# ECOLOGICAL STATUS OF THE SAND RIVER AFTER THE DISCHARGE OF SEWAGE EFFLUENT FROM THE POLOKWANE AND SESHEGO WASTEWATER TREATMENT WORKS

by

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**DECLARATION**

I declare that **ECOLOGICAL STATUS OF THE SAND RIVER AFTER THE DISCHARGE OF SEWAGE EFFLUENT FROM THE POLOKWANE AND SESHEGO WASTEWATER TREATMENT WORKS** is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other institution.

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Seanego K.G (Ms) Date

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## DEDICATION

In memory of my dear aunt Khomotso Seanego

## SAPSE ACCREDITED PAPERS PUBLISHED FROM THESIS

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2. Seanego K.G., Moyo, N.A.G. and Rapatsa, M.M. 2014. The status of heavy metal contamination in the sediment and biota of the Sand River. (Drafted)

**ABSTRACT**

Population growth in urban areas is putting pressure on sewage treatment plants. The improper treatment of sewage entering the aquatic ecosystems causes deterioration of the water quality of the receiving water body. The effect of sewage effluent on the Sand River was assessed. Eight sampling sites were selected, site 1 and 2 were upstream of the of the sewage treatment plant along the urbanised area of Polokwane, whilst sites 3, 4, 5, 6, 7 and 8 were downstream. The physico-chemical parameters and coliform counts in the water samples were determined. Macroinvertebrate abundances and diversity (H') was determined at the different sites during the dry and rainy season. The water quality status of the Sand River with respect to the South African scoring system (SASS) scores and average score per taxon (ASPT) was determined. A linear regression was performed to test the correlation of the SASS scores with abundance and H'. Heavy metal concentrations in water, sediment, grass (*Ishaemum fasciculatum*) and fish (*Oreochromis mossambicus*) at the sites were evaluated. The suitability of the Sand River and surrounding borehole water for irrigation was also determined.

Hierarchical average linkage cluster analysis produced two clusters, grouping two sites above the sewage treatment works and six sites downstream of the sewage effluent discharge point. Principal component analysis (PCA) identified total nitrogen, total phosphorus, conductivity and salinity as the major factors contributing to the variability of the Sand River water quality. These factors are strongly associated with the downstream sites. Canonical correspondence analysis (CCA) indicated that Chironomidae family was found on the nitrogen gradient during the dry season. However during the rainy season, Chironomidae was found in the centre of the ordination which indicated that it was ubiquitous. *Escherichia coli* levels (1463.73 counts/100ml) in the maturation ponds of Polokwane wastewater treatment works could potentially lead to contamination of the Polokwane aquifer. High diversity was recorded at the sites before discharge and the sites further downstream. There was significant correlation ($P<0.05$) between the SASS scores and macroinvertebrate diversity during the dry season ($R^2=0.69$) and the rainy season ($R^2=0.77$). Fish samples had significantly higher ($P<0.05$) iron and copper concentrations, while the

sediment had significantly higher lead concentration (P>0.05). The United States salinity laboratory (USSL) diagram indicated that the sodium hazard (SAR) and alkalinity hazard (conductivity) was low and the Sand River and borehole water was suitable for irrigation. The residual sodium carbonate (RSC) was below 1.24 meq/l, also indicating that both the Sand River and borehole water is still suitable for irrigation. The total phosphorus concentrations fluctuated across the different site. Total nitrogen concentrations showed a gradual decrease downstream from the point of discharge. The coliform levels also showed a gradual decrease downstream. This shows that the river still has a good self-purification capacity.

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# CHAPTER 1: GENERAL INTRODUCTION

Rapid urbanisation is a major problem faced by most developing countries in Southern Africa. Industrial, agricultural and mining activities are important economy drivers in these countries. Rapid urbanisation is associated with increased population growth, which has led to the increase in the consumption of energy and raw materials. This has led to the generation of greater volumes of waste, with some being more complex and potentially hazardous. Most of the waste is deposited into rivers, thus making many of these rivers a repository for waste. The pollution of rivers and aquifers is a growing threat to waters in most parts of Southern Africa (Amadi, 2010; Dan-Hassan *et al*., 2012). The major sources of pollution include domestic and industrial wastewater discharges, mining, surface runoff and agro-chemicals (Murray *et al*., 2005). The contaminants associated with these sources include organic chemicals (pesticides and herbicides), inorganic chemicals (acids, alkalis, salt and metals), nutrients (nitrogen and phosphorus), pathogens (bacteria, viruses and parasites), radioactive materials (uranium, thorium, caesium, iodine and radon), sediment (soil and silt) and solid waste (Moyo and Mtetwa, 2002; Feng *et al*, 2004; Quibell, 2011).

In recent years, there has been an upsurge in water pollution especially in developing countries. Moyo and Mtetwa (2002) identified pollution hot spots in the Southern Africa. These include Lake Chivero in Zimbabwe, Kafue River in Zambia, Hartbeespoort Dam in South Africa and several other urban rivers in Southern Africa. Much emphasis is placed on point source pollution, particularly municipal wastewater. This is the major pollutant that impacts negatively on water quality, with the rapid population growth, overloading the existing sewage plants is inevitable. Most sewage treatment plants in Southern Africa therefore treat large volumes of sewage way beyond their design capacity. The discharge of substandard sewage effluent usually with high levels of nitrogen and phosphorus is usually unavoidable. These two nutrients (nitrogen and phosphorus) are the major drivers of the freshwater aquatic ecosystem and have been a major threat to aquatic ecosystems globally.

The nutrient retention capacity of rivers ensures that the problem of wastewater disposal and diffused pollution does not lead to eutrophication (Bere, 2007). However, due to a river's unidirectional nature, much of the concern lies in their ability to retain and transform these nutrients to usable forms. The retention of these nutrients is a function of the river surface area, river flow, temperature and oxygen. Microorganisms also play a key role in the degradation of organic matter and the conversion of toxic nitrite to nitrate. This capacity of nutrient retention or river self-purification is however, strained by persistent pollution overloads (Mbuligwe and Kaseva, 2005).

The discharge of poorly treated sewage effluent results in algal blooms. The die-off of algae results in increased organic waste causing depletion in oxygen levels by decomposition. This causes a reduction in the diversity of aquatic ecosystem (Gray, 1997). Many rivers in South Africa are already under threat of eutrophication (de Villiers and Thiart, 2007). The rate of the country's development, does not allow sufficient time for improvement of water treatment facilities. As a result, the majority of the sewage treatment plants fall short of the water quality standards stipulated by the Department of Water Affairs and Forestry (Morrison *et al*., 2001; Samie *et al*., 2009; Britz *et al*., 2013).

Nutrient loading of rivers is not the only problem associated with sewage effluent as microbial contamination has also posed threat to the aquatic environment over the years (Fatoki *et al* 2001; Briz *et al*., 2013). Major factors affecting microbiological quality of surface waters are discharges from sewage works and runoff from informal settlements. Many of the microbial pollution incidences in some African countries such as Tanzania have been attributed to the malfunction of sanitation system (Mbuligwe and Kaseva, 2005). In some areas in Tanzania, raw sewage was even discharged (Mbuligwe and Kaseva, 2005). Continuous presence of raw sewage gives greater chances for the flourishment of virulent strains of bacteria and viruses (Cash 1974). Microbial pollutants serve as indicators of water quality. Enteric bacteria, such as coliforms, *Escherichia coli*, and faecal streptococci are used as indicators of faecal contamination in water sources (Akpor and Muchie, 2011; Bordalo *et al*., 2002). Faecal coliforms are used primarily to indicate the presence of bacterial pathogens such as *Salmonella spp*., *Shigella spp*., *Vibrio cholerae*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica* and *Escherichia coli* (Kanu and Achi, 2011). It is now apparent that most rivers in South Africa are heavily polluted with faecal organisms and when these rivers are used for irrigation of food products they will pose a potential threat to human health (Arkermann, 2010). In most cases the faecal coliform count levels exceeded the stipulated South African guidelines (Fatoki *et al*. 2001; Lin *et al.* 2002, Mthembu *et al*. 2012).

Along with sewage effluent, concentrated industrial waste such as brewery effluent and waste food processing companies creates serious disposal problems. The effluent from such industries is characterised by high turbidity, conductivity, increased chemical and biological oxygen demand as well as suspended solids (Fakayode, 2005). The amount and type chemicals (e.g. caustic soda, phosphoric acid, nitric acid etc) used are usually responsible for low pH levels in brewery effluent. In some cases low pH is due to the chemical preservatives such as sulphur dioxide and carbon dioxide which in turn form trioxosulphate and carbonic acid on reaction with water (Fakayode, 2005). Effluents of this type received into communal sewers. They are however seldom treated efficiently by aerobic methods such as activated sludge or biological filtration processes. This is because the effluent requires excessive dilution to make them amenable to aerobic breakdown (Ross, 1989). In most developing African countries industries dispose of their effluent without pre-treatment due to economic and technological constraints (Sweeney, 1993). In South Africa, the division of Water and Technology in collaboration with other municipalities have employed the treatment of winery, brewery, maize processing, abattoir and apple processing waste by anaerobic digestion (Ross, 1989). The outcome has been that there has been lower surplus sludge production. Ikhu-Omoregbe *et al*. (2005) indicated that brewery effluent that was treated together with domestic effluent in Bulawayo municipality (Zimbabwe) impacted negatively on the quality of the discharged effluent because of high chemical oxygen demand, biological demand and suspended solids.

Heavy metals contamination is another problem in aquatic ecosystems. Sewage effluent may contribute to trace amounts of heavy metals. High industrial activity is the major contributor of heavy metals in aquatic ecosystems. The mining sector has been the major contributor of heavy metals in aquatic systems. Cadmium, copper, nickel, lead, iron, manganese and lead are some of the heavy metals found associated effluent discharged by the mining industry (Wepener *et al*., 2011). Zinc corrugated roof tops, petrol filling stations, waste disposal sites, atmospheric deposition, surface runoff from the urbanised area, informal settlements and the weathering of rocks are some of the heavy metal sources (Gibbs and Cartwright, 1982). Heavy metals cannot be degraded, and are therefore continuously accumulated in sediment, plants and aquatic organisms (Ogoyi *et al*. 2011). They may become concentrated in aquatic organisms to levels that affect their physiological state (Hussain *et al*., 2002). Despite some of the metals being essential (Cu, Mn, Zn, Fe) for the growth of many aquatic organisms, high levels of these metals may be toxic. Others such as cadmium, lead, mercury and other nonessential metals may be toxic even at low concentrations.

Metal uptake by organisms is influenced by water temperature, ionic strength, pH, the physiology and life cycle of the aquatic organism (DWAF, 1996). The pH determines the state in which the metal occurs in the water. A decrease in pH decreases the solubility of essential metals such as selenium, while it increases the solubility of others such as aluminium, copper, cadmium, mercury, manganese and iron (DWAF, 1996). In rivers, large proportions of metals are bound to organic and inorganic particulate matter. These are usually deposited at the river bottom, increasing heavy metal concentrations in sediment. Disturbances in the aquatic environment can cause re-suspension and redistribution of heavy metals. The Narri River of Nigeria is a typical example of areas that have experienced heavy metal pollution as a result of the mining activity (Ayenimo *et al*., 2005). Many rivers in South Africa including the Mooi, Diep, Tyume, Buffalo, Plankenburg River have been reported to be contaminated with heavy metals (lead, zinc, copper). The heavy metals found in these rivers were associated with sewage effluent, mining and industrial activity (Awofolu *et al*., 2004; Jackson *et al*., 2009). The Vaal River in the Gauteng area has been described as the hardest working regional river in South Africa (Wepener *et al*., 2011). It is one of the most polluted rivers, with 10 million people residing in the catchment and with 13600 industries. Mining activity in the Vaal River catchment has caused high levels of total dissolved solids and heavy metal concentrations (including zinc, lead, and manganese). These were found to be above the South African standards (Wepener *et al*., 2011).

Salinization is major problem in many semi-arid regions such as Southern Africa. The ecological effect of salinization on aquatic ecosystems involves the decrease in biodiversity and reduced agricultural activity. The salt contained in the effluent accumulates in the roots of plants over time posing harmful impacts on plant and soil health. Wastewater often contains high dissolved amounts of salts from domestic sewage (Morrison *et al*., 2001). Other sources of salts include municipal storm water drains. Sodium chloride and potassium sulphate are some of the salts that can pass through conventional wastewater treatment plants unaltered. High concentration of these salts can lead to increased salinity levels in river water and ultimately affect aquatic life (Fried 1991; Morrison *et al*., 2001).The leaching of these salts can also cause ground water pollution (Hussain *et al*., 2002).

The problem of groundwater contamination is more acute in areas which are densely populated and highly industrialized (Jasmshidzadah and Mirbagheri, 2011). The increase in water demand which is associated with rapid urban development and expansion of agricultural lands has led to the exploitation of groundwater (Jasmshidzadah and Mirbagheri, 2011) where excessive abstraction of water takes place. The risk associated with excessive ground water abstraction is contamination of aquifers (Tredoux *et al*., 2009). This occurs as a result of the dissolution of chemicals. It has been estimated that once groundwater pollution occurs, it remains concealed for years and eventually disperse over wide areas of the aquifer. Some groundwater analyses done in Zimbabwe (Moyo, 2013) and Malawi (Msilimba and Wanda, 2013) have already indicated chloride contamination. There are few aquifers in South Africa including the Atlantis (Cape) and Polokwane aquifer which are recharged by river water containing both storm water runoff and sewage effluent (Murray, 2009). This becomes a potential hazard to ground water quality as the quality of the sewage effluent discharged continues to deteriorate.

The reuse of sewage water in agriculture provides nitrogen, phosphorus and other macronutrients required by crops. These nutrient elements in the treated sewage effluent can increase crop yield and reduce the need for fertilizers (Scott *et al*., 2000). However, irrigation with sewage effluent puts immense strain on natural water resources. The two factors that are taken into consideration when using sewage effluent for farming are; the effect of sewage effluent on chemical, physical and biological soil characteristics and the soil contamination status both biologically and chemically (El-Ashry *et al*., 2011). Soil characteristics and associated soil hydrology is essential for a better prediction of long-term treatment of subsurface effluent disposal systems.

Gradual adverse changes in physical soil functions, e.g., hydraulic conductivity, leaching of nutrients and structural integrity, may not be noticed until sometime after soil degradation occurs (Dawes and Goonetilleke, 2004). Irrigated soils present lower water entry and infiltration rates, and higher values of runoff and soil losses. Furthermore, the organic matter content is increased, while the bulk density of the soil is decreased (Mojiri, 2011).The variation in chemical and physical soil properties show that within 10 years of supplementary irrigation a slight process of sodication and alkalinisation occurs (Mon *et al*., 2007). Irrigation with wastewater decreases soil pH due to the decomposition of organic matter and production of organic acids (Mollahoseini, 2013). The role of wastewater in reducing soil pH can be effective in increasing extractable Zn. Electrical conductivity of soils irrigated with wastewater increased because of higher electrical conductivity of wastewater (Mollahoseini, 2013).

Upgrading of sewage treatment plant is important for the improvement the quality of sewage effluent. One way in which this could be done is to divert effluent received from industries to a separate treatment system. This allows for proper and more efficient treatment of the industrial waste, resulting in the reduced contamination by industrial effluent. In South Africa there have been several attempts to improve the quality of effluent discharged into rivers. The efficiency of sewage treatment plants which were underperforming in Western Cape was improved by upgrading the plants. In some municipalities, membrane technologies have been incorporated in the treatment process (Hendricks and Pool, 2012). In the Mpumalanga Province, wastewater treatment plants have been renovated. They have adopted biological treatment processes which have been effective in reducing the nutrient levels and bacterial pathogens (Samie *et al*., 2009).

In the Limpopo Province, South Africa, the Polokwane and Seshego wastewater treatment works (WWTW) are located in the city of Polokwane. The Polokwane WWTW is a macro size plant which uses the activated sludge treatment process. Biofiltration is used to treat domestic effluent, while upflow anaerobic sludge blanket (UASB) is used for brewery effluent. Upflow anaerobic sludge blanket reactor system is a high rate anaerobic treatment system. Activated sluge is used as a suitable seed sluge for the startup of the USAB reactors as methanogenic bacteria can be cultivated under anaerobic conditions. The advantages of using UASB is that it has a high COD removal efficiency, low energy demand, short retention time and the methane produced can be used as an energy source (Weiland *et al*., 1991). The Seshego WWTW uses the biofiltration process.

The effluent from the Polokwane WWTW is discharged into the Sand River and the Seshego WWTW discharges its effluent into the Blood River. The Sand River flows by the western edge of this town and is joined up north of the city by the Blood River (Figure 3.5). The city of Polokwane like most cities in South Africa is experiencing rapid urbanisation. The sewage treatment plant is operating beyond its designed capacity, subsequently discharging effluent which is substandard into the Sand River (SABMiller plc, GIZ and WWF-UK, 2011). It has been estimated that the population of Polokwane increases annually by 6%, investment in infrastructure and treatment is therefore required to prevent further deterioration of the water quality of the Sand River and possible contamination of the Polokwane aquifer (SABMiller plc, GIZ and WWF-UK, 2011). Other sources that contribute significantly to the deterioration of the Sand River include surface runoff from storm water drains from the city, runoff from rural and the farmland surrounding the river. Suspended solids, coliforms, heavy metal (including zinc, lead and copper) are some of the pollutants associated with surface runoff. The major industrial source of pollution is from brewery effluent.

The treated sewage effluent which is discharged into the Sand River is artificially recharging the aquifer. This aquifer has been operational since the 1970s and it supplies the city with water in times of water shortages (Murray and Tredoux, 2002). Most of the area is covered by alluvium, which extends to about 300m on either side of the Sand River drainage channel reaching depths of up to 25 m. The deposits consist of upper clayey sand, overlying permeable coarse sand and gravel boulder layers near its base. The alluvium is underlined by granite gneiss rocks. The granite geneisis rocks have been exposed to various phases of granitization, with pegmatitic and amphibolitic rocks being common.

The long term impact of the wastewater recharge on the water quality of the Polokwane aquifer is of concern. In recent years the Polokwane WWTW has not been meeting the discharge standards because the maturation ponds have not been well maintained. Elevated nitrate concentrations during certain periods of the year particularly the rainy season have been evident and could pose a health threat to infants (Connelly and Taussig, 1995). The ground water can also be contaminated by bacteria, viruses and parasites that survive band infiltration. Water from the river and from boreholes is used for irrigation purposes and could cause health related issues if contaminated.

This study was aimed at identifying the key water quality stressors that are associated with the effluent discharge into the Sand River and how they impact on aquatic life.

### 1.1 Dissertation layout

The effect of Sewage effluent on the Sand River was assessed. This dissertation has been divided into seven chapters, each addressing how the discharge of sewage effluent has affected the ecology of the Sand River

### Chapter 2

In this chapter, relevant literature on the effect of sewage on physico-chemical and biological characteristics of rivers was reviewed. The impact of the sewage effluent on aquatic macroinvertebrates and their use as biomonitoring tools was also reviewed. The self purification capacity of rivers together with the suitability of borehole and river water for irrigation was also reviewed.

### Chapter 3

In this chapter, the physico-chemical and biological characteristic of the Polokwane Municipality maturation ponds and the Sand River were determined. The water quality parameters that were responsible for the variation in the Sand River water were also established. The self purification capacity of the Sand River with respect to total nitrogen and phosphorus was established.

**Chapter 4**

The effect of sewage effluent on macroinvertebrate abundance and diversity were determined. This was determined by assessing the relationship between the water quality variables and the abundance of macroinvertebrate communities.

**Chapter 5**

The heavy metal contamination in the Sand River was evaluated. The bioaccumulation of the heavy metals in sediment, grass and fish were determined.

**Chapter 6**

The suitability of Sand River water and the surrounding boreholes for irrigation was investigated.

**Chapter 7**

The nutrient status of the Sand River was highlighted. Alternative methods to reduce the nutrient concentrations in the river were recommended.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 The effect of sewage effluent on the physico-chemical and biological characteristics of rivers.

Sewage effluent has been identified as the major point source of pollution in many river ecosystems (Mazrouh and Mahmoud, 2009; Madanire-Moyo and Barson, 2010 Ijeoma and Achi 2011). Sewage effluent that has not been properly treated affects the water quality of the receiving water bodies and has been linked to high conductivity, suspended solid, salts, nitrogen, phosphorus and low dissolved oxygen levels in rivers (Fatoki *et al*. 2001; Morrison *et al*. 2001; Ijeoma and Achi 2011). The Mirongo River in Tanzania has been turned into an open sewer which transports 160 tonnes of BOD, 400 tonnes of COD, 10 tonnes of total phosphorus and 60 tonnes of nitrogen in Lake Victoria every year (Moyo and Mtetwa, 2002). Much of this has been attributed to the Mirongo River receiving sewage from squatter settlements because of the breakdown of pumps and stabilization ponds (Moyo and Mtetwa, 2002). Many South African sewage treatment plants fall short of the stipulated guidelines (Fatoki *et al*., 2003; Odjadjare and Okoh, 2010). The quality of the Keiskamma River in the Estern Cape has been well documented. Morrison *et al.* (2001) reported on the impact of Keiskammahoek sewage treatment plant on the Keiskamma River water quality Fatoki *et al*. (2003) evaluated the physicochemical quality of Keiskamma River in Eastern Cape (South Africa) and found that the level of electrical conductivity, nitrate, orthophosphate and oxygen-demanding substance were above the South Africa guideline values. Igbinosa and Okoh (2009) also found elevated conductivity, nitrate and orthophosphate in the Keiskamma River. In the Umhlathuze River (Estern Cape), high sulphide, nitrate and ammonia concentrations have been linked to sewage effluent discharges (Mthembu *et al*., 2012).

Inorganic and organic nutrients in rivers are further increased by industrial effluents that are received by municipal sewers or disposed off into rivers. Ross (1989) found that in South Africa the COD concentration in industrial effluent from brewery and various food manufacturing companies vary from 2 to 200 g/l as compared to domestic sewage effluent with a COD value of less than 1 g/l. The effect of brewery effluent on river has been studied extensively in Nigeria. Fakayonde (2005) found high levels of turbidity, conductivity, COD, suspended solids and BOD in the Alaro River of Nigeria. High levels of suspended solids and BOD in the Omoku Creek Rivers were also recorded (Etim and Onianwa, 2013). In some cases the effluent has been reported to be affecting the self-purification capacities of these rivers (Adeogun *et al*, 2011). In certain instances, microorganisms such as *Echerichia coli*, *Lactobacillus species* and *Bacillus species* have been isolated from brewery effluent (Ibekwe *et al*., 2004). Similar cases of microorganisms found in brewery effluent have also been reported in Botswana (Emongor *et al*., 2005). In South Africa however, despite the several breweries located in the country few investigation have been undertaken to evaluate the effect of the effluent on the aquatic environment.

Eutrophication resulting from nutrient enrichment has been regarded as the most serious threat to aquatic systems (Pieterse *et al*., 2003; de Villiers and Thiart, 2007). The major nutrients of concern are nitrogen and phosphorus. The major sources of phosphorus in domestic effluent are human excreta and detergents. Additional levels of phosphorus are brought into the aquatic ecosystem by industrial wastewater generated from feedlots, meat processing, milk processing and commercial laundries (Wiechers and Heynike, 1986). Orthophosphate is the most usable form of phosphate assimilated by algae and aquatic plants. Incidences of algal blooms therefore occur because the conventional activated sludge and biological filter wastewater treatment processes do not sufficiently remove phosphorus but increase the orthophosphate percent from 50 to 90 percent. This is further exacerbated by the overloading of many of the sewage treatment plants. The contribution of detergent phosphate to the total phosphorus load on sewage works in South Africa was documented in the early 80s to be between 35 to 50 percent (Wiechers and Heynike, 1986).

There has been a substantial increase in nutrient enrichment in South African rivers in the recent years. The status of nutrient of 20 of the largest river catchment in South Africa based on dissolved inorganic nitrogen and phosphates have been evaluated by de Villiers and Thiart (2007). These include some of the most polluted river catchments, the Vaal, Oliphants, Berg and Keiskama. These authors found that the dissolved phosphorus at most of the rivers, exceed the recommended water quality guidelines for aquatic life. These were attributed to sewage effluent discharges and informal settlements. The Vaal River is situated in the mining and industrial heartland of South Africa. It receives treated wastewater from the largest metropolitan area in South Africa. Incidences of mass fish kills have been reported due to low dissolved oxygen (Wepener *et al*., 2011). These low oxygen levels were attributed to increased algal growth in response to increased nutrient loads. Momba *et al*. (2006) found eutrophic conditions in the Tyme and Kate River of the Eastern Cape Province (South Africa). The orthophosphate and total nitrogen levels in ranged between 3.7 to 11.5 and 2.9 to 6.9 mg/l respectively. In Zambia for instance, inadequate sewage treatment and sanitation has led to widespread eutrophication of water bodies near towns and cities. An estimated 20 percent of sewage collected in many Zambian towns by sewage treatment plants is not adequately treated. The remaining 80 percent is lost in storm drains because of leakages and blockages (Moyo and Mtetwa, 2002).

Seasonal changes can sometimes play a role in the levels of nutrients found in a river. Point source pollution provides a relatively constant input throughout the year. de Villiers and Thiart (2005), have identified areas with dominant point sources for elevated dissolved nitrate and nitrite concentrations (>400 µg N/l) in South Africa. These include the Phongola, Komati, Lower Orange, Limpopo, Harts and Upper Orange rivers. Seasonal variations of nutrient levels occur with higher levels nutrients sometimes occurring during the rainy season because of seepage of effluent and surface runoff from agricultural fields (Odjadjare and Okoh, 2010). Nutrient concentrations can however decrease due to dilution. de Villiers and Thiart (2007) also indicated that areas such as Berg, Breede, Tugela, Wigle, Upper Vaal and Swartkop rivers as typical examples of areas with seasonal nutrient profiles consistent with agricultural activity. Odjadjare and Okoh (2010) reported elevated nitrate levels and high turbidity in the Eastern Cape during summer when the surface runoff was high. In some cases no seasonal variation in water quality parameters occurs. Johnes (1996) found less seasonal variation of phosphorus and nitrogen concentrations in the Windrush (UK).

The Seshego and Polokwane Sewage treatment plants are the major sources of pollution of the Sand River. The surrounding informal settlements also contribute to further deterioration of the river water quality. However, the contribution of the sewage treatment plant and the informal settlements towards the contamination of the Sand River has not been investigated.

**2.2 Effect of sewage effluent on microbial load**

Many South African rivers are contaminated with faecal coliforms. The faecal coliform counts in these rivers exceed the South African guidelines (Fatoki *et al*. 2001; Lin *et al.* 2002, Mthembu *et al*. 2012). Faecal contamination in water resources is highly related to disposal of inadequately treated sewage effluent (Fatoki *et al*., 2001; Germs *et al*., 2004). These coliforms are indicative of pathogenic bacteria and viruses. Enteric bacteria, such as coliforms, *Escherichia coli*, and faecal streptococci are used as indicators of faecal contamination in water sources (Akpor and Muchie 2011, Bordalo *et al*. 2002). Faecal coliforms are used primarily to indicate the presence of bacterial pathogens such as *Salmonella spp*., *Shigella spp*., *Vibrio cholerae*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica* and *Escherichia coli*.

Several studies in South Africa have been undertaken to determine the status of the rivers with respect to microbial contamination. Nkokonbe Municipality in the Eastern Cape (South Africa) was found to discharge effluent which was not in compliance with the limit of effluent discharge (Momba *et al*, 2006). These authors found that the levels of *Salmonella*, *Shingella*, *Vibrio cholera* and coliphages were high. These pathogens have been known to cause severe diarrhoea in children and adults, leading to mortality. The outbreaks of *Shigella dysenteriae* and *Vibrio cholera* is the major cause of many fatalities (Momba *et al*, 2006). Similar outbreaks of diarrhoea have also been reported in the Mpumalanga Province where the water source was contaminated with faecal matter (Germs *et al.*, 2004). The Plankenburg and Eerste River in the Eastern Cape (South Africa) have been reported to be contaminated with faecal coliforms (Paulse *et al*., 2009; Britz *et al*., 2013). The bacterial pathogens that have been isolated particularly in the Plankenburg River include *E. coli*, *Salmonella*, *Staphylococcus* and *Listeria*. This is a serious concern because in certain rivers such the Palmiet and Umgeni River resistant strains of *E.coli* already exist (Olaniran *et al*., 2009).

Many of the river water in South Africa are a potential threat to human health because they are used for crop irrigation (Arkermann, 2010). The transfer of river bound microorganisms via irrigation water to fresh produce can lead to vegetable contamination (Fatoki *et al*., 2001). Several foodborne disease outbreaks have been attributed to the consumption of vegetables irrigated with contaminated water from sewage treatment works. Islam *et al*. (2005) reported contamination of enterotoxigenic *Escherichia coli*, *Shigella spp*. and *hepatitis A.* in carrots and, to a lesser extent, onions irrigated with sewage water in the USA. These authors reported *E. coli* O157:H7 (enterohaemorrhagic strain) levels far greater than what would likely be found on an agricultural field. The concern is that, under natural conditions, a low infective dose could present a human health hazard (Islam *et al*. 2005). Gemmel and Schmidt (2010), reported that the microbial load of Baynespruit River (KZN, South Africa) in water was higher than the values recommended by WHO and South African guidelines for safe use of wastewater. Both irrigation water and vegetable samples showed the presence of faecal coliforms and *E. coli*. Treatment of the water prior to irrigation is very crucial as the pathogens present in raw wastewater can survive extended periods of time in the soil and crops. The pathogens are able to survive harvesting and subsequently processing (Gemmel and Schmidt, 2010).

The Sand River flow is maintained by the constant discharge of effluent from the Polokwane sewage treatment plant. This effluent further recharges the Polokwane aquifer and the continuous discharge of substandard effluent into the river could eventually lead to contamination of the aquifer. Farmers downstream utilize this water for irrigation purposes. It is therefore important to determine the faecal coliform level in the Sand River and the presence of *E. coli* in vegetables irrigated with this water. There are relatively few studies that have been done on the carry-over loads from irrigation water to plants.

**2.3 Effect of sewage effluent on heavy metals**

Generally, lower concentrations of heavy metals occur in African aquatic systems compared to other areas of the world (FAO, 1994). The concentrations in inland and coastal environments on a continental level, in the four geographical areas Northern, Western, Eastern and Southern Africa have similar low levels (FAO, 1994). Generally low levels of heavy metals exist in surface water as a result of the geological background of the area (de Villiers and Mkwelo, 2009). The major sources associated with heavy metal pollution include the mining industry, petrol filling stations and metal smelters, agricultural sectors and wastewater treatment plants. Contamination by heavy metals may severely influence organisms and soil activities.

The mining of heavy metals in South Africa, especially in Pretoria and Johannesburg has been taking place for several years (Gzik *et al*., 2003). The extraction of mineral resources such as gold, platinum, chromium, nickel, copper, molybdenum resulted in immense pollution of the environment. High concentrations of heavy metals were found in soils of the Rustenburg area (North-west of Pretoria) that is well known for its important mining industries (Gzik *et al*., 2003). Contamination of the Vaal River has been reported as a result of these mining activities (Wepener *et al*., 2011). The Olifants River in South Africa has been described as one of the most polluted rivers in South Africa due to anthropogenic activities including mining industry, petrol filling stations and metal smelters, agricultural sectors and wastewater treatment plants (Grobler *et al*., 1994). Although the river is affected by a number of variables, the most prominent are heavy metals. The metals that have been recorded at toxic levels included aluminium, iron and manganese. Several crocodile and fish mortalities have been recorded in this river as a result (de Villiers and Mkwelo, 2009). The Tyme River in the Eastern Cape (South Africa) has been reported to have elevated cadmium and lead (Awofolu *et al*., 2004). However not all rivers in South Africa are experiencing heavy metals concentrations that may be toxic to aquatic organisms. In less urbanised areas such as Thohoyandou, certain rivers such as the Dzindi, Madanzhe and Mvudi, lead, zinc and copper concentrations were reported t be in a non-liable fractions where no serious damage to aquatic organism can occur (Okonkwo and Mothiba, 2005).

Sediment has been found to be a major depository of metals in some cases, holding more than 99 percent of total amount of a metal present in the aquatic system (Odiete, 1999). Accumulation of heavy metals in sediment is associated with the sedimentation of suspended solids carried by the effluent discharge (Milenkovic *et al*., 2005). FAO (1994), reported elevated levels of heavy metals in the sediments of the Papenkuil River in South Africa, the Niger Delta in Nigeria, Nile River in Egypt and the Mcllwaine Lake in Zimbabwe. Heavy metal concentrations in sediments of the River Ngada in Nigeria were above the WHO limits (Akan *et al*., 2010). The concentrations of heavy metals increased with sediment depth indicating long term accumulation. The levels of heavy metals reported by Van Vuuren *et al*. (1994) in the Olifants River were not in compliance with the recommended South African guidelines. Higher levels of metals were detected in sediment than in water due to the absorption of metals on sediment particles. The metal concentrations followed the order Fe>Mn>Cr>Ni>Zn>Sr>Pb>Cu (Van Vuuren *et al*., 1994).

Sediments serve as a growth media for aquatic plants. The plants accumulate the metals directly from the sediments and surrounding water. Ying and Raitt (2005), reported high concentrations of Cd, Cr, Ni, Zn and P in *Typha capensis,* this is a perennial aquatic weed which is widely distributed across the Bottlelary River in the Western Cape (South Africa). The Cu, Zn and Cd were above the permissible limits. Ayeni *et al*. (2012) reported heavy metal accumulation in the roots and shoots of *Phragmites australis,* which is the most widely distributed plant across the river banks of the Diep River, Cape Town (South Africa). The abundance of heavy metal were in the order Al>Pb>Cd>Co>Ni>Cr for macronutrients and Fe>Mn>Zn>Cu for micronutrients.

The increase in heavy metal contamination is a concern as these metals have the ability to bioaccumulate in the tissues of various biota and may affect the distribution and density of benthic organisms, as well as the diversity in faunal communities (Milenkovic *et al*., 2005). Wepener *et al*. (2011) report disturbances in fish community structure of the Vaal River (South Africa) that was related to exposure to copper and nickel. Several studies have indicated that fish accumulate heavy metals to concentrations much higher than their surrounding environment (Yehia and Sebae, 2011; Babatunde *et al*., 2012). Several endemic fish species have become threatened, leading to the depletion of fish and reduction in their nutritive value (Babatunde *et al*., 2012). The fish species *Clarias gariepinus* and *Oreochromis mossambicus* are widely distributed across South Africa. Heavy metal accumulation of the *Clarias gariepinus* has been extensively studied in southern Africa (Crafford and Avenant-Oldewage, 2010; Babatunde *et al*., 2012). Crafford and Avenant-Oldewage (2010), have reported heavy metal accumulation in *Clarias gariepinus.* These authors have indicated that accumulation is dependent on particular organs.

High concentration of strontium, aluminium, lead and nickel were found in the gills followed by the muscle and liver. Few studies have been done to investigate the accumulation of heavy metals by *O. mossambicus* despite its wide distribution, culture and consumption by rural communities across South Africa.

The city of Polokwane generally does not have major industries that pose a potential threat in terms of heavy metal contamination. However, heavy metal sources such as the Polokwane smelter, petrol filling stations and agricultural farms and the Polokwane WWTW may pose a potential threat to the Sand River. The neighbouring communities of the Sand River, particularly those of poor backgrounds rely on the fish as a food source. The increase in population and socio-economic activities increases the need to identify the sources and quantify the heavy metals concentrations of the Sand River, as they could be harmful to fish and humans.

## 2.4 Effect of sewage effluent on macroinvertebrates

Macroinvertebrate communities are greatly affected by episodic perturbation such as droughts, floods and spills of untreated wastewater (Canobbio *et al*., 2009). Changes in the presence, absence, numbers, morphology, physiology or behaviour of these organisms indicate that the physical and chemical conditions are outside their preferred limits (Mandaville, 2002).

Macroinvertebrate families vary in their sensitivity to pollution. Their relative abundance is used to understand the nature, load and severity of contamination (Wenn, 2008). Macroinvertebrates of the order, Plecoptera, Ephemeroptera and Trichoptera are considered sensitive to environmental stress (Wenn, 2008; Canobbio *et al*., 2009; Dube *et al*., 2010). Although the Ephemeropterans are considered pollution sensitive, the Baetidae family is known to dominate in poor environmental conditions (Wenn, 2008). Some families such as those Hydrophysidae from the order Trichoptera are affected to a lesser extent. Macroinvertebrate of the family Chironomidae and Tubrificidae are considered to be tolerant to organic pollution (Wenn, 2008).

Macroinvertebrates have been used for the rapid assessment of stream and river water quality (Mandaville, 2002). The Empirical Biotic index is the earliest form of river water quality assessment tool in South Africa using macroinvertebrates as bioindicators (Chutter, 1972). This method was carried out on stones in current. The macroinvertebrate were allocated a score depending on their sensitivity towards pollution. The scores ranged from 0 to 10, with 10 being a score of highly sensitive species and 1 being highly tolerant. Chutter (1972) indicated that this method had certain limitations as the presence of macroinvertebrates is not only affected by pollution, but rather by the habitation, flow of river and flooding of rivers. It was through these observations that Chutter (1994) modified the Biological Monitoring Working Party (BMWP) scoring system to the South African Scoring System (SASS) which is now at its fifth version. It includes three biotopes namely; sand in current, rock in current and vegetation in current.

The development of the SASS has resulted in a number of studies being carried out in South African rivers. Vos *et al*. (2002) reported that the biotic indices used in association with the SASS4 scores; average score per taxon (ASPT), total score and number of taxon reflect changes in the community structure. These authors further reported that the qualitative family level data provided an adequate classification of sites for biomonitoring. In a study on the Louren River, South Africa, the SASS5 was effective in demonstrating water quality deterioration downstream of the pollution affected sites. Tafangeyasha and Dube (2008) report that the SASS indices provides a better measurement of water quality since they integrated seasonal changes in rivers, whereas chemical analysis only reflect the condition of the river water at the time which the sample are taken. Williams *et al*. (2004) reported that sewage discharged into the Umbilo River in Durban, South Africa adversely affected the macroinvertebrate community.

There are however certain limitations to SASS. Ndebele-Murisa (2012) reported that SASS cannot be extensively applied at regions with lower numbers of macroinvertebrates. This author further indicated that this limitation occurs because the SASS works best when a varied number of organism scores are calculated. The SASS cannot distinguish between different kinds of pollutants and is largely dependent on habitat availability (de la Rey *et al*., 2008). Therefore, one of the objectives of the present study will be to determine the effect of sewage effluent on macroinvertebrate in the Sand River. The factors that influence the SASS scores reported in this study will also be identified.

