USE OF TREATED WASTEWATER FOR IRRIGATION AND ITS EFFECTS ON SOIL AND PLANT HEALTH UNDER *NATUURBOERDERY* FARMING SYSTEM

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[redacted]

THESIS SUBMITTED FOR THE DEGREE DOCTOR OF PHILOSOPHY IN AGRICULTURE (SOIL SCIENCE), DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING, SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

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DECLARATION

I, Pholosho Mmateko Kgopa, declare that this thesis hereby submitted to the University of Limpopo for the degree Doctor of Philosophy in Agriculture (Soil Science) has not been previously submitted by me or anybody for a degree at this or any other University, that this is my work in design and in execution and that all material contained herein had been acknowledged.

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DEDICATION

To my late father Ramolangoane Kanyane,

My beloved late mother Nthakwedi Kanyane,

My sisters Shatadi and Hunadi Kanyane.

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ABSTRACT

Due to high incidents of drought in the semi-arid regions of Limpopo Province, South Africa, the potential feasibility of using treated wastewater for irrigating vegetable crops had been researched and developed under the best practices of *Natuurboerdery* farming system. The investigation aimed at the development of soil and plant health management strategies for crops irrigated with treated wastewater that could ameliorate the ever-increasing demand for fresh irrigation water in the Province. Seven specific objectives, reduced to three overall objectives under *Natuurboedery* farming system were intended to investigate (1) the chemical and biological quality of treated wastewater used in irrigation with respect to disposal points and sampling period, (2) the responses of soil physico-chemical properties, heavy metal distribution and biological-soil-health indicators to irrigation with treated wastewater under field conditions and (3) the partitioning of cations and heavy metals in root, stem and leaf tissues of onion, tomato and a selected weed plant irrigated with treated wastewater. In each objective, unless otherwise stated, treatment effects were described at the probability level of 5%. In Objective 1, relative to the borehole water, treated wastewater had lower concentration of Ca, Mg, K, Na, Cl, HCO_3^-, SO_4^-, Zn, Cr and Pb, with temporal and spatial tendencies in accumulation of certain elements. In contrast, the treated wastewater had higher loads of pathogenic microbes that included bacteria (*Escherichia coli, Salmonella* spp., *Shigella* spp. and *Vibrio spp.),* protozoa *(Entamoeba histolytica)* and two helminths (*Schistosoma mansoni; Ascaris lumbricoides),* with temporal and spatial tendencies. In Objective 2, Al and Cr tended to be high in deeper soil layers (60-100 cm), whereas most essential nutrient elements and essential heavy metals (Cu, Fe, Mn, Ni, Zn, As, Cr and Pb) were contained in the upper soil levels (0-40 cm). The upper soil levels were also characterised by having substantial attributes of root health, namely, soil organic carbon, active carbon and potentially mineralisable nitrogen. In Objective 3, root, stem and leaf tissues of horseweed (*Conyza canadensis* L.), onion (*Allium cepa* L.) and tomato (*Solanum lycopersicum* L.) plants had different accumulation abilities for different metals, except for As. Contrary to the expectation, the onion bulb contained less test cations and heavy metals. In conclusion, in terms of chemical component at the time of sampling, as depicted from the water and soil samples, the treated wastewater was suitable for irrigation. However, in terms of pathogenic microbes, the treated wastewater was not suitable for irrigating crops intended for human consumption due to significantly higher loads than the permissible standards. Amongst all observed challenges, the reduction of pathogenic microbes should be given priority since it contravened existing national and international standards for using treated wastewater for in irrigation of crops intended for human consumption.

REVIEWED JOURNAL ARTICLES FROM THESIS

3. KGOPA P.M., MASHELA P.W. and A. MANYEVERE. 2018. Accumulation of heavy metals in onion (*Allium cepa*) plants irrigated with treated wastewater under field conditions. *Research on Crops* 19(1):62-67.
2. KGOPA P.M., MASHELA P.W. and A. MANYEVERE. 2018. Suitability of treated wastewater with respect to pH, electrical conductivity, selected cations and sodium adsorption ration for irrigation in a semi-arid region. *WaterSA* 44(4): 1-7.
1. KGOPA P.M., MASHELA P.W. and A. MANYEVERE. 2017. Temporal concentrations of heavy metals in treated wastewater and borehole water for irrigation in a semi-arid region of South Africa. *Transylvanian Review* 21:5419-5425.

CONFERENCE PRESENTATIONS

1. KGOPA P.M., MASHELA P.W. and A. MANYEVERE. 2019. Post treatment disposal effects on microbial quality of treated wastewater used for irrigation in a semi-arid area. 21-25 January 2019. Bloemfontein, South Africa.
2. KGOPA P.M., MASHELA P.W. and A. MANYEVERE. 2018. *Escherichia coli* counts in treated wastewater and borehole water used for irrigation at University of Limpopo experimental farm. African Combined Conference, 14-18 January 2018. Cape Town, South Africa.
3. KGOPA P.M., MASHELA P.W. and A. MANYEVERE. 2018. Partitioning of essential heavy metals in onion plants (*Allium cepa*) irrigated with treated wastewater under field conditions. African Combined Conference, 14-18 January 2018. Cape Town, South Africa.

4. KGOPA P.M., MASHELA P.W. and A. MANYEVERE. 2017. Heavy metal accumulation in different organs of tomato (*Solanum lycopersicum* L.) plants grown on treated wastewater irrigated fields. Faculty of Science and Agriculture Research Day, 19 October 2017, Bolivia Lodge, Polokwane.
5. KGOPA P.M., MASHELA P.W. and A. MANYEVERE. 2017. Soil active and organic carbon stock as affected by cultivation and irrigation with treated waste water.1st World Conference on Soil and Water Conservation Under Global Change (CONSOWA). 12-16 June, 2017, Lleida, Spain.
6. KGOPA P.M., MASHELA P.W. and A. MANYEVERE. 2017. Spatial and vertical distribution of selected physico-chemical properties in treated waste-water-irrigated fields. Combined Congress. 23-26 January 2017. Bela-Bela, Limpopo.
7. KGOPA P.M., MASHELA P.W. and A. MANYEVERE. 2016. Distribution of selected physico-chemical properties in soils following cultivation and irrigation with treated waste-water. Faculty of Science and Agriculture Research Day, 29 September 2016, Bolivia Lodge, Polokwane.

CHAPTER 1

RESEARCH PROBLEM

1.1 Background

1.1.1 Description of the research problem

Climate change had since exerted dire pressure on the ability of agricultural land and water resources to sustainably feed humanity without using innovative practices. Generally, floods, drought, storms and other types of extreme weather that threaten to disrupt agricultural production systems, and over-time shrink global food supply, could as well threaten humanity to extinction. Currently, over half a billion people live in places with increased desertification rates (COP17/CMP7, 2011), with almost more than 10 percent global population remaining undernourished (COP17/CMP7, 2011). The likelihood that food shortages could in future lead to increased cross-border migration, is being witnessed globally. Climate change predictions, with undesirable climate for crop husbandry, in southern Africa (COP17/CMP7, 2011; Griffin, 2012; Steyn *et al*., 2016), suggested future drastic shifts in rain distribution. Generally, fossil energy (COP17/CMP7, 2011), halogenated fumigant pesticides and various industrial chemicals (Kgakatsi *et al*., 2007) – past and emergent, are being singled out as principal contributors to climate change (Deressa and Hassan, 2005). Inland South Africa, predictions suggested that temperatures would by 2030 be as high as from 39 to 46ºC maximum (Weather SA, 2015). Under such conditions, the atmospheric demand would far exceed the water absorption capabilities for most crops to meet their water requirements and therefore, necessitating frequent irrigation. However, South Africa is a water-scarce country, with most regions classified as being semi-arid to arid climates (DWAF, 2013).

Globally, more especially in sub-Sahara countries, irrigated agriculture has been playing a major role in food security, job creation and wealth generation (FAO, 2015). In South Africa, competencies for food security, job creation and wealth generation had been assigned to agriculture, mining and tourism (National Development Plan (NDP), 2012). However, most areas where the National Development Plan framework is agro-relevant could be classified as being historically-marginalised rural areas, where land lies fallow due to low rainfall and lack of resources for implementing agriculture-related projects (Khan, 2014). Water-scarcity, described as the imbalances between the availability and demand (FAO, 2015), threatens food security most since at a global scale almost 70% portable water is allocated to agriculture (FAO, 2015). Limpopo Province, inland South Africa, has since 2012 intermittently declared drought-stricken (Mabelane, 2016).

1.1.2 Possible causes of the research problem

In South Africa, agriculture, mining and tourism have been singled-out as being central in speeding up development, particularly in the previously neglected marginalised rural communities (Kepe, 1999; NDA, 2001). A large number of mines that use high quantities of water were established in Limpopo Province (Kepe, 1999). Proliferation of the mining sector resulted in the establishment of resettlement areas and expansion of towns and cities around the mines. All these developments have had enormous pressure on limited natural water resources in the Province. Agriculture became the victim since as an industry, it could not compete with mining and tourism (Kepe, 1999).

1.1.3 Impact of the research problem

Climate change predictions had also suggested that by 2030, temperatures in the coastal and inland areas of South Africa could increase by 2 and 6º C, respectively (IPCC, 2014), with inland regions having low and irregular rainfall (IPCC, 2014). Consequently, inland areas with increased drought incidents, could suffer from increased evaporation and evapotranspiration rates. Historically, since inland South Africa is climatically suitable for the production of tropical/subtropical fruits and vegetables, irrigated regions have been viewed as the most important contributors towards gross domestic product (Machete *et al*., 2004). Therefore, predicted water deficits could invariably increase the cost of producing most such crops (Pascale *et al*., 2011), with the result that the majority of people from marginalised communities, both in rural and urban areas, could hardly afford such commodities. As the high cost of irrigation water escalate, some farmers would reduce the labour force and land size used for producing such commodities. Therefore, water deficits in agriculture would negate the expected achievements of the National Development Programme (NDP) presidential outcomes, which are led by the agriculture sector, namely, job creation, wealth creation and food security (NDA, 2012).

Agriculture as a sector in South Africa, had been contributing approximately 5% in economic growth through job creation throughout the value chain (DWAF, 2013). Although its contribution to economic growth had been declining gradually in the past years, the agricultural sector remains a very crucial sector despite its relatively small contribution to the overall South African economy (AgriSA, 2018). Most disadvantaged communities in the province depend on agricultural produce for their livelihood. In areas where dryland agriculture had been practiced, drastic crop yield reduction due to droughts resulted in abandonment of large tracts of land with high agricultural potential. Generally, drastic crop yield reduction is inversely proportional to food prices, which could eventually destabilise food security in the entire region (Durán-Álvarez and Jiménez-Cisneros, 2014).

1.1.4 Proposed solutions

In context of climate-smart agriculture (FAO, 2010), various resilient technologies to manage water deficits in irrigated agriculture are being researched and developed. One such initiative is the use of treated wastewater (AL-Hamaiedeh and Bino, 2010), which had been investigated for decades with potential for success under sandy-deep soils (Durán-Álvarez and Jiménez-Cisneros, 2014). In *Natuurboerdery* farming system, which is being *Natuurboerdery* developed by one prominent commercial farmer in Limpopo Province, the system had been replicated at the University of Limpopo (UL) Experimental Farm (ULEF). In *Natuurboerdery* farming system an attempt is being made to produce a set of best soil management practices that focus on soil health, plant health, and human health (Taurayi, 2011). Originally, the *Natuurboerdery* farming system was researched and developed using high quality water, with its success being extrapolated to ULEF under *Natuurboerdery* farming system treated wastewater without empirically-based information.

1.1.5 General focus of the study

The ULEF project using treated wastewater under *Natuurboedery* farming system *Natuurboerdery* had been successfully practised for over a decade, with rotations that comprised a three-year crop cycle, followed by as six-year fallowing (Taurayi, 2011). The current study was intended to investigate the chemical and biological quality of treated wastewater, along with its 10-year effects on soil health and its effect on plant health for the existing crops in comparison with conventional farming system where portable borehole water was used on adjacent fields.

1.2 Problem statement

Irrigation with treated wastewater under *Natuurboedery* farming system *Natuurboerdery* could serve as a resilient strategy to ameliorate the pressure on irrigation using portable water in context of climate-smart agriculture. Worldwide, most farmers view treated wastewater as a reasonable alternative for ameliorating the scarcity of irrigation water. Thus, due to high incidents of drought in sub-Sahara regions, including southern Africa, treated wastewater is increasingly becoming an attractive resource for use in irrigation. However, treated wastewater, in addition to heavy essential and non-essential metals, is viewed as a potential carrier of pathogenic microbes such as bacteria, protozoans and helminths, with potential dire consequences in agricultural produce. In 2010, the World Health Organisation (WHO) estimated that 420 000 fatalities emanated from food-borne contaminants in different countries, with a substantial percentage associated with produce where crops were irrigated with treated wastewater.

In the treatment plant associated with the ULEF study, the major focus was on separating physical material from water, with limited attention to chemicals and pathogenic microbes post a series of 16 ponds where chemicals were expected to settle to the bottom of the ponds, with surfaces fortified using metal-trapping clay. Neither the owners nor the farmer had much interest on the infrastructure post-units that separated water from physical material. For instance, at the initiation of the current study, the chlorine pump in pond 1 of the 16 ponds was dysfunctional. Although such dysfunctionality could result in challenges that include the failure of the treatment plant to decontaminate treated wastewater from pathogenic microbes, due to lack of empirically-based information, there was no need to expect the owners or farmer to act on the challenge of chlorine pump. Most household products have high cations, which are used as adjuvants. Such adjuvant, for example high Na content, could be undesirable since it could affect soil aggregate stability, thereby resulting in challenges that could be costly to ameliorate. Unacceptable concentration of heavy metals and high loads of pathogenic microbes, with temporal fluctuations, could render the water unsuitable for use in irrigated agriculture since in the long-run the induced challenges could be costly to rehabilitate. Identifying the challenges that the treatment plant could pose to the soil-plant environment and consumers could require information from empirically-based studies beyond the treatment plant. The ULEF site was ideal since *Natuurboerdery* farming system under treated wastewater had been running for over 10 years, with an adjacent field which was under conventional chemical farming that was irrigated using borehole water for comparative purposes.

1.3 Rationale of the study

The successful use of treated wastewater could drastically ameliorate the pressures faced by irrigated agriculture, especially in semi-arid regions of Limpopo Province. The strategy would be important since agriculture could not be expected to successfully compete with highly competitive economic sectors such as mining and tourism for portable water (Adewumi *et al*., 2010). The investigation of the chemical and biological quality status of the treated wastewater, along with temporal and spatial fluctuations, could provide information on whether the treatment plant was effective in decontaminating the water through various phases of treatment, thereby providing information on what needed to be improved and done to improve the status quo. The ULEF project could also provide an excellent opportunity to investigate the effects of treated wastewater in context of *Natuurboerdery* farming system in comparison with the usage of borehole water under conventional best farming practices with continuous cultivation and use of chemical fertilisers.

Natuurboerdery directly translated as nature farming, was established by ZZ2 due to challenges associated with conventional methods of farming (Taurayi, 2011). Mainly it first focused on improving soil health by improving soil carbon content which influences factors which affect plant nutrient availability. The main challenges ZZ2 experienced were recurrent pests and diseases which were becoming difficult to control with inorganic pesticides, large decreases in yields and unsustainable production outputs or returns to support production costs mainly due to the escalating cost of inorganic pesticides and fertilisers. There was a declining trend in yield even when soil chemical nutrient requirements for tomato production were at optimum (Van Zyl *et al.,* unpublished). ZZ2 also became aware of the growing customer demand for healthy food produced by ethically accepted methods while minimising environmental degradation (Taurayi, 2011). In 1999 and 2000 ZZ2 began the process of transforming from a conventional and inorganic chemical agriculture system on all its farming enterprises to a more ecologically-balanced farming system based on downscaling inorganic and upscaling organic inputs (Taurayi, 2011) Irrigation system for *Natuurboerdery* is designed according to soil type, which includes water holding capacity of the soil. The irrigation scheduling depends on evapotranspiration (ET_0) rates generated daily using from the DFM probe software (DFM Technologies, 2017), and the crop coefficients (K_c) for water requirements (Allen *et al*..1998). The *Natuurboerdery* farming system provides a suitable model for assessing whether the use of treated wastewater in agriculture could be expanded to other Municipalities in Limpopo Province, where such a resource had not been tapped.

1.4 Purpose of the study

1.4.1 Aim

The establishment of soil health management strategies for open-field agricultural systems irrigated with treated wastewater.

1.4.2 Overall objectives

The seven specific objectives of the study were to determine whether the treated wastewater from Mankweng Wastewater Treatment Plant (MWTP) site would have:

1. Suitable chemical quality attributes for irrigation as sampled at various disposal points of the irrigation systems over a six-month period.
2. Suitable biological quality attributes for irrigation as sampled at various disposal points of the irrigation systems over a six-month period.
3. Effects on the physico-chemical properties of the soil health at the ULEF site.
4. Effects on the heavy metal distribution of the soil health at the ULEF site.
5 Effects on the biological indicators of the soil health at the ULEF site.
6. Effects on partitioning of cations in root, stem and leaf tissues of onion, tomato and weed plants at the ULEF site.

7. Effects on partitioning of heavy metals in root, stem and leaf tissues of onion, tomato and weed plants at the ULEF site.

The above seven specific objectives were grouped according to similarity and reduced to three overall objectives, namely, to:

1. Investigate whether the spatial and temporal chemical and biological quality of the treated wastewater from Pond-16 exit, through the night-dam entry and exit points at the ULEF, would be similar to the portable borehole water serving as a standard..
2. Determine whether the effects of treated wastewater on the (1) physical and chemical properties of soil, (2) distribution of heavy metals in irrigated fields and (3) biological-soil-health indicators under *Natuurboerdery* and conventional farming systems would be similar.
3. Establish whether the distribution of cations and heavy metals in (1) shoot and leaf tissues relative to root tissues in onion and tomato plants and (2) the related accumulation in onion, tomato and horseweeds (*Conyza canadensis* L.*)* leaf tissues in soil irrigated with treated wastewater under *Natuurboerdery* farming system would be similar.

1.4.3 Hypotheses

1. The spatial and temporal chemical and biological quality of the treated wastewater from Pond-16 exit, through the night-dam entry and exit points at the ULEF, would be similar to the portable borehole water serving as a standard.

2. The effects of treated wastewater on the (1) physical and chemical properties of soil, (2) distribution of heavy metals in irrigated fields and (3) biological-soil-health indicators under *Natuurboerdery* and conventional farming systems would be similar.
3. The distribution of cations and heavy metals in (1) shoot and leaf tissues relative to root tissues in onion and tomato plants and (2) the related accumulation in onion, tomato and horseweeds (*Conyza canadensis* L.) leaf tissues in soil irrigated with treated wastewater under *Natuurboerdery* farming system would be similar.

1.5 Reliability, validity and objectivity

Reliability was ensured by the use of statistical levels of significance as derived through the use of analysis of variance. Validity was achieved through conducting experiments using factorial arrangements (Little and Hills, 1978). Objectivity was achieved by ensuring that the findings were discussed on the basis of empirical evidence in order to eliminate all forms of subjectivity (Leedy and Omrad, 2005).

1.6 Bias

Bias would be minimised by ensuring that the experimental error in each experiment was reduced through adequate replications. Also, treatments would be assigned at random within the selected research designs (Leedy and Ormrad, 2005).

1.7 Scientific contribution of the study

Findings of this study would be used to assess the potential use of treated wastewater under *Natuurboerdery* farming system, which is intended to enhance soil health, plant health and human health, with the view of expanding the practices to other district municipalities in the semi-arid regions of Limpopo Province, South Africa. Also, the findings would be expected to provide information on specific factors that need to be addressed in order to enhance the potential success of *Natuurboerdery* farming system in South Africa, with the view of improving food security, job creation and wealth generation challenges in context of climate-smart agriculture.

1.8 Structure of thesis

The thesis was presented in six chapters, where Chapter 1 introduced the research problem and Chapter 2 the Literature Review on the work done on the problem statement. Subsequent chapters (3, 4 and 5) addressed the three respective objectives. In Chapter 6, the significance of the findings was summarised and integrated to provide their significance, followed by potential recommendations for future research. The synthesis and conclusion in each chapter attempted to provide the take-home message with respect to the major findings in sustainable agricultural practices (*Natuurboerdery* farming system) involving irrigation using treated wastewater relative to unsustainable agricultural practices depicted by the block used exclusively for research (research field) irrigated with borehole water. Citations in text and references adopted the Harvard style. In the next chapter, the researcher reviewed literature on the work done and not yet done on irrigation with treated wastewater on various agricultural systems in relation to *Natuurboerdery* farming system.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The University of Limpopo Experimental Farm (ULEF) uses treated wastewater from the Mankweng Wastewater Treatment Plant (MWTP). The MWTP is situated in a semi-arid area, with soils comprising Bainsvlei and Hutton forms. The MWTP focuses on separating water from physical material that include feces, tissues and other household wastes. The chemical and biological components are handled through a curing treated water in a series of 16 ponds, with the first pond equipped with a chlorine pump, which was not functional at the time of sampling. After Pond 16, most of the treated waste water is channelled to an earthen dam for further curing prior to discharging into the wild, whereas a small portion is channelled to the night-dam, for use at the ULEF. Consequently, the focus of this review was on the quality (chemical and biological) of treated wastewater, its potential effects on soil and plant health, with a view of establishing the potential ramifications of using such water for irrigation on fields under *Natuurboerdery*.

2.2 Synopsis of *Natuurboerdery* farming system

Natuurboerdery farming system is defined as farming in harmony with nature, using best science and technology. The first distinguishes it from industrial farming, the second from organic farming. *Natuurboerdery* farming system does not only comprise the cultivation methods, but the entire farming operation and indeed at the value chain level. Figure 2.1 shows a schematic diagram of how the *Natuurboerdery* farming system is differentiated from other mainstream farming approaches.

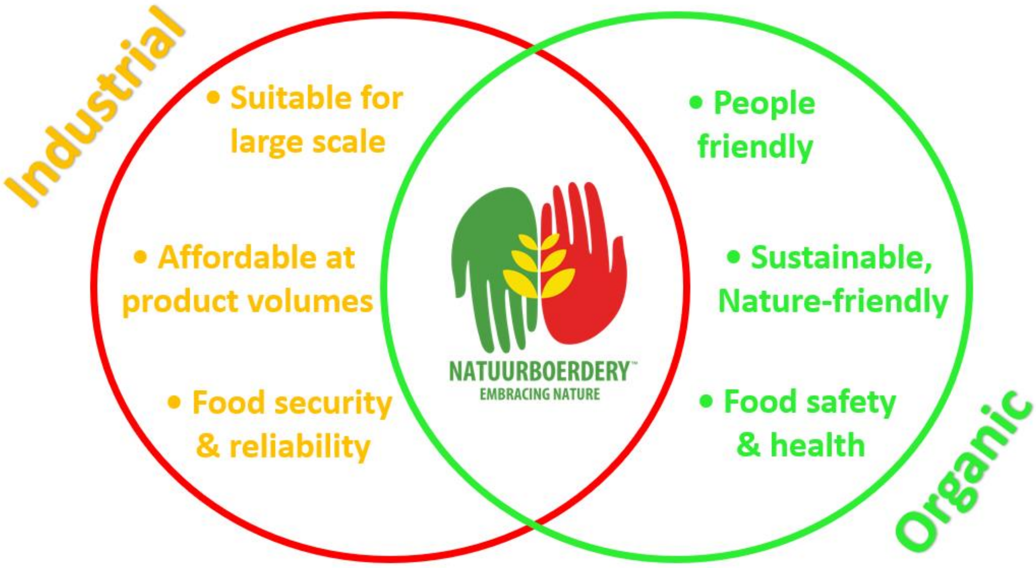

Figure 2.1. *Natuurboerdery* differentiated from Organic and Industrial agriculture (Provided by ZZ2).

2.2.1 Biodiversity

Natuurboerdery farming system focuses on the principles of biodiversity, from the microscopic scale, the landscape scale. Microbial diversity in the soil is enhanced with the use of compost, compost tea and probiotics. In the fields, green belts and buffer zones are maintained to serve as habitat for beneficial insects and other wildlife. At a landscape scale, conservation initiatives are protecting important and sensitive ecosystems from degradation. In some cases, degraded ecosystems are rehabilitated at great cost to recover the ecosystem services that support agriculture, such as sustainable water catchment areas. ZZ2 has been clearing invasive plant species, which are used for various purposes intended to improve soil health. The clearing of blue gum (*Eucalyptus grandis*) and black wattle (*Acacia mearnsii*) around water catchment areas enhanced the return of natural springs which had previously dried up, thereby increasing the available surface and underground water resources (Taurayi, 2011). The invasive noxious weed lantana (*Lantana camara*) is cleared for use in the production of EM lantana which is used for managing nematode population densities *in lieu* of synthetic chemical nematicides. The latter had been withdrawn from the agrochemical markets due to their environment-unfriendliness.

2.2.2 Water conservation

The adoption of conservation techniques as articulated above has improved water conservation and water use efficiency which has resulted in increased available surface and ground water for irrigation and domestic use by at least 30%. Under *Natuurboerdery* farming system, irrigation scheduling is strictly adhered to and is enhanced by empirically-based techniques that include uses of digitised weather stations, irrigation profile holes, tensiometers and moisture probe meters on all farms and cultivated fields. In recent years the use of GIS tools in drones and their integration with various data sensors in a cloud-based data management system has improved the ability to sustainably manage scarce water sources.

2.2.3 Plant protection and nutrition

The "nature friendly farming practices" component of *Natuurboerdery* farming system comprises, among other things, plant nutrition and protection that aims at reducing the use of inorganic fertilisers and chemical pesticides, while promoting soil health and plant health. Plant protection uses a holistic approach that involves pest and disease monitoring and management. Monitoring involves scouting, use of indicator plants and pest trapping devices. *Natuurboerdery* farming system manages pests and diseases to under economic population threshold levels rather than control (eliminate), which is the dominant practice under the purely chemical, conventional farming systems. The integrated management system of *Natuurboerdery* farming system include the use of biological agents such as predators and parasites, the use of natural products such as fermented plant extracts, compost teas, organic pest repellents and disease suppressive compost for soil-borne pathogens. However, when pests or diseases are escalating beyond the damage threshold levels, "soft" chemical pesticides are used for quickly reducing the population densities to below the damage threshold levels. The use of industrial farming agricultural chemicals is closely monitored and managed using a self-developed point-scoring system. Decisions on plant nutrient management are based on soil, soil water and leaf sap analysis for mineral ions, pH, EC and brix levels. Under *Natuurboerdery* farming system, operational decisions are based on careful analysis of large data sets.

2.2.4 Food health

High nutritional value and long shelf-life are intended to differentiate ZZ2 products in order to satisfy customer needs and demands. Tomatoes produced under *Natuurboerdery* farming system has a shelf-life of 21 days when compared to shelf-life of 7 days under conventional systems (Taurayi and Nzanza, 2010). At ZZ2, the slogan "healthy soil is our passion and food health our promise" (ZZ2, 2010), serves as an indelible guideline in farming operations. Food produced under

Natuurboerdery farming system aims at having zero chemical residues. The latter is achieved by a soft approach to plant protection that favours the use of organic or natural products and preferential use of the least toxic pesticides if and only if population densities escalate above the damage threshold levels. Should the need arise for a chemical pesticide to be used during harvesting, the waiting period is strictly adhered to. As a quality control measure, random samples are regularly collected unannounced from harvesting farms by the Marketing and Research and Development departments and submitted for real time analysis of chemical residues, with positive samples resulting in consequences. Also, sanitation on-farm, at packaging and processing facilities and during transit to markets, is practiced without compromise.

2.2.5 Carbon fixing and climate change

Soil health is the most fundamental theme of change to *Natuurboerdery* farming system practices that aim at building diverse and complex healthy soil ecosystems. Soil carbon content has significantly increased across ZZ2 farms from an average low content of 0.3 to 0.8% and has increased to approximately 1.5% on some of the tomato fields and 7% on avocado orchards, representing an increase of 80% to 85%, respectively. The increases had mainly been attributed to the widespread use of organic soil amendments, comprising compost, organic mulches, effective microorganisms (EM), compost teas and cropping systems that include fallowing and the use of cover crops. Generally, the philosophy of *Natuurboerdery* farming system farming is to achieve on average from 3 to 5% carbon content on all cultivated lands to encourage soil fauna and flora, improve soil structure and create a more stable system. Soil improvement activities under *Natuurboerdery* farming system are, thus, fixing a very large amount of CO_2 in the soil, contributing not only to soil health, but also towards mitigating the global climate crisis.

2.3 Suitability of treated wastewater for irrigation

Treated wastewater for use in irrigation has clearly defined standards for permissible limits of contamination (DWA, 2010; Pestcod, 1992). Above the set limit, treated wastewater could be harmful to soil by transforming its soil health characteristics (Jeong *et al*., 2016). In such cases plant produce could be affected, resulting in a health-hazard that affects the entire food chain, as shown previously in pesticides (Clarke, 1997). Consequently, irrigation with treated wastewater of acceptable quality is a pre-requisite for ensuring the sustainability of the soil (Mohammad and Mazahreh, 2003). Effective treatment plant facilities should be able to remove harmful chemical, physical and biological materials from the effluent prior to being used for irrigation purpose (Angelakis and Snyder, 2015). The treatment facilities should fully comply with the standards of the international bodies such as the World Bank, the Food and Agriculture Organisation (FAO) of the United Nations (UN) and the World Health Organisation (WHO), along with those of relevant local authorities, for example, the South African Department of Health (2004).

2.3.1 Chemical quality

Storage of wastewater post-treatment could motivate improvements in water management from the final point of treatment to the point of discharge (Qadir *et al*., 2010). Improvements could include segregation of chemical pollutants at different points of the plant, thereby reducing various risks on irrigated fields and cultivated plants. Chemical characteristics of irrigation water primarily refer to the content of salts in water and various health-threatening elements. Major chemicals in the form of salts include the chlorides, sulphates and carbonates (Zumdahl and Zumdahl, 2014), which have the potential of inducing soil salinity (Salcon, 1997; Watling, 2007), especially in areas such as Limpopo Province with high evaporation rates.

Salinity in treated wastewater effluent could be monitored by assessing the electrical conductivity of water (EC_w), where $EC_w \geq 3.0$ dS/m is deemed unsuitable for irrigation (FAO, 2010), depending on crop type (Maas and Grattan, 1999; Shannon and Grieve, 1999) and soil type (Schipper *et al*., 1996). Water pH could also serve as a guide for assessing its suitability for irrigation (Bauder *et al*., 2011; De La Mora-Orozco *et al*., 2017). The normal pH range for irrigation water is from 6.5 to 8.4 (Ayers and Westcott, 1985; Bauder *et al*., 2011). Irrigating with water outside the normal pH range might induce nutritional imbalances or could be indicative that such water contains carbonate salts, which could result in unwarranted soil risks. For instance, high carbonates cause Ca and Mg ions to form insoluble minerals, thereby leaving Na as a dominant ion in soil solution (Bauder *et al*., 2011), to the detriment of C3 plants which do not use Na as an essential element.

Treated wastewater could introduce imbalances in soil Ca, K, Mg and Na cations (Angelakis *et al*., 2003; Rusan *et al*., 2007). Such imbalances could result in risks such as fluctuation in pH of treated wastewater (NRCS, 2015; Rusan *et al*., 2007), thereby inducing high soil pH (Schipper *et al*., 1996). Under such conditions, Ca, Mg, N, Fe, Mn, B, Cu and Zn might be deficient (Mosse *et al*., 2011), whereas P, K, S and Mo could be available in phytotoxic quantities (Christen *et al*., 2010). High Na concentration in irrigation water could lead to water and soil characterised as having sodic properties, measured through Sodium Adsorption Ratio (SAR) (Ayers and Westcot, 1985). The SAR (milliequivalents/litre) defines sodicity in terms of the proportion of Na to the sum of Na, Ca and Mg in a given sample, using the formula:

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

Generally, should the soil be irrigated with water containing SAR higher than 9 milliequivalents/litre for years, Na would eventually displace Ca and Mg in the soil (Haritash *et al*., 2016). The latter would cause a decrease in the ability of the soil to form stable aggregates and the eventual loss of soil structure and tilt (Haritash *et al*., 2016). Also, forces that bind clay particles together are disrupted when too many Na ions interspersed them. Generally, when the latter occurs, clay particles expand, resulting in swelling and soil dispersion (Shainberg and Lete, 1984). The latter challenges would decrease infiltration and permeability of the affected soil, resulting in crop production-related challenges (Ayers and Bronson, 1975).

Treated wastewater from domestic uses is usually high in Cl^-, SO_4^{2-}, PO_4^{3-}, CO_3^{2-} and NO_3^{3-} anions and NH_4^+ ion (Wagner, 2015). The latter could be due to composition of human diets, household products and chemical residues from runoff water (Wagner, 2015). The traditional processing of effluents tends to retain Cl^- constant, but it could be decreased through ion exchange and reverse osmosis (USEPA, 1999). In municipality water, Cl^- ion above 10 $mg.l^{-1}$ was shown to be harmful to certain agricultural crops (Naidoo and Olaniran, 2014). The presence of most anions is harmful to plants, animals and the overall biodiversity of soil (De Almeida *et al*., 2015). Similarly, high NH_4^+ concentration causes plant injuries (Krupa, 2003), with increased

N in plant tissues resulting in loss of drought- and frost-tolerance (Krupa, 2003). Excess NH_4^+ in water bodies could be toxic to aquatic organisms and when ultimately converted to NO_3^-, the material could be harmful to humans (Dong and Reddy, 2012). Some risks include associations with ovarian and bladder cancers, with blue baby syndrome to babies less than six months and unborn foetus (Gao *et al*., 2012).

In contrast, as NO_3^--N in irrigation water, N could be regarded as an asset for crop production (DeLaune and Trostle, 2017). An increase in NO_3^- in soil could improve microbial activities (Zhen *et al*., 2014), as shown increased soil respiration rates, effective microorganisms and soil enzyme activities (Gilliam *et al*., 2011; Zhen *et al*., 2014). Cusack (2013) showed that there were some links between soil NO_3^- and decomposition enzyme activities. However, NO_3^- is a common contaminant of surface- and underground-water, with the potential of causing health problems in infants and animals, along with eutrophication that could wipe out animal life in dams (Wang *et al*., 2015). Also, high NH_4^+ and NO_3^- concentrations from treated wastewater could lead to soil acidification, with excessive NO_3^- leaching into underground water sources (Wallenstein *et al*., 2006).

Most risks associated with uses of treated wastewater include excessive additions of heavy metals in soil, posing risks to soil ecosystems (Khaskhoussy *et al*., 2015; Mapanda *et al*., 2005; Pinto *et al*., 2010). Heavy metals, technically termed environmental toxins, have high atomic density, usually above 6 $g.cm^{-3}$ (Duffus, 2002). A number of heavy metals are essential nutrient elements when occurring in trace quantities (Salem *et al*., 2014), but tend to be very toxic when concentrations are slightly above the maximum required limit (Tchounwou *et al*., 2012). High heavy metals in both soils and plants have undesirable effects to consumers (Khan *et al*., 2007).

Generally, Cu, Zn and Cr heavy metals are essential micronutrient elements with low limits towards toxicity in plants (Chronopoulos *et al*., 1997). Consequently, heavy metal in produce have FAO- or WHO-established toxicity limits (Chronopoulos *et al*., 1997). The overriding factor is that heavy metals are persistent pollutants and non-biodegradable in water and when added to soil through irrigation could be absorbed and compartmentalised in plant tissues (Sharma *et al*., 2009). Plants exposed to high levels of heavy metals experience oxidative stress that could lead to cellular damage (Singh and Agrawal*,* 2012), with human health being at risk when produce from such plants are consumed. Heavy metals also affect growth, morphology and metabolism of soil microorganisms, through functional disturbance, protein denaturation or destruction of the integrity of cell membranes (Leita *et al*., 1995). Soil microbes are principal decomposers of soil organic matter and any disturbance in biodiversity and abundance of microbes, reduces the rate of nutrient cycles and by extension, plant health (Xie *et al*., 2016). Parveen *et al*. (2014) observed that Fe, Zn and Mn concentrations in plant organs rose continuously when plants were irrigated with treated wastewater. Similarly, Mapanda *et al*. (2005) observed high Cu, Zn, Cd, Ni, Cr and Pb concentrations in soils irrigated with treated wastewater than in fields irrigated using other sources.

