



Midlands State University

# THE IMPACT OF SEWAGE EFFLUENT ON THE AQUATIC HEALTH OF SEBAKWE RIVER, KWEKWE, ZIMBABWE

BY MANHIRE BILLIE

R152027Y

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## **Approval Form**

This is to certify that the dissertation entitled “The impact of sewage effluent on the aquatic health of Sebakwe River, Kwekwe, Zimbabwe”, submitted in partial fulfilment of the requirements for the Bachelor of Science Honours Degree in Applied Biological Sciences and Biotechnology at Midlands State University, is a record of the original research carried out by Billie Manhire R152027Y under my supervision and no part of the dissertation has been submitted for any other degree or diploma.

The assistance and the help received during the course of this research have been duly acknowledged. I, therefore, recommend that it be accepted as fulfilling the dissertation requirements.

Name of supervisor .....

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## Abstract

Many of the sewage treatment plants in Zimbabwe have not been upgraded to accommodate the population increases that the cities have undergone. This has resulted in the release of raw or partially treated sewage into rivers, which negatively impacts the ecological health of these ecosystems. The aim of this study was to determine the impact of sewage effluent on the aquatic health of Sebakwe River, Kwekwe. Monthly surveys of macroinvertebrates and water variables were carried out from September 2018 to January 2019, to cover the wet and dry seasons. Three upstream and three downstream sampling sites were used to evaluate the effects of sewage effluent being discharged into Sebakwe River by the city's sewage treatment plant. Macroinvertebrate sampling was done according to South African Scoring System (SASS) 5 index protocol. *In situ* water analyses of electrical conductivity, DO, pH and temperature were carried out using metres. The macroinvertebrates present in the water were identified up to family level. The results showed no significant differences ( $p > 0.05$ ) in seasonal and spatial variation of the physicochemical variables. Species richness varied among the sites. The highest species richness was recorded at the first upstream site while the lowest species richness was recorded at the sampling site immediately after discharge of sewage effluent. The upstream and downstream sites had average ASPT scores of 7.64 and 4.23 respectively, indicating that the downstream sites were unhealthy. The first upstream site had pollution-sensitive taxa from the family of *Trichoptera* indicating good river health. Pollution sensitive taxa such as *Zygoptera* were common upstream while the downstream sites were dominated by pollution insensitive taxa such as *Oligochaetae*, *Hirudinea* and *Diptera*. The site immediately after point source pollution had an abundance of water hyacinth indicating nutrient pollution. The health downstream of Sebakwe River is very low since pollution-tolerant taxa showed low species richness but high evenness. The measured environmental variables were not important in describing the macroinvertebrate diversity. Future studies on rivers should include measurements on nutrient variables as these have a great impact on river health. SASS 5 index should be formally adopted and used to regularly assess the health of all rivers in the country to help keep organic pollution levels low.

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## **DEDICATION**

This work is dedicated to my late mother Salome, Kupurayi Manhire.

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## **1.0 CHAPTER ONE: INTRODUCTION**

### **1.1 Background**

Aquatic ecosystems are highly complex environments because they are a result of interactions between physical, chemical and biological variables (Dias-silva *et al.*, 2010). River health is defined by the ability of a river to recover after having experienced stress, presence of a diverse assemblage of aquatic biota, absence of distress defined by measured indicators, absence of risk factors (namely sewage and industrial effluents) and good physical habitats which are directly affected by water and sediment flows (Norris and Thoms, 1999). To some extent, riverine degradation occurs as a result of changes in physiological structure of the riverine ecosystem. The changes in riverine ecosystem occurs through dam construction and change in the flow regime (Norris and Thoms, 1999; Dube *et al.*, 2016). These changes alter the habitats that support aquatic organisms (Norris and Thoms, 1999). Activities such as river bank cultivation, gold panning contribute to direct river bank degradation. Riverine ecosystems are important for draining catchments, transportation of sediments, regulation of floods and supporting biodiversity (Bredenhand, 2005). Rivers are a cheap source of freshwater used for crop production, livestock rearing and energy creation (Priya *et al.*, 2016). Despite all these benefits, the ecological health of rivers is threatened by anthropogenic activities (Li *et al.*, 2010; Chikodzi, *et al.*, 2017). The most prevalent anthropogenic activity causing this decline, in developing countries, is disposal of untreated or partially treated sewage/wastewater into surface water bodies (Muisa *et al.*, 2015).

Many of the sewage treatment plants in Zimbabwe have not been upgraded to accommodate the population increases that the cities have undergone (Makwara and Tavuyanago, 2012). This has resulted in the release of raw or partially treated sewage into rivers, which negatively impacts the ecological health of these ecosystems (Muisa *et al.*, 2015). Inadequately treated sewage effluent is laden with nutrients like phosphates and nitrates which cause massive eutrophication-related problems in the water channel (Nhapi and Tirivarombo, 2004). These chemical and physical alterations have a direct impact on macroinvertebrate populations (M'Erimba *et al.*, 2014). Nutrient concentrations rise with increase in the volume of sewage effluent caused by increasing population density in a city (Dube *et al.*, 2014).

Good river health is assessed in terms of water quality and biodiversity, among other indicators (Priya *et al.*, 2016). Traditionally, water quality has been assessed through the

measurement of physicochemical variables (Gratwicke, 1998; Odume *et al.*, 2012) . A drawback of relying on these variables is that they do not show the full synergistic effects of pollution on the aquatic biotic community (Kasangaki *et al.*, 2006; Tazvivinga *et al.*, 2012; Chikodzi *et al.*, 2017). Furthermore, the physicochemical variables give a picture of the environment at that time only, but not random or periodic pollution events (Chikodzi *et al.*, 2017). Over time, biomonitoring has gained popularity in the assessment of aquatic health (Odume *et al.*, 2012). Biomonitoring is defined as the systematic use of living organisms or their responses to determine the condition or changes of the environment (Li *et al.*, 2010). The advantage of biomonitoring is that it can detect cumulative physical, chemical and biological impacts of adverse activities to an aquatic system (Li *et al.*, 2010; Tazvivinga *et al.*, 2012; Utete and Kunhe, 2013). Biomonitoring in aquatic ecosystems is implemented through the use of several biotic indices such as the Index of Biotic Integrity (IBI) for fish, Dragonfly Biotic Index (DBI), Wetland Zooplankton Index (WZI), the Ecological State Macrophyte Index (ESMI), River Macrophyte Index (RMI) and River Invertebrate Prediction And Classification System (RIVPACS) (Karr, 1981; Chikodzi *et al.*, 2017; Khalifa *et al.*, 2015; Ciecierska and Kolada, 2014; Kuhar *et al.*, 2011; Wright *et al.*, 1997).

Amongst these indices, macroinvertebrates are commonly used because their abundance and distribution is as a result of a river's physical, chemical and biological state hence they give an integrated ecological overview of the ecosystem (Hussain and Pandit, 2012). Furthermore, macroinvertebrates have varying tolerances to pollution such that their populations reflect the underlying abiotic and biotic conditions in stream ecosystems (Phiri, 2014). The macroinvertebrate indices are developed specifically for different regions: Tanzania River Scoring System (TARISS), Multimetric Macroinvertebrate Index Flanders (MMIF), Namibian Scoring System (NASS), Zambian Invertebrate Scoring System (ZISS) and the Okavango Assessment System (OKAS) (Kaaya *et al.*, 2015; Gabriels *et al.*, 2010; Shimba and Jonah, 2016; Dallas *et al.*, 2018; Lowe *et al.*, 2013).

In southern Africa, the South African Scoring System (SASS) developed in South Africa is used for the assessment of aquatic health (Chutter, 1995). The SASS index has been successfully used in Zimbabwe to assess the ecological health of Chiraura River, Gwebi, Manyame and Mukuvisi Rivers (Harare), Mazai Stream (Bulawayo), rivers of Mazowe Valley Catchment, Sakubva River and Muccheke River (Mutare) (Utete and Kunhe, 2013; Phiri, 2014; Muisa *et al.*, 2015; Dube *et al.*, 2014; Mapira, 2011; Gratwicke, 1998; Chikodzi

*et al.*, 2017). Although, SASS studies have been extensively carried out in Zimbabwe, the method is still to be used in the Midlands region of Zimbabwe. Inefficient waste treatment plants coupled with dumping of raw or partially treated sewage effluent are common in almost all Zimbabwean cities and this potentially threatens the ecological health of rivers (Makwara and Tavuyanago, 2012; Mapira, 2011). It is therefore important that every river should undergo biological monitoring to assess the extent of pollution.

## **1.2 Problem statement**

Point source pollution is defined as the pollution originating from a single identifiable source such as industrial effluents and wastewater effluent discharge from sewage treatment plants (Vadde *et al.*, 2018). Point source pollution is the most prevalent in affecting the sustainability of water resources in southern Africa (Masere *et al.*, 2012). Kwekwe was established in 1898 as a mining town became a municipality in 1934 and a city in 1997. The population in Kwekwe grew from 47.607 in 1982, to 75.425 (1992), to 93.072 (2002) and finally to 100 900 (2012) (Makwara and Tavuyanago, 2012). This increase in population increased the demand in water resources resulting in domestic water supply shortages (Matsa and Tapfuma, 2015). The growth in urban population is associated with an increase in volumes of domestic sewage (Mangizvo *et al.*, 2016; Mapira, 2011; Masere *et al.*, 2012; Muisa *et al.*, 2015). The Kwekwe City sewer reticulations have not been upgraded to accommodate the increase in population size over time. There is one sewage treatment plant for all of Kwekwe's residents. These sewer reticulation systems serving Kwekwe eventually feed to the Northern Sewage Works located about 1 km from north-east of Mbizo (Figure 1). The sewage works is comprised of a set of waste-stabilisation ponds and two Biological Nutrient Removal (BNR) activated sludge plants. The treatment capacity of these three units is 9000 kg COD/day. Analysis of sewage strength data for the period January 1989 to June 1993 showed that pollution load discharged to the works in 1993 was about 8200 kg COD/day (Stewart Scott Zimbabwe, 1999). This was significant overload resulting in a decline in effluent quality. The same analysis of sewage flows and loads indicated that the raw sewage strength had risen well above the original design of the sewage plant.

The volume of sewage effluent received by the treatment plant is therefore overloading the present equipment resulting in generation of poor quality effluent. The poor effluent quality from sewage systems is characterised by high levels of nitrogen and phosphate which lead to

algal blooms (Nhapi and Tirivarombo, 2004). The Kwekwe sewage treatment plant releases its effluent into Sebakwe River after processing.

Depending on concentrations, these affect humans and limit the agricultural use of the river water. Farmers in areas like Sherwood and Marivale where agriculture is done rely on water from Sebakwe River for irrigation. If it is contaminated by sewage effluent, plants may get diseases and the produce will be unfit to sell or eat. Pathogenic viruses, bacteria, protozoa and helminths may be present in raw municipal wastewater and will survive in the environment for long periods.

