INVESTIGATING THE EFFECTIVENESS OF A SIMPLE WATER-PURIFYING GADGET MADE OF *MORINGA OLEIFERA* SEEDS AS THE ACTIVE BEADS

Dineo Gladys Raphasha
219098838

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In
Ecological Science

Supervisor: Dr Z. Tsvuura
Co-supervisors: Dr A.R. Ndhlala
Dr D. Mbendana

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School of Life Sciences

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I, Dineo G. Raphasha student number: 219098838 declare that:

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We hereby declare that we acted as Supervisors of this MSc student:

Student full name: Dineo Gladys Raphasha

Student number: 219098838

Thesis title: Investigating the effectiveness of a simple water-purifying gadget made of *Moringa oleifera* seeds as the active beads.

Regular consultation took place between the student and ourselves throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the College of Agriculture, Engineering and Science Higher Degrees Office for examination by the University appointed examiners.

[redacted]

Supervisor: Dr Z. Tsvuura

[redacted]

Co-Supervisor: Dr A.R. Ndhlala

Co-Supervisor: Dr D. Mbendana

Declaration –Publications

DETAILS OF CONTRIBUTION TO PUBLICATION that form part of and/or include research presented in this thesis (include publications in preparation, submitted, in press and published and give details of the contributions of each author to the experimental work and writing of each publication).

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Abstract

Developing countries including South Africa are facing serious clean water challenges resulting in mortality and morbidity among children and adults. Shortage of clean water is mainly caused by faults in local water purifying systems which increases the risks of bacterial contamination. Moreover, the elevated price of purified water forces many rural communities to rely on easily accessible water sources of poor quality that usually expose them to waterborne diseases. Local indigenous plants may have active natural ingredients that can be used to purify contaminated water. The study was aimed at investigating the effectiveness of a simple water purification gadget made of *Moringa oleifera* Lam. seeds as the active beads. This was achieved by collecting water samples during winter and summer from three sites (Tierpoort, Mamelodi and Moretele) along the Pienaars River in North West and Gauteng Provinces of South Africa. Seeds of *M. oleifera* were obtained from Lefakong Farming (Pty) Ltd in Bosplaas (North West) and ground to three particle sizes (710 µm, 1000 µm and 2000 µm). The seed particles (20 g each) were fixed into a water filtering prototype containing 10 g of activated charcoal, sandwiching the *M. oleifera* particle seeds with 10 mm depth cotton wool on top. The experiment was done in triplicate and 500 ml each of river water samples were instantly filtered upon collection and analysed for amounts of microbiological determinants (total coliforms, *Escherichia coli*, *Salmonella* spp. and *Shigella* spp.) using the viable plate count method. The unfiltered samples were used as the controls. The physical, aesthetic, operational and chemical determinants of the filtered and unfiltered (control) water were also determined using inductively coupled plasma optical emission spectrometry for metal elements, anions using ion chromatography (IC), a pH meter and other standard methods. The data were compared among the sites using analysis of variance, and when significant, the means were separated using the Least Significant Difference. The physicochemical properties of water during winter and summer varied at different points, but the values did not exceed the limits set by the SANS 241:2015 standards for drinking water. There were notable differences ($p \leq 0.05$) in amounts of all the microbiological determinants across the collection points with samples from Moretele sampling point which is downstream, having the highest (17) colony forming units (CFU) of *E. coli* in summer while the least was detected in Mamelodi sampling point (7 CFU). However, a different trend was observed for winter results which were characterised by highest levels of *E. coli* in the Mamelodi samples (10 CFU in the control) and no CFU in the Moretele and Tierpoort samples. Total coliforms, *Salmonella* spp. and *Shigella* spp. (7.0×10^4, 3.9×10^4 and

3.2×10^4 CFU, respectively) were high in Mamelodi samples during summer. The winter results also exhibited higher levels of total coliforms, *Salmonella* spp., and *Shigella* spp. in the Mamelodi samples compared to the other two sites, this was the same trend for *Salmonella* spp. and *Shigella* spp. The number of CFU in the filtered samples and the controls remained higher than those recommended in the guidelines of the World Health Organisation and South African National Accreditation System, which requires zero CFU in drinking water. Control samples had higher level of microbial contaminants than the filtered samples, this reveals that the 20 g particles of *M. oleifera* may need to be revised by adding more to increase the filtering ability. Regardless of the quantity of microbial colonies still present in the filtered water, the study shows that *M. oleifera* seeds can be incorporated into the gadget for water purification with respect to reduction of microbial load.

Table of Contents

List of Figures

List of Table

List of Abbreviations

AC-Activated Charcoal

ANOVA-Analysis of Variance

AOAC-Association of official Analytical Chemists

C- Cotton

CDC- Centres for Disease Control Prevention

CFU- Colony Forming Units

CSIR-Council for Scientific and Industrial Research

DEA -Department of Environmental Affairs

DMRT-Duncan's Multiple Range Test

DWA- Department of Water Affairs

DWAF- Department of Water Affairs and Forestry

DWS-Department of Water and Sanitation

E.coli- *Escherichia coli*

EPA-Environmental Protection Agency

FAO-Food Agriculture Organization

GLAAS - Global Analysis and Assessment of Sanitation and Drinking-Water

ICP-OES-Inductively Coupled Plasma Optical Emission Spectrometric

ISTA-International Seed Testing Association

LSD- Least Significant Difference

M. oleifera-*Moringa oleifera*

MOCP-*Moringa oleifera* cationic protein

MSG-*Moringa* Seed Granules.

NGOs - Non-Governmental Organizations

NRF - National Research Foundation

OWQI-Oregon Water Quality Index

Pty Ltd-Proprietary Limited

Qi- Quality Index

SA - South Africa

STATS SA-Statistics South Africa

SANS-South African National Standards

SDGs - Sustainable Development Goals

Spp- Species

SPSS-Statistical Package for the Social Sciences

TC-Total Coliform

UN - United Nations

UP- University of Pretoria,

WAWQI-Weighted Arithmetic Water Quality Index

WHO-World Health Organization

Wi-Weighting factor

WMA-Water Management Area

WQI-Water Quality Index

WRC - Water Research Commission

List of Units & Symbols

B Boron

Ca Calcium

Cl Chloride

CFU Colony forming unit

EC Electrical conductivity

Ml Millilitre

mS/m Millisiemens/meter

Mg/l Milligram/litre

N Nitrogen

Na^- Sodium

NO_2^- Nitrite

NO_3^- Nitrate

P Phosphorus

PO_4^{3-} Phosphate

SO_4^{2}-Sulphate

µm micrometre

Glossary of terminology

Catchment: The area that drains into a certain point in a river system (DWAF, 2008, p. 8).

Coagulation: the growth of particles in a suspension and the separation or precipitation of those particles in a dispersed state. This can happen as a result of prolonged heating, the addition of an electrolyte (a coagulant), or a condensation reaction between a solvent and a solute (DWAF, 1996, p. 190).

Control: water in its untreated state. The meaning stated here is for this study. (DWAF, 1996, p. 191).

Flocculation: the process of adding chemicals (flocculants) to fine suspended particles to cause them to coagulate, aggregate, or undergo a biochemical reaction, resulting in flocs of small particles (DWAF, 1996, p. 190).

Hardness: determined by adding the calcium and magnesium concentrations, both of which are expressed as calcium carbonate, and measuring it in milligrams per litre (DWAF, 1996, p. 191).

Microbes: relatively small organisms such as bacteria or viruses (DWAF, 1996, p. 191).

Pathogenic: causing disease (DWAF, 1996, p. 192).

Pollution: Changing a water resource's physical, chemical, or biological characteristics directly or indirectly to make it (a) Unfit for any beneficial use for which it might reasonably be expected". The meaning stated here is for this study (DWAF, 1998, p. 8).

Runoff: water that flows over land as surface water instead of being absorbed into groundwater or evaporating (DWAF, 2008, p. 8).

Physical-chemical: characteristics of water include its temperature and electrical conductivity as well as its nitrate and mercury concentrations (DWAF, 1996, p. 192).

Water quality: refers to the physical, chemical, biological, and aesthetic characteristics of water that determine its suitability for a range of uses as well as for preserving the wellbeing and integrity of aquatic ecosystems. Many of these characteristics are influenced or controlled by substances that are suspended or dissolved in water. (DWAF, 1996, p. 2)

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Water is the most important resource on the planet and is considered an essential resource that is required by living organism. The most important resource on the planet and is essential for maintaininglife. Water scarcity is now a global issue as a result of the world population's steady growth and the demand for water has grown significantly. According to the World Health Organization(WHO) and UNICEF Joint Monitoring Programme (JMP) for Water Supply, Sanitation, and Hygiene, as of 2021, approximately 2.2 billion people worldwide do not have access to servicesthat manage drinking water safely (WHO, 2021).

The problem of water scarcity has been further compounded by climate change, which has caused alterations in meteorological patterns and intensified occurrence and magnitude of both droughts and floods in numerous regions across the globe (UNESCO, 2021). As a result, many countries are struggling to manage their water resources effectively, which has led to conflicts over water rights and access. Furthermore, it is estimated that half of the world's population will reside in water-stressed regions by 2025, making it essential to address the water crisis (Jenkins and Sugden, 2006).

Water has been acknowledged by the United Nations as a fundamental human right, and its importance has been emphasized through its inclusion in the Sustainable Development Goals (SDGs). Goal 6 highlights on ensuring access to water and sanitation for everyone and promoting sustainable management practices. This highlights the critical need to address water-related challenges at a global level (Delanka-Pedige *et al.,* 2020; Sasmito *et al.,* 2023).

Numerous efforts are being made at a global scale to solve the challenge of water shortage. One of these efforts is the UN-Water Global Analysis and Assessment of Sanitation and Drinking-Water (GLAAS), which provides a thorough assessment of water and sanitation conditions systems globally (WHO, 2017). Furthermore, various non-governmental organizations (NGOs) and governments are collaborating to enhance water and sanitation accessibility and attain sustainable water resources management (Yamauchi *et al.,* 2023; Wadström *et al.,* 2023).

There is a critical need to address the issue of water quality. Contaminated water can carry a high microbial load, potentially leading to waterborne diseases and widespread health issues, particularly in regions already struggling with water scarcity (Davis *et al.,* 2022). Therefore, efforts to reduce the microbial load of contaminated water through innovative solutions are needed (Wabatagore, 2022). These solutions can range from chemical disinfectants to more advanced techniques like ozonation and filtration (Velali *et al*., 2018).

1.2 Clean water shortage: The South African overview

Households and living organisms depends on water for survival (Yuliastri *et al.,* 2016). However, accessing clean water has become a constraint in South Africa. Water scarcity as well as pollution have led to a significant deterioration in the standard and quantity of water resources. Significant water shortages have developed in several parts of the country as a result of droughts, climate change, and inadequate water management. In South Africa, insufficient allocation of resources towards infrastructure or technological advancements for extracting water from rivers, aquifers, or alternative water sources is the cause of water scarcity. Additionally, it has been projected that up to 80% of infections and illnesses are caused by poor sanitation, contaminated water, or a lack of access to clean water (Demena *et al.,* 2003; Prüss-Ustün *et al.,* 2014). In terms of water availability per person, South Africa (SA) was placed the 26^{th} country with highest water availability out of the 164 countries with an estimated 822 cubic meters per person per year (DWS, 2022). South Africa is likely to face water scarcity challenges in the coming years, and the demand is projected to exceed supply by 17% in 2030 (Water Research Commission, 2017). This is due to several factors, including population growth, urbanization, and climate change.

1.3 Clean water shortage: The global overview

Many developing countries are facing serious challenges of lack of clean water resulting in deaths among people caused by contaminated water (Delelegn *et al.,* 2018). Approximately 159 million people globally consume surface water from streams and lakes without further treatment (WorldHealth Organization, 2017). Therefore, the release of untreated water from industrial and municipal wastewater to rivers puts populations with resource limitations at risk of waterborne diseases (Rosmawanie *et al.,* 2018). The World Health Organization (WHO) recommends a daily minimum of 2-3 L of clean drinking water for adults to maintain proper hydration and overall health (Popkin *et al.,* 2010). Therefore, the quality of drinking water should always meet the approved standard. The primary goal of water treatment, as stated by the Centre for Disease Control (CDC) (2012), is to create safe drinking water with no pathogenic microorganisms and toxic compounds.

Purifying water has become costly. Therefore, this forces poor people to collect water from contaminated sources such as rivers (Glennon, 2004; Chakma *et al*., 2020). According to the South African government, 64% of households can access piped water, with many rural areas still relying on unprotected water sources. Additionally, many of the country's water treatment facilities are old and in need of upgrades or repairs. Water obtained from these sources exhibits turbidity and is contaminated with microorganisms, leading to various diseases such as guinea worm infection and intestinal infections. Parliamentary Office of Science and Technology (POST, 2002) found out that waterborne diseases are one of the primary problems in developing nations. Approximately more than a million individuals, mostly children die every year from diarrhoea and other water-related diseases. The interest in understanding problems regarding water quality has grown extensively over the years due to widespread man-made activities affecting the natural water resources (Sharma and Bhattacharya, 2017; Wang, 2022).

The quality of water must meet certain standards to ensure that it is safe to use and consume. These standards are typically set by national or international organizations and may vary in accordance with the intended water use such as drinking water or industrial use. To ensure the safety of drinking water in South Africa, it is necessary for it to fulfil the specified criteria in the South African National Standard (Hodgson and Manus, 2006). Drinking water should possess specific characteristics such as being colourless, tasteless, odourless, pathogens-free, non-corrosive and chemical-free. This criterion was established to avoid waterborne diseases from occurring and spreading. To achieve this standard, coagulation-flocculation is a typical method applied in the process of water treatment. However, the water may still have pathogens, such as bacteria and viruses. Coagulation involves the addition of synthetic materials to neutralize charged colloidal particles, leading to the formation of a precipitate as a result of the gravitational force. Coagulants can be manmade materials including ferrous chloride, aluminium or aluminium sulphate and poly aluminium chloride (Şirin*et al*., 2012).

Natural elements obtained from tropical plants, such as *Moringa oleifera* Lam, can be used as coagulants for water treatment (Vieira *et al.,* 2010; Dzuvor *et al*., 2022; Rifi, 2022). Incorporating coagulants from local indigenous plants to clean contaminated water is not new (Mbogo, 2008; Sivaranjani, and Rakshit, 2016). Historically, herbs have been used as natural coagulants for river water treatment (Virk *et al*., 2019). Previous research (e.g. Stohs and Hartman, 2015) suggest that *M. oleifera* is not toxic and therefore has potential for use in water purification. This suggests that *M. oleifera* may be a viable and safe option for water treatment, potentially offering an alternative to traditional methods used in water purification. Ghebremichael *et al.* (1995) recommended *M. oleifera* seeds usage as a coagulant in under developed countries.

1.4 Aims and objectives of the study

The study seeks to investigate a simple water purification gadget made of *M. oleifera* seeds as the active beads for effective water treatment.

The study has the following objectives:

I. To determine the efficiency of different *M. oleifera* seed particle sizes in influencing the physicochemical parameters of contaminated water.
II. To investigate the efficiency of *M. oilefera* seed gadget in reducing the microbial load of contaminated water.
III. To determine suitability for human consumption of water purified using the *M. oleifera* seed gadget.

1.5 Structure of the thesis

Chapter 1 outlines clean water shortage in South Africa and beyond, and also sets out the aims and objectives of the study. **Chapter 2** is literature review on *M. oleifera,* its identification and uses, and in particular, the use of *Moringa oleifera* in water purification. The chapter also covers related topics such as low-cost water purification systems and the toxicology of *Moringa* seeds. In **Chapter 3**, the methodology used to collect water samples from three points along Pienaars River is presented. The chapter also provide a detailed report about the physico chemical parameters of water obtained from three sampling point along Pioneers River. In **Chapter 4**, the effect of filtration gadget on the microbial load of water samples collected from three points along Pioneers River is investigated, while **Chapter 5** evaluates the purified water's fitness for human consumption. Finally, **Chapter 6** summarises the results of the study and highlights suggestions for future research and policy.

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CHAPTER 2: LITERATURE REVIEW

2.1 Natural range of *Moringa oleifera*

The horseradish or drumstick tree, *Moringa oleifera* Lam., is part of the Moringaceae, a family of flowering plants that is made up of one genus (Fahey, 2005). There are 13 known cultivars within the family, in which *M. oleifera* is commonly known because of its widespread use and cultivation (Olusanya, 2018; Tshabalala *et al.,* 2019). Most of the species in the family are native to tropical and subtropical regions of Africa, while a few are found in South Asia and the Pacific Islands (Mogea *et al.,* 2019; Leblanc and Ndiaye, 2020). *Moringa oleifera* is genetically similar to two other species, namely *M. concanensis* and *M. peregrina*. In certain regions of the Indian subcontinent, *M. concanensis* is also used as a food source (Ravichandran *et al.,* 2009*). Moringa peregrinais* is found in areas in the vicinity of the Red Sea, extending northward to the Dead Sea and southern regions of the Arabian Peninsula (Olson and Carlquist, 2001). *Moringa stenopetala* and *M. drouhardii* are also among the species closely related to the *M. oleifera. Moringa stenopetala,* also known as the African *Moringa,* is native to Ethiopia and is renowned for its nutraceutical and medicinal characteristics (Abuye *et al.,* 2003;Seifu 2015). *Moringa drouhardii* is found in Madagascar (Olson and Razafimandimbison, 2000). Conversely, *M. hildebrandtii*, *M. ovalifolia* and *M. arborea* are among the distantly related species in the genus (Hausiku *et al.,* 2020). *Moringa ovalifolia* is distributed in Namibia and Angola whereas *M. hildebrandtii* is endemic to Madagascar. *Moringa oleifera*, *M. stenopetala, M. ovalifolia, M. drouhardii, M. hildebrandtii, M. peregrine and M. concanensis,* are found in different regions of Africa and are characterized by their unique physical and biochemical features (Fahey *et al.,* 2005; Mogea *et al.,* 2019).

Moringa oleifera is reputed to have exceptional social, environmental and economic benefits (Alegbeleye, 2018). The commercial cultivation of *M. oleifera* has drawn more attention in Limpopo, KwaZulu-Natal, Gauteng, North West, and Mpumalanga Provinces of South Africa (Mashamaite *et al.,* 2021) as the plant is considered to be a profitable crop for smallholder farmers. *Moringa oleifera* has acquired significance because of its multipurpose uses; nearly all parts of the plant are being used (Gandji *et al.*, 2018). The plant's high nutritional value makes it an attractive crop for subsistence and commercial farming (Leblanc and Ndiaye, 2020). The plant can adapt to different environmental and climatic conditions. However, higher yields can be expected when the plant is grown under warm and dry conditions supplemented with irrigation (Muhl *et al.,* 2011; Heuzé *et al.,* 2019).