2.3.2 Biological quality

Standards for treated wastewater use had been developed to minimise risks associated with soil, plant and human health (Al-Nakshabandi *et al.,* 2007; Biswas, 1986). The use of treated wastewater is associated with a wide range of pathogenic microbes, classified as bacteria, protozoans and helminths (Al-Lahham *et al.*, 2003; Margane and Steinel, 2011). The biodiversity and density of pathogenic microbes in treated wastewater can differ on a regional scale, depending on sources and prevalence of infections in the resident population that produces the wastes (Petterson and Ashbolt, 2003). Seasonal changes (Wemedo *et al*., 2012), socioeconomic conditions of resident communities (Gerba and Rose, 2003) and sampling time and frequency have been the major sources of variation on pathogenic microbe counts.

In treated wastewater, pathogenic coliform bacteria, *Escherichia coli*, S*almonella* spp., S*higella* spp. and enterococci, are the most common (Feachem *et al*.,1983). In addition to the influence of resident communities, a study on seasonal effects at the Dandora Sewage Treatment Plant in Kenya suggested that lower microbial loads could occur during the rainy seasons (Musyoki *et al*. (2013). In contrast, higher bacterial counts were observed during dry than wet seasons (Hodgson, 2007; Wemedo *et al*., 2012). Low counts during the rainy season could be due to the flowing water as opposed to stagnant water during dry seasons.

Helminths are intestinal parasites that include nematodes, tapeworms, hookworms, roundworms and whipworms (Feachem *et al*.,1983). Helminths produce ova with the ability to survive extended harsh conditions in water and soil (Crittenen *et al*., 2005). Helminths in treated wastewater can have fatal effects on humans when ingested through agricultural produce (Mara and Horan, 2003). As observed in other pathogenic microbes, helminthic load counts in treated wastewater also have seasonal variation (Halama *et al*., 2011).

2.4 Effects of treated wastewater on soil health indicators

In general, soil is a complex medium containing minerals and particles from chemical and physical weathering of rocks, humus, microorganisms, insects, water and air (Atlas and Bartha, 1997). Soil is evaluated on its capacity to support agriculture using concepts such as fertility, quality and health (Mursec, 2011). Soil fertility refers to the ability of a given soil to sustain agricultural plant growth, with sustainable agriculture encompassing the need to meet the current needs without compromising the productive potential for future generations (Cardoso *et al*., 2013). Cultural practices are potentially intended to promote the soil to attain economic and environment-sustainable yields, while retaining the recovery and management of soil health, intended to keep the soil alive and balanced. Soil health had been viewed as the continued capacity of soil to function as a vital living system, within the ecosystem and land-use boundaries. In order to sustain biological productivity, it is important to promote the quality of air and water environments and maintain plant, animal and human health (Doran and Safley, 1997), a view which was not different to that of describing soil quality (Karlen *et al*., 1997). Both soil health and quality were previously defined as the sustainability of a soil for a particular use (Gregorich *et al*., 1994).

Methods to quantify soil health include assessment of changes in selected soil characteristics over time (Doran, 2002). Soil health indicators should ideally correlate well with the ecosystem and integrate the chemical, physical and biological properties (Mursec, 2011). Typical soil fertility tests exclusively look at the chemical constituents, whereas soil health should attempt to integrate the physical, chemical and biological properties. Most soil health indicators had been descriptive and could be used in field assessment as part of a health card (Doran and Parkin, 1996). However, soil health indicators could be quantified using laboratory methods, with indicators being selected on the basis of soil use and management, soil characteristics, environmental circumstances and accessibility to a range of users (Cardoso *et al*., 2013).

2.4.1 Physical indicators of soil health

The soil physical properties include texture, aggregate stability, bulk density, hardness and porosity. The former could be correlated with hydrological processes such as erosion, aeration, water-holding capacity and water infiltration (Schoenholtz *et al*., 2000).

Soil texture: Soil texture comprises particle sizes that constitute the soil, with a mixture of differently sized minerals being described using textural classes (Murano *et al*., 2015). The relative amounts of clay, silt and sand affect most soil physical, chemical and biological processes (Roncucci *et al*., 2015). Size distribution of particles could also affect pore sizes that govern important processes of water and air movement in soil. Processes like water infiltration, permeability, water retention, aeration, nitrate leaching and denitrification could be affected greatly by pore size (Moebius-Clune *et al*., 2016). Although soil texture hardly changes over time, the total amount of pore space can be greatly affected by management processes (Gugino *et al.,* 2009). Application of wastewater to different loam soils within 2.5-19 years improved soil water retention capacity and reduced porosity (Dawes and Goonetilleke, 2004), which could be attributed to addition of organic matter from treated wastewater. Irrigation using treated wastewater in tomato fields with sandy soil, reduced soil hydraulic conductivity, porosity and water retention capacity, but increased bulk density of surface soil (Aiello *et al.*, 2007). The varying observations suggested that irrigation with treated wastewater could be beneficial or detrimental depending on the geographic region and the degree of treatment in treated wastewater (Razzaghi *et al*., 2016).

Aggregate stability: Aggregate stability of soil is a critical variable, affecting most physical properties such as water infiltration and water-air ratio, but also biological activities and subsequently, plant growth (Lynch and Bragg, 1985). Also, aggregate stability can be viewed as one of the principal indicators in assessing soil structure (Six *et al*., 2000) since management practices could influence soil structure (Albiach *et al*., 2001). Following short-term irrigation trials with treated wastewater in both pot and open field experiments, a decrease of 40% in aggregate stability was reported (Hasan *et al*., 2014). Similar results were observed inn a long-term irrigation with treated wastewater in Israeli, where aggregate stability was low when compared with tapwater (Schacht and Marschner, 2015). The reported decreases in aggregate stability were both correlated to the dispersing effects brought about by high salt concentrations that were added with treated wastewater (Hasan *et al*. 2014; Schacht and Marschner, 2015).

Field penetration resistance: Penetrometry and bulk density (BD) are the most common compaction measures (Freitag and Barnes, 1971). Generally, it had been shown that when the penetrometer reading was above 300 psi, most roots cannot penetrate the soil (Duiker, 2002). Increases in soil BD might alter root configuration and root-soil interactions (Lipiec and Stepniewski, 1995). Soil physical properties such as infiltration and water-holding capacity benefit from wastewater irrigation (Yerasi *et al*., 2013). The use of wastewater can improve soil physical properties such as BD, water retention and hydraulic conductivity (Kharche *et al*., 2011), with Mojiri (2011) reporting a decrease in BD after irrigation with wastewater. The increase in BD could be due to some total dissolved solids and total suspended solids that were added to soils through wastewater, with the two different solids causing a decrease in porosity (Abedi-Koupai *et al*., 2006).

2.4.2 Chemical indicators of soil health

Chemical indicators of soil health had been useful in terms of the availability or unavailability of essential nutrient elements to plants. Soil pH, cation exchange capacity, organic carbon and nutrient element levels are the primary driving force of nutrient availability in soil solutions in relation to production of high crop yield (Kelly *et al*., 2009; Schoenholtz *et al*., 2000). In a study undertaken in Morocco, increases in pH, EC, P and N were reported when treated wastewater was used for irrigation (AL-Jaboobi *et al*., 2014). Similarly, EC, SAR and cations (Ca, K, Na, Mg) increased following irrigation with treated wastewater on sandy loam soils of Riyadh, Saudi Arabia (Al-Othman, 2009).

Soil pH is an important soil variable impacting on crop nutrient availability and soil microbial activities (Hill, 2002). Therefore, careful monitoring of soil pH might assist in predicting crop productivity. Plants have an optimum soil pH range for effective growth and potential maximum productivity (Hill, 2002). Generally, when the pH of a soil solution is increased above 5.5, essential plant nutrient elements are made available in larger quantities to most crops to a certain extent (McLean, 1982). For instance, N, a chief nutrient element for most plant species could be available to plants in the nitrate-N form at specific soil pH (Spector, 2001). In highly acidic or alkaline soils, organic matter mineralisation could be slowed down or completely curtailed because of poor bacteria-linked microbial activities (Rietz and Haynes, 2003). Bacterial populations and activities decline at low pH levels, whereas fungi which could adapt to a wide range of pH could be least affected by pH changes. Most other microorganisms in soil and/or treated wastewater could also have optimum pH range for survival and function (Rietz and Haynes, 2003).

Some of positive effects that treated wastewater is known to confer are on chemical properties of the soil (Rusan *et al*., 2007). Treated wastewater affects macro-nutrient and micro-nutrient elements for plant growth, soil pH and cation exchange capacity (Mzini, 2013). For soils with known nutrient element deficiencies, application of wastewater becomes a remedial and necessary source. Kiziloglu *et al*. (2008) demonstrated a decrease in soil pH on calcareous soils where treated wastewater was applied. The decrease in pH was beneficial as it increased the solubility of exchangeable cations like Ca and Mg (Muhammad and Mazahreh, 2003). However, irrigation with wastewater could also result in disturbing chemical effects following irrigation (Abedi-Koupai *et al*., 2006; Al-Hamaiedeh and Bino, 2010). However, continuous irrigation with wastewater could lead to increased alkalinity overtime as pH values close to 8 were observed in a study meant to investigate effects of wastewater on soil and plants (Pinto *et al*., 2010). Alkalinity could disturb the release of exchangeable cations during mineralization of organic matter (Woomer *et al*., 1994). Others (Garcia *et al*., 1996; Vazquez-Montiel *et al*., 1996) observed an increase in salinity as a result of high EC in treated wastewater. An increase in EC from 0.89 to 0.94 dS/m was observed when following irrigation with wastewater in Morocco (AL-Jaboobi *et al*., 2014).

2.4.3 Heavy metal distribution

A number of studies (Ismail *et al*., 2014; Khan *et al*., 2008; Mapanda *et al*., 2005) were undertaken to assess the accumulation of heavy metals in soils subjected to irrigation with treated wastewater. Khan *et al*. (2008) investigated the potential accumulation of heavy metals in soils and food crops irrigated with treated wastewater in China and observed higher concentrations in both soils and plant tissues, which suggested potential health risks. Similarly, Mapanda *et al*. (2005) observed high concentrations of Cu, Zn, Cd, Ni, Cr and Pb in soils irrigated with treated wastewater than in fields irrigated with water from other sources. Heavy metal contamination in excess to the permissible levels could invariably be toxic to plants, humans and animals (NRCS, 2000). Globally, the most severe contamination related to heavy metals from irrigation with treated wastewater include Cd, Pb and Ni (Dere *et al*., 2006; Herselman and Steyn, 2001; McLaughlin *et al*., 2000; Rattan *et al*., 2005; Singh and Kumar, 2006). Contamination of soil by such heavy metals could invariably lead to elevated uptake by plants (Yerasi *et al*., 2013), which eventually affect internal quality of plant produce (Muchuweti *et al*., 2006).

2.4.4 Biological indicators

Biological indicators include organisms that form the soil food web and are responsible for decomposition of organic matter and nutrient cycling (Doran and Parkin, 1996). Biological indicators had been shown to be a function of living organisms that included flora and fauna, both external and internal to the soil (Gugino *et al*., 2009). Additionally, such microbes are necessary for recycling carbon to the atmosphere and assure the continuation of photosynthesis, along with nutrient mineralisation for plant and microbial nutrition (Doran and Parkin, 1996). Healthy soils have the capacity to keep the listed processes active in a sustainable manner. Generally, microbial indicators are more vulnerable than physical and chemical attributes to environmental changes such as soil use and management (Masto *et al*., 2009). Therefore, microbial indicators could predict any disturbance in the sustainability of the environment. Biological indicators comprise measures such as particulate organic matter, soil respiration, microbial activities and diversity (Doran and Parkin, 1996).

Soil organic matter is a nutrient sink and source that could enhance soil physical and chemical properties and could also stimulate biological activities (Mursec, 2011). Changes in land use, management practices and on-farm inputs, could affect stocks and storage of organic carbon in soils (Sardiana *et al*., 2017). Soil microorganisms are highly sensitive indicators of soil health since they respond to any stimulus in short-time scales relevant to land management (Mursec, 2011). The microbial activities could mostly be influenced by soil physio-chemical and ecological interactions (Mursec, 2011; Powlson *et al*., 2001). A number of studies (Bedbabis *et al*., 2014; Galavi *et al*., 2010; Hasan *et al*., 2014) reported contradicting results on organic matter accumulation under irrigation with treated wastewater. Bedbabis *et al*. (2014) reported an increase in organic matter after a four-year irrigation period. In contrast, decreases that correlated with aggregate stability were reported in a one-season experiment at the University of Jordan Research Station (Hasan *et al*., 2014).

Soil active carbon had been used as an indicator of the fraction of organic matter that could be readily available as an energy source for microbes. Active carbon content was shown to be the most sensitive and reliable indicator for assessing the impact of different soil management techniques on soil quality for short- and long-term basis

(Melero *et al*., 2009; Oyonarte *et al*., 2007). Microbial biomass in soil had been defined as that part of organic matter in soil which constitute living microorganisms smaller than the 5-10 μm^3 range (Yang *et al.*, 2016). The biomass can be dominated by fungi and bacteria which should therefore, be given much attention. Microbial biomass can have significant effects in soil health assessments as it had been singled out as one of the organic matter fractions that could be highly sensitive to management or pollution (Jenkinson *et al*., 1976; Mursec, 2011). Potentially mineralisable nitrogen (PMN) had been singled out as an indicator of the capacity of soil microbial community that could mineralise the N tied up in complex organic residues in dead entities into plant available forms (Gugino *et al*., 2009). The root health assessment has been viewed as the measure of quality and function of roots, providing symptoms and damage by some root pathogens, including *Fusarium oxysporum* and plant-parasitic nematodes (Murillo-Williams, 2007).

Schipper *et al*. (1996) could not find changes in microbial and biochemical activities in field irrigated with treated wastewater. However, Chen *et al*. (2008) observed that extended irrigation with treated wastewater could enhance enzyme activities in soil. Similarly, Truu *et al*. (2009) observed that soil irrigated with treated wastewater could have increased microbial activities, with the exception that microbes that displayed phosphatase activities did not respond significantly to treated wastewater. Heavy metal concentrations in soils from irrigation with treated wastewater are closely associated with the biological make-up of the soils (Abedi-Koupai *et al*., 2006). High concentration of toxic heavy metals in the soil alters the habitat of microbes and, thus reduce their population densities (Sharma *et al*., 2014), thereby negatively affecting decomposition and nutrient cycling.

2.5 Distribution of cations and heavy metals in organs of plants irrigated with treated wastewater

2.5.1 Cation distribution in organs of crops and weeds

Treated wastewater could, depending on various factors, increase crop yield due to added concentrations of essential macro-nutrients such as Ca, Mg and K (Day *et al*., 1981; Rusan *et al*., 2007). Different crops, including cotton and tomato plants, had improved yield and higher biomass at flowering stage (Day *et al*., 1981; Mirsa *et al*., 2009). Also, irrigation with treated wastewater led to a significant increase in Na content in consumable parts of sugar beet bulbs and potato tubers, in a study conducted in Czechoslovakia Republic (Zavadil, 2009). However, Al-Zu'bi and Al-Mohamad (2008) in Jordan did not observe increases in yield of tomato plants irrigated with treated wastewater. Similarly, in Ghana, onion plants were observed to have low Ca concentration under various levels of treated wastewater (Adotey *et al*., 2009). The reported contradictions could be due to different qualities of treated wastewater as affected by the employed treatment methods (Mzini, 2013). The quality of wastewater used does not only affect yield, but also the quality of plant produce (Mzini, 2013). Consumer satisfaction on quality of produce is dependent on sensory and observatory effects (Grunert, 2005). The effects include the firmness of a produce in question, including colour or appearance.

2.5.2 Heavy metal distribution in organs of crops and weeds

Heavy metals enter the human body mainly through inhalation or consumption of foodstuff, including vegetable crops (Khan *et al*., 2007). Information on heavy metal accumulation in vegetable crops irrigated with treated wastewater is available. Rusan *et al*. (2007) observed increases in Pb and Cd contents in barley crops irrigated with wastewater, with the concentration increasing yearly after 10 years of irrigation. Also, Cd previously measured on spinach and lettuce leaf tissues in Iran were observed to be eight times more than those permitted by international standards (FAO, 2010; Qishlaqi *et al*., 2008). Similarly, Cd, Pb and Zn concentrations in different vegetable crops in edible parts exceeded 0.1, 0.3-0.5 and 40 ppm limits, respectively, as stipulated in South African Legislation and regulations made under the Foodstuffs, Cosmetics and Disinfectant Act, Act number 54 of 197 (Government Gazette,1994; Malan *et al*., 2015). Ingestion of high amounts of heavy metals could result in various illnesses and toxicities in humans (Malan *et al*., 2015).

Weed species are often reported as good accumulators of heavy metals in general (Krishnasamy et al., 2005; Patel, 2013). *Fioria vitifolia* and *Ricinus communis* were reported to contain Pb and Ni in high concentrations in leaf tissues under irrigation with treated wastewater (Krishnasamy *et al*., 2005). High Pb accumulation was observed in shoot tissues of *Vetivieria ziznoides,* whereas *Typha latifolia* and *Acorus calamus* accumulated high Pb concentration in roots after irrigation with treated wastewater Patel, 2013). In contrast, Ni and Pb were reported to be below detectable limits in different organs of *Syringa amurensis* when irrigated with treated wastewater (Kordlaghari *et al*., 2015).

2.6 Work not yet done on problem statement

The efficacy of the biological and chlorine treatments in a series of 16 ponds at the MWTP, along with storage and the night-dam at the ULEF on quality of wastewater and soil and plant health have not been investigated under *Natuurboerdery* farming system. At the time of conducting the study, the chlorine station was dysfunctional, whereas the ULEF was under *Natuurboerdery* farming system a three-year continuous cropping under *Natuurboerdery* agriculture, with a subsequent six-year fallowing, with the focus being on soil health, plant health and human health (Taurayi, 2011). In the next chapter, the researcher investigated whether the spatial and temporal chemical and biological quality of the treated wastewater from Pond-16 exit, through the night-dam entry and exit points at the ULEF, would be similar to the portable borehole water serving as a standard.

CHAPTER 3
CHEMICAL AND BIOLOGICAL QUALITY OF TREATED WASTEWATER ALONG THE DISPOSAL SYSTEM TO THE IRRIGATED FIELDS

3.1 Introduction

Water quality is indispensable for soil health attributes that are prerequisites for the production of high quality crops (Kibblewhite *et al*., 2008). However, the increasing demand for quality water for irrigation and repeated incidents of drought in South Africa (DWA, 2010), dictate that other sources such as treated wastewater and poor quality borehole water be widely used as alternatives for irrigation in various cropping systems. Worldwide, most farmers view treated wastewater as a reasonable alternative for ameliorating the scarcity of irrigation water (Pinto *et al*., 2010). However, treated wastewater could carry excessive chemical wastes and a high load of pathogenic microbes, which could individually or collectively enter the food chains, with dire consequences (Gugino *et al*., 2009). Generally, the quality of treated wastewater depends to a great extent on the efficacy of the municipality treatment plants, the nature of chemical wastes added during usage, post-treatment handling and the subsequent disposal prior to use in irrigation (Pedrero *et al*., 2010). Effluents from households, restaurants and hospitals discharge acidic and basic chemical compounds, along with pathogenic microbes, which could eventually be detrimental to agricultural soils and consumers (Al Salem, 1987; Amouei *et al.,* 2014). Wastewater at the municipality treatment plants undergo physical, chemical and biological treatments (Kumar and Chopra, 2012), which are intended to debulk most of the undesirable entities, with the remaining water being referred to as treated wastewater, which have to be disposed-off from the treatment plants.

Microbial contamination limits for treated wastewater use in irrigation had been developed to minimise risks associated with health hazards in soils, plants and animals (Biswas, 1986). Consequently, all treated wastewater should be viewed as potential carriers of pathogens, with the potential ability to serve as source of contamination of the value-chain of agricultural products (Al-Nakshabandi *et al.,* 2007). A number of studies had since shown that there was a relationship between treated wastewater use and food-borne diseases such as cholera and gastroenteritis (Abakpa *et al*., 2013; Sou *et al*., 2011). In 2010, the World Health Organisation (WHO) had estimated 420 000 fatalities that emanated from food-borne diseases in different countries, with most associated with food contamination from crops irrigated with treated wastewater (WHO, 2012).

Due to high incidents of drought in semi-arid regions of Limpopo Province, certain commercial farmers had since resorted to using treated wastewater. The current case study involved the University of Limpopo Experimental Farm (ULEF), where treated wastewater was being used for irrigating crops such as onion (*Allium cepa* L.) and tomato (*Solanum lycopersicum* L.). Due to global warming, there is need to awareness and to protect the diminishing water resources through maintenance of ecosystem health. Initially, a dilution effect was used where wastewater was discharged directly into natural waterways. However, due to increased production of both domestic and industrial wastewater, the dilution effect escalated the pollution of surface and underground water resources (Okoh *et al*., 2010). The latter resulted in an increased need for the introduction of disposal points that would ameliorate pollution through including purification processes prior to discharge. Due to global warming with its high atmospheric demand on crops, treated wastewater disposal points could have the potential to serve as water resources under various conditions (WHO, 2012). At the MWTP, the post-treatment operation disposed treated wastewater through a combination of furrows and canals to the storage dams. Part of the treated wastewater on its way to the main disposal dams could be diverted for use, as had been the case at the ULEF. After a series of treatments that include 16 sequential ponds at MWTP, the treated wastewater from Pond-16 flows out into an open furrow from the initial point of discharge to the canal (ca. 2.9 km) prior to reaching the deep night-dam at the edge of the irrigated fields, which was originally intended to allow most of the heavy metals to settle to the bottom. In view of the depth, the water for irrigation would be scooped at the deep end from the surface water, thereby promoting the quality of treated wastewater prior to discharge for irrigation. Approximately 50 m down the slope of the night-dam is a borehole, originally intended to provide portable water for irrigating the research plots, whereas treated wastewater was intended for use in a citrus orchard and agronomic crops intended for use as animal feeds. The assumption had been that the “metal-less” treated wastewater was being delivered to the irrigated lands through the exit site which was on the opposite side of the entry site to the night-dam, which prompted a commercial farmer in the region to be interested in the rental of the facility that was not optimally used by the University. However, the quality of the treated wastewater in space and in time remained undocumented. The objective of this study, therefore, was to investigate whether the spatial and temporal chemical and biological quality of the treated wastewater from Pond-16 exit, through the night-dam entry and exit points at the ULEF, would be similar to the portable borehole water serving as a standard.

3.2 Materials and methods

3.2.1 Description of the study site

The study was conducted at Pond-16 exit of the MWTP (23° 50' 59'' S; 29° 42' 27'' E) throughout the irrigation network that delivers water at irrigated lands of the ULEF (23° 49' 58'' S; 29° 42' 27'' E). The sampling points were summarised (Figure 1). The MWTP received effluent from a number of industries in Mankweng Township (23° 53' 12'' S; 29° 43' 53'' E), namely, the University of Limpopo (23° 52' 51'' S; 29° 44' 18'' E), Mankweng hospital (23° 52' 51'' S; 29° 43' 33'' E), two local shopping centres, filling stations, various human settlements and from the runoff water. The pollutants from various places could include physical, chemical and organic pollutants such as faeces, hairs, food, paper fibres, plant material, pharmaceuticals, oils and fuel. The effluent underwent physical, biological and chlorine treatments prior to disposal into the furrow for conveying treated wastewater to the night-dam at the ULEF (Figure 3.1).

After physical treatment, excess water was disposed through a series of 16 ponds, each being 30 m × 90 m and technically referred to as maturation ponds. In Pond-01, the disposed water was subjected to the chlorine treatment, which primarily served as a biological treatment. After the disposed water had been temporarily stored in a series of 16 ponds, the disposed water moving through the furrow was technically referred to as treated wastewater. Water samples were collected in three replicates at four sites namely, (a) Pond-16 exit into the furrow, (b) night-dam entry, (c) night-dam exit to irrigated fields and (d) borehole exit to irrigated fields (Figure 3.2). Water samples were collected on the 15th of each month, starting from July to November.

3.2.2 Water sampling and analysis

pH and electrical conductivity (EC): Water samples were collected in three replicates in 1 l sterile bottles and immediately transported to UL Soil Science laboratory for determination of pH and electrical conductivity using pH and conductivity meters respectively. Total suspended solids and total dissolved solids were determined following APHA standard methods (APHA, 2005).

Water sampling and analysis for elements: Prior to sampling, the 1000 ml polypropylene containers were filled with a diluted hydrochloric acid and then rinsed several times with water collected form the sampling site. 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 Ca, Mg, Na, K, B, Zn, Cu, Cr, Pb, Cd and As (USEPA, 1996*a*). Bicarbonates (HCO_3), Chloride (Cl^-), phosphates (PO_4^-), sulphates (SO_4^-), nitrate-N (NO_3-N) and nitrite-N (NO_2-N) were determined by ion chromatography following methods by USEPA (1993). Sodium adsorption ratio was calculated using the following formula (Suarez *et al*., 2006):

$$SAR = (Na^+)/\sqrt{0.5(Ca^{2+} + Mg^{2+})}$$

where Na, Ca and Mg concentrations were expressed in milli-equivalents/litre.

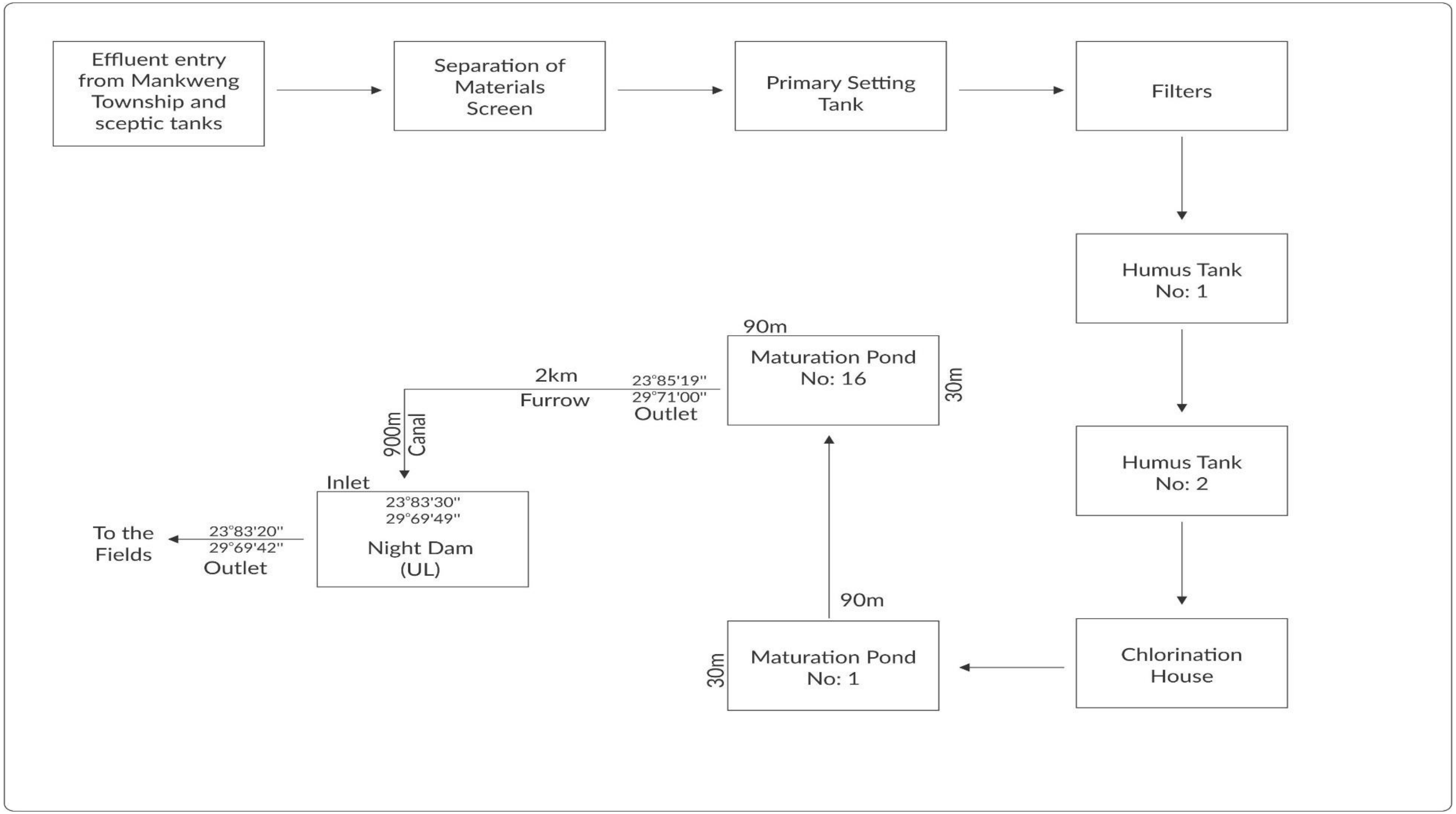

Figure 3.1 Schematic representation of the sampling points from the Mankweng Wastewater Treatment Plant in Mankweng and the receiving dam at UL Experimental farm.

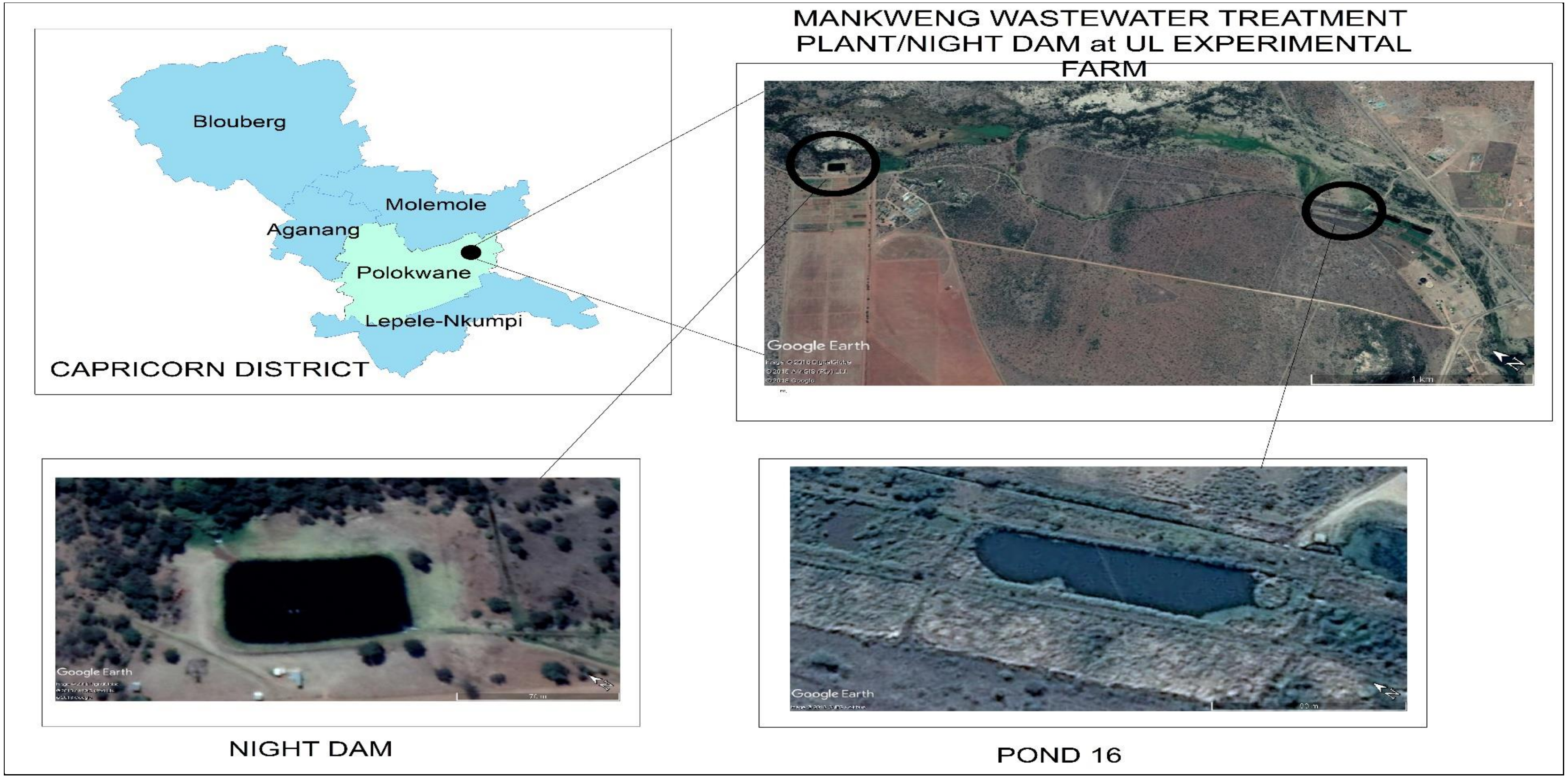

Figure 3.2 Map of the sampling points: Pond 16 at Mankweng Wastewater Treatment Plant and the night-dam at UL Experimental farm.

Water sampling and isolation of bacteria: Once a month, running treated wastewater samples were collected in 500 ml sterile glass bottles, with three samples collected per sampling site. Bottles containing samples were placed on ice in the cooler box and transported to the laboratory for immediate isolation and quantification of the pathogens. Each of the three samples per site were diluted at 10^5 in 100 ml bottles, which were brought to the mark and then filtered through 0.45 µm Whatmann micro filter using the water filtering manifold system (USEPA, 1996*b*). The membranes were aseptically placed on plates with appropriate selective media for isolation, while ensuring that air bubbles were not trapped (Mulamattathil *et al*., 2014). The selective medium for *Salmonella* spp*., Shigella* spp*.* and *Escherichia coli* were XLD agar and for *Vibrio fluvialis*, *Vibrio parahaemolyticus*, *Vibrio cholera* and *Vibrio alginolyticus* was thiosulfate-citrate-bile salts-sucrose (TCBS) and for fecal coliform was m-FC agar.

Water sampling and detection of helminths and protozoa: Wastewater samples were collected in sterile 5 L bottles and placed within ice in cooler boxes and transported to the Water Microbiology Laboratory, Council for Scientific and Industrial Research (CSIR), Pretoria. Samples were quantified for *Entamoeba histolytica, Schistosoma mansoni and Ascaris lumbricoides* (Feachem *et al*., 1983; Moodley *et al*., 2008).

3.2.3 Statistical analysis

Microbial data were log-transformed using $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984). All data were subjected to factorial analysis of variance (ANOVA) using Stata 12 software (StataCorp, 2011). Interactive effects of sampling site and sampling time were further assessed using the two-way matrix tables (Gomez and Gomez, 1984). The mean sum of squares (MSS) were used as source of variation to partition the treatment effects in total treatment variation (TTV) of the respective variables. Treatment means were separated using Duncan multiple range test ($P \leq 0.05$). In order to determine the relative impact of the treated wastewater, the mean chemical and microbe variables of samples collected from the borehole sampling site were used as a standard. The mean helminths and protozoa from samples collected from Pond-16 exit were used as a standard. Unless otherwise stated, treatment effects were described at the probability level of 5%.

3.3 Results

3.3.1 pH and electrical conductivity

The site × time interaction was highly significant for pH and EC of treated wastewater samples, contributing 14 and 12% in total treatment variation (TTV) of the respective variables (Appendix 3.1). The interaction results were further subjected to the two-way matrix table, where the magnitude and direction of the effects were provided. Relative to the borehole water, pH was significantly reduced during July in night-dam exit and Pond-16 exit by 38 and 71%, respectively, but then remained stable throughout the sampling period (Table 3.1). Generally, compared to the borehole water, the EC of the treated wastewater was also stable, with significant increases of the variable as affected by the sampling site and sampling time-frame.

3.3.2 Cations and SAR

The site × time interaction was highly significant on Ca, Mg and SAR, but magnitudes of the contribution in TTV of the respective variables were negligibly low, and were not described further (Appendix 3.2). Negligible effects related to the sampling period were also observed and not discussed. In contrast, the Sampling site had highly significant effects on Ca, Mg, K, Na and SAR, contributing 98, 100, 84, 55 and 70% in TTV of the respective variables (Appendix 3.2). Most cations were reduced along all sampling sites, with Ca being reduced from 83 to 88%; Mg by 96%, Na from 55 to 69%, except at the night-dam entry and K from 40 to 51% (Table 3.2), which were compared with the international standards (FAO, 1996). Relative to the borehole water, SAR in Pond-16 exit, night-dam entry and night-dam was increased by 520, 367 and 272%, respectively (Table 3.3). Compared to July as the initial sampling time, except in November where SAR of treated wastewater was increased by 37% and in August and September where the variable was reduced by 47 and 3%, respectively, in October the variable was not different to that in July (Table 3.5).

