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Treated domestic wastewater reuse

TOPIC:

Analysis of nature-based treatment of surface water runoff from an informal settlement to irrigate vegetables.

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DECLARATION

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ABSTRACT

An increasingly common feature among cities in the Global South (including South Africa) is informal urban settlements (IS). This study was conducted to analyse and make a comparative analysis of the physicochemistry of the soil, and nutrient concentration in crops following irrigation with biofiltration-treated surface runoff from IS. Municipal Treated Water (MTW) was used alongside Biofiltration-treated Water (BTW) to irrigate crops for nine months. Three soil treatments were prepared along each irrigation system mirroring each other: 1) control soil, 2) duckweed-treated soil, and 3) biochar-treated soil. Bulbous (belowground) and leafy (aboveground) crops were selected for the experiment. Following each successive harvest during the experimental period, soil samples from a depth of 10 cm were collected and analysed for Soil Organic Carbon (SOC), Sodium (Na+), Cations herein referred to as Exchange Capacity (CEC), Zinc (Zn+), and Iron (Fe+). In addition, the concentration of macro-and micronutrients (N, P, Ca, Mg, Na, Mn, Fe, and Zn) levels of the resulting produce were collected of each successive harvest and analysed at the end of the experiment. Compared with the concentration found in MTW-irrigated soil, the results from the experiment revealed that the concentration of Na +, Ca2 +, Zn+, and CEC was relatively high. Moreover, the results revealed that the concentrations of Zn and Na as measured by range in lettuce, spinach, and beetroot were high. These values were within the World Health Organization [WHO] guidelines and laboratory norms for the concentration of nutrients in vegetables for human consumption. However, the concentration of Na in spinach irrigated using BTW was higher than the laboratory norms and the WHO guidelines. Long-term monitoring and evaluation of soil physicochemistry and soil nutrient changes from BTW irrigation are required.

Keywords: Informal settlements, biofiltration treated water, municipal treated water, biochar, duckweed.

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CHAPTER 1: GENERAL INTRODUCTION

1.1.Introduction

An increasingly common feature among cities in the Global South (including South Africa) is informal urban settlements (IS) (Thatcher *et al*., 2022). Due to rapid urbanisation, and land "unavailability" among other factors, IS are densely populated living spaces and are characterised by the lack of, or inadequate delivery of basic services such as sewerage infrastructure, and energy challenges (Thatcher *et al*., 2022). Often, greywater which consists of dishwashing, bathing wastewater, urine, oil, and grease, is commonly disposed into makeshift stormwater drainage systems that discharge to nearby rivers with potential adverse human health, economic and environmental risks due to the nature of its composition (Salukazana *et al*., 2006; Carden *et al.,* 2007; Rodda *et al*., 2011; Winter, 2021; Thatcher *et al*., 2022). Reported in the literature are issues of downstream river contamination, disruptions of agricultural production due to crops and soil contamination from irrigation with contaminated water, unpleasant odours due to ponding in low-lying settlements (Kurian *et al*., 2013), groundwater contamination (Carden *et al*., 2007; Winter *et al*., 2023), disruption of recreational activities, and *e-coli* infections (Carden *et al*., 2007).

According to Carden *et al*. (2007), as cited in Winter *et al*. (2023), discarded greywater in South African non-sewered households accounts for more than 490 000 m^3. The recovery and reuse of wastewater is considered to have greater potential to expand the availability of water for the irrigation of edible crops, including the recovery of essential nutrients for plant growth and development (Salukazana *et al*., 2006; Carden *et al*., 2007; Bedbabis *et al*., 2014; Qureshi *et al*., 2016). Small-scale and subsistence food producers living in areas with limited services such as the provision of freshwater may potentially benefit from wastewater recovery and reuse, with positive socioeconomic and environmental benefits (Kurian *et al*., 2013). To achieve this, the utilisation of biofiltrations cells, a strategy founded upon the notion of Nature-based Solutions (NbS), offers a convenient, and an economically and environmentally sound technique to treat and/or improve the quality of contaminated surface runoff. Unlike energy intensive (Reverse osmosis) and chlorine-based water treatments that require immense financial investments, biofiltration systems are regarded as low cost as they rely on biological processes to improve the quality of water. Consequently, they are better suited for decentralisation with ease of operation especially in areas with limited water resources including IS (Kisser *et al*. (2020).

However, while the recovery and reuse of treated wastewater for irrigation of crops is a reliable source of nutrients (Poustie *et al.*, 2020), there are potential risks for the expected products and physicochemical properties of soil over time. These include the accumulation of heavy metals in edible parts of crops, and salts in soil, resulting in salinity issues (Qishlaqi *et al*., 2007; Rodda *et al*., 2011; Bedbabis *et al*., 2014).

Therefore, its continued application on crops is expected to occur at a rate at which the soil can naturally assimilate nutrients and salts, making them available for plants. The above points to the importance of developing healthy soil to ensure plant growth and development and achieve its provisioning and regulatory functions (Rhodes, 2017; Dent and Boicean, 2021a). Therefore, there is a need for explicit conditions and timeframes to regulate the suitability of recovered wastewater for safe reuse for various purposes (Reznik *et al*., 2019).

This study aimed to understand how the continued use of contaminated surface water runoff from IS retrieved from a river with and treated through NbS can be reused to irrigate crops by studying the effects of selected soil physicochemical parameters and nutrients that accumulate in edible crops. By adopting the principles of regenerative agricultural practices to enhance soil development in a peri-urban environment, the physicochemical properties of soil in different soil treatments were established and compared to those irrigated with MTW. A comparative analysis of micro-and macronutrient concentrations in crops under the two different types of water used for irrigation was performed to determine the suitability of BTW in growing crops for human consumption. The results were compared with the WHO guidelines and laboratory norms.

1.2.Contextualisation

From 2017 to 2019, Cape Town and the surrounding areas experienced water shortages driven by numerous factors, including less than average rainfall during the so-called Day Zero drought (Organisation for Economic Co-operation and Development [OECD], 2021). Subsequently, various departments responsible for water provision and management at national and local levels began exploring water augmentation strategies to reduce the dependence on surface rainwater. One of these strategies entailed exploring the potential of wastewater reclamation and reuse for non-potable uses, such as irrigation of edible crops. Domestically treated wastewater has been studied for its suitability to irrigate edible crops. Literature evidence reveals that, if not diligently utilised, wastewater reclamation and reuse carry potentially

harmful effects on humans and the environment given the purpose through which it is being utilised (Surdyk *et al*., 2010; Donnenfeld *et al*., 2018).

The treatment of wastewater from IS through NbS and its subsequent reuse for irrigation of edible crops has shown positive and promising results in producing quality crops comparable to those sold in the markets (Winter, 2021; Winter *et al*., 2023). Conceptually, when treated wastewater from an informal settlement is used for irrigation, it carries with it potentially harmful effects on the physicochemical properties of soil and has the potential to influence (negative or positive) the growth, quality, and yield of crops, depending on various factors that affect plant growth and development. When the uptake and safe reuse of wastewater treated through NbS has been achieved, the resilience of small-scale and subsistence farmers may be improved, as treated wastewater may serve as an alternative water supply where freshwater supply inefficiencies have been recognised. Reusing treated wastewater may reduce the use of external chemical inputs, with a significant impact on soil health and crop quality (Bedbabis *et al*., 2014).

The uptake of regenerative agricultural practices, including utilising duckweeds and biochar as soil amendment strategies, can affect various aspects of soil, including its physicochemical properties. Healthy soils are critical for excess nutrient assimilation, improved carbon storage, supporting plant growth and development, and encouraging the uptake of treated wastewater for irrigation (Musazura *et al*., 2018). This study aims to understand how the continued use of polluted runoff from IS retrieved from a contaminated river and treated through NbS to irrigate crops affects the physicochemical variables of soil and crop nutrient status.

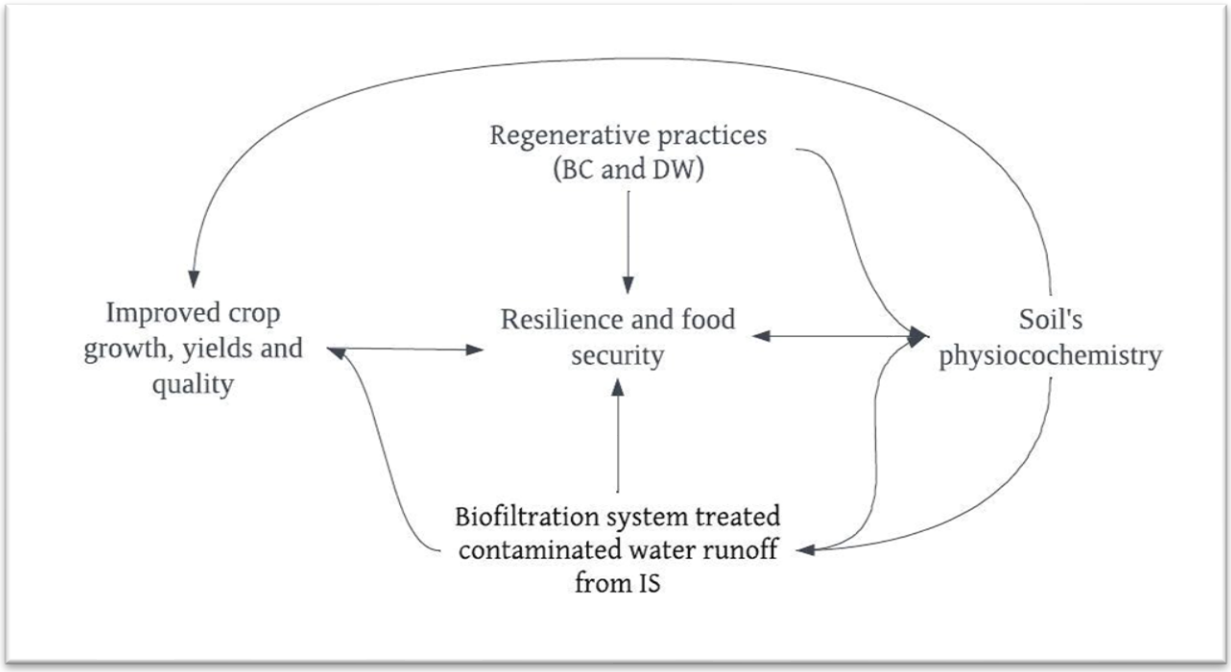

Figure 1: A conceptual framework with the introduction of BC (biochar) and DW (duckweed).

1.3.Motivation for the study

Wastewater emanating from domestic sources contains considerable amounts of inorganic and organic matter that are essential for plant growth and development in relatively low quantities (Bedbabis *et al*., 2014; Egbuikwem *et al*., 2020). They are indirectly or directly discharged into the environment with potential human health and ecological consequences. NbS using biofiltration systems offers an opportunity to treat contaminated water runoff from IS and have been proven significant in reducing nutrient (phosphate and nitrate) (Winter, 2021; Winter *et al*., 2023) loads, making it accessible for potential reuse for irrigation of edible crops. The treatment and reuse of contaminated water runoff, especially in peri-urban and IS and in drought-prone areas, has many possibilities, including improving access to water resources for subsistence agricultural practices, reducing dependence on the use of costly synthetic fertilisers, providing positive environmental benefits, and potentially improving the resilience of local food growers to future climate uncertainty, such as multiyear droughts (Gashaye, 2020).

Contaminated water runoff emanating from IS, retrieved from a contaminated river, and subsequently treated through NbS systems has been tested against growing edible crops and proven to be significant in providing quality and safe-to-eat crops comparable to those found in local markets (Winter *et al.*, 2023). Furthermore, work has been done by Winter (2021), who tested the potential of growing edible crops using BTW which contributed towards short-term relief meals in the nearby informal settlement in Franschhoek during the times when Covid-19

was most prevalent. While there are socio-economic opportunities for reusing BTW from IS, one should not overlook potential risks, such as the bioaccumulation of nutrients and salts in the soil and their subsequent transfer to edible parts of crops, and socio-ecological risks that may arise.

Therefore, the continued use of treated contaminated water runoff poses a risk of damage to soil and crops unless stringent measures and continuous proactive monitoring and research are conducted (Tunc & Sahin, 2014). Existing published literature has focussed on evaluating the effects of the reuse of untreated and diluted household wastewater from IS on the physicochemical properties of soil and the quality of resulting produce compared to that irrigated using municipal-treated tap water (Musazura *et al.,* 2018). Consequently, improving soil through regenerative practices (biochar and duckweed) may increase the soil's ability to assimilate nutrients for continued use of biofiltration-treated contaminated water runoff emanating from IS. Therefore, given the role that soils play in nutrient assimilation and cycling (Musazura *et al.,* 2018), there is a need for research and innovation in soil improvement practices to limit the potential risks of using treated contaminated water runoff from an IS. Regenerative agriculture, as coined by Robert Rhodale of the Rhodale Institute, holds great promise as it emphasises the restoration of degraded soils by enhancing soil health to improve soil nutrient assimilation, availability to support, and improved quality of crops.

1.4.Problem statement

The world's cities are expanding, and populations in peri-urban areas face challenges such as a lack of access to sewage infrastructure, inadequate access to freshwater resources and arable land, limited household electrification, and future climate uncertainty (Green, 2019). An accumulation of matter (nutrients) out of place in "peri-urban areas" carries with it potential negative socio-economic, ecological, and human health effects (Green, 2019). Much of this accumulation is brought upon by sewage spillages and discarding solid and liquid waste from the lack of provision of waste disposal services, especially in IS. The threat of damage to soil, freshwater resources, and ecological systems presents a risk of future food insecurity and human health issues (Rhodes, 2017; Anderson & Revera-Ferre, 2021) as downstream farming communities may potentially continue to use contaminated river water "unknowingly".

Treated wastewater, be it from an informal settlement or industrial environment, thus requires care and rigorous testing to meet the standards prescribed at national and international levels for potential reuse or disposal to the environment. Treated wastewater has potential positive

socioeconomic and environmental benefits (when safely disposed of) including environmental protection, improved access to water resources for non-potable purposes, improved resilience to droughts and water shortages (Capodaglio *et al.*, 2017; Dakkak, 2021) for small-scale subsistence farming communities and food security.

The decentralisation of wastewater treatment systems and the subsequent reuse of treated wastewater may be significant in overcoming the lack of access to water faced by small-scale subsistence farmers living on the fringes of major cities and small towns of underdeveloped and developing countries (Mena-Ulecia & Hernandez, 2015; Musazura *et al*., 2018). For instance, the treatment of contaminated water runoff from IS through biofiltration cells has been proven to be highly effective in decontaminating contaminated water runoff that emanates from informal household settlements, with up to a 90% reduction in nutrient concentration (Nitrates and phosphates) and a 99.99% removal of potential pathogens (Ghanashyam, 2018). The outcomes of this study present an opportunity for the decentralisation of water treatment and may serve as a water relief strategy for subsistence farmers during multiyear droughts and in instances where there is inadequate provision of water for crop irrigation. The impact of NbS, biofiltration systems, in improving the physiochemistry of contaminated water runoff from IS, and its subsequent suitability for reuse to irrigate crops for human consumption has been studied by Ghanashyam, 2018; Winter, 2021; and Winter *et al*., 2023.