Figure 2.1: *Moringa oleifera* trees at Lefakong Farm, Bosplaas, North West Province.Source: Dineo Raphasha

2.2 Description of the species

At maturity stage, the tree grows up to 12 meters in height, however *M. oleifera* trees can grow to different heights, depending on the variety, growing conditions, and management practices. The tree is maintained at 2-4 meters for easier management and harvest (Oliveira *et al*., 2013). The trunk is usually straight, and the bark is greyish-brown with corky, vertical fissures (Foidl *et al.,* 2001). The leaves are phyllotaxy alternate and compound, with 3-5 pairs of leaflets and a terminal one, and they are green to dark green in colour. The leaflets are 1-2 cm long and narrow, and they are covered with tiny hairs. The tree produces fragrant flowers that are white to cream-colored and are 10-25 cm long and 2.5 cm in diameter. The flowers are hermaphroditic, meaning they have both male and female reproductive organs, and they are pollinated by insects such as bees and butterflies (Fahey, 2005). *Moringa oleifera* seeds are contained in elongated seedpods that can measure up to 45 cm in length and contain 15-20 seeds per pod (Oliveira *et al.,* 2013). The seeds are brown when mature then become round or triangular with the loss of moisture during dormancy.

2.3 **Uses of *Moringa oleifera***

Some plants are rich in secondary metabolites that may be effective for the healing various illnesses in traditional medicine (Savithramma *et al.,* 2007). *Moringa oleifera* is a plant used for the treatment of various diseases in South Asia (Anwar *et al., 2007).* Various research affirm that *M. oleifera* is considered a food crop that is also used for its medicinal properties (Hassan and Ibrahim, 2013). Parts of *M. oleifera* such as leaves, roots, seeds, bark, and flowers, have all been utilized for their medicinal properties (Fahey, 2005; Mall and Tripathi, 2017). The seeds of *M. oleifera* are highly valued for their oil, which is rich in monounsaturated and polyunsaturated fatty acids. The oil is used in a variety of applications, including cooking, cosmetics and pharmaceuticals (Anzano *et al.,* 2021). Apart from this, the seeds has also utilized for water purification (Vlahov *et al.,* 2002; Sahay *et al.,* 2017).

Moringa oleifera seeds are commonly utilized for coagulation, flocculation, and sedimentation in water and wastewater treatment there by enhancing water quality, decreasing organic matter and microbial loads (Ghebremichael *et al.,* 2005; Alnawajha *et al.,* 2022). The plant has a unique role to play in intensive animal production processes, particularly in the field of aquaculture, where it is used for fish growth enhancement, improved feed utilisation, and disease control because of its immune-stimulatory characteristics (Brilhante *et al.,* 2017; Ikubanni *et al.,* 2017).The leaves and seeds of *M. oleifera* contains nutrients such as protein, vitamins, and minerals, making them a valuable supplement in animal diets (Saini *et al.,* 2016). Moreover, *M. oleifera* comprises of natural compounds like saponins, alkaloids, and flavonoids that havebeen shown to possess antimicrobial and antifungal activities, which can help prevent and control disease outbreaks in aquaculture systems (Ikubanni *et al.,* 2017). *Moringa oleifera* seeds have been shown to exhibit coagulation properties and possess a high capacity for removing bacterial contaminants in water, with reported removal rates ranging from 90% to 99%. As a result, the powdered form of hardened *M. oleifera* seeds has been utilized for watertreatment in both the water industry and in areas where water is heavily contaminated with various pollutants (Mumuni, 2013; Desta and Bote, 2021). The study of Kansal and Kumari (2014) states that only mature *M. oleifera* seeds that has brown seed coats exhibit significant coagulation properties. This finding supports earlier research by Jahn (1986), which showed that only ripe seeds of *M. oleifera* have high coagulation efficiency.

2.4 *Moringa oleifera* seed proteins as a natural coagulant for water purification

In regions where clean water for drinking is not readily available, *M. oleifera* offers a critical advantage through its ability to filter water (Megersa *et al.,* 2014; Muthuraman and Sasikala, 2014). Historical references suggest that Moringa species were used for water purification in ancient times (NRC, 2006). The dynamic flocculating agent in *M. oleifera* seeds is a protein that generates positive charges when the seeds are crushed and put in water, attracting negatively charged particles like clay, silt, and microbial contaminants (Broin *et al.,* 2002; Bodlund, 2013; Varkey, 2020). Previous research has identified two primary main constituents isolated from raw extract of *M. oleifera,* and confirmed the existence of more than one flocculating protein family in the seeds (Moulin *et al.,* 2019; Nouhi *et al.,* 2019; Wang *et al.,* 2021). Crushed *M. oleifera* seeds can be added to untreated river water for purification, with unsafe particles binding to the seed particles and sinking, allowing clean water harvest from upper part of the container (Delegn *et al.,* 2018; Landázuri *et al.,* 2018; Taiwo *et al.,* 2020). This simple yet effective technique has the potential to provide a biodegradable, less-toxic, affordable, and practical alternative to traditional water treatment methods (Al Juhaimi *et al.,* 2017; Okuda & Ali, 2019; Nhut, 2020). Collection of solid contaminations is the initial fundamental phase in water decontamination, this eliminates significant amount of pollutants and decrease turbidity (Cevallos-Mendoza *et al.,* 2022).

2.5 Reduction of microbial load from contaminated water after filtration

The reduction in *Escherichia coli* count on water treatment with *M. oleifera* seeds has been reported previously (Aboagye *et al.,* 2021; Alam et al., 2020; Dasgupta *et al.,* 2016). The *M. oleifera* seed extract inhibits bacteria and coliforms growth in raw contaminated water (Oloduro and Aderiye, 2007). The effectiveness of *M. oleifera* seed extract in reducing the *E. coli* count is believed to be from to its ability to destabilize colloidal particles (Alam *et al.,* 2020). Pritchard *et al.* (2009) discovered *M. oleifera* seed extracts decreases the turbidity and amount of faecal coliforms in water samples collected from shallow wells. Similarly, Amagloh and Benang (2009) reported that the seed powder significantly lowers the turbidity and coliform count, making the seed powder an effective method for water decontamination. Several studies have demonstrated that soluble peptides derived from crushed seed kernels are categorized as cationic peptides. These peptides are known for their antimicrobial activity mainly attributed to their interaction with negatively charged surfaces and possession of an amphiphilic structure, enabling them to be incorporated into cell membranes (Suarez *et al*., 2005; Moulin, 2019; Drayton *et al*., 2020). Zero *E. coli* count per 100 ml of water is deemed safe for consumption, based on standards of the World Health Organisation (Odonkor and Mahami, 2020).

2.6 *Moringa oleifera* dosage required for water purification

Determining the appropriate coagulation dosage is crucial in achieving optimal performance of coagulants during the coagulation and flocculation process (Patel and Vashi, 2013). Katayon*et al.* (2006) suggested that the powder produced from one *M. oleifera* kernel can purify 2 L of slightly turbid water, and 1 L when water is very turbid. The dosage required for coagulationfor non-shelled *M. oleifera* seeds is higher, at 500 mg/L, for waters with low turbidity (Bhattacharyya & Gupta, 2008). Varkey (2020), recommended a dosage of 0.25 g *M. oleifera* seed powder per litre of water to minimize raw water turbidity. Thakur and Choubey (2014), reported that a dosage of 200 mg/L of *M. oleifera* was efficient in water decontamination. Additionally, a mature *M. oleifera* plant can produce 3 kg of seeds, which can treat approximately 30 L of water (Schwarz, 2001). Therefore, it is worth noting that the different dosages reported are influenced by the condition of the water. The dosage required for effective coagulation and flocculation varies as per turbidity level water, as well as presence of other contaminants such as microorganisms. Thus, there is no single dosage that applies to all scenarios.

2.7 Shelf life of water after treatment with *Moringa oleifera* seeds

It should be noted that after several hours of storage bacterial regrowth inside the storage container can happen, therefore it is not guaranteed that the water will be 100% virus and/or bacteria-free immediately after treatment or storage, so further disinfection can be done (Sánchez-Martín *et al.,* 2012). The use of *M. oleifera* seeds to purify water can result in an increase in the content of organic matter, however this is dependent on the characteristics of the water before treatment and the conditions under which the treatment is carried out (Kabore *et al.,* 2013). To ensure microbiological stability, the recommended dissolved organic carbon in water should range from 0.5 and 2 mg/l (Lautenschlager *et al.,* 2010). Adversely, organic matter in treated water encourages microbial recontamination during storage. Therefore, processed water must be kept at low temperatures and utilized for drinking within 24 hours to maintain its microbiological consistency (Kabore *et al*., 2015).

2.8 The toxicology in *Moringa oleifera* seeds

Many traditional healers overlook toxicological testing of medicinal plants, assuming that plants are not poisonous. However, traditional products emanating from such cannot always be relied upon as a credible assurance of safety (Arora *et al*., 2013; Ekor, 2013; Mensah *et al*., 2019). Aqueous extracts of *M. oleifera* seeds has been considered safe based on numerous in vivo tests. However, Oluduro and Aderiye (2009) disputed these findings in their study and reported that the prolonged intake of water treated with 2 mg/ml of *M. oleifera* seed extract on rats could potentially result in liver infarction. Arora *et al.* (2013) conducted a study using methanolic extract of *M. oleifera* seed and found that it was relatively non-toxic for animals at low doses. However, the changes in different parameters measured at high doses indicated a dose toxicity when ingested regular for an extended periodof time. It has been reported that the seed powder of the *M. oleifera* is safe for human consumption and has coagulating properties used in water treatment for turbidity, alkalinity, total dissolved solids, and hardness, among other things (Arnoldsson *et al*., 2008; Meneghel *et al*., 2013; Valverde *et al.,* 2018). Toxicological investigations conducted by Berger *et al*. (1984) and Grabow *et al*. (1985) showed that the utilization of *M. oleifera* as a primary coagulant does not pose a risk to human health. However, toxicological testing of any seed material must be confirmed to obtain regulatory approval. Tripathi *et al.* (2012) stated that natural coagulantsfrom plants, animals, or microorganisms are generally considered to be safe and environmentally friendly, as they result in biodegradable sludge. *Moringa oleifera* biocoagulant is possibly the most studied coagulant amongst environmental scientists, and resulting from these studies, a number of researchers developed water and wastewater treatment prototypes (Yin, 2010; Camacho *et al.,* 2017; Andrade *et al*., 2020).

2.9 Effect of pH on water purification

Moringa oleifera seeds contain soluble cationic proteins. Hence, using *M. oleifera* seed powder as a coagulant will often increase the pH of treated water. According to Patel and Vashi (2013), coagulation efficiency can be reduced in cold waters due to the response of aluminium with the biocoagulant in water, which results in a decrease in pH. Therefore, the essential amino acids in *M. oleifera* protein draw positively charged hydrogen ion from water and release the OH-cluster, causing the solution to become basic (Nayana and Adi, 2019). The primary importance of pH in domestic water supplies lies in its impact on water treatment processes. Various factors, including temperature and discharge of pollutants, acidic precipitation, and microbial activity influence the natural pH level in water. The acceptable range of pH for drinking water should range 6.5 and 8.5 (WHO, 2007). Previous research of Gassenschmidt (1991) and Lea (2010) states that *M. oleifera* flocculants are polypeptides with a pH of 10 to 11 andmolecular weights from 6 to 16 kDa. Coagulation of *M. oleifera* is believed to involve the adhesion and neutralization of colloidal charges, as supported by studies of Ndabigengesere and Narasiah

(1998). The effects of acidic pH on various components of water, including heavy metals caused by the solubilisation of these substances and the deprotonating of other ions. The pH of most of untreated water sources falls between of 6.5 to 8.5.

2.10 Low cost water purification systems

Treatment plants are expensive in many developing countries, there is also a shortage of skills and innovative technologies (Ghebremichael, 2006). In these circumstances, safe piped water may not always be available, and numerous water sources may have pathogens (Chaudhuri and Sattar, 1990). Implementing conventional water treatment technologies in developing nations is extremely difficult due to the high cost and shortage of chemical coagulants and disinfectants. There are numerous methods for purifying different types of contaminated water, but they are costly and frequently inappropriate in poor regions (Pernitsky and Edzwald, 2006; Rezaei *et al*., 2018). Chemical precipitation, membrane filtration, adsorption, or ion exchange are some of the methods used for water treatment. However, these technologies are costly and often insufficient in meeting the required standard for drinking water. Ferric salts and synthetic polymers have been utilized as substitutes but they have limitations, such as significant change in the pH of treated water, increased non-target metal concentrations and the production of large volumes of sludge (Vijayaraghavan *et al.,* 2011).

The absence of electricity in most Sub-Saharan African communities makes it difficult to implement water treatment technologies that depend on electricity. On the other hand, coagulation and flocculation are energy efficient methods that can be relied upon for water treatment. Coagulation includes destabilization of smaller particles (0.01–1 µm), leading to the aggregation and formation of larger particles (Teh *et al.,* 2016*);* Hu *et al.,* 2021). Varsani *et al.* (2022), described coagulation as an effective method for reducing metal ion levels in water, achieved by introducing a coagulant, which can be either synthetic or bio-based, to destabilize colloidal particles. The resulting creation of flocs, can then be detached from the water through slow agitation followed by sedimentation.

Synthetic coagulants, such as polyaluminum chloride, and bio coagulants, derived from natural sources like plants or bacteria, can both be utilized to achieve the separation. The process of flocculation is when destabilized particles produce flocs (Metcalf, 2003). Following that, the particles can be filtered. Coagulation, however, cannot provide stable coliform elimination (Crump *et al*., 2014). The comparison of using *M. oleifera* and conventional water treatment technologies are mentioned in table 2.1.

Table 2.1 Comparison of *Moringa oleifera* and Conventional water treatment technologies

Aspect	**Moringa Treatment Technique**	**Conventional Treatment Methods**
Cost	Generally lower cost	Costs can be higher
Sustainability	Environmentally friendly	May require more resources
Effectiveness	Effective for some impurities	Effective for a wide range of impurities
Set-up and Maintenance	Simpler set-up and maintenance	May require more complex systems and maintenance
Energy Consumption	Lower energy consumption	May consume more energy
Residuals and by-products	Fewer residuals and by-products	May produce more waste
Flexibility	May be limited in some applications	Can be adapted to various treatment needs
Availability	Availability in certain regions	Widely available technologies
Natural coagulant Properties	Acts as a natural coagulant	May require synthetic coagulants

2.11 Evaluation of the water quality status in Pienaars River and its respective sampling locations

The Pienaars River holds significant importance as a major tributary of the Crocodile River, which is a classified crucial in Gauteng and South Africa as a whole (Tshivhase, 2019). The Crocodile River catchment, encompassing the Pienaars River, has unfortunately experienced extensive degradation over the years. There is a thriving manufacturing and commercial urban economy in the river catchment's urban portion, which gives it its distinctive character (Makanda *et al.,* 2022). This region encompasses several settlements, primarily townships such as Mamelodi and Moretele. The rural of the catchment is primarily characterized by agricultural practices and tourism (DWA, 2013). The river and its sampling point also provide a valuable habitat for wetland and riverside vegetation, which plays an important role in sustaining water quality and supporting ecosystem health (Mothowamodimo, 2011).

2.12 Sources of water pollution in Pienaars River

The Pienaars River is a dynamic water source for the surrounding residents and industries. However, the river has experienced pollution over the years, leading to significant environmental and health concerns (Brooks *et al.,* 2016). The River is situated in an area that has undergone significant urbanization and industrialization in recent years. As a result, the main cause of pollution in Pienaars River are anthropogenic activities, including industrial discharges, agricultural runoff, and urbanization (Dube *et al.,* 2017).

Research conducted by Mala *et al.* (2003) identified that the major source of pollution in the river was from urban runoff, which carried elevated concentration of nutrients, heavy metals, and biological pollutants. The discharge of untreated industrial effluent is also a significant contributor to water pollution in Pienaars River. The use of pesticides and fertilizers is also found to increase the levels of pollutants in the river (Oberholser and Ashton, 2008.). Farmers in the region uses a variety of chemicals to control pests and enhance crop yields, but the high use of these chemicals leads to soil erosion and contamination of the river through agricultural runoff.

2.13 Impact of water pollution in Pienaars River

The pollution of Pienaars River is a serious environmental problem that requires urgent action due to it being a water supply to the local residents and industries in the surrounding area. The river and its tributaries also provide a valuable habitat for wetland and vegetation by maintaining water quality and supporting ecosystem health (Flotemersch *et al.,* 2016: Das *et al.,* 2022). The water condition of the river has been found unfit for drinking due to the existence of high levels of coliform bacteria and heavy metals suchas lead, cadmium, and copper (Ashton *et al.,* 2001; Zulu, 2020). Pollution of the river has also impacted aquatic life, resulting in mortality of fish and various aquatic organisms. Moreover, the polluted water negatively impacts farmers who uses river water for irrigation purposes, as this leads to lower crop yields, poor crop quality, and even crop failures (Dotaniya *et al.,* 2023).

2.14 Water quality challenges in Moretele Municipality, South Africa

Moretele is a small rural village found in North West Province of South Africa. The village falls under jurisdiction of the Moretele Local Municipality, which is part of the Bojanala District Municipality. The municipality is situated in the heart of the province and is home to roughly 186,947 people (Stats SA, 2011). The water quality in Moretele municipality is influenced by various factors, including natural and anthropogenic activities (Clark, 2019). The Moretele local municipality manages a single drinking water supply system, which has been assigned a critical-risk rating score of 17.7% and an overall score of 53% in terms of water quality compliance assessment within the province. The findings of the DWS blue drop report (2022) state that 84% of the systems in the province fail to meet the microbiological determinants, pointing to notable deficiencies in ensuring microbiological safety. Furthermore, 88% of the systems do not comply with the chemical determinants, raising concerns about the immediate or potential long-term health risks involved. There have been some concerns raised by local communities about the quality of their drinking water in Moretele municipality (Nevondo and Cloete, 1999). These concerns are mainly related to the limited access to clean water, which is caused by poor infrastructure and maintenance of the water supply system.

2.15 Socioeconomic challenges and water quality concerns in Mamelodi, Gauteng, South Africa

Mamelodi is a crowded residential area located in the eastern part of the City of Tshwane Metropolitan Municipality in Gauteng Province, South Africa. The population in the area consists of nearly 410,000 local residents distributed among 54, 000 houses with around 50% of them residing in informal housing units (Nkosi *et al.,* 2019). The region is characterized by low-income communities, facing economic challenges, with an unemployment rate of approximately 20%. Nearly half of the households earn less than R 3200 per month (Ndzimbomvu, 2021). A significant proportion (55.6%) of households in Mamelodi utilize the stream area as adumping site for their domestic waste. Therefore, posing substantial risks to public health and contributing to extensive pollution of both land and water resources. According to DWS (2021), the quality of drinking water in Mamelodi, is generally satisfactory, meeting compliance levels of above 95%. However, local communities in Mamelodi have expressed concerns about the quality of their drinking water, primarily due to inadequate access to clean water resulting from poor infrastructure and preservation of the water supply system (Darkey *et al.,* 2010; Smit, 2018).

2.16 Ecological significance and water quality status in Tierpoort, Gauteng, South Africa

Tierpoort is a small geographical area found in Pretoria east Gauteng Province, South Africa. The population size of Tierpoort is 1,167 (Stats SA, 2011). The area is located in the Tiegerpoort area within the City of Tshwane Municipality. The Tierpoort sampling point passes through a recreational area and caravan park. The geology of the site primarily consists of Quartzite and Shale (Jollye, 2007). The site provides habitat for numerous animal species, including reptiles, mammals, birds, amphibians, and invertebrates. The unique ecological and physical features of the area make it worthy of protection from conventional urban development and other inappropriate uses. The water condition of this sampling point is influenced by numerous factors, including agricultural practices and industrial activities. The major contributors of pollution in this harvesting point are agricultural runoff and municipal storm water runoff. Therefore posing risk to human and environmental condition, in addition to economic viability of the agricultural sector in the area.

