THE ESTABLISHMENT OF A KNOWLEDGE HUB FOR CONTAMINANTS OF EMERGING CONCERN IN SOUTH AFRICAN WATER RESOURCES

Volume 2

Report to the
Water Research Commission

by

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The publication of this report emanates from a project entitled *The Establishment of a Knowledge Hub for Contaminants of Emerging Concern in South African Water Resources* (WRC Project No. C2021/2022-00256)

This report forms part of a set of two reports. The other report is *The Establishment of a Knowledge Hub for Contaminants of Emerging Concern in South African Water Resources. Volume 2* (WRC Report No. 3105/1/23)

EXECUTIVE SUMMARY

BACKGROUND

With population growth and shrinking fresh water sources, protecting water sources has come to the forefront of water resource management. Whilst substantial amounts of research is being conducted to identify pollutants in South African water sources, the research outputs and available information is not collated and presented to the science community and stakeholders in readily available formats and platforms. Current research outcomes need to be made known to regulators to develop environmental policies and laws. The CEC Knowledge Hub aims to collate all available data in literature and display these in a user-friendly online format to regulatory bodies as well as researchers to avoid duplication of studies. This access to information would not only reduce the possibility of duplication of research efforts but will also help identify CEC hotspots where research is urgently required as well as greatly enhance research efforts by allowing for research collaborations amongst researchers in the discipline. As part of the bigger project to develop a repository of CECs in SA (outlined in Volume I), two lab-scale studies were conducted to assess the impact of CECs on soil health (project 1) as well as to assess the level of microplastics and nanoparticles in selected waterways and establish the interaction of the selected CECs and model their fate (project 2). Upon completion, these lab-scale studies are anticipated to contribute CEC data to the ever-expanding data repository housed in the CEC knowledge hub.

AIMS

Project 1 (Emerging Pollutants in South African Surface Waters and their Associated Microbial Communities: Implications for Environmental Health and Sustainability) is aimed at understanding the impacts that emerging pollutants present in irrigation water have on soil health.
Project 2 (Characterization, interactions and modelling of contaminants of emerging concern in surface water from selected catchment areas in South Africa) is aimed at characterizing and quantifying the amount of microplastics and nanoparticles in water bodies and to use toxicity models to determine their fate.

METHODOLOGY

For project 1, smallholder farms in North West province were identified and selected. Selection was based on irrigation with stream water possibly getting deposition of CECs from upstream industries. Water and soil samples were aseptically collected in replicates and analysed for microorganisms and CECs. Microbial analyses involved DNA extraction, PCR and Next generation sequencing. Chemical analyses were conducted to identify and determine initial predominant CECs in the samples. After the initial analyses, the soil and water samples were used to set up a greenhouse experiment. The collected soil samples were used to plant spinach seeds that were appropriately irrigated using the collected water. Analysis of soil microbial and chemical abundance was done again after 6 weeks of spinach growth to evaluate the impact of CECs on soil microbiota and observed plant growth. For project 2, four contaminated rivers were selected from two provinces in South Africa, Gauteng and Limpopo Province. Water samples were collected and pH, dissolved oxygen, turbidity and temperature of the river water were measured using portable meters at the sampling point. Microplastics were extracted using the stacked sieve method and total suspended solids determined. Water samples were filtered and SVOC and nanoparticle levels determined.

RESULTS AND DISCUSSION

Preliminary results from project 1 revealed that higher concentrations of macronutrients in the soil are typically beneficial for soil productivity and plant growth. However, when pH is too high or too low, it can affect the soil functions and subsequently the availability of nutrients to the crop. Preliminary results

from project 2 revealed that all the rivers that the study focused on were highly contaminated with the exception of Mokolo River. The pH levels and electrical conductivity measurements for both winter and summer seasons at Mokolo River were within SAWQG specifications with turbidity readings being slightly higher than SAWQG specification. The pH levels during summer season at Blesbokspruit catchment were higher than all the pH levels that were obtained in this study during all seasons with the median of 9.69. Electrical conductivity and the plastic congestion at Hennops River were more than at all river sites sampled. It was also noted that Mokolo River, which does not pass through any informal settlements and has very few industrial activities around it exhibited better water quality in comparison to all other rivers studied that pass through urban areas (Hennops River, Blood River and Blesbokspruit River).

GENERAL CONCLUSIONS & RECOMMENDATIONS

The preliminary results from Project 1 suggests that the irrigation water quality could potentially have a negative impact on soil nutrients, and cause shifts in soil microbial community structure which may impact on nutrient mobilisation and nutrient availability to the plant. It is recommended that sampling be conducted upstream or directly from the dam, sediment sampling is also recommended during different seasons. The findings of the study may be expanded upon by analysing a wider range of CECs and using a wider variety of crops for the greenhouse experiment. Based on the results obtained thus far from this project it may be concluded that all rivers that were sampled were highly contaminated with the exception of Mokolo River. Moreover, surface water contamination differed with seasonal variations and areas. It is recommended that sampling should be conducted adjacent to the rivers to identify the sources of different contaminants in water. It is also recommended that the study in the selected rivers be carried out for more years in the future to observe the behaviour of the river over a longer duration.

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ACRONYMS & ABBREVIATIONS

AMD	Acid mine drainage
ARD	Acid rock drainage
CEC	Contaminants of Emerging Concern
DBP	Drinking Water Disinfection By-Products
EC	Electrical conductivity
EDC	Endocrine Disruptive Chemicals
EP	Emerging Pollutants
FET	Fish Embryo Test
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography – Mass Spectrometry
IT	Information Technology
KH	Knowledge Hub
KH DB	Knowledge Hub Database
MP	Microplastics
NGS	Next Generation Sequencing
NP	Nanoparticle
OCP	Organochlorine pesticides
PAH	Polycyclic Aromatic Hydrocarbon
PCB	Polychlorinated Biphenyls
PCR	Polymerase Chain Reaction
PGPR	Plant growth-promoting rhizobacteria
SAR	Sodium adsorption ratio
SAWQG	South African Water Quality Guidelines
SVOC	Semivolatile organic compound
TDS	Total Dissolved Solids
TSS	Total Suspended Solids

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CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

The importance of water on earth cannot be overemphasized as it is key to life on earth and available fresh water sources is dwindling due to pollution from anthropogenic sources (Rasheed et al., 2019; Department of Health, Australian Government, 2010). Globally, environmental pollution is a pressing issue (Pandey et al., 2019) that is aggravated by an increase in human population and their elevated qualities of life (Rasheed et al., 2019). This modern lifestyle has caused an increased production of chemical waste and waste from pharmaceuticals and personal care products as well as non-biodegradable materials especially plastics, fertilizers, pesticides, nanomaterials and a myriad of other products (Amin et al., 2014; Rimayi et al., 2019; Rasheed et al., 2019). Improper disposal of such products leads to pollution of fresh water (Amin et al., 2014). Some of the compounds contained in these products are not stated in the conventional list of contaminants nor controlled by any legislative body in the country; and such contaminants are of an emerging concern (Petrie et al., 2015). The existing methods used in water treatment and purification such as, activated sludge, reverse osmosis and trickling filters are not designed to successfully remove contaminants of emerging concern (CECs) and therefore treatment with such processes is ineffective (Amin et al., 2014; Petrie et al., 2015).

CECs are unregulated micro-contaminants that are found in water and mostly result from anthropogenic processes (Rasheed et al., 2019). These contaminants are made up of compounds that are unregulated, may be newly synthesized or new in the market, known but previously assumed to be existing in very low concentrations or discovered to be posing health hazards to both aquatic and human life (Moodley et al., 2016). According to Odendaal et al. (2015) and Halden (2015), a list of identified CECs includes endocrine disruptive chemicals (EDCs) brominated flame retardants, sunscreens/UV filters, drinking water disinfection by-products (DBPs), benzotriazoles, naphthenic acids, cyanobacterial toxins, perchlorate, dioxane, pesticides, microplastics, microorganisms, etc. A study conducted in 2012 stated an estimated increase of global CEC production from 1 million to 500 million tons, annually (Rasheed et al., 2019); and in 2015 Halden (2015) reported that the estimated number of discovered CECs is 40 000 wherein approximately six new contaminants of emerging concern are recorded daily around the globe. The presence of these CECs in water (even in trace amounts) poses a serious threat to human and aquatic lives because some of these compounds are non-biodegradable, chemically stable and may cause long term side effects (Thiebault et al., 2015).

As part of the bigger project to develop a repository of CECs in SA, two lab-scale studies were conducted to assess the impact of CECs on soil health and microbial community dynamics as well as to assess the level of microplastics and nanoparticles in selected waterways and establish the interaction of selected CECs and model their fate. Preliminary results from the ongoing studies are highlighted in this report.

1.2 PROJECT AIMS

The aim of **PROJECT 1** (*Emerging Pollutants in South African Surface Waters and their Associated Microbial Communities: Implications for Environmental Health and Sustainability*) is to understand the impacts that emerging pollutants present in irrigation water have on soil health.

The aim of **PROJECT 2** (*Characterization, interactions and modelling of contaminants of emerging concern in surface water from selected catchment areas in South Africa*) is to characterize and quantify the amount of microplastics and nanoparticles in water bodies and to use toxicity models to determine their fate.

1.3 SCOPE AND LIMITATIONS

PROJECT 1 – *Emerging Pollutants in South African Surface Waters and their Associated Microbial Communities: Implications for Environmental Health and Sustainability*

SCOPE: This study focuses on the microbial response of soils when irrigated with potentially contaminated water.

LIMITATIONS: The microbial communities identified in the study is limited to the microorganisms that can be identified by next generation sequencing (NGS) and therefore some will not be identified. Also, the contaminants of emerging concern targeted is limited to polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and phenols. Additionally, this study is qualitative and will not quantify the microbes but will give an indication of the variability amongst the microorganisms before and after irrigation with the sampled water.

PROJECT 2 – *Characterization, interactions and modelling of contaminants of emerging concern in surface water from selected catchment areas in South Africa*

SCOPE: This project focuses on the investigation of selected contaminants of concern (microplastics, SVOCs and nanoparticles) from four contaminated rivers in South Africa. The four rivers are Hennops River and Blesbokspruit catchment in Gauteng Province, Mokolo River and Blood River in Limpopo Province. Water samples were collected from the rivers in winter and summer for the determination of seasonal variations in terms of the differences in type and amount of the CECs. Physico-chemical parameters (pH, temperature, dissolved oxygen and turbidity) were measured at the sampling site. The collected samples were preserved accordingly and transported to the lab for further analysis. The scope of analyses conducted to characterise the water samples include:

Extraction of microplastics, nanoparticles and semi volatile organic compounds (SVOCs): The stacked-sieve method was used for the collection of microplastic containing sludge, while the extraction of microplastics was carried out using aqueous hydrogen peroxide, followed by washing with de-ionised water. SVOCs were isolated using solid-phase extraction method, whereas nanoparticles were settled by the use of coagulants and extracted through centrifugation.

Characterisation of microplastics, nanoparticles and SVOCs: The amount, size, shape and chemical profiles of the microplastics will be obtained using microscopy and spectroscopy. The size, morphology and surface chemistry of the nanoparticles will be characterised using spectroscopy and microscopy. The SVOCs will be profiled and quantified using GC-FID and GC-MS.

Modelling of the interaction between microplastics and SVOCs; nanoparticles and SVOCs: Simulated water in batch experiments will be used to study the interaction between microplastics and SVOCs as well as nanoparticles and SVOCs. FTIR, UV-vis, Raman spectrometry as well as microscopy will be used for characterisation.

Assess the toxicity effects of nanoparticles and microplastics using the zebrafish embryos assay: Fish embryos will be used to assess the toxic effects of nanoparticles and microplastics. Zebrafish embryos will be exposed to contaminated river water and simulated nanoparticles and microplastics solutions. From zebrafish embryotoxic responses, a hazard classification system will be developed.

LIMITATIONS: The study excludes the characterisation of other CECs in the water samples collected from the four rivers. Autumn and Spring seasons are also excluded from the study as they are very short seasons.

- Two abstracts were submitted for a poster presentation at the Combined Congress in January of 2023 and the Society of Environmental Toxicology and Chemistry Conference also in 2023.
- The Combined Congress conference was attended from the 23rd to the 26th of January 2023 where a poster was presented on preliminary results.
- Lab work for NGS is ongoing as well as the analysis of the greenhouse results.

CHAPTER 2: PROJECT 1 – Emerging Pollutants in South African Surface Waters and their Associated Microbial Communities: Implications for Environmental Health and Sustainability

A black South African female student, Ms. Fanelesibonge Vilakazi from KwaZulu Natal Province with Honours degree in Microbiology was recruited for the project. Ms. Vilakazi is studying towards a Master's degree in Microbiology at the North West University, Potchefstroom. Ms Vilakazi is supervised by Prof R. Adeleke, co-supervised by Dr M Bello-Akinosho and Dr EE Bamuza-Pemu. The training she would acquire during her masters studies would contribute to improve human capacity in the science/water sector in South Africa. At time of submission of this report, Ms Vilakazi's study is ongoing and preliminary results are outlined below.

2.1 INTRODUCTION

Globally, the interest in contaminants of emerging concern (CECs) or emerging pollutants (EPs) has been growing for the past 20 years (Noguera-Oviedo and Aga, 2016). Contaminants of emerging concern are best described as synthetic or naturally occurring unregulated chemicals or microorganisms commonly found in surface water sources as well as the environment in traces and are not typically detectable or monitored (Noguera-Oviedo and Aga, 2016). Poynton and Vulpe (2009) attributed this to a lack of sensitive analytical methods, which may lead to human and ecological health risks. Another reason for the poor monitoring of these pollutants is that they are not properly regulated, especially in developing countries, and therefore not detected, identified, and monitored in the environment (Chaukura et al., 2016). In previous studies, CECs have been suspected to induce endocrine-disrupting activity, promote carcinogenesis in humans and trigger physical as well as reproductive behavioural changes in aquatic animals (Grabicova et al., 2017). They also have been noted to possibly have negative impacts on composition and functional diversity of essential microorganisms inhabiting water and soil by altering environmental conditions.