Table 3.1 Distribution of pH and EC in treated wastewater relative to that from borehole water used for irrigation over five months at the University of Limpopo Experimental Farm.

Sampling site	July		August		September		October		November	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Ph										
Borehole	5.68^{bc}±0.07	–	7^{ab}±0.01	28	7.21^{ab}±0.02	45	7.01^{ab}±0.05	41	5.90^{abc}±0.14	4
Night-dam exit	3.54^{cd}±0.41	–38	8^{ab}±0.06	32	6.23^{ab}±0.03	10	7.23^{ab}±0.01	27	7.58^{ab}±0.03	34
Night-dam entry	6.11^{ab}±0.14	8	8^{ab}±0.00	32	6.86^{ab}±0.02	21	6.98^{ab}±0.02	23	6.87^{ab}±0.01	21
Pond-16 exit	1.63^{d}±0.02	–71	8^{ab}±0.01	35	6.49^{ab}±0.06	14	7.28^{ab}±0.01	28	7.78^{ab}±0.03	37
South African quality guidelines						< 6.5				
EC ($dS.m^{-1}$)										
Borehole	1.01^{fgh}±0.26	–	1.37^{fg}±0.09	36	1.22^{fgh}±0.18	20	1.42^{fg}±1.68	41	1.01^{fgh}±1.03	0
Night-dam exit	7.21^{a}±1.42	614	0.75^{gh}±0.64	–26	1.66^{f}±0.52	64	1.43^{fg}±0.14	42	3.22^{de}±4.77	219
Night-dam entry	5.18^{b}±10.58	413	1.19^{fgh}±0.05	17	2.82^{e}±0.26	179	1.81^{f}±0.90	79	3.74^{cd}±1.16	270
Pond-16 exit	5.81^{c}±6.58	475	0.43^{h}±0.06	–57	1.08^{fgh}±0.13	7	0.78^{gh}±0.65	–23	4.36^{c}±5.94	332

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Duncan multiple range test.

[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Table 3.2 Aggregated mean cation concentration ($mg.l^{-1}$) in treated wastewater relative to that from borehole water used for irrigation at the University of Limpopo Experimental Farm.

Sampling site[y]	Ca		Mg		Na		K	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Borehole	70.94^{a}±1.40	–	82.93^{a}±0.65	–	88.60^{a}±0.56	–	16.73^{a}±0.82	–
Night-dam exit	9.63^{c}±0.40	–86	3.48^{b}±0.57	–96	27.10^{b}±1.50	–69	8.27^{b}±0.11	–51
Night-dam entry	11.87^{b}±0.50	–83	3.19^{b}±0.24	–96	58.50^{ab}±9.92	–34	10.12^{b}±0.20	–40
Pond-16 exit	8.37^{c}±0.21	–88	3.04^{b}±0.31	–96	40.3^{b}±2.68	–55	8.52^{b}±0.18	–49
FAO-desired range	40-120		6-24		50-120		5-10	

[y]Column means followed by the same letter were not different (P ≤ 0.05) according to Fisher's least significant difference test.

[z]Relative impact = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Table 3.3 Relative impact (R.I.) of sodium adsorption ratio (SAR) of treated wastewater used for irrigation at the University of Limpopo Experimental Farm as affected by sampling site and time.

Sampling site	SAR[y]	R.I. (%)[z]	Sampling time	SAR	R.I. (%)
Borehole	$0.82^{c}\pm0.01$	–	July	$3.57^{b}\pm0.22$	–
Night-dam exit	$3.05^{b}\pm0.17$	272	August	$1.89^{d}\pm0.09$	–47
Night-dam entry	$3.83^{b}\pm0.15$	367	September	$2.46^{cd}\pm0.17$	–3
Pond 16 exit	$5.08^{a}\pm0.66$	520	October	$3.17^{bc}\pm0.25$	–11
			November	$4.88^{a}\pm0.42$	37
FAO-desired range	6.0–9.0				
South African Quality Guidelines	2.0–8.0				

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Duncan multiple range test

[z]Relative impact = R.I. (%) = [(wastewater/borehole) – 1] × 100

3.3.3 Anions

__Major anions__: The interaction was significant on Cl^- and SO_4^{2-}, contributing 1% and 8% in TTV, respectively, which were negligent and therefore was not described further. The interaction was not significant on $H_2CO_3^-$ (Appendix 3.3). The sampling site was highly significant to Cl^-, HCO_3^- and SO_4^{2-}, contributing 96, 99 and 86% in TTV of the respective variables (Appendix 3.3). The sampling time was significant on Cl, contributing 3% in TTV of the variable. However, the sampling time was not significant on HCO_3^- and SO_4^{2-} (Appendix 3.3). The interaction and months contributions to TTV of Cl^- were negligent and therefore, were not described further. Relative to the borehole water, the night-dam exit, night-dam entry and Pond-16 exit decreased Cl^- by 76, 70 and 68%, respectively. Relative to the aggregated mean borehole water, the night dam exit, night-dam entry and Pond-16 exit reduced HCO_3^- by 95, 54 and 95%, respectively (Table 3.4). compared to the borehole water in July, the night-dam exit increased SO_4^- from 10 to 54% during August through October. However, the night-dam entry increased SO_4^- from 38 to 86% during July through November, with a decrease from 29 to 71% in Pond-16 exit for July through November (Table 3.5).

Other anions: The interaction was highly significant on NO_3-N, NO_2-N and PO_4^-, contributing 1, 8 and 2% in TTV of the respective variables, which had negligent magnitudes and therefore not described further (Appendix 3.4). The sampling site had highly significant effects on NO_3-N, NO_2-N and PO_4^-, contributing 98, 75 and 95% in TTV of the respective variables (Appendix 3.4). The night-dam exit, night-dam entry and Pond-16 exit increased PO4- by 2414, 2283 and 2134%, respectively (Table 3.5). Relative to the aggregated mean borehole water, the night-dam exit and Pond-16 increased NO_3-N by 516 and 448%, respectively, whereas the night-dam entry decreased NO_3-N by 96% (Table 3.6). Borehole water, the night-dam exit did not have significant differences on NO_2-N. However, the night-dam entry and Pond-16 exit increased NO_2-N by 162 and 52%, respectively.

Table 3.4 Aggregated mean chlorine and bicarbonate concentration ($mg.l^{-1}$) of treated wastewater relative to that from borehole water.

Sampling site	Cl^-		HCO_3^-	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)
Borehole	$111.2^a \pm 1.87$	–	$245.80^a \pm 4.32$	–
Night-dam exit	$26.52^c \pm 1.57$	–76	$12.00^c \pm 0.87$	–95
Night-dam entry	$33.2^b \pm 1.01$	–70	$113.98^b \pm 3.23$	–54
Pond-16 exit	$35.6^b \pm 0.86$	–68	$11.40^c \pm 0.71$	–95

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Duncan multiple range test.

[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100, aggregated means over five months.

Table 3.5 Sulphates concentration ($mg.l^{-1}$) of treated wastewater relative to that from borehole water used for irrigation of various crops over five months in 2016.

Sampling site	July		August		September		October		November	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Borehole	21^{bc}±2.4	–	39^{ab}±0.8	86	45^{a}±1.0	114	29^{abc}±0.6	38	39^{ab}±1.0	86
Night-dam exit	32^{abc}±0.8	52	32.33^{abc}±0.3	54	29^{abc}±0.8	38	23^{bc}±0.5	10	13^{cd}±0.3	–38
Night-dam entry	31.33^{abc}±0.4	49	29^{abc}±1.0	38	31^{abc}±0.1	48	39^{ab}±0.3	86	39^{ab}±1.0	86
Pond-16 exit	9.67^{de}±0.1	–54	14^{c}±0.0	–33	6^{e}±0.0	–71	15^{c}±0.0	-29	10^{de}±0.5	–52

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Duncan multiple range test.

[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Table 3.6 Aggregated mean minor anion concentration ($mg.l^{-1}$) of treated wastewater relative to that from the borehole water.

Sampling site	NO_3-N		NO_2-N		PO_4^-	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)
Borehole	$49.00^{c}\pm1.13$	–	$0.15^{c}\pm0.01$	–	$0.19^{c}\pm0.02$	–
Night-dam exit	$323.80^{a}\pm6.72$	561	$0.20^{bc}\pm0.01$	38	$4.73^{a}\pm0.16$	2414
Night-dam entry	$1.88^{d}\pm0.13$	–96	$0.38^{a}\pm0.02$	162	$4.48^{ab}\pm0.10$	2283
Pond-16 exit	$268.47^{b}\pm6.40$	448	$0.22^{b}\pm0.01$	52	$4.20^{b}\pm0.07$	2134

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Duncan multiple range test.
[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100, aggregated means over five months.

3.3.4 Heavy metal concentrations

The sampling site × time interaction, sampling site and month of sampling were highly significant on Cu, Zn, Cr, Pb, Cd and As, contributing 1, 1, 2, 14, 9 and 6% in TTV of the respective variables (Appendix 3.5). The sampling site × time interaction for Cu, Zn and Cr were negligent and therefore, were not described further. Relative to the aggregated mean borehole water, the night-dam exit and Pond-16 exit increased Cu by 972, 424 and 713%, respectively. Relative to the aggregated mean borehole water, the night-dam exit and Pond-16 reduced Zn by 86, 98 and 94%, respectively. Relative to the borehole water, night-dam entry, night-dam exit and Pond-16 exit reduced Cr by 87, 89 and 92%, respectively (Table 3.7). Borehole water in August increased Pb by 59%, whereas in September, October and November Pb was decreased by 9, 9 and 23%, respectively. Relative to the borehole water in July, night-dam exit increased Pb by 2%, whereas the variable was reduced by 23, 14, 11 and 27% in July, August, October and November, respectively. Relative to the borehole water in July, the night-dam entry reduced Pb from 18 to 75% in July through November. Relative to the borehole water in July, the Pond-16 exit reduced Pb from 36 to 77% in August through October, but increased the variable by 5% in November (Table 3.8).

Relative to July, borehole water decreased Cd concentration by 50% in August and October, whereas, in September and November Cd was the same (Table 3.9). Relative to the borehole water in July, the night-dam exit reduced Cd by 100% during all the sampling timeframes. Cadmium in the night-dam entry was the same as that of the borehole, whereas the variable was reduced by 100% in August and November, but in September and October the variable was increased by 100%. Pond-16 exit reduced Cd by 50% in September, whereas in July, August, October and November the variable was increased by 100% in each month (Table 3.9). The borehole water increased As concentration by 24, 18 and 47% in August, September and October, respectively, whereas the metal was reduced by 97% in November. Relative to the borehole water in July, the night-dam exit, night-dam entry and Pond-16 exit reduced As by 97-98%, 98% and 97-98%, respectively (Table 3.9).

Table 3.7 Aggregated mean copper, zinc and chromium concentrations ($\mu g.l^{-1}$) of treated wastewater relative to that from borehole water.

Sampling site	Copper		Zinc		Chromium	
	Variable^{y}	R.I.(%)^{z}	Variable	R.I.(%)	Variable	R.I.(%)
Borehole	0.77^{d}±0.03	–	148.00^{a}±4.12	–	4.35^{a}±0.15	–
Night-dam exit	8.26^{a}±0.13	972	21.39^{b}±0.92	–86	0.58^{b}±0.03	–87
Night-dam entry	4.03^{c}±0.13	424	3.54^{c}±0.10	–98	0.50^{b}±0.02	–89
Pond-16 exit	6.26^{b}±0.24	713	8.17^{c}±0.16	–94	0.34^{b}±0.02	–92

^{y}Column means followed by the same letter were not different ($P \leq 0.05$) according to Duncan multiple range test.

^{z}Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100, aggregated means over five months.

Table 3.8 Lead concentration ($\mu g.l^{-1}$) of treated wastewater relative to that from borehole water used for irrigation of various crops over five months in 2016.

Sampling site	July		August		September		October		November	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Borehole	0.44^{bcd}±0.01	–	0.70^{a}±0.00	59	0.40^{bcd}±0.00	–9	0.40^{bcd}±0.01	–9	0.54^{ab}±0.02	23
Night-dam exit	0.34^{cde}±0.01	–23	0.38^{bcd}±0.01	–14	0.45^{bc}±0.00	2	0.39^{bcd}±0.00	–11	0.32^{cde}±0.01	–27
Night-dam entry	0.11^{f}±0.00	–75	0.20^{ef}±0.01	–55	0.36^{cde}±0.00	–18	0.30^{cde}±0.00	–32	0.11^{f}±0.01	–75
Pond-16 exit	0.21^{ef}±0.00	–52	0.10^{f}±0.00	–77	0.28^{de}±0.00	–36	0.20^{ef}±0.00	–55	0.46^{bc}±0.01	5

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Duncan multiple range test.

[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Table 3.9 Cadmium and As concentration (μg.l^{-1}) of treated wastewater relative to that from borehole water used for irrigation of various crops over five months in 2016.

Sampling site	July		August		September		October		November	
	Variable^{y}	R.I. (%)^{z}	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
					Cd					
Borehole	$0.02^{b}\pm0.00$	–	$0.01^{bc}\pm0.00$	–50	$0.02^{b}\pm0.00$	0	$0.01^{bc}\pm0.00$	–50	$0.02^{b}\pm0.00$	0
Night-dam exit	$0.00^{c}\pm0.00$	–100	$0.00^{c}\pm0.00$	–100	$0.00^{c}\pm0.00$	–100	$0.00^{c}\pm0.00$	–100	$0.01^{c}\pm0.00$	–100
Night-dam entry	$0.02^{b}\pm0.00$	0	$0.00^{c}\pm0.00$	–100	$0.04^{a}\pm0.00$	100	$0.04^{c}\pm0.00$	100	$0.02^{c}\pm0.00$	–100
Pond-16 exit	$0.00^{c}\pm0.00$	–100	$0.00^{c}\pm0.00$	–100	$0.01^{bc}\pm0.00$	–50	$0.00^{c}\pm0.00$	–100	$0.02^{c}\pm0.00$	–100
					As					
Borehole	$17.00^{c}\pm0.26$	–	$21.00^{b}\pm0.19$	24	$20.00^{b}\pm0.00$	18	$25.00^{a}\pm0.26$	47	$0.47^{d}\pm0.01$	–97
Night-dam exit	$0.34^{d}\pm0.00$	–98	$0.34^{d}\pm0.00$	–98	$0.42^{d}\pm0.00$	–98	$0.49^{d}\pm0.01$	–97	$0.34^{d}\pm0.01$	–98
Night-dam entry	$0.34^{d}\pm0.00$	–98	$0.36^{d}\pm0.00$	–98	$0.38^{d}\pm0.00$	–98	$0.40^{d}\pm0.00$	–98	$0.36^{d}\pm0.00$	–98
Pond-16 exit	$0.34^{d}\pm0.00$	–98	$0.43^{d}\pm0.00$	–97	$0.40^{d}\pm0.00$	–98	$0.39^{d}\pm0.00$	–98	$0.44^{d}\pm0.01$	–97

^{y}Column means followed by the same letter were not different ($P \leq 0.05$) according to Duncan multiple range test.

^{z}Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

3.3.5 Microbial counts

The site × time interaction was significant on *Salmonella* spp., contributing 1% in TTV of the variable, but did not have significant effects on *Shigella* spp., *E. coli* and fecal coliform (Appendix 3.6). Sampling site had highly significant ($P \leq 0.01$) effects on *Shigella* spp., *E. coli* and fecal coliform, contributing 87, 90 and 99% in TTV of the respective variables (Appendix 3.6). However, the sampling time did not have significant effects on any of the three variables. The TTV of the site × time interaction was negligent and was therefore, not described further. Relative to the borehole water, the night-dam exit, night-dam entry and Pond-16 exit increased *Salmonella* spp. by 243, 239 and 343%, respectively (Table 3.10). Fecal coliform was not detected in the borehole water. Relative to the borehole water, the night-dam exit, night-dam entry and Pond-16 exit increased *E. coli* by 88, 97 and 106%, respectively (Table 3.10). The night-dam exit, night-dam entry and Pond-16 exit increased *Shigella* spp. by 15, 65 and 64%, respectively (Table 3.10).

The site × time interaction and the sampling time were each not significant for any variable (Appendix 3.7). However, the sampling site had highly significant effects on *V. fluvaris*, *V. parahaemolytica*, *V. cholera* and *V. aginolytica*, contributing 17, 96, 18, 90 and 99.6% in TTV of the respective variables (Appendix 3.7). Relative to the borehole water, the night-dam exit, night-dam entry and Pond-16 exit reduced *V. fluvaris* by 51, 58 and 19%, respectively (Table 3.11). Relative to the borehole water, the night-dam exit, night-dam entry and Pond-16 exit increased *V. parahaemolytica* by 169, 180 and 191%, respectively (Table 3.11). Relative to the borehole water, the night-dam exit, night-dam entry and Pond-16 increased *V. cholera* by 169, 153 and 138%, respectively. The standard sample did not contain *V. aginolytica* (Table 3.11).

Table 3.10 Log-transformed mean counts of *Salmonella* spp., *Shigella* spp., *Escherichia coli* and fecal coliform in treated wastewater relative to that from the borehole water.

Sampling site	*Salmonella* spp		Fecal coliform		*Escherichia coli*		*Shigella* spp	
	Variable^{y}	R.I. (%)^{z}	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I.(%)
Borehole	0.65^{c}±0.05	–	0.00^{d}±0.00	–	1.45^{c}±0.04	–	1.63^{b}±0.04	–
Night-dam exit	2.24^{b}±0.03	243	2.01^{b}±0.03	–	2.73^{b}±0.03	88	1.87^{b}±0.06	15
Night-dam entry	2.22^{b}±0.04	239	1.78^{c}±0.04	–	2.86^{ab}±0.02	97	2.69^{a}±0.03	65
Pond-16 exit	2.90^{a}±0.02	343	2.92^{a}±0.01	–	3.00^{a}±0.02	106	2.67^{a}±0.02	64

^{y}Column means followed by the same letter were not different (P ≤ 0.05) according to Duncan multiple range test.

^{z}Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Table 3.11 Log-transformed mean counts *Vibrio fluvaris* (*ViFlu), Vibrio parahaemolytica (ViFlu), Vibrio cholera (ViCho) and Vibrio aginolytica (ViAgi)* as affected by sampling site along the wastewater treatment pathway from Pond-16 exit to night-dam exit relative to those in the borehole water.

Sampling site	*ViFlu*			*ViPar*			*ViCho*			*ViAgi*		
	Untrans[x]	Trans[y]	R.I. (%)[z]	Untrans	Trans	R.I. (%)	Untrans	Trans	R.I. (%)	Untrans	Trans	R.I. (%)
Borehole	2	0.42[a]±0.04	–	10	1.01[d]±0.02	–	22	1.06[d]±0.08	–	0	0.00[d]± 0.00	–
Pond-16 exit	1	0.20[b]±0.03	–51	533	2.72[c]±0.01	169	741	2.85[a]±0.02	169	995	2.97[a]±0.02	–
Night-dam entry	1	0.18[b]±0.03	–58	714	2.83[b]±0.02	180	494	2.68[b]±0.01	153	751	2.86[b]±0.02	–
Night-dam exit	2	0.34[ab]±0.04	–19	896	2.94[a]±0.01	191	324	2.52[c]±0.01	138	317	2.50[c]±0.01	–

[x]Untrans = Untransformed counts.

[y]Trans = transformed counts, Column means followed by the same letter were not different (P ≤ 0.05) according to Duncan multiple range test.

[z]Relative impact (%) = R.I. % = [(Wastewater/Borehole) – 1] × 100.

3.3.6 Helminths and protozoa counts

The site × time interaction was significant on *A. lumbricoides*, contributing 31% in TTV of the variable, but the interaction was not significant on *S. mansoni* and *E. histolytica*. The sampling site was highly significant on *S. mansoni* and *E. histolytica*, contributing 99.7 and 98%, respectively (Appendix 3.8). However, the sampling time did not have significant effects on *S. mansoni* and *E. histolytica* (Appendix 3.8). Relative to July, Pond-16 exit increased *A. lumbricoides* by 11, 14, 4 and 3% in August, September, October and November, respectively (Table 3.12). Relative to Pond-16 in July, the night-dam entry increased *A. lumbricoides* by 22, 20, 12 and 35 in July, August, September and November, respectively, but reduced the variable by 28% in October (Table 3.12). Relative to Pond-16 in July, the night-dam exit decreased *A. lumbricoides* by 5 and 10% in July and August, respectively, but decreased the variable by 5, 9 and 12% in September, October and November, respectively (Table 3.12). The night-dam entry and night-dam exit did not contain *S. mansoni* and *E. histolytica*, which, relative to Pond-16 exit, denoted a 100% decrease (Table 3.13).

Table 3.12 Log-transformed *Ascaris lumbricoides* counts as distributed along the wastewater treatment pathway from Pond-16 exit to night-dam exit for five months in 2016.

Sampling Site	July		August		September		October		November	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Pond-16 exit	1.10^{ab}±0.02	−	1.22^{ab}±0.02	11	1.26^{ab}±0.04	14	1.15^{ab}±0.03	4	1.13^{ab}±0.01	3
Night-dam entry	1.34^{a}±0.00	22	1.32^{a}±0.02	20	1.23^{ab}±0.03	12	0.79^{b}±0.06	−28	1.48^{a}±0.01	35
Night-dam exit	1.04^{ab}±0.02	−5	0.99^{ab}±0.03	-10	1.16^{ab}±0.01	5	1.20^{ab}±0.01	9	1.23^{ab}±0.00	12

[y]Column means followed by the same letter were not different (P ≤ 0.05) according to Duncan multiple range test.

[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Table 3.13 Distribution of log-transformed *Schistosoma mansoni* and *Entamoeba histolytica* ova in different treated wastewater sources and borehole water used for irrigation at UL Experimental Farm.

Sampling site	*S. mansoni*		*E. histolytica*	
	Variable[x]	R.I. (%)[y]	Variable	R.I. (%)
Pond16	$1.22^{a}\pm0.00$	–	$0.69^{a}\pm0.00$	–
Night-dam exit	$0.0^{b}\pm0.00$	−100	$0.0^{b}\pm0.00$	−100
Night-dam entry	$0.0^{b}\pm0.01$	−100	$0.0^{b}\pm0.02$	−100

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Fisher's least significant difference test.

[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100, aggregated means over five months.

3.4 Discussion

3.4.1 pH and EC

The site × time interaction was highly significant for pH and EC of test water. Unfortunately, limited work had been done to investigate the interactive effects of storage facilities or disposal points over time. However, results of spatial single-point sampling for pH and EC on treated wastewater effluents in the eThekwini Metropolitan, KwaZulu-Natal Province (Naidoo, 2013) confirmed the findings in the ULEF study, where significant differences on pH and EC were observed at different sampling sites.

pH of treated wastewater: Although pH was significantly reduced during July in night-dam exit and Pond-16 exit, the variable increased to alkaline level (pH 8) in August, and then stabilise to the neutral level throughout the sampling period. Although the reason for the prompt increase in pH of the treated wastewater in August was not clear in the ULEF study, in another study (Barron *et al*., 2006) the increase in ambient temperature was viewed as being responsible for increased pH through promotion of chemical reactions in treated wastewater. Detailed studies would be necessary to attempt to establish the variability in pH of treated wastewater during certain periods since it could eventually affect the soil pH (Hulme, 2012), since it is critical to the availability of nutrient elements to crops in terms of regulating deficiency and phytotoxic concentrations of mineral elements in soils (Oliveira *et al*., 2016). In the ULEF study, throughout the study period, pH values were similar to those observed in Iran (Abedi-Koupai *et al*., 2006) and were also safe for use in irrigation since the acceptable pH range in irrigation water, depending on soil type to be irrigated, could be from 6.5 to 8.4 (Jeong *et al*., 2016). The ULEF water pH was within the acceptable FAO (Pescod, 1992) and South African Quality Guidelines (DWAF, 1996) limits for vegetable production. Consequently, in terms of the variable under consideration, the ULEF treated wastewater was suitable for irrigating vegetable crops.

Electrical conductivity of treated wastewater: Generally, relative to the borehole water, the EC of the treated wastewater was also stable, showing significant increases of the variable as affected by the sampling site and the sampling period. The lowest EC at 0.43 dS/m was observed from samples collected at Pond-16 exit during August, whereas the highest at 7.21 dS/m was in samples from the night-dam exit. The source of the increased EC at the night-dam exit was not clear, but it could have been from the added salts when the treated wastewater moved through the open furrows in calcareous soils starting half-way from Pond-16 exit to the night-dam entry. Currently, from the used factorial experiment it was not feasible to explain why the increase in EC was in August as the experiment was not replicated in time due to its factorial nature. This observation was important because it could also explain differences in nutrient elements later on during the ULEF study. The EC had been associated with salinity and could cause damage to salt-susceptible crops if the EC was above the recommended threshold values (Kiziloglu *et al*., 2008), where values above 3.00 dS/m had been viewed as being severe (Ayers and Westcot, 1985; Jeong *et al*., 2016). Soils irrigated with high EC-containing water could invariably result in high soil EC and ultimately in soil salinity (Castro *et al*., 2011). In contrast, the EC results in the ULEF study, which were within the FAO (Pescod, 1992) standards, were much better when compared to those of treated wastewater in an Iranian study (Abedi-Koupai *et al.*, 2006).

3.4.2 Cations and SAR

Calcium: The observed lower Ca concentration in treated wastewater at all sampling sites relative to that from the borehole water, could be suggesting to an extent, (a) that the treatment plant was effective in reducing Ca in treated wastewater or (b) the borehole water was being contaminated with Ca-containing chemical compounds. In the ULEF study, Ca concentration at various sampling sites, regardless of sampling time was below the recommended maximum Ca guideline of less than 40 mg.l^{-1} for irrigation using treated wastewater (Alberta Environment, 2000; Al-Jasser, 2011; Ayers and Westcot, 1994). Water with low Ca concentration is generally being viewed as soft water, which is inherently suitable for irrigation (Swistock *et al*., 2017). The observed low Ca in treated wastewater automatically qualifies the water for use in best agricultural practices since such water could ameliorate soil hardness. Calcium concentrations in treated wastewater had been reported as being too high when it was within the 52 and 100 mg Ca.l^{-1} range (Al-Jasser, 2011). However, in other cases the permissible cation range could be from 15 to 84 $mg.l^{-1}$ range (Balkhair and Ashraf, 2016). The Ca range at the night-dam exit was at 5.2 to 13.03 mg $Ca.l^{-1}$, which was further below the permissible levels.

The observed Ca concentration from the borehole in the ULEF study at 59.7 to 89 mg $Ca.l^{-1}$ was considerably lower when compared to that in an Indian study on treated wastewater (Subramani *et al*., 2005). Irrigation water had been viewed as being moderately hard when Ca was within the 40-60 $mg.l^{-1}$ range (Swistock, 2017). However, in other countries, the below and above-surface water used for irrigation could have Ca concentrations above 280 $mg.l^{-1}$ (Orzepowski and Pulikowski, 2008). Generally, Ca-containing salts that leach deeper into the soil had been reported to be the major contaminants of underground water (Tandyrak *et al*., 2005). In a study undertaken by the Russian Academy of Sciences, high content of carbonates and gypsum in the soil profile were linked with high Ca in underground water (Gabbasova and Suleymanov, 2010). In the ULEF study, the high Ca concentration in the borehole water could be associated with a belt of calcareous/dolomitic soils that straddle the borehole and Pond-16 exit.

Magnesium: Relative to the borehole water (82.93 mg $Mg.l^{-1}$), Mg at all sampling sites was reduced by approximately 96%. Magnesium in the borehole water of the ULEF study was comparable to those from borehole water in other countries (Orzepowski and Pulikowski, 2008). Both Mg and Ca had been associated with the aggregate stability and friability of soil (Swistock *et al*., 2017), and their low concentration in treated wastewater was an ideal since they are required in large concentrations in soil. Notwithstanding, Mg concentration higher than 200 mg $Mg.l^{-1}$ in irrigation water could result in high soil pH values, with the resultant effect of reducing the availability of P, Cu and Zn for most crops (Khodapanah *et al*., 2009).

Potassium: Differences in K concentration across the sampling sites were observed. The lowest K concentration (8.27 mg.l^{-1}) in the night-dam exit could be associated with the settling of K to the bottom of that temporary storage dam prior to releasing the water into the irrigated field. The K value in borehole water (16.73 K mg.l^{-1}) was above the desirable range of 0-10 mg K.l^{-1} in irrigation water (Swistock *et al*., 2017), which could also be due to water seepage from the night-dam to the underground source for the borehole water. Although K is vital in plant tolerance to stress elicitors such as drought, low temperature and/or salinity (Tisdale *et al*., 1999), above the desirable range K could reduce Ca and Mg uptake by plants (Heinrich *et al*., 2018).

Sodium: Generally, Na concentration is considered moderate when it is just above 70 mg.l^{-1} (Pedrero *et al*., 2010), which was close to the concentration observed in the ULEF borehole water (88.60 mg Na.l^{-1}) study. High Na levels in treated wastewater would generally not be suitable to the recipient soils due to its ability to promote the adsorption of ions onto the soil cation exchange sites, thereby causing soil aggregates to disperse and seal the soil pores, with the resultant restriction to water penetration to lower soil horizons (Emongor and Ramolemana, 2004). Sodium concentration observed in the treated wastewater at the night-dam exit in the ULEF study was considerably lower when compared to 109.7 mg Na.l^{-1} from treated wastewater (Orzepowski and Pulikowski, 2008) and to 123.60 mg. Na.l^{-1} from water collected in contaminated rivers in other countries (Alobaidy *et al*., 2010). However, at 27.10 Na mg.l^{-1} for the treated wastewater at the ULEF night-dam exit, the water should still be used with circumspection in the short-term, as the build-up of Na in the soil could inevitably lead to sodicity as observed in other countries using treated wastewater for irrigation (Abrol *et al*., 1988).

Sodium adsorption ratio: Sodium adsorption ratio is often used as an indicator for the suitability of irrigation water. The higher the SAR (> 26 $mmol.dm^{-3}$), the less suitable is the water for irrigation purposes (Abrol *et al*., 1988). The water with SAR values above 26 $mmol.dm^{-3}$ had been viewed as not being suitable for irrigation since it would invariably lead to the deterioration of the soil physical structure (Ayers and Westcot, 1985; Shakir *et al*., 2016). In the ULEF study SAR of treated wastewater at the night-dam exit was3.05 $mmol.dm^{-3}$, under Class S1 (FSSA, 2007), which accommodates a low Na-hazardous irrigation water (Ayers and Westcot, 1985). All observed SAR values in the ULEF study were below the water quality desired SAR ranges as pronounced by FAO (Pescod, 1992) and SA (Department of Health, 2004). Despite the potential contamination of the underground sources for the borehole water, the borehole water had the lowest SAR (0.82) when compared to those at other sampling sites. The observed variability in SAR was mainly due to sampling times, which confirmed observations in similar treated wastewater studies in Iraq (Shakir *et al*., 2016).

3.4.3 Heavy metals

The sampling site × time interaction had highly significant effects on Cu, Zn, Cr, Pb, Cd and As of treated wastewater. However, there are limited similar studies for comparison purposes. A study in Northern Greece reported seasonal variabilities in Cd, Cr, Cu, Zn, Ni and Pb in treated wastewater (Spanos *et al*., 2016). In the ULEF study, the maximum concentrations for Cu, Zn, Cr, Pb, Cd and As were below the FAO recommended maxima in treated wastewater (Pescod, 1992). In the ULEF study, Cu was higher in treated wastewater than in the borehole water, suggesting that Cu was not leaching under gravity to contaminate groundwater. Basically, Cu has strong adsorption properties of Cu to cankiri bentonite, a natural clay, which is being used in lining storage dams (Altaher, 2001; Veli and Alyuz, 2007). In contrast, Zn concentration (184 $\mu g.l^{-1}$) in the borehole water, although it was still slightly below the internationally acceptable threshold level of 200 $\mu g.l^{-1}$ (Pratt, 1972; WHO, 1989), it was higher than that of treated wastewater at all sampling sites. The maximum Zn concentration at different water sampling sites in the ULEF study agreed was that observed in Iran (Taghipour *et al*., 2012) and Morocco (Al-Jaboobi *et al*., 2014), where the Zn was rather high, but still below the internationally recommended threshold levels (Pratt, 1972; WHO, 1989).

Relative to the borehole water, the treated wastewater at Pond-16 exit, night-dam entry and night-dam exit all had the lowest Cr concentration, which agreed with treated wastewater in the Moroccan study (Al-Jaboobi *et al*., 2014). Unlike other heavy metals which are non-essential to plants, Cr is an essential nutrient element to humans and could only be supplied through diet (Evert, 2013). On-farm inputs of Cr such as irrigation water could be viewed as being beneficial in adding the element in soils for subsequent absorption by plants (Stasinos and Zabetakis, 2013), but is naturally low in irrigation water (Torabian and Mahjori, 2003). Although Cr is an essential nutrient element to human beings, it is required in Nano-quantities due to its high level of cytotoxicity even in micro-quantities (Tchounwou *et al*., 2012). On the basis of the Cr concentration in the ULEF study, the treated wastewater was safe for irrigation. Lead concentration in all sampling points fluctuated with sampling time and site. For instance, the highest Pb concentration (5.70 µg.l^{-1}) in borehole water occurred in August, whereas the lowest (0.20 µg.l^{-1}) occurred in the night-dam entry in August and in the night-dam exit in September – with the changes appearing to be seasonal. Apparently, the main source for heavy metals could have been the Mankweng Hospital, where some seasonal illnesses could have increased the supply of medicines with heavy metals, thereby resulting in the observed seasonality of heavy metals. The temporal Pb variations in the ULEF study confirmed those in Brazil, where Pb variability was seasonal (Souza *et al*., 2016). The main sources of Pb are industries, mines and petrol stations, with most studies (Abdul-Jameel *et al*., 2012; Bichi and Bello, 2013) that reported high Pb being adjacent to such sources. However, sampling sites for the ULEF study were devoid of such sources. A further study would be necessary to trace the source of Pb at the ULEF.

Cadmium was the highest in the borehole water samples in August, whereas it was the lowest in the night-dam exit during both July and September, which were seasonal as observed in Brazil (Souza *et al*., 2016). According to the available guidelines (Ayers and Weststock, 1994), Cd in water should not exceed 10 µg.l^{-1} and therefore, the relatively low Cd concentrations in the ULEF study suggested that the water was, in terms of this variable, suitable for irrigation. However constant monitoring is vital given that the element has the potential to bioaccumulate. Generally, as shown in China where higher Cd concentrations than in the ULEF study were observed (Wu and Cao, 2010), the main sources of Cd pollution were viewed as mining, smelting and refining of nonferrous metals (WHO, 2000), along with heavy use of phosphate fertilisers

(Amfo-Otu, 2012). The ULEF site was devoid of Cd natural sources since the commercial farmer also practiced *Natuurboerdery* farming.

Borehole water samples had the highest As concentration at 25 $\mu g.l^{-1}$ relative to 0.49 As $\mu g.l^{-1}$ in treated wastewater from the night-dam exit, with the latter being the highest for all the treated wastewater sampling sites in the ULEF study. The internationally permissible lower level for As in irrigation water is 100 $\mu g.l^{-1}$ (Pratt, 1972). Due to its high mobility in soils (Barringer *et al*., 2011), As from the treated wastewater or other underground sources could find its way to the under-groundwater used for irrigation at the ULEF.

3.4.4 Microbial counts

Salmonella counts: The ULEF study, as supported by studies in Mexico (Palacios *et al*., 2017) and Georgia (Haley *et al*., 2009), demonstrated that the *Salmonella* counts were highly seasonal. During the five-month sampling period at the ULEF study, *Salmonella* counts started to decrease in the borehole water samples, whereas in treated wastewater sampling sites the counts increased, which confirmed a three-year cycle where *Salmonella* counts were strongly associated with seasonal variation (Vereen *et al*., 2013). In contrast, Salmonella counts in treated wastewater that was kept in storage tanks under constant conditions did not fluctuate with time (Palacios *et al*., 2012). The presence of *Salmonella* spp. in the ULEF borehole water, with its constant temperature, supported the view that the pathogen could survive for an extended period under conducive temperatures (Nevecherya *et al*., 2005). Based on observations in the previous three studies (Nevecherya *et al*., 2005; Palacios *et al*., 2012; Vereen *et al*., 2013), the changes in *Salmonella* counts over time in the ULEF study were probably an expression of the changing temperatures during the sampling period which was initiated in late winter to early summer. The survival abilities of *Salmonella* spp. had aslo been associated with predation by other organisms (Palacios *et al*., 2012), which could also be seasonal in their activities.

