of rivers around the world even though some models do not offer tangible solutions to the real-world situations. The most common water quality parameters according to Kney and Brandes (2007) include dissolved oxygen, temperature, pH, turbidity, temperature, nutrients, specific conductivity, alkalinity, hardness and bacteria. Davies and Day (1998) have identified the same parameters, but added that velocity and discharge of the water are also important. Seasonal variations in river flow and changes in habitat structure control the turbulence and mixing of rivers which control the concentration of pollutants in a river. Common physical aquatic factors that control the response of a river to pollutant input include the size, shape, depth, temperature conditions (degree of stratification), and the hydrological regime.

1.2 Statement of the problem

The Nzhelele River supports a variety of aquatic life forms ranging from unicellular to multicellular organisms. The biodiversity of these organisms has largely been threatened by the modification and dramatic changes in their habitats, through the utilization of rivers, indirect and direct deposition of toxic substances, and human use of the adjacent riparian and aquatic ecosystems. Subsistence and commercial agricultural activities seem to be the most common threats to the survival of most aquatic species along the Nzhelele River. The existence of subsistence and commercial farming, as well as settlements along the river have accelerated the rate of physical habitat deterioration, river eutrophication and altered aquatic species composition, biological productivity and the biological cycling of nutrient elements in many parts of the Nzhelele River. The problem is further worsened by other human activities such as water and sand harvesting for domestic purposes and inconsistent local management strategies.

1.3 Justification of the research

According to Narangarvuu *et al.* (2014), the overall quality of aquatic ecosystems is affected by land uses through the inadequate and improper use which lead to changes in channel structure, water quality and habitat and these changes pose threats to aquatic biological diversity. Understanding and prediction of the rate of species deterioration due to water quality and physical habitat changes is crucial for the management of water bodies and the preservation of aquatic biota. Previous studies put much emphasis on the management of water quality to protect human health, but recent emerging focus has been on management of water quality for aquatic ecosystems (Gyedu-Ababio and van Wyk, 2004). A study of the impact of water quality and physical habitat changes on aquatic biodiversity is important for determining the actual causes of aquatic species deterioration in different segments of a river characterised by different physical properties such as land-uses, gradient, discharge, turbulence and habitat characteristics (vegetated, rocky, sandy or muddy). This study examined how taxa composition changes with changes in the level of pollution and the magnitude of physical habitat modification or destruction. The study of river water quality and habitat conditions also helps in understanding the actual factors that affect aquatic species composition. This study therefore led to the development of the river water quality management model in order to help conserve the already threatened riverine ecosystems. It was therefore crucial to investigate the status of the Nzhelele River health by measuring and estimating water quality, macroinvertebrate diversity and habitat condition. This will generally improve developmental projects planning, river management planning and general environmental management.

1.4 Aim and objectives

1.4.1 Aim

The aim of the study was to assess the river health condition by using water quality, aquatic macroinvertebrate composition and aquatic habitat conditions.

1.4.2 Objectives

i. To assess water quality conditions in order to determine the magnitude of pollution impact.
ii. To correlate aquatic macroinvertebrate taxa diversity and water quality parameters.
iii. To measure the size of degraded area with respect to macroinvertebrate richness.
iv. To develop a model for managing river health conditions for the Nzhelele River that can be applied to other rivers in the region.

1.4.3 Research questions

i. How can the current water quality condition help in determining the magnitude of pollution impact?
ii. How can correlation of species diversity and water quality show the river health condition of the Nzhelele River?
iii. How does species diversity change with the scale of degraded aquatic area?
iv. Which model will best be applicable to the Nzhelele River in order to conserve aquatic biota?

1.6 Description of the study area

The Nzhelele River forms the main watercourse in the Limpopo Province of South Africa (Figure 1.1). The the catchment area of the Nzhelele River covers 2,436 square kilometers (DWAF, 2004). It runs through the Nzhelele Valley that is situated between Soutpansberg Mountains. The Nzhelele Valley lies between the Soutpansberg mountain ranges, roughly along latitudes 22º 20′ S and 23º 05′ S and longitudes 29º 45′ E and 30º 25′ E at an elevation of approximately 903 m above mean sea level. The valley is approximately 60 km East of Louis Trichardt and approximately 30 km west of Sibasa (Mathada, 2010). The Nzhelele River drains the north-eastern part of the Limpopo Water Management Area (WMA) (Figure 1.1) and flows into the Limpopo River (DWAF, 2003).

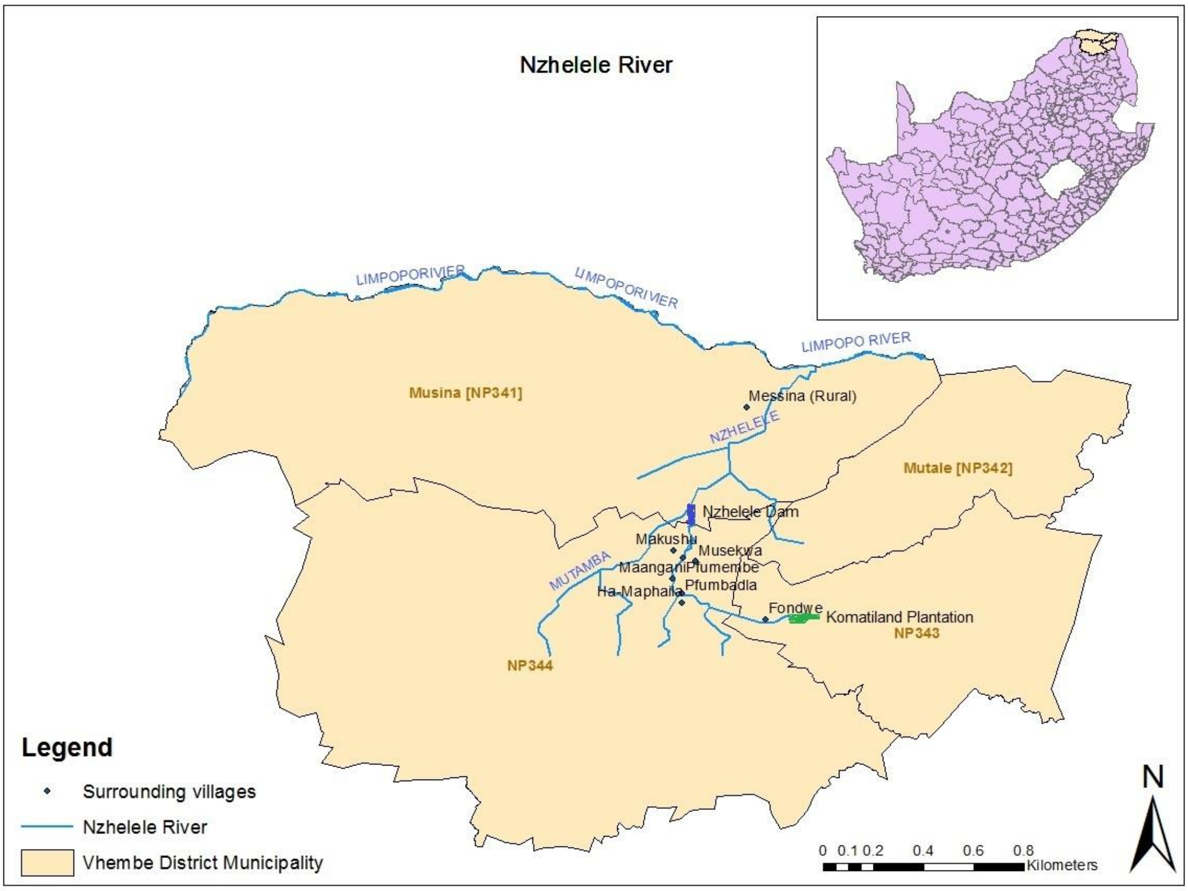


Figure 1.1 The Nzhelele River System and surrounding villages

1.6.1 Climate

The climate of the Nzhelele Valley is semi-arid and becomes arid eastward to the Kruger National Park (Mudau, 2002). Climate also plays an important role in determining the amount of rainfall necessary to trigger stream flow during wet and dry seasons. The amount of rainfall also controls the rate of surface erosion. Due to the east-west orientation of the Soutpansberg, the study area experiences orographic rainfall (Kabanda, 2003).

Temperatures vary dramatically according to topography and seasonal conditions. The summer months are warm, with temperatures ranging from 16-40°C. Winter temperatures are mild, ranging from 12-22°C. Minimum winter temperatures seldom drop below freezing point (Kabanda, 2003).

According to Maluleke (2003), rainfall is seasonal and is higher during the rainy season starting from October to March and its distribution is highly influenced by topography. The dry seasons are experienced between April and September (Kabanda, 2004). The peak rainfall months are in January and February. Average annual rainfall ranges from less than 300mm on the low - lying plains to more than 1800mm in the Soutpansberg mountainous areas. Rainfall is important in determining the mean surface flows of rivers, hence the mixing of pollutants in water.

1.6.2 Vegetation

The study area is characterised by the Central Bushveld and Mopane Bushveld vegetation units of the Savanna Biome (DEDET, 2009). The subtropical moist thicket, open savanna sandveld, mist belt bush and arid mountain bushveld form the main vegetation variations of the study area (Mucina and Rutherford, 2006).

1.6.3 Soils and geology

The area comprises red or brown shale, tuff, basalt, sandstone and quartzite, conglomerate and siltstone of the Soutpansberg Group of the Nzhelele Formations. Rocky areas with miscellaneous soils are a characteristic of the area with dystrophic to mesotrophic and sandy to sandy loamy soils. Common soil forms also include the Glenrosa and Mispah. The rate of erosion ranges from low to moderate (Mucina and Rutherford, 2006).

1.6.4 Hydrology and drainage

The Nzhelele dam and the Nzhelele reservoir form the major hydrological forms of the study area. These two water bodies are located in the eastern part of the Nzhelele Nature Reserve. The capacity of the Nzhelele dam is 51.2 million m^3 and its operation and management are the responsibility of the Department of Water Affairs. Its main water users are farmers and their utilization of the dam affects its water quality downstream of the dam (Cook, 2013).

1.7 Research design

Figure 1.2 shows the research design which was followed when collecting data on water quality, species diversity and physical habitat conditions. The design of the research approach can be summarised as follows:

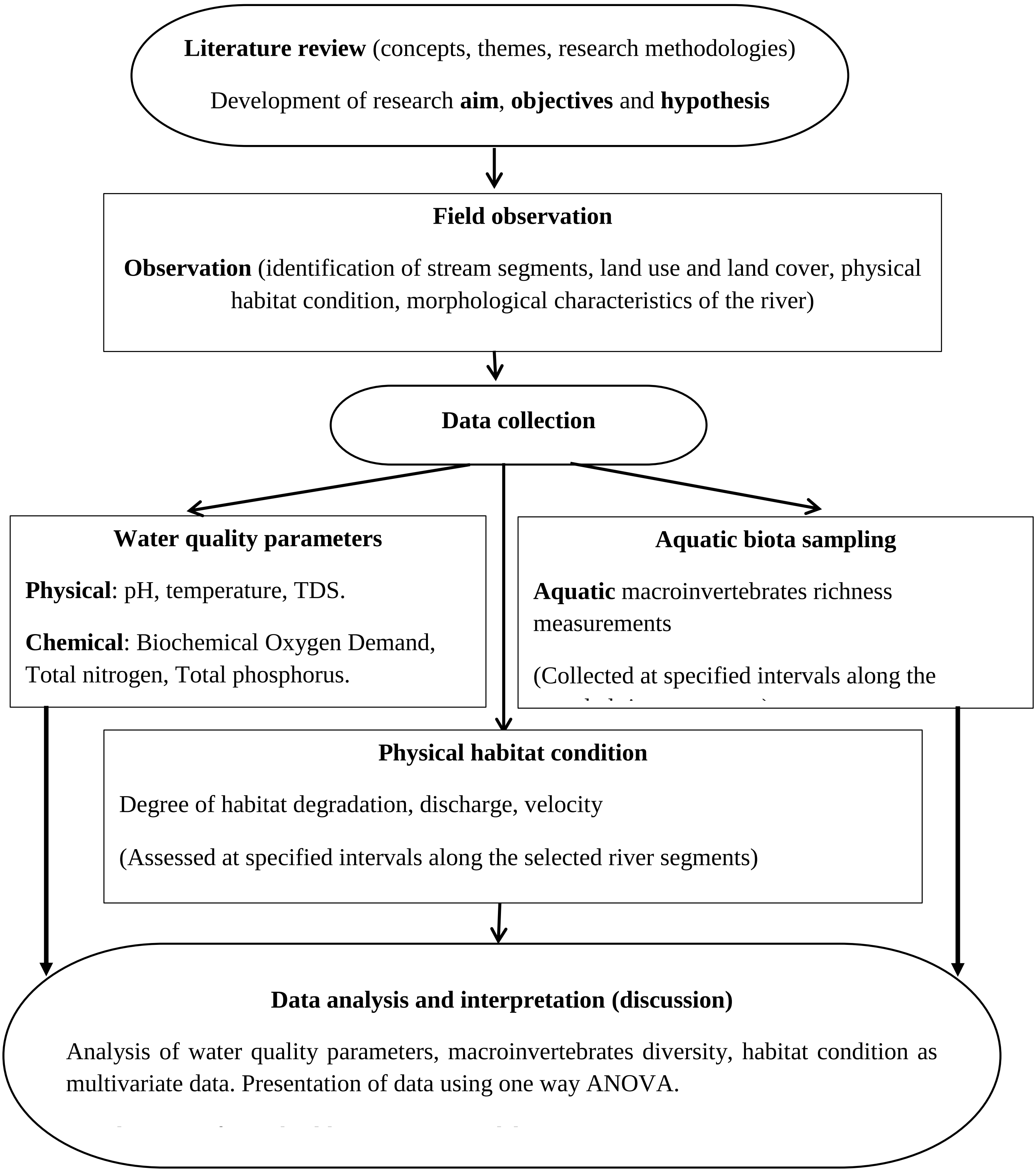


Figure 1.2 Research framework for collection of primary data

From Figure 1.2 the first step is observations, followed by the review of literature and the types of data and their collection. The approach is quantitative as most of the raw data were collected from the field and analysed.

1.8 Chapter breakdown

Chapter one: Comprises of the introduction and background to the problem, statement problem, justification, description of the study area aim and objectives, as well as research hypotheses.

Chapter two: Review of literature from past and current studies, research methodologies and management strategies.

Chapter three: Outlines research methodology. This chapter covers data collection methods on water quality parameters, aquatic macroinvertebrates diversity, and physical habitat characteristics, as well as data analytical methods.

Chapter four: Presentation and discussion of the findings.

Chapter five: Summary, conclusions, recommendations, water quality model development.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

This chapter presents a review of literature on the impacts of altered aquatic habitat and water quality on macro-invertebrate communities. Different methods that have been used to study macro-invertebrate assemblages in aquatic environments have are discussed in this chapter. Different arguments have been put forward regarding the causes of reduction in macro-invertebrate composition and in this chapter these causes and their impact on macro-invertebrate composition or assemblages have been discussed.

2.2 Indicators of river health

According to Pinto and Maheshwari (2014) the majority of river systems of developed and developing countries are faced with the problem of becoming irreversibly degraded. They further argued that the difficulty in developing comprehensive methodologies for river health assessment that could be applied to the world river system is created by enormous geographical differences, catchment characteristics and habitat-specific species attributed to river systems. Rowe (2014) observed that the response of macroinvertebrates to cumulative anthropogenic effects such as pollution, hydrological changes and changes in riparian vegetation has made macro-invertebrates suitable bioindicators of river health. Stark *et al*. (2001) also indicated that macroinvertebrate bioassessments are favoured because they are found in almost all freshwater environments and are relatively easy to collect and identify. Linares *et al*. (2013) further argued that existing the conditions of the environment such as hydraulic stress, temperature and the chemistry of water have been known to influence the abundance of aquatic invertebrates.

Huang *et al.* (2010) have argued that the health of stream ecosystems on many occasions indicates the aquatic biodiversity and also shows how streams are affected by water pollution due to alterations in land use practices of the terrestrial ecosystem which are affected by the natural geographic characteristics of the watershed. They further argued that the overall ecological integrity is reflected by resident biological communities which integrate the effects of a variety of stressors and provide a broad measure for their combined impacts. Therefore, the biotic approach of using aquatic assemblages of organisms such as phytoplankton, zooplankton, periphytic algae, macrozoobenthos, fish and bacterioplankton has been a common practice to monitor water quality for directly assessing the overall health of marine, coastal and estuaries, stream or lake ecosystems in relation to water pollution (Huang *et al.*, 2010). This means that aquatic species diversity depends on several factors such as water quality and physical habitat conditions which are a good measure of stream health. Miserendino *et al*. (2012) noted that fish and macroinvertebrate communities are affected by the extensive destruction of aquatic and riparian habitats.

Testi *et al*. (2012) note that the environmental quality of river ecosystems can be indirectly measured by river health indicators focusing on a variety of habitat components. Testi *et al*. (2009) observed that plants and animals are used as biological elements in many aquatic ecosystems to assess environmental quality based on their integration with chemical, physical, geomorphological and anthropogenic factors. Meng *et al.* (2009) argue that the common biological indicators of stream pollution include fish, plankton and benthic macroinvertebrates. Since a river is a complex ecosystem, the use of a single factor such as biological index for assessing river health would be unable to show a river regime (An *et al*. 2002). Roux (2000) had earlier made a similar observation that the advantages of integrated assessment methods based on multi-index are that the character of a river ecosystem can be generally reflected under the disturbance from human activities and it is helpful to reveal the inner relationships among different indices. Therefore, finding a proper multi-metric system for assessing river health based on the characteristics of a river basin is a necessity since different ecosystem structures and functions result from changes in land-use.

Yaping and Zongren (2012) stated that assessment of water quality involves the use of chemical energy, biological diversity, and others involve nutrient parameters of total nitrogen (TN) and total phosphorus (TP), temperatures, pH, biological oxygen demand (BOD), chemical oxygen demand (COD) and coliform. Astaraie-Imani *et al*. (2012) are of the view that the future of water quality of urban catchments is affected by climate change and urbanisation. Water quality deterioration is still common due to regular discharges of non-point sources (NPS) pollution into the water bodies (Lai *et al*. 2011). Irrigation return flows often carry NPS into receiving aquatic bodies. However, deterioration of water quality, according to Ouyang *et al*. (2010), results from NPD pollution of suspended solids (SS), nutrients, pesticides, fertilisers and a variety of sources of inorganic and organic matters which lead to the loading of water bodies. Nikolaidis *et al*. (2006) also noted that knowledge of transportation of pollutants into aquatic bodies is key to the management of NPS pollutant loads into water bodies. Wu and Chen (2013) assert that the aquatic ecosystem and water quality security are threatened by surface water impairment as a result of point source (PS) and NPS pollution. Municipal sewage discharges and industrial wastewater loads are common Point Source pollution problems. Soko and

Gyedu-Ababio (2015) have emphasised that substantial decrease in the quality of aquatic ecosystem surface water is a consequence of anthropogenic activities. These anthropogenic activities result from industry, urbanisation, agriculture, mining, afforestation, accidental freshwater pollution and the generation of power. Johnson and Ringler (2014) have pointed out that environmental factors such as dissolved oxygen, temperature and vegetation protection affect aquatic biota in addition to point source channel pollution.

The increasing impact of human activities on the fluvial system has led to the development of monitoring programs and bioassessment techniques for the detection and accounting for various effects in freshwater ecosystems (Alvarez-Cabria, *et al*., 2010). According to Erba *et al.* (2015), freshwater ecosystems have been subjected to a variety of human stressors including hydrogeomorphological alterations, accumulation of organic compounds and other contaminants. Alvarez-Cabria *et al.* (2010) have provided reasons for the use of macroinvertebrates as a biomonitoring tool thus:

- They are good indicators of numerous anthropogenic pressures such as water pollution and hydrological and geomorphological changes;
- They are widely distributed, and
- They provide cost-efficient results.

Bonada *et al*. (2006) have similarly argued that macroinvertebrates can be used across aquatic ecosystems owing to their large-scale capability. Gray and Delaney (2008) believed that riverine studies adopt the application of biological indices and diversity to measure the impact of pollutants through the use of macroinvertebrate communities which act as indicators of ecosystem health. However, Sidagyte *et al*. (2013) stated that macroinvertebrate metrics that have been developed to detect organic pollution which leads to depletion of oxygen in lotic systems may not be applicable to stagnant (lentic) waters. Leunda *et al.* (2009) have however, identified four key reasons for using benthic macroinvertebrate communities and biological indices for the assessment of water quality, namely:

- Sampling macroinvertebrates (qualitatively or semi-quantitatively) is relatively easy.
- A range of identification keys is available.
- The tolerance to pollution of many macroinvertebrate taxa is well-documented allowing biological indices to be developed, and
- The types of sampled macroinvertebrate communities integrate the state of the environment over preceding months.

Walters *et al.* (2009) further stated that indicators of stream health (biotic integrity) and stream stressors (sedimentation and water quality) have also become important tools for determining the mechanisms of impacts and for assessing stream condition. They further argued that responses of benthic macroinvertebrate assemblages to aquatic pollution are manifested by reduction in richness and diversity and increased abundance of organisms that are tolerant to urbanised streams. However, Simaika and Samways (2011) argued that a good biological indicator reflects the state of the environment, which also represents the impact of environmental change at various scales and is a beneficial substitute or umbrella of other taxa. They further stated that bioindicators can be used to measure three indicator categories, namely, biological diversity, environmental and ecological characteristics. For example, several aquatic plant bioassessment methods were developed through the use miscellaneous aspects of plant and vegetation attributes, through the consideration of the richness and abundance of species assemblages, vegetation structure species attributes and functional groups and the application of macrophytes species as trophic status indicators (Aguiar *et al*., 2011).

According to Varnosfaderany *et al*. (2010), the use of macroinvertebrates as bioindicators for assessing water quality has more advantages than those based on aquatic and riparian vegetation, diatoms, and fishes. This is because freshwater macroinvertebrates sensitivity to organic pollution differs and their occurrence and absence in aquatic environments helps to make inferences about pollution loads in water. Nguyen *et al.* (2014) indicated that the use of macroinvertebrates to assess water quality has been in practice for many years. According to Gabriels *et al.* (2010), evaluation based on the occurrence of species permits the detection of pollution in water. Gergel *et al*. (2002) have noted that since the diverse nature of biological, chemical, geophysical and hydrological components needs to be addressed during water quality assessment it becomes a challenge to detect human impacts on riverine ecosystems. Higher levels of nutrients in a stream are expected to accelerate primary productivity (Pearson *et al*., 1998). According to Pearson *et al*. (1998), this effect on the fauna frequently seems to be on the increase albeit with variable effects on diversity and composition.

Bird *et al*. (2014) stated that in South Africa, the South African Scoring System Version 5 (SASS 5) is a bioassessment procedure used to assess water quality in rivers. However, the SASS 5 is not applicable to lentic environments (Bird *et al.*, 2014). In South Africa, pollution sensitivity or tolerance levels for aquatic macroinvertebrate taxa ranges from 1 to 15, where 1 indicates tolerance to organic pollution and 15 intolerance to pollution (Gordon *et al*., 2015). The SASS metrics comprise the SASS score, which is the total of sensitivity ratings of those taxa sampled at site, the number of taxa (NOT) and lastly, the average score per taxon (ASPT) which is the SASS score divided by the NOT (Dickens and Graham, 2002; Fourie *et al*., 2014).

2.3 Causes of water quality degradation

2.3.1 Livestock practices

According to Miller *et al*. (2011), riparian zone soils, aquatic life, riparian wildlife, water quality, stream channel morphology, interim and bank vegetation are negatively affected by access of livestock to riparian zones. The deterioration in water quality due to livestock is caused by direct faecal release into water bodies, runoff of faecal matter from adjacent lands, increased streambank erosion as a result of cattle shearing and the resuspension of river sediments as a result of cattle trampling (Miller *et al*., 2011). This explains why Gyawali *et al*. (2013) argue that riparian buffers, particularly undisturbed vegetated riparian zones adjacent to rivers might have the ability to mitigate nutrients and sediment from surface and groundwater flow through deposition, absorption and denitrification. They further argued that riparian buffer zones are good filters of sediment, nutrients and pesticides which enter water bodies from agricultural and urban lands. However, Miller *et al*. (2010) have noted that stream bank fencing shields cattle from streams and riparian zones and this improves riparian health and water quality. Exclusion of livestock from riparian zones through fencing comes with a variety of benefits such as increase in growth and vigour of riparian vegetation, reduced bare ground exposure, shift plant communities and favour increase in willow shrubs or cotton-wood trees. Stream bank fencing, according to Ranganath *et al*. (2009), may also lead to changes in the physical characteristics of streams such as stream bank erosion while overhanging banks, river depth and channel particle size and riffle substrate may also increase.

2.3.2 Stream modification

Kwak and Freeman (2010) noted that the ultimate factor that affects the ecological integrity of aquatic ecosystems is human intervention. In a research undertaken by Miserendino *et al*. (2011) it was realised that extensive removal of the basin vegetation has altered flow characteristics, the amount of sediment introduced to stream systems, decreased infiltration and an increase surface runoff. They further argued that channelization and realignment of rivers produce substrate modification and description of the riffle or pool sequence that determine the diversity of aquatic habitats for species such as fish and invertebrates. The current and historical modifications of surface and subsurface water systems and land uses impact on downstream receiving water systems and threaten ecology (D'Ambrosio *et al*., 2014). Miserendino *et al.* (2011) also argued that further modification of the landscape habitats through fragmentation of uninterrupted riparian corridors and natural forests leads to habitat loss and an increase in isolation, decreasing colonisation and reduction in biodiversity. According to Carlisle *et al*. (2014), the most prevalent form of streamflow alteration is depletion through damming. Lessard and Hayes (2003), have observed that aquatic macroinvertebrates are affected by habitat changes caused by dams and always show shifts in community composition below dams. Lessard and Hayes (2003) argued that impoundment through dams can change numerous physical and chemical factors such as dissolved oxygen, stream substrate and water temperature.

Large dams alter the physical structure of rivers leading to changes in flow, water temperature and sediment regime which ultimately lead to degradation of freshwater ecosystems worldwide (Olden and Naiman, 2010). These changes in the physical template of dams lead to changes in riverine food webs, reduced biodiversity of algae, macroinvertebrates and fishes, and the extirpation of native species and facilitation of invasion by non-native species (Kelly *et al*., 2013). Changes in abundance and distribution of macroinvertebrate assemblages below dams are linked to shifts in temperature and flow regimes (Kelly *et al*., 2013). Dewson *et al*. (2007b) have observed that sensitive ETP and other native macroinvertebrate taxa are obliterated from streams with depleted or reduced flows. Van Vliet *et al*. (2013) have similarly observed that changes in stream flow through channelization or damming affect temperature of water, especially during warm, dry periods characterised by low river flows.

Almeida *et al*. (2013) argued that alteration of habitats is a major factor contributing to loss of biodiversity at the global scale. Postel and Carpenter (1997) have noted that many species are defined by specific temperature, water quality and other needs that determine their survival in any given river system. According to Wang *et al*. (2011), the construction of dikes, weirs and dams obstructs dispersal and migration routes of organisms and changes natural flow regimes, leading to the disturbance of refugees and trophic resources necessary for fauna, leading to the decrease in heterogeneity of the abiotic environment. They further argued that the regulation of flow may lead to loss of adaptive capacity to natural environmental fluctuations causing changes in function and structure of community, resulting from suboptimal and less heterogeneous abiotic environments. Lind *et al*. (2006) noted that physical characteristics at the microhabitat scale are often used to show changes between benthic macroinvertebrate assemblages under flow regimes. According to Postel and Carpenter (1997), the creation of a stable habitat by aquatic ecosystems depends on the dynamism of connectivity between water and land, physical processes (water and sediment flows), and a number of biophysical conditions (water quality, temperature and food webs relationships). They further contended that the supply of habitat for fish and other aquatic organisms is greatly affected by the volume, timing and quality of water that flows in its natural channel.

2.3.3 Land use and land cover changes

An interesting observation by Zhou *et al*. (2012) is that the quality of rivers and water flows of aquatic ecosystems is influenced by anthropogenic activities such as land use and land cover changes. Taylor *et al*. (2013) argued that the natural flow regimes of rivers have been found to be affected by their socio-economic uses which affect the ecosystem services. The land-use and land cover changes of rivers, according to Taylor *et al*. (2013), have the ability to modify geomorphological features and intensify the pollution sources. They also argued that there is a strong correlation between land-use and land cover changes and water chemistry parameters, biodiversity of freshwater fish and macroinvertebrates and the concentration of metal sediment. The conversion of land from forestry to agriculture or riparian buffer rehabilitation can show cascades of effects that impact on the physical-chemistry of streams or biota at different spatial scales (Minaya *et al.*, 2013). Agricultural activities such as forest harvesting cause a wide range of disturbances to adjacent streams, including changes in water quality, nutrient input, hydrology, habitat structure, food sources and channel morphology (Reid *et al*., 2010). According to Fahey *et al*. (2004), harvesting of overstorey riparian vegetation along stream banks leads to alteration of thermal regimes, increased autochthonous production and reduced allochtonous regime inputs. Impoundment, irrigation, diversion and groundwater extraction are associated with reduced stream flow in all regions of the world and these negatively impact on macroinvertebrate communities (Mackie *et al*., 2013).

Burcher *et al*. (2007) have observed that discharge of solute and seston load are a result of land use and landscape physiography at catchment-scale, but sediment, stream hydraulics, light and organic inputs are often in response to reach-scale geomorphology, conditions of the riparian zone and roughly land use. Erba *et al*. (2015) argued that changes in physical features of hydrological regimes of lotic environments through the clearing of riparian vegetation, opening of canopy, changing the timing, amount and inputs of water, organic matter and light are strongly linked to urbanisation. The removal of riparian canopy in agricultural and urban developments is a common mode of land-use which influences river ecosystem by altering hydrological regimes and creating resistant areas and increasing the input of sediments, nutrient loads and other pollutants (Narangarvuu *et al*., 2014). McCord *et al*. (2007) asserted that the clearing of riparian zones along rivers has the ability to alter river ecosystems functioning and disruption of fluxes of organic matter and energy. This can affect river biota directly and indirectly.

Wu and Liu (2012) have noted that the subsequent nutrient losses from agricultural fields into stream water and estuaries have led to eutrophication of many coastal and freshwater ecosystems worldwide. According to Meck *et al*. (2009), the excessive growth of bulrush in streams indicates high levels of phosphates which usually leads to stream eutrophication. This creates a choking aquatic ecosystem, reduction in light intensity and extreme fluctutations in oxygen, reduced habitat diversity and ultimately reduced invertebrate and and increased fish mortality in streams. According to Kney and Brandes (2007), high electrical conductivity, results from the high concentration of dissolved ions which give water elevated electrical conductivity. Iron, manganese and inorganic salts lead to elevated electrical conductivity (Meck *et al.,* 2009).

2.3.4 Flow characteristics

According to Eady (2011), stream flow is considered the primary driver of aquatic faunal distribution as it affects the biota in a variety of ways. Flow variability is one of the most important determinants of the ecological processes that control patterns of diversity and the disturbance of species in riverine ecosystems (Rocha *et al*., 2012). They further stated that the intermittence of flow allows for the coexistence of competing species and fragments the habitat temporally. According to Choudhury *et al*. (2014), macrophytes and stream flows play crucial roles in determining macroinvertebrate assemblages. However, Verdonschot *et al*. (2012) have noted that the positive correlation between macroinvertebrate abundances and flow velocity and macrophytes cover. Walker *et al*. (2013) further stated that high macroinvertebrate abundances are associated with plants that are characterised by finely dissected leaves and intricate branching. This means that stream flow velocity has the ability to indirectly influence the macroinvertebrate community by shaping the morphology of macroinvertebrates.

According to Erba *et al*. (2006), local scale macroinvertebrate communities are influenced by factors such as bank morphology, Froude number and Reynolds number, velocity, habitat types such as organic debris and leaf litter, periphyton abundance, riparian vegetation type, wood depth, percent sand and percent silt, substrate size, organic compounds and the degree of bank modification. D'Ambrosio *et al*. (2014) argued that straightened trapezoidal channels lead to increased sedimentation and these channels often lose their habitat structure and complexity that supports diverse aquatic biota. Dewson *et al*. (2007a) also stated that river discharge controls the volume of an aquatic habitat, variability conditions and stream connectivity that affect the aquatic biota distribution, density and life history patterns. According to Rolls *et al*. (2012), a 30% decline in average annual streamflow leads approximately 5.7% decline in macroinvertebrate taxa. According to Dewson *et al*. (2007a), macroinvertebrate assemblages do not show significant changes in reaches characterised by reduced flows. Taxa composition is also known to drop during low flows and declining water quality (Sheldon and Fellows, 2010), and the reduction in the available aquatic habitat.

2.3.5 Benthic habitat characteristics

According to Pan *et al.* (2015), in organic-rich silt bottom of eutrophicated rivers physico-chemical variables of water and substrate, as well as pollution are key factors structuring macroinvertebrate assemblages. Pan *et al.* (2015) further stated that sandy substrate is the poorest substrate for macroinvertebrates. This means that the macroinvertebrate assemblages will be lower where the substrate is sand. Pan *et al.* (2015) have argued that by improving habitat quality there is provision for superior living conditions, supporting higher abundances of inhabitants or dwellers. Macroinvertebrate assemblages are also said to be influenced by water velocity, substrate size, bank morphology and others (Allan and Castillo, 2007). Rezende *et al.* (2014) have similarly argued that substrate composition, input of detritus, and canopy cover make up the three major variables that account for the diversity of macroinvertebrate communities in lotic environments. According to Molokwu *et al*. (2014), substrate material can be a very important determining factor because some macroinvertebrates obtain nutrients from the substrate itself, utilise it for physical support and feed on deposited sediments that cover the substrate. Verdonschot *et al.* (2012) have similarly argued that in freshwater ecosystems habitat structure is the key determining factor for the occurrence and distribution of macroinvertebrates. They further argued that in lentic waters macrophytes provide habitat for macroinvertebrates. This might be true for extremely slow moving waters. According to Morse *et al.* (2007), since most aquatic macroinvertebrates inhabit benthic habitats for at least part of their life, relatively immobile and very sensitive, any disturbances in their environment may cause them to disappear or reduce diversity.

Rubach *et al*. (2011) have noted that biological tracts of macroinvertebrates respond specifically to certain sources of anthropogenic stressors resulting from effluents, sewage, land uses, and the periodic exposure to agricultural pesticides. Anthropogenic activities such as deforestation, construction, irrigation, urbanisation, drainage of wetlands and pollution have direct impacts on aquatic habitats (Al-Shami *et al*., 2011). Zhang *et al*. (2010) observed that hydrological conditions such as discharge and water disturbances always play roles in aquatic ecosystems. They argued that dramatic changes in environmental parameters such as oxygen conditions, light penetration, water flow, thermal structure and nutrient gradients are a result of high discharge of pollutants into receiving water bodies.