2.17 Importance of managing water pollution in Pienaars River

Managing water pollution is crucial for protecting public health, preserving aquatic ecosystems, supporting economic development and maintaining environmental sustainability (Wuijts *et al.,* 2019; Jourdain *et al.,* 2023). The South African Department of Water and Sanitation is responsible for monitoring and managing water conditions in the country, including Pienaars River (DWS, 2016). Regular monitoring of the water quality is necessary to ensure that the water is safe for drinking, recreational and other uses. Several measures have been taken to reduce water pollution in Pienaars River. These include the implementation of industrial wastewater treatment plants, the enforcement of environmental regulations, and the promotion of sustainable agricultural practices. Integrated water resources management (IWRM) approaches, including stakeholder participation and adaptive management are also helping to address the complex and interconnected nature of water pollution (Katusiime and Schütt, 2020).

2.18 Significance of the study

The elevated price of purified water compels most rural communities to utilize easily accessible water sources of poor quality that usually expose them to waterborne sicknesses (Ramírez-Castillo *et al*., 2015). South Africa is experiencing water scarcity, Mnisi (2020). According to Donnenfield *et al*. (2018), only one-third of South African rivers are used sustainably, while more than 60% of the rivers are overused. The model of International Futures (IFs) – predicts that demand in water supply will increase by to 2035.

Countries worldwide are concerned about the consequences of consuming contaminated water, as it leads to both illness and death due to waterborne diseases (Classen *et al*., 2007; WHO, 2010). Crapper *et al.* (1973) and Miller *et al.* (1984) stated that faults can occur during the water treatment process, where the chemicals used to purify water could pose serious health hazards. Several studies have highlighted various limitations of using aluminium salts as coagulants in water decontamination. These include the potential for residual aluminium in treated water, which has been linked to Alzheimer's disease, and the manufacture of high volumes of sludge. Additionally, use alum can result in the production of acidic water that can be unsafe to pregnant women and has been associated with predementia in some individuals (Patel and Vashi, 2013).

Chlorine and chlorine-based disinfectants have also been found to have potential drawbacks. They have been reported to form disinfectant by-products (DBPs), some of which can be carcinogenic and mutagenic, including tetra chloromethane (TCM) (Drogui and Daghrir, 2015). TCM has been found to produce a hormonal analogue that interferes with male fertility. DBPs have also been linked to cardiovascular disease, cancer, and birth defects (Eze and Ananso, 2014).

Efforts are made to purify water by boiling and chemical treatment, however, these methods are expensive for households (Anderson *et al*., 2010). Thus, a sustainable alternative for wastewater purification can be achieved by effectively using natural sources, low cost plants like *M. oleifera* seeds while keeping the environment within permissible limit (Adelodun *et al*., 2019). Water treatment utilizing natural coagulant from *M. oleifera* seeds is reported to be safe, because the seed contain organic natural polymer and biodegradable materials (Kalibbala, 2007; Zhigila *et al*., 2019). Traditionally, utilizing plant- based materials to purify contaminated surface waters has long been practiced (Choy *et al.,* 2014; Sulaiman *et al.,* 2017; Mohd-Salleh *et al.,* 2019).

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CHAPTER 3- EFFECT OF FILTRATION GADGET ON THE PHYSICO CHEMICAL PARAMETERS OF WATER COLLECTED FROM THREE SAMPLING POINTS ALONG PIENAARS RIVER

Abstract

Water scarcity and deteriorating water quality pose significant challenges to South Africa, affecting human health, economic development, and environmental sustainability. Consequently, providing residents with access to clean water becomes essential for their welfare. For drinking water to comply with the standard, it is essential to implement purification methods across some water sources where practical. Diverse techniques are implemented to ensure that water is safe for consumption. This chapter focused on evaluating the physico-chemical characteristics of water at different sampling sites along the Pienaars River, selected based on their potential sources of contamination. Samples were collected during winter and summer seasons to evaluate fluctuations in water quality based on the seasons, compare water quality data among the sampling points, and evaluate the water quality against South African National Standards. The research area encompasses the Pienaars River, which flows through Limpopo, Gauteng and North West provinces. Physicochemical parameters analysed were pH, Electrical Conductivity (EC), nitrate (NO_3^-), nitrite (NO_2^-), chloride (Cl^-), fluoride (F^-), bicarbonate (HCO_3^-) sulphate (SO_4^{-2}), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and boron (B).There was a notable differences in the water quality parameters between the seasons. The results of the chapter indicated that the water quality in the three sites analysed (Moretele, Mamelodi, and Tierpoort) is generally good and safe. However, slight variations were observed at the Mamelodi site in Gauteng, whereby the nitrite levels before filtration were higher than the set standards.

Keywords: Eutrophication, physicochemical parameters, seasonal variation, water pollution, water quality.

3.1 Introduction

There are several problems with water quality in South Africa, including salinity, eutrophication, microbial contamination, microcystins, radionuclide and heavy metal contamination (Griffin *et al.,* 2014). Fourteen years prior to the 2010 UN announcement, the government of post-apartheid South Africa listed water as an essential human right resource in the 1996 (South African constitution, 1996). However, South Africa is still facing a significant challenge in ensuring access to clean water for all its citizens (Yongabi *et al.,* 2011). Moreover, the degradation of freshwater sources in South Africa has been amplified due to heightened pollution stemming from industrialisation, urban expansion,afforestation, mining activities and agricultural practices (Ashton *et al*., 2008). Level of impacts and of knowledge & understanding of different water quality problems are shown in Figure 3.1.

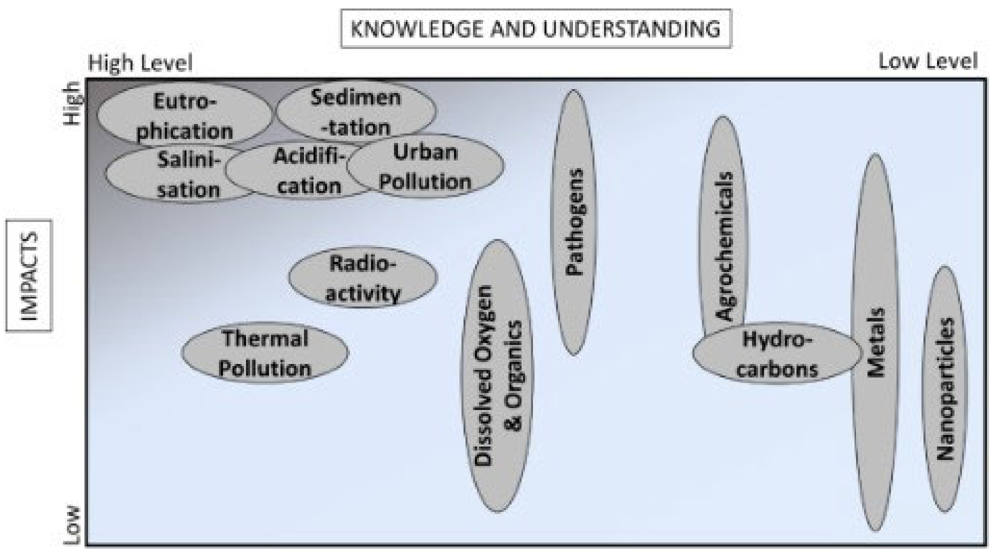

Figure 3.1: Prioritisation of water quality problems (Department of Water and Sanitation, 2016).

The South African Water Quality Guidelines for drinking water use are an essential requirement for the overall implementation of the water quality essential for different household purposes (DWAF, 1996). These guidelines include information that is similar to that found in the international literature. Policy guidelines such as the South African National Standards (SANS) are used to regulate and monitor drinking water quality (DWAF, 2005). Moreover, many countries around the world have made regulating water quality a priority (Chapman, 1996). The primary objective of formulating standards for the quality of drinking water is to safeguard the well-being of consumers (WHO, 2011).

Water quality for drinking purposes is determined by various indicators such as physical, chemical, and biological parameters (Shrestha and Basnet, 2018). The physical and chemical properties of water intended for drinking and other domestic purposes must not exceed specified limits (Dalen *et al.*, 2009; Wanda *et al*., 2016). Low basic services, including contaminated drinking water, contribute to 70% of diarrhoea in children under five in South Africa (Chola *et al.,* 2015).

Areas characterised by dense populations and restricted freshwater accessibility are more susceptible to experiencing severe water scarcity (Naik, 2017). Therefore, South Africa's population growth puts more strain on the water supplies. Migration from rural to urban areas is increasing in South Africa, with Gauteng Province leading with 97% of its population living in urban areas (Ntshidi, 2017; Ramuhulu, 2021). The Gauteng and KwaZulu-Natal Provinces are typical examples of rapid urbanisation in South Africa. Urban and rural landscapes are under a lot of stress as a result of rapid urbanisation. According to Statistics SA (2021), the 15.81 million residents of Gauteng constitute 26.3% from the entire residents of the country.

Gauteng has South Africa's highest density of residents. The most affected places by this population growth are the underdeveloped areas, like the black townships. Conditions for sustainable living are made more difficult by pressure on housing, where 38% of Gauteng's people live in substandard housing and suffer general lack of service delivery (Culwick and Patel, 2020).

As a mitigation measure to combat issues of lack of service delivery in terms of clean water, researchers produced a Moringa based filtration gadget (prototype) that can potentially be used to purify water. The research initiative not only showcases the potential of nature-inspired solutions but also offers a sustainable and cost effective means of addressing water scarcity. This innovative gadget contributes to achieving multiple Sustainable Development Goals (SDGs), such as promoting good health and well-being (SDG 3), ensuring access to clean water and sanitation (SDG 6), and fostering sustainable urban and community development (SDG 11) (Fonseca *et al.,* 2020).

3.2 Aims and objectives of this chapter

This chapter evaluates the general quality of water obtained from three points along Pienaars River. The chapter outlines the following objectives:

I. To determine the physico-chemical characteristics of water along Pienaars River before and after filtration.
II. To determine the efficiency of different particle sizes of *Moringa* seed powder in purifying river water
III. To evaluate the quality of water collected from Pienaars River against South African National Standards for Water quality after filtration.

3.3 Materials and methods

3.3.1 Study area

The Pienaars River traverses three provinces in South Africa: Gauteng, Limpopo, and the North-West (Figure 3.2). The river flows through these provinces and affects the surrounding communities,making it an important resource for the area's socio-economic and environmental health (Le Roux *et al.,* 2021). Pienaars River flows through the City of Tshwane and Bojanala Platinum District Municipality. The City of Tshwane is found in Gauteng Province, while the Bojanala Platinum District Municipality is found in the North-West Province. The watershed encompasses an approximate area of 7,000 km^2 and stretches from the north of Pretoria to the Limpopo River in the north. The Pienaars River constitutes a tributary of the Limpopo River, forming an integral component of the broader Limpopo River Basin.

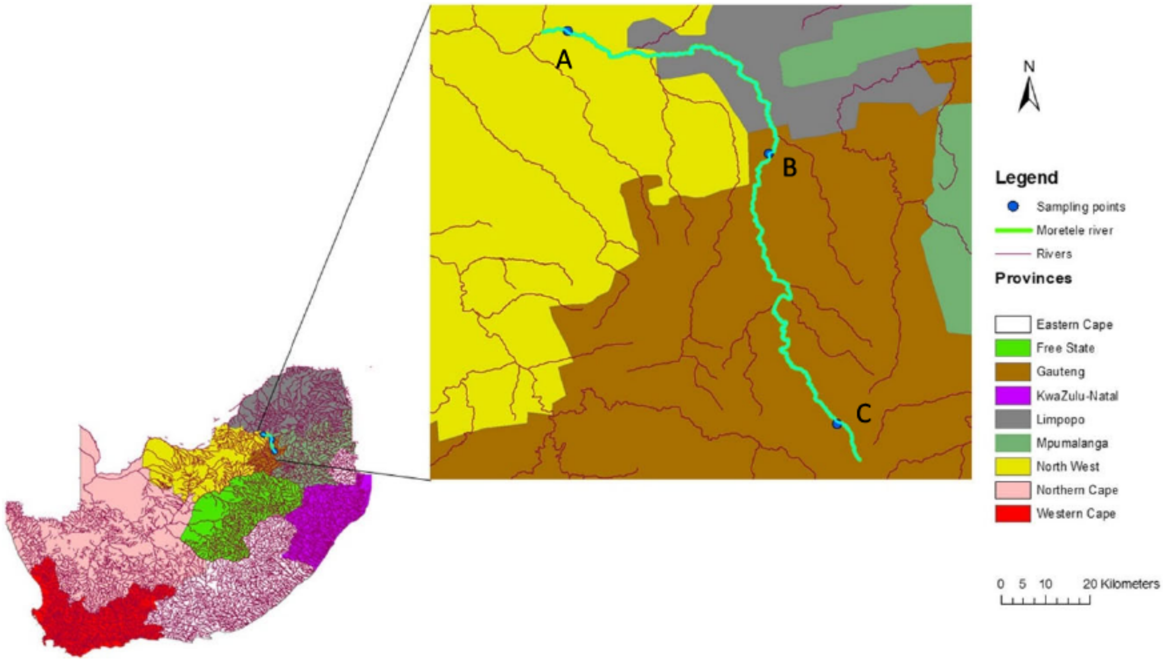

Figure 3.2: Study map to show sampling points along Pienaars River. (A) Moretele 25°07'50.1"S 27°57'34.5"E, (B) Mamelodi (25°40'43.0"S 28°21'27.0"E) and (C) Tierpoort (25°52'16.0"S 28°26'29.0"E)

3.3.2 Sampling points

Three sampling points were established along the Pienaars River. Two points were located within Gauteng province and one was located in Northwest province, all in South Africa (Figure 3.2). The water samples were collected during winter (June to August) and summer (December to February) seasons. Four replicate samples were collected once per season on the same spot at each sampling point. Tierpoort is located in the eastern part of Pretoria in Gauteng. Tierpoort is located approximately 30 km from Mamelodi, while Moretele is situated approximately 84 km from Mamelodi. The physico-chemical parameters of the samples were analysed prior to and following treatment using *M. oleifera* particle sizes.

Figure 3.3: Three sampling point along the Pienaars River at (A) Moretele, (B) Mamelodi and (C) Tierpoort.

3.3.3 Sampling procedures

Standard water collection techniques were carried out (Meybeck *et al*., 1996; DWAF, 2004). The sampling sites were carefully chosen to ensure accessibility and strategic coverage of various categories of land use or origins of contamination along the river. The samples were collected just below the water surface using 1L polyethylene sterile plastic bottles (Figure 3.4 A). Samples were collected in areas with gentle water movement against the current (Figure 3.4 B). As a precaution against contamination, the containers used to collect the samples were promptly sealed upon filling (Figure 3.4 C). Each container was appropriately coded and stored in a thermal container with ice before transportation to the laboratory (Figure 3.4 D). In the laboratory, water samples were refrigerated and analysed within 16 hours of collection (Figure 3.4 E).

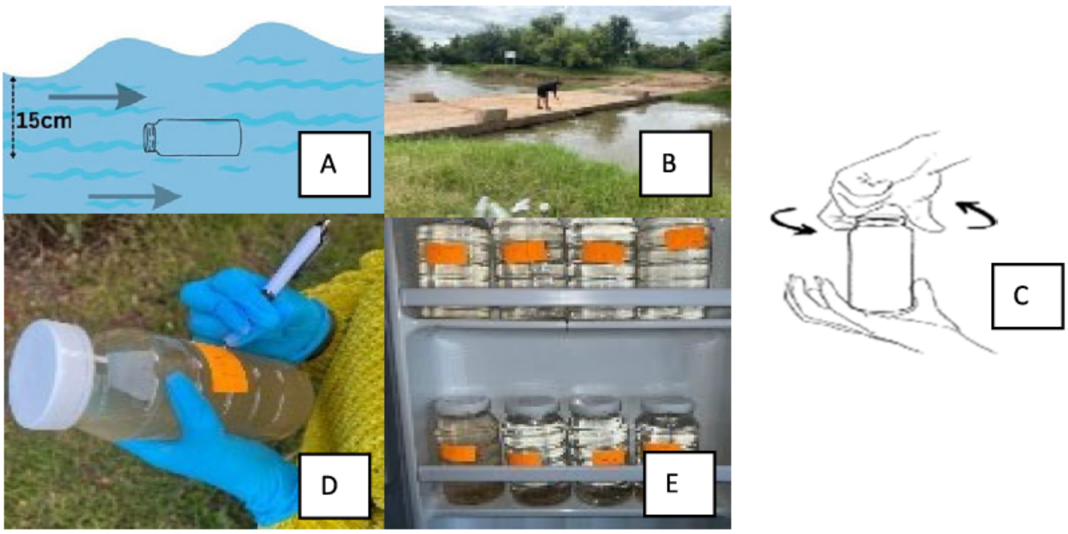

Figure 3.4: Process of collecting water samples from the sampling point (DWAF, 2009

3.3.4 Parameters analysed

All parameters, except for temperature, underwent examination at the laboratory. Temperature was measured in the field during sampling using a Portable Electronic Thermometer PET-250.1. The physical and chemical determinants analysed from winter and summer are illustrated on Table 3.1.

Table 3.1: Physicochemical parameters analysed in water samples

Parameter	Symbol	Method	Units
pH	pH	pH probe	
Electrical Conductivity	E C	E C meter	mS/m
Nitrate	NO_3^-	IC	mg/l
Nitrite	NO_2^-	IC	mg/l
Chloride	Cl^-	IC	mg/l
Flouride	F^-	IC	mg/l
Sulphate	SO_4^{2-}	IC	mg/l
Phosphate	PO_4^{3-}	IC	mg/l
Bicarbonate	HCO_3^-	Titration	mg/l
Sodium	Na	ICP-OES	mg/l
Potassium	K	ICP-OES	mg/l
Calcium	Ca	ICP-OES	mg/l
Magnesium	Mg	ICP-OES	mg/l
Boron	B	ICP-OES	mg/l

3.4 Moringa seeds preparation

Moringa oleifera seeds were obtained from Lefakong Moringa farm in Boosplaas (North West Province) and prepared immediately after harvesting. Brown seeds were used after grading, which is widely reported to reflect higher coagulation activity (Koul *et al.,* 2022; Al-Jadabi 2023). Green seeds have been reported not to have any coagulation activity (Ndabigengesere *et al.,* 1996). The seeds were oven-dried at ±25°C for 5 days. The *M. oleifera* seed kernels were obtained by cracking the seeds manually (Figure 3.5A and 3.5B). The kernels were crushed into powder using a mortar and pestle and sieved to three different particle sizes (710µm, 1000 µm and 2000 µm) (Figure 3.5 C, D, E). This approach is a minor alteration of the original method by Ghebremichael (2004).