At the ULEF study, *Salmonella* spp. were the highest in the night-dam-entry when compared to the other treated wastewater sampling sites. The decrease in *Salmonella* spp. in the night-dam exit could be ascribed to the settling ability of the pathogen in the night-dam since settling and exposure to heat are some of the ways in which *Salmonella* counts could be reduced (Pachepsky *et al*., 2011). The highest *Salmonella* counts at the ULEF study were in the same margin asof 1 000 cfu/100 ml as internationally proclaimed (WHO, 2000) and observed in other treated wasterwater studies (Pianetti *et al*., 2003; Ripabelli *et al*., 2004). Worldwide, *Salmonella* is the causal agent of gastroenteritis, with infection symptoms including fever, nausea and at times, vomiting (Kovačić *et al*., 2017). Consequently, *Salmonella*-contaminated irrigation water, such as reported at the ULEF study area, could contaminate vegetable produce, and should therefore cause a health-scare, particular in vegetables that are eaten raw (Balkhair and Ashraf, 2016).

<u>*Escherichia coli counts*</u>: Sampling sites had highly significant effects on the *E. coli* counts. Studies (Haley *et al*., 2009; Ulrich *et al.,* 2005; Vereen *et al*., 2013) that individually investigated the effects of sampling site or time, noted that the two factors could each significantly affect *E. coli* counts as observed in the ULEF study. At the ULEF study, *E. coli* was positive in samples collected at all sampling sites, with low counts depicted in borehole water samples. In the night-dam exit, *E. coli* counts were

above 1 000 cfu/100 ml, which suggested poor settling abilities of the pathogen as observed above for the *Salmonella* spp. The observed counts in the night-dam exit were within similar ranges as those observed in treated wastewater from the United Arab Unirates (Al Amimi *et al*., 2014). Treated wastewater standards for *E. coli* counts for agricultural uses had been set at the 5-300 cfu/100 ml range for various vegetables (Allende and Monaghan, 2015; Forslund *et al*., 2012). The *E. coli* counts observed in the ULEF study were much higher than the recommended standards and, therefore, render the ULEF treated wastewater unsuitable for agricultural irrigation purposes. *Escherichia coli* is the causal agent of gastroenteritis, which had been described as the inflammation of the gastrointestinal tract that comprises the stomach and the small intestine (Ciccarelli *et al*., 2014).

Fecal coliform counts: The sampling time × time interaction did not have significant effects on fecal coliform counts in the ULEF study. In contrast, streams are renowned to have fecal coliform loads that could have high seasonal variations (Sanders *et al.*, 2013). Fecal coliforms, conventionally used as the indicator bacteria in the reuse of treated wastewater, have permissible counts of less than 1000 cfu/100 ml water (WHO, 2000). Samples from Pond-16 exit at the ULEF site had slightly lower fecal coliform counts (930 cfu/100 ml) than the permissible limit, but the counts were lower than those (1 600 cfu/100 ml) observed in treated wastewater in Mexico (Sanders *et al.*, 2013). The absence of fecal coliform in the borehole water samples of the ULEF study could be ascribed to the sole association of the bacteria with the presence of fecal materials from humans and other animals (USEPA, 2012).

Shigella counts: The highest *Shigella* counts (> 500 cfu/100 ml) were observed in the night-dam entry and Pond-16 exit, whereas the borehole water samples had the lowest counts, followed by those at the night-dam exit. The lowest *Shigella* counts in the night-dam exit suggested that conditions in the night-dam did not favour the multiplication of this pathogen. The *Shigella* counts in the ULEF treated wastewater were much higher than those observed in treated wastewater (approximately 50 cfu/100 ml) in Dubai and Sharjah (Al Amimi *et al*., 2014). In the ULEF study, the low *Shigella* counts from samples in the borehole water could be ascribed to the fragility of *Shigella* spp. when exposed to unfavourable conditions as those in boreholes. *Shigella* spp. are being regarded as fragile organisms that could hardly survive outside their natural habitat, which is wastewater (Gil and Selma, 2006). In the North West Province, South Africa, *Shigella* counts in winter (176 cfu/100 ml) were higher than those insummer (49 cfu/100 ml) months (Kinge and Mbewe, 2012), thereby supporting the previous view which suggested that the pathogen was relatively a heat-sensitive organism (Frazier and Westhoff, 1988).

Vibrio species counts: Water from all treated wastewater sampling sites, including he borehole site, had positive (0.18-2.97 cfu/ml) *Vibrio* spp. counts, although the sampling period had no significant effects. The ULEF (late winter to early summer) counts agreed with those observed elsewhere with prevalence of high *V. fluvaris* (Bonnefont *et al*., 1990) *and V. parahaemolytica* (Martinez-Urtaza *et al*., 2013) counts in summer, when the water temperature was rising. The ULEF findings concurred with those in the Eastern Cape, South Africa, where seasonal effects on the prevalence of *Vibrio* spp. from the final effluents collected at the treated wastewater facilities were not detected (Okoh *et al*., 2015). However, others (De Pola *et al*., 2003) observed a decline in *Vibrio* spp. counts during winter when compared to summer time (De Pola *et al*., 2003).

The highest (995 cfu/100 ml) *V. aginolytica* counts in Pond-16 exit and the highest (890 cfu/100 ml) *V. cholera* counts in the night-dam exit, were all above the permissible counts (800 cfu/100 ml) in irrigation water (WHO, 2000). However, the *V. cholera* counts also decreased with the movement and storage of treated wastewater, as the lowest counts (324 cfu/100 ml) were observed in the night-dam exit. The observed *V. cholera* counts were still a health hazard since limited contamination of crops could cause cholera in consumers, with its acute diarrhoeal effects (WHO, 2017). The four species *V. fluvaris, V. parahaemolyticus, V. cholera* and *V. aginolytica,* each could cause diarrhoea, with entirely different bioactivities. For example, *V. parahaemolyticus* had been described as an invasive organism that primarily infected the colon, whereas *V. cholerae* was viewed as a non-invasive pathogen that preferrably infected the small intestine through secretions that contain enterotoxins (Todar, 2005).

3.4.5 Helminths and protozoa counts

The variability with sampling sites and sampling period were evident on the distribution of *A. lumbricoides* counts, without clear variability trends. In Pond-16 exit, the highest counts (1.26 cfu/100 ml) were observed during September, whereas both night-dam entry (1.48 cfu/100 ml) and night-dam exit (1.23 cfu/100 ml) exhibited the highest counts in early summer. Effects of sampling site and sampling periods on *A. lumbricoides* had not been properly documented, with some contradicting findings (Blumenthal *et al*., 2001). However, Amoah *et al*. (2016) in Ghana observed that high *A. lumbricoides* counts occurred during the dry season (April-October), with low counts occurring during the wet season (November-March). *Ascaris lumbricoides* counts were high in the night-dam entry and Pond-16 exit during different sampling periods, suggesting that the pathogen was reduced by the storage conditions during the passage of treated wastewater. However, the counts increased with the sampling period, suggesting that the variability of *A. lumbricoides* counts was seasonal as observed by others (Gupta *et al*., 2009; Mahvi and Kia, 2006).

Ascaris is one of the most resilient among the enteric-pathogens due to its resistance to external conditions (Crompton, 1989). *Ascaris lumbricoides* ova remain viable for long periods and could, therefore, be used as a parasitological indicator in irrigation water (Watson *et al.,* 1983). The presence of *A. lumbricoides* ova in treated wastewater could, invariably lead to contamination of irrigated vegetables and eventually this could result in the pathogen being consumed by people and/or animals. Globally, contamination of produce with *A. lumbricoides* under projects where crops are irrigated with treated wastewater had been increasing (Amoah *et al*., 2016; Gupta *et al*., 2009; Montero-Aguirre *et al*., 2016). Incidentally, in the ULEF study, *A. lumbricoides* ova were higher than the set standards of 1 ova/l water (WHO, 2012), which further disqualified the use of treated wastewater at the ULEF for irrigation purposes.

Counts of *S. mansoni* and ova were not detected in borehole water, night-dam exit and night-dam entry in the ULEF study, but were only present at the Pond-16 exit, suggesting that the transition conditions through which treated wastewater passed were not conducive for the survival of this pathogen. The pathogen has the permissible level of less than 1 ova/l water (WHO, 2006), with the limit being 0.1 ova/5 l water (WHO, 2000). The *S. mansoni* counts at Pond-16 exit were above the allowable limits.

Entamoeba histolytica ova were also detected at Pond-16 exit alone. The absence of *E. histolytica* in the other sampling sites were previously explained on the basis of their limited survival when exposed to different environments (Mortimer and Chadee, 2010), suggesting that from Pond-16 exit to night-dam exit, the micro-climates could be having diverse conditions for some of the pathogens. Limited transport of *E. histolytica* in the ULEF study could be a positive attribute since this pathogen is a serious human-intestinal protozoa that had been associated with contaminated water and food (Nyarango *et al.,* 2008). The parasite is responsible for amoebiasis disease, which has symptoms that include bloody diarrhoea (WHO, 1985). Consequently, the presence of this pathogen in irrigation water could lead to fatalities in consumers and labourers.

3.5 Synthesis and conclusion

At the time of conducting the ULEF study, the treated wastewater, on the basis of its chemical composition was suitable for irrigation. However, certain elements such as Ca and Mg, were low at Pond-16 exit, but were high in subsequent sampling sites. Due to the importance of Ca and Mg in regulating soil pH, regular monitoring of the two cations would be important and particularly an investigation of the source of the two, since existing evidence did not link them to Pond-16 exit and therefore, the treated wastewater. Analysis of the surrounding soil for Ca and Mg could provide some information on the origin of high Ca and Mg in treated wastewater and borehole water. Should the surrounding dolomitic soil be the source, it would increasing be imperative that an artificial canal be constructed since the impact of the two cation ions on soil in relation to changing soil pH could be costly to rectify. Additionally, although Na was still below the permissible limits, it appeared to be approaching the toxic level and it would, therefore, be imperative that regular sampling and monitoring be done in order to avoid the potential costly destruction of soil aggregates. Such a destruction would dramatically negate the benefits of organic farming as currently practiced by the commercial farmer. Also, the seasonality and/or sources of certain variables, for instance, EC and Cd, were identified as gaps for future research. Therefore, with regard to chemical composition, the findings support the hypothesis that the different disposal points of treated wastewater prior to irrigation contained suitable amounts of chemical constituents.

The major limiting factor to the use of the ULEF treated wastewater was in the composition and quantity of certain pathogenic microbes. *Salmonella spp., E. coli, Shigella spp., Vibrio spp., A. lubricoides and E. histolytica* were at unacceptable counts for treated wastewater in the ULEF study. In addition to spatial variation, the pathogenic microbes were significantly affected by temporal effects. The major finding in the ULEF study was that the night-dam, as shown by comparing microbial counts at the night-dam entry and night-dam exit points, played a major role in curtailing the counts. The current findings therefore contradicts with the hypothesis that the treated wastewater at different disposal points contaibned suitable counts of organisms. Due to the potential health-hazard that could be caused by the observed pathogenic microbes, it would be increasingly important that mitigation benefits such as erecting chlorine-station prior to the night-dam or at the night-dam exit, be further investigated. Additionally, regular sampling and monitoring for pathogenic microbes should be carried out prior to discharging the treated wastewater to the irrigated field in order to safe-guide the interest of workers and consumers. In the next chapter, the researcher investigated whether the effects of treated wastewater on the (1) physical and chemical properties of soil, (2) distribution of heavy metals in irrigated fields and (3) biological-soil-health indicators under *Natuurboerdery* and conventional farming systems would be similar.

CHAPTER 4

SOIL HEALTH IN FIELDS IRRIGATED WITH TREATED WASTEWATER

4.1 Introduction

Soil health primarily focused on chemical, physical and biological properties as depicted by the intersection of the three subsets (Gugino *et al.*, 2009; Trivedi *et al*., 2016). The soil health indicators had been integrated in such a way that they were virtually interdependent upon one another (Kibblewhite *et al*., 2008). For example, soil aggregates affect aeration and nutrient cycling, but also serve as habitat for soil fauna (Cardoso *et al*., 2013). Generally, biological indicators are more vulnerable than physical and chemical properties to environment-imposed external factors such as tillage and irrigation with poor quality water (Masto *et al*., 2009). Soil health management had been geared towards the integrated responses of the three components of soil health that could lead to favourable and reliable optimal support of cropping systems (Kibblewhite *et al*., 2008).

Irrigation with treated wastewater in arid areas could have both advantages and disadvantages, with the former including the reduced consumption of high quality water. Internationally, the potential effectiveness of municipal treated wastewater at relieving irrigation water pressures for different agricultural crops had been investigated, with mixed results (Abedi-Koupai *et al*., 2006; Khaskhoussy *et al*., 2015; Zhao *et al*., 2012). In such investigations, focus had been on the potential reuse of organic and inorganic mineral nutrients in wastewaters as fertilisers, thereby minimising the application of conventional fertilisers (Oliveira *et al.*, 2016). To date, reuse of treated wastewater had been focusing on improved crop productivity (Leal *et* *al*., 2009), along with satisfactory plant nutrition (Bedbabis *et al*., 2014). Furthermore, research has demonstrated positive alterations in soil physico-chemical characteristics, such as increase in fertility, reduction in soil acidity, increase in organic matter and improvement in particle aggregation when treated wastewater was applied (Leal *et al.*, 2009; Rusan *et al*., 2007; Silva *et al*., 2016; Tarchouna *et al.*, 2010; Xu *et al.*, 2010). Some studies demonstrated the effects of irrigation with treated wastewater in lowering toxic heavy metals concentrations to the lower horizons (Abdu *et al*., 2011; Zhao *et al*., 2012), whereas promoting the accumulation of essential heavy metals on the soil surface (Yao *et al*., 2014). The disadvantages of using treated wastewater on soil physical, chemical and biological properties have also been reported by a number of studies (Castro *et al*., 2011; Singh and Agrawal, 2012). Soil salinisation and sodification increase exchangeable Na that could compromise the soil structure (Krista *et al*., 2003) and deterioration of soil organic matter pools (Singh and Agrawal, 2012). Treated wastewater, as described previously (Chapter 3), had been used at the University of Limpopo Experimental Farm (ULEF) for the production of onions and tomato plants using best production practices that include *Natuurboerdery* farming and extended fallowing practices. However, the impact of the previously characterised treated wastewater (Chapter 3) on soils at the ULEF had not been investigated. The objective of this study, therefore, was to determine whether the effects of treated wastewater on the (1) physical and chemical properties of soil, (2) distribution of heavy metals in irrigated fields and (3) biological-soil-health indicators under *Natuurboerdery* and conventional farming systems would be similar.

4.2 Materials and methods

4.2.1 Site description and demarcation

The study coordinates of the ULEF study site were as described previously (Chapter 3). The area could be described as a semi-arid with approximately 80-87% rainfall occurring in summer (October-January) at less than 495 mm/annum. Minimum mean temperature ranged from 2.2 to 6.0°C during winter (May-July) and in spring (August-October) being from 9.0 to 16.7°C. In contrast, the maximum mean temperature from May to August ranged from 20.2 to 23.04°C and from September to April from 26.7 to 39.6°C (Weather SA, 2015).

Four 15 ha fields were identified for this study as virgin field (VF), cultivated field (CF), fallowed field (FF) and research block (RB). The VF was previously never cultivated and was, therefore, used as a reference point. The CF was in its second year of cultivation and being irrigated with treated wastewater as prescribed previously (Chapter 3). The field was cultivated once a year for a three-year cycle on onion (*Allium cepa)* production. Fertilisation was done as in commercial onion production systems (ARC, 2013). Irrigation was scheduled following the reference evapotranspiration (ET_o) which was estimated using the FAO Penman-Monteith equation using daily meteorological data observed from the DFM probe software collected daily (DFM Technologies, 2017). The crop water requirements (ET_c) over the growing season were determined by multiplying the ET_o values with the onion crop coefficients (K_c) given by Allen *et al*. (1998) as 0.7 for the 1st, 0.90 for the 2nd, 1.05 for the 3rd and 0.75 for the 4th growth stages as follows:

$$ET_c = K_c*ET_o$$

The FF (six-year-cycle) was in its fifth year of fallowing, which would after the six-year be followed by a three-year-cycle of onion cultivation – irrigated with treated wastewater. The RB, used for student trials with irrigation comprising borehole water, was continuously subjected to different synthetic chemical fertilisers. Soils at VF, CF and FF were classified as Bainsvlei or Plintustalfs (USDA), whereas that at RB was Hutton or Bruneel soils series (USDA) (Soil Classification Working Group, 1991; WRB, 1998). On average, in both soil forms, clay and sand contents were approximately 35% and 65%, respectively.

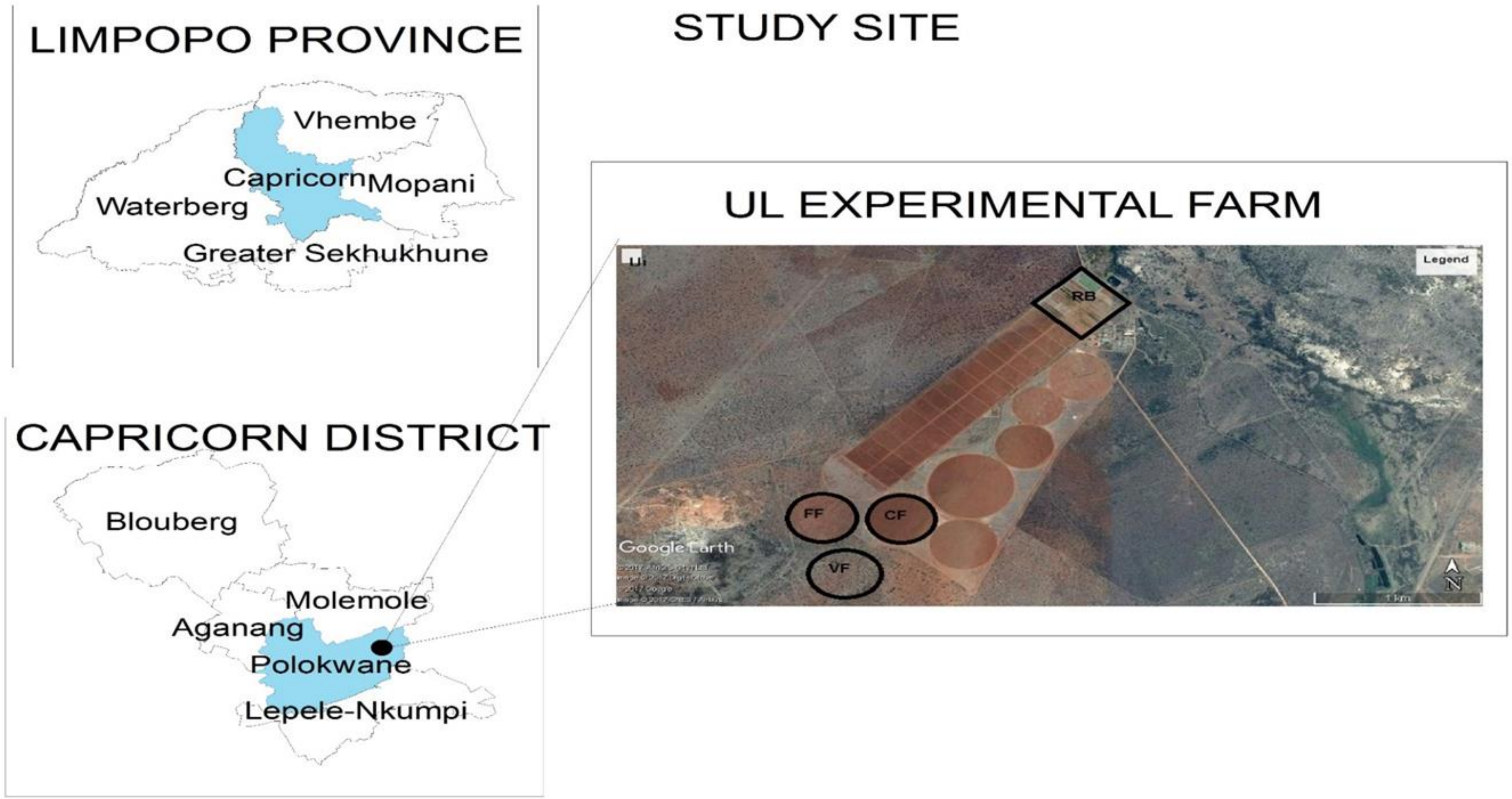

Figure 4.1 The University of Limpopo Experimental Farm, Limpopo Province.

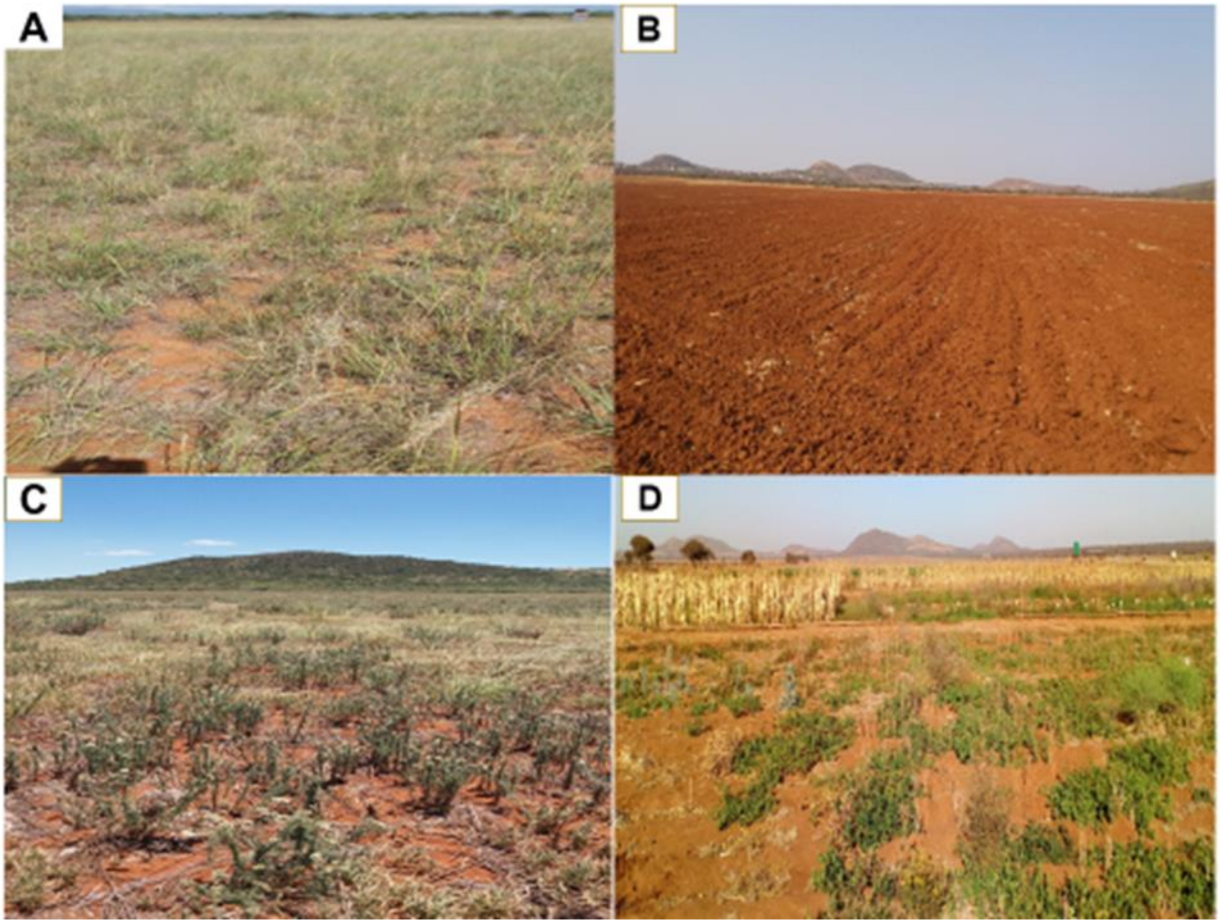

Figure 4.2 Demonstration of the sampled fields: (A) virgin field, (B) cultivated field, (C) fallowed field and (D) research block.

4.2.2 Experimental design

A 4 × 5 factorial experiment, with the first factor being four fields (CF, FF, CF, RB) and the second factor being five depths (0-20, 20-40, 40-60, 60-80 and 80-100 cm), were arranged in randomised complete block design, with 15 replications. Blocking was done for the moderate slope and undetected soil type variability.

4.2.3 Soil sampling and preparation

Each of the four 15 ha fields was divided into 15 equal plots. In the middle of each plot a soil profile was opened to the depth of 100 cm, where soil samples were collected from five equal depths at 20 cm interval, namely: 0-20, 20-40, 40-60, 60-80 and 80-100 cm. All soil samples were collected in sterile sampling bags, air-dried and crushed to pass through a 2-mm sieve, keeping enough for aggregate stability determination. Samples for biological indicators were stored in the refrigerator prior to analysis.

4.2.4 Data collection

Physico-chemical properties: Particle size distribution was determined using hydrometer method through differential settling densities of clay and sand particles (Bouyoucos, 1962). Aggregate stability was quantified using the modified (Kemper and Rosenau, 1986) wet-sieving method (Van Bavel, 1950). Briefly, 50 g of soil less than 4.75 mm soil samples were placed on stacked 2-, 1-, 0.5- and 0.25-mm opening sieves, with sieved samples immersed in distilled water and then sieved by moving the stacked sieves vertically. The soil particles retained by each sieve were dried at 105°C for 24 h, weighed and corrected for sand particles to obtain the proportion of water-stable aggregates by calculating the mean weight diameter (MWD) using the relation (Kemper and Rosenau, 1986):

$$\text{MWD (mm)} = \Sigma x_i w_i$$

where x is the mean diameter of aggregates separated by sieving and w is the weight fraction of aggregates in that size range and the total dry weight of soil used. Bulk density was determined by collecting undisturbed samples in 100 cm^3 soil cores, weighed, dried in an oven to constant weight, weighed and calculated using the formula (Carter, 1990):

$$\text{Bulk density (g/ml)} = \text{Dry soil weight (g) / Soil volume (ml)}$$

Soil pH(H_2O) and pH(KCl) were quantified using the 1:2.5 extracts through a benchtop pH meter (McLean, 1982). Soil electrical conductivity (EC) was quantified as in the pH(H_2O) procedure through a benchtop EC meter (McLean, 1982). Exchangeable cations (Ca, Mg, K, Na) and were extracted using 1 M ammonium acetate at pH 7 and quantified through the atomic absorption spectrophotometer (Chapman, 1965; Zhang *et al*., 2012). Cation exchange capacity (CEC) was determined by addition of the exchangeable cations (Chapman, 1965). The exchangeable sodium percentage (ESP) was calculated as follows with cations were expressed in milli-equivalents/100 g of soil:

$$ESP = Exchangeable \{(Na)/ (Ca + Mg + K + Na)\} \times 100$$

Selected essential nutrient elements: Nitrogen in the form of NH_4^+ and NO_3^- was quantified through the colorimetric method. For NH_4^+, 10.0 g of freshly sampled soil sample was extracted by adding 100 ml of 0.5 M K_2SO_4 extracting solution, filtered and added to a series of NH_4^+ standard solutions. The extract was then mixed with the first reagent that comprised 34 g sodium salicylate, 25 g sodium citrate and the second that comprised 25 g sodium tartrate, 0.12 g sodium nitroprusside and 10 ml sodium hypochlorite. The absorbance was measured at 655 nm after 1 h (Okalebo *et al*., 1993). The extraction of NO_3^- excluded addition of 10 ml sodium hypochlorite as it results in ionic interference with Cl^- (Freney and Wetselaar, 1969). Phosphorus was quantified through the Bray and Kurtz (1945) procedure, extractable B through the hot water extraction method (McGeehan *et al*., 1989). Sulphur was determined using the revised Anderson *et al*. (1992) procedure, which entailed extraction in 0.25 *M* KCl and heating at 40°C for 3 h prior to quantifying (Blair *et al.,* 1993). Available P, B and S were each quantified through ICP-MS.

Heavy metals: Bioavailable heavy metals from soil samples were extracted using the revised Kimbrough and Wakakuwa (1989) procedure (USEPA, 1996). Briefly, 15 ml NHO_3 was added to 1 g soil sample, heated for 2 h at 95°C. After cooling, 2 ml water and 10 ml 30% H_2O_2 were added to each sample, which was heated for 10 minutes at 95°C and then cooled at room temperature. The aliquot was then diluted to 100 ml with distilled water, filtered through Whatmann no. 41 filter paper prior to quantifying Al, As, Cd, Ni, Cr, Cu, Fe, Mn, Pb and Zn on ICP-MS, which were grouped as essential (Cu, Fe, Mn, Ni, Zn) and non-essential (Al, As, Cd, Cr, Pb) heavy metals.

Biological indicators of soil health: These indicators were quantified as described in the Cornell University Soil Health Handbook (Gugino *et al*., 2009). Organic carbon was quantified using the revised Walkely-Black method (Schumacher, 2002). Active carbon was quantified using the Blair *et al*. (1995) simplified method (Weil *et al*., 2003). Briefly, a 2.5 g air-dried soil samples were each mixed with 20 ml 0.02 M potassium permanganate ($KMnO_4$) to oxidise the active carbon and then centrifuged for 5 minutes prior to the absorbance measurement on the spectrophotometer (Gugino *et al*., 2009). Potentially minerilisable nitrogen (PMN) was measured from two 8 g soil samples which were each placed in 50 ml centrifuge tubes. The first tube was mixed with 40 ml 2.0 M potassium chloride (KCl) and mechanically shaken for one hour, centrifuged and quantified for NH_4^+ concentration as a starting time. In the second tube,10 ml distilled water was added, hand-shaken and incubated for 7 days at 30°C, thereafter, 2.67 M KCl was added to the mixture, mechanically shaken for 1 h, centrifuged and quantified for NH_4^+ (Benedetti and Sebastiani, 1996; Gugino *et al*., 2009). Soil samples collected in different fields (VF, CF, FF, RB) at the 0-20 cm depth were used for quantifying root health.

Root health rating: Soil samples from each field, serving as treatments, were placed in 200 ml cone-tubes, with one garden bean seed sown (Gugino *et al*., 2009). The tubes were placed on the greenhouse bench, with treatments arranged in a randomised complete block design, with four replications. Blocking was done for wind-stream variability which was created by heat-extracting fans. Plants were irrigated with 25 ml tapwater every other day (Figure 4.2). At four weeks after emergence, plants were removed from containers, with roots washed under running tapwater and then scored for root health rating on a 1 to 9 scale (Gugino *et al*., 2009).

Figure 4.3 Root health of garden bean at four weeks prior to termination.

Table 4.1 Root health rating numbers and description.

Rating number	Description
1	White and coarse textured hypocotyl and roots; healthy.
2	Light discoloration and lesions less than 10% of hypocotyl and root tissues.
3	Light discoloration and lesions covering up to a maximum of 10% of hypocotyl and root tissues.
4	Approximately 10-20% of hypocotyl and root tissue have lesions, but the tissues remain firm.
5	Approximately 25% of hypocotyl and root tissue have lesions, but the tissues remain firm.
6	There is little decay or damage to the root system.
7-9	50 to ≥ 75% of hypocotyl and roots severely symptomatic and at advanced stages of decay.

4.2.5 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Stata 12 software (StataCorp, 2011). Significant interactive effects for field data were further assessed using the two-way matrix tables (Gomez and Gomez, 1984). The mean sum of squares (MSS) were used to partition the source of variation and then express the treatment effects as total treatment variation (TTV) of the test variable (Gomez and Gomez, 1984). Mean separation of significant treatments was accomplished using the Tukey's Honestly Significant Difference (HSD) test at the probability level of 5%. Tukey's HSD test was used since the treatments did not include an untreated control (Gomez and Gomez, 1984). Descriptive statistics was used to assess root health to generate the minimum and maximum ratings, where the VF soil sample was used as a standard. Unless otherwise stated, treatment effects were described at the probability level of 5%.

4.3 Results

4.3.1 Physico-chemical properties

Field × depth interaction effects were not significant on all four test physical properties (Appendix 4.1). Field type had highly significant ($P \leq 0.01$) effects on clay, sand, aggregate stability and bulk density, contributing 58, 84, 90 and 98% in TTV of the respective variables (Appendix 4.1). Soil depth showed significant differences on bulk density, contributing 1% in TTV of the variable (not described further due to negligent TTV), but had no significant effects on clay, sand and aggregate stability (Appendix 4.1). Relative to VF; the CF, FF and RB effects increased clay content by 35, 35 and 19%, respectively, but decreased sand content by 20, 39 and 14%, respectively (Table 4.2). Virgin field, CF and FF decreased aggregate stability by 95 and 85%, respectively, whereas RB increased the variable by 230%, and consistently reduced bulk density by 89, 14 and 25%, respectively (Table 4.2).

Table 4.2 Effects of cultivated fields irrigated with (+) and without (-) treated wastewater on clay, sand, aggregate stability (AS) and bulk density (BD) relative to those on virgin field.

Treatment	Clay (%)		Sand (%)		AS		BD ($g.cm^{-3}$)	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Virgin field (VF)$^{-}$	26^{b}±0.53	−	70^{a}±0.55	−	0.2^{b}±0.00	−	1.79^{a}±0.02	−
Cultivated field (CF)$^{+}$	35^{a}±0.48	35	56^{b}±0.50	−20	0.01^{c}±0.00	−95	0.2^{d}±0.00	−89
Fallowed field (FF)$^{+}$	35^{a}±0.81	35	43^{c}±1.06	−39	0.03^{c}±0.00	−85	1.54^{b}±0.02	−14
Research block (RB)$^{-}$	31^{ab}±0.83	19	60^{b}±0.98	−14	0.66^{a}±0.03	230	1.34^{c}±0.02	−25

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey's HSD test.

[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Field × depth interaction effects were highly significant on soil pH(H_2O) and soil pH(KCl), contributing 2 and 1% in TTV of the respective variables, whereas the interaction was not significant on soil EC (Appendix 4.2). Similarly, soil depth effects were highly significant on soil pH(H_2O) and soil pH(KCl), contributing 4 and 3% in TTV of the respective variables, but had no effect on soil EC. In contrast, field type had highly significant effects on soil pH(H_2O), soil pH(KCl) and soil EC, contributing 93, 95 and 91% in TTV of the respective variables (Appendix 4.2). Generally, relative to VF at 0-20 cm soil depth, the trends for subsequent depths and field type at different depths were not clear for soil pH(H_2O) and soil pH(KCl) (Table 4.3), and were, therefore, not described further. Relative to VF, the effects of CF, FF and RB increased soil EC by 343, 319 and 116%, respectively (Table 4.4).

Table 4.3 Effects of cultivated fields irrigated with (+) and without (-) treated wastewater on soil pH relative to those on virgin field at different soil depths.

	Soil depth (cm)									
	0-20		20-40		40-60		60-80		80-100	
Treatment	Variable^{y}	R.I. (%)^{z}	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
					pH(H_2O)					
Virgin field (VF)$^{-}$	$5.29^{g}\pm0.03$	–	$5.11^{g}\pm0.02$	−3	$5.39^{fg}\pm0.03$	2	$5.60^{fg}\pm0.03$	6	$5.91^{ef}\pm0.05$	12
Cultivated field (CF)$^{+}$	$6.44^{de}\pm0.02$	22	$6.59^{cd}\pm0.03$	25	$7.01^{bc}\pm0.04$	33	$7.52^{ab}\pm0.05$	42	$7.82^{a}\pm0.04$	48
Fallowed field (FF)$^{+}$	$6.85^{cd}\pm0.01$	29	$6.76^{cd}\pm0.02$	28	$6.89^{cd}\pm0.01$	30	$6.81^{cd}\pm0.02$	29	$6.81^{cd}\pm0.02$	29
Research block (RB)$^{-}$	$7.84^{a}\pm0.01$	48	$7.64^{a}\pm0.02$	44	$7.60^{a}\pm0.01$	44	$7.63^{a}\pm0.01$	44	$7.67^{a}\pm0.02$	45
					pH(KCl)					
Virgin field (VF)$^{-}$	$4.23^{h}\pm0.02$	–	$4.30^{gh}\pm0.02$	2	$4.51^{gh}\pm0.02$	7	$4.63^{gh}\pm0.02$	10	$4.89^{fg}\pm0.03$	16
Cultivated field (CF)$^{+}$	$5.93^{cd}\pm0.03$	40	$5.89^{cde}\pm0.02$	39	$6.16^{bc}\pm0.03$	46	$6.50^{abc}\pm0.04$	54	$6.67^{ab}\pm0.03$	58
Fallowed field (FF)$^{+}$	$5.27^{ef}\pm0.02$	25	$5.38^{def}\pm0.02$	27	$5.51^{def}\pm0.02$	30	$5.88^{cde}\pm0.08$	39	$5.97^{cd}\pm0.04$	41
Research block (RB)$^{-}$	$7.09^{a}\pm0.02$	68	$6.69^{a}\pm0.01$	58	$6.85^{a}\pm0.02$	62	$6.84^{a}\pm0.01$	62	$6.87^{a}\pm0.03$	62

^{y}Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey's HSD test.