Lamouroux *et al*. (2004) have noted the correlation between macroinvertebrate traits (body form, feeding habitats, maximum size, mode of attachment to substrate, reproduction, lifespan and strategies of dissemination) and microhabitat variables (substrate, flow or trophic condition). This means that the number of macroinvertebrate assemblages of a river is determined by the physical integrity. Biesel *et al*. (2000) noted that there is a correlation between generalist species and unstable sites, but specialist species tend to favour more stable sites. Reid *et al.* (2010) noted that heterogeneous riverbeds reflect a higher variety of species than homogeneous beds, which are home to only one or two species. Species richness tends to be higher in patchy and heterogeneous environments because these habitats are characterised by diversity in substrate sizes and a greater number of niches. Sullivan *et al*. (2004) noted that there was a correlation between the percentage of macroinvertebrate community comprising the sensitive *Ephemeroptera, Plecoptera* and *Trichoptera* taxa and more stable habitats that were characterised by better geomorphic conditions and better quality habitats. However, some studies showed little correlation between heterogeneity of the physical environment and biotic assemblages (Sullivan *et al*. (2004). This means that macroinvertebrate assemblages respond to environmental conditions and they therefore integrate chemical, physical and biological aspects of ecosystems (Ligeiro *et al*., 2013). Therefore, resident freshwater communities are determined by the physical structure and architectural complexity of their habitats (Walker *et* *al*., 2013). This view is purported by Warfe and Barmuta (2004) who argued that the diversity and abundance of macroinvertebrates increases with an increase in the density of vegetation biomass.

Pan *et al.* (2015) argued that natural habitat conditions (substrate size, flow velocity, and bank morphology) regulate macroinvertebrate assemblages. This condition occurs where rivers are minimally impacted by human interference (Brooks *et al*., 2005). Problems associated with rivers affected by human interference such as flow regime changes as a result of dam construction and the impact on macroinvertebrates have been well documented (Ajuzie, 2012). According to Hilderbrand *et al*. (2005), the diversity and dynamics of the structure of aquatic habitats is the basis for biodiversity of river ecosystems. Cullum *et al*. (2008) have indicated that a system characterised by poor physical structure has a higher probability of creating a highly disturbed ecosystem. According to Reid *et al*. (2010), physical heterogeneity of a river results from geomorphic processes such as erosion, deposition, sediment sorting, hydraulic variability along with vegetation interaction. Heterogeneity is defined as variability in a process or pattern over space and time. Functional habitats are therefore structural components of the aquatic substrate and vegetation objectively identified as distinct by the macroinvertebrate assemblages which they embrace (Reid *et al*., 2010).

According to Pearson *et al*. (1998), changes in the dynamics of sediments can be one of the most detrimental impacts on stream ecosystems because of their effects on habitat, on ecological processes and on animals' survival. High sediments are likely to affect aquatic communities in many ways, like blanketing the substrate, reducing light penetration, thus limiting primary production, reducing oxygen concentrations and reducing the number of macroinvertebrates (Pearson *et al*., 1998). Rowe (2014) has shown that fast moving river water improves aeration and increases concentration of dissolved oxygen.

Kwak and Freeman (2010) have summarised the role of biological and physical factors which determine the integrity of aquatic ecosystems and how these variables are affected by human activities (Figure 2.1).

ECOLOGICAL INTEGRITY

Human Activities	
Land use changes	Water impoundment
Riparian alteration	Water extraction or diversion
Stream channel modification	Species introduction
Chemical or thermal effluent	Biomass extraction (fishing)
Diffuse pollution	Secondary and cumulative impacts

Physical conditions		Biotic conditions	
Water quality	Water quantity and dynamics	Assemblage properties	Population properties
Temperature Dissolved oxygen Nutrients Minerals Contaminants	Channel processes Flow regime Stochastic events Climatic	Richness Diversity Evenness Dominance Species composition Species interaction Stability Resilience	Productivity Abundance Survival Growth Reproductive Tolerance

Figure 2.1 Biological and physical factors that affect aquatic ecological integrity (Kwak and Freeman, 2010)

2.4.1 pH

Rowe (2014) noted that the analysis of macroinvertebrates to assess stream health is often complemented by measurements of stream temperature, pH, turbidity, river velocity and stream flow. For example, there is a strong correlation between water temperature and water quality because the temperature of water always influences the amount of dissolved oxygen in water, photosynthetic rates and the aquatic organisms metabolic rate (USEPA, 2003).

The pH of freshwater ecosystems affects the physiological functions of both aquatic flora and fauna (USEPA, 2003). According to latter, a pH range of 6.4-8.0 tends to favour most aquatic organisms. According to the DWAF (1996), most surface waters have a pH range of 4 and 11. DWAF (1996) further stated that most South African freshwater systems have a pH range of between 6 and 8 because they are well buffered and more or less neutral. According to Primbas (2005), each macroinvertebrate possesses its own pollution tolerance and pollution in water is often measured by pH, nitrate and phosphate concentrations. Macroinvertebrates that have low pollution tolerance include mayflies, water pennies and stoneflies. Moderately pollution tolerant macroinvertebrates include dragonflies, crane flies and damselflies. High pollution tolerant macroinvertebrates include black flies, aquatic worms and midges (Primbas, 2005). According to Vogel (2005), a pH value of less than 4 in river water signifies the absence of Plecoptera species. Heteropterans are associated with a positive relationship of pH tolerance (Vogel, 2005).

The pattern of the diversity of macroinvertebrate family and of the abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT) families is mostly structured by geographic characteristics and hydrology (Rolls *et al*., 2012). According to Tripole *et al*. (2008), acid formation in water due to low pH leads to various adverse effects on the benthic macroinvertebrate community. The lower pH values of less than 5.5 in aquatic environments leads to the exclusion of some macroinvertebrates (Tripole *et al*., 2008). However, Ernst *et al*. (2008) have stated that acidified reaches have fewer EPT taxa and fewer EPT individuals. Therefore, midge larvae which are tolerant to acidified conditions become dominant organisms. Plecoptera are less dominant in acidified water. The quality and quantity of biofilms decrease under acidic conditions and this will also limit the diversity or abundance of macroinvertebrate scrapers that feed on biofilms (Ernst *et al.*, 2008). According to Gaskill (2014), in order to maintain macroinvertebrate richness, it is important to improve the productivity and habitat structure of aquatic ecosystems by controlling pH not to drop significantly to create acidic conditions. Gaskill (2014) argued that as sensitive taxa (Trichoptera and Ephemeroptera) decline more pollution tolerant taxa increase in abundance to replace them. This has a potential to disrupt the upper levels of the food chain.

2.4 Water quality parameters

2.4.2 Turbidity

Dunlop *et al*. (2005) defined turbidity as "a measure of water clarity or cloudiness and it is defined further as the optical property of a liquid that causes light to be scattered and absorbed rather than transmitted in straight lines". Turbidity measures environmental health and indicates the occurrence of dissolved or suspended material, which also include organic matter, clay, silt, microscopic organisms, organic acids and dyes (USEPA, 2014). High turbidity is a result soil erosion, discharge of wastewater, urban runoff, or excessive growth of algae (USEPA, 2014). Stream velocity and the volume of flow can be affected by weather conditions, seasonality, water abstraction for irrigation or industrial use and the presence of dams (Walk *et al*., 1997). Stream velocity and turbidity are known to shape the ecosystem of streams (Walk *et al*., 1997). Velocity and flow rate impact on sedimentation in a river because fast-flowing rivers or streams carry suspended sediments for longer distances while slow-moving rivers are characterised by rapid settlement of sediments on the river bed or bottom (Rowe, 2014).

According to Dunlop *et al.* (2005), total dissolved solids (TDS) and total suspended solids (TSS) contribute to the measure of turbidity. Lower biomass and productivity downstream of the dams are associated with higher turbidity which leads to degraded food sources in aquatic environments. Filter feeders such as freshwater bivalves are affected by higher turbidity. The composition of benthic zone species is affected by increased turbidity and sediment deposition (Dunlop *et al*., 2005).

Schwartz *et al*. (2008) have argued that the impact of turbidity on aquatic life is controlled by duration, frequency and turbidity level. According to Borok (2014), turbidity is correlated through stream discharge, and also corresponds to storm events that carry and transport abundant sediments. Borok (2014) further noted that turbidity turns to be lower in dams because of direct input of clear water from tributaries and settling of solids due to reduced water mobility. In an interesting observation Kefford *et al.* (2010) noted that suspended sediments have less biological effect compared to the effects of deposited sediments. Borok (2014) further stated that turbidity is increased by anthropogenic inputs, erosion, inputs of turbid water and resuspension. Scherr *et al*. (2011) indicated that macroinvertebrate health is affected when turbidity values range from 4 to 10 NTU. Hubler (2002) noted a negative correlation between turbidity and macroinvertebrate density and diversity. Scherr *et al.* (2011)

have also noted a negative correlation between high turbidity values and decreasing number of sediment-sensitive macroinvertebrates. Borok (2014) argued that reduced primary productivity such as benthic algal production and the presence and growth of various macrophytes are a result of increased turbidity in water bodies such as streams, estuaries and lakes. Reduction in food availability for primary consumers due to elevated turbidity in streams limits primary productivity while turbidity and suspended sediments promote the drifting of macroinvertebrates due to clogging of benthic habitat (Borok, 2014).

2.4.3 Dissolved oxygen

According to Alexander *et al*. (2007), high dissolved oxygen levels are associated with the occurrence of caddisflies, riffle beetles, mayflies and stoneflies. These organisms prefer riffles which are characterised by cold temperatures and swift moving currents which increase dissolved oxygen in lotic waters (Molles, 2005). According to Molles (2005), warmer water temperature is associated with less dissolved oxygen. Leeches and aquatic worms are macroinvertebrates that are very tolerant to pollution, hence they do not require high dissolved oxygen levels (Alexander *et al*., 2007). According to Connolly *et al*., (2004), the availability of oxygen influences the composition of freshwater communities and it critically affects the distribution of numerous organisms. The variation in the levels of oxygen in water differ spatially and temporally because of photosynthesis by plants, respiration by organisms and atmospheric loses and gains, change in pressure and temperature and groundwater inflow (Dodds, 2002). Oxygen level below 2 mg/L has the ability to decrease the fitness and survival chances of numerous aquatic invertebrates. For example, caddisfly larvae are characterised by restricted locomotion and are therefore vulnerable to decreased oxygen levels in water (Dodds, 2002).

According to Nkwoji (2014) very low oxygen concentration occurs at the muddy bottom which serves as the habitat of the benthic organisms. Nkwoji (2014) also noted that increased biological productivity due to decomposition and biodegradation result in hypoxia. The senile nature of benthic macroinvertebrates makes them vulnerable to impacts of low dissolved oxygen (Nkwoji (2014). Dissolved oxygen concentration in water bodies characterised by dense macrophytes beds tends to be high during the day when photosynthesis is active and becomes lower at night when respiration is a dominant process (Teixeira *et al*., 2014).

Fluctuation in oxygen levels depends on plant architecture and stand size and density of plants, because they affect oxygen movement in water through water circulation and atmospheric exchange (Bunch *et al*., 2010). According to Fischer *et al*. (2012), plant structure influences macroinvertebrate composition through food availability and refuge efficacy against predation. Numerous physical and chemical characteristics influence aquatic fauna and interact with dissolved oxygen (DO) dynamics by determining the composition of aquatic communities (Teixeira *et al*., 2014).

2.4.4 Total Dissolved Solids (TSS)

Prolonged exposure to high concentrations of TSS alters the community structure of macroinvertebrates. Gordon *et al.* (2015) noted that accelerated loss Ephemeroptera and Trichoptera is caused by high TSS concentrations in water. Suspended solids trap heat from the sun, which causes an increase in water temperature and subsequent decrease in the levels of dissolved oxygen (Frondorf, 2001). According to Frondorf (2001), cloudy appearance in water occurs when TSS levels are between 40-80 mg/L, but when TSS levels are over 150 mg/L the water appears dirty (MIDEQ, 2000). According to Xu *et al*. (2014), poor water quality rich in pollutants threatens many aquatic species by reducing biodiversity to only pollution tolerant species. Pollution leads to low levels of dissolved oxygen in water. Nadushan and Ramezani (2011) noted that extremely high percentages of Olichaeta, Arachnida, and Gastropoda are a result of organic pollution contamination. Xu *et al*. (2014) have also noted that five biological indices: taxa richness (S), density (D), total Biological Monitoring Water Quality (t-BMWQ) score, average BMWQ score (a-BMWQ), and the family biotic index (FBI) are used for biological assessment of water quality.

2.4.5 Water temperature

Water temperature is a major factor in the distribution, abundance and richness of aquatic organisms along the gradients in latitude and altitude (Li *et al*., 2012a). Variation in climatic variables influences the concentration of TDS, nutrient concentrations, stream channel morphology, habitat stability, as well as connectivity of water bodies, and these will ultimately affect the community composition (Li *et al*., 2012b). According to Rivers-Moore *et al*. (2008), water temperature is a major species pattern driver in aquatic ecosystems. Ngodhe *et al*. (2014) have noted that water temperature, discharge, DO, pH, nutrients and specific conductivity are the main physico-chemical factors that affect aquatic environments. Water temperature and DO levels usually fluctuate seasonally and aid in the structuring of benthic communities, which vary from species to species (Shieh and Young, 2000). Increase in water temperature lowers the solubility of oxygen and vice versa. Rowe (2014) similarly argued that temperatures outside of the species' optimal range may stress or kill individuals. However, Ngodhe *et al*. (2014) argue that physico-chemical parameters of macroinvertebrates, high water temperature, pH, DO, Biological Oxygen Demand (BOD) and total nitrogen (TN) directly influence the composition and low abundance of macroinvertebrates. Species diversity dominance and richness are negatively influenced by low TN, high water temperature, low DO and high BOD. The temperatures of inland waters in South Africa generally range from 5 - 30˚C (DWAF, 2006). Flautt (2007) stated that water temperatures above 20°C and as high as 26°C can render some species extinct.

2.4.6 Nitrites

Kocour Kroupova *et al*. (2018) stated that the occurrence of nitrites in natural waters usually accompany nitrates and ammoniacal nitrogen forms, but is found in low concentrations. Nordin and Pommen (2009) stated that nitrite concentration for freshwater aquatic life is 0.02 mg. L and the maximum concentration is 0.060. mg.L. Corriveau (2010) noted that nitrite concentration in natural waters can reach levels that range from tenths up to 1 mg. L NO^{-}_{2}. Elevated nitrite concentration is a characteristic of intensive commercial farming, aquarium fish farming or other aquatic organism farming (Buric *et al*., 2016). Kalogianni *et al*. (2017) have also noted that he substantial increase in nitrite concentration is associated with organic matter respiration.

2.4.7 Nitrates

Enrichment and eutrophication of nitrate in water bodies are cause for concern because high concentrations of nitrogen lead to periodic phytoplankton blooms and changes in the natural trophic balance. Eutrophication by nitrogen occurs when its concentration ranges from 2.5 to 10 mg. L (DWAF, 1996). Water pollution by total nitrogen (TN) and total phosphorus (TP) affects the macroinvertebrate fauna the same way deforestation causes reduction in taxa richness by simplifying the insect community composition without changes in abundances (Couceiro *et al*., 2006).

2.4.8 Chlorine

High levels of chlorine in water are indicated by the absence or a decrease in macroinvertebrate levels (Kohli, 2012). Chlorine is usually used as poisoning to harvest large numbers of adult *Macrobrachium* (freshwater prawn) for commercial purposes and the use of chlorine, insecticides and traditional toxins for fish harvesting have been reported in Africa (Greathouse *et al*., 2005). Chlorine is known to impair water quality because of its ability to react with organic matter in aquatic environments leading to the formation of toxic disinfection by-products (State of California, 2006). Chlorine is known to be toxic to aquatic organisms and increases human health risk because its ability to produce hazardous disinfection by-products (USEPA, 2014). Sorokin *et al*. (2007) reported that 70% of chlorine in water will be present as HOCL at pH of 7 and a temperature of 25°C, but 80% will be present as OCL^- at a pH of 8. The effect of chlorine has been reported by the USEPA (2008) to be a challenge because it causes sublethal damage when concentrations are very low, making it difficult to detect its presence through conventional methods. Costa et al. (2017) also noted the toxic effects of residual chlorine on aquatic life (fish and macroinvertebrates). According to Rowe (2014) the survival of aquatic plants and inhibition of aquatic physiological processes takes place when total residual chlorine reaches concentrations of 1,000 ug/L or less for periods of one hour or less.

2.5 International research

An integrated approach to assess river health of the Liao River was used by Meng *et al.* (2009) by studying water quality, aquatic life and physical habitat. Twenty-five sampling sites were used to assess health conditions of Liao River, with water quality index, biotic index and physical habitat quality index. Cluster analysis method was used for water quality indices and it showed that sites that were heavily polluted along Liao River were located at the estuary and mainstream. Attached algae and benthic invertebrates were surveyed. The result showed that the diversity and biomass of attached algae and benthic index of biotic integrity (B-IBI) were degrading due to degrading chemical and physical water properties. Physiochemical parameters of BOD_5, COD_{Cr}, TN, TP, NH_3-N, DO, petroleum hydrocarbon and conductivity, were analysed statistically using principal component analysis (PCA) and correlation analysis. The statistical results were combined with integrated assessing water quality index, combining faecal coliform count, attached algae diversity, B-IBI and physical habitat quality score. From the findings of the study it was possible to develop a comprehensive integrated assessing system of river ecological health. Based on the systematic assessment, the assessed sites were categorized into 9 “healthy” and “sub-healthy” sites and 8 “sub-sick” and “sick” sites. Data analysis also revealed the correlation between water quality conditions and the impact on aquatic life. The results also revealed that water quality and physical habitat quality indices played crucial roles in river ecosystem health. Out of 17 studied sites 9 sites were categorized into healthy and sub-healthy levels. Eight sites with heavy organic and trophic pollution were at sub-sick and sick levels.

Huang *et* al. (2010) studied key environmental factors that affect the ecosystem health of streams in Dianchi Lake Watershed (DLW) in China. Streams in a lake watershed were considered to be important landscape corridors linking the lake and terrestrial ecosystems. Field surveys were conducted during July and August of 2009 to collect data on periphytic algal and macrozoobenthic biodiversity, and the monthly monitoring of water temperature, pH, TSS, DO, TN, TP, NH_3N, NO_3N, COD_{Mn}, BOD, TOC, and the heavy metals Zn (II), Cd (II), Pb (II), Cu (II), and Cr (VI) was done from January to December 2009. Field surveys were carried out in 29 streams flowing into Dianchi Lake. Factor analysis and canonical correspondence analysis were used as multivariate statistical techniques to analyse the structure of the aquatic community in relation to aquatic environmental factors in order to provide controlling objectives for integrated watershed management and improvement of stream rehabilitation in the DLW. The results showed that the structure of the macrozoobenthic communities and periphytic were dominated by pollution-tolerant organisms such as bacillariophytes *Navicula* and the annelids *Tubificidae* respectively, while TN, NH_3N and TP were found to be key aquatic environmental factors affecting the ecosystem health of streams in the DLW.

Miserendino *et al*. (2011) assessed the effects of land use on water quality, in-stream habitat, riparian ecosystem and biodiversity in Pantagonian northwest streams. The hypothesis for their study was that greater intensity of land-use will have negative effects on water quality, stream habitat and biodiversity. To test their hypothesis, they assessed benthic macro-invertebrates, riparian and littoral invertebrates, fish and birds from the riparian corridor and environmental variables of 15 rivers (Patagonia) subjected to a gradient of land-use practices (non-managed native forest, managed native forest, pine plantations, pasture, urbanization). A total of 158 macroinvertebrate organisms, 105 riparian/littoral invertebrate taxa, 5 fish species, 34 bird species, and 15 aquatic plant species, were recorded from all study sites. Urban land-use was associated with most significant changes in streams by impacting on physical features, conductivity, nutrients, habitat condition, riparian quality and invertebrate metrics. Pasture and managed native forest sites showed an intermediate situation. The disturbed sites were characterised by highest values of fish and bird abundance and diversity. The opportunistic behaviour by studied communities was highlighted by communities which took advantage of increased trophic resources of their environments. Non-managed native forest sites were characterised by higher integrity of ecological conditions and great biodiversity of benthic communities. Macroinvertebrate metrics that reflected good water quality were positively correlated with forest land cover and negatively correlated with urban and pasture land cover. Macroinvertebrates were found to be good indicators of land-use impact and water quality conditions as well as useful tools to detect early disturbances in streams. The greater ability of fish and birds to disperse and move quickly from disturbed environments always reflect changes at a higher scale.

Li *et al*. (2012b) conducted another study on the relationship of land-use and land cover on water quality on the Liao River Basin in China. A total of 76 sampling sites were selected in the Liao River basin (21.9×104km2). During the period of 2009-2010, 42 water samples were collected in 2009 and 58 were collected in 2010. Physical-chemical variables were analysed to investigate their spatial-temporal variability, in particular the relationship with land use or cover. Their results indicated that physical and chemical properties showed obvious spatial heterogeneity in the Liao River basin. Taizi River and Hun River are located in the southeast of the basin. The water quality for two sub-basins: water quality in upstream is better than that in downstream. Water quality level in downstream was classified into IV-V. There were no obvious features in the East Liao River basin, water quality in downstream was classified into III level. West Liao River ran for many years, water quality was classified into IV. Big Liao River basin was located in the middle and east of the Liao River basin. Water quality was classified into V. Correlation and regression analysis indicated that BOD5, COD, sediment, hardness and nitrate–nitrogen, total dissolved particulates (TDP) were significantly related to land use for forest and agriculture.

Testi *et al*. (2012) conducted a study on the characterisation of river habitat quality using plant and animal bio-indicators on the Tirino River in Italy. The aim of this study was to compare the five independently derived indices in order to stress their differences and similarities in the two river environmental compartments – aquatic and terrestrial. 14 sampling sites along the

Tirino River and two on the Pescara River (just off the confluence of the two rivers) were surveyed for the three biotic components of the ecosystem. Vegetation was surveyed by rectangular plots (20m wide) in two parallel belts: water and shore; aquatic macro-invertebrates were collected according to the extended biotic index standard method, and edaphic micro-arthropods were extracted in soil sampling according to the index of soil biological quality (QBS-ar) standard method. There was a very good agreement among the indices and Nonmetric Multi-Dimensional Scaling (NMDS) carried out on species, distinguishing an upper course with good environmental quality and a lower part of the riverine system with lower environmental quality. This division corresponds to the CORINE Land Cover class of the sites. Mann–Whitney test showed that ordination of sites was differentiated more by terrestrial than aquatic indices. Agreement among indices and species ordination highlighted the fact that bioindicators were related to habitat quality as a result of the multiple ecosystem interactions among the biotic components of the ecosystem.

Belmar *et al*. (2013) studied the effects of flow regime alteration on fluvial habitats and riparian quality in a semi-arid Mediterranean basin. The relationships between flow regime and fluvial and riparian habitats were studied at reference and hydrologically altered sites for each of the four types. Flow regime alteration was assessed using two procedures: (1) an indirect index, derived from variables associated with the main hydrologic pressures in the basin, and (2) reference and altered flow series analyses using the Indicators of Hydrologic Alteration (IHA) and the Indicators of Hydrologic Alteration in Rivers (IAHRIS). Habitats were characterized using the River Habitat Survey (RHS) and its derived Habitat Quality Assessment (HQA) score, whereas riparian condition was assessed using the Riparian Quality Index (RQI) and an inventory of riparian native or exotic species. Flow stability and magnitude were identified as the main hydrologic drivers of the stream habitats in the Segura Basin. With the indirect alteration index, main stems presented the highest degree of hydrologic alteration, which resulted in larger channel dimensions and less macrophytes and mesohabitats. However, according to the hydrologic analyses, the seasonal streams presented the greatest alteration, which was supported by the numerous changes in habitat features. These changes were associated with a larger proportion of uniform bank top vegetation as well as reduced riparian native plant richness and mesohabitat density.

Strauch *et al*. (2009) conducted a study on the impact of livestock management on water quality and stream bank structure in a semi-arid ecosystem of Zimbabwe. In their study, they examined riparian ecosystem structure and water quality to compare the environmental impact of this management to nearby communal lands during a drought. The results demonstrated that concentrating livestock on ephemeral stream standing pools resulted in reduced water quality and altered riparian ecosystem structure. These results were not significantly different from what was observed when wildlife utilized similar water resources without livestock influence. They concluded that when water is scarce, such as during extreme droughts, livestock usage of surface water resources must be weighed against community water needs.

Miller *et al.,* (2010) conducted a study on the influence of stream bank fencing with some cattle crossing on riparian health and water quality of the Lower Little Bow River in Southern Alberta, Canada. They conducted a four-year study (2004–2007) on a fenced 800-m reach of the river. Their hypothesis was that riparian health would be improved by streambank fencing, and that cattle exclusion would prevent water pollution within the fenced reach. Physical, chemical, and microbiological variables in the river were determined throughout the four years, and water quality variables at the upstream (control) and downstream (BMP-impact) sites during the post-BMP phase were evaluated using a paired t-test. The overall health of the riparian area, based on a visual assessment of vegetative, soils, and hydrologic features, was improved from a score of 65% (healthy but with problems) for pre-BMP phase in 2001 to 81% (healthy) for post-BMP phase in 2005. The majority of water quality variables were not significantly ($P > 0.10$) different at the downstream and upstream sites during stream bank fencing. The evidence from their study indicated that streambank fencing improved the riparian health, and that the BMP prevented the majority of water quality variables from increasing downstream.

2 .6 Southern Africa

2.6.1 South Africa

In South Africa, the South African Scoring System (SASS) is currently used for assessment of river health looking at the resident aquatic biota as well as water quality. The SASS was developed by Chutter (1998) but was later modified by Dickens and Graham (2002) as Version 5 and it is now generally used for Biological Monitoring Working Party (BMWP) (Bere and

Nyamupingidza, 2014; Gordon *et al*., 2015). In the SASS macroinvertebrates are assigned pollution tolerance level from 1 to 15, where 1 indicates highly tolerant to pollution and 15 indicates non tolerance to pollution. The results are later expressed as index score and average score per recorded taxon (ASPT) (Fourie *et al*., 2014). The DWAF (2008) provided a summary of the relationship (Figure 2.2) between important biological indicators and how they describe the condition of the environment. From Figure 2.2 solid lines indicate a strong relationship between parameters while broken lines show a weak relationship. An interesting point is that there is a strong relationship between invertebrates, fish, habitat integrity and ecosystem function and integrity. All the indices that have been mentioned in Figure 2.2 are used to assess river health in South Africa. According to DWAF (2008) the minimum suit to be used for River Health Program includes macroinvertebrates, fish, riparian vegetation and habitat integrity. These variables were considered for the assessment of the Nzhelele River health status except the fish variable because the state of the river at that time did not support fish due to the low water quantities.

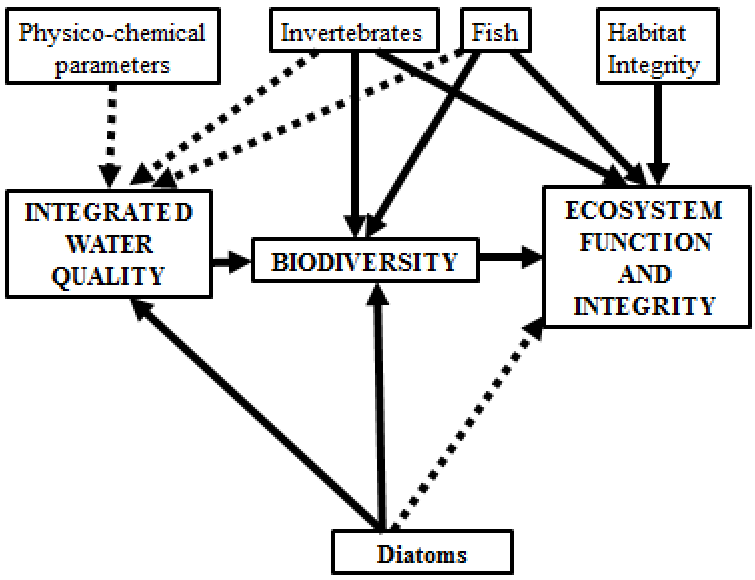


Figure 2.2 Relationship between biological factors (DWAF, 2008)

Rivers-Moore and Jewitt (2007) conducted another study on adaptive management and water temperature variability within a South African river system. Results showed that under broad scenarios of a 10% reduction in mean daily flow rates, or a 1°C increase in mean daily air temperatures, system variability was likely to increase relative to reference conditions.

Oberholster *et al*. (2010) studied the relationship between water quality and phytoplankton community within Lake Loskop during the late summer and autumn of 2008 to evaluate the impacts of acid mine drainage and high nutrient concentrations. The high nutrient concentrations (nitrogen: 17 mg.L and orthophosphate: 0.7 mg .L) during the mid-summer peak of rainy season were associated with the development of a bloom of the *Microcystis* species. Water quality data associated with the development of the *Microcystis* bloom suggested that the aquatic system of Lake Loskop had entered an alternate, hypertrophic regime. This change overshadowed the adverse effects of high concentrations of heavy metals, ion and low pH. High pH values were also recorded. The response of phytoplankton bioassays on integrated water samples from different sampling sites provided potential answers to the reasons for the absence of the algal group, *Chlorophyceae* in the phytoplankton community structure in the riverine zone of the lake.

Slaughter (2011) modelled the relationship between flow and water quality in South African Rivers. His study area was the Buffalo and Bloukrans Rivers and the QUAL2K model was used to assess water quality of the two rivers. Flow and water quality were investigated using the Department of Water Affairs (DWA) historical monitoring data. All data sets were collected when there was very little flow in the Buffalo and Bloukrans Rivers. Water quality of the point sources identified varied dramatically for some parameters. Nitrate concentrations in the King Williams Town waste water treatment works (WWTW) and Zwelitsha WWTW varied dramatically over the three sampling dates and dissolved oxygen (DO) varied greatly at the Zwelitsha WWTW over three sampling sites. The QUAL2K model appeared to have estimated DO and nitrate concentrations fairly accurately, with the model closing matching the first confirmation data set. It was suggested that the QUAL2K model is unlikely to be used routinely in South Africa to manage water resources because of the complexity of the model and high data requirements.

CHAPTER THREE: RESEARCH METHODOLOGY

3.1 Introduction

This chapter provides an overview of the methods that were used to collect data from the selected areas along the Nzhelele River. All data collection and analysis methods have been explained in this chapter. Sampling sites and sampling protocols have also been described thoroughly in this chapter. All instruments and units of measurements have also been described. The data that was collected included data on macroinvertebrates, water quality parameters and stream habitat integrity. The data was collected from six sampling sites named Dopeni, Fondwe, Maangani, Mphaila, Musekwa and Pfumbada. All these sampling sites were located upstream of the Nzhelele Dam. A brief description of these sampling sites as also been provided in this chapter.

3.2 Research design

The study adopted a mixed method-study approach because it was partly experimental, descriptive and correlational. This is because habitat evaluation was described based on observation over a period of 4 months (March to June, 2016). The relationships between macroinvertebrate communities, physico-chemical properties of water and habitat quality and habitat conditions as well as water quality parameters made the study correlational. The experimental approach involved the analysis of water samples while the identification of macroinvertebrates at family level was also descriptive.

3.3 Data collection

3.3.1 Field observation

Before the actual data collection in the field was undertaken, a reconnaissance survey was conducted between December 2014 and February 2015. The purpose of the field observation was to identify areas where data could be collected and also to conduct a general assessment of the environmental conditions and settings of the study area. This was done in order to identify suitable sampling sites from where data would be collected. Sampling points were identified and their locations were saved into a Trimble Juno SB GPS. Six sampling sites were identified based on their uniqueness and physical characteristics. The six sites were identified as Fondwe, Dopeni, Ha-Mphaila, Pfumbada, Maangani and Musekwa (Figure 3.1).

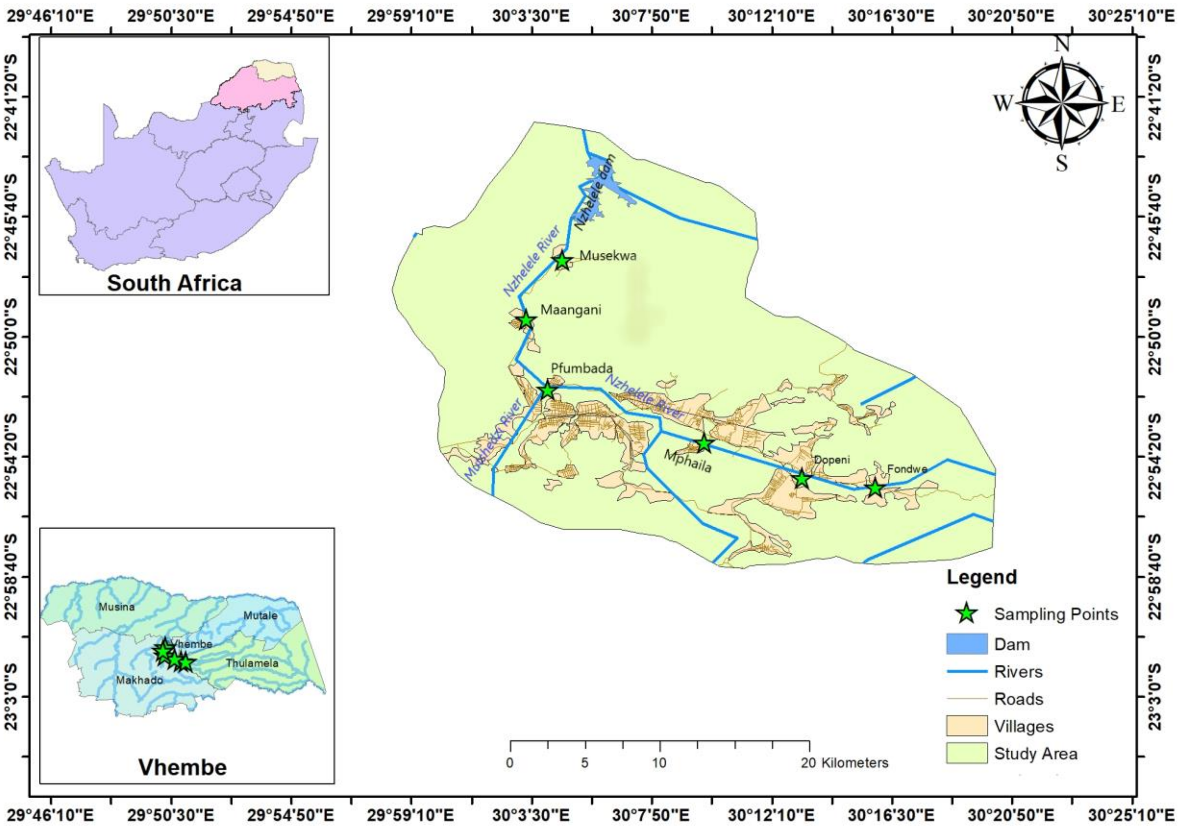


Figure 3.1 Sampling sites along the Nzhelele River

Fondwe lies at 22° 55’ 26” S and 30° 15’ 55” E. The sampling site has been named Fondwe because it lies very close to the village called Fondwe. It lies approximately 4.65 km upstream of the Dopeni sampling site. The sampling area is characterised by low flow, highly vegetated with stones making up the riverbed in some areas. The area is characterised by loose rocky bed characterised by riffles while in some areas the river is characterised by alcove conditions. It lies downstream of the Komatiland Plantation (pine trees) with some visible land-use activities such as subsistence farming in its vicinity. However, at certain points of the river community members use the river for laundry. Its flow runs from east to west. This sampling area lies downstream of the point where the river has its source. Its location downstream of the Komatiland Plantation (forestry) suggests that activities from the plantation and subsistence farming may lead to increased levels of nutrients and other organic material that might lead to cultural eutrophication. The average width of a river at Fondwe was 6.4 meters.