### **1.3 Justification of the study**

Contaminated riverine ecosystems are a threat to human health as they promote the spread of some water-borne diseases (Masere *et al*, 2012). Kwekwe is a highly industrialised city and the chemicals, nutrients and metals from these processes are a threat to the ecosystem, human and animal health. Water from Sebakwe River is used for domestic use by downstream communities, irrigation and livestock rearing. These communities stand the risk of contracting bacterial and viral disease-causing pathogens (Masere *et al*, 2012). Diseases like cholera, typhoid and dysentery have been reported in various Zimbabwean cities such as Mutare and Harare in the past (Mapira, 2011; Makwara and Tavuyanago, 2012). Bioaccumulation of heavy metals like silver, lead, cadmium, nickel and copper will also take place in animals, vegetation and humans resulting in both diseases and death. The river is of importance to people around it. The study will describe the current state of the physicochemical variables.

The study will enable the establishment of baseline biological and water quality data in Sebakwe River and this will be used to assess and track changes in water quality. It will also assist environmental protection law enforcers and policymakers like Environmental Protection Agency (EMA) and the government in formulating and establishing functional and efficient pollution abatement measures. This study will therefore benefit the Sebakwe River ecosystem and the information generated can be used for restoration of rivers.

## **1.4 Objectives**

### **1.4.1 General objective**

The general objective of the study was to determine the impact of sewage effluent on the aquatic health of Sebakwe River using the SASS index.

### **1.4.2 Specific objectives**

1. To determine the seasonal variation in physicochemical parameters in Sebakwe River
2. To determine the effect of sewage effluent on the physicochemical parameters (i.e. pH, dissolved oxygen (DO), electrical conductivity (EC) and temperature) of water in Sebakwe River
3. To determine the effect of sewage effluent on the diversity macroinvertebrates in Sebakwe River
4. To evaluate the overall health of Sebakwe River using the SASS index

## **1.5 Research hypothesis**

Sewage effluent has an effect on the water quality and aquatic biota of Sebakwe River.

## **2.0 CHAPTER TWO: LITERATURE REVIEW**

### **2.1 Decline in freshwaters**

Throughout history, rivers have been prone to pollution, due to their easy accessibility as a means to dispose of wastes (Bhat *et al.*, 2014). The most severe factor affecting the sustainability as well as availability of water resources in southern Africa is water pollution (Masere *et al.*, 2012). Zimbabwe is no exception with its water courses deteriorating in water quality because of regular inflows of poorly treated sewage effluents (Makwara and Tavuyanago, 2012). If uncontrolled, river pollution threatens the survival of natural ecosystems and human communities (Mapira, 2011); as it can affect human health, economic activities and biotic communities (Masere *et al.*, 2012). Good river health is reflected by a good ecological and chemical status (Gabriels *et al.*, 2010). This means chemical stability alone cannot stand alone as an indicator of river health. Water pollution is the discharge of a liquid, solid, gas, pathogenic organism(s) or other substances into the water that may create a nuisance, render the water harmful, or make it injurious to the health and/or welfare of the public and the environment (Masere *et al.*, 2012).

River pollution has seen an increase over time. It has been attributed to industrial and agricultural activities (Tazvivinga *et al.*, 2012). Mining and river bank cultivation were also observed as pollutants (Gratwicke, 1998). Effluent discharges and agricultural chemicals deplete river health (Yan *et al.*, 2015). Poor management of urban waste, sediments and urban runoff are other pollution factors (Masere *et al.*, 2012). Natural processes such as erosion and weathering of crustal materials affect river water quality and determine its use for various purposes (Bhat *et al.*, 2014). Effluents and toxicants generated in catchment areas also end up in river systems affecting their integrity (Tazvivinga *et al.*, 2012). Riverine and lake ecosystems are affected by multiple sources. Understanding the spatial and temporal variations in physicochemical and microbiological parameters is important for assessment, management and waterborne diseases prevention (Vadde *et al.*, 2018).

The ecological integrity of a river is its ability to sustain a balanced, integrated and adaptive community of physicochemical characteristics with a biological diversity (on a temporal and spatial scale) that are comparable to those of natural aquatic ecosystems in the region (Tazvivinga *et al.*, 2012). This integrity is threatened by pollution and eventually leads to compromised ecological functions (Odume *et al.*, 2012). Discharges not only influence the

quantity and quality of the water, they also impact on the ecological integrity and biodiversity of the system (Chikodzi *et al.*, 2017).

For a river to successfully self-depurate, it needs to be in a healthy state. This enables it to absorb the impact of various chemicals and organic matter which it receives (Gratwicke, 1998). Self-purification function of a river is therefore effective within a carrying capacity limit. With increased anthropogenic activity, the ability of a river system to self-purify also decreases (Gratwicke, 1998). Healthy riverine ecosystems serve the function of maintaining robust water quality and preserving rare or sensitive species which contribute to the overall biological diversity of the area (Gratwicke, 1998). Following the industrial revolution, carrying capacity of rivers and ability to process wastes reduced significantly (Bhat *et al.*, 2014). Rivers are very accessible as means to dispose of liquid and/or solid waste. Manufacturing and processing companies simply dump their partially treated or non-treated effluent into waterways. This effluent will be heavily laden with pollutants such as chemicals, metals and organic matter. Industrial waste was named as a possible cause for the high alkalinity in Kodhaiyar River in India (Priya *et al.*, 2016). Rivers close to industrial centres have been observed to have poor water quality (Phiri, 2014). The light industries around Chitungwiza, Norton and Harare were cited as having been releasing untreated and partially treated effluent to the environment leading to river health depreciation (Masere *et al.*, 2012).

Deteriorated water quality manifests in the form of reduced self-purification ability of the stream, reduced aesthetic qualities, disease outbreaks, fish deaths, blooms of water hyacinth (which cause evapotranspiration losses from a water body and increased costs of water purification for drinking purposes) (Gratwicke, 1998). A high concentration of water hyacinth plants is explicitly indicative of high water nutrient content (Mapira, 2011). The need for the development of methods to evaluate and monitor the state of riverine ecosystems was recognised as far back as 1972 when Chutter developed a biotic index (Dickens *et al.*, 2002). This need continued to be echoed later with further decline in abundance and/or quality of rivers and streams worldwide (Li *et al.*, 2010).

## **2.2 Impact of urbanisation on river systems**

Heavily industrialised catchments usually have poor water quality (Odume *et al.*, 2012). Surface water is under more threat than ground water because of pollutants, urbanization, industrialization and the use of pesticides in the agricultural sector (Priya *et al.*, 2016). Pollution of water bodies as a response to rapid urbanization has been observed in the Taihu watershed (third largest freshwater lake in China) (Vadde *et al.*, 2018). Declining water quality and ecosystem health as well as a risk on public health were some of the concerns (Vadde *et al.*, 2018).

During the last three decades, Zimbabwe has experienced massive rates of urbanization, which are comparable to those in other parts of Africa. While in 1982, the country's urban population was only 20% of the national total (7.6 million), by 1992, the figure had risen to 31% of the 10.4 million citizens (Mapira, 2011). Waste management problems have worsened as urban centres strive to maintain clean environments – a feat that is not easy due to limited budgets. Cholera, typhoid and dysentery (water borne diseases) have been reported in several urban centres including: Harare, Chitungwiza and Mutare (Mapira, 2011). Due to population growth in the Manyame Catchment, surface and groundwater quality were shown to increasingly degrade; industrial, agricultural activities, and domestic sewage being the main causative agents (Masere *et al.*, 2012). Most of the sewerage systems in these places are overloaded because they were not designed to cater for the current populations.

Rainfall events can further accelerate pollutant loadings due to storm water runoff from urban areas, as well as from agricultural areas where practices such as manuring, fertilization and livestock grazing are done near the water bodies (Vadde *et al.*, 2018).

## **2.3 Impact of sewage effluent on water resources**

Zimbabwe's urban settlements encounter several constraints in the delivery of services (power, water, sewage and solid waste management). Zimbabwe's urban centres experienced remarkable growth since independence in 1980 and were cited as being home to forty percent of the country's population in 2012 (Makwara and Tavuyanago, 2012). Municipal budgets in developing countries are often under strain and fail to cope with the demand of both spatial and demographic growth (Mapira, 2011). This is evidenced by irregular and/or non-existent disposal of solid wastes to proper dump sites (Mapira, 2011). During the rainy season, uncollected garbage and sewer effluent from burst pipes is washed into water sources leading to contamination of water (Makwara and Tavuyanago, 2012).

Surface water quality is altered by point and nonpoint sources of pollution. Point source pollution occurs from a single identifiable source. Examples of this are effluents from industries and wastewater treatment plants. Nonpoint sources include runoff associated with a particular land use pattern such as agriculture (e.g. fertilizers, animal manure), or forestry land uses e.g. stream bank cultivation (Tazvivinga *et al.*, 2012). The disposal of untreated or poorly treated sewage into surface water bodies in urban areas is common in Zimbabwe as in most developing countries (Muisa *et al.*, 2015). This effluent is rich in nitrogen and phosphorous and has been clearly shown to be a major cause of eutrophication problems in the country (Nhapi and Tirivarombo, 2004). Sewage discharges contribute to oxygen demand and nutrient loading. They promote toxic algal blooms and ultimately lead to a debilitated aquatic ecosystem (Morrison *et al.*, 2001). This threatens the survival of many species of fish and other aquatic life (Muisa *et al.*, 2015).

The use of rivers to drain (sometimes raw) wastewater generated in towns and cities has been cited in the Zimbabwean cities of Harare, Mutare and Chinhoyi (Makwara and Tavuyanago, 2012; Mapira, 2011; Muisa *et al.*, 2015). Pipe bursts which are frequent in occurrence because of old sewer systems are not attended to promptly due to lack/scarcity of funds and this raw sewage ultimately ends up in waterways (Mapira, 2011). Companies compound the problem by disposing of dangerous waste material into watercourses (Makwara and Tavuyanago, 2012).

The effects of disposing untreated sewage in aquatic systems are depletion of dissolved oxygen (Odume *et al.*, 2012). This is a result of the oxidation of organic matter (Masere *et al.*, 2012). Furthermore, increased nutrient loading with nitrogen and phosphorus in the waterway causes proliferation of invasive aquatic plant species like the water hyacinth *Eichhornia crassipes*. (Chikodzi *et al.*, 2017). The proliferation of *E. crassipes*, also reduces light penetration in aquatic environments. In Zimbabwe, *E. crassipes* has been observed in Sakubva River of Mutare, Shagashe River of Masvingo and Manyame River in Manyame Catchment (Mapira, 2011; Chikodzi *et al.*, 2017; Masere *et al.*, 2012). The water hyacinth plant can also increase evaporative water losses from reservoirs and water bodies which leaves less water available for economic production purposes like irrigation (Gratwicke, 1998; Masere *et al.*, 2012).

