EFFECTS OF TREATED WASTEWATER ON SELECTED SOIL NUTRIENTS AND BIOLOGICAL PROPERTIES

BY

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DECLARATION

I, Zaphania Mmadichaba Kganyago, hereby declare that this mini-dissertation submitted to the University of Limpopo for the degree, Master of Science in Soil Science, and has not been submitted previously by me or any other for the degree at this or any other University. This is my work in design and all material therein has been duly acknowledged.

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DEDICATION

I dedicate this work to my late father, Samson M., my late mother Asnath M. Kganyago, my grandmother Elizabeth M. Mackhoyasi, and my daughter Demetra R. Mabala.

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ABSTRACT

Water scarcity poses significant risks to global food security. The use of treated wastewater for irrigation could be a sustainable remedy for water scarcity in arid to semi-arid regions. Furthermore, it has been the most readily available source of water which can serve as an adaptation strategy to shortage of irrigation water. The objectives of the study were to determine (1) whether different disposal points following wastewater treatment could have effects on the quality of treated wastewater used for irrigation at the University of Limpopo (UL) experimental farm and (2) the response of selected critical nutrients, microbial and enzyme activities on soils irrigated with treated wastewater at the UL Experimental Farm. Water samples were collected monthly at three disposal points, namely, the exit point of treated wastewater from the treatment plant (Pond 16), the entry point into the night-dam and the exit point from the night-dam at the UL Experimental Farm. The water samples were analysed for pH, electrical conductivity (EC), sodium (Na), nitrate (NO_3^-), phosphate (PO_4^{2-}), sulphate (SO_4^{2-}), salinity, magnesium (Mg), calcium (Ca), potassium (K), chlorine (Cl), total dissolved solids (TDS), total soluble salts (TSS), lead (Pb), copper (Cu), cadmium (Cd), zinc (Zn), *Ascaris lumbricoides, Escherichia coli*, *Shigella* spp., *Salmonella* spp. and *Vibrio cholera*. A field experiment was conducted on a separate 4-ha virgin field (VF), cultivated field (CF) and fallowed field (FF), with soil samples collected from 0-5, 5-15 and 15-30 cm soil depth in each field and analysed for pH, EC, mineralisable P, NH_4^+, NO_3^-, organic carbon (OC), active carbon (AC), potential mineralized nitrogen (PMN), fluorescein diacetate (FDA) and phosphatase (PTS) enzyme activity. All data were subjected to ANOVA using Statistix 10.1. The treated wastewater had, at the three sampling points, significantly different EC, Na, NO_3^-, PO_4^{2-}, SO_4^{2-}, Cu, Zn, *Shigella* spp., *V. cholerae*, *A. lumbricoides* and *E. coli,* whereas salinity, pH, Mg, Ca, K, Cl and Cd were not affected by the sampling point. Generally, the night-dam entry and exit points had significant increases in PO_4^{2-}, K and Ca when compared to Pond 16 exit. In contrast, the flow of wastewater from Pond 16 through the furrow to the entry and exit of the night-dam resulted in decreases of Na, NO_3^- and Cl. The exit point of water from the night-dam at UL Experimental Farm had the least harmful materials, rendering it the safest point with the best water quality for irrigation. In the field study, NH_4^+, NO_3^- and PMN were significantly different in soil depth. However, NO_3^- field × depth interaction effects were not significantly different. In contrast to all soil parameters, FDA and PTS activity for both soil depth and field × depth interaction effects were highly significantly different. The EC and pH were not affected by soil depth or field type. The negative effects of treated wastewater were mainly observed in the cultivated field. In conclusion, treated wastewater with fallowing could be used as the best approach to overcome water shortages, with the uses having the potential to reduce the need to apply high synthetic chemical fertilisers.

Keywords: water reuse, disposal points, essential nutrients, microbial activities

CHAPTER 1

INTRODUCTION

1.1 Background

Water scarcity has been reported as a global problem with adverse effects on irrigated agriculture, thereby enhancing food insecurity and slow economic growth (Grau *et al*., 2017). Limpopo Province in the northern parts of South Africa is a water scarce region that has been experiencing drought once in every four years (DWAF, 2016). A state of drought was proclaimed by the Limpopo provincial government in 2015 and 2016, consecutively (Nyambeni, 2015). Water scarcity is described as water deficit resulting from higher demand than supply (FAO, 2015), which could lead to high competition for fresh water for different uses. In most cases, water for agricultural uses is deemed to be low priority (DWAF, 2016).

According to DWAF (2013), South Africa is located in arid and semi-arid zones, thereby necessitating the use of different alternatives to manage the water discrepancies in irrigated agriculture (FAO, 2010). One of the alternatives being considered for agriculture is the reuse of wastewater for irrigation (Durán–Álvarez and Jiménez–Cisneros, 2014; Gatica and Cytryn, 2013). Reuse of wastewater for irrigation had been commonly used in other countries such as Mexico, Brazil and South Korea (Durán–Álvarez and Jiménez–Cisneros, 2014). The practice was recently assessed for potential use in irrigation in the semi-arid areas of Limpopo Province (Kgopa *et al*., 2017). In light of the negative effects of treated wastewater such as containing pathogens, high concentration of salts and heavy metals (Durán–Álvarez and Jiménez–Cisneros, 2014; Jaramillo and Restrepo, 2017; Jiménez, 2006; Shakir *et al*., 2017), proper research was viewed as being necessary to ascertain the suitability of treated wastewater for irrigation.

In Limpopo Province, a large number of mines were established during the 21st century, resulting in an increase in demand for water as the population grew due to migration in search of employment (NDA, 2001; Rankoana, 2016). Water usage allocations for agriculture were reduced as demand shifted towards domestic consumption and industrial use (Kepe, 1999). In addition, there has been a steady decrease in rainfall received in the province over the last couple of years, a phenomenon attributed to climate change (Maluleke and Mokwena, 2017; Raneesh, 2014).

The use of treated wastewater has its own downturns especially if water quality is poor (Khan *et al*., 2011a). Inefficient water treatment systems result in discharge of poor quality water, which introduces undesirable elements into the soil (Mzini, 2013). A number of studies have reported adverse chemical effects following irrigation with wastewater irrigation (Abedi-Koupai *et al*., 2006; AL-Hamaiedeh and Bino, 2010; Asano, 1998; Disciglio *et al*., 2015; Friedel *et al*., 2000; Mekki *et al*., 2015; Romanos, 2016). Irrigation with wastewater could lead to increased alkalinity overtime with pH values increasing to over 8. Treated wastewater can also inhibit the release of exchangeable cations from organic matter during mineralisation (Woomer *et al*., 1994). Treated wastewater might contain excessive levels of heavy metals that could be toxic to plants, humans and other animals (Begum *et al*., 2014). Soil quality, as a function of the physical, chemical and biological properties, could be negatively affected under irrigation with treated wastewater. In the short-term, soil microbial activities could also be mostly affected as they are vulnerable to soil alteration, such as changes in pH facilitated by addition of cations and anions. Heavy metals could interfere with nutrient forms and could make it impossible for microorganisms to optimally perform activities such as transformation of organic phosphorus as well as being suppressive to other microorganisms (Chen *et al*., 2003; Stotzky and Norman, 1961).

Treated wastewater studies are necessary on land under different management practice and sampling depth in order to investigate which cannot tolerate the irrigation practice being employed (Shalinee and Ademola, 2014).

1.2 Problem statement

Irrigation with treated wastewater could serve as an alternative source to ameliorate the shortage of irrigation water. However, preliminary studies have shown that treated wastewater can increase levels of N, increase toxicity of heavy metals and chlorine and add *Escherichia coli* (Kgopa *et al*., 2017). Deposition of heavy metal frequently into the soil could lead to complex reactions especially with organic matter, thereby inhibiting the activity of soil microorganisms and soil fauna. The solubility and mobility of heavy metals were shown to have direct effects on soil biological components, since the method of binding heavy metals and their bioavailability could depend on several properties such as organic matter (Violante *et al*., 2010). Continuous deposition of chlorine might result in chlorinated hydrocarbons, which could interfere with microbial activities by limiting their energy source as well as inhibiting microbial growth, thereby binding the surface soil particles. Therefore, resulting in low enzymatic activities, which could affect the productivity of the soil. Detailed studies could be indispensable whenever treated wastewater was envisaged for use in agriculture.

1.3 Rationale of the study

Water scarcity poses significant risks to global food security and the use of treated wastewater for irrigation could be a remedy for water scarcity in arid to semi-arid regions. However, inefficient treatment plants could result in low quality water, which could pose threats to the natural ecosystem of the soil. There was therefore need to combat deterioration of natural ecosystems that could be caused by irrigation with treated wastewater. The current study sought to promote the use of treated wastewater and thereby providing amelioration strategies on negative effects that could be caused by treated wastewater on soil health properties, with the view of increasing crop productivity.

1.4 Purpose of the study

1.4.1 Aim

The aim of the study was the provision of information regarding the potential use of treated wastewater for irrigation under a semi-arid region in Limpopo Province, South Africa.

1.4.2 Objectives

1. To determine the quality of treated wastewater in the three post treatment disposal points at Mankweng Wastewater Treatment Plant and University of Limpopo Experimental Farm.
2. To investigate the response of selected critical nutrients, microbial and enzyme activities on soils irrigated with treated wastewater under different cultural practices.

1.4.3 Hypothesis

1. Treated wastewater in the three post treatment disposal points at Mankweng Wastewater Treatment Plant and University of Limpopo Experimental Farm will be of low quality.
2. The response of selected critical nutrients, microbial and enzyme activities on soils irrigated with treated wastewater will vary under different cultural practices.

1.5 Reliability, validity and objectivity

Reliability was ensured by the use of statistical levels of significance as derived with analysis of variance and multivariate analysis; validity was achieved through repeating the activities in time for Objectives 1 and in space for Objectives 2. Objectivity was achieved by ensuring that the findings are discussed on the basis of empirical evidence, as shown in the statistical analysis, in order to eliminate all forms of subjectivity (Leedy and Omrad, 2005).

1.6 Bias

Bias was minimised by ensuring that the experimental error in each experiment was reduced through replications in all objectives, and by the use of grid and systemic soil sampling in objectives 2.

1.7 Scientific contribution of the study

The problems associated with shortage of water could be addressed if wastewater can be depurated thoroughly. The study assessed the quality of the water and the findings of this study imposed ameliorating strategies on how the quality of the water could be maintained or should range in order to secure soil fertility, quality and productivity.

1.8 Structure of the mini-dissertation

The mini-dissertation consists of five chapters, where Chapter 1 outlines the description and the details of the research problem, Chapter 2 reviews the work done and work not yet done on the problem statement, Chapter 3 and 4 were derived from the work done to achieve Objective 1 and Objective 2, respectively, and Chapter 5 provided the summary of the findings, the significance of the findings, recommendations for future research and the conclusions drawn from the study. The mini-dissertation followed the Harvard referencing style of author alphabet in text and reference list as approved by the University of Limpopo Senate.

CHAPTER 2

LITERATURE REVIEW

2.1 Work done on the research problem

2.1.1 Quality of treated wastewater

Wastewater is any form of water whose quality has been adversely affected by human activities and originates from a mixture of industrial, domestic, commercial or agricultural activities, surface runoff (Tilley *et al*., 2011). About 80% of untreated wastewater around the world is being discharged into the environment, causing extensive water pollution (WWAP, 2017). Reuse of treated wastewater for irrigation improved the livelihoods of many farmers in arid- and semi-arid regions (Scott *et al*., 2004). The high amounts of nutrients in treated wastewater can positively benefit farmers and the environment by reducing the amount of chemical fertilisers being applied (Joeng *et al*., 2016).

Irrigation with treated wastewater could enhance the structure of the soil and increase the soil microbial activities due to organic matter within the irrigation water (Durán–Álvarez and Jiménez–Cisneros, 2014). However, treated wastewater might contain pathogens, which may result in outbreaks of diseases in consumers and salts that might have adverse effects on soil structure, soil aggregate, stability and permeability (Mateo-Sagasta *et al*., 2013). The salts might also affect growth and yield of crops since they could increase the osmotic potential of the soil, thereby resulting in an increase in the energy required by crops to take up water from the soil (Durán–Álvarez and Jiménez–Cisneros, 2014).

Wastewater treatment for irrigation

Wastewater treatment is the process of removing sludge from raw wastewater into treated wastewater that could eventually be discharged into the environment with minimal environmental disturbances (WWAP, 2017). The treatment is done in wastewater treatment plants, where combinations of physical, chemical and biological treatment processes are employed. The physical treatment uses physical processes such as filtration where large contaminants are trapped and only water pass through or sedimentation tanks, which allow the suspended particles to settle at the bottom as water flows slowly through the tank, thereby providing some degree of purification (SWBNO, 2013). Chemical treatment uses chemical reactions to treat disinfect the water. Biological treatment processes use biological matter and bacteria to break down waste matter (Gupta *et al.*, 2016), with the processes having the potential to alter the chemical status in different ways. The latter affect and/or modify soil water quality as levels of chlorine used during purification could contaminate treated wastewater and could also be harmful to effective soil microbes.

Treated wastewater disposal points are of great importance since water resources has constantly been stressed. There is a need to increase concern and protect diminishing water resources and maintenance of ecosystem health (USEPA, 2004). Initially, a dilution effect was used where wastewater was discharged directly into natural waterways. However, due to increased production of both domestic and industrial waste, the dilution effect could not ameliorate wastewater quality thus escalating pollution of water resource (Okoh *et al*., 2010). This resulted in an increased need for the introduction of disposal points that would aid purification process prior to discharge.

Sodium Adsorption Ratio

Sodium adsorption ratio (SAR) is an irrigation water quality parameter used in the management of sodium-affected soils. The SAR is the ratio of sodium to calcium and magnesium and it had been used as an indicator of the suitability of water for use in agricultural irrigation (Reeve *et al.*, 1954). Sodium end up being high in treated wastewater since it originate from household products such as detergents (DWAF, 1996). In addition, water treatment chemicals, such as sodium fluoride, sodium bicarbonate and sodium hypochlorite, together result in sodium levels as high as 30 mg/litre.

Generally, in order to remove sodium compounds in treated wastewater in the form of sodium chloride, reverse osmosis is usually applied (Abdul *et al*., 2010). Reverse osmosis system forces water through a semi-permeable membrane, with salts being left behind as the water passes through since the former are denser than water (Mouhanni *et al*., 2011). High SAR cause dispersion of clay aggregates in the soil, with sodium ions facilitating the dispersion of clay particles, whereas calcium and magnesium could promote the flocculation of clay particles (Reeve *et al.*, 1954). However, flocculation influences the soil structure and affect the permeability of the soil, directly reducing the water infiltration rate. Sodium in irrigation water could displace the calcium and magnesium in the soil, thus causing a decrease in the ability of the soil to form stable aggregates and a loss of soil structure and tilth (Belaid *etal*., 2012a).