Emerging pollutants encompass a variety of chemicals which include pharmaceuticals, personal care or household cleaning products, lawn care and agricultural products (Jurado et al., 2012). Due to their frequency of use, these pollutants regularly find their way into nearby water sources through runoff from either irrigation, storm water, or improper disposal. These water sources are typically local dams, rivers, lakes, and streams sustaining the livelihood of local communities. The lack of monitoring and detection of CECs has been observed to significantly affect low to middle-income countries that lack the necessary infrastructures. Chaukura et al. (2016) further explained that as a result, developing countries are currently using outdated conventional methods and technologies that still leave a wide array of undetected emerging pollutants going undocumented and unmonitored in the environment.

South Africa is a low to a middle-income country with limited availability of surface water sources, ranked 30th driest in the world (Owusu-Sekyere et al., 2016). It is reported to be arid to semi-arid with only 7% of the country region receiving enough rainfall per annum to sustain dry crop farming (Masubelele et al., 2015). The agricultural sector is among the most water dependent sectors in the country (De Villiers et al., 2004), utilizing an estimated 51-63% of the country's available surface water for irrigation and accounts for almost 30% of the country's crop production. It is for this reason that the agricultural sector is recognized as the single largest user of fresh water in the country (Van Averbeke et al., 2011). Furthermore, the agricultural sector promotes the use of fertilizers, pesticides, and insecticides for crop production enhancement (Chowdary et al., 2005) in order to cater to the demand of the rapidly growing population. Such practices may cause these excess chemical compounds to run off into the nearest water bodies during irrigation or rainy seasons. Runoff is common in agriculture but other industries, like the mining industry, also play a major role in water pollution by poor mode of waste disposal.

As a response to the growing industrialization (mining, agricultural and manufacturing), there is a rise in the amount and abundance of pollutants continuously making their way into water sources. These pollutants include heavy metals from acid mine drainage (Ahmed and Wohnlich, 2014), excess nutrients from fertilizer use, agricultural chemical residues and other industrial discharge chemicals (Verlicchi and Grillini, 2020). South Africa is widely known for its mineral richness with a total of 526 mines predominantly producing platinum, coal, gold, and diamonds. As per the Department of Water Affairs 2018 report, these mines and industries use approximately 5.7% of the country's freshwater ranking 3rd after urban use which accounts for 25,1%.

Both the agricultural and mining industries contribute towards the utilization and pollution of waterways (Verma, 2005) but because of weather extremes brought by climate variability such as drought conditions, the agricultural sector is constantly in need of irrigation water to achieve maximum crop yield. This water, however, harbours a wide spectrum of CECs that could be introduced into the soil during irrigation. Soil comprises various microbiota of bacteria, fungi, algae and protozoa that are important for soil formation, structure sustainability, and soil ecology (Verma, 2005). These soil microorganisms are crucial as they are responsible for multiple functions necessary in the regulation of soil nutrients thus enhancing plant nutrition, and improving plant growth, productivity, and quality (Joergensen and Emmerling, 2006). However, when the irrigation water is polluted, it can directly impact soil microbial biodiversity as polluted water may affect the functioning of the important microbes and cause changes in soil structure as well as crop yield (Yadav, 2017). It is therefore of utmost importance to identify, monitor and measure CECs present in the country's water systems so that their sources, environmental, human and overall impacts on the ecosystem can be better understood. With this approach, there can be improvements in the currently applied methods as well as the identification of better potential mitigation strategies.

2.2 PROBLEM STATEMENT AND SUBSTANTIATION

In several previous studies, CECs have been suspected to induce endocrine-disrupting activity, promote carcinogenesis in humans and trigger physical as well as reproductive behavioural changes in aquatic animals (Grabicova et al., 2017). They have also been noted to possibly have a negative impact on essential aquatic and terrestrial organisms due to them altering physical and chemical conditions. The growth of microorganisms is directly impacted by various intrinsic and extrinsic environmental factors. Beneficial soil microorganisms such as the plant growth-promoting rhizobacteria (PGPR) which makes up most of the rhizosphere microbiome are sensitive to biotic and abiotic changes thus changes in pH, temperature, moisture and oxygen availability affect their functions causing irregular enzyme production. The rhizobacteria does not only produce enzymes and other organic bioactive compounds (Hassan et al., 2019) but also play major roles in soil formation, structure as well as nutrient and water cycling in the soil (Hoorman, 2011). Although the rhizosphere is made up of different microbial communities including fungi, protozoa, archaea, and oomycetes that are all important for the growth and health of plants, there is little understanding of how CECs impact the rhizosphere microbes and how plants respond to these influences. Whilst the interaction between the plants and different rhizosphere microbial communities is better understood (Van der Putten et al., 2007), there is insufficient information on the interaction of CECs and the rhizosphere, thus it is vital for the soil and CECs interaction be studied to ensure better plant growth, better production quality, sustainable soils and environment.

2.3 RESEARCH AIMS AND OBJECTIVES

The aim of this study is to understand the impacts that emerging pollutants present in irrigation water have on soil health.

Objectives:

1. To identify and collect soil and water samples from three smallholder farms irrigating with water potentially containing CECs from nearby industrial sources
2. To determine certain CECs present in the collected samples by means of GC-MS and identification of microbial communities by PCR and NGS
3. To evaluate the impacts of soil and irrigation the water containing CECs on beneficial soil microbiota relevant to plant growth in a greenhouse experiment

2.3.1 HYPOTHESIS

Contaminants of emerging concern in irrigation water influence important microbial diversity and composition in agroecosystems

2.4 METHOD OF INVESTIGATION

Smallholder farms in North West province irrigating with stream water possibly getting deposition of CECs from upstream industries were identified and selected. Soil samples (rhizospheric) were collected in replicates using a hand spade. Collected soil samples were stored at -20 or -80°C until further analysis. Water samples were aseptically collected in replicates, stored in ice during transportation and stored at 4°C until further analysis. Microbial analyses involved (DNA extraction, PCR and Next generation sequencing) in the Microbiology laboratory. Chemical analyses was conducted to identify and determine initial predominant CECs in the samples. After the initial analyses, the soil and water samples were used to set up a greenhouse experiment. The collected soil samples were used to plant spinach seeds that were appropriately irrigated using the collected water. Analysis of soil microbial and chemical abundance was done again after 6 weeks of spinach growth to evaluate the impact of CECs on soil microbiota and observed plant growth.

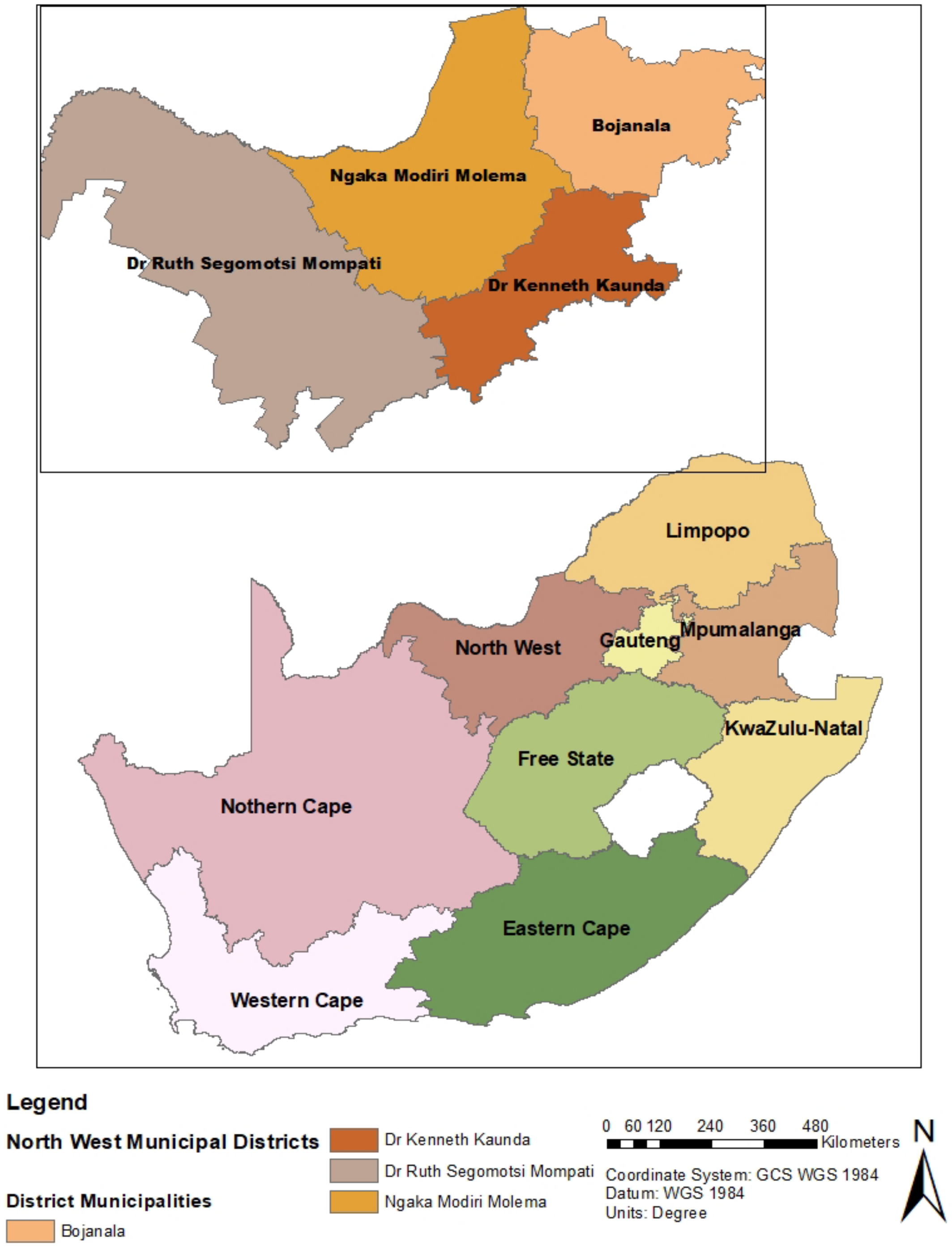


Figure 2.1a: *North West Province (categorized by districts) where the samples were collected*

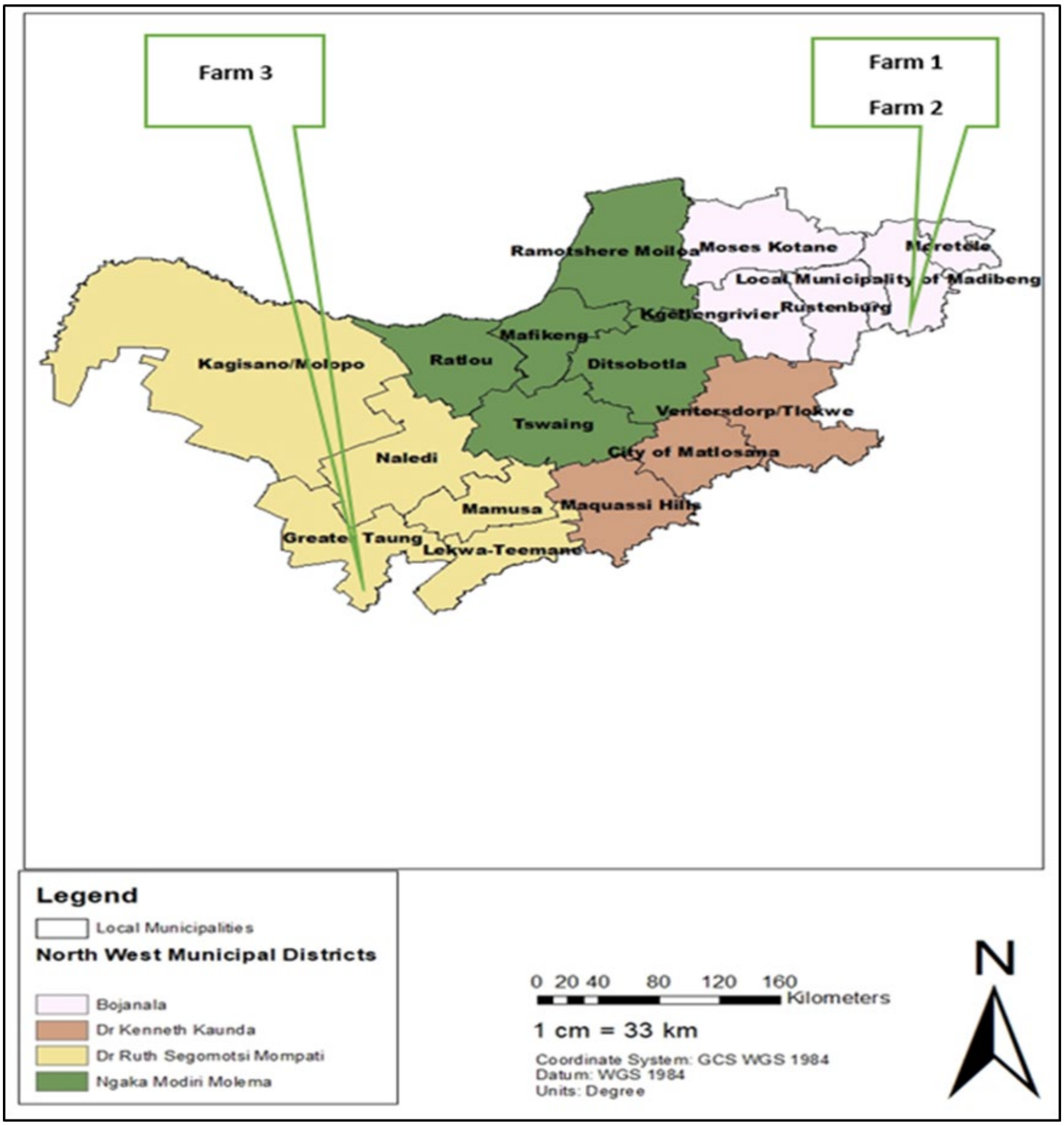


Figure 2.1b: *Locations of the crop farms where the soil and water samples were collected*