Peri-urban agriculture practiced by urban dwellers in Harare, Norton and some parts of Chitungwiza has been cited as a cause of nutrient loading in the rivers leading to

eutrophication (Masere *et al.*, 2012). In a study by Masere (2012); the presence of nitrates and phosphates was ultimately attributed to entry of untreated sewage into the river. Domestic wastewaters, particularly those containing detergents and fertilizer runoff, contribute to presence of phosphates in the water column (Bhat *et al.*, 2014). The problem is then compounded in areas where wastewater treatment systems are simple and not efficient (Morrison *et al.*, 2001). Aquatic plant growth is then accelerated at great rates at the expense of aquatic creatures. There are many factors responsible for distribution of organisms in various fresh water habitats according to their adaptations, which allow them to survive in a specific environment (Priya *et al.*, 2016). By depriving such organisms of oxygen, these plants upset the ecological balance of some rivers so that they eventually die (Mapira, 2011).

#### **2.4 Disease spread**

It is important that a river be in a healthy state when it is used for domestic uses by downstream communities (Morrison *et al.*, 2001). Few of these people take sanitary measures like boiling before use and this leads to the spread of some water-borne diseases. Cases of cholera, typhoid and dysentery have been reported in Southern African countries such as: Malawi, Mozambique and Zambia (Mapira, 2011).

Zimbabwe has also had outbreaks of cholera and typhoid in recent years in the large urban centres. The outbreaks were attributed to the disintegration of sewer systems, poor sanitation in the cities' high density suburbs, the decline and of health services and consumption of water from contaminated sources (Makwara and Tavuyanago, 2012).

The incidences of waterborne diseases were mainly experienced in Chitungwiza and Norton as a result of untreated sewage finding its way into drinking water sources (Masere *et al.*, 2012). In the city of Mutare, communities 10km downstream of Sakubva River were impacted by the condition of the water which was polluted by sewage, industrial and institutional waste (Mapira, 2011).

## **2.5 Interaction of sewage and other factors**

The physical disturbances of stream sediments such as sediment coring have been found to cause reductions in total the diversity of benthic organisms (Hussain and Pandit, 2012). In Njoro River of Kenya, it was observed that human and animal disturbances have an effect on macroinvertebrate populations, migrations and dispersal but only if they are persistent (M'Erimba *et al.*, 2014). The impact of the disturbances are more intense during periods of low water discharge (dry season) and became less during periods of high water discharge (wet season) (M'Erimba *et al.*, 2014). In the Njoro River, downstream of the Turkana site, there was a point where partially treated sewage was discharged. Another sewage discharge point was located several metres upstream of another site, Mary Joy. Macroinvertebrate communities in rivers receiving raw sewage from urban areas are often dominated by a few taxa that can tolerate low levels of oxygen associated with that kind of pollution (M'Erimba *et al.*, 2014).

Larger aquatic species are strongly negatively impacted by a wide range of stressors. Examples of these stressors include: drought, acidification, habitat loss and fragmentation (Woodward *et al.*, 2013). Many factors regulate the occurrence and distribution of stream dwelling macroinvertebrates. The most important of these are current speed, temperature, season, the substratum, vegetation, dissolved substances, liability of the river to drought and floods, food, competition between species, shade and zoogeography (Hussain and Pandit, 2012). Environmental alterations affect community structure leading to a decrease or increase of sensitive species (Dias-silva *et al.*, 2010).

Other factors that pose a threat to rivers and streams are metal pollutants such as zinc, lead, arsenic and mercury originating from industries as well as oil spills which drain into natural watercourses and wastes from hospitals (Mapira, 2011). Reduction in benthic macroinvertebrate diversity have been reported in streams contaminated with insecticides (M'Erimba *et al.*, 2014). Ultimately, there are a number of stressors which interact to diminish natural freshwater ecosystems, some ancient and others urban (Woodward *et al.*, 2013). The interactions among chemical, physical and biological pollutants emphasises the need to assess the biota as indicators of poor river health (Tazvinga *et al.*, 2012).

## 2.6 Use of physicochemical parameters to test river health

Historically, the assessment of water quality in freshwater ecosystems has been through the measurement of physicochemical parameters (Odume *et al.*, 2012). Such measurements, however, cannot on their own provide ecological information, because the synergistic effects of pollutants on aquatic biotic community may not be fully and easily assessed (Tazvivinga *et al.*, 2012). The interactions of the chemical and physical properties of water play a significant role in distribution, composition and diversity of aquatic organisms (Priya *et al.*, 2016). Chemical data, although instantaneous, only reveal the conditions of the stream or river at the time of sampling (Olomukoro and Dirisu, 2014).

Waters that are enriched with nutrients are characterized by high pH values (Masere *et al.*, 2012). The pH is an important parameter in evaluating the acid-base balance of water. The chief component regulating ion pH in natural waters is the carbonate, which is comprised of CO<sub>2</sub>, H<sub>2</sub>CO<sub>3</sub> and HCO<sub>3</sub><sup>-</sup>. Alkalinity of water increases with increase in dissolved carbonates and bicarbonates and this high concentration is attributed to sewage and industrial waste (Priya *et al.*, 2016). Aquatic macroinvertebrates are sensitive to the extremes in pH. Most of the aquatic macroinvertebrates tolerate pH range between 5 and 9 (Hussain and Pandit, 2012). Acidic rivers have fewer species and individuals (Thomsen and Friberg, 2002). Low pH values are associated with lower diversity of macroinvertebrates as they cause decreased emergence rates and physiological problems (Hall *et al.*, 1980).

A decrease in stream water pH can trigger the release of heavy metals and these are toxic to benthic macroinvertebrates. Acidification results in difficulty in ion regulation and reduced calcium absorption for exoskeleton formation (Thomsen and Friberg, 2002). Amphipods, isopods, crayfish, snails and bivalves are more common in hard than in soft waters (Hussain and Pandit, 2012). The higher the removal rate of free CO<sub>2</sub> during photosynthesis, the higher the alkalinity (Nhapi and Tirivarombo, 2004). High pH values may alter the toxicity of other pollutants in the river for example ammonia is much more toxic to aquatic biota at pH values greater than 8.5 because this is when it is in the oxidised form NH<sub>4</sub><sup>+</sup> (Morrison *et al.*, 2001). A decrease in pH results in decreased solubility of essential elements such as selenium while also increasing the solubility of elements such as aluminium, iron, copper, manganese, boron, cadmium and mercury (Morrison *et al.*, 2001).

Discharges to streams can change their electrical conductivity (EC). High conductivity values are indicative of pollution levels, particularly a high amount of dissolved inorganic

substances in ionized form. Conductivity is defined as the capacity of a substance or solution to conduct electrical current through water (Priya *et al.*, 2016). Electrical conductivity is an indicator of the salinity (total salt content) of water. Wastewater effluents (and sometimes industrial effluents) contain high amounts of dissolved salts (Morrison *et al.*, 2001). Salts like sodium chloride and potassium sulphate have the ability to build up and pass through conventional water and wastewater-treatment plants unaffected. These then increase the salinity of the receiving water which may result in adverse ecological effects on aquatic biota (Morrison *et al.*, 2001).

Warm conditions that prevail under dry weather conditions promote evaporation of oxygen from the water into the air and more plant biological activity in the water resulting in dwindling DO (Muisa *et al.*, 2015). Likewise, proliferation of algal blooms and invasive species like the water hyacinth reduces DO availability. In their study, Nhapi and Tirivarombo (2004) observed that waters upstream of sewage pollution had higher DO levels than downstream waters.

Proliferation of blue-green algae may be caused by high nutrient values, leading to the release toxic cyanotoxins into the water (Morrison *et al.*, 2001). Cyanotoxin poisoning and deaths have been observed in humans in China and Brazil (Codd *et al.*, 2005). Nitrates in waste effluents can originate from domestic and agricultural wastes, especially from nitrogen-containing fertilizers. High nitrate concentrations are frequently encountered in treated wastewater as a result of ammonium nitrogen being totally or partially oxidised to nitrate by microbiological action. High nitrate levels also contribute to eutrophication effects in freshwater (Morrison *et al.*, 2001).

Phosphates in sewage effluents arise from human wastes and domestic phosphate-based detergents. Phosphates are the chief growth-limiting factor in eutrophication. The potential health risk from nitrate in drinking water is methaemoglobinemia – a condition in infants and pregnant women (Morrison *et al.*, 2001). It occurs rarely and only in water containing more than 30 mg of nitrate per litre (Morrison *et al.*, 2001). Ammonium-N is extremely soluble and is readily transported by surface runoff from cultivated lands. It is also a major component of raw sewage. It occurs in water as a breakdown product of nitrogenous material (Morrison *et al.*, 2001). The benthic macroinvertebrates have evolved to live within a specific temperature range. Temperature affects their emergence patterns, growth rates, metabolism, breeding capacity and body size (M’Erimba *et al.*, 2014). Species vary in their tolerance to temperature

ranges, but few are able to tolerate temperatures beyond their upper tolerance limit (Hussain and Pandit, 2012).

Riverbed material characteristics are another important characteristic of physical habitat in riverine ecosystems. Spatial variations in riverbed material composition (size, shape, and sorting) impact macroinvertebrate responses in different ways. Coarse and strongly structured substrates are preferable to benthic fauna because they experience minimal disturbance during floods. Substrate type may be affected by presence or absence of riparian vegetation, which ultimately has an effect on biotic interactions (Dias-silva *et al.*, 2010). The type of substratum therefore controls the types of macroinvertebrates which are found in a river system (Hussain and Pandit, 2012). The fauna of clean stony runs is richer than that of silty reaches and pools. Synergistic effects of pollution on aquatic biotic communities, however, may only be fully investigated through biomonitoring of stream conditions. Biomonitoring is the most credible source of freshwater ecological status since it provides an integrated and comprehensive assessment of the health of a water body over time (Muposhi *et al.*, 2015).

## **2.7 Biomonitoring as a water assessment tool**

Aquatic biomonitoring is deducing the ecological condition of rivers, lakes, streams, and wetlands by examining the effects of xenobiotics to organisms (Tazvivinga *et al.*, 2012). In running waters, where changes in hydrology are rapid and difficult to estimate, they cannot reflect the integration of numerous environment factors of river ecosystems because of their instantaneous nature. Biomonitoring has proven to be necessary as the main method or a supplementary to the traditional monitoring techniques for assessment of aquatic health. Aquatic organisms, such as diatoms and benthic macroinvertebrates, can serve as bioindicators to integrate their total environment and their responses to complex sets of environmental conditions. They give an ecological overview of the status of streams or rivers (Li *et al.*, 2010). Ideally, indicator species should: be holistic but closely related to assessment goals, show a response to a range of environmental stresses, show an integrative potential in the long-term, be easily measured, quantified and interpreted (Nahmani *et al.*, 2006).