^{z}Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Table 4.4 Effects of cultivated fields irrigated with (+) and without (-) treated wastewater on soil electrical conductivity (EC) relative to those in virgin field.

	Variable[y]	R.I. (%)[z]
Virgin field (VF)$^{-}$	4.96^{c}±0.03	–
Cultivated field (CF)$^{+}$	22.00^{a}±0.57	343
Fallowed filed (FF)$^{+}$	20.77^{a}±0.97	319
Research block (RB)$^{-}$	10.70^{b}±0.24	116

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey's HSD test.

[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Cation distribution: The field × depth interaction effects were not significant on the test cations. However, field type had highly significant effects on Ca, Mg, K and Na, contributing 92, 96, 81 and 96% in TTV of the respective variables (Appendix 4.3). Soil depth did not have significant effects on any the cations. Relative to VF; the effects of CF, FF and RB increased Ca by 389, 944 and 465%, respectively (Table 4.5). The CF and FF effects significantly increased soil Mg by 44 and 70%, respectively, whereas the RB effects decreased Mg by 80% (Table 4.5). The CF effects increased soil K significantly by 2356%, whereas the VF, FF and RB effects on K were not significantly different (Table 4.5). The CF, FF and RB effects increased Na by 29, 181 and 232%, respectively (Table 4.5).

Table 4.5 Effects of cultivated fields irrigated with (+) and without (-) treated wastewater on soil exchangeable cation concentration ($cmol_c/kg$) relative to those in virgin field.

Treatment	Ca		Mg		K		Na	
	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Virgin field (VF)$^-$	0.01^c±0.00	–	0.12^b±0.00	–	0.01^b±0.00	–	0.01^c±0.00	–
Cultivated field (CF)$^+$	0.04^b±0.00	389	0.17^a±0.00	44	0.22^a±0.00	23	0.02^c±0.00	29
Fallowed field (FF)$^+$	0.08^a±0.00	944	0.20^a±0.01	70	0.01^b±0.00	−18	0.03^b±0.00	181
Research block (RB)$^-$	0.05^b±0.00	465	0.02^c±0.00	−80	0.01^b±0.00	−6	0.05^a±0.00	232

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey's HSD test.

[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Cation exchange capacity and exchangeable sodium percentage: The field × depth interaction did not have significant effects on both CEC and ESP (Appendix 4.4). However, the field type effects were highly significant on soil CEC and ESP, contributing 92 and 98% in TTV of the respective variables (Appendix 4.4). Soil depth effects were significant on soil CEC, but had no significant effects on ESP (Appendix 4.4). The CF and FF effects increased soil CEC significantly by 69 and 122%, respectively, whereas RB had no significant effects on the variable (Table 4.6). Relative to VF, the CF and FF effects on ESP were not significantly different, whereas the RB effects significantly increased ESP by 432% (Table 4.6). Relative to soil depth at 0-20 cm, soil depth at 20-80 cm did not have significant effects on CEC, whereas soil depth at 80-100 cm increased CEC by 25% (Table 4.7).

Table 4.6 Effects of cultivated fields irrigated with (+) and without (-) treated wastewater on soil cation exchange capacity (CEC) and exchangeable sodium percentage (ESP) relative to those in virgin field.

Treatment	CEC ($cmolc.kg^{-1}$)		ESP	
	Variable^{y}	R.I. (%)^{z}	Variable	R.I. (%)
Virgin field (VF)$^{-}$	0.14^{c} ±0.00	−	8.90^{b}±0.19	−
Cultivated field (CF)$^{+}$	0.24^{b}±0.00	69	7.38^{b}±0.23	−17
Fallowed field (FF)$^{+}$	0.32^{a}±0.01	122	10.78^{b}±0.41	21
Research block (RB)$^{-}$	0.13^{c}±0.00	−12	47.33^{a}±1.03	432

^{y}Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey's HSD test. ^{z}Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Table 4.7 Relative cation exchange capacity at different soil depths.

Depth (cm)	CEC ($cmolc.kg^{-1}$) Variable[y]	R.I. (%)[z]
0-20	$0.20^{b}\pm0.00$	−
20-40	$0.19^{b}\pm0.00$	−1
40-60	$0.20^{b}\pm0.00$	1
60-80	$0.21^{ab}\pm0.01$	6
80-100	$0.25^{a}\pm0.01$	25

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey's HSD test.

[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Nitrate and ammonium: The field × depth interaction effects were not significant on soil NO_3^- and NH_4^+, whereas field type was highly significant on soil NO_3^- and NH_4^+, contributing 52 and 70% in TTV of the respective variables, whereas soil depth had no significant effects on the two variables (Appendix 4.5).

Relative to VF; the CF effects decreased soil NO_3^- by 21%, but were not significantly different with the FF and RB effects on the variable (Table 4.8). The CF did not have significant effects on NH_4^+, whereas FF and RB significantly reduced the variable by 35 and 32%, respectively (Table 4.8).

Table 4.8 Effects of cultivated fields irrigated with (+) and without (-) treated wastewater on soil nitrate (NO_3^-) and ammonium (NH_4^+) concentrations relative to those on virgin soil.

Treatment	NO_3^- (mg.kg^{-1})		NH_4^+ (mg.kg^{-1})	
	Variable^y	R.I. (%)^z	Variable	R.I. (%)
Virgin field (VF)$^-$	52.11^a±2.22	–	8.88^a±0.37	–
Cultivated field (CF)$^+$	41.48^b±0.98	−21	7.36^a±0.18	−15
Fallowed field (FF)$^+$	50.75^a±0.97	−3	5.78^b±0.09	−35
Research block (RB)$^-$	53.18^a±1.10	2	6.01^b±0.10	−32

^{y}Column means followed by the same letter were not different ($P \le 0.05$) according to Tukey's HSD test. ^{z}Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

<u>*Phosphorus, boron and sulphur*</u>: The field × depth interaction was highly significant on P and B, contributing 1 and 4% in TTV of the respective elements (Appendix 4.6). The respective field × depth interaction TTV were negligent and were therefore not discussed. Field and depth were highly significant on P and B, contributing 91% and 4% in TTV of the respective elements (Appendix 4.6). The depth TTV for the respective variables were negligent and were therefore, not discussed further. The interaction, field and depth were not significant on S (Appendix 4.6). Relative to VF the increased in were not significant in CF and RB, however, FF significantly increased P by 969% (Table 4.9). The treatment effects of irrigation and cultivation decreased B significantly by 22 and 42 CF and FF, but increased the variable by 78% in RB (Table 4.9).

Table 4.9 Effects of cultivated fields irrigated with and without treated wastewater on soil phosphorus (P) and boron (B) concentrations in different soil depths relative to those on virgin soil at the plough-able soil depth.

	P ($mg.kg^{-1}$)		B ($mg.kg^{-1}$)	
Field	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)
Virgin field (VF)$^{-}$	$0.60^{b}\pm0.01$	–	$2.65^{b}\pm0.05$	–
Cultivated field (CF)$^{+}$	$1.22^{b}\pm0.07$	105	$2.06^{c}\pm0.03$	−22
Fallowed field (FF)$^{+}$	$6.36^{a}\pm0.37$	969	$1.55^{d}\pm0.02$	−42
Research block (RB)$^{-}$	$1.18^{b}\pm0.07$	99	$4.71^{a}\pm0.13$	78

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey's HSD test.

[z]Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

4.3.2 Heavy metals

Essential heavy metals: The field × depth interaction effects were significant on soil Ni and highly significant on soil Zn, contributing 2 and 1% in TTV of the respective variables (Appendix 4.7). However, the interaction effects were not significant on Cu, Fe and Mn (Appendix 4.7). Field type effects were significant on soil Cu, Fe, Mn and Cr contributing 89, 92, 98 and 33% in TTV of the respective variables (Appendix 4.7). Soil depth effects were not significant on soil Cu, Fe, Mn, Ni and Zn, but significant on Cr, contributing 37% in TTV of the variable. Using the depth-field matrix, relative to VF at 0-20 cm depth, depths at VF, the treatment effects in CF, FF and RB decreased Ni by 5-97% (Table 4.10).

Relative to VF, the treatment effects of cultivation and irrigation in CF, FF and RB reduced Zn by 22, 56 and 96%, respectively. The treatment effects of cultivation and irrigation in CF, FF and RB reduced Ni by 13, 25 and 95%, respectively. However, the treatment effects were not statistically different for Cu in CF and FF, but decreased the variable by 91%. The treatment effects were not statistically different for Fe in CF. However, the variable significantly increased by 63% in FF and deceased by 34% in RB. The treatment effects in RB were not statistically different for Mn, whereas in CF and FF Mn increased by 852 and 1636% (Table 4.10). Generally, the increase in soil depth increased soil Cr from 21 to 65% (Table 4.10). However, the increases were not significant.

Table 4.10 Effects of cultivated fields irrigated with (+) and without (-) treated wastewater on soil zinc (Zn), nickel (Ni), copper (Cu), iron (Fe), manganese (Mn) and chromium (Cr) concentrations relative to those on virgin soil.

Treatment	Zn (mg.kg^{-1})		Ni (mg.kg^{-1})		Cu (mg.kg^{-1})		Fe (mg.kg^{-1})		Mn (mg.kg^{-1})	
	Variable^{y}	R.I. (%)^{z}	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Virgin field (VF)$^{-}$	8.22^{a}±0.19	−	10.16^{a}±0.28	−	9.93^{a}±0.27	−	11.04^{b}±0.23	−	1.00^{c}±0.03	−
Cultivated field (CF)$^{+}$	6.44^{b}±0.14	−22	8.83^{ab}±0.22	−13	7.63^{a}±0.29	−23	12.16^{b}±0.39	10	9.55^{b}±0.37	852
Fallowed field (FF)$^{+}$	3.65^{c}±0.12	−56	7.58^{b}±0.27	−25	9.72^{a}±0.43	−2	17.94^{a}±0.23	63	17.40^{a}±0.04	1636
Research block (RB)$^{-}$	0.35^{d}±0.01	−96	0.46^{c}±0.02	−95	0.88^{b}±0.04	−91	7.31^{c}±0.31	−34	0.86^{c}±0.04	−15

^{y}Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey's HSD test.

^{z}Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Non-essential heavy metals: The field × depth interaction effects were not significant on soil Al, As, Cd, Cd and Pb (Appendix 4.8). Field type effects were highly significant on soil Al, Cd and Pb, contributing 84, 67 and 73% in TTV of the respective variables, but had no significant effects on As (Appendix 4.8). Soil depth effects were significant on soil Al, contributing 7 in TTV of the respective variables, but had no significant effects on As and Pb (Appendix 4.8). Relative to VF, the treatment effects in CF, FF and RB increased soil Cr concentration by 43 and 49%, respectively (Table 4.11). Relative to VF, the treatment effects in CF and FF increased soil Al by 24 and 58%, respectively, whereas those in RB decreased Al by 16% (Table 4.11). Relative to VF, the treatment effects in CF, FF and RB increased soil Cd by 120, 18 and 254%, respectively (Table 4.11). Relative to VF, the treatment effects in CF and RB increased soil Pb by 408 and 148%, respectively, whereas those in FF decreased the variable by 20% (Appendix 4.11).

Table 4.11 Effects of cultivated fields irrigated with (+) and without (-) treated wastewater on soil chromium (Cr), aluminium (Al), cadmium (Cd) and lead (Pb) ($mg.kg^{-1}$) concentrations relative to those on virgin soil.

Treatment	Cr ($mg.kg^{-1}$)		Al ($mg.kg^{-1}$)		Cd ($mg.kg^{-1}$)		Pb ($mg.kg^{-1}$)	
	Variable^{y}	R.I. (%)^{z}	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Virgin field (VF)$^{-}$	0.41^{b}±0.02	−	12.40^{c}±0.31	−	0.67^{b}±0.03	−	1.37^{bc}±0.18	−
Cultivated field (CF)$^{+}$	0.58^{a}±0.03	43	15.41^{b}±0.37	24	1.48^{b}±0.09	120	6.97^{a}±0.57	408
Fallowed field (FF)$^{+}$	0.51^{ab}±0.02	25	19.64^{a}±0.51	58	0.79^{b}±0.03	18	1.10^{c}±0.04	−20
Research block (RF)$^{-}$	0.61^{a}0.03	49	10.38^{d}±0.60	−16	2.38^{a}±0.21	254	3.41^{b}±0.30	148

^{y}Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey's HSD test.

^{z}Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

4.3.3 Biological indicators of soil health

The field × depth interaction effects were significant on soil SAC, contributing 11% in TTV of the variable, but had no significant effects on SOC and PMN (Appendix 4.9). Field effects were highly significant on SOC and PMN, contributing 100 and 90% in TTV of the respective variables (Appendix 4.9). Soil depth had no significant effects on both SOC and PMN (Appendix 4.10). Relative to 0-20 cm soil depth, subsequent depths in VF increased soil SAC from 2 to 10% (Table 4.12). Relative to VF at the 0-20 cm soil depth, treatment effects in CF increased SAC in 0-20 and 20-40 cm soil depths by 43 and 8%, respectively, but decreased the variable by 10, 15 and 7% in the 40-60, 60-80 and 80-100 cm soil depths, respectively (Table 4.12). Relative to VF at 0-20 cm soil depth, treatment effects in FF decreased SAC from 21 to 53% in all five soil depths (Table 4.12). In contrast, treatment effects in RB increased soil SAC by 19% in the 0-20 cm soil depth, but decreased the variable from 4 to 39% in subsequent soil depths (Table 4.12).

Table 4.12 Effects of cultivated fields irrigated with (+) and without (-) treated wastewater on soil active carbon (SAC) ($g.ha^{-1}$) in the cultivated field, fallowed field and research block relative to virgin field over five depths (cm).

	Soil depth (cm)									
	0-20		20-40		40-60		60-80		80-100	
Treatment	Variable^{y}	R.I. (%)^{z}	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Virgin field (VF)$^{-}$	457.83^{abc}±11.36	–	493.50^{abc}±11.73	4	467.47^{abc}±14.19	2	517.84^{abc}±9.41	13	503.67^{abc}±9.63	10
Cultivated field (CF)$^{+}$	678.56^{a}±16.85	43	515.18^{abc}±18.70	8	409.85^{abc}±18.98	−10	388.46^{abc}±19.12	−15	423.26^{ab}±17.73	−7
Fallowed field (FF)$^{+}$	377.11^{bc}±10.98	−21	285.56^{bc}±12.82	−40	266.34^{bc}±12.21	−42	270.80^{c}±12.69	−41	218.00^{abc}±12.42	−53
Research block (RB)$^{-}$	564.43^{abc}±14.20	19	456.79^{abc}±13.63	−4	346.26^{bc}±11.29	−24	277.46^{bc}±10.50	−39	278.54^{bc}±12.64	−39

^{y}Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey's HSD test.

^{z}Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Table 4.13 Effects of cultivated fields irrigated with (+) and without (-) treated wastewater on soil organic carbon (SOC) and potentially mineralisable nitrogen (PMN) per week relative to that in virgin soil.

Treatment	SOC (%)		PMN (mg.kg^{-1})	
	Variable^{y}	R.I. (%)^{z}	Variable	R.I. (%)
Virgin field (VF)$^{-}$	13.79^{a}±0.01	−	0.07^{c}±0.01	−
Cultivated field (CF)$^{+}$	1.69^{d}±0.03	−88	0.30^{b}±0.01	334
Fallowed field (FF)$^{+}$	3.57^{c}±0.02	−74	0.15^{c}±0.00	114
Research block (RB)$^{-}$	8.42^{b}±0.04	−39	0.52^{a}±0.03	642

^{y}Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey's HSD test. ^{z}Relative impact (%) = R.I. (%) = [(Wastewater/Borehole) – 1] × 100.

Field was significant to root health rating of dry bean, contributing 91% in TTV (Appendix 4.10), whereas other sources of variation had no effect on the variable. Relative to VF; the treatment effects in CF, FF and RB increased root health rating by 238, 65 and 115%, respectively (Table 4.14). Root health ratings at VF, CF, FF and RB had 1-3, 3-9, 1-5, 1-9 ranges, respectively (Table 2.14).

Table 4.14 Effects of cultivated fields irrigated with (+) and without (-) treated wastewater on root health rating of garden bean relative to that in virgin soil.

	Mean rating		Range	
Treatment	Variable[y]	R.I. (%)[z]	Minimum	Maximum
Virgin field (VF)⁻	2^{c}±0.09	–	1	3
Cultivated field (CF)⁺	6^{a}±0.22	238	3	9
Fallowed field (FF)⁺	3^{bc}±0.17	65	1	5
Research block (RB)⁻	4^{b}±0.29	115	1	9

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

[z]Column means followed by the same letter were not different (P ≤ 0.05) according to Tukey's HSD test.

4.4 Discussion

In all the variables, unless stated otherwise, the field × depth interaction effects were not significant, and therefore, the focus was primarily on the main factors. The field type, which could be viewed as representing a wide spectrum of management practices [virgin field (VF), cultivated field (CF), fallowed field (FF) and research block (RB = repeated cultivation)]. The VF constituted undisturbed soil, which was used as a standard, whereas RB comprised repeated cultivation with conventional fertilisation practices and therefore, poor agricultural practices, whereas CF and FF comprised *Natuurboerdery* farming, viewed as the best agricultural practices in context of the ULEF study.

4.4.1 Physico-chemical properties

Soil texture: Soil textural fractions of clay and sand percentage exhibited spatial variability in the four fields. Textural differences could have been due to the slope, where finer particles moved with time down the slope and accumulated at the bottom, thus, increasing sand content at the higher slope as observed elsewhere (Ceddia *et al*., 2009). Additionally, cultural practices could have also played a role in the observed differences. Relative to the VF; the CF, FF and RB fields all had increased clay content, but decreased sand content, which supported the view that management practices such as cultivation could also have significant effects on increasing clay content of the soil (Adugna and Abegaz, 2016; Mohammed, 2017; Yimam *et al*., 2014). In the current study, clay content was greatest in CF ≥ FF ≥ RB ≥ VF, where virgin field (VF) had the least clay content. Soil depth had no significant effects on clay and sand content in the current study, which contradicted observations in another study on treated wastewater in an arid region where clay content was reduced at 80-120 cm soil depth by 27% (Abedi-Koupai, 2006). The latter was justified through the concept of particle dispersion which was caused by the leached salts (Abedi-Koupai, 2006).

Aggregate stability: Generally, aggregate stability had been known to be highly sensitive to repeated cultivation practices and use of heavy farming machinery (Bidisha *et al*., 2010; Mohammed, 2017). Due to the sandy texture of the soil, the soil structure was weak, with micro-aggregates that were easily disturbed with accumulation of Na ions further decreasing the mean weight diameter of existing aggregates (Mohammed, 2017). Cultivation also subjected the soil aggregates to fragmentation and exposure of soil organic matter to microbial attack (Li *et al*., 2006; Yimam *et al*., 2014). Organic matter could be positively correlated with aggregate stability (Chaney and Swift, 1984), with extreme decomposition of organic matter resulting in weak aggregates. In the FF there was a slight increase in aggregate stability, probably due to organic matter build-up as observed in another five-year fallowed field (Beare *et al*., 1993). The research block (RB) with the highest aggregate stability than other field types, including the virgin field, had the mean weight diameter of 0.66 mm, which was still regarded as being weak. The RB was irrigated using borehole water; where the presence of Na salts could have contributed to the observed weak stability of aggregates as observed in another study (Tedeschi and Dell'Aquila, 2005). Soil depth, as observed in soil texture, did not have significant differences in aggregate stability. However, others (Bird *et al*., 2002; Miller and Jastrow, 1990) demonstrated that the top soil had aggregate stability with high mean weight diameters due to of the accumulation of higher organic matter.

Bulk density: The CF had the lowest bulk density (0.2 g/ml), followed by the FF (1.54 g/ml) and then the VF (1.79 g/ml), which were possibly due changes in cultural practices. In contrast, Ufot *et al*. (2016) observed a decrease in bulk density from 1.86 g/ml on cultivated field to 1.62 g/ml on fallowed field, which was attributed to the use of treated wastewater and shorter cultivation periods (Azouzi *et al*., 2016). The decrease could also be as a result of leaching of tiniest particles depending on volumes and frequency of water added to the soil. Abedi-Koupai *et al*. (2006) reported an increase in bulk density in n two depths of top soil that was irrigated with treated wastewater. Bulk density is a critical indicator of soil compaction and soil health (USDA-NCRS) and could affect rooting depth, water infiltration and the presence of microorganisms (Logsdon and Karlen, 2004).

Soil pH: Generally, soil pH(H_2O) in ULEF study increased with addition treated wastewater and soil depth, probably due to salts from the used water as observed in other studies (Tarchouna *et al.*, 2010), although others (Bedbabis *et al*., 2014) observed an opposite trend due to treated wastewater. Importantly, the pH values from 7.0 to 8.5 in the CF, FF and RB were still within the acceptable pH range for most agricultural crops (Gargouri, 1998).

Electrical conductivity: The interaction and soil depth did not affect soil EC. In contrast, a number of studies (Bedbabis *et al*., 2014; Rusan *et al*, 2007), due to the leaching effects, noted that high EC values were observed beyond the 60-cm soil depth with repeated application of treated wastewater. The significant effects of field type on EC at the ULEF study, confirmed observations in short-term studies where treated wastewater were applied in Europe and in Asia (Khurana and Singh, 2012). In contrast, the observed decrease in EC on the FF at the ULEF study could be attributed to leaching of salts during the rainy season as observed in other studies (Bedbabis *et al*, 2014). The magnitude of the decrease on the FF in the ULEF study in EC was not as high as in other studies (Bedbabis *et al*, 2014) since the rainfall at the ULEF location, which was in a semi-arid region, was relatively low. Most importantly, the observed EC values in all the test fields were still below the EC threshold of 4 $dS.m^{-1}$ (Qadir *et al*., 2010).

<u>Cation distribution</u>: Interaction and soil depth did not have any effects on cation concentrations in the ULEF study, whereas field type had highly significant effects on Ca, Mg, K and Na. Others (Oliveira *et al*., 2016; Parvan and Danesh, 2011) observed significant cation distribution with soil depth. For instance, Parvan and Danesh (2011) observed high Na and Mg on top than bottom soil horizons in a field irrigated with treated wastewater when compared with field irrigated with well-water. Similarly, Oliveira *et al*. (2016) observed an increase in Ca with soil depth that was accompanied by a decrease in K when the soil was irrigated with treated wastewater. Apparently, the *Natuurboerdery* farming practices in the ULEF study played a role in the uniform vertical distribution of cations, which was supported by the significant effects of field type on the distribution of the cations.

Comparing the VF; the effects of CF, FF and RB increased Ca with exceedingly high magnitudes of 389, 944 and 465%, respectively. The highest magnitude of Ca in the FF confirmed the poor mobility of Ca in soil (Sharpley, 2008), particular in soils with organic matter as in the ULEF study where *Natuurboerdery* farming was practiced. Additionally, the RB irrigated with borehole water that contained high Ca concentration, had comparatively high Ca in soil, which contradicted others (Heidarpour *et al*., 2007; Khaskhoussy *et al*., 2015), who observed that irrigation with groundwater invariably resulted in lower soil Ca than when irrigating with treated wastewater.

Relative to VF; the CF and FF field types increased soil Mg, but with far lower magnitudes than as observed in soil Ca. Both treated wastewater and borehole water, as shown previously (Chapter 3), had low Mg concentrations, which supported the relatively low soil Mg in the ULEF study. The differences in soil Mg in cultivated and fallowed fields in the current study could be attributed to either mobilization (Grzebisz, 2011; Schachtschabel, 1954) or absorption abilities of onions, which were cultivated on the field (Sullivan *et al*., 2001) during sampling.

The highest soil K in CF under the ULEF study could not be explained exclusively through additions from treated wastewater as a nutrient carrier and fertiliser as inferred in some studies (Galavi *et al*., 2010). Under *Natuurboerdery* farming at the ULEF, some fertilisers, depending on soil sample analysis results and the crop, are applied, with the organic matter being mainly for the improvement of the physical properties of the soil. In other studies, (Bedbabis *et al*., 2014; Kiziloglu *et al*., 2008; Urbano *et al*., 2007), claims were made that the increase in soil K in soils irrigated with treated wastewater were exclusively related to the presence of the cation in irrigation water. Generally, K increases as observed in the ULEF study are advantageous for crop growth and quality as the nutrient element is generally required in large quantities for proper growth and reproduction in plants (Arienzo *et al*., 2009).

Sodium increased in the CF, FF and RB, but when compared to other studies subjected to treated wastewater, the observed Na concentrations in all four fields were low (Horneck *et al*., 2011). The ULEF results contradicted observations where significantly high soil Na concentrations were observed in fields irrigated with treated wastewater in comparison to those irrigated with well-water or borehole water

(Bedbabis *et al*., 2014; Heidarpour *et al.,* 2007). In the ULEF study, it should be remembered that the borehole water had significantly high Na concentrations (Chapter 3). In another study of vertical distribution of cations in fields treated with wastewater in Western Cape, South Africa, Mzini (2013) observed high Na concentrations in such fields when compared with those that were irrigated with portable water. However, as seen in the ULEF study, due to the widespread availability of Na ions, both treated wastewater and borehole water, along with organic matter and fertilisers, could be the potential carriers of Na (Patterson, 1997). High Na in soils could be undesirable since it might cause dispersion of aggregates, resulting in prompt deterioration of the physical properties of soil, with undesirable consequences that might negatively affect plant growth and productivity (Emongor and Ramolemana, 2004).

Cation exchange capacity (CEC): The field type and soil depth each had highly significant effects on the variable. In a study where the effects of treated wastewater and borehole were compared on CEC (Al-Khamisi *et al*., 2015), the variable increased under irrigation with treated wastewater and decreased with borehole water. In the ULEF study, all CEC values were below 1 $cmol.kg^{-1}$, which had elsewhere been categorised as being extremely low (CUCE, 2007). However, the values were close to the sum of the exchangeable cations (Tarchouna *et al*., 2010), suggesting that the cations had the potential of being fixed on the exchange complex (Tarchouna *et al*., 2010). In the ULEF study the low CEC values could also be explained on the basis of the high sand content, which confirmed observations in other studies where sandy soils were irrigated with treated wastewater (Moore *et al*., 1998). In the ULEF study, the highest CEC values were observed in the 80-100 cm depth, with the lowest CEC values being in top soils, which confirmed other observations (Al-Khamisi *et al*., 2015), where higher CEC values were at least below 90-cm depth. In another study, Jim

(1998) observed a decrease in CEC with increase in soil depth, which was attributed to deficiencies in inorganic colloids that are required to provide exchangeable sites for nutrient adsorption. Similarly, relative to VF, the CF and FF in the ULEF study increased soil CEC, which could also be attributed to differences in concentrations of inorganic colloids with addition of treated wastewater and the use of best agricultural practices in the form of *Natuurboerdery* farming system.

Exchangeable sodium percentage (ESP): Comparing with VF, the CF and FF effects on ESP were not significantly different, whereas the relative effects in RB increased ESP over four-hundred fold. The latter agreed with the observed high concentration of Na in borehole water (Chapter 3). In the CF and FF, which were reliant on treated wastewater for irrigation, the ESP values were below 15%, which is the lower limit beyond which sodicity could become a threat (Kallel *et al*., 2012), whereas in the RB the 47.33% ESP was far above the 15% ESP limit. Generally, using treated wastewater increase ESP value to above 15% in flavisols (Kallel *et al*., 2012), which suggested that soil form could also play a role in changes of the test variable. In the ULEF study, the RB in comparison to the CF and FF under irrigation with treated wastewater, was already at high level of sodicity, which suggested a high Na concentration in the exchange complex (Leal *et al*., 2009). Consequently, the borehole water used in irrigating the RB, when compared with the treated wastewater used in irrigating the CF and FF, was, on the basis of the soil ESP, not suitable for irrigation purposes.

Nitrates and $NH_4^{\pm}$: Relative to VF; CF soil had reduced NO_3^-, but the variable was not different on FF and RB samples. Relative to VF; CF soil had significant effects on NH_4^+, which was relatively reduced in FF and RB soil samples. Generally, treated wastewater contain excessive sources of NO_3^- and NH_4^+ (Hernandez-Martinez *et al*., 2018), which could lead to high concentration of the variables in groundwater due to leaching (Tredoux *et al*., 2009). In contrast to observations in the ULEF study, in other cases where treated wastewater was used, there had been some evidence of increased NO_3^- and NH_4^+ concentrations in soil samples (Belaid *et al*., 2010; Matheyarasu *et al*., 2016). In most cases, agricultural activities had been associated with a high reduction of organic matter (Tiessen *et al*., 1982), with the potential of reducing the nitrogen dynamics in the soil (Knops and Tilman, 2000). The reduced NO_3^- and NH_4^+ concentrations in the ULEF study could be attributed to losses due to plant uptake since the two are the available forms of N in the soils and could have been leached out as observed in other investigations (Walworth, 2013). Additionally, the observed low values of NH_4^+ relative to NO_3^- observed in all the ULEF test fields, could imply that the rate of nitrification in conversion of the NH_4^+-N to the NO_3^- -N was low (Lamb *et al*., 2014).

Phosphorus: In the ULEF study soil P was higher in the treated wastewater irrigated fields than in VF and RB, with the highest concentration (6.36 mg P.kg^{-1}) observed in FF. Bedbabis *et al*. (2014) observed the same trend where treated wastewater resulted in higher P than well water. The observed increase in soil P in both treated wastewaters irrigated fields could have resulted from treated wastewater irrigation and fertilisation during onion production (Sullivan *et al*., 2001). Furthermore, the observed highest P concentration in FF, could be attributed to plant material return on soil and restoration of soil structure with the five year of fallowing (Selles *et al*., 2002). Depth effects on Soil P were significant but negligent. A study conducted in Ethiopia reported on concurring result where soil P decreased with depth (Emiru and Gebrekidan, 2013).

The negligent result in the ULEF study could be due to immobile attributes of P in soils (Balemi and Negisho, 2012).

<u>Boron</u>: Boron was the highest in RB, with the lowest concentration observed in FF. The parent material of the ULEF study site was granite, which is reported to comprise 10-30 mg B kg^{-1} (Ahmad *et al*., 2012). However, the highest concentration found in RB (4.71 mg $B.kg^{-1}$) was below the expected minimum B concentration as per parent material. The low B concentration in soil could be as a result of low B in treated wastewater that was used for irrigation in this study, and its relationship with K and Zn (Ref). Currently, it is not clear why B was the highest in the borehole water samples in August and the lowest in the night-dam during both July and September. It is likely that the presence of chemical elements in the borehole were influenced by those in treated wastewater. Apparently, the disposal of the unutilised wastewater in the forest further up the western side of the night-dam, along with the night-dam itself, would have some detrimental effects on the borehole water through downward movements of elements like B. Graham *et al*. (1987) reported that B uptake by barley (*Hordeum vulgare* L.) was lower when Zn was applied, compared to when it was absent. In the ULEF study Zn was low in the irrigated soils, however, the variable was high in the produce. The results were in contrast to those in another study (Tsadilas, 1997), where treated wastewater had a high B concentration (above 1 $mg.l^{-1}$) and increased B concentration in crop leaf tissues and soil. Ahmad *et al*. (2012) also indicated that when heavy K application was made, B had to be escalated to prevent reduction in crop uptake, which was not the case under *Natuurboerdery* farming system in the ULEF study, since B was observed to below the toxic level of 100 $mg.kg^{-1}$ (Ahmad *et al*, 2012) in all fields.

4.4.2 Heavy metals

<u>Essential heavy metals</u>: Field type effects were significant on soil Zn, Cu, Ni, Fe, Mn and Cr all with extremely high TTV magnitudes, whereas soil depth had no significant effects on any variable. The interactive effects of treated wastewater and soil depths had been well studied in various regions (Abdu *et al*., 2011; Ibrahim *et al*., 2016; Stietiya *et al*., 2014), with some studies having significant wastewater and soil depth interactions on Ni (Abdu *et al*., 2011) and Zn (Atanassova *et al.,* 2015; Stietiya *et al*., 2014). Also, Kebonye *et al*. (2017) in Botswana reported wastewater × soil depth interactive effects on Cu and Mn in a 20-year wastewater irrigated field. Additionally, Atanassova *et al*. (2015), observed significant effects of soil depth on Fe and Mn when the field was treated with industrial sludge in Bulgaria.

Low values of Zn were observed in all fields with the lowest being on the RB and the highest observed in VF. Generally, Zn is an abundant element that could get as high as 500 $mg.kg^{-1}$ of in most agricultural soils (Long *et al*., 2003). However, the observation is in contrast with previous work conducted in South Africa which confirmed that Zn is generally higher in irrigated systems than virgin land (Manyevere *et al*., 2017). In the current study, Zn was least reduced in best agricultural *Natuurboerdery* practices (CF), where treated wastewater was used, with situation worsening under unsustainable practices irrigated with borehole water (RB). The improvement in Zn on the CF could also be attributed to the addition of fertilisers during the production of onions. Relative to CF, in FF Zn decreased, suggesting the need to analyse the soil after the six year fallowing cycle under *Natuurboerdery* farming. In contrast, fallowing had no significant effects on soil Zn (Stanislawska-Glubiak *et al.*, 2012), supporting the view that the *Natuurboerdery* farming practices could be contributing to the observed soil Zn status quo. Soil Zn increased with an increase in depth of the FF, which could be attributed to Zn mobility as affected by soil texture and high soil pH in the test field. Generally, when pH increases, Zn concentration would also increase (Rutkowska *et al*., 2015). Contrary to the latter, in the RB Zn decreased despite the higher observed soil pH and irrigation borehole water. This could be associated to the different crops cultivated on the RB every year.

Relative to VF, the RB drastically reduced Cu, whereas the CF, FF and VF effects on the variable were not significantly different. Consequently, the best sustainable practices (CF, FF) in the ULEF study were suitable for soil Cu when compared with the unsustainable practices in the RB. In contrast to cultural practices, Cu distribution was not affected by soil depth as observed elsewhere, regardless of the crops and irrigation water (Abedi-Koupai *et al*., 2006). However, in the same study, Cu had increased with irrigation, which is in contrast with the ULEF results. In the ULEF study the Cu concentration was low in all test fields since the international threshold values had been set for Cu at 100 $mg.kg^{-1}$ of soil (Tóth *et al*., 2016). Granites are associated with Cu mineralisation (Blevin and Chappel, 1992). Low Cu in the Aconcagua River Basin study, north-central Chile averaging 30 $mg.kg^{-1}$ attributed to the geological makeup of the surrounding soil, which were primarily of the granite parent material, as was in the ULEF study (Aguilar *et al*., 2011). In addition to the parent material, the concentration of Cu in irrigation water could play a role in the accumulation of this heavy metal. Borehole water used in the RB had the lowest Cu concentration at 0.99 $mg\ Cu.l^{-1}$ (Chapter 3). Copper is an essential heavy metal; therefore, it is required for various plant functions. However, there are no mines and big industries around Mankweng area, therefore, low values were as a result geological makeup of the surrounding soils, as some granites are associated with Cu mineralisation (Blevin and Chappel, 1992).

Relative to VF, FF and RB reduced soil Ni, whereas the relative effects of CF to VF on the variable were not different. In all fields, Ni concentration on the surface was decreased with irrigation, as the highest was observed in VF. The decrease in CF was not statistically significant, but then fallowing was able to decrease Ni from 10.16 mg $Ni.kg^{-1}$ in VF to 7.58 mg $Ni.kg^{-1}$ in FF. A further decrease of 95% was observed in CF. The results in the ULEF were in contrast to those in a study by Abedi-Koupai *et al*. (2006), where there was a slight increase in Ni with irrigation using treated wastewater. Further contracting results were observed in another study where treated wastewater irrigation increased Ni from 0.3 to 1.42 mg $Ni.kg^{-1}$ (Balkhair and Ashraf, 2016). The decrease in Ni in the irrigated fields of the present study could imply that cultivation coupled with the sandy clay texture, were able to speed up Ni mobility towards deeper depths with the aid of irrigation as observed elsewhere (Agnieszka and Barbara, 2012).