***Figure 2.1c and d**: The farms where the soil and water collection was done in Taung and Brits, respectively*

2.5 PRELIMINARY RESULTS AND DISCUSSION

Water pH levels in irrigation water impact and influence the soil as well as the crop (production and quality) that the water is used to irrigate (Yermiyahu et al., 2007). The acidity or alkalinity of water determines the quality of water as acidic water is considered to be more polluted or contaminated. This is due to heavily polluted water being associated with lower pH levels, thus making it acidic. For example, even though acidic water exists naturally from disintegration of sulfide minerals, it is not as common as water that is acidic due to anthropogenic sources such as acid mine drainage common in industrialized countries (Quatrini and Johnson, 2018). Farm 1 had an average pH of 7.21, which is considered neutral level. Neutral pH is ideal for plants as well as the environment as most bacteria are neutrophiles (pH 5.5-8.5) which means they cannot thrive in acidic environments (Rosso et al., 1995). Farm 2 and 3 ranged between pH 8.75 and 8.53, respectively and farm 3 water was the most alkaline of all the farms. The recommended range for irrigation water as per South African irrigation water standards is pH 6.5-8.4. At this pH there is optimum crop yield, crop quality and better sustainability for environmental nutrients. When pH is higher or lower than the recommended range the implications for soil nutrients and crop production vary as tabulated (Table 2.1).

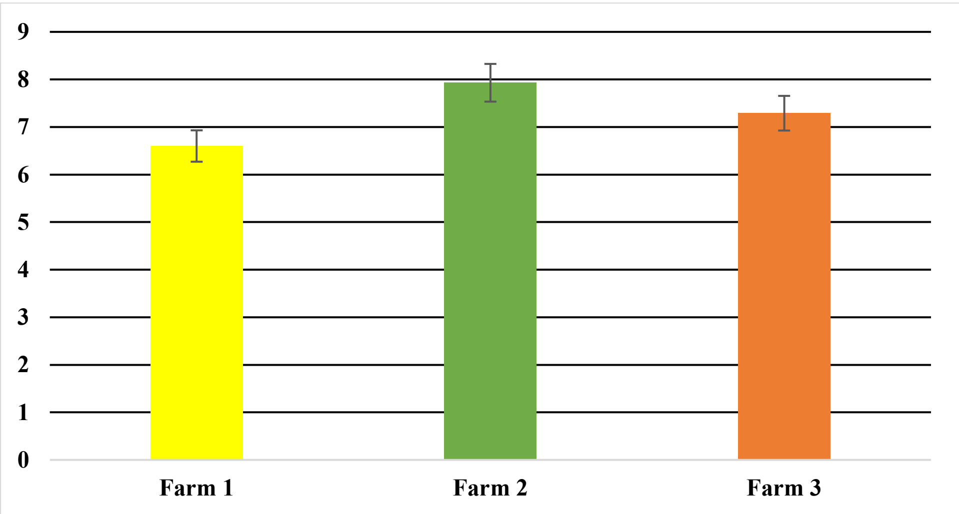


Figure 2.2: *Average pH levels of the water from the selected farms*

Table 2.1: *The different pH ranges of irrigation water as per South African irrigation water standards*

pH levels	**Effect on crop yield and quality**	**Effect on sustainability**
< 6.5	• Could cause foliar damage when crop foliage is wet • Reduction in crop yield • Decreased crop quality	• Could cause long term problems with the availability of various macro and micro-nutrients in toxic concentrations
Recommended range 6.5-8.4	• Reduced foliar damage even when crop foliage is wet • Improved crop yield • Better crop quality and marketability	• Reduced problems with both unavailability of nutrients and toxic levels of elements
> 8.4	• Foliar damage • Decreased yield • Decreased crop quality • Decreased crop marketability	• Long term unavailability of various nutrients

Electrical conductivity (EC) and total dissolved solids (TDS) of irrigation water impact crop quality and soil productivity. As total dissolved solids are inorganic salts and organic matter present in water bodies, it commonly refers to calcium, magnesium, sodium, potassium cations and carbonate, hydrogencarbonate, chloride, sulfate and nitrate anions detected in the water (Corwin and Yemoto, 2020). It is therefore common practice to compare EC to TDS due to the fact that EC measures the charge and TDS also encompasses charged particles such as Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, HCO_3^-, NO_3^-, SO_4^{-2} and CO_3^{-2} (Rhoades, 1996). As a result of both EC and TDS being charged, they tend to form a positive correlation. The recommended EC is 40 mS/m as indicated by the irrigation water standards of South Africa. When EC is at the recommended 40 mS/m it allows for salt-sensitive crops to grow with optimum yield even with low frequency irrigation. When EC increases to the range of 40-90 and 90-270 mS/m there can still be a 95%, 90% and 80% crop yield, respectively. Any EC above 540 mS/m may still have some yield especially for a crop that is not sensitive to salt but may result in increased salinity of the soil. Although almost 69% of South African soils are non-saline, there is a looming salinity problem as a result of anthropogenic activities in the country (Nell and Van Huyssteen, 2018).

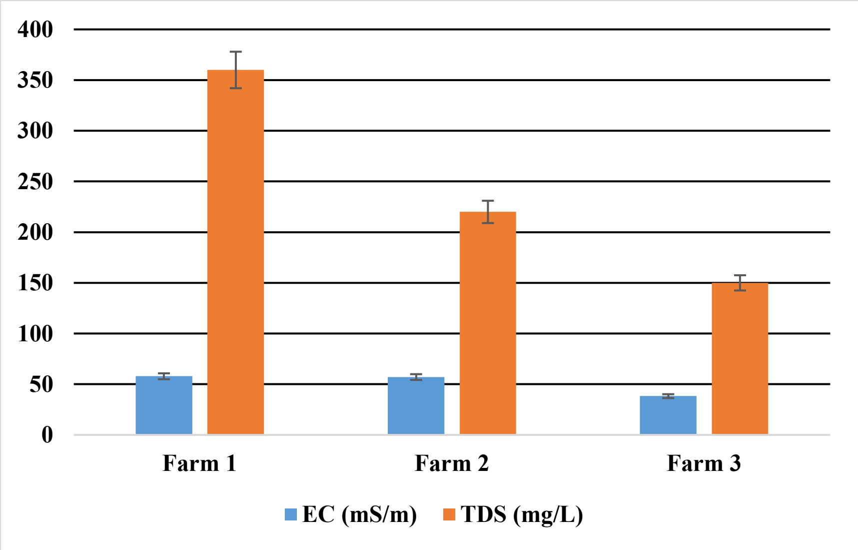


Figure 2.3: *Electrical conductivity (EC) and total dissolved solids (TDS) readings from water samples collected in North West*

The sodium adsorption ratio (SAR) measures the relative concentration of sodium (Na) to calcium (Ca) and magnesium (Mg). Increased SAR in irrigation water also increases soil sodification which often leads to poor soil structure as a result of high salt levels in the soil (Awan et al*.,* 2021). The recommended SAR is 2.0 and all the water samples had SAR below the recommended limit. Farm 1 had 1.73, Farm 2 had 1.71 and farm 3 had the lowest SAR of 1.13. Lower SAR aids in preventing toxicity in soils and plants that may occur due to higher sodium concentrations. When the SAR range is between 2.0-8.0 salt-sensitive crops may absorb excess salt through the roots and higher sodium concentrations ranging above 15.0 become even more toxic for sensitive plants. While sensitive crops may be harmed by high levels of sodium, crops that are more salt-tolerant may not be affected as much by similar sodium concentrations (Awan et al*.,* 2021).

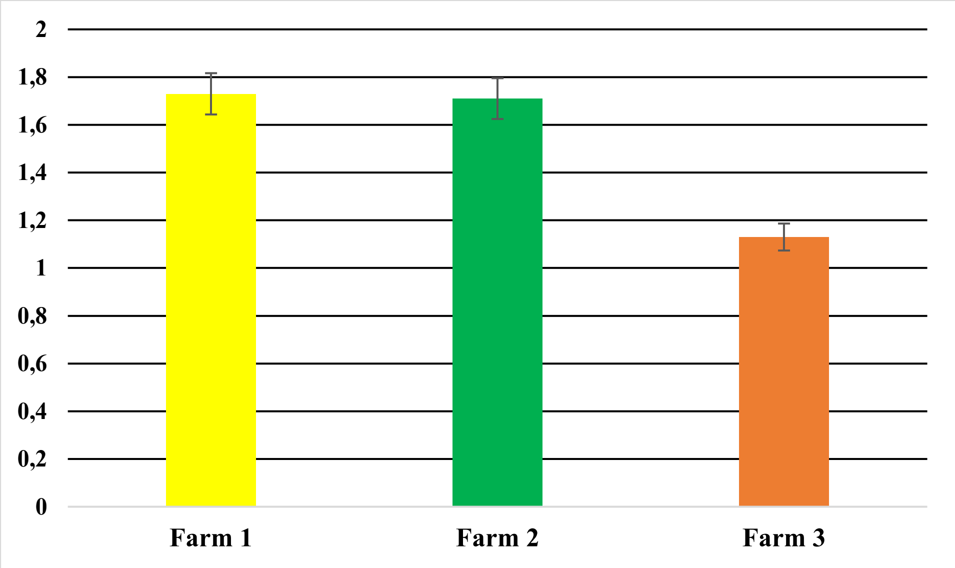


Figure 2.4: *Sodium adsorption ratios of water samples collected from the 3 farms in the North West province*

The Brits farms (farm 1 and farm 2) had the highest levels of bicarbonate (HCO_3) which was 141 and 137 mg/L, respectively. Bicarbonate was the highest concentrated nutrient across all water samples as it followed similar trend with the Taung farm (farm 3) as well with a concentration of 96.4 mg/L. Bicarbonate is as a result of dissolved CO_2 in water that produces carbonic acid, which further breaks down into bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) (Poschenrieder et al., 2018). Terrestrial cyanobacteria is able to use HCO_3^- dissolved in the surrounding water bodies for photosynthesis. Soils rich in HCO_3^- concentrations can harm other land plants particularly those that are carbonate sensitive (Poschenrieder et al*.,* 2018). Sulphate was the second highest across all water samples followed by chloride (Cl) and sodium (Na) which were 67.1 mg/L, 66.7 mg/L, 57.0 mg/L and 56.0 mg/L, 56.3 mg/L and 31,5 mg/L, respectively. Sulphate can occur naturally from soil and rocks containing sulphate as a mineral, however, it is commonly detected in surface water bodies from either waste or industrial disposal (Fernando et al*.,* 2018).

Metal sulphates contribute largely to acid mine drainage (AMD) also known as acid rock drainage (ARD) by oxidation of metal sulphides (Fernando et al., 2018). The recommended range of chloride is 100 mg/L and at this range the accumulation of chloride from reaching toxic levels although sensitive plants may be slightly affected. Plants that are tolerant to chloride may be able to withstand chloride concentrations above 700 mg/L. Sodium recommendations for optimum crop yield and quality highly depend on the type of crop and the crop's level of sensitivity, thus no actual standard has been set yet for South African irrigation water. Sensitivity of crop can be classified as sensitive, moderately sensitive, moderately tolerant and tolerant. High salinity in irrigation water may drive the phenomenon of soil salinity which currently a huge threat to food security in the world. Salinity in the root zone may be due to different factors such as poor irrigation water quality, limited leaching by soils, texture of soil (soil type) and limited drainage by soil (Le Roux et al*.,* 2007).