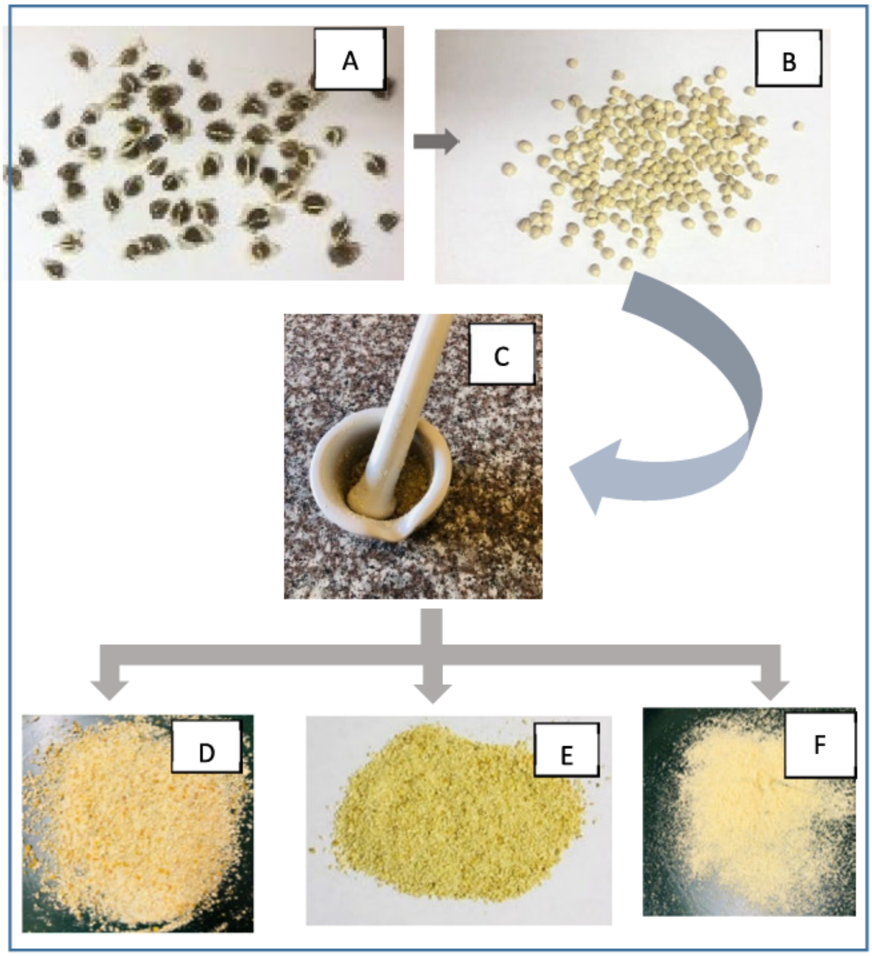

Figure 3.5: Process of preparing seeds for packing in the prototype (A) *Moringa oleifera* seeds with seed husks, (B) de-hauled seeds, (C) pestle andmortar used to mechanically grind the seed kernels, and Moringa seed power (D) 2000 µm, (E)1000 µm, (F) 710 µm. Source: Dineo Raphasha.

3.4.1 The prototype

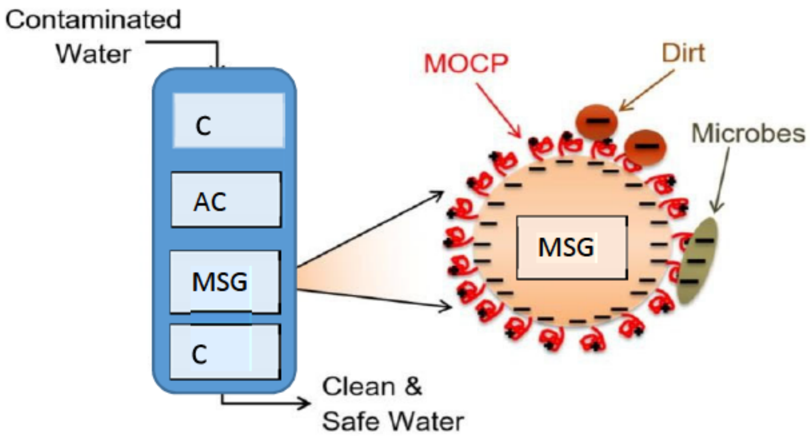

Figure 3.6: Simulated water filtering gadget where C = cotton, AC = activated charcoal, MSG = *Moringa oleifera* seed granules, MOCP = *Moringa oleifera* cationic protein. Source: Dineo Raphasha.

The prototype emanated from a large research project on a Moringa flagship program. River water samples were filtered using the prototype upon collection. A total of 500 ml of water sample was eluded through a gadget containing 20 g packed column of seed powder with 10 g activated charcoal and 9 mm depth of cotton wool (Figure 3.6). A 500 ml capacity polypropylene sterile syringe, manufactured by A AKRAF (Shenzhen, Guangdong, China) was used for the filtration process. The syringe's tube had an inner breadthof 6 mm, an outer breadth of 9 mm, and a length of 258 mm. The filtration process involved allowing water to pass through the prototype. Control samples were unfiltered.

3.4.2 Seed moisture content

A random sample of *M. oleifera* seeds were weighed to determine fresh weight. After that, the seeds were oven-dried (Scientific – economy oven 224 – 400l) at 120°C to constant weight (Reeb *et al*., 1999). The subsequent equation was utilized to compute the seed moisture content (ISTA, 2005).

Percent moisture content = 100 × (fresh seeds weight –dry seeds weight)/ fresh seeds weight

3.4.3 Bio-coagulant preparation

A pestle and mortar were used to obtain different particle sizes of *Moringa* seed powder. Dry high-quality seeds of *M. oleifera* were chosen prior to de-hauling to guarantee the use of appropriate and viable seed material.

3.5 Water quality tests

Water quality assessments were carried on the river water samples after filtration using a gadget in order to assess whether they meet specific quality standards, such as those required for human consumption. Experiments were performed to compare the water quality parameters with the generic water quality before and after filtration through the gadget. The microbial contamination, microelements, pH, and electrical conductivity were determined using standard methods (see Tables 3.1).

3.5.1 Determination of pH and electrical conductivity

The pH of the filtered and unfiltered water samples was measured by means of an HT-1202 Digital pH meter. Calibration of the pH meter was done using three buffer solutions of pH 4, 7, and 10 before measurement (Golnabi *et al*., 2009). Electrical conductivity was determined using a Bante901 Benchtop Conductivity Meter. Prior to being immersed into the sample for data collection, the probes were cleansed with distilled water, and a similar rinsing procedure was conducted before obtaining another reading from the subsequent sample.

Figure 3.7: Laboratory procedures for pH and EC analysis. Source: Dineo Raphasha.

3.5.2 Metal elements determination using inductively coupled plasma-optical emission spectroscopy (ICP-OES)

Metal elements from the water samples were analysed using an Agilent 725 Series simultaneous Inductively Coupled Plasma Optical Emission Spectrometric (ICP-OES) instrument. The chemical parameters analysed are listed in Table 3.1. Multiple elements were analysed across various wavelengths to ensure accurate results. This validation was achieved without prolonging the analysis time or using excessive amounts of the digest solution. Each element's concentration was measured at one or two emission wavelengths that were carefully selected for their sensitivity and lack of spectral interference (Lisev *et al*., 2015).

Calibration concentrations ranged from 0.0125 to 0.5 mg/L for Cd, Cr, Cu, Ni, and Pb, Mn and Zn standards spanned 0.05 to 5 mg/L, Sn ranged from 0.5 to 50 mg/L, Fe spanned from 0.1 to 10 mg/L, Hg was calibrated from 0.02 to 2 mg/L, and Al ranged from 0.2 to 2 mg/L. Standard solutions were prepared in 100 ml volumetric flasks by adding 20 ml of HNO_3^- and diluting to volume with deionised water.

Digestion was done using a 50 ml portion of each sample in a beaker. This was achieved by adding 10 ml of 55% v/v HNO_3^- and 3 ml of H_2O_2. The beaker was subsequently covered using a watch glass.The heating block's temperature was then adjusted to 120 °C, and this temperature was maintained for 1 hour to ensure thorough digestion. Following digestion, the resulting clear solutions were carefully transferred into a 50 ml volumetric flasks and subsequently diluted with deionised water to the mark. The digested samples were then stored in high- density polyethylene bottles until they were ready for analysis (Okem *et al*., 2012).

3.5.3 Determination of anions using ion chromatography (IC)

The concentration analysis of anions was carried out using the Dionex 5000^+ Ion Chromatography from Thermo Scientific, USA. The analysed anion parameters are listed in Table 3.1. The column temperature was maintained at 30°C, while the cell temperature was set to 35°C. A flow rate of 1 mL/min and an injection volume of 100 µL were used. For the separation of the target anions, the Dionex IonPac AS19 (4 mm x 250 mm) analytical column, along with the Dioex IonPac AG19 (4 mm x 50 mm) guard column, were utilized. Themobile phase consisted of potassium hydroxide (KOH) with a concentration ranging from 12 to 45 mm. All solutions were prepared using deionised water. Working standard solutions were prepared using certified 1000 mg/L solutions of chloride, fluoride, nitrate, nitrite, and sulphate purchased from Sigma Aldrich (St. Louis, United states) (Zhao *et al*., 2017).

3.5.4 Bicarbonate detection using titration

The bicarbonate ions concentration in water samples were determined using a 50 ml Erlenmeyer flask, phenolphthalein indicator solution, and standardised hydrochloric acid (HCl) solution. 50 ml of water sample was poured inside the Erlenmeyer flask, 2-3 drops of phenolphthalein indicator solution were subsequently added to the water sample, causing the solution to exhibit a pale pink colour. The addition of the HCl solution was continued drop by drop until the pink hue entirely disappeared and the solution turned colourless. This visual change indicated the completion of the bicarbonate ion reaction. The HCl solution was gradually titrated into the water sample. The volume of the HCl solution used for titration was carefully recorded (Goertzen, *et al.,* 2010). The amount of bicarbonate in the water sample was calculated using the following equation:

Bicarbonate concentration (mg/l) = (Volume of HCl solution used) × (Normality of HCl solution) × (Molar mass of HCO_3^-) / (Sample volume in litres)

3.6 Data analysis

A one-way analysis of variance (ANOVA) was done to evaluate the water quality at variations sampling points ($p \leq 0.05$). The least significant difference test was used to separate the means. Statistical analysis was conducted using R statistical package version 3.6.3 (R Core Team).

2020). Additionally, a Pearson correlation analysis was implemented to highlight potential relationships among water quality parameters.

3.7 Results

3.7.1 Conventional parameters

Notable variations ($p < 0.05$) in pH was observed on the particle sizes in the gadget and unfiltered water. In summer, the pH of the Tierpoort site for fine particles was higher than that of the Mamelodi site and the Moretele site. In winter, Moretele site had a higher pH compared to theMamelodi and Tierpoort sites for the fine particles. Overall, the summer pH was lower than the winter pH. In both seasons, notable variations were observed in the electrical conductivity (EC) values across the sites and particle sizes (Tables 3.2 and 3.3). In winter, the Moretele site exhibited higher EC and chloride on the unfiltered water, large and fine particle sizes. Sodium values were also high in winter on the unfiltered and particle sizes compared to the Mamelodi and Tierpoort sites (Table 3.3).

3.7.2 Mineral parameters (macro-elements)

Significant variations were noted in the mineral parameters between seasons. The mean Mg varied from 13.81 mg/l to 30.7 mg/L in summer and winter, respectively (Tables 3.2 and 3.3).

Table 3.2: Mean (±SE) values of physico-chemical determinants of water quality based on samples collected form the Moretele, Mamelodi and Tierpoort sampling points along Pienaars River in summer, 2021. Large, medium and fine represents the particles sizes of *M. oleifera* seed powder.

Parameter		Moretele site	Mamelodi Site	Tierpoort site	SANS 241:2015 limit	WHO limit for drinking water	conformity
pH	Unfiltered	6.98 ± 0.02[a]	6.77 ± 0.04[a]	6.76 ± 0.3[a]	≥5 to ≤9.7	6.5-8.5	Conforms
	Large	6.16 ± 0.04[a]	6.71 ± 0.01[b]	6.37 ± 0.07[a]			
	medium	6.64 ± 0.03[a]	5.61 ± 0.01[b]	6.62 ± 0.02[a]			
	Fine	6.02 ± 0.08[a]	6.01 ± 0.01[a]	6.23 ± 0.03[b]			
EC (mS/m)	Unfiltered	42.2 ± 0[b]	34.35 ± 0.15[a]	17.8 ± 0.2[c]	≤170	-	Conforms
	Large	99.75 ± 0.25[b]	80.38 ± 0.37[a]	17.85 ± 0.25[c]			
	Medium	108.5 ± 0.5[b]	92.5 ± 0.5[a]	107.5 ± 0.5[b]			
	Fine	70.5 ± 0.5[b]	65.55 ± 0.55[a]	23.9 ± 0.1[c]			
NO_3^- (mg/L)	Unfiltered	12.05 ± 0.05[a]	4.26 ± 0.53[b]	0.85 ± 0.01[c]	<49	50	Conforms
	Large	0.35 ± 0.01[a]	0.34 ± 0.02[a]	0.63 ± 0.01[b]			
	Medium	11.45 ± 1.45[a]	0.21 ± 0.01[b]	0.57 ± 6.01[b]			
	Fine	10.5 ± 0.5[a]	3.80 ± 0.8[b]	0.52 ± 0.01[c]			

NO_2^- (mg/L)	Unfiltered	0.07 ± 0.05[b]	**4.7 ± 0.24[a]**	0.05 ± 0.02[b]	<3	<3	Non-Conforms
	Large	0.02 ± 0.01[a]	0.02 ± 0.00[a]	0.03 ± 0.0[b]			
	Medium	0.02 ± 0.01[b]	0.02 ± 0.00[a]	0.01 ± 0.00[a]			
	Fine	0.05 ± 0.01[b]	0.03 ± 0.01[a]	0.02 ± 0.10[a]			
Cl^- (mg/L)	Unfiltered	36.85 ± 0.15[b]	17.35 ± 0.05[a]	8.57 ± 0.17[c]	≤300	≤500	Conforms
	Large	65.3 ± 0.05[b]	50.5 ± 0.5[a]	8.91 ± 0.01[c]			
	Medium	72.94 ± 0.05[b]	56.5 ± 1.5[a]	12.5 ± 0.5[c]			
	Fine	64.5 ± 0.5[b]	50.81 ± 0.19[a]	9.3 ± 0.01[c]			
F^- (mg/L)	Unfiltered	0.29 ± 0.05[b]	0.22 ± 0.10[a]	0.16 ± 0.01[c]	≤1.5	1.5	Conforms
	Large	0.05± 0.03[a]	0.05± 0.01[a]	0.15 ± 0.03[b]			
	Medium	0.06±0.01[a]	0.06 ± 0.02[a]	0.22 ± 0.01[b]			
	Fine	0.36 ± 0.10[b]	0.15 ± 0.03[a]	0.27 ± 0.02[c]			
SO_4^{2}- (mg/L)	Unfiltered	30.6 ± 0.1[b]	27.15 ± 0.05[a]	9.61 ± 0.02[c]	≤250/≤500	-	Conforms
	Large	26.55 ± 0.35[a]	25.75 ± 49.25[a]	11.46 ± 0.46[b]			
	Medium	29.5 ± 0.5[a]	25.5 ± 0.5[b]	8.05 ± 0.5[c]			
	Fine	19.5 ± 0.5[b]	13.7 ± 0.7[a]	8.05 ± 0.05[c]			
PO_4^{3-} (mg/L)	Unfiltered	1.9 ± 0.03[b]	0.75 ± 0.01[a]	0.1 ± 0.0[c]	-	-	Conforms

	Large	0.76 ± 0[ab]	0.13 ± 2.12[a]	0.1 ± 0.0[b]			
	Medium	0.95 ± 0.05[b]	0.05 ± 0.5[a]	0.1 ± 0[c]			
	Fine	0.14 ± 0.01[b]	0.58 ± 0.16[a]	0.1 ± 0[c]			
HCO_3^-(mg/L)	Unfiltered	149.5 ± 0.5[b]	140.5 ± 0.5a	86 ± 0.6c		-	Conforms
	Large	110.5 ± 0.5[a]	140.2 ± 1.5a	38.62 ± 38.62a			
	Medium	102.5 ± 0.5[b]	98.5 ± 0.5a	2.5 ± 0.5a			
	Fine	192.5 ± 0.5[b]	128.5 ± 0.5[a]	111.45 ± 0.45[c]			
Na (mg/L)	Unfiltered	31.65 ± 0.05[b]	15.05 ± 0.05[a]	5.92 ± 0.02[c]	≤200	20	Conforms
	Large	20.51 ± 0.49[a]	15.2 ± 0.02[a]	3.9 ± 0.3[b]			
	Medium	13.1 ± 0.2[b]	13.5 ± 0.5[b]	2.5 ± 0.5[a]			
	Fine	9. 3 ± 1[b]	9. 2 ± 2[b]	2.2 ± 0[a]			
K (mg/L)	Unfiltered	7.28 ± 0.20[b]	3.32 ± 0.02[a]	1.89 ± 0.02[a]	≤50	3000	Conforms
	Large	22.1 ± 4.89[c]	3.31 ± 0.02[a]	2.12 ± 0.12[a]			
	Medium	16.5 ± 0.50[b]	13.95 ± 0.05[b]	19.5 ± 0.5[c]			
	Fine	19.35 ± 0.05[c]	8.1 ± 0.01[b]	2 ± 0.10[a]			
Ca (mg/L)	Unfiltered	28.65 ± 0.05[b]	27.7 ± 0.03[b]	14.65 ± 0.05[a]	≤150	-	Conforms
	Large	28.95 ± 3.43[b]	27.85 ± 0.05[b]	15.5 ± 0.5[a]			
	Medium	31.5 ± 0.5[c]	28.01 ± 1.10[b]	31 ± 1.13[c]			
	Fine	29.55 ± 0.64[b]	30.08 ± 0.5[c]	15.8 ± 0.14[a]			
Mg (mg/L)	Unfiltered	14.4 ± 0.01[b]	17.45 ± 0.15[a]	9.7 ± 0.11[c]	≤70	≤50	Conforms
	Large	20.02 ± 1.17[a]	17.45 ± 0.05[a]	9.95 ± 0.03[c]			

	Medium	29.5 ± 0.5^{b}	21.5 ± 1.5^{a}	29.5 ± 0.5^{b}			
	Fine	29.55 ± 0.35^{b}	18.75 ± 0.25^{a}	8 ± 0.01^{c}			
B (mg/L)	Unfiltered	0.04 ± 0.03^{b}	0.02 ± 0.01^{a}	0.01 ± 0.00^{c}	<2400	2.4	Conforms
	Large	0.03 ± 0.01^{b}	0.01 ± 0.14^{a}	0.01 ± 0.00^{a}			
	Medium	0.03 ± 0.02^{b}	0.01 ± 0.11^{a}	0.01 ± 0.00^{a}			
	Fine	0.02 ± 0.03^{b}	0.01 ± 0.02^{b}	0.01 ± 0.00^{c}			

Note: "Conforms" means that the parameter meets both SANS 241:2015 Limit and WHO Guidelines for drinking water
Unfiltered = unfiltered water (control)
Means highlighted in bold means "non- conformance"
Means in the same row indicated by different letters are statistically significant ($p \leq 0.05$).