Total dissolved and suspended solids

Total dissolved solids (TDS) is a measure of the combined content of all inorganic and organic substances contained in a liquid in molecular, ionized or micro-granular suspended form. Generally, the operational definition is that the solids must be small enough to survive filtration through a filter with two-micrometre (nominal size or smaller) pores (Shrivastava and Mishra, 2014). However, total suspended solids (TSS) cannot pass through a sieve of two micrometres and yet are indefinitely suspended in solution (Abdul *et al*., 2010; EPA, 1999).

Total dissolved solids found in water are due to various sources. Natural occurrence of total dissolved solids arises from the weathering and dissolution of rocks and soils, from run-off rainwater, leaves, silt, or plankton (Wright, 2003). Chemicals from pesticides, road salts, and/or fertilisers can also be dissolved in water and contaminate different bodies of water, including irrigation effluents (Belaid *et al*., 2012b; Michaud, 1994). The most common chemical constituents of TDS are calcium, phosphates, nitrates, sodium, potassium and chlorine (Yeang and Woo, 2010).

Treatment plants use microfiltration to remove suspended solids and ion exchange demineralisation, which uses beds of cationic and anionic resin in a pressure vessel. High TSS can block light from reaching submerged vegetation (EPA, 2001). As the amount of light passing through the water is reduced, photosynthesis slows down. If light is completely blocked from bottom dwelling plants, the plants will stop producing oxygen and will die. High TSS can also cause an increase in surface water temperature, because the suspended particles absorb heat from sunlight and create hostile environments for crop to absorb nutrients and provoke specific ion toxicity

(EPA, 1999). Seanego and Moyo (2013) reported suspended solids as high as 59.98 mg/l due to overloading of the treatment plant. Suspended solids concentration of >25 mg/l is regarded to be high, according to standards by DWAF (2013).

Water pH

pH is a measure of the acidity or basicity of the water. pH is the negative logarithm (base 10) of the activity of hydronium ions (H^+ or H_3O^+aq) in a solution (Joeng *et al*., 2016). Water that has more free hydrogen ions is acidic, whereas water that has more free hydroxyl ions is basic (Buol *et al*., 2002). There are many factors that can affect pH in water, both natural and man-made. Most natural changes occur due to interactions with surrounding rock particularly carbonate forms and other materials (Hakanson, 2005). Carbonate materials and limestone can buffer pH changes in water, calcium carbonate ($CaCO_3$) and other bicarbonates can combine with both hydrogen or hydroxyl ions to neutralize pH (McNally and Mehta, 2004).

Anthropogenic causes of pH fluctuations are usually related to pollution. Point source pollution is a common cause that can increase or decrease pH depending on the chemicals involved. Sodium, urea, nitrogen and oxides could come from agricultural runoff, wastewater discharge, and industrial runoff or mining operations (McNally and Mehta, 2004). Wastewater discharge that contains detergents and soap-based products could cause a water source to become too basic (Griffiths *et al*., 2006).

Water with low pH values could affect the mobility of heavy metals in the soil and can be absorbed by crops and contaminate water bodies (Joeng *et al*., 2016). Soil pH specifically affects plant nutrient availability by controlling the chemical forms of the different nutrients and influencing the chemical reactions they undergo. Sudden change in pH either acidic or alkaline can cause a variety of stresses to plants, such as nutrient deficiencies (Hansson *et al.*, 2011). Acidic conditions could result in root growth inhibition, lateral roots and root tips thickening due to excess aluminium, because roots would be damaged and nutrient uptake reduced. pH is also known to interfere with enzyme activities (Rout *et al.*, 2001).

Physical and chemical parameters

Different countries have different protocols for maintaining the quality of wastewater in order to minimise contamination and increase agricultural production. The UN water quality standards advice that total solids should range from 350 – 700 mg/l (Table 2.1). Wastewater mainly comprises of 99.9% water and extremely low concentrations of suspended and dissolved organic and inorganic solids. The organic substances present in wastewater are carbohydrates, lignin, fats, soaps, synthetic detergents, proteins and their decomposition products, as well as various natural and synthetic organic chemicals from the process industries. Water is used as a solvent and carrier of the solids. Table 2.1 shows the levels of most common parameters in domestic wastewater and the threshold values of their concentration. The table below (Table 2.1) outlines that levels above the medium on each constituent could limit their significance in agricultural use (Uleimat, 2012).

Table 2.1: Wastewater protocols of the United Nations (United Nations Department of Technical Cooperation for Development, 1985).

Constituents	Concentration (mg/l)		
	Strong	Medium	Weak
Total solids	1200	700	350
Dissolved Solids (TDS)	850	500	250
Suspended solids	350	200	100
Nitrogen	85	40	20
Phosphorus	20	10	6
Chloride	100	50	30
Alkalinity	300	200	100

Biological parameters

The quantity of water in treatment plants are estimated based on community population size (EPA, 2001), the higher the population the more likely to have high contaminants since the plants would pass the design capacity and supply poor quality water (Alguacil *et al*., 2012; Ayers and Westcot, 1994). In agriculture, the use of wastewater for irrigation has raised concerned with the contamination by pathogenic micro and macro-organisms. Table 2.2 outlines the possibilities of pathogenic viruses, bacteria, protozoa and helminths that could be present in wastewater. Feachem *et al.* (1983) reported that the higher the amount of organisms the longer period they would survive in the environment.

Table 2.2: Allowed limits of pathogens in wastewater (Feachem *et al.*, 1983).

Type of pathogen		cfu/l in municipal wastewater
Viruses:	*Enteroviruses*	5000
Bacteria:	*Salmonella* spp.	7000
	Shigella spp.	7000
	Vibrio cholera	1000
Protozoa:	*Entamoeba histolytica*	4500
Helminths:	*Ascaris Lumbricoides*	600
	Hookworms	32
	Schistosoma mansoni	1
	Taenia saginata	10
	Trichuris trichiura	120

The Department of Water Affairs and Forestry (2016), outlined the regulatory compliance towards the quality of treated wastewater, that the treated wastewater should be analysed for *Escherichia coli* (*E. coli*), faecal coliforms, chemical oxygen demand (COD), ammonia, nitrate, ortho-phosphate, pH, suspended solids and electrical conductivity (EC) with the limits shown by Table 2.3 below. Inefficient treatment systems result in poor quality treated wastewater due to on point pollution. Furthermore, Seanego and Moyo (2013) reported that the quality of treated wastewater was negatively affected by the continued increase in population, which influence the overloading of the Polokwane treatment plant.

Table 2.3: Regulatory standards of treated wastewater by DWAF in South Africa.

Parameters	Regulatory limits
Escherichia coli (cfu/100ml)	1000
Faecal coliforms (cfu/100ml)	1000
Chemical oxygen demand (mg/l)	75
Ammonia (mg/l)	6
Nitrate (mg/l)	15
ortho-phosphate (mg/l)	10
pH	5.5 – 9.5
Suspended solids (mg/l)	25
Electrical conductivity (mS/m)	150

Treated wastewater could contain high loads of nutrients, salts, microorganisms, heavy metals and other pollutants as a result of poor maintenance in treatment plant (Friedel *et al*., 2000; Mekki *et al*., 2015). Treated wastewater was considered a deleterious practice since it facilitated pollution by resistant pathogenic agents (bacteria, protozoa, viruses and helminths) which would survive in the environment for a longer period (Juan and Blanca, 2008). Several studies had reported large populations of Total coliforms (TC) in treated urban wastewater (Romanos, 2016), while other researchers had also detected pathogens of genera *Salmonella*, *Streptococci*, *Clostridium*, *Shigella* spp. and *Vibrio* spp. (Abdul *et al*., 2010; Hagedorn, 2001; Jiao *et al*., 2010). Jiménez-Cisneros (2002) reported that helminths and pathogenic bacteria contaminated soils irrigated with wastewater.

2.1.2 Some critical nutrients, microbial and enzyme activities following irrigation with treated wastewater

There is not much research on the effect of wastewater irrigation on soil nitrogen organisms. Yasser *et al*. (2013) observed an increase in the activity of nitrifying bacteria accompanied by a low rate of denitrification in forest soils irrigated with wastewater. Nitrite oxidizing bacteria (NOB) and denitrifying bacteria (DB) decreased in soil irrigated with treated wastewater (Shang *et al.*, 2007) while soil bacteria such as ammonia oxidizing bacteria (AOB) and aerobic cellulose decomposing bacteria (CDB) increased with irrigation. The quantity of these bacteria were highly influenced by the pollutants concentration of sewage, effects of chemical degradation or microbial decomposition on pollutants and the other chemical or biological components in soils might lead to this trend (Xu *et al.*, 2012).

Xu *et al*. (2012) reported a decrease of nitrogen-fixing bacteria population with long - term wastewater irrigation. The quantity of bacteria was positively correlated with soil total nitrogen, ammonia nitrogen and organic substances. In addition, previous studies have proven the relationship between soil nutrition and the quantity of microorganisms (Shang *et al.*, 2007; Xu *et al.*, 2012; Yasser *et al*., 2013; Zhang *et al*., 2007). Han *et al*. (2006) emphasised that treated wastewater added phosphorus into the soil which increased the content of available phosphorus and decrease the quantity of AOB. Ammonium oxidizing bacteria plays an important role in the soil since it can mineralize organic nitrogen into ammonium which can be absorbed and utilized by plants and microorganisms. Elifantz *et al*. (2011) observed an increase in NO_3^- concentration than NH_4^+ as a result of nitrification potential activity influenced by higher soil temperatures.

Soil phosphorus

The concentration of soil phosphorus in the majority of studies had significantly increased as a result of irrigation with treated wastewater (Belaid *et al*., 2012b; Galavi *et al*., 2010; Kaboosi, 2017; Khaskhoussy *et al*., 2013; Mañas *et al*., 2009; Yassin *et al*., 2017). Al-Jaboobi *et al*. (2014) observed the same results. Furthermore, it was stated that the concentration of P increased significantly in treatments of treated wastewater than other treatments (Kordlaghari *et al*., 2013; Mohammed and Sidduraiah, 2016). However, Heidarpour *et al*. (2007), observed a significant negative effect on phosphorus as a result of irrigation with treated wastewater.

Soil organic carbon

Gao *et al*. (2015) and Guy *et al*. (2011) observed a significant increase in organic C as an increase in microbial diversity resulted from high loads of organic matter deposition to the soil by the treated wastewater. However, Heinze *et al*. (2014) reported a tendency of lower organic C content in soil irrigated with treated wastewater than fresh water, this trend was influenced by soil texture than irrigation water quality. Irrigation with treated wastewater showed no substantial effects on microbial biomass C on the top few millimetres of the soil, this is not only influenced by the input of organic matter (OM), nutrients, salts and heavy metals but also by harsh environmental conditions with extreme temperatures and soil type (Heinze *et al*., 2014). In soil with average clay content, microbial biomass C was the highest and lowest in the coarse soil. However, Guy *et al*. (2011) and Gao *et al*. (2015) observed an increase in microbial biomass C suggesting that they are sensitive to soil organic C changes.

Active carbon

Soil active carbon was defined as the active fraction of soil organic carbon which breaks down relatively quickly during microbial growth and it changes greatly after disturbance and management (Zoua *et al*., 2005). There is scarcity of studies done on active carbon following irrigation with treated wastewater (Li *et al*., 2015). Apps (1987) stated that an increase in the concentration of active carbon could be attributed to the increased organic matter additions from residues, active and diverse forage, crop, or cover crop growth carried within the treated wastewater. Similar trends of increase active carbon in soils irrigated with treated wastewater were observed by Liang *et al*. (2014), Belaid *et al*. (2012a) and Jogan *et al*. (2017), which were attributed to changes in soil organic carbon caused by irrigation management.

Potential mineralisable nitrogen

The concentration of potential mineralisable nitrogen (PMN) is the fraction of organic nitrogen easily decomposable and mineralisable by soil microorganisms (Kayikcioglu, 2012). Hoang *et al*. (2016) and Cordovil *et al*. (2007) indicated that the increase in the concentration of potential mineralisable nitrogen is a contribution of the soil organic matter of which is considered as a prime source of PMN which is continuously deposited through irrigation with treated wastewater.

However, AL-Jaboobi *et al*. (2014) reported a decrease in the concentration of PMN as a result of high heavy metals in soils limiting the availability of organic compounds.

Furthermore, Ghaemi *et al*. (2014) stated similar results of decreased PMN as attributed to decreased microbial activity for organic nitrogen breakdown and the decomposition rate of organic nitrogen as affected by heavy metal availability within the irrigation water. Gao *et al*. (2015) also stated that irrigation with treated wastewater decreased the concentration of potential mineralized nitrogen in comparison with river water irrigation.

<u>Enzyme activities</u>

Conflicting reports concerning the effect of treated wastewater on microbial activities has been observed. Tabari *et al*. (2011) reported that treated wastewater has no effect on soil quality and microbial activities. However, several enzyme activities (urease, protease and phosphatase) were adversely affected by irrigation with treated wastewater. Atashgahi *et al*. (2015) observed a decrease in microbial diversity and a negative effect on the cycling of nitrogen as a result of irrigation with treated wastewater.

Enzymes in soils catalyse the biogeochemical cycling of C, N, P, and S, and their activities reveal the degree of chemical and biological reactions in the soil (Ma *et al*., 2015). Soil enzyme activities could be affected by many factors, such as treated wastewater, soil characteristics, plant cover and irrigation practices (Wang *et al*., 2016). In addition, the higher organic C levels might provide a favourable environment for the accumulation of enzymes in the surface soil (Elifantz *et al*., 2011), since soil organic matter is considered important in forming stable complexes with free enzymes (Heinze *et al*., 2014). However, supplementary enrichment could inhibit soil enzyme activities, especially if the accumulation lead to the increase of water-soluble and exchangeable fractions of metals (Chen *et al*., 2008).

Gao *et al*. (2015), observed an increase in phosphatase activity in the top soil following irrigation with treated wastewater. The observed increased activities were positively correlated with increased organic C content and long-term accumulation of organic matter and nutrients (Carr *et al*., 2010). In contrast, Heinze *et al*. (2014) observed a decrease in phosphatase as a result of heavy metals accumulation, microbial biomass and enzyme activities decrease with an increase in heavy metal accumulation in soil. Heavy metals were reported to adversely affect enzyme activity by interacting with the enzyme substrate complex, denaturing the enzyme protein or interacting with its active groups (Chen *et al*., 2008). The fluorescein diacetate hydrolysis activity (FDA) increased during the irrigation season and was significantly higher in soils irrigated with treated wastewater as compared to those irrigated with freshwater (Elifantz *et al*., 2011). In contrast, the hydrolytic activity and nitrification potential showed distinctive seasonal patterns. FDA activity was usually higher in the summer and lower during winter, showing that FDA enzymes require high temperatures. The variation of these soil microbiological parameters might suggest that they were affected by seasonal trends in climate, crop system and agricultural practices (Gao *et al*., 2015).