### 2.5 Effect of sewage effluent on the self-purification capacity of rivers

There is a general trend of increasing self-purification capacity as the distance travelled by the nutrient load increases (Abdel-Satar, 2005). Continuous loading of rivers by sewage effluent hinders the self-purification capacity of rivers. There is a subsequent reduction in river flow after it has received sewage effluent. This favours nutrient enrichment and eutrophication (Longe and Omole, 2008). The Msimbazi River of Tanzania has high organic and nutrient concentrations, low dissolved oxygen and high coliform counts (Mbuligwe and Kaseva, 2005). This river has been regarded as polluted by these authors and it was further concluded that the river cannot cleanse itself adequately endangering aquatic life. Ndebele-Murisa (2012) reported that the self-purification of the Mukuvisi River was efficient as the water quality improved further downstream of the sewage discharge point. Longe and Omole (2008) reported that the reaeration of the River Illo was slow and full recovery from pollution was difficult. Bere (2007) found that the nutrients from the sewage effluent plant in the Chinyika River, Zimbabwe, were retained over a distance of 4 km from the point of discharge. Many of these authors have reported on the self-purification of these rivers, however, no measures have been taken to quantify the self-purification of the rivers with respect to different nutrients. The self-purification of the Nile River allows for sufficient dilution and degradation of the pollutants that are discharged (Abdel-Satar, 2005). The ability of the Sand River to sustain life is largely dependent on its self-purification capacity. Knowledge of status of the Sand River self purification capacity is therefore important.

### 2.6 Effect of sewage effluent on ground water and the suitability of water for irrigation

The discharge of sewage effluent does not only cause adverse effects to surface water but to groundwater as well. Artificial recharge is not a new concept in Southern Africa. The Windhoek, Atlantis Omdel, Karkams, Calvinia and Polokwane aquifers have been operational for over 15 years (Murray *et al*., 2005). The Windhoek and Atlantis aquifers are already showing evidence of contamination

(Murray *et al*., 2005). In the Western Cape (South Africa), there was evidence of pollution in the Cape Flats aquifer. Pollution with respect to elevated levels of ammonium, nitrite, potassium, alkalinity and chemical oxygen demand has been evident (Adelana and Xu, 2006). In the North West Province (South Africa), high nitrate concentrations in groundwater were ascribed to sewage effluent and sewage sludge disposal on land overlying aquifers. Several studies have been done in Botswana with nitrate concentrations being the variable of concern reaching levels of up to 200 mg/l (Vogel *et al*., 2006). Nitrate pollution was evident in most of the boreholes in Gaborone that aligned the Nsthe and Tati River. The water in these boreholes was declared unfit for human consumption. Nitrate in groundwater is of major concern because the exposure to high concentration of nitrate causes methaemogobin in infants (Fewtrell, 2004). The bacterial pathogens that contaminate groundwater pose a health risk to consumers. In 2005 and 2006 there were disease outbreaks in Delmas, South Africa, associated with ground water contamination (CSIR, 2010). Msilimba and Wanda (2013) found that the major sources of chemical and microbial contamination in Malawi wells are rock interactions and surface pollution, respectively.

Informal settlements have also contributed to ground water pollution. In a rural settlement near Makhado in the Limpopo Province (South Africa) nitrate levels in several boreholes were above the recommended levels for drinking purposes (Connelly and Taussig, 1995). In Kisauni, Nigeria, a high degree of groundwater contamination by microbial contaminants was experienced especially in the high density settlement. This contamination was attributed to on-site sanitation. The contamination levels were more severe during the rainy season when aquifer recharge was high (Mombase *et al*., 2006).

The city of Polokwane and the Seshego Township are dependent on surface water resources. The Polokwane aquifer is one of the major water storage sources in Polokwane. Recharge of the aquifer is achieved by the discharge of treated wastewater from the Polokwane WWTW into the Sand River. This water then infiltrates the sandy and gneissic aquifer. The Polokwane WWTW is now discharging effluent that is substandard and it is therefore prudent to assess the quality of this water for drinking purposes.

Dissolved solids concentration from sewage effluents are one of the factors that threaten aquatic systems by increasing the salinity of the receiving water. Effluents from urban and industrial areas are some of the factors responsible for the increase in the concentration of total dissolved solids in the Hartz River, South Africa (Allanson *et al*., 1990). Sewage effluent discharge into the Crocodile River (South Africa) resulted in chloride and sulphide increase of up to 178 and 151 % respectively (Marshall and Maes, 1994). The total dissolved solids in the Vaal River have been found to have increased from 500 mg/l to 700 mg/l from 1951 to 1964 (Marshall and Maes, 1994). Similarly in Lake Chivero, chloride levels have risen from 7 mg/l in the 1950s to 37 mg/l in 1991 because of sewage effluent discharge. Marshall and Maes (1994) indicated that salinity may not as yet limit fish production in southern Africa. However, it is a potential problem which is likely to increase particularly around urban areas.

Long term wastewater irrigation increases salts, organic matter and nutrients in soil. This increases soil salinity which is a major concern particularly in semi-arid and arid regions (Areola *et al*, 2011). The use of water that is polluted with domestic effluent for crop irrigation may result in high nitrate and chloride levels (Moyo, 2013). The sodium hazard to soils is important when evaluating irrigation water quality. The sodium hazard in the Notwane River in Gaborone was measured using the sodium adsorption ratio (SAR). The sodium content was found to be within the recommended limits for irrigation with the SAR ranging from 1-9. The sodium did not result in any toxic effects (Emongor *et al*., 2005). It is crucial to determine the salinity of water prior to irrigation because excess salinity reduces plants osmotic activity preventing the absorption of water and nutrients from the soil (Ratnam, 1996).

The Sand River is surrounded by several farms downstream. Many of the farmers utilize the water directly from the river. This not only poses a threat in terms of contamination of crops but could result in salinization related problems.

The paucity of information on a river that is important in the livelihoods of many people in Polokwane prompted this study. The general objective of this study was to determine the effect of sewage treatment works on the water quality, flora and fauna of the Sand River.

## CHAPTER 3: IMPACT OF THE SEWAGE EFFLUENT ON PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF THE SAND RIVER

### 3.1 INTRODUCTION

Polokwane is one of the fastest growing towns in South Africa. Many new residential and administrative buildings have been constructed. These new developments have inevitably led to an increase in domestic effluent. The Sand River flows by the western edge of the city and it is the main repository of domestic effluent and storm water (Murray *et al*., 2005). Despite the increase in population, the Polokwane municipality has not made any concomitant increases to the design capacity of the sewage treatment works.

Part of the Sand River is perennial because of the storm water and the sewage effluent that flows into it. The lower section of the river is ephemeral because of the seasonal nature of rainfall in South Africa. The Polokwane sewage treatment plant is authorised to discharge 25 462 $m^3$ per day of effluent, of which 14 000 $m^3$ per day is diverted to the Mogalakwena Platinum Mine. A minimum of 2 062 $m^3$ is further utilized by the municipality and 9 400 $m^3$ of effluent per day is being discharged into the river. In the western part of Polokwane there is an aquifer that is regularly recharged by 3-4 million $m^3$p/a of the 6 million $m^3$ p/a discharged by the wastewater treatment works (Murray and Tredoux, 2002). A major tomato producing company (ZZ2) and a few farmers use the Sand River water to irrigate their crops (Figure 3.1). Some communities who live near the river undertake recreation and subsistence fishing (Figure 3.2).

Polokwane is a water scarce area and in order to conserve water, artificial recharge of the local Polokwane aquifer using treated wastewater is practised. The treated wastewater is discharged into the alluvial and gneissic aquifer. Before discharging into the gneissic aquifer, the municipal wastewater goes through both primary and secondary treatment and has a retention time of three weeks in a series of maturation ponds. Poor maintenance of the maturation ponds in the last few years may lead to poor effluent being discharged into the aquifer. It is therefore prudent that the quality of the municipal waste be determined before discharge.

Despite the importance of the Sand River, no work has been done on the effect of discharging sewage effluent into it. The aim in this chapter is to provide baseline information on a river that plays a critical role in the lives of the surrounding communities.

**Figure 3.1**: Withdrawal of water for irrigation by farmers downstream of the Polokwane WWTW.

**Figure 3.2**: Rod and line fishing by residents in and around Polokwane at the Sand River at site 3, after sewage effluentdischarge.

## 3.2 OBJECTIVES

The objectives of this chapter are to determine;

I. The effect of sewage effluent on the physico-chemical parameters of the Sand River.

II. The microbial characteristics of the Sand River after the discharge of sewage effluent.

## 3.3 NULL HYPOTHESIS

I. Sewage effluent has no effect on the physico-chemical parameters of the Sand River.

II. Sewage effluent has no effect on the microbial characteristics of the Sand River.

## 3.4 MATERIALS AND METHODS

### 3.4. 1 Description of study area

The Sand River sub-catchment is a major tributary of the Sabie River Catchment and a right hand tributary of the Limpopo River (Figure 3.3). The Sand River has its source south of Mokopane and flows northwards across central Limpopo Province. The river flows by the western edge of the city of Polokwane (Figure 3.4). The Polokwane Pasveer Activated Sludge Wastewater treatment works (WWTW) discharges its effluent into the Sand River. The Blood River joins the Sand River from the left just north of Polokwane (Figure 3.3). Seshego Township has a population in excess of more than 83,863 people (Limpopo census, 2011). The Seshego WWTW discharges its effluent into the Blood River. The Sand River is surrounded by farmland and rangeland further downstream. Eight sampling sites were selected. Site 1 and 2 are upstream of the Polokwane WWTW, while Site 3, 4, 5, 6, 7 and 8 are downstream (Figure 3.5). Site descriptions are indicated in Table 3.1. Several boreholes are located downstream of the Polokwane WWTW (Figure 3.6).

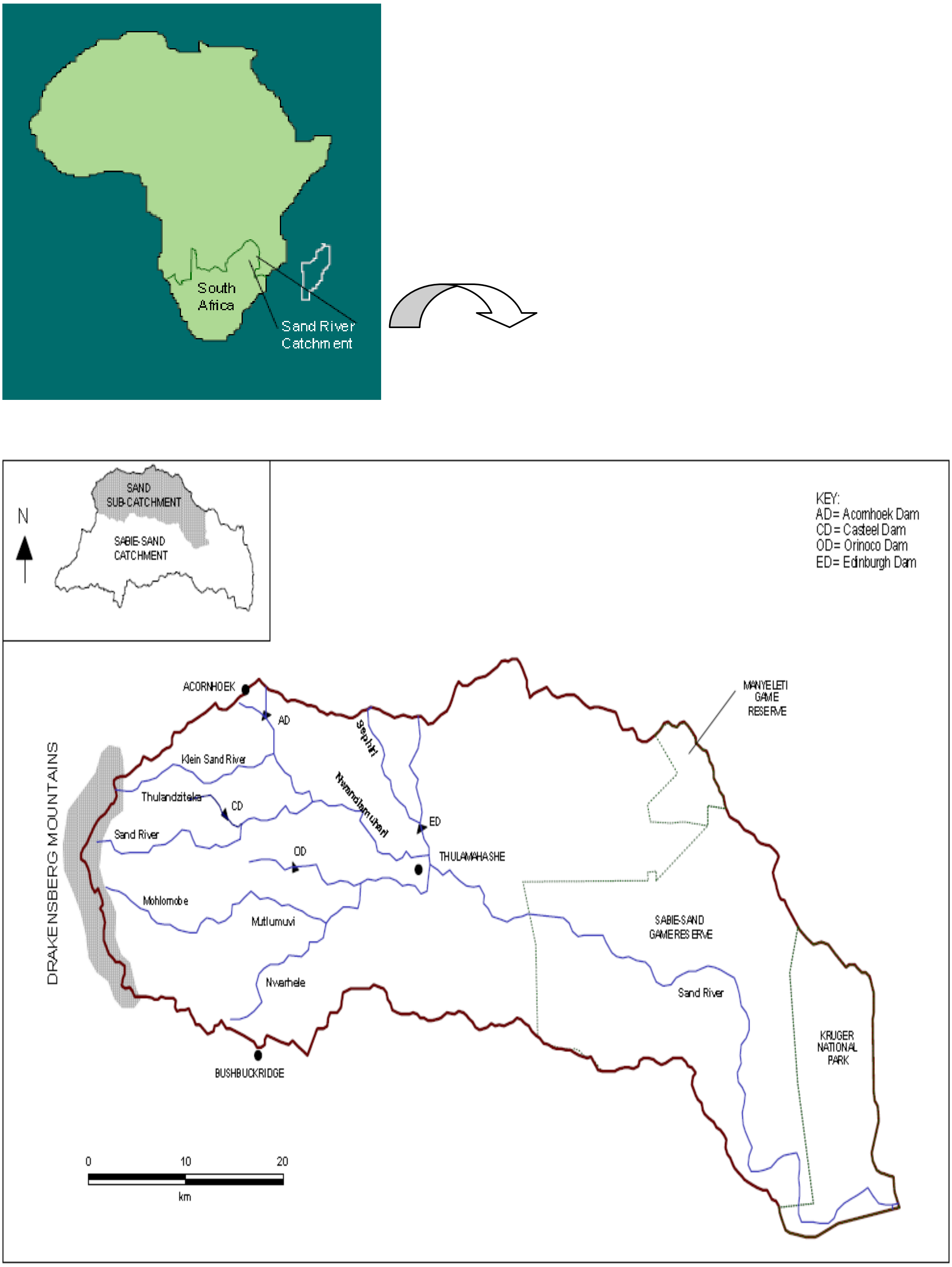


**Figure 3.3**: The Sand River sub-catchment indication catchment boundaries, major rivers, existing dams and game reserve boundaries (Pollard and Walker, 2000).

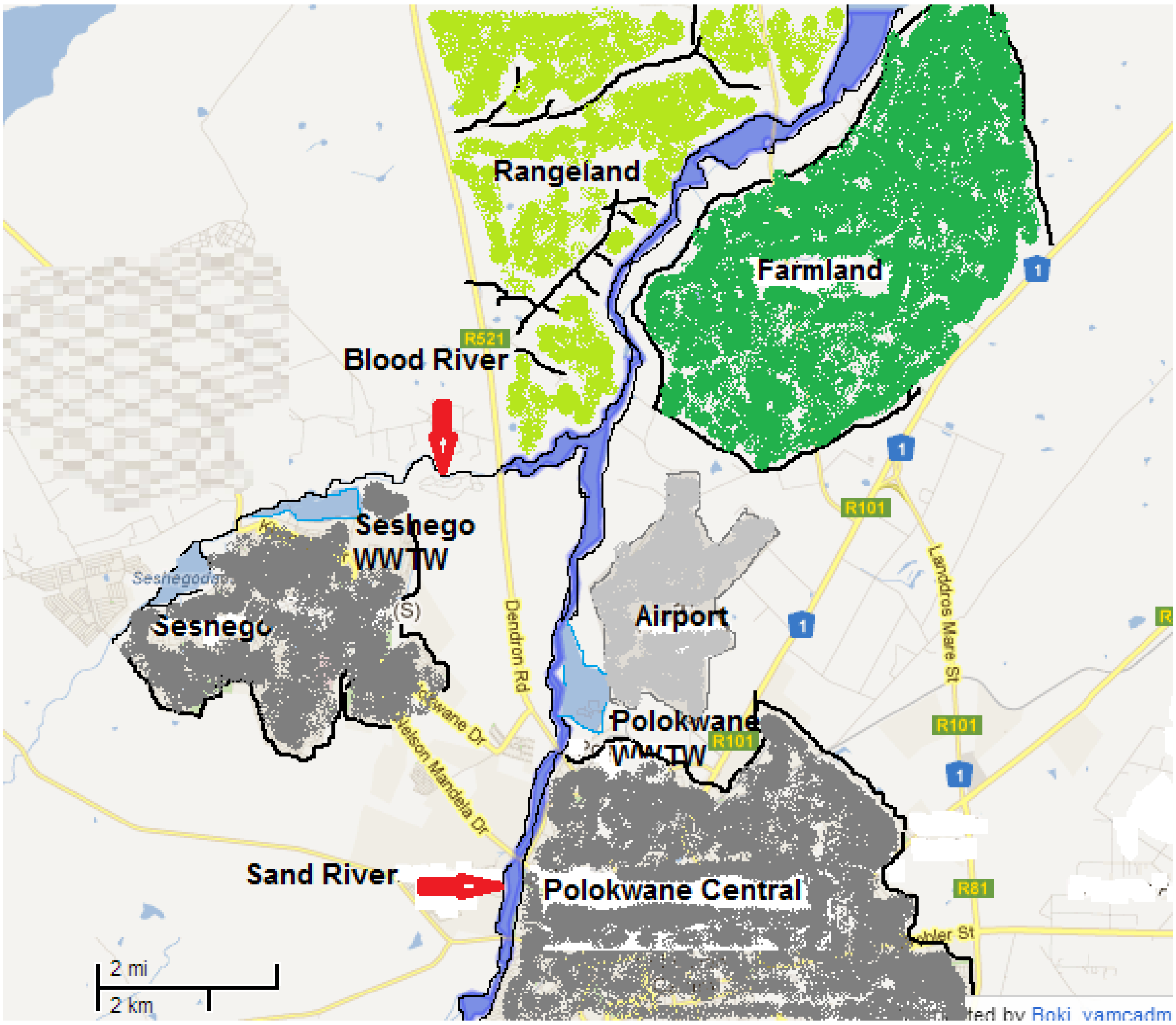


**Figure 3.4**: Map of the Sand River and the surrounding areas (http/www.gpsvisualizer.com/map?output_google).

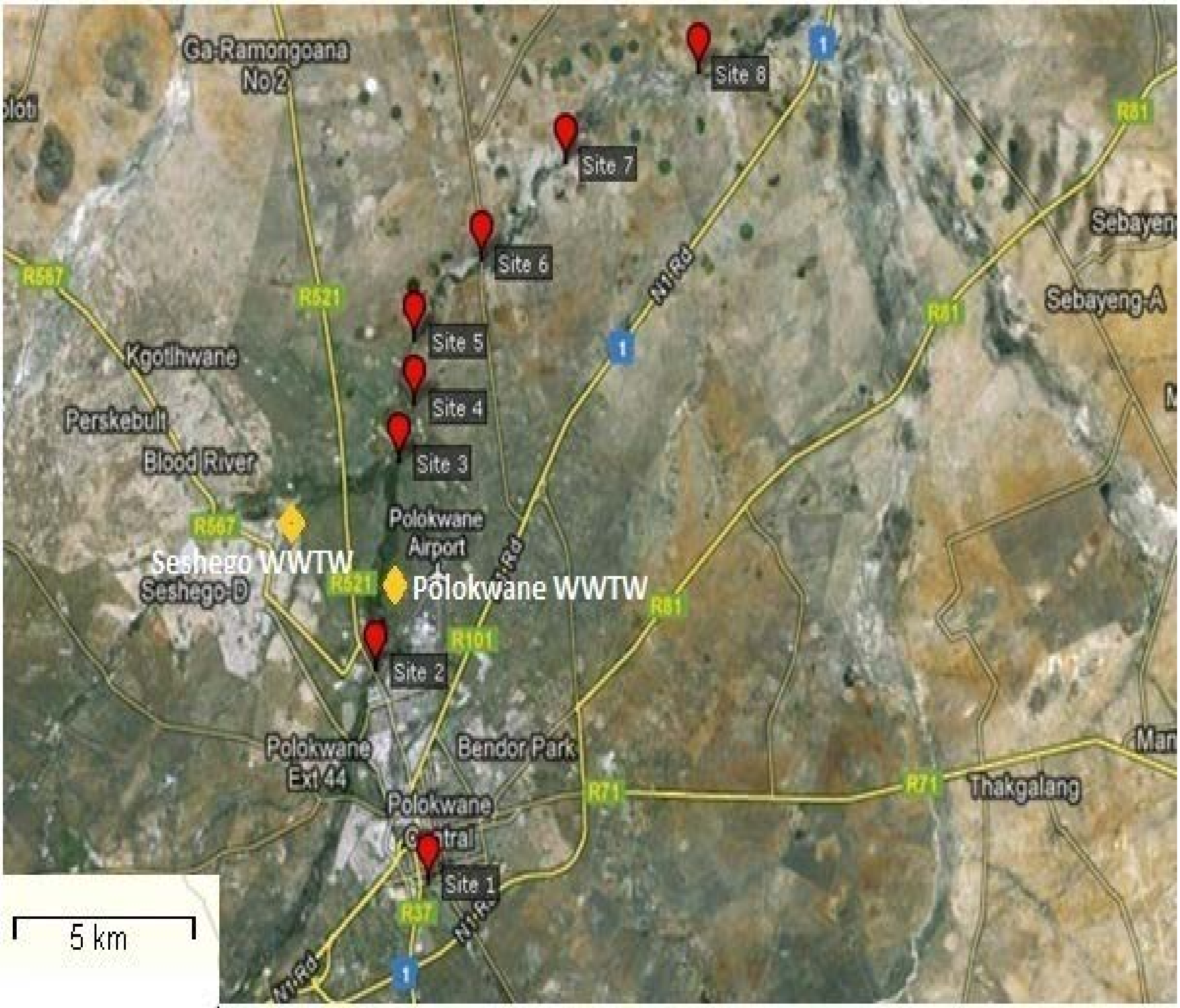


**Figure 3.5**: Map of the Sand River indicating Polokwane WWTW and Seshego WWTW and sites 1, 2, 3, 4, 5, 6, 7 and 8 (http/www.gpsvisualizer.com/map?output_google).

**Table 3.1**: Site description

| Site | Coordinates | Width (m) | Depth (cm) | Substrate | Vegetation | Special features |
|---|---|---|---|---|---|---|
| Site 1 | S23˚ 55' 16.2, EO 29˚ 27' 19.2 | 6 | 10.53 | Rocks, sediment and sand | Marginal vegetation | Receives water from storm water drains |
| Site 2 | S23˚ 52' 10.7, EO 29˚ 26' 10.5 | 16 | 22.04 | Rocks, sediment and sand | Marginal vegetation | Slightly within the urbanised area |
| Site 3 | S 23˚49' 08.2, EO 29˚ 26' 39.9 | 16 | 12.91 | Sediment and sand | Marginal vegetation, algae | Site after effluent discharge |
| Site 4 | S 23˚ 48' 18.1, EO 29˚ 27' 02.0 | 10 | 13.78 | Sediment and sand | Algae | Surrounded by farmland and rangeland |
| Site 5 | S 23˚ 47' 20.2, EO 29˚ 27' 02.1 | 18 | 8.41 | Sediment and sand | Marginal vegetation, algae | Surrounded by farmland and rangeland |
| Site 6 | S 23˚ 46' 11.3, EO 29˚ 28' 24.3 | 7 | 7.41 | Sediment and rocks | Marginal vegetation, wetland | Surrounded by farmland and rangeland |
| Site 7 | S 23˚ 44' 44.3, EO 29˚ 30' 11.5 | 22 | 12.56 | Rocks, sediment and sand | Macrophytes | Surrounded by farmland and rangeland |
| Site 8 | S 23˚ 43' 25.4, EO 29˚ 32' 59.6 | 14 | 10.50 | Rocks | Marginal vegetation | Surrounded by farmland and rangeland |

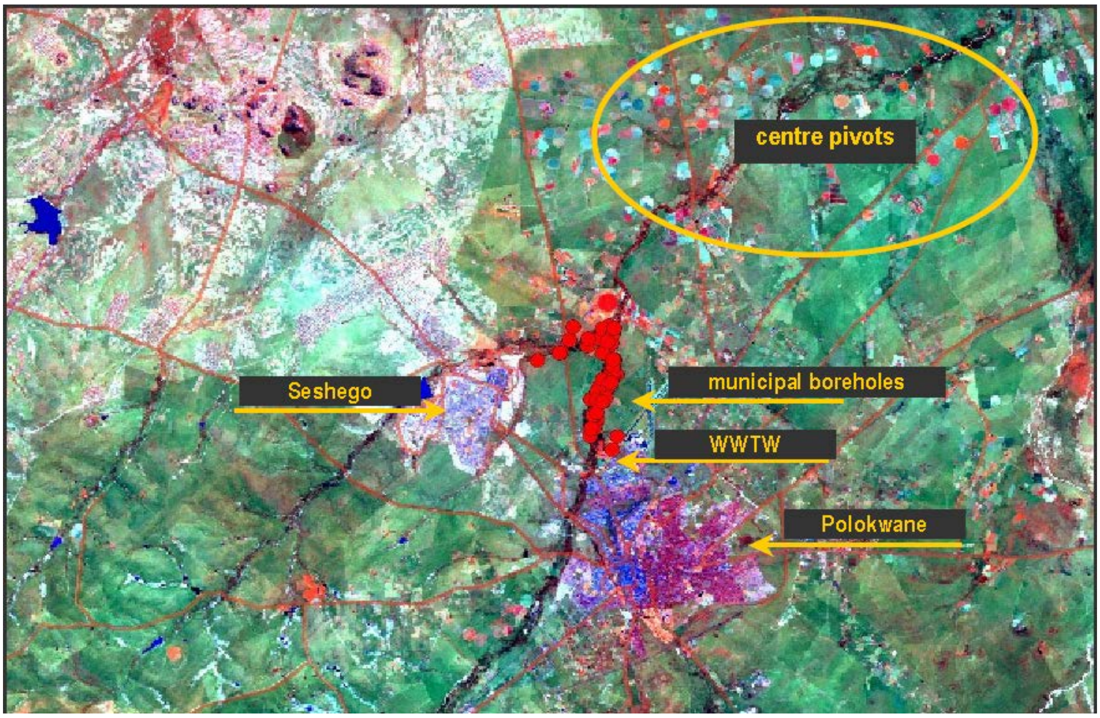


**Figure 3.6**: Map indicating Municipal boreholes around Polokwane (Murray *et al.,* 2005).

### 3.4.2 Physical and chemical parameters

Temperature, salinity, conductivity, pH and dissolved oxygen at each site were measured monthly from February, 2012 to November, 2012 on site using a YSI meter (MPS 556, Figure 3.7).

**Figure 3.7**: YSI meter (MPS 556) used for measuring some of the water quality parameters.

Total phosphorus and total nitrogen concentrations were determined in the water samples collected at each site using the Standard methods for the examination of water and wastewater (APHA, 2005). The samples were collected monthly in 250 ml polyethylene plastic bottles. They were kept in ice during transportation until analysis at the laboratory.

Two water samples of 250 ml were collected at each site. The dissolved oxygen in one of the bottles was measured on site where a meter probe was dipped into the samples and the dissolved oxygen (DO) was recorded. The other bottle was wrapped with an insulation tape on site, put in dark container and transported to the Aquaculture Research Unit (ARU) laboratory where it was incubated for five days at 20°C. After incubation the dissolved oxygen levels were recorded. The DO values were used in the equation below to determine the BOD (Viessman and Hammer, 1993).

$BOD^{20}_{5} = D1 - D2$

where:

D1 = dissolved oxygen (mg/l) on the first day

D2 = dissolved oxygen (mg/l) after five days

5 = number of days

20 = temperature (°C).

The total dissolved solids (TDS) were determined using the method adapted from Eaton *et al*., (1995). At each site, water samples were collected using 250 ml polyethylene bottles. At the ARU laboratory, 100 ml of the 250 ml was filtered through a filtration apparatus where a glass fibre filter (Whatman, 1.6 µm) was placed. To allow complete drainage, after draining the water sample, 10 ml of distilled water was filtered for three minutes. The filter paper was then removed and placed in a clean glass petri- dish and dried at 120° C. The TDS was calculated as follows:

Total dissolved solids (mg/l) = $\frac{(A\text{-}B)\times 1000}{\text{Sample volume, ml}}$

where, A = weight of dried residue + dish, mg

B = weight of dish, mg

The river velocity was determined using two points at each site. Two observers were at each point. A float was tossed and the time it took for the float to get from one point to the other point was recorded. This was repeated twice to get an average of the velocities. The river discharge at each site was determined by the velocity area method (Schumm, 1977):

$Q = VA$

where:

$Q$ = discharge ($m^3 \cdot s^{-1}$)

$V$ = mean velocity ($m \cdot s^{-1}$)

$A$ = area of cross-section ($m^2$), this was obtained by measuring the distance across the river channel and depths at equally spaced intervals. The depths and distances are then used to calculate the area within each cross-section segment.

Self-purification capacity with respect to total nitrogen and total phosphorus was determined as follows (adapted from, Bourne *et al*., 2002):

$$Sr\ (g \cdot s^{-1} \cdot m^{-1}) = \frac{Q_1C_1 + Q_SC_S - Q_2C_2}{L}$$

where:

$Q_1C_1$ = pollution load at the upstream station, $g \cdot s^{-1}$

$Q_2C_2$ = pollution load at the downstream station, $g \cdot s^{-1}$

$Q_sC_s$ = point source pollution load in the river reach, $g \cdot s^{-1}$

$L$ = distance between the two points 1 and 2, m.

### 3.4.3 Coliform isolation and enumeration

The total coliform, faecal coliforms, and faecal streptococcus coliforms were determined using the Membrane filtration method (Figure 3.8, WHO 1996).

Glass sample bottles of 1 litre were sterilized at 120°C and used to collect water from each site. They were filled with river water, one bottle per site and stored in ice during transportation to the ARU laboratory.

**Figure 3.8**: Membrane filtration apparatus

m-Endo agar plates were prepared using 36 g of m-Endo powder agar. The agar was suspended in 1 litre of distilled water. It was then autoclaved (15 min at 121°C) and poured into the plates. mFC agar plates were prepared using 52 g of mFC powder agar. The agar was suspended in 1 litre of distilled water and heated to boil and dissolve completely. An amount of 10 ml of 1% solution of rosolic acid in 0.2 N NaOH was added to the mixture. Heating was continued for 1 minute with frequent agitation. The solution was then allowed cool to 45-50°C. From the mixture, 4 ml amounts were dispensed into petri dishes (50-60 mm) and allowed to solidify.

KF–streptococci agar plates were prepared using 71.5 g of KF–streptococci powder agar. The agar was suspended in 1 litre of distilled water and then boiled with frequent agitation. It was then cooled to approximately 50°C and 10 ml of a 1% TTC solution (2, 3, 5 - triphenyltetrazolium chloride) was added, mixed and poured into the plates.

The m-Endo plates were used to enumerate colonies of total coliform (TC) count. From each site, a 100 ml of the sample water was used to make a serial dilution of 1 x $10^{-1}$ to 1 x $10^{-3}$ in three 100ml glass bottles containing 99 ml of distilled water. Using a sterile pipette, 1 ml of sample water was transferred into the first bottle. The water was serially transferred from one bottle to the other. The diluted and undiluted 100ml samples were then filtered through a 4.5 μl microfilter. The microfilters for each dilution were aseptically removed using sterilized forceps and placed in the M-Endo agar plates and incubated at 37°C for 24 hours. The faecal coliform (FC) counts were obtained using the mFC (m-faecal coliform) agar plates. Blue coloured colonies on the membrane filter were counted as faecal coliforms. The same filtration process as the one for the TC was carried out and the plates were incubated at 42°C for 24 hours. The bacterial colonies were greenish, with a metallic sheen in reflected light and with a blue-black centre in transmitted light. Faecal streptococci, the KF-streptococcal agar plates were incubated at 37°C for 48 hours. Red bacterial colonies were counted as faecal streptococci colonies. All colonies were counted manually.

The Polokwane municipality irregularly monitors pH, conductivity, suspended solids, ammonia, nitrite, phosphate, chemical oxygen demand and *E.coli* in the maturation ponds. Since the water from the maturation ponds is used to recharge the Polokwane aquifer. Samples were collected from maturation ponds. Water quality parameters determined from the maturation ponds include pH, conductivity, suspended solids, ammonia, nitrate, phosphorus, chemical oxygen demand and *E.coli*. Since the water from the Polokwane WWTW recharges the aquifer, it is prudent to look at the borehole water. Ten boreholes were sampled. The pH, conductivity, suspended solids, ammonia, nitrate and phosphorus were determined.

### 3.4.4 Statistical analysis

Normality and homogeneity of variance of water quality parameters were tested using the Shapiro-Wilk normality test. A one way analysis of variance (ANOVA) was used to determine any significant differences with respect to total nitrogen, total phosphorus (mg/l) and pH and coliform counts before and after discharge. Principal component analysis was used to determine the factors that contribute to water quality variation (using SAS version 1.9). PCA is an extension of fitting straight lines and planes by least-squares regression analysis. It extracts a set of eigenvectors, or theoretical variables, that minimise the total residual sum of squares after fitting straight lines to the data. PCA relates to a linear response model in which the abundances of species either increase or decrease along environmental gradients. Bartlett's test was used to assess the homogeneity of variables. Hierarchical cluster analysis was used to cluster the different sites of the Sand River in relation to the water quality parameters (IBM SPSS version 20.0). Hierarchical cluster analysis (HCA) is an exploratory tool designed to reveal natural groupings (or clusters) within a data set that would otherwise not be apparent. It is used to determine variables that are interrelated to one another according to maximum similarities (Tolulope, 2011).

## 3.5 RESULTS

### 3.5.1 Physico-chemical parameters

Nitrogen and phosphorus were highest at the point of discharge (Table 2a). Conductivity was also highest at the point of discharge and decreased further downstream (Table 3.2b). The levels of both nitrogen and phosphorus recorded during the dry season were higher than those recorded in the rainy season (Figure 3.9 and3.10). The flow rate showed an increase after effluent discharge at site 3 and the lowest rate recorded was at site 8 (Table 3.2a). The river depth fluctuated across the different sites, with the lowest depth at site 6 and the highest at site 2. Total dissolved solids fluctuated throughout the different sites. Dissolved oxygen levels ranged between 7.49 and 8.30 mg/l. The temperature during the study period ranged between 19. 34 °C and 24.54 °C (Table 3.2b). The levels of BOD increased after discharge and gradually decreased further downstream (Table 3.2b). Analysis of variance (ANOVA), indicated that there were no significant differences in temperature, conductivity, salinity, pH, TDS and flow rate between the upstream and downstream sites ($P>0.05$). Phosphorus and nitrogen were however significantly higher after the point of discharge ($P<0.05$).

**Table 3.2a**: Water quality parameters of the Sand River from February to November, 2012, the values are expressed as the mean ± SE.

| Site | Flow (m/s) | Depth (cm) | Nitrogen (mg/l) | Phosphorus (mg/l) | TDS (mg/l) |
|---|---|---|---|---|---|
| 1 | 0.09±0.02[a] | 10.53±1.31[a] | 0.50±0.21[a] | 0.29±0.00[a] | 140±0.00[a] |
| 2 | 0.21±0.14[a] | 22.04±2.54[b] | 1.15±0.01[a] | 0.12±0.05[a] | 155±15.00[a] |
| 3 | 2.64±2.23[a] | 12.91±0.41[a] | 13.85±0.62[b] | 2.83±2.95[b] | 145±0.00[a] |
| 4 | 1.22±0.92[a] | 13.78±1.73[a] | 11.45±0.39[b] | 3.09±2.55[c] | 146±0.00[a] |
| 5 | 0.30±0.16[a] | 8.41±1.81[a] | 8.60±0.02[b] | 2.69±0.20[d] | 170±0.00[a] |
| 6 | 2.03±1.60[a] | 7.41±2.99[a] | 5.85±0.46[c] | 2.65±2.35[d] | 155±0.00[a] |
| 7 | 2.62±2.19[a] | 12.56±2.15[a] | 3.55±0.26[b] | 1.94±0.05[d] | 155±0.00[a] |
| 8 | 0.21±0.02[a] | 10.50±1.50[a] | 3.00±0.29[c] | 1.56±1.80[d] | 141±1.00[a] |

**Table 3.2b**: Water quality parameters of the Sand River from February to November, 2012, the values are expressed as the mean ± SE.

| Site | Temperature (°C) | Conductivity (mS/cm) | Salinity (mg/l) | DO (mg/l) | pH | BOD(mg/l) |
|---|---|---|---|---|---|---|
| 1 | 19.34±1.97[a] | 0.703±0.02[a] | 0.44±0.09[a] | 4.54±0.66[a] | 7.49±0.41[a] | 1.04±0.005[a] |
| 2 | 22.43±3.65[a] | 0.728±0.09[a] | 0.40±0.01[a] | 4.90±1.11[a] | 8.48±0.27[a] | 1.05±0.005[a] |
| 3 | 24.14±4.27[a] | 1.072±0.62[a] | 0.52±0.05[a] | 4.52±0.05[a] | 8.00±0.09[a] | 5.06±0.550[a] |
| 4 | 23.35±4.90[a] | 1.050±0.72[a] | 0.55±0.02[a] | 6.51±1.59[a] | 8.10±0.18[a] | 5.44±0.005[a] |
| 5 | 24.54±4.13[a] | 0.986±0.10[a] | 0.48±0.07[a] | 5.80±0.80[a] | 8.30±0.15[a] | 5.20±0.005[a] |
| 6 | 24.52±7.84[a] | 0.926±0.10[a] | 0.56±0.01[a] | 4.53±1.00[a] | 7.82±0.21[a] | 4.88±0.070[a] |
| 7 | 23.78±8.58[a] | 0.929±0.10[a] | 0.54±0.02[a] | 5.27±1.14[a] | 7.68±0.35[a] | 4.28±0.005[a] |
| 8 | 21.99±10.54[a] | 0.905±0.13[a] | 0.54±0.15[a] | 4.44±0.58[a] | 8.20±0.27[a] | 4.28±0.010[a] |

Means with different superscripts are significantly different ($P<0.05$)

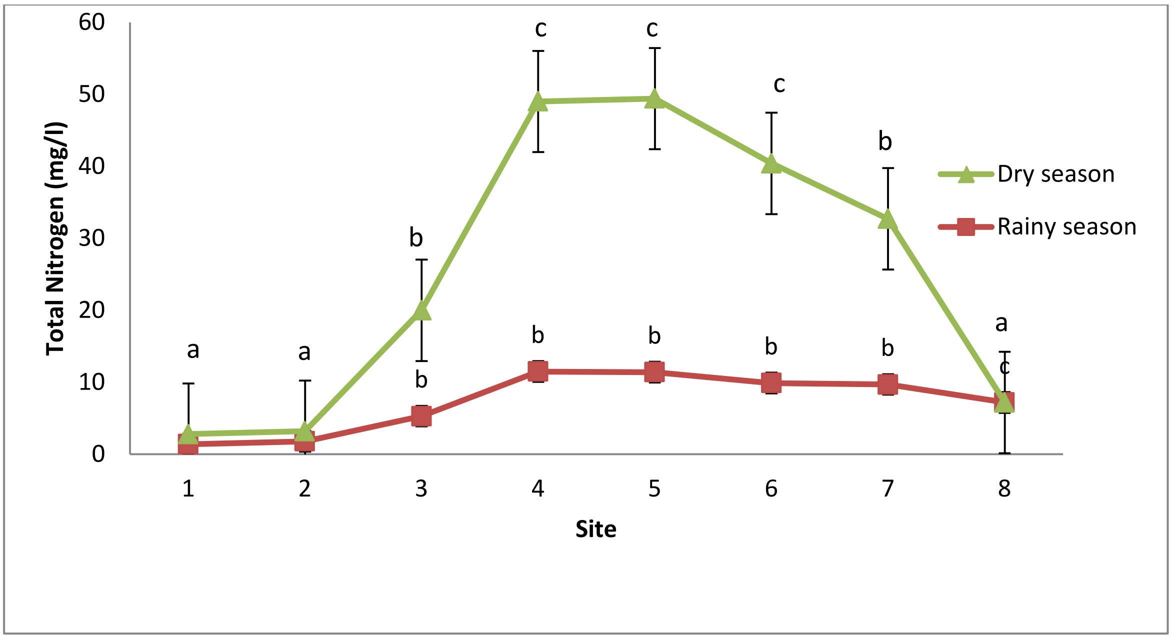


**Figure 3.9**: Total nitrogen levels during the rainy dry season (March and April, 2012) and (October and November, 2012). Points on a curve with different letters are significantly different (P<0.05).