Biomonitoring is advantageous in that it can detect cumulative physical, chemical and biological impacts of adverse activities to an aquatic system (Bonada *et al.*, 2006). The first practical application of water quality assessment using biota, the saprobic system, was devised by Kolkwitz and Marson (Gratwicke, 1998). It was used in Europe to indicate

oxygen shortages caused by biologically decomposable, organic pollution in running waters. They mainly used bacteria and sometimes algae, protozoans, rotifers, fish and some macroinvertebrates as indicator species. By the mid-1970s, these indices had been rejected by most European countries for their limits (Li *et al.*, 2010). In South Africa, Arthur Harrison presented a paper on the role of river fauna in the assessment of pollution and he noted that faunal data would be useful: when episodes of intermittent pollution (which would not always show in chemical tests) were suspected; where the pollutant was undetectable by chemical tests, and where the environmental impact was not toxic in nature (Gratwicke, 1998).

As the study of ecology evolved, community structure became more important and a host of indices which could be used to assess aquatic pollution became available. The Biological Monitoring Working Party (BMWP) in Britain developed a scoring system based on family-level identifications for rapid aquatic site appraisals (Chutter, 1995). This was based on the tolerance of aquatic invertebrates to organic pollution and an Average Score per Taxon (ASPT) could be calculated. The programme later became refined to species level identifications known as RIVPACS (River and Invertebrate Prediction and Classification System) (Bredenhand, 2005). The basic scores from the BMWP were adapted to South African conditions and following thorough testing the South African Scoring System (SASS) was created (Gratwicke, 1998).

Rapid bioassessment methods (RBMs) for assessing ecological condition in river ecosystems using macroinvertebrates have been developed and used worldwide (Kaaya *et al.*, 2015). They are valuable in water resource management. Six biotic indices which are based on aquatic macroinvertebrates have been developed in the southern region of Africa: the South African Scoring System (SASS) in South Africa (Dickens and Graham 2002), the Namibian Scoring System (NASS) in Namibia (Shimba and Jonah, 2016), the Okavango Assessment System (OKAS) in the Okavango Delta (Dallas and Mosepele, 2010), the Tanzanian River Scoring System (TARISS) in Tanzania (Kaaya *et al.*, 2015), and the Zambia Invertebrate Scoring System (ZISS) in Zambia (Lowe *et al.*, 2013). Four of the biomonitoring indices NASS, OKAS, ZISS and TARISS were modified from the SASS index (Kaaya *et al.*, 2015). The SASS index has proven its efficiency and reliability as an index for the assessment of water quality and general river condition (Kaaya *et al.*, 2015).

The assessment of biota in rivers and streams is a widely recognized means for determining the health of rivers (Dickens and Graham, 2002). Stressed water bodies are often dominated by tolerant organisms and reduction in the number of sensitive ones (Graham *et al.*, 2004). Parallel chemical monitoring should also be done under all circumstances to reveal causative actors. Biological monitoring therefore complements chemical and physical monitoring. The biomonitoring indicates whether a problem is present, and the chemical monitors identify its nature (Lowe *et al.*, 2013).

Biomonitoring is either passive or active. Passive biomonitoring uses organisms in their natural environment to evaluate environmental health, while in active biomonitoring organisms are introduced into controlled conditions and monitored (Muposhi *et al.*, 2015). Indigenous organisms are continuous monitors of environmental quality and can help in the detection of short-term environmental variations. Passive biomonitoring has been used to assess the water quality of Mazowe and Yellow Jacket Rivers, Chiraura River in Harare, Gwebi and Mukuvisi Rivers in Harare, Mucheke and Shagashe Rivers (Tazvivinga *et al.*, 2012; Utete and Kunhe, 2013; Phiri, 2014; Chikodzi *et al.*, 2017). In aquatic ecosystems, these assessments usually focus on invertebrates, algae, macrophytes, fish or amphibians. Passive biomonitoring is usually done together with habitat assessment. This is because the physical habitat influences macroinvertebrate distribution in rivers (Dias-silva *et al.*, 2010). Habitat integrity assessment is therefore an essential part of the ecological analysis of a river (Tazvivinga *et al.*, 2012).

Benthic macroinvertebrates are often used as indicator organisms in passive biomonitoring because they are abundant, inexpensive to sample, found in nearly all aquatic ecosystems (Tazvivinga *et al.*, 2012). Their sedentary nature helps in the detection of point source pollution or localized disturbances (Shimba and Jonah, 2016). Different taxonomic groups of the macroinvertebrates have different sensitivities to pollution (Hussain and Pandit, 2012). They are easy to collect and identify and act as continuous water quality monitors (Graham *et al.*, 2004). Biological monitoring can provide information about past and/or episodic pollution (Kasangaki *et al.*, 2006). Important inferences about the health of the river can be made by examining the relative abundance and diversity of macroinvertebrates. Contrary to fish and other aquatic fauna which are very mobile, benthic macroinvertebrates have less capability to escape the effects of sediment and other pollutants that reduce water quality (Chikodzi *et al.*, 2017).

## **2.8 Macroinvertebrate prevalent use**

For rivers one of the most applicable elements in water assessment are the benthic invertebrate fauna (macroinvertebrates). The parameters that are taken into account are taxonomic composition and abundance (Gabriels *et al.*, 2010). Among aquatic biota, macroinvertebrates are an important component of the ecosystems, particularly utilisation of energy and matter (Dallas and Mosepele, 2010). Individual taxa respond differently to pollutants and are able to provide an indication of water quality over varying time periods (Bonada *et al.*, 2006). They are therefore the most suitable, reliable, and the most widely acclaimed in ascertaining the overall health status of aquatic environments globally (Olomukoro and Dirisu, 2014).

Macroinvertebrate based biomonitoring approaches, including single biotic indices such as the South African Scoring System (SASS), multimetric indices such as the index of biotic integrity (IBI-12) and multivariate techniques such as the Australian River Assessment System (AUSRIVAS) have been developed and applied to assess water quality of rivers and streams. (Odume *et al.*, 2012). A multimetric index describes the state of an ecosystem by means of several individual variables (metrics). Each metric represents a different component of ecosystem quality and they are combined into one index value. Multimetric indices were first developed for fish communities and later also for other indicator groups, including macroinvertebrates (Gabriels *et al.*, 2010).

Macroinvertebrates play a central ecological role in nutrient cycling in aquatic ecosystems (Bredenhand, 2005). Most macroinvertebrates are important components of stream ecosystems. They graze periphyton (preventing blooms in some areas), assist in the breakdown of organic matter and cycling of nutrients and, in turn, may become food for predators (Hussain and Pandit, 2012). Each macroinvertebrate has a unique characteristic. They range from pollution tolerant, somewhat tolerant to pollution sensitive. Macroinvertebrates, which have been utilized in aquatic pollution studies include: mayflies (*Ephemeroptera*), caddisflies (*Trichoptera*), stoneflies (*Plecoptera*), beetles (*Coleoptera*), crayfish and amphipods (*Crustacea*), aquatic snails (*Mollusca*), biting midges (*Chironomidae*) and leeches (*Hirudinea*) in Nigeria, North America and Europe (Olomukoro and Dirisu, 2014).

## 2.9 The SASS method

The South African Scoring System (SASS), was developed by Chutter in the late nineties and over recent years the method has become the standard for the rapid bioassessment of rivers in South Africa; and is the backbone for their National River Health Programme (Dickens *et al.*, 2002). SASS 5, the latest version, is ISO 17025 accredited and has been proven to be suitable for the assessment of river health. The SASS is a warning system for detecting pollution events that has advantages over traditional approaches because it can give insights into the effect of stress on a community (Tazvivinga *et al.*, 2012). Unlike other indices of river health assessment, SASS provides clear sampling techniques, which makes it a boundless and practical choice (Graham *et al.*, 2004). The SASS method works best when the diversity of biotopes is wide and includes riffles or rapids, but it also produces valuable results from poor habitats (Dickens and Graham, 2002). The data is interpreted according to habitat quality, availability and diversity (Chutter, 1995).

Biomonitoring tools for water quality assessment are largely lacking for many developing countries including Zimbabwe, resulting in adoption of tools developed from other countries. Although there are variations in invertebrate assemblages and sensitivity levels amongst countries (Kaaya *et al.*, 2015), when directly applied to Zimbabwean rivers, the SASS method has been successful in assessing river health. It has shown not only potential, but, effectiveness (Gratwicke, 1998). The results obtained from a study by Chikodzi *et al* (2017), are in agreement with other studies by Phiri (1998), Tazvivinga *et al* (2012), Utete and Kunhe (2012) in Zimbabwe and show that the SASS 5 system can be easily implemented in rapid assessment of water quality of rivers found in Zimbabwe. Zimbabwe was cited as having similar occurrence of ubiquitous macroinvertebrate taxa with environmental tolerances as those recorded for South African systems. Physical and chemical water quality variables and SASS5 indices were found to be consistent with SASS5 scores (Chikodzi *et al.*, 2017). This shows that this index can be adopted for river health assessment for the entire country. Sustainable means of water quality management can then be created from this.

### 3.0 CHAPTER THREE: METHODS AND MATERIALS

#### 3.1 Study site

Sebakwe River is located in the Midlands Province of Zimbabwe in the city of Kwekwe and is 150 km from source to mouth (Figure 1). It is a tributary of Munyati River which it joins in Zhombe East. Sebakwe River has a very rich history as it has always been a source of drinking and irrigation water. Que Que, as the city was called then, developed from mining compound townships for Gaika and Globe and Phoenix mines and drinking water for the two mines was ferried from Sebakwe River by ox wagon until Globe and Phoenix mine was granted water rights to pipeline water. Sebakwe River supplies Munyati Power Station with water for boilers via a 23 km long canal. The 266 mega litre Sebakwe Dam along Sebakwe River supplies Kwekwe, Sebakwe Recreational Park and Redcliff town with water. Kwekwe City is now a busy industrial-commercial centre (which include iron and steel, ferro chrome, maltings and gold mines) situated halfway between Harare and Bulawayo on the main road and rail lines. The mean altitude is 1800 m above sea level (Matsa, 2014).