There was a reported increase in urease activity of 44.9% and 92.3%, when both wastewater and treated wastewater were used for irrigation, respectively (Wang *et al.*, 2016). The increase was attributed to increased inputs of microbial biomass into the soil by irrigation water and a positive correlation with total N, NO_3^- and total P in the soil. Chen *et al*. (2008) observed the same trend of increase in N cycling enzyme activity as a result of higher quantity of biodegradable organic matter and nutrients. Accumulation of organic C influence high microbial activity especially C cycling enzyme activity. However, Ma *et al*. (2015) reported a decrease in urease activity as affected by an increase in soil pH and toxic levels of Cr and Pb.

2.2 Work not yet done on the problem statement

Less work has been done on different disposal points and post treatment points in the quality of wastewater (Skosana, 2015). Teklehaimanot *et al*. (2014) only observed the quality of treated wastewater on three seasons. Different disposal points comprise different loads of organisms and compounds in the treated wastewater. Furthermore, the response of microbial activities to cultivation and irrigation with treated wastewater in semi-arid areas of Limpopo Province has not been studied.

CHAPTER 3

WASTEWATER QUALITY FOR IRRIGATION AS AFFECTED BY DISPOSAL POINTS

3.1 Introduction

Water quality is a measure of numerous factors such as the amount of salts and suspended material, bacteria levels, concentration of dissolved oxygen, microscopic algae and quantities of pesticides, herbicides and heavy metals (EPA, 2001). Wastewater quality varies spatially and temporally, depending on seasonal and climatic changes and with the types of soils, rocks and surfaces through which, it moves. Generally, water quality is also affected by human activities such as agricultural activities, urban and industrial development, mining and recreation (DWAF, 2016). Deteriorating water quality usually leads to decreased agricultural yields due to increased salinity and toxic heavy metals of irrigation water.

Wastewater post treatment at Mankweng wastewater treatment plant (MWTP) effluent travels by canal for approximately 2.9 km to a receiving night-dam at University of Limpopo Experimental Farm (ULEF). The water is then stored in the dam prior to release for irrigation at the UL fields. This chapter focused on the quality of treated wastewater at MWTP and the receiving dam at the ULEF, at three different disposal points. Therefore, the objective of this chapter was to determine the quality of treated wastewater in the three post treatment disposal points at MWTP and ULEF.

3.2 Materials and methods

3.2.1 Description of the study site

The study was conducted at the Mankweng Wastewater Treatment Plant (23°51'23.61"S; 29°42'27.24"E) and at the University of Limpopo Experimental Farm (23°50'42.86"S; 29°42'44.35"E). The MWTP and the ULEF are adjacent to each other (Fig 3.1) and located west of Mankweng area of Capricorn district, Limpopo Province, South Africa. The climate of the study site is classified as semi-arid with an annual precipitation of approximately 405 to 500 mm received mostly in the summer months of October to March (Weather SA, 2017). The mean annual temperature of 25^{o}C (max) and 10^{o}C (min). The study site averages 170 frost-free days extending from late October to mid-April. The MWTP obtain their water from Mankweng hospital, filling stations, surrounding settlements, University of Limpopo, two shopping centers, run-off and agricultural fields.

3.2.2 Treatments and research design

The study comprised of three treatments being the disposal points: 1 – pond 16 exit, which is the final stage of water that leaves the MWTP; 2 – night-dam entry, which is the point where water enters the dam at ULEF and 3 – exit, where water leaves the dam at ULEF and released to the irrigation site (Figure 3.1). The samples were collected once a month in triplicates per disposal point.

3.2.3 Water sampling for chemical properties

Treated wastewater samples were collected in 1000 ml sterilized sampling bottles at 10 cm depth of the sampling point once per month. For heavy metals, samples were preserved by adding 2 ml concentrated nitric acid, stored in a cooler box to minimise further chemical reactions and transported to University of Limpopo Soil Science laboratory where they were immediately analysed.

3.2.4 Water sampling for biological properties

Treated wastewater samples were collected in 1000 ml sterilized sampling bottles at 10 cm depth from the sampling point once monthly, transported to University of Limpopo Microbiology laboratory where they were immediately analysed.

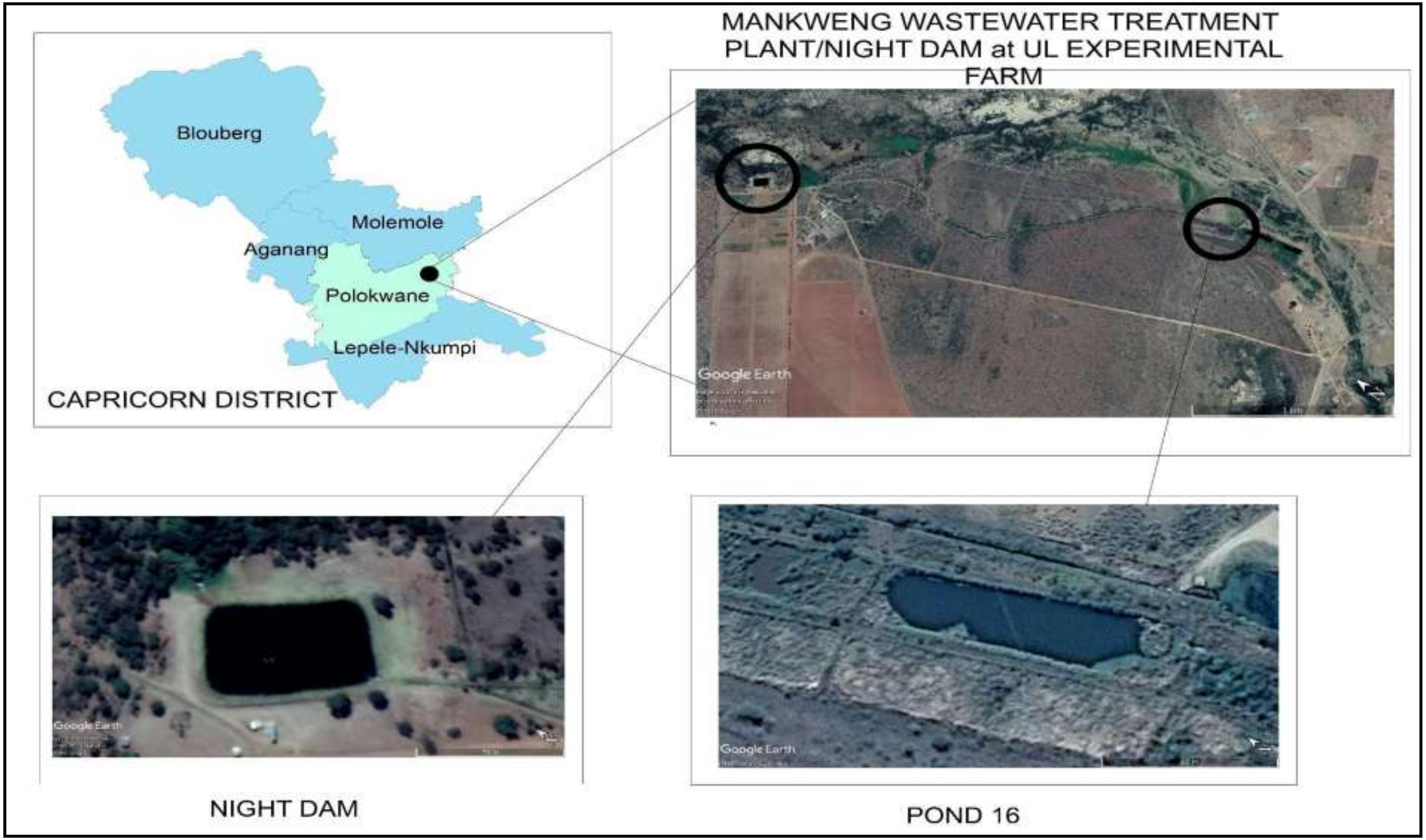

Figure 3.1: Pathway from pond 16 of MWTP to ULEF Night dam along a 2.9 km canal.

3.2.5 Data collection

Physico-chemical parameters

Prior to analysis, pH and electrical conductivity (EC) were determined using the electrode method. Total dissolved solids (TDS) were determined by slowly evaporating 100 ml of the filtrate from total suspended solids (TSS) in a pre-weighed beaker and after the sample dried, reweighed the beaker (Howard, 1933). For the determination of nitrates, a volume of 50 ml of the sample was pipetted into a porcelain dish and evaporated to dryness on a hot water bath, 2 ml of phenol disulphonic acid was added and concentrated solution of sodium hydroxide and distilled water was added while stirring to make it alkaline. Filtered into a test tube and made up to 50 ml with distilled water and the absorbance was read at 410 nm using a UV/Visible spectrophotometer after colour development (Armstrong, 1963).

The determination of phosphates, was done following the procedure outlined by Sletten and Bach (1961). In summary, 4 ml of ammonium molybdate reagent and 5 drops of stannous chloride reagent were added to the filtered sample of 50 ml. The absorbance was read at 690 nm using a UV/Visible spectrophotometer after colour development. For sulphates, 100 ml of filtered sample was transferred into a test tube containing 5 ml of conditioning reagent, and 0.2 g of barium chloride crystals was added with continued stirring for 2 minutes (Manivanan, 2006). For the determination of chlorides, 50 ml of a filtered sample was taken in a conical flask, to which 0.5 ml of potassium chromate indicator was added and titrated against standard silver nitrate till silver dichromate ($AgCrO_4$) started precipitating (Sawyer *et al.*, 2000).

Determinations of cations

Water samples were kept at less than 4°C prior to analysis and pre-treated using ultrapure HNO_3 for 16 hours to reduce pH to less than 2 and then samples subjected to ICP-OES analysis of Na (Hesse, 1971). For the determination of potassium and sodium, samples were analysed using a Jenway PFP7 flame photometer (Junsomboon and Jakmunee, 2011).

For determination of magnesium and calcium 250 ml of deionized water was added to five dry beakers each and using a pipette, 0.0, 0.5, 1.0, 1.5 and 2.0 ml of calcium and magnesium stock solutions were added to the beakers, respectively and mixed thoroughly. The concentrations of calcium and magnesium in the standards were calculated and the flame atomic spectrophotometer was set and the full set of standards and unknown samples were measured (Ramakrishna *et al*., 1966). Sodium absorption ratio was calculated through the ratio of Na^+ to Ca^{2+} and Mg^{2+}, following the equation:

$$SAR = \frac{Na^+}{\sqrt{\frac{1}{2}(Ca^{2+} + Mg^{2+})}} \qquad (1)$$

Heavy metals

Prior to sampling, 1000 ml polypropylene containers were filled with a diluted hydrochloric acid and then rinsed several times with water collected form the sampling site (Hesse, 1971). Containers were kept at less than 4°C prior to analysis. Water samples were pre-treated using ultrapure HNO_3 for 16 hours to reduce pH to less than 2 and then samples subjected to ICP-OES analysis of Pb, Cu, Cd, Cr and Zn (USEPA, 1996).

Biological parameters

Culturing methods were used for isolation of *Salmonella* spp. and *Shigella* spp. through Salmonella shigella (SS) agar*, Vibrio cholera* and *Escherichia coli* through thiosulfate citrate bile salts sucrose (TCBS) agar as shown by Figure 3.2. *Ascaris lumbricoides* was determined using methods described by Feachem *et al.* (1983) at CSIR Pretoria, where three batches of five water samples (500 ml) each were seeded with 1, 5, or 10 *A. lumbricoides* eggs (five replicates per sample).

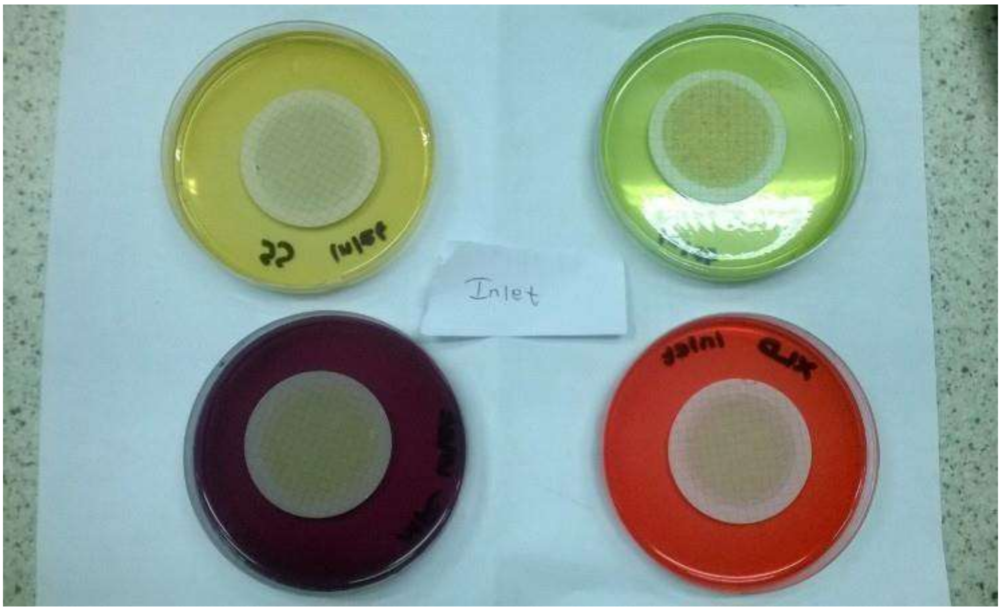

Figure 3.2: Isolation of biological parameter using respective media agars.

3.2.6 Data analysis

Microbial variables were log transformed by $\log_{X+D}$ prior to analysis, to homogenise the whole numbers and no outliers were found. The results of water analyses were subjected to analyses of variance (ANOVA) as applicable to a completely randomized design. The statistical analysis was performed for each parameter, and the treatment means were compared using the Least Significant Difference (LSD) test at 5%

confidence interval for different treatments. All statistical analyses were carried out using the SAS program, Version 10.0.

3.3 Results

3.3.1 Exchangeable cations

Treated wastewater disposal points had highly significant ($P \leq 0.01$) effects on Na, Ca and K. However, the concentration of Mg among the three disposal points was not significantly different (Table 3.1). Relative to pond 16, the quantity of Na at inlet and outlet decreased by 36 and 56%, respectively whilst, Ca decreased by 40 and 60%, respectively. Similarly the concentration of K decreased by 23 and 42%, respectively (Table 3.2). The results are pooled means of the samples collected across the year.

Table 3.1: Partitioning of variation for Na, Ca, Mg and K at different disposal points.