Treated contaminated water runoff from IS (as a reliable source of water and nutrients to produce consumer-quality crops) carries potential risks and opportunities for the expected products, and physicochemical properties of the soil in the long term (Bedbabis *et al*., 2014; Khan *et al*., 2018; George, 2022), and the bioaccumulation of Na and the potential loss of soil's reproductive potential. According to the National Academies of Sciences, Engineering, and Medicine [NASEM] (2024), elevated sodium disperses the soil and suspends silt in the soil water solution. This reduces seed germination, promotes runoff and topsoil loss through erosion, and clogs the pores in the soil, capping it and preventing water from passing through it. Again, the loss of organic matter-rich topsoil and clogged soil pored reduces crop root penetration and impacts the soil's reproductive potential. Therefore, the continued application is expected to occur at a rate at which the soil can naturally assimilate nutrients and salts, thereby reducing their bioavailability for safe utilisation by crops. This may be achieved by developing healthy soils that support crop growth and development and play a regulatory role for nutrients emanating from treated wastewater from IS (Musazura *et al*., 2018). The state of soil and water affects more than just agriculture, as they are inextricably linked to one another; they impact

biodiversity, soil carbon concentrations, and crop quality (Green, 2019). Therefore, the risks associated with the long-term use of treated wastewater from an informal settlement on various socioeconomic and environmental factors, including soil, require special attention.

The reuse of treated wastewater and the rehabilitation of previously unproductive land and/or soil through regenerative agricultural practices hold great promise for expanding access to water and land for improved resilience to water shortages and food insecurity. Investigations of site-specific and comprehensive approaches that consider heterogeneity and go beyond simple technocratic solutions in food production are of greater importance, as the world is rapidly changing (Gassner *et al*., 2019; Wise, 2021). The above encourages the need for traditional or conventional ways of food production such as "working with nature" as means to sustainable food production with greater emphasis on soil quality for long-term uptake of treated wastewater and climate resilience is required (Burns, 2021).

1.5.Aim and objectives of the research.

This study aims to understand how the continued use of polluted runoff from an informal settlement retrieved from a contaminated river and treated through NbS to irrigate crops affects selected soil physicochemical variables and the concentration of nutrients in crops compared to MTW. The following objectives were determined.

- The physicochemical composition of the water used for irrigation was determined.
- The physicochemical properties and mineral composition of soil media irrigated with BTW were determined and compared with those of media irrigated with MTW.
- Comparative analysis of macro-and micronutrient concentrations in crops under different irrigation conditions.
- Determination of the allowable limits of micronutrient concentrations in vegetables.

1.6. Limitations of the study

The lack of control of prevailing environmental and climatic conditions had an impact on the experimental period, given that some harvests took 4 to 5 weeks in summer, while during winter, crops took about 100 days to grow to a mass acceptable for laboratory analysis. This led to a situation with which harvest time and subsequent cropping were disrupted because crops took longer to be ready for harvest, with a significant impact on data collection. Variations in temperature have had significant effects on crop growth and development rates. Consequently, cropping and harvests reduced and further contributed to reduced data collected and subsequent limited data points during the cleaning process to standardise the data.

Therefore, the margin of error in the results from analysing the data increased because of the reduced harvests which were initially planned. Again, limited data points had an impact in establishing the statistical significance of the difference between soil treatment conditions between the two irrigation conditions.

In addition, the aim was to conduct an experiment during the dry period, as it would allow a more comprehensive analysis without external influences, such as rainfall. However, the experiment extended to the rainy period (March to August); as a result, irrigation with BTW was limited during this period which may have influenced soil conditions owing to dilution and nutrient washout. In addition, rainfall may have had a significant impact on the expected results, particularly in the comparative analysis of soil physicochemistry between the two water treatment technologies.

The experimental area covered $9x6m^2$ while the combined data used (historical and present) in the study was for a period of less than one year. This was "far" less than what many published studies have considered. For instance, Erel *et al*. (2019) and Bedbabis *et al*. (2014) conducted field-scale experiments over 2–15 years of data collection. Therefore, the limited data impeded further analysis of whether soil amendment strategies used in combination with biofiltration wastewater improved soil physiochemistry and nutrition levels in vegetables in the long term.

Soil nutrient balance in the duckweed and biochar plots was not monitored. This would have ensured that there was constant consistency in the nutrients derived from biochar and duckweed in the soil. Also, the concentration of nitrogen in the soil was not tested and/or monitored during the experiment.

CHAPTER 2: LITERATURE REVIEW

2.1. Nature-based solutions for treatment and reuse of contaminated water runoff from an informal settlement

According to Qi *et al*. (2020), the broader concept of NbS originated in Europe in the early 2000s. It was coined around the idea of holistically investigating alternatives that seek to integrate traditional engineering methods with "Blue-Green" techniques to create "merged systems of Blue-Green-Grey infrastructure". To put it into perspective, these solutions may tackle environmental urban concerns, such as managing water resources, and climate change resilience, and economic and social issues including public health, social fairness, environmentally friendly economics, and human well-being. Among other things, NbS comprises interventions that employ ecosystem services and natural processes for functional objectives such as water purification. Carden *et al*. (2007) and Kisser *et al*. (2020) consider NbS to be reliable, low-cost, and economically viable comprising a suite of methods including biofiltration cells and phytoremediation to decontaminate wastewater.

Compared to water treatment systems such as chemical treatment and conventional filtration, biofiltration systems are increasingly recognised as sustainable and effective strategies for treating various types of contaminated water and offer numerous advantages (Sinha & Mukherji, 2022). For instance, by utilising natural processes and microorganisms to decontaminate water, these systems can reduce the reliance on chemical additives, making them suitable and environmentally friendly alternatives to traditional water treatment techniques (Tanwir *et al.*, 2021). Furthermore, biofiltration systems have become recognised for their effectiveness in eliminating contaminants and impurities from water, such as organic compounds, nitrogen, pathogens, and metals (Winter *et a*l., 2023) and less hazardous water is the consequence of these toxins being efficiently broken down and removed by the biological processes involved in the process of biofiltration (Tanwir *et al.,* 2021). Relative to conventional water treatment systems, biofiltration systems frequently require less energy and are low maintenance, which could result in long-term cost benefits (Egbuikwem *et al.,* 2020; Mena-Ulecia & Hernández, 2015). With their ability to effectively decontaminate wastewater, and provide environmental advantages and cost-saving benefits, biofiltration water treatment systems offer an alternative to conventional techniques (Sinha & Mukherji, 2022).

The uptake and implementation of these systems may be crucial to ensure that wastewater treatment is brought closer to the communities that produce the waste, especially in peri-urban areas and IS at reduced operation costs. The living conditions found in developing countries,

such as South Africa, make the uptake and advancement of NbS to treat and reuse wastewater a practical alternative (Winter *et al.,* 2023). IS lacks appropriate disposal methods for liquid waste and treatment at local municipal wastewater treatment facilities, lack access to land, and has limited access to water (Winter *et al.,* 2023) for irrigation of edible crops for subsistence or small-scale production. Therefore, public initiatives to treat and reuse contaminated water runoff for irrigation of edible crops could be economically significant, especially with reduced exposure to potentially harmful contaminants (Winter 2021).

Biofiltration systems are one of the most promising NbS for treating contaminated water runoff from an informal settlement (Winter *et al.,* 2023). They are designed to reduce sedimentation, allow chemical precipitation and plant adsorption, and create a thriving environment for microorganisms to initiate biodegradation and the biotransformation of contaminants (Winter 2021; Sinha & Mukherji 2022). As a primary decontamination technique, the system has been proven to decontaminate between 80 and 90% of nutrient-based contaminants, such as phosphates and nitrates, and further eliminate approximately 99.99% of pathogens contained within contaminated water runoff emanating from an IS (Egbuikwem *et al.,* 2020). Moreover, successive treatments have been shown to improve nutrient decontamination by up to 90% over time, depending on various factors such as retention time, influent composition packing media, type of filter media (biochar or gravel), and local environmental conditions (Winter, 2021; Sinha & Mukherji, 2022). Biofiltration cell's potential to decontaminate heavy metals and other persistent compounds, including emerging pollutants has been understudied. Therefore, monitoring and testing soil and the resulting produce for contamination is vital to protect public health and prevent bioaccumulation through established soil and plant monitoring strategies.

According to Langergraber *et al*. (2020), NbS can offer various ecosystem services to the advantage of urban ecosystems and communities, such as controlling microclimates, preventing floods, water treatment, and food supply. However, most NbS can be implemented for a specific purpose. For instance, treating contaminated water runoff from an informal settlement requires a biofiltration cell design with a filter media and a design suited to target specific pollutants or the composition of the wastewater treated. Adopting the idea of a circular economy, which advocates for the decentralisation of wastewater treatment technologies, and the recycling and reuse of resources may promote human health and well-being and increase resilience in the face of uncertainty. For instance, treating contaminated water runoff and its subsequent reuse may increase water availability for non-potable purposes, including the production of edible crops for household sustenance (Musazura *et al.,* 2018).

2.2.Potential for the recovery of runoff from an informal settlement

Contaminated water runoff from an informal settlement consists of polluted greywater and is rich in organic nutrients, which are essential for plant growth and development when recovered with care (Tunc & Sahin, 2014; Egbuikwem *et al.,* 2020; Alnaimy *et al*., 2021). Recovered wastewater is rich in potassium (K), nitrogen (N), and micronutrients, and its reuse may be found in agriculture, particularly for crop irrigation (Carden *et al*., 2007; Wiem *et al*., 2023). Consequently, the use of decontaminated wastewater for irrigation reduces the need for synthetic fertilisers, with potential financial benefits (Wiem *et al*., 2023) in small-scale crop production. In cities and urban, peri-urban, and IS, many of these nutrients end up in terrestrial and aquatic environments through direct or indirect disposal (Wiem *et al.,* 2023) due to a lack of sewage infrastructure, inadequate or inefficient wastewater treatment technologies, and spillage due to aging sewage infrastructure (Winter, 2021).

According to Carden *et al.* (2007), emergency water provision and a lack of liquid waste management contribute to significant volumes of greywater being generated. As cited in Winter *et al.* (2023), Carden *et al.* (2007) maintain that the discarded greywater in South African non-sewered households accounts for more than 490 000 m^3. Greywater is often generated in downstream rivers and water bodies because of its disposal into stormwater drainage systems and leaching into groundwater from discharge into unpaved surfaces, with potential ecological and human health effects (Carden *et al*., 2007).

Even though wastewater reclamation in the agricultural sector has been growing and gaining ground globally, one of the key challenges for the recovery and reuse of wastewater is the lack of a formalised sewage infrastructure system to channel liquid waste discarded to wastewater treatment facilities for successful recovery (Egbuikwem *et al.,* 2020). Therefore, significant quantities of contaminated water runoff must be captured and channelled for the treatment of decentralised wastewater treatment plants in the vicinity of settlements that produce liquid waste in non-sewered areas. However, greywater management, treatment, and reuse may be feasible according to the needs and specific purposes, such as small-scale irrigation of edible crops (Musazura *et al.,* 2018). However, lack of investment remains a limiting factor for successful large-scale implementation of wastewater reclamation in various environmental settings, especially in developing and third-world countries (Egbuikwem *et al.,* 2020). Observations from published materials in academic and popular literature focusing on the potential reuse of greywater from an informal settlement for irrigation of edible crops have relied on storing and actively transporting greywater from households producing liquid waste

for experimental purposes. This is because of the lack of space and formalised infrastructure to channel greywater for full-scale implementation in high-density areas (Carden *et al*., 2007).

The successful implementation of greywater management, treatment, and reuse requires consideration of several factors. These include availability of infrastructure, land availability, distance from the settlement to the treatment system, quantities of wastewater generated, associated costs and practicality, and public perception (Carden *et al*., 2007). Although greywater quality may be variable, it has, however, been observed to have a composition similar to that of municipal sewage effluent that has been passed through tertiary treatment and is highly biodegradable (Carden *et al*., 2007). Therefore, biological treatment systems remain a suitable alternative for greywater emanating from IS (Carden *et al*., 2007). NbS for the treatment and safe reuse of greywater from an informal settlement, as promising, effective, and economically sustainable wastewater decontamination methods (Winter *et al.*, 2023), may play a significant role in ensuring that wastewater treatment, recovery, and reuse are brought closer to communities that produce liquid waste at relatively low operating costs (Carden *et al*., 2007; Musazura *et al*., 2018).

2.3.The effects of the reuse of treated household wastewater.

Wastewater reclamation and/or reuse have been practiced globally for various purposes, such as agriculture for the irrigation of edible crops and other products (Bedbabis *et al*., 2014). Although limited, evidence of the quality of edible crops irrigated using contaminated water runoff from IS treated using NbS as a primary treatment method has been established by Egbuikwem *et al*. (2020), Winter (2021), and Winter *et al.* (2023). These studies tested the potential for the reuse of biofiltered contaminated water runoff for edible crops by monitoring plant phenological and physiological parameters. The studies found no significant compromise in the quality of the edible parts of crops. However, studies have noted possible ecological and human health risks caused by the bioaccumulation of persistent organic and inorganic compounds in edible parts of crops and soil over time. There is limited literature documenting evidence which aims to evaluate the effects of the use of river contaminated with runoff from an informal settlement treated through NbS on the physicochemical properties and nutrient status of the soil. Several published studies have focused on establishing the effects of the use of untreated greywater from domestic sources on crops and soil physicochemical quality, including Rodda *et al*. (2009), Mzini *et al.* (2015) and Mustapha *et al.* (2020).

Concerns shared by Winter (2021), Ganjegunte *et al.* (2017), and Egbuikwem *et al*. (2020) of increased salinity of the soils have been reported by Erel *et al.* (2019). Using "standard fertilisation" practices, Erel *et al*. (2019) evaluated the performance of olive cultivars (Barnea and Leccino) from continued use of reclaimed treated wastewater for irrigation for 8 years. This study revealed that soils irrigated with reclaimed wastewater had relatively high salinity, compared to those irrigated with freshwater. Moreover, the study highlighted the possibility of deterioration of physical and chemical properties in soils irrigated with reclaimed wastewater due to the steady rise in the sodium absorption ratio, triggering an increase in the exchangeable sodium percentage compared to soils irrigated with freshwater. The limited literature on the effects of irrigation with wastewater from an informal settlement treated through NbS on soil physicochemical and chemical properties forms the basis of this study.

Emerging pollutants, such as those of pharmaceutical origin, heavy metals, and soluble salts, have been found to have evaded wastewater treatment (Yan *et al*., 2020) and have been introduced to the natural environment from household wastewater among other sources. During irrigation, the utilisation of wastewater treated through a certain level of treatment has potentially detrimental effects on the physicochemical properties of the soil and the nutrient status of crops (Bardhan *et al*., 2016; Alnaimy *et al.,* 2021). Wiem *et al.* (2023) reported structural deterioration, increasing heavy metal concentrations, and decreasing soil pH. Consequently, excessive salts in agricultural soils are one of the main factors affecting land abandonment and crop output. This represents one of the main concerns for wastewater recovery and reuse in environments with depleted or underdeveloped soils (Wiem *et al.*, 2023).