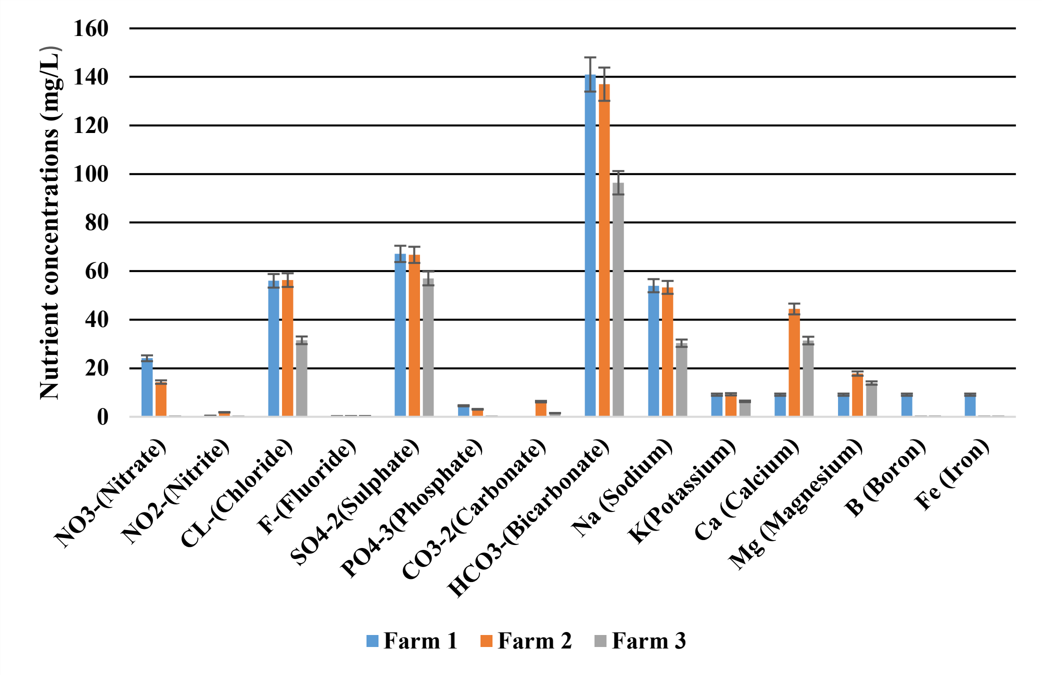


Figure 2.5: *Water nutrients concentrations in the collected water samples from the North West province*

Table 2.2: *Some of the commonly grown crops in South Africa and their level of tolerance to salinity (Adapted from Irrigation water standards of South Africa, Vol 4)*

Sensitive	Moderately sensitive	Moderately tolerant	Tolerant
Onion	Cabbage	Beetroot	Asparagus
Beans	Cauliflower		
Peas	Maize		
	Cucumber		
	Lettuce		
	Pepper		
	Potatoes		
	Spinach		
	Sweet potatoes		
	Tomatoes		
	Watermelon		

The soil samples were analysed and grouped according to particle size. Based on particle size and abundance and distribution the soil was classified based on the sand to silt to clay ratio. Using the triangular textural chart (USDA) method, which is commonly used for agricultural soil classification (Park and Santamarina, 2017).

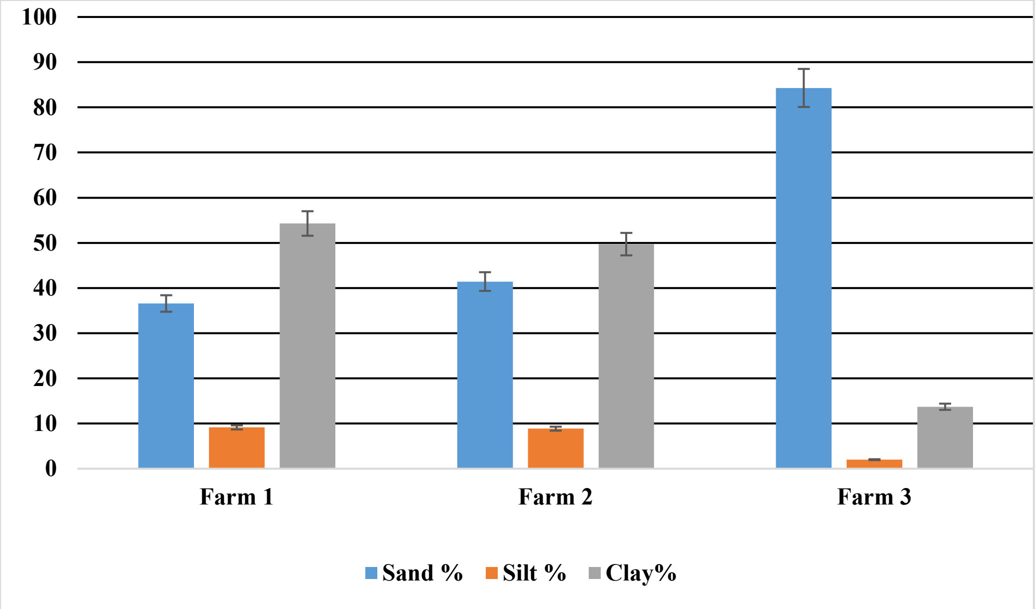


Figure 2.6: *Particle sizes of the soil samples collected from different farms in North West Province*

Farm 1 and farm 2 which were both Brits farms had very high percentages of clay with 45.28% and 49.71% clay content. Clay soil is made up of very fine particles <0.002 mm and as such has a high water holding capacity as a result of being able to easily retain water (Rasa et al., 2018). Clay rich soils have high permeability thus water can access through the particles but because clay soils also have high retention and low permeability there is less water released to the crop (Abu-Khasan and Egorov, 2020). As water accumulates in clay the nutrients are deposited as well but just like water they remain in the soil and are not easily accessible to shallow plants such as crops (Rasa et al., 2018). Farm 3 soils were classified as sandy loam because of having 84.28% sand content (medium to coarse ranging between 0.5-2 mm), 13% clay and only 2.0% silt (0.002-0.05 mm). Sandy loam soils are very light with good drainage and subsequently low on nutrients compared to clay soils.

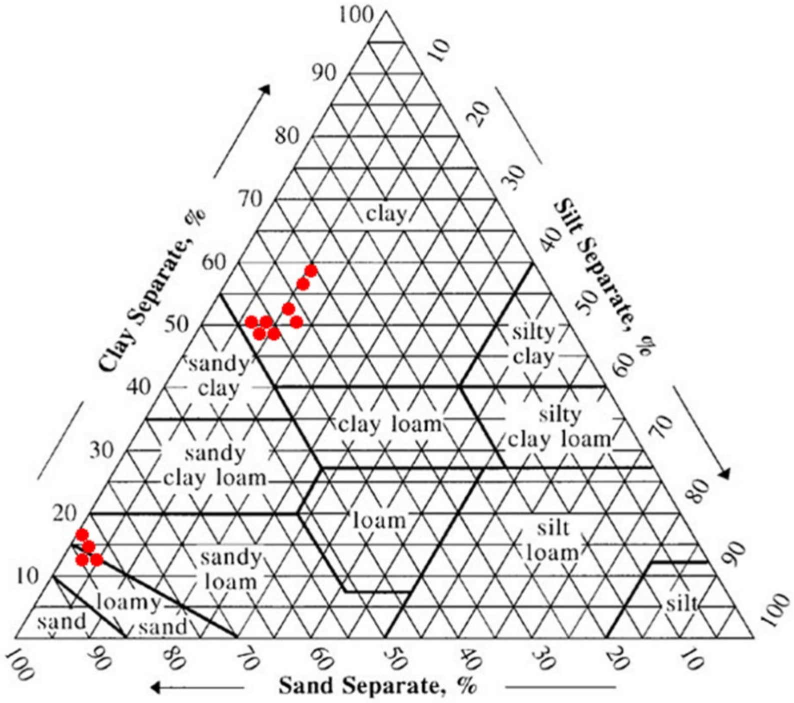


Figure 2.7: The USDA triangle for soil classification based on particle size

Farm 1 had the highest pH level of 7.93, slightly alkaline. High pH in soils is associated with high levels of calcium or calcium carbonate (Poschenrieder et al., 2018). Such soils known as calcareous soils tend to have all the other nutrients in low concentrations as they precipitate into either oxides or carbonates (Poschenrieder et al., 2018). Crops grown on calcareous soils are affected by a nutritional disorder known as lime-induced chlorosis (Sahin et al., 2017). Farm 1 and farm 3 had pH in the near-neutral and neutral range and such pH is more conducive for soil bacteria compared to acidic soil whilst alkaline soils have a less significant impact on soil microorganisms (Zhang et al., 2017).