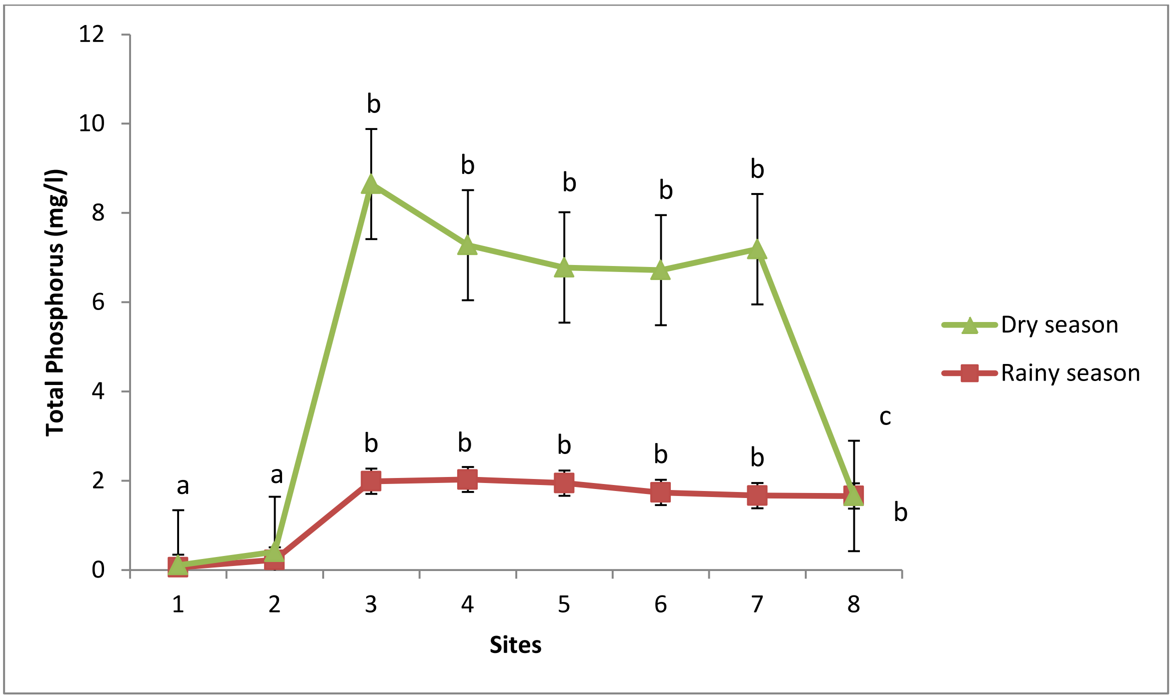


**Figure 3.10**: Total phosphorus levels during the dry season (March and April) and rainy (October and November). Points on a curve with different letters are significantly different (P<0.05).

Bartlett’s test of sphericity indicated high significance ($P<0.001$), indicating sufficient correlation between variables to proceed with the PCA analysis (Table 3.3). All the extracted communalities are reasonably high and acceptable (>0.5), except slightly lower values of depth (Table 3.4). Eigenvalues showed that the first three principal components are the most significant (>1). These extracted components explain 83 % of the total variance in the water quality of the Sand River (Table 3.5). Principal component (PC) 1, explained 46 % of the total variance. This component incorporates the major water quality variables that are characteristic of wastewater discharges with strong positive loading of nitrogen, phosphorus, conductivity, BOD, salinity, temperature and flow rate (Figure 3.11, Table 3.6). These parameters are strongly correlated with one another. PC 2 explained 20% of the total variance, with strong positive loading of oxygen, TDS and pH. PC 3 explained 17% of the total variance, with a strong positive loading of depth (Figure 3.11, Table 3.6). The first and second component separates the sites (site 3, 4, 5, 6, 7) with high nutrient loading that indicate deterioration of water quality from those with low nutrient loading (site 1, 2 and 8, Figure 3.11).

**Table 3.3**: KMO and Bartlett's Test.

| | |
|---|---|
| Kaiser-Meyer-Olkin Measure of Sampling Adequacy. | 0.371 |
| Bartlett's Test of Sphericity | |
| *Approx. Chi-Square* | 92.146 |
| *Df* | 36.000 |
| *Sig.* | 0.000 |

**Table 3.4**: Communalities of the water quality parameters.

| Parameter | Initial | Extraction |
|---|---|---|
| Temperature | 1.000 | .841 |
| Conductivity | 1.000 | .881 |
| Salinity | 1.000 | .707 |
| Oxygen | 1.000 | .719 |
| pH | 1.000 | .769 |
| Flow | 1.000 | .686 |
| Depth | 1.000 | .482 |
| Phosphorus | 1.000 | .964 |
| Nitrogen | 1.000 | .863 |
| BOD | 1.000 | .976 |
| TDS | 1.000 | .626 |

**Table 3.5**: Eigenvalue of water quality components.

| | | Rotation sum of squares | |
|---|---|---|---|
| Component | Eigenvalue | % variance | % Cumulative |
| 1 | 5.871 | 45.704 | 45.764 |
| 2 | 2.175 | 20.103 | 65.867 |
| 3 | 1.084 | 17.136 | 83.003 |
| 4 | 0.936 | 0 | 0.000 |

**Table 3.6**: Correlation (structure) of water quality components.

| Variable | Principal Component | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Temperature | 0.821 | 0.339 | 0.166 |
| Conductivity | 0.966 | 0.019 | 0.091 |
| Salinity | 0.808 | -0.284 | -0.297 |
| Oxygen | 0.395 | 0.669 | 0.100 |
| pH | -0.275 | 0.846 | -0.252 |
| Flow | 0.679 | -0.416 | 0.389 |
| Depth | -0.464 | 0.281 | 0.781 |
| Nitrogen | 0.848 | -0.003 | 0.299 |
| Phosphorus | 0.987 | 0.055 | -0.070 |
| BOD (mg/l) | 0.970 | 0.071 | -0.173 |
| TDS (mg/l) | 0.258 | 0.746 | -0.050 |

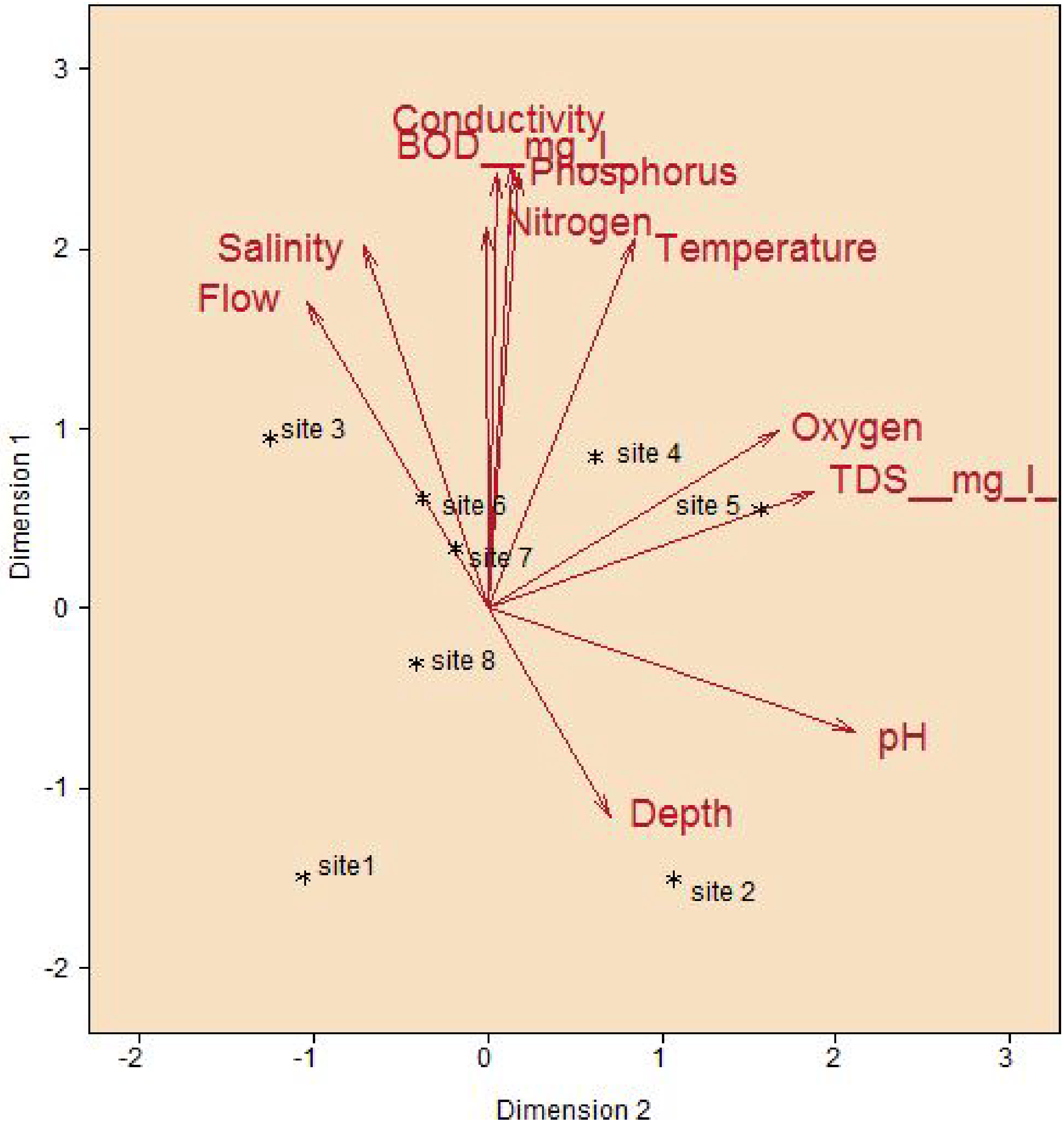


**Figure 3.11**: Component plot in rotated space for water quality parameter distribution with respect to component 1, 2.

Hierarchical method average linkage cluster analysis produced two clusters, grouping two sites above the sewage treatment works and six sites downstream of the sewage effluent discharge point (Figure 12). The two clusters were joined using the Ward's Method with Squared Euclidean metric. The downstream sites were characterised by the high phosphorus and nitrogen levels.

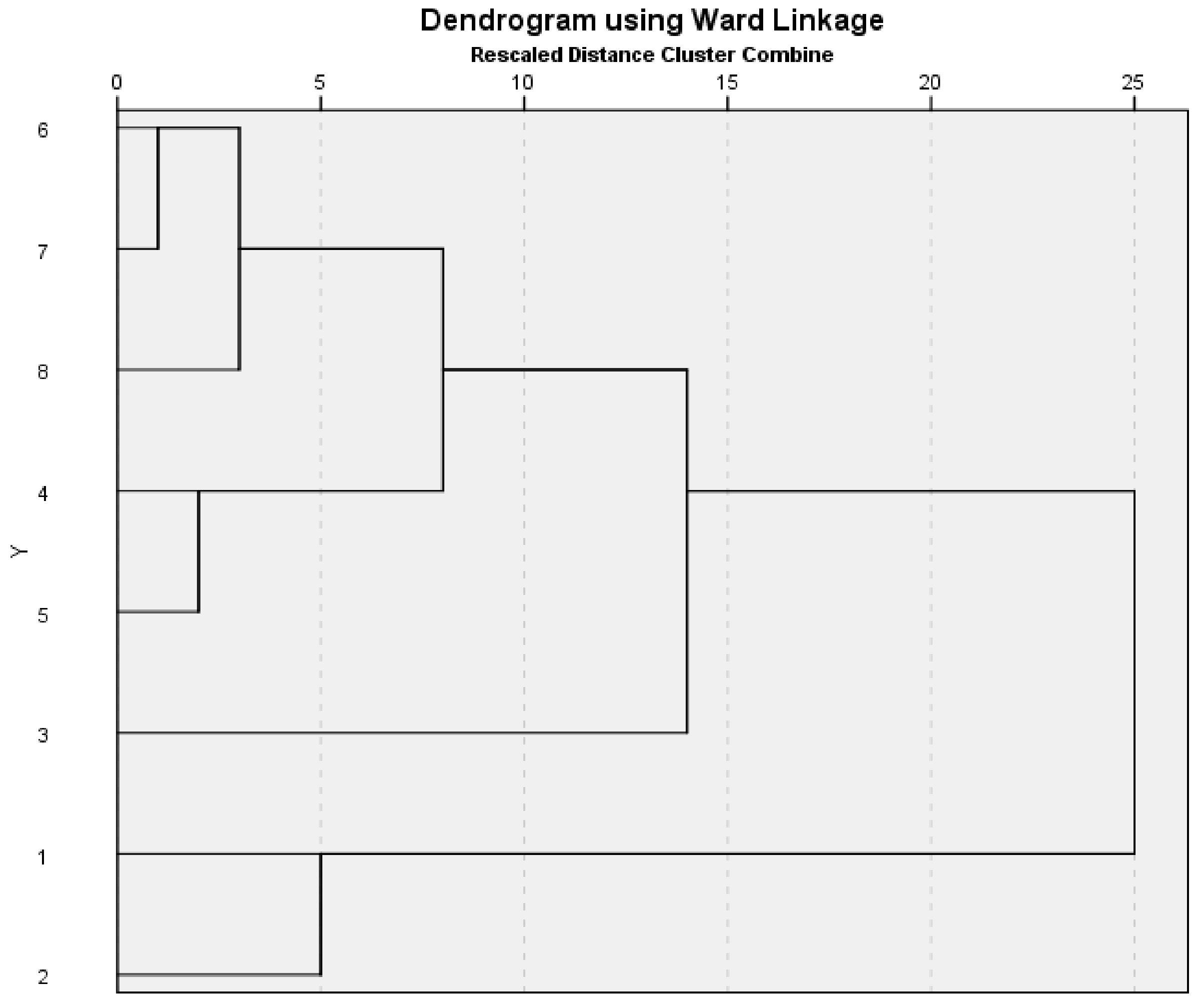


**Figure 3.12**: Dendrogram showing sampling sites (Y-axis) clusters of water quality parameters of the Sand River.

The self-purification rate with respect to total phosphorus fluctuated throughout the different sections of the river, with the highest self-purification rate between sites 3 to 6 (Table 3.7). Similarly the self-purification of the total nitrogen fluctuated throughout the different sections however, it declined further downstream (3-7, 3-8) (Table 3.7).

**Table 3.7**: Self-purification of total nitrogen and phosphorus across the different sites of Sand River.

| site | Distance (Km) | TP load(g/s) | TN load(g/s) | TP self-purification rate (g/s/km) | TN self-purification rate (g/s/km) |
|---|---|---|---|---|---|
| 3-4 | 13.431 | 5.059467 | 27.79757 | 0.376701 | 2.069658 |
| 3-5 | 15.221 | 1.753796 | 18.66903 | 0.115222 | 1.226531 |
| 3-6 | 18.375 | 7.473754 | 37.36878 | 0.406735 | 2.033675 |
| 3-7 | 22.430 | 2.530277 | 36.97504 | 0.112808 | 1.648464 |
| 3-8 | 27.777 | 7.019533 | 36.61398 | 0.252710 | 1.318140 |

TP- total phosphorus, TN- total nitrogen

### 3.5.2 Coliform isolation and enumerations

Total coliform (TC) levels gradually increased from the upstream sites to the downstream sites (Figure 3.12). There was however a decrease at site 6 and 8 (Figure 3.13). Faecal coliforms (FC) levels started increasing at site 3 (after discharge) and then decreased at the sites further downstream. Faecal matter was observed at site 3 (Figure 3.16). Faecal streptococci (FS) levels were highest at site 2. The levels then decreased gradually from the point of discharge (Figure 3.13). During the rainy season, the TC levels fluctuated across the different sites however; there was a distinct increase at the point of discharge and the last two sites (Figure 3.14). Faecal coliform levels increased after discharge and decreased further downstream (site 5 to 8). Faecal streptococci levels were highest at the upstream site and they gradually decreased to the last site (Figure 3.14).

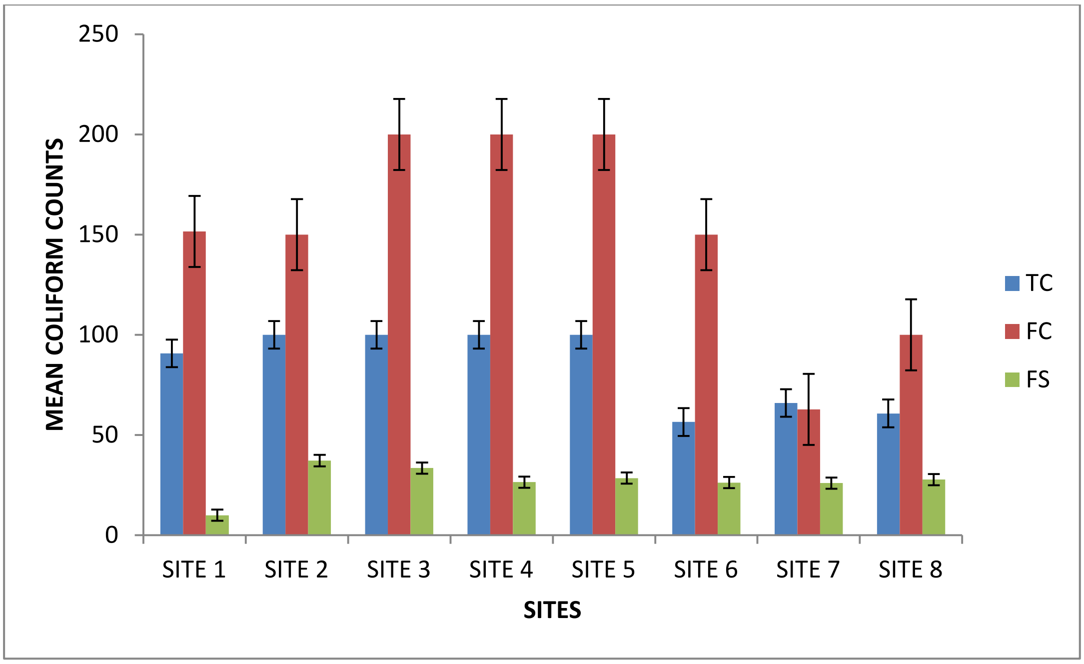


**Figure 3.13**: Mean values of the total coliforms, faecal coliforms and faecal streptococci coliform counts across at different sites of the Sand River during the dry season.

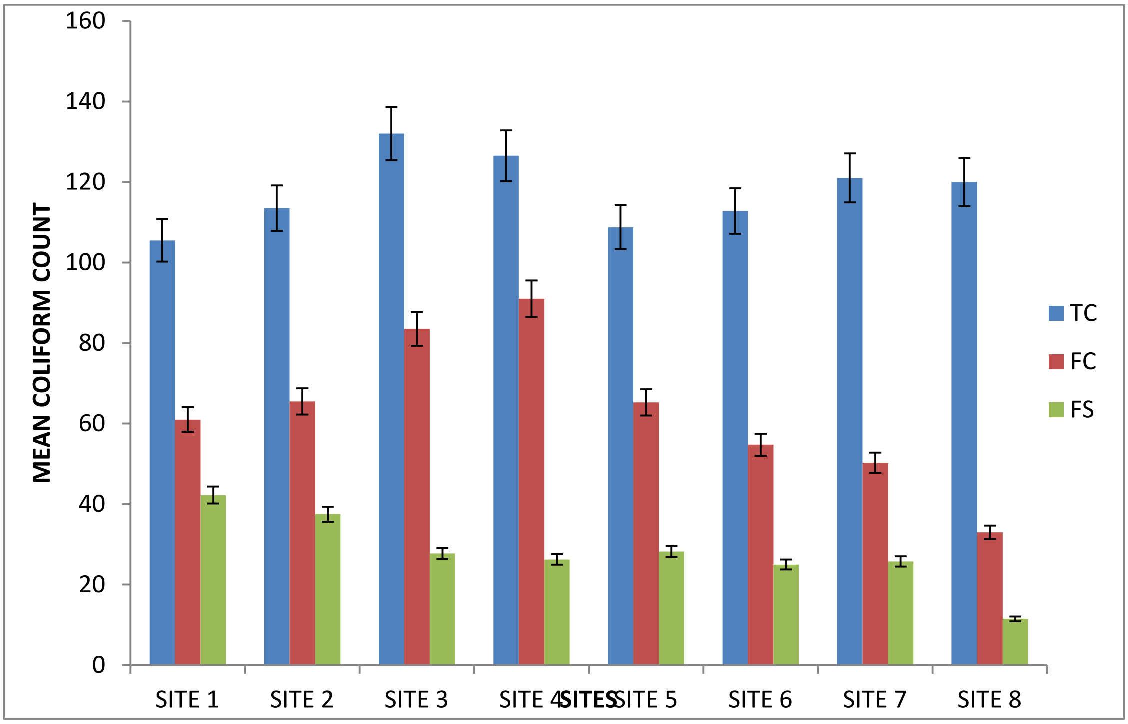


**Figure 3.14**: Mean values of the total coliforms, faecal coliforms and faecal streptococci coliform across at different sites of the Sand River during the rainy season.

**Figure 3.15**: Foam indicating high ammonia levels at site 3, after discharge.

**Figure 3.16**: Evidence of Faecal matter along the Sand River.

### 3.5.3 Water quality of Polokwane WWTW maturation ponds and boreholes along the Sand River.

The mean values for EC, SS, $NH_3$, $PO_4$, COD, *E.coli* were much higher than those of the licence requirements. However, $NO_3$ and pH levels were within the licence requirements (Table 3.7).

**Table 3.8**: Mean of water parameters of the final effluent determined in the maturation ponds of the Polokwane WWTW.

| Parameter | Mean ± SE | Licence Requirements |
|---|---:|---:|
| PH | 7.30 ± 0.035 | 5.5-9.5 |
| EC (mS/cm) | 109.90±4.335 | 150.0 |
| SS (mg/l) | 59.98±7.166 | 25.0 |
| $NH_3$ (mg/l) | 38.90±4.132 | 3.0 |
| $NO_3$ (mg/l) | 1.85±0.395 | 15.0 |
| $PO_4$ (mg/l) | 7.16±0.449 | 1.0 |
| COD (mg/l) | 93.17±3.589 | 75.0 |
| *E.coli* (counts/ 100ml) | 1463.73±52.63 | 1000.0 |

Ammonia levels in the maturation ponds were significantly higher ($P<0.05$) than those recorded in all the boreholes (Table 3.8). The effluent discharged from the maturation ponds created foam at the site after discharge, as an indication of the high ammonia levels (Figure 3.15). Significantly higher nitrate and significantly lower suspended solids were recorded in the maturation ponds than all the boreholes ($P<0.05$). The conductivity fluctuated at the different boreholes. The maturation pond conductivity was significantly lower than all the boreholes except borehole 14 (Table 3.8).

**Table 3.9**: Water quality parameters of Polokwane wastewater treatment works maturation ponds and boreholes along the Sand River.

| Source | pH | $NH_3$ (mg/l) | $NO_3$ (mg/l) | SS (mg/l) | EC (mS/cm) |
|---|---|---|---|---|---|
| maturation ponds | 7.3±0.14$^{a}$ | 38.9±0.1$^{a}$ | 1.85±0.02$^{a}$ | 59.98±0.03$^{a}$ | 109.92±0.10$^{a}$ |
| BH5 | 7.4±0.14$^{a}$ | 0.22±0.01$^{b}$ | 1.4±0.03$^{b}$ | 845.7±0.01$^{b}$ | 130.12±0.55$^{b}$ |
| BH6 | 7.4±0.14$^{a}$ | 0.32±0.01$^{b}$ | 1.4±0.03$^{b}$ | 794.3±0.01$^{b}$ | 122.19±0.10$^{c}$ |
| BH7 | 7.4±0.14$^{a}$ | 2.63±0.01$^{b}$ | 1.4±0.02$^{b}$ | 799.5±0.01$^{b}$ | 123.20±0.10$^{c}$ |
| BH8 | 7.4±0.14$^{a}$ | 2.24±0.01$^{b}$ | 1.4±0.04$^{b}$ | 800.8±0.01$^{b}$ | 123.21±0.10$^{c}$ |
| BH10 | 7.6±0.14$^{a}$ | 0.29±0.01$^{b}$ | 1.4±0.03$^{b}$ | 692.9±0.01$^{b}$ | 106.59±0.10$^{d}$ |
| BH11 | 7.5±0.14$^{a}$ | 0.25±0.01$^{b}$ | 1.4±0.00$^{b}$ | 682.5±0.01$^{b}$ | 105.26±0.10$^{d}$ |
| BH12 | 7.5±0.14$^{a}$ | 0.28±0.01$^{b}$ | 1.4±0.02$^{b}$ | 733.2±0.01 | 112.82±0.10$^{d}$ |
| BH13 | 7.3±0.14$^{a}$ | 3.98±0.01$^{d}$ | 1.4±0.01$^{b}$ | 722.8±0.01$^{b}$ | 111.24±0.10$^{d}$ |
| BH14 | 7.6±0.14$^{a}$ | 0.59±0.01$^{b}$ | 1.4±0.02$^{b}$ | 710.5±0.01$^{b}$ | 109.34±0.01$^{a}$ |
| BH16 | 7.4±0.14$^{a}$ | 0.76±0.01$^{b}$ | 1.4±0.2$^{b}$ | 678±0.01$^{b}$ | 104.34±0.1$^{d}$ |

### 3.6 DISCUSSION

Principal component analysis showed that nitrogen and phosphorus were associated with the first component which explained 45% of the total variation in the water quality of the Sand River. The sites before discharge (Site 1 and 2) form their own cluster and had significantly lower phosphorus and nitrogen levels than the downstream sites. The main reason for the elevated nitrogen and phosphorus levels in the downstream site is the substandard effluent being discharged from the Polokwane and Seshego WWTW. Both sewage treatment works are overloaded. Polokwane WWTW has a design capacity to discharge 28 ML/day and yet it is discharging 30 ML/day. Similarly the Seshego WWTW is exceeding its designed capacity by 0.8% (DWAF, 2009). The South African guidelines for total nitrogen (nitrate + nitrite as N) for domestic use is <0.6 mg/l (DWAF, 1998). The target water quality range for total nitrogen in water for full contact recreational use is 6.0 to 10 mg/l. Total nitrogen levels at site 3 and 4 after discharge, exceeded both the domestic and recreational standards. Although the communities around fish in the river, the nitrogen results suggest that no recreational activity should be undertaken in the river. The levels of nitrogen and phosphorus in surface water that are likely to cause eutrophication are between 2.5 – 10 mg/l (DWAF, 1996) and 20 – 100 µg/l (de Villers and Thiart, 2007), respectively. The nitrogen levels recorded in the Sand River fell within these levels. The problem of sewage treatment works that are overloaded is common in South Africa (Morrison *et al.,* 2001; Fatoki *et al.,* 2001; Igbinosa and Okoh, 2009). The Vaal River services the most industrialized and populated area in South Africa (Johannesburg). Numerous studies have been done on the Vaal River and have indicated that it has reached eutrophication levels (Braune and Roger, 1987; Pieterse and Van Vuuren, 1997; Wepener *et al*., 2011). Phosphorus levels of up to 1.4 mg/l and nitrogen levels exceeding 2.5 have been reported. The nitrogen and phosphorus levels recorded in the Sand River ranged from 0.05 to 13.85 mg/l and 0.29 to 3.09 mg/l, respectively. The continuous discharge of high nitrogen containing sewage effluent into the Sand River is most likely to cause eutrophication. The rapid urban population growth in all these areas is the root cause of the problem. In the city of Polokwane the population increased from 20 500 in the 1950s to 631 318 in 2012 (DWAF, 2012). However, there was no concomitant increase in the design capacity of the Polokwane WWTW.

The Polokwane WWTW uses biofiltration to remove nutrients from the effluent prior to discharge. However, the reception of high nutrient wastewater from household, inns and beverage industries continues to hinder the treatment capacity of the Polokwane WWTW. This is because the design capacity has been exceeded. The continuous discharge of effluent into the Sand River could affect the river self-purification and inevitably lead to eutrophication. The use of organic detergents in households can therefore be suggested as an alternative to products which contain inorganic phosphorus. This is because the major phosphorus source in domestic effluent is detergents.

The Polokwane WWTW treats effluent received from the SAB brewery using Upflow Anaerobic Sludge Blanket (UASB). The separate treatment of the effluent from the brewery with that of the domestic effluent is due to the high hydraulic strength of the brewery effluent. Brewery effluent is associated with low dissolved oxygen, high suspended solids, high BOD and high nitrogen levels (Kanu and Achi, 2011). SAB brewery is the major production industry in the city of Polokwane. This makes it a major threat to the water quality of the Sand River. High suspended solids levels have been recorded in the maturation ponds. This is because of the brewery effluent. These levels were above the Polokwane WWTW licence limit. Treatment of the brewery effluent separately must be maintained because failure to do so will lead to sewage effluent that has excessively high levels of BOD and suspended solids. China is amongst some of the most industrialized countries. Most parts of this country use Upflow Anaerobic Sludge Blanket (UASB) to treat high strength industrial effluent (including brewery) which contains over 100 000mg/l of COD (Matsuo *et al*., 2001). The separate treatment of domestic effluent and industrial effluent resulted in less than 1000 mg/l of COD. In most developing African countries however, industries dispose of their effluent without pre-treatment due to economic and technological constraints (Sweeney, 1993). Ikhu-Omoregbe *et al*. (2005), indicated that brewery effluent that was treated together with domestic effluent in Bulawayo municipality (Zimbabwe) impacted negatively on the quality of the discharged effluent. It resulted in high COD, BOD and SS. Therefore, the use of Upflow Anaerobic Sludge Blanket by the Polokwane Municipality seems to be a reliable way of improving the quality of brewery effluent. However, proper management of the treatment plant should be practised in order to maintain effluent quality standards.

Oxygen levels recorded at all sites throughout the sampling period were not significantly different. They ranged between 4.4 to 6.5 mg/l. Dissolved oxygen concentration in unpolluted water normally ranges between 8 and 10 mg/l. The oxygen levels (4.4 to 6.5 mg/l) recorded in this study was typical of most rivers receiving sewage effluent. The above assertion is corroborated by similar findings from other authors (Longe and Omole, 2008; Igobinosa and Okoh, 2009; Kosamu *et al*., 2011) who have found oxygen levels ranging between 3.5 to 6 mg/l in South African rivers receiving effluent discharge. This is not surprising as even the most polluted river in South Africa, the Vaal River has been reported to receive sewage from extensive formal and informal settlements causing great reduction in oxygen levels (Wepener *et al*., 2011).

The BOD levels in this study increased after effluent discharge and gradually decreased further downstream. There is no guideline in South African for BOD in effluent. The European Union stipulated target limits of BOD is 3.0 to 6.0 mg/l, this is for the protection of fisheries and aquatic life (Chapman, 1996). The BOD levels recorded from the Sand River fell within the recommended limits. This is probably because of the separate treatment that's given to the brewery effluent. Higher BOD levels were recorded elsewhere in South Africa and Africa (Momba *et al*., 2006; Nhapi *et al*., 2011; Kamusoko and Musasa, 2012). This indicates that the Sand River in comparison with other rivers in and around South Africa is still able to sustain aquatic life. Brewery effluent being the major contributor of high BOD should be well managed in order to maintain low BOD levels.

Conductivity levels recorded from Sand River were below 250 mS/cm. This is the South African recommended standard for conductivity of effluent that is allowed to be discharged into rivers (Government Gazette, 1984). It was also below the South African accepted limit for conductivity in domestic water supply (DWAF, 1998). However, the increase in conductivity at the sites after discharge particularly site 3, 4 and 5 indicates that sewage effluent contributes significantly to the increase in conductivity. This is because the effluent that is discharged from the maturation pond contains high levels of conductivity which is not in compliance with the licence limit. These high conductivity levels in the maturation pond indicated inability of the Polokwane treatment plant to effectively remove the dissolved salts. Similar increases in conductivity after effluent discharge were recorded in the Keiskamma River (South Africa) (Morrison *et al*., 2001).

The self-purification capacity of the Sand River with respect to total phosphorus and nitrogen fluctuated across the different stretches of the river. Self-purification capacity is dependent on a number of factors which include; flow rate, quantity and quality of loaded nutrients, river surface area, oxygen, temperature, turbulence, river velocity, distribution of vegetation across the river and bacterial degradation (Ifabiyi, 2008). The fluctuation of the self-purification rates in this study are a result of increasing and decreasing surface area and velocity across the different stretches of the river. The diversion of effluent to the Mogalakwena Platinum Mine reduces the amount of nutrient received by the Sand River. This allows sufficient dilution and distribution of nutrients across the river that can be degraded by bacteria and utilized by the surrounding vegetation. However, the self-purification capacity of the river is still compromised by sewage effluent from the Seshego WWTW. The diversion of effluent to the Mogalakwena Platinum Mine could also play a role in the reduction of the self-purification capacity of the Sand River. The reduction of effluent volume causes a reduction in the river velocity. This is one of the factors responsible for increased self-purification as it facilitates the exchange of dissolved oxygen from air to river water. This probably indicates that there may not be sufficient oxygen for nutrient biodegradation and oxidation of ammonia. However, due to the discharge of substandard effluent into the Sand River, it is most probable that if fifty five percent of the effluent from the Polokwane WWTW was not diverted to Mogalakwena Platinum Mine, the water quality would have deteriorated even further. Moss *et al.* (2005) conducted a study on how the Rostherne Mere and Mere Lake were affected after diversion of sewage effluent. These authors concluded that the nutrient levels declined as a result of steady dilution, which resulted from the river receiving lower levels of nutrients. These studies show that diversion of sewage effluent from sewage plants is beneficial in the recovery of the river water quality downstream as it allows sufficient self-purification.

Faecal coliform levels increased from the point of discharge (site 3, 4 and 5) and gradually decreased further downstream (Site 6, 7 and 8). The elevated levels of faecal coliforms particularly at the sites just after discharge are mainly due to sewage effluent. The faecal and total coliform levels were above the South African guideline of 0 counts/ 100ml faecal coliform and 130 counts/100 ml total coliforms for domestic use. The faecal coliforms were also above 130 counts/100 ml for full contact recreational use and 200 counts/100 ml for livestock watering (DWAF, 1996). Failure of the plant to comply with coliform standards can be attributed to the inefficiency of both the Polokwane and Seshego sewage treatment plants to remove the microbes.

The efficiency of treatment plants to remove organic, inorganic and microbes is a major concern in South Africa (Samie *et al*., 2009). Mbuligwe and Kaseva (2005) reported high faecal and total coliforms in the Msimbazi River (Dar es Salam). These authors concluded that these high faecal coliform levels were attributed to on-site sanitation systems which cater for more than 80% of the urban population. Faecal streptococci levels recorded in this study were fairly uniform throughout the entire river but, with higher levels recorded at site 1. The coliforms at the upstream sites were a result of storm water. Reduction of all the coliforms further downstream implies that there was may have been some dilution and degradation of organic matter. This is also an indication of the self-purification capacity of the river. Fatoki *et al*. (2001) also reported high levels of faecal and total coliforms downstream of the Umtata River in South Africa. High microbial counts have also been recorded in the Vaal River (Wepener *et al*, 2011). These studies indicate that sewage effluent generally contains high levels of coliforms.

It is common practise for most sewage treatment plants to chlorinate effluent prior to discharge, as a way of killing bacterial pathogens (Samie *et al*., 2009). However, if there is a build-up of sludge in the maturation ponds, such as in the case of the Polokwane WWTW, chlorination will not be effective in the removal of the bacteria. Cleaning of the maturation pond is therefore of great importance as it will enhance the effectiveness of the chlorine in reducing the levels of bacterial pathogens. The *E.coli* levels in the maturation pond exceed the licence limit and could potentially pose a health threat to people from the neighbouring communities that consume fish from the Sand River. It may also lead to contamination of the aquifer. This has already been experienced in certain parts of South Africa, such as Delmas where cholera outbreaks occurred due to groundwater contamination (Griesel *et al*., 2006).

There has been some query about the effectiveness of chlorination in terms of bacteria removal (Miranzadeh, 2005). Miranzadeh (2005), found that the substitution of chlorine disinfection by a maturation pond with 10 days hydraulic retention time after activated sludge system is able to produce effluent which is suitable for reuse in irrigation. This was due to the exposure of the effluent to sunlight throughout the retention period. The effluent from the Polokwane WWTW is retained for a period of three weeks prior to discharge. The effluent is also chlorinated prior to discharge into the maturation ponds. The implication of Miranzadeh (2005) findings is that the retention time in Polokwane WWTW is supposed to be effective considering that it is also chlorinated prior to discharge. However, the *E.coli* levels in the maturation ponds and the coliform counts in the surface water indicate otherwise. It is therefore crucial to clean up the maturation ponds. The water from the Sand River flows over the Polokwane gneissic aquifer, possible contamination of the aquifer could occur if the coliform levels continue to increase. The aquifer is of great importance to the city of Polokwane because of its reliability as water storage source during the dry/drought season (Murray *et al*., 2005). Direct discharge of substandard effluent into the Sand River increases the chances of microbial contamination of the aquifer.

There is also a need to assess the transmission of these pathogenic bacteria from water to crops. The use of polluted river water for irrigation is practised nationally. The water needs to be free of pathogens particularly if the crops to be watered are eaten raw. The major vegetables that are grown in the farms surrounding the Sand River include onions and tomatoes, which can be consumed raw. Therefore a lot of precaution needs to be taken when irrigating crops with this water.

The borehole water has significantly nitrate than the Polokwane maturation ponds. However, the levels recorded for nitrate in the borehole water indicates ground water contamination. This is in accordance with the guidelines (nitrate + nitrite as N, <0.6 mg/l) of the Department of Water Affairs and Forestry (DAFF, 1996). The major concern is that nitrate can be reduced to nitrite, which has been linked to the condition known as methaemoglobinemia in infants and pregnant women (Fatoki, 2001). The high nitrate levels may have been because of the recharge of the aquifer with substandard effluent. The surrounding farms also contribute in the nitrogen levels found in the river. In the Western Cape (South Africa), contamination of the Cape Flats aquifer has been reported. Sewage disposal has been proven as the cause of contamination of the aquifer with respect to elevated levels of ammonium, nitrite, potassium, alkalinity and chemical oxygen demand (Adelana and Xu, 2006). In some cases, such as in the North West Province (South Africa), high nitrate concentrations in groundwater were ascribed to sewage effluent and sewage sludge disposal on land overlying aquifers. The contamination of the boreholes within the Sand River catchment area emphasizes the need to efficiently treat effluent to the recommended standards as it endangers the livelihood of the residential communities.

This chapter has indicated that due to increased population in the city of Polokwane, the Polokwane and Seshego WWTW is now discharging effluent which is substandard. This sewage treatment works is not well maintained, evidence of this is seen in the quality of the effluent from the maturation ponds. Nitrogen and phosphorus levels are above the South African limits. The coliform levels provide a potential threat to the downstream communities and may result in contamination of the Polokwane aquifer. Despite the deterioration in water quality, the Sand River is still able to maintain a good self-purification capacity.