Table 3.3: Mean (±SE) values of the physico-chemical determinants of water quality based on samples collected form the Moretele, Mamelodi and Tierpoort sampling points of Pienaars River in winter, 2021. Large, medium and fine represents the particles sizes of *M. oleifera* seed powder.

Parameter	Treatment	Moretele site Results	Mamelodi Site	Tierpoort	SANS 241:2015 Limit	WHO Guidelines for Drinking Water	conformity
pH	Unfiltered	7.63 ± 0.01^{b}	7.51 ± 0.01^{a}	7.73 ± 0.01^{c}	≥5 to ≤9.7	≤6.5-8.5	Conforms
	Large	6.14 ± 0.12^{ab}	5.57 ± 0.32^{b}	7.73 ± 0.02^{c}			
	Medium	7.19 ± 0.06^{a}	7.03 ± 0.02^{a}	7.5 ± 0.24^{a}			
	Fine	6.06 ± 0.04^{a}	5.35 ± 0.02^{b}	6.01 ± 0.14^{a}			
EC (mS/m)	Unfiltered	74.78 ± 1.93^{a}	70.53 ± 0.47^{a}	21.5 ± 1.5^{b}	≤170	-	N/A
	Large	100.3 ± 0.7^{b}	80.75 ± 0.75^{a}	21.9 ± 1.9^{c}			
	Medium	71.8 ± 1.7^{a}	66.45 ± 1.25^{a}	22.15 ± 1.65^{b}			
	Fine	74.1 ± 0.9^{b}	109.5 ± 0.5^{a}	$94.3 \pm 5.5a^{b}$			
NO_3^- (mg/L)	Unfiltered	27.5 ± 1.5^{b}	2.16 ± 0.01^{a}	0.7 ± 0.05^{a}	<49	<50	Conforms
	Large	39.55 ± 1.15^{a}	0.35 ± 0.01^{b}	0.64 ± 0.11^{b}			
	Medium	36.25 ± 1.05^{a}	0.30 ± 0.65^{b}	0.54 ± 0.00^{b}			
	Fine	0.88 ± 0.03^{a}	33.05 ± 1.05^{b}	0.21 ± 0.0^{a}			
NO_2^- (mg/L)	Unfiltered	2.9 ± 0.01^{b}	0.7 ± 0.01^{a}	0.02 ± 0.01^{c}	<3	<3	Conforms
	Large	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}			

	Medium	0.02 ± 0.02[a]	0.02 ± 0.02[a]	0.02 ± 0.02[a]			
	Fine	0.02 ± 0.02[a]	0.02 ± 0.02[a]	0.02 ± 0.02[a]			
Cl^- (mg/L)	Unfiltered	71.2 ± 1.7[b]	52.75± 0.35[a]	8.89 ± 0.39[c]	≤300	<500	Conforms
	Large	65.35 ± 1.15[b]	52 ± 1.1[a]	8.91 ± 0.41[c]			
	Medium	64.55 ± 1.35[b]	50.65± 1.25[a]	9.66 ± 0.34[c]			
	Fine	11.35 ± 0.45[c]	73.55± 1.05[b]	58.4 ± 0.5[a]			
F- (mg/L)	Unfiltered	0.31 ± 0.02[a]	0.26 ± 0.01[a]	0.15 ± 0.03[b]	≤1.5	≤1.5	Conforms
	Large	0.02 ±0.01[a]	0.03 ± 0.02[a]	0.16 ± 0.04[b]			
	Medium	0.36 ± 0.01[b]	0.16 ± 0.01[a]	0.28 ± 0.01[c]			
	Fine	0.04 ± 0.00[a]	0.04 ± 0.00[a]	0.03 ± 0.01[a]			
SO_4^{2}- (mg/L)	Unfiltered	27.5 ± 1.5[b]	2.16 ± 0.01[a]	0.7 ± 0.05[a]	≤250/≤500	-	Conforms
	Large	296.5 ± 2.5[b]	196.5 ± 1.5[a]	11.95 ± 1.85[c]			
	Medium	60.8 ± 0.2[b]	64.55± 0.45[a]	13.35 ± 0.45[c]			
	Fine	170.50 ± 5.5[b]	133 ± 0.5[a]	130 ± 0.5[ab]			
PO_4^{3}- (mg/L)	Unfiltered	8.7 ± 0.34[b]	3.88 ± 0.07a	0.1 ± 0.00 [c]	-	-	Conforms
	Large	23.4 ± 0.4[b]	10.65± 0.55[a]	0.1 ± 0.00[c]			
	Medium	7.21 ± 0.22[b]	4.75 ± 0.2a	0.1 ± 0.00[c]			
	Fine	41.75 ± 0.05[b]	24.75± 0.15[a]	0.10 ± 1.25[c]			
HCO3-	Unfiltered	171.5 ± 11.5[a]	204 ± 4.03[a]	92.25± 5.35[b]			Conforms

(mg/L)							
	Large	90.36 ± 1.20^{b}	77.25± 1.65^{a}	110.5 ± 1.5^{c}			
	Medium	193.5 ± 1.5^{b}	218.5 ± 1.5^{a}	113.5 ± 1.5^{c}			
	Fine	104 ± 1.10^{a}	99.9 ± 0.1^{a}	32.15± 0.55^{b}			
Na (mg/L)	Unfiltered	83.95 ± 1.35^{b}	62.19± 2.01^{a}	9.45 ± 0.53^{c}	≤200	≤200	Conforms
	Large	77.85 ± 0.65^{b}	61.2 ± 1.1^{a}	21.8 ± 1.3^{c}			
	Medium	94.5 ± 5.2^{a}	94.55± 4.65^{a}	22.6 ± 0.5^{b}			
	Fine	10.04 ± 0.05^{c}	84.4 ± 0.9^{b}	65.2 ± 0.7^{a}			
K (mg/L)	Unfiltered	13.74 ± 1.34^{a}	9.28 ± 0.25^{a}	0.95 ± 0.03^{b}	≤50	3000	Conforms
	Large	15.95 ± 1.65^{b}	39.1 ± 0.2^{a}	2.26 ± 0.25^{c}			
	Medium	9.55 ± 0.05^{b}	8.27 ± 0.01^{a}	2.13 ± 0.02^{c}			
	Fine	14.52 ± 0.48^{b}	20.3 ± 0.5^{a}	3.25 ± 0.45^{c}			
Ca (mg/L)	Unfiltered	39.55 ± 6.65^{b}	30.48± 1.42^{a}	20.3 ± 0.8^{a}	≤150	-	Conforms
	Large	41.5 ± 1.4^{b}	34.15± 1.35^{a}	25.05 ±0.75^{a}			
	Medium	40.55 ± 0.65^{b}	46.8 ± 0.5^{b}	35.35± 0.45^{a}			
	Fine	13.75 ± 0.85^{c}	32.3 ± 0.6^{b}	21.8 ± 0.5^{a}			
Mg (mg/L)	Unfiltered	21.8 ± 1.1^{ab}	22.05± 1.45^{a}	13.8 ± 1^{c}	≤70	0.08	Conforms
	Large	21.25 ± 0.55^{a}	28.85± 1.55^{b}	17.15 ± 0.65^{c}			

	Medium	26 ± 0.8^{a}	22.55± 0.35^{a}	18.11± 0.31^{b}			
	Fine	21.2 ± 0.6^{a}	30.7 ± 0.7^{b}	23.35± 0.45^{a}			
B (mg/L)	Unfiltered	0.07 ± 0.01^{ab}	0.01 ± 0.00^{b}	0.03 ± 0.01^{a}	<2400	2.4	Conforms
	Large	0.05 ± 0.01^{b}	0.01 ± 0.00^{c}	0.02 ± 0.01^{a}			
	Medium	0.06 ± 0.01^{b}	0.01 ± 0.00^{c}	0.03 ± 0.01^{a}			
	Fine	0.05 ± 0.02^{b}	0.01 ± 0.0^{a}	0.01 ± 0.01^{a}			

Note: "Conforms" means that the parameter meets both SANS 241:2015 Limit and WHO Guidelines for Drinking Water
Means in the same row indicated by different letters are statistically significant ($p \leq 0.05$).
Unfiltered = unfiltered water (control)

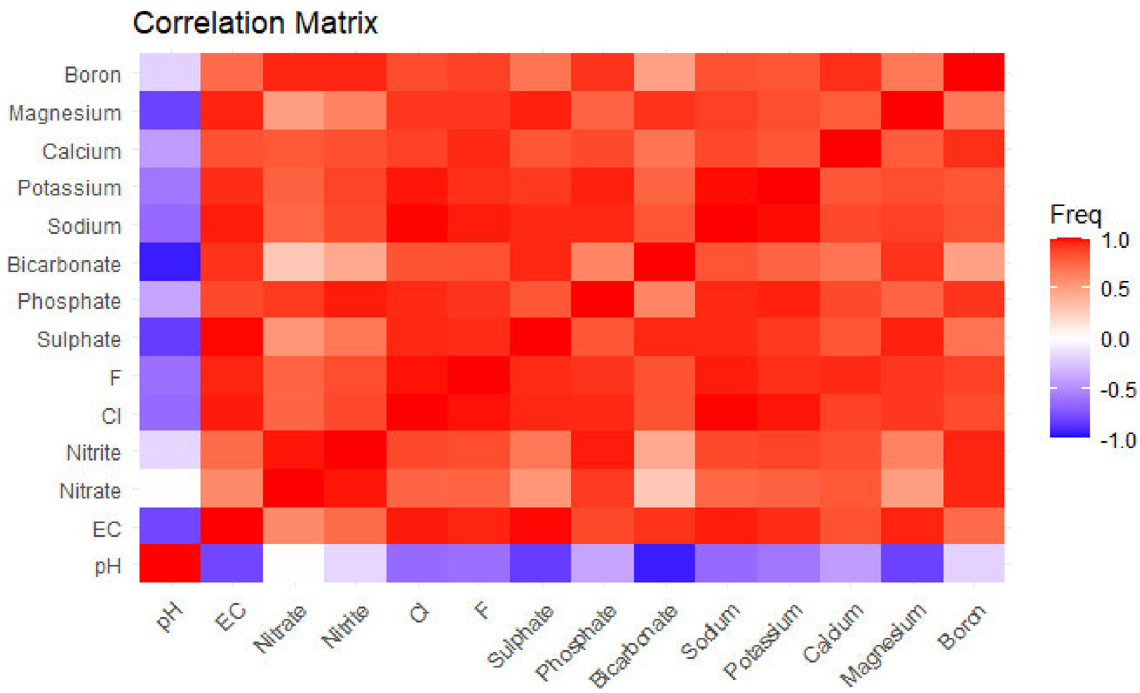

Figure 3.8: Correlation matrix among different physico chemical parameters in the three sampling points along Pienaars River in summer, 2021. Red colour (r = 1.0) represents a strong positive correlation; faint red (r = 0.5) represents weak positive correlation; white colour (r = 0.0) represents no correlation; faint blue(r = -0.5) represents weak negative correlation, and blue (r = -1.0) represents strong negative correlation.

Correlation analysis was done to determine the relationship of the physicochemical parameters after filtration. The different particle sizes data from the three sampling points in summer were combined to calculate the correlation matrix shown in Figure 3.8. Correlation coefficient on different physico-chemical parameters revealed that pH exhibited a strong negative correlation with bicarbonate (r = -0.946) and strong to moderate negative correlations with sulphate (chloride, and sodium (range in r -values: 0.646 – 0.838). Conversely pH exhibited a moderate positive correlation with EC (r = -0.796) and a slight positive correlation with nitrite (r = -0.175). EC showed strong positive correlations with sodium, chloride, and fluoride (range in r -values: 0.961 – 0.975). EC also displayed a moderate positive correlation with nitrate (r = 0.601) and nitrite (r = 0.733).

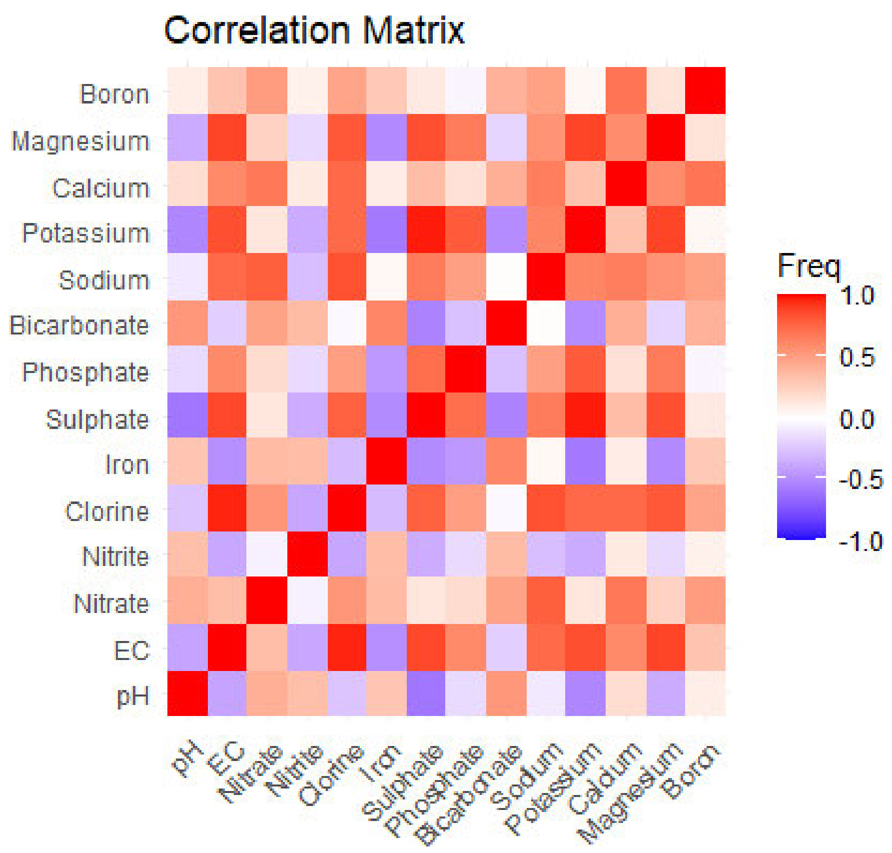

Figure 3.9: Correlation matrix analysis of water quality parameters in three sampling points along the Pienaars River in winter, 2021. Red colour (*r* = 1.0) represents a strong positive correlation; faint red (*r* = 0.5) represents weak positive correlation; white colour (*r* = 0.0) represents no correlation; faint blue (*r* = -0.5) represents weak negative correlation, and blue (*r* = -1.0) represents strong negative correlation.

The correlation results for the water quality parameters revealed several significant relationships. The pH exhibited weak to moderate positive correlations with nitrate, chlorine, bicarbonate, and calcium (range in *r* = 0.178 to 0.532), while showing negative correlations with sulphate, potassium, and magnesium (range in *r* = -0.592 to -0.359). Electrical conductivity displayed strong positive correlations with pH (*r* = -0.397) and iron (*r* = -0.482). In summer, EC exhibited strong positive correlations with sodium (*r* = 0.973), chloride (*r* = 0.975), and fluoride (*r* = 0.961).

3.8 Discussion

The results of the three sampling points along Piernaars river for various chemical quality parameters including pH, EC, NO_3^- , Cl^- , F^-, SO_4^{2-}, HCO_3^- Na, K, Ca, Mg and B were compliant with the set standards for drinking water in both seasons. However, there was high concentrations of nitrite obtained from the Mamelodi site before filtration, which may have been a result of contamination from fertilized soil since the area is situated near Roodeplaat and Cullinan agricultural farms. Other sources may be from animal feedlots, septic systems, or urban drainage. Mamelodi is situated in an industrial area and waste from these areas may contribute nitrate concentrations of up to 5 mg/L. Normal surface water nitrate concentrations should be from 0 to 18 mg/L, but can increase due to contamination by agricultural activities and human or animal wastes (WHO, 2007). In cases where the river is supplied by nitrate rich sources, the concentration may rise and fall often with the seasons. Moreover, use of nitrogen-containing compounds in households and industries may also contribute to higher nitrite in water. Elevation of nitrite can also occur from natural sources such as soil and water, and in some cases, it may be present in river water because of decomposition or nitrogen fixation. Environmental conditions may also elevate the level of Nitrite in water, environmental conditions such as low oxygen levels, can promote the conversion of nitrate to nitrite in river water. These results of high nitrite are similar to past study of Maruapula (2020). By observation, the Mamelodi site also depicted considerable human activities including African and spiritual rituals, waste dumping and heavy pollution from the nearby communities.

Figure 3.10: Spiritual rituals at the Mamelodi sampling point. Source: Dineo Raphasha

There was a reduction in pH values as the crushed *M. oleifera* seed increased in both seasons. Similar results were obtained by Semanka *et al.* (2022), where the investigation of the influence of *M. oleifera* seeds on the physicochemical characteristics and microbiological safety of borehole water from Botswana was studied. The observed lower pH values during the summer season could potentially be attributed to rainfalland run-off, as suggested by Shabalala *et al.* (2013). The finding of high EC, sodium and chloride in Moretele water is consistent with the results obtained by Mpenyana-Monyatsi and Momba (2012), who identified high sodium content in water from Moretele and Madibeng area. Additionally, the study found that filtration with *M. oleifera* seed particle size of 710 µm resulted in higher concentrations of Mg, as recorded in a previous study by Morgan *et al.* (2020). This suggests that the addition of *M. oleifera* seed powder causes the concentration of metals in treated water to increase, which is inconsistent with the findings of Nand *et al*. (2012), who found that *M. oleifera* seed has the potential to adsorb metals in the treatment of turbid water. Agricultural run-off throughout the rainy season could lead to an increase in conductivity, primarily stemming from the existence of chloride, phosphate, and nitrate compounds within the water. It is also important that chloride does not impose any health-related implications; however, the primary concern lies in its propensity to elevate the corrosion rate of household appliances (WHO, 2011). Furthermore, the Ca concentrations of the water samples increased after filtration with *M. oleifera* seed powder in both seasons. This phenomenon could be ascribed to the existence of potassium, sodium, magnesium, calcium, and other elements present in *M. oleifera* seeds as noted by El-

Hack *et al.* (2018) and Liang *et al*. (2019).

The positive correlation observed from the winter season indicates that as the pH of river water increases, the concentration of chlorine also tends to increase. This relationship suggest that higher pH levels affect the behaviour of chlorine as a disinfectant in the water (Doederer *et al.,* 2014). The increase in sulphate, chloride, and sodium may have been from the anthropogenic influences happening around the Mamelodi area such as industrial discharges, sewage effluents, and use of fertilizers (Van der Merwe, 2003). The moderate negative correlation between pH and EC and a weak negative correlation between pH and nitrite implies that higher pH values are associated with increased EC and nitrite levels. Moreover, elevated EC levels signify the presence of dissolved salts or minerals in the river water. This suggests that the mineral composition of the water changed during the seasons.