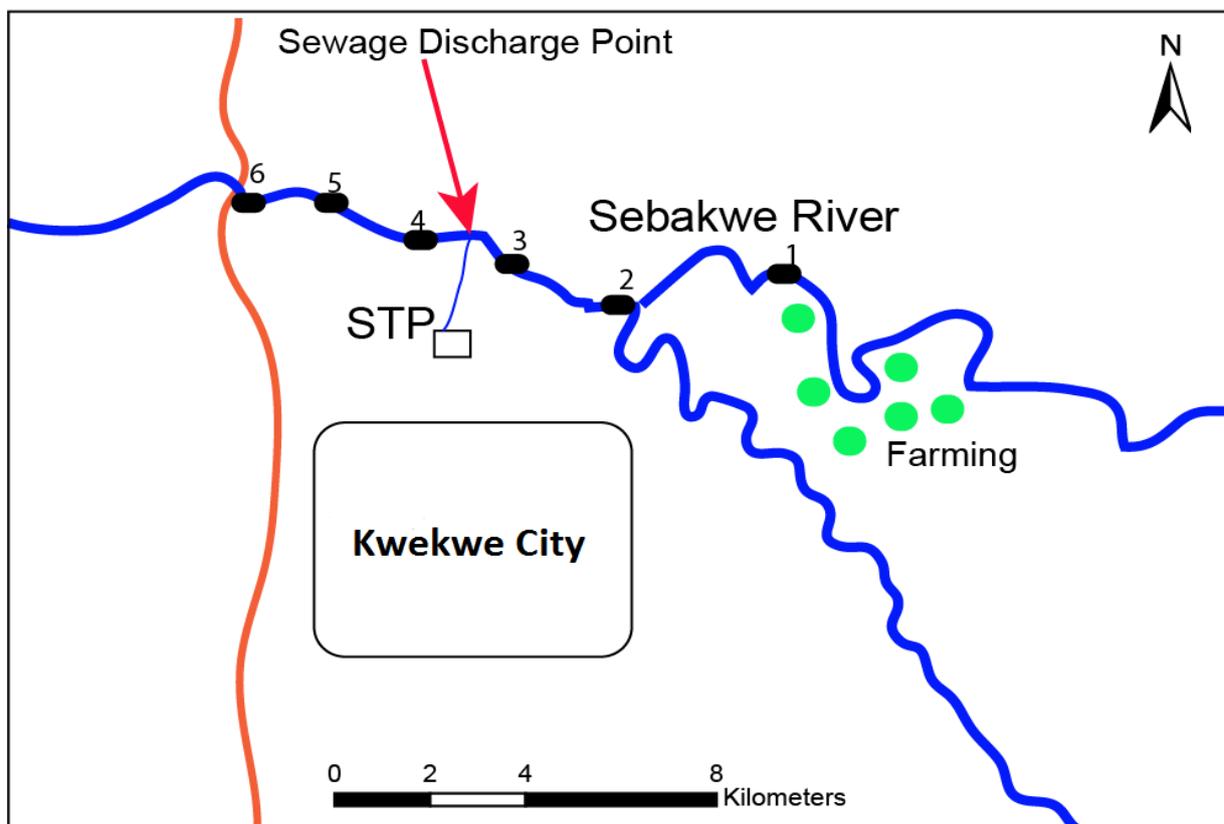


Figure 1: Map of Sebakwe River showing the location of sampling sites (1 - MP; 2 - CP; 3 - BP; 4 - AP; 5 - RB; 6 - SB; STP – Sewage Treatment Plant).

### **3.2 Sampling design**

Sampling points were chosen to evaluate the impact of sewage effluent on the aquatic health of Sebakwe River. Three of the sampling points served as reference sites as they were upstream of the sewage effluent discharge point and these were 1: Mlala Park (MP), 2: Confluence Point of Sebakwe and Mbembeswani rivers (CP) and 3: Before Point-source (BP). Downstream of the sewage treatment plant were also three points: 4: After Point-source (AP), 5: Railway Station (RS) and 6: Sebakwe Bridge (SB). The sampling points were chosen according to their accessibility.

### **3.3 Field sampling**

Monthly surveys of macroinvertebrates and water variables were carried out from September 2018 to January 2019, once per month to cover the wet and dry seasons. At each site, additional information required on the SASS5 sheet was captured (i.e. site code and description, signs of disturbance e.g. cattle and/or other animal activity, human activity like bathing, swimming and clothes-washing).

#### **3.3.1 Macroinvertebrate collection**

The macroinvertebrate samples were collected using the SASS5 kick-sampling method (Davies, 2001; Letovsky *et al.*, 2012). At each site, three biotopes: stones (S), gravel, sand and mud (GSM) biotope and vegetation (Veg) biotope were sampled separately for the macroinvertebrates. Hand picking and visual observations were also done, noting down dominant taxa and dominant vegetation onto the SASS sheet.

##### **3.3.1.1 Stones (S) biotopes**

The stones biotope included stones in current (SIC) and stones out of current (SOOC). Stones and bedrock were sampled for approximately one minute by kicking, turning and/or scraping them with the hands and feet, whilst continuously sweeping the net through the disturbed area. Samples collected both in and out of current were combined into a single Stones (S) biotope sample.

##### **3.3.1.2 Vegetation (Veg) biotopes**

The vegetation biotope included i) marginal vegetation which is vegetation hanging into or growing at the edge of the stream both in current (MVegIC) and out of current (MVegOOC) and ii) aquatic vegetation. A total length of approximately two metres of vegetation was

sampled, spread over one or more locations, especially where different kinds of marginal vegetation were present (e.g. reed, grasses, floating vegetation) in different flow velocities. The net was pushed vigorously into the vegetation, moving backwards and forwards through the same area. The dominant plant species was recorded whenever possible. Aquatic vegetation was for the most part submerged. It included algae and the roots of floating aquatics such as water hyacinth. Samples collected in and out of current were combined into a single Vegetation (Veg) biotope sample.

### **3.3.1.3 Gravel, sand and mud (GSM) biotopes**

The GSM biotope specifications were small gravel stones < 2 cm in size, sand (grains were <2 mm diameter), mud (silt and clay particles of < 0.06 mm diameter). The GSM was stirred by shuffling the feet, whilst continuously sweeping the net over the disturbed area to catch dislodged biota. Samples collected in and out of current were combined into a single gravel, sand and mud (GSM) biotope sample.

Samples from all three biotopes where available in a variety of water currents and were sampled for approximately one minute total. The contents of each sample from each biotope were washed down to the bottom of the net separately and tipped into a tray by inverting. The net was flushed out with a washbottle to transfer all the sticky macroinvertebrates into the tray. Viewing and identification was done for a maximum of 15 minutes per biotope. Samples were placed in 70% ethanol and labelled awaiting further laboratory analysis.

### **3.3.2 Physicochemical variables**

*In situ* water analyses were carried out at each of the six sampling sites. A HANNA Instruments HI 8633 metre was used to measure electrical conductivity; EcoSense ® pH100 metre for pH and temperature; and, HI 9143 Microprocessor Auto Cal Dissolved Oxygen metre for DO.

## **3.4.0 Macroinvertebrates identification**

### **3.4.1 Analytical procedure**

The macroinvertebrates present in the water were identified up to family level using macroinvertebrate identification guides. Taxa seen were ticked off on the SASS score sheet under the appropriate biotope heading before combining the three columns into a single total column. The abundance of organisms within each taxon was roughly estimated.

Comparison on species diversity for upstream and downstream were made. Evaluation of the diversity of the biotopes, and thus their of ability to support a diverse invertebrate population at the site was gained by rating them, with a value of 1 for a biotope of limited diversity, up to 5 for a biotope with wide diversity.

### **3.5.0 Data analysis**

#### **3.5.1 SASS scoring**

Three principal indices were calculated for SASS: SASS Score, Number of Taxa and Average Score per Taxon (ASPT). The calculation of results was done by noting any families seen irrespective of abundance, in any of the biotopes, in the Total column (TOT) of the scoring sheet. Each taxon has a quality score, based on its susceptibility or resistance to pollution and disturbances; lowest scores assigned to the taxa that are resistant and the highest score to those susceptible to pollution. Quality scores for each taxon noted in the Total column were as assigned on the scoring sheet and were summed to provide the SASS Score. Score ranges of 1 - 5 indicating highly polluted areas, 6 - 10 indicating moderate and 11 - 15 indicating low tolerance to pollution. The total SASS score divided by the number of taxa provides the ASPT. This value was the species richness i.e. the number of different species represented in an ecological community. (Dickens and Graham, 2002).

#### **3.5.2 Seasonal variation of physicochemical parameters in Sebakwe River**

PAST version 2.17, a software for scientific data analysis was used to analyse seasonal variation of physicochemical parameters in Sebakwe River. Analysis of variance (One way ANOVA, SPSS version 21) was used to test whether the differences in environmental variables amongst the months were significant. The data did not conform to normality (Shapiro-Wilk;  $p < 0.05$ ). A non-parametric test, Kruskal-Wallis test was used to test for the seasonal variation of environmental variables amongst the six sites in Sebakwe River. PCA was used to explore the seasonal and spatial variation of physicochemical variables among the sites.

#### **3.5.3 Effect of sewage effluent on the physicochemical parameters and species richness**

Kruskal-Wallis test was used to test for the effect of sewage effluent on the physicochemical parameters and one way ANOVA was used to test the variability in species richness along the Sebakwe River. Box plots were created to show this variability.

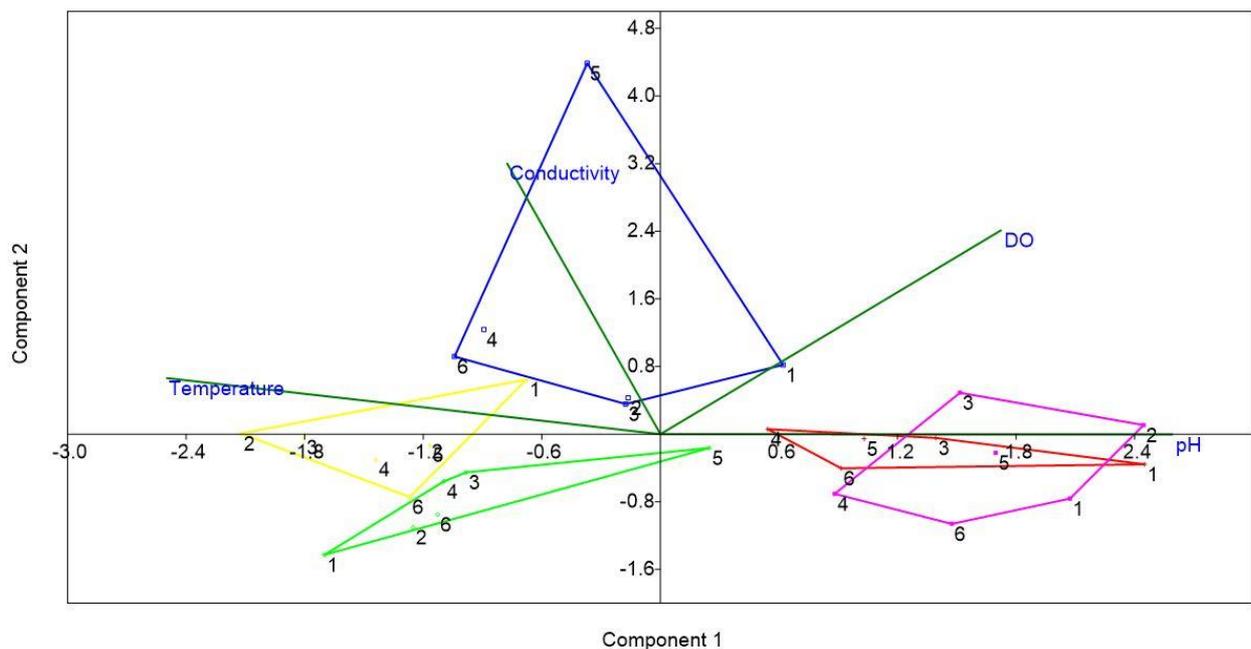
#### **3.5.4 Effect of physicochemical parameters on macroinvertebrate diversity in Sebakwe River**

Multiple regression analysis was used to determine the effect of physicochemical variables on the macroinvertebrate richness in Sebakwe River. The data was log transformed to improve the normality of residuals.