Dopeni lies at 22° 55’ 06” S and 30° 13’ 15” E. It lies at the foot of Dopeni and Mandala villages and the Nzhelele River lows parallel to a number of subsistence farms belonging to the local community members. The sampling site lies approximately 4.65 km downstream of Fondwe sampling site and approximately 7.75 km upstream of Mphaila sampling site. The average width of the river at this sampling site was 8.6m. The Nzhelele River at this point flows parallel to the R523 road. Many cultural activities like sand mining, washing, brick making and baptism take place along this section of the river. The riverbed is largely sandy with noticeable stone bed along some sections of the river. Solid waste disposal was evident along some parts of the river. The banks are permanently covered with thick grass of *Trianda themeda* which acts as a buffer to surface erosion from the mainland. This section of the river flows in a westerly direction. Flow was generally lower at the time of research. The site was chosen because there were many cultural activities taking place and assessing the overall river health at this point was of prime importance.

Mphaila sampling site lies at 22° 53’ 49” S and 30° 09’ 45” E. The sampling site lies just immediately to the north-east of Ha-Mphaila village. Current developments indicate that the area has been earmarked for cultural activities such as subsistence farming. Some sections adjacent to the river have been fenced while other areas have been cleared for future human activities. The river bed is characterised by loose rocks while the river banks are characterised by tall grass. Tall trees provide shade to large parts of the river and flow is moderately low. This section of the river also runs from east to west. The presence of modified banks next to the bridge, agricultural activities and irrigation pipes made the site to be a priority for assessing overall river health. The average width at this sampling site was 12m.

Pfumbada lies at 22° 51’ 54” S and 30° 04’ 06” E. This section of the Nzhelele River lies to the northeast of the Pfumbada village and the river flows from east to west. This sampling site lies approximately 10.85 km downstream of Mphaila sampling area. There were no visible land use activities at the sampling site except for cattle grazing and washing by local community members. Some parts of the river banks lacked vegetation while others were covered by grass and small shrubs. It was characterised by small rocks that covered larger section of the riverbed at some points. At some points the banks had been steeply carved by water and were inaccessible to livestock and humans. Flow was extremely low along the river. At this point the river meanders to create changes in velocity and bank structure. The average width of the river at this sampling site was 7.9m.

Maangani lies immediately to the east of Maangani village at 22° 49’ 22” S and 30° 03’ 19” E. It lies approximately 5.43 km downstream of Pfumbada sampling site. Its average width was 26.4m. This section of the river flows from south to north and the riverbed is a stepped solid rocky surface that stretches from one side of the bank to the other. The banks are vegetated and are very steep. Vegetation along the banks includes trees, grass and shrubs. Flow is moderately low. Washing or laundry and agricultural activities seemed to be dominant in the area and pipes for drawing water from the river were also visible. This section of the river is rich in algae that hinders the visibility of some parts of the riverbed.

Musekwa lies at 22° 47’ 14” S and 30° 04’ 38” E. It lies approximately 4.65 km downstream of Maangani sampling site. The average width of the river at this side was measured as 11.5m. The riverbed is composed of gravel and sand with numerous subsistence farming plots along the river. Water extraction pipes are visible in some parts of the river and livestock watering is intense. This sampling site lies approximately 200 m from the Musekwa village. The river banks are gentle and cover is not continuous. This section of the river lies approximately 500m from the Nzhelele Dam. Its flow is north-easterly and flow is also low at this section of the Nzhelele River.

3.3.2 Primary data collection

Data for different water parameters was collected monthly between February and December of 2016. The length of the river between the sampling areas was 33.3 km. Data that were collected *in situ* along the Nzhelele River included water quality parameters such as pH, water temperature, electric conductivity (EC), TDS, stream velocity and dissolved oxygen. Water samples were also collected for analysis of nitrates, nitrites and chlorine. Field survey also included the collection of macroinvertebrates and habitat integrity evaluation. Data could not be collected and interpreted seasonally because there were very few macroinvertebrates that were collected each day of the month per sampling site because some seasons (winter and spring) were characterised by extremely low water and few patches of water due to 2015/2016 drought.

Data were collected monthly during the months of February and December of 2016 (early autumn to early-summer) because this was the period when rainfall was at its lowest and allows macroinvertebrates to settle in a river. Data could not be collected during the rainy season from January to February because some of the macroinvertebrates that would have been sampled could have been those which were washed from upstream and not belonging to the area. This could have made the results not to be representative of the macroinvertebrate assemblages of sampled sites because samples would have included macroinvertebrates that have been brought into the sampled site by river runoff. According to the DNR (2000), fall or autumn sampling allows for easy detection of the extent of organic enrichment problems which are associated with low dissolved oxygen (DO). This is why the sampling was done between autumn and early-summer. Akaahan (2014) also noted that macroinvertebrate diversity is higher during dry season because of low wash-off effects. Therefore, it was considered appropriate to collect data when rainfall was lower or absent. Again, the sampling period was kept as short as possible because there was a gradual decrease in river water quantity and flow due to the effect of 2015/ 2016 drought. Hill *et al*. (2016) have confirmed through their study that sampling in autumn had greater macroinvertebrate richness than in spring and summer. Therefore, it was absolutely necessary to keep the sampling period shorter because by November 2016 most sections along the river had begun to decrease in flow velocities due to 2015/2016 drought.

3.3.2.1 Macroinvertebrate sampling

A 40 cm D-frame kick net with mesh size of 500 microns was used to sample macroinvertebrates. Stark *et al*. (2001) have recommended a 500 microns net size because it is sufficient for most biomonitoring purposes. Samples were collected from each sampling site at an interval of 10 metres within a reach of 150 metres for each sampling site. This 10m interval points along a 150m reach from each sampling site were referred to as sampling points. This means that all six sampling sites that were chosen were sampled fifteen times at an interval of 10m. This means that all the six chosen sampling sites were sampled 90 times per visit. Sampling sites were visited once per month from February to November 2016. The macroinvertebrates were sampled between 10H00 and 16H00 on sunny days when biological activity was very high.

The macroinvertebrates were sampled within an area of 2 m^2 at each interval point across the river, but care was taken not to sample outside the selected area. The sampling points were carefully marked off with four rods that covered an area of 1 m x 2 m and samples were collected in each area for a maximum of 2 minutes. This was done to avoid catching macroinvertebrates that could have floated from upstream into the sampling points. A stop watch was used to regulate time during sampling. The D-net was placed downstream of the river flow to collect samples that were dispatched from river surface through kicking the bottom and small rocks.

Kick-sampling method (Dickens and Graham, 2002) was used only where the riverbed was lightly disturbed upstream and macroinvertebrates were collected using the D-net. This was done to release some of the macroinvertebrates that could have hidden beneath the rocks. This method was used in areas where **riffles** were present. In areas containing **aquatic plants** sampling was done by carefully shifting vegetation and scooping the area.

The samples were then emptied into a clean tray with clean water for easy separation from grass, leaves and twigs. After separation was done the macroinvertebrates were stored in a l.5 litre plastic container containing 70% ethanol. The ethanol was used to conserve the macroinvertebrate for longer periods. All containers were clearly marked to avoid mixing the samples. The labelling on the containers included information such as name of the site, sample month and habitat type (e.g. riffle, alcove).

The macroinvertebrates were later identified in the laboratory to family level to categorise them in terms of pollution tolerance levels and the total numbers for all organisms per sampling area were then recorded. A magnifying glass was also used to identify some of the macroinvertebrates that were difficult to identify through a naked eye. Such macroinvertebrates included the tiny larvae and nymphs. Samples were stored for a period of two weeks before sorting. Abundance measures were done during the sorting process in order to determine the total number of macroinvertebrates per sampling site. Abundance measures were considered in terms of Percent Contribution of Dominant Family because the sampled macroinvertebrates that were collected seasonally were very few and the use of other indices such as EPT Index or Ratio of EPT and Chironomidae Abundances would have been inefficient in this study

The pollution tolerance levels of the sampled macroinvertebrates were interpreted based on the guidelines provided by Gerber and Gabriel (2002) as follows:

- 1-5: Highly tolerant to pollution
- 6-10: Moderately tolerant to pollution
- 11-15: Very low tolerance to pollution

The pollution tolerance score was provided by a line graph which indicated the actual pollution tolerance level for each organism. Figure 3.2 shows the tolerance scale similar to the one provided by Gerber and Gabriel (2002):

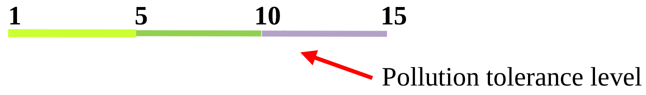


Figure 3.2 Pollution tolerance levels

The pollution tolerance levels were also confirmed or verified from the South African Scoring System Version 5 (SASSV5) protocol as described by Dickens and Graham (2002).

3.3.2.2 Water quality parameters

Water samples were collected at the same 15 sampling points where macroinvertebrate samples were collected. This was done in order to ensure that the water quality parameters that were measured were representative of the points where macroinvertebrates were collected. Water samples were collected the very same day that macroinvertebrate sampling was done. Samples were collected in 500ml glass bottles that were prewashed with hydrochloric acid and stored for a day before collection of water samples. The samples were collected for the analysis of nitrates, nitrites and chlorine. Other water quality parameters that were measured *in situ* included pH, water temperature, conductivity, river velocity and dissolved oxygen. Water temperature, pH, conductivity and Total dissolved solids (TDS) were measured using the hand-held multi-parameter metre. The probe of the instrument was submerged under water and readings were recorded only after the readings on the instrument had stopped counting. Readings were then recorded in a fieldwork book and later exported to excel spreadsheet for analysis.

pH was measured on a scale of 1 to 14 where 7 represented neutral and any value less than seven was considered acidic and values above 7 were considered alkaline. **Stream temperature** was measured in degrees Celsius (°C). Temperature was also measured between 10H00 and 16H00. **Electrical conductivity** (EC) was measured in μm/cm. **TDS** was measured in mg/l. **Dissolved oxygen** was measured as percentage of air saturation using Jenway 970 DO_2 meter. Dissolved oxygen was measured in the morning between 05H30 and 06H00 as required by guidelines set out by DWAF (1996). This was done in order to obtain the correct oxygen concentration as it decreases as temperature increases. Therefore, measurements were done before sunrise before biological activity became intense.

To measure other water quality parameters (nitrates, nitrites and chlorine) samples were immediately transported to the University of Venda laboratory (Department of Ecology and Resource Management) for further analysis using the Metrohom 850 Chromatograph that was equipped with two columns namely Metrosep A Supp 5-100/4.0 with flow rate of 0.7 mL/min and Metrosep C4-250/4.0 with low rates were and 0.9 mL/min for anion and cation, respectively. For the analysis of anions carbonate eluent was used and for the cations, dipiclonic acid eluent was used. Prior to analysis, the column was equilibrated for 60 minutes. Other water quality parameters such sulphates and phosphates were not measured because there were no visible effluent discharges from commercial agriculture and mining activities along the Nzhelele River where samples were collected.

For **sample preparation** four (4) stands of 1ppm, 5ppm, 10ppm and 20ppm were prepared by appropriate dilutions from 1000 ppm multi-component standard of analytical grade using ultra-pure water (18.2 MΩ/cm). All samples were filtered through 0.25 syringe filter before analysis.

3.3.2.3 Environmental data

For measurement of environmental data such as river velocity and discharge a Model FP 111 flow probe that measured velocity and depth was used. Stream velocity was measured in $m.s^{-1}$. In order to be precise about discharge, velocity measurements across the river were measured at an interval of 50 centimetres (cm). This means that depths across the river were measured at an interval of 50 cm using centimetres as units of measurement. The measurements were later converted to metres. To calculate discharge, depth measurements were multiplied by width (50 cm = 0.5 m) and discharge for every point at an interval of 50cm (0.5m) was then recorded in a fieldwork sheet. This means that the cross-sectional area for each 50cm interval was calculated and the velocity at that point was also measured. The individual discharges per cross-sectional area across the river were then added together to give the average discharge of the river at a selected point at intervals of 10m. This means that discharge and velocity records were also measured fifteen times at an interval of 10 metres to cover the total length of 150 m per sampling site. Velocity and discharge were measured at the same points were macroinvertebrate and water samples were collected.

The degree of **environmental degradation** along the Nzhelele River was interpreted based on Index of Habitat Integrity as defined by Kleynhans *et al*. (2008). Since the river traversed a number of villages it was not surprising that many sections of the river were characterised by cultural activities such as subsistence farming, livestock watering, water extractions and ritual activities. These activities seemed to have had direct and indirect impact on the habitat quality and therefore accounted for macroinvertebrate assemblages at any given time. For example, Van Rensburg (2012) has observed that the presence of bridges increases the rate of riverbed erosion. The Index of Habitat Integrity, as proposed by Kleynhans *et al*. (2008) takes the quality of instream and riparian habitat index values into consideration (Table 3.1). Criteria that were used to assess habitat integrity of the selected points of the Nzhelele River were based on the following table adapted from Kleynhans (1996) (Table 3.1).

Table 3.1 Criteria used in the assessment of habitat integrity (Kleynhans, 1996)

Criterion	**Relevance**
Water abstraction	Measurement of direct impacts on the type of habitat, riparian vegetation, size, abundance, water flows, bed, water quality and channel characteristics.
Flow modification	Focuses on the impacts of water abstraction and regulations through impoundments, which lead to alterations in temporal and spatial flow characteristics (duration and season of low flows, reduction of heterogeneous habitats and water.
Bed modification	Regarded to be the result of increased sediment input from the catchment or decreased ability for sediment transport in a river (Gordon *et al.,* 1992). Indicators of sedimentation include catchment and bank erosion. Deliberate streambed alteration results from removal of rapids for navigation (Hilden & Rapport, 1993).
Channel modification	Caused by changes in river flow which lead to changes in channel characteristics. This leads to alteration of marginal instream and riparian habitat. It is also caused by deliberate modification of channel to improve river.
Water quality modification	This results from point and diffuse point sources such as human settlements, agricultural activities and industrial activities. Water quality modification is also worsened by a decrease in the volume of water due to low or no flow conditions.

Criterion	Relevance
Inundation	This is caused by obstruction of aquatic fauna movement or migration which affects water quality and sediment movement. Destruction of rapids, riparian zone habitat and ripples also lead to inundation (Gordon *et al.*, 1992).
Exotic macrophytes	This depends on the type of invasive species and the magnitude of infestation. Obstruction of flow due to habitat alteration may impact on water quality.
Exotic aquatic fauna	The disturbance of the stream bottom during feeding may influence the water quality and increase turbidity. Depends on the type of invasive species and their nature of disturbance. Streambed disturbance during feeding may also influence water quality of a stream and increased turbidity.
Solid waste disposal	This results from direct anthropogenic impact may lead to structural changes in habitats. Solid waste disposal also manifests the misuse and mismanagement of the river.
Indigenous vegetation removal	This involves the impairment of vegetation as the buffer zone which prevents sediment movement and other catchment runoff products into the river (Gordon *et al.*, 1992). Involves physical eradication of vegetation due to fuelwood collection, farming purposes and overgrazing.
Exotic vegetation encroachment	This excludes the encroachment of natural vegetation into riparian zones due vigorous growth leading to instability of banks and reduction in the buffering function of the riparian zone. This leads the reduction in the diversity of the riparian zone habitat. A change in the input of allochtonous organic matter input will also occur.
Bank erosion	Decreased bank stability leads to river sedimentation and possible river bank collapse. This will result in loss or modification of both instream and riparian habitats. Accelerated erosion can result from removal of natural vegetation, exotic vegetation encroachment or overgrazing.

Descriptive class that were used to assess modifications to habitat integrity were also adapted from Kleynhans *et al*. (2008) (Table 3.2):

Table 3.2 Descriptive classes for the assessment of modifications to habitat integrity (Kleynhans *et al*., 2008)

Impact class	Description	Score
None	No noticeable impact or modification that can be located to have discernible impact on habitat size, quality, variability and size.	0
Small/ Minimal	The modification exists in very few localities and there is very small impact on habitat size, quality, variability and diversity.	1-5
Moderate	The modifications exist at very few localities and there is limited impact on habitat size, quality, variability and diversity.	6-10
Large	The modification is largely present, but does not cover large areas. There are however, noticeable detrimental impacts on habitat size, quality, variability and diversity	11-15
Serious	The modification occurs frequently and covers large areas. There are noticeable detrimental impacts over a large area on habitat size, quality, variability and diversity	16-20
Critical	There is high intensity modification. There are noticeable detrimental impacts over the entire area on habitat size, quality, variability and diversity.	21-25

3.4 Data analysis

Descriptive statistics and Principal Component Analysis (PCA) were used to analyse data from the field. Data for macroinvertebrates per sample area were firstly depicted at abundance levels using line and bar graphs while stream temperature, pH, conductivity, nitrates, nitrites, chlorines and environmental data were also compared per sample area. All physico-chemical parameters were compared with the South African Target Water Quality Range (TWQR) as defined by water quality guidelines by DWAF (1996).

To compare the diversity of species across all six sampling areas the Simpson Diversity Index was used. The diversity index was calculated based on the following formula:

$$\text{Simpson Diversity Index (D)} = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

Where $\sum$ = sum of (total)

n = the number of individuals of each different species

N = the total number of individuals of all the species

Diversity index was considered important because it gives more weight to the dominant species while considering that rare or few species will not affect the diversity that is being measured. It was therefore important to compare the six areas because even if an area had more species than others that did not mean that such an area was diverse. Therefore, only the diversity index such as the Simpson's Diversity Index could show such a diversity among the six sampling areas in addition to the abundance values that were obtained directly from the field.

PCA was used to analyse all the results from all sampling sites. This was done in order to determine the relationship between measured variables. This is because PCA helps to identify patterns of data by showing relationships between them and how they similar to one another. It also helps to determine whether variables are correlated and whether the correlation is weak or strong. Therefore, PCA shows strength of relationships between variables where +1 indicates perfect positive linear relationship and -1 indicates perfect negative linear relationship and zero (0) indicates existence of no relationship between variables. With PCA the dimensionality of data is reduced to create new sets of data called principal components. Smith (2002) defined PCA as a way to identify patterns in data in order to identify similarities and differences in data. PCA covers standard deviation, covariance and eigenvectors in data set (Karamizadeh *et al*., 2013). Biplots were used to show the relationship between water quality parameters and sampling sites. This was done to establish whether the relationships between water quality parameters and sampling sites were uniform and strong or whether the relationships differed from one sampling site to another.

3.4.1 ANOVA analysis

One way ANOVA was used to analyse the effects of aquatic substrate on different types of macroinvertebrates. The ANOVA was used to show the differences between the means of the macroinvertebrate from different sampling sites and differences between water quality parameters amongst the six sampling sites. This is because substrate characteristics of the sampling sites differed and therefore, the water quality parameters and macroinvertebrates abundances will be affected by substrate characteristics and the types of land uses. Therefore, *F*-tables were used to test hypotheses about different groups from the study sampling sites. The macroinvertebrates were collected under different habitat conditions due to substrate characteristics. The hypotheses to be tested were as follows:

The first null hypothesis stated that there was no difference between the six sampling sites in terms of macroinvertebrate abundances:

H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu$

The alternative hypothesis stated that there were significant differences in abundance values of the six sites

H_1: not H_0

The second null hypothesis stated that there was no difference between the values of water quality parameters from the six sampling sites:

H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu$

The alternative hypothesis stated that there were significant differences amongst the values of the water quality parameters from the six sampling sites

H_1: not H_0

The level of significance for the two tests was chosen as $\alpha = .005$

The degrees of freedom were determined as follows:

$df_{betw} = k - 1$, where k represented number of columns in a data set.

df_{betw} was calculated when comparing the means of a set of k groups. This means that the between-comparison was done for k groups.

$df_{with} = n - k$, where n represented the total number of scores of all the groups together.

This was done to evaluate the degrees of freedom of the within-comparison by assessing the degrees of freedom of each single group and added them all.

The critical value (F_{crit}) was determined after calculating the degrees of freedom and was determined from the F-Table.

The summary of the ANOVA was summarised in a table of results as follows:

Source of variation	**Degrees of freedom**	**Sums of Squares**	**Mean Squares**	**F**
Between group or treatment	$df_{betw} = k - 1$	SS_{betw}	$\frac{SS_{betw}}{k - 1}$	$MS_{betw} \div MS_{with} = F_{obs}$
Within groups or error	$df_{with} = n – k$	SS_{with}	$\frac{SS_{with}}{n - k}$	
Total	$Df_{tot} = n – 1$	SS_{tot}		

Where SS_{with} represents the sum of squared deviations within groups and SS_{betw} represents the sum of squared deviations between group means.

MS represents Mean Squares for each group (between and within groups). These were determined by diving each SS by its degrees of freedom. These were determined as follows:

$$MS_{with} = SS_{with} \div df_{with}$$

$$MS_{betw} = SS_{betw} \div df_{betw}$$

The F-ratio (F_{obs}) was determined by dividing MS_{betw} by MS_{with}.

Conclusions of the F-tests were based on the following rule:

If *p-value* < *significance value* (0.05), then the null hypothesis (H_0) is rejected.

If *p-value* ≥ *significance value* (0.05), then the H_0 is not rejected.

CHAPTER FOUR: DATA PRESENTATION AND DISCUSSION

4.1 Introduction

This chapter presents data on the abundance of macroinvertebrates and relationships between macroinvertebrate abundance and the selected water quality parameters. Relationship between macroinvertebrate abundance and physico-chemical properties of water has been analysed using one-way ANOVA. Abundance values per sampling site were compared and conclusions were drawn from the interpretation of abundance and ANOVA results. Biplots were used to show the relationships between water quality parameters and resident macroinvertebrates. Biplots were also used to show the relationships between water quality parameters and sampling points from six sampling sites.

4.2 Macroinvertebrate family abundance

Table 4.1 shows abundance values of the macroinvertebrates from the six sampling sites, namely, Dopeni, Fondwe, Maangani, Mphaila, Musekwa and Pfumbada. The results show that all six sampling sites had eight (8) orders with nine (9) different families of macroinvertebrates that were sampled. The total number of macroinvertebrates across all six sampling sites was 674. Maangani had the highest number of macroinvertebrates (178) while Musekwa recorded the lowest number of macroinvertebrates of 72. The family Thiaridae (193) were the most abundant and were found in all areas except Mphaila. This family recorded the highest abundance value of 76 at Maangani. The presence of the family Thiaridae indicated polluted environments because this family of macroinvertebrates are highly tolerant to pollution.

Table 4.1 Macroinvertebrate abundances (totals) from sampling sites (Field data, 2016)

Family	Dopeni	Fondwe	Maangani	Mphaila	Musekwa	Pfumbada	Total
Aeshnidae	29	25	35	28	18	30	165
Chironomidae	0	17	0	6	0	9	32
Coenagrionidae	0	30	28	0	0	0	58
Ecnomidae	0	0	0	17	0	0	17
Elmidae	24	0	0	13	11	7	55
Heptageniidae	30	0	30	41	0	21	122
Nepidae	0	6	9	0	0	7	22
Potamonautidae	0	0	0	0	10	0	10
Thiaridae	21	25	76	0	33	38	193
Total	**104**	**103**	**178**	**105**	**72**	**112**	**674**

The family Aeshnidae (Odonata) (165), which included dragonfly and damselfly nymphs were common across all sampling sites, with Maangani recording the highest abundance value of 35 and Mphaila the lowest abundance value of 18. Based on the South African Scoring Systems 5 (SASS5) as depicted by Dickens and Graham (2002), Aeshnidae are moderately tolerant to pollution. This means that they survive in moderately polluted water of lotic environments. The pollution tolerance level that was based on the SASS5 according to Gerber and Gabriel (2002) was found to be 8, which was described by SASS5 as moderately tolerant to pollution. This means that some sections of the Nzhelele River were moderately polluted. However, this condition was likely to change from one season to another due to changes in environmental conditions. Table 4.2 below shows different macroinvertebrate families and their pollution tolerance levels that were based on the SASS5.

Table 4.2 Pollution tolerance level of sampled macroinvertebrate (Field data, 2016)

Family name	**Order/ Class**	**Abundance values**	**Percent (%)**	**Pollution tolerance level**
Aeshnidae	Odonata	165	24.5	Moderately tolerant (8)
Chironomidae	Diptera	32	4.7	Highly tolerant (2)
Coenagrionidae	Odonata	58	8.6	Highly tolerant (4)
Ecnomidae	Trichoptera	17	2.5	Moderately tolerant (8)
Elmidae	Coleoptera	55	8.2	Moderately tolerant (8)
Heptageniidae	Ephemeroptera	122	18.1	Low tolerance (13)
Nepidae	Hemiptera	22	3.3	Highly tolerant (3)
Potamonautidae	Decapoda	10	1.5	Highly tolerant (3)
Thiaridae	Gastropoda class	193	28.6	Highly tolerant (3)
Total		**674**	**100**	

The family Heptageniidae (Ephemeroptera) (122) were also common across all sampling sites except at Fondwe and Musekwa. However, the family Heptageniidae were found to be many at Mphaila with an abundance value of 41 (Table 4.1). Mphaila also recorded a total of 105 macroinvertebrates, which was the third highest value after Maangani (178) and Pfumbada (112). However, the Heptageniidae was the dominant macroinvertebrate family at Mphaila. The tolerance level of Heptageniidae was found to be low with a score of 13. This implies that during the time of sampling some sections of the Nzhelele River had lower pollution levels and supported macroinvertebrates that did not tolerate pollution such as flat headed mayfly nymphs.

Unlike the Aeshnidae family that constituted 24% of the total sampled macroinvertebrates, Heptageniidae constituted 18% of the total sampled macroinvertebrates.

The lowest abundance values for all sampled macroinvertebrates were for the Potamonautidae (Decapoda) (10) and were recorded at Musekwa. The family Potamonautidae (crabs) have a pollution tolerance level of 3, indicating that they are highly tolerant to pollution. Musekwa also recorded the lowest macroinvertebrate abundance value of 72 compared to all other sampling sites. Table 4.2 above shows that Potamonautidae are highly tolerant to pollution. Their presence signified a polluted stream with low water quality conditions. The family Potamonautidae only constituted 1.5% of the total sampled macroinvertebrates. The Musekwa sampling site was characterised by high human activity such as laundry, water abstraction and livestock watering. This might be a factor towards polluted water which favoured the presence of Potamonautidae. Thiaridae (Gastropoda) (193) were also found at Musekwa sampling site and this family of macroinvertebrates favours polluted waters, and their pollution tolerance level is higher (3). The presence of Chironomidae, Coenagrionidae, Nepidae, Potamonautidae and Thiaridae families would mean that the river is polluted as these families are highly tolerant to pollution. Thiaridae recorded the highest number of all sampled macroinvertebrates from all six sampling sites.

Family Nepidae (Hemiptera) (water scorpions) were only found at Fondwe (6), Maangani (9) and Pfumbada (7). This family of aquatic insects is highly tolerant to pollution with a pollution tolerance score of 3. However, Nepidae accounted for just 3% of the total sampled macroinvertebrates. The total number of sampled Nepidae (water scorpions) was 22 from all six sampling areas.

The family Ecnomidae (Trichoptera) (caddisflies) were only found at Mphaila with an abundance value of 17. The pollution tolerance level for this family is 8 which means that they are moderately tolerant to pollution. The presence of caddisflies indicated that some sections of the river at the point where they were collected were moderately polluted. The family Potamonautidae was also restricted to Musekwa area. However, their presence also indicated that some sections of the river were polluted because Potamonautidae family can tolerate polluted environments (Table 4.2).

The family Chironomidae (Diptera) and Coenagrionidae (Odonata) accounted for 4.7% and 8.6% respectively of the total number of all sampled macroinvertebrates. The two families are highly tolerant to pollution with pollution tolerance score of 2 and 4 respectively. The family Chironomidae (midges) was found at Fondwe, Mphaila and Pfumbada while the family Coenagrionidae (damselflies) existed at Fondwe and Maangani. Jacob *et al.* (2017) indicated that few Coenagrionidae organisms exist in excellent water quality bodies compared to medium and good water quality conditions. Since the water quality at Mphaila might be characterised as excellent due to the high number of pollution-intolerant families the existence of Coenagrionidae was therefore possible but their abundance was lower indicating their low tolerance to excellent water conditions. The family Elmidae, which are moderately tolerant to pollution were found in all sampling areas except Fondwe and Maangani and they constituted 8.2% of the total number of sampled macroinvertebrates. Generally, pollution tolerant organisms constituted 46.7% of the total sampled macroinvertebrates. The remaining 53.3% was for pollution intolerant (18.1%) and moderately tolerant (35.2%) organisms. It could therefore be concluded that pollution intolerant organisms constituted 53.3% of the total number of sampled macroinvertebrates across the six sampling sites.

4.3 Macroinvertebrate abundances (totals) per sampling site

4.3.1 Dopeni

Table 4.3 below shows percentages of abundance values of macroinvertebrates per sampling site. From Table 4.2 above, of the total number of macroinvertebrates that were sampled from the Dopeni site, only the Heptageniidae family (29%) was found to be intolerant to pollution. Their tolerance level to pollution is 13. Macroinvertebrate families that were found to be somewhat tolerant or moderately tolerant to pollution included both Aeshnidae (28%) and the Elmidae (23%) families respectively. This means that approximately half (51%) of the total macroinvertebrates sampled from the Dopeni site were moderately tolerant to water pollution, with only the family Thiaridae (20%) being highly tolerant to pollution. The total percentage of organisms that were moderately tolerant and intolerant to pollution at Dopeni site was 80%. This means that the river water at Dopeni did not support the majority of pollution tolerant organisms. This shows that the river water was moderately polluted, hence the higher percentage of pollution intolerant organisms that made up a total of 80%. This suggested that the existence of agricultural fields along the Nzhelele River at Dopeni area did not significantly impact on the water quality of the river. However, since the samples were collected during low rainfall season direct impacts could not be directly reflected by the resident macroinvertebrates. Odume (2013) has indicated that organic inputs from agriculture often lead to depletion of oxygen which impacts on diversity of EPT group. However, the absence of other EPT organisms could be attributed to the flow conditions of the river at the time of sampling but not to agricultural fields. This explains why only Heptageniidae (Ephemeroptera) which is sensitive to pollution, existed at Dopeni site. An interesting observation by Keke *et al.* (2017) was that the occurrence of Coleoptera, Plecoptera, Trichoptera, Anisoptera and Odonata in aquatic environments showed that the river water quality was good. For Dopeni, Odonata (Aeshnidae) and Coleoptera (Elmidae) were present in addition to Ephemeroptera (Heptagennidae) an indication of good river water conditions at Dopeni which allowed the survival of pollution intolerant organisms.

Table 4.3 Abundance of macroinvertebrates per sampling area (Field data, 2016)

	Abundance values and percentage (%) per sampling area						
Family(Tolerance level)	**Dopeni**	**Fondwe**	**Maangani**	**Mphaila**	**Musekwa**	**Pfumbada**	**Total**
Aeshnidae (**8**)	29 (**28**)	25 (**24**)	35 (**20**)	28 (**27**)	18 (**25**)	30 (**27**)	165
Chironomidae (**2**)	0	17 (**17**)	0	6 (**6**)	0	9 (**8**)	32
Coenagrionidae (**4**)	0	30 (**29**)	28 (**16**)	0	0	0	58
Ecnomidae (**8**)	0	0	0	17 (**16**)	0	0	17
Elmidae (**8**)	24 (**23**)	0	0	13 (**12**)	11 (**15**)	7 (**6**)	55
Heptageniidae (**13**)	30 (**29**)	0	30 (**17**)	41 (**39**)	0	21 (**19**)	122
Nepidae (**3**)	0	6 (**6**)	9 (**5**)	0	0	7 (**6**)	22
Potamonautidae (**3**)	0	0	0	0	10 (**14**)	0	10
Thiaridae (**3**)	21 (**20**)	25 (**24**)	76 (**42**)	0	33 (**46**)	38 (**34**)	193
Total	**104 (100)**	**103 (100)**	**178 (100)**	**105**	**72 (100)**	**112 (100)**	**674**
Tolerance level	**1-5 = Highly tolerant**		**6-10 = Moderately tolerant**		**11-15 Very low tolerance**		

4.3.2 Fondwe

Fondwe area had a total of 103 macroinvertebrates that were sampled. Of the 103 macroinvertebrates 76% were found to be tolerant to pollution except the Aeshnidae family

(24%) which was moderately tolerant to pollution. There were no EPT organisms that were intolerant to pollution. These families were Chironomidae, with tolerance level of 2, Coenagrionidae (4) and Thiaridae (3). The presence of organisms highly tolerant to pollution indicated that the river at Fondwe was polluted. This might be due to the presence of agricultural fields adjacent to the river and the frequent use of the river for laundry and livestock watering. Figure 4.1 below shows the location of typical agricultural fields along the Nzhelele River at Fondwe. Activities along the Nzhelele River at Fondwe were highly associated with cultural eutrophication and only pollution tolerant organisms were present. From Figure 4.1 below it can be observed that the river bank had been modified to prevent bank erosion and the slippage of agricultural soil directly into the river. However, utilisation of the river for laundry, car washes and agriculture could be the possible causes of higher abundance values of pollution tolerant organisms. For example, Morrison and Bohlen (2010) have noted that Diptera organisms such as Chironomids tend to increase in numbers when vegetation is cleared or clipped for purposes of livestock grazing. Since some sections of the river at Fondwe lacked vegetation it might be argued that they partly contributed to the occurrence of Chironomids.

Figure 4.1 Location of an agricultural field at Fondwe (Field data, 2016)

4.3.3 Maangani

Sixty-three (63%) percent of macroinvertebrates sampled at Maangani were highly tolerant to pollution with tolerance levels of 3 and 4 and these were Coenagrionidae (Odonata), Nepidae (Hemiptera) and Thiaridae (Gastropoda) families. Only the Heptageniidae family is sensitive to pollution, but constituted only 17% of the total macroinvertebrates that were sampled at Maangani. Aeshnidae which were moderately tolerant to pollution made up the remaining 20% of the sampled macroinvertebrates at Maangani. This means that some sections of the river at Maangani were not polluted, hence the existence of Heptageniidae family. This was because some sections of the river were inaccessible due to the presence of steep banks that prevented livestock and humans from accessing the river. However, the presence of pollution tolerant organisms might be explained by the close proximity of the Maangani settlement to the river, which was approximately 50 m from the river banks. Just like at Fondwe, residents at Maangani utilise the Nzhelele River water for laundry and agricultural irrigation and there was evidence of water extraction from the river to adjacent agricultural fields. This explains the presence of algae along some parts of the river at Maangani where samples were collected. Chironomid larvae and *Tubifex* tend to increase in abundance due to organic inputs which deprive the aquatic environments of dissolved oxygen but high organic enrichment (Mustapha and Yakubu, 2015). For example, Egler *et al.*(2012) cited nitrogen concentrations as the determining factors in the decline of macroinvertebrate diversity. Since many agricultural activities are associated with organic inputs it is not surprising that algal blooms were visible at Maangani. However, algae has been known to be source of food for macroinvertebrates, including pollution sensitive organisms such as the family Heptageniidae. This explains the presence of these organisms in an area dominated by agricultural activities (Griffin *et al*., 2015) which are associated with pollution tolerant organisms that make up 63% of the total number of macroinvertebrates sampled at Maangani.

