EVALUATION OF HEAVY METAL AND MICROBIOLOGICAL CONTAMINATION AND ASSESSMENT OF THE SUITABILITY OF THE SAND RIVER WATER FOR IRRIGATION IN THE LIMPOPO PROVINCE

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WATER RESEARCH COMMISSION

TT 835/20

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Report to the
Water Research Commission

by

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WRC Report No. TT 835/20
November 2020

Obtainable from

Water Research Commission
Private Bag X03
Gezina
PRETORIA, 0031

orders@wrc.og.za ordownload from www.wrc.org.za

ISBN 978-0-6392-0211-2

Printed in the Republic of South Africa

EXECUTIVE SUMMARY

Water scarcity is now a global challenge and climate change has exacerbated the situation. It is thus important for stakeholders to optimally utilize this scarce resource. Globally, 70% of the freshwater resources are used by the agricultural sector (Pimentel et al., 2004). In recent years some provinces in South Africa have experienced critical water shortages such as the Western Cape, Eastern Cape, Northern Cape and Limpopo Province. Optimal utilisation of water in agriculture must now be a top research priority. This then calls for government, communities and researchers to collaborate and ensure that water is viewed as an economic good. This study is a situation analysis of the Sand River which passes through a fast developing urban area, Polokwane City. This river is extensively used for irrigation of vegetables. However, the possibility of heavy metal contamination and microbial contamination of crops harvested after use of sewage effluent has not been investigated before. Evaluation of heavy metal and microbial contamination and assessment of the suitability of Sand River water for irrigation, was undertaken between March, 2017 and December, 2019.

The project report consists of six chapters. Chapter 1 is an overall introduction that provides background and contextualization, rationale, aims and objectives of the whole project. Chapter 2 is a literature review that highlights some of the work that has been done in the evaluation of heavy metals and microbial contamination of South African rivers. Furthermore, the work that has been done in microbial and heavy metal contamination of fresh produce is also reviewed. The gaps in the literature that justify the current project are identified. Chapter 3 gives the nutrient status of the Sand River and specifically looks at both spatial and temporal variation of physico-chemical parameters in the Sand River. Chapter 4 focuses on heavy metal contamination of the Sand River water and sediments. Significantly, in this chapter, the geo-accumulation index is used to determine the extent to which the Sand River sediment is contaminated with heavy metals. This chapter also looks at spatial and seasonal variations of heavy metals in Sand River water. It was deemed important to look at spatial variation of the heavy metals in order to assess the self-purification capacity of the river. Chapter 5 focuses on the microbiological contamination of the Sand River water. The chapter also focuses on spatial and temporal variations of the microbial load in the Sand River. It was also deemed important to look at the spatial variations of the microbial load so as to determine to what extent the Sand River can self-purify. Temporal variations were also investigated between the rainy and dry season because it is normally assumed that physico-chemical parameters concentrations will vary between the rainy and dry season. Chapter 6 investigates the suitability of Sand River and borehole water for irrigation of tomatoes and onions. The effect of Sand River and borehole water on soils was also assessed. The contamination of tomatoes and onions is reported on in this chapter. Significantly, the hazard quotient which assesses the health risk associated with consumption of tomatoes and onion irrigated with sewage effluent was assessed. The last part of the project report gives recommendations to local farmers, vegetable consumers and the academia.

Chapter 3 shows that the Sand River can self-purify because all the physico-chemical parameters declined downstream of the discharge point. Phosphorus levels were above the recommended limits for irrigation water. It was observed that Sand River water can be used to boost vegetable production because of its high nitrogen and phosphorus levels. The major ions in the Sand River water namely, sodium, potassium, chloride, calcium, magnesium and sulphate were mostly within acceptable limits at both international and local levels. The only nutrient that exceeded acceptable limits was phosphorus. The most suitable site for water abstraction that would optimize plant growth was identified as site 5. This is the site where the large commercial tomato producing company abstracts water. There were no detectable seasonal variations in the nutrient status of the Sand River between the rainy and dry seasons. This was attributed to intermittent discharge of poor quality effluent that probably obliterated any seasonal variations. It was therefore suggested that stakeholders using Sand River water to irrigate crops must closely monitor the quality of the water they abstract.

The situation analysis of heavy metal contamination of the Sand River water showed that iron, manganese, cadmium, copper, nickel and zinc all fell within the target for irrigation. However, lead levels exceeded the target range at two sites. Heavy metal concentration in the sediment followed the order iron>manganese>nickel>copper>lead>chromium>cadmium. The geo-accumulation index showed that the sediment was not contaminated with any of the trace metals before and after discharge of sewage effluent since the index was below 1 for all heavy metals.

Most *E. coli* bacteria are normal non-pathogenic inhabitants of the intestinal tracts of humans and animals and are, therefore, commonly used as hygiene indicator (faecal origin). However, some pathogenic strains (i.e. disease-producing) are known. The type of disease-producing *E. coli* bacteria are known as STEC (Shiga toxin-producing *Escherichia coli)* because they produce a potent toxin called Shiga toxin. In this study, the *E. coli* levels rose significantly after the discharge of sewage effluent. There were only marginal differences in the microbial load between *E. coli* levels during the rainy and dry seasons. No seasonal differences were observed in the levels of heterotrophic count, total coliforms and faecal coliforms. This again is attributed to the intermittent discharge of poor quality sewage effluent. Total coliforms, faecal coliforms and *E. coli* levels were above the stipulated South African water guidelines for irrigation use.

A field study was undertaken to determine the suitability of Sand River and borehole water for irrigation of tomatoes and onions. The field study was undertaken at the Aquaculture Research Unit. A risk assessment study was also undertaken for people consuming vegetables that are irrigated with Sand River water. The sodium adsorption ratio (SAR) and sodium soluble percentage (SSP) all fell within the target range for irrigation water. The hazard quotient for tomatoes was less than 1 for all the trace metals except lead. This indicates that lead can potentially be hazardous to people consuming tomatoes irrigated with Sand River water. However, further studies must be conducted because the reference dose for lead used in this study was very low. The hazard quotient for lead in onions was also above 1. This again highlights the importance of carrying out more work so as to fully establish the potential health impacts of lead. Microbial analysis of the tomatoes irrigated with Sand River water showed no heterotrophic, coliform, faecal and *E. coli* bacteria on the inside of the tomatoes. Total coliform, faecal coliforms and *E. coli* were also not detected outside the tomatoes irrigated with Sand River water. However, total heterotrophic bacteria were detected outside the tomatoes. No microbes were detected inside the onions. However, heterotrophic bacteria and total coliforms were detected outside the onions. The presence of coliforms outside the onions is not a major concern as coliforms are normally expected on plants.

This project is the first study to assess the suitability of Sand River water for irrigation. The health risk assessment showed that lead is potentially hazardous to people consuming tomatoes and onions irrigated with Sand River water. It is thus important for more studies to be undertaken in other rivers and dams where sewage effluent is used to irrigate crops.

ACKNOWLEDGEMENTS

We are grateful to the Water Research Commission for providing funding for this project. Staff and students at the Aquaculture Research Unit assisted us in innumerable ways during the implementation of this project, and we would like to register our appreciation for all their efforts. We thank Mr Gavin Geldenhuys for the technical support and Ms Eva Nephale for assisting with data collection. The researchers at ZZ2 attended the stakeholders meeting and their input during the meeting is gratefully acknowledged. The department of Agriculture in Limpopo Province cooperated immensely with the research team and their valuable input is gratefully acknowledged. The University of Limpopo Research Office handled the financial aspects of this project and we thank the office for expeditiously processing our requisitions.

We are equally grateful to the following reference group committee members:

- Prof. S Mpandeli who chaired the first few steering committee meetings and gave valuable inputs and guidance to the research team;
- Dr SN Hlophe-Ginindza who chaired the steering committee meetings after taking over from Prof. S Mpandeli and gave valuable guidance to the research team;
- Prof. S Schmidt (School of Life Sciences, University of KwaZulu-Natal) for his constructive comments and expertise during the course of the investigation that improved the quality of this report;
- Prof. O Sigge (Department of Food Science, University of Stellenbosch) is also appreciated for his constructive criticism during the course of the investigation that improved the quality of this report. The efforts of both Prof. O Sigge and Prof. S Schmidt are gratefully acknowledged.
- Dr LS Mudau for her input.
- Dr L Nhamo is also greatly appreciated for his valuable comments.

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LIST OF ACRONYMS

AOAC	Association of Official Analytic Chemistry
ARU	Aquaculture Research Unit
BH	Borehole
BOD	Biological Oxygen Demand
CBD	Central Business District
CCM	Canadian Council of Ministers
CFU	Colony Forming Unit
DF	Dilution Factor
DIR	Daily Intake Rate
DO	Dissolved Oxygen
DWAF	Department of Water Affairs
FAO	Food and Agriculture Organisation
FC	Faecal coliforms
HQ	Hazard quotient
MPN	Most Probable Number
PAR	Potassium Adsorption Ratio
PCA	Principal Component Analysis
PN	Number of Plates Counted
PSTW	Polokwane Sewage Treatment Works
PTF	Plant Transfer Factor
QA	Quality Assurance
QC	Quality Control (QC)
RfD	Reference Doses
SAB	South African Breweries
SANS	South African National Standards
SAR	Sodium Adsorption Ratio
SD	Standard Deviation
SSP	Sodium Soluble Percentage
SSTW	Seshego Sewage Treatment Works
STEC	Shiga toxin-producing *Escherichia coli*
TC	Total Coliforms
TDS	Total Dissolve Solids
TNC	Total Number of Colonies Counted
TPCa	Average Total Plate Count
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation
WQI	Water Quality Index
WRC	Water Research Commission

CHAPTER 1: INTRODUCTION

Globally, only 2% of water resources can be used as viable freshwater source (Shklomanov, 1993). Many African countries will face serious water scarcity by the year 2025 (Falkenmark, 1989). The demand for freshwater is growing yet its availability per person is reaching a critical point. Increased demand for water is occurring in four key areas:

- Human needs for safe drinking water and sanitation
- Agricultural needs
- Industrial needs
- Environmental needs

Agriculture uses more water than any other sector and in order to enhance the sustainable utilization of this scarce resource. It is important to develop water conservation strategies in agriculture.

South Africa is a water scarce country and water security issues have further been compounded by climate change. Drought have become more frequent in recent years along with unusual weather patterns including stormy weather (Dai et al., 2018). There is thus general consensus that South Africa is a water insecure country. Furthermore, the water quality in both rivers and dams has deteriorated. This has largely been attributed to dysfunctional sewage treatment works and unsewered informal human settlements (Gumbo et al., 2016; Mamugize et al., 2018). It is important for South Africa to come up with sustainable solutions to the water insecurity. One of the sustainable solutions is to ensure quality wastewater treatment that can then be recycled and reused. The critical research question in this project is: Is Sand River water in Limpopo Province suitable for irrigation after sewage effluent discharge?

Seasonal water shortages in Limpopo Province have severely affected crop growth and development (Hoffman et al., 2018; Rankoana, 2019). It is thus important to develop enterprise innovations which can lead to improved water security in the province. Use of sewage effluent is one way of optimally utilizing scarce water resources but this is only recommended when the sewage effluent meets stipulated guidelines before discharge into the river. The national state of water resources was last reviewed by the Department of Water and Sanitation in 2014. They reported that Limpopo province had the highest contaminated water. The most contaminated water was from the Crocodile West and Marico river systems. Four major water quality problems facing Limpopo province were identified: eutrophication, trace metal contamination, salinization and faecal contamination. Excessive use of nitrates and phosphates in agriculture ultimately leads to deterioration of water quality resulting in eutrophication. High nitrates and phosphate levels have also been discharged into water courses through poorly treated sewage effluent. Malfunctioning and overloaded sewage treatment plants are the main reason for the sub-standard sewage effluent discharged in rivers within Limpopo province. However, there is very little data available on the trophic status of rivers within the province. The little data that is available on trophic status have mainly focused on dams (Dabrowski, 2014). It is therefore prudent to investigate the trophic status of rivers in Limpopo province, particularly those used for crop irrigation.

The Sand River in Limpopo Province receives sewage effluent from Polokwane sewage treatment works and Seshego sewage treatment works. The river also receives storm water from Polokwane central business district. Furthermore, the river is also close to an informal settlement. Thus, organic pollution is a major concern for all stakeholders using Sand River water. Most rivers passing through urban areas in developing countries like South Africa, are now heavily polluted (Lin et al., 2017). Urbanization and population growth is putting unprecedented pressure on these rivers. Sewage effluent has been identified as a major point source of pollution in lotic ecosystems. Poorly treated sewage effluent has been linked to high conductivity, salinity, nitrogen, phosphorus and high biological oxygen demand (BOD) levels (Moyo and Mtetwa, 2002; Brion et al., 2015). The Polokwane sewage treatment works (PSTW) and Seshego sewage treatment works (SSTW), like many sewage treatment works in South Africa discharge sewage effluent that does not always meet the guidelines from the Department of Water Affairs (Seanego and Moyo, 2013). Thus, eutrophication resulting from nutrient enrichment is a serious threat to the Sand River. Additional sources of nutrients for the Sand River come from the surrounding farms where farmers use inorganic fertilizers on their crops. The nutrient status of 20 of the largest river catchments in South Africa based on dissolved inorganic nitrogen and phosphates have been evaluated by De Villiers and Thiart (2007). However, their study did not include the Sand River. In this study, the nutrient status of the Sand River was assessed because it is used for irrigation by farmers after effluent discharge.
Use of sewage effluent in the irrigation of crops is now widely practised (Jimenez-Cisneros, 1995). The rationale for using sewage effluent in the irrigation of crops is the high nutrient content of the effluent, particularly, nitrogen and phosphorus which can be used as fertilizers. Thus, the use of sewage effluent will lead to a reduction in the use of chemical fertilizers which are expensive. In South Africa, sewage effluent is used to irrigate crops in provinces that are water stressed, such as the Western Cape, Eastern Cape and Limpopo Province. The use of sewage effluent in these provinces is a water saving strategy that must be encouraged if proper water quality management strategies are put in place. Treated sewage effluent can also be used for the recharge of ground water. The Polokwane sewage effluent is used to recharge the Polokwane aquifer (Seanego and Moyo, 2013). Several boreholes have been sunk around the Polokwane aquifer and these are used to irrigate crops. No studies have been undertaken to evaluate the suitability of the borehole water from the Polokwane aquifer for irrigation purposes.

One of the disadvantages of using sewage effluent to irrigate crops is that the sewage effluent may be contaminated with heavy metals. Heavy metals are highly persistent and non-degradable contaminants. Many studies have shown that the uptake of metals is increased in plants in rivers receiving effluent (e.g. Samecka-Cymerman and Kempers, 1996). Heavy metals can also be airborne and the Sand River is in close proximity to the Polokwane Smelter. It is thus important to establish the hazard quotient associated with people consuming vegetables irrigated with Sand River water. Another disadvantage of using sewage effluent for irrigation of vegetables is the potential pathogenic microbial contamination of the vegetables. The microbiological quality of water in South Africa has been deteriorating because of the overloaded sewage treatment works. Thus, the presence of bacteria like *E. coli* in water indicates faecal contamination which may pose a potential risk to consumers of fresh produce such as tomatoes and onions due to some pathogenic strains such as STEC. As already indicated, farmers downstream of the Polokwane and Seshego treatment works use Sand River water to irrigate tomatoes and onions. However, the potential risk associated with use of Sand River water has not been assessed before.

The aim of this study was to assess the suitability of Sand River water for use in irrigation. The objectives were to:

i) Determine the nutrient status of the Sand River
ii) Determine the level of heavy metal contamination of Sand River water and sediments
iii) Investigate microbial contamination of Sand River water
iv) Assess the suitability of Sand River water and surrounding boreholes for irrigation of tomatoes and onions
v) Determine human risk assessment on people consuming tomatoes and onions irrigated with Sand River water and borehole water

CHAPTER 2: LITERATURE REVIEW

Poorly treated sewage effluent has been identified as a major source of organic pollution in many lotic ecosystems (Mazrouh and Mahmoud, 2009; Wepener et al., 2011; Moyo and Rapatsa, 2016). Substandard sewage effluent affects the water quality of receiving water bodies and normally leads to high conductivity, suspended solids, salts, nitrogen, phosphorus, high BOD and low dissolved oxygen (Morrison et al., 2001). Many South African sewage treatment plants discharge effluent that does not meet the stipulated guidelines (Fatoki et al., 2003; Mtetwa and Schutte, 2003; Van Vuuren, 2005). Poor quality sewage effluent does not only affect water quality but it also affects the biota that is found in the rivers. Many studies have shown the effect of poor quality sewage effluent on macroinvertebrates (Chutter, 1994) and fish (Kleynhans, 1999). Rivers passing through urban areas are now degraded due to the high pollution load coming from substandard sewage effluent and runoff from the built-up areas (Seanego and Moyo, 2013; Lin et al., 2017).

The nutrient status of rivers in South Africa was last extensively reviewed by De Villiers and Thiart in 2007. They noted that the most polluted river catchments were the Vaal, Olifants, Berg and Keiskama. The Vaal receives wastewater from the largest metropolitan area in South Africa, Johannesburg. Incidences of mass fish kills have been reported due to the deteriorating water quality (Wepener et al., 2011). The nutrient status of the Sand River has largely been ignored and unlike the other major rivers, there is no monitoring of the nutrient status of the Sand River by the Department of Water Affairs. Intermittent monitoring of the Sand River is done by the Polokwane Municipality but this data is not easily accessible to the general public. The only study that gives some indication on the nutrient status of the Sand River was undertaken by Seanego and Moyo (2013). This study assessed the suitability of Sand River water for sustaining aquatic life and used the Water Quality Index (WQI) to determine the water quality status of the river. The WQI indicated that the water quality of the Sand River was very poor. No other studies were carried out on the Sand River after 2013 although anecdotal evidence shows that the water quality in the Sand River continues to deteriorate. Local press reports have also highlighted the poor water quality of the Sand River. In this study, the spatial and temporal variations of the nutrient status of the Sand River will be examined. Unlike Seanego and Moyo (2013), this study looks at the nutrient status in relation to the suitability of the water for irrigation.