Various studies including Rodda *et al*. (2009), Christou *et al*. (2014), Bardhan *et al*. (2016) Mzini *et al.* (2015) and Mustapha *et al.* (2020) have given rise to varying responses in soil physicochemical properties, crop yields, and nutrient status of crops owing to several factors. These include the level of wastewater treatment, type of soil in the experimental area, selection of crops, soil treatment strategies, and prevailing climatic conditions. Thus, it is crucial that treated wastewater that meets the requirements for all trace metal levels serves as an irrigation source for edible crops. In Israel, the observed negative response of tree growth and yields of *Citrus sinensis* and *Persea americana* may have been attributed to the negative response of the physicochemical and hydraulic properties of clay soil from irrigation with wastewater passed through secondary treatment for 15 years (Bardhan *et al*., 2016). By contrast, Christou *et al*. (2014) and Bardhan *et al.* (2016) evaluated the impact of wastewater that has been subjected to tertiary treatment on the geochemical properties of sandy clay loam soil, and *Solanum*

lycopersicum fruit safety and productivity in three (3) years and eight successive harvests. The study found no compromise to the quality of produce and soil, as heavy metals in soil and fruits were below the bioavailability limits. There is a noticeable gap in the timeframes through which both studies were conducted; however, comparing both studies revealed that irrigation of crops with wastewater subjected to secondary treatment is highly likely to cause a negative response in soil physicochemical properties and further deterioration in crop yields and quality.

Again, more specifically, Bedbabis *et al*. (2014) studied the impact of using treated wastewater for irrigation of an olive orchard on sandy soil. This study concluded that there was an observed response in the key physicochemical properties of the soil. Notably, there was a significant decrease in soil pH and infiltration rate. According to Jaramillo and Restrepo (2017), the response in pH is a product of the type of soil cover, texture of the soil, level through which wastewater is treated, and period through which the soil has been irrigated with treated wastewater. Among other key findings by Bedbabis *et al.* (2014), there was a significant increase in electrical conductivity, sodium adsorption ratio, and soil organic matter. However, the study did not emphasise or detail the nature of the wastewater treatment technology, the extent to which the water was treated, and the soil amendment strategies utilised during the experiment. There is evidence to suggest that varying degrees of wastewater treatment and the treatment technology utilised, the nature of the composition of wastewater, the type of soil, the time of exposure, and the amount of treated wastewater used for irrigation of crops are important factors for deducing the feedback in soil physicochemical parameters and the quality and quantity of harvest (Bedbabis *et al.*, 2014).

Unlike clay and loam soil, nutrient accumulation in sandy soil is significantly low because of larger particle sizes and a lack of surface area for nutrient absorption (Pluer *et al.*,2020). Consequently, there is an increased leaching of soil water, and soil nutrients due to low CEC as opposed to clay or a mixture of clay/sand/silt or clay soils which have a relatively high CEC (Pluer *et al.*,2020).

By contrast, Bedbabis *et al*. (2014), and Tunc and Sahin (2015) investigated the changes in the physicochemical properties of loam soil from irrigation with wastewater that passed through the secondary treatment in a semi-arid region for two years. Red cabbage and cauliflower were cultivated and used for analysis. This study found that soil electrical conductivity and organic carbon content were higher in wastewater-irrigated soils. Moreover, the sodium exchange rate was low in the irrigated plots. Suspended solids in wastewater resulted in the clogging of soil

pores and a reduced soil infiltration rate. Bedbabis *et al*. (2014) and Tunc and Sahin (2015) did not indicate the nature of the soil treatment strategies used. However, both studies revealed similarities, including reduced soil infiltration and improved soil electrical conductivity and organic carbon content. Evidence from the literature suggests that irrigation with wastewater subjected to a certain level of treatment triggers a response (positive or negative) in the soil's physicochemical properties and the resulting quality of produce (Levy *et al*., 2014; Bedbabis *et al*. (2014); Tunc & Sahin (2015); Farhadkhani *et al*., 2018). However, the response in the soil may be influenced by various factors, including a combination of biotic and abiotic factors (Bedbabis *et al*., 2014). For instance, wastewater of similar composition used for irrigation in different soil types, growing conditions, and experimental periods may aid in varying responses in soil physicochemical properties and crop nutrition value.

Conventional water treatment systems including coagulation, sedimentation, filtration, and disinfection provide the most reliable and well-established wastewater treatment technology to effectively eliminate harmful substances, such as pathogens and chemicals (Pakharuddin *et al.*, 2021). Biofiltration systems are promising economical, energy-efficient, and ecologically sustainable strategies for decontaminating contaminated water runoff from IS. However, their efficiency in decontaminating pharmaceuticals, heavy metals, and emerging pollutants remains understudied (Egbuikwem *et al.*, 2020). Therefore, there is a need for extensive wastewater reclamation and reuse programs to ensure that various streams of wastewater treated using biofiltration technology are rigorously tested to meet the regulatory standards for various uses (Egbuikwem *et al.*, 2020) including the irrigation of edible crops. In addition, although soils play a vital role in contaminant regulation, the reuse of biofiltered contaminated runoff from IS poses a challenge, especially in instances where treatment efficiencies remain below screening criteria for the irrigation of edible crops.

2.4.The need for wastewater treatment and reuse in South Africa

Driven by economic growth, freshwater use and demand are predicted to grow by 2035, especially in industries such as agriculture and manufacturing (Donnenfeld *et al*., 2018). Currently, the agricultural sector accounts for 70% of water consumption in South Africa and is set to rise considerably owing to the increasing scale of food production (Ghanashyam, 2018). The World Wildlife Fund (2017) estimated that South Africa will face a water deficit of 17% because of less-than-average annual rainfall by 2030. Consequently, small-scale food producers and subsistence farmers situated in peri-urban areas are at greater risk of being exposed to water shortages, with a potential impact on food production at the local level.

However, the problem is not unique to South Africa but a global phenomenon (Wiem *et al.*, 2023).

As part of a long-term water augmentation strategy in water resources planning, the reclamation and reuse of wastewater is positioned as a viable strategy with the potential to assist countries such as South Africa to offset the impact of multi-year droughts and achieve their development objectives (Bardhan *et al*., 2016; Kalebaila *et al*., 2021). Thus, the integration of domestic wastewater treated through NbS into a long-term water augmentation strategy offers an opportunity to improve access to water (Donnenfeld *et al*., 2018) and the resilience of subsistence and small-scale farmers, among other benefits (Salukazana *et al*., 2006; Rodda e*t al*., 2010), as the demand for freshwater resources is set to increase by the year 2030 (Donnenfeld *et al*., 2018). According to Adewumi *et al.* (2010), many communities struggle to secure access to water resources for various purposes because of the lack of service delivery, placing emphasis on the recognition of the potential of wastewater reclamation and reuse programs as measures to improve access to water for these communities. Nonetheless, in South Africa, wastewater reclamation and reuse, particularly for crop irrigation, is not a widespread practice (European Union, 2019; Kalebaila *et al*., 2021).

Kalebaila *et al*. (2021) stressed the need for urgency as to the reclamation of wastewater and reuse given that South Africa is renowned as a water scarce nation. European Union (2019) states that while the technology, knowledge, and systems required to safely integrate treated wastewater into supply systems, public perception and acceptance are what stand in the way of South Africa's uptake of treated wastewater for reuse and reintegration into the potable water supply system and other alternative uses. Kalebaila *et al*. (2021) revealed that a combination of socio-political, technical, and economic factors is in the way of accelerated implementation of treated wastewater re-use within the water sector. Kalebaila *et al*. (2012) recalls the halting of the implementation of a direct potable water scheme in South Africa's eThekwini Metropolitan Municipality, which includes the city of Durban, where public perception took prominence in 2012. Following the widely reported 2015–2019 multi-year drought and subsequent water shortages driven by factors such as below-average rainfall in Cape Town, South Africa, the need for wastewater treatment and reuse as an alternative water augmentation strategy has been closely investigated (OECD, 2021).

Although water reclamation and reuse are not common practices in South Africa (European Union, 2019), the National Water and Sanitation Master Plan (NW&SMP) aims to expedite

the implementation of key measures, such as water reclamation and reuse, to manage existing and future water shortages that may impact the nation's development goals (Kalebaila *et al*., 2021). There are also stories of local and regional successes. The Karoo settlement of Beaufort West incorporates approximately 20% recycled water into its water supply to nearby dam systems (Kalebaila Union, 2019; Kalebaila Union, 2021). However, South Africa's existing policy guidelines often refer to water that has been reclaimed and treated using technologies such as reverse osmosis and other chemical-based water treatment technologies such as chlorination (European Union, 2019). Biological treatment, referred to as phytoremediation, can be integrated into technocratic solutions as a tertiary water treatment strategy.

2.5.Regenerative agriculture as a soil management strategy

According to de la Bellacasa (2015), one of the most prominent risks of future food crises could potentially be due to multi-year droughts and the loss of soil's self-regenerative capacity in the face of urban expansion, intensive utilisation, and the application of agrochemical inputs. Loss of soil's regenerative capacity is related to the loss of the ability of the soil to naturally rehabilitate and perform its regulatory role in the assimilation of nutrients to reduce the impact of elevated nutrient concentrations in the soil and subsequently reduce the quality and quantity of the resulting produce. When the ability of soil to assimilate nutrients and salts is enhanced, including enhancing soil organic matter, improving soil drainage, and soil microorganisms, offers an opportunity for the reuse of biofiltered contaminated water runoff from IS for the irrigation of crops with minimal risks to the environment, human health, soil, and crops (Green, 2019). However, as wastewater goes through a series of tests to achieve the standard needed for reuse for a specific purpose, it is important to note that its use in soils may not pose an immediate risk because soil does not lose its self-regenerative capacity overnight; it is a result of a combination of factors that interact with one another over a long period (Green, 2019). Consequently, previously productive soils may not be able to achieve regulatory, supportive, and provisioning functions. This period may be characterised by soils having reached their "maximum threshold" which has historically been recorded and referred to as soil exhaustion (de la Bellacasa, 2015).

Robert Rodale of the Rodale Institute coined the term regenerative agriculture with the aim of developing a holistic approach to revitalise the soil and achieve greater production at lower costs while ensuring that carbon sequestration is improved in the long term (Rhodes, 2017). Accordingly, by focusing on soil, regenerative agriculture seeks to achieve regulatory, supportive, and provisioning potential at relatively low-energy inputs. However, a review by

Newton *et al*. (2020) revealed a lack of an established definition of regenerative agriculture. Nonetheless, proponents of regenerative agriculture believe that the current dominant and globalised food systems driven by increasing production and capital accumulation are vulnerable to shocks and may potentially compromise soil functions in the long term (Newton *et al.,* 2020). Consequently, the vulnerability of communities, especially those residing in peri-urban areas, IS, and rural areas, remains relatively high (Rahimi *et al.,* 2021).

Again, advocates for regenerative food production systems consider regenerative agriculture as a multidisciplinary and holistic approach or strategy that accounts for the human, social, climatic, ecological, and economic aspects of food production, distribution, and consumption (Giller *et al.,* 2021). It emphasises the notion that reducing hunger and food insecurity may not necessarily be achieved through a globalised system of food production, but rather a localised system that fosters resilience and values different contexts through which the intervention seeks to make a difference (Anderson & Rivere-Ferre, 2021). Humans are not isolated from nature in this context, and caring for nature and soil is a prerequisite for civilisation. For instance, the decentralisation of wastewater treatment facilities and the subsequent reuse of treated wastewater and the uptake of regenerative agricultural practices may play a significant role in encouraging nutrient cycling, improving access to water resources through the re-use of treated wastewater, and fostering the resilience of subsistence and small-scale food producers.

Agroecology represents the foundation through which regenerative agricultural systems have been formulated (Anderson & Rivere-Ferre, 2021). Both entail nature-derived strategies to improve functional diversity at the genetic, species, ecosystem, and landscape levels, to promote responsible food production (Anderson & Rivere-Ferre, 2021). Furthermore, it encourages the design of farm systems to promote circular recycling of materials and, ultimately, energy efficiency. Similarly, agronomic practices must promote soil health, thereby sequestering carbon and preserving soil biodiversity and other natural resources to promote human health and well-being (Anderson & Rivera-Ferre, 2021). Table 1 presents the most common regenerative agricultural practices and their potential for restoring soil health. In particular, the use of biochar and green manure is essential for building soil organic carbon (Giller *et al.,* 2021) which in turn plays a role in biomass production. In this study, duckweed green manure was used as a source of organic soil carbon and plant nutrients. There is limited recently published literature on the use of duckweed for agricultural purposes, which will be discussed in Subsection 2.5.2.

Table 1: Regenerative agricultural strategies and practices with potential contributions to the restoration of soil health adapted from Giller *et al*. (2021).

Principle	**Practice**
Minimal soil tillage	Zero and reduced soil tillage.
Build soil organic carbon and encourage water percolation	The use of green manures, Biochar, compost, and animal manure
Avoid the use of pesticides and foster plant diversity.	Diverse crop rotations, multi species cover crops

2.5.1. Biochar

According to Glodowska and Lyszcz (2016), the use of biochar for soil improvement in agriculture has historically been practiced through the incorporation of charcoal. Of note is the Terra Treta, a practice of the pre-Colombian natives, where charred residues of organic and inorganic matter were combined with soil to yield organic-matter-rich soils in the Amazon. However, the terminology has been transformed to categorise certain charcoal production processes as yielding a different kind of charcoal called biochar, of a specific composition depending on the conditions of formation, such as temperature and the nature of feedstock (Gou *et al*., 2020). Wood can be transformed into biochar through a process called pyrolysis, which involves the transformation of various plant residues into bio-oils and syngas (Woolley & Hallowell, 2018). Through optimisation, pyrolysis can produce a range of products with varying compositions depending on the production temperature (Garcia *et al*., 2022). Of particular interest in this study is biochar which can potentially serve a variety of purposes, such as filter media for pollutants in wastewater, as a reducing medium for landfill leachate pollutant load, and for direct application in the soil to improve nutrient retention and water holding capacity (adsorptive) (Woolley & Hallowell, 2018; Garcia *et al*., 2022).

The physical and chemical composition of biochar can be understood based on the raw material used during its production and the conditions under which it is transformed (Jindo *et al*., 2014; Guo *et al*., 2020; Qian *et al*., 2023; Karim *et al*., 2021). Pyrolysis optimisation may yield biochar of a specific composition depending on the purpose through which the final product is applied (Guo *et al*., 2020). When analysed under a microscope, biochar is porous with a rough morphological surface that resembles a honeycomb-like anatomical structure (Guo *et al*., 2020). The porous nature of biochar can improve the capacity of soil to support plant growth and development by improving the soil water retention capacity and nutrient retention, reducing the bioavailability of heavy metals in the soil, and accumulating microorganisms

(George, 2022; Guo *et al*., 2020; Qian *et al*., 2023). Biochar from woody feedstock is a rich source of macronutrients, such as potassium, carbon, iron, phosphorus, and nitrogen, which are essential for plant growth and development (Woolley & Hallowell, 2018). The composition of different proportions of macronutrients in biochar is dependent on various factors, including the production temperature and feedstock (Karim *et al*., 2021). Gou *et al.* (2014) reviewed the literature on the mechanisms, processes, and effectiveness of biochar as a soil amendment strategy and revealed that the pH range of biochar derived from a variety of sources and conditions of formation ranges from 5.4 and 12.4 while ash and mineral contents ranged from 1.1% to 82%. Furthermore, the review discovered that the relatively higher pH in biochar contains a high mineral ash content and occurs at high pyrolysis temperatures (more than 600 °C). Consequently, its application to soil increases pH and CEC, resulting in improved efficiency of nutrient utilisation by crops (Kang *et al*., 2021). However, wood and plant residues generally have low mineral ash content, and therefore, lower pH (Gou *et al*., 2014).