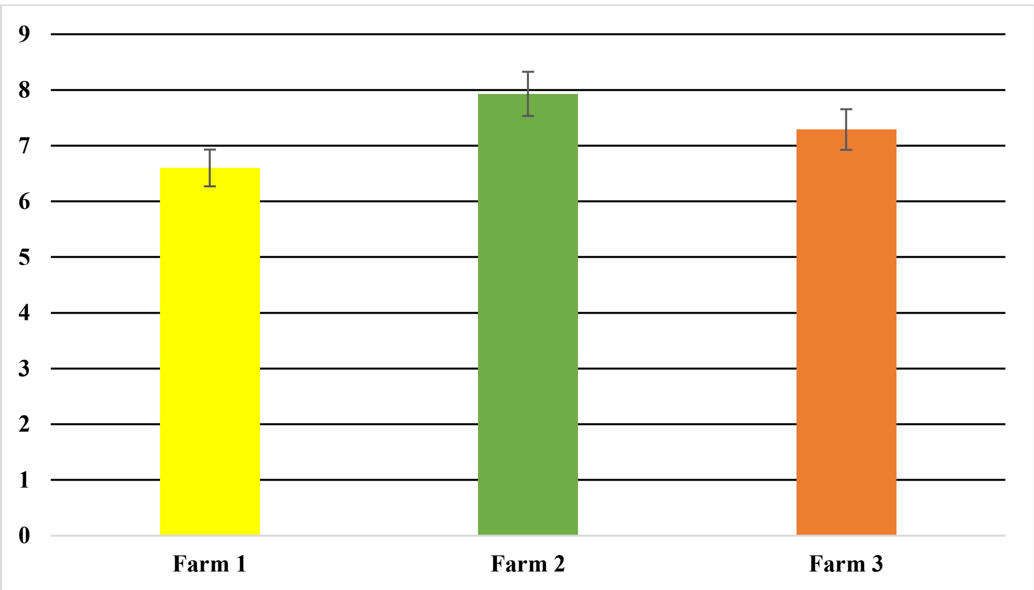


Figure 2.8: Soil pH levels of collected soil samples

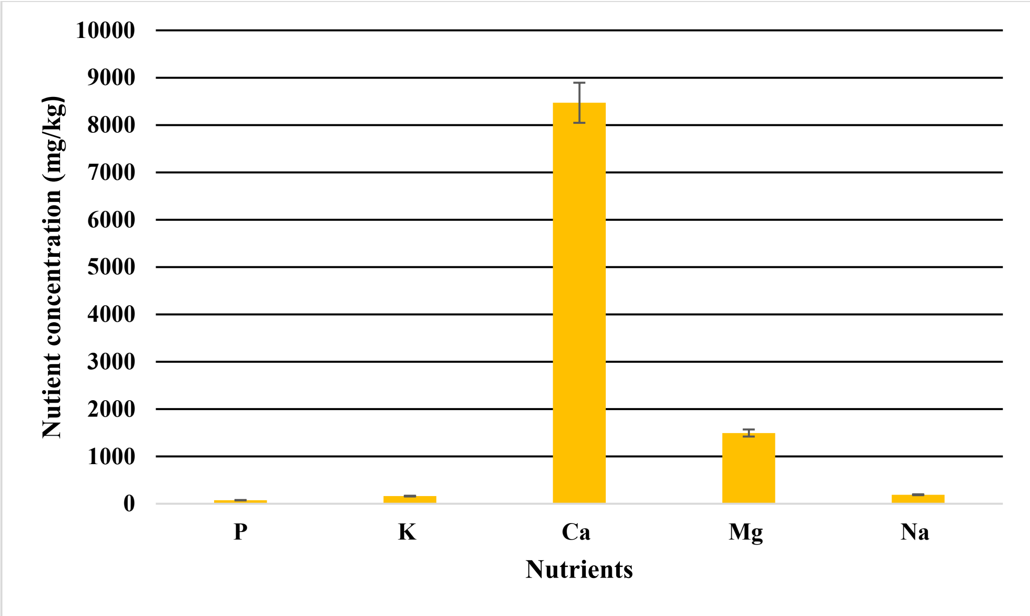


Figure 2.9: *Soil nutrient levels from Farm 1 samples*

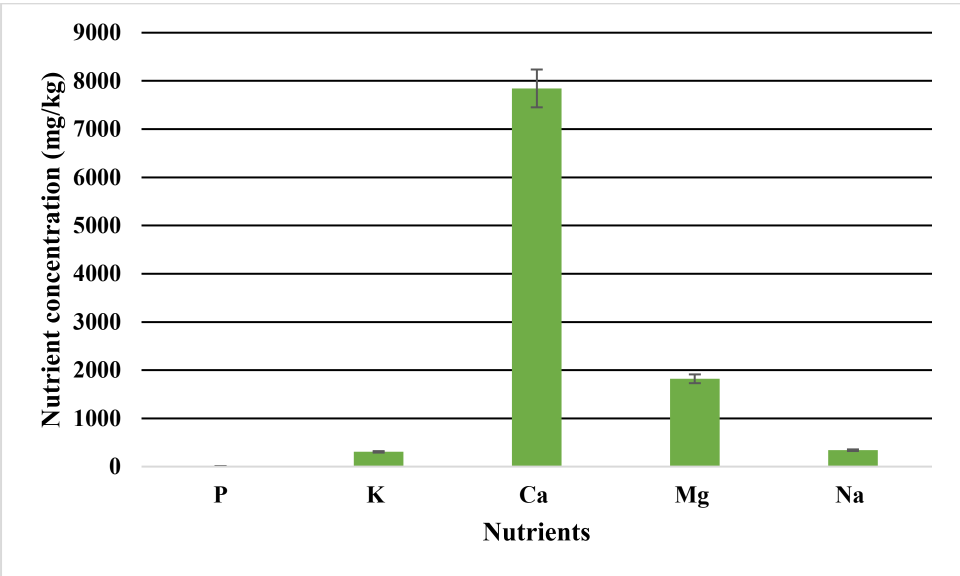


Figure 2.10: *Soil nutrient levels from Farm 2 samples*

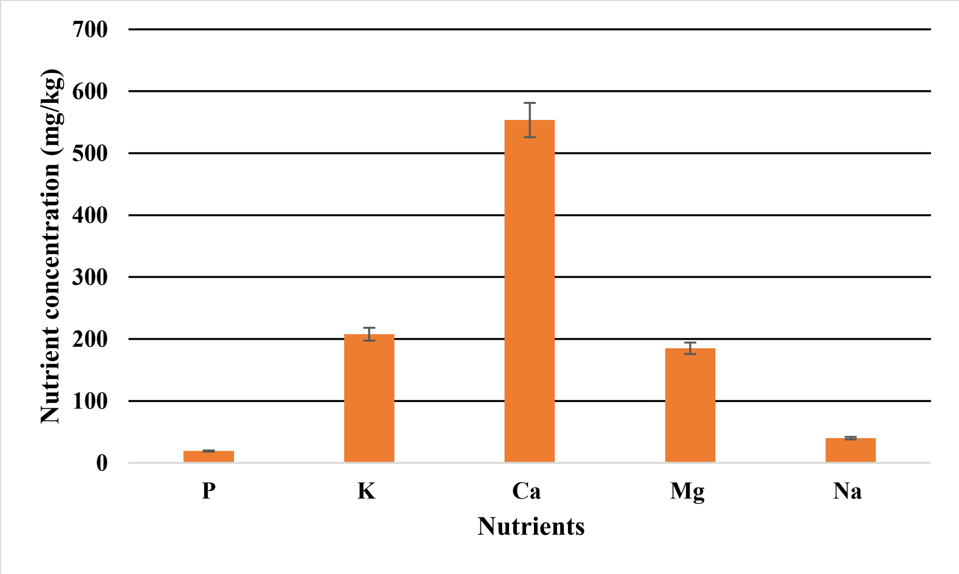


Figure 2.11: *Soil nutrient levels from Farm 3 samples*

Farm 1 and 2 (figure 2.9 and figure 2.10) had the highest nutrient contents compared to farm 3 (figure 2.11) and this can be attributed to the fact that both farms were classified to be clay. Because farm 3 was classified as sandy loam, the low levels of nutrients therefore exhibited the traits of sandy loam soils. Throughout all the soil samples there was high calcium content with farm 1 and 2 having 8470 mg/kg and 7844.28 mg/kg, respectively. Farm 3 only had 553.57 mg/kg calcium. Calcareous soils typically have high calcium and lower essential nutrients (Poschenrieder et al., 2018) such as phosphorus (P), potassium (K) and magnesium (Mg) as exhibited across all the soil samples. In coherence with the Mulder's chart, calcium proven to indeed have an antagonistic effect on magnesium (Mg), phosphorus (P) (although P stimulates Ca), and potassium (K) which explains why they are all lower than Ca (Mori et al., 2018).

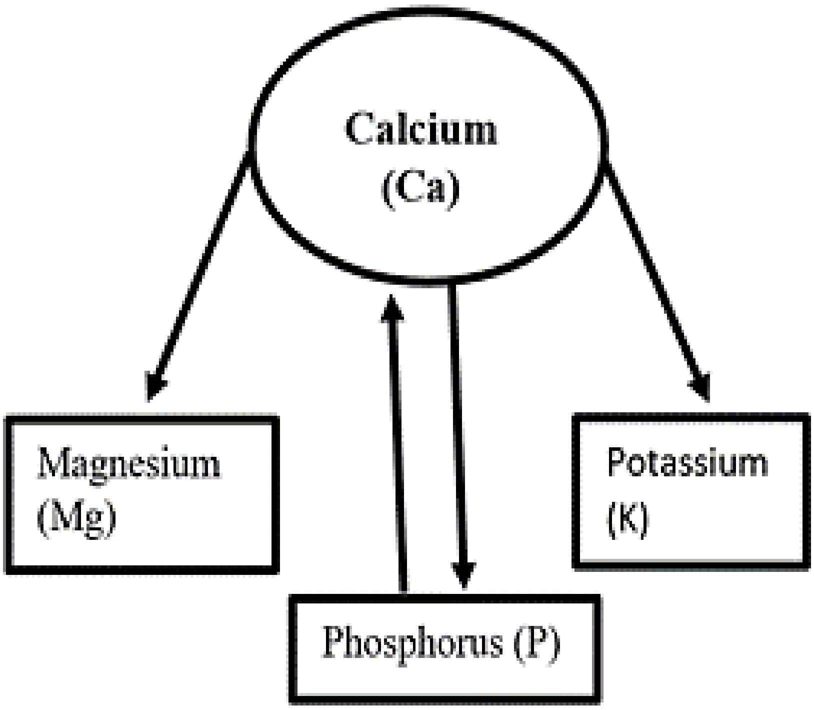


Figure 2.12*: The antagonistic and stimulative relationship between soil nutrients as exhibited in Mulder's chart*

Based on the locations of the sampling sites, the soils can be further classified as vertic for farm 1 and 2 (Brits) and calcic for farm 3 (Taung). Vertic soils are high in clay and behave like clay by swelling when wet and cracking when dry (Fey, 2010). Calcic soils are high in carbonate or gypsum and are more common in arid climates (Fey, 2010).

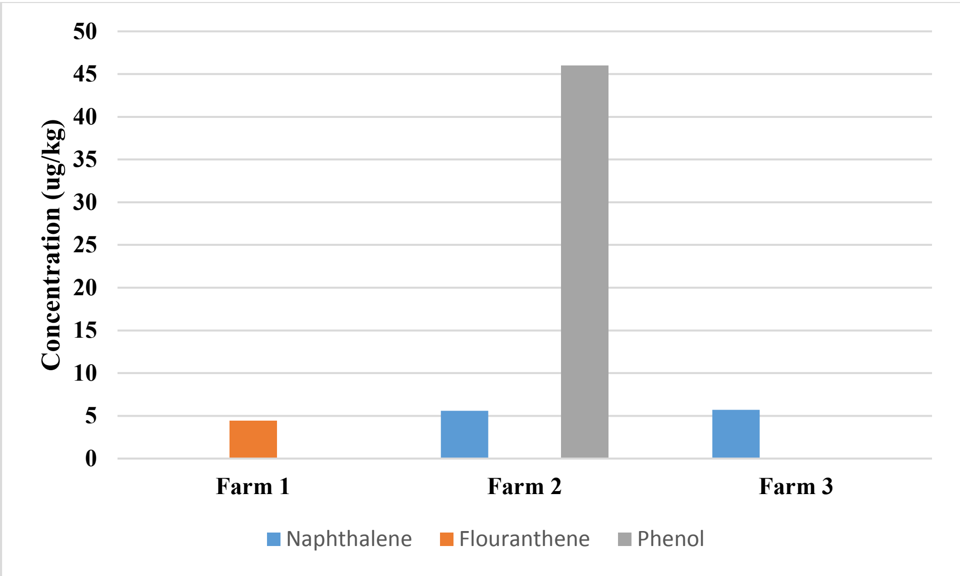


Figure 2.13: *The GC-MS results for 3 compound soil samples analysed for agricultural CECs (ug/kg)*

Table 2.3: *The experimental set up of the greenhouse experiment*

Site no.	**Treatment 1 (SW+SS)**	**Treatment 2 (SW+PS)**	**Treatment 3 (DW+SS)**	**Control (DW+PS)**	**Soil per pot**	**Seeds per pot**
Site 1	5 replicates	5 replicates	5 replicates		1 kg	4
Site 2	5 replicates	5 replicates	5 replicates	5 replicates	1 kg	4
Site 3	5 replicates	5 replicates	5 replicates		1 kg	4
Total	**15**	**15**	**15**	**5**	**Approx. 50 kg**	**200**

Figure 2.14: *The different treatments used in the greenhouse experiment*

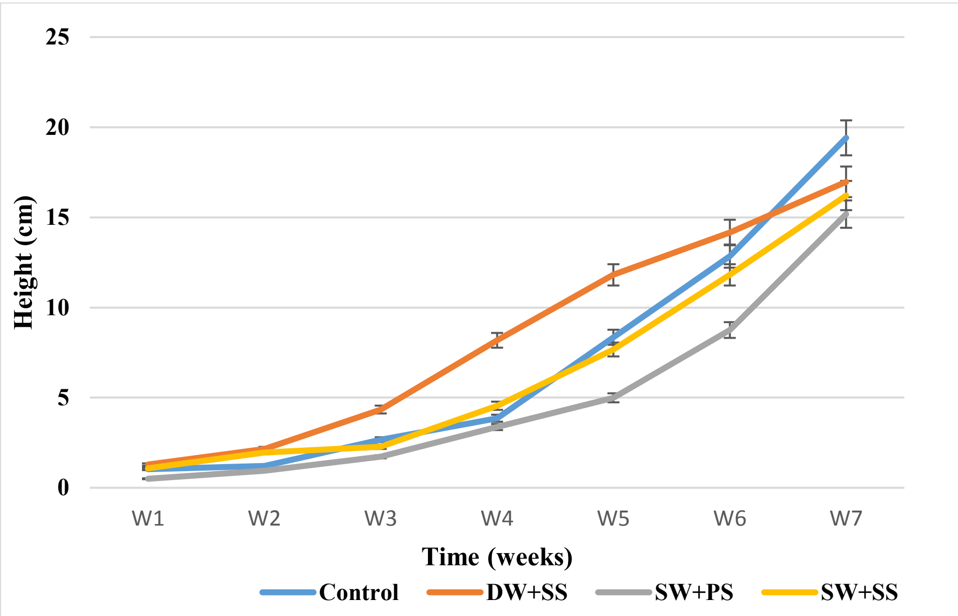


Figure 2.15: *Average heights (cm) of spinach plants over 7 weeks*

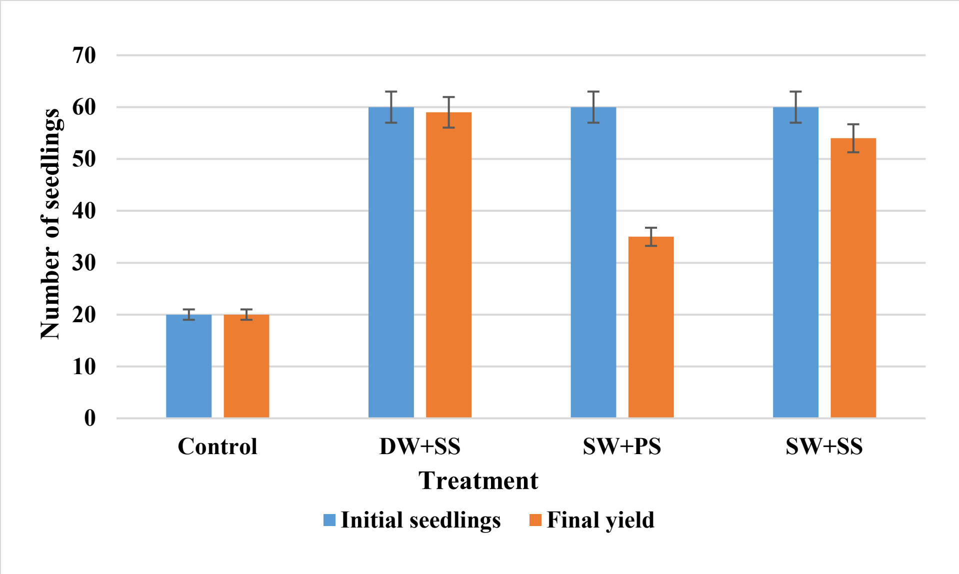


Figure 2.16: *The total number of initial seedlings planted against the final grown spinach after 7 weeks*