# CHAPTER 4: EFFECT OF SEWAGE EFFLUENT ON MACROINVERTEBRATE COMMUNITIES.

## 4.1 INTRODUCTION

Sewage effluent has put immense strain on the water quality of the Sand River. The effluent discharged is characterised by high levels of nitrogen and phosphorus and these could negatively impact the macroinvertebrate community of the Sand River. Changes in the water quality of rivers have a major impact on aquatic fauna and flora. The discharge of effluent into rivers has been known to be detrimental to many aquatic organisms (Wynes and Wissing, 1981; Ollis, 2005). The major water quality parameters that play a key role in aquatic ecosystems are nitrogen, phosphorus. These are the growth limiting factors in aquatic systems. Aquatic organisms are sensitive to changes in their environment and have therefore been used as biomonitoring tools to assess the severity of pollution.

Macroinvertebrates have been effectively used in the rapid assessment of river water quality in several studies (Monda *et al*., 1995; Wenn, 2008; Canobbio 2009; Odume *et al*., 2012). Macroinvertebrates are always present in a river system with high diversity under pristine conditions. The diversity is sufficient enough to indicate small changes in the river conditions. This is because, different families are variably sensitive to stress, therefore allowing the assemblage to indicate the conditions found in the river (Dicken, 2009). They have limited mobility and are thus representative of the site being sampled. This allows effective spatial analyses of disturbances to the river. Changes in their presence, absence, abundance, morphology and physiology of these organisms indicate that the physical and chemical conditions are outside of their preferred limits. Macroinvertebrate life spans range from several weeks to several years. This provides an indication of the water quality over a period of time and not just the sampling window (Dickens and Graham, 2002). The life cycles are relatively longer in comparison to microscopic organisms and shorter in comparison to fish. According to Dickens (2009), such a life cycle fits in well with the temporal scale suited for management of rivers.

Chutter (1994) developed the South African Scoring System (SASS) which essentially looks at the response of different macroinvertebrates to organic pollution.

SASS is a qualitative, multi-habitat, rapid, field-based method where macroinvertebrates are identified to family level. Sensitivity weightings are used to calculate the biotic index. SASS is now on the fifth version (SASS5) and is used by the South African Department of Water Affairs as a standard method for River Health Assessment (Dallas, 2007).

There are however, some limitations to SASS as a biomonitoring tool. The main problem is that SASS is qualitative and fails to incorporate the measure of abundance of the macroinvertebrate families (Brown, 2001).

## 4.2 OBJECTIVES

The objectives of this chapter were to determine:

I. The relationship between water quality parameters and macroinvertebrates communities of the Sand River.

II. The spatial and temporal variation in the macroinvertebrates abundances in the Sand River.

III. The spatial and temporal variation of macroinvertebrates diversity in the Sand River.

## 4.3 NULL HYPOTHESES

I. Macroinvertebrate communities of the Sand River are not affected changes in water quality.

II. There is no spatial and temporal variation in macroinvertebrates abundances in the Sand River.

III. There is no spatial and temporal variation in macroinvertebrate diversity in the Sand River.

## 4.4 MATERIAL AND METHODS

### 4.4.1 Macroinvertebrate assemblage

Macroinvertebrates samples were collected at different sites along the Sand River. Samples were collected at site 1and 2, upstream of the effluent discharge point and site 3 to 8 downstream (Figure 3.1: Chapter 3). The samples were collected in replicates during the dry season (February, April and May 2012) and rainy season (August, October and November, 2012).

### 4.4.2 Determination of SSAS, diversity and ASPT scores

Macroinvertebrates were collected according to the SASS-5 (South African Scoring System, Version 5) sampling protocol (Dickens and Graham 2002) at each sampling site along the Sand River. Approximately 20 m to 25 m of river length were sampled at each site. Kick and sweep sampling was undertaken, using a hand net of 2 mm mesh size (Figure 4.1). Samples were collected and analysed separately from the three pre-defined SASS-5 biotope groups (i.e. stones in current; marginal and aquatic vegetation and sand in current), where present. As per the protocol, stones-incurrent (SIC) were sampled for two minutes, and gravel, sand and mud (GSM) for a total of one minute, while 2 m of marginal vegetation were sampled for two minutes. Sampling of each biotope was conducted over the whole sampling area available. At each sampling site, macroinvertebrate samples collected from each of the biotope groups were placed in separate sampling trays for sorting and identification. Plastic, white-coloured trays, approximately 30 cm by 45 cm in size with a depth of 10 cm, were used. After adding river water from the site to each tray, and carefully removing debris, the macroinvertebrates collected from each biotope group were added to 5 L buckets and preserved with 10% formalin and identified at the laboratory. In the laboratory, uncertain family-level identifications were confirmed using optical microscopes. Identifications were undertaken to the family level, using a photographically illustrated identification guide (Gerber and Gabriel 2002.) for aquatic invertebrates of South African rivers.

Species diversity at each was calculated using the Shannon-Wiener diversity index.
The Shannon-Wiener Diversity Index (Shannon Wiener, 1949), H´, was calculated using the following equation:

$$H' = \frac{n \log n - \sum (fi \log fi)}{n}$$

where:

H' = Shannon's diversity index

n = total number of frequencies

fi = frequency of occurrence

The SASS-5 Score, Number of Taxa and Average Score per Taxon (ASPT), none of which take the abundance estimates into account, were calculated for each biotope group present at a sampling site and for all the biotope groups combined. Scoring of the macroinvertebrates was done for 15 minutes, but discontinued if no new species were found. The Sand River falls under eco-region level 1 and the SASS scores and ASPT were interpreted based on the biological band classification of this (Table 4.1):

**Table 4.1**: Ecological Categories (A-F) that were used to describe Sand River site water quality conditions for Eco region level 1 (Dallas, 2007).

| Biological Band | Water quality category name | Description Range of SASS5 Scores | Range of ASPT values |
|---|---|---|---|
| A | Natural Unmodified | 149 – 180 | 7.1-8 |
| B | Good largely natural with few modifications | 100 - 148.9 | 6.0-7.0 |
| C | Fair moderately modified | 82 - 99.9 | 5.4-5.9 |
| D | Poor largely modified | 63 - 81.9 | 5.1 - 5.3 |
| E/F | Seriously/ critically modified | < 62.9 | < 5 |

**Figure 4.1**: Sampling on site using a hand net of 2 mm mesh size.

### 4.4.3 Statistical analysis

Canonical correspondence analysis (CCA) was used to analyze macroinvertebrate and water quality relationship in order to identify environmental factors potentially influencing macroinvertebrate assemblages. A Monte Carlo permutation test was performed to assess the statistical significance of the environmental parameters (Heino, 2000). The environmental factors used were pH, water temperature, dissolved oxygen and conductivity salinity, flow and depth. CCA was carried out on macroinvertebrates collected at the sites over the sampling period. Taxa with less than 10 individuals were removed from the analysis, which was carried out using the CANOCO 5 program (Ter Braak and Šmilauer, 2012). Hierarchal Cluster analysis was used to group the different sites of the Sand River in relation to the macroinvertebrate abundances to enable the verification of differences in assemblage composition (IBM SPSS version 20.0). Significant differences ($P<0.05$) in the SASS5 scores, average score per taxon (ASPT), Shanon Weiner diversity indices between sampling sites were tested using Kruskal-Wallis multiple comparison. The Kruskal-Wallis test was conducted using the SPSS version 20.0. A regression analysis was done on the SASS scores and in relation to macroinvertebrate diversity.

## 4.5 RESULTS

### 4.5.1 Macroinvertebrate assemblage

During the dry season the density of macroinvertebrates was 6300.54/$m^2$ (Table 2). The macroinvertebrate community was dominated by Chironomidae at the sites after the discharge of effluent (site 3 and site 4) (Table 4.2). Potamonautidae and Baetidae dominated the macroinvertebrate fauna before discharge (site 1 and 2). At site 5 Simulidae and Dytiscidae dominated the macroinvertebrate fauna. Simulidae dominated the macroinvertebrate fauna at site 6. Simulidae and Hydrophilidae were the dominant families at site 7. There was no obvious dominant family at site 8 (Table 4.2). During the rainy season, Chiromidae still dominated the macroinvertebrate community after the discharge of effluent (Table 4.3). Baetidae and Simulidae dominated the macroinvertebrate community before discharge.

Cluster analysis divided the macroinvertebrate communities into two groups during the dry season (Figure 3.2). Site 4 and 5 form their own cluster whilst site 1, 2, 3, 6, 7 and 8 form another cluster. During the rainy season site 3, 4 and 5 formed their own cluster. On the other hand site 2, 6, 7 and 8 formed another cluster (Figure 3.3).

**Table 4.2**: Macroinvertebrate abundances along the different sites of the Sand River during the dry season (number/$m^2$).

| Family | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 |
|---|---|---|---|---|---|---|---|---|
| Aeshnidae | 1.00 | 9.00 | 0.25 | 3.50 | 67.20 | 0.00 | 0.00 | 0.00 |
| Ancylidae | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Baetidae | 24.40 | 55.80 | 6.50 | 16.00 | 2.00 | 0.00 | 0.00 | 0.00 |
| Belastomatidae | 1.75 | 1.00 | 21.25 | 17.25 | 0.00 | 0.00 | 0.00 | 0.00 |
| Calopterygrionidae | 0.00 | 53.66 | 0.00 | 0.00 | 13.33 | 0.66 | 29.00 | 36.33 |
| Chaoborus | 2.00 | 11.40 | 44.50 | 56.40 | 74.80 | 3.60 | 7.00 | 5.20 |
| chironomidae | 6.20 | 31.80 | 894.40 | 1876.20 | 0.40 | 1.60 | 4.20 | 1.20 |
| Chlorolestidae | 0.00 | 0.00 | 1.00 | 0.00 | 3.00 | 5.00 | 41.00 | 10.00 |
| Cilucidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5.00 | 0.00 |
| Coenagrionidae | 4.80 | 17.60 | 7.00 | 9.25 | 1.40 | 2.40 | 17.60 | 0.50 |
| Corixidae | 0.00 | 0.00 | 0.00 | 0.00 | 7.00 | 0.00 | 0.00 | 0.00 |
| Dytiscidae | 0.00 | 0.00 | 1.00 | 0.00 | 459.00 | 0.00 | 0.00 | 0.00 |
| Gerridae | 2.66 | 2.33 | 0.00 | 0.00 | 23.00 | 8.33 | 2.00 | 0.00 |
| Gomphidae | 0.00 | 3.50 | 1.50 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Gyrinidae | 0.00 | 0.00 | 0.66 | 0.00 | 0.33 | 0.00 | 0.33 | 0.00 |
| Helodidae | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Hirunidae | 0.50 | 0.00 | 86.20 | 5.50 | 2.20 | 10.40 | 35.60 | 0.00 |
| Hydraenidae | 0.00 | 0.00 | 0.50 | 0.50 | 0.50 | 5.50 | 22.50 | 0.00 |
| Hydrophilidae | 0.00 | 0.33 | 0.33 | 0.00 | 6.66 | 11.33 | 208.33 | 0.00 |
| Lymnaedidae | 2.00 | 0.00 | 0.00 | 3.00 | 21.00 | 6.00 | 0.00 | 0.00 |
| Nepidae | 0.00 | 0.00 | 1.00 | 0.00 | 3.50 | 5.50 | 1.00 | 0.00 |
| Notonectidae | 0.66 | 3.50 | 2.33 | 9.33 | 0.00 | 0.00 | 0.00 | 0.00 |
| Oligochaeta | 11.2 | 29.60 | 38.80 | 62.00 | 0.20 | 1.40 | 0.60 | 0.60 |
| Oligonueridae | 0.50 | 0.00 | 0.00 | 0.00 | 15.00 | 10.00 | 12.50 | 1.50 |
| Physidae | 0.50 | 0.25 | 0.00 | 0.50 | 0.00 | 1.00 | 2.40 | 23.0 |
| Potamonautidae | 32.00 | 4.40 | 5.00 | 2.40 | 4.50 | 0.50 | 20.66 | 0.00 |
| Pyrulide | 0.00 | 0.00 | 0.00 | 0.00 | 45.00 | 0.00 | 47.00 | 0.00 |
| Simulidae | 21.2 | 0.00 | 0.00 | 86.50 | 579.66 | 173.5 | 443.50 | 0.00 |
| Syrphidae | 0.00 | 0.00 | 0.00 | 0.00 | 26.00 | 2.00 | 3.00 | 0.00 |
| Teloganodidae | 4.50 | 0.00 | 0.00 | 0.00 | 19.00 | 52.00 | 2.50 | 0.00 |
| Thiaridae | 0.50 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 36.00 | 0.00 |

**Table 4.3**: Macroinvertebrate abundance along the different sites of the Sand River during the rainy season (number/$m^2$).

| Family | site 1 | site 2 | site 3 | site 4 | site 5 | site 6 | site 7 | site 8 |
|---|---|---|---|---|---|---|---|---|
| Aeshnidae | 2.00 | 9.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Baetidae | 114.00 | 137.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 2.00 |
| Belastomatidae | 0.00 | 3.00 | 0.00 | 0.00 | 1.00 | 7.00 | 0.00 | 0.00 |
| Chaoborus | 2.00 | 7.00 | 41.33 | 81.33 | 67.00 | 48.66 | 4.50 | 6.00 |
| Chironomidae | 30.66 | 61.00 | 1213.33 | 1587.00 | 1578.50 | 537.00 | 355.33 | 21.50 |
| Coenagrionidae | 6.00 | 26.33 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Corixidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 6.00 |
| Culicidae | 4.00 | 1.00 | 0.00 | 2.00 | 631.00 | 39.33 | 0.00 | 5.00 |
| Gerridae | 4.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Gomphidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 |
| Hirunidae | 0.00 | 0.00 | 3.00 | 0.00 | 2.00 | 0.00 | 0.00 | 1.00 |
| Hidrophilidae | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 5.00 |
| Muscidae | 2.00 | 2.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| Notonectidae | 7.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Oligochaetae | 37.00 | 111.00 | 75.33 | 63.33 | 142.50 | 156.33 | 17.50 | 2.50 |
| Physidae | 0.00 | 27.00 | 0.00 | 0.00 | 1.66 | 12.00 | 81.00 | 20.50 |
| Potamonautidae | 6.00 | 4.66 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 |
| Pyralidae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| Simulidae | 216.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Syphiridae | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |

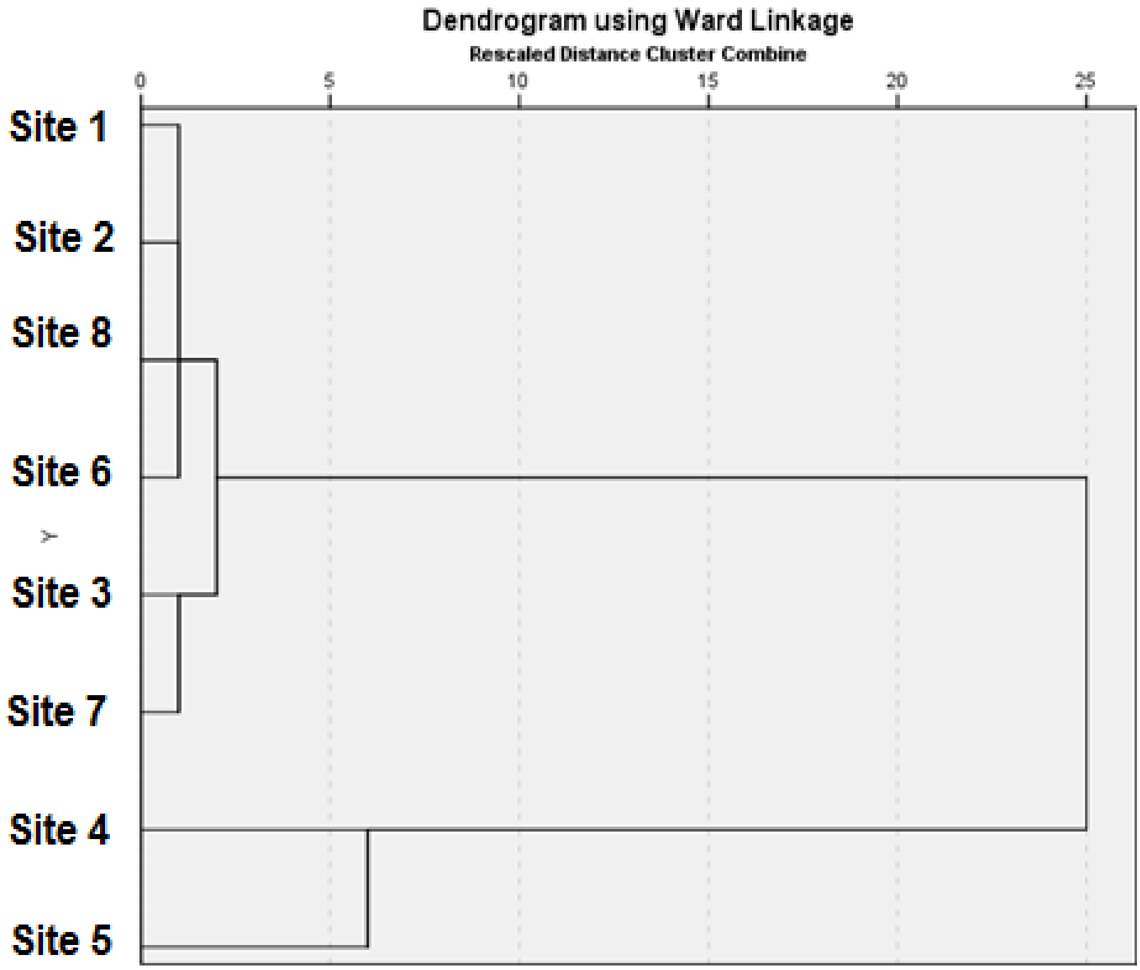


**Figure 4.2**: Dendrogram showing sampling sites (Y-axis) clusters of macroinvertebrate families of the Sand River during the dry season.

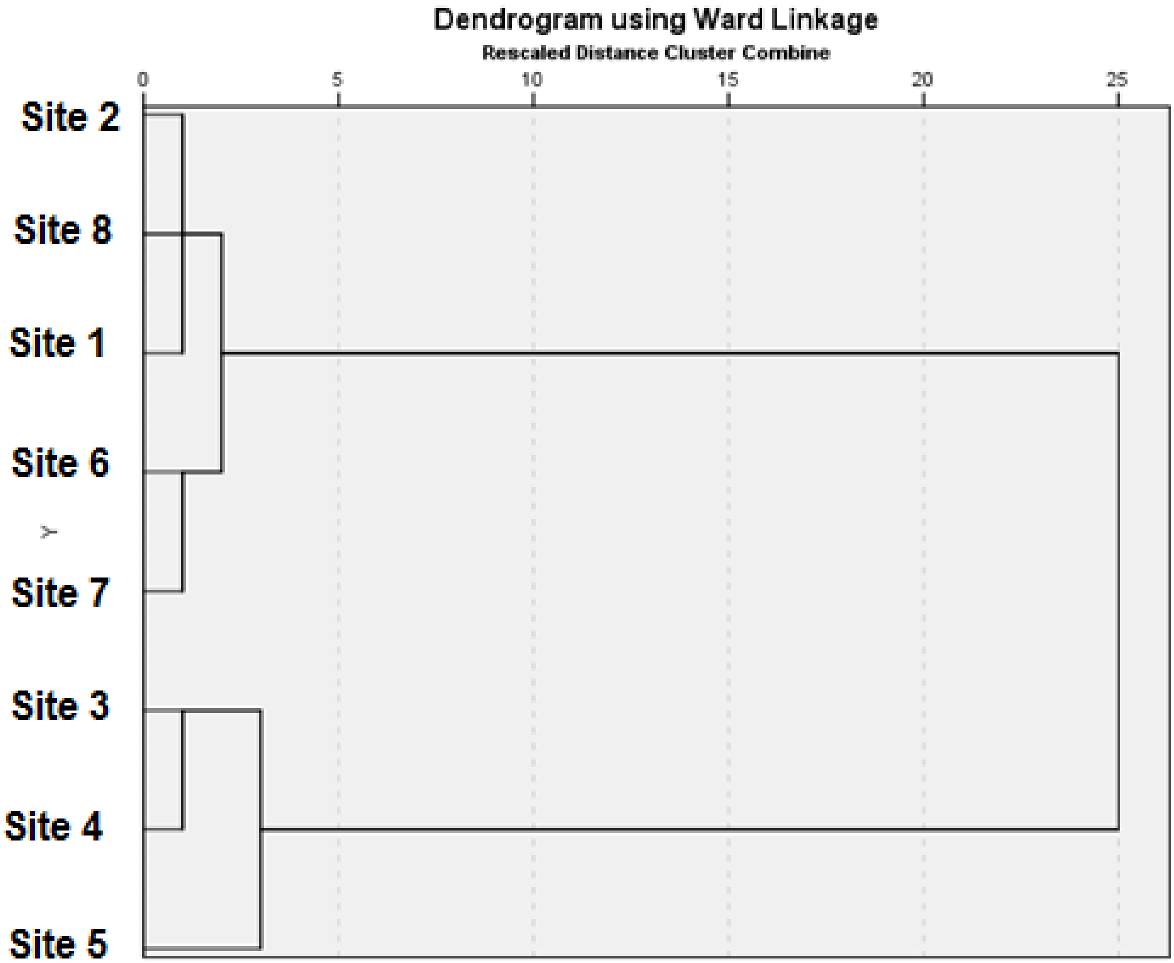


**Figure 4.3**: Dendrogram showing sampling sites (Y-axis) clusters of macroinvertebrate families of the Sand River during the rainy season.

### 4.5.2 Canonical correspondence analysis of macroinvertebrate and water quality parameters during the dry season

Canonical correspondence analysis axes 1 and 2 accounted for 56.6 % of the variation in macroinvertebrate assemblage (Table 4.4). These two axes were therefore used for data interpretation for the dry season. Canonical correspondence analysis axis 1 explained 29.79% of the total variance of macroinvertebrates distribution (Table 4.4). Total nitrogen, total phosphorus, temperature and conductivity were correlated with axis 1 (Table 4.5). Chironomidae were found on the total nitrogen gradient but they were also close to the centre of the ordination. Hirunidae and Belastomatidae were found on the temperature gradient (Figure 4.4). Coenagrionidae distribution was not defined by any gradient, as it was found at the centre of the ordination. Aeshnidae, Baetidae and Potamonautidae were not found on any of the water quality parameter gradients (Figure 4.4). Canonical correspondence analysis axis 2 explained 26.70% of the total variance of macroinvertebrate distribution (Table 4.4). Dissolved oxygen was correlated to this axis (Table 4.5).

**Table 4.4**: Canonical correspondence analysis of water quality parameters and macroinvertebrates.

| Axis | Eigenvalues | Pseudo-canonical correlations | Cumulative percentage variance of response data |
|---|---|---|---|
| 1 | 0.073 | 1.000 | 29.8 |
| 2 | 0.065 | 1.000 | 56.5 |
| 3 | 0.047 | 1.000 | 75.6 |
| 4 | 0.037 | 1.000 | 90.9 |

**Table 4.5**: Canonical correspondence analysis (CCA) based on the physico-chemical parameters macroinvertebrate data collected during the dry season.

| Parameter | Axis1 | Axis 2 |
|---|---|---|
| Temperature | -0.85 | -0.03 |
| Conductivity | -0.73 | -0.24 |
| Salinity | -0.43 | 0.14 |
| Oxygen | -0.05 | -0.74 |
| PH | 0.24 | 0.03 |
| Flow | -0.51 | 0.01 |
| Depth | 0.33 | -0.12 |
| Nitrogen | -0.76 | -0.39 |
| Phosphorous | -0.77 | -0.24 |

### 4.5.3 Canonical correspondence analysis of macroinvertebrate and water quality parameters during the rainy season

During the rainy season, canonical correspondence analysis axes 1 and 2 accounted for 55.9 % of the variation in macroinvertebrate assemblage (Table 4.6). These two axes were therefore used for data interpretation for the rainy season. Canonical correspondence analysis axis 1 explained 37.1% of the total variance of macroinvertebrate distribution (Table 4.7). Total phosphorus, total nitrogen, conductivity and salinity were correlated to axis 1 (Figure 4.5). Chironomidae was not defined by the nitrogen gradient during the rainy. However, it was close to the centre of the ordination, which indicated it was ubiquitous. Simulidae and Baetidae were not on any of the water quality gradients. Oligochaetes were found on the flow gradient.

**Table 4.6**: Canonical correspondence analysis (CCA) of water quality parameters and macroinvertebrates.

| Axis | Eigenvalues | Pseudo-canonical correlations | Cumulative percentage variance of response data |
|---|---|---|---|
| 1 | 0.397 | 1.000 | 37.1 |
| 2 | 0.201 | 1.000 | 55.9 |
| 3 | 0.170 | 1.000 | 71.8 |
| 4 | 0.143 | 1.000 | 85.2 |

**Table 4.7**: Canonical correspondence analysis (CCA) based on the physico-chemical parameters and macroinvertebrate data collected during the rainy season.

| Parameter | Axis 1 | Axis 2 |
|---|---|---|
| Temperature | -0.06 | -0.87 |
| Conductivity | -0.96 | 0.08 |
| Salinity | -0.97 | 0.10 |
| DO | 0.12 | -0.08 |
| PH | 0.33 | -0.25 |
| Flow | -0.63 | -0.30 |
| Depth | 0.22 | -0.46 |
| Phosphorus | -0.92 | 0.18 |
| Nitrogen | -0.89 | -0.13 |

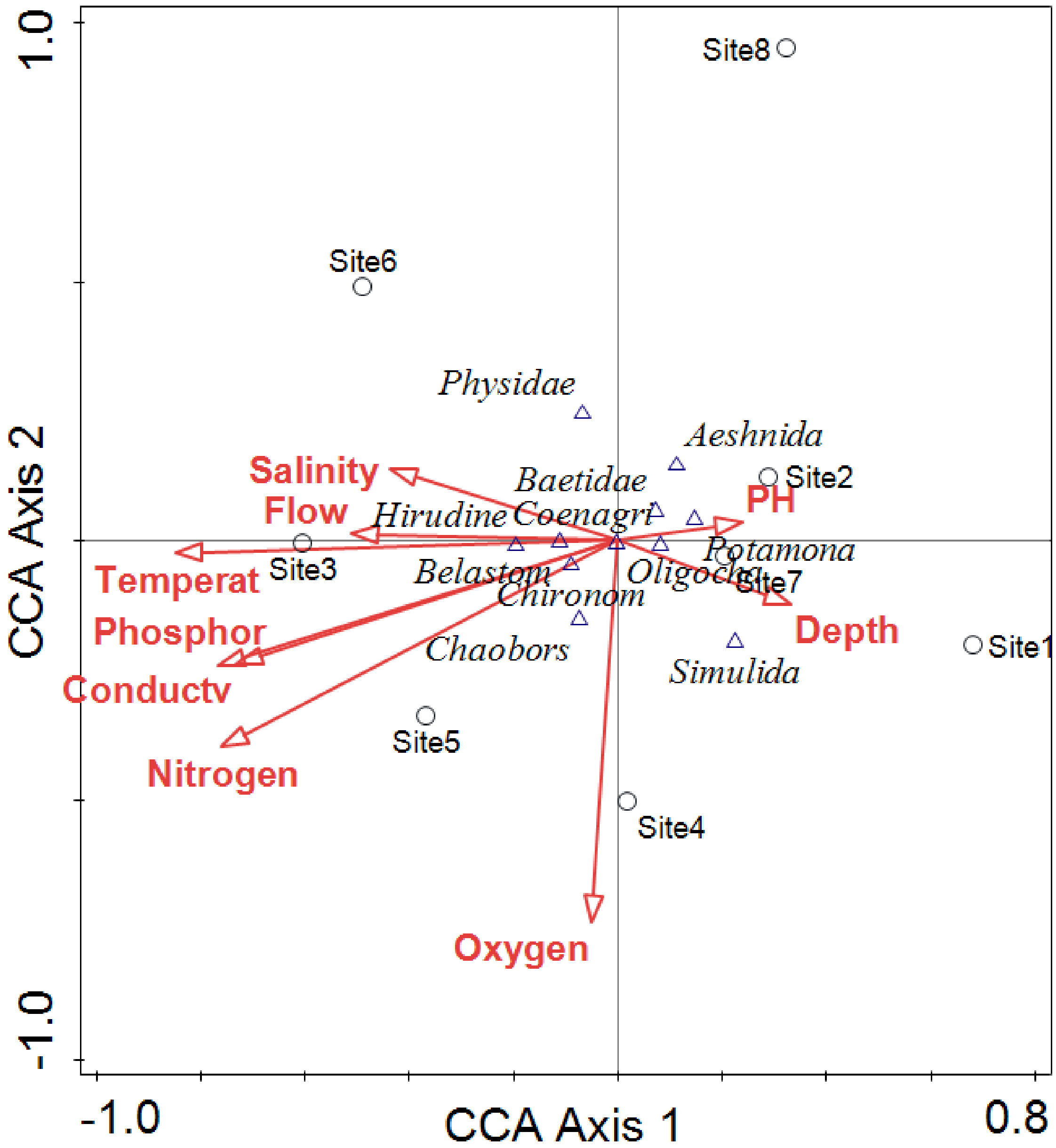


**Figure 4.4**: Triplot of Canonical correspondence analysis (CCA) depicting the relation between the physico-chemical parameters (arrows), macroinvertebrates (triangles) and sampling sites (circles) during the dry season. (Temperat - temperature; Phosphor - phosphorus; Conductv - conductivity; Aeshnida - Aeshnidae; Potamona - Potamonautidae; Oligocha - Oligochaeta; Hirudine - Hirunidae; Belastom - Belastomatidae; Chironom - Chironomidae; Chaobors - Chaoborus; Simulida - Similidae).

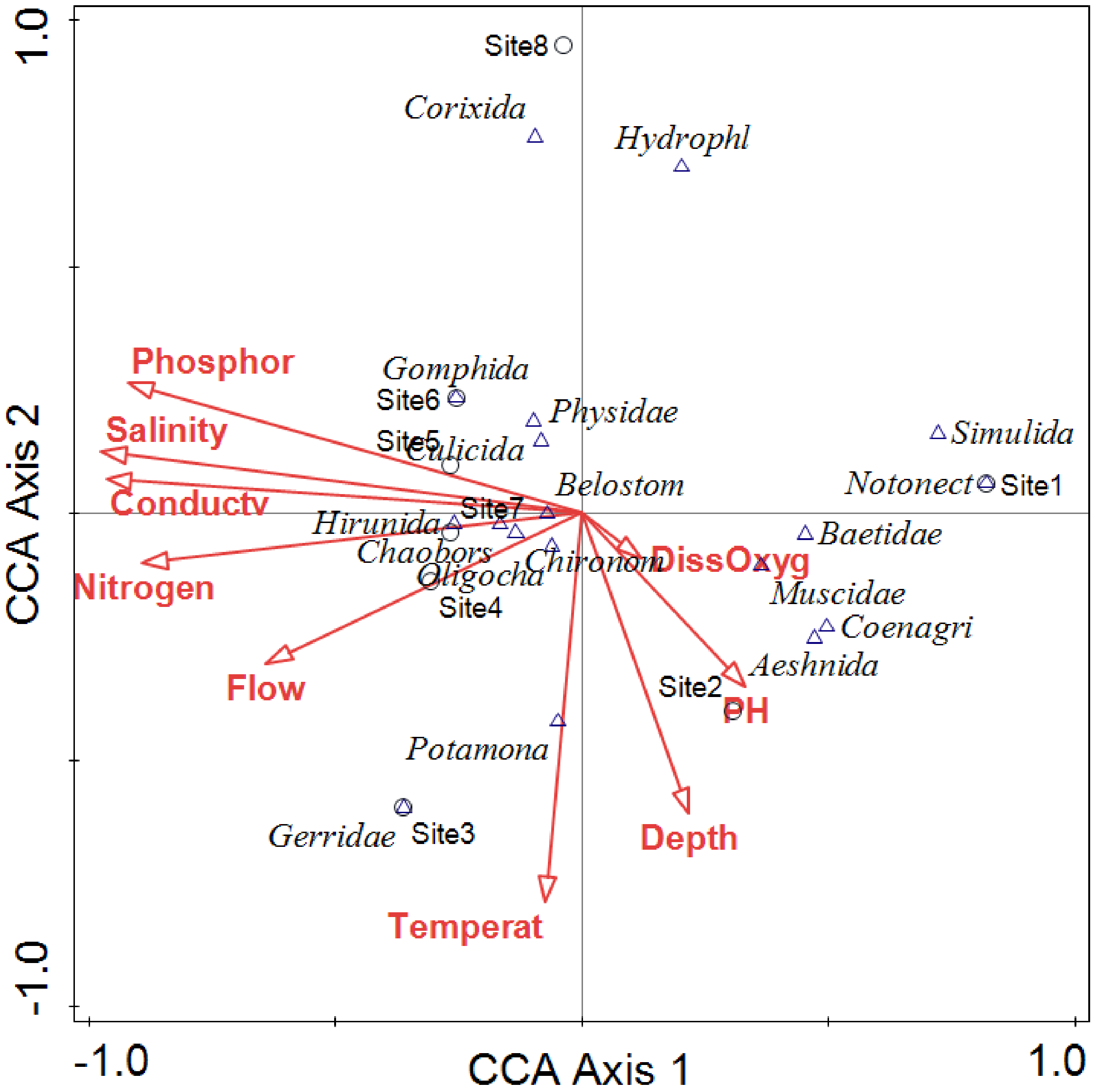


**Figure 4.5**: Triplot of Canonical correspondence analysis (CCA) depicting the relation between the physico-chemical parameters (arrows), macroinvertebrates (triangles) and sampling sites (circles) during the rainy season. (Temperat - temperature; Phosphor - phosphorus; Conductv - conductivity; Aeshnida - Aeshnidae; Potamona - Potamonautidae; Oligocha - Oligochaeta; Hirudine - Hirunidae; Belastom - Belastomatidae; Chironom - Chironomidae; Chaobors - Chaoborus; Simulida - Similidae; Gomphida - Gomphidae; Culicida - Culicidae; Notonec - Notonectidae).

### 4.5.4 Diversity, evenness, South African scoring system scores and average score per taxon of macroinvertebrate during the dry season.

The Shannon Weiner diversity index was significantly higher at site 8, 1 and 2 ($P<0.05$, Table 4.8). ASPT scores were highest at upstream at site one, however the ASPT scores were not significantly different at all sites. The SASS scores were highest at site 8, theses scores were however not significantly different ($P>0.05$).

**Table 4.8:** Diversity, evenness, South African scoring system scores and average score per taxon of macroinvertebrate during the dry season.

| | SASS (±SE) | ASPT (±SE) | H' (±SE) |
|---|---|---|---|
| Site 1 | $47.5\pm2.32^{a}$ | $7.0\pm0.23^{a}$ | $1.63\pm1.00^{a}$ |
| Site 2 | $48.0\pm0.01^{a}$ | $4.7\pm1.29^{a}$ | $1.21\pm0.23^{a}$ |
| Site 3 | $29.0\pm4.12^{a}$ | $4.3\pm0.00^{a}$ | $0.69\pm0.05^{a}$ |
| Site 4 | $28.0\pm0.03^{a}$ | $4.7\pm1.03^{a}$ | $0.57\pm0.00^{b}$ |
| Site 5 | $32.0\pm0.02^{a}$ | $3.6\pm0.96^{a}$ | $0.52\pm0.01^{b}$ |
| Site 6 | $28.5\pm2.00^{a}$ | $4.1\pm1.00^{a}$ | $1.06\pm0.01^{c}$ |
| Site 7 | $50.0\pm1.23^{a}$ | $6.8\pm0.36^{a}$ | $1.08\pm0.02^{c}$ |
| Site 8 | $56.52\pm0.12^{a}$ | $5.4\pm2.13^{a}$ | $2.11\pm0.02^{a}$ |

SASS = South African Scoring System, ASPT = Average Score Per Taxon, H' = Shannon Weiner Diversity index.

### 4.5.5 Diversity, South African scoring system scores and average score per taxon of macroinvertebrate during the rainy season.

During the rainy season the Shannon Weiner diversity index was highest at site 1, 8 and site 2. However there was no statistical significant difference between the sites ($P>0.05$). ASPT score were lowest after effluent discharge (Site 3, 4 and 6). The ASPT scores started rising at site 7. The upstream sites had higher ASPT scores.

**Table 4.9:** Diversity, evenness, South African scoring system scores and average score per taxon of macroinvertebrate during the rainy season.

| Site | SASS (±SE) | ASPT (±SE) | H' (±SE) | J' (±SE) |
|---|---|---|---|---|
| 1 | 44.0±1.54[a] | 6.2±0.00[a] | 1.39±0.10[a] | 1.25±0.03[a] |
| 2 | 32.3±3.69[a] | 5.0±0.10[a] | 1.28±0.05[a] | 1.15±0.01[a] |
| 3 | 6.7±1.00[b] | 3.3±0.12[a] | 0.34±0.02[a] | 0.32±0.00[a] |
| 4 | 7.0±0.00[b] | 3.1±0.01[a] | 0.49±0.01[a] | 0.47±0.02[a] |
| 5 | 17.7±0.01[a] | 2.9±0.00[a] | 0.39±0.02[a] | 0.37±0.00[a] |
| 6 | 18.7±1.23[a] | 3.3±0.00[a] | 0.95±0.01[a] | 0.86±0.23[a] |
| 7 | 20.0±0.01[a] | 5.2±0.01[a] | 0.47±0.03[a] | 0.41±0.04[a] |
| 8 | 30.7±0.02[a] | 4.7±0.00[a] | 1.35±0.13[a] | 1.13±0.39[a] |

SASS = South African Scoring System, ASPT = Average Score Per Taxon, H' = Shannon Weiner Diversity index.

### 4.5.6 Linear regression between SASS and abundance during the dry season

The relationship between the SASS scores and the macroinvertebrate abundance during the dry season was weak, with a correlation coefficient of $R^2 = 0.41$. The relationship was described by the equation; SASS score = 47.88 – 0.01abundance (Figure4.6).