Biochar and treated wastewater from an informal settlement are regarded as sources of nutrients essential for plant growth and development (McHenry, 2011; Winter, 2021) and pollutants that can potentially damage the environment. However, there are uncertainties regarding the direct effects of biochar application on soil ecology, surrounding environment, and residence time (McHenry, 2011). Consequently, care must be taken when using treated wastewater to irrigate crops in soils treated with biochar, as the potential risk of nutrient and salt accretion may be expected (Xiang *et al*., 2021). For instance, Xiang *et al*. (2021) found that the release of harmful elements from biochar to the soil may have resulted in chemical reactions and changes in surface properties and pH when biochar was used for soil amendment.

2.5.1.1.Plant nutrient availability in biochar made from woody feedstock.

According to Jindo *et al.* (2014), biochar, such as derived wood, may potentially yield less biomass during the first cycle of soil treatment at relatively high temperatures. However, this depends on biotic and abiotic factors. One of the many reasons advanced by Jindo *et al*. (2014) and Qian *et al*. (2023) is that fresh biochar is highly adsorptive and, therefore, may limit crop nutrient availability and result in a decrease in plant yields. Nutrient retention in the soil enhances the availability of nutrients that are essential for plant growth and development, including labile carbon, phosphates, and nitrates in the soil, and improves the mineralisation rate of organic matter (Jindo *et al*., 2014; Qian *et al*., 2023) for improved soil fertility (George, 2020). Guo *et al*. (2020) confirmed the findings of Jindo *et al*. (2014) who studied the selection of organic raw materials for biochar production. The study found that biochar obtained from

woody feedstock contained high levels of carbon with a high absorptive capability. Plant residue-derived biochar is a rich source of carbon (McHenry 2011). Furthermore, the study found that biochar pyrolysed at relatively low temperatures (300 °C) produced easily accessible organic carbon and plant nutrients needed for plant growth in the soil. Similar observations were made by Guo *et al*. (2020), who reported that different biochar feedstocks may yield varying amounts of plant nutrients, including 0.5 to 48.0, 0.1 to 198.0, and 1.4 to 91.5 g/kg Nitrogen, Phosphorus, and Potassium, respectively. Furthermore, the study concluded that the improved adsorption capacity of woody biochar was a result of the increased temperature of formation during pyrolysis.

2.5.1.2.Effects of biochar use on soil physicochemical parameters and crop quality. The high nutrient retention potential of biochar produced from woody materials can enhance soil aeration, further limiting runoff and leaching of chemical compounds (Jindo *et al*., 2014). Qian *et al*. (2023) has noted that soil has been shown to have retained about 33% and 53% more $NO3^-$N and $NH4^+$-N, respectively after the addition of biochar. An extensive literature review by Guo *et al.* (2020) aimed to understand the potential of biochar to immobilise chemical contaminants in the soil, and found a positive outcome when biochar was used as a soil amendment strategy aimed at mitigating soil contamination by immobilising heavy metals and organic compounds. For instance, through enhanced sorption and chemical precipitation, soil-treated biochar has been shown to stabilise chemical compounds such as Zn and Cu and reduce their bioavailability in soil to restore basic soil functions, such as producing edible crops that are safe for human consumption (Gou *et al*., 2020; George, 2022). George (2020) and Qian *et al*. (2023) found evidence of improved soil water and nutrient retention following the application of biochar in soil.

Figure 2 was adapted from Qian *et al*. (2023) and presents some of the most basic effects of biochar when applied to the soil. However, different contexts and environmental conditions may play a significant role in establishing the level of response for the respective soils and the quality of production where biochar has been applied (Jeffery *et al*., 2011).

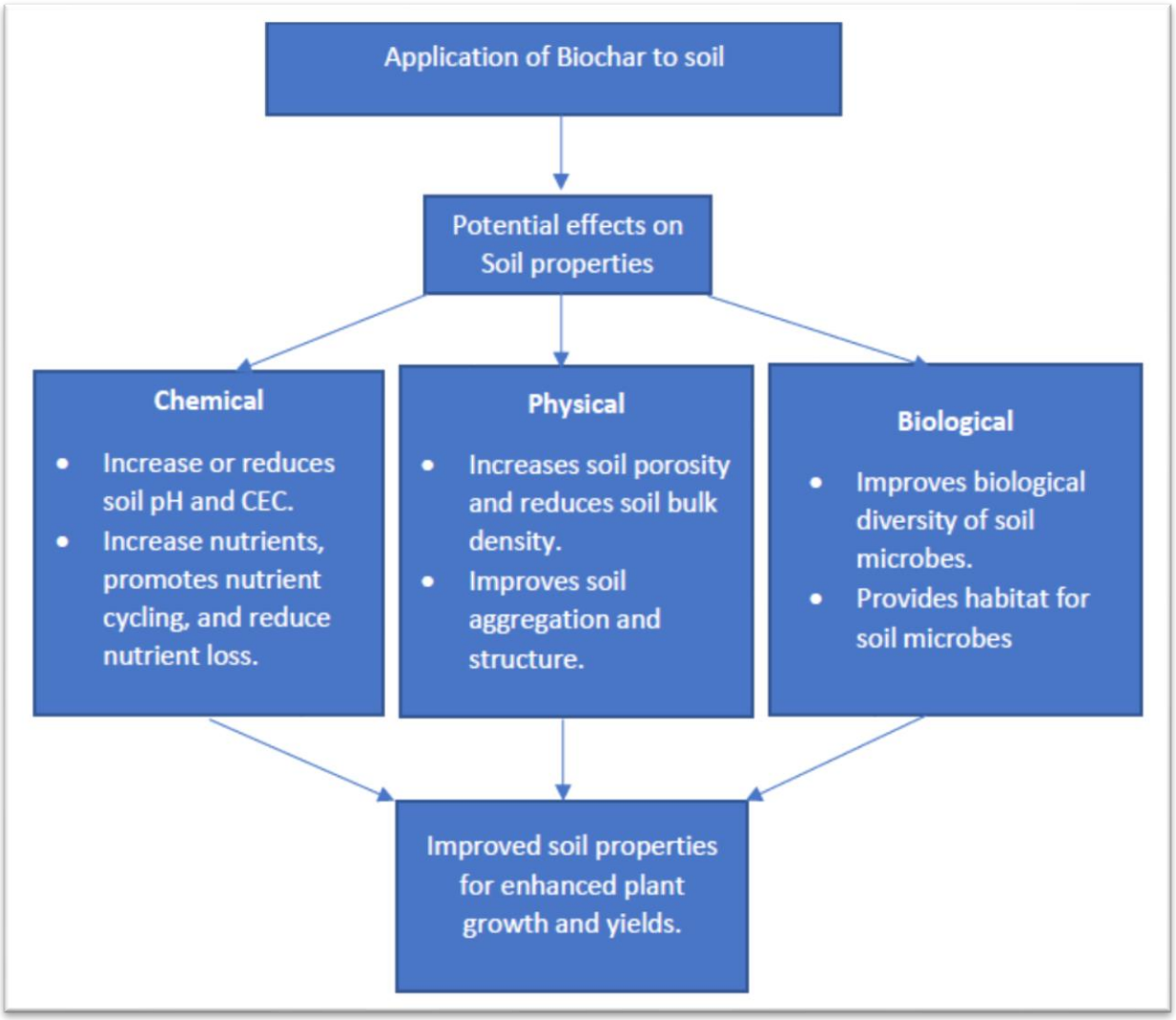

Figure 2: A summary of basic influence of biochar on soil's physicochemical properties adapted from Qian *et al*. (2023).

Various laboratory and field experiments have been conducted to investigate the potential of biochar as a soil amendment strategy to improve soil physicochemical and biological properties, improve crop yields, and maintain the bioavailability of heavy metals and organic contaminants in the soil, which have produced variable but positive outcomes (Guo *et al*., 2020). Gholamahmadi *et al*. (2023) conducted a global systematic meta-analysis to quantify and interpret the effects of biochar application on runoff and soil erosion. The study concluded that following the application of biochar, soil runoff and erosion were reduced to varying degrees, depending on the prevailing environmental and climatic conditions. Moreover, the study concluded that there was considerable improvement in soil structure which directly influenced the erodibility of the soil. On the other hand, Qian *et al*. (2023) observed improved crop yields especially on tropical soils. Jeffery *et al.* (2011) evaluated the relationship between the use of biochar as a soil management strategy and improved crop productivity across 14

studies. The study found the relationship to be statistically significant, with up to a 10% improvement in crop production. Although the relationship was found to be statistically significant, the study further cautioned that the effect may be variable, and the conclusion made may not always reflect the reality of different environmental conditions and contexts.

The porous nature of biochar also plays a significant role in allowing biological activity to thrive, given that pores serve as habitats for microorganisms that facilitate biodegradation and biotransformation of organic and inorganic compounds in contaminated soils (Qian *et al*., 2023). Moreover, pore surfaces are equally important in facilitating the adsorption of heavy metal ions and their conversion into hydroxide, carbonate, and phosphate precipitates (Gou *et al*., 2020). Therefore, improved biological activity may translate to improved biodegradation and biotransformation of chemical contaminants in the soil, guided by various factors such as prevailing climatic conditions. According to George (2022) and Qian *et al*. (2023), literature evidence suggests that treating soil with biochar reduces soil bulk density and improves its porosity. Consequently, the soil water-holding capacity is significantly enhanced (George, 2020; Qian *et al*., 2023). However, the improvement in the soil water retention capacity is a function of the amount of biochar applied to the soil (George, 2020). Moreover, the application of biochar to soil influences CEC, EC, and pH literature evidence reveals (Qian *et al*., 2023).

However, biochar derived from different feedstocks has diverse abilities and efficiencies in stabilising soil contaminants under different contexts and environmental conditions (Guo *et al*., 2020; Qian *et al*., 2023). According to Gou *et al*. (2020), biochar treatment can potentially elevate soil pH when applied regularly. This is a particularly important remediation strategy for soils that are exposed to heavy metal contamination. Gou et al. (2020) revealed that increasing the pH of the soil is one of the ways to promote the hydrolysis of heavy metals in the soil to reduce the concentration of water-soluble metal ions.

2.5.2. Duckweed

Duckweeds are free-floating monocotyledons belonging to the Lemnaceae family (Baek *et al*., 2021). They comprise five (5) genera (*Spirodela, Landoltia, Lemna, Wolffiella, and Wolffia*) under the subfamilies *Lemnoideae* and *Wolffioideae,* with 16 and 21 species, respectively. Duckweeds are widely distributed and may be found worldwide (Chenga & Stomp, 2009), except for some species that are endemic to specific regions (Sree *et al*., 2016). Duckweeds are not endemic to South Africa; nonetheless, they are found in various parts of the country (including the Eastern Cape, Limpopo, Western Cape, and Kwa-Zulu Natal), where nutrient

contamination is most prevalent in aquatic ecosystems (Mtshali *et al*., 2017). The number of species recorded in various journals and grey literature varied between 36 and 38 globally. This variation was addressed by Sree *et al*. (2016), who contended that the correct number of species is 37. Through the explanation of the supposed "incorrect" citing by various authors including Klaus *et al*. (2013) and Wong *et a*l. (2010), is that one of the species (*Lemna ecuadoriensis*) identified before the year 2000 who's similarly referred to as *L. obscura* has been mistaken for a different species of duckweed.

In addition to the short reproduction period (approximately two days per generation), the rapid replication and proliferation rate of duckweed makes it adaptable to various aquatic environments (Xiao *et al*., 2013). Duckweed has been found to thrives in ponds and lakes of water contaminated with industrial and domestic pollutants owing to its phytoremediation potential (Ali *et al*., 2016). Consequently, duckweed has been extensively studied as a tertiary water treatment strategy for various industrial and domestic pollutants. However, there is limited literature on the effects of duckweed on the physicochemical properties of soil and the quality of edible crops following irrigation with BTW from an informal settlement. Duckweeds have been found to grow in aquatic environments with minimal disturbance from conditions such as wind and rapidly moving water currents (Ali *et al.*, 2016). Furthermore, Dudley *et al.* (2009) reported that in less than 1 ha of nutrient-rich lagoons, the maximum dry biomass production of duckweed ranges between 10 and 12 metric tons, especially in regions with a growing season of up to 9 months, with an average of more than 1.5 tons a month.

Moreover, the growth conditions and chemical composition of duckweed may be optimised to increase biomass production and improve various chemical components, in line with opportunities for its utilisation (Cheng & Stomp, 2009). For instance, optimised growth conditions to enhance starch and protein content in duckweed enhance its use in producing ethanol for fuel and animal feed (Cheng & Stomp, 2009).

2.5.2.1.Review of duckweed use in agriculture.

Duckweeds have been extensively studied and evaluated for their effects on the treatment of domestic and industrial wastewater (Cheng & Stomp, 2009). Most studies have focused on the ability of duckweed to remove various inorganic and organic nutrients from industrial and domestic wastewater (Cheng & Stomp, 2009), pharmaceuticals (Allam *et al*., 2015), and heavy metals (Zhu *et al*., 2016). However, some studies have explored the potential of duckweed as

a food source for humans and animals (Iqbal, 1999), and others have recently explored the potential of duckweed in biofuel energy production (Cui & Cheng, 2015).

According to Iqbal (1999), duckweed has numerous applications in various sectors including agriculture, aquaculture, and domestic and industrial wastewater treatment works among others. For instance, the potential use of duckweed in the agricultural sector is due to its high moisture content (up to 94% for desiccating duckweed) and nutrient (nitrogen and phosphorus) content, with ease of direct application in the soil. When used in phytoremediation for wastewater treatment, duckweed has high nutrient adsorption capacity (Xiao *et al*., 2013). *S. polyrhiza* and *L. minuta* may serve as valuable sources of plant nutrients when used as green manure in agriculture (Xiao *et al*., 2013). However, its large surface-area-to-volume ratio has direct implications for the potential large-scale utilisation and commercialisation of organic fertilisers. Depending on the prevailing environmental and climatic conditions, growing media, and nutrient availability and tolerance, duckweed can attain near-exponential growth rates with relatively high biomass (Cheng & Stomp, 2009; Bartosova *et al*., 2015).

Cheng and Stomp (2009) reviewed the rates and mechanisms of nitrogen and phosphorus uptake and the growth of duckweed in nutrient-deficient environments. This review found that wastewater provides duckweed with essential nutrients to support its growth. Once growing conditions have been attained, duckweed stores excess nutrients on the tissues for use when nutrients in wastewaters have been "depleted" overtime. The latter may potentially impact soil nutrient availability, given the limited uptake and use of synthetic fertilisers which regenerative agriculture advocates.

In contrast, the use of highly contaminated duckweed sourced from industrial phytoremediation processes poses a threat to the environment (Xiao *et al*., 2013) and soil, and compromises the quality of crops where duckweed is used to improve soil organic matter. However, care, ongoing risk assessment, and mitigation strategies are required (Baek *et al*., 2021). According to Cheng and Stomp (2009), the desire to commercialise the use of duckweed in agriculture may be due to the following reasons: 1) the high moisture content of duckweed makes large-scale composting an energy-intensive exercise that may not be economically viable, and 2) it may take a relatively long period to achieve under natural conditions. Duckweeds can be utilised and directly applied to soil to improve the capacity of the soil to support crops and to assist in building soils (Cheng & Stomp, 2009). For instance, the use of *Lemnaceae* as green manure has been shown to have a positive impact on soil texture, improving water and cation

exchange, with approximately four (4) harvests being recorded for vegetables and corn (Cheng & Stomp, 2009). However, biomass production in different parts of the world varies depending on factors such as experimental conditions, climate, size of the growing area, crop variety, and nutrient supply and management.