Source	DF	Na		Ca		Mg		K	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Treatment	2	753.52	98***	94.91	99***	0.80	44ns	25.65	33***
Error	12	16.25	2	1.66	1	1.01	56	50.96	67
Total	14	769.78	100	96.58	100	1.81	100	76.61	100

TTV (%) = Total treatment variable = (MSS/Total) × 100

*** Highly significant at $P \leq 0.01$, ns Not significant at $P \leq 0.05$.

Table 3.2: Distribution of major soluble and exchangeable cations (mg/l) of treated wastewater effluent at different disposal points.

Sampling points	Na	R.I. (%)	Ca	R.I. (%)	K	R.I. (%)
Pond 16	43.30^{a}	–	14.28^{a}	–	10.76^{a}	–
Inlet	27.78^{b}	–36	8.58^{b}	–40	8.24^{b}	–23
Outlet	19.06^{c}	–56	5.72^{c}	–60	6.24^{c}	–42

$^{a,\ b\ \text{or}\ c}$ Treatment means followed by the same letter were not significant ($P \le 0.05$) according to Fisher's least significant difference

Relative impact [R.I. (%)] = [(Point/Pond 16) – 1] × 100.

3.3.2 Physicochemical properties

Treatments had no significant effect on pH, EC and TDS (Table 3.3). However, the treatments had highly significant effect ($P \le 0.01$) on SAR contributing 96% in TTV (Table 3.3). Relative to pond 16; the quantity of SAR at inlet and outlet decreased by 31 and 60%, respectively (Table 3.4).

Table 3.3: Partitioning of variation for pH, EC, TDS and SAR at different disposal points.

		pH		EC		TDS		SAR	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Treatment	2	1.18	21^{ns}	2880.2	5^{ns}	26255	4^{ns}	139.94	96^{***}
Error	12	4.48	80	50549.4	95	659610	96	5.506	4
Total	14	5.66	100	53429.6	100	685865	100	145.44	100

TTV (%) = Total treatment variable = (MSS/Total) × 100

*** Highly significant at $P \leq 0.01$, ^{ns} Not significant at $P \leq 0.05$.

Table 3.4: Distribution of physicochemical properties of treated wastewater effluent at different disposal points.

Sampling points	SAR	R.I. (%)
Pond 16	18.80[a]	–
Inlet	12.98[b]	–31
Outlet	7.60[c]	–60
Standard limits	10	

[a, b or c] Treatment means followed by the same letter were not significant ($P \leq 0.05$) according to Fisher's least significant difference

Relative impact [R.I. (%)] = [(Point/Pond 16) – 1] × 100.

3.3.3 Anions

Treatments had highly significant effect ($P \leq 0.01$) on CO_3^-, HCO_3^-, NO_3^- and SO_4^{2-}, contributing 82, 99, 99 and 96% in TTV, respectively (Table 3.5). However, treatments had no significant effect on PO_4^{2-} and Cl^- (Table 3.5). Relative to pond 16, the quantity of CO_3^- at inlet and outlet decreased by 100 and 78%, respectively. Relative to pond 16, the quantity of HCO_3^- and SO_4^{2-} increased, where the quantity of HCO_3^- at inlet and outlet increased by 900 and 5%, respectively (Table 3.6), the quantity of SO_4^{2-} at inlet and outlet increased by 217 and 137%, respectively. In contrast, the quantity of NO_3^- was fluctuating with a 27% decrease at the inlet and a 24% increase at the outlet (Table 3.6).

Table 3.5: Partitioning of variation for CO_3^-, HCO_3^-, NO_3^-, SO_4^{2-}, PO_4^{2-} and Cl^- at different disposal points.

		CO_3^-		HCO_3^-		NO_3^-		SO_4^{2-}		PO_4^{2-}		Cl^-	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Treatment	2	44670	82^{***}	17435.8	99^{***}	145862	99^{***}	700.47	96^{***}	0.29	31^{ns}	110.69	57^{ns}
Error	12	10000	18	170.5	1	1047	1	32.23	4	0.64	69	84.18	43
Total	14	54670	100	17606.3	100	146909	100	732.7	100	0.93	100	194.88	100

TTV (%) = Total treatment variable = (MSS/Total) × 100

*** Highly significant at $P \leq 0.01$, ^{ns} Not significant at $P \leq 0.05$.

Table 3.6: Distribution of anions (mg/l) of treated wastewater effluent at different disposal points.

Sampling points	CO_3^-	R.I. (%)	HCO_3^-	R.I. (%)	NO_3^-	R.I. (%)	SO_4^{2-}	R.I. (%)
Pond 16	0.018[a]	–	11.40[b]	–	261.80[b]	–	10.80[c]	–
Inlet	0[b]	–100	113.98[a]	900	190.80[c]	–27	34.20[a]	217
Outlet	0.004[b]	–78	12.00[b]	5	323.80[a]	24	25.60[b]	137
Standard limits	5		10		10 – 18		20	

[a, b or c] Treatment means followed by the same letter were not significant ($P \leq 0.05$) according to Fisher's least significant difference

Relative impact [R.I. (%)] = [(Point/Pond 16) – 1] × 100.

3.3.4 Heavy metals

Treatments were highly significant ($P \leq 0.01$) on Pb, Cu and Zn, contributing 80, 92 and 97% in TTV, respectively. However, treatments were not significantly different on Cd (Table 3.7). Relative to pond 16; the quantity of Pb at inlet and outlet decreased by 37 and 42%, respectively (Table 3.8). Relative to pond 16, the quantity of Cu at inlet and outlet decreased by 25 and 50%, respectively. Relative to pond 16, the quantity of Zn at inlet and outlet decreased by 62 and 83%, respectively (Table 3.8).

Table 3.7: Partitioning of variation for Pb, Cu, Cd and Zn at different disposal points.

Source	DF	Pb		Cu		Cd		Zn	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Treatment	2	0.04	80^{***}	21.93	92^{***}	60670	81^{ns}	432.63	97^{***}
Error	12	0.01	20	1.83	8	14000	19	11.84	3
Total	14	0.05	100	23.76	100	74670	100	444.47	100

TTV (%) = Total treatment variable = (MSS/Total) × 100

*** Highly significant at $P \leq 0.01$, ^{ns} Not significant at $P \leq 0.05$.

Table 3.8: Distribution of heavy metals (mg/l) of treated wastewater effluent at different disposal points against regulatory standards limits.

Sampling points	Pb	R.I. (%)	Cu	R.I. (%)	Zn	R.I. (%)
Pond 16	7.60[a]	–	8.31[a]	–	21.5[a]	–
Inlet	4.80[b]	–37	6.26[b]	–25	8.17[b]	–62
Outlet	4.40[b]	–42	4.12[c]	–50	3.60[c]	–83
Standard limits	<5.0		<0.20		<2.0	

[a, b or c] Treatment means followed by the same letter were not significant ($P \leq 0.05$) according to Fisher's least significant difference

Relative impact [R.I. (%)] = [(Point/Pond 16) – 1] × 100.

3.3.5 Categorisation of water into the water suitability class

The water suitability class among all the post treatment disposal points were categorised under different classes. Among the disposal points pond 16 had the highest salinity and sodium than the other points. The water at pond 16 was categorised in C2–S3 class, which indicates medium salinity and high sodium, inlet fell in the category C2–S2 class, which indicated medium salinity and medium sodium whilst outlet was categorised in the class C1–S1 which indicated low salinity and low sodium (Table 3.9). The class are clearly outlined in Appendix 3.4.

Table 3.9: Water salinity class of treated wastewater effluent at different post treatment disposal points

Sampling points	Suitability class
Pond 16	C2–S3
Inlet	C2–S2
Outlet	C1–S1

3.3.6 Bacterial counts

Treatments were highly significant ($P \leq 0.01$) on *Shigella* spp, *E. coli*, *V. cholerae*, Faecal coliform and *A. lumbricoides*, contributing 92, 91, 95, 99 and 66% in TTV, respectively (Table 3.10). Relative to pond 16; the count of *Shigella* spp at inlet and outlet decreased by 8 and 38%, respectively. Relative to pond 16, the count of *E. coli* at inlet and outlet decreased by 2 and 6%, respectively (Table 3.11). Relative to pond 16, the count of *V. cholerae* at inlet and outlet decreased by 6 and 12%, respectively. Relative to pond 16, the count of faecal coliform at inlet and outlet decreased by 31 and 46%, respectively. Relative to pond 16, the count of *A. lumbricoides* at inlet and outlet decreased by 64 and 65%, respectively (Table 3.11).

Table 3.10: Partitioning of variation for *Shigella* spp., *Escherichia coli*, *Vibrio cholerae*, Faecal coliform and *Ascaris lumbricoides* at different disposal points.

		Shigella spp		*E. coli*		*V. cholerae*		Faecal coliform		*A. Lumbricoides*	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Treatment	2	1.66	92***	663087	91***	0.14	95***	2.46	99***	0.03	66***
Error	12	0.15	8	66777	9	0.01	5	0.02	1	0.01	34
Total	14	1.80	100	729864	100	0.15	100	2.48	100	0.04	100

TTV (%) = Total treatment variable = (MSS/Total) × 100

*** Highly significant at P ≤ 0.01, ^{ns} Not significant at P ≤ 0.05.

Table 3.11: Distribution of microbes at different disposal points.

Sampling points	*Shigella* spp	R.I. (%)	*E. coli*	R.I. (%)	*V. cholerae*	R.I. (%)	Faecal coliform	R.I. (%)	*A lumbricoides*	R.I. (%)
Pond 16	2.89^{a}	–	3.08^{a}	–	2.85^{a}	–	2.97^{a}	–	3.23^{a}	–
Inlet	2.66^{a}	–8	3.01^{ab}	–2	2.69^{b}	–6	2.04^{b}	–31	1.17^{b}	–64
Outlet	1.79^{b}	–38	2.89^{b}	–6	2.52^{c}	–12	1.60^{c}	–46	1.12^{b}	–65

$^{a,\ b\ \text{or}\ c}$ Treatment means followed by the same letter were not significant ($P \leq 0.05$) according to Fisher's least significant difference

Relative impact [R.I. (%)] = [(Point/Pond 16) – 1] × 100.

3.4 Discussion

3.4.1 Distribution of cations and sodium hazard of treated wastewater

The quantity of Na in pond 16 was 36 and 56% higher than that of inlet and outlet, respectively. This could have resulted from poor treatment at the plant. El-Nahhal *et al.* (2014) reported that high concentration of Na was influenced by the fact that a lot of surfactants, cosmetics, and soaps contain Na or K in their chemical structure. Furthermore, Ambily and Jisha (2012) elucidated that high Na in treated wastewater results from the transfer of Na into inorganic forms such as NaCl. Dakoure *et al.* (2013) reported sodium concentration above the standard limit by 40% due to the fact that frequently wastewater does not frequently comply with the standards of WHO and FAO for irrigation water. Gattaa *et al.* (2015) observed an increase in Na as a result of high deposition of ionic liquids from nearing commercials farms.

Shakir *et al*. (2017) reported high degree of restriction of Na concentration (261.75 mg/L) in treated wastewater use for irrigation. Furthermore, Al-Khashman *et al.* (2013) reported Na concentration of 94.76 mg/L in the summer season, which resulted from the evaporation of wastewater in ponds. However, the concentration of Na at the inlet and outlet are low in comparison to pond 16 and this could be attributed to dilution with rain water. Al-Khashman *et al.* (2013) and Almeida *et al.* (2015) indicated that low concentration of Na was mainly observed during the winter and summer seasons which showed the dilution of wastewater by rain water.

Among the three disposal points at the treatment, the concentration of Ca and K was significantly different, with pond 16 having the highest concentration of Ca and K (Table 3.2). Al-Khashman *et al.* (2013) reported Ca concentration as high as 140.5

mg/l, and the higher concentrations were observed in the wet season due to release of calcium from the sedimentary rocks were the water originated. However, Ines *et al.* (2016) observed that the concentration of almost all elements was higher in treated wastewater than in tap water, with the exception of Ca^{2+} as a result of minimum time for ion exchange softening process and settling in the treatment plant.

Pond 16 of the MTWP fell in the category C2–S3, which indicated medium salinity and high sodium, which was not ideal for irrigation (FSSA, 2007). High concentration of sodium in irrigation water is likely to influence sodicity and soil dispersion (Almeida *et al.* (2015). Night-dam receiving inlet was categorised in the class C2–S2, which indicated that the water had medium salinity and medium sodium which can be used for irrigation on most crops and night dam exit fell in the category C1–S1 class, which can be used for irrigation on almost all crops since they had low salinity and low sodium hazard (FSSA, 2007).

3.4.2 Distribution of physicochemical properties at different disposal points

The SAR of the pond 16 was high with the value of 18.80 and outlet with the lowest value of 7.6, as a result of high accumulation of Na which were above the stipulated standards for irrigation water quality. The reduction in the concentration of sodium could be attributed to the self-purification capacity of the dam. Self-purification is influenced by a lot of factors such as flow rate, distribution of vegetation across the dam, temperature (Seanego and Moyo, 2013). The SAR indicated that the water was not suitable for irrigation purposes because their values were above 3.0 and such values are likely to results in water infiltration problems by clogging the micro-macro pores in soils (DWAF, 2016). Abdul *et al.* (2010), observed high sodium concentrations in treated wastewater that varied from 123.60 to 221.0 mg/L, while Qian and Mecham (2004), reported 481% higher SAR than the recommended standard for irrigation water.

3.4.3 Distribution of anions of treated wastewater

The concentration of NO_3^- at the disposal points was 94% higher than the standards limits with the outlet having the highest concentration among the three points. This was attributed to on point pollution, whereby there was an external source of pollution directly to outlet. Morrison *et al.* (2001) observed NO_3^- values were both low in influent and in the effluent. The values ranged from 0.6 to 1.2 mg in the influent and effluent. The high values of nitrates in the treated wastewater might possibly be due to domestic waste and fertiliser run off. Shalinee and Ademola (2014) observed a higher concentration of NO_3^- in the effluent than in the influent as a result of low advanced methods of wastewater treatment or low conventional biological secondary treatment. Omar *et al.* (2013) reported high concentration of NO_3^- in treated wastewater as attributed to lack of prior ammonia removal by conventional secondary treatment processes.

The concentration of SO_4^{2-} in all the sampling points was below the standard limit set by WHO (limit of 250 mg/l) and there was no significant difference in SO_4^{2-} among the three sampling points as indicated by ANOVA. Hamid *et al.* (2013), observed SO_4^{2-} concentration of 272 mg/l which was within the standard limits as a result of low sulphur pollutants in the treatment plant.