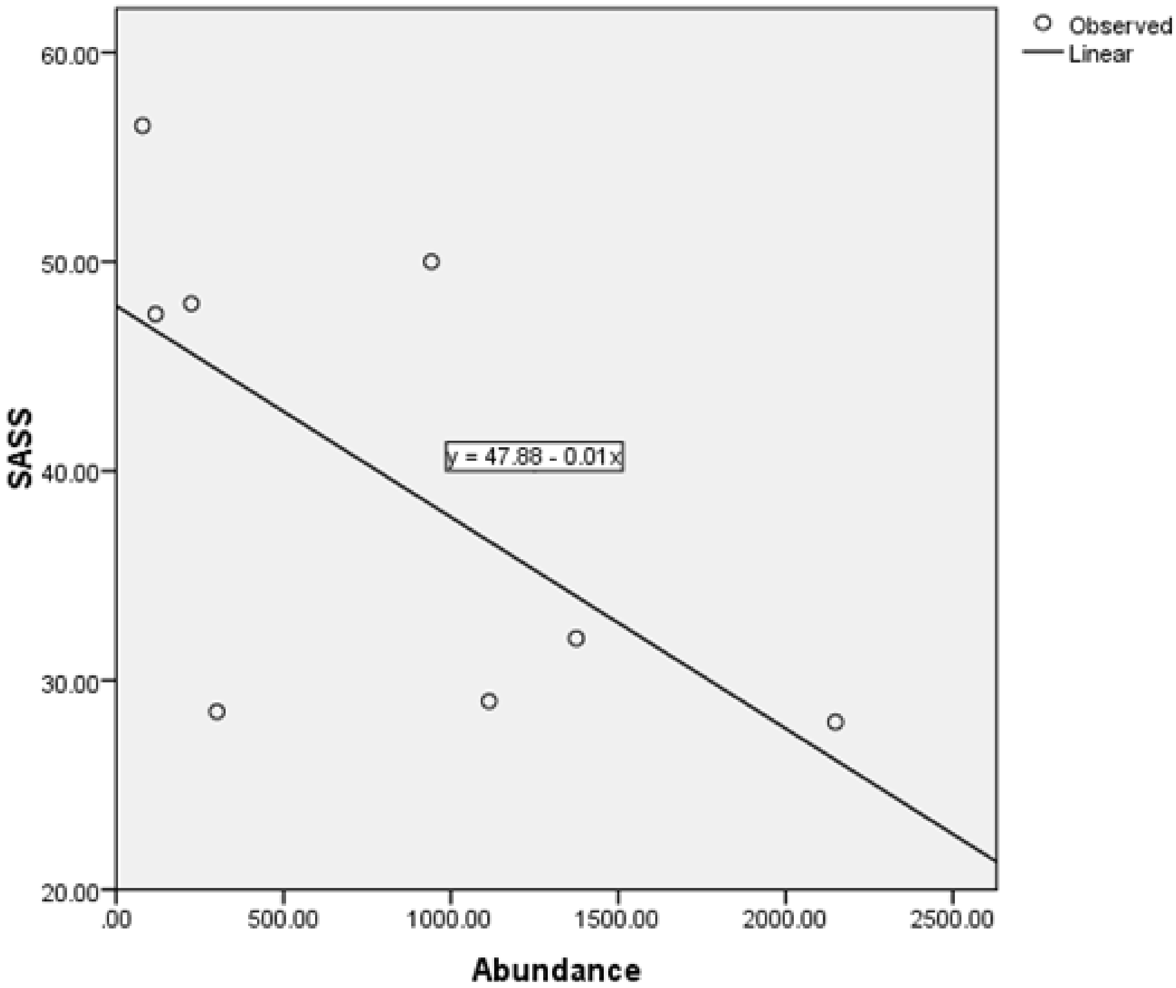


**Figure 4.6**: The linear regression of the abundance on the SASS scores during the dry season.

### 4.5.7 Linear regression between SASS and abundance during the rainy season

The relationship of the SASS scores and the macroinvertebrate abundance during the rainy season was also weak ($R^2$ = 0.41; Figure 7). The equation; SASS score = 32.07 – 0.01abundance adequately described the relationship (Figure 4.7).

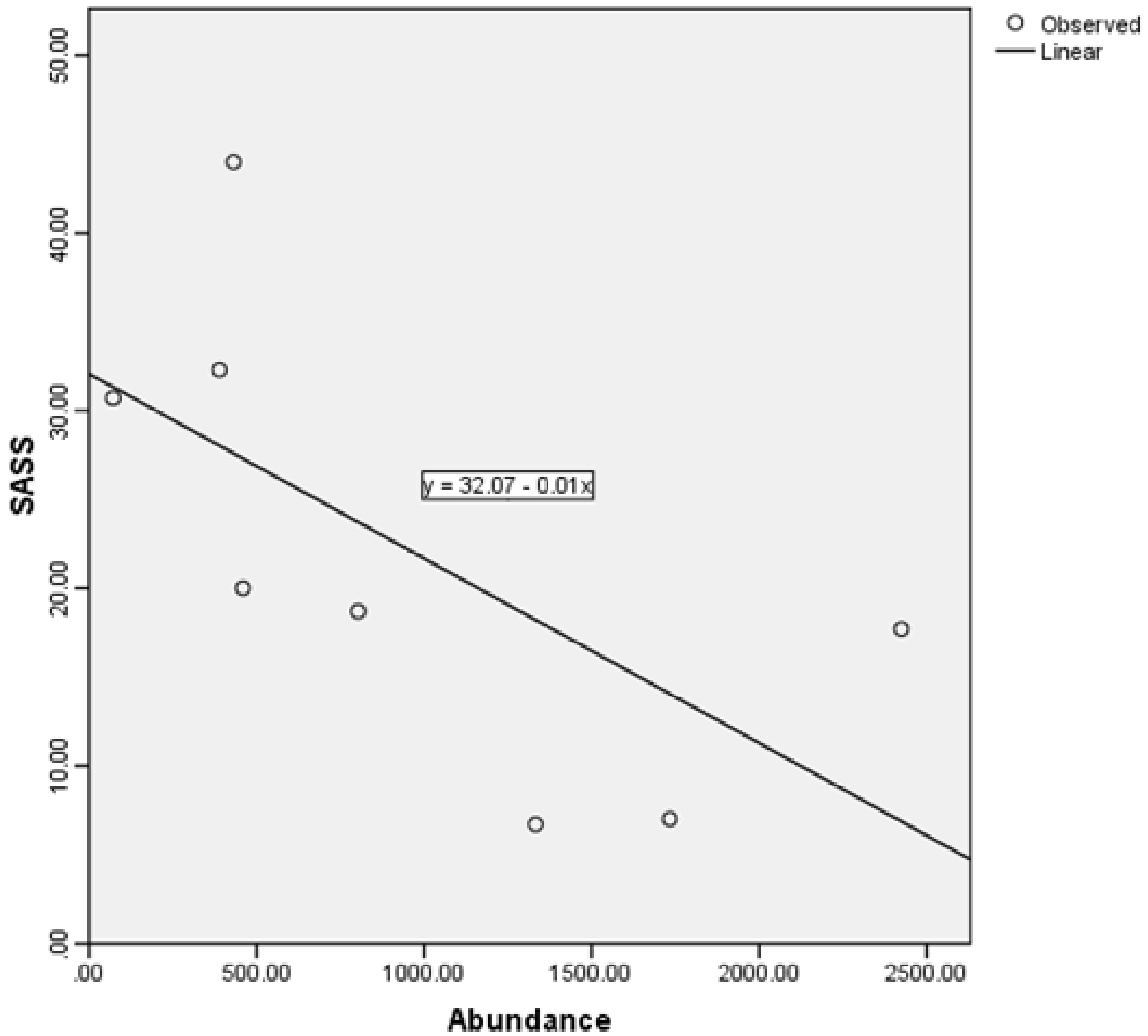


**Figure 4.7**: The linear regression of the abundance on the SASS scores during the rainy season.

**4.5.8 Linear regression between SASS and diversity during the dry season**

There was a significant correlation ($R^2$ = 0.69) between SASS scores and macroinvertebrate diversity during the dry season (P<0.05). The relationship between SASS scores and macroinvertebrate diversity was adequately described by the equation; SASS score = 20.25 + 17.76H' (Figure4.8).

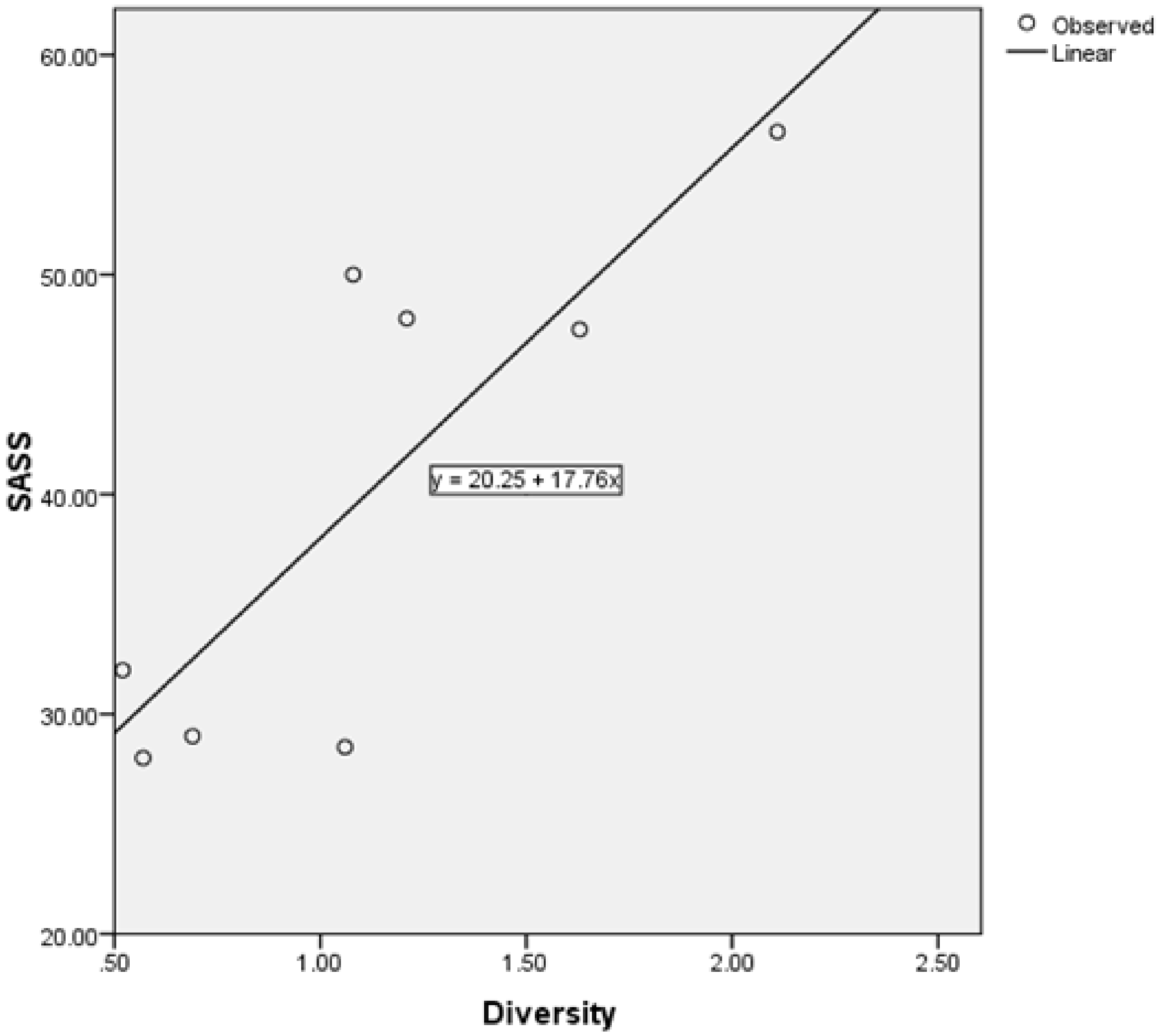


**Figure 4.8**: Linear regression of SASS scores and macroinvertebrate diversity index during the dry season.

### 4.5.9 Linear regression between SASS and diversity during the rainy season

During the rainy season, the correlation coefficient ($R^2$ = 0.77) between the SASS scores and macroinvertebrate diversity was also significant ($P<0.05$). The relationship was described by the equation; SASS score = 1.72 + 24.52H' (Figure 4.8).

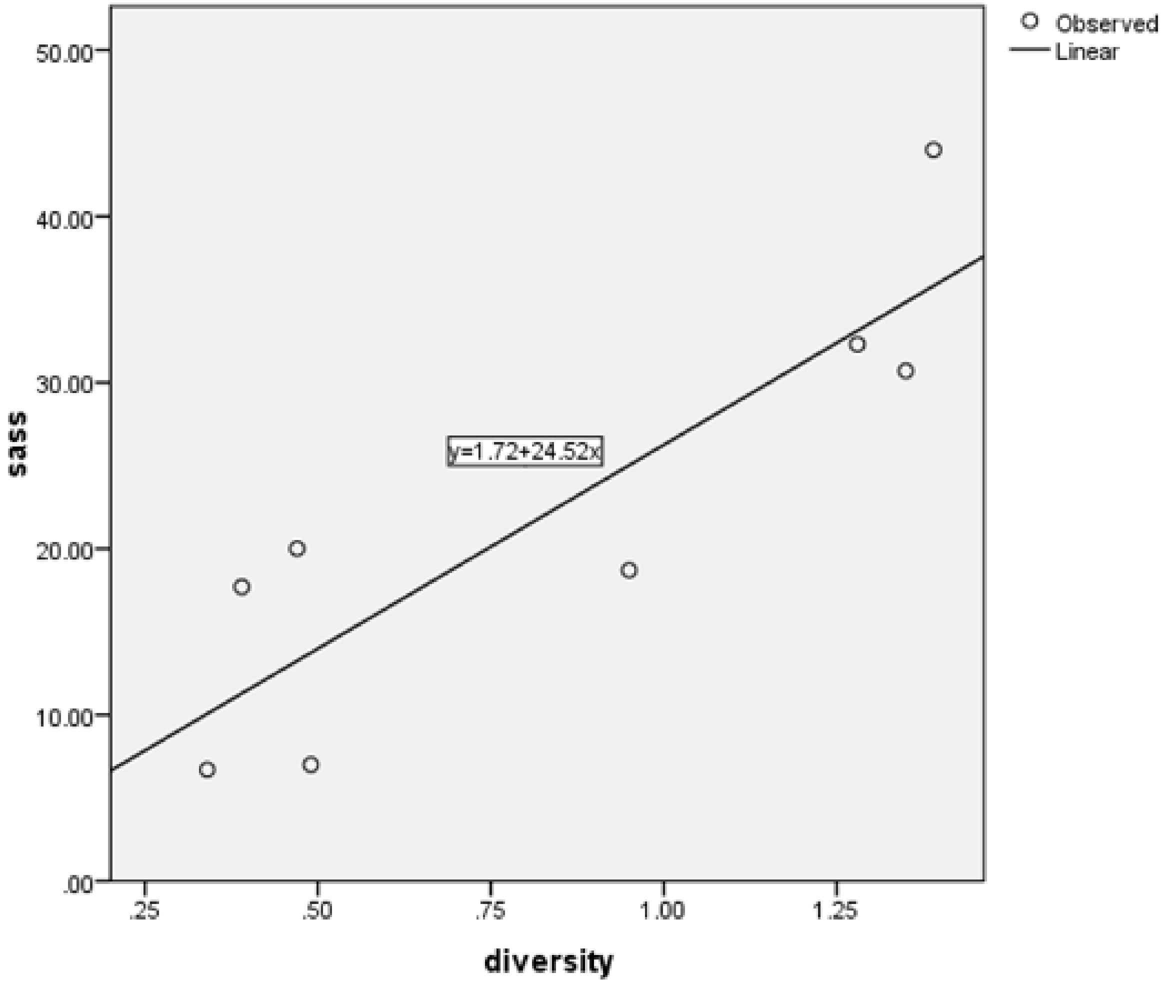


**Figure 4.9**: Linear regression of SASS scores and macroinvertebrate diversity index during the rainy season.

Some of the macroinvertebrate families recorded at the sites are shown in Figure 4.10a, b and c.

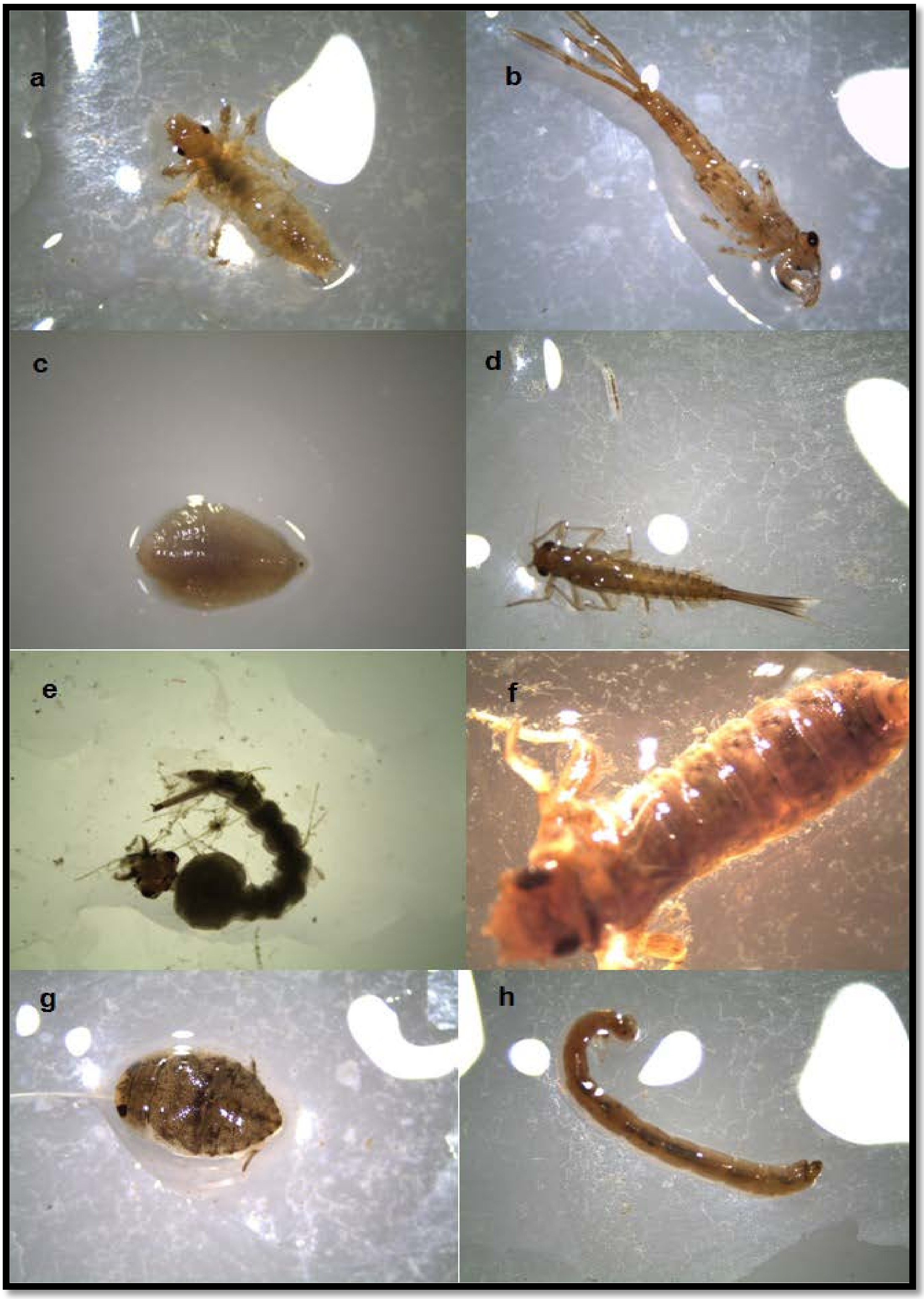


**Figure 4.10a**: Illustrations of some of the macroinvertebrates families found at the Sand River sites. (a and f - Aeshnidae, b - Coenagrionidae, c - Hirunidae, d - Baetidae, e - Culiicidae, g - Belastomatidae, h - Chironomidae).

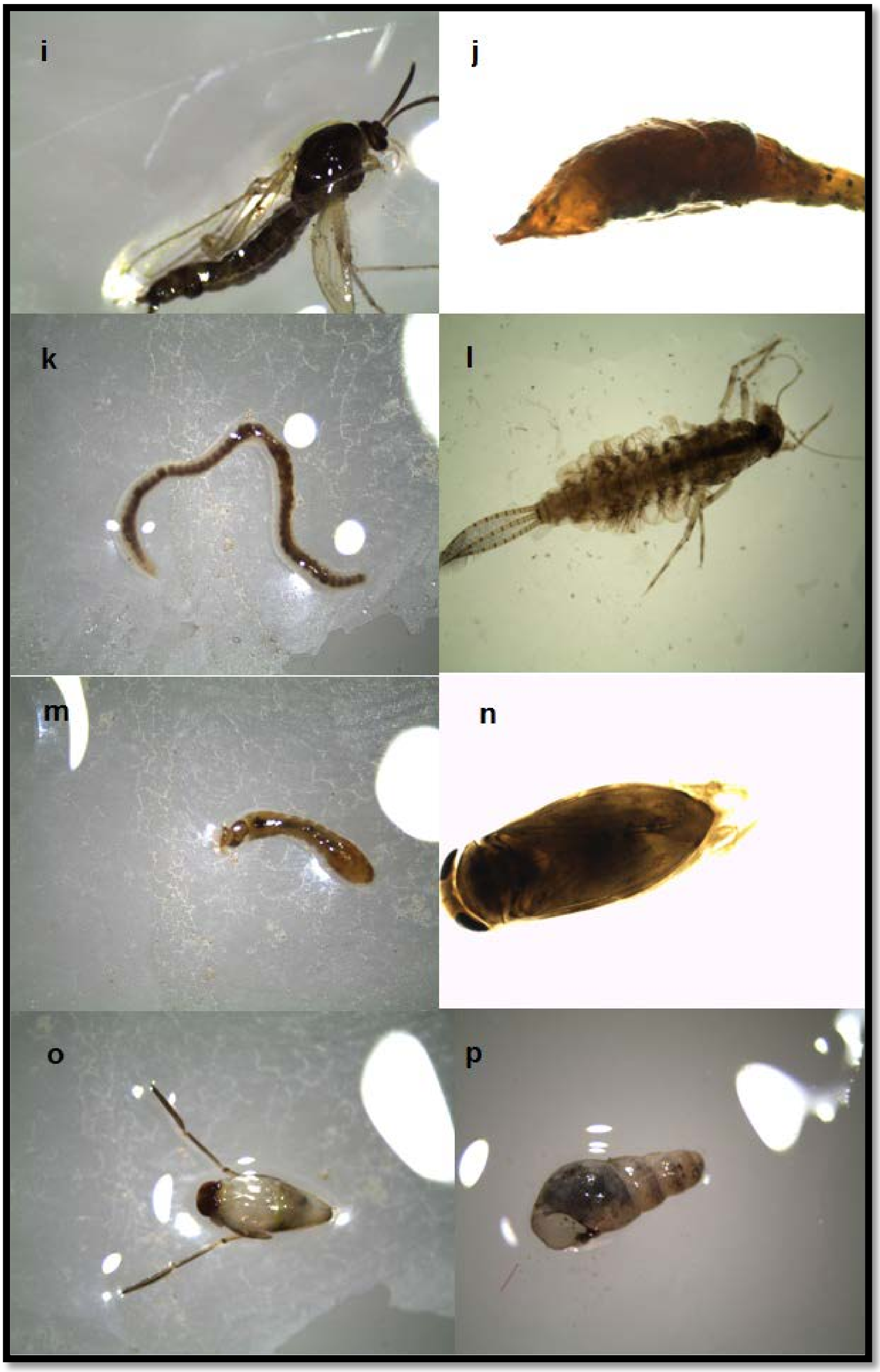


**Figure 4.10b**: Illustrations of some of the macroinvertebrates families found at the Sand River sites. (I - Chaoborus (adult), j - Muscidae, k - Oligochaeta, l - Baetidae , m - Simuliidae, n - Corixidae, o - Notonectidae, p – Thriaridae).

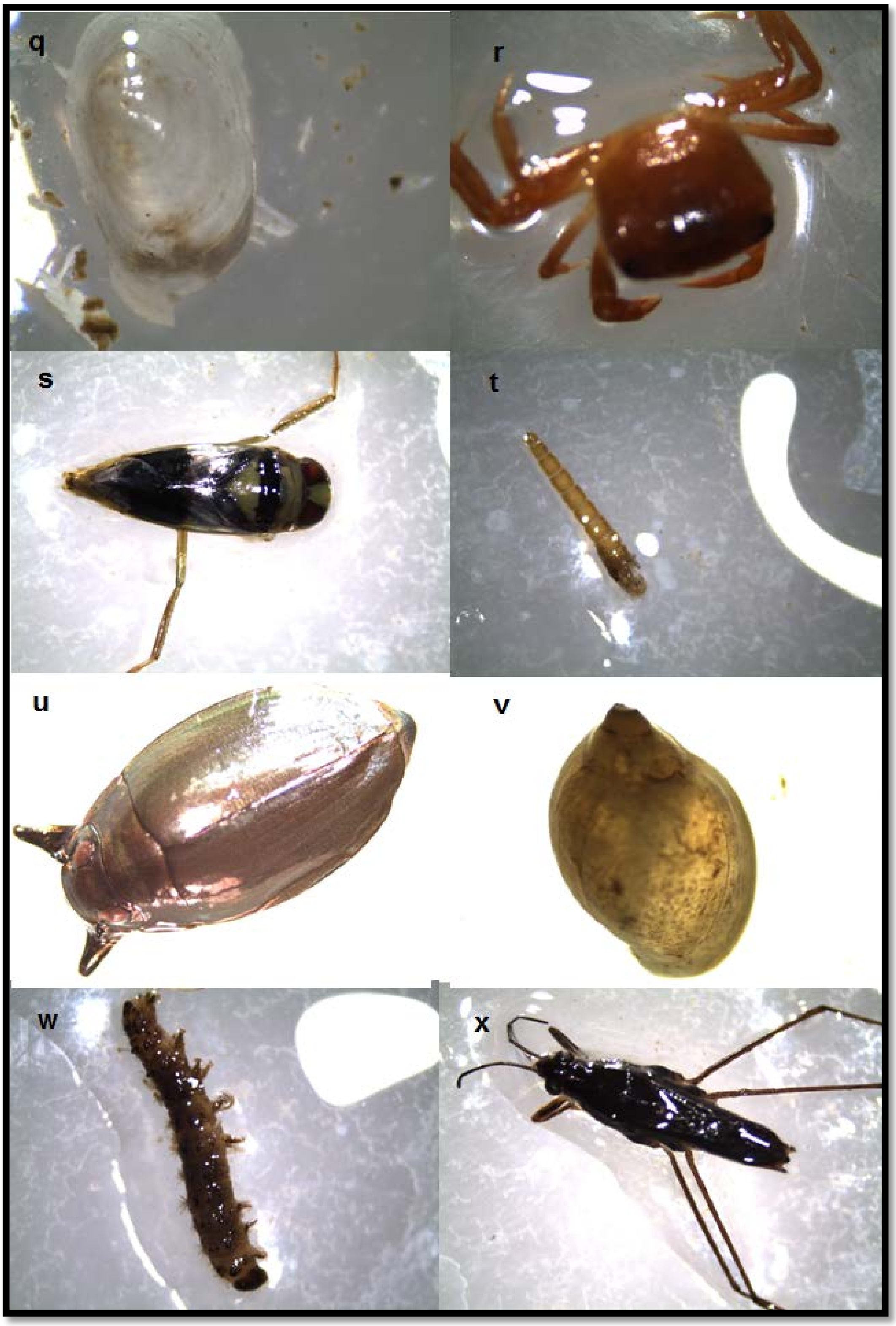


**Figure 4.10c**: Illustrations of some of the macroinvertebrates families found at the Sand River sites. (q - Sphaeriidae, r - Potamonautidae, s - Notonectidae, t - Chaoborus, u - Gyrinidae, v - Physidae, w - Pyralidae, x - Gerridae).

## 4.6 DISCUSSION

During the dry season, the cluster analysis showed that the sites after discharge had a higher density of pollution tolerant macroinvertebrates and were therefore separated from those upstream. These downstream sites were characterised by high nutrient levels and dominated by macroinvertebrates of the Chironimidae family. The upstream sites were dominated by the Baetidae family, while those further downstream were dominated by Simulidae. The main reason for the dominance of pollution tolerant species downstream is the continuous discharge of substandard sewage effluent throughout the year. Continuous loading of substandard effluent in rivers results in substantial decline of oxygen levels and results in the depletion of pollution sensitive macroinvertebrates (Parr and Manson, 2003). Nitrogen and phosphorus are the main driving factors of many aquatic ecosystems and changes in their concentrations ultimately affect macroinvertebrate abundance. Nitrogen in the form of ammonia can be very toxic to aquatic organisms, particularly at a pH of above 8. On the other hand, at pH of 6 to 7, ammonia is converted to ammonium which is not toxic to aquatic organisms.

Chironomidae are known to tolerate a wide range of pollutants (Bhattacharyay *et al*., 2006; Ghane *et al*., 2006, Luoto, 2010). Chironomus species larvae possess the oxygen transport pigment haemoglobin, which allows them to absorb dissolved oxygen more efficiently, hence their survival under harsh environmental conditons (Odume *et al*., 2012). Simulidae like Chironomidae are pollution tolerant. The presence of these macroinvertebrates at the sites downstream can be linked with the presence of organic matter. Simulidae and Chironomidae filter feed on organic matter (Abbaspour *et al*., 2013). Odume *et al*., (2012) explains that the mouth parts of Chironomidae are always in close contact with sediment and contaminates since they are detritus feeders. The family Baetidae, despite its dominance at the upstream sites, is also considered pollution tolerant. Baetidae have been known to dominate areas of poor environmental conditions and are tolerant of nutrient enrichment (Hall *et al*., 2006). The dominance of these macroinvertebrates at these sites probably indicates that the water quality at the sites is slowly deteriorating. This is a result of runoff from the urbanised area of Polokwane. A wide variety of wastes are deposited into the river through storm water drains, including animal faecal matter, suspended solids, heavy metals and solid waste from refuse dumps.

Regardless of season, the sites after sewage effluent discharge were dominated with Chiromomidae and those further downstream with Simulidae. Similar macroinvertebrate dominance during the rainy season as the dry season is probably because of the low dilution factor. This is expected because river flow is maintained mainly by sewage effluent. It is important to note that the maturation ponds overflow during the rainy season. However, the macroinvertebrate community did not change drastically because of the dilution factor. Nutrients and suspended solids may also be introduced at the downstream sites of the Sand River from surrounding farms. Cannobio *et al*. (2009) also found that during high rainfall, spillage of untreated wastewater occurred along the Laura stream (Italy), the very high diluting capability of the high flows were reduced by greater polluting loads from surface runoff.

The accumulative variation of macroinvertebrate assemblage explained by CCA axis 1 and 2, for both seasons, was low (± 55 %). This suggests low correlation between the macroinvertebrates and water quality variables. Most of the macroinvertebrates families were found close to the centre of the ordination. High variation of macroinvertebrate is usually above 70 % total variance of macroinvertebrate assemblage explained by the first two axes (Hump and Pivnicka, 2006; Benetti *et al*., 2012). Macroinvertebrate communities may have been impacted by the chlorine levels in the effluent rather than the measured water quality parameters. The However, during the dry season, the Chironomidae family was found on the total nitrogen gradient. Odume and Muller (2011) concluded that the Chironomidae family can be used to assess environmental water quality status of South African fresh water ecosystems. This was concluded after the CCA showed associations of this macroinvertebrate family with BOD, DO, EC, $PO_4$ and total nitrogen.

High macroinvertebrate diversity indices during the both seasons were recorded at the upstream sites and the sites further downstream. Lower diversity was however recorded at the sites after discharge (site 3, 4 and 5). Loss of diversity at these sites may have resulted from the elevated nitrogen levels recorded at these sites. The absence of pollution sensitive macroinvertebrates serves an indication that the Sand

River water is unsuitable for sustaining pollution sensitive aquatic organisms. Organic pollution generally reduces macroinvertebrate diversity, resulting in a community dominated by Chironomidae and Oligochaetes (Wright, 1995; Pinel-Alloul *et al*., 1996). The significantly higher diversity at the last site downstream than the sites just after discharge, may be due to reduction of nutrient along the river. This serves as an indication of river self-purification with gradual recovery of macroinvertebrates. High macroinvertebrate diversity has been reported as being reflective of diverse biological productivity and habitats (Booth, 2005). Arimoro *et al*. (2008) related high diversity at the upstream sites in a study on the Orogodo River (Nigeria) to high dissolved oxygen. On the other hand, lower diversity scores of less than 1 are indicative of high stress and poor environmental conditions (Wenn, 2008).

Chlorine is widely used in South African sewage treatment works as an oxidizing agent and disinfectant (William *et al*., 2004). The Polokwane WWTW also chlorinates its effluent prior to discharge. This may be the other reason for the loss of macroinvertebrates diversity at the sites after discharge. William *et al*. (2004) found that in both the Umsunduze and the Umbilo Rivers (KZN), chlorinated sewage effluent killed entire communities at the point of discharge, and it was only some distance downstream before the communities were restored. Hayes *et al*. (2001) found reduced macroinvertebrate downstream as a result of effluent chlorination. However, these authors found that the diversity was higher downstream than upstream of the effluent discharge point. They attributed these findings to relatively even distribution of macroinvertebrate abundances downstream, whereas the upstream sites had more taxa which were not evenly distributed.

The Sand River sites further downstream experiences reduced flow rates and at times dry up during the dry season. This is largely due to low rainfall in this region and the extensive use of this water for irrigation purposes. In such a case where the river dries up (site 8) usually has no water, until the Polokwane WWTW discharges effluent. As mentioned earlier, this site has a higher diversity of macroinvertebrates, despite it drying up from time to time. The recovery of these macroinvertebrates may be due to their life cycles and it may also be that the macroinvertebrates are now adapted to such conditions. Davies *et al*. (1994) confirmed in his finding that the biota of dry-land rivers, as in South Africa, evolved in an environment that is highly variable, particularly due to the very low conversion ratio of rainfall to runoff. These authors further indicate that the macroinvertebrates are more opportunistic than those from areas of higher rainfall. Davies *et al*. (1994) also found a wide overlap in the diet of Ephemeropteran and Trichopteran in the Buffalo River. They suggested that these macroinvertebrates have adapted to changes in their environment. Chadwick and Huryn (2007) however indicated that the intermittent stream flow, seems to negatively affect the macroinvertebrate communities. Mantel *et al*., (2010), reported that the reduction in river flow supports the dominance of macroinvertebrate from the family Baetidae and Simulidae and the reduction of macroinvertebrates from the Teloganodidae family. Cortes *et al*., (1998) explains that the dominance of Ephmeropterans where reduced river flow occurs may be a result of increase in periphyton, which most of them feed on. The CCA results in this study did not indicate any correlation of the Baetidae and Simulidae with the river flow. However, the dominance of these macroinvertebrates at some of the downstream sites suggests the availability of periphyton as a result of high nutrient levels. This therefore indicates that macroinvertebrates in the Sand River may have adapted to the intermittent flow conditions.

The ASPT scores during the dry season were highest at the first site before discharge. This indicates that this site was not critically modified and the water quality was good. There was however a decline in ASPT scores after discharge, indicating deterioration in water quality and an increase further downstream, indicating recovery of macroinvertebrates. Increase in the ASPT further downstream was in agreement with the increase in diversity. The water quality of site 7 was good, while that of site 8 was fair with moderate river modifications. During the rainy season, ASPT were highest upstream, decreased after discharge and increased further downstream. The ASPT scores however, indicated that the water quality was good only at the first upstream site, while those further downstream still indicated poor water quality with large river modification. This was further supported by the macroinvertebrate diversity as it was much lower than the dry season. The reason for lower ASPT scores during the rainy season can be because of the destruction of habitats at increased flow rate conditions. Thiere and Schulz (2004), reported ASPT scores at the upstream sites of the Lourens River (South Africa) as indicative of good water quality, with a deterioration of water quality further downstream. Mantel *et al*.

(2010) however found no difference in ASPT scores between sites of high density and those of lower density. These authors attributed this to the replacement of macroinvertebrate families with other families which were allocated the same SASS score.

The regression analysis indicated that macroinvertebrates diversity influences the SASS scores. Increase in diversity during both seasons resulted in an increase in the SASS scores. High diversity is reflective of the availability of a variety of habitats. Failure of the CCA to indicated high correlation of water quality with macroinvertebrates may indicate that much of the variation is accounted for by habitat availability. The SSAS scores takes into account the different biotopes present at a particular site. Availability of all the biotopes causes an increase in the SASS scores. Brown (2001) report that the increase in SASS score at sites further away from the discharge point may be due to a slight increase in marginal vegetation. The assessment of combined macro-habitats presented at a site, therefore provide accurate measure of the water quality (Fontoura and De Pauw, 1994). Measures of abundance may also be useful in estimating the severity of site destruction. There was some moderate correlation of the SASS scores with the macroinvertebrate abundance during both seasons. Increase in pollution tolerant macroinvertebrates resulted in a decrease in the SASS scores. However, a low SASS scores can also occur with the dominance of one pollution sensitive macroinvertebrate family. This therefore implies that measures of abundance may not significant in SASS interpretation.

Macroinvertebrate within the Sand River have indicated that sewage effluent from the Polwane WWTW is impacting on the abundance and diversity of these organisms. A decline in pollution sensitive macroinvertebrates after effluent discharge serves as an indication that the water quality conditions are outside of their preferred limits. Despite the some evidence of self-purification with regards to increased diversity further downstream. The ASPT scores indicated the first site as having good water quality for both seasons. It further indicated in the dry season that the water quality further downstream was fair with moderate river modifications. In order to restore the diversity of these indigenous aquatic fauna, it is therefore essential for the Polokwane Municipality to adhere to effluent discharge standards.

# CHAPTER 5: EVALUATION OF HEAVY METAL CONTAMINATION IN THE SAND RIVER.

## 5.1 INTRODUCTION

Heavy metal contamination of rivers passing through urban areas is a growing major concern in Africa. Industrial and domestic effluents are the significant sources of heavy metals of rivers passing through this urban areas.The main problem with heavy metal contamination in these rivers is not only the health risk associated with some of these metals but also the damage that heavy metals cause to aquatic life (Canli *et al*., 1999). Sediments are an important sink for these heavy metals and several factors affect the metal mobility between the sediment and the water. These include pH, conductivity, redox potential and bioturbation (Bryan and Langston 1992; Caussy *et al*., 2003). Metals in unpolluted sediments are mainly bound to silicates and primary minerals, and these metals are most immobile and are not biologically available to living organisms (Pardo *et al*., 1990). However, in polluted sediments the metals are mobile and bound to different phases of the sediments. Most of the metals discharged into rivers flowing through urban areas are found in bottom sediments. Remobilization of these heavy metals may be due to natural or anthropogenic factors (Akan *et al*., 2010).

Living organisms in the rivers where industrial and domestic effluent is discharged have the ability to bioaccumulate organic and inorganic substances in their tissues. Heavy metals that may be non toxic in the water may be accumulated in food chains and affect predators. Bioaccumulation is an important environmental transport process. It was observed in the Sand River that some people catch fish for personal consumption and resale. There are four possible routes for heavy metals to enter a fish, that's the gills, food, drinking water and skin (Heath, 1987). The importance of each of these routes varies but the significant factor is the availability of the heavy metal for bioaccumulation. There is no work that has been carried out at the Sand River in relation to heavy metal contamination and bioaccumulation in grass and fish.

## 5.2 OBJECTIVES

The objectives of this chapter are to determine:

I. Cadmium concentrations in water, sediment, grass and fish along the Sand River.
II. Copper concentrations in water, sediment, grass and fish along the Sand River.
III. Iron concentrations in water, sediment, grass and fish along the Sand River.
IV. Manganese concentrations in water, sediment, grass and fish along the Sand River.
V. Lead concentrations in water, sediment, grass and fish along the Sand River.
VI. Zinc concentrations in water, sediment, grass and fish along the Sand River.

## 5.3 NULL HYPOTHESES

I. There are no spatial variations in cadmium concentrations in the Sand River.
II. There are no spatial variations in copper concentrations in the Sand River.
III. There are no spatial variations in iron concentrations in the Sand River.
IV. There are no spatial variations in manganese concentrations in the Sand River.
V. There are no spatial variations in lead concentrations in the Sand River.
VI. There are no spatial variations in zinc concentrations in the Sand River.