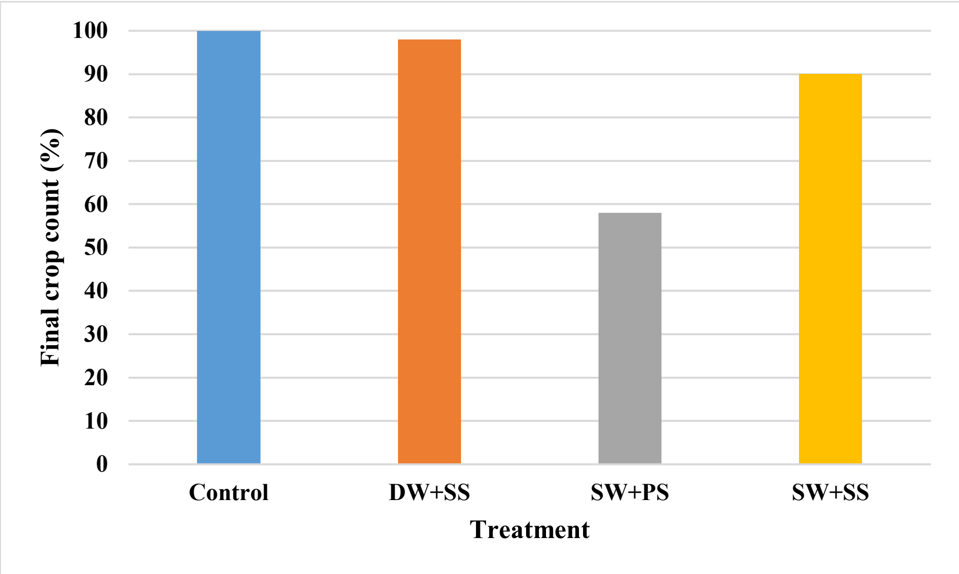


Figure 2.17: *The percentage final crop count of spinach after 7 weeks*

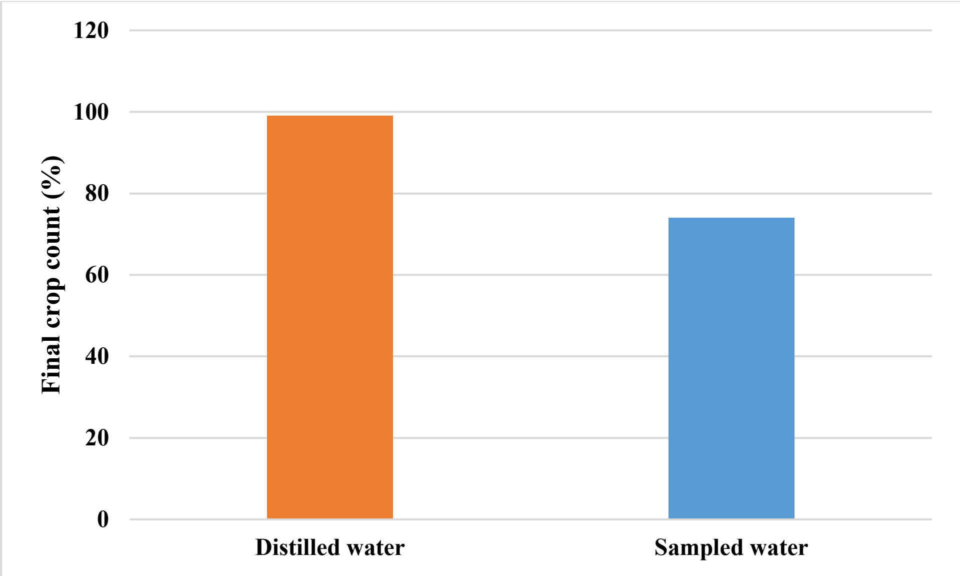


Figure 2.18: *The percentage final crop count relative to water type*

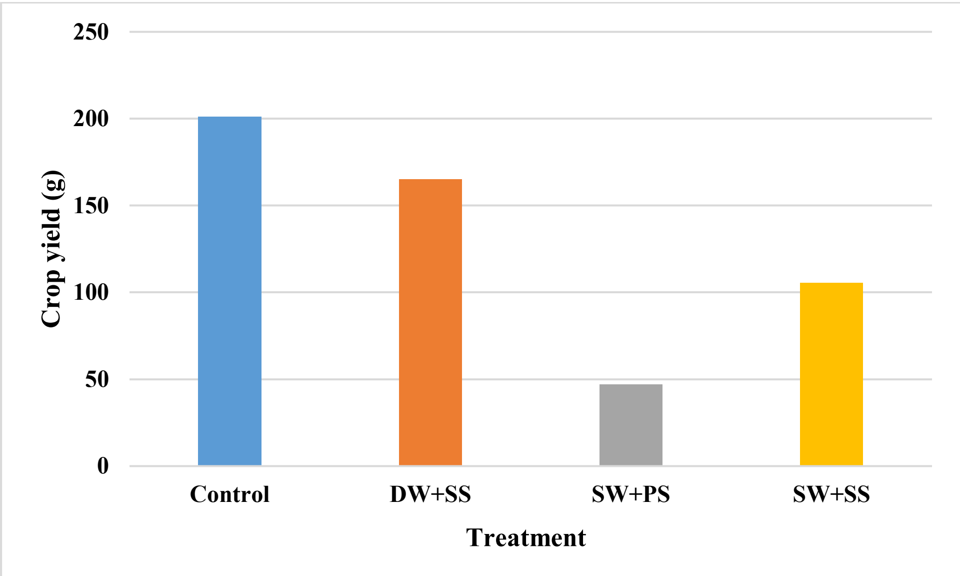


Figure 2.19: *The average crop yield (g) by each treatment*

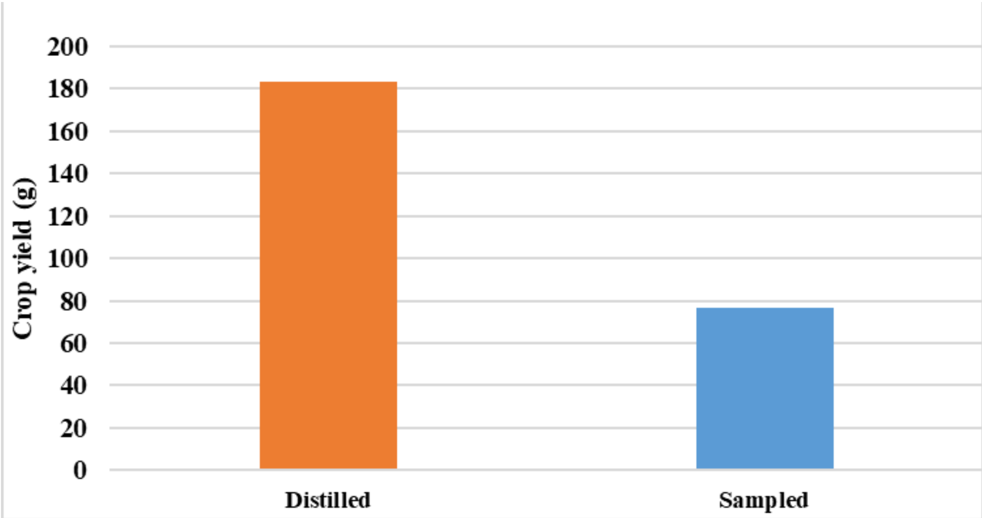


Figure 2.20: *Total crop yield (g) per water type*

2.6 PRELIMINARY CONCLUSIONS

Higher concentrations of macronutrients in the soil are typically beneficial for soil productivity and plant growth. However, when pH is too high or too low it can affect the soil functions and subsequently the availability of nutrients to the crop. These preliminary results suggest that the irrigation water quality could potentially have a negative impact on soil nutrients, and cause changes in soil microbial composition responsible for nutrient mobilisation. Results obtained from the greenhouse indicated that water quality had a potential impact and contribution towards plant growth and possibly the soil microbial communities.

2.7 RECOMMENDATIONS

- Sampling upstream or directly from the dam
- Sediment sampling
- Seasonal and spacial variations
- Wider range of CECs to be analysed
- Using different crops for the greenhouse experiment

CHAPTER 3: PROJECT 2 – Characterization, interactions and modelling of contaminants of emerging concern in surface water from selected catchment areas in South Africa

A black South African female student, Ms. Phedisho Mphahlele from Limpopo Province with an M Tech degree in Chemistry was recruited for the project. Ms Mphahlele is conducting her doctoral studies at the Department of Chemistry, Tshwane University of Technology (TUT), Pretoria under the supervision of Dr EE Bamuza-Pemu, co-supervised by Prof N Mokgalaka. Ms Mphahlele is expected to acquire training and key skills in analytical and environmental chemistry relevant to the water sector. At time of submission of this report, her study is ongoing and preliminary results are outlined below.

3.1 INTRODUCTION

Microplastics – Plastics have a wide range of application and because of their versatility, their production globally was approximated to 359 million tons in 2018 (Shen et al., 2020). Plastics are immensely useful in industries during production; they are favoured because they are inexpensive, light, easily manufactured, do not rust and are poor conductors of electricity (Tien et al., 2020). However, the downside of mass production of plastics is that they are often speedily and improperly disposed (due to their lightweight) and can be consumed in high amounts as tiny plastics or/and plastic contaminated food by both aquatic and terrestrial animals (due to their non-biodegradable nature) (Zhang et al., 2020). Tien et al. (2020) reported that between 1950 and 2015, 4900 million tons of plastic waste was disposed globally. Literature reports that in 2010, South Africa ranked 11th position on a global scale of countries with the highest mismanaged plastic waste. An estimation of 0.63 million matrix tonnes of mismanaged plastic waste per year was reported within 50 km of the coastline (Williams-Wynn et al., 2020; Jambeck et al., 2015). Notwithstanding, PlasticSA (2019) pride itself in having collected 519 400 tonnes of plastic waste in 2018 and that the plastic recycling rates in South Africa (46.3%) outperformed those in Europe (31.1%). According to Shen et al. (2020), out of 359 million tons of plastic produced in 2018, 13 million tons ended up in oceans; and they also estimated that by 2025, a total of 250 million tons of plastic will be disposed into the oceans. However, due to environmental factors such as sunlight (UV radiation), temperature variations (Weinstein et al., 2020); and anthropogenic processes (Chen et al., 2020), plastics slowly degrade into microplastics (Shen et al., 2020). Furthermore, due to disparities in environmental conditions between different regions and differences in human activities, patterns of microplastics contamination remain heterogeneous among different locations (Chen et al., 2020).

Microplastics are smaller fragments of plastics with particle sizes between 0.1 μm and 5 mm, while plastics of < 0.1 μm are referred to as nanoplastics (Shen et al., 2020). Because they are widespread and they have accumulated significantly over the past decades, microplastics have become contaminants of concern and an emerging field of study among scholars globally (Tien et al., 2020). There are intensive studies about the abundance of microplastics in oceans, lakes and rivers, however, there is limited information of microplastics in fresh waters, the source of drinking water for humans and animals (Shen et al., 2020). Zhang et al. (2020) reported that the exposure of goldfish (Carassius auratus) to microplastics in the laboratory had a negative impact on the fish health resulting in weight loss and damage to gastrointestinal system organs. Ingestion of polystyrene microplastics by zebrafish caused lipid accumulation and liver inflammation in the fish (Zhang et al., 2020).

Nanoparticles – Particles that have three dimensions all within 1-100 nm in diameter are classified as nanoparticles (Li et al., 2020; Madkour, 2020). Nanomaterials do, however, have the ability to agglomerate and can reach sizes much larger even up to the mm range. Nanoparticles are further divided as soluble/biodegradable and insoluble/bio-persistent. Biodegradable nanoparticles are regarded as less harmful because they can easily be discharged from the body, while bio-persistent nanoparticles are classified as toxic and are often simulated or engineered from metal compounds while some are carbon-based (Pacheco-Blandino et al., 2012). Due to their unique properties, engineered nanoparticles have been used as catalysts, adsorbents and antimicrobial agents in the medical field, for water purification, cosmetics, personal care products, pharmaceuticals and paints (Bundschuh et al., 2018; Saidi et al., 2017; Honda et al., 2014). This is due to their physicochemical properties such as high surface area, small particle size, high surface reactivity, charge and shape (Jiang et al., 2020; Bundschuh et al., 2018). The high surface-to-volume ratio of nanoparticles make them very reactive and catalytic species (Madkour, 2020). In addition, nanoparticles have different physical and biological properties as compared to the same bulk material with similar chemical composition and quantity (Madkour, 2020; Khurana et al., 2019). According to Pacheco-Blandino et al. (2012), there were over 300 products containing nanoparticles in the market in 2012. Taylor et al. (2020) reported that for the past two decades, the production of nanoparticles has been on the rise with the advancement of nanotechnology and nanoscience, however, nanoparticles can end up contaminating freshwater and thus affect aquatic life. For example, Xiao et al. (2020) reported that due to the emergent use of silver nanoparticles (AgNPs) in food, water purification, textile and medicine as antibacterial agent, AgNPs will surely end up in water treatment plants and other water bodies. Inasmuch as nanoparticles possess such unique physicochemical properties and complex structures, their characterization, fate and toxicity to marine and terrestrial life are not yet well understood by environmentalists (Bundschuh et al., 2018; Pacheco-Blandino et al., 2012). According to Honda et al. (2014), nanoparticles can end up in surface water through recycling, disposal and from nanoparticles applications in water purification. Water treatment plants are failing to remove or to significantly reduce the amount of nanoparticles in drinking water, thus leading to a significant amount of these particles ending up in human and animals (Taylor et al., 2020). The smaller the particle size of nanomaterials, the higher the cytotoxicity and phototoxicity effects (Taylor et al., 2020). Furthermore, nanoparticles have a potential of forming reactive oxygen species (ROS) in living organisms causing harm to their biological structure (Bundschuh et al., 2018).

3.2 PROBLEM STATEMENT AND SUBSTANTIATION

South Africa is a semi-arid country with intermittent droughts ultimately resulting in shortage of water supply within the country (Masante *et al*., 2018; Edossa *et al*., 2014). The country is transitioning rapidly into urbanization as well as experiencing rapid growth in population; however, infrastructural development is not being implemented at the same rate Archer *et al*. (2020). Like in most African countries, South Africans dwelling in rural areas still rely on water from the rivers for domestic needs. Although there are wastewater treatment plants in urban areas, the effluent thereof is still directed into the rivers thus affecting people in rural areas and informal settlement downstream who depend solely on river water for domestic purposes (Mhuka *et al*., 2020). Emerging contaminants (ECs) also called contaminants of emerging concern (CECs) are widely distributed in environment and are a threat to South African water resources (Gani et al., 2021). Contaminants of emerging concern include a broad range of natural and chemical compounds, such as pharmaceuticals, hormones, personal care products, fire retardants, coatings, surfactants, microplastics, plasticisers, insect repellents and nanomaterials amongst others (Amin *et al*., 2014; Rimayi *et al*., 2019; Rasheed *et al*., 2019).

In 2014, plastic producing industries in South Africa were reported to have employed approximately 60 000 people and there were an estimated 1 800 companies involved in the plastic supply chain. In a report by Williams-Wynn *et al*. (2020), an estimated 1.876 million tons of plastic was manufactured and/or imported into the country in 2018. The use of plastics has broadened to all sectors in South Africa (Verster *et al*., 2017). With little to absent waste management infrastructure in rural areas and informal settlements, most of the plastics used in these communities are poorly disposed and some are washed into water bodies. According to Verlicchi *et al*. (2020) although wastewater in urban areas undergo treatment, the river system in South Africa overtime will be heavily contaminated because in rural areas and informal settlements wastewater is discharged into surface water with little or no treatment (Figure 3.1).