4.3.4 Mphaila

Fifty-five percent (55%) of the macroinvertebrates sampled at Mphaila were moderately tolerant to pollution and these included Aeshnidae, Ecnomidae and Elmidae families. Their pollution tolerance level is 8. Only the family Coenagrionidae (6%) were highly tolerant to pollution with the tolerance level of 2. Thirty-nine percent (39%) of the remaining macroinvertebrates (Family Heptageniidae) were highly sensitive to pollution. This means that the river at Mphaila was less polluted compared to all other five sites due to a higher number of pollution intolerant organisms. The pollution level at Mphaila could have been due to the lack of anthropogenic activities at the points where samples were collected. Signs of future development in the form of fencing off areas earmarked for new agricultural fields show that the area will in the near future drastically change its current status. Intensive land uses, more often lead to alteration of the watershed functionality which leads to alteration of stream system (Currinder, 2017). Once anthropogenic impacts such as intense subsistence farming and water abstraction become intense at Mphaila site changes in stream functionality will be inevitable. These changes will automatically alter macroinvertebrate assemblages because even trophic levels would have also changed. The current composition of 94% of pollution intolerant families (Aeshnidae, Ecnomidae, Elmidae and Heptageniidae) suggested that the Mphaila area was still minimally or moderately polluted. Therefore, Mphaila site was found to be the least polluted sampling site along the Nzhelele River where the river transcends villages. This was evident from the number of pollution tolerant organisms that constituted just 6% of the macroinvertebrates sampled from Mphaila sampling site.

4.3.5 Musekwa

Musekwa had the lowest abundance values of macroinvertebrates (72) and 60% of them were found to be highly tolerant to pollution. These were the families Potamonautidae (14%) and Thiaridae (46%) with tolerance level of 3. The dominance of the Thiaridae family organisms could be attributed to the fact that these organisms are known for their ability to colonise quickly and survive under a variety of habitats due to their strong and thick shells (Strzelec and Królczyk, 2004; Flores and Zafaralla, 2012). The remaining 40% of the macroinvertebrates at Musekwa were moderately tolerant to pollution and these included Aeshnidae (25%) and Elmidae (15%) families. The absence of pollution intolerant organisms showed that the water quality of the river at Musekwa had deteriorated to a point where EPT groups were eliminated or could not colonise the area. This might be due to the intense utilisation of the river for water abstractions, laundry, livestock watering and the proximity of the Musekwa village and agricultural fields to the river. Just like at Maangani, residents of the Musekwa village also used the river as a waste dumping area. However, the utilisation of the river for laundry, bathing, livestock watering and location of agricultural fields along the river could have degraded the water quality and favoured pollution tolerant organisms such as the family Thiaridae. Musekwa was the only area with a highly degraded environment where large parts of the river lacked vegetation and agricultural fields have replaced some of the adjacent riparian vegetation. Figure 4.2 below shows some of the activities along the Nzhelele River at Musekwa site. From Figure 4.2 below it can be seen that the location of the subsistence agricultural field is very close or is at the margin of the formally inundated area. This suggests that agricultural inputs might be intense, depending on the type of fertilisers used by local subsistence farmers. The utilisation of the river for laundry, livestock watering and grazing, as well as water abstraction suggests further anthropogenic impacts at the Musekwa sampling site. From Figure 4.2 below it could also be seen that river flow was at its lowest, suggesting the absence of the Heptageniidae because these organisms are a characteristic of a mobile water body (Flores and Zafaralla, 2012).

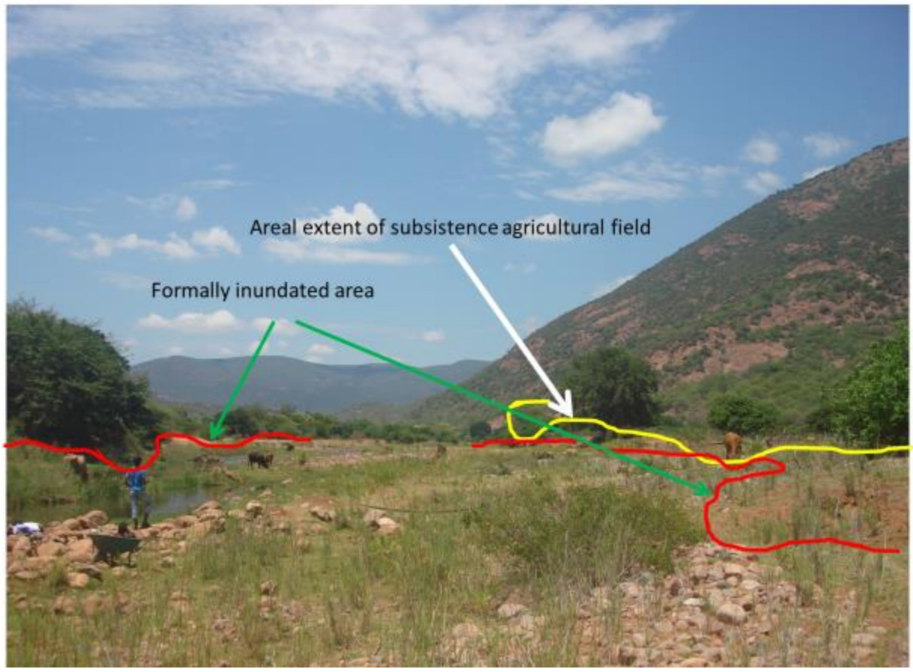


Figure 4.2 Activities along the Nzhelele River at Musekwa (Field data, 2016)

Just like Dopeni and Fondwe, Musekwa water quality seemed to have been altered by intense agricultural activities and the frequent utilisation of the river by community members. Given the absence of steep river banks and heavy utilisation, pollution is inevitable at Musekwa. Higher abundance values for the families Potamonautidae (Decapoda) and Thiaridae (Gastropoda) suggested that eutrophication was relatively high compared to the Mphaila sampling site. Low flows at the time of sampling could have also accounted for higher numbers of pollution tolerant organisms and a decline in the family Heptageniidae because these organisms are a characteristic of flowing water (Gillespie *et al*., 2015; White *et al*., 2017).

4.3.6 Pfumbada

Of the 112 macroinvertebrates sampled from the Pfumbada site 48% were highly tolerant to pollution and these were Chironomidae, Nepidae and Thiaridae families. Their tolerance level ranged from 2 to 3. Thirty-three percent (33%) were found to be moderately tolerant to pollution and these included Aeshnidae and Elmidae families with a tolerance range of 8. The remaining 19% consisted of Heptageniidae family were found to be sensitive to pollution. From the information contained in Table 4.2 above, the majority of sampled macroinvertebrates at Pfumbada were highly tolerant to pollution. Just like Dopeni, Musekwa, Maangani and Fondwe, Pfumbada site was utilised by local residents for laundry, water extraction and livestock watering. As it has been stated earlier, these activities are known to be the leading causes of cultural eutrophication which limits the diversity of pollution intolerant organisms.

Figure 4.3 below shows the percentage of all macroinvertebrates from the six sampled areas, whereby a total of 674 macroinvertebrates was recorded. From Figure 4.3 below, it is clear that organisms that were sensitive to pollution made up a total of 18% (Family Heptageniidae) across all six sampling sites. A total of 47.5% of the sampled macroinvertebrates were highly tolerant to pollution and these included Chironomidae, Coenagrionidae, Nepidae and Thiaridae families. However, 34.5% of the remaining macroinvertebrates were moderately tolerant to pollution and these included Aeshidae, Ecnomidae and, Elmidae families. The family Thiaridae (29%) were found to be the dominant organisms that were highly tolerant to pollution while the family Aeshnidae (24%) were the most dominant organisms that were moderately tolerant to pollution. A total of 82% had a tolerance range of moderately tolerant to highly tolerant. This means that the pollution status of the Nzhelele River ranged from moderate to severe, with few sections of the river that were not polluted or insignificant to allow the survival of pollution intolerant organisms such as Heptageniidae.

As long as people utilise the Nzhelele River the water bodies will continue to be polluted. However, Helson and Williams (2013) reported that the presence of the orders Ephemeroptera (flat-headed mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies), (EPT) indicates good water quality. This is why these orders have been used as indicators of water quality. From the results of the study, only Ephemeroptera and Trichoptera organisms were found from some of the sampling sites. No Plecoptera organism was found from any of the six sampling sites. The results indicated that both Ephemeroptera (Family Heptageniidae) and Trichoptera

(Ecnomidae) organisms made up a total of 20.5% of the total number of sampled macroinvertebrates from the six sites. However, the Trichoptera were restricted to Mphaila site, while Ephemeroptera family (Heptageniidae) were found at four sites, namely Dopeni, Maangani, Mphaila and Pfumbada. This says a lot about the river conditions at Fondwe and Musekwa because of the dominance of pollution tolerant organisms which made up a total of 76% (Fondwe) and 60% for the Musekwa sampling sites. Conditions at Fondwe and Musekwa could have been exacerbated by intense anthropogenic activities along the river.

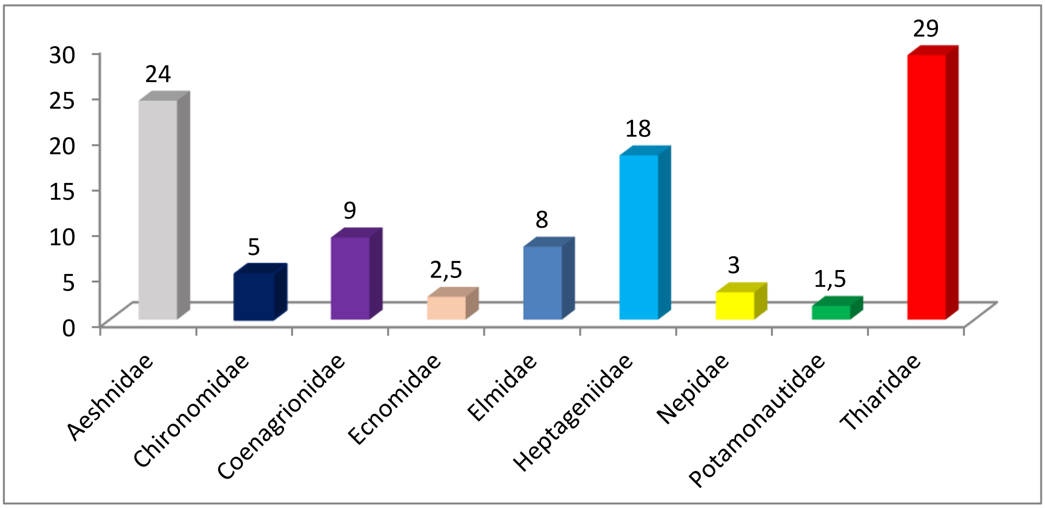


Figure 4.3 Percentage of macroinvertebrate families from six sampling sites (Field data 2016)

The diversity of macroinvertebrates per site was calculated to acertain whether the sites were evenly distributed in terms of macroinvertebrate composition. The Simpson Diversity Index was used. Table 4.4 below shows the diversity results. Interestingly, Maangani had the highest number (abundance) (178) but was not the most diverse sampling site. The calculated results indicated that Fondwe, despite recording an abundance value of 103 it was found to be the most diverse of all the sampling sites. However, the diversity indices indicated that all sites were more diverse but Fondwe recorded a higher diversity value of 0.78, followed by Pfumbada (0.77), Dopeni (0.75), Mphaila (0.74) and Maangani (0.73). Musekwa was the least diverse of all the six sampling sites with a diversity index of 0.69.

Table 4.4: The diversity results for the six sampling sites (Field data, 2016)

Family	**Dopeni**	***n*-1**	***n*(*n*-1)**	**Fondwe**	***n*-1**	***n*(*n*-1)**	**Maangani**	***n*-1**	***n*(*n*-1)**
Aeshnidae	29	28	812	25	24	600	35	34	1190
Chironomidae	0	-1	0	17	16	272	0	-1	0
Coenagrionidae	0	-1	0	30	29	870	28	27	756
Ecnomidae	0	-1	0	0	-1	0	0	-1	0
Elmidae	24	23	552	0	-1	0	0	-1	0
Heptageniidae	30	29	870	0	-1	0	30	29	870
Nepidae	0	-1	0	6	5	30	9	8	72
Potamonautidae	0	-1	0	0	-1	0	0	-1	0
Thiaridae	21	20	420	25	24	600	76	75	5700
Total	**104**		**2654**	**103**		**2372**	**178**		**8588**
Family	**Mphaila**	***n*-1**	***n*(*n*-1)**	**Musekwa**	***n*-1**	***n*(*n*-1)**	**Pfumbada**	***n*-1**	***n*(*n*-1)**
Aeshnidae	28	27	756	18	17	306	30	29	870
Chironomidae	6	5	30	0	-1	0	9	8	72
Coenagrionidae	0	-1	0	0	-1	0	0	-1	0
Ecnomidae	17	16	272	0	-1	0	0	-1	0
Elmidae	13	12	156	11	10	110	7	6	42
Heptageniidae	41	40	1640	0	-1	0	21	20	420
Nepidae	0	-1	0	0	-1	0	7	6	42
Potamonautidae	0	-1	0	10	9	90	0	-1	0
Thiaridae	0	-1	0	33	32	1056	38	37	1406
Total	**105**		**2854**	**72**		**1562**	**112**		**2852**

(Dopeni) Simpson Diversity Index (D) = 1 - ∑n(n-1) ÷ N(N-1), D= 1- 2654 ÷ 10712, D= 1- 0,25, D= **0.75**

(Fondwe) Simpson Diversity Index (D) = 1 - ∑n(n-1) ÷ N(N-1), D= 1- 2372 ÷ 10506, D= 1- 0,22, D= **0.78**

(Maangani) Simpson Diversity Index (D) = 1 - ∑n(n-1) ÷ N(N-1), D= 1- 8588 ÷ 31506, D= 1- 0,27, D= **0.73**

(Mphaila) Simpson Diversity Index (D) = 1 - ∑n(n-1) ÷ N(N-1), D= 1- 2854 ÷ 10920, D= 1- 0,26, D= **0.74**

(Musekwa) Simpson Diversity Index (D) = 1 - ∑n(n-1) ÷ N(N-1), D= 1- 1562 ÷ 5112, D= 1- 0,31, D= **0.69**

(Pfumbada) Simpson Diversity Index (D) = 1 - ∑n(n-1) ÷ N(N-1), D= 1- 2852 ÷ 12432, D= 1- 0,23, D= **0.77**

4.4 ANOVA results

4.4.1 Macroinvertebrate abundance

The ANOVA results presented and discussed in this section showed that the degree of variations of physico-chemical properties of water did not have significant difference between their averages. It was hypothesised that the sampling sites or groups did not differ in their physico-chemical properties which also affected macroinvertebrate composition. Table 4.5 below, (*F*-table) shows ANOVA results for macroinvertebrates from Dopeni, Fondwe, Maangani, Mphaila, Musekwa and Pfumbada sampling sites.

Table 4.5 ANOVA results for macroinvertebrate abundance (Field data, 2016)

Family groups						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Dopeni	4	104	26	18		
Fondwe	5	103	20,6	88,3		
Maangani	5	178	35,6	607,3		
Mphaila	5	105	21	188,5		
Musekwa	4	72	18	112,6667		
Pfumbada	6	112	18,66667	174,6667		
***F*-table**						
Source of Variation	***SS***	***df***	***MS***	***F***	***p-value***	***F crit***
Between Groups	1089,577	5	217,9154	1,043801	0,416154	2,64
Within Groups	4801,733	23	208,771			
Total	**5891,31**	**28**				

From the *F*-table results it can be concluded that there was no significant difference between the six sampling sites in terms of macroinvertebrate abundances. However, Maangani area had a slightly higher average of 35.6. Since the critical value (2.64) was greater than the F_{obs} (1.04) the null hypothesis stated in Chapter 3 was not rejected. Again, the *p*-value was also more than the significance level (0.05) and therefore the H_0 hypothesis was not rejected. There was no significant difference between the means of the groups for the macroinvertebrates from the six areas. The groups contained a more or less equal number of species. The diversity of organisms could have been affected by the low flows at the time of sampling due to the 2015/2016 drought. Since water was scanty and flows were low the diversity of macroinvertebrates was also low. Only nine (9) families were sampled. The diversity of macroinvertebrates at the time of sampling could have been affected by the aquatic conditions. Again, low flows could have made conditions impossible for other groups of macroinvertebrates to survive. Rolls *et al*.

(2012) stated that richness and diversity of macroinvertebrates shows a decline when periods of low flows are extended for longer periods. The same can be concluded about macroinvertebrate diversity along the Nzhelele River in the sense that the 2015/2016 drought could have impacted negatively on macroinvertebrate richness by altering river flows.

4.4.2 River velocity

Table 4.6 below shows ANOVA results for river velocity from six sampling sites. It was hypothesised that there was no significant difference between physico-chemical properties of water from six different sites of the Nzhelele River. Therefore, it was expected that there would be no significant difference in velocity data between the studied sites. Table 4.6 below shows that slow velocity rates varied from one area to another and ranged from an average of 0.11 to 0.42 m. s^{-1}.

Table 4.6 River velocity data (Field data, 2016)

Groups	**Count**	**Sum**	**Average**	**Variance**		
Dopeni	17	2,13	0,125294	0,000926		
Fondwe	13	1,44	0,110769	0,000358		
Maangani	47	20,02	0,425957	0,041064		
Mphaila	24	2,88	0,12	0,001487		
Musekwa	23	7,47	0,324783	0,007662		
Pfumbada	16	2,74	0,17125	0,012745		
F-table						
Source of Variation	***SS***	***df***	***MS***	***F***	***p-value***	***F crit***
Between Groups	2,584843	5	0,516969	30,09292	2,08E-20	2,281814
Within Groups	2,301997	134	0,017179			
Total	**4,88684**	**139**				

Results from the *F*-table above indicated that the *p-value* was greater than the *significance value* (0.05). This means that the null hypothesis for velocity data was not rejected and that there was no significant difference in velocity rates between the six sites. This means that the low velocity of the river reduced the turbulent flow which was important in the distribution and mixing of pollutants along the Nzhelele River. Pollutants usually have enough resident time in streams with low velocities as was the case with the Nzhelele River. This also explains why pollution tolerant organisms were in abundance across all sampling sites. The velocity data from the ANOVA table suggested that the Nzhelele River at the time of sampling was characterised by velocities which had an impact on macroinvertebrate communities. The lowest average river velocity was recorded at Fondwe (0.11 $m.s^{-1}$) and the highest was recorded at Maangani (0.42 $m.s^{-1}$). According to the DWAF (2007), river velocities for different macroinvertebrates range from very slow to moderately fast. Different macroinvertebrate communities prefer certain flow velocities of the river for their survival (DWAF, 2007). The families Elmidae, Coenagrionidae, Potamonautidae and Heptageniidae survive under moderately fast streams ranging from 0.3 to 0.6 $m.s^{-1}$. However, Ephemeroptera (mayflies) are also known to occur in both fast and slow moving water bodies (Bauernfeind and Soldán, 2012, Vilenica *et al.,* 2017). This explains their occurrence in four out of six sampling sites (Dopeni, Maangani, Mphaila, and Pfumbada). Organisms that prefer low river velocities are the families Ecnomidae and Chironomidae (0.1-0.3 $m.s^{-1}$) while the families Nepidae and Thiaridae (highly tolerant to pollution) are found along streams characterised by very slow velocities (<0.1 $m.s^{-1}$). Of the six sampling sites, Fondwe (0.11 $m.s^{-1}$), Mphaila (0.12 $m.s^{-1}$), Dopeni (0.13 $m.s^{-1}$) and Pfumbada (0.17 $m.s^{-1}$) were characterised by slow flow conditions. Musekwa (0.33 $m.s^{-1}$) and Maangani (0.43 $m.s^{-1}$) were characterised by moderately fast flows. This explains the absence of the Family Chironomidae with the highest pollution tolerance level of 2 at Maangani and Musekwa. This family of macroinvertebrates are found in rivers characterised by low velocities. Even though the Nepidae and Thiaridae families are found in very low flow velocities their abundance values indicate that they were sampled in pools which were dominant along all sampling points because they were found to exist even at Maangani and Musekwa. The Nepidae family was absent from Musekwa. However, there was no sampling site which had an average of below 0.1 $m.s^{-1}$.

Macroinvertebrates that tolerated moderately fast conditions (Maangani and Musekwa) included the Elmidae, Coenagrionidae, Potamonautidae and Heptageniidae families. Family Elmidae was found at Musekwa, but not at Maangani. Family Coenagrionidae was found at Maangani but absent at Musekwa. Family Potamonautidae was found at Musekwa. Family Heptageniidae was found at Maangani but absent at Musekwa.

According to DWAF (2007), the families Ecnomidae and Chironomidae are found in rivers characterised by slow velocities of 0.1 to 0.3$m.s^{-1}$. All studied areas except Musekwa and Maangani had slow velocities. Chironomids were found at Fondwe, Mphaila and Pfumbada while the family Ecnomidae was found at Mphaila only. From the results above, it can be concluded that river velocities were low along many sections of the river because no sampling site recorded velocities above 0.6 $m.^{s-1}$ (very fast). Due to low river velocities it was not surprising that 46.7% of the sampled macroinvertebrates (Families Chironomidae, Coenagrionidae, Potamonautidae and Thiaridae) were highly tolerant to pollution while 35.2% (Aeshnidae, Ecnomidae and Elmidae) where moderately tolerant to pollution and the remaining 18.1% (Family Heptageniidae) were sensitive to pollution. This means that the majority of pollution tolerant organisms could be related to low stream velocities which are associated with increased deposition and poor self-cleaning capability.

4.4.3 Dissolved Oxygen data

Table 4.7 below shows dissolved oxygen results from the six sites along the Nzhelele River. However, the same could be said about the dissolved oxygen results from the *F*-table (Table 4.7). From Table 4.7 F_{crit} (2.32) is less than the F_{obs} (2.41). The average dissolved oxygen content of the Nzhelele River at Dopeni was 59% and the highest average recorded was 65% (Musekwa). The degree of impairment in aquatic organisms due to DO levels becomes acute at concentrations of 27 to 35% at temperature regimes of 10 to 23ºC (Ausseil, 2013). According to Ausseil (2013), impairment becomes slight at DO concentrations ranging from 53 to 70% and there is no impairment at values above 70%. The oxygen concentration indicated that it was capable of slight impairment on macroinvertebrates since it ranged from 58.53 and 65.06%. The lower concentrations at Mphaila could be explained by the presence of high densities of submerged and marginal aquatic vegetation which use up ogygen during photosynthesis. Low concentrations at Mphaila also favoured the presence of Aeshnidae and Chironomids which were capable of surviving under low DO concentrations. However, Connolly *et al*. (2004) have noted that macroinvertebrates such as mayflies (Family Heptageniidae) cannot survive between the ranges of 15 and 48% saturation. Since the average was higher than this range it explained the presence of mayflies along some sections of the river. The presence of the Family Heptageniidae in four of the six sampled sites (Dopeni, Maangani, Mphaila and Pfumbada) suggested that DO did not impact negatively on macroinvertebrates but other factors did. Hypoxia has been negatively correlated with mayflies and the current oxygen concentration along the Nzhelele River was still within the required range for the survival of a variety of macroinvertebrates. From the ANOVA results, the *p-value* was less than the *significance value* (0.05) which meant that there was significant difference in DO concentrations across the six sampling sites. Therefore, the null hypothesis stated in Chapter 3 that stated that there was no difference between the six sampling sites in terms of macroinvertebrate abundances, was rejected since there was a significant difference in the averages of dissolved oxygen concentration across the six sampling areas.

Table 4.7 Dissolved oxygen (Field data, 2016)

Groups	***Count***	***Sum***	***Average***	***Variance***
Dopeni	15	892	59,46667	101,6952
Fondwe	15	960	64	75,28571
Maangani	15	912	60,8	40,88571
Mphaila	15	878	58,53333	18,26667
Musekwa	15	976	65,06667	5,209524
Pfumbada	15	930	62	0,714286

***F*-table**						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	487,8222	5	97,56444	2,418382	0,042401	2,323126
Within Groups	3388,8	84	40,34286			
Total	**3876,622**	**89**				

4.4.4 Chlorine

The average chlorine concentration of water ranged between 11.9 to 14.18 µg/l of Total Residual Chlorine (TRC). The Acute Effect Value (AEV), according the South African Water Quality Guidelines for Aquatic Ecosystems (DWAF, 1996), is 5µg/l, but the proposed Target Water Quality Range (TWQR) by DWAF (1996) is 0.2 µg/l TRC. DWAF (1996) further suggested that 90% of all chlorine readings at study sites should be within the TWQR but all readings should fall below the Chronic Effect Value (CEV) of 0.35 µg/l TRC. The chlorine concentration was very high and it was found to be an important determinant of the type of macroinvertebrates that were found to be present in the river. It was similarly observed by Bradley *et al*. (2002) that organisms that were sensitive to pollution (Heptageniidae) decreased in abundance in areas of high chlorine concentrations while those that were highly tolerant to pollution survived and increased in abundance. In the case of the Nzhelele River the highest chlorine concentration average was found at Dopeni (14.18 µg/l) (Figure 4.4) and the lowest concentrations were experienced at Maangani (11.97 µg/l). All chlorine values from all sampling sites were above the suggested CEV of 0.35 µg/l and the AEV of 5µg/l. Table 4.8 below shows the chlorine information of river water from all six sampling sites. From the *F*-table results it was clear that there was difference between groups in terms of chlorine concentration and this also determined the composition of macroinvertebrates. However, from the ANOVA table, (Table 4.5) above, it could be concluded that chlorine concentrations differed significantly from one area to another. However, the concentration was also higher than the AEV of 5 µg/l indicating elevated concentration values. Since chlorine is known to negatively affect mayflies (Clements and Kotalik, 2016), it was true with the Nzhelele River since these organisms accounted for 18.1% of the total sampled macroinvertebrates. They were the third largest groups after the families Thiaridae (28.6%) and Aeshnidae (24.5%) suggesting the impact of chlorine on these families. Interestingly, the highest abundance value of macroinvertebrates at Dopeni, which recorded the highest average value of 14.18 µg/l, was for the family Heptageniidae (29%). This value was higher than all abundance values for other taxa recorded at Dopeni. This could have been due to variation in chlorine concentration along sampling points of Mphaila site. Therefore, the causes and effects of chlorine on mayflies and other macroinvertebrates, depending on its form, needs to be further investigated. For example, Williams *et al*. (2003) have observed that free forms of chlorine are more lethal to aquatic life if chlorine is in the form of hypochlorous acid (HOCl) or hypochlorite ion (OCl^-). Therefore, the null hypothesis that there is no significant difference between water quality parameters of different sampling sites was not rejected, since the *p*-value (2.32) was greater than the significance value of 0.05. However, it is worth noting that the concentration of chlorine from all sampling sites was approximately over three times the AEV of 5µg/l suggesting a decline in macroinvertebrate family diversity and abundance. This explains why the six sampling areas had a total of 647 macroinvertebrates that were sampled in a period of 10 months. Since chlorine is known to affect mayflies its concentrations explains why mayflies constituted only 18.1% of the total macroinvertebrates that were sampled from the six sampling sites.

Table 4.8 Chlorine data (Field data, 2016)

Groups	***Count***	***Sum***	***Average***	***Variance***		
Dopeni	15	212,788	14,18587	0,080606		
Fondwe	15	208,04	13,86933	0,109278		
Maangani	15	179,69	11,97933	0,233292		
Mphaila	15	181,894	12,12627	2,254763		
Musekwa	15	202,789	13,51927	0,362611		
Pfumbada	15	189,174	12,6116	0,148066		
***F*-table**						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	65,60257	5	13,12051	24,68879	3,25E-15	2,323126
Within Groups	44,64064	84	0,531436			
Total	**110,2432**	**89**				

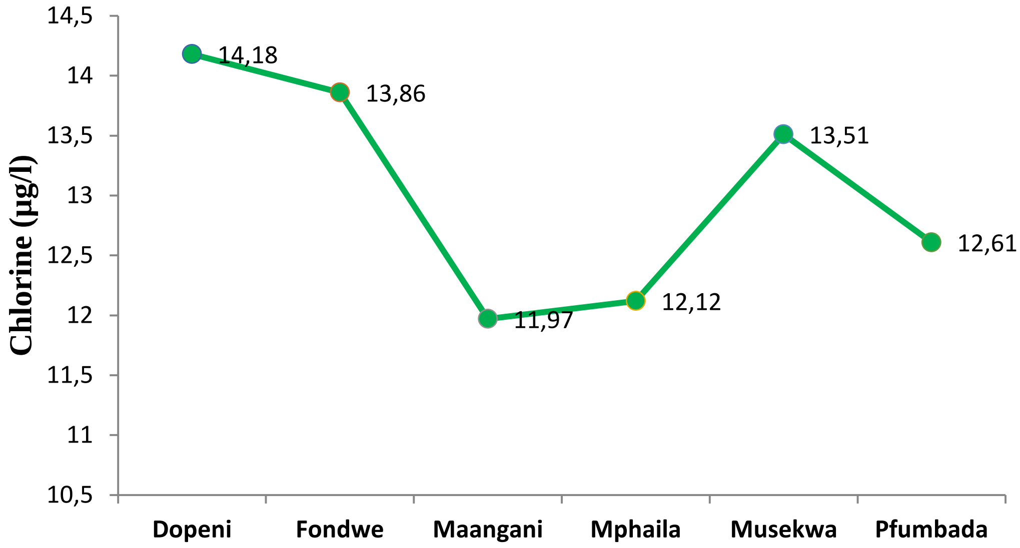


Figure 4.4 Chlorine concentration average per sampling area (Field data, 2016)

Even though chlorine concentrations are associated with macroinvertebrate diversity, an important point to note was the one raised by Williams *et al*. (2003) that the effect of chlorine on macroinvertebrates depends on the various forms of chlorine in water. This explains why high values above TWQR value did show significant impact on mayflies (Family Heptageniidae) since they are known to be sensitive to elevated chlorine levels. Therefore, all forms of chlorine should be defined to determine how various forms of chlorine impact on macroinvertebrate assemblages.

4.4.5 Nitrite ($NO^{-}{}_{2}$)

Table 4.9 below shows ANOVA results for nitrates from the six sampling sites. From Table 4.9 below it can be noted that nitrite concentration ranged from 0.26 to 0.28 mg/L. According to Nordin and Pommen (2009), the 30 day the maximum nitrite concentration for protecting aquatic life is 0.060 mg/L if chloride is more than 10 mg/L. High nitrite concentrations in aquatic environments are common in rivers that are intensively used for farming of commercial and aquarium fish because these activities are associated with ineffective biological filtering processes (Kocour-Kroupová *et al*., 2016).The average nitrite concentrations for the six sampling sites were lethal to macroinvertebrates because the averages for the six sites fell above 0.060 mg L^{-1}, suggesting elevated concentrations. Willingham *et al.* (2016) notes that nitrite toxicity displays a wide range of tolerance with Diptera families which survive at concentrations of 123 mg/l. Willingham further argued that even if the exposure has been prolonged, Diptera families (chironomids) can still survive nitrite concentration ranges of between 0.25 and 2.4 mg/l. This shows that nitrite concentrations at given locations along the Nzhelele River seemed to have played an important role in the diversity and abundance of macroinvertebrates. Potamonautidae appeared to have been negatively affected by higher values of nitrites since they are well known for their sensitivity to higher nitrite values. Potamonautidae were recorded at Musekwa sampling site which recorded the highest nitrite concentration of 0.28 mg.L. High nitrite concentrations impact on oxygen transport in crustaceans than fish and Thiaridae (Kocour-Kroupová *et al*., 2016). This explains the abundance of Thiaridae in five of the six sampling sites.

From the *p*-value (0.02) it was concluded that there was a significant difference in the nitrite averages of the six sampling sites. The null hypothesis formulated in Chapter 3, that there was no significant difference in the physico-chemical properties of water from the six sampling areas was therefore rejected. Since the *p*-value was less than the significance value (0.05) there were strong reasons to reject the null hypothesis. The F_{obs} (2.82) was also greater than the F_{crit} (2.32) which also made it possible to reject the null hypothesis.

Table 4.9 Nitrite data (Field data, 2016)

NO_3						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Dopeni	15	4,011	0,2674	5,2E-05		
Fondwe	15	4,047	0,2698	1,76E-05		
Maangani	15	4,109	0,273933	3,22E-05		
Mphaila	15	4,062	0,2708	0,000219		
Musekwa	15	4,257	0,2838	0,000701		
Pfumbada	15	4,002	0,2668	0,000237		
***F*-table**						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	0,002961	5	0,000592	2,823328	0,020917	2,323126
Within Groups	0,01762	84	0,00021			
Total	**0,020581**	**89**				

From the ANOVA results of all physico-chemical properties of water, it can be concluded that in seven out of nine instances, the *p-value* was higher than the *significance value*. This meant that the null hypothesis in many instances was not rejected and that there was no significant difference between the six studied sites in terms of physico-chemical properties of water. Although there was no significant difference between macroinvertebrate abundance from the six studied sites the diversity could be explained by a host of different factors, including the general set up or the environmental conditions of the study area, such as the nature of the slope and the degree of human impact.

4.4.6 Nitrate (NO^-_3)

Table 4.10 below shows nitrate concentration from the sampling sites. The average nitrate concentration ranged from 18.7 to 28.2 ppm. According to Nordin and Pommen (2009), in order to protect freshwater aquatic life the average concentration of nitrate is 3.0 mg.L and the maximum concentration is 32 mg.L. However, oligotrophic conditions are considered to have nitrate concentrations of less than 0.5 mg L^{-1} (Mwangi, 2014). However, the acute trigger value of 20 mg/L and the chronic trigger value of 1.0 mg/L, 1.7 mg/L, and 2.4 mg/L have been recommended for ecosystem protection levels of above 80% (Hickey and Martin, 2009). Since nitrate is considered to be less toxic than nitrite due to its limited uptake (McGurk *et al*., 2006), its toxicity from the Nzhelele River was therefore negligible. However, the occurrence of algal growth at Maangani could be strongly associated with increased levels of nitrate at certain points along the river. This also explains the high values (42%) of occurrence of Thiaridae from this sampling site which tolerate more polluted environments. Pfumbada recorded the highest nitrate concentration average and was also the second sampling site after Maangani to have recorded high macroinvertebrate abundance values. Niyogi *et al*. (2007) have similarly observed that macroinvertebrate density increases when nutrient concentrations and fine sediments in a river are high. Some river sections at Maangani had algae blooms to indicate that the river was loaded with nutrients. This also explains why Maangani recorded the highest number (178) of macroinvertebrates than other sampling sites. This also explains why 63% of the macroinvertebrates sampled at Maangani were highly tolerant to pollution while only 17% were very sensitive to pollution. The remaining 20% was moderately tolerant to pollution. In total, Maangani had 37% of organisms that were intolerant to pollution. It could be argued that nitrite concentration was positively correlated with higher abundance values of pollution tolerant macroinvertebrates Therefore, the role of nitrate in the determination of macroinvertebrate communities was found to be minimal because of its average concentration values of below maximum of 32 mg.L and it has been considered less toxic than nitrite. An interesting observation by Guevara-Mora *et al*. (2017) was that the concentration of nitrogen and phosphorus in water bodies does not always lead to drastic environmental conditions, but McKinney (2012) have stated that elevated nitrates in streams have effects on some aquatic invertebrates. There was no significant difference in nitrate concentration between the sampling sites. This difference might be caused by the location of subsistence agricultural fields along the river and the utilisation of the river for various purposes. The presence of algae at Maangani area indicated a problem of cultural eutrophication. From the *F*-table, since the *p-value* was greater than *significance value* (0.05) the null hypothesis was therefore accepted because there was no significant difference in the concentration of nitrites from the sampling sites.