## 5.4 MATERIALS AND METHODS

Water, sediment, grass, and fish samples were collected at different sites along the Sand River for heavy metal analysis. Samples were collected at site 1, upstream of the effluent discharge point. Site 4 (1.67 km after discharge) and sites 6 and 7 (6.60 and 10.67 km after discharge) (Figure3.1: Chapter 3). The mean concentrations of the heavy metals at the sites were regarded as the concentration of the heavy metals.

### 5.4.1 Determination of heavy metal in water samples

Polyethylene sampling bottles (250 ml) were used to collect water samples monthly from each site. The samples were taken at a depth of 10cm and stored in ice during transportation. At the Aquaculture Research Unit (ARU) Laboratory, 10 ml of 65% nitric acid was added to preserve the samples. The samples were then stored at 4°C until analysis. Samples were analysed as described by Ogoyi *et al*. (2011), where a 100ml of each sample was used. About 10ml of aqua regia $HNO_3$: HCl in the ratio of 3:1 and 1 ml of perchloric acid was added in a culture test tube.The solution was incubated at 80°C for 5 minutes. After subsequent cooling, the solution was diluted to 50ml and analysed for zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd), iron (Fe) and manganese (Mn) in a closed system using atomic absorption spectrophotometry. Heavy metal concentrations in the water were compared to the South African aquatic guidelines (Table 5.1; DWAF, 1996).

**Table 5.1**: Water Quality target for water use in Aquatic ecosystem.

| Heavy metal | Concentration (mg/l) |
|---|---|
| Cd | 0.4 |
| Cu | 1.4 |
| Fe | - |
| Mn | 180 |
| Pb | 0.01 |
| Zn | 2 |

- There is no stipulated guideline for iron for aquatic ecosystems (DWAF, 1996)

### 5.4.2 Determination of heavy metal in sediment samples

Sediment samples from each site were collected 20cm below the river bank vegetation and placed in 250 ml polyethylene bottles using a hand trowel. The samples were kept in ice during transportation to the ARU Laboratory where they were placed in a freezer until analysis. For analysis, each frozen sample was thawed separately and the dry weight determination. Each sample was dried at 105°C. The remaining samples were freeze-dried, finely crushed and homogenized using mortar and pestle, about 0.5g of the homogenized sample was digested in 10 ml aqua regia $HNO_3$: HCl in the ratio of 3:1 and 1 ml perchloric acid in a culture test tube. The mixture was then incubated in a water bath at 80°C. After total digestion and subsequent cooling, the solution was diluted to 50ml and analyzed for, copper, lead, cadmium, iron and manganese in a closed system by atomic absorption spectrophotometer as described by Ogoyi *et al*. (2011).The metal concentrations in sediment were compared to those found in granitic igneous rocks (Table 5.2; Krauskopf, 1967; Rose *et al*., 1979; Alloway *et al*., 1997)

**Table 5.2**: Metal concentration of igneous granite rocks (mg/kg) (Krauskopf, 1967; Alloway *et al*., 1997, Rose *et al*., 1979).

| Heavy metal | Concentration in Igneous granite rocks (mg/kg) |
|---|---|
| Cd | 0.01 to 0.60 |
| Cu | 0.013 |
| Fe | 50 000 |
| Mn | 400 |
| Pb | 0.024 |
| Zn | 0.052 |

### 5.4.3 Determination of heavy metal in grass (*Ischaemumfasciculatum*) samples

A stratified random sample of *Ischaemum fasciculatum* grass (Figure 5.1) samples were collected at each sampling site along the sides of the river. The grass was collected into clean transparent plastic bags, previously soaked in dilute nitric acid and thoroughly rinsed with deionised-distilled water. In the laboratory, the grass was washed carefully with distilled water and placed in the freezer (−20°C) until analysis.

Each frozen sample was thawed separately and a sampled for dry weight determination was dried at 105°C. The other portion of the samples was freeze-dried, finely crushed and homogenized using mortar and pestle, 0.5g of the homogenized sample was digested in 10 ml aqua regia $HNO_3$: HCl in the ratio of 3:1 and 1 ml perchloric acid in a culture test tube. The mixture was then incubated at 80°C in a water bath, after total digestion and allowed to cool, the solution was then diluted to 50 ml. It was then analysed for, copper, lead, cadmium, iron and manganese in a closed system by atomic absorption spectrophotometer as described by Ogoyi *et al*. (2011).

**Figure 5.1**: *Ischaemum fasciculatum* grass of the Sand River.

### 5.4.4 Determination of heavy metals in fish (*Oreochromismossambicus*) samples

*Oreochromis mossambicus* samples (Figure 5.2) were collected at all sites using a siene net (50 mm mash size). The fish samples were placed in 5L buckets. The fish were sacrificed by severing the spinal cord. Fish muscle was examined because they contribute the greatest mass of flesh that is consumed as food. Muscle tissue were excised, transferred to pre-cleaned polyethylene tubes and frozen at 20°C pending metal analyses. A similar procedure as that of the grass samples was carried out after thawing the samples.

**Figure 5.2**: *Oreochromis mossambicus* fish collected along the different sites of the Sand River.

### 5.4.5 Bioaccumulation of heavy metals

The bioaccumulation of heavy metals in grass (*I. fasciculatum*), fish (*O. mossambicus*) and sediment samples was determined using the bioaccumulation factor (BAF) as described by Deforest *et al*., 2007.

BAF = (P ⁄ E) i

where:

i= heavy metal.

BAF= the bioaccumulation factor and is dimensionless.

P= the trace element concentration in plant tissues or fish (mg / kg dry weight)

E = the trace element concentration in the sediment (mg / kg dry weight).

A larger ratio implies better accumulation capability.

**5.4.6 Suitability of Sand River water for sustaining aquatic life**

The water quality of the Sand River with respect to sustaining aquatic life was measured using the Water Quality Index (WQI) (Brown, 1970). The heavy metal standards used were those recommended by the Department of Water Affairs and Forestry (DWAF, 1996). The WQI was calculated using the following expression

$$WQI = \Sigma (QnWn) / \Sigma (Wn)$$

where:

Wn = unit weight for the nth parameter

$Wn = k / Sn$

Sn = standard value for the nth parameter

K = constant for proportionality

Qn = quality rating for the nth water quality parameter

$$Qn = 100 (Vn – Vio) / (Sn – Vn)$$

where:

Vn = estimated value of the nth parameter at a given sampling point

Sn = standard permissible value of the nth parameter

Vio = ideal value of the nth parameter in pure water (i.e. 0 for all the parameters except pH and dissolved oxygen).

**Table 5.3**: The status of the river with respect to water quality index values was determined using the following scaling (Brown *et al*., 1970).

| Water quality index | Description |
|---|---|
| 0 – 25 | Excellent |
| 26 – 50 | Good |
| 51 – 75 | Poor |
| 76 – 100 | Very poor |
| >100 | Unfit for drinking |

### 5.4.7 Statistical analysis

Normality and homogeneity of variance was tested using the Shapiro-Wilk normality test. A one way analysis of variance (ANOVA) was used to determine any significant differences of heavy metal concentrations in sediment, grass and fish between the sites on the Statistical Package and Service Solutions (SPSS version 20.0). Kruscal Walis test was used to test significant differences of the bioaccumulation factors between the sites.

## 5.5 RESULTS

### 5.5.1 Heavy metal concentration in water from Sand River

There was no significant difference ($P>0.05$) in cadmium, copper and lead concentrations in the water among the different sites (Figure 5.3, 5.4 and 5.7). There was a general decrease in iron concentrations in the water at different sites. It is however, significant to note that iron concentration at site 7 was higher than that at site 1(Figure 5.5). Manganese concentrations significantly increased at the sites after the point of effluent discharge (Figure 5.6). Zinc concentrations remained constant in all sites at 0.01 mg/l, except at site 6 where it rose to 0.02 mg/l.

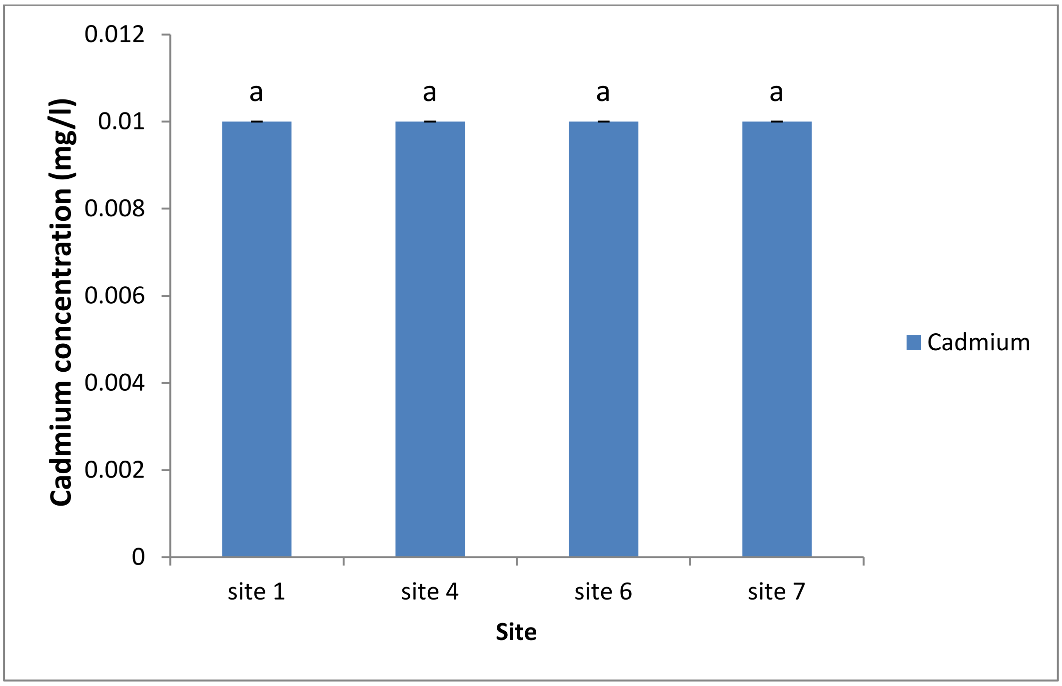


**Figure 5.3**: Cadmium concentrations in water at the different sites of the Sand River. Means with different superscripts in the column are significantly different ($P<0.05$).

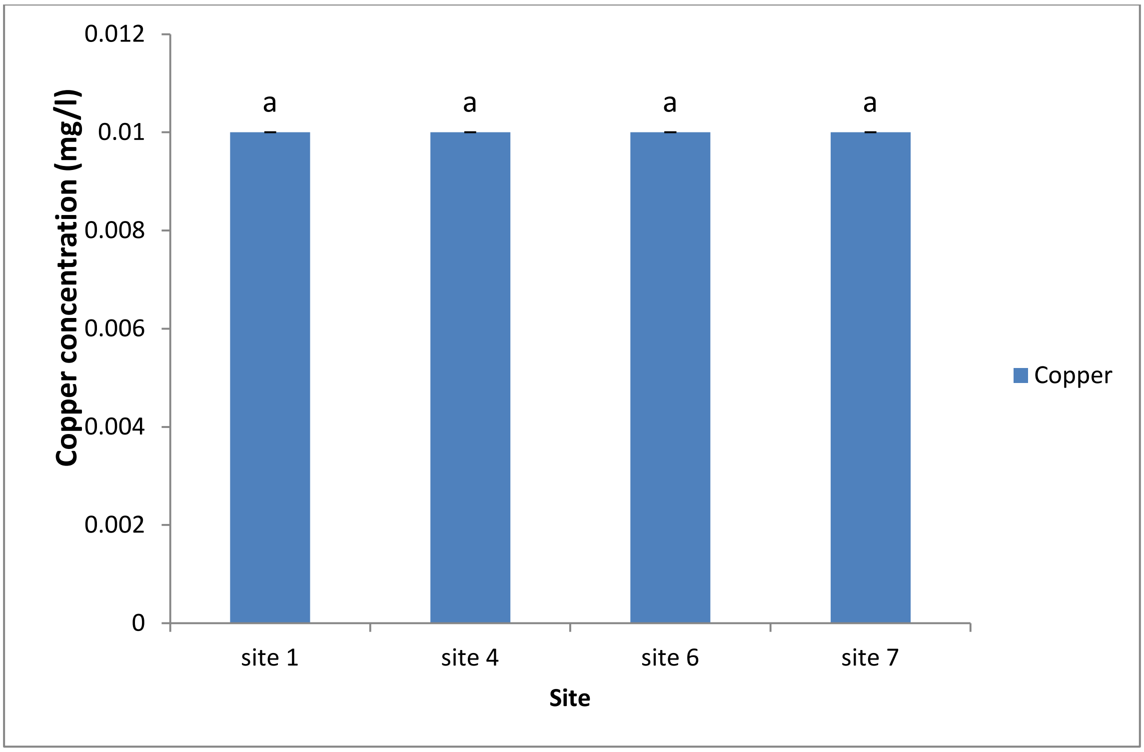


**Figure 5.4**: Copper concentrations in water at the different sites of the Sand River. Means with different superscripts in the column are significantly different (P<0.05).

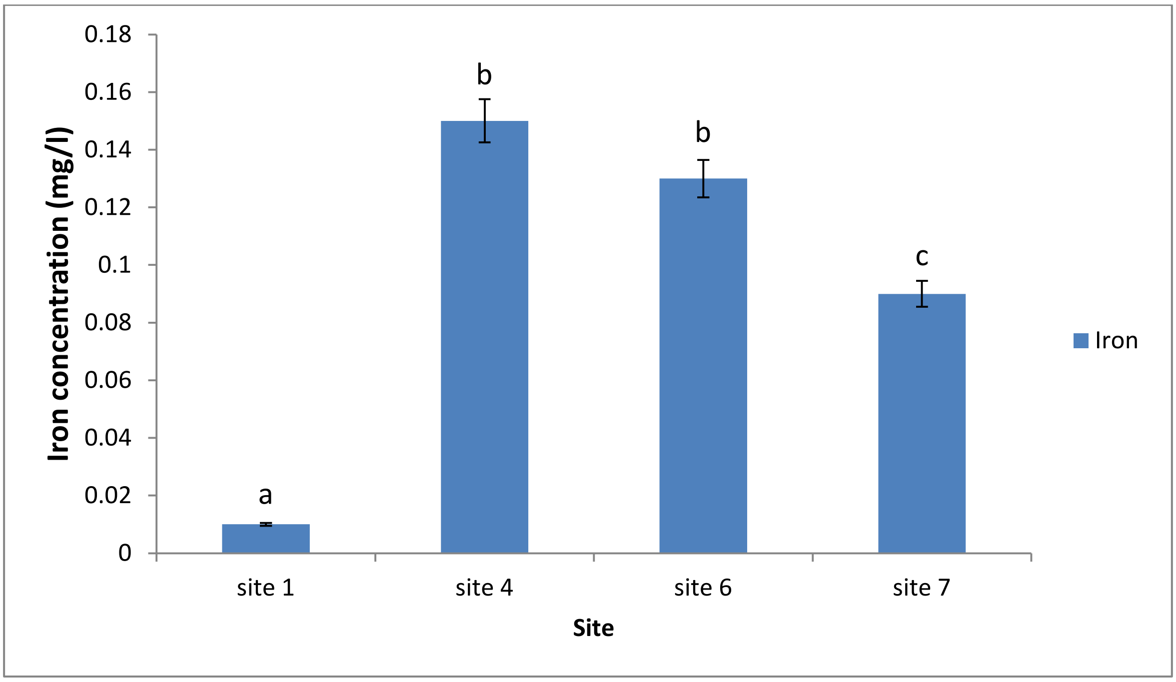


**Figure 5.5**: Iron concentrations in water at the different sites of the Sand River. Means with different superscripts in the column are significantly different (P<0.05).

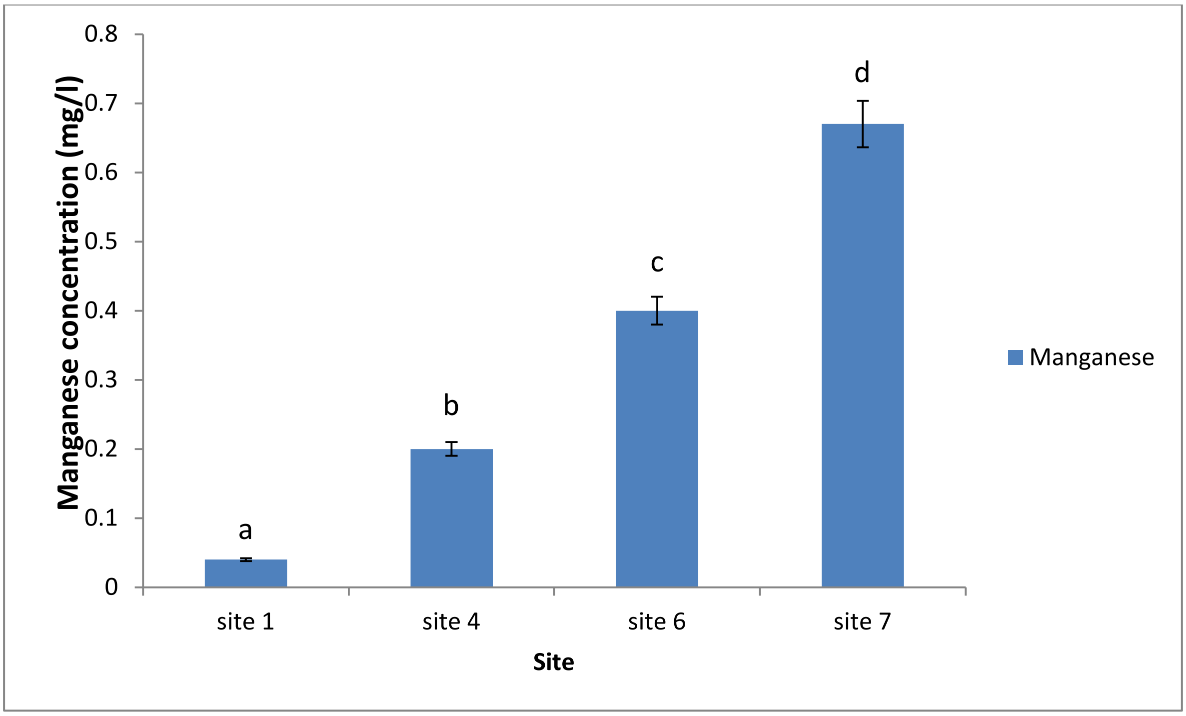


**Figure 5.6**: Manganese concentrations in water at the different sites of the Sand River. Means with different superscripts in the columns are significantly different (P<0.05).

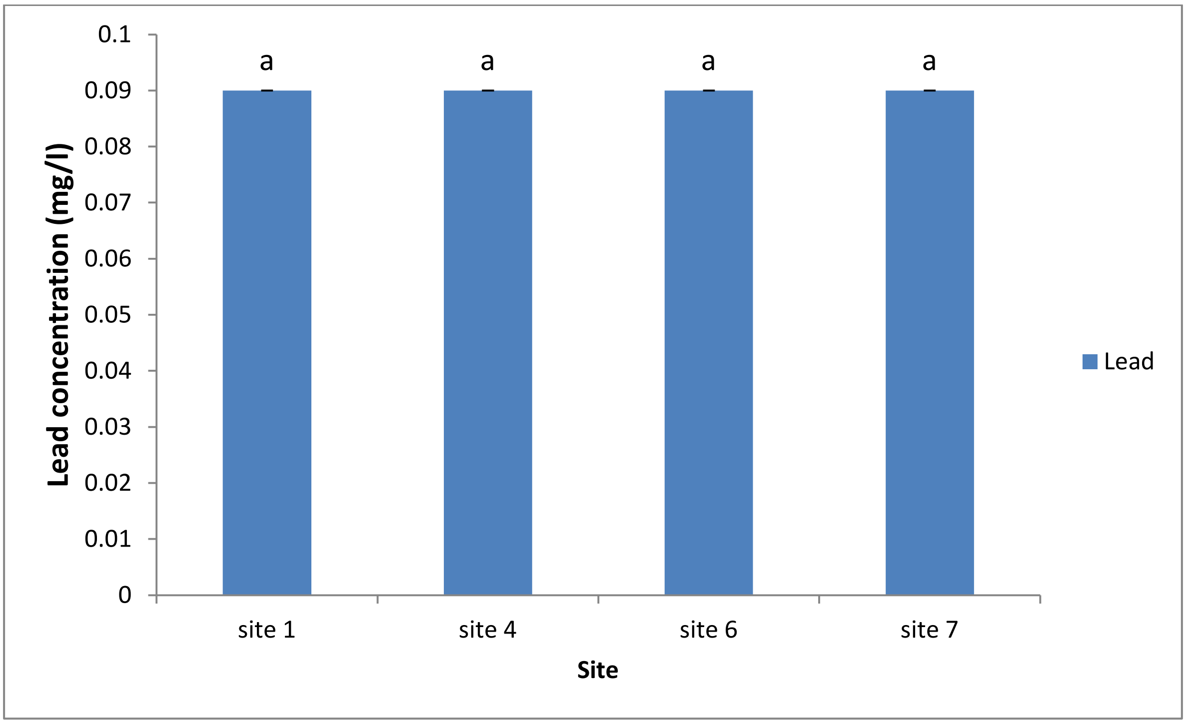


**Figure 5.7**: Lead concentrations in water at the different sites of the Sand River. Means with different superscripts in the columns are significantly different (P<0.05).

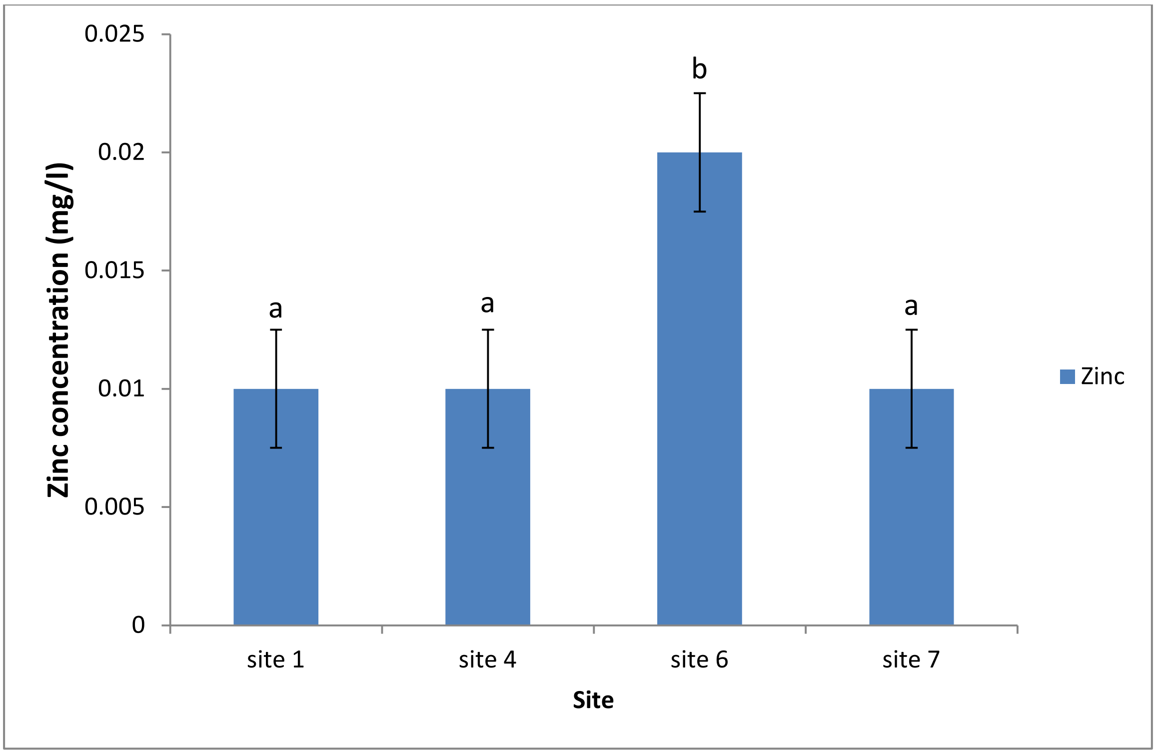


**Figure 5.8**: Zinc concentrations in water at the different sites of the Sand River. Means with different superscripts in the columns are significantly different (P<0.05).

### 5.5.2 Heavy metal concentrations in the sediment, grass and fish from the Sand River

Cadmium concentration in the sediment were not significantly different (P>0.05) at all the sampling sites (Figure 5.9). The cadmium concentrations increased in grass and in fish, however the concentrations were also not significantly different (P>0.05). However, in grass significantly higher (P<0.05) cadmium concentrations were recorded at site 7. Copper concentrations indicated increase from sediment, grass throught to fish. Copper concentrations in the sediment did not vary (P>0.05) at all sites (Figure 5.10). The copper concentrations in grass were higher (P<0.05) at site 1 than all the other sites. In fish samples, there was no difference (P>0.05) in the copper concentrations at sites 4, 6, 7. However, significantly lower concentrations were recorded at site 4 (Figure 5.10). The iron concentrations in the sediment were also the same at site 4, 6, 7 (Figure 5.11). Significantly lower iron concentrations were recorded at site 1 (P<0.05). Iron was highest at site 1 in the grass samples. The iron concentration increased sharply in the fish (Figure 5.11). The iron concentrations in fish were significantly higher at sites 1 and 6 ($P<0.05$) than at sites 4 and 7 (Figure 5.11). Manganese concentrations in the sediment were significantly different ($P<0.05$) at all sampling sites (Figure 5.12). The highest manganese concentration in the sediment was recorded at site 6. Manganese concentration increased in grass with significantly higher concentration at site 4 and 6. Manganese concentrations in fish were lower than in gass. The highest lead concentrations in the sediment were recorded at site 6 and 7. However, the lead concentrations in the grass and fish did not differ significantly ($P<0.05$) with site (Figure 5.13). There were no major spatial variations in the zinc concentrations in the sediment, grass and fish (Figure 5.14). The heavy metal concentrations were in the order, iron>manganese>zinc>copper>lead>cadmium in sediment, grass through to fish.

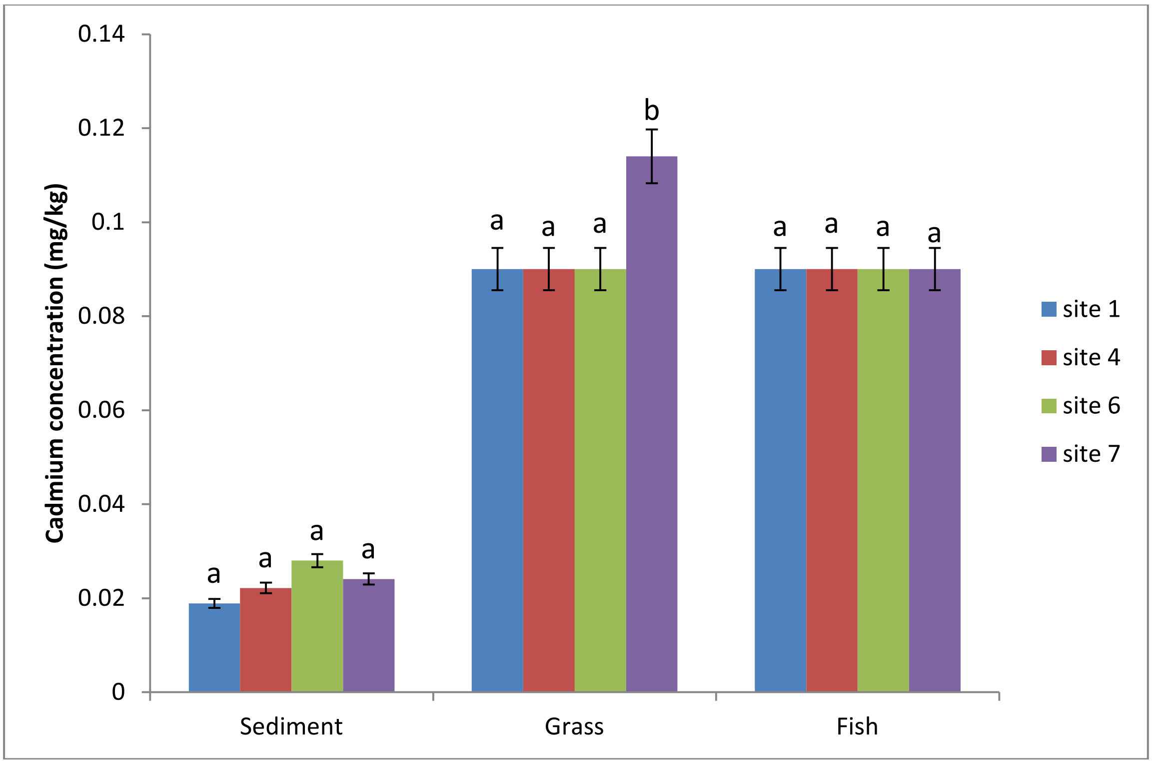


**Figure 5.9**: Mean cadmium (Cd) concentrations in sediment, grass and fish samples during the dry season. Different bars in the sediment, grass and fish with different letters are significantly different ($P<0.05$).

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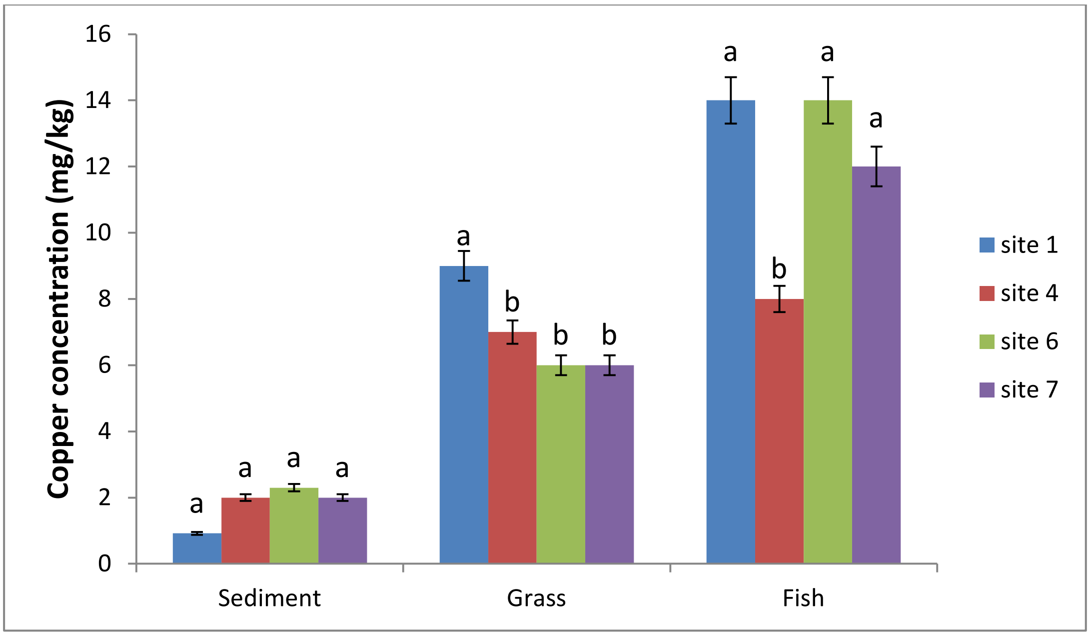


**Figure 5.10**: Mean copper (Cu) concentrations in sediment, grass and fish samples during the dry season. Different bars in the sediment, grass and fish with different letters are significantly different (P<0.05).

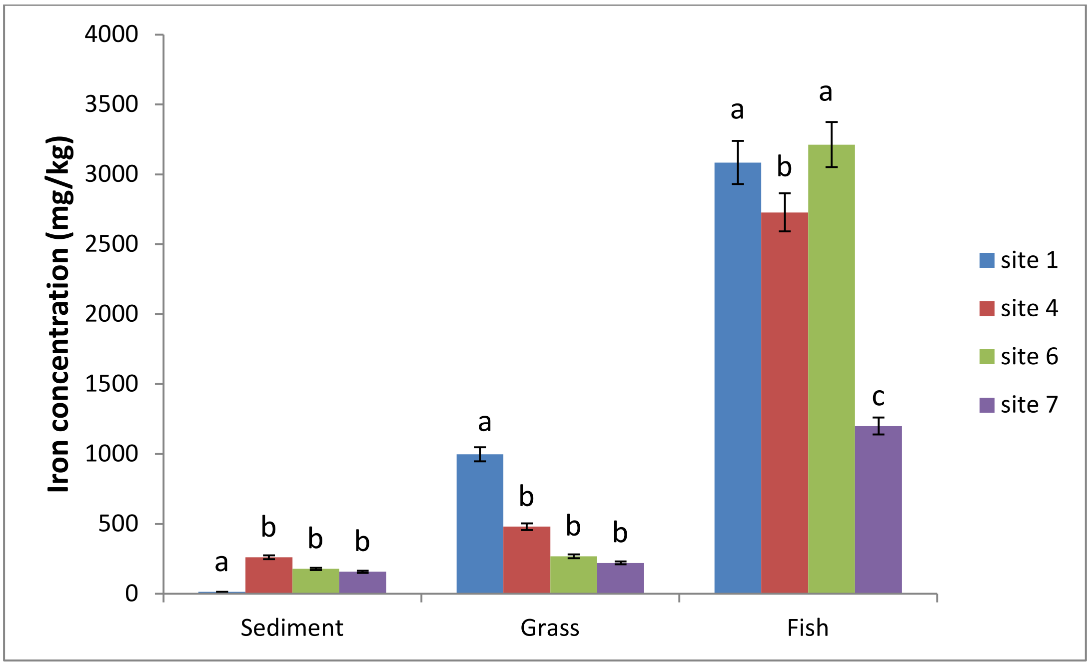


**Figure 5.11**: Mean iron (Fe) concentrations in sediment, grass and fish samples during the dry season. Different bars in the sediment, grass and fish with different letters are significantly different (P<0.05).

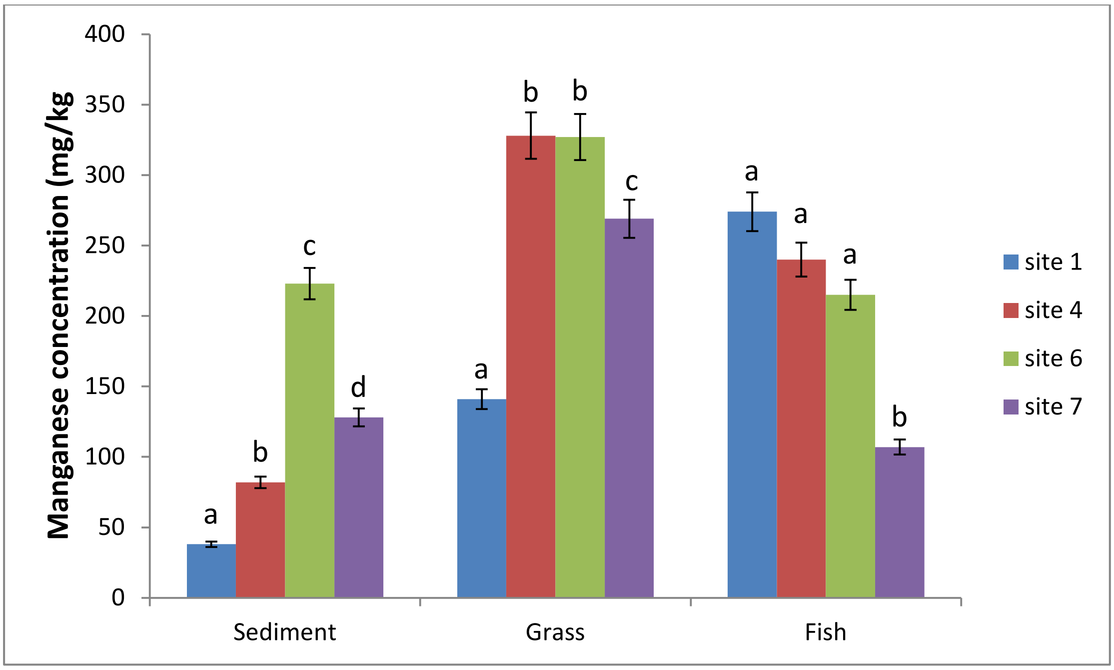


**Figure 5.12**: Mean manganese (Mn) concentrations in sediment, grass and fish samples. Different bars in the sediment, grass and fish with different letters are significantly different (P<0.05).

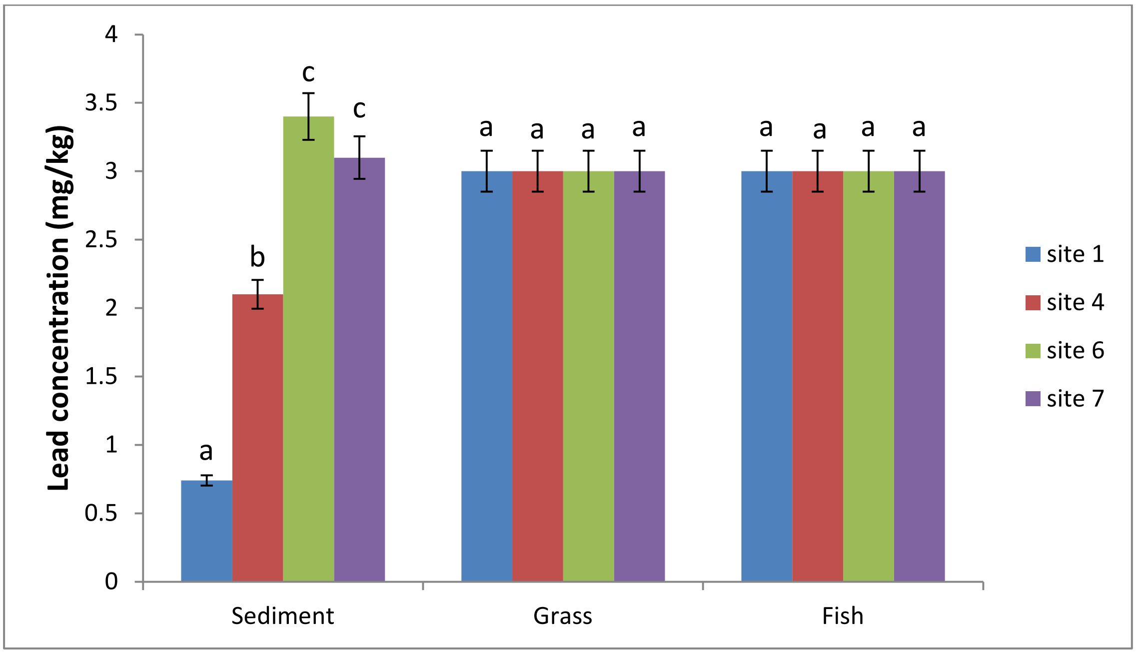


**Figure 5.13**: Mean lead (Pb) concentrations in, sediment, grass and fish samples during the dry season. Different bars in the sediment, grass and fish with different letters are significantly different (P<0.05).