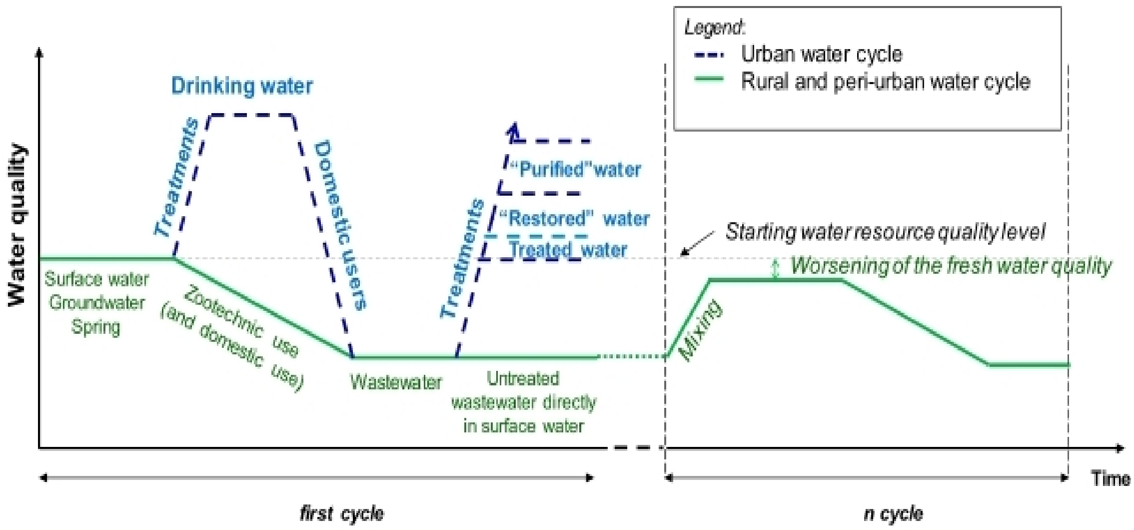


Figure 3.1: *Deterioration of fresh water quality over time in the presence of no treatment of wastewater produced by anthropic activities (Verlicchi* et al.*, 2020)*

The introduction of nanoparticles to the environment does not mainly occur during production period but rather during application/utilization and disposal stages. Nanoparticles that are used in cosmetic industries are found in wastewater in high concentrations (Bundschuh *et al*., 2018). According to

Khurana *et al*. (2019) wastewater from industries are contaminated with huge concentrations of nanomaterials and other hazardous matter and there are no stern comprehensive regulations in place focusing on nanoproducts and industries producing them. Bumbudsanpharoke *et al*. (2015) reported the same statement regarding to South African food and agriculture sectors. Khurana *et al*. (2019) further explained that nanoparticles contaminated wastewater gets discharged into wastewater treatment plants from industries and households. The under-treated wastewater effluent in South Africa (Verlicchi *et al*., 2020), as well as run-offs containing nanomaterials due to improper disposal during rainy seasons could form part of inlet water to water purification plants. That is a pathway that nanoparticles are introduced into organisms' system.

The fate of these emerging contaminants, microplastics and nanoparticles, is still not yet well understood by scientists and there are still gaps (Wirnkor *et al*., 2019; Bundschuh *et al*., 2018). This study will focus on identifying, characterizing and quantifying microplastics and nanoparticles in selected South African water systems. Outputs from this research will give insight to researchers as well as South Africa's water quality regulators concerning the degree to which surface water sources are affected by these CECs. Such information will guide wastewater treatments plant managers and policy makers into formulating new methods for water quality preservation and thus preventing potential health risks associated with the use of contaminated water.

3.3 RESEARCH AIMS AND OBJECTIVES

The aim of this study is to characterize and quantify the amount of microplastics and nanoparticles in water bodies and to use toxicity models to determine their fate.

Objectives:

1. Develop and optimize a method to extract microplastics and nanoparticles from water samples;
2. Obtain profiles of semi-volatile organic compounds in water samples using gas chromatography;
3. Characterize and quantify extracted microplastics and nanoparticles from water using microscopy and spectroscopy;
4. Model the interaction and impact of microplastics and semi-volatile compounds contaminants on the size, morphology, surface chemistry and toxicity of nanoparticles in simulated water;
5. Assess the toxicity risks posed by microplastics and nanoparticles contaminated waters by investigating the effects of exposure towards the development of zebrafish embryos; and to
6. Develop a microplastics and nanoparticles hazard classification system for freshwater bodies using zebrafish embryotoxic responses.

3.3.1 HYPOTHESIS

South African freshwater contains a significant amount of microplastics and nanoparticles; and the fish embryo test (FET) using the Zebrafish model can be used to assess the toxicity of these contaminants of emerging concern.

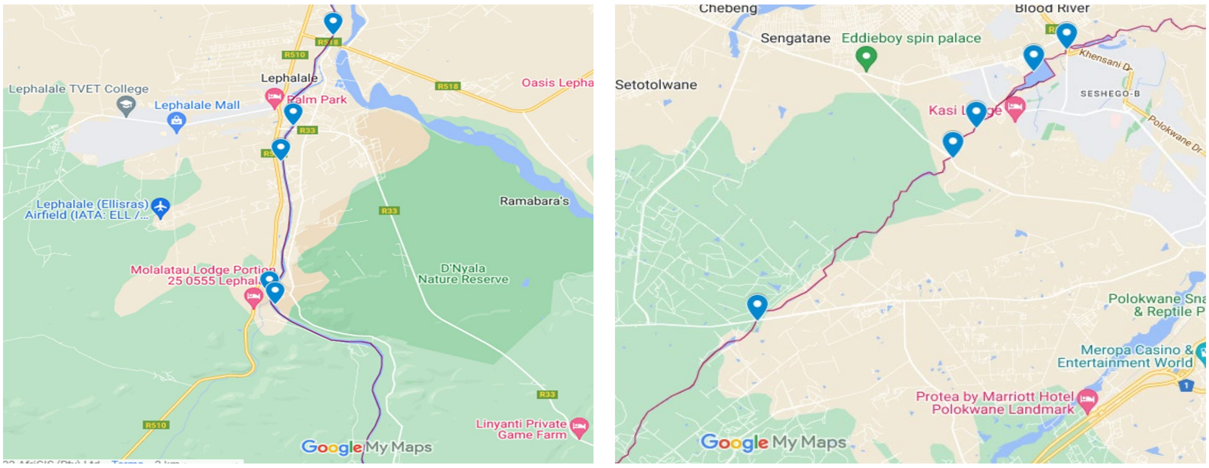
3.4 METHOD OF INVESTIGATION

Sampling sites – Four contaminated rivers were selected from two provinces in South Africa, namely: Gauteng Province (Figure 3.2a: Hennops River and Blesbokspruit catchment) and Limpopo Province (Figure 3.2b: Mokolo River and Blood River).

Hennops River

Blesbokspruit Catchment

Figure 3.2a: *Maps of the sampling sites in Gauteng Province*



Mokolo River

Blood River

Figure 3.2b: *Maps of sampling sites in Limpopo Province*

Figure 3.3: *Images of sampling sites at the Hennops river during the dry season*

Sampling – Water samples were collected in triplicate in 1 L amber bottles for both SVOCs and nanoparticles assays at all five sampling locations in each river studied. Immediately after sampling, the sampling bottles were placed in cooler boxes containing ice. The sampling process happened in one day and on the same day, the samples were transported to the laboratory and were stored in a refrigerator at 4 °C. At the sampling points, pH, dissolved oxygen, turbidity and temperature of the river water were measured using portable meters.

Microplastics extraction and total suspended solids determination – A stacked sieve method described by Masura *et al*. (2016) was used for collection of microplastics in a slurry. Four sieves of different sizes (4750, 600, 45 and 5 μm) were used to filter 90 L of river water. After the filtration, the slurry was transferred into clean 750 mL Consol glass bottles with metal lids. The sample bottles were also placed in the cooler box with ice. Upon arrival at the lab, sample bottles were placed in the oven at 50°C for five days until the slurry was completely dry. Total suspended solids (TSS) were measured at the range 4.75 mm ≤ TSS ≥ 5 μm.

After drying, 35% aqueous H_2O_2 was added to the dried solids and kept at 35˚C for 48 h to digest organic matter to obtain isolated microplastics. Microplastics were collected using a 0.47 μm membrane filter.

SVOCs and nanoparticles water sample filtration – Water samples were placed on a platform shaker at 150 rpm for 2 h before filtration. Initially, the water samples were filtered using the 2.5-5 μm filter paper followed by filtering using 0.22 μm nylon membrane filter papers.

SVOCs extraction – Solid-phase extraction method was used to for this assay following the protocol described by Li *et al*. (2018) and US EPA 525 (1995). C18 cartridges fused with 500 mg silica were used. Cartridges were sequentially conditioned with 6 mL dichloromethane, 6 mL ethyl acetate, 6 mL (1:1 of v/v) dichloromethane and ethyl acetate, 6 mL methanol and 6 mL Milli-pore water. Flow rate was adjusted to 10 mL/min using a vacuum pump. A 1 L aliquot of filtered water sample was passed through the cartridge. SVOCs were eluted using 4 mL each of the same solvents used for conditioning the cartridge. The samples were evaporated using nitrogen gas to dryness. Samples are kept at 4 ˚C until the time of analysis.

Nanoparticles extraction – The jar method was used for obtaining nanoparticles colloids (Honda *et al*., 2014). After the filtration of sampled water, 1 L of filtered water was measured and poured into 1 L amber bottles. Thereafter, 5.00 mL of each coagulant [$FeCl_3$, and $Al_2(SO_4)_3$] was pipetted into the filtered water and the bottles were placed on a platform shaker for 5 min at 300 rpm. Flocculation process was performed on a platform shaker at 50 rpm for 1 h. Thereafter, a sedimentation step was implemented, wherein the water sample was left to rest overnight. The resulting sample was transferred into prior weighed centrifuge tubes and then centrifuged at 6000 rpm for 10 min. The collected nanoparticles were washed with de-ionized water 3 times and rinsed with ethanol 2 times, then dried in the oven at 45˚C, weighed and stored at 2-4˚C, away from the sunlight until further analysis.

3.5 PRELIMINARY RESULTS AND DISCUSSION

Physical-chemical properties of river water

According to South African Water Quality Guidelines (SAWQG), domestic water should have the pH levels of between 7-9, turbidity readings of 0-1 NTU and electrical conductivity range of 0-70 μS/cm. The pH levels (Table 3.1) obtained at Hennops River both in winter and summer seasons were within SAWQG specification, however winter pH levels were slightly higher than summer pH levels. This is because of the dilution of water due to increased rainfalls in summer compared to winter season (Xu et al., 2019). The dilution of water is also observed with the turbidity difference between the two seasons (Table 3.1), wherein, summer turbidity readings were lower than winter turbidity readings. However, Liu *et al*. (2023) reported the contrary results stating that increased rainfall results in increased turmoil in water causing the turbidity of water to increase. Electrical conductivity measurements observed at Hennops River (Table 3.1) were much higher (with the maximum reading of 869 μS/cm) than the maximum recommended electrical conductivity of 70 μS/cm in domestic water. Sun *et al*. (2023) reported that increased electrical conductivity measurements are a result of the inflow of industrial effluent, sewage and other human activities. Oxygen saturation percentages at Hennops River (Table 3.1) in summer were lower compared to winter percentages. Xu et al. (2019) reported that urban pollutants can cause a drop in dissolved oxygen because of microbiological activities. They further explained that summer samples tend to have lower oxygen saturation percentages because temperature affects oxygen saturation percentages in water, lower temperature results in higher oxygen saturation percentage.

Table 3.1: *Physico-chemical properties of sample water from Hennops River in two different seasons (winter and summer)*

Hennops River	Winter sampling (18/08/2021)				Summer sampling (25/02/2021)				
	% Oxygen saturation	pH	Turbidity (NTU)	Temperature (°C)	Electrical conductivity (µS/cm)	% Oxygen saturation	pH	Turbidity (NTU)	Temperature (°C)
Point 1	30.8	7.87	111	16.2	658	13.2	7.49	24.00	23.3
Point2	32.1	7.65	103	19.2	647	11.2	7.48	17.76	24.4
Point 3	20.2	7.95	43.6	17.6	670	13.1	7.53	13.47	24.3
Point 4	60.2	8.10	213	18.1	700	12.4	7.51	19.02	24.9
Point 5	50.5	8.06	93.5	17.5	869	4.20	7.47	21.00	26.1

The pH levels at Blesbokspruit catchment (Table 3.2) in winter seasons were within the SAWQG specification and lower than summer pH levels which were higher (maximum of 9.84) than the maximum recommended SAWQG specification. These results were contrary to the reported findings (Xu et al., 2019; Romanescu *et al*., 2014) wherein summer pH levels were generally lower than winter pH levels in water. The turbidity readings in summer (Table 3.2) were higher than the turbidity readings in winter, with sampling point 5 having the highest turbidity reading of 128 NTU. Although the turbidity readings obtained at Blesbokspruit catchment for both seasons were higher than SAWQG specification, their trend was incoherent with Liu *et al*. (2023) report. There was no major difference in electrical conductivity measurements observed at Blesbokspruit catchment (Table 3.2) between the two seasons, nonetheless, all the electrical conductivity measurements were higher than SAWQG specification.