## 4.0 CHAPTER FOUR: RESULTS

### 4.1 Seasonal variation of physicochemical parameters in Sebakwe River

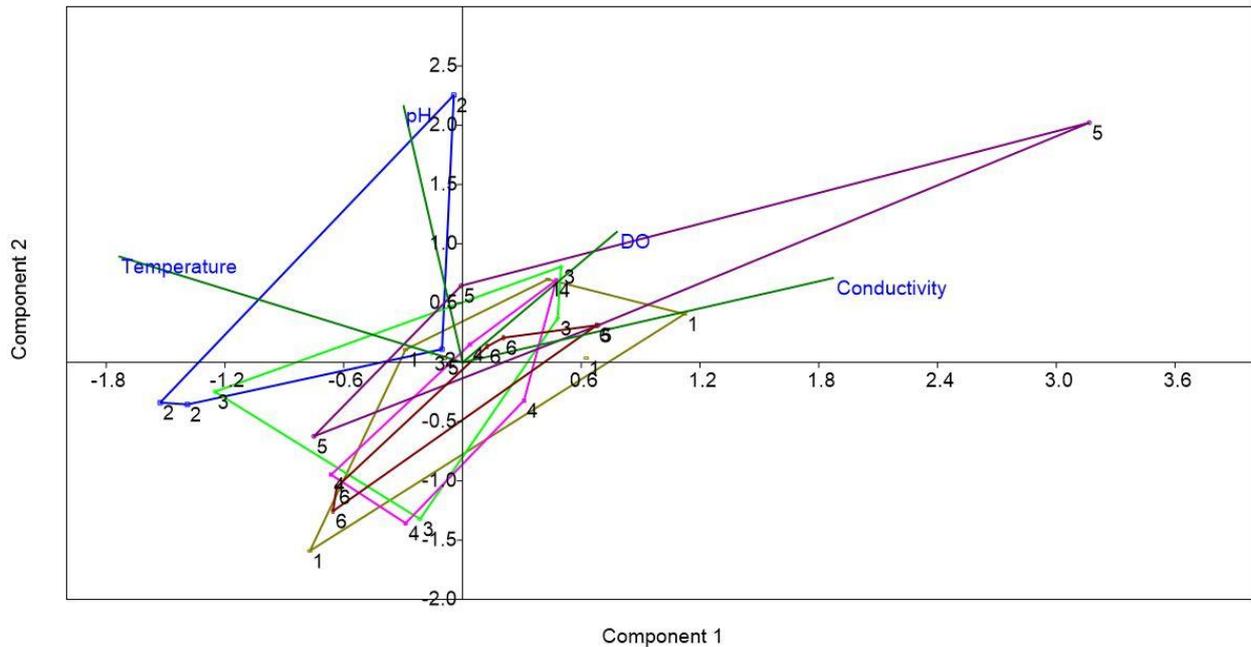
The PCA explained a total of 94.5% variation of the environmental variables among the months (Figure 2). The months of January (brown), September (red) and October (pink) were separated along PCA1 due to differences in pH and temperature. The pH was high in September and October but low in January. Conductivity was high in the month of December (blue) and low in the months of October and September. PCA2 explained a total of 32% variation in environmental variables and separated December from November due to differences in conductivity and DO. DO was low in November. Temperature was high in January (Figure 2). There were no significant differences in DO (Kruskal Wallis;  $p = 0.416$ ) (Appendix 1-5), conductivity (Kruskal Wallis;  $p = 0.416$ ) (Appendix 6-10), pH (Kruskal Wallis;  $p = 0.416$ ) (Appendix 11-15) and temperature (Kruskal Wallis;  $p = 0.416$ ) (Appendix 16-20) among all the months.



**Figure 2: PCA of the physicochemical variables among the months: red – September, pink – October, green – November, blue – December, yellow – January; Numbers 1-6 on polygons represent sampling sites.**

## 4.2 Spatial variation of physicochemical parameters in Sebakwe River

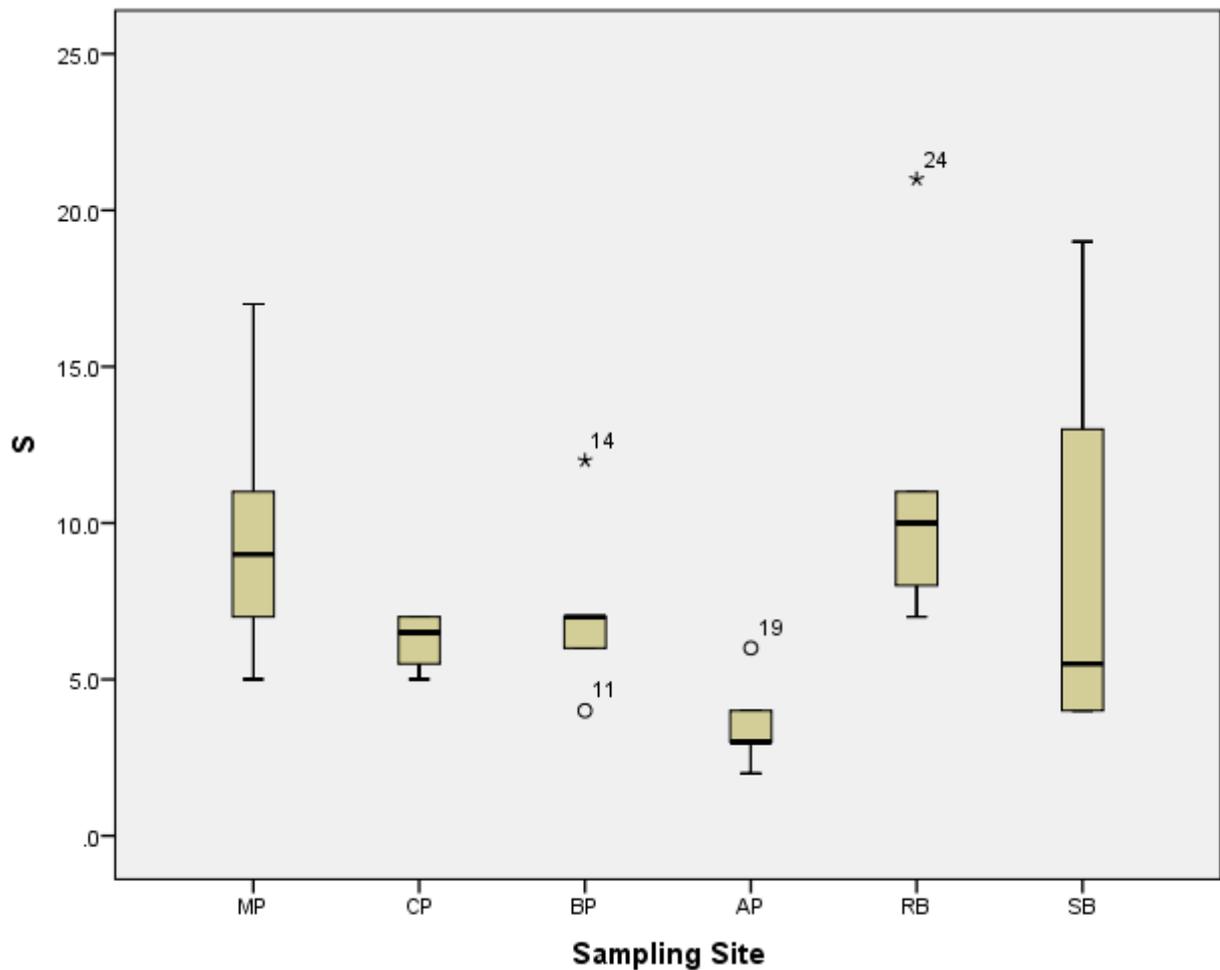
The PCA explained a total of 57.8% variation in the environmental variables among the sites (Figure 3). The temperature among the sites ranged from 25.5 °C - 29.6°C. pH ranged from 5-9 from September to January. There were no significant differences in physicochemical variables among the sites (Kruskal Wallis;  $p = 0.406$  for September) (Appendix 21) and (Kruskal Wallis;  $p = 0.416$  for October to January, (Appendix 22-25).



**Figure 3: PCA of the physicochemical differences between sites: dark green = site 1, blue = site 2, light green = site 3, pink = site 4, purple = site 5, brown = site 6; Numbers 1-6 on polygons represent sampling months September to January.**

## 4.3 Effect of sewage effluent on macroinvertebrate diversity in Sebakwe River

Species richness varied among the sites. The lowest species richness was recorded at site AP ( $3.6 \pm 1.1$ ) while the highest was at site MP ( $9.8 \pm 1$ ) (Figure 4). There were significant differences in species richness among the sites (ANOVA;  $p = 0.012$ ) (Appendix 26). A Tukey post-hoc analysis showed that sites MP and AP ( $p = 0.007$ ), MP and RB ( $p = 0.027$ ), BP and MP ( $p = 0.007$ ) differed significantly in species richness (Appendix 27).