Table 4.10 Nitrate data (Field data, 2016)

NO_3-						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Dopeni	15	281,64	18,776	0,073183		
Fondwe	15	306,26	20,41733	2,553535		
Maangani	15	310,57	20,70467	0,571627		
Mphaila	15	291,618	19,4412	2,639219		
Musekwa	15	282,421	18,82807	2,961959		
Pfumbada	15	423,913	28,26087	36,66884		
***F*-table**						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	978,1453	5	195,6291	25,81519	1,08E-15	2,323126
Within Groups	636,557	84	7,57806			
Total	**1614,702**	**89**				

4.4.7 Water temperature

Table 4.11 below shows water temperature ranges from the study sites. The average temperature from the six sampling sites ranged from 16.15 to 20.9°C. Temperature ranges differed from one area to another. Temperature has been identified as one of parameters that affects the distribution and abundance of macroinvertebrate communities. According to Eady (2011), some macroinvertebrates such as Hydrophilinae, Chironomidae, Veliidae families and others tend to increase in abundance between winter and autumn while other families such as Chironomidae and Coenagrionidae are found throughout all seasons. The findings indicate that stream temperature played a significant role on the abundance of other macroinvertebrates. Since the samples were collected between February and December of 2016 when the temperatures ranged from less favourable to favourable due to seasonal changes, it was not surprising that only 9 different families of macroinvertebrate were sampled across all six areas. From the observation by Eady (2011) it could be concluded that more families of macroinvertebrates could have been sampled if sampling was done in late spring and summer. A finding by Grab (2014) showed that abundance of macroinvertebrate families, especially Family Heptageniidae, decreased with decreasing temperature. The lower diversity or abundance of the Heptageniidae family along the Nzhelele River could be attributed to the time of sampling. While temperature could be directly linked to the abundance of pollution intolerant organisms such as Heptageniidae, it might have also determined the abundance of this family along the Nzhelele River. The average temperature at Dopeni was 18.11 ºC, and this was the sampling area which hosted 80% of pollution intolerant organisms (Families Ashnidae, Elmidae and Heptageniidae). The Family Heptageniidae (Ephemeroptera) were found to be the most abundant at Dopeni, suggesting that they survive well at these temperatures. Ausseil (2013) posited that Ephemeroptera and Plecoptera are sensitive to increased temperature ranges. This explains the dominance of the Family Heptageniidae (29%) at Dopeni sampling site. However, a higher value for Heptageniidae was recorded at Mphaila which recorded 6% of pollution tolerant organisms (6%) (Family Chironomidae). The Family Heptageniidae constituted (39%) of the total number of macroinvertebrates sampled at Mphaila. The average temperature for this sampling area was 18.74 ºC which resembled the Dopeni average temperature. Fondwe recorded the lowest average temperature of 16.15 ºC and it was characterised by 76% of pollution tolerant families Thiaridae, Chironomidae, Coenagrionidae and Nepidae. All of these organisms tolerate temperature ranges of 8-30 ºC with the Nepidae, Heptagennidae and Ecnomidae families reflecting a drop in diversity at temperatures below 12 ºC and above 25 ºC (Dallas, 2009). It was however, surprising to find the occurrence of the Family Heptageniidae at Maangani which recorded an average temperature of 21.72 ºC.

At a maximum temperature of 21.5 ºC Ephemeroptera starts to decline in diversity and abundance (Ausseil, 2013). Ephemeroptera was absent at Musekwa sampling site which recorded an average temperature of 22 ºC, suggesting the order sensitivity to elevated temperatures. Since Musekwa had numerous indigenous agricultural fields next to the river it was not surprising for the area to have higher temperature averages because of lack of vegetation along the banks of the river as well as the continuous utilisation of the river for various purposes. Deborde *et al*. (2016) noted that warmer temperatures due to decreased riparian vegetation cover are a characteristics of agricultural and mixed areas. It was also not surprising to find the Family Aeshnidae (Odonata) across the six sampling sites and also being the second highest group after the Family Thiaridae (Gastropoda) because these organisms together with Coleoptera, Diptera and Hemiptera families are less affected by significant changes in temperature (Fulan *et al*., 2011). The results from the *F*-table suggested that the null hypothesis was accepted since the *p-value* was greater than the significance value (0.05). It was therefore concluded that there was no significant difference between temperature averages across the six sampled sites along the Nzhelele River.

Table 4.11 Water temperature data (Field data, 2016)

Temperature						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Dopeni	15	271,7	18,11333	0,006952		
Fondwe	15	242,3	16,15333	0,041238		
Maangani	15	325,8	21,72	0,087429		
Mphaila	15	281,1	18,74	1,106857		
Musekwa	15	330	22	0,371429		
Pfumbada	15	314,17	20,94467	0,363841		
***F*-table**						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	403,3645	5	80,67291	244,742	1,64E-48	2,323126
Within Groups	27,68844	84	0,329624			
Total	**431,053**	**89**				

4.4.8 pH

Table 4.12 below shows the pH range of the Nzhelele River at six different sites. The average pH values ranged from 7.04 to 8.62. According to Balachandran *et al*. (2012), the pH of natural waters ranges from 6 to 8.5 and values above 7 are considered alkaline and indicate the presence of CO_2 and more organic matter content. Figure 4.12 below shows pH range across the six sampled sites. From Figure 4.5 it can be seen that the pH of all studied areas was between 6 and 8.5, which is conducive for the survival of many macroinvertebrates. At this pH range it can be concluded that many macroinvertebrate families survive because their abundance depends on the pH values of water. Tripole *et al*. (2008) have indicated that the Family Aeshnidae have a pH tolerance range of between 6.7 and 8.9. This explains their abundance and occurrence across all sampled sites. However, the Elmidae and Chironomidae families have been found to have a pH tolerance range of 3.6-9.5. These families have a broader pH tolerance range than other families. The pH range falls between 6 and 9 and at this range many macroinvertebrates survive, even though ranges for the Elmidae and Chinoromidae families are lower than 6. This means that these two families can survive under saline environments. According to USEPA (1997), most macroinvertebrates survive at a pH range of 6.5-8.0. Maangani, Musekwa and Pfumbada recorded pH averages that were slightly alkaline, ranging from 7.89 to 8.62. It was not surprising that the Family Thiaridae recorded the higher abundance values for each of the three sampling sites, an indication that these organisms thrive well under alkaline aquatic conditions. A similar observation was made by Sharma, *et al*. (2013) who noted in their study that alkaline conditions favour abundance of molluscan populations. The same was true for Maangani, Musekwa and Pfumbada. Mphaila, which also recorded an alkaline pH of 7.73 but did not record the presence of a single Gastropod specimen. Dopeni and Fondwe, with pH values of 7.04 and 7.03 respectively recorded low abundance values of the Family Thiaridae of below 25. From the ANOVA results, the null hypothesis that there was no significant difference between physico-chemical properties of water between six areas was accepted. This was because the *p-value* was greater than the *significance value* (0.05).

Table 4.12 pH data (Field data, 2016)

Groups	**Count**	**Sum**	**pH range**	**Variance**		
Dopeni	15	105,64	7,042667	0,019135		
Fondwe	15	105,59	7,039333	0,058721		
Maangani	15	129,37	8,624667	0,003284		
Mphaila	15	116,04	7,736	0,123783		
Musekwa	15	118,42	7,894667	0,08127		
Pfumbada	15	124,39	8,292667	0,047435		
***F*-table**						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	31,24806	5	6,249613	112,3938	1,08E-35	2,323126
Within Groups	4,670787	84	0,055605			
Total	**35,91885**	**89**				

Dopeni and Fondwe had lower pH values than other study areas. The pH values for the two areas are in the region of 7, which is considered neutral. However, the pH from six study sites fall within the acceptable ranges of 3.6 and 9.5 which is suitable for many types of macroinvertebrates.

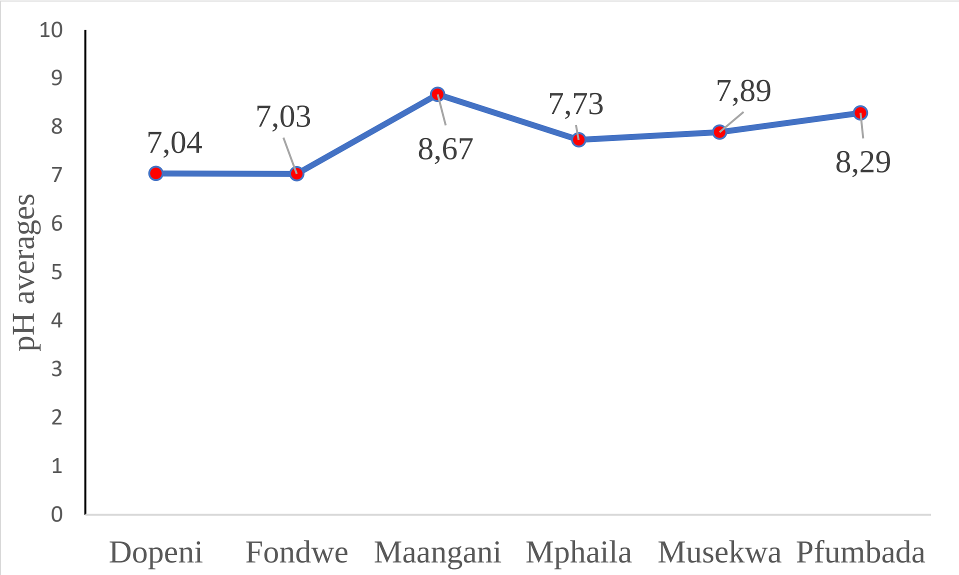


Figure 4.5 pH range per sampling point per sampling site (Field data, 2016)

An interesting observation by Hussain (2012) was that values below 5 and greater than 9 are regarded as lethal or harmful to macroinvertebrates. Since pH range across all six sampling sites fell between 6 and 9, it was evident that these ranges were suitable for the existence of a variety of macroinvertebrates. The nine (9) families could have not been directly influenced by pH but water temperature because sampling was done between February and November of 2016 when temperatures differed seasonally. However, seasonal variations in water temperature and pH was not considered in this study but averages for the sampled periods were considered. Since the average pH ranges fell within the suitable limits for macroinvertebrate survival it can therefore be asserted that pH did not negatively impact on macroinvertebrate diversity. Since samples were collected during the dry season due to drought it was not surprising that no extreme values were recorded. Souto *et al*. (2011) noted that lower pH values are a characteristic of dry seasons while high DO and conductivity values are common during the rainy season. This explains the relatively lower TDS and conductivity values which were a result of the 2015/ 2016 drought period.

4.4.9 Total Dissolved Solids (TDS)

Tables 4.13 and 4.14 below show TDS and electric conductivity data from the six sampling sites along the Nzhelele River. The two variables are considered the same hence they have been interpreted simultaneously. The average TDS values ranged from 74.37 (Fondwe) to 549.93 mg.L (Pfumbada). High conductivity and TDS readings are often associated with nutrient inputs from agricultural fields (Al-Shami *et al*., 2011; Piggott *et al*., 2012). TDS measurements were considered because it has been noted that spatial distribution of macroinvertebrates is sometimes a function of the TDS. Timpano *et al.* (2010) note that elevated levels of TDS are known to be stressors for aquatic life. However, Olson and Hawkins (2017) have noted that several genera of Plecoptera and Trichoptera are not strongly affected by TDS concentrations. A study by Timpano *et al.* (2010) showed that mayfly taxa (Ephemeroptera) abundance did not respond to an increase in TDS. This suggests that elevated TDS affects mayfly richness but not overall order abundance (Green *et al*., 2000; Pond, 2004; Pond *et al.*, 2008; Timpano *et al.,* 2010).

Maangani, Musekwa and Pfumbada recorded higher TDS averages than all other sampled sites. Interestingly, Pfumbada recorded 48% of pollution tolerant organisms, suggesting that the higher TDS values did not adversely affect pollution intolerant organisms. It was therefore, not surprising that Elmidae were common at Dopeni, Mphaila, Musekwa and Pfumbada because these sampling sites recorded TDS averages of 100,87 $\mu S.cm^{-1}$ and above. Mazzoni *et al*. (2014) reported that Elmidae and Simuliidae families are associated with higher conductivities in the region of 100 $\mu S.cm^{-1}$. The same is true with these four sampling areas. The Family Elmidae were absent at Fondwe and Maangani sampling sites, with TDS averages of 74.37 and 476.06 $\mu S.cm^{-1}$ respectively. Even though Maangani recorded a higher TDS value the absence of this family suggested that other environmental variables could have accounted for their absence. Again, the presence of the Family Chironomidae at Mphaila and Pfumbada was strongly linked to high TDS values but their absence at Maangani could have been the effect of other environmental variables. This is because organisms such as gastropod, Family Baetidae, Chironomidae and Culicidae are to known perform better at higher TDS ranges of above 300 $\mu S.cm^{-1}$ (Olson and Hawkins, 2017). The higher percentage of the Family Thiaridae (Gastropoda) at Maangani, Musekwa and Pfumbada sampling sites suggested that these organisms tolerated higher TDS concentrations and a wide range of environmental parameters and survived in many environments as noted by Adeogun and Fafione (2011). Flores and Zafaralla (2012) also noted that freshwater gastropods are organisms that are tolerant of habitat diversity and variability, enabling them to colonise habitats quickly. Mphaila was the only sampling area where the Family Thiaridae was not recorded even though a higher TDS average of 304 μS.cm^{-1} was recorded. Even though the Family Ecnomidae (Trichoptera) are known to be the least affected by variations in the concentration of TDS (Kefford *et al*., 2010) their presence in only one sampling area suggested that other environmental variables were responsible for their absence. From the ANOVA results, the null hypothesis was accepted since the *p-value* was greater than the *significance value* (0.05) and the results from the ANOVA table indicated that there was no significant difference in the TDS concentration averages from the six sampling sites.

Table 4.13 TDS data (Field data, 2016)

TDS						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Dopeni	15	1513,1	100,8733	0,126381		
Fondwe	15	1115,6	74,37333	0,01781		
Maaangani	15	7141	476,0667	21,92381		
Mphaila	15	4560	304	15255,14		
Musekwa	15	6915	461	5,428571		
Pfumbada	15	8249	549,9333	691,4952		
***F*-table**						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	3080335	5	616066,9	231,3992	1,47E-47	2,323126
Within Groups	223637,9	84	2662,356			
Total	**3303972**	**89**				

Table 4.14 Electric conductivity data (Field data 2016)

Conductivity						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Dopeni	15	2530	168,6667	0,238095		
Fondwe	15	1861,6	124,1067	0,174952		
Maangani	15	11913	794,2	56,74286		
Mphaila	15	7873	524,8667	65753,41		
Musekwa	15	11521	768,0667	32,06667		
Pfumbada	15	13674	911,6	2725,114		
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	8480418	5	1696084	148,4153	3,69E-40	2,323126
Within Groups	959948,4	84	11427,96			
Total	**9440366**	**89**				

4.5 Environmental degradation

The degree of **environmental degradation** along the Nzhelele River was based on Index of Habitat Integrity as defined by Kleynhans *et al*. (2008). Since the river and its immediate environment were being utilised for various purposes such as settlement, agriculture, water extraction, laundry, and livestock watering, it was therefore necessary to study the habitat integrity in order to determine its influence on water quality and macroinvertebrate composition. For example, degraded environments are associated with pollution tolerant organisms. Table 4. 15 below shows the results of an assessment of modification of instream habitat integrity from the six studied sites (Dopeni, Fondwe, Maangani, Phaila, Musekwa and Pfumbada). The degree of impact has been shown in the third column of Table 4.15 below while the second column provided a brief description of the assessment results. The criteria used to assess modification of instream habitat has been provided in the first column.

Table 4.15 Assessment of modification of instream habitat integrity (Field data, 2016)

Criterion	Assessment per sampling area	Modification impact class and score
Water abstraction	**Dopeni**: Characterised by many agricultural fields along the stream. Water extraction pipes and water collection activities via trucks and vehicles were present along the river.	Serious (16-20)
	Fondwe: Agricultural fields were present and water extraction furrows were also present.	Moderate (6-10)
	Maangani: Few but small agricultural fields were present. Water extraction furrows along the river were present	Moderate (6-10)
	Mphaila: New small subsistence agricultural fields were developing. No visible water extraction activities along the river.	None (0)
	Musekwa: Many agricultural fields were present and water abstraction activities through generators, channels and pipes were present. Evidence of water collection activities through vehicles was also present.	Critical (21-25)
	Pfumbada: Water extraction through generators was evident due to presence of agricultural fields. Water collection activities via vehicles and wheelbarrows were also present.	Serious (16-20)

Criterion	Assessment per sampling area	Modification impact class and score
Flow modification	**Dopeni:** Water channels were present. **Fondwe:** Water extraction furrows were present but very few. **Maangani:** Big water extraction furrows were present. **Mphaila:** No modifications were observed. **Musekwa:** Presence of water channels. **Pfumbada:** Channel straightening at some sections was evident.	Large (11-15) Small/ Minimal (1-5) Large (11-15) None (0) Serious (16-20) Moderate (11-15)
Bed modification	**Dopeni:** Sedimentation and sand extraction were evident along some sections of the river. **Fondwe:** Sediment transport was curtailed along some points due to reduced river velocity. No sand extractions activities were observed. **Maangani:** Sedimentation occurred along some sections of the river due to reduced river velocity. No sand extraction activities were observed. **Mphaila:** No bed modifications were observed. **Musekwa:** Sand extraction activities were high. **Pfumbada:** Sedimentation present along some sections of the river due to reduced river velocity.	Serious (16-20) Small/ Minimal (1-5) Small/ Minimal (1-5) Small/ Minimal (1-5) Large (11-15) Small/ Minimal (1-5)
Channel modification	**Dopeni:** Presence of stream straightening channels **Fondwe:** No visible channel modification but banks at one point were deliberately carved to be steep next to the agricultural field. . **Maangani:** Small artificial channels were observed. . **Mphaila:** River bed reinforced with cement where river crosses a bridge. **Musekwa:** Channel straightening ridges were observed. Small artificial channels were also observed. **Pfumbada:** Channel straightening ridges were observed	Large (11-15) Small/ Minimal (1-5) Large (11-15) Small/ Minimal (1-5) Serious (16-20) Moderate (11-15)

Criterion	Assessment per sampling area	Modification impact class and score
Water quality modification	**Dopeni:** Too many agricultural fields were present to impact on water quality. **Fondwe:** Presence of agricultural fields and river utilisation for laundry and livestock watering suggests impact on water quality. **Maangani:** Evidence of river utilisation, proximity of the settlement of Maangani, water extraction presence of agricultural fields and livestock watering suggest an impact on water quality. **Mphaila:** Very few agricultural fields suggest minimal impact on water quality. **Musekwa:** Presence of many subsistence agricultural fields, water extraction, river utilisation for laundry and livestock watering suggest impact on water quality. **Pfumbada:** Presence of subsistence agricultural fields and river utilisation for laundry and livestock watering suggest impact on water quality.	Moderate (6-10) Moderate (6-10) Moderate (6-10) Small/ Minimal (1-5) Large (11-15) (Moderate) (6-10)
Inundation	Inundation is caused by the presence of bridges at Dopeni, Fondwe, Mphaila, Musekwa and Pfumbada.	Small/ Minimal (1-5) for all areas. None (0)
Exotic macrophytes	No exotic macrophytes were observed from all six areas.	None (0)
Exotic aquatic fauna	No exotic aquatic fauna were observed in all areas.	None (0)
Solid waste disposal	**Dopeni:** Illegal solid waste dumping occurs along the river where sand mining is also active. **Fondwe:** Solid waste disposal occurs along some parts of the river but very minimal. **Maangani:** Solid waste disposal is present along the stream. **Mphaila:** There was no evidence of solid waste disposal. **Musekwa:** Solid waste disposal is present along various parts of the river. **Pfumbada:** There were no visible signs of solid waste disposal.	Serious (16-20) Small/ Minimal (1-5) Small/ Minimal (1-5) None (0) Serious (16-20) None (0)

4.5.1 Water abstraction

From the results in Table 4.15 above, water abstraction activities at Dopeni were found to be serious or intensive, with a rating of 16-20. Several activities such as water collection, laundry activities, livestock watering, water extraction via pipes and dirt roads across the river create water quality challenges at certain points or sections of the river. The same results or observations were recorded at Pfumbada. The rate of water extraction was serious due to proximity of agricultural fields and livestock watering. The existence of generators and extraction pipes indicates the seriousness of extraction activities. This poses a serious challenge on the quality of water and the resident macroinvertebrates in some sections of the river. Water abstraction activities can be directly linked to point source pollution. This also explains why Pfumbada had pollution tolerant families (Chironomidae, Nepidae and Thiaridae), making a total of 48% of all macroinvertebrates that were sampled at Pfumbada site. However, since water abstraction cannot always be linked with water pollution some abstraction activities were carried out in areas where macroinvertebrates were not sampled. Interestingly, the activities around the Dopeni sampling site might not have impacted on the abundance of pollution intolerant organisms because the percentage of macroinvertebrates that were intolerant to pollution was found to be 80%, with only the Family Thiaridae (pollution tolerant) making the remaining 20%.

However, Musekwa's situation was found to be critical because of the high rate of water abstraction to nearby indigenous agricultural fields. This could have been created by the proximity of Musekwa village to the river, which was approximately 150 m. The situation was so critical that the area was dominated by the majority of pollution tolerant macroinvertebrate families (Potamonautidae and Thiaridae). Musekwa also recorded the lowest number of macroinvertebrates than all other sites. Pollution tolerant organisms constituted 60% of the total number of macroinvertebrates that were sampled from this site. The extremely low flows, due to previous droughts of 2015/ 2016 were suspected to have been exacerbated by excessive water abstraction. Musekwa and Dopeni were found to be the only villages with many agricultural fields close to the river. Conditions of water abstraction at Maangani and Fondwe were found to be moderate (11-15). Even though the two sites were characterised by low flows the rate of water abstraction was considered moderate due to fewer agricultural fields compared to Musekwa. However, the composition of pollution tolerant organisms at these two sites was found to be 63 and 76% respectively. Mphaila did not have any water abstraction activities and even the agricultural fields that were present were still new because they existed in areas which never existed during field observation in 2015.

4.5.2 Flow modification

The severity of flow modification was found to be serious at Musekwa. The river flow was modified through the creation of water channelling ridges to channel water to subsistence agricultural fields. It is the only site with many agricultural fields that lie adjacent to the Nzhelele River. Modification might have directly affected macroinvertebrate composition along the river. This is because many sections of the river from all sampled sites were characterised by very slow to slow velocities. This explains the presence of organisms that favoured slow velocity conditions such as Ecnomidae (Trichoptera), Chironomidae (Diptera), Nepidae (Hemiptera) and Thiaridae (Gastropoda) families. Even though the Family Chironomidae were associated with low river velocities, an interesting observation by Everaert *et al*. (2014) was that Chironomids also existed in fast flowing waters making it difficult to correlate them with environmental variables. Changes to river channels will also change flow characteristics which changes macroinvertebrate composition. For example, Thirion (2016) has noted that runs develop into riffles when conditions are changed to low flows. This supports the view that macroinvertebrate richness and diversity decrease with low flows (Rolls *et al*., 2012). Musekwa site had 60% of macroinvertebrates that were highly tolerant to pollution (Families Thiaridae and Potamonautidae) while the remaining 40% were moderately tolerant to pollution (Families Aeshnidae and Elmidae). It was not surprising that the Family Heptageniidae and other ETP groups were missing from Musekwa site because these organisms are known to be dominant in moderately fast moving waters (Thirion, 2007). This shows that continuous and prolonged utilisation of the river will change its water quality so much so that it favours pollution tolerant organisms. The site lacked a single organism from the Order Ephemeroptera such as Family Heptageniidae. The level of modification of the river at Dopeni and Maangani were considered large because like with Musekwa, the two areas were characterised by the presence of channelling ridges but at a smaller scale compared to Musekwa site. Fondwe site was considered to have a small modification problem because flow modification was confined to one area along the entire sampled site. Mphaila was found to be the only area without any modification to the flow characteristics of the river. It is not surprising that this area has the highest number of pollution intolerant organisms (Family Heptageniidae). Only 6% of the total number of macroinvertebrates was highly tolerant to pollution. Any changes to flow characteristics of the river will ultimately alter aquatic diversity, including macroinvertebrates. Caletkova *et al.* (2012) have noted that extremely low flows are associated with a loss in aquatic habitat, which leads to decreased biological diversity. The same was true with the Musekwa site.

4.5.3 Bed modification

Dopeni site was found to have a serious bed modification problem due to activities such as sand mining and the creation of short-cut dirt roads across the river. In some areas where samples were collected it was evident that the riverbed was modified through the creation of artificial riffles which were built by the assemblage of rocks on the river bed. In some places dirt roads which were created through rocks altered the riverbed and flow characteristics at that particular sampling points. This changed runs into riffles and determined the resident macroinvertebrate such as pollution intolerant families Aeshnidae, Elmidae and Heptageniidae. This suggested that bed modifications created turbulent conditions which minimised the effects of pollutants, hence the dominance of pollution intolerant organisms at Dopeni sampling site. The Family Thiaridae also existed because they are known to master a variety of environments. At Musekwa, the bed modification was considered to be large. The only cause of bed modification was sand mining by the local community. In areas where sand was excessively mined the runs became pools and at some sampling points the velocities were significantly reduced. Just like Maangani, Musekwa village was found to be closer (approximately 150 m) to the Nzhelele River. This means that in years to come the level of bed modification will change from large to critical. Interestingly, bed modification at Fondwe, Maangani, Mphaila and Pfumbada was considered to be small or minimal due to river sedimentation at some points which was caused by reduction in river velocity. The only noticeable bed modification at Mphaila was the cement river bed that was built during the construction of a bridge in order to curb surface erosion in the vicinity of the bridge to prevent it from collapsing. Generally the degree of bed modification from all the studied sites was on average small (minimal) but this poses future threats to the hydrodynamics of the river as long as the river is continuously utilised by members of the community. The reduction in flow velocities impacts on the distribution or presence of macroinvertebrates. For example, Pan *et al*. (2015) have noted that rivers with stable and heterogeneous habitats favour the assemblages of benthic taxa and the existence of cobbles create a variety of habitats that favour a variety of macroinvertebrates. Duan *et al*. (2011) have similarly noted that the presence of many cobbles in a river increases the attachment area for benthic macroinvertebrates such as Heptageniidae (Ephemeroptera), Elmidae (Coleotera), Potamonautidae (Decapoda), Aeshnidae (Odonata) and Ecnomidae (Trichoptera). However, cobbles substrate was observed at Fondwe, Mphaila and Pfumbada. Bedrock streams like at Maangani, support macroinvertebrates such as Heptageniidae (Ephemeroptera), Chironomidae (Diptera), Elmidae (Trichoptera) and Ecnomidae (Trichoptera) (DWAF, 2007; Duan *et al*., 2011). An interesting observation was that from the above listed macroinvertebrates many were absent from the substrates that were listed to favour them. For example, at Maangani, only the Family Heptagennidae was among the listed group of organisms that favours bedrock streams. Duan *et al*. (2011) have noted that streambeds of degraded rivers have substratum made of cobbles, gravel and sand and these rivers are more often unstable. This situation was observed at Dopeni and Musekwa. The state of the river is well reflected by the abundance values at Musekwa. Musekwa recorded the lowest abundance values (72) to show the severity of a degrading stream. These types of substrates often support families such as Heptageniidae (Ephemeroptera), Chironomidae (Diptera), Ecnomidae (Trichoptera) and Elmidae (Coleoptera). From the listed macroinvertebrates only families Elmidae and Heptageniidae existed at Dopeni. The Elmidae family was recorded at Musekwa site only.

4.5.4 Channel modification

The degree of channel modification has been likened to the degree of flow modification. The situation at Musekwa was considered serious due to the existence of numerous artificial channel ridges along the river. However, the situation at Pfumbada was considered moderate because water channelling through artificial ridges was confined to fewer areas. However, at Dopeni and Maangani the degree of channel modification was considered large due to the existence of numerous channelling ridges. The situation at Fondwe and Mphaila was considered small because channel modification was confined to one specific area along the river. For example, at Mphaila, the bank was fortified with rocks to minimise mass wasting along the banks. This was meant to reduce lateral erosion of the river at that point. At Fondwe, the bank at one point was steeply carved to prevent water from flooding the adjacent agricultural field. Alouch (2012) has indicated that channelization of rivers leads to a decrease in aquatic habitat. Alouch (2012) further argued that channelization creates peak stream flow and leads to reduced benthic substrate heterogeneity. Channelization therefore, reduces habitat size quality and the abundance of the resident macroinvertebrates. The same was true with the

Musekwa site where channel modification was serious. This means that the connectivity of the river had been disturbed and formerly inundated areas became dry, leading to a reduction in macroinvertebrate habitat and abundance. An interesting observation by Hill *et al*. (2016) was that channelization, flow regulation and embankment disconnect floodplains from rivers. Floodplains, according to Gerken (2015), are considered to be biologically diverse habitats and have many ecological benefits such as retention of important nutrients for macroinvertebrates and other aquatic biota. The low water quantities and low flow regimes along the river at Musekwa cut water from reaching the floodplains making it impossible for floodplains to provide nutrients to macroinvertebrates during flooding or flash floods. However, channel modification at Musekwa and Pfumbada showed signs of aquatic habitat fragmentation. This seemed to have been exacerbated by low flows and low water quantities. The formerly inundated riverbeds have become isolated islands in a river. These isolated fragments now serve the adjacent water with nutrients that are precipitated on them, which are a good source of macroinvertebrates and pollution intolerant taxa such as Heptageniidae. The creation of isolated pools in a river due to drought changes lotic conditions into lentic conditions and this leads to the absence of EPT groups and Family Simuliidae, but Heteroptera and Odonata increase in numbers (Bogan *et al*., 2013; Barrios, 2015). This explains the omnipresence of the Family Aeshnidae (Odonata) at all sampling sites. However, the Family Coenagrionidae (Odonata) were found in extremely flowing sections of Nzhelele River even though they are known to proliferate in moderately fast waters (Thirion, 2007). This makes the reliance on water velocities to categorise macroinvertebrate assemblages problematic.

4.5.5 Water quality modification

The deterioration of water quality at Dopeni, Fondwe Maangani and Pfumbada were considered to be moderate despite the fact that Dopeni had large scale water abstraction activities. Dopeni, Maangani, Mphaila and Pfumbada had the Ephemeroptera family (Heptageniidae) which were highly intolerant to pollution, suggesting that some sections of the river along these sampling sites were not severely affected by water abstraction activities. The deterioration of water quality at Mphaila was found to be small because the area had the highest number of Ephemeroptera (Family Heptageniidae) specimen. The presence of these organisms signified conditions of good water quality. This was because the current agricultural fields along the area where samples were collected were zoned after the 2015 field observation. As a result, the impact of agriculture had not fully manifested itself. The water quality condition at

Musekwa was found to be of serious concern and this was evident in the number of pollution tolerant organisms which constituted 60% of the total number of macroinvertebrates sampled at Musekwa. The remaining 40% were moderately tolerant to pollution. Not a single pollution intolerant organism (EPT) was found at Musekwa, suggesting that the situation needs immediate attention. This suggests that agricultural activities along the river contributed to the types of resident macroinvertebrates due to changes in water quality. Pracheil (2010) has indicated that land-use for agricultural purposes has been associated with a decline in the quality of water, biological diversity and the complexity or heterogeneity of the aquatic habitats.

Agricultural development leads to clearing of vegetation and increased runoff into the river. Litvan *et al*. (2008) noted that sediments that are deposited into the riverine ecosystems due to surface runoff often cover rocky substrates and these substrates form habitats for macroinvertebrate families such as Heptageniidae (Ephemeroptera), Potamonautidae (Decapoda), Aeshnidae (Odonata) and Ecnomidae (Trichoptera) (DWAF, 2007). According to Litvan *et al*. (2008) these substrates reduce in diversity meaning that the diversity of the macroinvertebrates that depend on each substrate will decline in abundance. Interesting, the Chironomidae family which are known to prefer and survive under degraded or polluted environments were restricted to Fondwe, Mphaila and Pfumbada, but were absent from Musekwa area which was characterised by numerous indigenous agricultural fields. This was not surprising because, Chironomids survive in a variety of environmental conditions and are tolerant to disturbance (Mereta *et al.* (2013). This makes them to have weak association with environmental variables (Everaert *et al.*, 2014). Adeogun and Fafione (2011) noted that Chironomids survive in polluted environments because they possess haemoglobin, a pigment which transports dissolved oxygen, enabling them to proliferate and colonise effectively. Maangani, which had the highest abundance values (178) (Table 4.1, 4.3 above) than all other sampling sites did not record a single Chironomid specimen. This sampling site had 63% of pollution tolerant families (Thiaridae, Nepidae and Coenagrionidae). This can be assumed that the water quality had been altered because this was the only area where algae was observed. The existence of algae however, did not inhibit the occurrence of the Family Heptageniidae because it has been noted earlier in this chapter that agricultural inputs favour a variety of aquatic insects.

4.5.6 Inundation

Inundation was caused by the presence of bridges in all sampled sites. The degree of inundation in all areas was found to be small because inundation occurred only at sampling sites where bridges existed. Inundation often leads to sediment deposition at sampling points where the cemented bottom which acts as a small levee, is slightly raised under the bridge. This is the area where a run is converted into a riffle due to flow disturbance. Lind *et al*. (2006) have noted that once flow regimes have been changed by anthropogenic activities water quality and macroinvertebrate communities decline. However, the degree of inundation in all studied sites was considered not to have had considerable impact on the diversity of macroinvertebrates. This is because each site had a single bridge and due to low flow regimes as a result of the 2015/ 2016 drought there was no considerable inundation of high impact observed. The only minor change was that at the point of contact with the bridge bottom the runs were converted to riffles and the impact was however very small or insignificant.

4.5.7 Exotic macrophytes and aquatic fauna

No exotic macrophytes and exotic fauna were observed in all the six sites. Even though the Nzhelele River is known to have been invaded by invasive species such as *Lantana camara* there were no exotic macrophyte species from all sampled sites. This means that the problem of invasion by exotic macrophytes and aquatic fauna was absent from the six sampling sites.

4.5.8 Solid waste disposal

The problem of solid waste disposal was found to be serious at Dopeni and Musekwa. There was a large volume of solid waste along the Nzhelele River at Dopeni, even along the riparian zones. Residues from agricultural fields and domestic waste were evident along the river at Musekwa. The situation was considered serious due to the volumes of visible solid waste. Fondwe and Maagnani had minimal solid waste disposal problems. Solid waste was scanty and occurred in one point along the sampling points and this explains why it was considered small. However, the situation at Mphaila was different because there was no evidence of solid waste in areas where samples were collected. However, the amount of solid waste seemed to have had an impact on water quality and macroinvertebrate composition at Musekwa which was characterise by low abundance values. Despite Dopeni having a serious solid waste disposal problem along the river, many pollution intolerant organisms were sampled from this site. It could be argued that the severity of impact of solid waste depends on its nature, volume and decomposition, explaining less impact at Dopeni. The direct impact will therefore not be immediately manifested.