3.9 Conclusion

The findings of the chapter indicate that the water quality in the three sites analysed (Moretele, Mamelodi, and Tierpoort) is generally good and safe. However, slight variations were observed at the Mamelodi site in Gauteng, whereby the nitrite levels before filtration were higher than the set standard, which can be as a result of urbanisation and industrialisation in the area. Filtration with all particle sizes of *M. oleifera* seed powder decreased the nitrite level in Mamelodi sampling point. Despite these variations after filtration with Moringa seed powder, the water quality parameters, including pH, EC, NO_3^-, Cl^-, F^-, SO_4^{2-}, Na, K, Ca, Mg, and B, all fell within the acceptable limits for drinking water as prescribed by SANS241:2015 and WHO (2011).

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CHAPTER 4: EFFECT OF FILTRATION GADGET ON THE MICROBIAL LOAD OF WATER OBTAINED FROM THREE POINTS ON THE PIENAARS RIVER

Abstract

Water should not contain pathogens and toxic chemicals in order to be considered fit for household use, such as cooking and drinking. *Moringa oleifera* seeds have been found to contain natural coagulants that can effectively remove contaminants from water. In this study, a water purification gadget using *M. oleifera* seeds was developed as a cost efficient option for providing safe drinking water in resource-limited settings. Seeds of the plant contain a natural coagulant protein, *Moringa oleifera* cationic protein (MOCP) that can bind with impurities and suspended particles in water. The MOCP can attract negatively charged water particles such as soil, clay, and organic matter, and turn them into larger particles that can be easily removed. The collection of water samples was done in winter (July) and summer (February) from the Tierpoort, Mamelodi and Moretele sites along the Pienaars River in North West and Gauteng Provinces of South Africa. The experiment involved using three particle sizes of ground *M. oleifera* seeds to sieve the water specimens, which were filtered and evaluated for microbiological determinants by means of the viable plate count technique. Results showed that the quantity of coliform forming units (CFU) in the filtered samples and the controls were higher than those recommended in the guidelines of the World Health Organisation (2010) and South African National Accreditation System (SANS 241:2015), which both indicate the requirement of CFU values of no greater than zero. Treatment through fine particles of *M. oleifera* seeds resulted in lower microbial counts compared to the medium and large particles. The fine particles size higher surface area-to-volume ratio provides more attachment sites for microorganisms. The increased surface area can facilitate the adsorption and retention of microorganisms, leading to lower microbial counts in the water sample.

Keywords: Bio-coagulant, cationic protein, microbiological determinants, Pienaars River,water standards.

4.1 Introduction

Water is an essential resource for human survival, and the quality of that water is vital to human health. However, water from natural sources such as rivers may contain a high microbial load, which can lead to waterborne diseases (Okwelle *et al.,* 2022; Fatima *et al.,* 2022; Mussa and Kamoto, 2023). Microbial contamination of water is a frequent issue that results to poor public health (Lapworth *et al.,* 2022). Worldwide ecological harm and threats to human livelihoods have been linked to river pollution from untreated sewage and industrial effluent (Hoque *et al.,* 2021). Therefore, filtration gadgets are devices that have been designed to remove impurities in water, including microorganisms.

While achievements have been made in terms of expanding access to safe drinking water, millions of people still suffer from a number of health-related, treatable diseases as a result of drinking contaminated water (Edokpoyi *et al.,* 2020). In many remote areas of Southern Africa, boreholes are still being used as a source for drinking water. Water from these origins is often consumed without further treatment (Obi *et al*., 2002; Nogueira *et al.,* 2003). The quality of borehole water can vary widely based on various factors, including the geographical and hydrological features of the area, the depth and location of the borehole, and anthropological activities in the surrounding area. Thus, the health of the consumers from this source is potentially at considerable risk. For example, Taonameso *et al.* (2019) revealed the dangers of consuming water from boreholes, which may not always provide clean water and may contain bacteria and other contaminants.

Although the South African Constitution declares that everyone has the right to access clean and safe drinking water, millions of South Africans do not have sustainable access to a supply of drinkable water (South African Government, 1996; du Plessis, 2023). The Department of Water and Sanitation (DWS) in South Africa oversees regulation and monitoring of the quality of borehole water. The department has established various guidelines and standards forthe quality of drinking water, including the South African National Standards (SANS), which set the drinking water quality standards and guidelines for South Africa (SANS:241:2015).

Despite these guidelines and regulations, the efficacy of borehole water intended for drinking in South Africa is often a concern due to contamination by various sources such as agricultural activities, mining, and waste disposal. Additionally, some rivers may be located in areas with high levels of natural contaminants such as fluoride or arsenic, which can pose a health risk if consumed in excess (Edokpayi *et al.,* 2018; Odiyo *et al.,* 2020).

The three sites selected for this study are known to have varying levels of pollution, which can affect the microbial load in the water (Gowar, 2016; Mlotshwa, 2018). The Pioneers River is a significant water source for domestic and agricultural use in Mamelodi and Moretele areas (Orton, 2010). The river receives wastewater and runoff from different origins, comprising of industrial and agricultural activities. Therefore, the microbial load in the river water may be high, resulting in pollution of water sources downstream.

Utilizing *Moringa oleifera* seeds for water purification has been shown to be effective in various parts of the world and may be a viable solution to address the worldwide water crisis (Varkey, 2020). Filtration gadgets are a low-cost technology that can be used to eliminate microbial pollutants from water.

4.2 Aims and objectives

This study sought to determine the effect of a filtration gadget on the microbial load of water obtained from three sampling point along Pioneers River. The chapter addresses the following specific objective:

I. To determine the effectiveness of the gadget in reducing the microbial load.

4.3 Materials and methods

4.3.1 Sample collection

Water collection was done using sterile water bottles as highlighted in section 3.2. Samples were collected once in June (winter) and once in February (summer) in 2021. The biological parameters of water collected from the summer and winter are illustrated on Table 4.1.

Table 4.1: Biological parameters analysed and the method of analysis

Parameter	Units	Method of Analysis
Total coliforms	CFU/100 ml	Viable plate count
Escherichia coli	CFU/100 ml	Viable plate count
Salmonella	CFU/100 ml	Viable plate count
Shigella	CFU/100 ml	Viable plate count

The microbial contaminants present in river water samples were evaluated before and after filtration with *Moringa oleifera* seed powder. The analysis of the water samples included the examination of microbiological determinants such as total coliforms and amounts of each of *Escherichia coli, Salmonella* spp. and *Shigella* spp. The method used for this analysis was the Plate Count Method (Table 4.1).

4.3.2 Microbiological analysis of samples

The samples were subjected to dilution series to obtain various dilutions. From each dilution, aliquots were plated on agar plates. Semi-automatic digital device was used to count the colonies. The microbial population was quantified in terms of bacterial count per millilitre (CFU/ml).

4.3.3 Sample dilution

To prepare the initial dilution, 1 ml of the sample was added to a 99 ml sterile saline solution, making a 1/100 (or 10^{-2}) dilution. The dilution was vigorously mixed to disperse the bacteria and dissolve any aggregates. Then, 1 ml from the 10^{-2} dilution was moved to a second 99 ml saline solution, creating a 10^{-4} dilution. This procedure was continued until a 10^{-8} dilution was obtained.

4.3.4 Plating and incubation

One petri dish received 1 ml of each dilution sample, while another received 0.1 ml. To support bacterial growth for *E. coli* and total coliforms the samples were thoroughly blended with nutrient agar which was previously heated at 48-50°C in a water bath. Xylose Lysine Deoxycholate (XLD) agar was used to determine the presence of *Salmonella* and *Shigella* in water samples. To promote microbial growth, the plates were then turned over and kept at 25°C for 48 hours (Rolfe *et al.,* 2012).

4.3.5 Colony counting

The treatments analysed were 4, replicated 4 times per site. The petri plates with 30 to 300 colonies were chosen for counting after incubation. Plates with more than 300 colonies were deemed too many for an accurate count, while plates with fewer than 30 colonies were deemed insufficient for a reliable statistical analysis. An automated colony counter was used to count the colonies on each plate (ZR-1101) (Zhu *et al.,* 2018). The following equation was used to calculate:

Number of bacteria (CFUs) = Dilution × Number of colonies on plate

4.4 Data analysis

A one way analysis of variance (ANOVA) was done to determine the water quality at different sampling points ($p \leq 0.05$). The Least Significant Difference (LSD) was used to separate the means. A statistical analysis was conducted using the R statistical package version 3.6.3 (R core team, 2020).

4.5 Results

Figures 4.1 to 4.8 show the microbiological results of the water samples at the three locations along Pienaars River in winter and summer during 2021. In both seasons, the analysis of unfiltered water samples (Figures 4.1 and 4.8) revealed elevated counts of total coliforms, *Salmonella* spp., *Shigella* spp., and *E. coli.* Mamelodi had the highest amounts of *Salmonella* spp., *E. coli* and total coliforms (3.9×10^4, 7 and 7.0×10^4 CFU) in summer and winter (2.3×10^4, 11 and 7.0×10^4, 1.5×10^4 CFU). After filtration with the gadget, the microbiological load and values of the physicochemical parameters of the water samples decreased. There were significant variations in the microbiological load between the filtered samples and the unfiltered in all test samples ($p < 0.05$), except for Mamelodi, on the values for *Shigella* and *E. coli*. Among the three sites, the finest particle size resulted in the lowest microbial counts (Figure 4.1 to 4.8) in both seasons. There was a high level of *Shigella* at the Mamelodi sampling point in winter (Figure 4.8) and Tierpoort in summer (Figure 4.7).

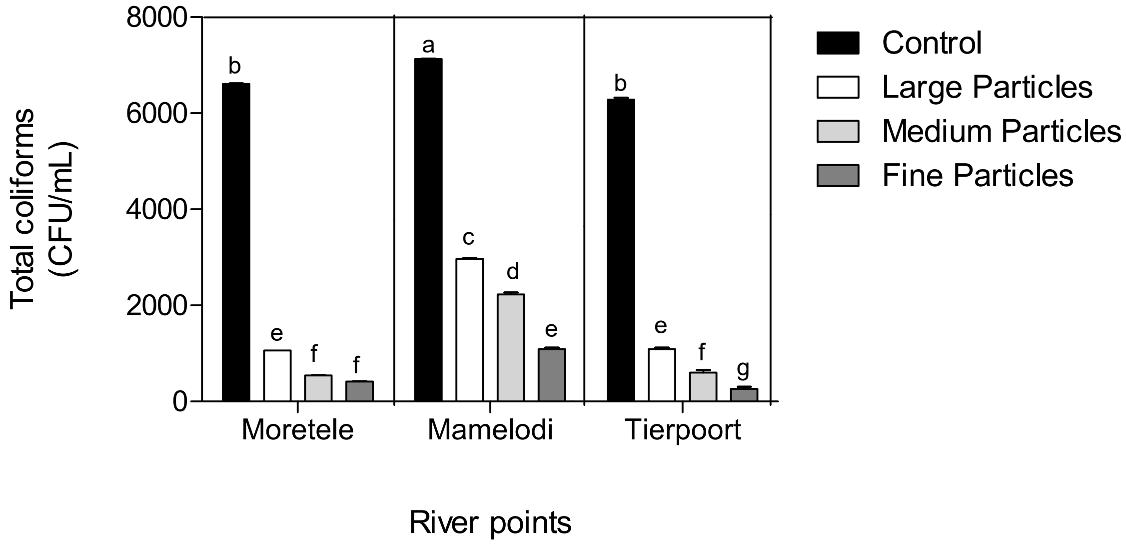

Figure 4.1: Total coliform counts in water collected from three sampling points along the Pienaars River during the summer. Means with varying letters differ significantly ($p \leq 0.05$). Control was unfiltered.

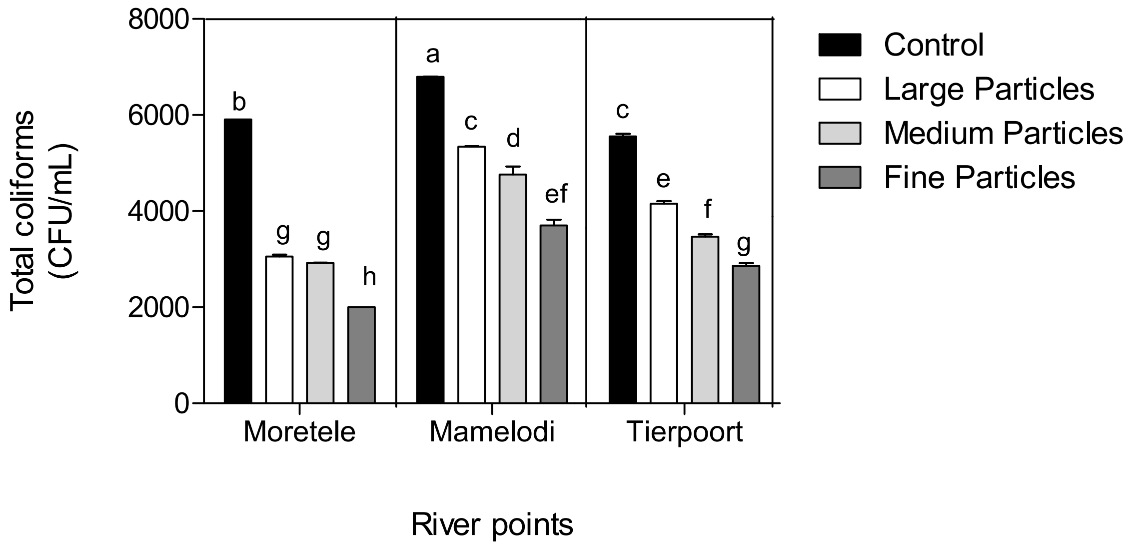

Figure 4.2: Total coliform counts in water collected from three sampling points during the winter season. Means with varying letters differ significantly ($p \leq 0.05$). Control was unfiltered.

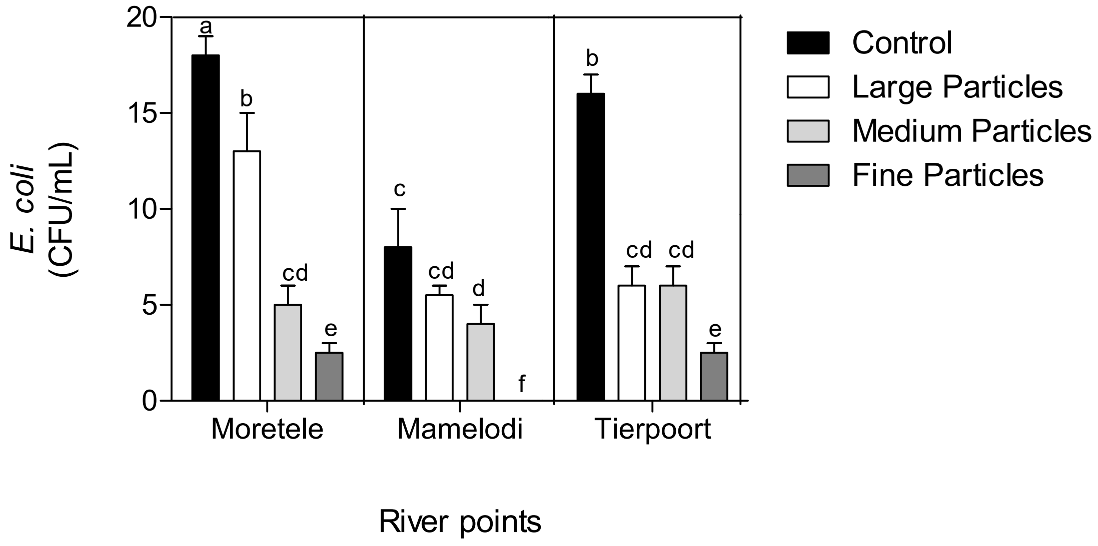

Figure 4.3: *Escherichia coli* counts in water collected from three sampling points along Pienaars River during summer. Means with varying letters differ significantly ($p \leq 0.05$). Control was unfiltered.

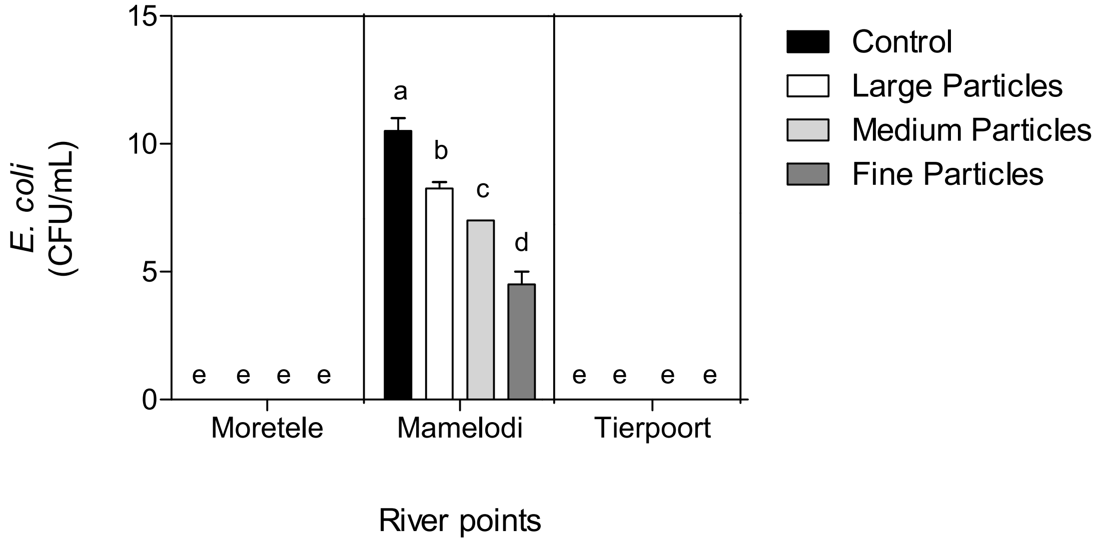

Figure 4.4: *Escherichia coli* counts in water collected from three sampling points along Pienaars River during the winter season. Means with varying letters differ significantly ($p \leq 0.05$). Control was unfiltered.

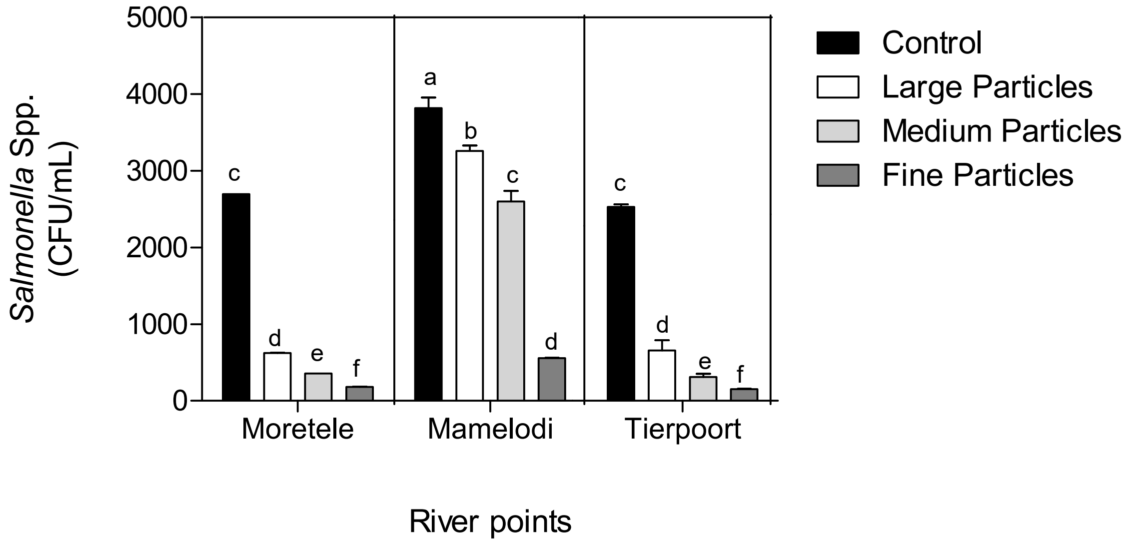

Figure 4.5: *Salmonella* counts in water collected from three sampling points along Pienaars River during the summer. Means with varying letters differ significantly ($p \leq 0.05$). Control was unfiltered.