There are very few industries in Polokwane and the assumption is made that the Sand River will not experience heavy metal contamination. Thus, there is hardly any information on heavy metals found in Sand River water, sediment and biota. However, physico-chemical data is particularly important since Sand River water is used for irrigation. The possible sources of heavy metal contamination for the Sand River is urban run-off, fertilizer applications and sewage effluent. Another possible source of heavy metal contamination for the Sand River is the Polokwane Smelter which is located 15 Km away from the Sand River. Airborne pollutants from the Polokwane smelter can potentially contaminate the Sand River. The importance of airborne pollutants in water pollution was recently elucidated by Berger et al. (2019), Te Chien et al. (2019) and Liu et al. (2018).

Trace metal contamination of rivers passing through urban areas is a growing major concern globally because of its environmental toxicity (Yuan et al., 2011; Islam et al., 2015). Rivers passing through urban areas are a major repository of industrial and domestic effluent (Moyo and Phiri, 2002; Venugopal et al., 2009). The problem of trace metal contamination in Africa has been exacerbated by the discharge of untreated domestic and industrial effluent into rivers passing through urban areas (Tshibanda et al., 2014; Sibanda et al., 2015). In the aquatic ecosystem, sediments are a major repository of trace metals. It has been shown that sediments may accumulate trace metals a thousand times higher than the overlying water (Haller et al., 2009). Trace metals are adsorbed to sediment particles and may be resuspended into the water column. Elevated levels of trace metals in sediments are a good indicator of pollution. Several factors affect metal mobility between the sediment and water. These include pH, conductivity, redox potential and bioturbation (Bryan and Langston, 1992; Caussy et al., 2003). The release of trace metals is to a large extent affected by changes in water pH and bioturbation than changes in redox potential (Atkinson et al., 2007). The resuspended trace metals in the water may be toxic to aquatic flora and fauna. It is thus important to understand the ecological risk associated with trace metal contamination. Many studies have shown that the uptake of trace metals is increased in plants growing in rivers receiving sewage effluent (e.g. Samecka-Cymerman and Kempers, 1996).

The Department of Water Affairs monitors heavy metal contamination in some major rivers and dams. However, there is no monitoring of heavy metal contamination in sediments. A number of studies have shown that sediments in rivers and some dams are contaminated (Binning and Baird, 2001; Greenfield et al., 2007; Edokpayi et al., 2016; Dahms et al., 2017). DWAF does not have any guidelines on maximum permissible limits of heavy metals in sediments. Thus, in some instances the Canadian Council of Ministers (CCM) Guidelines on sediments have been used. Edokpayi et al. (2016) used those guidelines and concluded that cadmium, chrome and copper exceeded the permissible range as suggested by CCM. Despite the importance of the Sand River as a source of irrigation water, no studies have been undertaken to determine the heavy metals in the sediments.

One of the major challenges associated with the use of sewage effluent is that heavy metals in the effluent may accumulate in the soils and can thus be readily taken up by plants. The long term impact of sewage effluent irrigation on soil properties and the risk in relation to human food chain has received considerable attention on a global scale (Teng et al., 2014) but very little has been done in most developing countries. In India, Meena (2016) investigated the effect of sewage effluent on groundwater, soils and plants (rice & wheat grain). They noted that sewage irrigation resulted in significant build-up of Zn, Cu, Fe, Ni and Pb in sewage irrigated soils. A similar build up was observed under the rice crop. They indicated that the hazard quotient for the intake of toxic trace metals by humans through consumption of rice and wheat irrigated with sewage effluent were within permissible limits. Meng et al. (2016) reported that concentrations of heavy metals in soils irrigated with sewage effluent in Tianjin, China were significantly higher than those of the control. They also reported that Cd, Pb and As in vegetables exceeded permissible limits. More recently, Xue et al. (2019) showed that Cd, Pb and Hg were significantly higher in soils irrigated with sewage effluent in comparison to the control. They further showed that that the target hazard quotient was greater than 1 for Cd, Pb and Hg in wheat from sewage irrigated areas. In Morocco, the use of wastewater for irrigation is widely practiced. A recent study however showed that both the irrigated soil and crops were contaminated with heavy metals (Chaoua et al., 2019). It was recommended that preventative measures must be introduced to reduce heavy metal pollution in soils and thus protect both human and animal health in Morocco. Whereas, many studies have been done in different countries on heavy metal contamination of soils and crops from sewage effluent irrigated areas, there are very few studies that have been undertaken in South Africa.

Malan et al. (2015) investigated the concentration of heavy metals in irrigation water, soils and vegetables in Philippi in the Western Cape Province, South Africa. It was noted that a number of heavy metals in the soils and fresh produce exceeded permissible limits. The source of the heavy metals was not identified but the authors suggested that the major agronomic sources of heavy metals in the Philippi horticultural area be identified. Furthermore, they did not calculate the risk associated with the consumption of the vegetables. Gupta et al. (2018) undertook a comprehensive analysis of heavy metal concentration in food stuffs including vegetables in Durban, South Africa. They concluded that the hazard quotient values were arranged in the order Pb>Mn>Cu>Ni>Zn>Cr. They indicated that manganese in pineapple and lead in bananas, oranges, guava and kiwi fruit posed a potential risk to children. However, their study was based on collected fruit and vegetables from local mall and food courts in Durban city. Thus, their work was not experimental but a survey and the source of heavy metals could not conclusively be established.

The discharge of substandard sewage effluent can result in microbiologically contaminated water. Use of this water for irrigation can result in disease outbreaks (Craun, 2006; Greene et al., 2008). South Africa does not specify the heterotrophic bacterial count for use of water for irrigation. However, the DWAF (1996) guidelines for domestic use specify a heterotrophic plate count of ≤100 cfu/ml. Furthermore, these guidelines indicate that the water for domestic use must contain ≤ 5 total coliforms per 100 ml and no faecal coliforms per 100 ml. There is scarcity of information on the potential risks associated with consuming vegetables which have been irrigated with sewage effluent in South Africa. The use of sewage effluent to irrigate crops is now widely practised in several countries (Shanmuganathan et al., 2015; Anwar et al., 2016). In Israel microbiological contamination of vegetables irrigated with sewage effluent was investigated by Sadovski et al. (1978). They indicated that use of drip irrigation substantially reduced the possibility of microbial contamination. Irrigation water quality and microbial safety of vegetables was reviewed by Jongman and Korsten (2017). They indicated that there has been a decline in the microbial quality of surface water on a global scale because of faecal contamination. However, their review did not specifically focus on South Africa. Qadir et al. (2010) also reviewed the challenges of wastewater irrigation in developing countries. They noted that many farmers using wastewater for irrigation are exposed to health risks from viruses, bacteria and parasites. However, most of the studies done on microbial contamination in South Africa has mainly focused on the quality of the water used for irrigation and not its effect on the vegetable quality and human health. Gemmell and Schmidt (2013) assessed the microbiological quality of the Msunduzi River which is used for irrigation of some crops. They did not look at the microbial contaminants found in the vegetables. Edokpayi et al. (2015) also investigated the microbiological characteristics of Mvudi River in Limpopo Province and concluded that the microbiological parameters exceeded the established guidelines. However, this study did not focus on the microbial quality of the Mvudi River water with respect to irrigation.

Gemmell and Schmidt (2012) investigated the effect of irrigation water from the Baynespruit River in Sobantu on fresh produce. They looked at spinach, cauliflower and parsley, and recorded *E. coli* in all the vegetables. This indicates that *E. coli* was in the river water and was transferred via irrigation to fresh produce. Gemmell and Schimdt (2012) did not conclusively identify the source of the faecal matter but speculated that it could have been either an overflowing sewage pipe, or illegally dumped faecal matter. It is not clear from their study if treated sewage is discharged into the river.

The health consequences of consuming fresh produce irrigated with sewage effluent is generally not appreciated in most developing countries. WHO (2007) indicated that there are risks associated with consuming vegetables harvested from sewage effluent irrigated places. Yabe et al. (2010) reported that African populations are at risk from exposure to toxic metals. It is therefore prudent to carry out a situation analysis on heavy metal and microbiological contamination of vegetables harvested from sewage irrigated areas. A situation analysis on the suitability of Sand River water for irrigation will bring to fore the risk associated with use of sewage effluent for crop irrigation. Pollution abatement measures can only be taken by stakeholders after the identification of the risks.

CHAPTER 3: NUTRIENT STATUS OF THE SAND RIVER

3.1 Rationale

The Sand River receives sewage effluent from Polokwane sewage treatment works and Seshego sewage treatment works. The river also receives storm water from Polokwane central business district. Furthermore, the river is also close to an informal settlement. Thus organic pollution is a major concern for all stakeholders using Sand River water. Most rivers passing through urban areas in developing countries like South Africa, are now heavily polluted. Substandard treated sewage effluent has been linked to high conductivity, salinity, nitrogen, phosphorus and high biological oxygen demand (BOD) levels (Moyo and Mtetwa, 2002; Brion et al., 2015). The Polokwane sewage treatment works (PSTW) and Seshego sewage treatment works (SSTW), like many sewage treatment works in South Africa discharge sewage effluent that does not always meet the guidelines from the Department of Water Affairs. Thus, eutrophication resulting from nutrient enrichment is a serious threat to the Sand River. The major nutrients of concern are nitrogen and phosphorus and the major sources of these nutrients in domestic effluent are human excreta and detergents. Additionally, sources of nitrogen and phosphorus for the Sand River may come from the surrounding farms where farmers use inorganic fertilizers on their crops. The nutrient status of 20 of the largest river catchments in South Africa based on dissolved inorganic nitrogen and phosphates have been evaluated by De Villiers and Thiart (2007). However, their study did not include the Sand River. In this study, the nutrient status of the Sand River was assessed over a period of a year.

There are number of farmers that use Sand River water to irrigate their crops. Limpopo Province is a water scarce area and the use of sewage effluent to irrigate crops ensures optimal utilization of the scarce water resources. Furthermore, sewage effluent water is rich in plant nutrients and thus farmers can cut costs since they do not need to apply organic fertilizers. Despite the widespread use of the Sand River for irrigation, very few studies have been undertaken to determine its nutrient status. One of the studies (Seanego and Moyo, 2013) done on the Sand River looked at the effect of sewage effluent on ecological interactions in the Sand River. The sewage effluent discharged from the Polokwane sewage treatment works is also used to recharge the Polokwane aquifer (Seanego and Moyo, 2013). A situation analysis on the nutrient status of a river that is used for crop irrigation is thus imperative.

3.2 Objectives

The objectives in this chapter were to determine:

a) Spatial variations in the physico-chemical parameters of the Sand River.

3.3 Materials and Methods

3.3.1 Study Area

3.3.1.1 Sewage treatment works

The Polokwane sewage treatment works is a macro-size plant which uses activated sludge treatment process. This process involves separation of solid/grit material and a series of aerobic, anoxic, sedimentation and maturation. The effluent is chlorinated before discharge. Bio-filtration is used to treat domestic effluent. On the other hand, up-flow anaerobic sludge blanket is used for brewery effluent. The brewery effluent is the only major industrial effluent and it is from South African Breweries (SAB). The Polokwane sewage treatment works is authorized to discharge 25 462 m^3/day of effluent, of which 14 000 m^3/day is diverted to Mogalakwena platinum mine. Approximately 9 400 m^3/day is discharged into the Sand River.

The Seshego sewage treatment works also uses the bio-filtration process. The design capacity of the Seshego sewage treatment works is 7 ML/day but its current discharge rate is not known. Anecdotal evidence based on water quality suggests that Seshego sewage treatment works is seriously overloaded.

3.3.1.2 Site selection

Preliminary visits were made to the Sand River before site selection. The land uses around the Sand River were mainly, farmland and rangeland (Figure 3.1; Table 3.1). The urbanized areas were also identified. Eight sites were eventually identified along the Sand River (Figure 3.2). The first 3 sites are above the Polokwane sewage treatment works. These sites receive mostly storm water drains from Polokwane central business district. Site 1 is in a non-industrialized area. Site 2 and 3 are in an industrialized area. Site 4 is soon after the point of discharge of sewage effluent from the Polokwane sewage treatment works. At this site, the Blood River drains into the Sand River. The sewage treatment works in Seshego (township) discharges into the Blood River. Site 4 is a confluence of two rivers carrying sewage effluent from the Polokwane treatment works and Seshego treatment works. Site 5,6, 7 and 8 are surrounded by farms and rangeland. Sand River receives run-off from the surrounding farms ad rangeland. The farms are mostly commercial farms where crop cultivation takes place. A major tomato growing company, draws water from site 5 and uses the water to irrigate tomatoes and onions (Figure 3.3). Several other commercial farmers draw water downstream of site 5. For its part, the tomato growing company uses the drip irrigation method which involves the lateral spread of water irrigated surface through drippers.

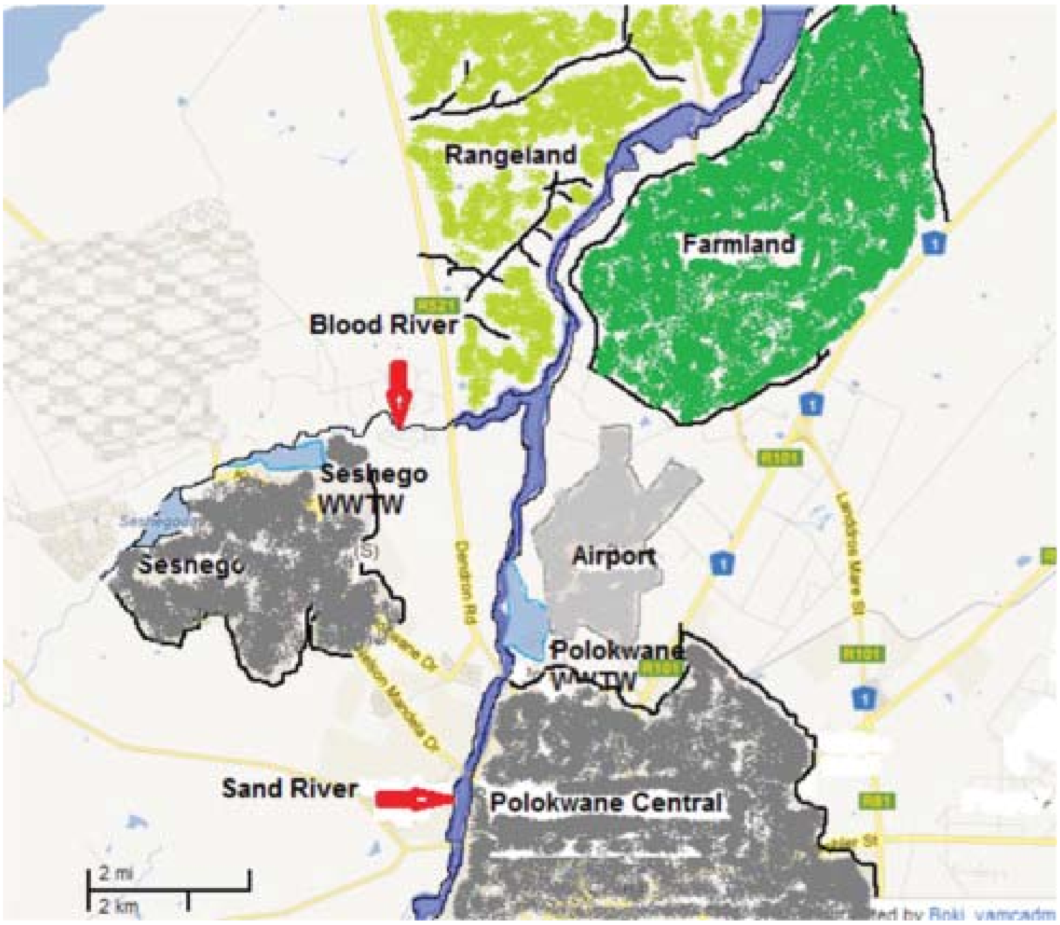

Figure 3.1: Map of the Sand River with the Polokwane and Seshego wastewater treatment works (WWTW) together with the surrounding rangeland and farmland.

Table 3.1: Substrate and vegetation at the different sampling sites.

Site	Substrate	Vegetation	Special features
Site 1	Rocks, sediment and sand	Marginal vegetation and eucalyptus trees	Receives water from storm water drains in non-industrialized area
Site 2	Rocks, sediment and sand	Marginal vegetation dominated by phragmites	Slightly within the urbanised area
Site 3	Sediment and sand	Marginal vegetation dominated by phragmites, algae	Slightly within the urbanised area
Site 4	Sediment and sand	Marginal vegetation dominated by panicum sp., algae	Site after effluent discharge and confluence of Sand and Blood Rivers
Site 5	Rocks, sediment and sand	Marginal vegetation dominated by Ishaemum sp., algae	Surrounded by farmland and rangeland
Site 6	Rocks and sediment	Marginal vegetation, algae, wetland	Surrounded by farmland and rangeland
Site 7	Rocks and sand	Limited marginal vegetation	Surrounded by farmland and rangeland
Site 8	Rocks and sand	Limited marginal vegetation	Surrounded by farmland and rangeland

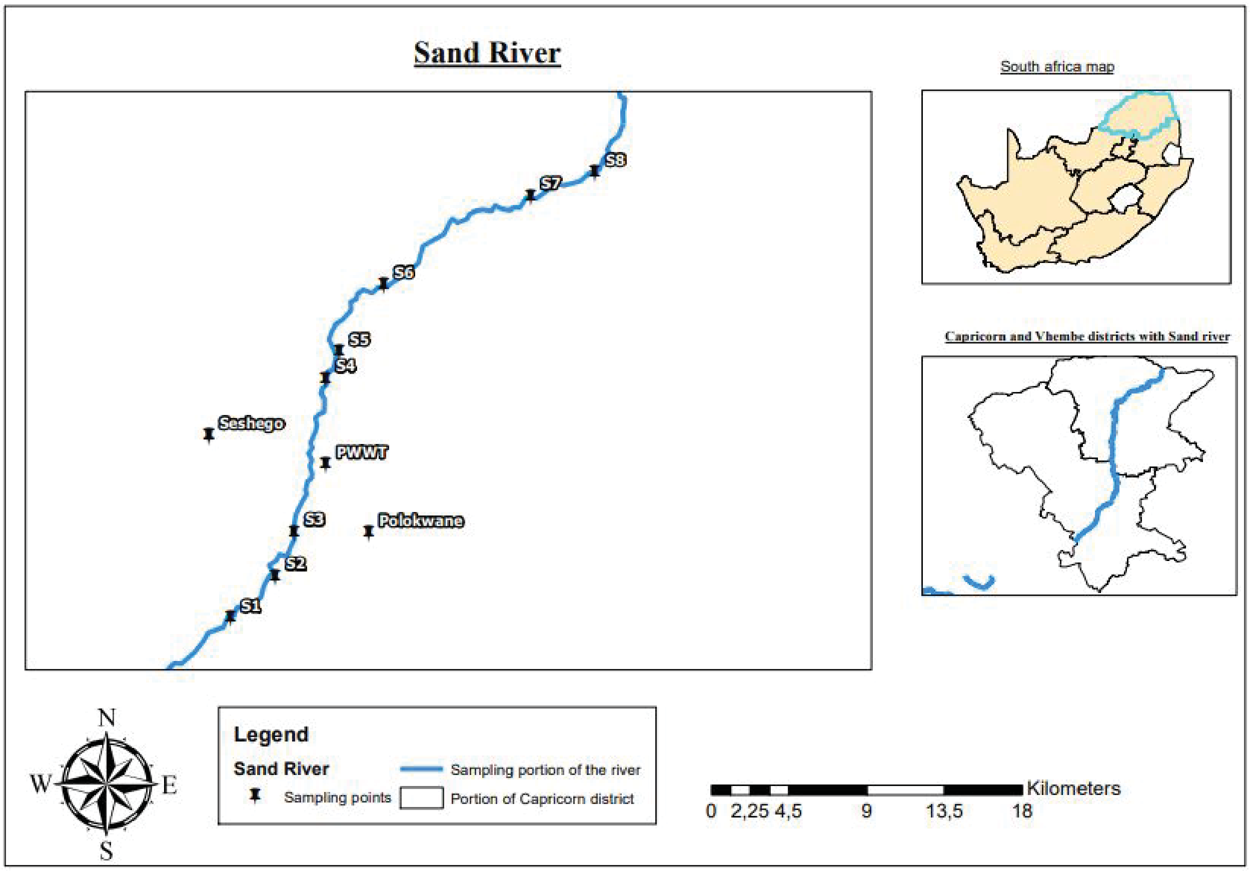

Figure 3.2: Sampling sites on the Sand River before and after the sewage treatment works

Figure 3.3: Water abstraction point for a large commercial farming enterprise

3.3.1.3. Sample collection

Water samples were collected at the different sites between March, 2017 and March 2018. The months were grouped as dry (May-August) and rainy (October-December) seasons. Polyethylene sampling bottles (250 mℓ) washed with de-ionized water were used to collect water samples from each site. Water samples were collected in duplicates just below the surface at a depth of 10 cm 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.