Table 4. 16 below also shows the results of an assessment of modification of riparian zone habitat integrity from the six studied sites of Dopeni, Fondwe, Maangani, Mphaila, Musekwa and Pfumbada. Riparian zone habitat is important in providing marginal vegetation as habitat for other organisms that require riparian zone for reproduction and feeding. Riparian zone habitat also provides shelter for other macroinvertebrates. The alteration or modification of riparian zones would mean a direct impact on the river water quality and the organisms found in a river. It was necessary to assess habitat integrity because degradation of streams due to modification in the pattern of land use is reflected in changes in flows, river water temperature, bank erosion and deposition of silt (Cordero-Rivera *et al*. (2017). Assessing riparian zone modification helped to determine the severity or magnitude of change in physical parameters.

Table 4.16 Assessment of modification of riparian zone habitat integrity (Field data, 2016)

Criterion	**Assessment per sampling area**	**Modification impact class and score**
Indigenous vegetation removal	**Dopeni:** Riparian vegetation removal is evident along various parts of the river due to sand mining, water extraction and large agricultural fields.	Serious (16-20)
	Fondwe: Some parts along the river lack vegetation due to the presence of agricultural fields.	Moderate (11-15)
	Maangani: Few areas have minimal vegetation removal.	Small/ Minimal (1-5)
	Mphaila: Removal of indigenous vegetation is evident along the banks of the river where a bridge is present. Development of new agricultural fields along the river has also led to the removal of vegetation.	Small/ Minimal (1-5)
	Musekwa: Large parts of the indigenous vegetation along the river have been replaced by agricultural fields.	Serious (16-20)
	Pfumbada: Few sections of the river along the banks lack indigenous vegetation due to paths created by water abstraction and agricultural fields.	Moderate (11-15)

Criterion	Assessment per sampling area	Modification impact class and score
Exotic vegetation encroachment	**Dopeni:** Very few or limited sections of the river have *Opuntia engelmannii* (small round-leafed prickly pear)	Small/ Minimal (1-5)
	Fondwe: Presence of *Lantana camara* and *Opuntia engelmannii* in some limited areas.	Small/ Minimal (1-5)
	Maagnani: No exotic vegetation encroachment was recorded or observed.	None (0)
	Mphaila: No exotic vegetation encroachment was recorded or observed.	None(0)
	Musekwa: Presence of *Lantana camara* and *Opuntia* species (*Opuntia engelmannii*) along the riparian zone.	Small/ Minimal (1-5)
	Pfumbada: No exotic vegetation was observed.	None (0)
Bank erosion	**Dopeni:** Due to reduced water quantity and low stream velocity there was no evidence of active bank erosion.	Small/ Minimal (1-5)
	Fondwe: Minimal bank erosion occurs where the river becomes narrow.	Small/ Minimal (1-5)
	Maangani: Bank erosion is evident along some parts of the river bank but is very minimal.	Small/ Minimal (1-5)
	Mphaila: Bank erosion minimal where the river meanders along agricultural fields.	Small/ Minimal (1-5)
	Musekwa: No observable bank erosion.	None (0)
	Pfumbada: Bank erosion is minimal where the river meanders.	Small/ Minimal (1-5)
Channel modification	**Dopeni:** excavated banks increased river width at some sampling points.	Moderate (6-10)
	Fondwe: Excavation of banks was evident at some sampling points	Small/ Minimal (1-5)
	Maangani: No visible channel modification.	None (0)
	Mphaila: Banks have been reinforced by rocks at sampling points were bridges were present.	Small/ Minimal (1-5)
	Musekwa: Banks have been excavated to widen the river at some sampling points. Artificial ridges have been erected to channel water at some points.	Moderate (6-10)
	Pfumbada: Construction of ridges at some sampling points to straighten the channel was evident.	Moderate (6-10)

Criterion	Assessment per sampling area	Modification impact class and score
Water abstraction	**Dopeni:** Water abstraction for indigenous agricultural irrigation happens along the banks. . **Fondwe:** Water collected through small pipes. **Maangani:** Water is collected through small pipes and furrows. **Mphaila:** Water collection points were not observed. **Musekwa:** Abstraction takes place through generators, vehicles and pipes. **Pfumbada:** Water abstraction happens through pipes and channel modification.	Serious (16-20) Moderate (6-10) Moderate (6-10) Small/ Minimal (1-5) Critical (21-25) Large (11-15)
Inundation	Inundation is caused by the presence of bridges at Dopeni, Fondwe, Mphaila, Musekwa and Pfumbada.	Small/ Minimal (1-5)for all areas. None (0)
Flow modification	**Dopeni:** Water channels are present and they alter river flow and create low flows. **Fondwe:** Channelling furrows are present and the affect river flow. **Maangani:** Furrows to extract water change river direction resulting in low flows **Mphaila:** No flow modification was observed. **Musekwa:** River channelling and ridges created low flows and pools. **Pfumbada:** Presence of water channelling affected flow changed flow regimes to low flows.	Large (11-15) Small/ Minimal (1-5) Large (1-5) None (0) Serious (16-20) Moderate (6-10)
Water quality	**Dopeni:** Non-point and point source pollution from neighbouring agricultural fields, laundry activities and livestock watering was observed. **Fondwe:** Non-point and point source pollution from neighbouring agricultural fields and laundry activities were observed. **Maangani:** Point source pollution from laundry activities was observed. **Mphaila:** No form of pollution was observed. **Musekwa:** Point source pollution from laundry activities, water abstraction and livestock watering was observed. **Pfumbada:** Point source pollution from agricultural fields.	Moderate (6-10) Moderate (6-10) Moderate (6-10) Small/ Minimal (1-5) Large (11-15) (Moderate) (6-10)

4.5.9 Indigenous vegetation removal

The extent of indigenous vegetation removal along the riparian zones differed from one place to another. The assessment results indicated that the state of vegetation removal at Dopeni and Musekwa was serious. Musekwa and Dopeni are characterised by indigenous agricultural fields along the river and a vast amount of vegetation, including the riparian vegetation have been removed. The absence of riparian vegetation means that the river's capacity to filter pollutants has been reduced. Claeson *et al.* (2014) observed that riparian vegetation produces detritus that serves as source of energy for aquatic ecosystems. Changes in the riparian plant communities will therefore alter macroinvertebrate composition along a river. Cohen *et al.* (2012) found that changes in the composition of riparian plants can alter aquatic food webs when inputs from detritus differ in intrinsic properties. The conditions at Musekwa were manifested by the lack of pollution intolerant organisms such as the Family Heptageniidae but conditions at Dopeni were not as serious as at Musekwa area. This might have been the result of lack of vegetation and extremely gently sloping landscaping connecting the river which could have accelerated the deposition of pollutants and sediments into the river. Hepp *et* al. (2010) have noted that the removal of riparian vegetation eliminates the natural protection mechanisms of rivers against erosion, increased sediment inputs and other pollutants. This can be seen by the presence of the Family Heptageniidae (29%) and organisms that were moderately tolerant to pollution which constituted a total of 51%. The remaining 20% were pollution tolerant organisms of Thiaridae family. Bruno *et* al. (2014) have argued that riparian ecosystems are important in determining the structure and functioning of aquatic organisms. The extent of indigenous riparian removal at Fondwe and Pfumbada was considered moderate as removal was not continuous along the river just like at Dopeni and Musekwa. The situation at Fondwe and Pfumbada could have been regarded as critical if the area did not have some vegetated corridors along some parts of the river. Even though the conditions at Fondwe and Pfumbada were considered moderate, Fondwe recorded the highest number (76%) of pollution tolerant families Chironomidae (Diptera), Coenagrionidae (Odonata), Nepidae (Hemiptera) and Thiaridae (Gastropoda). The remaining 24% were moderately tolerant to pollution, suggesting the absence of pollution intolerant organisms like the Order Ephemeroptera. However, conditions at Pfumbada showed that the presence of vegetation at some points along the river allowed for the existence of the pollution intolerant families such as Heptageniidae, Aeshnidae and Elmidae.

Maangani and Mphaila had a minimal vegetation removal problem and the extent of indigenous vegetation removal was considered small. However, even though the extent of removal was small at Maangani many pollution tolerant organisms (63%) were recorded while Mphaila had the lowest number of pollution tolerant organisms (6%). It can therefore be argued that conditions at Maangani resulted from the direct input of pollutants from adjacent agricultural fields and sand mining. Maangani was the only sampling site where the river was wider due to the presence of trees along numerous sections of the banks. Hussain (2012) indicated that rivers that are characterised by forests along their riparian corridors tend to be two and half times wider than rivers with deforested riparian zones like Musekwa which was characterised by a flat terrain but narrow water channel.

4.5.10 Exotic vegetation encroachment

The extent of exotic vegetation encroachment was considered small in the three sites of Dopeni, Fondwe and Musekwa. The other three sites where samples were collected had no exotic vegetation. There were no exotic vegetation observations at Maangani, Mphaila and Pfumbada at sampling points where sampling was conducted. The presence of *Opuntia* species (*Opuntia engelmannii*) (small round-leafed prickly pear), *Lantana camara* (tick-berry) and *Dodonaea angustifolia* (sand olive) at Dopeni, Fondwe and Musekwa indicated that these species could have colonised the areas after the clearing of riparian zone and the adjacent land for agriculture. McNeish *et al*. (2017) similarly observed that the invasion of riparian zones by invasive species leads to near-monocultures along river systems and often enables opportunistic species to colonise some sections of the river and the ecological functions of the riparian zone at the point where invasive biota are recorded are partly disturbed or altered.

The role of indigenous vegetation such as the provision of shade and organic matter has been altered by the presence of this invasive biota. Stockan and Fielding (2013) have noted that riparian vegetation often provides shading to adjacent water bodies and reduce the proliferation of algae. This was evident at Maangani where algal bloom was observed along the sections parallel to the agricultural fields while on the eastern part of the river banks where dense trees were present algal blooms were not observed. No other site recorded the presence of algal blooms except Maangani. Cordero-Rivera *et al*. (2017) have noted that the presence of *Eucalyptus* trees leads to the reduction of macroinvertebrates in streams characterised by small forests. However, the diversity of macroinvertebrates at Dopeni, Fondwe and Musekwa has not been directly linked to the effects of invasive biota but rather, the water quantity, quality and flow regimes of the 2015/2016 drought. This was because the density of these invasive species from the three places was considered too low to have a significant impact on the stream functioning. The main threats were flow regimes, water quantity, quality, and land-use activities along the river. However, the presence of these alien species was directly linked to agricultural activities along the river. Turpie *et al*. (2008) have indicated that the alien plants that have invaded the Cape fynbos originated from commercial plantations and woodlots on farms. It can therefore be argued that there is a strong link or relationship between invasive alien plants and anthropogenic activities such as agriculture. The low flows at Musekwa, the presence of *Lantana camara* and *Opuntia* species, as well as low macroinvertebrates abundance values were complex to understand but the impact of very few alien species could not have not accounted for such low macroinvertebrate diversity. Figure 4.6 below shows *Opuntia* species that was found to be common along the riparian zones and adjacent landscapes of the Dopeni, Fondwe and Musekwa.

Figure 4.6 *Opuntia engelmannii* (Prickly pear) that was found to be common at Dopeni, Fondwe and Musekwa (Field data: 2016)

4.5.11 Bank erosion

There were no visible signs of active bank erosion at Musekwa because the site was characterised by a flat terrain (Figure 4.7 below). However, bank erosion in the other five sites (Fondwe, Maangani, Mphaila Musekwa, and Pfumbada) was very minimal due to the low flow nature of the stream. The river water was at its lowest and only small areas along the river suffered bank erosion which was generally associated with sedimentation in the river. Lack of erosive power led to poor bank erosion. The situation at Fondwe, where an agricultural field was located at the meander belt meant that should the river receive water inputs from precipitation bank erosion would be severe and more sediments and increased cultural eutrophication will take place. Channel instability and bank erosion are directly influenced by deforestation and agricultural land use patterns (Simpson *et al*., 2014). Once riparian vegetation has been removed for agricultural purposes, bank stability becomes weakened, making the river to be unstable. Sullivan *et al*. (2004) noted that unstable rivers more often change their course of flow by metres per annum and create new channels. Such streams are characterised by bed degradation, channel widening and platform adjustment process (Sullivan *et al*., 2004). The degraded beds were only observed at Musekwa and Dopeni, with Musekwa having a wider channel but low water quantity. Maangani's wetted perimeter was greater than in all studied sites. Sites that suffered minimal bank erosion were Fondwe, Maangani, Mphaila and Pfumbada. Even though there was no evidence of bank erosion at Musekwa due to its flat terrain, it can be concluded that the water surface area was very low, leading to a reduction in aquatic habitat heterogeneity. Mukundan *et al*. (2010) have noted that bank erosion leads to a reduction in the physical and biological functions of streams. This could not be true for six studied areas which had minimal bank erosion. The effect of bank erosion from the six sampling sites was considered to be insignificant because there was no evidence of active bank erosion at the time of sampling due to reduced river flow.

Figure 4.7 Flat terrain and reduced river flow of the Nzhelele River at Musekwa (Field data: 2016)

4.5.12 Channel modification

At Dopeni the river had been widened along the banks where soil extraction for brick-making activities was visible. The excavated banks increased river width and bank calving at some sampling points. Bank calving increases river sedimentation that leads to bed modification. This will ultimately lead to decreased flow velocity. However, the impact of bank erosion is still very small due to low flows that were experienced during drought. At Fondwe, excavation along the riparian zones where marginal vegetation plays an important role in the supply of litter to macroinvertebrates occured in only two areas. However, serious modification of the channel could have negative impact on river dynamics and macroinvertebrate composition. The same problem has been observed at Musekwa but the excavation problem occurs in numerous places along the river. The presence of ridges and furrows for channelling river water away from the river also exacerbated the low flow conditions at Musekwa.

Channel straightening at Pfumbada also impacted on riparian zone because fragmented or isolated ridges impact on the dynamics of the river and the assemblages of aquatic biota. The erection of dams, levees and river channelization change the timing, magnitude, and the duration of high flows, and the reduction and elimination of floodplain connectivity in large rivers (Hill *et al*. (2016). This situation leads to a decline in the number of native species and the reduction in macroinvertebrate organisms (Poff and Zimmerman, 2010). However, even though channel straightening at Pfumbada was found to be moderate it seemed not to have affected species diversity because Pfumbada recorded the second highest diversity index of 0.77 after Fondwe (0.78) (Table 4.4 above). However, at Mphaila, the replacement of bank soil with rocks increased risks of mass wasting should the river overflow its banks and once rocks settle at the bottom they are likely to change river flow from runs to riffles. This will also change macroinvertebrate assemblages at that point where the area would be characterised by organisms that favour riffles such as the families Heptageniidae (Ephemeroptera), Elmidae (Coleoptera), Potamonautidae (Decapoda), Aeshnidae (Odonata) and Ecnomidae (Trichoptera). This is because the rocks are likely to trap sediments, pollutants and other debris that will change the composition of the macroinvertebrate at that particular point along the river. Reduction in floodplain connectivity like at Musekwa and Pfumbada due to channelization was moderate but the loss in connectivity meant that the diversity of adjacent habitat was curtailed. Even though channelization was also moderate at Dopeni there was no evidence of channel modification impacting on macroinvertebrates and water quality because Dopeni recorded 20% of pollution tolerant organisms (Family Thiaridae). The Potamonautidae family of Musekwa site was restricted to sampling points that were close to river banks, riparian vegetation and small rocky substrate.

4.5.13 Water abstraction

At Dopeni, Fondwe, Maangani, Musekwa and Pfumbada water abstraction through pipes affected the riparian conditions. No water collection points were observed at Mphaila. The abstraction of water through pipes affects the riparian zone because of continuous disturbances along places where furrows and water pipes were located. The plastic water pipes are likely to create small channels that carry runoff water into the stream causing further sedimentation. This will also affect the composition of macroinvertebrates at the point. It has been noted earlier that sedimentation will lead to aggradation of the river covering bedrock or cobbles and change flow regimes from riffles to runs. The removal of vegetation during pipe and channel creation for water abstractions will gradually worsen the sedimentation problem along the Nzhelele River. Hepp *et al*. (2010) indicated that the practice of removing vegetation for agricultural developments leads to the weakening of the stream capacity to shield itself from erosion by surface runoff, thereby leading to the problem of increased sedimentation, nutrients and pollutants. This will also affect the composition of the resident macroinvertebrates. However, even though other sampling sites had high abundance values for pollution intolerant organisms it suggested that the effects of agricultural practices alone were not manifested by the resident macroinvertebrates. This also suggested that even though agricultural fields were not located far from the river, the types of fertilisers or the lack of use of fertilisers led to the good water conditions at certain sampling points along the river. This meant that indigenous agriculture could not have been the only causes of water quality deterioration at the six sampling sites. Alternatively, the utilisation of fertilisers might be limited to cause substantial impact on macroinvertebrate communities. Cortelezzi *et al*. (2015) noted that the effects of fertilizers on macroinvertebrates require a continuous period of three years for the impacts to be noticeable. This is not noticeable at the six sampling sites.

4.5.14 Inundation

Inundation is caused by the presence of bridges at Dopeni, Fondwe, Mphaila, Musekwa and Pfumbada. Inundation at the places where rivers crossed over the cemented or artificial rock layer seemed to have caused changes in the flow mechanisms of a river. At Mphaila, the narrowed bottom of the bridge will exacerbate inundation during floods and lateral cutting will be severe and followed by bank collapse and sedimentation at those points. At the points of inundation bank collapse will eradicate riparian vegetation and the removal of vegetation will automatically change river dynamics and macroinvertebrate assemblages. The impact of inundation was discussed under section 4.5.6. However, no inundation problems were observed along the riparian zones due to low water quantity at the time of sampling.

4.5.15 Flow modification

The presence of water channels at Dopeni, Fondwe, Maangani Musekwa and Pfumbada modified the flow characteristics of the river. No modification was observed at Mphaila. The presence of artificial channels can lead to reduced or increased river velocities. Increased low flows as a result of flow modification will lead to sedimentation due to reduced sediment-carrying capacity of the river. Flow modification can increase bank erosion during floods which will also remove marginal vegetation which will accelerate the rate of bank collapse and sedimentation. This will ultimately have a direct impact on macroinvertebrate communities. Belmar *et al.* (2013) have noted that flow regulations and land use changes lead to hydromorphological alteration of rivers and this often leads to the modification of freshwater biological communities like macroinvertebrates. Growns *et al.* (2017) have noted that rivers with reduced flow increase siltation of the stream bed. For example, the low flows at Musekwa could have accounted for the existence of the substrate characterised by sand and gravel. Brooks *et al*. (2011) also noted that changes in river flow change macroinvertebrate assemblages and their trait structure. The situation at the five sampled sites might lead to these problems in the near future and the composition of macroinvertebrates will completely change from what it is currently.

4.5.16 Water quality

Point-source pollution from laundry activities and livestock watering were observed at Dopeni, Fondwe, Maangani, Musekwa and Pfumbada. No observable forms of pollution were recorded at Mphaila. Pollution from agriculture will alter riparian biota such as the proliferation of invasive plant communities. The presence of *Oputia* species, *Lantana camara* and sand olive (*Dodonaea angustifolia*) is a clear indication of agricultural activities. The proliferation of this alien biota will alter the types of macroinvertebrates in the river. This means that the type of detritus that comes from these alien plant species will require specific macroinvertebrates that will feed on this new form of dead organic matter. This will mean that a new group of macroinvertebrates will have to replace the resident ones that do not feed on litter from these invasive plant species. Polluted waters, especially with low pH are associated with pollution tolerant organism. For example, Ernst *et al*. (2008) have noted that streams that have elevated levels of acidity have fewer EPT taxa while the Family Chironomidae (Diptera) that tolerate pollution become the dominant organisms. Interestingly, all average pH values recorded from the six sampling sites ranged from neutral to alkaline. The neutral values for Fondwe seemed to have allowed for the higher abundance values of Diptera (Chironomids) compared to Mphaila and Pfumbada which had higher average pH values of 7.7 and 8.29 respectively. These two areas recorded lower abundance values of chironomid organisms of 6 and 9. Three sampling sites (Dopeni, Maangani and Musekwa) did not record a single chironomid specimen. The water quality of the studied areas could have been altered by low flows at the time of sampling, but could also change after water input from rains. Riparian communities seemed not to have directly impacted on water quality of the river, even though sites such as Dopeni, Fondwe, Maangani and Musekwa where characterised by the presence of solid waste disposal along riparian and aquatic zones.

4.6 Principal Component analysis

Figure 4.8 below shows correlation between sampling sites and macroinvertebrates. The first principal component (P1) accounted for 42.08% of variance in the data while the second component (P2) accounted for 28.85% variance. The combination of the two components explained 70.94% of the variation in macroinvertebrate composition among the six sampling sites. Maangani, Fondwe and Pfumbada were positively defined by the families Aeshnidae (Odonata), Nepidae (Hemiptera), Chironomidae (Diptera), Coenagrionidae (Odonata) and Thiaridae (Gastropoda) with the first component, but these sampling sites were negatively correlated with Potamonautidae (Decapoda), Elmidae (Coleoptera), Ecnomidae (Trichoptera) and Heptageniidae (Ephemeroptera) which positively defined Mphaila and Dopeni with the second principal component. Musekwa had a negative value for the Family Potamonautidae suggesting a degraded sampling site since they are strongly linked to polluted environments. These families are highly tolerant to pollution with a tolerance level of 3. It can therefore, be asserted that the water quality at Musekwa was degraded. This is because Musekwa area constituted 60% of pollution tolerant organisms families while the remaining 40% represented pollution intolerant organisms (Table 4.1, 4.3).

From Figure 4.8 below, just like Musekwa, Fondwe area was strongly defined by the presence of Coenagrionidae (Odonata), Thiaridae (Gastropoda) and Chironomidae (Diptera) families. All these families of macroinvertebrates are highly tolerant to pollution, with a pollution tolerance range of 2 to 4. These include organisms such as midges, damselflies and snails. Therefore, a strong association of pollution tolerant organisms with Fondwe suggests that the river water during the time of sampling did not favour the presence of ETP groups which indicate good water quality.

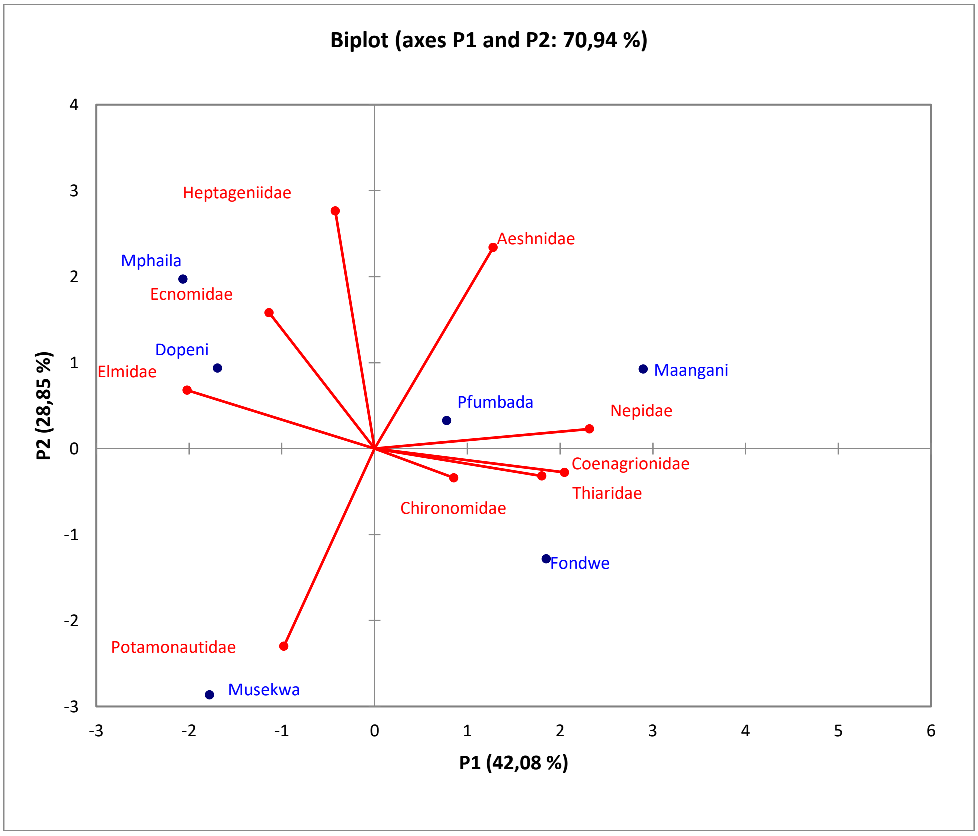


Figure 4.8 Relationship between macroinvertebrate families and sample sites (Source: Field data, 2016)

Pfumbada and Maangani formed a group of sampling sites characterised by the families Aeshnidae (Odonata) and Nepidae (Hemiptera), but were not strongly linked to the family Potamonautidae. Nepidae families, however, showed higher values of representation with the first principal component. Only Family Nepidae were found to be highly tolerant to pollution at Maangani and Pfumbada. While the Family Aeshnidae are moderately tolerant to pollution, Nepidae families are highly tolerant. Therefore, the moderately and highly polluted water bodies along the Nzhelele River defined these two sampling sites.

Dopeni and Mphaila were well defined by the pollution intolerant organisms which characterised all other four (4) sampling sites of Fondwe, Maangani, Musekwa and Pfumbada. These two areas were well represented by Ecnomidae (Trichoptera), Elmidae (Coleoptera) and Heptageniidae (Ephemeroptera) families which are pollution intolerant, suggesting a generally good water quality from these sites. This includes caddisflies, riffle beetles and flat-headed mayflies. These groups strongly define the water quality of the two sites as ranging from moderately polluted to non-polluted. This is because these families tolerate moderately polluted (Ecnomidae and Elmidae) and non-polluted waters (Heptageniidae). However, at

Dopeni and Mphaila, Heptageniidae (pollution intolerant) was the dominant family with higher abundance values (Table 4.1, 4.3 above) than Ecnomidae and Elmidae families (moderately tolerant to pollution). The ephemeropteran families are well-represented with the second principal component to show their strong correlation with the two sampling sites. This is a strong indication of the least polluted water condition from these two sampling sites. The percentage of all pollution intolerant organisms from Dopeni and Maangani was 80% and 94% (Table 4.3 above) respectively indicating good water condition.

Figure 4.9 below shows relationships between sampling sites and physico-chemical properties of water along the Nzhelele River. In Figure 4.9 below, the first principal component (P1) accounted for 59.85% of variation while the second component (P2) accounted for 19.45% of variance. Therefore, the first two components accounted for 79.30% of variation in water quality parameters among the six sampling areas. Dopeni and Mphaila were poorly represented by nitrates, DO, temperature, TDS, conductivity, discharge, pH and nitrites, suggesting good water conditions. These sites were strongly defined by the presence of pollution intolerant organisms and were negatively influenced by pollution tolerant families such as Coenagrionimidae, Nepidae, Chironomidae, and Potamonautidae (Figure 4.8, Table 4.3 above). However, Dopeni, Fondwe and Mphaila had a strong positive influence of chlorine, with the first principal component which accounted for 59.85% of variation in the data, but chlorine negatively defined Maangani, Musekwa and Pfumbada with the first principal component. Maangani, Musekwa and Pfumbada were positively defined by DO, nitrate, temperature, TDS, discharge, pH and nitrites with the first principal component. These correlations define the water quality of these sites which was degraded and supported the majority of pollution tolerant families (Table 4.1, 4.3 above). Maangani recorded 63% of pollution tolerant organisms, while Musekwa recorded 60% of pollution tolerant organisms. Interestingly, Pfumbada recorded 48% of pollution tolerant organisms suggesting that the water quality condition was moderately degraded. This could be true because this sampling site had the second highest diversity index value of 0.77, which was the second highest after Fondwe (0.78).

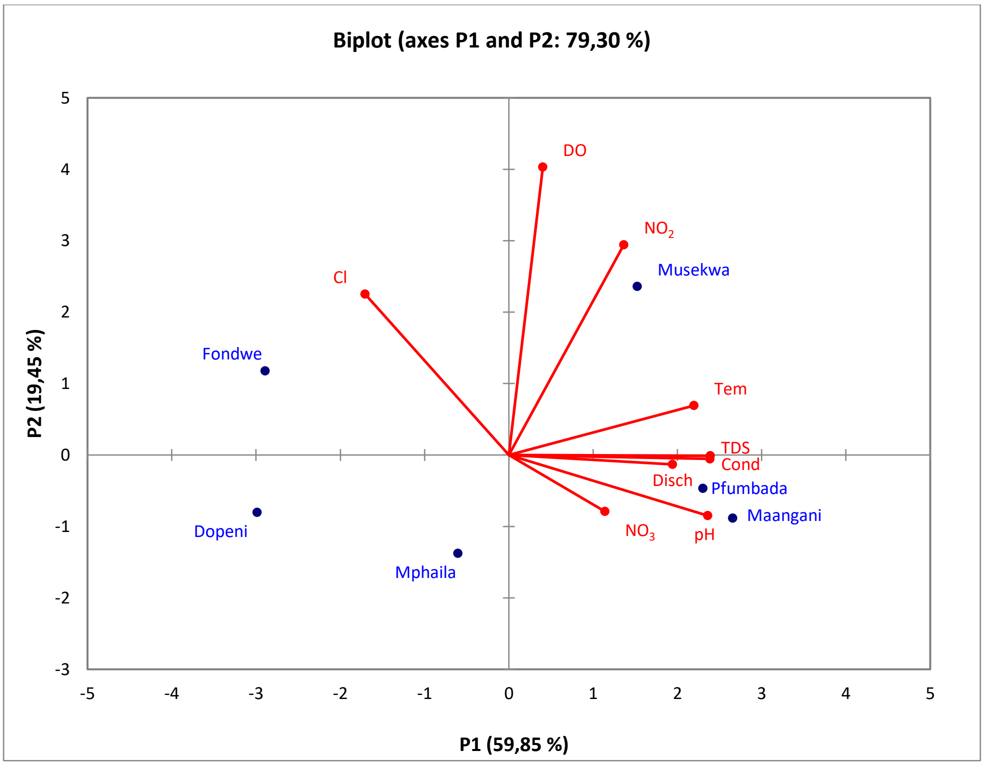


Figure 4.9 Relationship between water quality parameters and sampling sites (Source: Field data, 2016)

Pfumbada and Maangani which were associated with pollution tolerant Nepidae family and pollution intolerant Aeshnidae family was well-defined by the presence of nitrates, pH, river discharge, conductivity and TDS which were all positively correlated with these two sampling sites with the first principal component (P1). Nitrates, for example, can be a source of polluted water and increased temperature. Musekwa, which was well-defined by the Family Potamonautidae (highly tolerant to pollution) was strongly defined by DO, temperature and nitrites. High DO concentrations (Table 4.7) along the Musekwa sampling site also suggested that the sampling site had less anaerobic microbial activity due to lower abundance values of macroinvertebrates (Table 4.1 and 4.3) that were sampled at the site. Musekwa sampling site was also located along many indigenous agricultural fields which were characterised by heavy utilisation of the river such as water abstraction, laundry and agricultural irrigation. This explains low abundance values and diversity of macroinvertebrates. From Tables 4.10 and 4.14 below, it can be seen that Musekwa recorded the highest temperature and nitrate averages, suggesting nutrient input from adjacent subsistence agricultural fields. This explains a lower diversity index value of 0.69 compared to other sites.

Fondwe was not well represented by all other water quality parameters except chlorine. This sampling site was positively defined by Chironomidae, Coenagrionidae and Thiaridae families (Figure 4.8 above). The presence of chlorine in water is known to be negative on pollution intolerant organisms which show a decline when chlorine concentrations increase (Bradley *et al*., 2002). The average chlorine concentration at Fondwe was the second highest (13, 86 mg/L), suggesting that its concentration along most sampling points was relatively higher. This explains the high percentage (76%) of pollution tolerant families (Chironomidae, Coenagrionidae, Nepidae, and Thiaridae) and total absence of pollution intolerant families (EPT group), suggesting a negative association of chlorine with these families. Chlorine has influenced the absence of EPT group at Fondwe.

From Figure 4.9 above, it is evident that Musekwa was well represented by DO with the second principal component while Pfumbada and Maangani were positively defined by pH, discharge and nitrite with the first principal component. The positive correlation of nitrite and the two sampling sites could be explained by the higher abundance values of macroinvertebrates from these two sites. Maangani and Pfumbada recorded nitrite averages of 20.70 and 28.38 mg/L respectively and they happened to be the only two sampling sites with higher abundance values of macroinvertebrates.

The presence of macroinvertebrates from all sampling sites was also strongly associated with the physico-chemical properties of water at the time of sampling. This also means that these physico-chemical properties strongly defined the presence of macroinvertebrates at each sampling point of the sampling sites. Therefore, physico-chemical properties strongly defined the presence of the families of macroinvertebrates present in water and the presence of Ecnomidae, Elmidae and Heptagennidae families from Dopeni and Mphaila was a good indication of non-polluted or moderately-polluted aquatic environment. These two sampling sites were negatively correlated with parameters such as nitrates, temperature and TDS with the first principal component. These two sampling areas were characterised by the majority of pollution intolerant organisms, suggesting that these areas had the least polluted environments compared to the other four (Fondwe, Maangani, Musekwa and Pfumbada). Mphaila recorded the highest percentage (94%) of pollution intolerant families, while Dopeni recorded the second highest (80%).

When 15 sampling points from each sampling site were compared with regards to macroinvertebrates an interesting observation was that each site was defined by its own distinct family of macroinvertebrates. Figures 4.10*a-f* below depict the relationships between sampling points and macroinvertebrates per sampling site. Figures 4.10*a-f* below show these relationships from six sampling sites in alphabetical order as Dopeni (Figure 4.10*a*), Fondwe (Figure 4.10*b*), Maangani (Figure 4.10*c*), Mphaila (Figure 4.10*d*), Musekwa (Figure 4.10*e*) and Pfumbada (Figure 4.10*f*).

Figure 4.10*a* below shows the relationships for the Dopeni site. From Figure 4.10*a* the first two principal components accounted for 71.08% of variation in data from Dopeni, with the first principal component (P1) accounting for 45.53% of the variation and the second component (P2) accounting for 25.55% of variance. Four sampling points were strongly defined by the presence of Thiaridae and Heptageniidae families. Seven sampling points (S1, S3, S6, S9, S12, S13 and S15) had a strong positive correlation with the Family Aeshnidae (Odonata), Heptageniidae (Ephemeroptera), and Thiaridae (Gastropoda) families with the first principal component, but negatively correlated with the Family Elmidae. Sampling points S4, S5, S10 and S14 were positively correlated with Family Elmidae but S2, S6, S8 and S11 had negative values for their representation, suggesting that they were poor performers in macroinvertebrate composition. The low abundance values of macroinvertebrates from these sampling points could have been attributed to extremely low flows at the time of sampling. Sampling point 12 (S12) was positively defined by Family Heptageniidae, suggesting that this sampling point was not polluted. However, only three sampling points (S1, S13 and S15) from Dopeni were positively associated with the Family Aeshnidae, suggesting that water quality at these sites was moderately or less polluted.