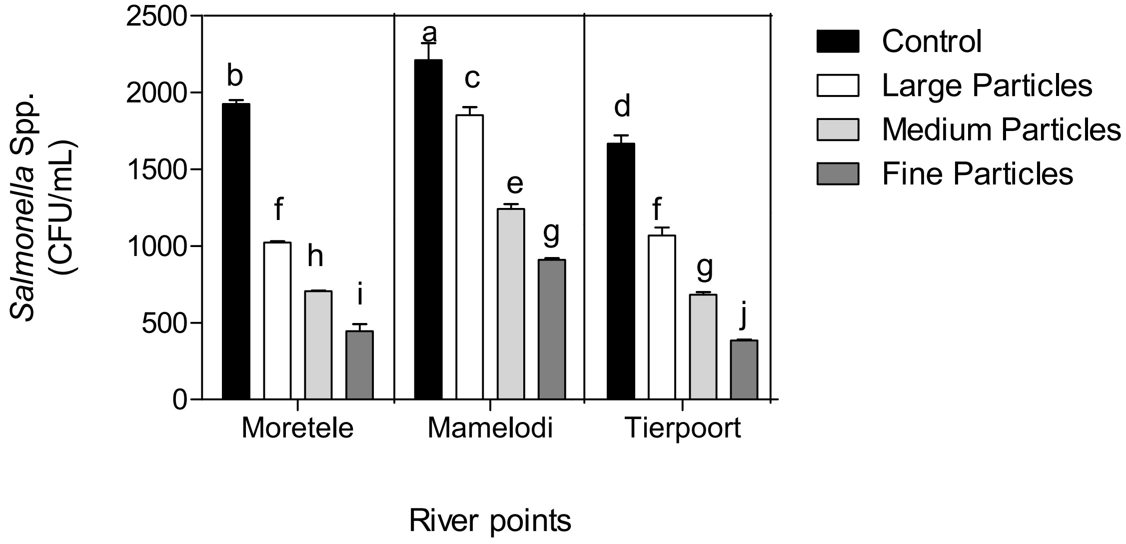

Figure 4.6: *Salmonella* counts in water collected from three sampling points along Pienaars River during the winter season. Means with varying letters differ significantly ($p \leq 0.05$). Control was unfiltered.

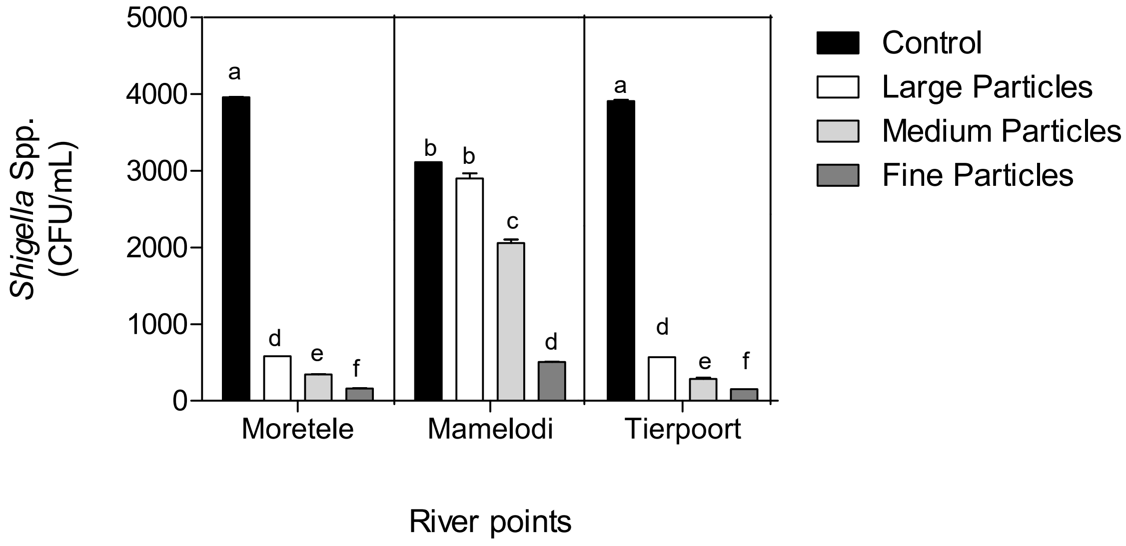

Figure 4.7: *Shigella* counts in water collected from three sampling points along Pienaars River in summer. Means with varying letters differ significantly ($p \leq 0.05$). Control was unfiltered.

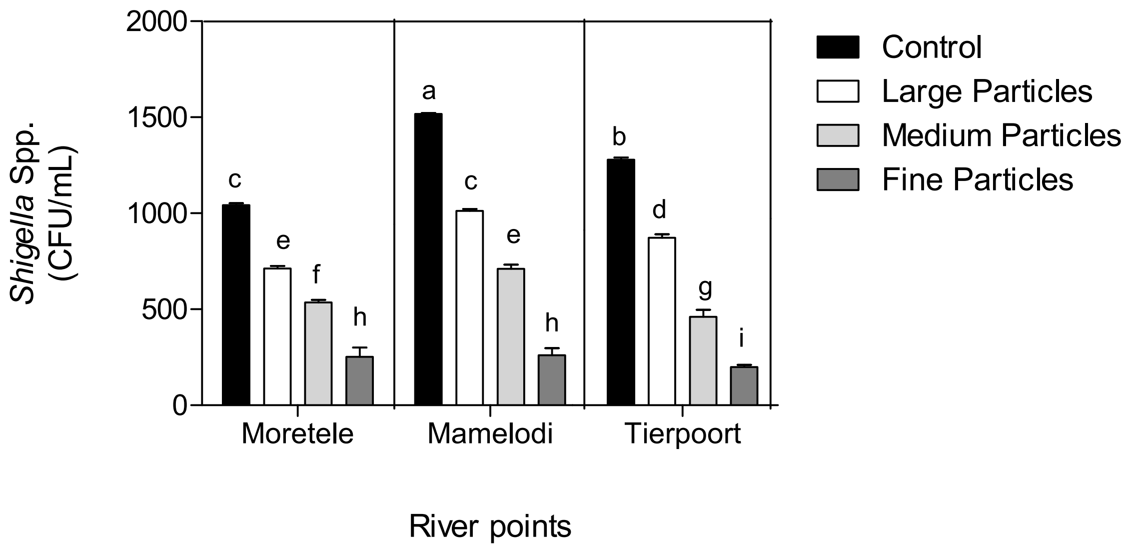

Figure 4.8: *Shigella* counts in water collected from three sampling points along Pienaars River during winter season. Means with varying letters differ significantly ($p \leq 0.05$). Control was unfiltered.

4.6 Discussion

The samples from Moretele, which is downstream, had the most colony forming units (CFU) for *E. coli* in summer, this could be due to high rainfall, leading to increased runoff from surrounding areas. This runoff can carry pollutants, including faecal matter containing *E. coli,* into the sampling point. The results obtained by the DWS (2022) correlates with the results obtained in this study where Moretele sampling point had the highest counts of *E. coli.*

The winter results were characterised by high levels of *E. coli* in the Mamelodi samples (10 CFU in the control) and no CFU in the Moretele and Tierpoort samples. The winter results also exhibited higher levels of total coliforms in the Mamelodi samples compared to the other two sites. This was the same trend for *Salmonella* spp. and *Shigella* spp.

The microbiological results of the water samples collected before purification with *M. oleifera* seeds showed a high microbial count. The overall CFU for *Salmonella*, *Shigella* and *E. coli* were reduced after treatment with *M oleifera* seed powder. However, the overall CFU in water was higher than the WHO (2017) limit of 0 CFU/100 ml for the quality of drinking water, which indicated the presence of pathogens and unsuitability of the water for drinking. Delelegn *et al*. (2018) reported similar findings in which they observed a decrease in microbial growth in water samples treated with *M. oleifera* seed powder. Higher level of *E. coli* and *Salmonella* present in the samples could be due to sewage spills from municipalities, septic leachate, agricultural runoff, storm water runoff, wildlife, and nonpoint sources of human and animal waste. There high level of *Shigella* at the Mamelodi site in winter and Tierpoort in summer is likely due to contamination by human activities. Activities such as swimming, fishing, and boating, can introduce *Shigella* into the river water. This could be because the Mamelodi sampling point feeds from Roodeplaat dam, and Tierpoort sampling point passes through Nkwe resort where these recreational activities are carried out. Other sources may be environmental such as warm water temperatures and low flow rates. These can promote the growth and survival of *Shigella* bacteria in river water (Chua *et al.,* 2021; Hazra *et al.,* 2022).

4.7 Conclusion

This chapter confirms the second objective of the study, which is to demonstrate the water purification potential of the gadget using *M. oleifera* seeds. Findings revealed that the gadget reduced the number of bacterial colonies in the filtered water. However, is should be noted that that all samples, both filtered and unfiltered, contained CFU counts higher than the recommended values set by the World Health Organisation and South African National Accreditation System. Based on the particle sizes tested in this study, the finest particle size of 710 µm was the most effective in reducing the CFU count in the water. Therefore, further improvements are needed to enhance the filtering ability of the gadget, possibly by decreasing the particle size further and increasing the quantity of *M. oleifera* seeds used.

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CHAPTER 5: SUITABILITY OF WATER PURIFIED WITH *MORINGA* SEED POWDER FOR HUMAN CONSUMPTION

Abstract

Economists suggests that population growth has the potential to contribute positively to a nation's economic growth. However, achieving this outcome hinges on the ability of the state to create a supportive environment that enables the population to effectively utilize its productive potential. Innovative solutions like harnessing the natural bio-coagulant properties of *Moringa oleifera* seeds can play a crucial role. These seeds has the ability to remove contaminants from water, making it safer to drink, thereby ensuring the well-being of the growing population. The research aimed at investigating the suitability of water purified with *Moringa* seed powder for human consumption. A total population of 96 water samples were collected from three sites along Pienaars River during winter and summer. Thereafter, filtered using a prototype containing *M. oleifera* seed powder. The physicochemical parameters of the treated water samples were analysed and compared with World Health Organization (WHO) drinking water standards. Microbial load analysis was alsoperformed to determine the effectiveness of *M. oleifera* seed powder as a water purifier. The results showed that the *M. oleifera* seed powder effectively reduced microbial load and physico-chemical determinants in all water samples. The treated water also met the WHO standards for pH, electrical conductivity and amounts of nitrate, sulphate, and chloride. Moreover, water purified with *M. oleifera* seed powder was generally suitable for human consumption in winter in Moretele and Tierpoort sampling point, as it was effective in reducing the bacterial count and *E.coli* levels were zero. However, the summer water samples were not suitable for drinking because they did not meet the WHO standard for *E. coli* levels. Therefore, further studies may be necessary to investigate the optimal dosage of *M. oleifera* seed powder to achieve a consistently lower bacterial count that meets the WHO standards, including those for *E. coli* levels.

Keywords: Drinking water quality, health risk assessment, microbial contaminants, Water Quality Index, Moretele, Tierpoort, Mamelodi.

5.1 Introduction

Water is one of the most vital resources for human well-being, yet access to clean and safe drinking water remains a significant challenge in many parts of the world including South Africa. With the growing global population and increasing water pollution, the demand for clean water is becoming more pressing. The use of *Moringa oleifera* seed powder for water purification has gained attention recently due to its low cost, availability, and effectiveness (Bilal *et al*., 2021; Benettayeb *et al.,* 2022; Pandey and Khan, 2023). However, there is less data on the suitability of water purified with *M. oleifera* seed powder for human consumption. Therefore, this study aims to investigate the suitability of water purified with *M. oleifera* seed powder for human consumption by evaluating its chemical, physical, and microbiological properties.

Assessing water quality is crucial to determine its suitability for various uses, as water quality often determines its fitness for purpose (Edokpayi *et al*., 2020). *Moringa oleifera* seeds are advocated to be an excellent source of functional protein isolate, as they contain a high protein content of approximately 52%, with all essential amino acids (Jain *et al.,* 2019). *Moringa oleifera* seeds have also been found to have water purifying properties. Therefore, its seeds can be used to treat contaminated water. Polluted water usually contains coliform bacteria which is unacceptable for drinking water (Mathipa, 2016).

The United Nations (2010) has acknowledged that having access safe and clean water for drinkingis an inherent and essential human right. Despite initiatives to increase the availability of waterworldwide, as of 2023, billions of people worldwide still do not have access to it (Holmes *et al.,* 2023; Quentin Grafton *et al.,* 2023). Widespread diseases such as cholera, typhoid, and diarrhoea are prevalent in regions with limited access to clean water. Moreover, exposure to chemical pollutants and heavy metals through contaminated water sources can have severe health consequences. The cost of setting up and maintaining water treatment facilities can be expensive, especially for communities in developing countries. Therefore, it is essential to explore alternative methods of water purification that are affordable, effective, and environmentally friendly.

Despite the potential benefits of using *M. oleifera* seed powder for water decontamination, there are still many unanswered questions about its suitability for human consumption. Some studies have reported that water purified with *M. oleifera* seed powder has a high turbidity level, a bitter taste, and a distinctive odour (Abo-Zaid, 2019; Dhakad *et al.,* 2019). Concerns have been raised in relation to the potential toxicity of *M. oleifera* seed powder if utilized in excessive amounts.

5.2 Aims and objectives

This chapter aims to investigate the suitability of water purified with *M. oleifera* seed powder for human consumption. The chapter has the following objectives:

I. To assess the physico-chemical and microbial parameters of the water after purification with *M. oleifera* seed powder.
II. To identify any potential health risks associated with consuming water purified with *M. oleifera* seed powder.

5.2 Materials and methods

The mean values of selected water quality parameters in water that had been filtered through a prototype filter consisting of *M. oleifera* seeds ground to a fine particle size from the three sampling points in both seasons were evaluated using the South African Water Quality Guidelines (SAWQGs) as well as the international guidelines to assess the water's fitness for drinking. The fine particle size of 710 µm was selected because it resulted in lower microbial counts and improved physicochemical values following filtration with *M. oleifera* seed powder in chapter 3 and 4. This comparison aimed to evaluate whether the water quality parameters met the recommended criteria for drinking water.

5.3.1 Determination of water quality index

The Weighted Arithmetic Water Quality Index (WAWQI) was used to assess the overall water quality of Piernaars River by combining various physico-chemical parameters (Chidiac *et al.*, 2023). The first step involves selecting specific parameters according to their values and significance to water quality standards (Akter *et al.,* 2016). Ten physico-chemical parameters, including pH, EC, $NO3^-$, $NO2^-$, Cl^-, F^-, $SO4^{2-}$, Na, Ca and Mg were selected based on domestic water quality standards endorsed by the by the World Health Organization (WHO) and South African WaterQuality Guideline (SAWQGs) (see Table 5.1).

Table 5.1: Guideline values for drinking water in South Africa.

Parameter	Unit	WHO drinking water guideline	Water quality guidelines values inSouth Africa
pH	pH units	NS	6.5- 8.5
EC	mS/m	NS	< 70
CL^-	mg/l	NS	≤ 100
$N03^-$	mg/l	50	≤ 6
NO_2^-	mg/l	3	≤ 6
Ca	mg/l	NS	< 80
F^-	mg/l	1.5	≤1. 5
Mg	mg/l	NS	< 70
Na	mg/l	NS	< 100
SO_4^{2-}	mg/l	NS	< 100
E. coli	CFUs/100 ml	0	0
Total coliforms	CFUs/100 ml	NS	< 10
Salmonella	CFUs/100 ml	NS	NS
Shigella	CFUs/100 ml	NS	NS

Note- NS: Not Specified

The second step involves developing a rating scale with five distinct classes ranging from excellent to unsuitable based on the fitness for use criteria for water use (Uddin *et al.,* 2021) (Table 5.2). Each parameter had a quality rating scale (Qi) assigned to it based on its concentration (Ci) and respective standard (Si), using the South African Water Quality Guideline for Domestic Use and the WHO's guidelines for drinking water.

The following formula was used to calculate the quality rating scale:

$$Qi = \left(\frac{Ci}{Si}\right) \times 100$$

Table 5.2: Criteria for evaluating the suitability of water for drinking (Uddin *et al.,* 2021).

Quality Rating Scale (Qi)	**Range**	**Description**
<50	$0 \leq Qi < 50$	Excellent
50 -100	$50 \leq Qi < 100$	Good
100 -200	$100 \leq Qi < 200$	Moderately polluted
200 -300	$200 \leq Qi < 300$	Severely polluted
>300	$Qi \geq 300$	Unsuitable for human consumption

Note: Qi is the quality rating scale for each parameter, Ci is the concentration of the water quality parameter, and Si, adheres to the respective standards set by the South African Water Quality Guideline for Domestic Use and the World Health Organization (WHO) guidelines for drinking water.

The third step determines the relative weight (Wi) of each parameter, based on its respective standard (Si).
The relative weight (Wi) of each parameter was determined using the following equation:

$$Wi = \frac{1}{Si}$$

Where:

Wi represents the weight assigned to each water quality parameter.

Si denotes the respective standard for each parameter.

On the last step, the total WQI was calculated as the sum of the product of each quality rating scale (Qi) and its respective weight (Wi), divided by the sum of all weights (Wi). This mathematical expression can be represented as:

$$\text{WQI} = \sum \text{Wi X Qi} / \sum \text{Wi}$$

Where:

Wi is the weight assigned to the i-th parameter and

*Q*i is the quality rating scale for each parameter

5.3 Results

Water samples collected from Moretele, Mamelodi, and Tierpoort in summer were found to be suitable for human consumption after filtration. In winter, the water from Moretele and Tierpoort was suitable for drinking. However, the water quality index at the Mamelodi sampling point was 174.64, which indicates moderately polluted water.

5.4.1 Water quality index after filtration for Moretele sampling point in summer

The calculated WQI value of Moretele sampling point was 60.07 (Table 5.3), which falls in the "good" water quality range of 50-100 on the Qi scale. This indicates that the water quality at the sampling point is generally acceptable for domestic use. However, it is essential to highlight that although the water is within the acceptable limit, consuming river water directly is not advisable for health purposes. This cautionary statement is in accordance with the understanding that the water quality in rivers can vary on a daily basis due to various factors, including natural processes and anthropocentric activities occurring in and around the river.