Challenges, such as aging sewage infrastructure from lack of investment and lack of basic services, such as wastewater disposal facilities in peri-urban areas (Winter, 2021), may situate these environmental settings at the highest level of planning for cropping systems to increase duckweed biomass production for utilisation at the local level for various uses, including soil regeneration. This may be achieved through the construction of ponds and lakes to capture and treat wastewater runoff through NbS, and by integrating duckweed to improve water quality and biomass production for subsequent utilisation as green manure by local small-scale and subsistence farmers.

2.6.Summary

NbS offer an opportunity to treat irrigation standards and reuse wastewater for irrigation of edible crops. However, its reuse carries risks to the physicochemical properties of the soil, quality of the resulting produce, and human and ecological health. Through the uptake of regenerative agricultural practices in crop production, which include the use of biochar and duckweed as soil amendment strategies, there is potential for improved soil quality and crop nutrient availability. Therefore, through improved soil quality, the uptake of wastewater from an informal settlement treated through NbS may be expanded to limit the risk of soil contamination and improve water availability to subsistence and small-scale food producers located within peri-urban and IS. However, there are shortcomings, such as the rate at which soils develop to achieve expected crop yields.

CHAPTER 3: STUDY DESIGN AND METHODOLOGY

The Methods section provides a description of the procedures employed during data collection and analysis. Therefore, it offers detailed procedures and processes followed during the data collection and analysis process to enable the reader to visualise and replicate the study. This section is divided into four subsections: the study site, which provides a detailed account of the study site; study design, which provides a strategy to satisfy the research objectives and research questions; data collection methods, to describe procedures and protocols when collecting data; statistical analysis; the transformation of data to facilitate its analysis, including visual representation; and data quality and assurance, which describes procedures and protocols followed to ensure the credibility of the data collected.

3.1. The study site

The Water Hub is an abandoned wastewater treatment plant which has been turned into a water research facility located in a Mediterranean climate in Franschhoek, approximately 60 km east of the Cape Town Central Business District (CBD) in the Western Cape Province, South Africa. Built in the 1960s on land owned by the municipality, the vacant site presents a chance to establish a resource recovery centre and conduct cutting-edge research (University of Cape Town, 2024). The Stiebeuel River, which divides the study site in half, must have its contaminated surface water runoff treated first. The location of the Water Hub, where wealthy landowners and picturesque vineyards coexist with IS and the urban poor, is an excellent test site that reflects many of South Africa's waterways. Franschhoek Valley attracts thousands of domestic and foreign tourists who come to save the region's fine dining and wine harvest. On the other hand, 64% of residents in the residential settlement upslope of the Water Hub, 2.5 km from the town of Franschhoek, live in unofficial housing, with 1,800 homes sharing 150 toilets (University of Cape Town, 2024) According to the OECD (2021), the Western Cape region is

characterised by dry summers and wet winters with an average annual rainfall of less than 100 mm to 500 mm. The rainfall season varies from April to the end of September each year.

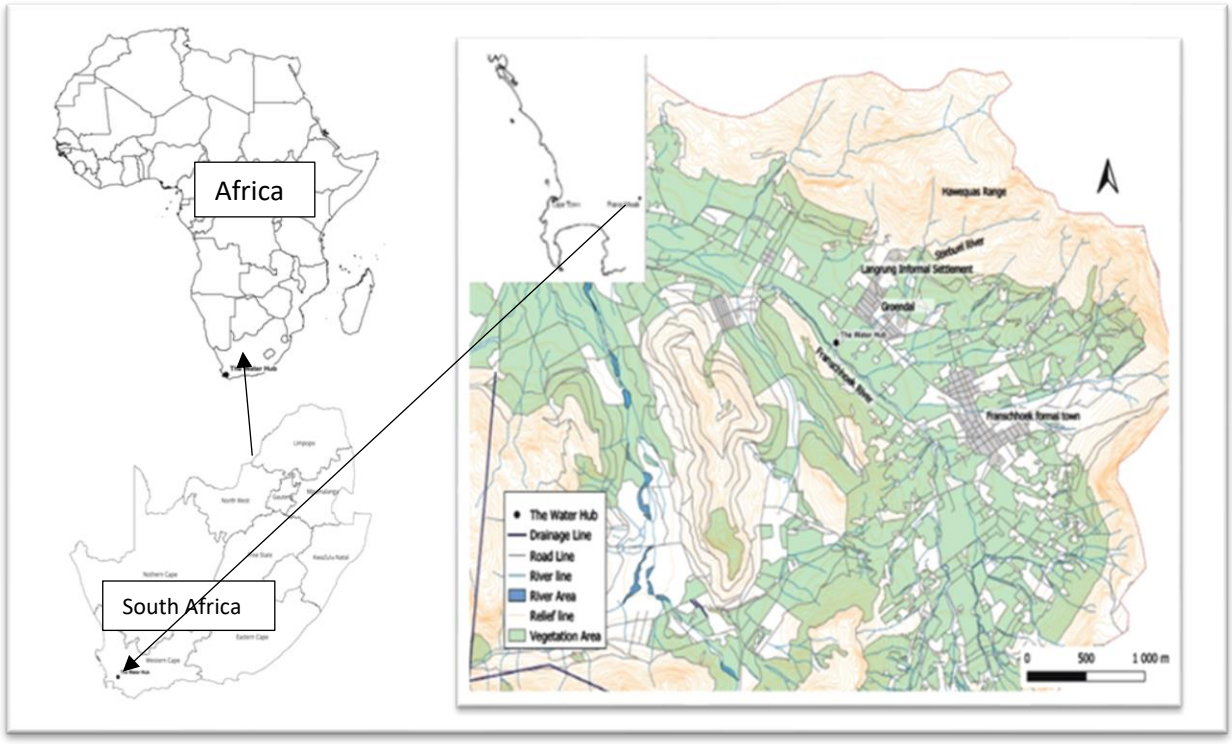

Figure 3: Map of the study site and the surrounding areas.

Contaminated water was pumped from the Stiebeuel River and stored in 10000l tanks located on the north portion of the site. Thereafter, the influent water was released via a series of valves and flowed into large biofiltration cells comprising vegetated and unvegetated cells with varying sizes of natural media (stone aggregates, carbon sources comprising peach pips, and biochar). Water is usually retained in each cell between 3 and 5 days, during which nutrients, such as phosphate, are captured within the bio-slime within the cells. In addition, elevated concentrations of ammonia are released into the atmosphere during the culmination of the treatment through identification. Similarly, bacteria and pathogens are exposed to microbes in an anaerobic environment and to ambient conditions in the environment. During the retention, and eventual discharge of treated water from the cells, researchers took regular samples and tested the chemical, biological, and physicochemical parameters. Approximately 100 Kl of water was added each week. Most of this water is discharged into the Stiebeuel River, which flows through a Water Hub research site. A small percentage was diverted to a holding tank, which was stored and used for irrigating a series of experimental gardens that were used in this study.

At the site, wastewater contaminated water was pumped from the Stiebeuel River and stored in 5000l tanks located on the north side of the site. Thereafter, raw contaminated river water is released from the tanks via a valve and flows to fill the bio-filtration cells which comprise both vegetated and unvegetated cells with varying gravel sizes constructed using bricks and lining materials at the bottom. Water is then left to settle on bio-filtration cells for a given period and is continuously tested for chemical, biological, and physicochemical parameters before being released into the environment via a series of pipes from a valve located on the southern end of the bio-filtration cells and/or reused to irrigate crops onsite. However, the water used for irrigation of crops was pumped from the release point of the biofilter cells to an onsite storage tank which was then fed into the irrigation system.

3.1. Study design

Crops utilised for the experiments were selected based on their ability to grow in less than 90 days under the prevailing climatic and environmental conditions and suitable for cultivation throughout the year in the study location. Again, the treatment and the reuse of contaminated water runoff from IS form part of the poverty alleviation strategy and the potential expansion of water resources to disadvantaged communities (Kalebaila *et al*., 2021). Again, as part of risk assessment and the potential uptake of biofiltered contaminated water runoff for the irrigation of crops, the selection of crops needed to be suitable for human consumption. In addition, given that the accumulation of salts and nutrients occurs within the soil during irrigation, a combination of both bulbus (below-ground) and leafy (above-ground) crops needed to be selected for the study. This was to establish which crop was suited to be grown under BTW irrigation conditions based on the degree of the uptake of heavy metals from the soil. In essence, bulbous plants are at the highest risk of accumulating potential contaminants in treated wastewater before soil remediation for uptake at relatively low concentrations (Saruchi & Khanna, 2011). However, as a function of the irrigation system utilised and the accumulation potential of each heavy metal by crops, there is a greater chance that the accumulation of nutrients in crops may not differ (Saruchi & Khanna, 2011). Because of the varying growth rates for each successive harvest, the experiment was extended from 120 days initially planned to 210 days, with three harvests in 2023. Bulbus and leafy crops were subjected to two water treatments, MTW, and BTW from IS via a sprinkler irrigation system (figure 5). Cropping was carried out on three different soil treatments namely: duckweed, control, and biochar.

Prior to cropping, spinach, carrot, beetroot, and lettuce seeds were grown separately, irrigated with MTW (outdoors) until they germinated, and were at an appropriate height (5 cm from

ground level) for transfer to the respective planting beds. These were selected because they can grow and mature in less than 90 days when growing conditions such as temperature, soil nutrients are favourable. This would provide enough time for sufficient data to be collected. This was done to achieve a comparative analysis of crops and soils subjected to different irrigation conditions. MTW remained the suitable water source for the irrigation of safe to eat crops and for drinking purposes at the study site. Therefore, the use of MTW for irrigation during seed cultivation was to establish suitable irrigation conditions for comparative analysis to be carried out. Furthermore, before seedlings were transferred to their respective beds, and after each successive harvest and soil amendment, i.e. the addition of duckweed or biochar, soil samples were collected at the depth of 10 cm on each planting bed and irrigation condition using zip lock bags and preserved on an ice cooled cooler box for transport to the laboratory for analysis of physicochemical parameters. Most importantly, additional soil treatments following each successive harvest were to improve the level of SOC. Essentially, at more than 3% SOC, agricultural soils are regarded as productive while significant losses result reduced biomass (du Plessis *et al*., 2011). Seeds were cultivated according to an established standard suitable for each crop and as guided by the Starke Ayres seed and garden care catalogue first edition published in 2019.

Thereafter, edible parts of the crops on each planting bed and irrigation conditions were sampled, rinsed with deionised water, packed on appropriately labelled zip lock bags, transferred to a cooler box with ice packs, and transported to the laboratory for testing. Icepacks were used to preserve and reduce the degradation of analytes while in transit to the laboratory. Sampling, packing and dispatch of successive harvest were carried out on the same day of sample collection in September 2022, November 2022, February 2023 May 2023, and August 2023. Also, two additional soil and crop quality data (September 2022 and November 2022) were sourced from the Water Hub's management systems and studied for inclusion to the report. However, the experiment commenced on the same date; that is, the planting of seeds and seedlings was carried out on the same day for all crops selected for the experiment. This was done to ensure that all crops used in the experiment were harvested and sampled at the same time for dispatch to the laboratory for analysis.

3.1.1. Planting bed preparation and seed cultivation

Three plots (3 m2 each) were prepared on either side of the irrigation system from a 9 m2 plot area, one irrigated using BTW and the other with MTW (Figure 5). Both BTW and MTW were stored in 2500L tanks and subsequently released from a control valve into each irrigation

system via gravity. Control soil planting beds were prepared using compost sourced from an onsite soil composting pit produced by compacting a variety of garden waste, including grass, wood clippings, and vegetable waste. The soil used for the experiment was transferred from sludge drying beds located onsite and subsequently prepared as a control. However, two of the three planting beds were further treated with duckweed and biochar (crushed to a 2 mm diameter). Biochar was sourced locally (Mamre) from an organic fertiliser processing factory and subsequently mixed with soil and chicken manure to activate microbial activity. Molasses (a by-product of sugar production) was utilised to bind the mixture which was left to inoculate for 3 days prior to transfer into the soil. Furthermore, duckweed was sourced from maturation ponds situated at the Water Hub, rinsed with MTW to remove any excess contaminants, sun dried and transferred into the soil.

During the first and third planting cycles, biochar and duckweed were applied to the soil by spreading over the soil surface and mixing with the soil to a depth of 5 cm before transferring seedlings into the soil. This was done to achieve consistency in mixing throughout the planting beds and the soil profile. For each 3 m^2 planting area, approximately 2-wheelburrow loads (with a 120L bucket) of biochar, and duckweed were applied. However, during the 2^{nd} planting cycle, mixing was revised and achieved by digging 8 cm deep and 10 cm wide rows with a base filled with duckweed and biochar to a thickness of approximately 3 cm to reach the root systems of the plants. According to George (2022), the application of biochar to the soil is highly contentious. However, "deep banding" biochar in the soil ensures that losses due to wind erosion are minimised. George (2020) regards this method as the most appropriate for improving soil water holding capacity as compared to surface mixing. Furthermore, soil treatments by spreading on the soil surface were performed to further improve soil organic matter, and field observations noted inefficiencies in crop growth. Moreover, each row on each of the three planting beds on either side of the irrigation system contained both bulbus and leafy crops (Figure 5).

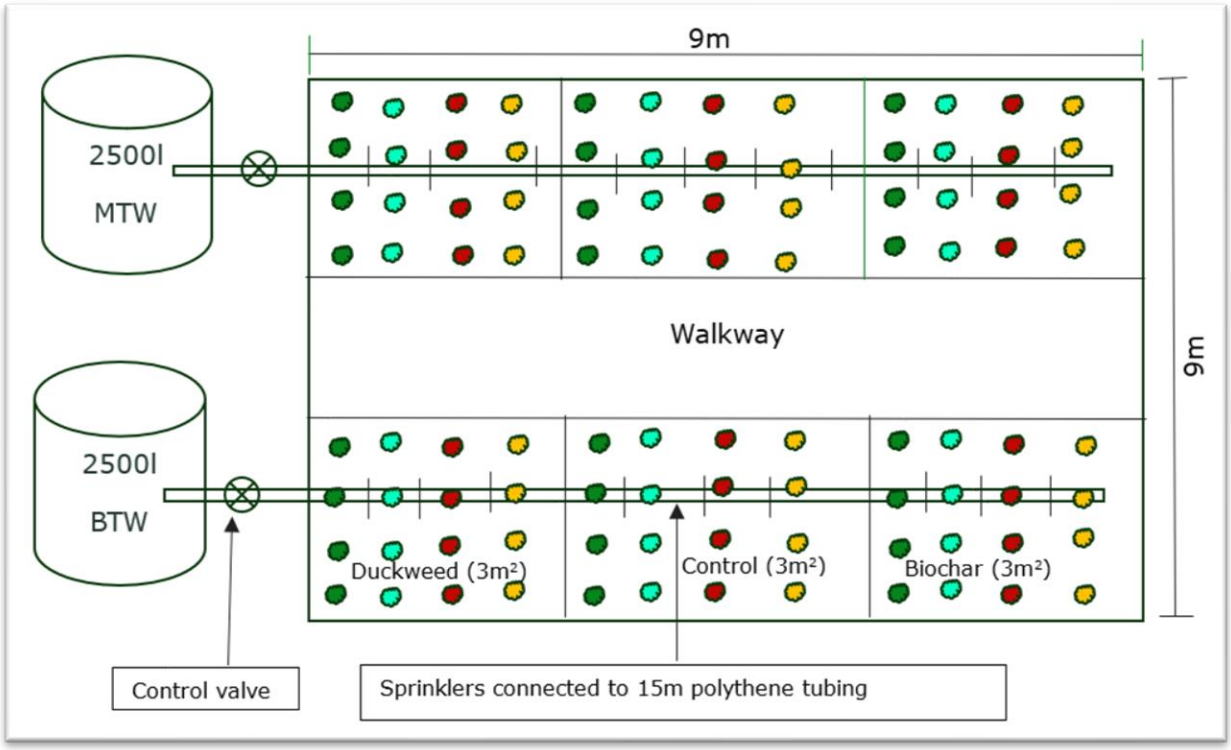

Figure 4: The layout of the experimental area. Spinach (green), lettuce (blue), beetroot (red), and carrots (orange). NB: The layout was not drawn to scale.