3.4.4 Distribution of heavy metals at different disposal points

The amount of Zn, Cu and Pb in all sampling sites were above the standard limits in pond 16, with Zn as high as 91% over the standard limits. High accumulation of heavy metals could be attributed to industrial waste disposal (mainly batteries, filling stations, domestic waste). Lower pH favour availability, mobility and redistribution of heavy metals. Khaskhoussy *et al.* (2015) stated that availability of heavy metals especially Zn was increased at pH lower than 6.5. The pH of pond 16 was roughly 6.13 and the concentration of Zn was highest, which was very interesting since pH of 6.13 and 6.5 are within the same group class. Ramesh and Damodhram (2015) reported zinc as high as 7.22 mg/l, which was 58.4% above the permissible limits of irrigation water quality. Long *et al*. (2003) stated that high levels of zinc could be phytotoxic and cause plant death since it is involved in the process of chlorophyll synthesis. Furthermore, Abdul *et al*. (2016) reported that too much zinc could create a hostile environment for microorganisms. It could also cause nausea, stomach cramps, diarrhoea and headaches in human beings.

Analysis of variance indicated that the concentration of Cu in all the sampling sites was significantly different and was also above the standard limits set by FAO. Pond 16 had the highest concentration among all the sampling points and it was 98% above the standard limits. Khan *et al.* (2011b) observed high concentration of Cu, which was 4 times above the standard level in treated wastewater as a result of numerous industries in the study area. Ramesh and Damodhram (2015) reported that the concentration of Cu was above the permissible limits in all the sampling site and the concentration of Cu ranged from 0.41 to 2.98 mg/l. Hamid *et al*. (2013), observed low concentration of cations in the treatment plant but high concentration of Cu, as high as 72% above the recommended levels for irrigation water. Roy and Gupta (2016) reported high concentration of Cu in wastewater to exceed the FAO/WHO standards for irrigation water.

Analysis of variance indicated that there was a significant difference in the concentration of Pb between pond 16 and the other two sampling points, while there was no significant difference between inlet and outlet. The concentration of Pb at pond 16 sampling site was 35% above the recommended standards set by FAO for irrigation water. Ramesh and Damodhram (2015) reported maximum concentration of Pb to be above the permissible limits in all the sampling sites as a results of source in the water containing Pb such as batteries, paint and petroleum. The three categories of water samples were not suitable for irrigation because the high concentration of heavy metals (Saini *et al*., 2014). Saini *et al*. (2014) further revealed that the levels of heavy metals were higher than the permissible limits of Indian Standards for Irrigation water.

3.4.5 Distribution of microbial variables

Escherichia coli and faecal coliform count was abundant at pond 16 and decreased at night dam outlet. The levels of *E. coli* were observed to be more than the licenced requirements. Reduction in faecal coliform and *E. coli* at the outlet could be an indication of a decline in organic matter, which could have served as food for *E. coli*, as well as the presence of chlorine which inhibits microbial growth. However, *A. lumbricoides* were much accumulated at the night dam inlet, which implies that there was an external addition source of supply from either air or the surrounding. Makoni (2014) observed the value as high as 5836 cfu/100ml for faecal coliform in wastewater, which were 5 times above both WHO and national recommended standards for irrigation as a result of inefficiency of the ponds in removing bacteria. *E. coli* produces powerful toxin and could cause severe illnesses and food borne illnesses. Similarly, the study outlined values 3 times above the standard limits set by WHO which could be explained by ineffective treatment system.

The DWAF (2016) reported that compliance regarding monitoring microbiological and chemical properties was rated as unacceptable at Mankweng wastewater treatment plant since it was below 80%. Momba *et al.* (2006) indicated that the microbial quality of effluent discharged did not comply with the standard limits set by the South African authorities especially for *Salmonella* spp., *Shigella* spp., *Vibrio cholera* and coliphages. Furthermore, Giorgis *et al.* (2014) elucidated that the overall quality of effluents and the quality of the receiving bodies did not meet the standard limits set by South African regulatory limits for irrigation purposes.

3.5 Conclusion

The study indicated that the night dam outlet water was better than that of pond 16, as a result of water storage since it facilitated settling of materials. This study indicated that the treatment plant discharged high concentration of unwanted materials within the water and less time for settling solids. This was observable by the level of nitrogen, phosphorus and salts in the water that were above the stipulated standards. The level of *E. coli* was also high indicating that Pond 16 provided a suitable habitat for their survival and this indicated that Pond 16 water was not suitable for irrigation, the continued deposition of Pond 16 water at the night dam outlet would results in poor irrigation water at the outlet despite the self-purification capacity of the dam. The study proves that there was an external addition of pollutants to the night dam since there was a lot fluctuation in terms of the concentration within the three post treatment disposal points. Among the sampling points, Pond 16 was observed to have the highest concentration for most of the parameters as a result of inefficient systems. This could also be attributed to overflow or over capacitation of the treatment plant. However, outlet was observed to have acceptable concentration of the tested parameters and could not pose significant risk to the irrigation site. It is recommended that outlet water be used for irrigation purposes since the water quality of outlet complied with the permissible levels set for irrigation by WHO, FAO and the South African regulatory limits for irrigation.

CHAPTER 4

SELECTED SOIL ESSENTIAL ELEMENTS AND MICROBIAL ACTIVITIES IN FIELDS IRRIGATED WITH TREATED WASTEWATER

4.1 Introduction

The use of treated wastewater for irrigation is practised worldwide mainly in developing countries, due to of water shortages (Gatica and Cytryn, 2013). Treated wastewater has been proven to be beneficial since it could serve as a source of nutrients for crops (Xu *et al*., 2012). However, irrigation with treated wastewater could introduce unwanted material that interferes with the activities of microbes and limit the availability of nutrients within the soil (Trivedi *et al*., 2016). Continuous use of treated wastewater could also alter the pH of the soil affecting microbial activities and introducing heavy metals that results in complexation reaction with organic nutrients, which mobilises or immobilises them depending on the pH levels (Ge *et al*., 2009).

The study of microbial and enzyme activity and ecology in the soil play an important role in determining many soil characteristics, and activities such as the decomposition of organic matter facilitated by soil organisms (Haynes and Graham, 2004). Soil biological activities influence soil fertility, soil structure, carbon sequestration and plant growth (Godde *et al*., 2016). Low availability of native soil biological activities could act as a limiting factor for good soil health increased crop productivity (Dani and Tecon, 2017). Soil microbial communities are abundant in the top soil due to the presence of organic matter that provide a source of energy necessary for their activity and survival. Therefore, it is important to understand how microbial communities function within the heterogeneous soil landscape (Lehman *et al*., 2015). The objective of this study was to determine the response of some critical nutrients, microbial and enzyme activities on soils irrigated with treated wastewater at the UL Experimental Farm.

4.2 Materials and methods

4.2.1 Description of the study site

The study was conducted at the University of Limpopo Experimental Farm (ULEF) (23°50'42.86"S; 29°42'44.35"E). The soil in the study site was classified as Bainsvlei soil form, developed from a granite parent material (Moshia *et al*., 2008), the soil displayed luvic properties with increasing clay content moving down the profile. Three fields of 4 ha in size were identified for this study. The first one was virgin field (VF) and has never been cultivated or irrigated. The second was a cultivated field (CF) in its third year of onion cultivation and irrigated with treated water, and lastly was fallowed field (FF) and has been fallowed for 5 years following 3 years of cultivation of tomatoes and irrigated with treated wastewater (Figure 4.1). The study site has been further described in Chapter 3.

4.2.2 Treatments and research design

The study was composed of two factors namely, the fields and the sampling depth. The first factor had three 4 ha fields namely, virgin field (VF), cultivated field (CF) and fallowed field (FF) (Figure 4.1). The second factor comprised of three top soil depths (0-5, 5-15 and 15-30 cm).

4.2.3 Soil sampling procedure

Each plot was divided into 20 equal grids of 50 × 40 m. Soil samples were collected from three top soil depth (0-5, 5-15 and 15-30 cm) in each of the field. Eight reference soil samples were collected from the centre of each grid at 10 m intervals and bulked to make a composite sample. The composite samples were then transferred into a sterile sampling bag, and then transported in cooler boxes to University of Limpopo Soil Science Laboratory for further preparation and analysis.

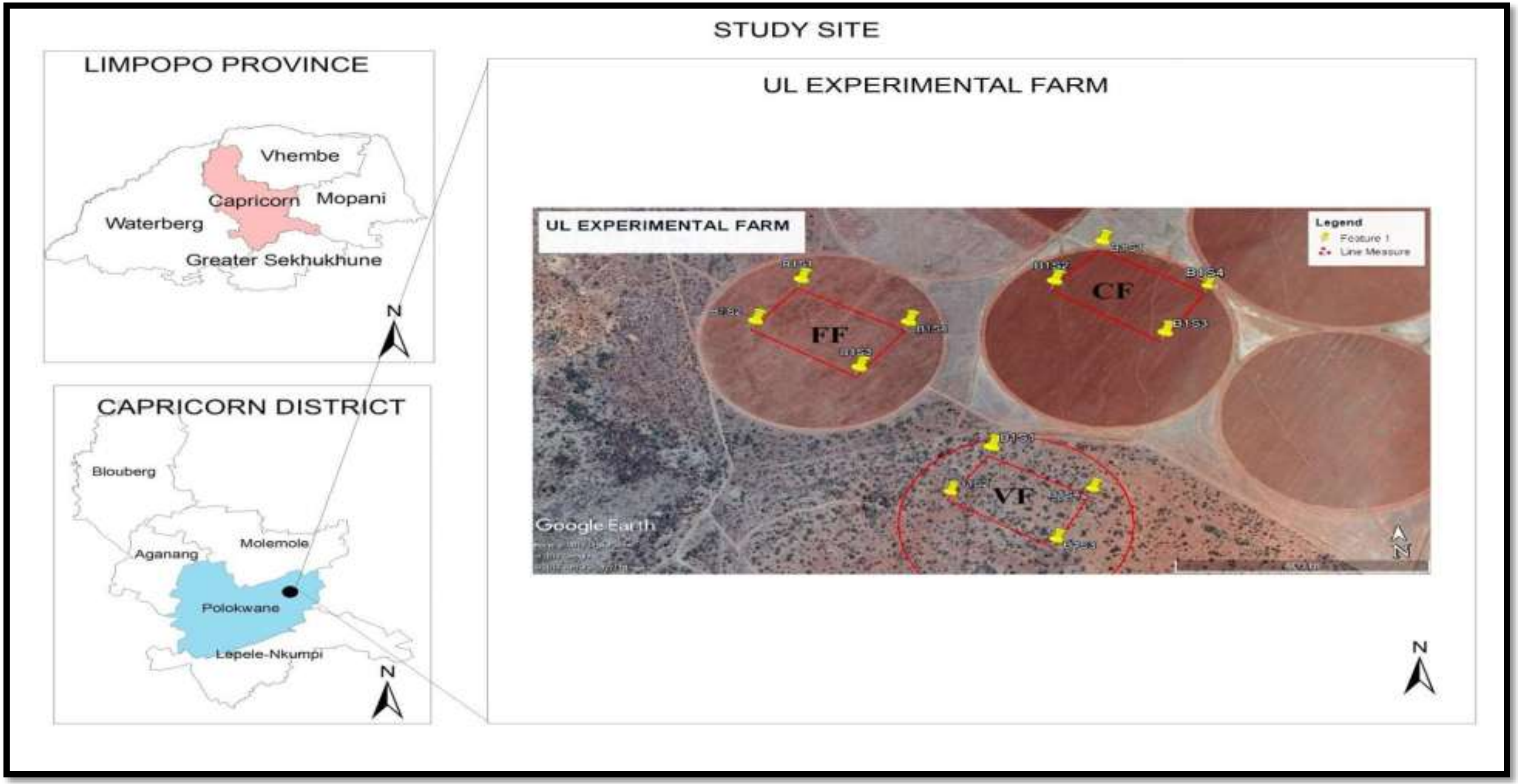

Figure 4.1: Study site map showing the three experimental fields within the University of Limpopo Experimental farm.

4.2.4 Data collection

Physicochemical properties and selected nutrients

Soil was analysed for particle size distribution using the hydrometer method. For calculation of particle size distribution, the following equations were used (Bouyoucos, 1962). For % clay:

$$\% \text{ clay} = \frac{corrected\ hydrometer\ reading\ at\ 6hrs,52\ min \times 100}{weight\ of\ sample} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

For % silt:

$$\% \text{ silt} = \frac{corrected\ hydrometer\ reading\ at\ 40\ sec \times 100}{weight\ of\ sample - \%\ clay} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (2)$$

For % sand:

% sand = 100% - % silt - % clay …………….……………………………………... (3)

Soil pH was determined by weighing 10 g of dried soil into a glass beaker into which 25 cm^3 of KCl solution or H_2O (distilled or de-ionised) was added. The contents were stirred rapidly for 5 seconds with a glass rod and stirred again after 50 minutes and allowed to stand for 10 minutes. pH was determined with a calibrated pH meter with the electrode positioned in the supernatant (Reeuwijk, 2002). Electrical conductivity (EC) was determined using the electrode method, were 10 g dried soil was weighed in a glass beaker and 25 cm^3 of H_2O (distilled or de-ionised) was added. The contents were stirred rapidly for 5 seconds with a glass rod, was stirred again after 50 minutes, and was allowed to stand for 10 minutes. EC was determined with a calibrated conductivity meter with the electrode positioned into the supernatant (Rhoades, 1982).

Phosphorus (P) was determined through the use of Bray 1 extraction method, where 6.67 g of soil sieved with a 2 mm sieve were transferred into the extracting bottles and

50 ml of bray 1 extracting solution was added and hand shaken for 1 minute. The mixture was then filtered through a 42 Watchman filter paper, 5 ml of the sample extract was transferred into a test tube, 3 ml of distilled water and 2 ml of reagent B were added. The absorbance was read after 30 minutes at a wavelength of 882 nm with a spectrophotometer (Bray and Kurtz, 1945). Ammonium (NH_4^+) and nitrate (NO_3^-) determined using colorimetric method, were 10.0 g of soil was weighed into a plastic extracting bottle and 100 ml of 0.5 M K_2SO_4 extracting solution was added and shaken for 1 hour using a horizontal shaker. The mixture was filtered through a 42 Watchman filter paper. For ammonium, a micro-pipette was used to transfer 0.2 ml of the sample extract into a test tube, 5.0 ml of reagent N1 was then added and allowed to stand for 15 minutes and vortexed. 5.0 ml of reagent N2was then added and vortexed, the absorbance was measured after 1 hour at wavelength of 655 nm using a spectrophotometer.