Table 5.3: Calculated water quality index in Moretele sampling point in summer

Parameter	Value (Q)	SA Water Quality Limit for Domestic Use	Qi	Wi	WQI
pH	6.02	6.5-8.5	63.38	0.11	6.97
EC	70.5	< 70	100.71	0.01	1.01
NO_3^-	10.5	< 6	175.00	0.17	29.75
NO_2^-	0.05	< 6	0.83	0.17	0.14
Cl^-	64.5	< 100	64.50	0.01	0.65
F^-	0.36	< 0.7	51.43	1.00	51.43
SO_4^{-2}	19.5	< 200	9.75	0.01	0.10
Na	9.30	< 100	9.3	0.01	0.09
Ca	29.55	< 80	36.94	0.03	1.11
Mg	26.55	< 70	42.21	0.03	1.27
				1.54	**92.52.**
			Overall	**WQI**	**60.07**

5.4.2 Water quality index after filtration for Mamelodi sampling point in summer

Table 5.4 presents the water quality results for Mamelodi sampling point. For each parameter, the corresponding South African Water Quality Limit for Domestic Use has been compared with the measured value to calculate a Water Quality Index (WQI) value. The total WQI value for Mamelodi sampling point was 27.73, indicating excellent water, quality with reference to the rating scale of less than 50 on the rating scale (Table 5.2). However, it should be noted that although the water condition falls within the "excellent "range, changes in the river flow, rainfall patterns, and the introduction of pollutants can also impact the water quality. These dynamic factors can lead to fluctuations in water parameters and potentially affect its suitability for human consumption.

Table 5. 4: Calculated WQI for the Mamelodi sampling point in summer

Parameter	Value (Q)	SA Water Quality Limit for Domestic Use	Qi	Wi	WQI
pH	6.01	6.5-8.5	63.26	0.11	6.95
EC	65.55	< 70	93.64	0.01	0.93
NO_3^-	3.8	< 6	63.33	0.17	10.76
NO_2^-	0.03	< 6	0.5	0.17	0.08
Cl^-	50.81	< 100	50.81	0.01	0.50
F^-	0.15	< 0.7	21.42	1.00	21.42
SO_4^{-2}	13.7	< 200	6.85	0.01	0.06
Na	9. 2	< 100	9. 2	0.01	0.09
Ca	30.08	< 80	37.6	0.03	1.12
Mg	18.75	< 70	26.78	0.03	0.80
				1.54	**42.71.**
			Overall	**WQI**	**27.73**

5.4.3 Water quality index after filtration for Tierpoort sampling point in summer

Based on the water quality index (WQI) value of 37.70, the water quality at the Tierpoort sampling point is classified as "excellent". This means that the water is suitable for domestic use. Based on the observations, the excellent water quality observed at the Tierpoort sampling point is likely due to the absence of pollutants or contaminants that could have a significant impact on its quality. This finding is particularly noteworthy considering the lower population density and reduced industrial activities in the area. The lower human settlements and limited industrial operations are likely contributing factors to the favourable water conditions. Although the results of the Tierpoort sampling point indicates that the water can be used for drinking, it is important to note that the water might still require treatment before consumption.

Table 5.5 Calculated WQI in Tierpoort sampling point in summer

Parameter	Value (Q)	SA Water Quality Limit for Domestic Use	Qi	Wi	WQI
pH	6.23	6.5-8.5	65.57	0.11	7.21
EC	23.9	< 70	34.14	0.01	0.34
NO_3^-	0.52	< 6	63.33	0.17	10.82
NO_2^-	0.02	< 6	0.33	0.17	0.05
Cl^-	9.3	< 100	9.3	0.01	0.09
F^-	0.27	< 0.7	38.57	1.00	38.57
SO_4^{-2}	8.05	< 200	4.02	0.01	0.04
Na	2.2	< 100	2.2	0.01	0.02
Ca	15.8	< 80	19.75	0.03	0.59
Mg	8.00	< 70	11.42	0.03	0.34
				1.54	**58.07**
			Overall	**WQI**	**37.70**

5.4.4 Water quality index after filtration for Moretele sampling point in winter

The calculated WQI value of Moretele sampling point in winter was 28.25 (Table 5.6), which falls in the "excellent" water quality range of 0-50 on the Qi scale. This indicates that the water quality at the sampling point is generally acceptable for drinking.

Table 5.6: Calculated WQI in Moretele sampling point in winter

Parameter	Value (Q)	SA Water Quality Limit for Domestic Use	Qi	Wi	WQI
pH	6.06	6.5-8.5	63.78	0.11	6.95
EC	109.5	< 70	156.42	0.01	0.94
NO_3^-	33.05	< 6	550.83	0.17	10.76
NO_2^-	0.02	< 6	0.33	0.17	0.09
Cl^-	73.55	< 100	73.55	0.01	0.51
F^-	0.04	< 0.7	5.71	1.00	21.42
SO_4^{-2}	170.50	< 200	4.02	0.01	0.07
Na	84.4	< 100	85.25	0.01	0.85
Ca	32.3	< 80	40.37	0.03	1.13
Mg	30.7	< 70	43.85	0.03	0.80
				1.54	**43.52.**
			Overall	**WQI**	**28.25**

5.4.5 Water quality index after filtration for Mamelodi sampling point in winter

The results showed that the WQI for Mamelodi in winter season was 174.64, indicating moderately polluted water on the rating scale of 100-200 (Table 5.3). Moreover, the pH value of 5.35 obtained after filtration with the fine particle size was lower than the above mentioned set standards for drinking water purposes of (6.5-8.5) and therefore, considered acidic. Drinking water with a pH of 5 may have a sour taste and may also indicate the presence of other contaminants or issues with the water source (Akinbile and Yusoff, 2011).

Table 5.7: Calculated WQI in Mamelodi sampling point in winter

Parameter	Value (Q)	SA Water Quality Limit for Domestic Use	Qi	Wi	WQI
pH	5.35	6.5-8.5	61.57	0.11	6.77
EC	74.1	< 70	34.14	0.01	105.87
NO_3^-	0.88	< 6	63.33	0.17	14.67
NO_2^-	0.02	< 6	0.33	0.17	0.33
Cl^-	11.35	< 100	9.3	0.01	11.35
F^-	0.04	< 0.7	38.57	1.00	5.714
SO_4^{-2}	133.5	< 200	4.02	0.01	66.75
Na	10.04	< 100	2.2	0.01	10.04
Ca	13.75	< 80	19.75	0.03	17.19
Mg	21.2	< 70	11.42	0.03	30.28
				1.54	**268.96**
			Overall	**WQI**	**174.64**

5.4.6 Water quality index after filtration for Tierpoort sampling point in winter

Based on the water quality index (WQI) value of 28.14, the water quality at the Tierpoort sampling point is classified as "excellent". Therefore, there water is fit for drinking purposes. Based on the observations, the excellent water quality observed at the Tierpoort sampling point is likely due to the absence of pollutants or contaminants that could have a significant impact on its quality. However, several limitations were encountered during the development of the WQI, particularly in the steps of water quality indicators selection and the classification methods applied to determine the absolute index value (Lukhabi *et al.*, 2023). Therefore, considering these limitations, it is important to interpret the WQI value cautiously and recognize its inherent limitations.

Table 5.8: Calculated WQI for the Tierpoort sampling point in winter

Parameter	Value (Q)	SA Water Quality Limit for Domestic Use	Qi	Wi	WQI
pH	6.01	6.5-8.5	63.26	0.11	6.95
EC	94.3	< 70	93.64	0.01	0.94
NO_3^-	0.21	< 6	63.33	0.17	10.76
NO_2^-	0.02	< 6	0.5	0.17	0.09
Cl^-	58.4	< 100	50.81	0.01	0.51
F^-	0.03	< 0.7	21.42	1.00	21.42
SO_4^{-2}	130.05	< 200	65.02	0.01	0.65
Na	65.2	< 100	9. 2	0.01	0.09
Ca	21.8	< 80	37.6	0.03	1.13
Mg	23.35	< 70	26.78	0.03	0.80
				1.54	**43.34**
			Overall	**WQI**	**28.14**

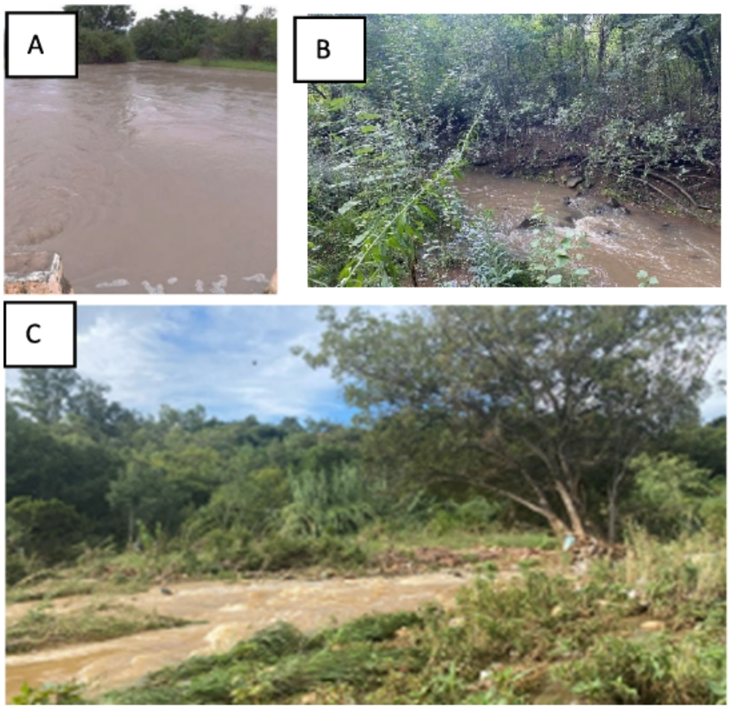

Figure 5.1: Physical observation of the water quality at (A) Moretele, (B) Tierpoort and (C) Mamelodi. Source: Dineo Raphasha.

Based on the observations, the colour of the water was muddy on all collection points (Figure 5.1 A, B and C). The Tierpoort site (Figure 5.1 B) yielded low levels of microbial contaminants and excellent water quality index on both seasons.

5.4 Discussion

The muddy colour of the water in all collection points is likely due to high levels of suspended sediments, which can be caused by various aspects including erosion, land use changes, and agricultural practices around the Mamelodi area. Farmers often till the soil and leave it exposed, which makes it vulnerable to erosion by wind and rain. When it rains, the runoff from the fields can carry large amounts of sediment and nutrients into the sampling points. The Tierpoort site yielded low levels of microbial contaminants and excellent water quality index on both seasons. The results are due to the influence of its unpolluted surroundings and limited industrial activities in the area as compared to Mamelodi and Moretele. These factors are instrumental in promoting the overall health and quality of the water, making it a valuable resource for both humans and the ecosystem.

The results indicated that when using Moringa seed powder for water purification, the water quality in Pienaars River complies with the drinking water standards set by South African Water Quality Guidelines for Domestic Use and the WHO guidelines. However, Mamelodi sampling point in winter had slightly polluted water. The Mamelodi point has been studied extensively over the past few years to assess its water quality and the influence of pollution on the ecosystem and human well-being (Mlotshwa, 2018; Desta and Bote, 2021). A recent study found that the water was heavily polluted with elevated concentrations of bacteria, nitrates, and phosphates (Dube *et al.,* 2017). Seeteram *et al.* (2019) found that high nitrite levels in the water led to a decrease in fish and macroinvertebrate populations. Nitrite is a toxic compound that can reduce blood's ability to carry oxygen and result in methemoglobinemia, or "blue baby" condition (Richard *et al.,* 2014). The non- conformity of nitrite in the Mamelodi sampling point indicates that the water may not be safe for drinking without treatment. One of the main causes of high nitrite levels in the Mamelodi point is the excessive use of agricultural fertilizers. These fertilizers contain high levels of nitrogen compounds, which when applied to fields, can leach into nearby rivers and water bodies.

The presence of microbial contaminants such as *E. coli, Shigella,* and *Salmonella* in the three sampling points as observed from chapter four results can pose a significant risk of contracting various infections and diseases (Cabral, 2010). Epidemics of shigellosis occur in crowded communities as observed in Mamelodi during summer (WHO, 2011). These contaminants are associated with illnesses such as bacillary dysentery, respiratory infections, urinary tract infections, and gastroenteritis, which can occur through exposure to or consumption of contaminated water (Ashbolt, 2004). Enteric pathogens, including *E. coli*, which was found in all sampling points in summer and only in Mamelodi in winter, are leading causes of diarrhoea and bacillary dysentery worldwide (Samie *et al.,* 2009; WHO, 2011). WQI does not represent the actual water quality or the level of pollution in an absolute way (Adelagun *et al*., 2021).

5.5 Conclusion

The water quality assessment of the filtered water reveals that the Moretele and Mamelodi sampling points along Pienaars River demonstrate "good" water quality, which indicates that the water purified with Moringa seeds powder is acceptable for domestic use. Similarly, the filtered water at Tierpoort sampling point displayed "excellent", making it highly suitable for domestic purposes. However, it is important to recognize that rivers are dynamic systems where water quality can potentially fluctuate due to various factors, including weather patterns, seasonal changes, human activities, and natural processes because the effectiveness of water purification with Moringa seed powder depends on the level of contamination in the water. These factors can lead to fluctuations in water quality over time. For example, heavy rainfall or storm events can result in increased runoff and the introduction of pollutants into the river system. Similarly, agricultural activities, industrial discharges, or improper waste management practices can contribute to water pollution and affect its quality.

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CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Background

Water pollution poses significant environmental and health challenges in South Africa, particularly in water scares areas. Water supplies that are contaminated contribute to the transmission of waterborne diseases, resulting in higher rates of illness and mortality within the population. Addressing this issue requires innovative and sustainable solutions for water treatment. *Moringa oleifera,* a tree commonly found in various regions, has emerged as a potential natural coagulant in water purification. Extensive research has revealed that the seeds of natural coagulants found in *M. oleifera* can effectively remove pollutants from water including bacteria and other microorganisms. This natural coagulation process involves the aggregation and subsequent settling of suspended particles, allowing for their removal from the water.

6.2 Review of research aim and objectives

The aim of this study was to examine the efficacy of a simple water purification device utilizing *Moringa oleifera* seed powder as the active beads. The investigation focused on three sampling points along the Pienaars River by considering the physical, chemical, and microbiological aspects of water purification. The study addressed the following objectives:

I. To determine the efficiency of different *M. oleifera* seed particle sizes in influencing the physicochemical parameters of contaminated water.

Water samples were collected from three designated locations along Pienaars River, namely Tierpoort, Mamelodi, and Moretele, during winter and summer seasons. These sites were selected to represent different locations along the river and to capture potential variations in water quality. Filtration with *M. oleifera* seed powder using different particle sizes resulted in a notable reduction in nitrite levels in Mamelodi water samples. This reduction is indicative of the effective removal of nitrite contaminants, which can have adverse effects on water quality. The findings suggest that *M. oleifera* seed powder, when used as a filtration medium, can play a vital role in improving the physicochemical parameters of contaminated water.

II. To determine the efficiency of *M. oleifera* seed particle sizes in reducing the microbial load and influencing the physicochemical parameters of contaminated water.

Experiments were conducted using different particle sizes of *M. oleifera* seed powder to determine the impact of the particle sizes on the reduction of microbial load in contaminated water. *Moringa oleifera* seed powder has been reported to have anti-microbial properties and can be used as a natural coagulant to remove suspended solids and microorganisms from water. The study found that the smaller particle size (710µm) of *M. oleifera* seed powder had a higher surface area and better ability to adsorb bacteria, leading to a more effective reduction in microbial load. However, the results of the study showed that the number of colony forming units (CFU) in the filtered water samples, even though reduced, remained higher than the recommended guidelines set by the World Health Organization and the South African National Accreditation System for drinking water. By revising the gadget design, such as exploring alternative filtration techniques, it may be possible to improve the filtering efficiency and achieve a lower CFU count in the filtered water.

III. To determine suitability for human consumption of water purified using the *M. oleifera* seeds gadget

The determination of purified water's suitability for human consumption is an important process that involves a series of tests to verify that the water meets certain quality standards. In the study, the quality of purified water for drinking was assessed based on its physical, chemical, and microbiological properties. The water from Pienaars River after filtration with *M. oleifera* seed powder was deemed unsuitable for drinking purposes without undergoing pre-treatment .This determination is primarily based on the presence of *E. coli, Salmonella, shigella* and total coliform bacteria in water after filtration.

However, the suitability of water purified with Moringa seed powder for human consumption depends on several factors, including the water source's quality, the purity of the *M. oleifera* seed powder, and the effectiveness of the purification process. If the water source is heavily polluted with harmful pathogens, such as bacteria's, *M. oleifera* seed powder alone may not be sufficient to make the water safe for consumption. In such cases, additional treatment, such as boiling or chemical disinfection, may be necessary.

6.3 Conclusion

The study's results indicate that the water-purifying gadget utilizing *M. oleifera* seeds has promising potential as a straight forward, cost-effective, and environmentally friendly approach to water purification, especially in regions with limited access to clean water. The water-purifying gadget was found to effectively remove contaminants from the three sampling points along Pienaars River. However, additional research is required to optimize the performance of the gadget by exploring the most effective parameters, such as the amount of seed powder, the particle size and contact time required for efficient water purification. Additionally, the long-term effects of using *M. oleifera* seeds for water purification on human health and the environment need to be studied. Overall, the use of indigenous plants such as *M. oleifera* seeds as a water purifier could have significant implications for improving access to clean water in resource constraint countries.

Incorporating *M. oleifera* seeds into the gadget offers an environmentally friendly and sustainable solution as the seeds are readily available and do not require any expensive equipment or chemicals. Their natural coagulant properties make them effective in removing contaminations from water, including bacteria and microorganisms. By harnessing the potential of this indigenous tree, communities can enhance their water treatment capabilities in a sustainable and environmentally friendly manner. Continued research and implementation efforts will further contribute to the development of affordable and accessible water treatment solutions, ultimately improving the well-being and health outcomes of communities impacted by water pollution.

Addressing water quality challenges requires adherence to legislated norms and standards, such as the South African National Standards, and the implementation of comprehensive water treatment and purification methods. The study's results contribute to understanding of the present water quality conditions in the Pienaars River and can guide policymakers and stakeholders in formulating appropriate strategies for sustainable water resource management, protecting human health, and ensuring the well-being of both urban and rural communities in South Africa.

6.4 Recommendations

Based on the findings and limitations of the study, several suggestions can be made for further investigation and practical applications. Firstly, additional studies should explore methods to enhance the filtering capacity of the *M. oleifera* seed gadget to ensure compliance with microbial guidelines. This may involve adjusting the quantity of *M. oleifera* particles or exploring alternative combinations with other filtering agents. Studies aimed at the stability and shelf life are essential to evaluate the durability and effectiveness of the gadget over extended periods.

Furthermore, comprehensive assessments of the economic feasibility and scalability of the *M.*

oleifera seed gadget should be conducted. Cost-effectiveness analyses and studies on large-scale production and distribution of the gadget will provide valuable insights for its implementation in resource-constrained areas.

Additionally, further studies can be on the sustainability and long-term impacts and sustainability of *M. oleifera* seed-based water purification systems. This includes evaluating the durability of the gadget, assessing its performance in various water sources and environmental conditions, and conducting comprehensive health impact assessments to make sure of the safety of the purified water for human consumption. The gadget could be revised by lengthening the size, thereby increasing the quantity of the particles. The diameter of the gadget can also be increased to increase the volume and the surface area of the filtering particles.