3.1.2. Irrigation system design and frequency of irrigation

Along either side water treatment conditions, a 20 mm wide and 15 m long polythene pipeline was used to construct the irrigation system. Fitted along the pipeline to reach each planting bed to dispense water were Tap Micro 10 x Peg and 360 Degree Sprinkler Orange. This is a "ready to use" micro irrigation system for small scale irrigation. The 20 mm polyethene piping was connected to 2500l tanks, with each filled with MTW and BTW on the other. On each 3 m2 plot, six (6) water sprinklers were connected to the 15 m long polythene pipeline to achieve a uniform coverage of the planted area.

Through physical observation, irrigation was monitored to prevent excess nutrient input, and soil nutrient washout from irrigation with BTW, and MTW respectively. Crops were irrigated once a day (i.e. in the early mornings for an average of 1 hour. To prevent, over irrigation and nutrient leakage, the soil was kept wet only as deep as the roots. The experiments were conducted in the field (i.e. with no control of climatic and environmental conditions), and soil saturation was probable where rainfall was forecast. Therefore, water was applied at a rate that allowed it to penetrate the soil profile to prevent crusting of the soil surface and subsequent water runoff.

3.2. Data Collection Methods

3.2.1. Soil and water sample collection and testing

After each successive harvest before soil application with duckweed and biochar, the soil, from the surface to a depth of 10 cm was sampled using a hand trowel on each soil treated bed and irrigation condition. Each time, a new pair of nitrite gloves were used to prevent cross-contamination of the soils. The topmost soil to a depth of 10 cm was sampled because “root-soil" penetration was up to 15 cm. To achieve homogeneity in the sample, two soil samples were collected on the opposite edges of the planting beds and one in the middle of the bed, transferred to an appropriately labelled zip-lock plastic bag, transferred to an ice-cooled cooler box for the preservation of analytes and subsequently transported to the laboratory to determine soil Zn, Fe+, Na+, SOC, and CEC. For soil sampling procedures and guidelines, USEPA (2024) standard operating procedures section 2, subsection 2.1. This was done to make a comparative analysis against similar soil treatment conditions found in MTW and BTW irrigation. Soil pH was regularly tested by pushing and penetrating the soil surface to 10 cm at different locations using a handheld portable soil pH meter (PH328) to compare against laboratory-measured recorded tests.

Studies evaluating the effects of irrigation with untreated greywater from IS (Salukazana *et al*., 2006), the use of biofiltration cells to filter contaminated water from a slum (Ghanashyam, 2018) and that evaluating the influence of biofiltration-treated contaminated runoff from IS on crops (Winter *et al*., 2023) have emphasised the risk posed by bioaccumulation of Na to the soil and subsequent potential long-term effects of reduced soil's reproductive potential. The above formed the basis through which the soil physicochemical parameters analysed were selected through studies within the South African context focusing on using wastewater from IS.

At the point of control valves, samples of MTW and BTW were collected through a grab sampling procedure using 200 ml glass containers. At this point, water was released to flow for three minutes to achieve homogeneity in water before the sample was collected. Thereafter, the glass container was rinsed three times using the sample water before it was filled to less than 1% of its volume and preserved on an ice-cooled cooler box for transport to the laboratory and testing of physicochemical properties and nutrient concentrations before the commencement of the experiment period (see United States Environmental Protection Agency [USEPA], 2016).

3.2.2. Crop sampling and quality testing

During the harvesting period, edible parts of the fruit and leaf were sampled, rinsed with deionised water, stored in appropriately labelled zip-lock plastic bags of crops were collected preserved in ice-cooled cooler box, and transported to the laboratory for analysis of nutrient status by establishing the concentration of macronutrients (N, P, Ca, and Mg) and micronutrients (Na, Zn, Mn, and Fe). Section 7 of the Standard Operating Procedures on Sampling and Analysis of Agricultural Products of Plant Origin developed by the Department of Agriculture Forestry and Fisheries [DAFF] (2018) was used as a guide to sample, preserve, and dispatch fruit and leaf samples to the laboratory for testing. Sampling the edible part of the crop was crucial because it would inform whether crops grown under BTW irrigation conditions would be suitable for human consumption. This assisted in making a comparative analysis of the nutrient concentrations in crops grown using different water treatment technologies i.e. MTW and BTW. The selection of vegetable parameters assessed in the study was guided by research in Winter *et al.* (2023) that previously focussed on using BTW to irrigate vegetables and compare the nutrient concentration to those found in the local market. This forms part of the extension of the published works by Winter *et al.* (2023).

3.2.3. Secondary data.

The data from WHO, retrieved from Olayiwola *et al.* (2017) details guidelines of the concentration for various micronutrients in edible vegetables for human consumption. This data was used to compare against the detections and/or concentrations of micronutrients from vegetables irrigated using BTW. Furthermore, data on macronutrient laboratory norms, and macronutrients sufficiency ranges were retrieved Campbell (2000) to compare against those in crops irrigated using BTW.

3.3. Statistical analysis

Laboratory data for soil and selected crops for the experiment were statistically analysed using Microsoft Excel version 2016 to manipulate, graphically represent, and provide descriptive statistics for data interpretation. Specifically, range, standard deviation, mean and skewness of the data was computed to provide a descriptive analysis of the relationship between the soil treatments, and crops under the two irrigation conditions. The data was further visualised by constructing box and whisker plots to show differences between water treatment conditions.

CHAPTER 4: RESULTS AND DISCUSSION

This section provides an analysis of the results, concerning the aim of the study described in Section 1.5. Due to data limitations and the scope of the research, the results and discussion section were combined to provide the audience with ease of reading and comprehension. Section 4.1 provides an analysis of the measured condition of the water used at the beginning of the experiment compared to the water quality guidelines provided by the Department of Water Affairs and Forestry [DWAF] (1996). Sections 4.2 and 4.3 present a comparative analysis of the concentration of nutrients in soil and crops, respectively. The box and whisker plots illustrate the concentrations of nutrients in the soil and crops during the study period, followed by an explanation for each case.

4.1. Characterisation of irrigation water utilised for irrigation of crops.

Table 2: Characteristics of selected physicochemical properties of water used for irrigation of crops during the experimentation period.

Measured Parameters	MTW	BTW	DWAF target range for crop irrigation.
Physical and Aesthetic determinants.			
pH at 25°C	7.2	8.0	6.5-8.4
Electrical Conductivity (EC) at 25°C	6.4 mS/M	52.8 mS/M	≤40 mS/M
Macro and micro chemical determinants (mg/L).			
Chloride (Cl)	9.4	47.0	≤100
Dissolved Sodium (Na)	4.3	47.1	≤70
Dissolved Calcium (Ca)	4.1	25.2	-
Dissolved Magnesium (Mg)	<0.50	2.9	-
Dissolved Potassium (K)	<1.0	8.8	-
Dissolved Sulphur (S)	0.65	8.6	
Sulphate (SO_4)	2.0	25.9	-
Dissolved Iron (Fe)	<0.10	0.20	≤5.0
Dissolved Manganese (Mn)	<0.05	0.06	≤0.02

The target pH range, as guided by DWAF (1996), is set between 6.5 and 8.4 for water to be considered suitable for irrigation purposes. The pH of BTW was 8.0 which is higher than that of MTW (7.2). Measured in mg/L, BTW contained five (5) times more chloride than MTW

did. Again, the national guidelines of water for irrigation of crops provide that the threshold for the concentration of Na, Fe, and Mn is advised to be ≤ 70 mg/L, ≤5.0 mg/L, and ≤0.02 mg/L, respectively for detrimental effects on soil and crops to be minimised in the long term (DWAF,1996). The pH, Cl^-, Na, and Fe+ were within the DWAF guidelines for water used to irrigate crops in MTW and BTW. However, manganese for BTW was slightly high (0.06 mg/L) as compared to MTW (<0.05), however, these were both high comparable to the national target range for irrigation of crops (≤0.02 mg/L).

The results for BTW showed that electrical conductivity was 8 times higher than that of MTW, 52.8 mS/M, and 6.4 mS/M respectively. The observed electrical conductivity in the BTW was above the target range of ≤40 mS/M, as presented in the DWAF (1996) guidelines. Consequently, the Na concentration in BTW was 10 times higher than that in MTW, 47.1 mg/l, and 4.3 mg/l respectively. This was possibly due to agricultural runoff and wastewater from household sources (DWAF 1996). Therefore, the concentration of Na may be high in the runoff of leachates from soils irrigated with treated wastewater, with potentially adverse effects on the physical conditions of the soil and its ability to support crops in the long term (Jeong *et al.,* 2016). Moreover, in BTW, dissolved Ca+, Mg+, K+ and $SO4^+$ was 25.2 mg/L, 2.9 mg/L, 8.8 mg/L and 8.6 mg/L, respectively. However, these were relatively higher compared to MTW, 4.1 mg/L, <0.50 mg/L, <1.0 mg/L, and 0.65 mg/L, respectively. Again, BTW contained 12 times more SO4+ compared to MTW, 25.9 mg/L and 2.0 mg/L respectively. The measured constituents in BTW were within the DWAF target range for crop irrigation.

4.2. Analysis of soil physicochemical properties.

This section presents a comparative analysis of soil's physiochemistry between BTW and MTW. Abbreviated, CsB (Control and BTW irrigated soil); CsM (Controlil and MTW irrigated soil); DwB (Duckweed treated and BTW irrigated soil); DwM (Duckweed treated soil and MTW irrigated); BcB (Biochar treated and BTW irrigated soil); BcM (Biochar treated and MTW irrigated soil). Along with the median, the interquartile range (IQR), herein referred to as the range, was analysed to provide an overview of where most of the values were situated, how clustered they are, and how the concentration compares in the 25^{th} and 75^{th} percentiles. Working with range was because it is based on the middle half of the distribution and is not influenced by extreme (high and low) values in the data compared to the mean. Therefore, it reflects the concentrations compared to different irrigation conditions. Furthermore, skewness was analysed to estimate where most values were related to the group median.

4.2.1. Soil Organic Carbon (SOC)

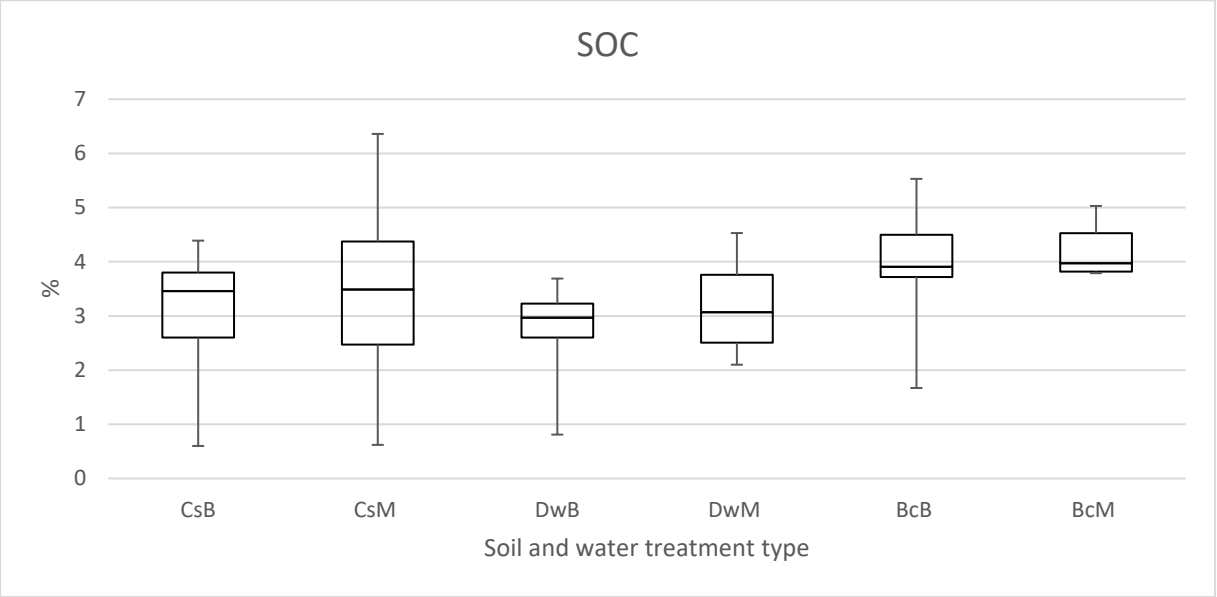

Figure 5: Box and whisker plots of SOC.

Measured from the soil surface to a depth of10 cm to the soil profile, the SOC concentration ranged between more than 2% and less than 6% (Figure 6). As shown in Table A1, the median SOC concentrations were comparable between CsB (3.46%) and CsM (3.49%), DwB (2.97%), DwM (3.07%), BcB (3.91%), and BcM (3.97%). Except for BcB, the range of CsM (4.4-2.5%) and DwM (3.8-2.1%) was comparatively higher than that of CsB (3.8-2.6%) and DwB (3.2-2.6%). Therefore, the difference between the values in the 75^{th} and 25^{th} percentiles measured for SOC in MTW-irrigated soil was significant, spread out about the median, and comparatively high. Moreover, the distributions of SOC in CsB, DwB, and BcB were negatively skewed and asymmetrical, indicating that most SOC values were clustered at the lower end of the scale. Meanwhile, most measured SOC values, CsM, DwM, and BcM, were concentrated at the higher end of the scale and positively skewed.