Sediment samples from each site were collected in duplicates 20 cm below the river surface and placed in 250 mℓ polyethylene bags using a hand trowel. The samples were immediately kept in ice and transported to the ARU Laboratory, where they were kept in a freezer (-20°C) until analysis.

3.3.1.4 In-suite sampling

Temperature, conductivity, pH and dissolved oxygen were measured at each site using a YSI meter (MPS-556). The flow rate was determined using a flow meter (Model PS2000). Three replicate samples were taken at each site. Self-purification capacity with respect to ammonia, sulphate, TDS and total phosphorus was determined using Bourne et al. (2002):

***Equation 1*: $\mathbf{Sr\ (mg/l/km) = Q_1C_1 + Q_sC_s - Q_2C_2/L}$**

Where:
Q_1C_1 = pollution load at the upstream station (mg/ℓ·km)
Q_2C_2 = pollution load at the downstream station (mg/ℓ·km)
Q_sC_s = point source pollution load in the river reach (mg/ℓ·km)
L = distance between the two points (km)

3.3.2 Laboratory analysis

Total phosphorus, total nitrogen and ammonium were determined using calometric methods adopted from APHA, 1995. Biological oxygen demand was determined using Viessman and Hammer (1993). Two water samples were collected from each site using 250 ml bottles. An oxygen meter probe was used to determine dissolved oxygen in one of the bottles. The other bottle was wrapped with a black insulation tape on site before being placed in a dark container and transported to the ARU laboratory. It was then incubated for 5 days at 20°C. Dissolved oxygen level were determined after the 5-day period. The BOD was determined using the equation:

***Equation 2*: $BOD^{20}{}_5 = D1 - D2$**

Where D1 is equal to dissolved oxygen (mg/l) on the first day and D2 equal to dissolved oxygen (mg/l) after 5 days. Total dissolved solids were determined using the methods adopted from Eaton et al. (1995). Sodium, chloride, potassium, calcium, magnesium and sulphate were determined using standard methods by APHA (1995).

3.3.3 Statistical Analysis

Hierarchical cluster analysis was used to group the different sites of the Sand River in relation to water physicochemical parameters. This was carried out using IBM SPSS Version 20. Principal component analysis (PCA) was used to determine the physico-chemical factors that account for most of the variation in water quality. PCA was carried out using CANOCO 5.

3.4 Results and Discussion

Temperature and pH did not vary much with sites. However, BOD, phosphorus, ammonium, conductivity, flow rate, TDS and nitrogen varied significantly across sites (Table 3.2). All these physico-chemical parameters increased significantly after discharge (S4). Most of these parameters declined steadily between site 4 and site 8 (Table 3.2). This is attributed to the self-purification capacity of the Sand River. TDS had the highest self-purification capacity in comparison to sulphate, total phosphorus and ammonia (Table 3.3). The concentration of unionized ammonia in a water body is determined by dissolved oxygen, pH and temperature. The dissolved oxygen increased slightly after the discharge of sewage effluent. This is why ammonia registered a low self-purification capacity. Phosphorus is a relatively immobile nutrient in comparison to nitrogen and thus tends to have a lower self-purification capacity (Han et al., 2014).

The pH ranged between 7.68 at site 7 to 8.42 at site 2 (Table 3.2). These are normal pH values in most aquatic ecosystems in South Africa. These pH values will not have a negative effect on aquatic life. However, pH is one of the important values that will affect the bioavailability of heavy metals. It is thus an important variable that has to be monitored. The pH range for irrigation water is normally between 6.0 to 8.5 from the South African guidelines. FAO irrigation guidelines recommend a pH range of 6.5 to 8.4 (Ayers and Westcot, 1985). The problem with irrigation water which is outside this range is that it may cause nutritional imbalance (DWAF, 1996; Mutengu et al., 2007). Furthermore, the irrigation water may contain toxic ions. Another hazard of an abnormal pH in the water is its impact on irrigation equipment. A pH of less than 6.5 will promote leaching while a pH greater than 11 generally inhibits the movement of heavy metals through the soil (Mutengu et al., 2007). The Sand River water pH falls within the target range for irrigation.

The lowest dissolved oxygen values were recorded at site 8 (4.44 mg/ℓ). This is largely because the water was no longer flowing at that site. It must also be noted that this site dried out completely at times because the farmers upstream would have used up all the water. The dissolved oxygen levels at all sites were not lethal to aquatic life. There are no recommended guidelines on the levels of dissolved oxygen in water that is used for irrigation from both South Africa and FAO. However, water that has high oxygen levels is normally indicative of good quality water. South African guidelines recommend oxygen levels above 5 mg/ℓ for aquatic life. On that basis, the quality of water drawn from site 4 and site 8 is not as good as water from the other sites.

The biological oxygen demand (BOD) was low between site 1 to site 3 but rose significantly after the discharge of sewage effluent (Table 3.2). The increase in BOD is largely because of the organic load from the sewage treatment works. High levels of BOD is typical of sewage effluent. However, there was a general decline in BOD levels between site 4 and site 8. It is not immediately clear if the decline can be attributed to the self-purification capacity of the Sand River. None of the abstraction points exceeded the recommended levels. The FAO guidelines (Ayers and Westcot, 1985) recommend the maximum level of 10 mg/l of BOD for irrigation. This then suggests that the effluent quality is acceptable for irrigation with respect to BOD.

Total dissolved solids (TDS) rose significantly after discharge of sewage effluent and subsequently declined between site 5 and site 8 (Table 3.2). The TDS values are not a threat to crops that are grown downstream. The target range for TDS in irrigation water is 0-2000 mg/ℓ. The TDS after discharge ranged between 532-581 mg/ℓ. Excessive levels of TDS greater than 2000 mg/ℓ may result in a build-up of salts in the root zone and this may in turn lead to alteration of soil properties. Although, the maximum TDS values deemed to be harmful are up to 2000 mg/l. Moyo et al. (2015) indicated that effluent with TDS ranges between 450-2000 mg/ℓ may be moderately used for irrigation. It is suggested that a monitoring programme for salts in soils irrigated with sewage effluent be implemented. Both the BOD and TDS levels are not a threat to aquatic life. The brewery effluent from South African Breweries (SAB) is a major source of TDS and high BOD levels. The domestic effluent and brewery effluent are treated separately because of the high hydraulic strength of the brewery effluent. Typical brewery effluent has BOD levels of between 1200 and 3600 mg/ℓ, nitrogen levels of between 25-80 mg/ℓ, phosphorus levels of between 10-50 mg/ℓ and TDS 2712 mg/ℓ. South African Breweries has put in place some pre-treatment facilities to reduce the nutrient strength of its effluent. The effectiveness of this pre-treatment facilities is yet to be evaluated. It is thus suggested that an evaluation and monitoring programme of SAB effluent be implemented.

Conductivity values were low at the upstream sites and significantly rose after the discharge of sewage effluent (Table 3.2). The conductivity levels marginally declined after discharge. Conductivity is a measure of the ions in the water and measures salinity hazards. After discharge conductivity ranged between 130-140 mS/cm. The FAO guidelines (Ayers and Westcot, 1985) recommend a maximum of 300 mS/cm for irrigation water. It must also be noted that electrical conductivity is also a good indicator of TDS in the water. The conductivity values recoded in this study show that Sand River is suitable for irrigation and thus causes no salinity challenges. However, it is again recommended that the salt levels in the soil be monitored to avoid any possible alterations in the soil.

Total nitrogen encompasses all forms of nitrogen which can be found in sewage effluent, namely ammonia, nitrate, nitrite, and organic nitrogen. The total nitrogen ranged from 0.6 mg/ℓ at the first site to 51.0 mg/ ℓ at site 4. Site 1 receives storm water from a non-industrialized area, and this probably explains the low nitrogen levels. Site 4 is the site after discharge of sewage effluent and the nitrogen levels are inevitably quite high. The nitrogen levels after discharge declined slightly between site 4 and site 8. This may be an indication of the self-purification capacity of the Sand River (Table 3.3). Despite the decline in nitrogen levels from the point of discharge, these levels still remained high enough and thus the Sand River water can be used as a fertilizer for the crops that are grown. It has been reported that total nitrogen concentrations of less than 5 mg/ℓ do not affect crops while total nitrogen concentrations in excess of 30 mg/ℓ may be harmful to some crops depending upon the developmental stage of the crop (Girovich, 1996). Although the nitrogen values after discharge are above 30 mg/ℓ, no negative impacts of tomatoes and onions have been reported by the farmers. This suggests that the Sand River water has no negative effect on the growth of tomatoes and onions.

Phosphorus along with nitrogen are important in the fertilization of crops. The phosphorus levels in the first 3 sites before discharge were quite low at ≤0.14 mg/ℓ (Table 3.2). After discharge, the phosphorus levels rose significantly and ranged between 4.25 and 4.94 mg/ℓ. Between site 4 and site 6, there appears to have been some self-purification (Table 3.3) with respect to phosphorus but this appears to have been lost between site 7 and site 8 (Table 3.2). Phosphorus is normally a limiting nutrient in soils and is thus supplied as a fertilizer. Its availability in the Sand River water will certainly be beneficial for the tomatoes and onions. The maximum recommended level for phosphorus is 2 mg/ℓ (Ayers and Westcot, 1985). The Sand River water used for irrigation had phosphorus levels above the recommended limit and although this may cause environmental damage, it is highly unlikely that these levels would be detrimental to the growth of vegetables. It has been reported that excess phosphorus reduces zinc, copper and iron availability. It also increases the need for iron, calcium and magnesium (Ayers and Westcot, 1985).

Two of the major cations sodium and potassium significantly increased after the discharge of sewage effluent (Table 3.4). Sodium levels before discharge ranged between 37 mg/ℓ at site 1 and 60 mg/ℓ at site 3. After discharge, the sodium levels ranged between 142 to 146 mg/ℓ. The South African guidelines for irrigation water has a target of 70 mg/ℓ for sodium. Sensitivity to high sodium levels varies among different crops. Tomatoes are regarded as moderately tolerant to high sodium levels of between 115-230 mg/ℓ (DWAF, 1996). Although, the sodium levels were above the target range for South African irrigation guidelines, they are still within acceptable limits for tomatoes. Tomato foliage can absorb sodium which may become toxic if it accumulates in the leaves and stems. High sodium levels can also reduce the rate of water infiltration in soils. Furthermore, high sodium levels may also cause the water to be alkaline and soils irrigated with alkaline water have reduced availability of some micro nutrients such as iron, copper and manganese. Along with major ions, sodium might lead to the plugging of dripline emitters and corrosion of metal fittings.

Potassium levels ranged between 5.6 mg/ℓ to 6.3 mg/ℓ at sites before discharge (Table 3.4). The potassium levels increased significantly after discharge and were ranging between 17 to 19 mg/ℓ. The FAO (Ayers and Westcot, 1985) recommended limit for potassium in irrigation water is 2 mg/ℓ. The South African guidelines do not stipulate any limits for potassium in irrigation water. The potassium levels in the Sand River were way above the stipulated 2 mg/ℓ. Excess potassium can cause soils to be alkaline and also causes high osmotic pressure in soils. Chloride levels ranged between 17 and 55 mg/ℓ at the sites upstream the sewage treatment works (Table 3.4). The lowest value was recorded at site 1. After discharge, the chloride levels ranged between 127.5 to 129.1 mg/ℓ (Table 3.4). The South African guidelines stipulate a target of 100 mg/ℓ of chloride in irrigation water. However, for less sensitive crops like tomatoes values less than 140 mg/ℓ are still acceptable. The highest chloride level after discharge was below 140 mg/ℓ and this suggests that Sand River water is suitable for the irrigation of tomatoes. The problem normally associated with high concentrations of chloride is that it increases the salinity of the water and if highly saline water is used for irrigation the process of osmosis will reverse. Thus, water will move from the roots to the surroundings. The symptoms of high salt damage in crops are similar to those of moisture stress. Furthermore, chloride ions can be taken up the roots or by direct contact on the leaves. This can also stress the plants. In irrigation equipment, high chloride concentration will lead to corrosion of metals in the distribution system.

Sulphate levels ranged between 4 and 19 mg/ℓ in the upstream sites (Table 3.4). The lowest sulphate values were recorded at site 1. The highest sulphate levels of 50 mg/ℓ were at site 4 which is the point of discharge and declined to 12 mg/ℓ at site 7 and site 8. This decline is probably due to the self-purification capacity of the Sand River (Table 3.3). There are no stipulated irrigation sulphate concentrations in the South African guidelines. However, FAO has a wide range for sulphate at 0-20 meq/ℓ. The sulphate levels in the Sand River are acceptable for irrigation purposes.

The calcium levels ranged between 36 and 43 mg/ℓ between site 1 and site 8 (Table 3.4). The sewage effluent did not affect the calcium concentration in the Sand River. There are no stipulated guidelines for calcium in irrigation water for South Africa. The main problem with high levels of calcium is that it decreases the availability of nitrogen, phosphorus, iron, manganese, zinc and potassium (Cristelo et al., 2012). Magnesium levels were highest at site 1 and declined after discharge of sewage effluent (Table 3.4). Both calcium and magnesium cause water hardness. The problem of hard water in irrigation is that they destroy irrigation equipment because they're corrosive. Excess magnesium increases the need for phosphorus and also cements clay soils tightly preventing air retention during dry periods (Cakamak et al., 1994). There are no stipulated guidelines for magnesium in the South African water guidelines.

The Sand River had the highest self-purification capacity with respect to TDS and self-purification for other constituents was quite low (Table 3.3). This is probably due to the relatively short distance between the sampling points and the low velocity.

The Sand River water can be used to boost vegetable production because of its high nitrogen and phosphorus levels. The major ions in the Sand River water namely, sodium, potassium, chloride, calcium, magnesium and sulphate are mostly within acceptable limits (international and local).

Table 3.2: Physico-chemical parameters of the Sand River from the different sampling sites between March, 2017 and March, 2018. Values are presented as means ± standard deviation.

Sites	Temp (°C)	pH	DO (mg/ℓ)	BOD (mg/l)	P (mg/ℓ)	Ammonium (mg/ℓ)	Conductivity (mS/cm)	Flow rate (m/s)	TDS (mg/ℓ)	N (mg/ℓ)
Site 1	21.2±1.9	8.2	5.3±1.2	1.04±0.23	0.18±0.02	0.09±0.01	63.0±2.2	0.02±0.001	293.0±10.1	0.67±0.01
Site 2	22.4±3.5	8.42	5.0±1.4	1.01±0.05	0.18±0.03	0.13±0.02	71.0±2.8	0.35±0.001	328.0±8.2	11.2±0.80
Site 3	21.0±2.2	8.0	6.1±0.09	1.2±0.05	0.14±0.01	0.26±0.07	71.0±3.2	0.34±0.02	321.0±13.2	12.7±0.30
Site 4	24.1±3.5	7.8	4.52±0.08	5.44±0.07	4.48±0.12	45.0±6.80	140.0±8.1	3.62±0.13	581.0±18.3	51.0±2.30
Site 5	23.3±3.2	8.1	6.51±1.9	5.20±0.6	4.34±0.05	44.78±6.80	134.0±7.4	2.80±0.24	552.0±20.1	50.1±2.10
Site 6	24.5±2.9	8.30	5.80±1.3	5.06±0.2	4.25±0.05	35.0.0±3.49	133.0±9.2	2.80±0.25	546.0±14.3	48.2±3.90
Site 7	22.5±3.2	7.68	5.27±1.8	4.28±0.08	3.97±0.01	32.60±5.80	133.0±2.1	0.23±0.18	546.0±22.3	40.0±2.10
Site 8	23.7±3.4	8.2	4.44±1.4	4.28±0.06	4.01±0.01	36.50±4.70	130.0±1.9	0.21±0.005	532.0±17.1	43.2±1.30

Table 3.3: Self-purification amounts (mg/ℓ/km) with respect to selected water variables in the Sand River.

Variable	S_m(mg/ℓ/km)
Total Phosphorus	0.32
Sulphate	1.10
Ammonia	0.04
TDS	16.63

Table 3.4: The concentrations (mg/ℓ) of major ions in the Sand River at different sites. Values are presented as means ± standard deviation.