Table 3.2: *Physico-chemical properties of sample water from Blesbokspruit catchment in two different seasons (winter and summer)*

Blesbokspruit Catchment	Winter sampling (01/07/2022)				Summer sampling (01/12/2022)			
	Electrical conductivity (µS/cm)	pH	Turbidity (NTU)	Temperature (°C)	Electrical conductivity (µS/cm)	pH	Turbidity (NTU)	Temperature (°C)
Point 1	365.6	6.98	3.42	9.7	385.0	9.69	9.97	22.4
Point 2	401.0	7.03	3.40	9.1	496.0	9.84	9.55	23.4
Point 3	504.0	7.90	1.48	8.7	527.0	9.71	12.0	24.0

Blesbokspruit Catchment	Winter sampling (01/07/2022)				Summer sampling (01/12/2022)			
	Electrical conductivity (µS/cm)	pH	Turbidity (NTU)	Temperature (°C)	Electrical conductivity (µS/cm)	pH	Turbidity (NTU)	Temperature (°C)
Point 4	475.3	7.82	14.28	7.2	380.0	9.49	42.9	25.0
Point 5	364.3	7.62	28.50	12.0	359.0	9.43	128	25.5

Although the summer pH levels at Mokolo River (Table 3.3) were slightly higher than winter pH levels, all the pH levels obtained in this river were within the SAWQG specification. There was no clear trend in turbidity readings between summer and winter samples, moreover, the readings from both seasons were higher than SAWQG specification. Summer samples in Mokolo River (Table 3.3) had the electrical conductivity measurements that are within SAWQG specification at all sampling points and winter samples had 3/5 sampling points that had the electrical conductivity measurements that are within SAWQG specification. In general, winter samples had slightly higher electrical conductivity measurements as compared to summer samples.

Table 3.3: *Physico-chemical properties of sample water from Mokolo River in two different seasons (winter and summer)*

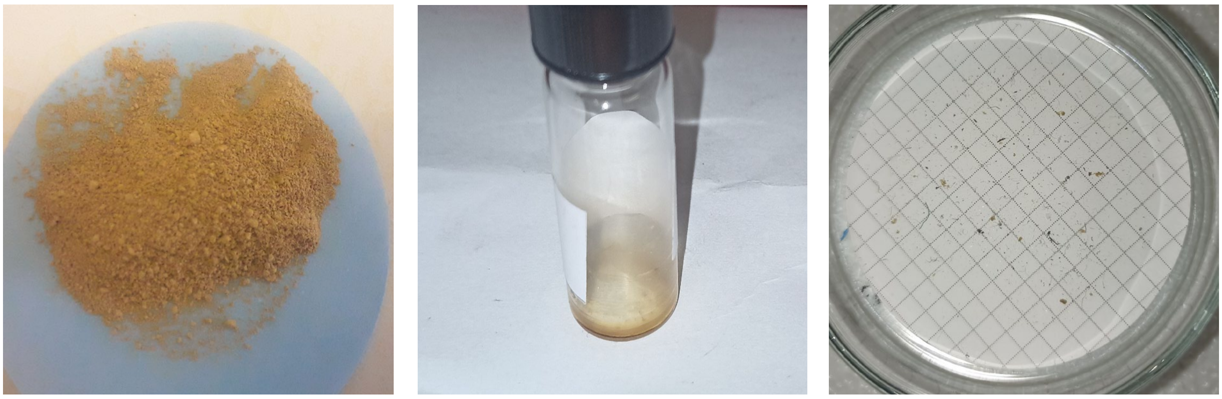
Mokolo River	Winter sampling (26/08/2022)				Summer sampling (12/12/2022)			
	Electrical conductivity (µS/cm)	pH	Turbidity (NTU)	Temperature (°C)	Electrical conductivity (µS/cm)	pH	Turbidity (NTU)	Temperature (°C)
Point 1	78.8	7.64	4.18	16.9	54.8	6.71	2.64	25.4
Point 2	67.3	6.23	5.74	17.9	54.0	7.30	7.74	26.2
Point 3	94.2	6.64	10.59	19.6	53.8	7.44	6.61	26.1
Point 4	52.6	6.54	6.77	19.3	59.4	6.63	7.07	26.8
Point 5	58.9	6.42	4.24	18.2	53.7	6.86	3.67	25.6

Summer samples in Blood River (Table 3.4) at all sampling points had the pH levels that were within SAWQG specification, while 4/5 sampling points in winter season had the pH levels that were within SAWQG specification. There was no clear trend in pH level difference between summer samples and winter samples. On average, the turbidity readings in summer were higher than the turbidity readings in winter. These results are incoherent with Liu *et al*. (2023) findings. However, the turbidity readings for both seasons were higher than SAWQG specification. On average, electrical conductivity measurements at Blood River in winter season was higher in comparison to electrical conductivity measurements obtained in summer season. Nonetheless, the electrical conductivity measurements obtained in both seasons were higher than SAWQG specification.

***Table 3.4:** Physico-chemical properties of sample water from Blood River in two different seasons (winter and summer)*

Blood River	Winter sampling (27/08/2022)				Summer sampling (13/12/2022)			
	Electrical conductivity (µS/cm)	pH	Turbidity (NTU)	Temperature (°C)	Electrical conductivity (µS/cm)	pH	Turbidity (NTU)	Temperature (°C)
Point 1	398	7.25	86.9	12.2	200.8	7.71	104	23.3
Point 2	343	6.67	17.25	11.1	209.1	7.62	71.5	23.5
Point 3	541	8.05	71.2	16.3	223	7.76	103	26.6
Point 4	322	8.21	38.1	16.5	289	7.75	22	28.3
Point 5	297	9.12	0.38	18.04	301	7.98	4.42	28.9

Characterisation of nanoparticles, SBOVs and microplastics extracted from Hennops river water samples



Suspended solids SVOCs Microplastics

***Figure 3.4:** An illustration of successfully extracted samples from different assays*

Nanoparticles

In summer seasons, river water gets diluted from rainfalls and this leads to reduced contaminants in water (Xu et al., 2019) Again, due to lower temperatures in winter, microbial activity usually decreases resulting in little to no degradation of matter in water (Zaidi, 2008). These reports are incoherent with the nanoparticles obtained in this study (Figure 3.5) wherein winter samples had higher amounts of nanoparticles as compared to summer samples. The nanoparticle results were also in agreement with turbidity readings at Hennops River (Table 3.1) wherein winter turbidity readings were higher than summer readings. It was also observed that sampling Point 2, which is adjacent to an informal settlement had the highest amount of nanoparticles in winter. The reason for this occurrence could be due to little to no waste management plan in place around such areas.

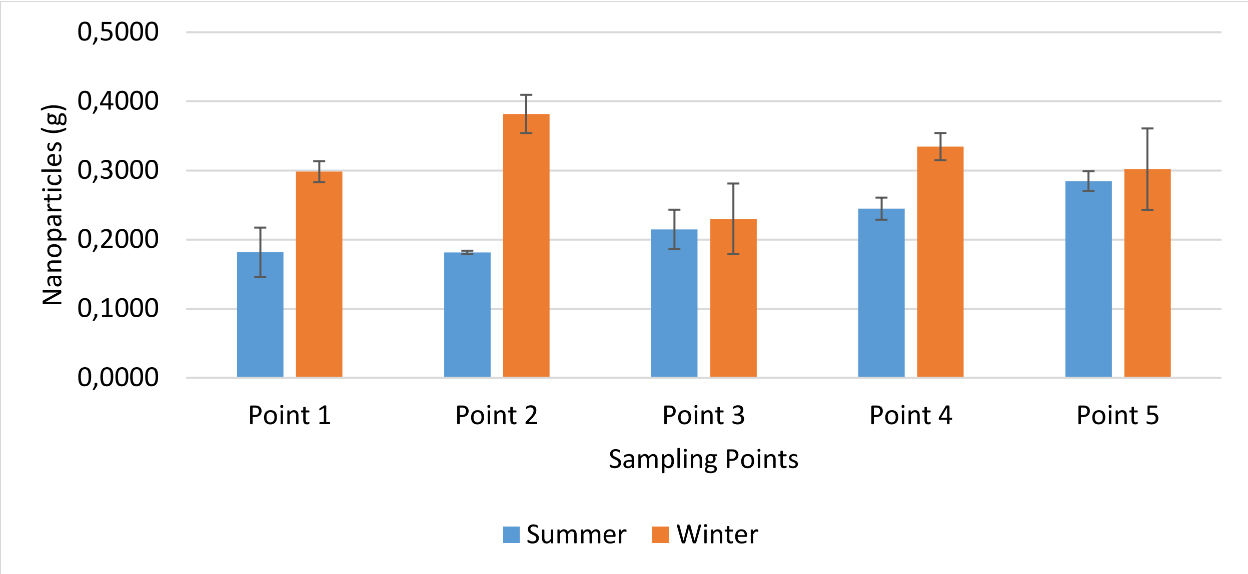


Figure 3.5: *Comparison of nanoparticles masses extracted from Hennops River water in two different seasons (winter and summer)*

Microplastics

Microplastics were successfully extracted from river water as illustrated in Figure 3.6a & 3.6b.

600 µm sieve | 45 µm Sieve | 5 µm sieve

Dry season samples

600 µm sieve | 45 µm Sieve | 5 µm sieve

Figure 3.6a: *Microplastics after extraction*

Figure 3.6b: Visualization of microplastics under FTIR imager

FTIR spectra of the isolated microplastics is shown below in Figure 3.7. Using literature, microplastic B was identified as polystyrene. When comparing the two spectra (A and B), it was observed that they have similar stretches and peaks at the same frequency. Thus, microplastic A was identified as one of polystyrene derivatives. A spectral library will be used to confirm these results.

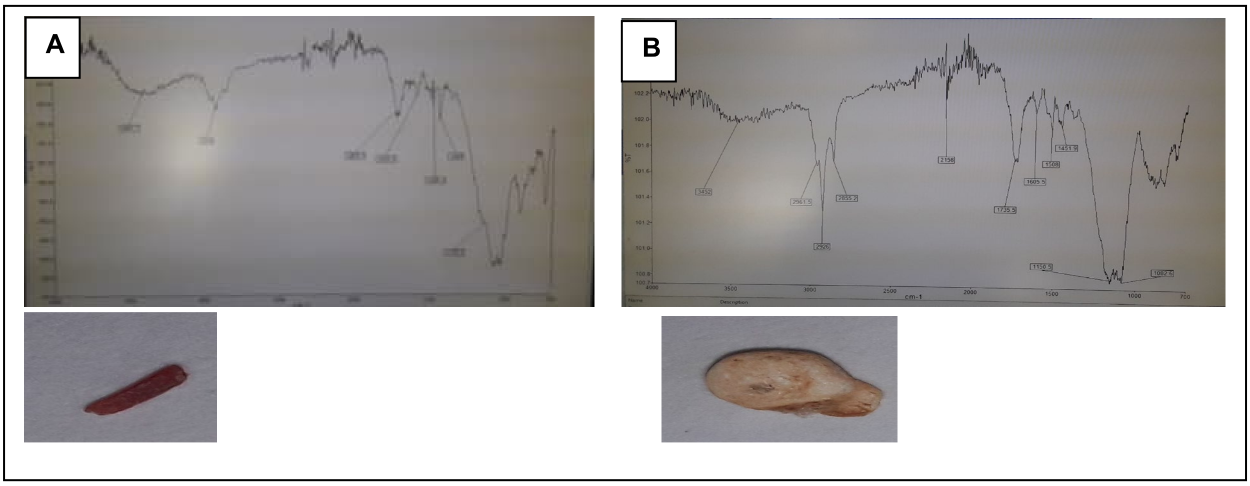


Figure 3.7: *FTIR spectra of microplastics extracted from Hennops River using 600 µm sieve*

3.6 PRELIMINARY CONCLUSIONS

The preliminary results indicate that the water quality of all the rivers studied, with the exception of Mokolo River, was poor. The pH levels and electrical conductivity measurements for both winter and summer seasons at Mokolo River were within SAWQG specifications with turbidity readings being slightly higher than SAWQG specification. The pH levels during summer season at Blesbokspruit catchment were higher than all the pH levels that were obtained in this study during all seasons with the median of 9.69. Electrical conductivity and the plastic congestion at Hennops River were more than at all river sites sampled. It was also noted that quality of the water of Mokolo River, which does not pass through informal settlements and has few industries around was better in comparison to rivers that pass through urban areas (Hennops River, Blood River and Blesbokspruit River). After extraction and optimisation of methods of analysis the presence of SVOCs, microplastics, nanoparticles and suspended solids was detected in all the river water samples. Based on physico-chemical properties and mass of suspended solids, it can be concluded that water contamination of the surface waters studied differed with seasonal variations and location.

3.7 RECOMMENDATIONS

A case study should be carried out in the areas adjacent to the rivers to identify the sources of different contaminants in water. Minimal waste management plans in informal settlements adjacent to the river decreases the quality of the river water, thus it is recommended that the responsible authorities be informed about this arising issue. It is also recommended that the study in the selected rivers be extended over several years to monitor the levels of pollutants and behaviour of the pollutants over time. Water quality, fauna and flora might be impacted by interactions of pollutants due to climate change.

CHAPTER 4: *PROJECT 3* – Computer science internship

A black South African male, Mr Akani Mushwana, an intern (computer science/IT) was recruited for the project. Mr Mushwana worked closely with the IT team on the project and acquired skills for setting up and maintenance of websites and all related activities. This contributed to human capital in the fast growing IT/virtual industry in South Africa.

Progress and training

Mr Mushwana worked on the Knowledge Hub website where he was assigned to create the database of the website. Mr Mushwana installed XAMPP and Notepad++ as the programs he needed to create the database for the Knowledge Hub. As he needed to create the database tables and forms, he first created a connection to the server in order for the site to locate the database stored. The forms created will be used to store the information to the database from where it can be retrieved for later viewing or use.

Skills Developed

- Using MYSQL WorkBench and XAMPP:
- Create the Knowledge Hub Database (KH DB)
- Create the tables needed for the KH DB
- Create the connection to the MySQL server from PHP
- Also, installing / setting up all relevant additional software needed for the connection

Using PHP

- Developing user friendly forms to capture data supplied by the user
- Research / develop procedures to import / store user supplied info to the relevant tables
- Research / develop procedures to export / view user supplied info from the relevant tables to a web-based form
- How to fix errors / debugging of code by running it step by step

Other skills developed

- Setting up the server to act as a host server.
- Exposure to APTANA Studio. (IDE used for development)

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