Nitrate was determined following protocols described by Okalebo *et al*. (1993), where a micro-pipette was used to transfer 0.5 ml of the sample extract into a test tube and 1.0 ml of salicylic acid was added, mixed well and allowed to stand for 30 minutes. 10 ml 4M sodium hydroxide was added and mixed well; the absorbance was measured after 1 hour at wavelength 419 nm using a spectrophotometer. Organic carbon (OC) was determined using Walkley-Black method, where 1 g of soil was transferred to a 500 cm^3 Erlenmeyer flask and 10 cm^3 of $K_2Cr_2O_7$ solution was added. The flask was swirled to disperse the soil in the solution and rapidly was added with 20 cm^3 concentrated sulphuric acid and the flask was swirled vigorously for 1 minute. The flask was allowed to cool on a sheet of asbestos for 30 minutes and added with 150 cm^3 of de-ionised water and 10 cm^3 of concentrated orthophosphoric acid. The flask was swirled gently and added with 1 cm^3 indicator and titrated the excess dichromate with ammonium ferrous sulphate solution until the solution changed colour to a sharp green. Organic C% was calculated using the following formula by Walkley and Black (1934):

$$\text{Organic C \%} = \frac{[cm^3 blank - cm^3 sample] \times M \times 0.3 \times f}{soil\ mass\ (g)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Soil biological activities

Active carbon (AC) was determined using permanganate oxidisable carbon, by adding 18 ml of deionised water and 2 ml of 0.2 M KM_nO_4 in soil sample of 2.5 g. The mixture was then sealed and shaken at 240 oscillations per minute for 2 minutes, removed the cap and placed the sample in a dark area for 10 minutes. After 10 minutes, 0.5 ml of supernatant was transferred to a second tube containing 49.5 ml of water, it was capped and mixed and the absorbance was read at 550 nm using a UV/Visible spectrophotometer (Weil *et al.*, 2003).

Potential mineralisable nitrogen (PMN) was determined using phenyl hypochlorite method where 40 ml of KCl was added to 8 g of oven dried soil in a centrifuge tube, sealed and shaken at 200 rpm for 1 hour and settled for 10 minutes, then filtered. A second set of centrifuge tube where 10 ml of distilled water and N gas for 30 seconds was added to 8 g of soil, sealed with para-film and incubated for 7 days at 27°C. After incubation, 30 ml of KCl was added and then filtered. Approximately 1 ml was pipetted from the filtrates into a centrifuge tube separately and 24 ml of distilled water, 1 ml of phenol solution, 1 ml of sodium nitroprusside and 2.5 ml oxidising solution was added and sealed with para-film and stored for an hour in a dark place. The absorbance was read at 640 nm using UV/Visible spectrophotometer (Solorzano, 1969).

Soil enzyme activities

Fluorescein di-acetate hydrolysis (FDA) activity was determined by measuring the fluorescein released following hydrolysis of fluorescein di-acetate. Where 1 g of soil was placed into a 150 ml plastic bottle and 50 ml of THAM buffer (0.1 M, pH 7.6) and 0.5 ml of 47.6 µm FDA was added. The samples were incubated at 37°C for 3 hours, following which 2 ml of acetone was added. The samples were then filtered and the absorbance read at 490 nm using UV/Visible spectrophotometer, the FDA hydrolytic activity was calculated (Prosser *et al.,* 2011). Phosphatase activity was determined by measuring the released p-nitrophenol following p-Nitrophenol Phosphate solution, where 50 ml Erlenmeyer flask containing 1 g of soil was added with 0.2 ml toluene, 4 ml MUB (pH 6.5) and 1 ml p-nitrophenyl phosphate solution. The flask was capped, swirled and incubated for 1 h at 37°C, after incubation 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH was added and swirled. The sample was filtered and absorbance read at 405 nm using a UV/Visible spectrophotometer (Tabatabai and Bremner, 1969).

4.2.5 Data analysis

All data were subjected to analysis of variance (ANOVA) as applicable to factorial analyses. Mean separation was done for significant means using Tukey's multiple range test at the probability level of 5%. All statistical analysis was carried out using the Statistical Analysis Software (SAS) program, Version 10.0 (SAS Institute, 2013). Unless otherwise stated, treatment effects were discussed at the probability level of 5%.

4.3 Results

4.3.1 Soil physicochemical properties

Field (A) × soil depth (B) interaction and depth were not significant on pH and EC in total treatment variation (TTV) of the respective variables (Table 4.1). However, field effects were highly significant on pH and EC, contributing 83 and 98% in TTV of the respective variables (Table 4.1). Relative to VF; the combined effects of treated wastewater and cultivation in CF and FF increased percentage of pH by 1 and 7%, respectively. Relative to VF; the combined effects of treated wastewater and cultivation in CF and FF increased percentage of EC by 2158 and 212%, respectively (Table 4.2).

Table 4.1: Partitioning of variation for pH and EC under irrigation with treated wastewater.

		pH		EC	
Source	DF	MSS	TTV (%)	MSS	TTV (%)
Block	19	0.20	5	29809	1
Field (A)	2	2.98	83^{***}	5382881	98^{***}
Depth (B)	2	0.27	7^{ns}	41081	1^{ns}
A × B	4	0.06	2^{ns}	29960	0^{ns}
Error	152	0.10	3	15324	0
Total	179	3.60	100	549905	100

TTV (%) = Total treatment variation = (MSS/Total) × 100

***Highly significant at $P \leq 0.01$, ^{ns}Not significant at $P \leq 0.05$.

Table 4.2: Effects of treated wastewater on soil pH and EC of cultivated and fallowed fields relative to those of virgin field.

Treatment	pH	R.I. (%)	EC (mS/cm)	R.I. (%)
Virgin field	6.25[b]	–	25.11[c]	–
Cultivated field	6.31[b]	1	566.90[a]	2158
Fallowed field	6.66[a]	7	78.44[b]	212

Relative impact [R.I. (%)] = [(Field/Virgin field) – 1] × 100.

The mean values of clay and sand in VF were 31.83 and 54.33, respectively. (Table 4.3). Clay % of CF had a range of 4% to 32% and an average of 13.35. Sand % ranged from 50% to 84%. Both the percentage of clay and sand at FF were high with minimum values of 15.2 and 33.6, respectively, and maximum values of 51.2 and 73.6. The mean values were 34.03 and 53.33, respectively (Table 4.3).

Table 4.3. Descriptive statistics for soil texture in the three fields.

Basic soil properties	Virgin field				Cultivated field				Fallowed field			
	Min	Max	Mean	St Dev	Min	Max	Mean	St Dev	Min	Max	Mean	St Dev
%Clay	8	76	31.83	12.29	4	32	13.35	6.48	15.2	51.2	34.03	6.32
%Sand	12	83	54.34	16.31	50	84	56.53	22.22	33.6	73.6	53.33	7.46

Min = minimum, Max = Maximum, St Dev = Standard deviation.

4.3.2 Selected essential nutrients

Field (A) × soil depth (B) interaction were highly significant on NO_3^-, contributing 18 % in TTV of the variable. However, A × B interaction were not significantly different on NH_4^+ and P (Table 4.4). Field effect was highly significant on NH_4^+, NO_3^- and P, contributing 44, 13 and 99% in TTV of the respective variables (Table 4.4). Depth effect was highly significant on NH_4^+ and NO_3^-, contributing 35 and 60% in TTV of the respective variables. However, depth did not have significant difference on P (Table 4.4). Relative to VF; the combined effects of treated wastewater and cultivation in CF and FF increased percentage of NH_4^+ by 50 and 52%, respectively (Table 4.5). Relative to VF; the combined effects of treated wastewater and cultivation in CF and FF increased percentage of P by 274 and 123%, respectively (Table 4.5). Relative to the first depth; the concentration of NH_4^+ decreased in both second and third depths by 26 and 29%, respectively (Table 4.6).

Relative to VF; the combined effects of treated wastewater and cultivation in CF and FF increased percentage of NO_3^- by 63 and 8%, respectively, in the 0-5 cm depth (Table 4.7). The combined effects in CF increased percentage of NO_3^- in the second depth (5-15 cm) by 7%, whereas the combined effects in FF decreased percentage of NO_3^- by 18% in the same depth (Table 4.7). The combined effects of treated wastewater and cultivation in CF and FF decreased percentage of NO_3^- by 52 and 49%, respectively, in the 15-30 cm depth (Table 4.7).

Table 4.4: Partitioning of variation for NH_4^+, NO_3^- and P under irrigation with treated wastewater.

Source	DF	NH_4^+		NO_3^-		P	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	19	26.74	7	1475.8	7	1.68	0.16
Field (A)	2	175.50	44^{***}	2755.0	13^{***}	1033.24	99^{***}
Depth (B)	2	140.02	35^{***}	13090.9	60^{***}	2.24	0^{ns}
A × B	4	40.46	10^{ns}	4006.6	18^{***}	1.30	0^{ns}
Error	152	17.65	4	438.6	2	0.83	0
Total	179	400.37	100	21766.9	100	1039.29	100

TTV (%) = Total treatment variation = (MSS/Total) × 100

***Highly significant at $P \le 0.01$, ^{ns}Not significant at $P \le 0.05$.

Table 4.5: Effects of treated wastewater on the concentration of NH_4^+ and P of cultivated and fallowed fields relative to virgin field.

Treatment	NH_4^+	R.I. (%)	P	R.I. (%)
Virgin field	5.81^b	–	3.01^c	–
Cultivated field	8.72^a	50	11.27^a	274
Fallowed field	8.82^a	52	6.70^b	123

Relative impact [R.I. (%)] = [(Field/Virgin field) – 1] × 100.

Table 4.6: Effect of soil depths on the concentration of NH_4^+ (mg.kg^{-1}).

Treatment	NH_4^+ (mg.kg^{-1})	R.I. (%)
0 – 5	9.54^a	–
5 – 15	7.08^b	–26
15 – 30	6.73^b	–29

Relative impact [R.I. (%)] = [(Depth/First depth) – 1] × 100.

Table 4.7: Concentration of NO_3^- (mg.kg^{-1}) among fields in three soil depths as compared to virgin field.

	0-5		5-15		15-30	
Treatment	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Virgin field	50.27^b	–	49.35^b	–1	48.61^b	–3
Cultivated field	82.04^a	63	54.03^b	7	24.10^c	–52
Fallowed field	54.32^b	8	40.78^{bc}	–18	25.28^c	–49

Relative impact [R.I. (%)] = [(Field/Virgin field) – 1] × 100.

4.3.3 Biological activities

Field (A) × soil depth (B) interaction had no significant effect on OC, AC and PMN (Table 4.8). However, the effect of field had highly significant effect on OC, AC and PMN, contributing 100, 86 and 86% in TTV of the variable (Table 4.8). Effects of depth on OC and AC were not significant (Table 4.8). However, depth had significant effect on PMN, contributing 8% in TTV of the variable (Table 4.8). Relative to VF, the combined effects of treated wastewater and cultivation in CF and FF increased AC by 43 and 93%, respectively (Table 4.9). The combined effects of treated wastewater and cultivation in CF decreased PMN by 56%, whereas the combined effects in FF increased PMN by 94% (Table 4.9). The combined effects of treated wastewater and cultivation in CF and FF increased percentage of OC by 86 and 73%, respectively (Table 4.9). Relative to the first depth; the concentration of PMN in both second and third depths (5–15 and 15–30 cm) decreased by 23 and 36%, respectively (Table 4.10).

Table 4.8: Partitioning sources of variation for organic carbon (OC), active carbon (AC) and potential mineralisable nitrogen (PMN) in three fields over three top soils.

Sources	DF	OC		AC		PMN	
		MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	19	0.22	0	95966	6	0.02	3
Field (A)	2	2197.79	100***	1470353	86***	0.86	86***
Depth (B)	2	0.29	0 ns	39042	2ns	0.079	8**
A × B	4	0.43	0 ns	36558	2ns	0.01	1ns
Error	152	0.25	0	67536	4	0.02	2
Total	179	2198.97	100	1709455	100	1.00	100

TTV (%) = Total treatment variation = (MSS/Total) x 100

*** Highly significant at P ≤ 0.01, **Significant P ≤ 0.05, ns Not significant at P ≤ 0.05.

Table 4.9: Effects of treated wastewater on OC, AC and PMN of cultivated and fallowed fields relative to virgin field.

Treatment	OC	R.I. (%)	AC	R.I. (%)	PMN	R.I. (%)
Virgin field	13.87[c]	–	337.17[c]	–	0.16[b]	–
Cultivated field	25.8[a]	86	482.31[b]	43	0.07[c]	–56
Fallowed field	23.99[b]	73	651.56[a]	93	0.31[a]	94

Relative impact [R.I. (%)] = [(Field/Virgin field) – 1] × 100.

Table 4.10: Effect of depth on the concentration of PMN (mg/kg).

Treatment	PMN	R.I. (%)
0 – 5	0.22[a]	–
5 – 15	0.17[ab]	–23
15 – 30	0.14[b]	–36

Relative impact [R.I. (%)] = [(Field/Virgin field) – 1] × 100.

4.3.4 Enzyme activities

Field (A) × soil depth (B) interaction had highly significant effect on FDA and phosphatase, contributing 2 and 3% in TTV of the respective variables (Table 4.11). Field effect had highly significant effect on FDA and phosphatase, contributing 96 and 86% in TTV of the respective variables. Depth were highly significant on FDA and phosphatase, contributing 2 and 11% in TTV of the respective variables (Table 4.11).

Table 4.11: Partitioning sources of variation for enzyme activities.

Sources	DF	FDA		Phosphatase	
		MSS	TTV (%)	MSS	TTV (%)
Block	19	0.04	0	188	0
Field (A)	2	22.41	96***	100279	86***
Depth (B)	2	0.47	2***	12218	11***
A × B	4	0.42	2***	3196	3***
Error	152	0.02	0	103	0
Total	179	23.36	100	115984	100

TTV (%) = Total treatment variation = (MSS/Total) x 100

*** Highly significant at $P \leq 0.01$, ^{ns} Not significant at $P \leq 0.05$.

Relative to VF, the combined effects of treated wastewater and cultivation in CF decreased FDA activity in the first, second and third depth (0-5, 5-15 and 15-30 cm) by 90, 92 and 85%, respectively (Table 4.12). The combined effects of treated wastewater and cultivation in FF decreased percentage of FDA activity in the first, second and third depth by 35, 40 and 43%, respectively (Table 4.12). Relative to VF; the combined effects of treated wastewater and cultivation in CF decreased phosphatase in the first, second and third depths (0-5, 5-15 and 15-30 cm) by 93, 96 and 97%, respectively (Table 4.13). The combined effects of treated wastewater and cultivation in FF decreased phosphatase in the first, second and third depths by 74, 90 and 97%, respectively (Table 4.13).

Table 4.12: FDA hydrolysis activity among cultivated and fallowed fields in three soil depths relative to virgin field.

	0-5		5-15		15-30	
Treatment	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Virgin field	1.61^{a}	–	1.38^{b}	14	1.13^{c}	-30
Cultivated field	0.16^{e}	–90	0.13^{e}	–92	0.24^{e}	–85
Fallowed field	1.05^{cd}	–35	0.97^{d}	–40	0.92^{d}	–43

Relative impact [R.I. (%)] = [(Field/Virgin field) – 1] × 100.

Table 4.13: Phosphatase (PTS) activity among cultivated and fallowed fields in three soil depths as compared to that of virgin field.