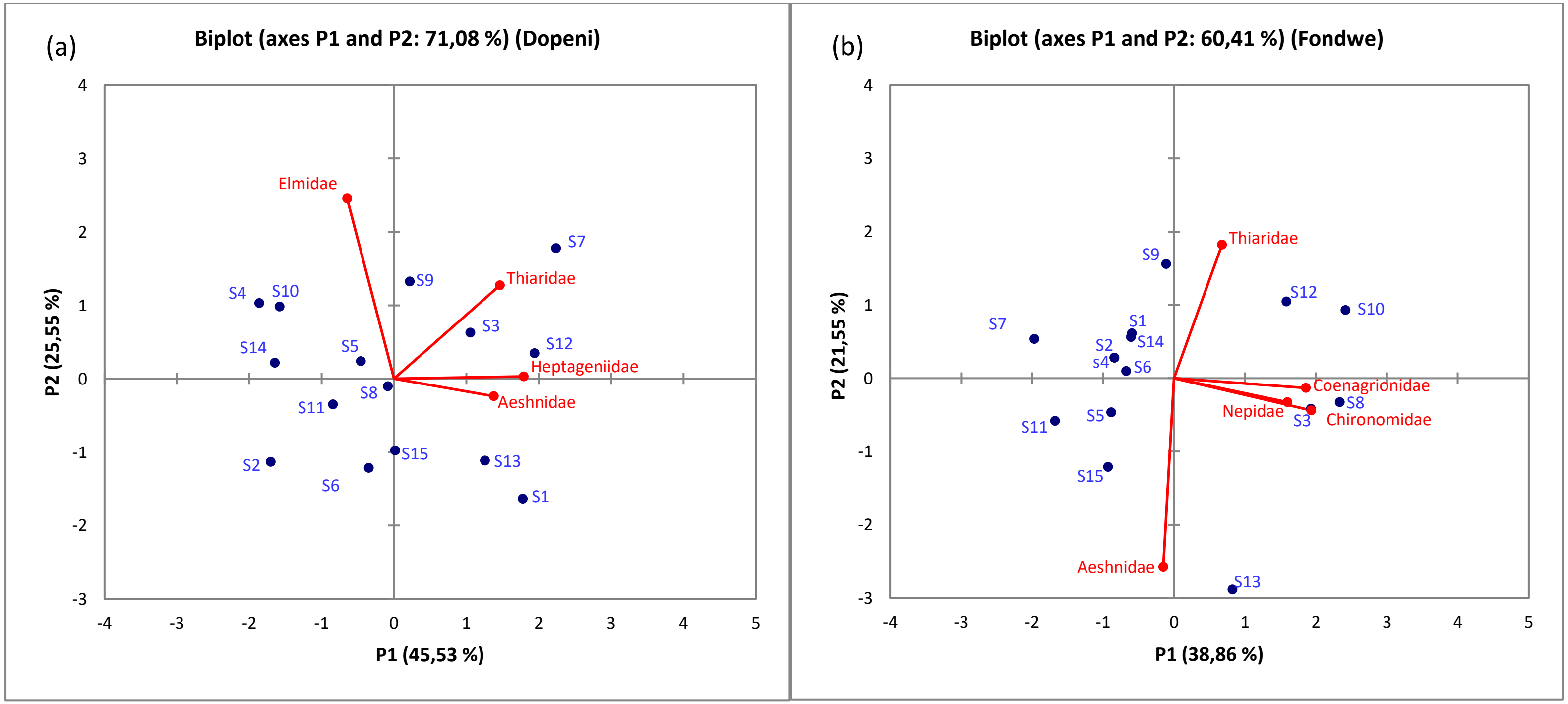


Figure 4.10*a,b* Correlation between sampling points and macroinvertebrate families for Dopeni (*a*) and Fondwe (*b*)

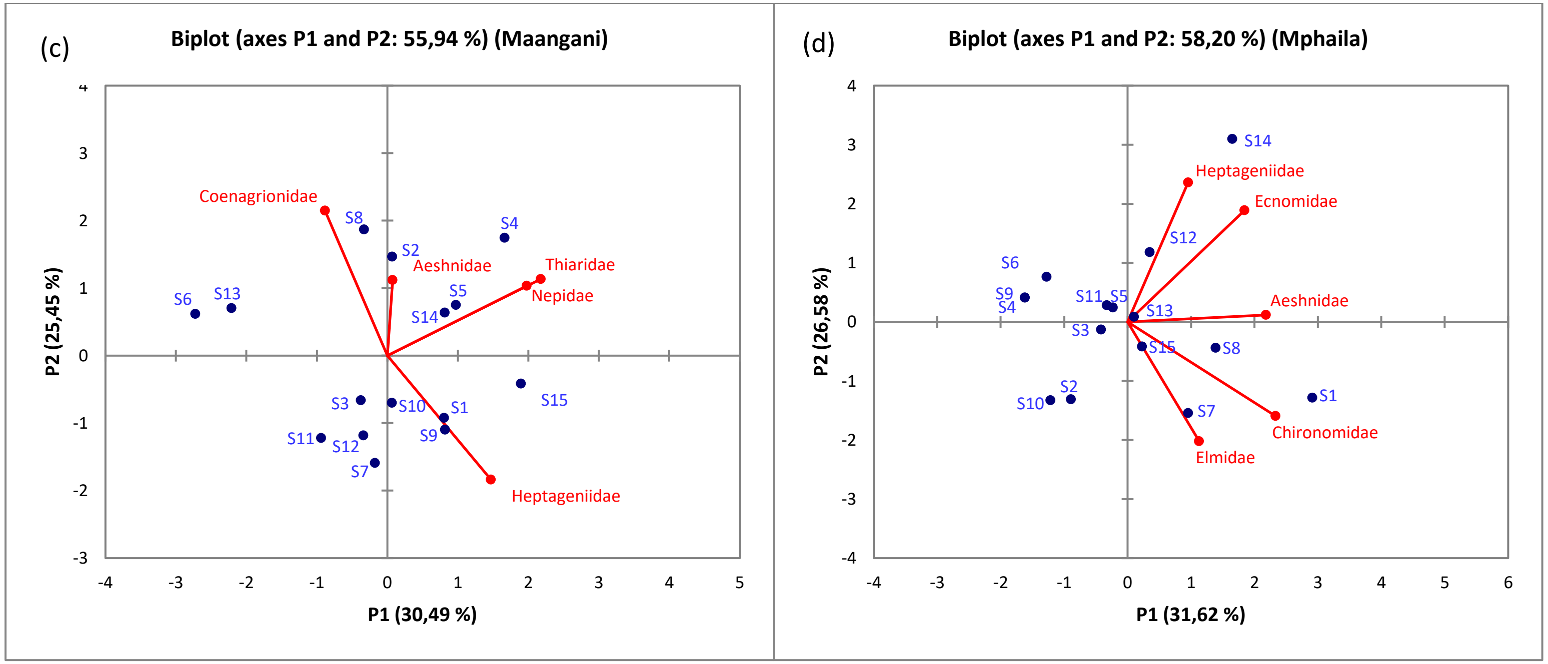


Figure 4.10*c*,*d* Correlation between sampling points and macroinvertebrate families for Maangani *(b)* and Mphaila (*c*)

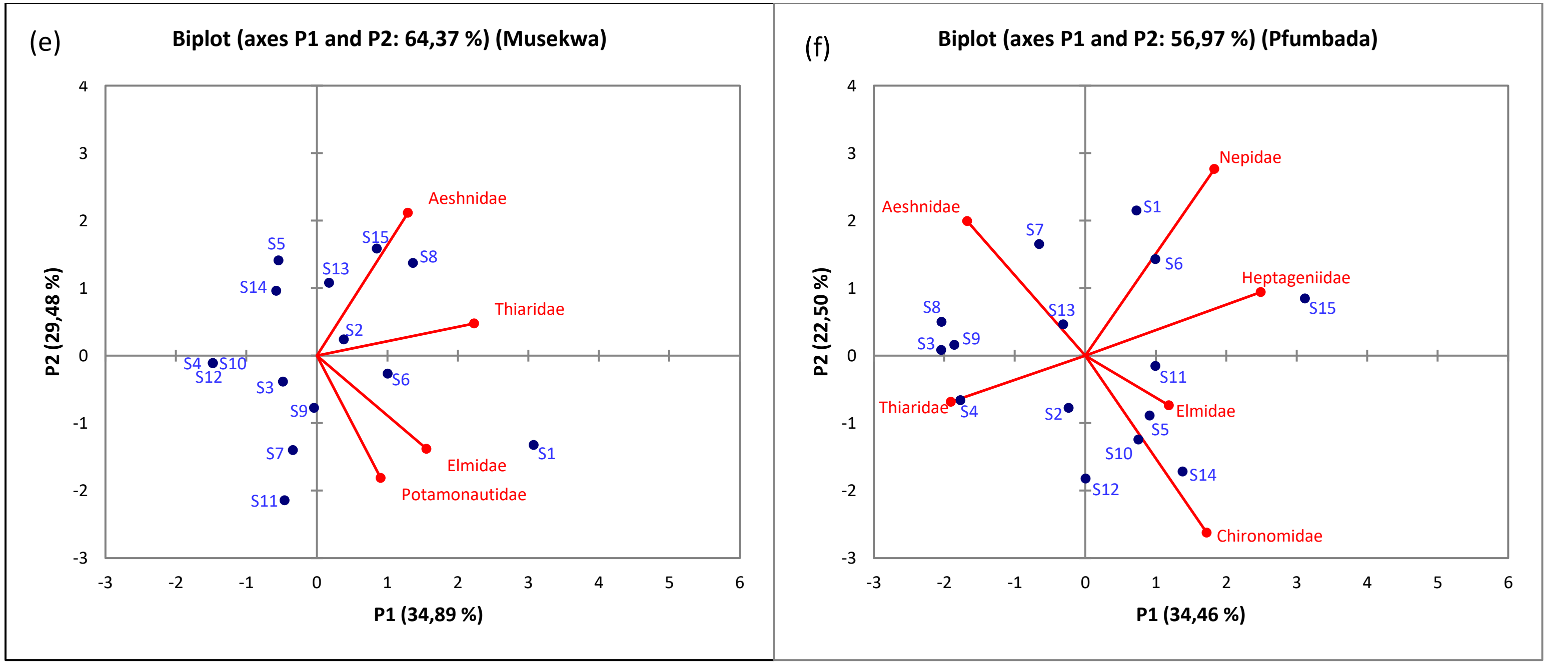


Figure 4.10*e, f* Correlation between sampling points and macroinvertebrate families for Musekwa (*e*) and Pfumbada (*f*)

However, the distribution of macroinvertebrates along the sampling points was related to the water quality of sites at the time of sampling because these variables change from time to time. Therefore, the macroinvertebrates sampled from each sampling point were directly related to the condition of the river at a particular point in time. This means that if samples were collected daily for 52 weeks at the same spots it would have been easy to identify macroinvertebrates that would have colonised certain areas through drifting or other forms of movement, especially those that could colonise an area through drifting such as the Chironomidae family. Graca *et al.* (2004) have noted that colonisation of new substratum takes place through drifting mechanism rather than by active movement of organisms within the substratum. Since chironomids, which were a characteristic of Fondwe, Mphaila and Pfumbada, possess the ability to move by drifting, their occurrence in some parts of the river along the Nzhelele River suggests that some locations were colonised through this mechanism. This explains their occurrence at Mphaila which was characterised by 94% of pollution intolerant taxa. Again, it has been noted by Lencioni *et al*. (2012) that chironomids also survive under pristine aquatic conditions, suggesting their presence at the Mphaila sampling site.

For Fondwe (Figure 4.10*b*), the first two principal components accounted for 60.41% of variance with the first component (P1) accounting for 38.86% of variation while the second principal component (P2) had 21.55% variance. Five sampling points (S3, S8, S10, S12 and S13) were positively correlated with Thiaridae, Coenagrionidae, Nepidae and Chironomidae families with the first principal component but negatively correlated with the family Aeshnidae. This suggests that very few sampling points at Fondwe were positively defined by pollution sensitive organisms. This means that the water quality at Fondwe was degraded. This explained a composition of 76% of pollution tolerant organisms that were sampled at Fondwe. Three sampling points (S5, S11 and S15) recorded negative values for the Family Aeshnidae but the remaining seven sampling points (S1, S2, S4, S6, S7, S9, and S14) were positively correlated with Aeshnidae but negatively correlated with the Thiaridae, Coenagrionidae, Chironomidae and Nepidae families with the first principal component. This correlation defines the water quality of these sampling points as being less polluted. From Figure 4.8, it can be seen that Fondwe was positively correlated with the Chironomidae, Coenagrionidae and Thiaridae families. These are all pollution tolerant organisms and this explained 76% of pollution tolerant organisms found at Fondwe sampling site. Three other sites (S3, S8 and S13) were well represented by three pollution tolerant families (Chironomidae, Coenagrionidae and Nepidae). The results indicated that the level of pollution differs significantly from one sampling point to another, suggesting that changes in the level of pollution along the river at Fondwe occurred over short distances since these points were 10 m apart over a distance of 150 m.

For Maangani (Figure 4.10*c*), the first principal component (P1) accounted for 30.49% of variation in data and 25.45% for the second component (P2). From Figure 4.10*c* eight (8) sampling points (S1, S2. S4, S5, S9, S10, S14 and S15) were positively correlated with the Aeshnidae, Thiaridae, Nepidae and Heptageniidae families with the first principal component but negatively correlated with Coenagrionidae. Sampling points S6, S8, and S13 were positively defined by pollution tolerant family Coenagrionidae with the second principal component. This meant that these sites were more polluted than S1, S2, S9, S10 and S15 which were defined by families Aeshnidae and Heptageniidae respectively. This suggested that sampling points at Maangani differed in the level of pollution. Interestingly, Family Heptageniidae (Ephemeroptera) which is known to be highly intolerant to pollution was also represented at Maangani with percentage value of 17% of all sampled macroinvertebrates (Table 4.3). However, a higher percentage (63%) of pollution tolerant organisms at Maangani suggested that other sites were degraded and were able to host many pollution tolerant organisms. Four sampling sites (S3, S7, S11, S12) were poor performers in the composition of macroinvertebrates. Four other sites (S1, S9, S10, S15) (lower right) were well represented by pollution intolerant Heptageniidae family. Four other sites (S2, S4, S5, S14) were well defined by the families Aeshnidae, Nepidae and Thiaridae. However, Aeshnidae were poorly represented due to the position in the graph. The remaining three (3) sampling points were positively correlated with pollution tolerant Coenagrionidae family.

At Mphaila (Figure 4.10*d*), the first two principal components accounted for 58.20% of variation with the first principal component accounting for 31.62% of variation in the data and the second component 26.58% of variance. For Mphaila site, Heptageniidae, Ecnomidae, Aeshnidae, Elmidae and Chironomidae families were positively correlated with seven (7) sampling points (S1, S7, S8, S12, S13, S14 and S15) with the first principal component. Even though the represented organisms from the seven sampling points suggested non-polluted or moderately polluted environments the presence of chironomids suggested that it was omnipresent in all types of water ranging from polluted to less polluted environments. Mazzoni *et al*. (2014) have noted in their study, that the Family Chironomidae occurred from clean to critically polluted sites and it was often absent from degraded environments. This explains their occurrence at Mphaila site which was characterised by good water quality. Even though the Family Chironomidae are pollution tolerant, the total reliance on these organisms to assess water quality should be approached with caution. Their presence at Mphaila suggested that the water condition was of good quality since 94% of organisms at Mphaila were pollution intolerant. Interestingly, at Mphaila a total of 8 samplinng points out of fifteen (15) had poor representation of macroinvertebrates suggesting lower abundance values of macroinvertebrates. Four sampling points (S1, S7, S8 and S15) represented Family Chironomidae (pollution tolerant) and Family Elmidae (moderately tolerant) well. S1 had good representation of these macroinvertebrates given its position in the graph. The remaining three (3) sampling points (S12, S13,S14) were good performers in the composition of Aeshnidae, Ecnomidae and Heptageniidae families with the tolerance levels ranging from moderate to non-tolerant suggesting good water quality.

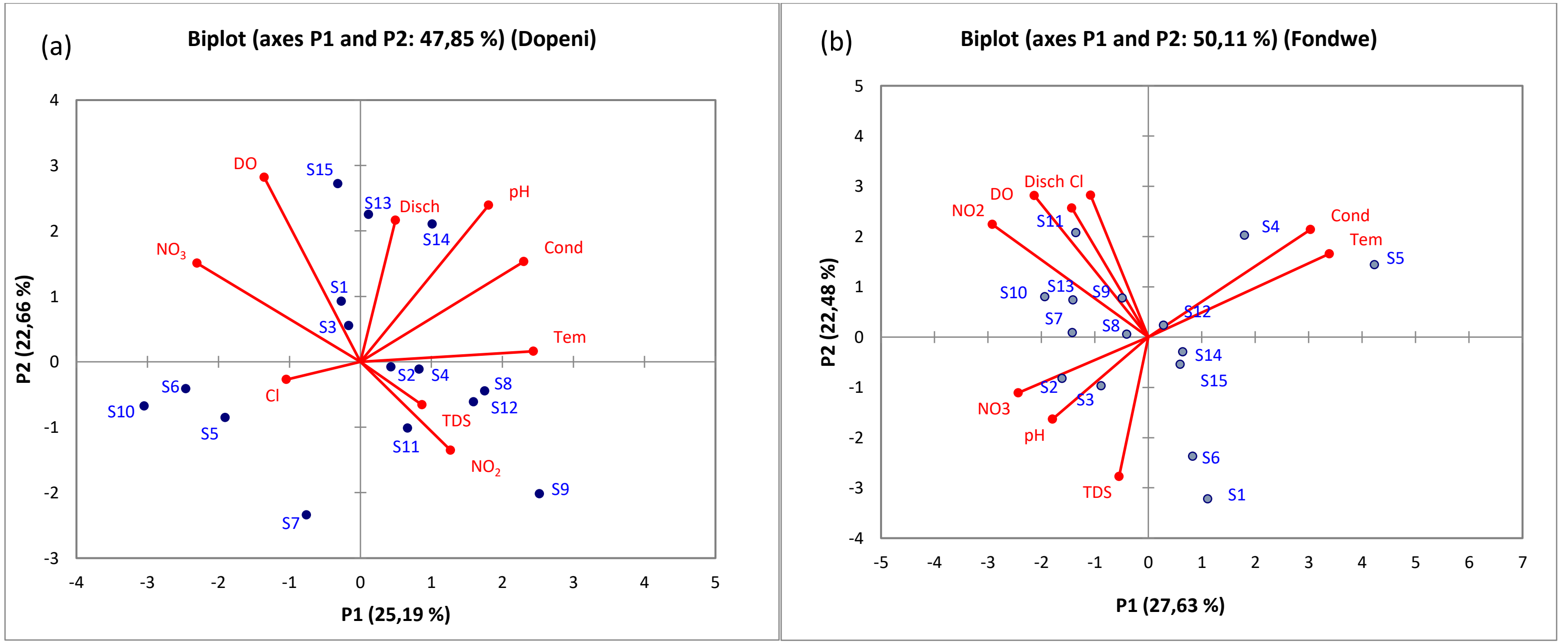
From the Musekwa sampling site (Figure 4.10e) the first two principal components showed 64.37% of variation in data and the first component (P1) accounted for 38.89% of variation of data from different sampling points while the second principal component (P2) accounted for 29.48% of the variation. The Aeshnidae, Elmidae Thiaridae, and Potamonautidae families positively defined six sampling points (S1, S2, S6, S8, S13 and S15) with the first principal component. The remaining nine (9) sampling points were negatively correlated with the Aeshnidae, Elmidae, Potamonautidae and Thiaridae families with the first principal component. For Musekwa, 9 out of 15 sampling points were poor performers in macroinvertebrates composition. Only two sampling points (S1and S6) performed well in the representation of Elmidae and Potamonautidae families with S1 showing the best representation of macroinvertebrates. The remaining four sampling points (S2, S8, S13, and S15) were best performers in the representation of Aeshnidae and Thiaridae families. These sampling points can be assumed to be moderately polluted and highly polluted because of their resident macroinvertebrates at the time of sampling. It can be concluded that the pollution levels along any river differed from one point to another and this could depend on the activities and environmental characteristics at the time of sampling.

At Pfumbada (Figure 4.10*f*), the first two principal components accounted for 56.97% of variation with the first principal component (P1) accounting for 34.46% of variation in the data and the second component (P2) 22. 50% of variance. Eight (8) sampling points (S1, S5, S6, S10, S11, S12, S14 and S15) were positively correlated with the Nepidae, Heptageniidae, Elmidae and Chironomidae families with the first principal component but were negatively correlated with Aeshnidae and Thiaridae families. The results show that there is a balance in the assemblages of pollution tolerant and pollution intolerant organisms. The remaining seven sampling points (S2, S3, S4, S7, S8, S9 and S13) were positively defined by Aeshnidae and Thiaridae families with the second principal component suggesting variations in the level of pollution at these sampling points. Two sampling points (S2, S4) recorded negative values for the Family Thiaridae (pollution tolerant) but were poorly represented by other five families that were also a distinguishing characteristic of Pfumbada sampling area. Five sampling points were a good representation of Chironomidae (pollution tolerant) and Elmidae (moderately tolerant) families. Only S14 had a good representation of both Chironomidae and Elmidae, but Elmidae were the least represented in all five sampling points. The families Nepidae (pollution tolerant) and Heptageniidae (pollution intolerant) were well represented by three sampling points with S15 having the best representation of Heptageniidae while S1and S6 had the best representation of Nepidae families. The Family Aeshnidae (moderately tolerant) was best represented by five sampling points with S7 and S13 showing good representation of Aeshnidae. Heptageniidae had the highest positive coefficient (0.794) with the first principal component, suggesting that this family was well represented at Pfumbada. Family Aeshnidae (Odonata) were found to be common across all sampling sites suggesting that they too, like chironomids which were found in both degraded and non-degraded environments can adapt in a variety of aquatic environments. Family Aeshnidae are known to use a wide range of habitats from stagnant to flowing water (Gupta and Veeneela, 2016). This explains their occurrence in all six sampling sites.

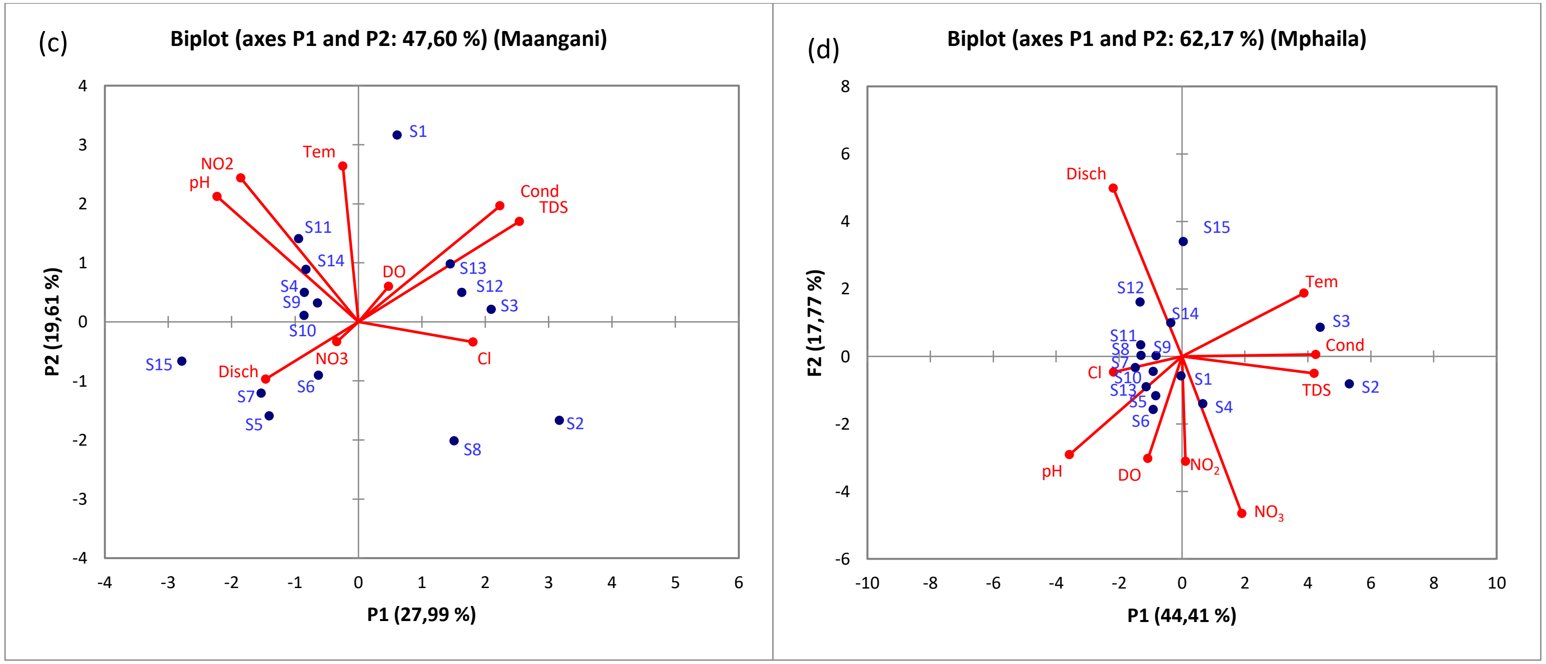
Figure 4.11 below shows the relationships of water quality parameters and sampling points from the six sampling sites of Dopeni, Fondwe, Maangani, Mphaila, Musekwa and Pfumbada. Figures 4.11*a-f* show physico-chemical property representations per sampling point from six sampling sites.

From Figure 4.11*a* (Dopeni) the first two principal components accounted for 47.85% of variation in data, with the first component (P1) accounting for 25.19% and the second component (P2) 22.66%. Eight sampling points (S2, S4, S8, S9, S11, S12, S13, and S14) were positively correlated with pH, discharge, conductivity, temperature, TDS and nitrites with the first principal component but negatively correlated with DO, nitrates and chlorine. This correlation explains more about the water quality status which is in good condition since low dissolved oxygen content, nitrates and chlorine are associated with degraded environments. This explains why only 20% of pollution tolerant organisms were found at Dopeni (Tables 4.2 and 4.3). From Figure 4.11*a* below, only S9 and S12 were positively correlated with both Heptageniidae and Thiaridae families (Figure 4.10a) suggesting that Thiaridae family exists in all types of polluted and non-polluted waters. The diversity, distribution and persistence of molluscs such as Family Thiaridae in all types of freshwater is very common (Contreras-Arquieta, 1998; Flores and Zaffaralla, 2012; Sharma, *et al*., 2013). The other six sampling points were correlated with pollution intolerant organisms with S2, S8 and S11 being negatively correlated with Thiaridae family with the first principal component. The remaining seven sampling points (S1, S3, S5, S6, S7, S10 and S15) were positively defined by DO and nitrates and chlorine suggesting that these sites were partly degraded or degraded and these were the sampling points that could have accounted for the existence of Aeshnidae, Elmidae, Heptageniidae and Thiaridae families. For example, Sharma *et al*. (2013) have noted in their study that the chloride content of water favours the survival of molluscs. Sampling points S5, S6, S7 and S10 were negatively correlated with chlorine suggesting that the chlorine did not have significant impact on the assemblages of macroinvertebrates from these sampling points. For example, from Figure 4.10*a* S5, S6 and S10 were positively correlated with Elmidae with the second principal component and S7 was positively correlated with Thiaridae families respectively with the first principal component. Only two sampling points (S13 and S14) were positively correlated with discharge, pH, conductivity and temperature. These sampling points were positively correlated with Elmidae and Aeshnidae families in Figure 4.10*a* suggesting moderately good water quality from these two sampling points. Six sampling points (S2, S4, S8, S9, S11 and S12) were positively correlated with TDS and NO_2. These sampling points were positively correlated with Aeshnidae, Heptageniidae and Thiaridae families (Figure 4.10*a*) with the first principal component suggesting good and poor water quality. Despite the occurrence of many subsistence agricultural fields, sand mining and livestock watering, Dopeni recorded the second highest percentage (80%) of pollution intolerant organisms while Mphaila recorded the highest percentage of 94%. These included organisms that were moderately tolerant to pollution.

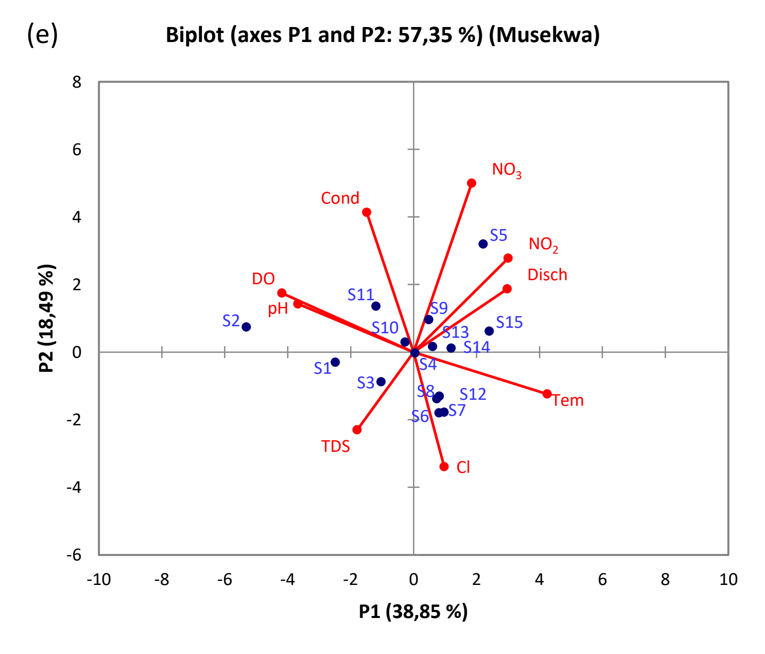
From Figure 4.11*b* (p.111) (Fondwe), the first two principal components accounted for 50.11% of variation in data with the first component (P1) accounting for 27.63% and the second component (P2) 22.48%. From Figure 4.11*b* seven sampling points (S1, S4, S5, S6, S12, S14 and S15) were positively correlated with conductivity and temperature with the first principal component. Since these sampling points were strongly defined by temperature and conductivity it means they were defined by good water quality since they were negatively correlated with chlorine, discharge DO, nitrites, nitrates, pH and TDS. These sites were also positively correlated with the Family Aeshnidae (moderately tolerant) in Figure 4.10*b* with the second principal component. Therefore, this supports the view that these sampling points were characterised by good water quality, suggesting that they were characterised by temperature and conductivity regimes that allowed the proliferation of Aeshnidae family. An important point to note is that Aeshnidae are known to use a wide range of flowing and stagnant water bodies (Gupta and Veeneela, 2016). Therefore, their occurrence in stagnant water bodies means that they can also survive under slightly elevated temperature regimes, which is a characteristic of stagnant water, depending on its size and depth. Sampling points S7, S8, S9, S10, S11 and S13 were positively correlated with chlorine, discharge, DO, and nitrite, nitrates, pH and TDS with the second principal component but negatively correlated with conductivity and temperature. S7 and S9 were positively correlated with the Family Aeshnidae (Figure 4.10*b*) with the second principal component suggesting good water quality. Sampling sites S8, S10 and S13 were positively correlated with Chironomidae, Coenagrionidae, Nepidae and Thiaridae families with the first principal component (Figure 4.10*b*). These sites could be described as degraded sampling points given the macroinvertebrates that defined them during sampling. Three (3) sampling points (S4, S5, and S12) were positively correlated with conductivity and temperature but negatively correlated with nitrate, pH and TDS. This suggests good water quality since sampling point S12 was positively defined by the Family Heptageniidae while S4 and S5 were negatively correlated with Chironomidae (which occur in both polluted and non-polluted water) with the first principal component (Figure 4.10*b*). Only two sampling points were negatively defined by NO_3, pH and TDS, suggesting that they were characterised by pollution tolerant organisms as depicted by Figure 4.10*b*. Six (6) sampling sites (S7, S8, S9, S10, S11, and S13) had a good representation of Cl, DO, NO_2, and river discharge. However, sampling points S8, S10, and S13 were positively correlated with pollution tolerant families Chironomidae, Coenagrionidae, Nepidae and Thiaridae. This suggests that these organisms survive in aquatic environments characterised by reduced flows since Fondwe had an average river velocity of 0.11 $m.s^{-1}$. The presence of chlorine at Fondwe also explains the absence of the Family Heptageniidae (Ephemeroptera) because these organism are known to be sensitive to the presence of chlorine (Bradley *et al*., 2002). However, the presence of chlorine at other sampling points did not impact on the presence of mayflies (Family Heptageniidae) which were present at Dopeni, Maangani, Mphaila and Pfumbada. Four sampling points (S1, S6, S14, S16) were defined by lower values of nitrite, DO, discharge and chlorine suggesting minimal pollution condition. These were sampling points that were negatively defined by Chironomidae, Coenagrionidae and Nepidae families (Figure 4.10*b*).

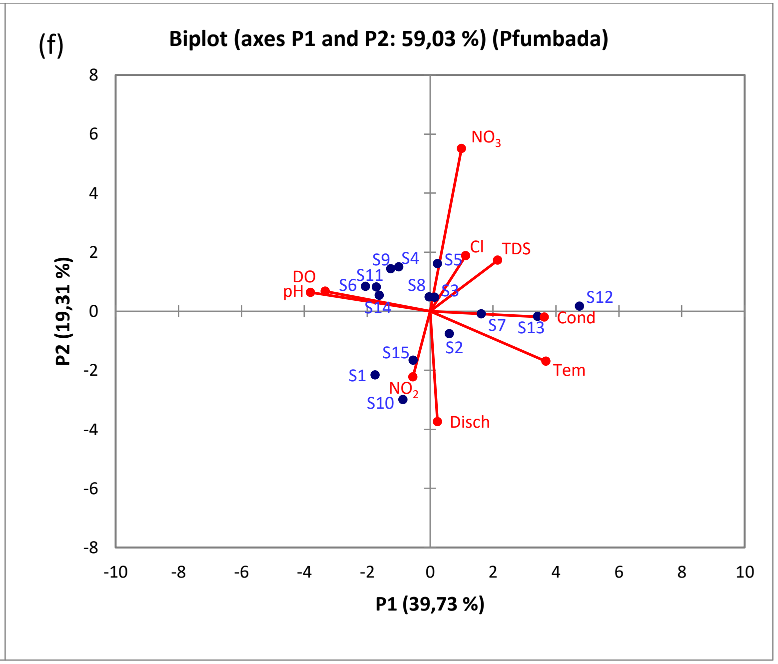


Figures 4.11*a,b* Correlation between sampling points and water quality parameters for Dopeni (*a*) and Fondwe (*b*)



Figures 4.11*c*,*d* Correlation between sampling points and water quality parameters for Maangani (*c*) and Mphaila (*d*)





Figures 4.11*a,b* Correlation between sampling points and water quality parameters for Musekwa (*e*) and Pfumbada (*f*)

Figure 4.11*c* shows the first two principal components for Maangani which accounted for 47.60% of variation in the data with the first component (P1) accounting for 27.99% of variation and the second component (P2) accounting for 19.61% of variance. Six sampling points (S1, S2, S3, S8, S12, and S13) were positively correlated with conductivity (TDS), chlorine and DO with the first principal component but were negatively defined by temperature, pH nitrites, nitrate and discharge. For example, in Figure 4.10*c* S1 and S2 were positively correlated with Aeshnidae and Heptageniidae, suggesting good water quality for these two sampling points. This explains the low quality water conditions for some of these six sampling points because other sampling points were correlated with Coenagrionidae which are associated with stressed or polluted habitats. Pollock (2012) observed that high TDS values indicate a stream that is stressed, suggesting stressed sampling points. Olson and Hawkins (2017) have observed that the Family Chironomidae and Order Gastropoda perform poorer under EC values of between 150 and 300 μS cm^{-1}. This was true with regard to Maangani which had higher abundance value of the Family Thiaridae (76) and a higher EC value of 794.2 µS.cm^{-1} (Figure 4.14) which was higher than 150 and 300 µS.cm^{-1}. This explained why Thiaridae were the most abundant family at Maangani. The remaining nine (9) sampling points were positively correlated with temperature, pH nitrites, nitrate and discharge but negatively defined by TDS DO and chlorine with the first principal component. Two sampling points (S2, S8) were positively defined by chlorine with S2 strongly defined by chlorine. DO (poorly represented), conductivity and TDS (well represented) were associated with four sites (S1, S3, S12, S13). However, S3 and S12 had poor representation of macroinvertebrates while S13 was strongly linked to the Family Coenagrionidae (Figure 4.10*c*). S1 was however, strongly linked to the Family Heptageniidae (Figure 4.10*c*). Discharge and nitrates were well represented by four sampling points (S5, S6, S7, S15). S7 had a weak representation of all types of water parameters, while S5 and S6 (Figure 4.10*c*) were associated with pollution tolerant families Thiaridae, Nepidae and Coenagrionidae respectively. These two sampling points were strongly linked to nitrates and discharge, suggesting poor water quality.