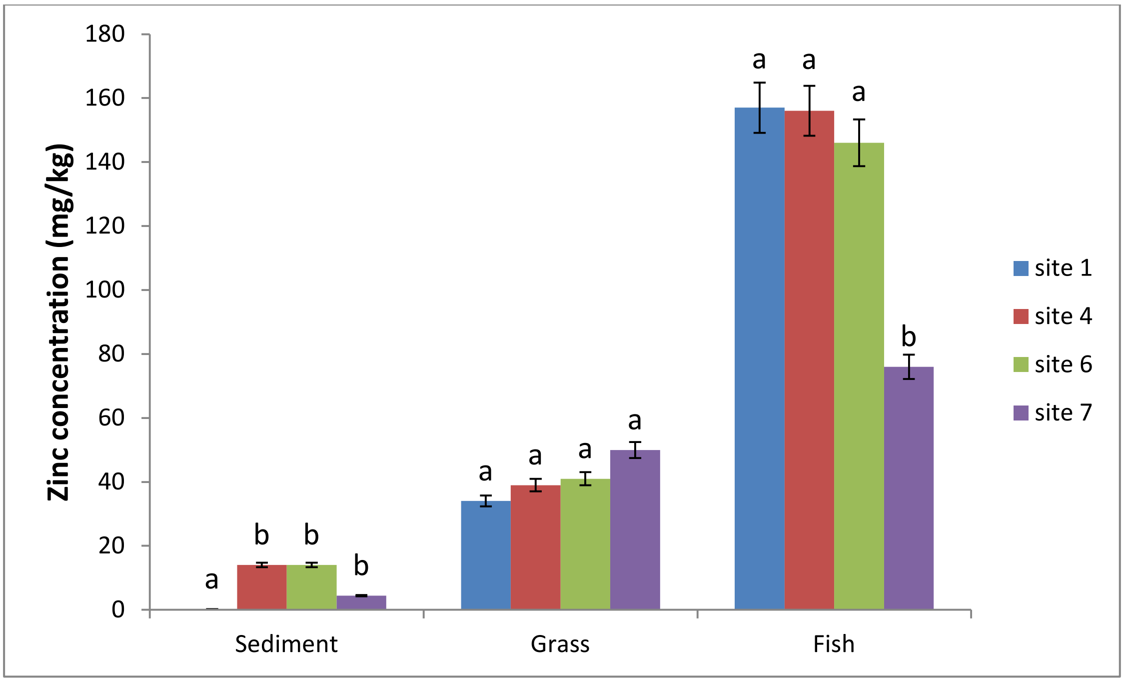


**Figure 5.14**: Mean zinc (Zn) concentrations in water, sediment and grass samples during the dry season. Different bars in the sediment, grass and fish with different letters are significantly different (P<0.05).

### 5.5.3 Bioaccumulation factor from sediment to grass (*Ischaemum fasciculatum*).

Bioaccumulation factors of cadmium were not significantly different (P<0.05) at all sampling sites (Table 5.4). Copper, iron and zinc bioaccumulation factors fluctuated across the different sites. Bioaccumulation factors of these metals were high at site 1. There was no particular trend in manganese bioaccumulation factors. However, manganese bioaccumulation was lowest at site 6 and highest at site 4 (Table 5.4). There was a decrease in lead bioaccumulation in grass at the sites after the point of effluent discharge (Table 5.4).

**Table 5.4**: Mean bioaccumulation factors (±SD) from sediment to grass *(Ischaemumfasciculatum).*

| site | Cadium | Copper | Iron | Manganese | Lead | Zinc |
|---|---|---|---|---|---|---|
| 1 | $4.76\pm0.00^{a}$ | $9.78\pm0.18^{a}$ | $71.29\pm23.23^{a}$ | $3.71\pm0.06^{a}$ | $4.05\pm0.90^{a}$ | $157.40\pm10.23^{a}$ |
| 4 | $4.05\pm0.00^{a}$ | $3.50\pm0.03^{b}$ | $1.83\pm0.00^{b}$ | $4.00\pm0.01^{a}$ | $1.43\pm0.21^{b}$ | $2.79\pm0.00^{b}$ |
| 6 | $3.21\pm0.00^{a}$ | $8.30\pm2.231^{a}$ | $2.10\pm0.05^{c}$ | $1.47\pm0.05^{b}$ | $0.88\pm0.06^{b}$ | $2.93\pm0.00^{b}$ |
| 7 | $4.73\pm0.01^{a}$ | $3.00\pm0.00^{b}$ | $1.40\pm0.05^{b}$ | $2.10\pm0.00^{c}$ | $0.97\pm0.01^{b}$ | $11.36\pm1.02^{c}$ |

### 5.5.4 Bioaccumulation factor from sediment to fish (*Oreochromismossambicus*).

There was a significant difference ($P<0.05$) in the bioaccumulation of copper and zinc in fish at the different sampling sites (Table 5.5). The highest bioaccumulation of these metals was recorded at site 1 and the lowest at site 4. Cadmium, iron, manganese and lead bioaccumulation was also high in fish found at site 1.

**Table 5.5**: Mean bioaccumulation factors (±SD) from sediment to *Oreochromis mossambicus* (fish).

| Site | Cadmium | Copper | Iron | Manganese | Lead | Zinc |
|---|---|---|---|---|---|---|
| 1 | 4.76±0.02$^{a}$ | 9.33±1.54$^{a}$ | 79.10±5.49$^{a}$ | 2.98±0.42$^{a}$ | 3.26±0.02$^{a}$ | 92.35±2.57$^{a}$ |
| 4 | 1.50±0.01$^{b}$ | 2.42±0.63$^{b}$ | 10.18±3.05$^{b}$ | 0.79±0.12$^{b}$ | 0.11±0.02$^{b}$ | 2.17±0.12$^{b}$ |
| 6 | 3.21±0.01$^{a}$ | 6.08±0.00$^{c}$ | 18.05±0.25$^{b}$ | 0.96±0.00$^{b}$ | 0.88±0.00$^{c}$ | 10.43±0.01$^{c}$ |
| 7 | 1.91±0.00$^{b}$ | 4.80±0.01$^{d}$ | 2.76±0.01$^{c}$ | 0.53±0.00$^{c}$ | 0.83±0.12$^{c}$ | 36.19±1.02$^{d}$ |

### 5.5.5 Water quality index

Water quality index with regards to metal contamination in the Sand River was 0.00, which was within the scale of 0 to 25. This was regarded as good according to the classification by Brown (1970).

## 5.6 DISCUSSION

The heavy metals in the Sand River water were within the recommended standards for sustaining aquatic organisms and irrigation of crops (DWAF, 1996). The only metal that exceeded the recommended irrigation levels was manganese. Polokwane has very few industries that can possibly discharge heavy metals into the Sand River. It is therefore, not surprising that most of the heavy metals in the water were at very low concentrations. The most probable cause of the high manganese concentration in the Sand River water is the type of rock found in the Sand River catchment area. Granite is one of the major basement rocks found in the Sand River catchment area (Polokwane Municipality SDF, 2011). On weathering, this rock produces iron and manganese.

Manganese is the only heavy metal in the water that showed notable spatial variation. This again is probably due to the lithology of the area as there is no clearly identifiable source of manganese in Polokwane municipality. Manganese is known to penetrate through soils more readily than the other metals (L'Herroux *et al*., 1997). This tendency in part explains the spatial variations. Part of the Sand River flows through agricultural land where the application of fertilizers is widespread. The main problem with fertilizer application is that it may lead to a decrease in pH which inevitably leads to an increase in heavy metal availability (Taboada-Castro *et al*., 2010). The pH range was between 7.49 and 8.4. In chapter 3 pH values that were less than 10 were recorded at site 6, 7 and site 1. It is important to note that site 6 and site 7 are within the agricultural zone.

Heavy metal concentration in sediment followed the order iron > manganese> zinc > copper > lead > cadmium. These metal levels in the sediment were higher than in the water. These results suggest that iron and manganese precipitated much faster that the other heavy metals. The overall redox state of the sediments plays an important role in the precipitation of iron and manganese. Anoxic conditions will alter the chemistry of iron and manganese which in turn may affect other metals that would have been bound to oxides of iron and manganese. Oxidizing conditions tend to favour the release of iron from the water (Groblelak *et al*., 2012). Three main redox conditions exist in the sediment namely oxidizing, reducing and transitional. The prevailing condition is determined by the river morphology, thermal conditions and nutrient flux. Many investigators have shown that ferric iron in sediments influences the exchange of phosphate from the sediments to the overlying waters (Fijalkowski *et al*., 2012). The physical nature of the sediment is also important in sedimentation and transport processes of heavy metals. Sediments are characterized as coarse material, clay/silt and sand fractions, this classification is based on particle size. The clay/silt fraction has a high surface area and it is more likely to adsorb heavy metal contaminants. At most sites the sediment was mostly clay/silt and this may account for the high iron and manganese levels found in the sediment of the Sand River. Iron (III) oxides will be reduced to more soluble iron (II), while hydrous manganese oxides will be reduced to soluble manganese (II). Manganese is readily more reduced than iron. The rate of oxidation of manganese is slower than that of iron hence it is more readily transported through aerobic environments. Discharge of sewage effluent decreases oxygen levels in the Sand River and this affected the redox potential.

The concentration of heavy metals in the grass also followed the same order (iron > manganese > zinc > copper > lead > cadmium) as observed in the sediments. This suggests that *I. fasciculatum* is tolerant of heavy metals and can thus be used in the abatement of heavy metal pollution. The use of plants in the control of water pollution is now widely accepted (Chaney *et al*., 1997; Cunningham *et al*., 1995). Vetiver grass has been shown to be tolerant of heavy metals concentrations (Roongtanakiat *et al*., 2003). Although, the tolerance of *I. fasciculatum* too heavy metals has not been tested, it appears to be a good candidate for heavy metal pollution control. It is therefore, suggested that heavy metal toxicity experiments be undertaken on this plant.

Heavy metal concentration in the fish followed the same order (iron > manganese > zinc > copper > lead > cadmium) as in the sediment and grass. Heavy metals enter the fish through the gills, skin and diet of the fish. *Oreochromis mossambicus* is a herbivorous fish that feeds on phytoplankton and detritus. There is very little phytoplankton in rivers and *O. mossambicus* does not feed on *I. fasciculatum*. It therefore, appears that the major sources of heavy metals in *O. mossambicus* are either directly from the water or the sediment in the form of detritus. The accumulation of heavy metals depends on the concentration of the metal, time of exposure, water temperature, pH, water hardness and conductivity. Some important intrinsic factors that may play a role in the uptake of heavy metals are the age of the fish and the feeding habits. Most of the heavy metals accumulate in liver, kidney and gills. Fish muscles usually contain the lowest levels of heavy metals. In this study, fish muscles were used because some people consume the fish that they catch from the Sand River. It was also evident from these results that the various metals accumulated in the fish muscle in different amounts. These differences are a result of the different affinity of metals to fish tissues, different uptake deposition and excretion rates. Metals levels in fish usually follow the following ranking: iron > zinc > lead > copper > cadmium > mercury (Jezierska and Witeska, 2001). Generally, this ranking was confirmed in this study. It has also been shown that the concentrations of cadmium and lead are considerably higher in fish from acid lakes and rivers (Horwitz *et al*., 1995; Haines and Brumbaugh, 1994). Copper accumulation is also higher at lower pH (Cogun and Kargin, 2004). It is therefore, important to ensure that the pH of the Sand River does not decrease as this may lead to greater bioavailability of copper, cadmium and lead. Water hardness also plays an important role in the uptake of metals across the gills. Playle *et al*. 1992 reported that increases of calcium in water reduced copper accumulation in the gills. This is attributed to calcium competing with other metals for binding sites on the gill surface. This suggests therefore, that hard water will reduce bioaccumulation of heavy metals in fish. The Polokwane municipality must closely monitor this important water quality parameter.

Intermetallic concentrations may also affect bioaccumulation in fish (Pelgrom *et al*., 1995). Interactions among the different metals are related to their different affinities to various organs. A number of studies have shown the synergistic and additive effect of metals. Antagonistic effects may also occur (Jezierska and Witeska, 2001).

The Sand River is not polluted with heavy metals. This has also been confirmed by the water quality index. Only iron and manganese were recorded in high concentrations in the water, sediment and biota. There are no heavy industries discharging in the Sand River. Despite the low levels of heavy metals concentrations in the Sand River, it is suggested that the Polokwane municipality implement a heavy metal monitoring programme.

# CHAPTER 6: SUITABILITY OF WATER FROM THE SAND RIVER CATCHMENT AND SURROUNDING BOREHOLES FOR IRRIGATION PURPOSES

## 6.1 INTRODUCTION

In semi-arid areas where rainfall is inadequate, sewage effluent is used for crop irrigation. In South Africa, nearly half the effluent produced by local authorities is used for irrigation (Akpor and Muchie, 2011). In recent years the quality of effluent discharged from sewage treatment plant has deteriorated. This is largely attributed to the increased rate of urbanisation (Morrison *et al*., 2001; Samie *et al*., 2009; Britz *et al*., 2013). The use of water that is polluted with domestic effluent for crop irrigation may result in high nitrate and chloride levels (Moyo, 2013). High salt levels in river waters in South Africa are exacerbated by the predominance of saline underlying geology (Halls and Gorgens, 1978). The suitability of Sand River water for irrigation was evaluated in this study.

Ground water plays an important role in agriculture. However; it is continually threatened by substandard sewage effluent that is discharged into rivers. The contamination of ground water can disperse over wide areas and persist for several years making the water unsuitable for both irrigation and human consumption. The Polokwane wastewater treatment works discharges its effluent into the ephemeral Sand River. This river flows over a 20 m thick by 300 m wide layer of alluvium. Underlying the alluvium are granite-gneiss rocks that are weathered and fractured to depths of 60 m. The municipal production boreholes are found along the Sand River (Murray and Tredoux, 2002). The Sand River water is recycled through artificial recharge and subsequent abstraction and also pumped from the river by other users downstream of the discharge point for irrigation. It is therefore essential to determine whether this water is suitable for irrigation.

## 6.2 OBJECTIVES

I. To determine the effect of sewage effluent on the suitability of the Sand River water for irrigation purposes.

II. To determine the suitability of the borehole water in Sand River catchment area for irrigation

## 6.3 HYPOTHESES

I. The suitability of the Sand River water for irrigation is not affected by effluent discharge

II. The borehole water in the Sand River catchment is not suitable for irrigation.

## 6.4 MATERIALS AND METHODS

### 6.4.1 Suitability of Sand River water for irrigation

Suitability of Sand River water for irrigation was determined at sites 4 and 7 (where irrigation takes place). Samples were collected at a depth of about 30 cm using polyethylene water bottles. The bottles were pre-rinsed with river water three times before the final sample was taken. The calcium and magnesium content of the water at the downstream sites were determined by EDTA titration using Mordant black 11as an indicator. Sodium and potassium content were determined using a flame photometer. Chloride concentrations were measured by silver nitrate titration (APHA, 2005). Carbonate and bicarbonate content was measured by acid-base titration (APHA, 2005). Total dissolved solids were determined using the method described in Chapter 3.

### 6.4.2 Suitability of Sand River catchment borehole water for irrigation

Borehole water samples were collected at random along the Sand River from ten boreholes (Figure 3.6; Chapter 3). Water was pumped from each borehole and similar parameters as those in the river water were determined. For the purpose of the borehole water type characterization, sulphate ($SO_4^{2-}$) and chloride ($Cl^-$) were also determined using ion chromatography.

### 6.4.3 Determination of the Sodium adsorption ratio (SAR)

Sodium adsorption ratio also expressed as sodium hazard is important for determining the quality of water used for irrigation purposes. The AquaChem 2012.1 software was used to plot United States salinity laboratory (USSL) diagram. It is used widely for rating the irrigation waters. The diagram is a simple scatter plot of SAR on the Y-axis versus electrical conductivity on the X-axis (in log scale).

Interpretation of the USSL diagram was done using the following Table 6.1:

**Table 6.1**: Classification of USSL diagram.

| Salinity Hazard (Conductivity) | | Sodium hazard (SAR) | |
|---|---|---|---|
| C1 | Low | S1 | Low |
| C2 | Medium | S2 | Medium |
| C3 | High | S3 | High |
| C4 | Very high | S4 | Very high |

### 6.4.4 Determination of the suitability for irrigation of the water from the Sand River and the borehole.

High concentrations of $HCO_3$ in irrigation water have a tendency of causing $Ca^{2+}$ and $Mg^{2+}$ precipitation. The effect of $CO_3$ and $HCO_3$ ions on the quality of water was expressed in terms of the Residual Sodium Carbonate (RSC) (Ragunath, 1987). Residual sodium carbonate (RSC) is calculated as follows:

$RSC = (CO_3^{-} + HCO_3^{-}) – (Ca^{++} + Mg^{++})$

Permeability index (PI) was also used to determine the suitability for both the Sand River water and borehole water. It was used to indicate whether the soil permeability will be affected by long-term use of irrigation water as influenced by $Na^{+}$, $Ca^{2+}$, $Mg^{2+}$, and $HCO_3$ contents of the soil. Doneen (1964) classified irrigation water based on the Permeability index:

$PI = (Na^{+} + \sqrt{HCO3} *100) \backslash (Ca^{++} + Mg^{++} + Na^{+})$ all ions are in equivalents per million

Water can be classified as Class I, II and III.

Class I and II water are categorized as good for irrigation with 75% or more of maximum permeability.

Class III water is unsuitable with 25% of maximum permeability.

### 6.4.5 Statistical analysis

Normality and homogeneity of variance was tested using the Shapiro-Wilk normality test. A one way analysis of variance (ANOVA) was used to determine any significant differences in anions and cations concentrations between the Sand River sites. It was also used to determine significant differences in anions and cations concentrations the between borehole sites.

## 6.5 RESULTS

### 6.5.1 Suitability for irrigation (Sand River and borehole water)

Calcium, sodium, bicarbonate, potassium and conductivity were not significantly different between sites 4 and 7 (Table 6.2; $P>0.05$). However, the magnesium and total dissolved solids concentrations between the sites were significantly different ($P<0.05$).

The calcium, potassium and conductivity of the borehole water were also not significantly different ($P>0.05$). Sodium concentrations at BH13 were significantly higher ($P<0.05$) than those at all the other sites. Sodium concentrations were significantly lower at BH10. Bicarbonate concentrations were significantly lower at BH13 and BH15 ($P<0.05$). Magnesium concentrations fluctuated across the different boreholes. However, significantly higher concentrations were recorded at BH6 ($P<0.05$). Total dissolved solids also fluctuated at the different boreholes. Potassium concentrations were higher at BH5 and lower at all the other borehole sites (Table 6.2; $P<0.05$)

**Table 6.2**: Concentration of anions and cations in the Sand River and borehole water.

| Source | Ca (mg/l) | Na (mg/l) | HCO3 (mg/l) | Mg (mg/l) | K (mg/l) | TDS (mg/l) | Conductivity (mS/m) |
|---|---|---|---|---|---|---|---|
| *River water* | | | | | | | |
| Site 4 | 27.28±1.01$^{a}$ | 70.59±1.01$^{a}$ | 206.99±1.01$^{a}$ | 13.98±1.01$^{a}$ | 09.14±0.01$^{a}$ | 144.99±1.01$^{a}$ | 102.44±1.26$^{a}$ |
| Site 7 | 31.52±1.01$^{a}$ | 68.57±1.01$^{a}$ | 212.99±1.01$^{a}$ | 18.45±1.01$^{b}$ | 10.56±0.01$^{a}$ | 149.49±5.51$^{b}$ | 107.34±6.14$^{a}$ |
| *Borehole water* | | | | | | | |
| BH5 | 49.69±1.01$^{a}$ | 5.51±1.01$^{a}$ | 400.99±1.01$^{a}$ | 52.73±0.51$^{a}$ | 155.61±0.01$^{a}$ | 499.34±346b$^{ba}$ | 111.99±1.51$^{a}$ |
| BH6 | 50.41±1.01$^{a}$ | 5.55±1.1$^{a}$ | 440.99±1.01$^{a}$ | 73.15±21.49$^{b}$ | 141.34±0.01$^{b}$ | 818.99±24.69$^{b}$ | 119.99±9.49$^{a}$ |
| BH7 | 49.81±1.01$^{a}$ | 7.73±1.01$^{b}$ | 427.49±0.51$^{a}$ | 44.83±1.01$^{a}$ | 141.25±0.01$^{c}$ | 795.89±3.61$^{b}$ | 126.99±2.51$^{a}$ |
| BH8 | 50.13±1.01$^{a}$ | 7.95±1.01$^{b}$ | 406.99±1.01$^{a}$ | 43.41±0.99$^{a}$ | 140.05±0.01$^{c}$ | 799.14±1.66$^{b}$ | 112.39±12.11$^{a}$ |
| BH10 | 39.43±1.01$^{a}$ | 4.11±1.01$^{a}$ | 373.49±0.51$^{a}$ | 38.10±1.10$^{a}$ | 132.55±0.01$^{c}$ | 740.84±47.94$^{b}$ | 100.14±0.16$^{a}$ |
| BH11 | 37.71±1.01$^{a}$ | 7.79±1.01$^{b}$ | 362.99±1.01$^{a}$ | 37.47±1.01$^{c}$ | 128.03±0.01$^{c}$ | 686.69±4.19$^{b}$ | 101.24±1.24$^{a}$ |
| BH12 | 44.41±1.01$^{a}$ | 7.61±1.01$^{b}$ | 396.99±5.01$^{a}$ | 42.71±1.01$^{a}$ | 133.39±0.01$^{c}$ | 706.84±26.36$^{b}$ | 105.49±2.99$^{a}$ |
| BH13 | 49.51±1.01$^{a}$ | 13.51±1.01$^{c}$ | 326.99±1.01$^{b}$ | 33.85±1.01$^{c}$ | 120.89±0.01$^{c}$ | 726.99±4.19$^{b}$ | 110.04±1.54$^{a}$ |
| BH14 | 36.69±1.01$^{a}$ | 4.19±1.01$^{a}$ | 372.99±1.01$^{a}$ | 38.85±1.01$^{d}$ | 132.53±0.01$^{c}$ | 715.64±4.14$^{b}$ | 108.79±2.81$^{a}$ |
| BH15 | 44.79±1.01$^{a}$ | 8.29±1.01$^{b}$ | 338.99±1.01$^{b}$ | 36.39±1.01$^{d}$ | 119.90±0.10$^{c}$ | 693.74±15.74$^{b}$ | 108.79±2.79$^{a}$ |

River sites and borehole sites with different superscript are significantly different

### 6.5.2 Suitability for irrigation of the water from the Sand River and the borehole

The USSL diagram indicated that the Sand River water (site 4 and 7) and borehole water (BH5 – BH15) were within the C1 and S1 category, where both sodium (SAR) and salinity (Conductivity) hazard was low (Figure 6.1).

The permeability index indicated both borehole and Sand River water was suitable for irrigation. The RSC values also indicated that both borehole and Sand River water were suitable for irrigation (Table 6.3).

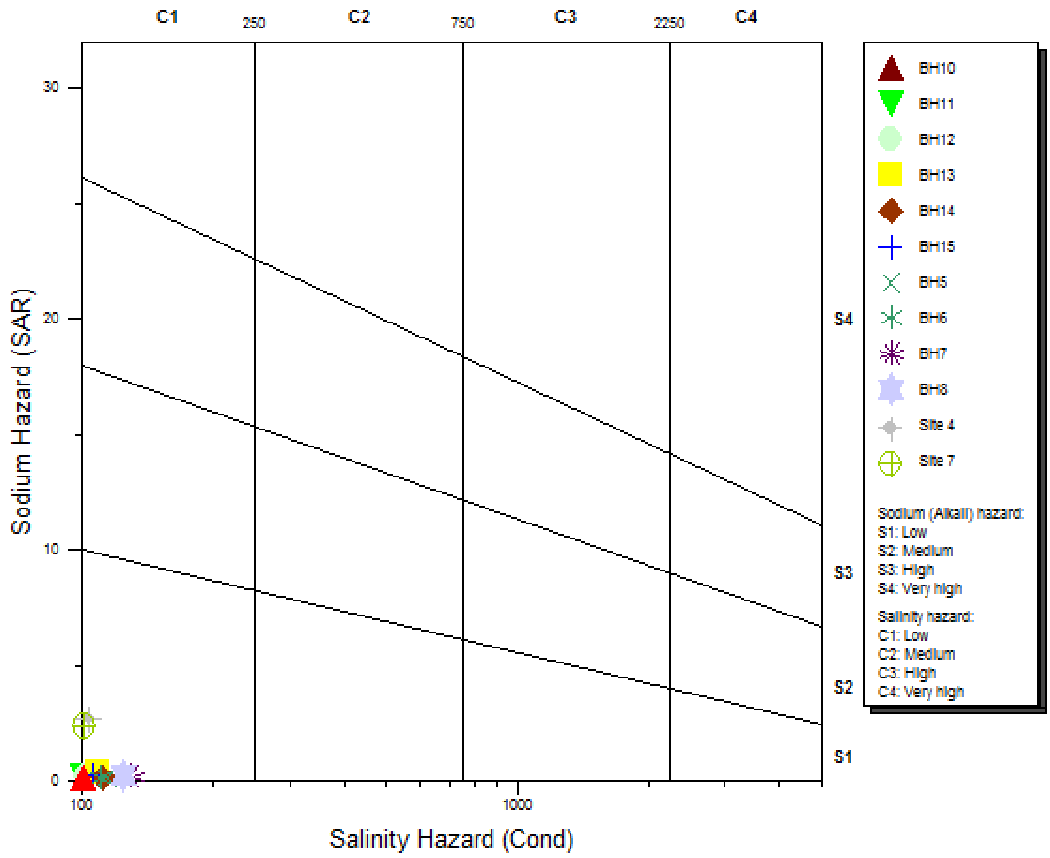


**Figure 6.1**: USSL diagram indicting the sodium (SAR) and salinity (Cond) hazard of Sand River water (Site 4 and 7) and borehole water (BH5, BH6, BH7, BH8, BH10, BH11, BH12, BH13 and BH15).

**Table 6.3**: The suitability for irrigation of the Sand River water and the borehole water with respect to Residual sodium carbonate and Permeability index (PI) values.

| Source | %PI | RSC |
|---|---|---|
| site 4 | 82.81 | 0.26 |
| site 7 | 67.2 | 0.75 |
| BH5 | 68.43 | -0.52 |
| BH6 | 65.84 | -1.08 |
| BH7 | 68.27 | -0.75 |
| BH8 | 68.21 | -0.91 |
| BH10 | 72.4 | -0.38 |
| BH11 | 72.5 | -0.39 |
| BH12 | 69.3 | -0.64 |
| BH13 | 68.72 | -0.11 |
| BH14 | 72.82 | -0.31 |
| BH15 | 69.09 | -0.95 |

## 6.6 DISCUSSION

Calcium, sodium, bicarbonate and potassium were not significantly different between sites 4 and 7. However, the magnesium concentrations and total dissolved solids between the sites were significantly different. The Sand River catchment consists mainly of granite rocks. Calcium, sodium, potassium and magnesium occur as a result of the weathering of these rocks (Wanda *et al*., 2011). The differences in the magnesium and total dissolved solids concentrations may have resulted from surface runoff from agricultural fields and waste disposal sites. The $HCO_3$, Na, K, TDS in the borehole water was however significantly different at some of the borehole sites, while calcium levels were not significantly different at all sites. The geology of the Sand River catchment area plays a significant role in terms of the mineral content of the borehole water. The rise and fall of water levels through groundwater recharge processes causes changes in the water chemistry due to dissolution of chemicals. Tredoux *et al*. (2009) highlighted in their study that the abstraction of ground water in accompanied by the ingress of oxygen. This promotes oxidation reactions in groundwater. Some of the salts found in the borehole water in this study may therefore have resulted from the oxidation reactions that occur as a result of the abstraction of water.

When irrigating with water containing sewage effluent, more carbonates are added to the soil than divalent cations. The calcium in the soil is thus lost during the formation of calcium carbonate deposits (Hopkins *et al*., 2007). These lime deposits cause precipitation of phosphorus and other macronutrients required in fruits and vegetables. The salt contained in the effluent accumulates in the roots of plants over time posing harmful impacts to the soil health and crop yield. Some areas in the Eastern Cape Province (South Africa) have already encountered problems relating to salinization. Some of the impacts of excess salinisation that have been evident on water resources include reduced crop yield and increased scale formation (Igbinosa and Okoh, 2009). It is therefore essential that the carbonates and other salts that are discharged with the sewage effluent into the Sand River be maintained so that they do not deprive the plants of essential macronutrients.

The SAR and the conductivity levels of the Sand River water and the borehole fell within the $S_1$ and $C_1$ category. This indicated low salinity and alkalinity hazard and the water was therefore considered suitable for irrigation. The problem associated with high levels of salinity in irrigation water is that it affects the permeability of soil and causes infiltration problems, and results in breakdown of soil aggregates (Kallergis, 2001). High conductivity levels in irrigation water causes an increase in soil solution osmotic pressure (Thorne and Peterson, 1954). Despite the Sand River water being suitable for irrigation, it is located in high temperature region with low rainfall. In such an area, evaporation rates are usually high. The levels of dissolved salts in the water increase with increased evaporation rate. This can be problematic because there are a number of farms along the Sand River who solely rely on this water for irrigation. The major vegetables planted in the area include tomatoes and onions. Maas (1986) indicated that onions sensitivity to conductivity is evident at 1.2 ds/m, while that of tomatoes is at 1.7 ds/m. An increase in Sand River water conductivity could therefore affect the production of these vegetables.

The residual sodium carbonate levels in both the Sand River and borehole water have also indicated that water is suitable for irrigation. RSC values less than 1.25 meq/l are good for irrigation, a value between 1.25 and 2.5 meq/l is of doubtful quality and a value more than 2.5 meq/l is unsuitable for irrigation (Eaton, 1950). Hopkins *et al*., (2007) further found that an increase in RSC, above zero meq, increases the sodium hazard to the soil structure. Water with high RSC has high pH, which causes water infertility and deposition of sodium carbonate (Wanda *et al*., 2013). Calcium and some magnesium precipitate when such water is applied to the soil. This increases the sodium percentages and the rate of sorption of sodium on soil particles. This also increases the potential for a sodium hazard and restricts water and air movement through soil (Eaton *et al*., 1950). Several areas in South Africa have already been identified as high risk areas, including the lower Vaal River (Van Rensburg *et al*., 2008), Breed, Crockodile and Olifants River because of the salinity levels (CSIR, 2010).

Soil permeability is affected by the long term use of irrigation water which contains Na, Ca, Mg and $HCO_3$ (Wanda *et al*., 2013). The permeability index indicated that the soil permeability will most likely not be affected by long-term use of Sand River and borehole water. Supplementary irrigation can show slight evidence of sodication and alkalinisation as early as 10 years (Mon *et al*., 2007). High Na, Ca, Mg and $HCO_3$ causes lower water entry and infiltration rates, and high surface runoff and soil losses. However, low salts in water can also result in poor soil permeability. Water which is low in salt has the capacity to dissolve and remove calcium and other solutes from the soil (Ayers and Westcot, 1976).

This chapter indicated that the water quality of the Sand River and that of the borehole water was suitable for irrigation. The salinity and alkalinity hazard were low and the soil permeability will most likely not be affected by long-term use of Sand River and borehole water.

## CHAPTER 7: GENERAL DISCUSSION, RECOMMENDATIONS AND CONCLUSION

### Nutrient status of the Sand River

The Sand River passes through an urban area. This has inevitably led to it being used to discharge sewage effluent and industrial waste. Most rivers in Africa that pass through urban areas are now degraded. There are high levels of phosphorus and nitrogen in the form of ammonia (de Villier and Thiart, 2007). The levels of nitrogen in the Sand River after the discharge of effluent of the sewage effluent exceed the South African recommended guidelines. It is important to note that the phosphorous level of the effluent that is discharged is not above the recommended guidelines. Most river in South Africa that pass through urban areas have phosphorous levels way above the recommended levels e.g Vaal, Oliphant and Klien River (de Villier and Thiart, 2007).

Polokwane is a relatively small city, but growing at unprecedented levels. In the past few years, the Polokwane Sewage Treatment Works discharged effluent that always meets the standard. Indeed, the city of Polokwane was awarded the blue status of 92. 61 % in 2011, it however dropped in 2012 to 86.52 % (DWA, 2012). Despite recent efforts to upgrade the sewage treatment plant, the city has failed to get the blue status. The decline in the diversity of macroinvertebrates after the discharge is clear testimony of the detrimental effects of the sewage effluent. Only pollution tolerant macroinvertebrate species are found at site near the point of discharge. Although this study did not investigate the fish species found in the Sand River, casual observation during sampling showed that only hardy species such as *Clarias gariepinus* and *Oreochromis mossambicus* are found in the river. The Cyprinidae species seem to have disappeared. It is recommended that further studies be done focussing of the effect of sewage effluent on the fish species.

The city of Polokwane irregularly monitors the Sand River. It is recommended that a proper monitoring schedule be adopted. The monitoring should not only focus on physco-chemical parameters but it must also look at the biological integrity of the river. It is thus important for municipal workers to undertake training on the use of SASS.

**Microbial status of the Sand River**

There has been a tendency to focus on the nutrient status of rivers and ignore the microbial status after sewage effluent. There is indeed a dearth of information on the microbes found in the different urban rivers in Africa. As already indicated, the Sand River is used by some farmers to irrigate their crops. The key question being asked and debated on in South Africa at the moment is the safety of the crops / vegetables that are irrigated with sewage effluent. Tomatoes and onions are the main vegetables that are irrigated with Sand River water. No studies have been undertaken to determine their suitability for human consumption. It is now prudent to look at the potential hazards associated with use of Sand River water for irrigation. Although the *E. coli* levels were within the recommended levels, they were rather on the high side. Several studies have investigated the carryover of pathogen particularly *E.coli* from polluted rivers to crops (Barnes and Taylor, 2004; Germs *et al*., 2004; Griesel and Jagals, 2002; Olaniran *et al*., 2009; Paulse *et al*., 2009). Foodborne disease outbreaks have been attributed to the consumption of vegetables irrigated with contaminated water from sewage treatment works. The *E. coli* O157:H7 (enterohaemorrhagic strain) has been found to be very problematic and even at a low infective dose, could present a human health hazard (Islam *et al*., 2005). Disease outbreaks due to contamination in the Sand River catchment could be disastrous considering that the agricultural sector is of great economic importance to the city of Polokwane. It is therefore important to further investigate the potential contamination of crops irrigated with water from the Sand River. *E.coli* levels in the Polokwane maturation ponds were already above the licence limit. Persistent increase in these levels could ultimately result in possible contamination of crops.

**Heavy metal contamination**

There are no major industries in Polokwane that discharge heavy metals in the Sand River. Heavy metal concentration in the water did not exceed the recommended levels. However, as already indicated, petrol stations garages and scrap metal dump sites can be sources of heavy metal contamination. It is recommended that continuous monitoring of heavy metal be undertaken by the Polokwane municipality. At the present moment, no routine heavy metal monitoring is being done.

There was significant bioaccumulation of iron in fish. Although iron is not a toxic metal, excessive levels of it can lead to hemochromatosis in people. The main problem with hemochromatosis is that it is not specific and its symptoms are vague. People who suffer from this disease may not know it. They complain of flu-like symptoms, aching of joints and fatigue. It is recommended that people be banned from catching fish in the Sand River for human consumption. This study clearly demonstrated the bioavailability of iron.

Lead and Cd are toxic metals and their levels were relatively low in fish. This study did not look at intermetallic relations, but these are very important as it will indicate the pathways through the aquatic system that the metal follows in relation to others. It is also further recommended that a study be undertaken to compare heavy metal concentrations during the dry and the wet season. The diversion of effluent to Mogalakwena platinum mine is certainly helping in reducing the heavy metals concentrations in the river.

**Ground water contamination**

The Polokwane aquifer acts as a reservoir for the drought prone Polokwane area. It is important to protect this important natural asset for the people of Polokwane. The recharging of the Polokwane aquifer with sewage effluent that has been well treated is the only way that the aquifer can be maintained. However, poorly maintained maturation ponds have led to substandard sewage effluent being discharged into the Polokwane aquifer. There are very limited pollution abetment measures that can be put in place in respect to ground water. Furthermore, groundwater pollutants can travel for long distances. It is recommended that the Polokwane aquifer must only be recharged with water the meets that recommended standard. As already indicated, the Cape Flats aquifer in the Western Cape is already polluted (Adelana and Xu, 2006). This clearly emphasises the importance of preventing pollutants from entering the Polokwane aquifer.

**What can be done to reduce the pollution loads of the Sand River?**

The Polokwane WWTW is currently being upgraded. The Polokwane population is increasing at a rate of 2.13% per annum (DWA, 2012). In most urban areas in Africa the problem of overloading sewage treatment plants has never been solved by upgrading. The rate at which these countries develop, does not allow sufficient time for improvement of water treatment facilities (Mthembu *et al*., 2013). Although upgrading may seem as a viable option, it requires frequent upgrades which may become costly. The rate of upgrading is being surpassed by the rate urbanisation (Mthembu *et al*., 2013). The need for frequent upgrading can be minimised by incorporating more effective treatment technologies. These include the biological nutrient removal process which has proven effective in nutrient removal (Barnard, 2005). The Polokwane WWTW is currently using activated sludge technology to treat sewage effluent, while the Seshego WWTW uses biological filters. The effectiveness of the treatment for both processes with respect to nitrogen and phosphorus removal is may not be adequate. This is cited as the main reason most rivers in South Africa have reached eutrophication is since conventional activated sludge and biological filter treatment processes do not sufficiently remove phosphorus and nitrogen (Wiechers and Heynike, 1986). The use of biological treatment can be more beneficial in the removal of most inorganic and organic nutrients. Biological nutrient removal (BNR) processes have been found to remove between 0.1 and 0.15 mg/l of phosphorus reducing the use of chemicals used to remove phosphorus (Barnard, 2005). The incorporation of BNR may be a feasible alternative to the current technologies employed by the Polokwane municipality and may aid in reducing the high nitrogen levels in the effluent discharged into the sand River.

The use of natural wetlands may also be a viable strategy that can be employed to reduce high nutrient loads in the sewage effluent discharged into the Sand River. Naturally, wetlands aid in improving the self purification capacity of the rivers. Unlike convention methods, wetlands have been proven to have the potential to meet the required effluent treatment standards (Mthembu *et al*., 2013). Wetlands are characterised by high organic matter accumulation due to high rate of primary production (Britx, 1993). The incoming nutrients support the growth of vegetation. Inorganic chemicals are converted into organic materials on the basis of the wetland food chain. Many natural and constructed tropical wetlands have a net primary production of more than 1000 g ($m^{-2}$/yr) greater than most ecosystems (Neue *et al*., 1997). However, natural wetlands are being destroyed because of the construction of urban centres. In the planning of these urban areas, the effective use of these wetlands as natural water purifiers is ignored. In this study, site 6 was a wetland and the water quality inevitably started improving from this site. The construction of wetlands in the sites after sewage effluent discharge would work effectively and efficiently in the Sand River as the volumes of sewage effluent received by the river are not very large. This is because a substantial amount (14 000 $m^3$ per day) of the sewage effluent is diverted to the Mogalakwena Platinum Mine. Large volumes of effluent may hinder the nutrient retention capacity and the wetland may become waterlogged (Lee *et al*., 2009). A typical example of the efficiency of a constructed wetland in South Africa is in the Lourens River, the Western Cape (Schulz and Peall, *et al*., 2001). These authors reported that up to 70% nitrates and 54 % orthophosphate were retained in the wetland. Although their study was not focusing on point source pollution, it clearly demonstrates the efficacy of wetlands for nutrient removal. Some wetlands may even function as discharge areas for surfacing groundwater, allowing groundwater to sustain base flow stream during dry seasons (Kivaisi, 2001). Mthembu *et al*., (2013) stated the with the increasing eutrophication status of many South African rivers, constructed wetlands maybe the most suitable alternative to treatment technologies. Theses authors further highlighted that wastewater treatment will always pose problems if there are no new alternative technologies in place to replace the currently available technologies. It is estimated that developing countries will run out of water by 2050 (Mthembu *et al*., 2013). This is a course for concern not only to the communities but also a challenge to scientist to find new ways of wastewater recycling

**Outcomes of this study**

The findings in this study provide baseline information for the water quality status of the Sand River. Sewage effluent containing high levels of nitrogen is currently discharged into the Sand River. In the long run, this may result in eutrophication. The river is however still able to maintain self-purification. The coliform levels and *E.coli* levels do not as yet pose a threat, however continuous increase could ultimately result in levels that could be unsuitable for irrigation and recreational use. The river is dominated by pollution tolerant macroinvertebrates. This indicates that the conditions in the river are no longer suitable for pollution sensitive macroinvertebrates. Heavy metal concentrations in the Sand River water were low, however elevated iron concentrations were found in fish. The levels found in fish may cause health related issues to consumers. In terms of suitability for irrigation, the sodium and alkalinity levels in the borehole and river water was low. The water was found to be suitable for irrigation and long term use of this water is not likely to cause soil infiltration problems unless if the quality of the water deteriorates.