The effects of CF on Fe were not different, whereas RB significantly reduced the variable. Silva *et al*. (2016) could not detect significant differences between treated wastewater and fresh water on Fe concentration under non-organic farming. Consequently, the observed positive attribute of treated wastewater and *Natuurboerdery* farming at the ULEF study, count as one such attribute for the system under evaluation.

Generally, treated wastewater had been shown to increase Mn (Abedi-Koupai *et al*., 2006). However, under the ULEF study, caution should be taken to avoid Mn phytotoxicities in crops. Generally, Mn phytotoxicities in most crops occur when Mn in irrigation water and soil are above 0.20 $mg.l^{-1}$ and 2000 $mg.kg^{-1}$, respectively (Ayers and Westcot, 1985). In the ULEF study, under *Natuurboerdery* farming irrigation soil contained 9.55 (CF) and 17.40 $mg.kg^{-1}$ soil (FF), respectively.

Generally, the concentration of Cr increased with soil depth. Fallowing was able to reduce Cr concentration from 0.58 $mg.kg^{-1}$ in CF to 0.51 $mg.kg^{-1}$. The decrease FF indicated the advantages of fallowing, as Cr in high concentration could be toxic to crops. The observed Cr values in the ULEF study were far below the minimum range of 100 $mg.kg^{-1}$ soil (Crommentuijn *et al*., 1997). High values of Cr could lead to phytotoxicities with severe chlorosis, necrosis and disturbed enzyme activities (Samantaray *et al*., 1998). In the ULEF study under *Natuurboerdery* farming, the low Cr values possibly due to low concentration of Cr in irrigation water (Chapter 3), with high concentrations emanating from industries (Vodyanitskii *et al*., 2015). The increased accumulation of Cr with soil depth could have been due to organic matter which promote Cr mobility (Banks *et al*., 2006), therefore, it could have been leaching stimulated by organic matter presence and organo-complexes.

<u>*Non-essential heavy metals*</u>*:* Generally, Al concentration increased with soil depth. In the ULEF study, Al accumulation could have been added through treated wastewater since aluminium sulphate is part of the coagulant used in treatment of treated wastewater (Sahu and Chaudhari, 2013). Generally, Al values above 10 $mg.kg^{-1}$ are considered toxic (Edmeades *et al*., 1983), with the toxicity being more likely in acidic soils, as Al is available at pH less than 5.5 (Silva *et al.*, 2016). In the ULEF study, *Natuurboerdery* fields irrigated with treated wastewater were having soil pH of 5.93 for CF, which implies the need for some caution with respect to this non-essential element. However, since Al increased with increasing soil depth in the ULEF study, the element might be out of reach for most roots. The increased Al with soil depth

contradicted others (Oliveira *et al*., 2016), who observed a decreasing trend with soil depth restricted to 0-35 cm under treated wastewater.

Field type had highly significant effects on Cd, increasing Cd by 254% in RB, whereas the VF, CF and FF effects on the variable did not differ. The Cd maximum permissible level is 3 $mg.kg^{-1}$ in agricultural soils (Mapanda *et al*., 2005), and above this level Cd could accumulate in plant produce, with resulting health hazards in consumers (Godt *et al*., 2006). Therefore, all Cd concentrations were below the recommended standard. Although in the current ULEF study Cd accumulation was not affected by soil depth, others (Mapanda *et al*., 2005) observed that the metal could accumulate at various depths. Cadmium is derived from weathering of rocks and minerals or from numerous anthropogenic sources (Alloway and Steinnes, 1999), Cd has proved to have a positive correlation with phosphorus fertilisers (Roberts, 2014). Accumulation of Cd, a biotoxic heavy metal, in the food chain is detrimental as plant uptake of Cd from fertilized soils could result in entry of cadmium into the human food chain (Mapanda *et al*., 2005).

The VF, FF and RB effects on the variable were not significant. Other studies on Pb distribution on treated wastewater irrigated fields without *Natuurboerdery* farming did not observe significant responses on Pb (Khaskhoussy *et al*., 2015; Rattan *et al*.,2005). Generally, Pb is adsorbed strongly on fine clay and organic matter particles, which could explain high Pb CF. Although borehole water used to irrigate RB was low (0.44 $mg.l^{-1}$) in Pb (Chapter 3), Pb concentration from RB soil samples was relatively high (3.41 $mg.kg^{-1}$). The high soil Pb could be due to soil texture and soil pH of 7.84. High concentrations of Pb could occur in soil with pH above 6.5 (Holmgren *et al*., 1993).

4.4.3 Biological indicators of soil health

Four biological indicators of soil health, namely, soil organic carbon (SOC), soil active carbon (SAC), potentially minerilisable nitrogen (PMN) and root health rating were, unless stated otherwise affected by field type alone, which showed the potency of cultural practices on the variables.

Soil organic carbon: Change in land use from virgin to cultivated land was previously shown to decrease SOC by about 50% (Celik *et al*., 2004). Al-Hamaiedeh and Bino (2010) also observed a decrease in SOC from 2.83 to 0.81% when field was irrigated using treated wastewater. In contrast, fallowing increased SOC from 1.69 to 3.57 %. A significant increase in SOC with a natural fallow of 3 years has been observed, which was also related to increased crop yields (Tian *et al*., 2005), supporting the 6 year fallowing practices in the ULEF study. Organic carbon relates greatly with aggregation of the soil (Chaplot and Cooper, 2015). Therefore, fallowing in the ULEF study was able to recuperate the aggregate stability, with SOC being highly increased when compared with other cultural practices.

Soil active carbon: Soil active carbon in the first three depths was not statistically different, but increased with depth from 60 cm, with the highest accumulation of SAC at the 80-100 cm soil depth. Soil active carbon as an important indicator of soil health comprises that fraction of soil organic matter which is readily available as a carbon and energy source for the soil microbial community (Jogan *et al*., 2017). In terms of cultural practices, the CF at 0-20 cm depth, increased SAC, whereas in the VF the variable was decreased with depth, with the lowest SAC content (388.46 g. ha^{-1}) being at 60-80 cm soil depths. Fallowing decreased SAC content throughout the soil profile, with the lowest recorded at 80-100 cm depth. The fractions of SAC have high rates of decomposition and short occupation times in the soil (Haynes, 2005). Because of this fast cycling, SAC is a more sensitive and a relevant fraction for assessing changes caused by agricultural practices than SOC (Campos *et al*., 2011). Research block exhibited high SAC content, even higher than the reference field denoting a decrease in depth, with the lowest content reported in 60-100 cm depths.

Potentially mineralisable nitrogen: The PMN values reported were the mineralised nitrogen in one week, nevertheless, the varied PMN indicated the abilities of the soil to mineralise. The PMN test indicates the capacity of the soil microbes to recycle organic nitrogen into the plant available forms. Although there is scarcity of information on effects of treated wastewater on PMN, a study on seasonal variation of PMN in four cropping systems, had significant differences per cropping system and seasons (Torben *et al.*, 1988). Hernandez *et al*. (2018) study on nitrogen mineralisation under different textures and amendments with sewage sludge, reported significant treatment difference. Singh *et al*. (2005) reported that measured N mineralization depended on the C:N ratio, which could be a valid case as different materials decompose differently due to their C:N ratio (Pal *et al*., 1975).

Root health: Root health assessment is a tool used in estimating expected effects of health of a particular soil on root development (Gugino *et al*., 2009). Healthy roots are essential for substantial plant growth and high yield by being efficient in absorption of plant nutrients and water. Development of healthy roots is an indication of good soil structure, low populations and activities of root pathogens and pests (Murillo-Williams, 2007). The CF and RB had a maximum rating of 9, which suggested that 75% of hypocotyl and roots were severely damaged and at advanced stages of decay (Gugino *et al*., 2009). In most cases the rating 5, which was observed in soil collected from FF suggested that there could be little decay or damage to the root system whereas the tissues remained firm (Gugino *et al*., 2009). Several parameters that have been used the literature to describe characteristics of root systems such as these include root length, root extension, root mass, root volume and root diameter, could be easily affected by physical properties of the soil such as penetration, compaction and bulk density (Atkinson, 2000).

4.5 Synthesis and conclusion

Generally, results observed in the four fields which were managed differently and irrigated with different types of water indicated the positive and the negative effects depending on the cultural practices. In most cases, fallowing of the field that was exposed to *Natuurboerdery* farming enhanced the mitigation of the potential negative responses observed mostly under bad cultural practices such as when monoculture fields were irrigated with treated wastewater. The *Natuurboerdery* FF and the unsustainable RB exhibited a positive effect towards soil health physical indicators such as aggregate stability and bulk density, whereas CF with sustainable practices exhibited negative attributes. However, for pH and EC, the three fields (CF, FF, RB) responded positively. All four fields irrespective of the irrigation water source, displayed positive effects on soil Ca, Mg, K and Na, although Na ion was high in both treated wastewater and borehole water (Chapter 3), the variable was within acceptable limits in the CF, FF and RB. However, it would be important that continuous monitoring of the variable be done because its deleterious effects on soil structure are costly to mitigate. It can be concluded that fallowing was able to restore the negative attributes.

Fields amended with *Natuurboerdery* materials displayed negative effects on NO_3^- and NH_4^+, with the block where unsustainable agricultural practices occurred (RB) being the most affected. Essential heavy metals were also reduced in the RB field, whereas field amended with organic materials had the cations within the recommended standards, possibly due to strong adsorption. In contrast, the organically-amended fields had the highest concentration of the non-essential heavy metals, probably for the same reasons as the essential cations. In general, fields irrigated with treated wastewater displayed more positive attributes than those in the research block (RB), where borehole water was used for irrigation, without the use of organic amendments. In conclusion, treated wastewater, in terms of its chemical composition, when used under properly managed soil systems that include soil amendments and fallowing, could have negative effects on chemical composition of the soil. Therefore, the findings support the hypothesis that indicated that treated wastewater from MWTP would have effects on physico-chemical properties, heavy metal distribution and biological indicators of soil health at the ULEF site. In the next chapter the researcher investigated whether the distribution of cations and heavy metals in (1) shoot and leaf tissues relative to root tissues in onion and tomato plants and (2) the related accumulation in onion, tomato and horseweeds (*Conyza canadensis* L.) leaf tissues in soil irrigated with treated wastewater under *Natuurboerdery* farming system would be similar.

CHAPTER 5

CATIONS AND HEAVY METALS IN ROOT, STEM AND LEAF TISSUES OF PLANTS IRRIGATED WITH TREATED WASTEWATER

5.1 Introduction

Treated wastewater had been receiving attention in irrigation of various crops (Pinto *et al*., 2010). In some instances, the water served as a significant plant nutrient source for soils of low fertility by providing abundant essential nutrient elements (Parveen *et al*., 2014). However, certain studies indicated that treated wastewater could serve as a potential source of environmental pollution with respect to cations and heavy metals, which could eventually find themselves in food chains and therefore, these challenges should be taken into consideration when using such water (Khan *et al*., 2008; Matović *et al*., 2015). Due to disproportionate imbalances of nutrient elements supplied through treated wastewater (Chapter 3), crops could be favoured either positively or negatively (Pedrero *et al*., 2010). Cations and heavy metals taken up in excess by plants could stimulate, show no effect or inhibit plant growth in context of density-dependent growth (DDG) patterns (Salisbury and Ross, 1992), thereby, disturbing a wide-range of physiological processes (Parveen *et al*., 2014).

Plants tend to accumulate nutrients and heavy metals disproportionally in different organs through absorption from contaminated soils through root interception mechanisms (FSSA, 2007), with distribution to different parts through the vascular bundle. Furthermore, high concentrations of heavy metals in tissues of edible produce invariably expose consumers to potentially hazardous chemicals (Antonious *et al*., 2011; Matović *et al*., 2015). Heavy metals such as zinc (Zn) and iron (Fe) are essential elements, but when absorbed in excess, could result in phytotoxicity in certain plant species (Nagajyoti *et al*., 2010).

In plants, lead (Pb), cadmium (Cd) and arsenic (As) heavy metals do not have any essential role and are therefore, regarded as a health hazard to both plants and animals (Skipper *et al*., 2016). Cadmium could accumulate in the body and cause challenges such as kidney failure and fertility dysfunction in both humans and animals (Skipper *et al*., 2016). *In vivo* studies have demonstrated that Cd could affect male reproduction at a concentration as low as 1 ppm body mass (Rossman *et al*., 1992; Skipper *et al*., 2016). Lead had been associated with liver, brain and the central nervous system dysfunction and has been classified as a probable human carcinogen (Assi *et al*., 2016). The partitioning of cations and heavy metals in below and above ground plant organs in crops irrigated with treated wastewater had not been documented. The objective of the study was two-fold, namely, to establish whether the distribution of cations and heavy metals in (1) shoot and leaf tissues relative to root tissues in onion and tomato plants and (2) the related accumulation in onion, tomato and horseweeds (*Conyza canadensis* L.) leaf tissues in soil irrigated with treated wastewater under *Natuurboerdery* farming system would be similar.

5.2 Materials and methods

5.2.1 Description of the study site

The study was conducted at the University of Limpopo Experimental Farm (ULEF), Limpopo Province, South Africa (23°83'31''S, 29°69'46''E). Soils in the study area were characterised as Hutton, with an average of 38% clay and pH of 7.78 (Chapter 4). Two cultivated fields, described previously (Chapter 4), were used for the production of onion (*Allium cepa* L.) and tomato (*Solanum lycopersicum L.*) plants during 2015, 2016 and 2018, with samples collected during the first two cropping seasons (2015: Experiment 1, 2016: Experiment 2. Treated wastewater used for irrigation on the cultivated fields was as described previously (Chapter 3). Weeds were collected from a fallowed field that was also described previously (Chapter 4).

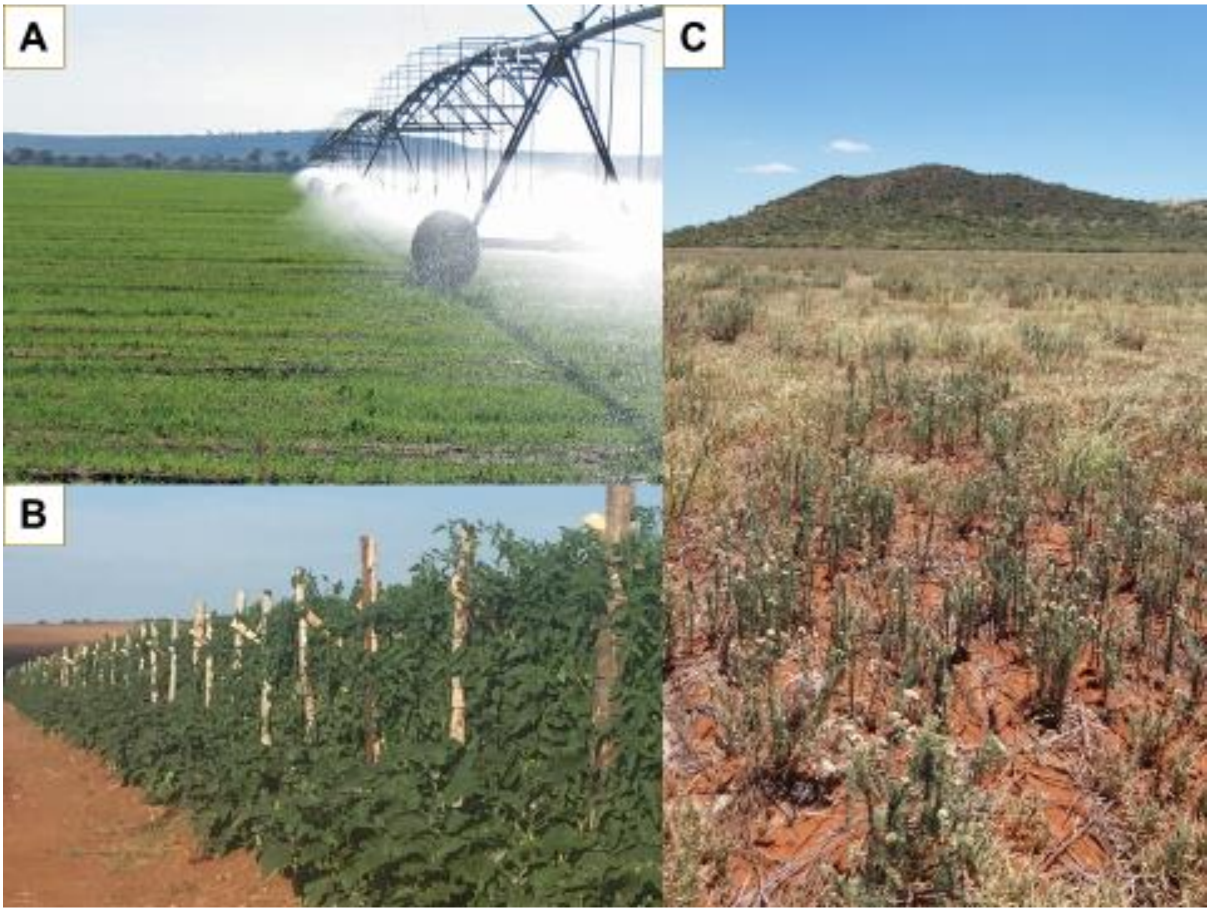

Figure 5.1: Representation of (A) the onion field, (B) the tomato field and (C) the fallowed field with weeds in 2015.

5.2.2 Planting and sampling of test plants

Onions: Approximately 600 000 to 800 000 seeds were sown on 1.2 m wide ridges/ha, giving a total planting density from 104 to 140 seedlings/m on a commercial scale. The farming system comprised of a *Natuurboerdery* farming system (Taurayi, 2011). Bulbs of cv. 'Star 5516' were generally borne above the soil surface, with only roots penetrating into the soil. Plants were irrigated using the 36 m wide centre pivot irrigation system, which covered a total field of 15 ha. Fertiliser and pest management were as in commercial onion production systems under *Natuurboerdery* farming system (Nzanza *et al*., 2013). Briefly, during soil preparation, 2:3:2 (22) NPK fertiliser was applied at the rate 100 g/m^2. Limestone ammonium nitrate (LAN) and KCl were applied at 3 and 6 weeks after planting. At full maturity, a 1 ha land was selected at random among the 15 ha field for sampling. The 1 ha was divided into 8 and 7 blocks for sampling in August 2015 (Experiment 1) and validated in August 2016 (Experiment 2), respectively. Five onion plants were selected randomly and manually pulled out of the soil. The different onion organs were separated using a knife, washed in running tap water and rinsed with deionised water. The outer scales of the bulb were peeled and bulbs sliced into pieces to facilitate drying. Roots, bulbs and leaves were dried in air-forced ovens at 65°C for five days (Udiba *et al*., 2015).

<u>*Tomato*</u>: Tomato cv. 'Topacio' seedlings were transplanted in 40 ridges/ha, with inter-row spacing of 2.5 m and intra-row spacing of 0.30 m, irrigated using inline drip irrigation system. Fertilisers and pest management were done as in *Natuurboerdery* farming system in commercial tomato production systems (Nzanza *et al*., 2013). A one-hectare field was selected at random among the 15 ha for sampling, divided into 8 and 7 plots for sampling in March 2016 and 2017, respectively. At full maturity, five tomato plants were randomly pulled out of the soil to form a sample. Roots, stems and leaves were separated, washed in tap water and rinsed in distilled water prior to drying as described for onions (Udiba *et al*., 2015).

<u>*Weeds*</u>: Leaves of *C. canadensis* were collected from a 15 ha fallowed field. A 1 ha plot was randomly selected, divided into 8 and 7 plots for samples in 2016 and 2017,

respectively. Representative plants were randomly collected from each plot. Leaves were separated from 5 plants to form a sample/plot, slightly washed in tapwater and rinsed with distilled water prior to drying as described for onions.

5.2.3 Sample preparation extraction and analysis

All samples were ground to pass through a 0.5 mm sieve and stored in sealed plastic bags prior to analysis. Powdered 0.5 g samples were transferred into 50 ml centrifuge tubes, each digested with 10 ml of HNO_3 and 3 ml of H_2O_2 using the microwave extraction EPA Method 3052 (USEPA, 1996). Samples were cooled at room temperature and then filtered using Whatmann 42 filter paper. The samples were quantified for Ca, Mg, K, Na, Zn, Fe, Cu, Mn, Al, As, Cd, Cr and Pb using ICP-OES (Stephan and Hineman, 2012).

5.2.4 Statistical analysis

All data were subjected to analysis of variance (ANOVA) using Statistix 10 software, normality of data was assumed due to the sample size (Elliott, 2007; Pallant, 2007). The mean sum of squares (MSS) were used to partition the treatment effects in total treatment variation (TTV) of the respective variables (Gomez and Gomez, 1984). Significant treatment means were separated using Fisher's Least Significant Difference test at the probability level of 5%. Unless stated otherwise, treatment effects were discussed at the probability level of 5%

5.3 Results

5.3.1 Accumulation of cations and heavy metals in onion

Major cations: In Experiment 1, the treatment effects were highly significant ($P \leq 0.01$) on Ca, Mg, K and Na, contributing 91, 88, 77 and 90% in TTV of the respective variables, whereas in Experiment 2 the treatment effects contributed 92, 85, 74 and 96% in TTV of the respective variables (Appendix 5.1). Relative to root tissues, Ca in leaf tissues was increased by 144 and 85%, in Experiment 1 and Experiment 2, respectively. However, Ca was decreased in bulb tissues by 42 and 44%, respectively (Table 5.2). In both experiments, the partitioning of Mg in root and leaf tissues did not differ. However, Mg in bulb tissues was decreased by 66 and 68%, in Experiment 1 and Experiment 2, respectively (Table 5.1). Potassium in leaf tissues in Experiment 1 and Experiment 2 was increased by 46 and 64%, respectively, but was decreased by 36 and 10% in bulb tissues, respectively (Table 5.1). Sodium in leaf and bulb tissues in Experiment 1 was decreased by 49 and 89%, respectively. In Experiment 2, Na in leaf and bulb tissues was decreased by 61 and 86%, respectively (Table 5.1).

Essential heavy metals: The treatment effects were highly significant for Zn, Fe, Cu and Mn in onion tissues for both experiments, contributing 78, 86, 94 and 84% in TTV of the respective variables in Experiment 1 and then 67, 79, 96 and 88% in TTV of the respective variables in Experiment 2 (Appendix 5.2). Relative to onion root tissues, Zn in leaf tissues was increased by 37 and 36% in Experiment 1 and Experiment 2, respectively, whereas Zn in root and bulb tissues were not different (Table 5.2). Iron was not different in leaf tissues, but was reduced by 50% in bulb tissues in Experiment 1 (Table 5.2). In contrast, Fe was reduced by 19 and 32% in leaf and root tissues, respectively, in Experiment 2. Copper was increased by 76% and 85% in leaf tissues in Experiment 1 and Experiment 2, respectively, but was reduced by 64 and 72% in bulb tissues of the respective experiments. In Experiment 1, Mn in root and leaf tissues did not differ, whereas the element was reduced by 64% in bulb tissues. In contrast, in Experiment 2, Mn was reduced in leaf and bulb tissues by 39 and 72%, respectively (Table 5.2).

Table 5.1 Accumulation of calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) in leaf and bulb relative to root tissues of onions irrigated with treated wastewater.

Plant organ	Ca (ppm)		Mg (ppm)		K (ppm)		Na (ppm)	
	Variable^{y}	R.I. (%)^{z}	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Experiment 1								
Root	1129.50^{b}±113.33	−	933.00^{a}±88.82	−	3496.30^{b}±298.98	−	3628.80^{a}±230.15	−
Leaf	2761.30$^{a±}$221.51	144	1158.40^{a}±62.24	24	5095.00^{a}±422.64	46	1860.90^{b}±270.39	−49
Bulb	656.00^{c}±37.04	−42	319.50^{b}±32.08	−66	2235.00^{c}±163.85	−36	391.40^{c}±12.74	−89
Experiment 2								
Root	1256.1^{b}±112.33	−	1200.7^{a}±75.84	−	2471.4^{b}±228.10	−	3068.6^{a}±171.77	−
Leaf	2322.9^{a}±118.24	85	1233.4^{a}±108.65	3	4058.6^{a}±307.12	64	1206.7^{b}±89.77	−61
Bulb	702.9^{c}±41.84	−44	382.9^{b}±28.07	−68	2221.4^{b}±100.17	−10	444.7^{c}±19.47	−86

^{y}Column means followed by the same letter were not different (P ≤ 0.05) according to Fisher's least significant difference test.

^{z}Relative impact [R.I. (%)] = [(Plant organ/Root) – 1] ×100.

Table 5.2 Accumulation of zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) in leaf and bulb relative to root tissues of onions irrigated with treated wastewater.

Plant organ	Zn (ppm)		Fe (ppm)		Cu (ppm)		Mn (ppm)	
	Variable	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
				Experiment 1				
Root	$156.50^{b}\pm2.94$	−	$557.63^{a}\pm29.28$	−	$2.22^{b}\pm0.35$	−	$134.82^{a}\pm12.35$	−
Leaf	$214.03^{a}\pm17.54$	37	$412.00^{a}\pm19.76$	−26	$3.90^{a}\pm2.05$	76	$115.59^{a}\pm4.65$	–14
Bulb	$107.84^{b}\pm7.71$	−31	$281.38^{b}\pm7.62$	−50	$1.50^{c}\pm0.28$	−64	$48.21^{b}\pm0.77$	−64
				Experiment 2				
Root	$145.70^{b}\pm5.22$	−	$442.86^{a}\pm20.69$	−	$2.22^{b}\pm0.73$	−	$180.96^{a}\pm16.19$	−
Leaf	$198.86^{a}\pm11.81$	36	$356.71^{b}\pm9.81$	−19	$4.10^{a}\pm1.53$	85	$109.87^{b}\pm3.80$	–39
Bulb	$133.00^{b}\pm2.68$	−9	$300.00^{b}\pm5.08$	−32	$1.70^{c}\pm0.29$	−72	$50.30^{c}\pm0.87$	−72

[y]Column means followed by the same letter were different ($P \leq 0.05$) according to Fisher's least significant difference test.

[z]Relative impact [R.I. (%)] = [(Plant organ/Root) – 1] ×100.

Non-essential heavy metals: Treatment effects were significant on Al, As, Cd, Cr and Pb in onion tissues in Experiment 1, contributing 79, 73, 80, 76 and 81% in TTV of the respective variables, whereas in Experiment 2 treatment effects contributed 77, 83, 80, 74 and 78% in TTV of the respective variables (Appendix 5.3). In Experiment 1 and Experiment 2, relative to root tissues, Al in leaf tissues was decreased by 17 and 20%, respectively, whereas in bulb tissues the variable was decreased by 46 and 40%, respectively. In Experiments 1 and Experiment 2, As in leaf tissues was increased by 185 and 126%, respectively, but was decreased in bulb tissues by 20 and 51% in the respective experiments. In Experiment 1 and Experiment 2, Cd in leaf tissues was increased by 101% and 32%, respectively, but in bulb tissues the variable was decreased by 60% and 71%, respectively. In Experiment 1 and Experiment 2, Cr in the leaf tissues was increased by 358 and 107%, respectively, but was decreased in bulb tissues by 47 and 87, respectively. In Experiment 1 and Experiment 2, Pb in the leaf tissues was increased by 106 and 93%, respectively, but was decreased in the bulb tissues by 46 and 37%, respectively (Table 5.3).

Table 5.3 Accumulation of aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr) and lead (Pb) in leaf and bulb relative to root tissues of onions irrigated with treated wastewater.

Plant organ	Al (ppm)		As (ppm)		Cd (ppm)		Cr (ppm)		Pb (ppm)	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
					Experiment 1					
Root	$7.49^{a}\pm1.04$	−	$0.86^{b}\pm0.06$	−	$3.72^{b}\pm1.31$	−	$2.11^{b}\pm1.18$	−	$1.12^{b}\pm0.13$	−
Leaf	$6.20^{a}\pm1.01$	−17	$2.46^{a}\pm0.38$	185	$7.46^{a}\pm1.98$	101	$9.68^{a}\pm1.82$	358	$2.30^{a}\pm0.29$	106
Bulb	$4.01^{b}\pm0.99$	−46	$0.69^{b}\pm0.01$	−20	$1.49^{b}\pm0.37$	−60	$1.13^{b}\pm0.30$	−47	$0.61^{b}\pm0.10$	−46
					Experiment 2					
Root	$6.89^{a}\pm1.66$	−	$1.02^{b}\pm0.83$	−	$4.93^{ab}\pm0.45$	−	$3.87^{a}\pm0.69$	−	$1.03^{b}\pm0.6.8$	−
Leaf	$5.52^{ab}\pm1.05$	−20	$2.31^{a}\pm0.25$	126	$6.53^{a}\pm1.78$	32	$8.01^{ab}\pm1.34$	107	$1.99^{a}\pm0.23$	93
Bulb	$4.16^{b}\pm0.85$	−40	$0.50^{b}\pm0.06$	−51	$1.43^{b}\pm0.31$	−71	$0.50^{b}\pm0.10$	−87	$0.65^{b}\pm0.32$	−37

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Fisher's least significant difference test.

[z]Relative impact [R.I. (%)] = [(Plant organ/Root) – 1] × 100.

5.3.2 Accumulation of cations and heavy metals in tomato plants

Major cations: The treatment effects were highly significant on Ca, Mg, K and Na in tomato tissues, contributing 99, 99, 75 and 76% in TTV of the respective variables in Experiment 1, but were significant in Experiment 2, contributing 99, 59, 81 and 98% in TTV for the respective variables (Appendix 5.4). In Experiment 1 and Experiment 2, Ca and Mg in both the stem and leaf tissues were increased under treated wastewater. Calcium in stem tissues was increased by 217 and 157% in the respective experiments, whereas in leaf tissues Ca was increased by 414 and 370% in respective experiments (Table 5.4). Magnesium was increased by 483 and 85% in stem tissues and by 527 and 119% in leaf tissues in Experiment 1 and Experiment 2, respectively (Table 5.4). In Experiment 1, K in stem and leaf tissues was increased by 34 and 29%, respectively. In Experiment 2, the partitioning of K in root and leaf tissues did not differ. However, the variable in stem tissues was increased by 25% (Table 5.4). In Experiment 1, relative to root tissues, Na in stem and leaf tissues was decreased by 54 and 37%, respectively. In Experiment 2, Na in stem tissues was increased by 59%, but in leaf tissues was reduced by 23% (Table 5.4).

Table 5.4 Accumulation of calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) in stem and leaf relative to root tissues of tomato plants irrigated with treated wastewater.

Plant organ	Ca (ppm)		Mg (ppm)		K (ppm)		Na (ppm)	
	Variable[y]	R.I (%)[z]	Variable	R.I (%)	Variable	R.I (%)	Variable	R.I (%)
				Experiment 1				
Root	5487.50^{c}±46.9	–	1643.75^{c}±11.05	–	16868.75^{c}±135.00	–	1018.39^{a}±81.42	–
Stem	17387.50^{b}±139.40	217	9581.25^{b}±38.73	483	22637.50^{a}±261.10	34	472.11^{b}±37.76	−54
Leaf	28225.00^{a}±225.08	414	10310.31^{a}±69.53	527	21687.5^{b}±173.50	29	639.11^{c}±51.12	−37
				Experiment 2				
Root	9478.57^{c}±89.03	–	2442.86^{c}±105	–	19258.93^{b}±13.49	–	794.31^{b}±5.51	–
Stem	23900.00^{b}±169.97	152	4528.57^{b}±87.03	85	24092.86^{a}±16.87	25	1264.90^{a}±88.54	59
Leaf	44500.00^{a}±356.02	370	5345.36^{a}±95.03	119	19928.57^{b}±13.95	3	613.77^{c}±14.96	−23

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Fisher's least significant difference test.

[z]Relative impact [R.I. (%)] = [(Plant organ/Root) – 1] ×100.

Essential heavy metals: In Experiment 1, the treatment effects were highly significant on Zn, Fe and Mn, contributing 99, 77 and 80% in TTV of the variables, but had no effects on Cu. In Experiment 2, the partitioning of essential heavy metals in the three organs of tomato plants was highly significant on Zn, Fe, Cu and Mn, contributing 98, 83, 97 and 96% in TTV of the respective variables (Appendix 5.5).

In Experiment 1, relative to root tissues, Zn in stem and leaf tissues of tomato plants was increased by 401 and 186%, respectively. In Experiment 2, the accumulation of Zn between root and stem tissues did not differ. However, Zn in leaf tissues was increased by 335% (Table 5.10). In Experiment 1, relative to root tissues, Fe in stem and leaf tissues was decreased by 19 and 39%, respectively. In Experiment 2, accumulation of Fe between root and stem tissues did not differ. However, the variable in leaf tissues was decreased by 34% (Table 5.5). In Experiment 1, relative to root tissues, Cu in stem tissues was increased, but in leaf tissues was decreased. However, in Experiment 2, the variable was increased in stem and leaf tissues by 109 and 93%, respectively (Table 5.5). In Experiment 1, the partitioning of Mn between root and stem tissues did not differ. However, relative to root tissues, the variable was increased by 31% in leaf tissues. In Experiment 2, relative to root tissues, Mn in stem and leaf tissues was increased by 52 and 39%, respectively (Table 5.5).

Table 5.5 Accumulation of zinc (Zn), Iron (Fe), copper (Cu) and manganese (Mn) in stem and leaf tissues, relative to that of roots in tomato plants irrigated with treated wastewater.

Plant organ	Zn (ppm)		Fe (ppm)		Cu (ppm)		Mn (ppm)	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Experiment 1								
Root	26.40^{c}±1.10	−	1736.40^{a}±14.77	−	14.49^{ab}±0.60	−	53.97^{b}±2.25	−
Stem	132.38^{a}±5.52	401	1403.80^{ab}±11.94	−19	15.77^{a}±0.66	9	55.16^{b}±2.30	2
Leaf	75.59^{b}±3.15	186	1059.20^{b}±9.01	−39	12.58^{b}±0.52	−13	70.46^{a}±2.94	31
Experiment 2								
Root	19.73^{b}±0.82	−	1557.50^{a}±13.25	−	6.98^{b}±0.29	−	54.74^{c}±2.28	−
Stem	22.47^{b}±0.94	16	1337.50^{a}±11.38	−14	14.57^{a}±0.61	109	83.45^{a}±3.48	52
Leaf	85.83^{a}±3.58	335	1022.90^{b}±8.70	−34	13.44^{a}±0.56	93	76.34^{b}±3.18	39

[y]Column means followed by the same letter were not different ($P \le 0.05$) according to Fisher's least significant difference test.

[z]Relative impact [R.I. (%)] = [(Plant organ/Root) – 1] ×100.

Non-essential heavy metals: In Experiment 1, treatment effects were highly significant on Cd, Cr and Pb, contributing 84, 99 and 85% in TTV of the respective variables, whereas in Experiment 2, treatment effects contributed 73, 98 and 89% in TTV of the respective variables. Treatment effects were not significant on As in all plant tissues (Appendix 5.6). In both Experiment 1 and Experiment 2, Cd in root and stem tissues of tomato plants did not differ. However, relative to root and stem tissues, Cd in leaf tissues was reduced by 34 and 31%, in Experiment 1 and Experiment 2, respectively (Table 5.6). In Experiment 1 and Experiment 2, Cr in stem tissues was increased by 85 and 87%, whereas Cr in leaf tissues was increased by 217 and 227%, respectively (Table 5.6). In Experiment 1 and Experiment 2, the partitioning of Pb in root and leaf tissues of tomato plants did not differ. However, Pb in stem tissues was increased by 80% in Experiment 1, whereas in Experiment 2 the variable was increased by 143% (Table 5.6).

Table 5.6 Accumulation of cadmium (Cd), chromium (Cr) and lead (Pb) in stem and leaf relative to root tissues of tomato plants irrigated with treated wastewater.

Plant organ	Cd (ppm)		Cr (ppm)		Pb (ppm)	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)
			Experiment 1			
Root	0.23^{a}±0.05	−	2.87^{c}±0.53	−	0.23^{b}±0.03	−
Stem	0.21^{a}±0.05	−8.6	5.31^{b}±1.00	85	0.40^{a}±0.07	80
Leaf	0.15^{b}±0.03	−34	9.11^{a}±1.75	217	0.15^{b}±0.03	−35
			Experiment 2			
Root	0.25^{a}±0.76	−	2.62^{c}±0.43	−	0.14^{b}±0.23	−
Stem	0.24^{a}±1.52	−4	4.91^{b}±1.98	87	0.34^{a}±0.04	143
Leaf	0.17^{b}±0.30	−31	8.57^{a}±0.23	227	0.13^{b}±0.02	−7

[y]Column means followed by the same letter were not different ($P \le 0.05$) according to Fisher's least significant difference test.