At Mphaila (Figure 4.11*d* above) the first two principal components accounted for 62.17% of the variation in data. Only four sampling points (S2, S3, S4 and S15) were positively linked to temperature, conductivity, TDS, NO_2 and NO_3 with the first principal component (P1). The high positive values for temperature, TDS and conductivity suggested good aquatic environment for the survival of pollution intolerant organisms. Almost all sampling points at Mphaila were associated with pollution intolerant organisms (Figure 4.10*d*). For example, the average temperature at Mphaila area was 18.74ºC which is good for the persistence and diversity of Aeshnidae, Chironomidae, Ecnomidae, Elmidae and Heptageniidae families. However, Chironomidae are known to persist throughout all seasons (Eady, 2011). This explains their presence at Mphaila even though they constituted 6% of the total number of macroinvertebrates from this sampling area. The remaining eleven sampling points were negatively correlated with temperature, conductivity, nitrites, nitrates and TDS, with the first principal component. Of the eleven sampling points, five (S4, S8, S9, S11, and S12) were positively defined by discharge with the second principal component. The average river velocity at Mphaila was 0.12 $m.s^{-1}$ but the area had a higher biodiversity index (0.74) than Musekwa which was characterised by higher velocities but lower biodiversity index value (0.69). Therefore, it can be argued that river velocity did not encourage pollution tolerant organisms at Mphaila. It can also be argued that due to the minimal utilisation of the river where samples were collected the occurrence of pollution intolerant organisms indicated a good aquatic environment. However, six of the eleven sampling points were negatively defined by chlorine, pH and DO, suggesting stressed habitats. However, only S1 was defined by chironomids (Figure 4.10*d*), suggesting that all the other sampling points were characterised by pollution intolerant organisms. This explains the 94% occurrence of pollution intolerant organisms from Mphaila (Table 4.3). Mphaila was the only sampling site which lacked noticeable active indigenous agricultural fields. It means that subsistence agriculture along sections of the Nzhelele River for the other sampling sites contributed to the high abundance values of pollution tolerant organisms.

For Musekwa (Figure 4.11*e* above), the first two principal components accounted for 57.37% of variation in water quality data among the sampling points. The first principal component (P1) accounted for 38.85% of variation and the second component (P2) accounted for 18.49% of variance. Ten sampling points (S4, S5, S6, S7, S8, S9, S12, S13, S14 and S15) were positively correlated with NO_3, NO_2, discharge, chlorine and temperature with the first principal component. The occurrence of chlorine, nitrates and nitrites is an indication of a stressed environment. These ten sampling points were negatively correlated with conductivity, DO, pH and TDS with the first principal component. However, from Figure 4.10*e* sites S4, S5, S7, S9, S10, S12 and S14 were negatively correlated with the Aeshnidae, Elmidae, Potamonautidae and Thiaridae families with the first principal component. This might have been due to lower diversity and abundance of macroinvertebrates from Musekwa. Sampling points S1 and S3 were negatively defined by TDS while S2, S10 and S11 were positively defined by conductivity, DO and pH with the second principal component, but were negatively correlated with temperature and chlorine, suggesting polluted environments. For example, S2 was positively correlated with Thiaridae with the first principal component (Figure 4.10*e*), suggesting a stressed habitat. Musekwa was the only sampling site that did not contain a single EPT organism, suggesting adverse aquatic conditions for this sampling area. The total absence of these taxa suggested that Musekwa was the most degraded of all sampling sites. The Family Aeshnidae, was surprisingly found in all studied sites. Therefore, their occurrence at Musekwa suggests that they too survive in all types of water.

At Pfumbada (Figure 4.11*f* above) the first two principal components accounted for 59.03% of variation in water quality data. The first principal component (P1) accounted for 39.73% of variation while the second component (P2) accounted for 19.31% of variance. Six sampling points (S2, S3, S5, S7, S12, and s13) were positively correlated with nitrates (NO_3), chlorine, TDS, conductivity, temperature and discharge with the first principal component. This suggested a moderately and highly polluted habitats for these six sampling points. These sites were negatively correlated with DO, pH, nitrites (NO_2) with the first principal component. Sampling point S1, S10 and S15 were negatively correlated with nitrites, with the second principal component, suggesting non-polluted environments. Sampling points S2, S10 and S11 were positively defined by DO, conductivity and pH with the second principal component. At Pfumbada, the average values for these parameters were 62% saturation for DO, higher conductivity value of 911µm.S^{-1} and a pH of 8.29. Surprisingly, high conductivity values and pH did not affect the occurrence of the Family Heptageniidae. However, the Thiaridae (34%) and Aeshnidae (27%) families recorded the highest abundance values indicating their tolerance to high conductivity and alkaline conditions. This explains the occurrence of the Aeshnidae family in all six sampling sites. An interesting observation was that Pfumbada recorded a higher percentage (52%) of pollution intolerant organisms and a low percentage (48%) of pollution tolerant organisms. This means that despite continuous river utilisation pollution intolerant organisms dominated the Pfumbada sampling sites, indicating minimal anthropogenic impacts. This finding further highlights the use of the Chironomidae and Thiaridae families as reliable indicators of poor water quality only.

CHAPTER FIVE: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This chapter provides summary, conclusions and recommendations drawn from the findings of the results from the six sampling sites of the Nzhelele River. Conclusions and recommendations are therefore, based on the findings of research. The summary of the findings include findings on water quality parameters per sampling site, sampling points and macroinvertebrate assemblages. The summary of the ANOVA and PCA results have also been provided. However, the conclusions and recommendations are expected to guide future researches and provide suggestions regarding the use of biomonitoring indices and macroinvertebrate taxa to assess aquatic ecosystem health. Suggestions for future research have been highlighted in this chapter in order to improve on the findings of present research because approaches to studying change in river health status change when more and more data is being collected to supplement the existing knowledge from current and past researches. It is necessary to continuously monitor river health status to ensure that conditions in a river do not deteriorate to an extent of inhibiting aquatic ecosystem functioning. It is also important to keep on designing improved biomonitoring tools. The current use of approaches such as SASS5, Percent Contribution of Mayflies, Ratio of Ephemeroptera, Plecoptera, Trichoptera (EPT) and Chironomidae Abundance, EPT Index and Percent Contribution of Dominant Family which was used in this study still have challenges of justifying the co-existence with other family groups. A more improved biomonitoring approach which strongly addresses land use-macroinvertebrate relationships is necessary.

5.2 Summary and conclusions

The conclusions have been based on the findings derived from the objectives. The first objective was to assess water quality conditions in order to determine the magnitude of pollution impact. The results from ANOVA suggested that the water quality parameters significantly differed from one sampling site to another and this explained variations in the diversity and types of macroinvertebrates that were found in each sampling site. There were no significant differences in chlorine, temperature, pH, river velocity, nitrate and nitrites from all studied sites except in the case of DO and TDS where ANOVA results indicated that significant differences occurred amongst the six studied sites. Based on the differences in water quality parameters the results indicated that pollution tolerant organisms constituted a total of 46.7% while pollution intolerant families constituted 53.3% of the total macroinvertebrates from the six sampling sites. Of the 53.3% only 18.1% had low tolerance to pollution while the remaining 35.2% were moderately tolerant to pollution, suggesting that large sections of the river were polluted and moderately polluted.

The occurrence of organisms that were moderately tolerant to pollution could serve as an early warning system of a continuously degrading stream. The low percentage of pollution sensitive families raises an alarm regarding the river health status of the Nzhelele River. River velocities ranged from slow (0.11 $m.s^{-1}$) to moderately fast (0.43 $m.s^{-1}$). Dopeni, Fondwe, Mphaila and Pfumbada were characterised by low flows ranging from 0.11 to 0.17 $m.s^{-1}$ and these low velocities favoured the occurrence of the Ecnomidae and Chironomidae families. However, these organisms were absent from Dopeni, but present at Fondwe, Mphaila and Pfumbada. Musekwa and Maangani were characterised by moderately fast waters with velocities ranging from 0.33 to 0.43 $m.s^{-1}$. These velocities favoured the occurrence of Elmidae, Coenagrionidae, Potamonautidae and Heptageniidae families. However, Maangani only recorded the families Coenagrionidae and Heptageniidae, while Musekwa recorded Elmidae and Potamonautidae families. These families were also found at slow velocity sampling sites of Dopeni, Fondwe, Mphaila and Pfumbada. The Family Coenagrionidae had the highest abundance values at Fondwe, suggesting that the site was more degraded. The Family Elmidae was also recorded at Dopeni, Mphaila and Pfumbada. Heptageniidae were present at Dopeni and they were the most abundant organisms. At Mphaila, the family Heptageniidae was also the most abundant macroinvertebrate family. Fondwe, Mphaila and Pfumbada were characterised by chironomids which are a characteristic of fast flowing rivers and poor water quality yet they were found to occur also in slow flowing river sections of Nzhelele River. From the results it could be concluded that river velocity could not be directly linked to macroinvertebrate assemblages along the Nzhelele River. River velocity, therefore, was not a primary determinant of the macroinvertebrate assemblages from the six sampling sites. Of the six sampling sites, the Fondwe site had the highest percentage (76%) of pollution tolerant organisms suggesting that it was the most polluted sampling site because it also did not record a single EPT organism. The pollution tolerant organisms at Fondwe could not be directly linked to the upstream location of Komatiland Plantation (commercial forestry) because the anthropogenic activities along the river were so intense that they were directly linked to the condition of the river at the time of sampling.

The concentration of dissolved oxygen (DO) from the six sampling sites ranged from 58.53% to 65.06%. This meant that the magnitude of impairment due to DO was found to be slight because its range fell within the impairment range of 53 to 70% as stipulated by Ausseil (2013). DO seemed not to have directly affected macroinvertebrate composition because Musekwa site had an average concentration of 65% but it recorded lower abundance values while Mphaila site had a lower concentration value of 58.53% but recorded higher abundance values than Musekwa site and also recorded more sensitive organisms than all other sampling sites. However, it could still be argued that DO concentration at Musekwa site was higher than at Mphaila site because more macroinvertebrates would mean more oxygen demand for Mphaila site. Therefore, the Family Chironomidae for Mphaila site could not be interpreted as representing pollution tolerant organisms or a stressed stream because this family is known to inhabit all types of water (Adeogun and Fafione, 2011; Everaert *et al.*, 2014). Given their pollution tolerance level of 2, chironomids were therefore found not to be reliable organisms to be used as indicators of non-degraded streams because they were well represented at Fondwe which recorded 76% of pollution tolerant organisms. Dopeni was also the least polluted site because it recorded 20% of organisms (Family Thiaridae) that were highly tolerant to pollution.

Averages for chlorine were higher than the Target Water Quality Range (TWQR) of 0.2 mg/L and also above the CEV of 0.35 mg/L, as well as the AEV. However, the chlorine concentration could be directly linked to the impact on EPT assemblages (Ecnomidae and Heptageniidae families) at Fondwe than in other sampling sites. This was evident in the abundance of mayflies at Dopeni, Maangani, Mphaila and Pfumbada sites because mayflies are known to be sensitive to increased chlorine concentrations (Williams *et al.*, 2003). Therefore, it can be concluded that chlorine concentration should always be described in its actual form which was not the case in this study. If the form of chlorine had been expressed as either chloride then its role in the determination of macroinvertebrate assemblages could have been argued differently. Again, the use of macroinvertebrates at family level restricted the full investigation into the role of chlorine on macroinvertebrates at species level. However, in this study its concentration could be directly linked to a decrease in the abundance of mayflies (Ephemeroptera).

Nitrate concentration was found not to be lethal because it was positively associated with higher abundance values of macroinvertebrates. Maangani recorded a higher average of 20.70

mg/L and the highest number of sampled macroinvertebrates, including pollution sensitive Heptageniidae. Pfumbada recorded the highest average of 28.38 mg/L and also recorded the second highest total number of macroinvertebrates. It can be concluded that higher abundance values of macroinvertebrates were associated with higher concentrations of nitrates. Higher nitrate values seemed not to have severely affected Heptageniidae organisms since they were found at Maangani and Pfumbada which were dominated by pollution tolerant organisms. Nitrate impact was also considered insignificant because all values across the six sampling sites were below the suggested maximum value of 32 mg/L NO_3. Therefore, nitrate seemed to have had little role in the diversity of macroinvertebrates along the Nzhelele River. However, the higher nitrite values seemed to have had impact on diversity of Potanaumotidae because they were recorded at Musekwa site (10) only which had a slightly higher average concentration of 0.28 mg/L NO_2. This is because crustaceans are known to be more sensitive to elevated nitrites than the gastropod family.

The pH seemed to have favoured the Family Thiaridae abundance because it was positively correlated with Maangani, Musekwa and Pfumbada with the first principal component (Figure 4.10). This, therefore means that Thiaridae abundance will increase with increasing alkalinity. Just like with pH and nitrate, elevated TDS readings for Maangani, Musekwa and Pfumbada seemed to have also favoured the occurrence of Thiaridae family because these three areas recorded the highest abundance values for this family.

Mphaila recorded only 6% of pollution tolerant organisms suggesting that this sampling site was the least polluted of the six sampling sites because the area also recorded the highest percentage of Heptageniidae (Ephemeroptera) (39%). The results for Dopeni also suggested that the area was moderately polluted since it recorded the highest number of organisms that were moderately tolerant and sensitive to pollution. Only 20% were pollution tolerant. Interestingly, Dopeni and Mphaila were positively correlated with pollution intolerant families such as Ecnomidae (Figure 4.8), Elmidae and Heptageniidae. Maangani recorded the highest number of pollution tolerant organisms of 63%, suggesting a degraded or stressed environment. A higher number of pollution tolerant families associated with Maangani were Chironomidae, Coenagrionidae, Nepidae and Thiaridae families, suggesting a polluted sampling site. Musekwa had the lowest count of macroinvertebrates but also recorded 60% of pollution tolerant organisms. This also suggested a degraded aquatic environment. Pfumbada registered 48% of pollution tolerant organisms suggesting a moderately polluted environment with some sections being pristine due to the abundance of Heptageniidae (19%) as the third highest count of macroinvertebrates at this sampling site. Fondwe was found to be the most polluted sampling site because it registered 76% of pollution tolerant organisms. These were Chironomidae, Coenagrionidae, Nepidae and Thiaridae families. Fondwe site was characterised by laundry activities and subsistence agriculture. These could have altered the water quality to favour more pollution tolerant organisms. The presence of agricultural fields along Dopeni site suggested minimal anthropogenic impact. Mphaila lacked active agricultural activities and other anthropogenic activities such as water abstraction, making it the least polluted sampling site. Maangani and Musekwa were characterised by anthropogenic activities such as subsistence agriculture, water abstraction, laundry and livestock watering. This explains the high number of pollution tolerant organisms from these sites. Pfumbada site had very few subsistence agricultural fields, though water abstraction and laundry activities were intensive. This suggests that these activities account for the poor water quality of this site, hence the resident macroinvertebrates.

From the results on water quality, it could therefore be argued that the Nzhelele River is somewhat polluted due to the low percentage of pollution sensitive families (Family Heptageniidae) even though the percentage of pollution intolerant families such as Heptageniidae and Ecnomidae was only 18.1%. The remaining organisms were moderately tolerant to pollution with many EPT families missing from Nzhelele River. The land-use activities and utilisation of the river seemed to have deteriorated the river water quality because the Mphaila site which was not characterised by active agricultural practices recorded a higher percentage of pollution intolerant organisms. The level of pollution has been manifested by the resident macroinvertebrates of the sampling sites. In areas of active indigenous agriculture and heavy river utilisation the resident macroinvertebrates were found to be pollution tolerant. Musekwa, which was characterised by heavy river utilisation yielded lower abundance values of macroinvertebrates and it was found to be the least diverse sampling site.

The second objective was to correlate species diversity and water quality parameters. The PCA data showed positive and negative correlations between macroinvertebrates and water quality parameters. The number of macroinvertebrates that were sampled from the six sites was generally low but the macroinvertebrates were a true representation of the conditions of the river at the time of sampling. Even though it can be argued that more taxa and water quality parameters were needed to show a true composition of the macroinvertebrate groups to represent the river health, the available sampled macroinvertebrates and water quality parameters were able to reflect the condition of the river for each sampled site. Species correlation indicated that pollution tolerant organisms were positively correlated with parameters such as pH, low DO levels, chlorine and elevated TDS. Temperature, nitrate and river velocity seemed to have had no effect on the composition of macroinvertebrates. However, some macroinvertebrates such as the families Chironomidae, Thiaridae and Heptageniidae, seemed to have occurred in many sampling points while the Family Aeshnidae were common across all sampling sites indicating another family group which survives under different types of water bodies. From the results, it could be argued that Chironomidae and Thiaridae families cannot be regarded as trustworthy water quality indicators because they occurred in both polluted and non-polluted waters. Therefore, their use as reliable water quality indicators becomes more and more confusing and the precision of the findings regarding the quality of water is therefore minimised. The omnipresence of the Family Thiaridae in all seasons suggests that they tolerate a variety of aquatic habitats that differ in pollution levels. Therefore, their use in assessing river health should be approached with caution. The Family Aeshnidae was found in all sampling sites indicating its tolerance to a variety of environmental conditions. They are also known to tolerate changes in water temperature (Fulan *et al*., 2011). A more rigorous study and review of the use of the Family Aeshnidae as indicators of fair water quality is necessary.

The presence of chironomids at Mphaila cannot be assumed to represent a stressed habitat or a degraded habitat because they too, like the Family Thiaridae, inhabit polluted and non-polluted environments. Their use as water quality indicators also needs to be approached with caution. Their ability to drift to nearby locations or habitats in water gives them the advantage of inhabiting all types of aquatic environments. Therefore, their existence in water bodies cannot always be associated with polluted or degraded environments. The challenge which therefore arises is whether to regard them as representatives of polluted environments or whether their coexistence with other organisms should be re-examined. The examination should also detect whether they occur by chance or their coexistence with pollution intolerant species is significant at all times.

Even though low abundance values were recorded from the six sampling sites it was interesting to observe that the macroinvertebrates that were sampled were a reflection of the land use activities along the river even though the existence of Chironomidae, Thiaridae and Heptageniidae families should always be interpreted with care. However, the macroinvertebrates that were sampled were a true representation of the health status of the environment from where they were harvested. However, a new approach to bioassessment is necessary taking into consideration the land-use types, seasonality, river velocity, resident macroinvertebrates and agricultural inputs. These variables should altogether form the basis for an improved biomonitoring tool.

The results on habitat integrity suggested that the level of modification of the river and riparian zones differ from one sampling site to another, with Musekwa being characterised by a highly degraded environment. The habitat integrity for each sampling site corresponded with the diversity and types of macroinvertebrates that were sampled from each site. Musekwa was found to be the most degraded site as it was found to have lower species diversity and lower abundance values. Musekwa, Dopeni and Fondwe were the only sites that were characterised by invasive plants, suggesting the direct impact of land use along the river. Mphaila site was found to be the least degraded and recorded the lowest percentage (6%) of organisms that were highly tolerant to pollution. The land-use activities also seemed to have played a role in the level of degradation of the Nzhelele, with Mphaila being the least degraded because of lack of active indigenous agricultural activities and river utilisation for various purposes. Conditions at Mphaila could change in the near future due to the presence of areas that have been demarcated for future agricultural practices. The existence of human-made buffers in the form of a fence, and the partial clearing of trees along the river is an indication of future agricultural activities because agricultural fields along the river were a prominent feature from all other sampling sites.

Habitat integrity assessment indicated that water abstraction activities were serious at Dopeni and Pfumbada, but critical at Musekwa. These activities put pressure on water bodies especially during low rainfall periods or drought. This explains why flows were critically low during the time of sampling which were also compounded by the 2015/2016 drought events. Continued uncontrolled water abstraction activities will lead to the end of or permanent change of the Nzhelele river system and aquatic biota will be confined to species that survive under degraded environments such as Chironomidae and Thiaridae.

Modification of flow was found to be serious at Musekwa due to the existence of water extraction pipes and channelization which led to altered flow velocities. However, Musekwa and Maangani recorded higher velocities of 0, 32 and 0.42 $m.s^{-1}$. Generally, modification of flow was found to be minimal at Fondwe. There was no evidence of flow modification at

Mphaila, while it was considered large at Maaangani and Dopeni. This explains more about the level of habitat deterioration which will in future alter macroinvertebrate composition. However, modification of flow at Dopeni favoured the abundance of pollution intolerant macroinvertebrates which constituted 80% of the total sampled macroinvertebrates at Dopeni.

Bed modification was generally minimal because it was only serious and large at Dopeni and Musekwa respectively. The same was true with channel modification where it was found to be serious at Musekwa, but large at Dopeni and Maangani.

Water quality modification across the six sampling sites ranged from minimal to moderate, suggesting that the effect of indigenous agricultural activities and other activities did not severely affect the quality of water. Inundation was found to be minimal except at Pfumbada, suggesting insignificant impact of this activity. No exotic aquatic fauna and macrophytes were recorded across the six sampling sites.

Solid waste disposal was only serious at Dopeni and Musekwa and minimal at other sites, but absent at Mphaila. Solid waste for Dopeni and Musekwa could be explained from the location of the river near the villages which encouraged illegal solid waste disposal. The extent of indigenous vegetation removal for agricultural purposes was also serious at Dopeni and Musekwa, suggesting direct impacts on stream shading effects and water temperatures. Musekwa recorded the highest temperature, averaging of 22ºC and it also did not record a single EPT organism, suggesting a direct impact of temperature on macroinvertebrate assemblages at Musekwa. According to Ausseil (2013) mayflies (Ephemeroptera) start to decline at temperatures above 21.5ºC. Exotic vegetation encroachment was generally minimal at Dopeni, Fondwe and Musekwa suggesting minimal impact of these species along the river. The other three sites (Maangani, Mphaila and Pfumbada) did not record a single exotic vegetation species. This means that the environmental problems associated with exotic species is still at a lower phase and will manifest itself fully if no remedial actions are taken in time before these species could fully colonise a vast area along the river, leading to drastic changes in water quality and macroinvertebrate assemblages.

Bank erosion was found to be absent at Mphaila, but minimal in all other sampling sites, suggesting that this process has been minimised or halted by extremely low flows. This means that it is a periodic or ephemeral problem. From the habitat integrity assessment it can be concluded that the only area which experienced a high degree of modification was Musekwa, while Dopeni and Maangani were progressively being degraded and, may soon reach a critical stage of habitat modification. Generally, the results of the assessment of the habitat integrity showed that all the six sampled sites were experiencing continued modification that differed in scale and magnitude. Due to different land use activities along the river as well as their different magnitude of impact it was not surprising to discover that macroinvertebrate assemblages were characterised by the severity of impacts along the Nzhelele River. Therefore, macroinvertebrates need to be studied through a biomonitoring tool or approach that extensively highlights or fuses land use activities and biomonitoring.

In order to improve biomonitoring approach a framework has therefore been proposed as the last objective for guiding researchers and conservationists for improving biotic integrity. The framework has taken into consideration the use of different biomonitoring approaches which are guided by the habitat integrity of lotic environments. Integration of these variables is important because a generic approach to biomonitoring often encounters some limitations and confusion regarding the use of certain macroinvertebrate families. This framework does not necessarily replace the existing approaches or protocols to macroinvertebrate sampling for biotic integrity studies but attempts to strengthen the existing biomonitoring tools. The integration of habitat integrity assessment approach and land use activities approach will strengthen the current biomonitoring approaches. The inclusion of land use assessment and habitat integrity prior to macroinvertebrate sampling is considered important because these parameters will guide a researcher or conservationists on the type of biomonitoring approach to be used instead of a combination of a variety of approaches. It is from the results of these two parameters that a researcher will know which appropriate biomonitoring tool to use so that it does not always depend on the preferred approach by the researcher but rather, on the habitat integrity and land use assessment outcomes. Soko and Gyedu-Ababio (2015) have also argued that in order to protect the needs of the environment there is a need to develop tools that can monitor environmental conditions as well as setting ecological objectives that will ensure that there is proper and sustainable management of the resource. For the purpose of this study it was therefore, necessary to propose a framework for biomonitoring in subtropical regions. Figure 5.1 represents a proposed framework for biomonitoring and indicates the use of habitat integrity results and land-use approaches to determine the appropriate biomonitoring tool to be chosen when conducting research.

The main reason for starting with land use assessment is the fact that once land-uses have been identified, habitat integrity can thus be easily studied, guided by the type of land-use. It is easy to assess the integrity of habitat before the actual sampling process because the known

condition of the aquatic environment will aid the researcher to select an index for studying the resident macroinvertebrates. It is of no use to use the Percent Contribution of Mayflies or EPT index in a highly degraded stream because the results will always reflect low EPT values. The habitat integrity results will categorise the levels of pollution of the river or a stream and once a stream or river has been found to be degraded the Percent Contribution of Dominant Family together with Simpson's Diversity Index or Shannon-Weiner Diversity Index and Evenness would be appropriate for research. Proposed indices (Percent Contribution of Chironomidae, Thiaridae and Heptageniidae), and Ratio of EPT and Chironomidae-Thiaridae Abundaces were found to be a necessity because they define the relationship between pollution tolerant and pollution-intolerant organisms. These will help to determine whether the co-existence of certain macroinvertebrates of a particular tolerance level occurs by chance or their existence is significant.
The second index was just the addition of the Family Thiaridae as it tends to survive a variety of aquatic environments under higher alkalinity, pH and temperature. This proposed framework is suitable for subtropical streams which are characterised by higher biodiversity of macroinvertebrates. The Percent Contribution of Chironomidae, Thiaridae and Heptageniidae families has been considered important because the abundances of these families could be compared under different water bodies characterised by different levels of pollution. Conclusions about river health could be drawn from comparisons of these organisms. This will therefore, assist to review the use of Chironomidae and Thiaridae families as indicators of poor water quality.

The approaches in Figure 5.1 will determine the abundance values of pollution tolerant organisms even though there will be pollution intolerant organisms, the assumption being that they will automatically be lower in abundance. Having used the Percent Contribution of Dominant Family like in this study, it is easy to compare the diversity of these organisms in terms of locations or sampling points along a river or stream. For moderately polluted and non-polluted streams any index of biodiversity is suitable given the fact that for a moderately polluted stream it is possible to find a higher abundance value for EPT and Chironomidae which colonise any type of lotic environments. The ratio of EPT and Chironomidae will be easily compared. The biotic indices under degraded streams make it easier to identify and sort sampled macroinvertebrates according to Percent Contribution of Dominant Family. Even though family level is used for these degraded streams each family could also be identified at species level or trait characteristics as suggested by other authors such as Odume (2014).

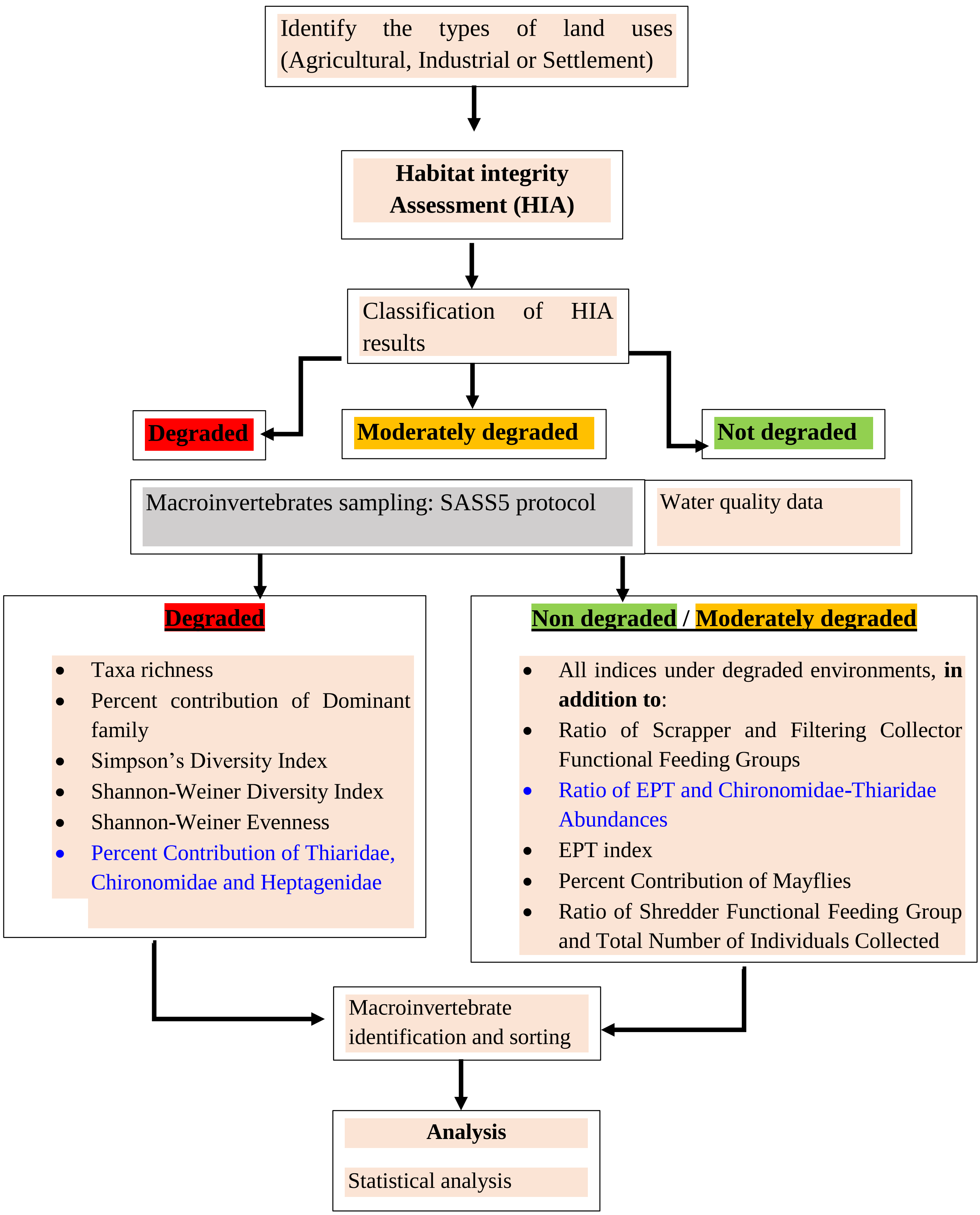


Figure 5.1 Schematic representation of biomonitoring framework for different types levels of degradation of rivers

5.3 Limitation of the study

This study was conducted during unfavourable conditions of drought that had significant impact on the abundance values of macroinvertebrates and some physico-chemical properties of water. The abundance values were lower and the number of families found across all six sampling sites were only nine, suggesting that species diversity of macroinvertebrates was directly influenced by these conditions. The study also covered areas where the Nzhelele River transcends rural villages and subsistence farms. Only the Fondwe site was located immediately downstream of commercial agriculture of Komatiland Plantation characterised by blue gum tree plantations. Therefore, the direct impact of commercial agriculture in this study could not be fully assessed. The other limitation was that the use of Percent Contribution of Dominant Family did not break down family levels down to species or trait characteristics of the sampled organisms to determine the response of different species of macroinvertebrates to pollution levels in the river. Since family level approach gives a generic approach to responses of a dominant group of organisms to pollution it cannot provide differences in tolerance levels of individual species belonging to the same family. However, the approach showed the dominant families per sampling site. Therefore, the results of this study indicate river water quality and habitat integrity in terms of dominant family according to tolerance levels to pollution. Therefore, based on these limitations the following recommendations are made for further future research purposes. The other limitation of the study is the water quality study which did not test other parameters such as phosphates, sulphates and potassium to see how they also had direct link to macroinvertebrate diversity and river health even in the absence of noticeable effluent discharges.

5.4 Recommendations

- There is a strong need to review biomonitoring approaches because the levels of pollution in a river can be determined before sampling of macroinvertebrate communities. Future biomonitoring approaches should begin by assessing land uses and habitat integrity before applying the SASS5 protocol for macroinvertebrate sampling.
- The hydrological characteristics of a river should be considered before the actual macroinvertebrate sampling and there should be a strict protocol adopted for each section of a stream with distinct hydrological characteristics and habitat integrity. This calls for frequent review of the SASS5.

- The SASS5 protocol should be reviewed and should consider setting minimum standards for studying both degraded and non-degraded lotic environments such as the required minimum number of organisms per type of hydrological characteristics, such as low flows or moderately fast flows, etc. The habitat conditions can be assessed through conducting Habitat Integrity Assessment prior to the application of SASS5. This approach will assist in guiding a researcher on the appropriate biotic index to be used for each type of lotic environment.
- Since it is possible to sample macroinvertebrates in any lotic environment such as fast and slow flowing rivers, degraded and non-degraded streams, there should be strict protocols regarding the procedures for sampling these types of lotic environments.
- Trait and species level approach should also be incorporated into the SASS5 protocol since they clearly define the state of the river and tolerance levels of the resident macroinvertebrates.
- Percent Contribution of Thiaridae, Chironomidae and Heptageniidae should be added to the current list of biotic indices since the occurrence of these groups of macroinvertebrates is on many occasions present in all types of waters. Under the proposed framework this proposed biotic index could be used under degraded and non-degraded rivers, including the moderately degraded rivers. This will also assist in reviewing the roles or importance of these groups in assessing river health status.
- Ratio of EPT and Chironomidae Abundances should be reviewed to read as Ratio of EPT and Chironomidae-Thiaridae Abundances. This is a good measure of EPT against common pollution families since they exist under various water conditions. The results from this study have shown that Thiaridae exist in all types of water and for the purpose of improved biotic indices these families should be added to Ratio of EPT and Chironomidae Abunances. The proposed ratio will strengthen the use of EPT and masters of the environments (Chironomidae and Thiaridae families) to effectively make conclusions about river water quality.
- There is also need to review the use of the Family Aeshnidae because it has been found to occur in all types of aquatic environments. Their occurrence in all six sampling sites suggests that they are capable of inhabiting different types water bodies and their tolerance level needs to be reviewed.
- To minimise river pollution, local authorities should consider coming up with effective water supply strategies that will limit water abstraction rates from the rivers. There is

need for proper Environmental Impact Assessment (EIA) which will assist local authorities when zoning areas for agriculture and businesses in rural areas. This means that agricultural practices should be forbidden along riparian zones of all rivers.

- Regional Planning should consider rezoning subsistence agricultural fields since their current location seem to be directly linked to the Nzhelele River degradation.
- Subsistence farmers should also be educated on how to utilise natural resources sustainably, while being encouraged to improve their rural livelihood. This could be done through Adult Basic Education and Training which was introduced in the early 1990s in South Africa.

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