Site	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	SO_4^{2-}
Site 1	37.0±1.2	5.6±0.5	17.0±4.2	43.0±3.2	41.0±1.3	4.0±0.8
Site 2	58.0±3.2	6.3±0.7	54.6±8.1	39.0±2.8	34.0±2.4	19.0±1.9
Site 3	60.0±2.8	5.8±0.8	55.0±9.3	37.0±4.2	36.0±2.8	19.0±1.0
Site 4	142.0±5.3	19.0±1.3	127.5±12.3	39.0±6.9	24.0±1.8	50.0±2.3
Site 5	143.0±4.8	17.0±1.4	130.5±13.2	36.0±4.2	24.0±3.2	47.0±4.1
Site 6	145.0±3.2	18.0±2.0	131.1±8.9	37.0±2.1	24.0±4.2	37.0±2.3
Site 7	146.0±3.7	18.0±2.3	129.1±9.3	38.0±3.1	25.0±3.2	12.0±0.8
Site 8	143.0±2.8	17.0±1.9	128.3±12.1	40.0±1.8	25.0±2.5	12.0±1.0

Cluster analysis identified three major groups. Site 4 was on its own and this is because it is the site immediately after discharge and thus heavily polluted (Figure 3.4). Site 1,3,7,8 and 6 form another cluster. Site 1 and 3 are before discharge and sites 6,7 and 8 indicate recovery of the Sand River from the high pollution loads. The last cluster shows site 2 and site 5, both these sites show moderate pollution. As already indicated, site 5 is a major water abstraction site for a large commercial tomato farming company. Selection of this site by the company appears to be appropriate because the site is as polluted as site 4 and nutrient levels are high enough to sustain agricultural production. Nutrient levels declined after site 5. Farmers abstracting water after site 5 are probably not deriving maximum benefits of the nutrient from the Sand River.

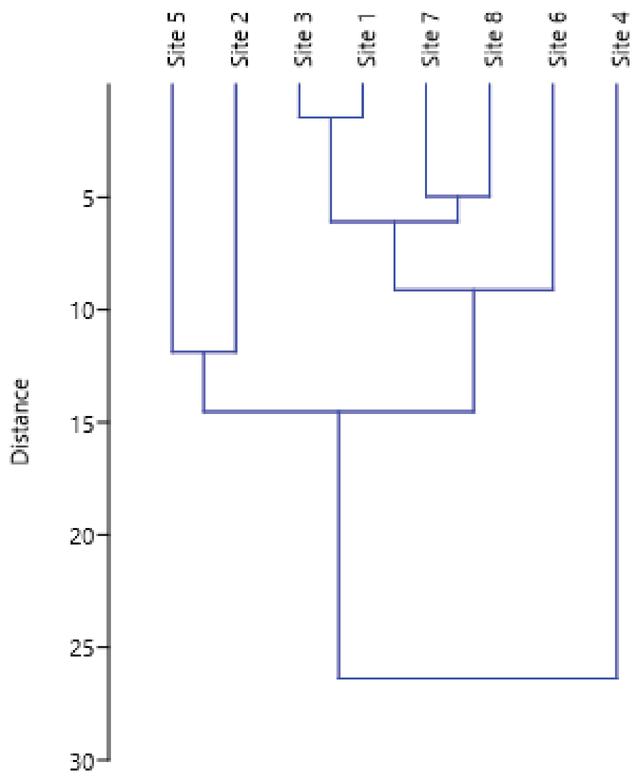

Figure 3.4: Hierarchical cluster analysis dendogram of sampling sites at the Sand River

Principal component analysis showed that sites 1, 2 and 3 were not defined by any nutrient gradient (Figure 3.5). On the other hand, site 4 was defined by TDS, ammonium, conductivity, and salinity. Site 5 was defined by phosphorus, nitrogen, and BOD (Figure 3.5). This confirms the suitability of site 5 as an abstraction point for irrigation. Most of the variation in the water quality was explained by ammonia, TDS, conductivity, salinity, BOD, nitrogen, and phosphorus. All these parameters are associated with PCA 1 which explained 87% of the variation in the water quality (Table 3.5).

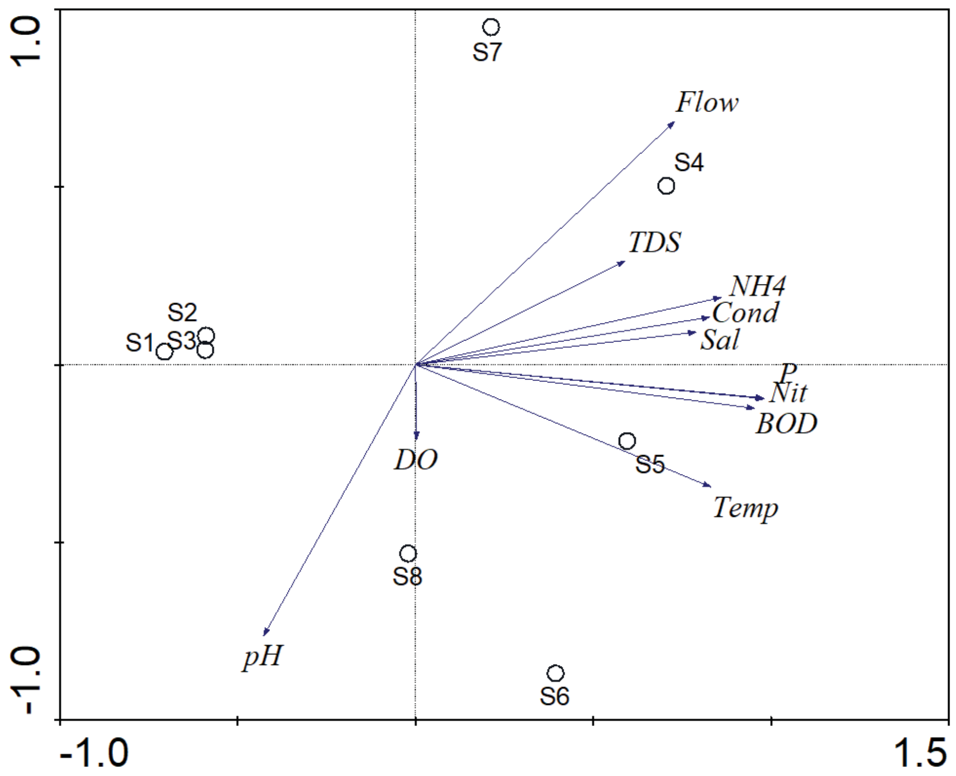

Figure 3.5: PCA plot of the relationship between physico-chemical parameters and sampling sites of the Sand River.

Table 3.5: Eigenvalues for the relationship between physico-chemical parameters and sampling sites

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.872	0.084	0.032	0.032
Cumulative percentage variance	87.2	95.6	98.6	99.7
Total variance				1.000

Site 4 recorded the highest nitrogen and ammonia levels during both the rainy and dry seasons (Figure 3.5; Figure 3.6). The levels of both nitrogen and ammonia were within the same magnitude during the rainy and dry seasons. This is contrary to Seanego and Moyo (2013) who recorded high nitrogen and phosphorus levels during the dry season. They attributed the seasonal difference to the dilution factor. However, seasonality is no longer playing a major role because of the intermittent discharge of very poor quality sewage effluent into the Sand River. No differences were also noted between the rainy and dry season with respect to phosphorus and BOD (Figure 3.6; Figure 3.7). This also could be an indication of intermittent release of substandard effluent into the Sand River. In some instances, it is possible for high nitrogen, ammonia and phosphorus levels to be recorded during the rainy season because of the increased overloading of sewage effluent systems that are already operating beyond their design capacity (Wilen et al., 2006). Total dissolved solids and conductivity also did not vary with season (Figure 3.6; Figure 3.7). Both these parameters are normally expected to increase during the rainy season because of the increase in soil erosion from surrounding farmlands. However, in this study the intermittent discharge of sewage effluent into the Sand River probably obliterated any differences.

Spatial variation analysis showed that Sand River still has self-purification capacity. Site 5 was identified as a suitable site for abstracting irrigation water. However, the lack of seasonal variation in the nutrient status suggests intermittent discharge of poor quality sewage effluent in the Sand River. It is thus important for stakeholders using Sand River water for irrigation to monitor the water they abstract from the river because the water may be heavily polluted at times. There is no temporal variation in the physico-chemical parameters. Except for phosphorus, most of the other nutrients were within the acceptable international and national guidelines.

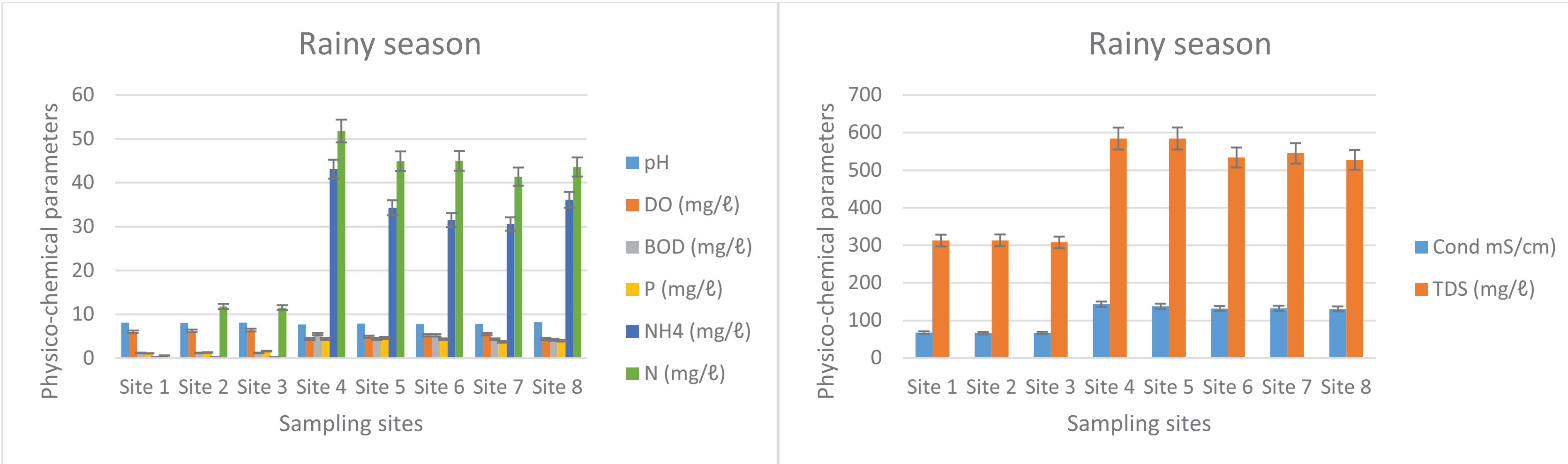

Figure 3.6: Physico-chemical parameters at different sampling sites of the Sand River during the rainy season (October-December, 2017).

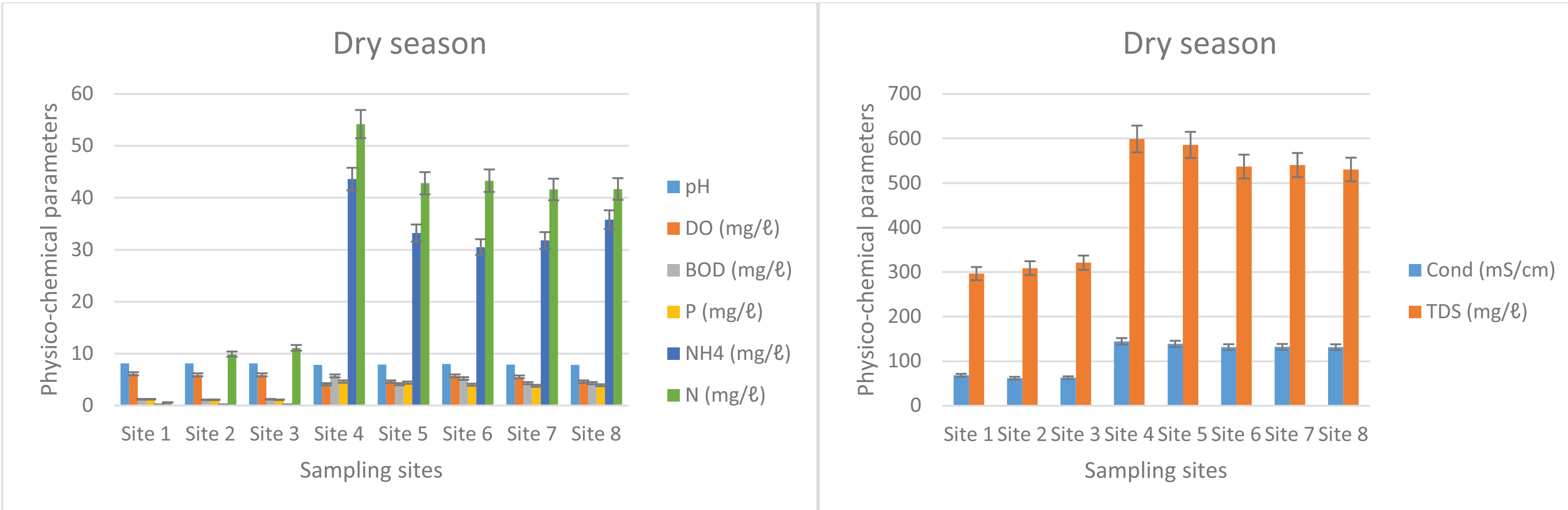

Figure 3.7: Physico-chemical parameters at different sampling sites of the Sand River during the dry season (dry season (May-August 2017).

CHAPTER 4: HEAVY METAL CONTAMINATION OF SAND RIVER WATER AND SEDIMENTS

4.1 Rationale

Trace metal contamination of rivers passing through urban areas is a growing major concern in Africa. Industrial and domestic effluents are the significant sources of trace metals of rivers passing through these urban areas (Moyo and Phiri, 2002). The Sand River is one such river that passes through the rapidly growing city of Polokwane in Limpopo Province, South Africa. This river is a major repository of wastewater. Many industries in developing countries do not have wastewater pre-treatment facilities. They discharge poor quality effluent in the municipal system. A number of industries are associated with both organic and inorganic pollutants. For example, the tanning and leather industry is associated with high levels of chromium. The sugar industry is associated with high level chemical oxygen demand. Textile industry is associated with high suspended solids, mercury and phenolics. The fertilizer manufacturing industry is associated with high levels of cadmium and lead. Poultry and red meat industry are associated with high levels of suspended solids, BOD and TDS. With rapid urbanization the problem of pollution has become more urgent, particularly trace metal contamination. The main problem with trace metal contamination in these rivers, is not only the human health risk associated with some of these metals, but also the damage that trace metals cause to aquatic life (Kalay et al., 1999). Sediments are an important sink for these trace metals and several factors affect metal mobility between the sediment and 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 mostly immobile and are not biologically available (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 from sewage effluent, industrial effluent and storm water and precipitate at the bottom of rivers in the sediment.

The city of Polokwane is one of the fastest growing urban areas in South Africa (Seanego and Moyo, 2013). A number of new industries have been commissioned in the city in recent years. Both domestic and industrial effluents are discharged into the Sand River. Whilst the effect of organic pollutants discharged into the Sand River have been evaluated (Seanego and Moyo, 2013), no work has been undertaken on heavy meatal contamination. This is despite that a number of local people catch fish from the Sand River for household consumption. Furthermore, the Sand River is in close proximity to a metal processing plant, the Polokwane Smelter. Smelting processes can cause high levels of pollution in the soils and rivers (Ettler, 2016). A number of trace metals such as manganese, iron, lead, arsenic, chromium, cadmium, nickel and copper may be released from smelting plants. These metals may be released as fine particles into water bodies via a chimney or emissions from general operations. Dust particles bearing metals can travel long distances to pollute the soil and surface water ways. Thus, it is important to investigate the pollution levels in the Sand River because of its proximity to the Polokwane smelter. A situation analysis of the heavy metal contamination in the Sand River water and sediments is important because the water is used to irrigate vegetables.

4.2 Objectives

The objectives in this chapter were:

a) To determine the heavy metal contamination of Sand River water and sediment.
b) To determine the geo-accumulation index of trace metals in the sediments of the Sand River.

4.3 Materials and Methods

4.3.1 Sample collection

Polyethylene sampling bottles (250 mℓ) washed with de-ionized water were used to collect water samples from each site. Water samples were collected in duplicates just below the surface at a depth of 10 cm 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.

Sediment samples from each site were collected in duplicates 20 cm below the river surface and placed in 250 ml polyethylene bags using a hand trowel. The samples were immediately kept in ice and transported to the ARU Laboratory, where they were kept in a freezer (-20°C) until analysis.

4.3.2 Laboratory analysis

Sediment samples were dried at 80°C for 48 hours and ground into a powder. The samples were passed through 60 µm mesh sieve to separate large fractions. The sieved samples were then used for determination of trace metal analysis. Each sediment sample was placed in a beaker with 20 mℓ aqua regia (1:3 $cHNO_3$: $HClO_4$) and allowed to stand overnight. The mixtures were heated until dry before 20 ml of a 5M HNO_3 solution was added. The samples were allowed to stand overnight and then filtered through Whatman No. 41 filter paper. The filtrates were transferred to a 100 mℓ volumetric flask and made up to the mark with 0.5M HNO_3. Trace metal determinations were performed on a sequential plasma spectrophotometer (ICP-OES) using the calibration curve method. A similar protocol was used for water samples [reverse aqua regia (3:1 $cHNO_3$: $cHCl$)]. Quality assurance (QA) and quality control (QC) procedures were conducted by using standard references materials. Each batch of samples had one blank and one standard. The accuracy of the analysis was determined using certified standards (De-Bruyn spectropic solutions 500 MUL20-

50STD2) and recoveries fell within 10% certified values. The chemicals used were analytical reagent grade. All samples were subjected to the same QA/QC.