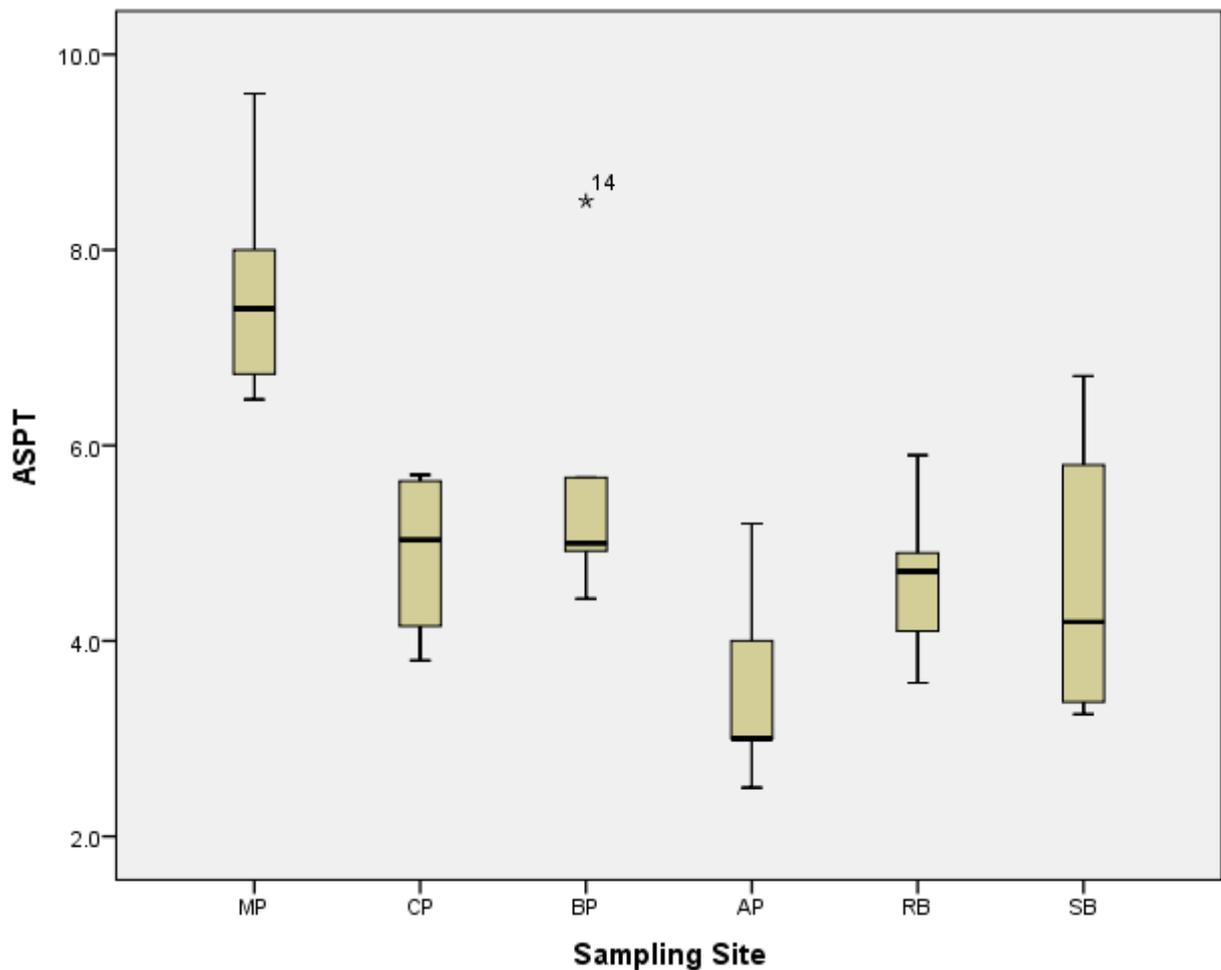


**Figure 4: Mean values of species richness at different sampling points: MP = site 1, CP = site 2, BP = site 3, AP = site 4, RB = site 5, SB = site 6. \* = outlier. o = outlier. S = species richness.**

#### 4.4 Overall health of Sebakwe River

The ASPT and SASS scores decreased where Sebakwe River meets with Mbembeswane River. Occurrence of pollution-tolerant families like the *Oligochaetae*, *Hirudinea* (leeches), backswimmers and the Gastropoda *Lymnaeidae*, *Planorbidae* increased at the sampling sites downstream of the point source pollution. The first upstream site had abundant pollution-sensitive taxa like the *Trichoptera* and *Zygoptera*. *Mollusca* were found in all the sites in small numbers.

ASPT scores decreased where Sebakwe River meets with Mbembeswane River. The highest ASPT scores were recorded in Mlala Park (MP), with a range of 6.47-9.6 and a mean of  $7.64 \pm 1$  (Figure 5). The lowest ASPT scores were recorded at site AP, immediately the point source pollution with a range of 2.5-5.2 and mean  $3.54 \pm 1.1$  (Figure 5). There were significant differences in ASPT scores among the sites (ANOVA;  $p < 0.05$ , Appendix 23).



**Figure 5: Mean ASPT values for sampling points: MP = site 1, CP = site 2, BP = site 3, AP = site 4, RB = site 5, SB = site 6. \* = outlier.**

#### **4.5 Effect of physicochemical parameters on macroinvertebrate diversity in Sebakwe River**

Multiple regression analysis showed that the measured environmental variables (electrical conductivity, DO, pH and temperature) were not significant in explaining the diversity of macroinvertebrates among the sites ( $F = 2.089$ ;  $p = 0.115$ ) (Appendix 29). The model explained 26.6 % of the variability in macroinvertebrate richness (Regression;  $R^2 = 0.266$ ) (Appendix 30).

## **5.0 CHAPTER FIVE: DISCUSSION**

### **5.1 Seasonal variation of the physicochemical parameters**

The results showed that there was no significant seasonal variation in the environmental variables. The high DO in September and October may be explained by difference in water flow levels. The expected rainfall for the rainy season in the months of November, December and January did not come and this resulted in decreased water levels in Sebakwe River. As a result, the re-aeration due to high water flow that was experienced in September and October depleted in later sampling months resulting in lower DO levels (Muisa *et al.*, 2015). The high conductivity in the month of December can be explained by increased levels of inorganic dissolved solids, chloride, phosphate and nitrate in the sewage effluent (Vadde *et al.*, 2018). A study on the impacts of agricultural runoff on water quality found that conductivity and dissolved solids have high correlation (Tafangenyasha and Dube, 2008). The low conductivity in the months of October and September can therefore be explained by the dilution effects that were occurring due to high water volumes in the river (Tafangenyasha and Dube, 2008).

### **5.2 Spatial variation of physicochemical parameters**

The physicochemical variables between the sampling sites had no significant variation. The slight acidity may have been due to presence of metals from industrial effluent which are not removed by sewage treatment (Tazvivinga *et al.*, 2012). pH values in this study were consistent with those in a study of Mucheke and Shagashe Rivers in Masvingo (Chikodzi *et al.*, 2017). The temperatures did not vary significantly among the sites. There was no significant differences in DO concentration between upstream and downstream sites and this was due to reaeration of the water downstream of the discharge point near site RB where there is a small waterfall. DO levels in the first upstream site, although high, were affected by anthropogenic activities in the area such as fetching water for domestic use, cattle activity and bathing. A Kenyan study on the effects of daily activities on a river found that anthropogenic activities increase turbidity and thus lower DO levels (Mathooko, 2001). Spatial DO levels for upstream and downstream sites therefore had no significant difference because the upstream sites had a variety of anthropogenic activities.

### 5.3 Effect of sewage effluent on macroinvertebrate occurrence and abundance in Sebakwe River

The ASPT, was generally high for the first sampling point upstream of the point source pollution and this indicates good river health (Gratwicke, 1998). Despite having a habitat diversity of 3.5 due to lack of stones biotope and a wide variety of vegetation types in the vegetation biotope, the Mlala Park site scored high ASPT  $> 7$  indicating very good health. Sites upstream of the point source pollution contained diverse assemblages of macroinvertebrate families despite having low habitat diversity. The site just before point source pollution, BP, had more of moderately tolerant taxa like the *Dytiscidae* and *Odonata*. Pollution sensitive taxa like the *Zygoptera* were present in low numbers and this is because of the backflow of effluent as it enters Sebakwe River. Upstream site, MP, had pollution-sensitive taxa from the family of caddisflies namely *Leptoceridae*, *Pisuliidae*, *Sericostomatidae*, *Barbarochthonidae* and mayflies (*Baetidae*, *Heptageniidae* and *Oligoneuridae*) indicating good river health (Dube *et al.*, 2014; Nhiwatiwa and Dalu, 2017).

The sampling point immediately after the point source pollution, site AP, had the least ASPT scores ( $< 3.5$ ) indicating poor water quality of Sebakwe River at this point (Gratwicke, 1998). The ASPT score for site CP, the confluence between Sebakwe and Mbembeswane Rivers was low due to the inflow of water from Mbembeswane River. This suggests that the water quality of Mbembeswane River is of poor quality. The sites downstream of the entry of sewage effluent were dominated by annelids (namely *Oligochaetae* and *Hirudinea*), diptera families (*Simuliidae*, *Culicidae*, *Muscidae*, *Dixidae*) and backswimmers (*Corixidae*, *Gerridae*) and other taxa found in areas of high pollution (Gratwicke, 1998). The backswimmers are found in a variety of biotopes: *Corixidae* along the margins of shallow water, *Gerridae* and *Veliidae* on the water surface of lentic pools and among aquatic vegetation (*Notonectidae*, *Nepidae* and *Naucoridae*) (Dias-silva *et al.*, 2010) and these were available in the sites sampled at Sebakwe River. Sites with high numbers of annelids and chironomids are often organically polluted (Chikodzi *et al.*, 2017). Chironomids can withstand very low oxygen levels and very high pollution levels (Ndebele-Murisa, 2012).

Although, downstream sites RB and SB had moderate ASPT scores of 4.6, it is not an indication of good water quality. This is because the site RB had a habitat scoring of 4 out of 5 because it has all three biotopes (S, V and GSM), in and out of current. Site SB on the hand, has only two biotopes (S and V) and a habitat score of 2.5 out of 5. Site SB has low family richness and therefore has low water quality (Gratwicke, 1998). Due to presence of all three

biotopes at site RB and presence of a waterfall adding oxygen to the water, the site had a high assemblage of families and consequently a high ASPT score. ASPT scores are less affected by poor habitat diversity because the present taxa may have high SASS scores (Bredenhand, 2016). Moderately pollution-tolerant families like the *Odonata* were found in low numbers in the downstream sites with the sensitive families like the *Zygoptera* in extremely low numbers or absent.

Site AP, immediately after point source pollution had an abundance of the water hyacinth plants, *Eichhornia crassipes*, a clear indication of nutrient enrichment (Mapira, 2011). These findings were similar to results from studies done in other Zimbabwean rivers receiving discharges of sewage effluent (Phiri, 2014; Makwara and Tavuyanago, 2012). Other abundant macrophytes were (*Phragmites australis*) and *Azolla pinnata* (red algae). Macrophytes create better habitat conditions through production of DO, providing attachment and oviposition sites and reducing predation pressure from predators and other invertebrates (Dube *et al.*, 2016). This environmental heterogeneity enables the co-occurrence of families with widely differing niche requirements thereby increasing macroinvertebrate abundance (Hussain and Pandit, 2012). This explains abundance of *Hemiptera* at the sites CP, BP, AP and RB. Dark weed, *Lemna* spp., was also present in downstream sites and this is because it was introduced as a way to reduce nutrient levels (Utete and Kunhe, 2013). The health downstream of Sebakwe River particularly at sites AP and SB is very low since pollution-tolerant taxa were also showing low species richness but high evenness (Dube *et al.*, 2014). These results are consistent with findings from a study on Ngamo River whereby *Diptera* families increased downstream as a direct result of sewage spillages (Nhiwatiwa and Dalu, 2017). Resurgence of moderately-sensitive *Coleoptera* and *Mollusca* families in downstream sites was due to the dilution effect and presence of a waterfall just after the first downstream site, AP, (Dube *et al.*, 2014).