## CHAPTER 8: REFERENCES


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# APPENDIX A

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# The effect of sewage effluent on the physico-chemical and biological characteristics of the Sand River, Limpopo, South Africa

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ABSTRACT

Population growth in urban areas is putting pressure on sewage treatment plants. The improper treatment of sewage entering the aquatic ecosystems causes deterioration of the water quality of the receiving water body. The effect of sewage effluent on the Sand River was assessed. Eight sampling sites were selected, site 1 and 2 were upstream of the sewage treatment plant along the urbanised area of Polokwane, whilst sites 3, 4, 5, 6, 7 and 8 were downstream. The physico-chemical parameters and coliform counts in the water samples were determined. The suitability of the water for irrigation was also determined. Hierarchical average linkage cluster analysis produced two clusters, grouping two sites above the sewage treatment works and six sites downstream of the sewage effluent discharge point. Principal component analysis (PCA) identified total nitrogen, total phosphorus, conductivity and salinity as the major factors contributing to the variability of the Sand River water quality. These factors are strongly associated with the downstream sites. Canonial correspondence analysis (CCA) indicated the macroinvertebrates, Chironomidae, Belastomatidae, Chaoborus and Hirudinea being strongly associated with nitrogen, phosphorus, conductivity and temperature. *Escherichia coli* levels in the Polokwane wastewater treatment works maturation ponds, could potentially lead to contamination of the Polokwane aquifer. The Sodium Adsorption Ratio was between 1.5 and 3.0 and residual sodium carbonate was below 1.24 Meq/l, indicating that the Sand River water is still suitable for irrigation. The total phosphorus concentrations fluctuated across the different site. Total nitrogen concentrations showed a gradual decrease downstream from the point of discharge. This shows that the river still has a good self-purification capacity.


## 1. Introduction

Many cities in South Africa have experienced rapid urbanisation and population growth, the city of Polokwaneis no exception. The continued increase in population has resulted in the overloading of the Polokwane sewage treatment plant. This has consequently led to the discharge of substandard effluent into the Sand River. The discharge of sewage effluent that has not gone through proper treatment processes causes adverse effects on the aquatic ecosystem. The major problems associated with the discharge of substandard effluent include, oxygen depletion due to oxidation of organic matter, increase in nutrients such as nitrogen and phosphorus and faecal contamination (Moyo and Mtetwa, 2002).

Inadequately treated sewage effluent that is continuously discharged into rivers leads to eutrophication. Moyo and Mtetwa (2002) reported that inadequate sewage treatment has resulted in the eutrophication of several water bodies near towns and cities in Zambia. Poor bacteriological qualities of river water in Blantyre, Lilongwe and Zomba (Malawi) due to effluent discharges have been reported (Moyo and Mtetwa, 2002). Such incidences are also common in South Africa, as it is now apparent that most rivers are heavily polluted with faecal organisms (Barnes and Taylor, 2004). The faecal coliform count levels in many rivers across the country have exceeded the South African guidelines (Fatoki et al., 2001; Lin et al., 2002; Mthembu et al., 2011). There have been reports of major fish killings in the Vaal River (Wepener et al., 2011) and cholera outbreaks in the Mpumalanga Province where the river water was suspected to be contaminated with faecal matter (Momba et al., 2006). Some rivers such as the Berg River in the Western Cape were at the verge of eutrophication (de Villiers, 2007).

Polokwane wastewater treatment works (WWTW) discharge sewage effluent into the Sand River. The Sand River water is used extensively by farmers downstream for irrigation. However, no studies have been done previously on the Sand River to determine the quality of the river water. Polokwane is a water scarce area and in order to conserve water, artificial recharge of the local Polokwane aquifer using treated wastewater is practised. The treated wastewater is discharged into the alluvial and gneissic aquifer. Before discharging into the gneissic aquifer, the municipal

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wastewater goes through both primary and secondary treatment and has a retention time of three weeks in a series of maturation ponds. Poor maintenance of the maturation ponds in the last few years may lead to poor effluent being discharged into the aquifer. It is therefore prudent that the quality of the municipal waste be determined before discharge.

The objectives of this study were to provide baseline physico-chemical and biological data on the Sand River and also to determine the suitability of the water for sustaining aquatic life and irrigation. The quality of the effluent for recharge of the aquifer was also investigated.

## 2. Materials and methods

### 2.1. Description of study area

The Sand River sub-catchment is a major tributary of the Sabie River Catchment and is a right hand tributary of the Limpopo River. The city of Polokwane is located 200 km upriver from its mouth. It flows by the western edge of this town. The Polokwane Pasveer Activated Sludge WWTW discharges its effluent into the Sand River. The Seshego WWTW discharges its effluent into the Blood River. The Blood River joins the Sand River from the left, just north of Polokwane (Fig. 1). Eight sampling sites were selected. Site 1 and 2 are upstream of the Polokwane WWTW, while Site 3, 4, 5, 6, 7 and 8 are downstream (Fig. 2).

### 2.2. Chemical and physical parameters of water from the Sand River

Total phosphorus and total nitrogen at each site were determined using calorimetric methods adapted from APHA (1995). Temperature, salinity, pH and dissolved oxygen from at each site were measured monthly using a YSI meter (MPS 556). The flow rate was determined using a flow meter (model PS2000). Suspended solids (SS), *E. coli*, chemical oxygen demand (COD) of the water samples from the Polokwane WWTW maturation pond were determined according to Standard Methods procedures (APHA, 1989). The nitrite and ammonia were also analysed according to Standard Methods procedures using ammonia selective electrode (APHA, 1989).

The discharge at each site was estimated by the velocity area method (Schumm, 1977):

$$Q = VA$$

where $Q$ is the discharge ($m^3\ s^{-1}$); $V$ the mean velocity ($m\ s^{-1}$); $A$ is the area of cross-section ($m^2$)

Self purification capacity was determined, according to Cooper et al., 1919 where:

$$Sr\ (g\ s^{-1}\ m^{-1}) = (Q_1C_1 + Q_SC_S - Q_2C_2)/L$$

where $Q_1C_1$ is the pollution load at the upstream station, $g\ s^{-1}$; $Q_2C_2$ the pollution load at the downstream station, $g\ s^{-1}$; $Q_sC_s$ the point source pollution load in the river reach, $g\ s^{-1}$; $L$ is the distance between the two points 1 and 2, m.

### 2.3. Microbiological analysis

One litre glass bottles were sterilized in the autoclave (Model HA 300MD, Hirayama Manufacturing Corporation) at 105 °C for 30 min and used to collect water samples monthly at each site. The bottles were stored in ice during transportation. Coliform counts were determined using the Membrane Filtration Method (WHO, 1996). The total coliform (TC), faecal coliforms (FC), and faecal streptococcus (FS) coliforms were isolated using M-Endo, m-FC and K-F agar respectively. The M-Endo and K-F agar plates were incubated at 37 °C for 24 and 48 h respectively. The m-FC agar plates were incubated at 44 °C for 24 h. *E. coli* counts/100 ml were determined according to Standard Methods procedures (APHA, 1989).

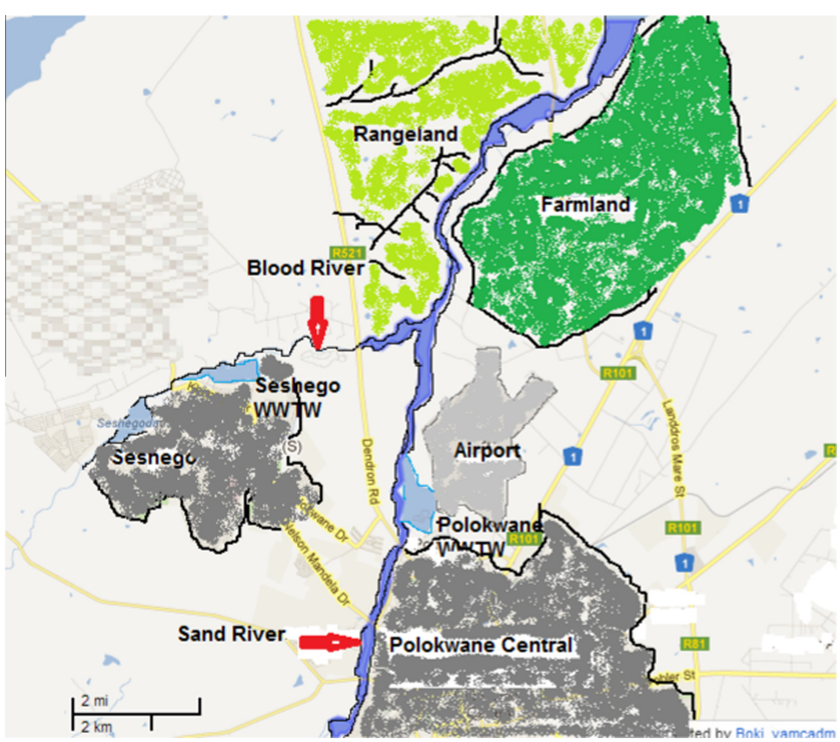


**Fig. 1.** Map of the Sand River with the Polokwane and Seshego wastewater treatment works (WWTW) together with the surrounding rangeland and farmland. (GPS Visualizer, 2002).


**Fig. 2.** Map of the Sand River indicating Polokwane and Seshego wastewater treatment works (WWTW) and sites 1, 2, 3, 4, 5, 6, 7 and 8 (GPS Visualizer, 2002).

### 2.4. Macroinvertebrates

Macroinvertebrates were sampled monthly from February, 2011 to June, 2012. They were collected with a hand net of 2 mm mesh size from three different biotopes namely soil in current, rock in current and sand in current. The macroinvertebrates were identified to family level using the illustrations from Aquatic Invertebrates of South African Rivers (Gerber and Gabriel, 2002) and enumerated.

### 2.5. Suitability of Sand River water for sustaining aquatic life and irrigation

The water quality of the Sand River with respect to sustaining aquatic life was measured using the Water Quality Index (WQI) (Brown et al., 1970). The water quality standards used were those recommended by the Department of Water Affairs and Forestry (DWAF, 1996a). The WQI was calculated using the following expression

$$\mathrm{WQI} = \Sigma Q_n W_n / \Sigma W_n$$

where $W_n$ is the unit weight for the nth parameter; $W_n$ the k/Sn; $S_n$ the standard value for the nth parameter; $K$ the constant for proportionality; $Q_n$ is the quality rating for the nth water quality parameter

$$Q_n = 100(V_n - V_{io})/(S_n - V_n)$$

where $V_n$ is the estimated value of the nth parameter at a given sampling point; $S_n$ the standard permissible value of the nth parameter; $V_{io}$ the ideal value of the $n$th parameter in pure water (i.e. 0 for all the parameters except pH and dissolved oxygen). River water quality status was classified using the scaling values (Table 1) by Brown et al., (1970).

Suitability of Sand River water for irrigation was determined at sites 4 and 7(where irrigation takes place). The calcium and magnesium content of the water at the downstream sites were determined by EDTA titration using eriochrome black T as an indicator. Sodium and potassium content were determined using a flame photometer. Chloride concentrations were measured by silver nitrate titration. Carbonate and bicarbonate content was measured by acid–base titration. The suitability of irrigation water was determined using the sodium adsorption ratio which was calculated from the ratio of sodium to calcium and magnesium.

The sodium adsorption ratio (SAR) was calculated using the following formula:

$$\mathrm{SAR} = \mathrm{Na}^+/(\sqrt{\mathrm{Ca}} + m^{++}/2)$$

The residual sodium carbonate (RSC) was determined as follows:

$$\mathrm{RSC} = (\mathrm{CO}_3^- + \mathrm{HCO}_3^-) - (\mathrm{Ca}^{++} + \mathrm{Mg}^{++})$$

### 2.6. Statistical analysis

The Shannon–Wiener diversity index was used to determine the diversity of the benthic fauna before and after the sewage effluent discharge (Shannon and Weaver, 1949). One-way analysis of variance (ANOVA) on the Statistical Package and Service Solutions (IBM SPSS version 20.0) was used to determine if there was any significant difference in water quality parameters. Principal component analysis (PCA) was used to determine the factors that contribute to water quality variation (using SAS version 1.9). Canonial correspondence analysis (CCA) was used to determine the relationship between the water quality parameters and the macroinvertebrates (CANOCO version 5). Hierarchal Cluster analysis was used to group the different sites of the Sand River in relation to the water

**Table 1**
The status of the river with respect to Water Quality Index values was determined using the following scaling (Brown et al., 1970).

| Water quality index | Description |
|---|---|
| 0–25 | Excellent |
| 26–50 | Good |
| 51–75 | Poor |
| 76–100 | Very poor |
| >100 | Unfit for drinking |

quality (IBM SPSS version 20.0). It was also used to cluster the site in relation to the macroinvertebrate abundances.

## 3. Results

The suspended solids, ammonia, chemical oxygen demand and *E. coli* levels in the Polokwane WWTW maturation ponds were above the licence guidelines (Table 2).

Analysis of variance (ANOVA) indicated that there were no significant differences for temperature, conductivity, salinity, pH, oxygen and flow rate between the upstream and downstream sites (ANOVA, $P > 0.05$). There were however significant differences in the phosphorus and nitrogen concentrations (ANOVA, $P < 0.05$), with higher phosphorus and nitrogen values at the sites after discharge (Table 3).

Eigenvalues showed that the first three principal components are the most significant (>1). These extracted components explain 85% of the total variance in the water quality of the Sand River (Table 4). Principal component (PC) 1, explains 54% of the total variance. This component incorporates the major water quality variables that are characteristic of wastewater discharges with strong positive loading of nitrogen, phosphorus, conductivity, salinity, temperature and flow rate (Fig. 3, Table 5). These parameters are strongly correlated with one another. Principal component 2 explains 20% of the total variance, with strong positive loading of oxygen and pH. Principal component 3 explains 11% of the total variance, with a strong positive loading of depth (Fig. 3, Table 5). The first and second component distinguishes the sites (site 3, 4, 5, 6 and 7) with high nutrient loading that indicate deterioration of water quality from those with low nutrient loading (site 1, 2 and 8) (Fig. 3).

All the eight sampling sites were grouped into two major clusters (Fig. 4). The two clusters were joined using Ward's Method with Squared Euclidean metric. Cluster 1, consisted of site 1 and 2, which are the upstream sites. Cluster 2, consisted of site 3, 4, 5, 6, 7 and 8, which are the sites downstream. These sites are characterised by high phosphorus and nitrogen levels.

Canonial correspondence analysis axis 1 explained 29.79% of the total variance (Table 6). Salinity and flow showed a moderately positive correlation to this axis. Temperature, phosphorus, nitrogen and conductivity showed strong negative loading on CCA axis 1 and was associated with Belastomatidae, Hirudinea and Chironomidae (Fig. 5). Canonial correspondence analysis axis 2 explained 26.70% of the total variance (Table 6). Depth and pH were positively correlated with CCA axis 2. Similarly, temperature, phosphorus, nitrogen and conductivity show strong negative loading on this axis with macroinvertebrate association similar to those of axis 1 (Fig. 5).

The eight sampling sites were clustered into two groups which represented the abundances of macroinvertebrates (Fig. 6). Cluster 1 consisted of site 4 and 5 which were dominated by pollution tolerant species of the family Chironomidae, Simulidae, Oligochaeta and Chaoborus. Cluster 2 consisted of sites 1, 2, 3, 6, 7 and 8, which consisted of both pollution tolerant and sensitive species.

Total coliform (TC) levels gradually increased from the upstream sites to the downstream sites, there was however a decrease at site 6 and 8 (Fig. 6). Faecal coliforms levels (FC) started increasing at site 3 (after discharge) until site 5. The levels then decreased at the sites further downstream. Faecal streptococci levels (FS) were lowest at site 1 and started to increase at site 2. The levels decreased gradually from site 3 to 4, increased at site 5 and further decreased at site 6 and 7 and increased at site 8 (Fig. 7). A

**Table 2**
Mean of water parameters of the final effluent determined in the maturation ponds of the Polokwane WWTW.

| Parameter | Mean ± SE | Licence requirements |
|---|---|---|
| pH | 7.30 ± 0.035 | 5.5–9.5 |
| Electrical conductivity (mS/cm) | 109.90 ± 4.335 | 150 |
| Suspended solids (mg/l) | 59.98 ± 7.166 | 25 |
| Ammonia (mg/l) | 38.90 ± 4.132 | 3 |
| Nitrate (mg/l) | 1.85 ± 0.395 | 15 |
| Phosphate (mg/l) | 7.16 ± 0.449 | 1 |
| Chemical oxygen demand (mg/l) | 93.17 ± 3.589 | 75 |
| *Escherichia coli* (cfu/100 ml) | 1463.73 ± 52.63 | 1000 |

**Table 3**
ANOVA table of water quality parameters.

| Parameter | *F* | *P* |
|---|---|---|
| Temperature | .076 | .999 |
| Conductivity | 2.190 | .147 |
| Salinity | 1.623 | .256 |
| Oxygen | .462 | .837 |
| pH | 1.620 | .256 |
| Flow | .763 | .633 |
| Depth | 7.823 | .005[a] |
| Phosphorus | 10.388 | .002[a] |
| Nitrogen | 8.231 | .004[a] |

[a] The mean difference is significant at 0.05 level.

**Table 4**
Eigenvalue of water quality components.

| Component | Eigenvalue | Difference | Proportion | Cumulative |
|---|---|---|---|---|
| 1 | 4.896 | 3.134 | 0.544 | 0.544 |
| 2 | 1.762 | 0.718 | 0.196 | 0.740 |
| 3 | 1.044 | 0.416 | 0.116 | 0.856 |
| 4 | 0.628 | 0.227 | 0.070 | 0.926 |
| 5 | 0.400 | 0.153 | 0.045 | 0.970 |
| 6 | 0.246 | 0.222 | 0.028 | 0.997 |
| 7 | 0.024 | 0.024 | 0.003 | 1.000 |
| 8 | 0 | | 0 | 1.000 |
| 9 | 0 | | 0 | 1.000 |

Extraction method: principal component analysis.

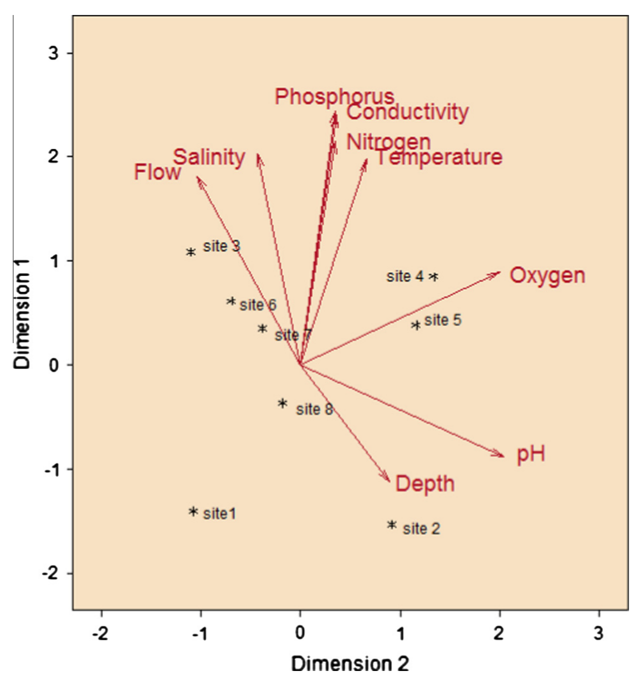


**Fig. 3.** Component plot in rotated space for water quality parameter distribution with respect to dimension (component) 1, 2.

**Table 5**
Correlation (structure) of water quality components.

| Variable | PC1 | PC2 | PC3 |
|---|---|---|---|
| Temperature | 0.7928 | 0.2656 | 0.1520 |
| Conductivity | 0.9624 | 0.1457 | 0.0335 |
| Salinity | 0.8127 | −0.1711 | −0.3264 |
| Oxygen | 0.3578 | 0.7990 | −0.0022 |
| pH | −0.3536 | 0.8159 | −0.2758 |
| Flow | 0.7254 | −0.4153 | 0.3999 |
| Depth | −0.4509 | 0.3557 | 0.7784 |
| Nitrogen | 0.8614 | 0.1390 | 0.2339 |
| Phosphorus | 0.9779 | 0.1370 | −0.1277 |

Extraction method: principal component analysis, rotation method: varimax, PC – principal component.

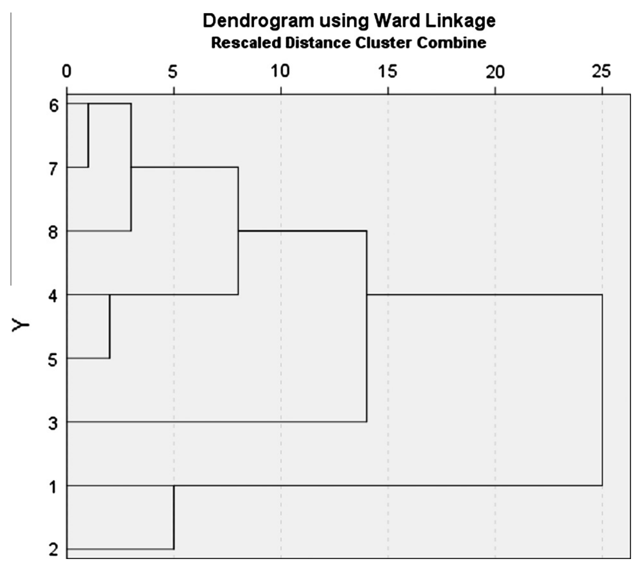


**Fig. 4.** Dendrogram showing sampling sites (*Y*-axis) clusters of water quality parameters of the Sand River.

similar trend as that of the faecal coliforms was observed for the *E. coli* counts (Fig. 8).

The upstream sites had higher macroinvertebrate diversity than the downstream sites (Table 7). However, the diversity was highest further downstream at the last site (site 8) and lowest at site 5. There were no significant differences in the diversity between site 1, 2, 3, 4, 6 and 7 (ANOVA, $P > 0.05$). There were however significant differences between site 5 and 8 (ANOVA, $P < 0.05$). The macroinvertebrates were more evenly distributed at site 1 (Table 7).

The self purification rate with respect to total phosphorus fluctuated throughout the different sections of the river, with the highest self purification rate between sites 3 and 6 (Table 8). The self purification rate was not directly proportional to the total phosphorus loading, indicating that other factors played a role in the purification rate. Similarly the self purification of the total nitrogen fluctuated throughout the different sections however, there was a

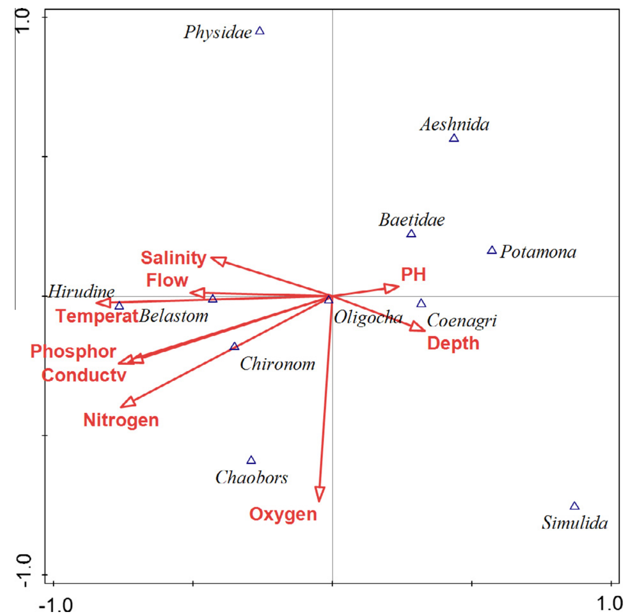


**Fig. 5.** Biplot of Canonial correspondence analysis (CCA) depicting the relation between the physico-chemical parameters and macroinvertebrates. (Temperat = temperature, Phosphor = phosphorus, Conductv = conductivity, Aeshnida = Aeshnidae, Potamona = Potamonautidae, Oligocha = Oligochaeta, Hirudine = Hirunidae, Belastom = Belastomatidae, Chironom = Chironomidae, Chaobors = Chaoborus, Simulida = Similidae).

steady decline further downstream (3–7, 3–8). The self-purification rates for both nutrients were positive, indicating that none of the river sections acted as prominent sources of nutrients or acting as river sinks.

The WQI indicated that the water quality of the Sand River was very poor and unfit for human consumption (Table 9), this is in accordance with Brown et al. (1970). In terms of the Sand River water fitness for irrigation, the sodium adsorption ratio (SAR) for site 4 (which is the site where most irrigation takes place) was good and that of site 7 was fair. This was determined in accordance to the irrigation water classification by Koegelenberg (2004) (Table 9). The Residual sodium carbonate for both site 4 and 7 do not pose any potential hazard to the physical properties of the soil (Table 9).

## 4. Discussion

Principal component analysis showed that nitrogen and phosphorus were associated with the first component which explained 54% of the total variation in the water quality of the Sand River. The sites before discharge (Site 1 and 2) form their own cluster and had significantly lower phosphorus and nitrogen levels than the downstream sites. The main reason for the elevated nitrogen and phosphorus levels in the downstream site is the substandard effluent being discharged from the Polokwane and Seshego WWTW. The phosphorus and ammonia levels from the Polokwane sewage treatment plant maturation ponds are above the stipulated licence

**Table 6**
Canonial correspondence analysis of water quality parameters and macroinvertebrates.

| Component | Eigenvalues | Pseudo-canonial correlations | Explained variation (cumulative) |
|---|---|---|---|
| 1 | 0.073 | 1.000 | 29.8 |
| 2 | 0.065 | 1.000 | 56.5 |
| 3 | 0.047 | 1.000 | 75.6 |
| 4 | 0.037 | 1.000 | 90.9 |

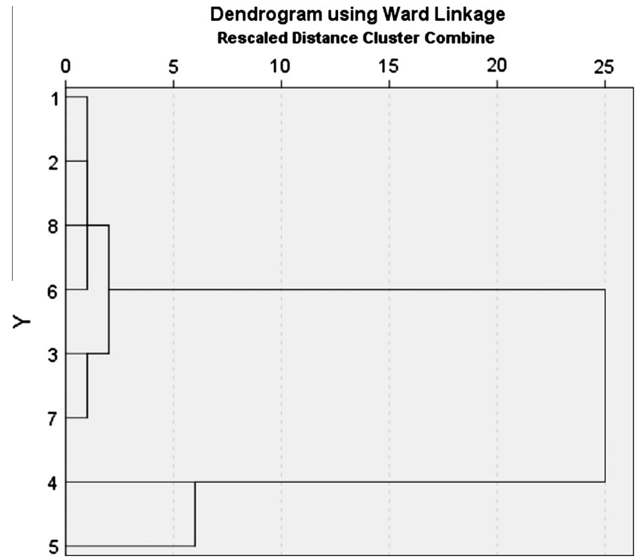


**Fig. 6.** Dendrogram showing sampling sites (Y-axis) clusters of macroinvertebrate families of the Sand River.

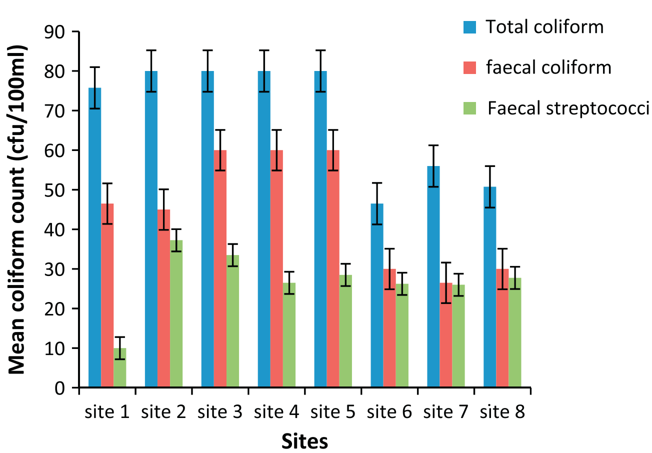


**Fig. 7.** Mean values of the total coliform counts, faecal coliform counts and faecal streptococci coliform counts across at different sites of the Sand River.

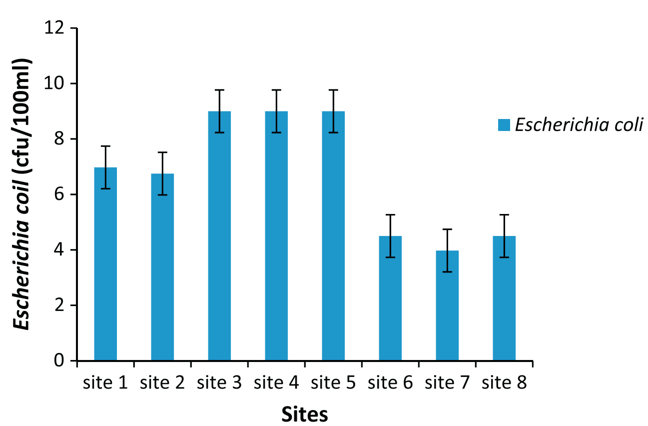


**Fig. 8.** Mean *Escherichia coli* counts at the different sampling sites across the Sand River.

guidelines. Both sewage treatment works are overloaded. Polokwane WWTW has a design capacity of 28 ML/day and yet it is discharging 30 ML/day (DWA, 2009). Similarly the Seshego WWTW is exceeding its designed capacity by 0.8% (DWA, 2009). The problem of sewage treatment works that are overloaded is common in South Africa (Morrison et al., 2001; Fatoki et al., 2001; Igbinosa and Okoh, 2009). The rapid urban population growth in all these areas is the root cause of the problem. In the city of Polokwane the population increased from 20,500 in the 1950s to 631,318 in 2012 (DWA, 2012). However, there was no concomitant increase in the design capacity of the Polokwane WWTW.

**Table 7**
Diversity of macroinvertebrates at the different sites of the Sand River. Data are mean values ± SE.

| Site | *H′* | *E* |
|---|---|---|
| Site 1 | 1.62 ± 0.100 | 0.22 ± 0.131 |
| Site 2 | 1.32 ± 0.177 | 0.07 ± 0.012 |
| Site 3 | 0.90 ± 0.667 | 0.15 ± 0.099 |
| Site 4 | 0.72 ± 0.470 | 0.04 ± 0.014 |
| Site 5 | 0.56 ± 0.115* | 0.03 ± 0.008 |
| Site 6 | 1.07 ± 0.194 | 0.06 ± 0.009 |
| Site 7 | 1.16 ± 0.629 | 0.06 ± 0.017 |
| Site 8 | 2.33 ± 2.507* | 0.09 ± 0.065 |

*H′* – Shannon Wiener diversity index, *E* – Shannon wiener evenness.
* Mean difference is significant at 0.05 levels.

CCA axes 1 and 2, showed high negative loadings of total phosphorus, total nitrogen, conductivity and temperature. These parameters were strongly associated with Hirudinea, Belastomatidae, Chironomidae and the Choaborus families. This then indicates that these macroinvertebrates are highly tolerant to pollution. The South African Scoring System (SASS) is the most widely used monitoring tool in Southern Africa. According to SASS5 all the species recorded in the study are pollution tolerant except for Aeshnidae which was recorded at site 8. This implies that the Sand River has a high nutrient load, leading to pollution, altering the biotic diversity.

High macroinvertebrate diversity indices were recorded at the upstream sites and the last downstream site. The macroinvertebrates at these sites were evenly distributed. Lower diversity was however recorded at the sites after discharge (site 3, 4 and 5). Loss of diversity at these sites could have resulted from the elevated nitrogen levels recorded at these sites. Nitrogen in the form of ammonia can be very toxic to aquatic organisms, particularly at a pH of above 8. At pH of 6–7, ammonia is converted to ammonium which is not toxic to aquatic organisms (DWAF, 1996a). The absence of pollution sensitive macroinvertebrates such as those of the Ephemeropteran family, serves an indication that water is unsuitable for sustaining pollution sensitive aquatic organisms including fish. The effects of sewage effluent on macroinvertebrates have also been investigated by Moyo and Phiri (2002), Dube et al. (2010), Canobbio et al. (2009) and several others. The main reason for the dominance of pollution tolerant species is the continuous discharge of substandard sewage effluent throughout the year. The Sand River is not a perennial river but, it flows throughout the year because of the sewage effluent. Some of the effluent is diverted to Mokgalakwena Platinum Mine (Bradford and Salmon, 2008). It is most probable that if there was no diversion, the water quality would have deteriorated even further.

Evidence of increasing self-purification was seen where *E. coli*, nitrogen and phosphorus levels in the water gradually declined from the point of discharge. Self purification capacity is dependent on a number of factors which include; flow rate, quantity and quality of loaded nutrients, river surface area, oxygen, temperature, turbulence, distribution of vegetation across the river and bacterial degradation to name but a few (Ifabiyi, 2008). In this study, surface area and reduced nutrient load (due to water diversion) may have played an important role in the self-purification capacity of the Sand River. Like most rivers it can lose its ability to retain nutrients

**Table 8**
Self purification of total nitrogen and phosphorus across the different sites of Sand River.

| Site | Distance (km) | TP load (g/s) | TN load (g/s) | TP self purification rate (g/s/km) | TN self purification rate (g/s/km) |
|---|---|---|---|---|---|
| 3–4 | 13.431 | 5.059467 | 27.79757 | 0.376701 | 2.069658 |
| 3–5 | 15.221 | 1.753796 | 18.66903 | 0.115222 | 1.226531 |
| 3–6 | 18.375 | 7.473754 | 37.36878 | 0.406735 | 2.033675 |
| 3–7 | 22.430 | 2.530277 | 36.97504 | 0.112808 | 1.648464 |
| 3–8 | 27.777 | 7.019533 | 36.61398 | 0.252710 | 1.318140 |

**Table 9**
The suitability of the Sand River water with respect to Soduim adsorption ratio (SRC), Residual sodium carbonate (of sites 4 and 7) and Water quality index (WQI) values.

| | Site 4 | Site 7 | Overall sites |
|---|---|---|---|
| SAR | 1.154 | 2.373 | – |
| RSC (meq/l) | 0.259 | 0.745 | – |
| WQI | – | – | 143.44 |

SAR – sodium adsorption ration, RSC – residual sodium carbonate, WQI – water quality index.

following continuous reception of substandard effluent. If there is no sufficient oxygen to degrade and transform the nutrients, it is most likely to become eutrophicated. Such an incident has been observed on the River Illo in Nigeria where self-purification factors revealed slow reaeration of the river with no pollution recovery (Longe and Omole, 2008). Reduction of the river velocity resulted in enrichment of the water body, oxygen deficit and eutrophication (Longe and Omole, 2008). Nhapi and Tirivarombo (2004) have reported nitrogen and phosphorus reduction levels of different magnitudes across the different stretches of the Marimba River in Zimbabwe. Even with the fluctuation of nutrient load levels, the river was able to reduce most of the nutrients. Similar results were also reported by Bere (2007) on the Chinyika River in Zimbabwe. These studies indicate that there is a general trend of increased self purification downstream of the discharge point.

*E. coli*, faecal coliform and total coliform levels increased from the point of discharge (site 3, 4 and 5) and gradually decreased further downstream (Site 6, 7 and 8). The elevated levels of and faecal coliforms particularly at the sites just after discharge, are mainly due to effluent discharge. Faecal coliforms were above the stipulated guidelines for both domestic (0 counts/100 ml). The faecal streptococci levels were fairly uniform throughout the entire river but, with lower levels at site 1. The water from the storm water drains contains both animal and some human faecal matter from the urbanised area and surrounding informal settlements, thus contributing to the levels of TC, FS and FC found in the river water. Reduction of the all the coliforms further downstream implies that there was some dilution and degradation of organic matter, a further indication of self purification of the Sand River. Despite the reduction in coliform levels this water still poses a potential threat if used for recreational and human consumption. The *E. coli* levels in the maturation pond exceed the licence limit and could potentially pose a health threat to people from the neighbouring communities. However, as a result of dilution and self purification the levels were much lower further downstream.

In Polokwane the most significant factor in groundwater yield is the recharge of the Sand River North Aquifer from treated sewage effluent. If the sewage treatment plant continues to discharge substandard effluent, this could lead to contamination of the aquifer.

The WQI indicated that the water quality of the Sand River is very poor. This is further confirmed by the high nitrogen and phosphorus levels. It is unsuitable for human consumption and could result in health related issues. However, the SAR and RSC indicated that the Sand River water is suitable for irrigation. According to DWAF (1996b), SAR values obtained between 1.5 and 3.0 are likely to cause water infiltration problems, however because the EC is <90 mS/m no problem is to be expected. This is because soil sodicity increases with increasing EC (DWAF, 1996b). Long term discharge of effluent which has not gone through the proper treatment couldhowever result in increased SAR values. If the residual sodium carbonate (RSC) increases above zero, it increases the sodium hazard to the soil structure (Hopkins et al., 2007). When irrigating with water containing sewage effluent, more carbonates are added to the soil than divalent cations. The calcium in the soil is thus lost during the formation calcium carbonate deposits (Hopkins et al., 2007). These lime deposits cause precipitation of phosphorus and other macronutrients required in fruits and vegetables. The salt contained in the effluent accumulates in the roots of plants over time posing harmful impacts on the soil health and crop yield. The leaching of these salts may cause soil and ground water pollution (Hussain et al. 2002). Some of the impacts of excess salinisation on water resources in the Eastern Cape province (South Africa) include reduced crop yield, increases formation of scale and added corrosion in domestic and increased requirementsfor pre-treatment of water (Igbinosa and Okoh, 2009). This is of great importance because there are a number of farms along the Sand River who rely solely on this water for irrigation.

## 5. Conclusion

This study has indicated that due increased population in the city of Polokwane, the Polokwane WWTW is now discharging effluent which is substandard. The sewage treatment works is not well maintained, evidence of this is seen in the quality of the effluent from the maturation ponds. Nitrogen and phosphorus levels are above the South African limits. The coliform levels pose a potential threat to the downstream communities and may result in contamination of the Polokwane aquifer. The macroinvertebrate communities are greatly affected by the deteriorating water quality as there is absence of pollution sensitive species and a decline in the diversity downstream. Despite the deterioration in water quality, the Sand River is still able to maintain a good self purification capacity and the water is still suitable for irrigation purposes.

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