The geo-accumulation index of heavy metals was determined based on four sites. Site 1 before discharge, site 4, site 5 and site 7. The geo-accumulation index of trace metals in sediment was calculated using Muller (1969)'s formula given below:

$$\textit{Equation 3}: Igeo = \log_2 \frac{C_n}{1.5\, B_n}$$

Where C_n is the measure of the metal concentration in the sediment, B_n is the background concentration of the element, and 1.5 is the factor compensating background data (correction factor) due to the lithogenic effect. The geo-accumulation index consists of seven grades (0-6) indicating the degrees of metal accumulation and ecological risk with 0 indicating uncontaminated and 6 indicating extremely contaminated

4.4 Results and discussion

Iron dominated the heavy metal profile in the Sand River water and averaged 0.43 mg/ℓ after discharge (Table 4.1). Average concentrations of manganese in the water were 0.05 mg/ℓ before sewage effluent discharge and rose to 0.25 mg/l after discharge. Thus, iron and manganese dominate the heavy metal composition of Sand River water. This is consistent with Edokpayi et al. (2016) who also showed that iron and manganese dominated the trace metals composition of the Mvudi River in Limpopo Province, South Africa. The dominance of iron is because of its high occurrence in different geological formations in Limpopo Province. The most probable cause of the high iron and manganese concentrations in the Sand River is the type of rock found in the catchment areas. Granite is one of the major basement rocks found in the Sand River catchment areas (Polokwane Municipality SDF, 2011). When granite withers it produces iron and manganese. After discharge of sewage effluent, the levels of iron and manganese increased and this probably indicates that sewage effluent may be increasing the concentration of iron and manganese in the Sand River. The solubility of iron and manganese is determined by the pH and redox potential of the water (Allen and Minear, 1982).

The manganese levels after discharge exceeded the DWAF (1996) regulations for aquatic ecosystems (0.18 mg/ℓ). DWAF (1996) does not have any recommended limits for iron in aquatic ecosystems. For irrigation purposes, the DWAF (1996) regulations stipulate that the iron levels must be between 5 to 20 mg/ℓ (DWAF, 1996). The levels of iron in the Sand River are below the target suggested by (DWAF, 1996). DWAF (1996) suggest that manganese levels for irrigation must range between 0.02 to 10 mg/ℓ. The manganese levels are also within the target range. Cadmium levels in the water remained the same before and after discharge. Cadmium levels also fell within the DWAF target range of 0.01 to 0.05 mg/ℓ for irrigation. Copper levels were also the same before and after effluent discharge. Most copper minerals are insoluble and adsorbed to solid phases and this is the reason there is normally low concentration of copper in natural waters (Allen and Minear, 1982). For copper, the DWAF (1996) guidelines for irrigation are between 0.2 to 5 mg/ℓ. In this study the copper concentrations were 0.01 mg/ℓ and fell within the target range. Lead concentrations were highest at site 2 followed by site 4 and site 3 (Table 4.1). Site 2 and site 3 are in an area where there is a taxi rank and garages and this probably explains the high levels of lead in these areas. Site 4 is at the confluence of Sand River and Blood River and it is probable that Blood River also contributes to the high concentrations of lead at this site. Between site 5 and site 8 the lead levels substantially declined (Table 4.1) and this may be due to the fact that there are no industries at these sampling sites, which are all surrounded by farms. The lead levels at site 2 and site 4 fell out of the target range (0.2 mg/ℓ) for irrigation. Lead is a minor element in the earth's crust. Its occurrence in high concentrations in water systems is normally a result of atmospheric input and industrial activities. Leaded fuel was banned in South Africa in 1996 and thus the source of the lead is probably from industrial activity. Zinc levels remained the same before and after effluent discharge. The zinc levels were way below the target range (1-5 mg/ℓ) for irrigation water. Zinc is also a minor constituent in the earth's crust and is normally found in low concentrations because of the low solubility of the metal and its oxides. However, it is a common constituent of industrial effluent. Nickel concentrations remained the same before and after discharge at 0.025 mg/ℓ (Table 4.1). Nickel fell within the target range for irrigation water (0.2-2.0 mg/ℓ).

Heavy metal concentration in the sediment followed the order Fe>Mn>Ni>Cu>Pb>Cr>Cd (Table 4.2). This to a large extent might be a reflection of the underlying geology of the area. These results are also consistent with most previous studies (Butu and Iguisi, 2013; Ladigbolu and Balogun, 2013). Iron levels rose significantly after discharge. Manganese levels also significantly rose after discharge. The rise in iron and manganese is consistent with the previous observations in the water. Chromium levels were highest at site 1 and site 2. It is not immediately obvious as to what the source of chromium is at the two sites. No cadmium levels were detected at all the sites except site 3. This is the site where there is a taxi rank, bus station and some garages, and this may be the source of cadmium. Zinc levels were highest at site 1 and site 2 (Table 4.2). Both sites receive storm water from the Polokwane CBD. Lead, nickel and copper levels were highest before discharge and declined after discharge (Table 4.2). This may simply be a reflection of the absence of any industries after discharge. However, total metal concentration in sediments provide little insight on their bioavailability and toxicity. It was thus important to calculate the geo-accumulation index.

Geo-accumulation index showed that the sediment was not contaminated with any of the trace metals before and after discharge because the index was below 1 for all trace metals (Table 4.3). This confirms our previous observations and probably indicates that the industries in and around Polokwane do not discharge significant amounts of trace metals into the Sand River. Thus, the ecological risk to the aquatic ecosystem is very low. Pheiffer et al. (2014) also used the geo-accumulation index to determine metal contamination of sediments in the Vaal River in South Africa. Except for copper the geo accumulation index for iron, manganese, cadmium and zinc were below 1. Although the sediment in the Sand River is uncontaminated, it is important to regularly monitor trace metals in sediments because metals that are bound to the sediment layer in an aquatic system can be released through changes in pH and resuspension of particles into the water body (Soares et al., 1999).

Table 4.1: Heavy metal concentration of the Sand River water (mg/ℓ) between February and June, 2018. Values are presented as means ± standard deviation.

Sites	Fe	Mn	Cr	Cd	Zn	Pb	Ni	Cu
Site 1	<0.1	<0.05	<0.025	<0.003	<0.025	0.049	<0.025	<0.01
Site 2	0.21±0.02	0.19±0.02	<0.025	<0.003	0.126±0.03	0.254±0.07	<0.025	<0.01
Site 3	0.27±0.021	0.24±0.05	<0.025	<0.003	<0.025	0.194±0.08	<0.025	<0.01
Site 4	0.47±0.09	0.25±0.01	<0.025	<0.003	<0.025	0.240±0.06	<0.025	<0.01
Site 5	0.36±0.04	0.22±0.03	<0.025	<0.003	<0.025	<0.01	<0.025	<0.01
Site 6	0.50±0.01	0.29±0.04	<0.025	<0.003	<0.025	<0.01	<0.025	<0.01
Site 7	0.43±0.06	0.25±0.06	<0.025	<0.003	<0.025	<0.01	<0.025	<0.01
Site 8	0.39±0.02	0.27±0.01	<0.025	<0.003	<0.025	<0.01	<0.025	<0.01

Table 4.2: Heavy metal concentration of the Sand River sediment (mg/kg) between February and June, 2018. Values are presented as means ± standard deviation.

Sites	Fe	Mn	Cr	Cd	Zn	Pb	Ni	Cu
Site 1	1320±50.5	190±12.0	0.40±0.05	0.000	40±4.0	0.38±0.02	0.89±0.03	0.85±0.02
Site 2	1405±37.0	185±7.0	0.33±0.09	0.000	10±0.5	1.70±0.04	2.60±0.04	1.50±0.01
Site 3	1800±20.0	194±15.0	0.15±0.01	0.001	6.39±0.4	1.20±0.02	0.70±0.08	1.50±0.03
Site 4	2720±27.0	298±19.0	0.16±0.01	0.000	6.79±0.8	0.33±0.04	0.45±0.05	0.53±0.04
Site 5	2500±57.0	251±10.0	0.12±0.02	0.000	6.00±0.5	<0.021	0.22±0.03	0.22±0.05
Site 6	2510±50.0	240±5.0	0.13±0.01	0.000	4.39±0.2	<0.021	0.26±0.04	0.26±0.01
Site 7	2800±43.0	252±16.0	0.29±0.04	0.000	4.40±0.7	<0.021	0.22±0.05	0.22±0.08
Site 8	2800±18.8	281±12.0	0.23±0.08	0.000	4.00±0.5	<0.021	0.45±0.01	0.45±0.03

Table 4.3: Geo-accumulation index of trace metals in sediment in the Sand River.

Sites		Cd	Cu	Fe	Mn	Pb	Zn
Before discharge	Sediment	-1.58	-2.96	-5.56	-2.67	-3.58	-2.83
After discharge	Sediment	-1.68	-2.35	-4.71	-2.12	-3.32	-2.68

CHAPTER 5: MICROBIAL CONTAMINATION OF WATER IN THE SAND RIVER

5.1 Rationale

Globally, numerous reports indicate that incidence of foodborne pathogens on fruits and vegetables possibly as a result of consuming contaminated fresh food produce is increasing. Such incidence reports vary by region and can be extremely high in some developing countries (Pachepsky et al., 2011). In South Africa, several studies have been undertaken to determine the status of some rivers with respect to microbial contamination (Momba et al., 2006; Gemmell and Schmidt, 2012; Ijabadeniyi et al., 2011). Momba et al. (2006) found high levels of *Salmonella*, *Shigella*, *Vibrio cholerae* and coliphages in a river in the Eastern Cape. These authors attributed the presence of these pathogens to the release of effluent by the Nkokonbe Municipality in the river. Similarly, the Plankenburg and Eerste Rivers in the same province have been reported to be contaminated with faecal coliforms (Paulse et al., 2009; Britz et al., 2013). The microbial load of Baynespruit River in KwaZulu-Natal was reported to exceed acceptable levels stipulated by WHO and South African guidelines for safe use of wastewater (Gemmell and Schmidt, 2010). In Limpopo Province, Traore et al. (2016) investigated the impact of human activities on microbial quality of 6 rivers in the Vhembe District. The rivers investigated were Mutale, Sambandou, Mbwedi, Tshinane, Madadzhe and Luvhu Rivers. They reported that the microbial quality of the rivers was poor because the total coliforms were found to be above acceptable limits set for drinking water by the South African Department of Water and Sanitation. Most of the work done on microbial quality of river water in Limpopo Province focused on its suitability for drinking purposes and not irrigation (Obi et al., 2003; Edokpayi et al., 2015; Edokpayi et al., 2018). In this study the focus is on the use of sewage effluent in the irrigation of crops. It is important to determine the microbial load of a river that receives sewage effluent. Whereas the studies carried out in Limpopo Province found out that total coliform and *E. coli* count exceeded the target range for drinking water, the situation with respect to microbial quality of irrigation water from rivers receiving sewage effluent might be different. This is the first study that looks at the microbial load in a river that receives sewage effluent in Limpopo Province. It is very important to identify the source of pollution because that will enable stakeholders to put pollution abatement measures into place.

5.2 Objectives

The objectives in this chapter were to:

a) Determine spatial variation of microbial load in the Sand River.
b) Determine temporal variation of microbial load in the Sand River.

5.3 Materials and Methods

5.3.1 Sample collection

Detailed descriptions and special features of each site are given in Table 3.1 in chapter 3. Water samples were collected at different sites from March, 2018 through to November, 2019. The collection of water samples was done in accordance with German standard DIN 38402-15 for running waters. Samples (1 litre) were collected in triplicate using sterile sampling bottles. The water samples were aseptically transported to the laboratory at the Aquaculture Research Unit where they were analysed within 12 hours.

5.3.2 Laboratory analysis

Samples were analysed for total coliform bacteria, faecal coliform bacteria, *E. coli* and heterotrophic bacteria. Analyses were carried out according to the SANS 5221:2011 Edition 4.4. These are standard methods describing the detection and enumeration, in potable water, non-potable water and effluent water, of total coliform bacteria, faecal coliform bacteria, *E. coli* and heterotrophic bacteria.

5.3.2.1 Heterotrophic count

A Standard plate count was used to estimate the total number of viable heterotrophic bacteria in water samples as described in the SANS guidelines (5221:2011 Edition 4.4). The water sample was thoroughly mixed by rapidly inverting and righting the sample container approximately ten times (by rapid movement of the wrist). Aseptically, 1 mL of appropriate ten-fold dilutions of the sample was transferred in a diluent (tryptone water) in two Petri Dishes. Within 20 min of transferring the samples to the Petri dishes, 15 mL of plate count agar previously melted and cooled to 45°C to 50°C was added to each of the Petri dishes. This was done in triplicates. While the Petri dishes were kept flat on the bench, the contents of each dish were immediately mixed by a combination of rapid but gentle to-and-fro and rotary movements for a period of 10 s. The petri dishes were then allowed to stand until the agar had solidified after which the petri dishes were inverted and incubated at 35°C ± 1 °C for 48 hours. Plates that contained between 30 and 300 colonies were counted. The number of counts obtained was used to calculate the average total plate count (TPCa) per millilitre of sample as follows:

***Equation 4*: *TPCa = TNC/ PN X DF*,**

$$TPCa = TNC / PN \times DF$$

Where TNC = total number of colonies counted; PN = number of plates counted; DF = dilution factor.

5.3.2.2. Total coliforms, faecal coliforms and E. coli

Total and faecal coliforms and *E. coli* were enumerated according to the most probable number (MPN) method due to the turbidity of the effluent water as specified by in the SANS guidelines as summarised below. This involved preparation of tenfold dilution series from samples under aseptic conditions. Briefly, water samples were thoroughly mixed by rapidly inverting and righting the sample container approximately 10 times. One millilitre was removed using a sterile pipette and added to 9 mℓ of sterile peptone water. This primary dilution was thoroughly mixed by inverting and righting the container approximately 10 times. One millilitre was transferred from this dilution using a fresh pipette into another bottle containing 9 mℓ of sterile peptone water. Precautions were taken to avoid contact between the pipette and diluent. The suspension was mixed thoroughly. This was repeated severally to obtain a tenfold dilution series. A sufficient number of dilutions was prepared to ensure that all bottles containing the final dilution yielded a negative result. Prior to carrying out of dilutions, first 10 mℓ of the original sample was added to each of three tubes containing 10 mℓ of double strength lauryl tryptose broth (LTB). Then 1 mℓ of original water sample was transferred to each of three tubes containing 10 ml single strength LTB. Then, 1 mℓ of each of the subsequent dilutions was transferred into each of three tubes or bottles containing single-strength lauryl tryptose broth. A fresh sterile pipette was used for each dilution. Tubes were then incubated at 37°C ± 1,0°C for 48 hours. After 48 hours of incubation, cultures were examined. Tubes showing turbidity were regarded as positive reactions due to bacterial growth and gas formation. Gas formation was indicated by an amount of gas at least sufficient to fill the concavity of the top of the fermentation (Durham) tube. For each dilution, the number of tubes or bottles showing a positive reaction were counted and recorded. Gas production and visible growth indicated the presence of bacteria.

5.3.2.2.1 Total coliforms

For coliform MPN, subcultures were made from each tube of lauryl tryptose broth giving a positive result into a tube or bottle of brilliant green bile broth and incubated at 37°C ± 1°C. Cultures were examined for gas formation within 48 hours and the formation of gas was used as confirmation for the presence of coliform bacteria.

5.3.2.2.2 Faecal coliforms

For faecal coliform determination, a similar process was followed, but now cultures were incubated in a waterbath maintained at 45°C ± 0,5°C, for 24 hours. Gas production after incubation confirmed the presence of faecal coliform bacteria.

5.3.2.2.3 Escherichia coli

Subcultures were made from each tube of lauryl tryptose broth giving a positive result into a tube of tryptone water. Cultures were grown at 45°C ± 0,5°C for 24 hours. After incubation, 0,3 mL to 0,5 mℓ of Kovacs reagent was added to each tube of inoculated tryptone water and allowed to stand for 10 min. The development of a red colour denoted the presence of indole. Tubes showing gas formation and the presence of indole were used to confirm the presence of *E. coli.*

5.3.2.2.4 Enumeration coliform bacteria, faecal coliform bacteria and E. coli.

For each dilution for each test (coliform bacteria, faecal coliform bacteria and *E. coli.*), the number of tubes showing a positive reaction were counted and recorded. The tubes were then used to calculate the most probable number (MPN) of coliform bacteria, faecal coliform bacteria and *E. coli*. The number of coliform bacteria, faecal coliform bacteria and *E. coli* per 100 mL of water sample was calculated by multiplying the MPN index by the reciprocal of the lowest dilution selected times 100. When the lowest dilution selected corresponded to the tubes inoculated with 10 mℓ, the MPN index was divided by 10.

5.4 Results and discussion

Total heterotrophic, total coliforms (TC), faecal coliforms (FC) and *E. coli* levels drastically increased from the upstream sites to the downstream sites (Figures 5.1, 5.2, 5.3 and 5.4). The highest levels in total heterotrophic, total coliforms, faecal coliforms and *E. coli* were recorded at site 4 (Figures 5.1, 5.2, 5.3 and 5.4), which is the site after discharge of sewage effluent. The lowest values of total coliform, faecal coliforms and *E. coli* were recorded at site 1, 2, 3, 7 and 8 (Figures 5.2, 5.3 and 5.4). Faecal coliforms were highest at site 4 (after discharge). During sampling, faecal matter was occasionally observed at site 4. No seasonal variation in microbial load were observed during the rainy and dry seasons (Figures 5.1, 5.2, 5.3 and 5.4). This again is attributed to the intermittent discharge of poor quality sewage effluent which will obliterate any seasonal variations. The sampling of the microbes was not done concurrently with that of nutrients yet no seasonal variations were observed in both instances. The Polokwane sewage treatment works and Seshego sewage treatment works handle sewage effluent beyond its design capacity. As long as these two sewage treatment works are not upgraded, the discharge of poor sewage effluent will continue unabated. The problem has been further compounded by the mushrooming of an unsewered informal settlement, Disteneng close to the Sand River.

The elevated levels of faecal coliforms and *E. coli* at the sites after discharge are mainly due to the release of partially treated sewage effluent in the river. This can be attributed to the inefficiency of both the Polokwane and Seshego sewage treatment plants to remove the microbes prior to releasing the effluent into the river. A large commercial tomato farm company abstracts water from the Sand River after the point of discharge. According to the South African Water Guideline (DWAF, 1996), the water after discharge is not suitable for irrigation (Table 5.1). The total coliform, faecal and *E. coli* levels were above the recommended 0 counts/ 100 mℓ faecal coliform and 130 counts/100 mℓ total coliforms for irrigation use. Faecal coliform levels exceeding 50 000 counts/100 mℓ are likely to cause damage to irrigation systems. At elevated levels, faecal coliforms may block drip irrigation systems. On the other hand, *E. coli* levels after the point of discharge exceeded the 1 MPN *E. coli*/100 mℓ recommended for irrigation of crops (DWAF, 1996). The guideline limit for the incidence of *E. coli* in irrigation water is ≤1000 CFU/100 mℓ for crops not to be eaten raw and allowed to dry (DWAF, 1996). According to the guidelines, there is an increase in the likelihood of contamination if water contaminated with *E. coli* between 1-1 000 CFU/100 mℓ is used to irrigate vegetables and other crops eaten raw. The guidelines suggest that such water may be used to irrigate fruit trees and grapes provided that the fruits are not wetted. The guidelines further states that anything above 1 000 *E. coli*/100 mℓ should not be used on fruits or vegetables consumed by humans. No contact is allowed to take place with humans. Such water can only be used in irrigation for the production of fodder, tree plantations, nurseries, parks, etc. (DWAF, 1996). According to these standards, the Sand River water is not acceptable for irrigation of fresh produce to be eaten raw because of the high probability of humans becoming infected with pathogens when consuming crops irrigated with such water.