Multiple linear regression analysis showed no relationship between physiochemical variables and macroinvertebrate richness in the river. The unmeasured environmental variables namely nutrient pollution levels may be the cause for the diversity of macroinvertebrates in Sebakwe River (Odume *et al.*, 2012). This is consistent with findings by (Nhapi and Tirivarombo, 2004; Chikodzi *et al.*, 2017). With a population increase of 34% (from 1993 to 2012) since the water treatment was upgraded and another population increase from 2012 up to date, the

sewage treatment plant is receiving more effluent volumes than it can treat (Makwara and Tavuyanago, 2012; Stewart Scott Zimbabwe, 1999).

#### **5.4 Conclusion**

Measured environmental variables were not successful in describing the macroinvertebrate diversity. The SASS 5 index was successfully used to assess the aquatic health of Sebakwe River. The index can therefore be used to monitor other Zimbabwean rivers. With an average ASPT score of 7.64 upstream of Sebakwe and an average of 4.23 in the downstream sites, Sebakwe River is healthy upstream of sewage effluent and unhealthy downstream.

#### **5.5 Recommendations**

The overall quality of Sebakwe River downstream of the released sewage effluent is not good therefore the sewage needs to be properly treated. Continuous biomonitoring of the river would help prevent further degradation of the river. Future studies on rivers should include measurements on nutrient variables as these have a great impact on river health. SASS 5 index should be formally adopted and used to regularly assess the health of all rivers in the country. This will help to keep organic pollution levels low. River corridors and the overall riverine structure should be maintained and where necessary improved in order to maintain and/or create river flows that support a diverse assemblage of macroinvertebrate taxa.

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## 4.7 Appendices

### Appendix 1: SPSS output of variation in DO in September

#### Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
September	5	61.5000	5.14976	52.80	65.70
sampling site	6	3.50	1.871	1	6

#### Test Statistics<sup>a,b</sup>

	September
Chi-Square	4.000
df	4
Asymp. Sig.	.406

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

### Appendix 2: SPSS output of variation in DO in October

#### Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
October	6	56.1333	17.15187	35.00	81.00
sampling site	6	3.50	1.871	1	6

#### Test Statistics<sup>a,b</sup>

	October
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

### Appendix 3: SPSS output of variation in DO in November

#### Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
November	6	31.4967	18.39504	3.78	53.00
sampling site	6	3.50	1.871	1	6

#### Test Statistics<sup>a,b</sup>

	November
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

### Appendix 4: SPSS output of variation in DO in December

#### Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
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December	6	62.1000	21.29366	30.00	86.60
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	December
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 5: SPSS output of variation in DO in January**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
January	6	45.9667	12.48578	28.70	66.30
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	January
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 6: SPSS output of variation in conductivity in September**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
September	5	.1800	.07842	.05	.26
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	September
Chi-Square	4.000
df	4
Asymp. Sig.	.406

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 7: SPSS output of variation in conductivity in October**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
October	6	.5450	.33267	.15	.90
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	October
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 8: SPSS output of variation in conductivity in November****Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
November	6	9.6250	8.24280	.75	25.00
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	November
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 9: SPSS output of variation in conductivity in December****Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
December	6	93.0000	102.63333	8.00	270.00
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	December
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 10: SPSS output of variation in conductivity in January****Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
January	6	15.5000	8.04363	3.00	26.00
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	January
Chi-Square	5.000
df	5
Asymp. Sig.	.416

- a. Kruskal Wallis Test  
b. Grouping Variable:  
sampling site

**Appendix 11: SPSS output of variation in pH in September**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
September	5	7.4540	.39659	6.90	8.00
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	September
Chi-Square	4.000
df	4
Asymp. Sig.	.406

- a. Kruskal Wallis Test  
b. Grouping Variable:  
sampling site

**Appendix 12: SPSS output of variation in pH in October**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
October	6	8.0250	.78790	7.20	9.30
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	October
Chi-Square	5.000
df	5
Asymp. Sig.	.416

- a. Kruskal Wallis Test  
b. Grouping Variable:  
sampling site

**Appendix 13: SPSS output of variation in pH in November**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
November	6	6.2233	.61983	5.60	6.98
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	November
Chi-Square	5.000
df	5
Asymp. Sig.	.416

- a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 14: SPSS output of variation in pH in December**

Descriptive Statistics					
	N	Mean	Std. Deviation	Minimum	Maximum
December	6	6.6983	.32177	6.25	6.98
sampling site	6	3.50	1.871	1	6

Test Statistics <sup>a,b</sup>	
	December
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test  
b. Grouping Variable:  
sampling site

Descriptive Statistics					
	N	Mean	Std. Deviation	Minimum	Maximum
January	6	6.0033	.19086	5.76	6.25
sampling site	6	3.50	1.871	1	6

**Appendix 15: SPSS output of variation in pH in January**

Test Statistics <sup>a,b</sup>	
	January
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test  
b. Grouping Variable:  
sampling site

**Appendix 16: SPSS output of variation in temperature in September**

Descriptive Statistics					
	N	Mean	Std. Deviation	Minimum	Maximum
September	6	25.3833	.91305	23.70	26.50
sampling site	6	3.50	1.871	1	6

Test Statistics <sup>a,b</sup>	
	September
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test  
b. Grouping Variable:  
sampling site

**Appendix 17: SPSS output of variation in temperature in October**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
October	6	21.9000	1.06395	20.80	23.40
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	October
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 18: SPSS output of variation in temperature in November**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
november	6	25.0667	.94587	24.20	26.90
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	november
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 19: SPSS output of variation in temperature in December**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
december	6	25.3833	.91305	23.70	26.50
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	december
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 20: SPSS output of variation in temperature in January**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
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January	6	27.2667	1.55005	25.50	29.60
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	January
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 21: SPSS output of spatial variation of physicochemical parameters in September**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
September	5	61.5000	5.14976	52.80	65.70
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	September
Chi-Square	4.000
df	4
Asymp. Sig.	.406

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 22: SPSS output of spatial variation of physicochemical parameters in October**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
October	6	21.9000	1.06395	20.80	23.40
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	October
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test

b. Grouping Variable:  
sampling site

**Appendix 23: SPSS output of spatial variation of physicochemical parameters in November**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
november	6	25.0667	.94587	24.20	26.90
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	november
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Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test  
b. Grouping Variable:  
sampling site

**Appendix 24: SPSS output of spatial variation of physicochemical parameters in December**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
december	6	25.3833	.91305	23.70	26.50
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	december
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test  
b. Grouping Variable:  
sampling site

**Appendix 25: SPSS output of spatial variation of physicochemical parameters in January**

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
January	6	27.2667	1.55005	25.50	29.60
sampling site	6	3.50	1.871	1	6

**Test Statistics<sup>a,b</sup>**

	January
Chi-Square	5.000
df	5
Asymp. Sig.	.416

a. Kruskal Wallis Test  
b. Grouping Variable:  
sampling site

**Appendix 26: SPSS output of species richness among the sites**

**ANOVA**

Species Richness

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35.960	5	7.192	3.841	.012
Within Groups	41.191	22	1.872		
Total	77.151	27			

**Appendix 27: SPSS output of species richness among the sites**

**Multiple Comparisons**

Dependent Variable: Species\_Richness  
Tukey HSD

(I) sampling site	(J) sampling site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound

MP	CP	2.72000	.91791	.068	-.1394	5.5794
	BP	1.99400	.86541	.234	-.7019	4.6899
	AP	3.44000*	.86541	.007	.7441	6.1359
	RB	2.94000*	.86541	.027	.2441	5.6359
	SB	2.09000	.91791	.245	-.7694	4.9494
CP	MP	-2.72000	.91791	.068	-5.5794	.1394
	BP	-.72600	.91791	.966	-3.5854	2.1334
	AP	.72000	.91791	.967	-2.1394	3.5794
	RB	.22000	.91791	1.000	-2.6394	3.0794
	SB	-.63000	.96756	.985	-3.6441	2.3841
BP	MP	-1.99400	.86541	.234	-4.6899	.7019
	CP	.72600	.91791	.966	-2.1334	3.5854
	AP	1.44600	.86541	.564	-1.2499	4.1419
	RB	.94600	.86541	.879	-1.7499	3.6419
	SB	.09600	.91791	1.000	-2.7634	2.9554
AP	MP	-3.44000*	.86541	.007	-6.1359	-.7441
	CP	-.72000	.91791	.967	-3.5794	2.1394
	BP	-1.44600	.86541	.564	-4.1419	1.2499
	RB	-.50000	.86541	.992	-3.1959	2.1959
	SB	-1.35000	.91791	.685	-4.2094	1.5094
RB	MP	-2.94000*	.86541	.027	-5.6359	-.2441
	CP	-.22000	.91791	1.000	-3.0794	2.6394
	BP	-.94600	.86541	.879	-3.6419	1.7499
	AP	.50000	.86541	.992	-2.1959	3.1959
	SB	-.85000	.91791	.935	-3.7094	2.0094
SB	MP	-2.09000	.91791	.245	-4.9494	.7694
	CP	.63000	.96756	.985	-2.3841	3.6441
	BP	-.09600	.91791	1.000	-2.9554	2.7634
	AP	1.35000	.91791	.685	-1.5094	4.2094
	RB	.85000	.91791	.935	-2.0094	3.7094

\*. The mean difference is significant at the 0.05 level.

#### Appendix 28: SPSS output of ASPT differences among the sites

##### ANOVA

ASPT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35.960	5	7.192	3.841	.012
Within Groups	41.191	22	1.872		
Total	77.151	27			

#### Appendix 29: SPSS output of macroinvertebrates among the sites

##### ANOVA<sup>a</sup>

Model	Sum of Squares	df	Mean Square	F	Sig.
1 Regression	.423	4	.106	2.089	.115 <sup>b</sup>
1 Residual	1.163	23	.051		
Total	1.586	27			

a. Dependent Variable: RichnessS2\_log

b. Predictors: (Constant), Conductivity, pH, DO, Temperature

#### Appendix 30: SPSS output of variability in macroinvertebrate richness

##### Model Summary<sup>b</sup>

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics		
					R Square Change	F Change	df1
1	.516 <sup>a</sup>	.266	.139	.22488	.266	2.089	4