According to Saha *et al.* (2023), the proportion of carbon remaining in the soil following the decomposition of organic matter content is represented by SOC. Increasing carbon in the soil has benefits such as improving soil health and crop productivity, contributing to the reduced need for inorganic or synthetic fertiliser inputs (McHenry, 2011; Saha *et al*., 2023). However, Chaoua *et al.* (2019) argued that wastewater irrigation improves SOC and organic complexing molecules of low molecular weight. These molecules may act as carriers of micronutrients, particularly, heavy metals, and augment their absorption in crops. Leifeld and Fuhrer (2010) analysed datasets from 32 peer-reviewed publications comparing SOC contributions between conventional and organic farming. This review revealed that the addition of organic fertilisers

resulted in a relative rate of change in SOC greater than zero. However, while increasing SOC remains a function of several factors, the addition of organic fertilisers remains the single most crucial driver that raises the levels of SOC at an annual rate of 2.2 %. Raising the percentage of SOC through conventional agricultural practices requires patience and persistence which may be impossible in certain instances (McHenry, 2011; Leifeld & Fuhrer, 2010). This study discovered that the amount of SOC in soils across MTW irrigation was comparable and increasing (although there were limited data to provide evidence of the increasing trend graphically). Despite the SOC contribution from BTW and organic fertilisers, the above anomaly and indifference in SOC may be attributed to the loss of organic matter from microbial activity intensification due to labile C contributions (Tarchouna *et al.* (2010); Farhadkhani *et al.*, 2018), resulting in the formation of humic substances and discharge of minerals (Gatta *et al.*, 2020). This finding coincides with that of Leifeld and Fuhrer (2010), Christou *et al*. (2014), Farhadkhani *et al.* (2018), and Gharaibeh *et al.* (2016), whereby contributions from organic fertiliser and irrigation with treated wastewater, respectively, led to an increase in SOC over time. However, the levels of SOC in soil treatment and water irrigation conditions over an extended period remain unclear.

However, according to Olson and Al-Kaisi (2015), soil management practices, i.e. tillage systems influence the distribution of SOC within the soil profile. Consequently, SOC retention within the soil profile may be altered at different depths in the soil profile owing to soil disturbance.

4.2.2. Sodium (Na+)

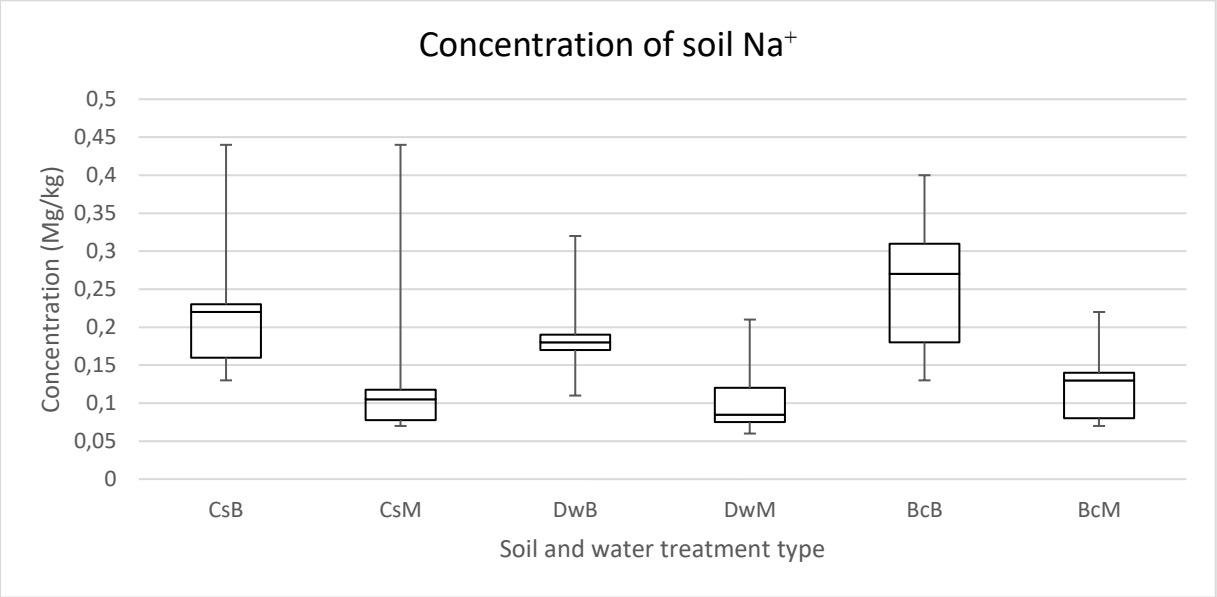

Figure 6: Box and whisker plots of soil Na+.

The concentration of exchangeable Na+ in the soil increased following irrigation with BTW relative to irrigation with MTW (Figure 8). Notable, was the median concentration of Na+ on CsB, DwB, and BcB measured at 0.22 mg/kg, 0.18 mg/kg, and 0.27 mg/kg respectively (Table A3). The ranges within which the measured concentrations of Na+ were for CsB, DwB, and BcB were 0.23-0.16 mg/kg, 0.19-0.17 mg/kg, and 0.31-0.18 mg/kg respectively. This indicated that the concentration of Na+ between the 25th and 75th percentiles was relatively high. According to Jeong *et al*. (2016), the presence of salts in treated wastewater is determined by its electrical conductivity. Therefore, the above equates to elevated concentrations of salts in the treated wastewater. Rodda *et al.* (2011) found that soil irrigated with greywater from an IS exhibited increasing electrical conductivity signifying the accrual of salts, particularly of Na+ over time. Consequently, the continued reuse of treated and untreated wastewater increases the risk of Na+ accumulation to toxic levels, leading to saline and sodic soil conditions (Jeong *et al*., 2016). The latter may affect the ability of crops to access water and nutrients from the soil and limit the ability of the soil to support crops in the long term (Rodda *et al*., 2011; Jeong *et al.*, 2016). Shamyov *et al.* (2023) investigated the effects of irrigation with municipal treated wastewater on vineyard soil and grapevines for nine years. A reduction in yield by approximately 12 to 49% was established and attributed to the accumulation of Na+ in the soil. Similarly, Garcia-Orenes *et al.* (2015) found that the prolonged use of treated wastewater for irrigation of orange tree orchards triggered an increase in soil salinity owing to the accumulation of Na+ in the soil. In this study, it was discovered that irrigation with BTW

increased the concentration of Na+ at depths of 0 to 10 cm as opposed to soils irrigated with MTW. A similar finding was established by Neilsen *et al.* (1989), who found which irrigation with treated wastewater increased the concentration of Na+ in the soil at a depth of 0.0 to 0.3 cm. According to Shamyov *et al.* (2023), rainwater is crucial for enhancing the leaching of Na+ in the soil and is regarded as one of the main controls for reducing its concentration in the topmost soil. Likewise, the use of MTW and the subsequent low concentrations of Na+ in the soil may be due to increased leaching and relatively low contributions of Na+ to irrigation water. However, the accumulation of Na+ in soil irrigated with BTW may have been due to insufficient leaching and enhanced evaporative accumulation (Shamyov *et al.*, 2023). Moreover, Bass *et al*. (2011) and Abideen *et al*. (2020) observed a high probability of water retention in soil treated with biochar. Consequently, in this study, the observed high Na+ concentration in soil treated with biochar and irrigated with BTW may be attributed to insufficient leaching due to the high water-holding capacity in biochar-treated soil and the subsequent contribution of Na+ from BTW and enhanced evaporative accumulation.

4.2.3. Cation Exchange Capacity (CEC)/ Total cations.

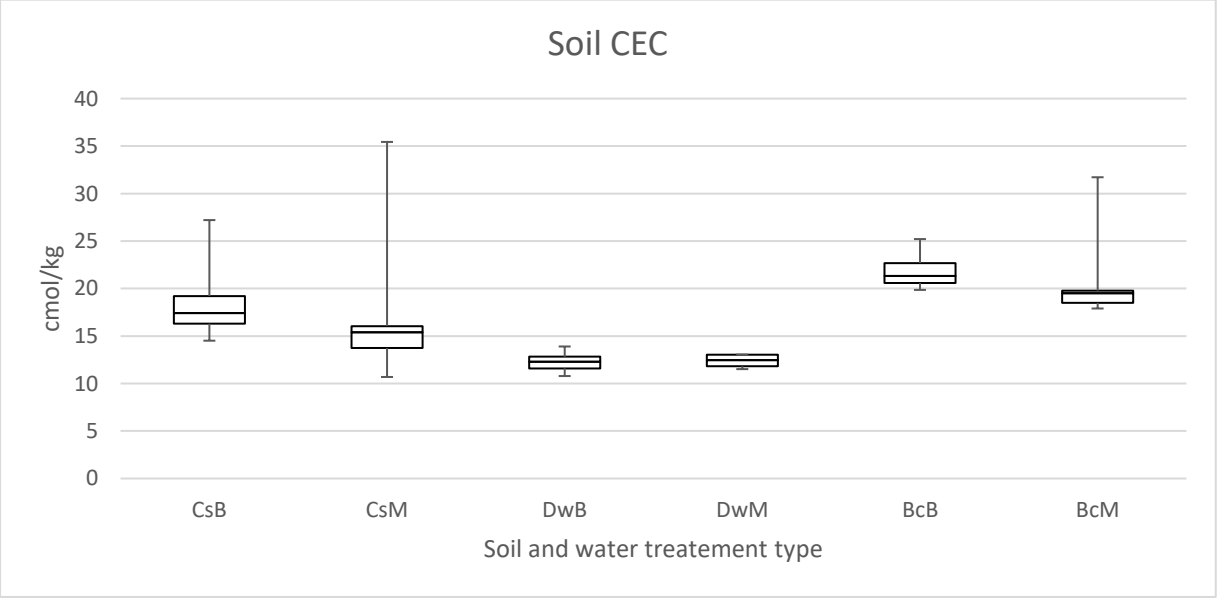

Figure 7: Box and whisker plots of soil CEC.

The measured values of soil CEC for BcB and CsB were positively skewed with less pronounced asymmetry, indicating that most of the values measured for CEC were comparable to the group median. The median CEC in soils irrigated using BTW were high, particularly BcB (21.85 cmol/kg) and CsB (17.4 cmol/kg) relative to soils irrigated using MTW, 15.4 cmol/kg, and 19.5 cmol/kg (Table A4). The range within which values in the 25th and 75th percentile is located was relatively high for CsB (19.2-16.3 cmol/kg), and BcB (21.9-20.8

cmol/kg) compared to that of MTW irrigated soil, 16.25-13.7 cmol/kg, and 19.8-18.5 cmol/kg respectively (Figure 9).

CEC represents the ability of the soil to retain nutrients and make them readily available to crops (Guo *et al.*, 2020). Irrigation with wastewater enhances soil organic matter which, in turn, creates more sites for cation adsorption on the surface of soil colloids (Asirifi *et al*. 2023). Consequently, an increase in the CEC of the soil irrigated with treated wastewater may be due to the enhanced Na+ ions in the treated wastewater. In this study, there was evidence of improved CEC in soils irrigated with BTW (CsB and BcB). These findings are in line with those of Farhdkhani *et al*. (2018), who studied the effects of irrigation with secondary-treated wastewater on soil physicochemical properties and crops. This study revealed an increase in CEC in soils irrigated with secondary treated wastewater, as opposed to those irrigated with MTW. The increase was attributed to the improvement of soil organic matter owing to the contribution from treated wastewater and organic soil amendment strategies (Angin *et al*., 2005; Hashem & Qi, 2021). Moreover, biochar-treated soils on either side of the irrigation conditions had a relatively high CEC compared with other soil treatments and irrigation water conditions. Tarchouna *et al.* (2010) attributed the enhanced CEC to the contribution of particulate organic matter from the hydrolysed organic substances of biochar. Kang *et al.* (2021) reported that the application of biochar to soil positively influences the pH and CEC.

4.2.4. Zinc (Zn+)

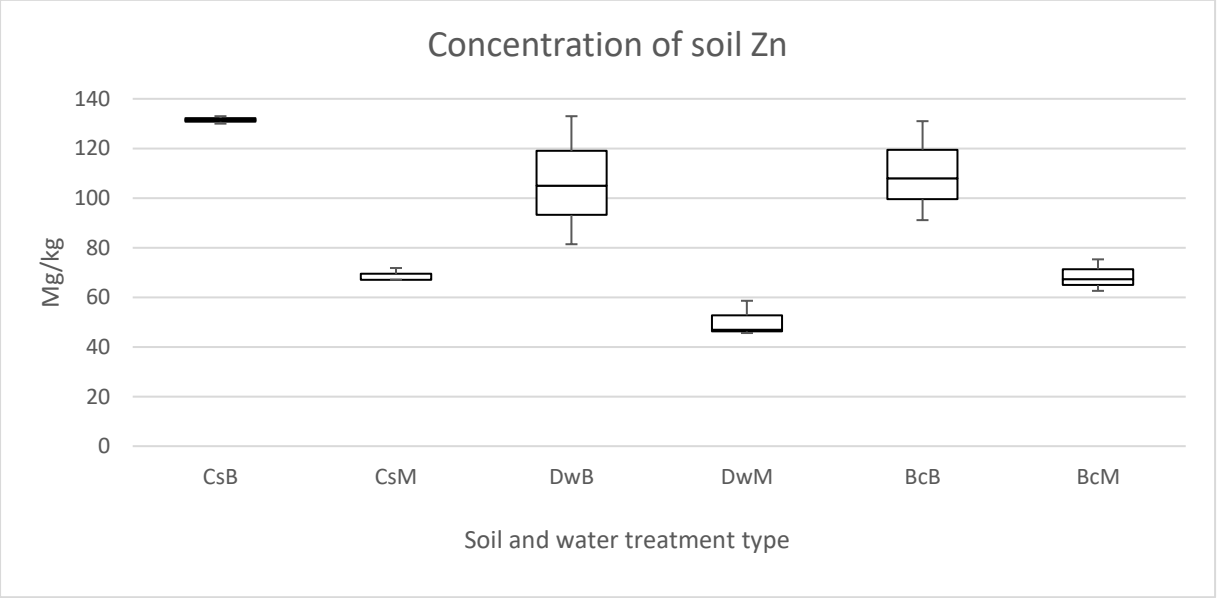

Figure 8: Box and whisker plots of Zn+ in the soil.

The median concentrations of CsB, DwB, and BcB were 133, 105, and 108 mg/kg, respectively, which were higher than those reported for MTW-irrigated soils (Table A5). Again, the range in CsB (134.5-131.5 mg/kg), DwB (119-93.2 mg/kg) and BcB (119.5-99.6 mg/kg) were higher than that of municipal irrigated soil, 69.5-67.1 mg/kg, 52.5-46.3 mg/kg, and 71.3-64.95 mg/kg respectively (Figure 13). Therefore, the concentration of Zn in the considered range was high in the soils irrigated with BTW. Meanwhile, Zn in soils irrigated using BTW were positively skewed towards normality with a symmetric distribution (CsB), and a less pronounced asymmetry (DwB and BcB) signalling that most values of Zn measured concentrated on the lower end of the scale and comparable to the group median.

In this study, the concentration of Zn was found to be elevated in soil treatments irrigated with BTW compared to those irrigated with MTW. This is consistent with the findings of Urbano *et al*. (2017), who investigated the effects of treated wastewater irrigation on the soil properties and lettuce yield. Consequently, the high Zn concentration in the soil may have been due to cumulative addition from irrigation with treated wastewater (Bedbabis *et al.,* 2015), relatively high retention by the soil, or low uptake by crops (Hussain *et al.*, 2019).