The poor quality of Sand River water requires stakeholders to effect on farm treatment of the water to reduce microbial load. It is important for farmers to take these mitigation measures because large scale outbreaks of food-borne diseases can occur particularly when fresh produce is consumed raw.

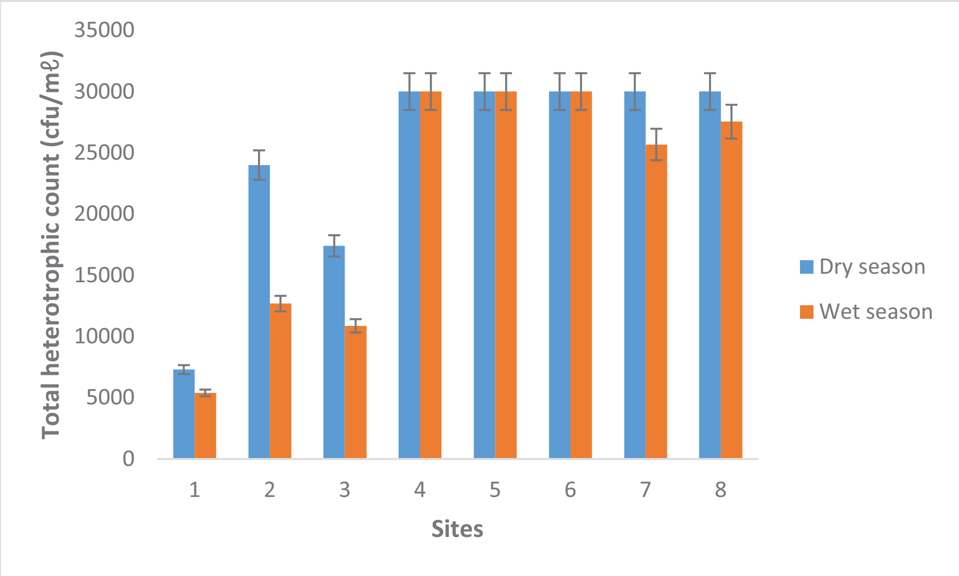

Figure 5.1: Total heterotrophic count (cfu/mℓ) during the dry season (May-August, 2018) and wet season (October-December, 2018).

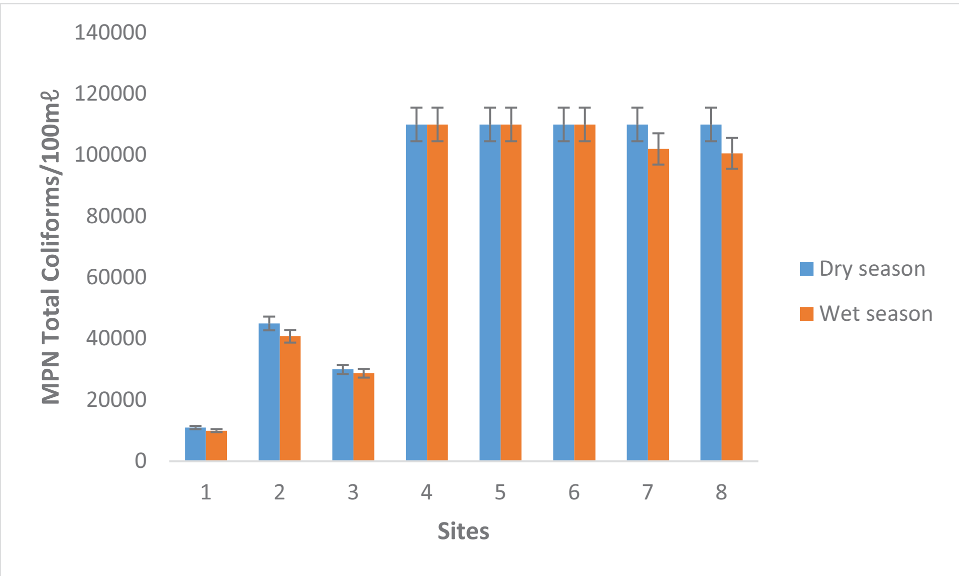

Figure 5.2: MPN Total Coliforms/100 mℓ during the dry season (May-August, 2018) and wet season (October-December, 2018).

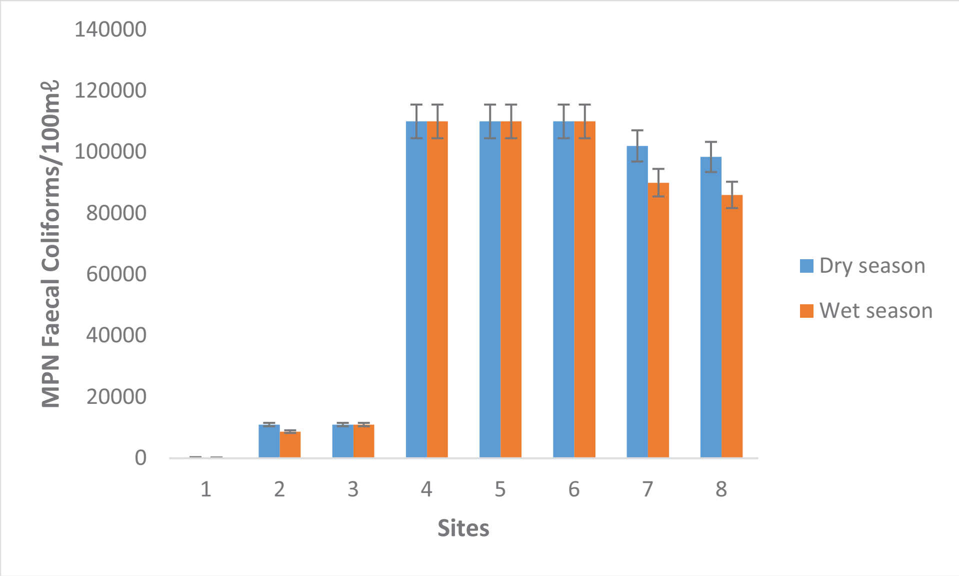

Figure 5.3: MPN Faecal Coliforms/100 mℓ during the dry season (May-August, 2018) and wet season (October-December, 2018).

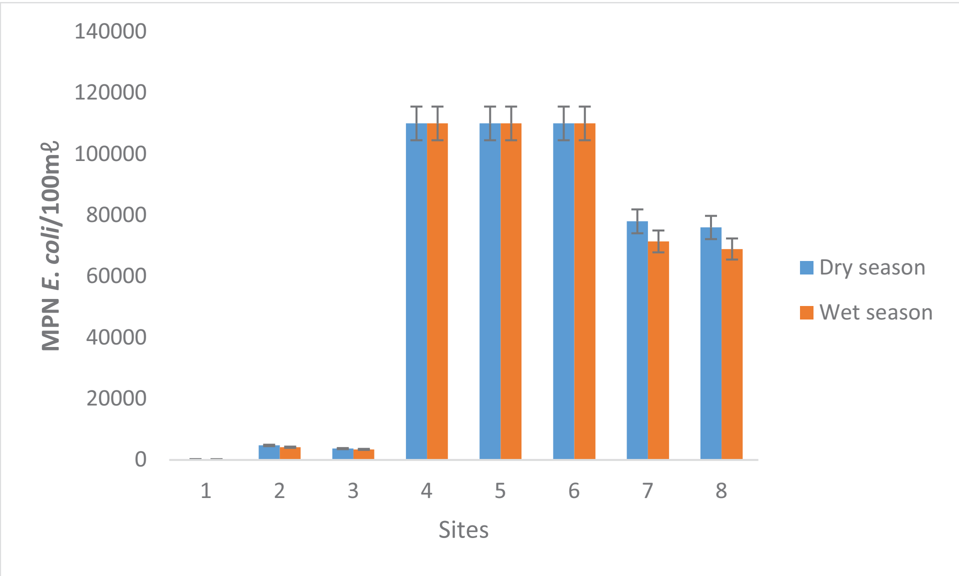

Figure 5.4: MPN *E. coli*/100 mℓ during the dry season (May-August, 2018) and wet season (October-December, 2018).

Table 5.1: DWAF (1996) guidelines indicating acceptable levels of total heterotrophic count, total coliform, faecal coliform and *E. coli* for irrigation of crops.

Bacteria	**Target Water quality range**	**Crop Quality**
Total heterotrophic count (cfu/mℓ)	N/A	
MPN Total Coliforms/100 mℓ	N/A	
MPN Faecal Coliforms/100 mℓ	10 000	Practically no problems with clogged drip irrigation
	10 000-50 000	Slight to moderate likelihood of clogging of drip irrigation equipment
	>50 000	Increasingly likely that clogging of drip irrigation equipment will occur
MPN *E. coli*/100 mℓ	<1	Any irrigation method on any crop-safe
	1-1 000	Likelihood of contamination from vegetables and other crops eaten raw Fruit trees and grapes may be irrigated provided that the fruits are not wetted
	>1 000	No contact is allowed to take place with humans, water can be used in irrigation for the production of fodder, tree plantations, nurseries, parks, etc.

CHAPTER 6: SUITABILITY OF SAND RIVER AND BOREHOLE WATER FOR IRRIGATION

6.1 Rationale

South Africa is a water scarce country and the use of sewage effluent to irrigate crops is widely practiced by both big commercial enterprises and small holder farmers. Wastewater irrigation is preferred because of its high nutrient content which sometimes eliminates the need to use fertilizers. Sixty percent of sewage effluent in SA is now used for irrigation (Akpor and Munchie, 2011). With climate change and frequent droughts, it is most likely that this figure will rise. Long term use of sewage effluent to irrigate crops can lead to heavy metal contamination in the soils. Thus, crops grown from such soils can accumulate high quantities of these metals which can cause clinical problems in humans and other animals who may consume metal rich foods. Monitoring of these heavy metals can provide valuable information for promoting food safety. There is a growing concern worldwide on exposure to heavy metal contamination due to the use of sewage effluent that may be rich in heavy metals (Singh et al., 2012). Unfortunately, in most developing countries, there are no national standard limits on heavy metals in crops.

As already mentioned, Polokwane is one of the fastest growing towns in SA and the urban population has significantly increased in the last 10 years (Seanego and Moyo, 2013). The rapid population growth has resulted in the overloading of the sewage treatment works. Thus, over the last few years poorly treated effluent has been discharged into the Sand River. Farmers downstream of the sewage treatment works use the Sand River water for irrigation of vegetables. Significantly, a large commercial farming enterprise uses the water to irrigate tomatoes. Other farmers with small holdings also use this water to irrigate tomatoes, onions and cabbage. Heavy metal contamination in both the soils and vegetables is thus a distinct possibility. No studies have been undertaken to investigate the effect of heavy metal contamination on the soils and vegetables irrigated with Sand River water. It is important to monitor heavy metal contamination in the vegetables because the vegetables may become a major sink for some toxic heavy metals. This study investigated the state of heavy metal contamination in soils and vegetables irrigated with Sand River water. The risk associated with consumption of these vegetables was also be determined. This is particularly important as no previous studies have been done on the risk associated with consumption of vegetables irrigated with Sand River water.

Apart from heavy metal contamination, use of sewage effluent for irrigation can also lead to microbial contamination of crops. Sewage effluent may have pathogenic bacteria that can lead to enteric diseases such as cholera (*Vibrio cholerae*), typhoid fever (*Salmonella typhi*), salmonellosis (salmonella spp.) and bacillary dysentery (*Shigella* spp. (McLain et al., 2011; Wu et al., 2011). These diseases usually indicate that the water used to irrigate the consumed crop is contaminated with faecal matter. There is a high possibility that the Sand River might have high levels of faecal coliforms that can be transferred to crops. It is, therefore, important to determine if the bacterial level in the Sand River water is within acceptable ranges for irrigation of crops.

The sewage from the Polokwane Sewage Treatment Works is also used to recharge the Polokwane aquifer. Alluvial deposits along the Sand River are important sources of ground water (Vegter, 2003). Numerous boreholes have been installed along the Sand River and these boreholes are also used for irrigations of crops in the same areas where sewage effluent is used for irrigation. The effect of borehole water on soils and crops along the Sand River have not been investigated before. A comparative study on the effect of the sewage effluent and borehole water on soils and crops was also undertaken.

6.2 Objectives

The objectives of this chapter are to determine the:

a) Suitability of Sand River and borehole water for irrigation.
b) Effect of Sand River and borehole water on irrigated soils.
c) Concentration of heavy metals in tomatoes and onions.
d) Effect of microbial contamination on tomatoes and onions.

6.3 Materials and Methods

6.3.1 Experimental Design

Field experiments were conducted at the University of Limpopo, Aquaculture Research Unit. Plots of 3 x 3 meters were used to investigate the effect of Sand River and borehole water on soils and vegetable quality. One aspect of quality that was investigated was based on heavy metal contamination of tomatoes and onions. The experimental design for both onions and tomatoes was a randomized complete block design, replicated 3 times. The background soil was also analysed for heavy metals. The tomatoes and onions were watered once every 3 days. The vegetative growth of tomatoes under the two treatments was determined by measuring plant height and leaf numbers. At the end of the experiment the tomatoes and onions were harvested and analysed for proximate composition, heavy metal and microbial contamination.

6.3.2 Sample collection

6.3.2.1 Suitability of Sand River and borehole water for irrigation

Water samples from the Sand River were collected at a depth of 20 cm at each site using polyethylene water bottles. There are 67 boreholes located along the Sand River. Ten boreholes were randomly selected to test the suitability of borehole water for irrigation.

6.3.2.2 Soil samples

Soil samples were randomly taken from the different treatments at a depth of 25 cm using a stainless-steel hand trowel and put in plastic bags.

6.3.2.3 Vegetative structure and quality

The plant height (cm) and the number of leaves on each plant were counted in the two different treatments. From each plot, a total of 5 fresh, firm and undamaged tomatoes were randomly selected. The vegetative growth of tomatoes was determined by measuring plant height, leaf numbers and number of fruits. The tomatoes were also scored on a scale of 1-5 for quality assessment, with 5 being the best quality.

The tomatoes and onions were also harvested aseptically and samples were separately packaged into different sterile containers, labelled and transported to the laboratory immediately for bacteriological analysis. The elapsed time between sample collection and analysis did not exceed 2 hrs.

6.3.3 Laboratory analysis

6.3.3.1 Suitability of Sand River and borehole water for irrigation

Calcium and magnesium content of the water was determined by EDTA titration using Mordant black 11 mixture as an indicator. Multiple aliquots were collected and stored for the different type of analysis. 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). Sodium adsorption ratio (SAR) was calculated using the equation below:

$$\textit{Equation 5:} \; SAR = Na/(\sqrt{Ca + Mg}/2)$$

where the ionic concentrations of sodium, calcium and magnesium are expressed in meq/ℓ. Soluble sodium percentage (SSP) was calculated by using the equation below:

$$\textit{Equation 6:} \; SSP = (Na)\,(100)/(Ca+Mg+Na+K)$$

where the ionic concentrations of sodium, calcium, magnesium, potassium are expressed in meq/ℓ.

6.3.3.2 Vegetative quality of tomatoes and onions

Five tomatoes were sampled from each treatment for proximate analysis. Crude protein (Method 954.01), carbohydrates (Method 989.03) and fat (Method 954.02) were determined using procedures stipulated by the Association of Official Analytic Chemistry (AOAC 2003). Ash content was determined by burning the samples in a muffle furnace at 550°C for 4 hours.

6.3.3.3 Heavy metal analysis in soils, tomatoes and onions

Soil, tomatoes and onions samples were dried at 80°C for 48 hours and ground into a powder. The soil samples were passed through 60 µm mesh sieve to separate large fractions. The sieved samples were then used for determination of trace metal analysis following the AOAC (2003) method. Each soil sample was placed in a beaker with 20 mℓ aqua regia (1:3 HNO_3: $HClO_4$) and allowed to stand overnight. The mixtures were heated until dry before 20 mℓ of a 5M

HNO_3 solution was added. The samples were allowed to stand overnight and then filtered through Whatman No. 41 filter paper. The filtrates were transferred to a 100 mℓ volumetric flask and made up to the mark with 0.5M HNO_3. Trace metal determinations were performed on a sequential plasma spectrophotometer (ICP-OES). Soil pH and conductivity were determined in a soil-water suspension at the ratio of 2:1.

Approximately 0.5 g of tomato sample was placed in a beaker with 20 mℓ reverse aqua regia (3:1 HNO_3: HCl) and digested on a hot plate for 3 hours at 125 °C (Bourioug et al., 2015). A solution of sulphuric acid 65%, perchloric acid 70% and nitric acid 70% with a ratio of 1:1:5 was prepared and used for digestion of tomatoes. One g of powder of each sample was digested in 100 mℓ pyrex glass beaker by adding 15 mℓ of the aforementioned acid mixture for 1 hour. The mixture was filtered through a Whatman No. 41 filter paper. The filtrate was then reconstituted with deionized water after digestion. Total metal concentration was determined using a sequential plasma spectrophotometer (ICP-OES). A similar protocol was followed for onions.

6.3.3.4 Microbiological analysis

In the laboratory, the tomatoes and onions were cut into two equal halves. A sterile swab stick was then used to sample the microbes on the inside and outside of the cut tomatoes and onions. For the presence of heterotrophic bacteria, swabs were streaked onto a plate count agar and incubated at 35°C for 48 hours. For coliforms, the swabs were then streaked onto m-Endo agar and incubated at 37°C for 18-24 hours. To confirm the presence of *E. coli*, cultures of faecal coliforms plated on mFC agar were subcultured into tubes of tryptone water and incubated at 45°C for 24 hours. After incubation, the colonies of the different culture media were examined and recorded based on the shape, colour, border, texture and general appearance of individual bacterial colonies on each plate.