	0-5		5-15		15-30	
Treatment	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Virgin field	110.39^{a}	–	73.71^{b}	–33	56.43^{c}	–49
Cultivated field	8.09^{e}	–93	4.43^{e}	–96	3.28^{e}	–97
Fallowed field	28.59^{d}	–74	11.29^{e}	–90	3.68^{e}	–97

Relative impact [R.I. (%)] = [(Field/Virgin field) – 1] × 100.

4.4 Discussion

4.4.1 Distribution of pH and EC across fields

The soil pH values ranged from slightly acid to neutral among the three fields, the pH of cultivated and fallowed fields were 1 and 7% higher than that of the control field (virgin field), respectively. The increase in pH is associated with application of treated wastewater for irrigation; increased soil pH was perhaps due to basic components included within wastewater, which was then converted to basic compounds and/or salts, which led to increased pH value. The irrigation water in the study had conductivity levels as high as 294.74 μS/cm and SAR values as high as 18.80, which could have facilitated the rapid increase in soil pH. The findings were in line with those of Bedbabis *et al*. (2015), who observed an increase in soil pH as a consequence of irrigation with wastewater. Sudden changes in pH values, either an increase or a decrease affect microbial activities negatively since the organisms are not familiar with the environment (Wang *et al*., 2006). Furthermore, Wafula *et al*. (2015) indicated that the increase in soil pH as resulted from irrigation with treated wastewater could be related to high organic carbon content, which bound aluminium ions and reduced their activity within the soil solution and increased the saturation of exchange site and soil pH.

The conductivity among the three fields was significantly different, with cultivated field consisting of conductivity of 566.90 mS/m which was 2158% above the control (virgin field) and fallowed field with 78.44 which was 212% above the control and in general cultivated field was highly saline (EC > 5 dS/m). Significant difference between the fields indicated that the application of treated wastewater had a direct influence on soil electrical conductivity. The increase of EC in both cultivated and fallowed fields were of the high EC reported in the treated wastewater. Adhikari *et al*. (2011) indicated that the significant difference between irrigated and unirrigated plots was influenced by non-uniform application of wastewater. Furthermore, the results were consistent with those of Mohammed and Sidduraiah, (2016) who indicated an increased soil electrical conductivity as a result of irrigation with wastewater. However, Khaskhoussy *et al*. (2015) indicated that the significant increase in the electrical conductivity of soil irrigated with treated wastewater was attributed to higher concentration of cations such as Na and K within wastewater. Soil salinity results in low soil biological activities as a result of osmotic stress and toxic ions, however soil microbes tolerant to salinity counteract osmotic stress by producing osmolytes which allowed them to maintain their cell turgor and metabolism (Yan *et al*., 2015). Irrigation water with high salt content is not ideal for irrigation since it interferes with the productivity of the soil by limiting the activities of microorganisms, which then adversely affect the process of nutrient transformation and availability.

4.4.2 Distribution of essential elements

<u>Nitrogen</u>

Nitrogen exists in the soil in different forms, transforms very easily and is biologically influenced. The significance of nitrogen in biological studies is that it can be used to estimate the biological activity of the soil since its transformation and soil microbes (Lamb *et al*., 2014) facilitate availability. Ammonium and nitrate were the two main forms of N analysed in this study. Ammonium within the cultivated and fallowed fields increased by 50 and 52%, respectively, which was higher than the control. The concentration of NH_4^+ fluctuated with depth in the cultivated field however, in the fallowed field the concentration of NH_4^+ decreased with depth. Similar fluctuations were observed by Disciglio *et al*. (2015) who indicated a significantly higher concentration of NH_4^+ in the upper layer and third layer, which might be due to the movement patterns of water along the soil profile. The water movement patterns determine the position or location at which nutrient elements are available within the soil solution since these elements are mainly dissolved in water, also higher amount of ammonium relative to nitrates could be due to the fact that nitrates leach faster than ammonium. Furthermore, Jemai *et al*. (2013) observed that after irrigation with treated wastewater the NH_4^+ distribution pattern within the soil significantly changed. However, Zhang *et al*. (2014) indicated an increased concentration of NH_4^+ by 43.6% after irrigation with treated wastewater. It is well known that treated wastewater contains excessive amounts of NH_4^+ and NO_3^- as a result of run-off water from agricultural site (Omar *et al*., 2013; Shalinee and Ademola, 2014). The high levels of NH_4^+ in treated wastewater effluent should be taken into consideration during irrigation since it could result in damages to plants cells.

Nitrate in cultivated field was increased by 8% having stark difference with the fallowed field which decreased by 19%. The reduction in NO_3^- indicated the possibility of nitrification inhibitors within the treated wastewater, which slowed down the conversion of NH_4^+ to NO_3^-. For the increased NO_3^-. The low levels of NO_3^- could be caused by decreased organic matter inputs; NO_3^- is an available form of nitrogen, which could have been easily absorbed by plants and results in losses within the soil solution. Zhang *et al*. (2014) observed similar results of 95.2% increase in NO_3^- following irrigation with treated wastewater. Furthermore, Belaid *et al*. (2010) indicated a systematically higher NO_3^- content in the soil irrigated with wastewater than in the non-irrigated soils, which was also consistent with the observed outcomes of Qi *et al*.

(2010) who indicated that NO_3^- concentration in soil increased substantially after irrigation with wastewater. The concentration of NO_3^- decreased with depth in both cultivated and fallowed field this could be attributed to low nitrifying bacteria down the profile. Similarly, Jemai *et al*. (2013) observed that soil NO_3^- content was significantly higher in surface layers during preliminary studies which might be attributed to the application of NPK fertilisers, thus treated wastewater have to be monitored since it serves as a form of nutrient amendment.

Phosphorus

The concentration of phosphorus (P) in both cultivated and fallowed fields had a relative impact of 274% and 123%, respectively. This might be attributed to continuous deposition of phosphoric materials such as magnesium ammonium phosphate and calcium phosphate fertilisers within run-off water from agricultural site, which were carried within the treated wastewater. Al-Jaboobi *et al*. (2014) indicated that average values of phosphorus were high in soil irrigated with wastewater. Furthermore, Mohammed and Sidduraiah, (2016) indicated an increased concentration of P as a consequence of irrigation with wastewater which increased significantly in treatments of treated wastewater than other treatments. The results were consistent with those of Kordlaghari *et al*. (2013) who stated that soil available phosphorus in treatment of treated wastewater was increased in comparison with the control.

4.4.3 Distribution of organic carbon, active carbon and potential mineralizable nitrogen

Organic carbon

Organic carbon percentage varied widely across fields, with organic carbon content being increased in comparison with VF. The change in organic carbon, averages to

81% increase across fields, this could be attributed to continuous addition of soluble organic compounds within the irrigation water. The findings were similar to those of Belaid *et al*. (2012b) and Galavi *et al*. (2010) reported a significant increase in the percentage of organic carbon as a result of irrigation with wastewater. The organic carbon content did not have significant difference among the three soil depths. However, these findings were contradicting with those of Hamid and Hamid (2012), who observed an increase in soil organic carbon in all the soil depth. Jemai *et al*. (2013) observed that irrigation with treated wastewater had reduced the OC content in surface layer and has increased it in the deeper layer.

<u>Active carbon</u>

Soil active carbon is the active fraction of soil organic carbon, which breaks down relatively quickly during microbial growth and it changes greatly after disturbance and management (Zoua *et al*., 2005). It is an active source of nutrition and a major food source for soil microbes, it is an indicator of change in the soil (Li *et al*., 2015). The concentration of active carbon (AC) in both cultivated and fallowed fields increased significantly with a relative impact of 43% and 93%, respectively, these could be attributed to the increased organic matter additions from various sources or the treated wastewater. Various sources include amendments, residues, active and diverse forage, crop, or cover crop growth, with living roots providing active carbon to soil microbes for as much of the year as possible (Apps, 1987). Gao *et al*. (2015) observed a significantly higher active carbon in soils irrigated with treated wastewater as attributed to the built up of organic load in the irrigation water. Similar trends of increase active carbon in soils irrigated with treated wastewater was observed by

Belaid *et al*. (2012a), Jogan *et al*. (2017) and Liang *et al*. (2014) which was attributed to changes in soil organic carbon caused by irrigation management.

Potential mineralizable nitrogen

Potential mineralizable nitrogen is the fraction of organic nitrogen easily decomposable and mineralizable by soil microorganisms, it is considered as a good indicator of N cycle and measure of nitrogen availability from soil during the crop-growing season (Kayikcioglu, 2012). The concentration of mineralizable nitrogen (PMN) in cultivated field decreased significantly by 56% and in fallowed field, the concentration increased by 94%. Hoang *et al*. (2016) indicated that the increase in the concentration of potential mineralizable nitrogen (PMN) is a contribution of the soil organic matter of which is considered as a prime source of PMN. Further, Cordovil *et al*. (2007) indicated that the improvement of PMN in soil is facilitated by the application of organic residues which increased microbial biomass and organic nitrogen, addition of N fertilizer as well as soil properties and soil management practices that affect organic matter and organic N dynamics affect available N and PMN levels. The significant decrease (56%) in PMN of CF could be attributed to the presence of foreign material such as heavy metal that restrict the availability and decomposition of organic carbon. The results were consistent with those of AL-Jaboobi *et al*. (2014), who observed a decrease in the concentration of PMN as a result of high heavy metals in soils limiting the availability of organic compounds. Furthermore, Ghaemi *et al*. (2014) observed similar results of decreased PMN as attributed to decreased microbial activity for organic nitrogen breakdown and the decomposition rate of organic nitrogen as affected by heavy metal availability within the irrigation water.

4.4.4 Distribution of enzyme activity

Fluorescein diacetate

Soil enzymes are large protein molecules produced by soil microorganisms that act on substrates to mediate biogeochemical reactions and are left intact at the end of the reactions (Iasur-Kruh *et al*., 2010). Fluorescein diacetate are enzymes responsible for the decomposition of organic matter and release carbon and other various nutrients. Contrary to all other soil parameters, FDA hydrolysis activity in both cultivated and fallowed field decreased by 87% and 28%, respectively. Klose *et al*. (2006) indicated that insecticides or herbicides in soils due to agricultural practices might disturb the activities of soil enzymes such as fluorescein diacetate hydrolysis. Velmourougane and Rajeev, (2012), observed similar results of decreased FDA activity due to herbicides in treated wastewater. However, Burns and Dick (2002) observed a decrease in FDA hydrolysis activity as attributed to abundance of saprophytic bacteria from the irrigation water and Li *et al*., (2017) observed a decrease in FDA activity as associated with high salinity that disperse the structure and facilitate leaching of finer particles, were most enzymes are bound.

Phosphatase

Phosphatase enzyme are responsible for hydrolyses and/or transformation of a variety of organic phosphorus compounds providing effective phosphorus, which could successively be assimilated by plants (Baddam *et al*., 2016). Soil phosphatase activity characterizes the status of soil fertility; it can however, be associated with P stress and plant growth (Efsun *et al*., 2017). Similar to FDA, phosphatase activity in both cultivated and fallowed fields decreased by 93% and 82%, respectively. There was a negative correlation between phosphatase activity and inorganic P, because phosphatase enzymes are responsible for catalysing the mineralisation of organic phosphorus to inorganic P. Klose *et al*. (2006) noted that a decrease in phosphatase activity might be attributed by soil fumigation and also pesticides in soils due to agricultural practices, such could disturb the activities of soil enzymes such as phosphatase. Kayikcioglu (2012) reported that the decrease in phosphatase activity was attributed to the inhibition of microbial communities and growth after irrigation with wastewater, probably by the fact that besides nutrients also contaminants, such as heavy metals, are being supplied. Furthermore, Wang *et al*. (2016) stated that the decrease in phosphatase activity was due to different agricultural practices (mono cropping and crop rotation).

4.5 Conclusion

The study indicated that the combined effect of treated wastewater and cultivation caused a huge increase on electrical conductivity of the soil and the soil pH were within acceptable level. Furthermore, a 50% increase in NH_4^+ was observed in both CF and FF, also significant increase of P level of which is beneficial since it is known to be immobile and in most cases in low concentration. However, the concentration of nitrogen (NH_4^+ and NO_3^-) decrease with increase in depth in both CF and FF. Irrigation with treated wastewater resulted in significant increase in OC, AC and PMN, however, a slight decrease in PMN in CF due to cultivation. Furthermore, the concentration of PMN decreased with depth. Irrigation with treated wastewater displayed a significant negative effect on the activity of soil enzymes (FDA and phosphatase) in all the study fields due to addition of toxic substances/inhibiting agents. With all that said, it can be concluded that treated wastewater could be recommended for irrigation of agricultural fields because it displayed more positive effects than negative effects. Therefore, irrigation with treated wastewater coupled with fallowing can be recommended since it allows the soil rest and recover.

CHAPTER 5

SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary of findings

The study focused on soil biological properties following irrigation with treated wastewater through assessing the quality status of the water used for irrigation with the view of improving water quality in water scarce areas of Limpopo Province. Furthermore, the study assessed the chemical and biological properties of the soil and the benefits of fallowing to soil microbial and enzymatic activities following irrigation with treated wastewater. The results of the study disclosed that treated wastewater contained foreign materials, which are not suitable for irrigation purposes, it contains high amount of nutrients (nitrogen nitrate) which were above the permissible level, heavy metals (Pb, Cu, Cd, Cr and Zn) and other toxic compounds as well as bacteria such as *E. coli*. The findings further demonstrated that treated wastewater needs further processing and thorough depuration/purification prior to irrigation since it did not meet the permissible limit for irrigation water standard. The study demonstrated that the use of treated wastewater as an irrigation source had deleterious effects on the soil. The soil had increased concentrations of nutrients, salts and hindered soil microbial and enzymatic activities as a result of continuous deposition of these compounds including heavy metals. Findings suggested that fallowing for a period of five years improves the quality of the soil in relation to virgin field, with the results showing that the increased levels of unwanted compounds in cultivated field decreased with years of fallowing, which was observed through the increase of microbial activities (active carbon and potential mineralisable nitrogen) after years of fallowing.

5.2 Significance of findings

The study proved that the movement and different points of treated wastewater storage post treatment could result in different chemical and microbial loads. Outlet performed better than Pond 16, where majority of the parameters were in adequate levels in conjunction with the permissible level for irrigation water. In the three studied disposal points, night-dam outlet was the best point with less pathogens such as *E. coli*, while Pond 16 was the worst due to the highest loads of *E. coli*, total dissolved solids, heavy metals and bacterial counts. The study furthermore revealed in Objective 2, that irrigation with treated wastewater could lead to increases in chlorine, heavy metals concentrations and decreased microbial activities. However, the study was able to show the benefits of fallowing as a strategy with treated wastewater irrigation. Fallowed fields performed better than cultivated fields, where all the negative outcomes of irrigation with treated wastewater were minimised. The most harmful parameter such as nitrogen (too much nitrogen can result in acidic pH), salt accumulation was decreased with fallowing. Therefore, the use of treated wastewater for irrigation coupled with fallowing can play a vital role in sustainable agricultural and soil preservation.