[z]Relative impact [R.I. (%)] = [(Plant organ/Root) – 1] ×100.

5.3.3 Cations and heavy metals in weed and crop tissues

Major cations: In Experiment 1, the treatment effects (weed, onion, tomato) were highly significant on Ca, Mg, K and Na, contributing 98, 94, 85 and 94% in TTV of the respective variables. Similar effects were observed in Experiment 2, with treatments contributing 97, 96, 83 and 95% in TTV of the respective variables (Appendix 5.7). Relative to weeds, in Experiment 1 and Experiment 2, Ca in onion leaf tissues was increased by 167 and 370%, respectively, whereas in tomato leaf tissues the variable was increased by 566 and 839%, respectively (Table 5.7). In Experiment 1, Mg accumulation in onion and tomato leaf tissues was increased by 720 and 1486%, respectively, whereas in Experiment 2 the variable was increased by 806 and 1212% in onion and tomato leaf tissues, respectively (Table 5.7). In Experiment 1, K accumulation in onion and tomato tissues was increased by 149 and 144%, respectively, whereas in Experiment 2 the variable was increased by 54 and 97%, respectively (Table 5.7). In contrast, in Experiment 1 Na in onion and tomato leaf tissues was reduced by 96 and 31%, respectively, whereas in Experiment 2 the variable was reduced by 95 and 10% in the respective crops (Table 5.7).

Table 5.7 Accumulation of calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) concentrations in leaf tissues of tomato and onion, relative to that of weeds irrigated with treated wastewater.

Plant	Ca (ppm)		Mg (ppm)		K (ppm)		Na (ppm)	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
				Experiment 1				
Weeds	4238^{c}±36.04	−	1138^{c}±9.68	−	14063^{c}±119.61	−	932.63^{a}±7.93	−
Onion	11295^{b}±96.07	167	9330^{b}±79.35	720	34963^{a}±297.37	149	36.29^{c}±0.31	−96
Tomato	28225^{a}±240.06	566	10310^{a}±87.69	806	21688^{b}±184.46	54	639.11^{b}±5.44	−31
				Experiment 2				
Weeds	2671^{c}±22.72	−	757^{c}±6.44	−	10114^{c}±86.02	−	681.86^{a}±5.80	−
Onion	12561^{b}±106.83	370	12007^{a}±102.12	1486	24714^{a}±210.20	144	30.69^{c}±0.26	−95
Tomato	25086^{a}±213.36	839	9929^{b}±84.45	1212	19929^{b}±169.50	97	613.77^{b}±5.22	−10

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Fisher's least significant difference test.

[z]Relative impact [R.I. (%)] = [(Plant/Weeds) – 1] ×100.

Essential heavy metals: In Experiment 1, the treatment effects (weed, onion, tomato) were highly significant on Zn, Fe, Cu and Mn, contributing 95, 89, 95 and 98% in TTV of the respective variables. Similar significant effects were observed in Experiment 2, with treatments contributing 99, 96, 98 and 95% in TTV of the respective variables (Appendix 5.8).

Relative to weeds, in both Experiment 1 and Experiment 2, Zn in onion leaf tissues, was reduced by 74 and 68%, whereas in tomato leaf tissues, Zn was increased by 124 and 280% in tomato leaf tissues, in Experiment 1 and Experiment 2, respectively (Table 5.8). The accumulation of Fe in tomato leaf tissues did not differ in Experiment 1 and Experiment 2. However, the variable was reduced by 99% in onion leaf tissues in both experiments (Table 5.8). In Experiment 1 and Experiment 2, Cu in onion leaf tissues was reduced by 82 and 84%, respectively, whereas in tomato leaf tissues the variable was increased by 48 and 65% in Experiment 1 and Experiment 2, respectively. Relative to leaf tissues in weeds, Mn in onion leaf tissues was reduced by 87 and 85% in Experiment 1 and Experiment 2, respectively, whereas in tomato leaf tissues the variable was increased by 25 and 62% in Experiment 1 and Experiment 2, respectively (Appendix 5.8).

Table 5.8 Accumulation of zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) (ppm) in leaf tissues of onions and tomato plants relative to that of weeds irrigated with treated wastewater.

Plant	Zn (ppm)		Fe (ppm)		Cu (ppm)		Mn (ppm)	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Experiment 1								
Weeds	3375.00[b]±28.70	−	1092.90[a]±9.30	−	850.00[b]±7.23	−	564.0[b]±4.80	−
Onion	866.00[c]±7.37	−74	560.00[b]±4.76	−48	157.00[c]±1.34	−82	74.90[c]±0.64	−87
Tomato	7559.00[a]±64.29	124	1059.20[a]±9.01	-3	1258.00[a]±10.70	48	704.60[a]±5.99	25
Experiment 2								
Weeds	2257.00[b]±23.45	−	873.60[a]±7.43	−	814.00[b]±7.40	−	470.00[b]±4.88	−
Onion	720.00[c]±7.48	−68	440.00[b]±3.74	−50	133.00[c]±1.21	−84	68.9[c]±0.72	−85
Tomato	8583.00[a]±89.19	280	1022.90[a]±8.70	17	1344.00[a]±12.22	65	763.40[a]±7.93	62

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Fisher's least significant difference test.

[z]Relative impact [R.I. (%)] = [(Plant/Weeds) – 1] ×100.

<u>Non-essential heavy metals</u>: In both experiments the treatment effects (weed, onion, tomato) were highly significant on As, Cd, Cr and Pb, contributing 100, 94, 98 and 93% in TTV of the respective variables in Experiment 1, whereas in Experiment 2 the treatments contributed 95, 81, 98 and 93% in TTV of the respective variables (Appendix 5.9).

In Experiment 1 and Experiment 2, relative to leaf tissues in weeds, As accumulation in onion leaf tissues was increased by 522 and 758%, respectively, whereas in tomato leaf tissues the variable was increased by 44 and 116% in the respective experiments (Table 5.9). In Experiment 1 and Experiment 2, relative to leaf tissues in weeds, the accumulation of Cd in tomato leaf tissues did not differ. However, in onion leaf tissues, Cd accumulation was increased by 1307 and 2480% in Experiment 1 and Experiment 2 (Table 5.9). Chromium in onion leaf tissues was reduced by 90 and 86% in Experiment 1 and Experiment 2, respectively, whereas in tomato leaf tissues, the variable was reduced by 32 and 34%, respectively (Table 5.9). In Experiment 1, the accumulation of Pb in tomato leaf tissues did not differ. However, in onion leaf tissues, the variable was increased by 514%. In Experiment 2, Pb accumulation in onion leaf tissues was increased by 362%, whereas the variable in tomato leaf tissues was reduced by 50% (Table 5.9).

Table 5.9 Accumulation of arsenic (As), cadmium (Cd), chromium (Cr) and lead (Pb) in leaf tissues onions and tomato plants relative to that of weeds, irrigated with treated wastewater.

Plant	As (ppm)		Cd (ppm)		Cr (ppm)		Pb (ppm)	
	Variable[y]	R.I. (%)[z]	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Experiment 1								
Weeds	$0.18^{c}\pm0.00$	−	$0.15^{b}\pm0.00$	−	$13.39^{a}\pm0.14$	−	$0.14^{b}\pm0.00$	−
Onion	$1.12^{a}\pm0.01$	522	$2.11^{a}\pm0.04$	1307	$1.35^{c}\pm0.02$	−90	$0.86^{a}\pm0.01$	514
Tomato	$0.26^{b}\pm0.00$	44	$0.15^{b}\pm0.00$	0	$9.11^{b}\pm0.09$	−32	$0.15^{b}\pm0.00$	7
Experiment 2								
Weeds	$0.12^{c}\pm0.00$	−	$0.15^{b}\pm0.00$	−	$13.01^{a}\pm0.14$	−	$0.26^{b}\pm0.00$	−
Onion	$1.03^{a}\pm0.01$	758	$3.87^{a}\pm0.02$	2480	$1.81^{c}\pm0.01$	−86	$1.20^{a}\pm0.01$	362
Tomato	$0.26^{b}\pm0.00$	116	$0.17^{b}\pm0.00$	13	$8.57^{b}\pm0.09$	−34	$0.13^{c}\pm0.00$	−50

[y]Column means followed by the same letter were not different ($P \leq 0.05$) according to Fisher's least significant difference test.

[z]Relative impact [R.I. (%)] = [(Plant/Weeds) – 1] ×100.

5.4 Discussion

5.4.1 Accumulation of cations and heavy metals in onion

Major cations: Calcium, Mg, K and Na each responded significantly to the partitioning of elements in various organs, with high contributions in TTV of the respective variables. In the review of the partitioning of nutrient elements in different organs, Marschner (2012) supported the observation that there were significant differences in the partitioning of most cations in onions, with different accumulation rates in various organs. The observed cation content distribution was critical in the test vegetable crops, especially in contaminated soils, as the major cations are required for various plant metabolic processes, but as well as for human health (Kitata and Chandravanshi, 2012). The significant partitioning of the cations in edible parts of the test crops in the current study, except for tomato fruit where the cations were not quantified, the accumulation under treated wastewater agreed with those of others (Wuana and Okieimen, 2011). These observations supported the view that the major cations are widely used in detergents and other household commodities which end up being disposed-off through wastewater (Wu and Cao, 2010).

Calcium concentration in the different organs gradually decreased from the bulb, then root and leaf tissues in both Experiment 1 and Experiment 2. In the bulb tissues, the Ca content values were higher than the permissible level of 23 mg/100 g (Yara US Crop Nutrition, 2017). As demonstrated earlier, it appeared that the source of Ca was actually not the treated wastewater, but the calcareous soil, through which the water passed through earth canal from Pond 16 to the night dam (Chapter 3). Also, it is important to remember that the soil irrigated with treated wastewater had low Ca, thereby suggesting that the cation was directly from treated wastewater used for irrigation. Although Ca is responsible for maintaining vigorous and healthy leaf growth, good bulb firmness and quality in onions and the integrity of cell membranes (Gupta *et al*., 2017), at high levels in soil solutions, Ca could be absorbed luxuriously, which appeared to have been the case in the current study. In contrast, Ca deficiency in onion plants could result in brown tissues in bulbs, with leaves appearing lumpy (Yara US Crop Nutrition, 2017). In Ghana onion plants were observed to have low Ca concentration (< 0.60 ppm) under various levels treated wastewater (Adotey *et al*., 2009). In animals, Ca is required for strong bones, with Ca deficiencies directly affecting the bone density, thereby increasing susceptibility to osteoporosis and fractures (Rupavate, 2015). Currently there is limited information on Ca distribution in onion organs, suggesting that findings in the current study would be providing important such information under treated wastewater.

The highest Mg concentration of 1158 ppm was observed in onion leaf tissues, whereas the lowest at 320 ppm occurred in bulbs. Incidentally, the values observed in this study were also high when compared to the allowable levels of Mg in onions which is at 15.27 mg/100 g (Yara US Crop Nutrition, 2017). Generally, onions are being grouped as low Mg foods (Pennington and Wilson, 1990) and could contain as low as 0.59 ppm (Adotey *et al.,* 2009). In other countries, values as low as 516 and 407 $\mu g.kg^{-1}$ of Mg content were also observed in onions irrigated with well- and lake-water, respectively (Kitata and Chandravanshi, 2012). As observed in Ca, treated wastewater could have been the source of Mg since the soil had low Mg concentrations (Chapter 4), whereas treated wastewater had high Mg (Chapter 3).

The highest concentration of 5095 ppm of K was observed in leaf tissues of onions, whereas the lowest value of 2235 ppm K was in the bulb tissues. The observed values were 56 to 127 higher than the 40 ppm set for the composition in onion bulb (Maaloufa *et al*., 2015). The high K concentration in onion bulbs could be due to treated wastewater-added K and fertilisation (Chapter 3). Sodium was in the range of 391. 40-3629.80 ppm in Experiment 1 and 444.7-3068.6 ppm in Experiment 2, with the maxima being much higher than the permissible 2500 ppm (Adotey *et al*., 2009) and minima permissible values of 3.13 and 3.30 ppm (Kitata and Chandravanshi, 2012). Apparently, the source of high Na in onion plants was also the treated wastewater. The high Na in onions as observed in the current study could not be desirable since it could result in diseases such as hypertension in consumers (Adotey *et al.,* 2009).

Essential heavy metals: Zinc was found to be dominant in the onion leaf tissues in both Experiment 1 and Experiment 2, with bulb tissues having the lowest concentration of 107.84 and 133.00 ppm, respectively. Generally, Zn in leaf tissues was previously shown to have a positive relationship with chlorophyll in some crops (Nguyen-Deroche *et al.,* 2012), which could explain the accumulation of the metal in onion leaf tissues in the current study. The observed Zn concentrations in bulb tissues were high when compared to the typical Zn concentration for onion nutrition at 1.89 mg to 0.07 mg/ 100 g serving (Yara US Crop Nutrition, 2017). The availability of Zn in crops is controlled by a number of factors, including soil reaction and mineralisation. Although the soil pH in the current study was in agreement with high potential mobility Zn, but with the contradiction that the abundance of Zn ions in the soil limits the absorption of this essential heavy metal by root hairs (Yoneyama *et al*., 2015). Ironically, also Zn deficiency in the soils leads to Zn deficiency in plant tissues.

In the current study Fe accumulated in onion root tissues at 557.63 and 442.86 ppm in Experiment 1 and Experiment 2, respectively. Plants acquire Fe from the rhizosphere in low quantities. Although Fe is one of the most abundant elements in the soil, in most cases it is in unavailable forms due to its high sensitivity to changes in soil pH (Morrissey and Guerinot, 2009). In the ULEF study with soil pH at 7.78 (Chapter 4), the latter could have limited the availability of this heavy metal for absorption by roots since at that pH it is oxidised, thereby becoming insoluble (Morrissey and Guerinot, 2009). In the current study, most Fe accumulated in root tissues than in leaf or bulb tissues. Generally, almost 75% Fe is attached to the apoplast in root cells, where the cell wall serves as a cation sink, which is gradually mobilised into the symplast when the plant activates signals for Fe deficient (Zhang *et al*., 1991). In plants, Fe is involved in protein synthesis, along with redox and electron transport complexes that are associated with pigment formation in plants (Zhang *et al*., 1991). In the current study, there was no evidence of Fe deficiency in onion leaf tissues.

The highest Mn concentration of 135 and 181 ppm also occurred in onion root tissues for Experiment 1 and Experiment 2, respectively, with 48.21 and 50.30 ppm Mn occurring in bulb tissues of the respective experiments. In contrast to onion plants in the current study, Mn in tissues of other plants is higher in stem tissues than in leaf or root tissues (Marschner, 2012). The Mn concentration in bulbs was relatively higher than the safe limit for daily adult intake permitted in food products (2-9 ppm) (WHO, 2004), with Mn in humans associated with proper functioning of connective tissues, formation of bones, blood clotting factors, fat and carbohydrate metabolism and blood sugar regulation (St Hilaire, 2015). In plants, Mn plays various key roles in photosynthesis (Hakala *et al*., 2006). The observed high values of Mn in onion tissues of the current study could also be linked with high soil pH (Chapter 4) since both high pH and redox reactions affect the availability of this heavy metal in soils. In contrast, at low soil pH (< 5.5), the oxides could be reduced in the exchangeable sites of soil solutions, increasing Mn^{2+} concentration – which is the available form for plants (Watmough *et al*., 2007). In contrast to findings in the current study, under high soil pH high Mn ions result in high adsorption of the heavy metal into soil particles, thereby decreasing its availability to plants (Fageria *et al*., 2002).

Copper in onion bulb tissues was at 1.50 and 1.70 ppm in Experiment 1 and Experiment 2, respectively, which was lower than the recommended limit of 5.0 ppm (Food and Nutrition Board, 2001) and the maximum Cu intake for an adult of 3.0 mg a day (Reilly, 2006). Additionally, the current Cu concentration was lower than that observed in onion bulb tissues at 7.7 to 15 ppm (Badilla-Ohlbaum *et al*., 2001), but higher than that observed from different field at 0.67 and 1.06 ppm in onion bulb tissues (Bystrická *et al.*, 2015). In plants Cu is associated with a number of enzyme activities such as those linked to the synthesis of lignin (Yamasaki *et al*., 2008). Copper is also required in photosynthesis and respiration processes, with deficiencies in plants related to leaf chlorosis and necrotic spots on young leaves, since Cu is relatively immobile (McCauley *et al*., 2011). In human nutrition, Cu and Fe are required in the formation of red blood cells (Collins *et al*., 2010). The onion bulbs in the current study could be viewed as having inadequate supply of Cu for human consumption – with the metal being a critical essential element.

Non-essential heavy metals: Like the essential heavy metals, Al was the highest in onion root tissues, with the range being at 4.01−7.49 and 4.16−6.89 ppm in Experiment 1 and Experiment 2, respectively. The latter values were low when compared to the provisional tolerance weekly Al intake of 7 ppm of body weight (WHO, 1989). Basically, the tolerance weekly Al intake of 7 ppm of body weight means that for an average 50 kg body weight person, the daily consumption of 50 mg of Al would be allowed (Mohammad *et al.,* 2012). Greger (1993) reported that on average an adult could consume approximately 2-25 mg dietary Al daily. Aluminium, like Fe, is one of the most abundant metals on the earth's crust (Encyclopedia of Earth, 2008) and is widely used in food additives such as baking powder since it serves as pH adjusting agents (Krewski *et al*., 2007). The most important health hazard of high Al in food is dialysis encephalopathy, which can lead to tremors, convulsions, psychosis and other related neurological problems (Health Canada, 2003).

In both experiments, As had the lowest concentration (0.60 ppm) in onion bulb tissues, whereas the highest was in leaf tissues. The latter contradicted the assertion that in plants, As accumulation in is restricted to root tissues (Rofkar and Dwyer, 2011; Wolterbeek and Van der Meer, 2002). Although the mean concentration of 0.34 ppm As was reported in treated wastewater (Chapter 3), it could be speculated that repeated irrigation using the current source could have resulted in gradual accumulation of As in onion bulb and leaf tissues. Currently there is no threshold limit for As in the South African Department of Health and Codex Alimentarius Commission's Joint Steering for FAO/WHO for vegetable and fruit produce. However, a maximum level of 0.1 ppm As was proposed for edible oils from vegetable produce (South African Department of Health, 2004). The accumulation of As in plant tissues was shown to be influenced by a number of factors, including soil pH and the presence of Fe oxide and the unavailability of phosphates in soils (Moreno-Jiménez *et al*., 2012). The accumulation of Cd in plant organs could be encouraged by low Zn in organs, which in turn is a factor of Zn deficiencies in the soil (Liphadzi and Kirkham, 2005). In the current study, low Zn in the different organs of onion plants could have led to the plant absorbing high amounts of Cd as observed in other plants (Mench *et al*., 1997). The permissible Cd limit in fruit and vegetable produce in South Africa had been set at 0.05 ppm Cd (South African Department of Health, 2004), which is below the international limit of 0.1 ppm Cd as pronounced in the Codex Alimentarius Commission Report (FAO/WHO, 2014). In the current study, onion leaf and bulb tissues contained 7.46 and 1.49 ppm Cd, respectively, with the latter being high when compared to both the national and international standards. High Cd in human beings had been associated with health hazards such as shortness of breath, kidney damage and cancer (Godt *et al*., 2006; Waalkes *et al*., 1988).

In the two experiments, Cr in bulb tissues was at 1.13 and 0.50 ppm, with both being lower than the ranges of 4.9 to 6.6 ppm Cr in Ethiopia (Kitata and Chandravanshi, 2012) and 3.87 to 8.87 ppm Cr in Nigeria (Abdullahi *et al*., 2008). The observed high Cr in root tissues agreed with other findings which suggested that Cr accumulated mainly in root and leaf tissues (Oliveira, 2012). Unlike other heavy metals, Cr is an essential element that is exclusively derived from food such as fruit and vegetable produce (Evert, 2013), with suggestions that the element be included as an agricultural input since in some cases soils could be deficient of the metal (Stasinos and Zabetakis, 2013).

Lead concentrations in the present study were higher than the threshold limit of 0.3 ppm (South African Department of Health, 2004), with bulb tissues having 0.61 and 0.65 ppm Pb in Experiment 1 and Experiment 2, which was more or less similar to that reported elsewhere in onion bulb tissues (Kitata and Chandravanshi, (2012). However, values ranging from 9.1 to 336.00 ppm Pb were reported in cabbage, onions and tomatoes (Okoroigwe, 2011). In vegetable plants, high Pb content causes reduction in growth and biomass production (Sharma and Dubey, 2005) due to its ability to inhibit carboxylating enzymes responsible for photosynthesis (Nagajyoti *et al*., 2010). High concentration of Pb in plants could be due to its competition with Ca^{2+} on permeable membranes (Pourrut *et al*., 2012) by which Pb enters the root system. After penetrating the epidermis in roots, Pb follows apoplastic movement with water streams to the endodermis, where accumulation occurs and then compete with Ca^{2+} for symplastic movement into the vascular bundle (Pourrut *et al*., 2012). Due to higher Pb content in leaf tissues than in onion root tissues, it could be that most accumulated on leaves during irrigation using the sprinkler irrigation systems since high Pb concentrations were detected in treated wastewater (Chapter 3), with some accumulating in soils (Chapter 4). High Pb in produce is undesirable since the ingested Pb in humans could cause severe health effects, particularly in women of childbearing age as Pb could be transferred to the foetus (Finster *et al*., 2003).

5.4.2 Accumulation of cations and heavy metals in organs of tomato plants

Major cations: In the current study, the partitioning of cations in root, stem and leaf tissues of tomato plants were highly significant, which supported other observations in the nutrient partitioning study under different fertiliser programmes in Zentsuji, Japan (Kinoshita *et al*., 2014). Also, Halder *et al*. (2015) observed significant differences on the partitioning of Ca, K and Cd in organs of vegetable plants grown under field conditions.

The concentration of Ca in tomato organs in both experiments was the lowest in root < stem < leaf tissues. The highest concentration of Ca (44 500 ppm ≈ 4.45%) in leaf tissues of tomato plants Experiment 2 was relatively high when compared to observations in other studies (Martel-Valles *et al.*, 2017). Generally, Ca^{2+} ions in plants are transported through the plant mainly through the apoplastic pathway from where they are taken into the vascular bundle via protein-mediated transport (Conn and Gilliham, 2010). The current study suggested that soil Ca could have been in excess to the requirements as a result of irrigation with treated wastewater which had high concentration of Ca (Chapter 3). In overabundance, Ca^{2+} ions move down the gradient following the apoplastic pathway (Hayter and Peterson, 2004). In the vascular bundle, Ca movement from root to leaf tissues is dependent upon the xylem sap flow, which is a function of the leaf transpiration stream (De Freitas *et al*., 2011). Most ions in the xylem sap end up in the leaf tissues and are returned to other parts of the plant through the phloem in relatively small quantities, with the unused being compartmentalised in vacuoles of leaf cells (De Freitas *et al*., 2014), which could provide some explanation of the high Ca concentrations in leaf tissues of tomato in the current study.

The Mg concentration, as in Ca, was the highest in leaf tissues, with the concentration of 10310.31 ppm (≈ 1.031%) and 5345.36 ppm (≈ 0.5345%) in Experiment 1 and Experiment 2, respectively. Generally, in tomato plants most mineral elements are the highest in leaf tissues than in tissues of other organs (Martel-Valles *et al.,* 2017). Magnesium is a component of the chlorophyll molecule (Rehm *et al*., 1994) and therefore, it is important for the element to be in abundance in the leaf tissues for photosynthesis to occur optimally.

Potassium was the highest in the leaf tissues and in the stem tissues in Experiment 1 (22637.50 ppm ≈ 2.264%) and Experiment 2 (24092.86 ppm ≈ 2.409%), respectively. The K concentration in the current study was relatively high when compared to the 2000 ppm K in tomato leaf tissues where the plants were also irrigated using treated wastewater (Alghobar and Suresha, 2017). The high K concentrations in tomato plant organs when compared with those in onion plant tissues (50.95 ppm K), highlighted the absorption abilities of different plant species. High K content in plant tissues in the current study could be attributed to high K content in the soil irrigated using treated wastewater and fertilisation since the leaves were not contaminated during irrigation using drip irrigation system (Chapter 4). Crops differ in their ability to take up and utilise K from a given soil relating to the type of root system, root density and metabolic activities that affect K uptake (Anonymous,1998). Also, some plant species resort to luxury consumption of K due to their immediate growth requirements during shoot flushes (Van Wijk *et al*, 2003).

Sodium was the highest in root and stem tissues in Experiment 1 and Experiment 2, respectively. In Experiment 1, relative to root tissues, Na decreased by 54 and 37% in the stem and leaf tissues, respectively. In contrast, relative to root tissues, in Experiment 2, Na increased by 59% in stem tissues, but was reduced by 23% in leaf tissues. The highest Na concentration in Experiment 1 was 1018.39 ppm Na, whereas in Experiment 2 it was 1264.90 ppm Na. The Na concentration in the current study could have been due to high Na in soils as a result of irrigation water (Mashela *et al*.,

1992) as poor-quality water could lead to saline conditions depending on the soil texture. The observed Na concentration in tomato leaf tissues in various organs were higher than recommended 5 mg/100 g (USDA, 2017), but in tomato fruit could be essential since Na improves fruit quality (Mashela *et al*., 1992). The observed Na concentration could interfere with physiological activities of tomato plants. Crops such as citrus that do not require Na as an essential element, could respond negatively to salinity ions at much lower Na concentrations (Mashela and Nthangeni, 2002). Generally, in onion (Adotey *et al.*, 2009) and cauliflower (Kiziloglu *et al*., 2008) Na is inherently lower in various organs. High Na ions in root tissues as observed in citrus (Mashela and Nthangeni, 2002) was safer and could have been due to the passive transport from root to leaf tissues (Cuartero and Fernandez-Munoz, 1999). High Na in leaf tissues could reduce the number of shoots in certain plants (Cruz *et al.*, 1990) and could results in Cu, Mn or Zn deficiency (Mashela and Nthangeni, 2002). Intermittent salinity that include Na was shown to increase nematode population densities (Mashela *et al*., 1992).

Essential heavy metals: The concentrations of Zn, Fe and Mn were different in various organs of tomato plants in Experiment 1, with Cu being an exception. In Experiment 2, the four essential heavy metals were affected by the treatment tomato organs, confirming observations in another study (Alghobar and Suresha, 2017), with another study (Andal, 2016) demonstrating that tomato plants have the potential of serving in phytoremediation of soils contaminated with essential heavy metals (Andal, 2016).
Zinc in Experiment 1 was the highest in stem tissues with a concentration of 132.38 ppm, while in Experiment 2, the variable was the highest in leaf tissues at 85.83 ppm. A study in Mardan, Pakistan reported on both contrasting and comparable results to

the ULEF study results (Amin *et al*., 2013). The study explored heavy metal accumulation from three regions where treated wastewater was used for irrigation. In on region, Zn was the highest (42.65 ppm) in the stem tissues, while in two others the metal was the highest (70.4 and 11.5 ppm) in the leaf tissues. It is clear that Zn does not have a stable trend on accumulation in a certain organ, however, could be controlled by different absorption and transport factors. Studies confirm Zn accumulation differ significantly at flowering and post flowering stages (Samardjieva *et al*., 2014; Waters and Grusak 2008). Zinc was not deficient in soils of the present study (chapter 4). However, morphological features such as root length, density of lateral roots, root cover in soil, age and root biomass, in overall affect the Zn absorption by roots (Gupta *et al*., 2016).

Iron was the highest in root tissues of tomato plants with concentrations being as high as 1736.40 ppm and 1557.50 ppm in Experiment 1 and Experiment 2. In both experiments, Fe was the lowest in leaf tissues at 1059.20 ppm and 1022.90 ppm, respectively, which were much higher than the 300 ppm toxicity threshold in tomato plants (Li *et a*l., 2006), which are susceptible to Fe toxicities (Grant, 2017). Also, the two Fe concentrations were higher than the ones at 32.70-65.40 ppm and 13.15-86.70 ppm observed in edible organs of various vegetables irrigated with treated wastewater (Amin *et al.,* 2013). Generally, leaves are the major sink of Fe that plays a major role in the synthesis of chlorophyll molecules (Ravet *et al.,* 2009). The high Fe in leaf tissues of tomato plants in the current study should be of great concern if the metal finds its way into tomato fruit and the potential accumulation in fruit where plants are irrigated with treated wastewater should be investigated.

In the current study, Cu concentrations were at 12.58-15.77 ppm and 6.98-14.57 ppm in Experiment 1 and Experiment 2, respectively, with the highest concentrations being in stem tissues. The observed Cu in tomato tissues in the current study was much higher than the 0.127 ppm in tomato plants irrigated with treated wastewater in West Bengal (Roy and Gupta, 2016). However, Amin *et al*. (2013) observed much higher Cu concentration at 62.95 ppm in leaf tissues of tomato plants irrigated with treated wastewater. Generally, the average Cu content in plant tissues is 10 ppm (Baker and Senef, 1995). Consequently, Cu observation in the current study was above the average and therefore in a toxic state since it is highly toxic due to its high redox properties (Yruela, 2005).

Partitioning of Mn in tomato plants was high in all organs, with the highest concentration being in leaf tissues in Experiment 1, confirming observations in leaf than in root tissues of rice, *Oryza sativa* plants (Lidon, 2001). In Experiment 2 Mn was the highest in stem tissues. The findings in the current study were in agreement with those of Amin *et al.* (2013), where the highest Mn concentration in leaf tissues were at 128 ppm when compared with 24.3 ppm in root tissues. The findings in the current study are in contrast with the reported low Mn concentration of 1.65 ppm (Roy and Gupta, 2016) and 37.02 ppm (Adotey *et al*., 2009). The Mn content in leaf tissues differed between plant species from 30 to 500 ppm Mn (Clarkson, 1988). The Mn concentrations in tissues of tomato plants were below the toxicity threshold levels. Generally, Mn tends to accumulate predominantly in leaf tissues than in root tissues (Millaleo *et al*., 2010), with its main role being in the splitting of water molecules in Photosystem II system of photosynthesis by providing electrons necessary for photosynthetic electron transport (Goussias *et al*., 2002).

Non-essential heavy metals: The partitioning of Cd, Cr and Pb was significantly affected by the organs of tomato plants, without affecting the partitioning of As. Different plant species have different degrees of accumulative abilities in different organs for the non-essential heavy metals as shown in different vegetables irrigated with treated wastewater (Adotey *et al*., 2009; Amin *et al*., 2013; Fontes *et al*., 2012).

Cadmium concentrations in root and stem tissues of tomato plants in the current study were not different, but was the highest in leaf tissues. In leaf tissues Cd was at 0.15 ppm and at 0.17 ppm in Experiment 1 and Experiment 2, which were comparable to those in leaf tissues of lettuce cultivars (Fontes *et al*., 2012). Generally, Cd in leaf tissues of plants increased with an increase of Cd in the soil (Fontes *et al*., 2012). Consequently, the presence of Cd in in tomato plant tissues could be due to the previously observed levels of Cd in the soil (Chapter 4), added through irrigation with treated wastewater. Generally, Cd is the highest in roots due to the existence of apoplastic pathways, but is limited in shoots since the endodermis serves as a poor symplastic pathway for non-essential heavy metals (Benavides *et al*., 2005).

As observed in onion plants, Cr was dominant in leaf tissues in both experiments at the respective concentrations of 9.11 ppm and 8.57 ppm. The current findings were in agreement with the 6-8 ppm Cr in leaf tissues of tomato plants irrigated with treated wastewater (Amin *et al*., 2013). In all cases the Cr concentrations were higher than the 0.1 ppm limit allowed in plants tissues (WHO, 1989). With the exception of the work by Amin *et al*. (2013), there is limited information on the partitioning of Cr in tomato plants. In ornamental plants, higher Cr concentration was partitioned in leaf tissues than in root tissues (Budak *et al*., 2011). The Cr-polluted soil had been identified as the most common source for the accumulation of Cr in plant tissues (Budak *et al*., 2011). Since Cr is not an essential nutrient element, its accumulation in plant tissues could be toxic at minute concentrations (Oliveira, 2012), with human exposure in edible plant organs resulting in pulmonary irritant effects (USEPA, 1998).

Lead in root and leaf tissues were not statistically different, but was the highest in stem tissues in Experiment 1 and Experiment 2 at 0.40 ppm and 0.34 ppm, respectively. Lead in most plants, Pb tends to be partitioned in higher concentration in root tissues than in leaf tissues (Sharma and Dubey, 2005), probably due to the restrictions imposed by symplastic pathways in the endodermis (Verma and Dubey, 2003). In some cases, the highest accumulation of Pb was reported in leaf tissues (Amin *et al*., 2013; Sêkara *et al*., 2005), whereas in the current study the metal was considerably low in leaf tissues of tomato plants. In plant nutrition, Pb had been shown to have the potential to block the uptake of other ions at the absorption sites of roots, therefore inducing ionic imbalances in plants (Sharma and Dubey, 2005).

5.4.3 Cations and heavy metals in weed and crop tissues

Major cations: The composition of the four major cations varied in the different plants that were sampled on treated wastewater fields in Experiment 1 and Experiment 2. Treatment effects, namely, onion, tomato and horseweed, had significantly high contributions in total treatment variation of the respective major cations. Comparative effects on between horseweed and vegetable crops in field irrigated with treated wastewater are scarce, with some information on acquisition of minerals and chlorophyll content in weeds being available (Glenn, 1987).

Calcium in both experiments exhibited similar trends, with the variable being the highest in tomato and the lowest in weed tissues. Horseweed plants in the current study emerged under fallowing following irrigation with treated wastewater for three years. In Experiment 1 Mg was the highest in tomato leaf tissues, while the variable was the highest in onion leaf tissues. In Experiment 1 and Experiment 2, the lowest Mg concentrations were at 1138 ppm and 757 ppm, respectively. Potassium was also the lowest in horseweed, with the highest concentration observed in tomato leaf tissues. The low cation concentrations in horseweed could be justified by the fact that there was no addition of fertilisers in the particular fallowed field, although the plants had been growing for three years, which could provide some explanation of the low Ca, Mg and K values in soil solutions of the fallowed field (Chapter 4). Generally, horseweed is a strong competitor to most agricultural crops (Glenn, 1987). Sodium was less in both onion and tomato by 96 and 31%, respectively, in Experiment 1, and 95 and 10% respectively, in Experiment 2. The Na concentrations were 932. 63 ppm (Experiment 1) and 681.86 ppm (Experiment 2). The concentration is in line with the Na concentrations of 736 ppm in maize as observed in a study that explored the distribution of major cations in different crops in Ekiti State, Nigeria (Adeyeye, 2005). The latter findings demonstrate that horseweed was able to compete for Na the same as other agricultural crops. The major cation distribution in weeds was K > Ca > Mg > Na, for both Experiments 1 and 2, for onion was K > Ca > Mg > Na in both experiments, and for tomato was Ca > K > Mg > Na for both experiments.

Essential heavy metals: The accumulation of Cu, Fe, Cu, Mn and Zn in leaf tissues of horseweeds, onion and tomato varied significantly in concentration in both experiments. Information on absorption abilities of heavy metals in weeds growing on fields previously irrigated with treated wastewater. In contrast, literature is replete with comparative studies on absorption abilities of heavy metals in agricultural crops and weeds on fields irrigated with treated wastewater (Barman and Lal, 1994; Barman *et al.*, 2000). Barman and Lal (1994) evaluated the accumulation abilities of heavy metals in weeds and vegetables on fields irrigated with industrial wastewater and observed that the accumulation abilities of heavy metals were species-specific. Also, Sonmez *et al*. (2008) observed that Zn in leaf tissues of *Avena sterils* (24-264 ppm), *Isatis tinctoria* (10-101 ppm) and *Xanthium strumarium* (28-48 ppm) was species-specific. Generally, weeds had higher absorption abilities than vegetables, which disagreed with observations in the current study. Barman *et al*. (2000) studied the distribution of heavy metals in wheat, mustard and weeds on fields irrigated with industrial wastewater and observed that weeds had the highest accumulative abilities.