6.3.4. Risk assessment of heavy metals in vegetables.

Daily intake rate (DIR) was calculated using the following equation:

Equation 7: DIR= C (metal) x D (food intake) / B (average weight)

Where C (metal), D (food intake) and B (average weight) are the heavy metal concentrations in plants (mg/g), daily intake of vegetables (kg/person) and average body weight (kg/ person), respectively. The average daily vegetable intake rate was calculated by doing a survey in which 20 people with an average body weight of 60 kg were asked for their daily intake of tomatoes. The assessment of health risk after consumption of vegetable by the local inhabitants was determined by calculating hazard quotient (HQ) following the methodology described by USEPA (2000). A hazard quotient (HQ) for each metal was calculated comparing the expected exposure of the population to the reference doses (RfD) for the respective metals as follows:

Equation 8: HQ= DIR/RfD

where HQ<1 indicates that adverse health effects are unlikely and HQ>1 indicates high probability of adverse health effects. The same method was followed for onions.

6.4 Results and discussion

6.4.1 Suitability of Sand River and borehole water for irrigation

The calcium levels averaged 36.63 mg/ℓ in the river and ranged between 37.10 to 51.90 mg/ℓ in the boreholes (Table 6.1). There are no stipulated guidelines for calcium in irrigation water in South Africa. There are two main problems with calcium, it decreases the availability of some key nutrients such and phosphorus and nitrogen. Calcium can also affect water reticulation in an irrigation system as it can block pipes.

Sodium levels were much higher in the river averaging 109.25 mg/ℓ whilst in the boreholes they ranged between 5.97 to 8.95 mg/ℓ (Table 6.1). The major problem of high sodium levels is that it affects the permeability of the soil and thus causes water infiltration problems. This is attributed to the fact that when sodium is present in the soil in an exchangeable form, it tends to replace calcium and magnesium. This causes dispersion of soil particles. The dispersion then results in breakdown of soil aggregates. Thus, the soil becomes hard and compact when dry resulting in poor infiltration of water and air. The other problem associated with sodium is the formation of crusting seed beds, high pH, increased potential of diseases, weeds, lack of oxygen and soil erosion (Herrera-Melian et al., 2010). The South African guidelines for irrigation water has a target of 70 mg/ℓ (DWAF, 1996). However, it must be noted that the sensitivity to high sodium levels varies among different plants. Borehole water had low sodium concentration and will thus not affect plant health. The mean sodium concentration was high in river water and this probably does not affect tomatoes as they have a tolerance for sodium levels between 115-230 mg/ℓ (DWAF, 1996).

The mean concentration of magnesium in the river was 29.13 mg/ℓ whereas in the boreholes it ranged between 34.80 to 74.20 mg/ℓ (Table 6.1). The problems with high magnesium is that it can block the absorption of calcium. Thus the limited availability of calcium can result in low fruit production. There are no stipulated guidelines in South Africa for calcium concentration in irrigation water. For the Sand River the ratio of Ca/Mg is above 1 and this suggests that Sand River water does not pose any problems in irrigation with respect to magnesium. The ratio was also above 1 at the lower range of borehole water. However, at the high range of magnesium for borehole water the ratio was 0.7 and this suggests potential negative effects of magnesium on plant growth. It must however be noted that the role magnesium plays in the plant nutrient dynamics is poorly understood (Ayers and Westcot, 1985).

Potassium levels in the river averaged 13.34 mg/ℓ whereas in the boreholes the levels ranged between 120.83 to 165.20 mg/ℓ (Table 6.1). The FAO recommended limits for potassium is 2 mg/l (Ayers and Westcot, 1985) while the South African irrigation guidelines do not have any stipulated limits for potassium. The use of wastewater for irrigation increases the levels of potassium in soils. However, the effect of potassium on soil hydraulic properties has received little attention because sodium concentrations are much higher than potassium in soils irrigated with wastewater (Smith et al., 2015). It has also been generally accepted that potassium has little adverse effects on physical properties of soils (Smith et al., 2015). However, in recent years the negative effects of potassium in soils have been highlighted (Smith et al., 2015). The negative effects of potassium in soils has led to the development of the potassium adsorption ratio (PAR) (Rengasamy and Marchuk, 2011). However, there are no guidelines based on PAR related to irrigation water. A comparison between PAR and SAR is normally used to determine the effects of potassium on soils. The main problem with high levels of potassium is that they alter the pH of the soil by making it alkaline. An alkaline pH above 9 can cause the precipitation of calcium and magnesium (Goulding, 2016). This will affect plant growth. Some trace elements like copper also become less available at high pH.

pH is an important water quality parameter for irrigation water because it will determine the success or failure of a crop. The general accepted pH for irrigation water is 6.5 to 8.4 (DWAF, 1996). As already indicated pH values greater than 9 can lead to precipitation of calcium and magnesium. On the other hand, acidic water with a pH of 4 can lead to soil acidification and may also be corrosive to the water reticulation system. The pH for Sand River was within the normal range at all sites (Table 6.1). The pH values that are above 8.5 can lead to soil salinization. The pH of borehole water was also within the normal range (Table 2).

Sodium adsorption ratio (SAR) has been traditionally used as a useful indicator of the suitability of water for use in irrigation. The mean sodium adsorption ratio (SAR) for Sand River was 4.51. The South African guidelines stipulate that SAR should range from 2-15 and it is put in 3 categories. The categories are 2-8, this is for the most sodium sensitive crops; 8-15 is the range for most crops that can tolerate fairly high sodium concentrations; the third category is for crops that are very tolerant to very high sodium concentrations (SAR>15) (DWAF, 1996). Tomatoes are fairly tolerant to high sodium levels and thus they can tolerate SAR values above 15 (ANZECC, 2000). The SAR for all boreholes was below 2 (Table 6.2), thus there is no possibility of sodium toxicity if borehole water is used to irrigate the tomatoes. However, these values also suggest that borehole water has very little sodium which may affect plant growth since plants need supply of sodium. The soluble sodium percentage (SSP) also evaluates sodium toxicity and the mean was 58.98% in the river. This percentage is very close to the target limit for SSP which is 60%. This suggests that the Sand River is suitable for irrigation but there is need to monitor the sodium levels in the water. The SSP was low in boreholes ranging between 2.0 to 20.6% (Table 6.2) and this indicates that there is no possibility of sodium toxicity when borehole water is used for irrigation.

Table 6.1: Mean concentration of anions, cations and pH in Sand River and borehole water. *Collected between Sep, 2017-March, 2018. Values are presented as means ± standard deviation.

Source	Site	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	SO_4^{2-}	pH
Sand River*	Site 1	37.0±1.2	5.6±0.5	17.0±4.2	43.0±3.2	41.0±1.3	4.0±0.8	8.2
	Site 2	58.0±3.2	6.3±0.7	54.6±8.1	39.0±2.8	34.0±2.4	19.0±1.9	8.42
	Site 3	60.0±2.8	5.8±0.8	55.0±9.3	37.0±4.2	36.0±2.8	19.0±1.0	8.0
	Site 4	142.0±5.3	19.0±1.3	127.5±12.3	39.0±6.9	24.0±1.8	50.0±2.3	7.8
	Site 5	143.0±4.8	17.0±1.4	130.5±13.2	36.0±4.2	24.0±3.2	47.0±4.1	8.1
	Site 6	145.0±3.2	18.0±2.0	131.1±8.9	37.0±2.1	24.0±4.2	37.0±2.3	8.30
	Site 7	146.0±3.7	18.0±2.3	129.1±9.3	38.0±3.1	25.0±3.2	12.0±0.8	7.68
	Site 8	143.0±2.8	17.0±1.9	128.3±12.1	40.0±1.8	25.0±2.5	12.0±1.0	8.2
Borehole	BH1	6.51±0.80	9.85±0.10	-	50.30±3.50	16.22±1.20	-	7.20
	BH2	6.55±1.10	165.20±8.10	-	51.90±1.80	50.60±3.90	-	7.60
	BH3	7.50±0.92	143.52±6.20	-	49.81±3.70	74.20±4.50	-	7.50
	BH4	8.95±1.20	140.20±5.80	-	50.60±2.50	44.90±1.80	-	7.60
	BH5	5.97±0.57	142.07±7.50	-	39.50±2.30	42.50±1.80	-	7.60
	BH6	7.79±1.20	132.61±3.20	-	39.50±1.50	40.20±1.90	-	7.50
	BH7	7.80±1.40	127.40±2.30	-	43.10±2.50	39.50±2.30	-	7.40
	BH8	8.90±1.80	133.89±3.40	-	48.10±3.40	40.70±3.10	-	7.50
	BH9	6.80±0.90	125.35±2.90	-	37.10±2.80	34.80±1.00	-	7.30
	BH10	8.00±1.30	130.01±2.10	-	43.00±2.80	37.86±1.50	-	7.40

Table 6.2: The suitability for irrigation of the Sand River water and the borehole water with respect to SAR and SSP values.

Source	SAR	SSP (%)
Sand River water (Mean)	4.51	58.98
Site 4	4.63	59.8
Site 5	4.38	58.9
Site 6	4.52	58.8
Site 7	4.42	58.6
Site 8	4.38	58.5
BH1	0.13	2.2
BH2	1.53	20.6
BH3	0.19	3.3
BH4	0.19	3.4
BH5	0.11	2.1
BH6	0.22	3.9
BH7	0.19	3.5
BH8	0.36	6.6
BH9	0.11	2.0
BH10	0.22	4.1

6.4.2 The effect of Sand River and borehole water on soils

6.4.2.1 Soils under tomatoes

The targeted heavy metals namely Zn, Cu, Fe, Mn, Pb and Cd were all present in the soils where tomatoes were cultivated using Sand River water (Table 6.3). Fe had the highest concentration (96.90 mg/kg) in soils under tomatoes irrigated with Sand River water. Iron is an important in the biosynthesis of chlorophyll in plants (Al-Lahham et al., 2007). However, excess iron can lead to iron-toxicity and tomatoes are prone to iron-toxicity (Kupper and Andresen, 2016). There was no evidence of iron toxicity in this study and it must be pointed out that iron toxicity is rare. The availability of this micro nutrient to the plant depends largely on the pH of the soil. Iron is more bioavailable in soils with low pH (Mirecki et al., 2015). There are no South African guidelines that limit the amount of Fe that should be in soils. The control registered 7.42 mg/kg of iron in soils (Table 6.3) which was less than in soils irrigated with Sand River water and this indicates that the sewage effluent is the source of excess iron in the soils. In the soils irrigated with borehole water, the iron concentration was 10.5 mg/kg (Table 6.3). This again probably confirms that sewage effluent is the source of the high iron levels. However, not all heavy metals in soils are a result of human activity. Heavy metals in soils can be from the net effects of geological and soil forming processing of these elements (Kabata-Pendas and Adriano, 1995). The concentration of heavy metals in the soil is largely determined by the parental material. Sandy soils from granite rocks, normally contain lower concentrations of heavy metals than clay soils (Ross, 1994). The irrigated area around the Sand River is mostly loam soil.

Manganese had the second highest concentration in soils irrigated with Sand River water (Table 6.3). Just like iron, manganese plays a significant role in a number of metabolic processes including the formation of chlorophyll (Huang et al., 2019). There was more manganese in the Sand River irrigated soils than the control and borehole water (Table 6.3). This again suggests that the major source of manganese in the soils may be sewage effluent.

Zinc is one of the micronutrients that is required by tomato plants for enzyme activation of oxidative metabolic processes. The zinc levels in soils irrigated with Sand River water were 23.80 mg/kg (Table 6.3). The maximum permissible limit of zinc in soils in South Africa is 46 mg/kg (WRC, 1997). The zinc levels are within the permissible limits. Excess uptake of zinc can lead to reduction in plant growth as it may inhibit some metabolic functions (Prasad et al., 1999; Lewis et al., 2001; Romero-Puertas et al., 2004). Soils irrigated with borehole water and tap water had more or less similar zinc concentrations (Table 6.3). The sewage effluent also appears to be contributing to the zinc levels in the soils.

The copper concentration in soils irrigated with Sand River was 13.80 mg/kg (Table 6.3). Copper is also considered as an essential micronutrient in plants because of the significant role it plays in number of enzymatic processes in plants and is key in the formation of chlorophyll, although required in small quantities (Rai et al., 2016). Excess copper, however, can inhibit a number of metabolic functions (Ebbs and Kochian, 1997). The maximum permissible limit of copper in soils is 6.6 mg/kg (WRC, 1997). The copper levels in soils irrigated with Sand River water exceeds the permissible limit. This again indicates that sewage effluent may be a significant source of copper because the concentration of the control was 4.2 mg/kg.

The lead levels in soils irrigated with Sand River water were 9.2 mg/kg (Table 6.3). Lead is a plant toxin and its uptake by plants affects the morphology of the plant and metabolic processes (Sharma and Dubey, 2005). The maximum permissible levels of lead in soils is 6.6 mg/kg (WRC, 1997). However, this level was exceeded in soils irrigated with Sand River water. The control (1.6 mg/kg) and borehole treatment (0.03 mg/kg) soils had much lower levels of lead in comparison to soils irrigated with Sand River water. This further indicates that sewage effluent may be a significant source of lead.

Cadmium is also a well-known plant toxin, whose uptake can have adverse effects on plant growth because it will interfere with biochemical processes (Sharma and Dubey, 2005). The cadmium concentration in soils irrigated with Sand River water was 0.73 mg/kg (Table 6.3). WRC (1997) suggested permissible limit for cadmium in soils is 2.0 mg/kg. Thus, cadmium fell within the acceptable limits. Soils irrigated with borehole water and control had lower level of cadmium than soils irrigated with Sand River water (Table 6.3). Sewage effluent again appears to be the source of cadmium.

6.4.2.2 Soils under onions

In soils where onions were planted and irrigated with Sand River water, the heavy metal concentrations followed the order Fe>Zn>Mn>Pb>Cu>Cd (Table 6.3). This closely mirrors the order under tomatoes. The physiological effects of these metals are to a large extent the same in tomatoes and onions. The concentration of the heavy metals in soils under tomatoes and onions are of the same magnitude. The sensitivity of onions and tomatoes was not investigated in this study. It is thus recommended that experiments be carried out to investigate the sensitivity of onions and tomatoes to the target heavy metals. No studies have been done in South Africa on this aspect.

Table 6.3: Heavy metal (mg/kg) analysis of soils under tomatoes and onions irrigated with Sand River and Borehole Water. Values are presented as means ± standard deviation.

Sand River water	Tomatoes	Onions	Control
Zn	23.80±4.6	30.60 ±7.8	16.2 ±1.3
Cu	13.80 ±5.4	10.60 ±5.6	4.2 ±0.8
Fe	96.90± 16.9	106 ±20.9	74.2 ±9.8
Mn	18.60± 3.3	26.8± 8.9	10.3 ±2.9
Pb	9.2± 0.9	10.8± 1.2	1.6 ±0.7
Cd	0.73± 0.60	0.82 ±0.4	0.5± 0.2
Borehole Water			
Zn	15.4 ±2.4	18.9 ±3.2	
Cu	3.2± 0.6	2.6 ±1.6	
Fe	10.5 ±2.4	15.8± 2.5	
Mn	12.8 ±1.8	18.0 ±1.9	
Pb	0.03-	0.03	
Cd	0.01-	0.01	

6.4.2.3 Nutrient composition of experimental soils

The nutrient composition of soils under the vegetables (tomatoes and onions) irrigated with sewage effluent was compared to soils irrigated with borehole water. The pH of soils irrigated with sewage effluent was slightly lower than that irrigated with borehole water (Table 6.4). The target pH range on South Africa is 6.5-8.5 (DWAF, 1996; WRC, 1997). Soil pH plays an important role in the bioavailability of heavy metals to plants. An increase in soil pH results in alkaline soils and in such instances the heavy metals become tightly bound to the soils (Prasad, 2004). However, when pH becomes more acidic, all these metals bound to soils can become immediately available and the metals may become toxic to the plants. Soil pH from both borehole water (pH 7.8) and sewage effluent (pH 7.5) met the target range. Nitrogen, potassium and phosphorus were higher in soils irrigated with sewage effluent than borehole water (Table 6.4). The build-up of nitrogen and phosphorus in sewage effluent irrigated soils is the reason why farmers use sewage effluent since they act as a fertilizer. It is however important to note that borehole water had fairly high levels of nitrogen, phosphorus and potassium and this may be due to the lithology of the area.

Table 6.4: Nutrient composition of soils, electrical conductivity and pH irrigated with Sand River and Borehole Water. Values are presented as means ± standard deviation.

	Sand River water	Borehole water
pH	7.5	7.8
Electrical conductivity dS/m	1.3 ±0.3	0.6± 0.1
Nitrogen (mg/kg)	70.4 ±6.4	78.0 ±3.9
Phosphorus (mg/kg)	32.0 ±14.8	30.1± 2.8
Potassium (mg/kg)	102 ±15.6	39.4 ±2.9

6.4.3 Heavy metal contamination of tomatoes and onions

6.4.3.1 Heavy metals in tomatoes

The mean zinc concentration of tomatoes irrigated with sewage effluent was 30.2 mg/kg and 19.2 mg/kg in tomatoes irrigated with borehole water (Table 6.5). According to FAO/WHO (1984, 1989), the safe limit for zinc is 99.4 mg/kg. Thus, the concentration of zinc in these tomatoes complied with the international standards. It must be pointed out that zinc is an essential element required by the body. However, excessive amounts can cause gastrointestinal complications (Gupta et al., 2018). Copper concentration in tomatoes irrigated with sewage effluent was 3.2 mg/kg and 1.6 mg/kg for tomatoes irrigated with borehole water (Table 6.5). Permissible standards from FAO/WHO (1984, 1989) is 73.3 mg/kg. Copper is an essential element in human physiology because it is involved in a number of enzymatic reactions and hair pigmentation (Fink et al., 2018). However, excess amounts can lead to malfunctioning of the kidneys and anaemia. Iron concentration in tomatoes irrigated with sewage water was 25.8 mg/kg and 20.2 mg/kg for tomatoes irrigated with borehole water (Table 6.5). The permissible limit for iron is 20 mg/kg (FAO/WHO, 1984). Thus, iron was above the permissible limits. Iron is essential in the synthesis of haemoglobin. Slight deficiencies of iron can cause anaemia. However, excess iron can also cause autism and allergies in children (Padhye, 2003). Accumulation of iron in the brain and liver can be fatal. It is possible that iron was easily absorbed by the root of the plants and translocated to the tomatoes. Gebrekidan et al. (2013) indicated that iron had the highest transfer factors among other trace metals. However, plant transfer factor (PTF) at any particular time is influenced by a number environmental conditions including pH and redox potential. The concentration of a heavy metal in the soil is a major factor in determining the concentration of the same metal in the plant. Iron levels were high in the soils and this explain the high levels of iron in tomatoes.