5.3 Recommendations

Irrigation is important in agricultural production and most smallholder farmers and arid to semi-arid areas have water shortages thus limiting their production and yields. It is necessary for water institutions to provide necessary information on the benefits of irrigation with treated wastewater in order to overcome water scarcity. Majority of people are unaware of the benefits of using treated wastewater as an irrigation source. Therefore, it is vital that relevant information be dispersed to all in order to improve yield and food security. Soil biologist should make it a point in improving the information bank, especially of microbial and enzymatic activities, since these are complex and vital activities that indicates the soil capability (Ademir *et al*., 2009). Encouraging soil biology related studies with treated wastewater use, could be a vital process since majority of people are not familiar with the importance of biological properties to both yield and soil sustainability. More awareness and knowledge should be generated in relation to amelioration strategies towards water usage and soil quality. Furthermore, fallowing after irrigation with treated wastewater is recommended to allow the soil to regain its soil health. Deposition of salt in dams carried from upstream, the organic matter content within the water and dilution by rain water should further be investigated.

5.4 Conclusions

Agricultural production serves as the main source of income in many rural families in Limpopo Province. Irrigation with treated wastewater serves as an ameliorating strategy to water shortages and reduce the cost of inputs since it supplies elemental nutrients to the soil. Fallowing also serves as smart agriculture since it preserves the little that is available, Limpopo Province is known to have less arable land and it is necessary to preserve the little land available. Continuous cultivation can result in marginal soils, where food production and security will decline significantly. The use of treated wastewater for irrigation and fallowing practice can help in preserving the soil, help eradicate food insecurities and reduce the emission of greenhouse gases as well as saving large amount of inputs costs. The current study proved that night dam outlet water was better and can be used for irrigation purposes, and that fallowing is the best way to preserve soil from degradation since it provides time for soil to recover.

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APPENDICES

Appendix 3.1 Analysis of variance for pH in response to different water sampling points.

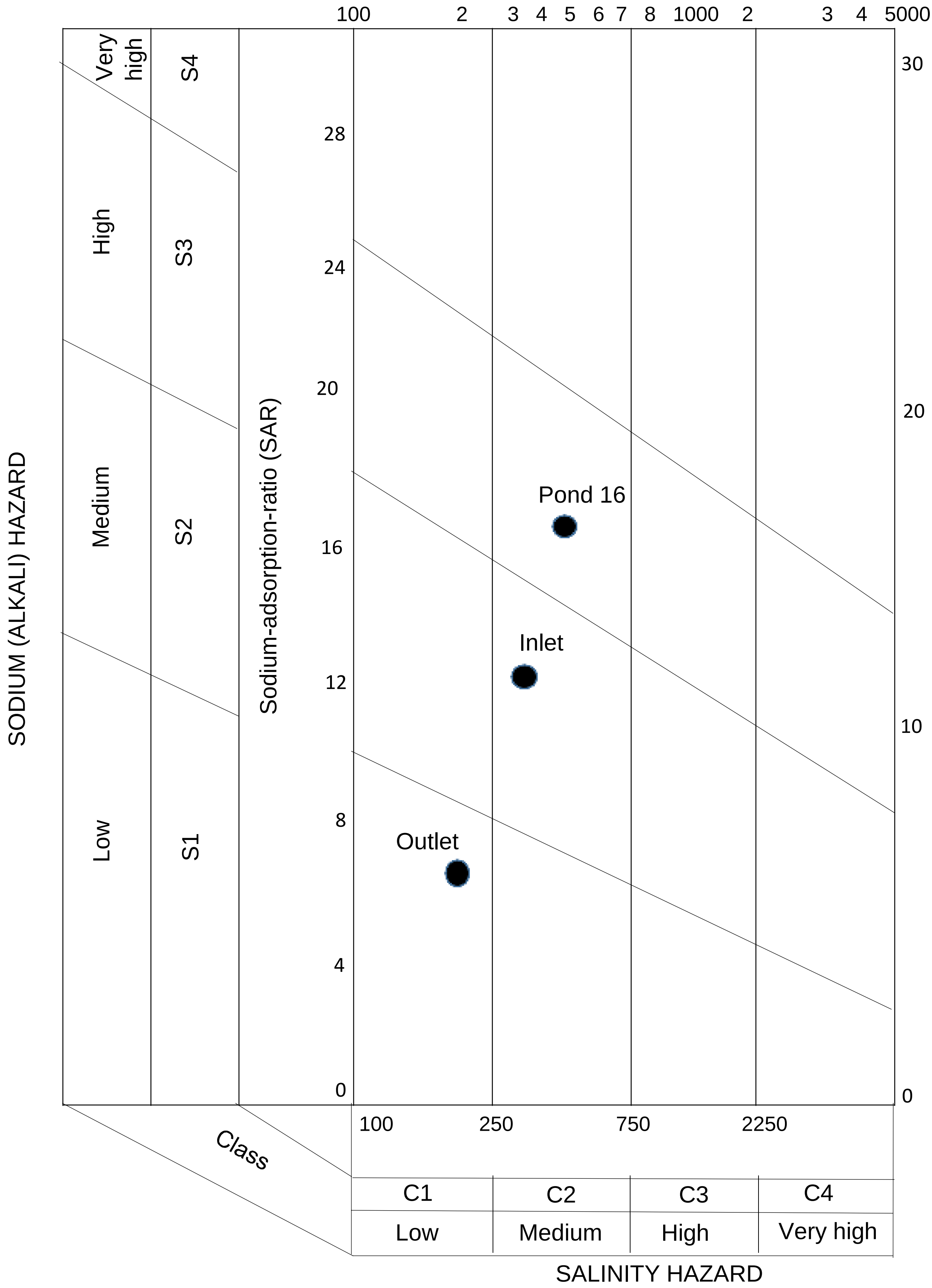
Source	DF	SS	MS	F	P
Treatment	2	2.19	1.09	0.25	0.79
Error	12	53.58	4.47		
Total	14	55.96	5.56		

Appendix 3.2 Analysis of variance for electrical conductivity (EC) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	5760	2880.2	0.06	0.94
Error	12	606593	50549.4		
Total	14	612353	53429.6		

Appendix 3.3 Analysis of variance for total dissolved solids (TDS) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	52510	26255	0.04	0.96
Error	12	7915316	659610		
Total	14	7967826	685865		



Appendix 3.4 Salinity diagram for water quality of different sampling points.

Appendix 3.5 Analysis of variance for sodium (Na) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	1507.04	753.52	46.36	0.00
Error	12	195.02	16.25		
Total	14	1702.06	769.77		

Appendix 3.6 Analysis of variance for calcium (Ca) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	189.83	94.91	57.02	0.00
Error	12	19.98	1.66		
Total	14	209.80	96.57		

Appendix 3.7 Analysis of variance for magnesium (Mg) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	0.50	0.25	0.17	0.84
Error	12	17.61	1.47		
Total	14	18.11	1.72		

Appendix 3.8 Analysis of variance for potassium (K) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	51.30	25.65	26.64	0.00
Error	12	11.56	0.96		
Total	14	62.86	26.61		

Appendix 3.9 Analysis of variance for sodium absorption ratio (SAR) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	279.87	139.94	25.42	0.00
Error	12	66.07	5.51		
Total	14	345.94	145.45		

Appendix 3.10 Analysis of variance for nitrate (NO_3^-) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	291725	145862	139.36	0.00
Error	12	12560	1047		
Total	14	304285	146909		

Appendix 3.11 Analysis of variance for sulphate SO_4^{2-} in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	1400.93	700.47	21.73	0.00
Error	12	386.80	32.23		
Total	14	1787.73	732.7		

Appendix 3.12 Analysis of variance for phosphate (PO_4^{2-}) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	0.58	0.29	0.45	0.65
Error	12	7.68	0.64		
Total	14	8.26	0.93		

Appendix 3.13 Analysis of variance for carbonate (CO_3^{2-}) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	8,933E-04	4,467E-04	4.47	0.04
Error	12	1,200E-03	1,000E-04		
Total	14	2,093E-03	5.467E-04		

Appendix 3.14 Analysis of variance for bicarbonate (HCO_3^-) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	34871.6	17435.8	102.24	0.00
Error	12	2046.4	170.5		
Total	14	36918.0	17606.3		

Appendix 3.15 Analysis of variance for chlorine (Cl) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	221.38	110.70	1.31	0.30
Error	12	1010.21	84.18		
Total	14	1231.59	194.88		

Appendix 3.16 Analysis of variance for zinc (Zn) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	865.25	432.63	36.53	0.00
Error	12	142.13	11.84		
Total	14	1007.39	444.47		

Appendix 3.17 Analysis of variance for copper (Cu) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	43.86	21.93	11.98	0.00
Error	12	21.97	1.83		
Total	14	65.82	23.76		

Appendix 3.18 Analysis of variance for lead (Pb) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	0.07	0.04	3.94	0.05
Error	12	0.11	0.01		
Total	14	0.19	0.05		

Appendix 3.19 Analysis of variance for chromium (Cr) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	0.15	0.08	2.92	0.09
Error	12	0.31	0.03		
Total	14	0.46	0.11		

Appendix 3.20 Analysis of variance for cadmium (Cd) in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	1.213E-03	6.067E-04	4.33	0.04
Error	12	1.680E-03	1.400E-04		
Total	14	2.893E-03	7.467E-04		

Appendix 3.21 Analysis of variance for *Escherichia-coli* in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	0.89	0.04	4.76	0.03
Error	12	0.11	0.00		
Total	14	0.20	0.05		

Appendix 3.22 Analysis of variance for *shigella spp* in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	3.32	1.66	11.44	0.00
Error	12	1.74	0.15		
Total	14	5.06	1.81		

Appendix 3.23 Analysis of variance for *Vibrio cholerae* in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	0.27	0.14	18.72	0.00
Error	12	0.09	0.00		
Total	14	0.36	0.15		

Appendix 3.24 Analysis of variance for faecal coliform in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	4.91	2.46	102.22	0.00
Error	12	0.29	0.02		
Total	14	5.20	2.49		

Appendix 3.25 Analysis of variance for *Ascaris lumbricoides* in response to different water sampling points.

Source	DF	SS	MS	F	P
Treatment	2	0.03	0.01	0.52	0.61
Error	12	0.34	0.03		
Total	14	0.37	0.05		

Appendix 4.1 Analysis of variance for pH(H_2O) in relation to different field and different sampling depth.

Source	DF	SS	MS	F	P
Blocks	19	3.74	0.20		
Field	2	5.96	2.98	29.17	0.00
Depth	2	0.54	0.27	2.64	0.07
Field*Depth	4	0.22	0.06	0.54	0.71
Error	152	15.52	0.10		
Total	179	25.98	3.61		

Appendix 4.2 Analysis of variance for pH(KCl) in relation to different field and different sampling depth.

Source	DF	SS	MS	F	P
Blocks	19	5.97	0.31		
Field	2	86.10	43.05	460.78	0.00
Depth	2	0.39	0.19	2.10	0.13
Field*Depth	4	0.27	0.07	0.73	0.57
Error	152	14.20	0.09		
Total	179	107.36	43.71		

Appendix 4.3 Analysis of variance for electrical conductivity (EC) in relation to different field and different sampling depth.

Source	DF	SS	MS	F	P
Blocks	19	566368	29809		
Field	2	1.077E+07	5382881	351.28	0.00
Depth	2	82161.0	41081	2.68	0.07
Field*Depth	4	119839	29960	1.96	0.10
Error	152	2329181	15324		
Total	179	13867549	5499055		

Appendix 4.4 Analysis of variance for phosphorus (P) in relation to different field and different sampling depth.

Source	DF	SS	MS	F	P
Blocks	19	32.00	1.68		
Field	2	2066.48	1033.24	1242.39	0.00
Depth	2	4.48	2.24	2.70	0.07
Field*Depth	4	5.19	1.30	1.56	0.19
Error	152	126.41	0.83		
Total	179	2234.56	1039.29		

Appendix 4.5 Analysis of variance for ammonium (NH_4^+) in relation to different field and different sampling depth.

Source	DF	SS	MS	F	P
Blocks	19	508.10	26.74		
Field	2	351.00	175.50	9.95	0.00
Depth	2	280.03	140.02	7.93	0.00
Field*Depth	4	161.85	40.46	2.29	0.06
Error	152	2682.28	17.65		
Total	179	3983.26	401		

Appendix 4.6 Analysis of variance for nitrate (NO_3^-) in relation to different field and different sampling depth.

Source	DF	SS	MS	F	P
Blocks	19	28039.4	1475.8		
Field	2	5510.0	2755.0	6.28	0.00
Depth	2	26181.8	13090.9	29.85	0.00
Field*Depth	4	16026.2	4006.6	9.13	0.00
Error	152	66668.6	438.6		
Total	179	142426	21766.9		

Appendix 4.7 Analysis of variance for organic carbon (OC) in relation to different field and different sampling depth.

Source	DF	SS	MS	F	P
Blocks	19	4.50	0.24		
Field	2	0.97	0.48	2.87	0.06
Depth	2	0.28	0.14	0.82	0.44
Field*Depth	4	0.25	0.06	0.38	0.83
Error	152	25.64	0.17		
Total	179	31.64	110		

Appendix 4.8 Analysis of variance for active carbon (AC) in relation to different field and different sampling depth.

Source	DF	SS	MS	F	P
Blocks	19	1823349	95966		
Field	2	2940706	1470353	21.77	0.00
Depth	2	78084.0	39042	0.58	0.56
Field*Depth	4	146230	36558	0.54	0.71
Error	152	1.027E+07	67536		
Total	179	15258369	1709455		

Appendix 4.9 Analysis of variance for potential mineralizable nitrogen (PMN) in relation to different field and different sampling depth.

Source	DF	SS	MS	F	P
Blocks	19	0.47	0.02		
Field	2	1.73	0.86	38.74	0.00
Depth	2	0.16	0.08	3.55	0.03
Field*Depth	4	0.04	0.01	0.47	0.76
Error	152	3.39	0.02		
Total	179	5.79	1.01		

Appendix 4.10 Analysis of variance for fluorescein diacetate (FDA) activity in relation to different field and different sampling depth.

Source	DF	SS	MS	F	P
Blocks	19	0.71	0.04		
Field	2	44.82	22.41	947.25	0.00
Depth	2	0.94	0.47	19.87	0.00
Field*Depth	4	1.69	0.42	17.90	0.00
Error	152	3.60	0.02		
Total	179	51.76	23.38		

Appendix 4.11 Analysis of variance for phosphatase (PTS) activity in relation to different field and different sampling depth.

Source	DF	SS	MS	F	P
Blocks	19	3573	188		
Field	2	200557	100279	970.71	0.00
Depth	2	24437	12218	118.28	0.00
Field*Depth	4	12786	3196	30.94	0.00
Error	152	15702	103		
Total	179	257055	115984		