In both experiments, the accumulation of Zn was the highest in tomato, followed by in weeds, with the lowest concentration occurring in onion bulb tissues. The Sonmez *et al*. (2008) result confirmed the observation where Zn in leaf tissues of three weed species had ranges 24-264 ppm in *Avena sterils*, 10-101 ppm in *Isatis tinctoria* and 28-48 ppm in *Xanthium strumarium (*Sonmez *et al*., 2008). In the ULEF study, horseweed retained more Zn in leaf tissues than in onion leaf tissues.

In the current study, results suggested that horseweed and tomato had the same accumulative abilities for Fe, which were better than that of onion. In a study with five weed species and maize (Ghasemi-Fasaei and Mansoorpoor, 2015), all had higher Fe accumulative abilities than maize. As in the current study, Zn, Mn and Cu accumulation ability had similar accumulation trends, namely, tomato > weeds > onion.

Also, Ghasemi-Fasaei and Mansoorpoor (2015) confirmed that weeds had higher accumulative abilities for Cu and Mn than maize. Similar higher accumulative abilities in the weed, *Rumex dentatus* (2.83 ppm) when compared to common wheat (0.93 ppm) plants had been reported under fields irrigated with treated wastewater (Barman *et al*., 2000).

In the current study and other cited studies, it was clear that weeds had higher accumulative abilities of heavy metals such as Cu, Fe, Cu, Mn and Zn than the cultigens. A number of factors could be at play, including the influence of root exudates in the rhizosphere. Generally, heavy metals are readily available at reduced soil pH and any root exudes that contain pH-reducing chemical compounds could inherently expose plants to high concentrations of such metals. Generally, during fallowing, grasses dominate the successions due to their interference nature (Kong *et al*., 2004), which render the soil in the rhizosphere unsuitable for other plants, thereby giving weeds a competitive advantage. Apparently, this competitive advantage is lost during the development of a plant into a cultigen.

Non-essential heavy metals: In the current study, the accumulation of non-essential heavy metals varied significantly in horseweed, onion and tomato leaf tissues. The accumulation of As was the highest (1.03-1.12- ppm) in onion plants and the least concentration (0.12-0.18- ppm) in horseweed. In both extremes for onion and horseweed with respect to As, the observed As concentrations were below those observed in sorghum (*Sorghum bicolor)* plants raised in field previously irrigated with treated wastewater (Darabi *et al*. (2016). Similarly, Cd was least accumulated in horseweed (0.15 ppm in both experiments) than in onion (2.11- 3.87 ppm). Generally, plants used in areas with high non-essential heavy metals on soils that were irrigated with treated wastewater are selected for their phytoremediation or phyto-extraction abilities for some of the heavy metals (Sonmez *et al*., 2008). For instance, *R. dentantus*, which previously accumulated 13.3 ppm Cd in its leaf tissues (Barman *et al*., 2000), could be viewed as a good phyto-remediator plant for Cd. As in Cd, the test plants had different accumulative abilities for Pb, with extremes as low as 0.14- 0.13 ppm Pb to as high as 0.86 and 1.2 ppm Pb, but were all lower than previously observed in leaf tissues of sorghum (Darabi *et al*., 2016). Sorghum could, therefore, be used as phytoremediation crop for Pb and As (see above).

In the current study, horseweed had the highest accumulation abilities for Cr (13.01-13.39 ppm) when compared to cultigens such as onion (1.35- 1.81 ppm) and tomato plants as observed in another study (Balkhair and Ashraf, 2016). However, in another study on cultigens high Cr concentrations were observed in leaf tissues when plants were irrigated with treated wastewater (Alghobar and Suresha, 2017).

5.5 Synthesis and conclusion

The ULEF fields irrigated with treated wastewater suggested some benefits and potential health hazards in terms of essential nutrient elements and heavy non-essential nutrient elements, respectively. For instance, the cation content and essential heavy metals were still in the sufficient range for the production of onion, although certain essential nutrient elements (K and Zn) were limiting in onion bulb tissues. In contrast, there were high Cd and Pb concertation in onion bulbs in the ULEF study and because they were higher than the recommended standards (DWAF, 1996; Ayers and Weststock, 1994), the two could be a health hazard to consumers. In tomato, leaf tissues had high concentration of Ca, Mg and K, which could, when transported to fruit, be beneficial to both consumers since it is well established that Ca is one of the malnutrition elements. Also, the high Ca could prevent a physiological disorder referred to as blossom-end rot on tomato fruit. In general, what came out strongly in the ULEF study was that although certain weeds such as horseweeds could be excellent phyto-remediator plants, they should not be viewed as “scavengers” of all non-essential heavy metals since they are quite specific in terms of accumulative abilities. Similarly, not all cultigens should be ruled out in selection of plants with phytoremediation attributes since cultigens such as sorghum were shown to possess good accumulative abilities, although they were also heavy element-specific. The current findings were in contrast with the hypothesis which stated that the partitioning of cations and heavy metals in root, stem and leaf tissues of onion, tomato and weed plants irrigated with treated wastewater at the ULEF site would be similar. In the next chapter the researcher provided the summary and significance of the findings, the recommended areas that still needed empirically-based information and conclusions based on the observed findings.

CHAPTER 6

SUMMARY OF FINDINGS, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSIONS

6.1 Summary of findings

Irrigation with treated wastewater could serve as a resilient strategy to ameliorate the pressures of extended droughts in context of climate-smart agriculture in certain regions of water-scarce South Africa. Treated wastewater could pose challenges related to excess essential nutrients, essential and non-essential heavy metals into the irrigated fields and contamination of produce by pathogenic microorganisms. The current study investigated the elemental and microbial quality of treated wastewater being used in commercial irrigation, the impact of treated wastewater on soil physico-chemical properties and the partitioning of various elements in plant organs.

Generally, essential nutrient elements and non-essential heavy metals were below the permissible allowed by South African Department of Health (2004) and Ayers and Weststock (1994) standards. In contrast, most microbial organisms, which were mostly, pathogenic, were outside the maximum range of the set standards. The borehole water, adjacent to the final storage dam, namely, the night-dam, had evidence of contamination from certain nutrient elements. For instance, Cl^- and HCO_3^- ions were low in treated wastewater than in borehole water. Similarly, certain microbial pathogens were detected in both treated and borehole water, suggesting that the borehole was wrongly positioned relative to the night-dam. Additionally, the large number of pathogenic microbes at the exit to the night-dam to the irrigated fields demonstrated that the treatment system was not effective in cleansing the treated wastewater of the pathogens.

The impact of irrigating with treated wastewater on the physico-chemical properties of the soil depended on soil form, soil uses such as repeated cultivation and/or fallowing. The four major cations, Ca, Mg, K and Na, were low in under all field conditions, whereas NO_3 and NH_4 was predominantly reduced in cultivated fields. Fallowing played a critical role with accumulation of soil P, whereas boron was low on all fields irrigated with treated wastewater. Treated wastewater and cultivation decreased Ni and Cu, but increased Zn, Fe, Mn, Cr and Al, Cd and Pb. The treatment effects of cultivation and treated wastewater irrigation favoured potentially minerilisable nitrogen (PMN) and active carbon (AC), whereas soil organic matter (SOC) was decreased. However, relative to repeated cultivation, fallowing increased SOC percentage by more than 100%. Root health under repeated cultivation rated poorly with a maximum of 9 units, suggesting severe damage to roots, whereas repeated cultivation improved the healthiness of roots to a rating of 5 units. Consequently, the soil health indicators were affected positively and negatively when treated wastewater was combined with poor cultural practices.

The three test plants, onion (*Aleum cepa* L.), horseweed (*Conyza canadensis* L.) and tomato (*Solanum lycopersicum* L.), had different abilities to assimilate essential and non-essential heavy metals in different organs. In onion plants, the bulbs had restricted accumulation of ions, whereas tomato leaves tendered to assimilate high concentrations of cations such as Ca, Mg and K, along with Cr. Among the three different plants, horseweed assimilated high nutrients and essential heavy metals, except for As, CD and Pb, under fallowed conditions and could therefore serve as a good remediation for Cr.

6.2 Significance of findings

Findings in the current study demonstrated that, except for the microbial pathogens, the test treated wastewater was suitable for use in irrigating crops under diverse cultural practices. Primarily, the chemical and microbial compositions of the treated wastewater were dependent either upon the time period and collection point from the settling ponds to the night-dam and then the exit to the irrigated field. The significance of the study was that in short-terms, most of the test chemicals, essential and non-essential elements for plant production, were suitable for irrigating fields at the study site, but in the long-run, due to gradual accumulation, could result in ion-toxicities to crops. The major drawback of the treated wastewater at the test site was primarily the high load microbial pathogens, which included *Shigella* spp., *Salmonella* spp., *Escherichia coli*, fecal coliforms, *Vibrio cholerae* and *Ascaris lumbricoides*. The transitional arrangement from the settling ponds to the exit of the night-dam, had high counts of microbial pathogens, which should be of great concern for potential contamination of produce where the crops were being irrigated with treated wastewater. In the current study, plant produce was not tested for contamination. Additionally, the proper location of boreholes intended for portable water relative to ponds and storage facilities such as night-dams, could ameliorate the potential contamination of underground water by microbial pathogens – observed in the current study, where the borehole was downslope to the night-dam. Due to the location of the borehole, counts of *E. coli*, *Shigella* spp. and *Salmonella* spp. were high in borehole water.

The findings demonstrated that the test treated wastewater was at the time of the study not posing serious challenges to irrigated soils of different forms and soil types under various cultural practices. However, due to the gradual accumulation of certain heavy non-essential elements, which were still below the national and international permissible standards, would eventually accumulate to toxic concentrations. However, cultural practices such as fallowing, would ameliorate the effects of certain heavy non-essential elements. Due to the fact that different plant species have different capabilities of assimilating heavy non-essential elements, appropriate plant species could be used during fallowing to remove such heavy elements and with appropriate harvesting of such plants ridding the soil of the heavy metals.

6.3 Gaps and related recommendations

Because not all the strains of *E. coli* are pathogenic (WHO, 2017), it would be important that *E. coli* strains from the treated wastewater and borehole samples be identified as a matter of urgency. Also, the produce from the farm where treated wastewater was used to irrigate the crops, especially on onions, should be regularly tested for microbial pathogens, including *E. coli.* Another immediate intervention could be the instalment of the chlorine-purification system after the night-dam exit in order to reduce the microbial counts to below the permissible national and international standards. In the current study, the chemical composition of tomato fruit, especially the non-essential heavy metals, was not tested, but these should be tested regularly, not only in tomato fruit, but in all produce. The proposed tests and adjustments of the transitional systems of the treated wastewater, particularly with the predicted scenarios of drought and high temperatures under climate change, would provide essential information as to whether the use of treated wastewater in Limpopo Province could be expanded to other semi-arid areas – but with best agricultural practices as was the case during the execution of the study.

6.4 Synthesis and conclusions

Inland South Africa, the consequences of climate change, primarily the extended drought periods and high temperatures, along with the demand for water in mining and expanded human settlements around the mines, demand innovative ways of looking at resources for irrigation water. Findings in the current study suggested that, with proper treatment of the microbial pathogens, the treated wastewater could have the potential for ameliorating such pressures. Generally, with best agricultural practices, the treated wastewater could be used for irrigating fields with different soil types. However, due to the inherent high concentrations of non-essential nutrient elements and the presence of high loads of microbial pathogens in treated wastewater, it would be necessary to invest in additional tests and infrastructure that would improve the quality of treated wastewater in order to avoid hazardous unintended consequences.

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Appendix 3.1 Partitioned sources of variation for pH and EC of treated wastewater used for irrigation at the University of Limpopo Experimental Farm.

		pH		EC	
Source	Df	MSS	TTV (%)^{y,z}	MSS	TTV (%)
Block	2	0.16	1^{ns}	0.06	0^{ns}
Sampling site (A)	3	2.32	8^{***}	9.69	22^{***}
Months (B)	4	21.77	76^{***}	29.87	67^{***}
A × B	12	3.93	14^{***}	5.25	12^{***}
Error	38	0.63	2	0.07	0
Total	59	28.81	100	44.93	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Appendix 3.2 Partitioned sources of variation for calcium, magnesium, potassium, sodium and sodium adsorption ratio (SAR) of treated wastewater used for irrigation at the University of Limpopo Experimental Farm.

Source	Df	Ca		Mg		K		Na		SAR	
		MSS	TTV (%)^{y,z}	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	2	23.39	0^{***}	9.50	0^{ns}	6.19	2^{ns}	2280.69	12^{ns}	0.54	1
Sampling site (A)	3	13978.02	98^{***}	23819.51	100^{***}	236.14	84^{***}	10641.99	55^{***}	48.14	70^{***}
Months (B)	4	155.32	1^{ns}	24.83	0^{***}	13.53	5^{ns}	3523.98	18^{ns}	15.60	23^{***}
A × B	12	103.73	1^{***}	21.32	0^{***}	8.19	3^{ns}	1400.03	7^{ns}	3.42	5^{***}
Error	38	3.10	0	6.82	0	15.42	6	1462.50	8	0.75	1
Total	59	14263.57	100	23881.98	100	279.47	100	19309.19	100	68.44	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Appendix 3.3 Total treatment variation (TTV) in a sampling site × month of collection factorial experiment on Cl^-, HCO_3^- and SO_4^- concentrations in borehole water and wastewater used for irrigation at University of Limpopo Experimental Farm.

Source	Df	Cl^-		HCO_3^-		SO_4^-	
		MSS	TTV (%)^{y,z}	MSS	TTV (%)	MSS	TTV (%)
Block	2	26.45	0^{ns}	23.33	0^{ns}	18.12	1^{ns}
Sampling site (A)	3	23878.61	96^{***}	184881.56	99^{***}	1812.33	86^{***}
Months (B)	4	828.79	3^{***}	616.29	0^{ns}	48.89	2^{ns}
A × B	12	194.99	1^{***}	760.38	0^{ns}	176.20	8^{***}
Error	38	20.45	0	365.68	0	40.15	2
Total	59	24949.29	100	186647.24	100	2095.69	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at $P \leq 0.01$; *Significant ≤ 0.05; ^{ns}Not significant at $P \leq 0.05$.

Appendix 3.4 Total treatment variation (TTV) in a sampling site × month of collection factorial experiment on NO_3-N, NO_2-N and PO_4^- of treated wastewater and borehole water used for irrigation at University of Limpopo Experimental Farm.

Source	Df	NO_3-N		NO_2-N		PO_4^-	
		MSS	TTV (%)^{y,z}	MSS	TTV (%)	MSS	TTV (%)
Block	2	832.95	0^{ns}	0.0001	0^{ns}	0.20	0
Sampling site (A)	3	379579.58	98^{***}	0.15	75^{***}	69.42	95^{***}
Months (B)	4	5251.48	1^{***}	0.03	14^{***}	2.00	3^{***}
A × B	12	2268.55	1^{***}	0.02	8^{***}	1.50	2^{***}
Error	38	620.42	0	0.00	2	0.23	0
Total	59	388552.98	100	0.21	100	73.34	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Appendix 3.5 Total treatment variation (TTV) in a sampling site × month of collection factorial experiment on copper (Cu), zinc (Zn), chromium (Cr), lead (Pb), cadmium (Cd) and arsenic (As) concentration of treated wastewater and borehole water used for irrigation at University of Limpopo Experimental Farm.

Source	Df	Cu		Zn		Cr		Pb		Cd		As	
		MSS	TTV (%)[y,z]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	2	0.93	1	108.86	0	0.69	1	0.00	0	0.0008	24	0.11	
Sampling site (A)	3	154.67	93^{***}	71206.01	98^{***}	56.63	96^{***}	0.24	79^{***}	0.0015	45^{***}	997.45	88^{***}
Months (B)	4	8.78	5^{***}	582.59	1^{***}	0.64	1^{***}	0.02	6^{***}	0.0004	13^{***}	68.30	6^{***}
A × B	12	2.24	1^{***}	817.44	1^{***}	1.02	2^{***}	0.04	14^{***}	0.0003	9^{***}	67.68	6^{***}
Error	38	0.40	0	70.17	0	0.12	0	0.00	1	0.0003	9	0.53	0
Total	59	167.01	100	72785.06	100	59.09	100	0.31	100	0.0033	100	1134.07	100

[y]Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

[z]***Highly significant at P ≤ 0.01; *Significant ≤ 0.05; [ns]Not significant at P ≤ 0.05.

Appendix 3.6 Total treatment variation (TTV) in a sampling site × month of collection factorial experiment on *Salmonella* spp., *Shigella* spp., *Escherichia coli* and fecal coliform log-transformed counts of treated wastewater and borehole water used for irrigation at University of Limpopo Experimental Farm.

Source	Df	*Salmonella spp.*		*Shigella spp.*		*E. coli*		Fecal coliform	
		MSS	TTV (%)^{y,z}	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	2	0.17	1^{ns}	0.23	4^{ns}	0.57	7^{ns}	0.01	0.04^{ns}
Sampling site (A)	3	13.64	90^{***}	4.46	87^{***}	7.63	90^{***}	22.38	99.6^{***}
Months (B)	4	0.56	4^{ns}	0.28	5^{ns}	0.03	0^{ns}	0.01	0.04^{ns}
A × B	12	0.14	1^{**}	0.11	2^{ns}	0.22	3^{ns}	0.01	0.04^{ns}
Error	38	0.64	4	0.08	2	0.04	0	0.05	0.22
Total	59	15.15	100	5.15	100	8.50	100	22.47	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

3.7 Appendix 3.7 Partitioning sources of variation in *Vibrio fluvaris (ViFlu), Vibrio parahaemolytica (ViPar), Vibrio cholera (ViCho)* and *Vibrio aginolytica (ViAgi)* at four treated wastewater sampling sites over five months sampling times.

		ViFlu		*ViPar*		*ViCho*		*ViAgi*	
Source	DF	MSS	TTV (%)^{y,z}	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	2	0.04	4 ^{ns}	0.00	0^{ns}	0.04	0^{ns}	0.02	0.04^{ns}
Sampling site (A)	3	0.19	17***	12.58	96***	10.18***	90***	29.55	99.6***
Month (B)	4	0.09	9^{ns}	0.45	3^{ns}	0.18	2^{ns}	0.01	0.04^{ns}
A × B	12	0.07	6^{ns}	0.02	0^{ns}	0.17	1**	0.01	0^{ns}
Error	38	0.70	64	0.02	0	0.76	7	0.01	0
Total	59	1.10	100	13.06	100	11.34	100	29.60	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Appendix 3.8 Total treatment variation (TTV) in a Sampling site × month of collection factorial experiment on *Schistosoma mansoni*, *Entamoeba histolytica* and *Ascaris lumbricoides* log-transformed counts of treated wastewater used for irrigation at University of Limpopo Experimental Farm.

Source	Df	*S. mansoni*		*E. histolytica*		*A. lumbricoides*	
		MSS	TTV (%)^{y,z}	MSS	TTV (%)	MSS	TTV (%)
Replication	2	0.01	0.1^{ns}	0.01	1^{ns}	0.06	20^{ns}
Sampling site (A)	2	7.42	99.7***	2.36	98***	0.04	15^{ns}
Month (B)	4	0.01	0.1^{ns}	0.01	0^{ns}	0.07	22^{ns}
A × B	12	0.01	0.1^{ns}	0.01	0^{ns}	0.09	31**
Error	28	0.00	0.0	0.01	1	0.04	12
Total	44	7.44	100	2.40	100	0.30	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Appendix 4.1 Total treatment variation (TTV) in a field × soil depth factorial experiment on percentage clay, percentage sand, aggregate stability (AS) and bulk density (BD) under irrigation with treated wastewater.

		Clay		Sand		AS		BD	
Source	Df	MSS	TTV (%)[y,z]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	14	464.24	21	928.88	8	0.10	1	0.19	0
Field (F)	3	1294.58	58***	9717.88	84***	6.72	90***	37.17	98***
Depth (D)	4	232.89	10ns	463.79	4ns	0.56	7ns	0.53	1***
F × D	12	115.17	5ns	260.97	2ns	0.06	1ns	0.09	0ns
Error	266	120.78	5	151.69	1	0.05	1	0.08	0
Total	299	2227.66	100	11523.20	100	7.49	100	38.05	100

[y]Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

[z]***Highly significant at P ≤ 0.01; *Significant ≤ 0.05; nsNot significant at P ≤ 0.05.

Appendix 4.2 Total treatment variation (TTV) in a field × soil depth factorial experiment on pH and electrical conductivity (EC) under irrigation with treated wastewater.

Source	Df	pH(H_2O) MSS	pH(H_2O) TTV (%)^{y,z}	pH(KCl) MSS	pH(KCl) TTV (%)	EC MSS	EC TTV (%)
Block	14	0.74	1	0.57	1	171.46	3
Field (F)	3	65.95	93***	77.59	95***	5023.88	91***
Depth (D)	4	2.77	4***	2.76	3***	79.35	1^{ns}
F × D	12	1.36	2***	0.56	1***	157.33	3^{ns}
Error	266	0.20	0	0.24	0	92.76	2
Total	299	71.02	100	81.72	100	5524.77	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Appendix 4.3 Total treatment variation (TTV) in a field × soil depth factorial experiment on soil exchangeable cations under irrigation with treated wastewater.

		Ca		Mg		K		Na	
Source	DF	MSS	TTV (%)^{y,z}	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	14	0.00847	2	0.00074	1	0.00017	4	0.00058	2
Field (F)	3	0.43100	92***	0.07245	96***	0.00363	81***	0.02400	96***
Depth (D)	4	0.01530	3^{ns}	0.00175	2^{ns}	0.00007	1^{ns}	0.00003	0^{ns}
F × D	12	0.00760	2^{ns}	0.00028	0^{ns}	0.00009	2^{ns}	0.00018	1^{ns}
Error	266	0.00766	2	0.00052	1	0.00052	12	0.00017	1
Total	299	0.47003	100	0.07574	100	0.00449	100	0.02496	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at $P \leq 0.01$; *Significant ≤ 0.05; ^{ns}Not significant at $P \leq 0.05$.

Appendix 4.4 Total treatment variation (TTV) in a field × soil depth factorial experiment on soil cation exchange capacity (CEC) and exchangeable sodium percentage (ESP) under irrigation with treated wastewater.

Source	Df	CEC		ESP	
		MSS	TTV (%)^{y,z}	MSS	TTV (%)
Block	14	0.01	1	210.18	1
Field (F)	3	0.61	92***	27666.94	98***
Depth (D)	4	0.03	4***	105.57	0.5^{ns}
F × D	12	0.01	1^{ns}	103.11	0.5^{ns}
Error	266	0.01	1	93.00	0
Total	299	0.66	100	28178.80	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Appendix 4.5 Total treatment variation (TTV) in a field × soil depth factorial experiment on soil nitrate (NO_3^-) and ammonium (NH_4^+) concentrations under irrigation with treated wastewater.

		NO_3^-		NH_4^+	
Source	Df	MSS	TTV (%)^{y,z}	MSS	TTV (%)
Block	14	741.56	18	27.92	13
Field (F)	3	2154.50	52^{***}	152.934	70^{***}
Depth (D)	4	534.28	13^{ns}	12.60	6^{ns}
F × D	12	96.48	2^{ns}	9.84	5^{ns}
Error	280	625.22	15	14.20	7
Total	300	3410.48	100	189.57	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at $P \leq 0.01$; *Significant ≤ 0.05; ^{ns}Not significant at $P \leq 0.05$.

Appendix 4.6 Total treatment variation (TTV) in a field × soil depth factorial experiment on soil phosphorus (P), boron (B) and sulphur (S) under irrigation with treated wastewater.

		P		B		S	
Source	Df	MSS	TTV (%)^{y,z}	MSS	TTV (%)	MSS	TTV (%)
Block	14	17.96	3	1.37	1	20.66	26
Field (F)	3	545.17	91***	144.38	91***	11.22	14^{ns}
Depth (D)	4	21.43	4***	6.58	4***	11.71	15^{ns}
F × D	12	6.99	1***	4.35	3***	18.57	21^{ns}
Error	266	5.04	1	1.39	1	16.11	21
Total	299	596.58	100	158.07	100	78.28	97

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Appendix 4.7 Total treatment variation (TTV) in a field × soil depth factorial experiment on soil copper (Cu), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn) under irrigation with treated wastewater.

		Copper		Iron		Manganese		Nickel		Zinc		Cr	
Source	Df	MSS	TTV (%)^{y,z}	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	14	111.55	7	47.73	3	43.43	1	44.81	3	18.42	2	0.25	14
Field (F)	3	1346.35	89^{***}	1455.10	92^{***}	4706.00	98^{***}	1403.62	93^{***}	885.35	95^{***}	0.61	33^{***}
Depth (D)	4	22.17	2^{ns}	13.01	1^{ns}	17.18	0.6^{ns}	23.01	1^{ns}	10.06	1^{ns}	0.67	37^{**}
F × D	12	4.20	0^{ns}	29.16	2^{ns}	11.19	0.2^{ns}	31.59	2^{*}	12.07	1^{***}	0.15	8^{ns}
Error	266	22.20	2	26.13	2	8.46	0.2	12.13	1	4.35	1	0.16	9
Total	299	1506.46	100	1571,13	100	4786.26	100	1515.15	100	930.25	100	1.83	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at $P \leq 0.01$; *Significant ≤ 0.05; ^{ns}Not significant at $P \leq 0.05$.

Appendix 4.8 Total treatment variation (TTV) in a field × soil depth factorial experiment on soil aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr) and lead (Pb) under irrigation with treated wastewater.

		Al		As		Cd		Pb	
		MSS	TTV	MSS	TTV	MSS	TTV	MSS	TTV (%)
Source	Df		(%)^{y,z}		(%)		(%)		
Block	14	45.75	3	37.94	37	13.30	19	159.74	21
Field (F)	3	1214.75	84***	38.35	38^{ns}	46.22	67***	550.32	73***
Depth(D)	4	105.75	7**	2.56	2^{ns}	2.70	4^{ns}	0.81	0^{ns}
F × D	12	37.55	3^{ns}	5.77	6^{ns}	3.19	5^{ns}	11.67	2^{ns}
Error	266	38.00	3	17.36	17	3.75	5	28.33	4
Total	299	1441.81	100	101.98	100	69.16	100	750.87	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Appendix 4.9 Total treatment variation (TTV) in a field × soil depth factorial experiment on soil organic carbon (SOC), soil active carbon (SAC) and potentially minerilisable nitrogen (PMN) under irrigation with treated wastewater.

Source	Df	SOC		SAC		PMN	
		MSS	TTV (%)^{y,z}	MSS	TTV (%)	MSS	TTV (%)
Block	14	0.22	0	145087.56	14	0.20	6
Field (F)	3	2197.79	100***	494290.72	46***	2.92	90***
Depth (D)	4	0.29	0^{ns}	258357.08	24***	0.03	1^{ns}
F × D	12	0.43	0^{ns}	123399.23	11***	0.03	1^{ns}
Error	266	0.24	0	53258.05	5	0.07	2
Total	299	2198.97	100	1074392.64	100	3.24	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Appendix 4.10 Total treatment variation (TTV) in selected fields on root health rating under irrigation with treated wastewater.

Source	Df	MSS	TTV (%)^{y,z}
Block	14	1.97	4
Field	3	45.84	91***
Error	42	2.80	6
Total	59	50.61	100

^{y}Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

^{z***}Highly significant at P ≤ 0.01; *Significant ≤ 0.05; ^{ns}Not significant at P ≤ 0.05.

Appendix 5.1 Total treatment variation (TTV) on calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) in onion plants irrigated with treated wastewater.

Source	DF	Ca		Mg		K		Na	
		MSS	TTV (%)[y,z]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Experiment 1									
Block	7	347060	3	99702	6	2633714	12	1429545	6
Treatment	2	9758517	91***	1507850	88***	16440000	77***	21020000	90***
Error	14	586193	5	103714	6	2222928	10	800774	3
Total	23	10691770	100	1711266	100	21296642	100	23250319	100
Experiment 2									
Block	6	168092	3	117858	6	1009638	11	177476	1
Treatment	2	4746472	92***	1625671	85***	6949386	74***	12750000	96***
Error	12	256305	5	161301	8	1372569	15	366765	3
Total	20	5170869	100	1904830	100	9331593	100	13294241	100

[y]Total treatment variation [TTV (%)] = (MSS/Total) × 100.

[z]***Highly significant at $P \leq 0.01$.

Appendix 5.2 Total treatment variation (TTV) on zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) in onion plants irrigated with treated wastewater.

		Zn		Fe		Cu		Mn	
		MSS	TTV	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Source	DF		(%)[y,z]						
Experiment 1									
Block	7	3488.4	12	18676	11	3488.4	12	2100.5	11
Treatment	2	22603.9	78***	152778	86***	22603.9	78***	16548.3	84***
Error	14	2767.6	10	6340	4	2767.6	10	1048.5	5
Total	23	28859.9	100	177794	100	28859.9	100	19697.3	100
Experiment 2									
Block	6	1702.45	15	6166.1	13	24.63	2	1538.4	5
Treatment	2	8544.82	74***	36219.5	79***	1117.75	96***	29952.1	88***
Error	12	1237.86	11	3520.6	8	23.25	2	2560.7	8
Total	20	11485.13	100	45906.2	100	1165.63	100	34051.2	100

[y]Total treatment variation [TTV (%)] = (MSS/Total) × 100.

[z]***Highly significant at $P \leq 0.01$.

Appendix 5.3 Total treatment variation (TTV) on aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr) and lead (Pb) in onion plants irrigated with treated wastewater.

Source	DF	Al		As		Cd		Cr		Pb	
		MSS	TTV (%)[y,z]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Experiment 1											
Block	7	4.14	13	1.74	17	8.50	9	27.65	12	0.77	10
Treatment	2	24.77	79***	7.62	73***	72.92	80***	175.01	76***	6.03	81***
Error	14	2.49	8	1.15	11	10.30	11	27.61	12	0.69	9
Total	23	31.39	100	10.51	100	91.72	100	230.26	100	7.48	100
Experiment 2											
Block	6	2.56	15	0.59	8	2.52	4	13.60	10	0.55	13
Treatment	2	13.07	77***	6.10	83***	47.61	80***	98.97	74***	3.32	78***
Error	12	1.33	8	0.64	9	9.71	16	20.97	16	0.42	10
Total	20	16.97	100	7.33	100	59.84	100	133.54	100	4.28	100

[y]Total treatment variation [TTV (%)] = (MSS/Total) × 100.

[z]***Highly significant at $P \leq 0.01$.

Appendix 5.4 Total treatment variation (TTV) on calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) in tomato plants irrigated with treated wastewater.

Source	Df	Ca		Mg		K		Na	
		MSS[y]	TTV (%)[z]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Experiment 1									
Block	7	8540952.38	1	754134.78	1	9552961.31	9	7352.10	1
Treatment	2	1034700000	99***	184859659	99***	76535104.20	75***	626900.50	96***
Error	14	4814702.38	0	712563.36	0	16372961.30	16	18497.06	3
Total	23	10480554.76	100	186326357.14	100	102461026.81	100	652749.66	100
Experiment 2									
Block	6	27172460.30	1	8876333.83	34	6548435.02	11	7423.20	1
Treatment	2	2168600000	99***	15682157.40	59***	48015989.60	81***	791006.03	98***
Error	12	7957460.32	0	1842050.50	7	4918524.31	8	4820.37	1
Total	20	2203729920.62	100	26400541.73	100	59482948.93	100	803249.60	100

[y]Total treatment variation [TTV (%)] = (MSS/Total) × 100.

[z]***Highly significant at $P \leq 0.01$.

Appendix 5.5 Total treatment variation (TTV) on zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) in tomato plants irrigated with treated wastewater.

		Zn		Fe		Cu		Mn	
Source	Df	MSS	TTV (%)^{y,z}	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Experiment 1									
Block	7	97.5	1	151985	13	10.6948	27	34.987	4
Treatment	2	22499.9	99***	917213	77***	20.5891	51^{ns}	676.804	80***
Error	14	91.6	0	117056	10	8.7361	22	130.406	16
Total	23	22689	100	1186254	100	40.02	100	842.197	100
Experiment 2									
Block	6	65.98	1	48863	8	1.414	1	46.15	3
Treatment	2	9788.88	98***	505225	83***	117.357	97***	1564.82	95***
Error	12	65.66	1	56086	9	2.142	2	25.32	2
Total	20	9920.52	100	610174	100	120.913	100	1636.29	100

^{y}Total treatment variation [TTV (%)] = (MSS/Total) × 100.

^{z}***Highly significant at P ≤ 0.01.

Appendix 5.6 Total treatment variation (TTV) on arsenic (As), cadmium (Cd), chromium (Cr) and lead (Pb) in tomato plants irrigated with treated wastewater.

Source	Df	As		Cd		Cr		Pb	
		MSS	TTV (%)[y,z]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Experiment 1									
Block	7	4.14E-03	33	0.001146	8	0.1491	0	0.01243	8
Treatment	2	3.45E-03	27[ns]	0.01283	84***	79.227	99***	0.13561	85***
Error	14	5.15E-03	40	0.00129	8	0.3205	1	0.01194	7
Total	23	1.27E-03	100	0.015266	100	79.6966	100	0.15998	100
Experiment 2									
Block	6	6.14E-03	35	0.0033	16	0.3882	1	0.00567	5
Treatment	2	7.39E-03	42[ns]	0.0149	73***	63.1454	98***	0.10013	89***
Error	12	4.13E-03	23	0.0021	11	0.4188	1	0.00674	6
Total	20	1.77E-03	100	0.0203	100	63.9524	100	0.11254	100

[y]Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

[z]***Highly significant at $P \leq 0.01$.

Appendix 5.7 Total treatment variation (TTV) on calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) in leaf tissues of weeds, onions and tomato plants irrigated with treated wastewater.

Source	Df	Ca		Mg		K		Na	
		MSS	TTV (%)[y,z]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Experiment 1									
Block	7	9181740	1	5471172	3	7.81E+07	7	51053	3
Treatment	2	1.22E+09	98***	2.03E+08	94***	8.95E+08	85***	1670619	94***
Error	14	1.81E+07	1	6963221	3	8.26E+07	8	50408	3
Total	23	1243311740	100	215434393	100	1.06E+09	100	1772080	100
Experiment 2									
Block	6	7164694	1	3830675	1	3.60E+07	8	26407	3
Treatment	2	8.83E+08	97***	2.51E+08	96***	3.88E+08	83***	896761	95***
Error	12	1.59E+07	2	5556627	3	4.56E+07	10	20989	2
Total	20	906294694	100	260187302	100	4.69E+08	100	944157	100

[y]Total treatment variation [TTV (%)] = (MSS/Total) × 100.

[z]***Highly significant at $P \leq 0.01$.

Appendix 5.8 Total treatment variation (TTV) on zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) in leaf tissues of weeds, onions and tomato plants irrigated with treated wastewater.

Source	DF	Zn		Fe		Cu		Mn	
		MSS	TTV (%)[y,z]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
					Experiment 1				
Block	7	223.2	2	196026	6	6.057	2	72.52	1
Treatment	2	11223.1	95***	3057916	89***	248.019	95***	8751.33	98***
Error	14	295.7	3	186876	5	6.89	3	60.9	1
Total	23	11742	100	3440818	100	260.966	100	8884.75	100
					Experiment 2				
Block	6	50.4	0	43136	2	2.999	1	234.14	3
Treatment	2	13676.1	99***	2117577	96***	258.171	98***	8508.16	95***
Error	12	73.1	1	37454	2	2.009	1	228.92	2
Total	20	13799.6	100	2198167	100	263.179	100	8971.22	100

[y]Total treatment variation [TTV (%)] = (MSS/TOTAL) × 100.

[z]***Highly significant at P ≤ 0.01.

Appendix 5.9 Total treatment variation (TTV) on arsenic (As), cadmium (Cd), chromium (Cr) and lead (Pb) in leaf tissues of weeds, onions and tomato plants irrigated with treated wastewater.

Source	Df	As		Cd		Cr		Pb	
		MSS	TTV (%)[y,z]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Experiment 1									
Block	7	0.01	0	0.28	3	2.42	1	0.06	4
Treatments	2	2.15	100***	10.27	94***	298.13	98***	1.37	93***
Error	14	0.01	0	0.28	3	2.76	1	0.05	3
Total	23	2.16	100	10.83	100	303.31	100	1.48	100
Experiment 2									
Block	6	0.06	3	3.82	9	4.19	2	0.04	2
Treatments	2	1.67	95***	32.13	81***	222.57	97***	1.62	94***
Error	12	0.04	2	3.89	10	3.67	1	0.07	4
Total	20	1.77	100	39.84	100	230.43	100	1.73	100

[y]Total treatment variation [TTV (%)] = (MSS/Total) × 100.

[z]***Highly significant at $P \leq 0.01$.