Manganese concentration was 23.5 mg/kg in tomatoes irrigated with sewage water and 19.6 mg/kg in tomatoes irrigated with borehole water (Table 6.5). The maximum permissible limit for manganese is 500 mg/kg (FAO/WHO, 1982, 1989). Manganese is an essential antioxidant in the human body but excessive uptake of manganese can cause complications in the nervous system (FAO/WHO, 2011). The manganese levels were way below the maximum permissible level. This contradicts Gupta et al. (2018) who found high manganese levels in fruits around Durban, South Africa. One of the major sources of manganese is automobile industry (Mirecki et al., 2015). In the area where the experiment was carried out, there was very low traffic volume and this may explain the low manganese levels in the fruit. Lead concentration in tomatoes irrigated with sewage water was 1.32 mg/kg and 0.04 mg/kg in tomatoes irrigated with borehole water (Table 6.5). This suggests that the source of lead is sewage effluent. The safe limit of lead in food is 0.3 mg/kg (FAO/WHO, 1982, 1989). The lead levels in tomatoes irrigated with sewage effluent exceeded the safe limits. High concentrations of lead can lead to lead poisoning which is characterized by renal failure, cardiovascular diseases and neurotoxicity (FAO/WHO, 2011). It must be noted that the lead levels in the soils also exceeded permissible limits. Cadmium concentrations were 0.11 mg/kg in tomatoes irrigated with sewage effluent and 0.05 mg/kg in tomatoes irrigated with borehole water (Table 6.5). The permissible limit for cadmium is 0.007 mg/kg (FAO/WHO, 2011). The cadmium levels were above acceptable levels in both sewage and borehole water irrigated tomatoes. Although, in tomatoes irrigated with borehole water the levels were marginally above. Cadmium toxicity is associated with kidney complication and may also lead to systolic high blood pressure.

6.4.3.2 Heavy metals in onions

The zinc concentration in onions both irrigated with sewage effluent and borehole water were within permissible limits (Table 6.5). However, zinc concentration was higher in onions than in tomatoes (Table 6.5). Copper concentration for both sewage effluent and borehole water irrigated onions were also within acceptable limits although the copper concentrations were higher in onions than tomatoes. Iron levels were above permissible limits for both onions irrigated with sewage effluent and borehole water. The concentration of iron was also higher in onions than tomatoes (Table 6.5). Manganese levels were within permissible levels in both sewage water and borehole irrigated onions. The manganese levels were also higher in onions than tomatoes. The lead levels were also above permissible limits in onions irrigated with sewage effluent water but within acceptable limits for onions irrigated with borehole water. Lead levels were marginally higher in onions than in tomatoes (Table 6.5). Cadmium levels were above permissible limits for both sewage effluent and borehole irrigated onions. It is thus clear that all the heavy metals were higher in onions than tomatoes. This suggests that the root system of onions is more efficient at taking up heavy metals than the tomato root system. It is also important to note that the onion bulb is in the ground which is the source of the heavy metals.

Table 6.5: Heavy metal (mg/kg) concentration in tomatoes and onions irrigated with Sand River and Borehole Water. Values are presented as means ± standard deviation.

	Tomatoes	Onions
Sand River water		
Zn	30.2± 6.8	42.3± 8.9
Cu	3.2± 1.2	8.2 ±4.3
Fe	25.8± 3.4	42.9± 6.8
Mn	23.5± 3.0	30.3 ±9.3
Pb	1.32± 1.6	1.79± 0.08
Cd	0.11 ±0.1	0.14 ±0.03
Borehole Water		
Zn	19.2± 1.4	30.6± 6.4
Cu	1.6 ±0.5	3.2± 1.3
Fe	20.2± 2.8	32.8 ±7.1
Mn	19.6± 3.8	22.3± 6.9
Pb	0.04	0.08
Cd	0.05	0.06

6.4.3.3 Plant growth and fruit quality

Tomatoes irrigated with Sand River water had higher vegetative growth (plant height and leaf numbers) than those irrigated with borehole water (Table 6.6; Figure 6.1; Figure 6.2). Fruit number was also significantly higher in the Sand River treatment than the borehole water treatment. Sand River water has high nitrogen, phosphorus and potassium levels. The mineral content is also higher in Sand River water than borehole water. A number of studies have also shown that tomatoes irrigated with sewage effluent show better vegetative growth (Al-Lahham et al., 2003; Bakari et al., 2019) The fruit quality as determined using firmness showed that Sand River irrigated tomatoes were of better quality with a score on 3.3 compared to borehole water irrigated tomatoes with a score of 2.8 (Table 6.6).

Table 6.6: Tomato plant height, fruit and leaf number under different irrigation treatments. Values are presented as means ± standard deviation

	Sand River water	Borehole water
No. of fruits	10.09±3.79	7.73±4.26
No. of leaves	22.26±7.89	19.59±8.71
Plant height (cm)	73.04±11.61	63.04±11.50
Fruit quality score	3.3	2.8

Figure 6.1 Tomatoes irrigated with Sand River water

Figure 6.2: Tomatoes irrigated with borehole water

The nutritional value of tomatoes irrigated with sewage effluent is not different from that irrigated with borehole water (Table 6.7). The dietary fibre, carbohydrates, protein, fat, and ash were within the same magnitude, although, tomatoes irrigated with sewage effluent had marginally higher values (Table 6.7).

The proximate composition of onions irrigated with sewage effluent and borehole water were within the same order of magnitude (Table 6.7). This indicates that for both onions and tomatoes sewage effluent does not affect the nutritional value of these vegetables.

Table 6.7: Proximate nutritional composition (g/100 g) of tomatoes and onions irrigated with Sand River and Borehole Water

	Tomatoes	Onions
Sand River water		
Dietary fibre	53.9	3.82
Carbohydrates	14.4	16.6
Protein	18.9	8.6
Fat	11.7	1.2
Ash	3.69	4.2
Borehole Water		
Dietary fibre	50.7	3.20
Carbohydrates	10.2	15.8
Protein	16.8	7.8
Fat	14.2	1.5
Ash	3.2	4.0

6.4.3.4 Health Risk Assessment

The hazard quotient was less than 1 in zinc, copper, iron, manganese and cadmium in tomatoes irrigated with sewage effluent (Table 6.8). However, the HQ of lead was more than 1 and this indicates that there are potential risks to people consuming tomatoes. One of the reasons for the high lead HQ is the low reference dose that was ascribed to lead. It is clear from Table 6.8 that the reference dose for lead is several magnitudes below all the other heavy metals. The HQ for tomatoes irrigated with borehole water also followed the same trend (Table 6.8). This suggests that the source of lead may not only be sewage effluent but might also be from other sources such as vehicle emissions, industrial air pollutants and incineration of waste material. It is thus possible that lead that is air borne might have been absorbed by the leaves thus entering the plant through foliage. The role played by leaves in absorption of heavy metals is only getting attention in recent years, but a number of studies have shown that foliar absorption of heavy metals can lead to contamination of the plant (Shahid et al., 2017).

The HQ in onions irrigated with sewage effluent was also less than 1 for all heavy metals except lead (Table 6.9). This is again attributed to the low reference dose ascribed to lead. The HQ in onions irrigated with borehole water followed the same trend and onions irrigated with sewage effluent (Table 6.9). It may be necessary for pollution abatement measures to be implemented that would reduce the amount of lead in the sewage effluent. Such measures can include phytoremediation.

Table 6.8: Hazard quotient for tomatoes assuming 140 g of tomatoes are consumed per day by a 60 kg person

Tomatoes	Zn	Cu	Fe	Mn	Pb	Cd
Sand River Water						
Metal concentration (mg/kg)	23.80	13.80	96.90	18.60	9.2	0.73
Average daily dose (µg/kg)	70.47	7.47	58.33	54.83	3.08	0.26
Reference dose (µg/kg)	300	40	700	140	0.057	3
Hazard quotient	0.2349	0.1868	0.0833	0.3916	54.035	0.0867
Borehole Water						
Metal concentration (mg/kg)	15.4	3.2	10.5	12.8	0.03	0.01
Average daily dose (µg/kg)	44.8	3.73	47.13	45.73	0.09	0.17
Reference dose (µg/kg)	300	40	700	140	0.057	3
Hazard quotient	0.1493	0.0933	0.0673	0.3266	1.5789	0.0567

Table 6.9: Hazard quotient for onions assuming a 70 g of onions are consumed per day by a 60 kg person

Onions	Zn	Cu	Fe	Mn	Pb	Cd
Sand River Water						
Metal concentration (mg/kg)	30.60	10.60	106.0	26.8	10.8	0.82
Average daily dose (µg/kg)	49.35	9.57	50.05	35.35	2.09	0.16
Reference dose (µg/kg)	300	40	700	140	0.057	3
Hazard quotient	0.1645	0.2393	0.0715	0.2525	36.6667	0.0533
Borehole Water						
Metal concentration (mg/kg)	18.9	2.6	15.8	18.0	0.03	0.01
Average daily dose (µg/kg)	35.7	3.73	38.27	26.02	0.09	0.07
Reference dose (µg/kg)	300	40	700	140	0.057	3
Hazard quotient	0.1190	0.0933	0.0547	0.1869	1.5789	0.0233

6.4.4 The effect of microbial contamination on tomatoes and onions

Total heterotrophic bacteria, total coliforms, faecal coliforms and *E. coli* were not detectible inside the tomatoes irrigated with Sand River and borehole water (Table 6.10). Total coliforms, faecal coliforms and *E. coli* were also not detected on the outside of tomatoes irrigated with both Sand River and borehole water. However, total heterotrophic bacteria were detected outside the tomatoes irrigated with both Sand River and borehole water (Table 6.10). The contamination of tomatoes with pathogenic bacteria is a distinct possibility when sewage effluent is used to irrigate crops. There are some studies that have been done to assess the microbiological quality of fresh produce harvested from sewage effluent irrigated areas (Gemmell and Schimdt, 2012; Jongman and Korsten, 2016). Gemmell and Schmidt (2012) indicated that *E. coli* contaminated fresh produce were prevalent in a sub-urban area in South Africa. The faecal counts exceeded WHO and South African limits for consumption of raw produce. In recent years, the sewage effluent quality discharged in South African rivers has deteriorated (Moyo and Rapatsa, 2019). It is thus important to rigorously monitor microbial contamination of fresh produce harvested from sewage effluent irrigated plots. The results from this study show that the inside of the tomato was not contaminated with any microbes. This is consistent with previous studies where drip irrigation method was used (Wen and Li, 2015). Drip irrigation is preferred over furrow or sprinkler irrigation because the risk of contamination is reduced. Drip irrigation minimizes the risk of contact between the plant and wastewater.

In the onions irrigated with Sand River and borehole water, no heterotrophic bacteria, coliforms and *E. coli* were detected inside the onions (Table 6.11). However, for both borehole water and sewage effluent, heterotrophic bacteria and coliforms were detected outside the onions. Significantly no faecal coliforms and *E. coli* were detected outside the onions (Table 6.11). Onions are known to have anti-microbial properties against some pathogenic bacteria and this may explain absence of *E. coli* on the inside of onions. Organo-sulphur compounds are the ones responsible for the antimicrobial properties that are found in onions (Martinez et al., 2007).

Table 6.10: The presence (+) or absence (-) of microbes on the outside and inside of tomatoes irrigated with Sand River and borehole water.

Sample type	Source of water	Total heterotrophic bacteria	Total Coliforms	Faecal coliforms	*E. coli*
Inside	Borehole	-	-	-	-
	Sand River	-	-	-	-
Outside	Borehole	+	-	-	-
	Sand River	+	-	-	-

Table 6.11: The presence (+) or absence (-) of microbes on the outside and inside of onions irrigated with Sand River and borehole water.

Sample type	Source of water	Total heterotrophic bacteria	Total Coliforms	Faecal coliforms	*E. coli*
Inside	Borehole	-	-	-	-
	Sand River	-	-	-	-
Outside	Borehole	+	+	-	-
	Sand River	+	+	-	-

CHAPTER 7: CONCLUSION AND RECOMMENDATIONS

The phosphorus levels in the Sand River exceeded the recommended limits for irrigation use. This is attributed to the discharge of poorly treated sewage effluent into the Sand River. However, phosphorus levels declined downstream of the sewage treatment works. The abstraction point used by a large commercial tomato producing company is appropriate because the water from that site has high nutrient levels that will not be deleterious to crops. Most of the heavy metals fell within the target range for irrigation except for lead. An analysis of the heavy metals in the sediments indicated that sediments were not contaminated with any of the heavy metals. Seasonal variations were not detected during the rainy and dry seasons. This is attributed to intermittent discharge of poorly treated sewage effluent. The Sand River water has extraordinarily high pathogenic bacterial loads and the water did not meet the target range for use in irrigation. Although no pathogen detection or quantification was done, the levels of faecal coliforms and *E. coli* (hygiene indicator) indicated that the water did not meet the target range for use in irrigation. Assessment of the suitability of Sand River and borehole water for irrigation was determined through SAR and SSP. Both these indices fell within the target range for irrigation water. The risk assessment study showed that lead was potentially hazardous to people consuming both tomatoes and onions irrigated with Sand River water.

It is recommended that local farmers monitor Sand River water because of the intermittent discharge of very poor quality effluent that takes place. It is further recommended that pre-treatment of the water be undertaken before it is used for irrigation. This can involve use of settlement ponds. Vegetable consumers must with time insist of quality assurance of tomatoes and onions from suppliers. This will protect consumers from eating vegetables contaminated with heavy metals and pathogenic microbes.

The problems identified in this report can be rectified if further research is done to reduce the pollution levels of the Sand River. It is thus recommended that phytoremediation studies be undertaken in the Sand River.

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Appendix

Capacity building

Ms VK Ramokone did her honours project in 2017 under the auspices of this project and looked at heavy metal contamination in the Sand River. She successfully completed her project within the stipulated time. Her study assessed heavy metals contamination in sediments and bank soil from the Sand River. Four sites were chosen. Sites 1 and 2 were upstream of the river before the Polokwane Wastewater Treatment Works and sites 3 and 4 were downstream. The metal concentrations were analysed using an inductively-coupled plasma optical emission spectrometer (ICP-OES) after nitric acid digestion. Sediments contamination was determined using the pollution index. The study found that physico-chemical parameters were within recommended standards for sustaining aquatic organisms and for domestic use. The values for turbidity were 5.06 mg/ℓ (± 1.91), 1.54 mg/ℓ (1.80), 15.25 mg/ℓ (26.52) and 44.75 mg/ℓ (27.93) for sites 1, 2, 3 and 4, respectively, which was more than the recommended value of 1 mg/ℓ. The pollution index showed that the sediments and bank soil were not contaminated.

Ms L Mogoboya also did her honours project in 2017 under the auspices of this project. Her study was undertaken because pollution of water resources is a major risk to human health and water quality throughout the world. The purpose of the study was to determine the microbiological contamination along the Sand River. Bacterial contamination was monitored using the membrane filtration technique. Results showed that there was spatial variation of the bacterial counts along the river. Bacterial contamination significantly increased after discharge of sewage effluent, exceeding recommended international and national guidelines for both irrigation and domestic use.

Ms Eva Nephale enrolled for an MSc by research and submitted her dissertation in March 2020. Ms Nephale worked on the Sand River and the Blood River. Her major findings indicated that a situation analysis of the nutrient status showed that physico-chemical parameters were elevated after discharge of sewage effluent from the Polokwane and Seshego Sewage Treatment Works. She used the water quality index to assess the suitability of Sand and Blood River water for irrigation and reported that the Sand River was highly polluted at the point of confluence between the Sand and Blood Rivers. Thus the water at that site is not suitable for irrigation. Further downstream, the quality of the water improved and could be used to irrigate crops. Ms Nephale also looked at the potential of using bio-indicators to monitor pollution in these rivers. She concluded that enzymes particularly acetyl cholinesterase can be useful bio-indicators in the Sand and Blood rivers.

Community engagement

This Water Research Commission sponsored project started in March, 2017. A workshop was organized on the 26th of July, 2018 to present the preliminary findings to stake holders based on the nutrient status, microbial contamination and heavy metal contamination of the Sand River. Letters of invitations to the following main stakeholders were sent out:

1. ZZ2
2. Polokwane municipality
3. Department of Agriculture
4. South Africa Agricultural Union
5. SAB
6. Nature conservation
7. Department of health
8. University of Limpopo, research office
9. Individual farmers downstream of the Sand River

All the stakeholders accepted the invitations but some of them did not pitch up for the meeting. A total of 11 people attended the workshop.

Professor Moyo presented the preliminary findings and there were extensive discussions after his presentation. The following issues emerged from the discussions:

- The quality of the water has deteriorated to unacceptable levels in the last few months.
- The stakeholders expressed concern that the Polokwane municipality did not send a representative since the issue of water quality of the Sand River is the domain of the Polokwane municipality.
- The stakeholders also expressed concern about the lack of monitoring of the effluent that is being directed to the Mogalakwena platinum mine.
- The stakeholders indicated that the Blood River is more contaminated than the Sand River because the Seshego sewage treatment plant is dysfunctional. The Blood River feeds into the Sand River.
- The stakeholders also indicated that the Polokwane municipality stated that they will build a new sewage treatment plant but this has materialized in the last 4 years.
- The farmers indicated that the water quality was not affecting their crops and soils.
- ZZ2 farmers indicated that they do not use Sand River water for irrigation, they only use it to moisten the soil before planting.
- The farmers indicated that they use drip irrigation and not spray irrigation

The stakeholders suggested that the Polokwane Municipality be represented at the next stakeholders meeting and this could be done through the Limpopo Research Forum. The stakeholders also suggested that trees around the Sand River be cut to increase the river flow. The stakeholders also suggested that the water being diverted to the mine must be monitored.

In conclusion, the stakeholders appreciated the interaction and indicated that they look forward to the next workshop.

Frame 1. Workshop proceedings

Frame 2: Workshop proceedings

Publications

The following paper has been published:

Moyo, N.A.G. and Rapatsa, M.M. 2019. Trace metal contamination and risk assessment of the Sand River, Limpopo Province South Africa. *Bulletin of Environmental Contamination and Toxicology* 102: 492-497

Two other papers are envisaged.