4.2.5. Iron (Fe+)

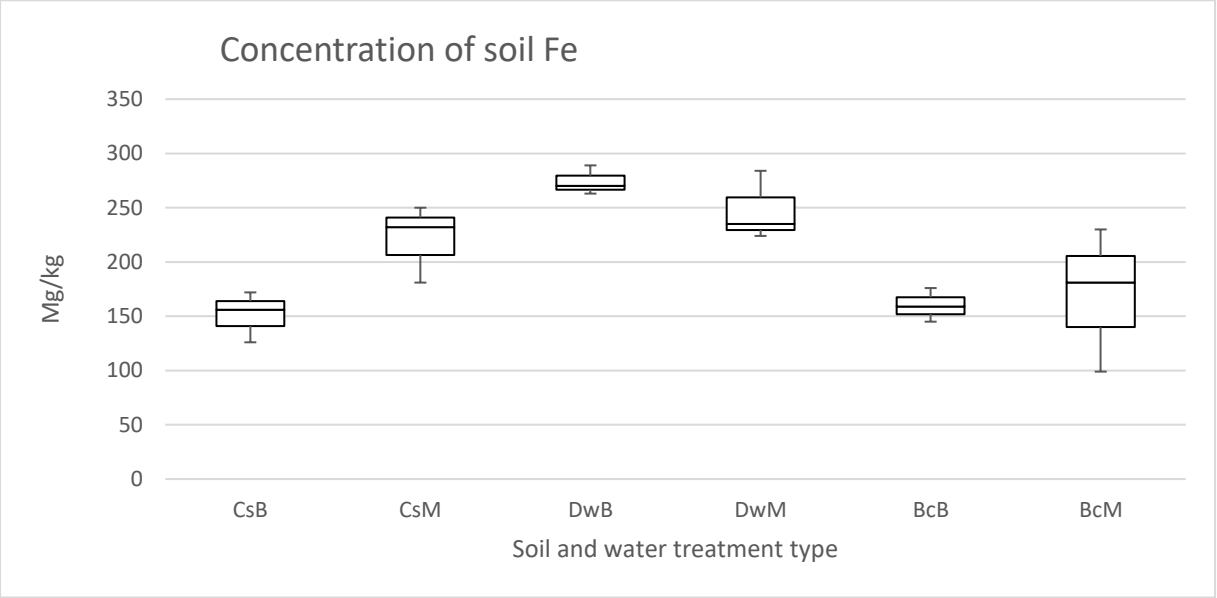

Figure 9: Box and whisker plots of soil Fe+.

Although Fe+ was most pronounced in BTW utilised during the experiment (Table 2), the results revealed that its concentration measured in soil was low, particularly in the control and biochar-treated soils irrigated using BTW. Consequently, the median concentrations of Fe+ in soil irrigated with BTW, namely CsB (156 mg/kg) and BcB (159 mg/kg), were lower than

those of MTW (232 mg/kg) and 182 mg/kg (Table A6). As shown by the range, the concentration of Fe+ in the considered in soil was relatively low in CsB (164-141 mg/kg), and BcB (167.5-152 mg/kg) compared to that irrigated using MTW, 241-206.5 mg/kg, and 205.5-140 mg/kg respectively. However, DwB (270 mg/kg) was found to have a higher median concentration with relatively less pronounced asymmetry signalling, and most measured values were concentrated around the group median. Elevated Fe+ in the soil may be due to the transfer of Fe+ from duckweed biomass (Gonzalez & Guo, 2018) and the contribution from BTW, given its high concentration compared to that of MTW.

4.3. Analysis of the concentration of macro and micronutrients in crops

This section presents the results from the findings of the effect on the nutrient concentrations of crops irrigated with BTW compared with those grown in MTW. In a pattern similar to that of soil physiochemistry, analysis of the results was based on the median, range, and skewness. In this section, B refers to BTW, whereas M represents MTW for the measured variables. Moreover, the concentration of Na in the crops was analysed and presented graphically for spinach, lettuce, beetroot, and carrot. This was done to ensure visual presentation to the reader given the relatively high values measured. Lt (B) (lettuce) and Sp (B) (spinach) plants were exposed to BTW irrigation, whereas Lt (M) and Sp (M) plants were exposed to MTW. Cr (B) (carrots) and Bt (B) (beetroot) have been exposed to BTW irrigation, while Cr (M) and Bt (M) were exposed to MTW.

4.3.1. Macronutrients

Table 3: Concentration of major macronutrients in vegetable crops

Element	Laboratory normal range (mg/100g)		Nutrient sufficiency range in Campbell (2000) (mg/100g)
	Lettuce	Spinach	Carrots
N	4.5-3	6-4	2.5-1.5
P	0.6-0.4	0.6-0.4	0.40-1.8
Ca	3-1.5	1.2-0.6	1.5-1.0
Mg	0.6-0.3	0.8-0.35	0.5-0.4

Table 3 presents the range within which previous studies have compared their findings on crop quality. The normal laboratory range is related to the nutrient sufficiency range and is useful in determining nutrient deficiency in vegetables.

4.3.1.1. Lettuce.

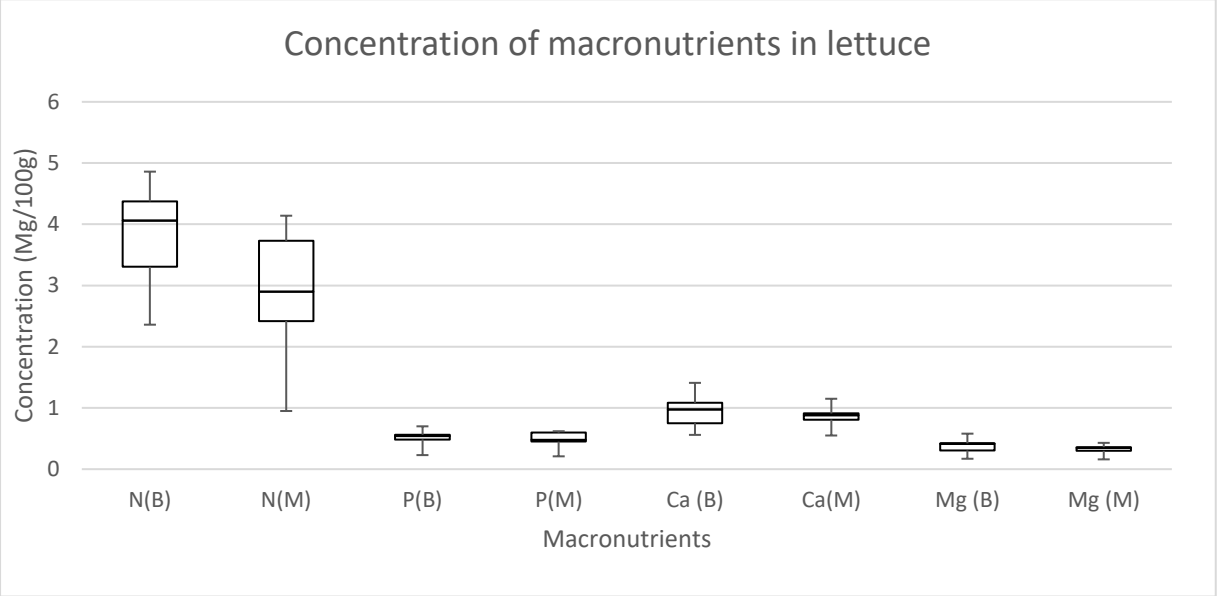

Figure 10: Box and whisker plots showing the percentage of available macronutrients in Lettuce of BTW and MTW.

The concentrations of P (B), Ca (B), and Mg (B) in lettuce were comparatively higher than those irrigated using MTW and were negatively skewed with less pronounced asymmetry, indicating that most measured values were higher than the group median. The median concentration of P (B) (0.53 mg/100 g), Ca (B) (0.97 mg/100 g), and Mg (B) (0.41 mg/100 g) in lettuce was therefore high compared to that grown in MTW irrigated soil, 0.49 mg/100 g, 0.87 mg/100 g, and 0.34 mg/100 g respectively (Table B1). However, the concentration of N (4.02 mg/100 g) in BTW-irrigated lettuce was 1.2 mg/100 g higher than in MTW, 2.9 mg/100 g as measured by the median. The range of N (B) (4.4-3.3 mg/100 g) was higher than that of N (M) (3.7-2.4 mg/100) giving an indication that measured concentration was located higher on the scale. When studying the effects of irrigation with treated wastewater on soil and lettuce, Mañas *et al.* (2009) found that the concentrations of Ca and Mg were comparatively different from those irrigated using drinking water after 3 years of irrigation. In contrast, N was significantly higher in treated wastewater-irrigated lettuce. The findings of Mañas *et al.* (2009) were consistent with the observations of this study. Maynard and Hochmuth (2006), cited by Mañas *et al.* (2009), N concentration between 3.5 mg/100 and 4.5 mg/100 in lettuce is considered adequate while that of less than 3.5 mg/100 shows deficiency. In this study,

irrigation with BTW achieved an adequate (4.02 mg/100) N concentration in lettuce, whereas that grown in MTW showed deficiency (2.9 mg/100).

4.3.1.2. Spinach

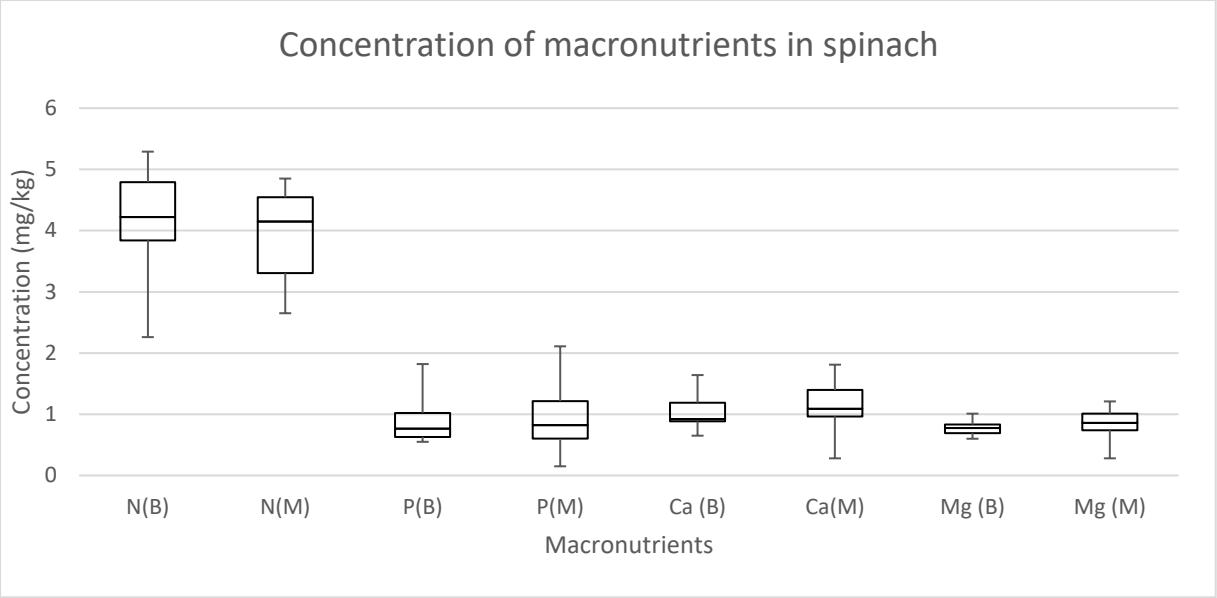

Figure 11: Box and whisker plots showing the percentage of available macronutrients in spinach in BTW and MTW.

Irrigation with BTW did not result in notable variations in the concentration of macronutrients in spinach compared to MTW, as shown by the median concentration. The concentrations of N (B) (4.3%), P (B) (0.68%), Ca (B) (0.9%), and Mg (B) (0.8%) in spinach under the two irrigation conditions were comparable (4.15 %, 0.74 %, 1.1 %, and 0.9 %, respectively) (Table B2). However, the concentration of N (B) (4.8-3.8 mg/100 g) was comparatively high compared to that grown using MTW, 4.5-3.3 mg/100 g as shown by the range (Figure 19). Alternatively, the quality of spinach is compromised due to elevated N concentrations in leaves following irrigation with domestic wastewater for 12 months (Mustapha *et al.*, 2020). However, it is still uncertain as to how irrigation with BTW may influence the accumulation of macronutrients during a period comparable to that of Mustapha *et al*. (2020), given the varying quality of wastewater utilised during the experiment.

4.3.1.3. Carrots

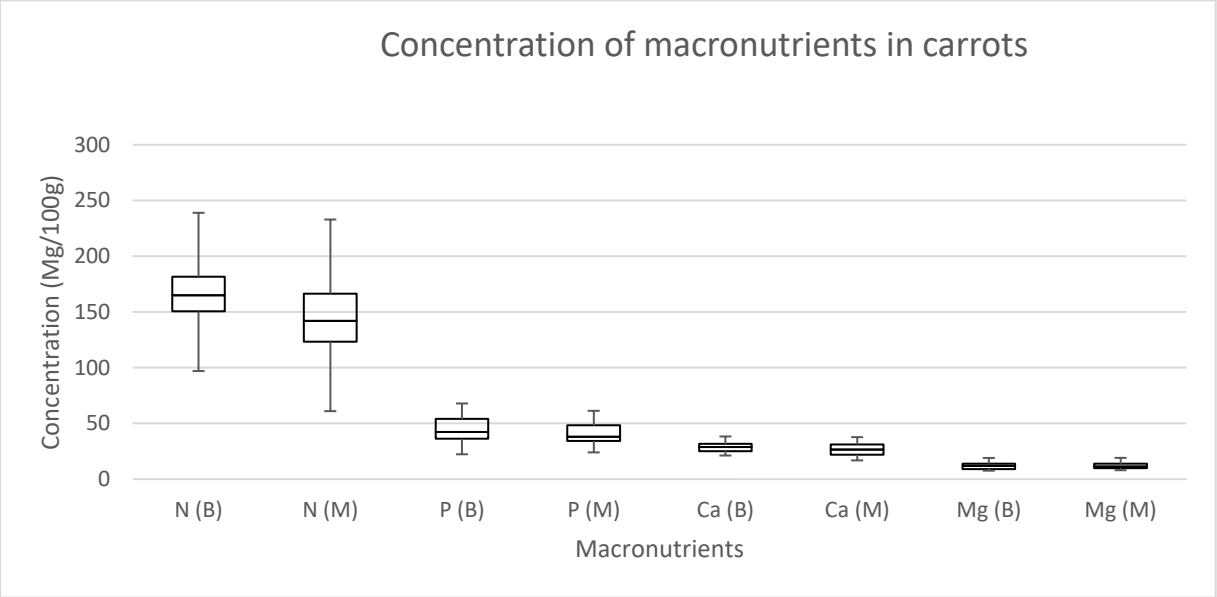

Figure 12: Box and whisker plots showing the concentrations of available macronutrients in BTW and MTW carrots.

Except for N, the concentrations of macronutrients (P and Ca) in carrots from BTW were higher than those in carrots from MTW. The median concentration of P (B) (42.4 mg/100 g), and Ca (B) (28.75 mg/100 g) in carrots irrigated using BTW was comparatively high to that of MTW, 38.05 mg/100 g, and 26.55 mg/100 g respectively (Table B3). However, there was a notable difference in the concentration of N (B) (165 mg/100 g) in the carrots compared to that of N (M) (142 mg/100 g). Relatively high range of N (B) (181.8-150.5 mg/100 g) gave an indication that the measured concentration was high compared to that of MTW irrigated carrots (166.5-123.3 mg/100 g). Considering the observed less pronounced asymmetry and positively skewed distribution, most of the measured values were comparable to the group median. Boskovic-Rakocevic *et al*. (2012) found that increasing the rate of nitrogen-based fertiliser use resulted in an accumulation of N in carrot roots after each successive application in the soil. Although N was not measured during the experimental period, the above results suggest that BTW may have contributed to the excessive nitrogen concentration in the soil and subsequent observed uptake by carrots.

4.3.1.4. Beetroot.

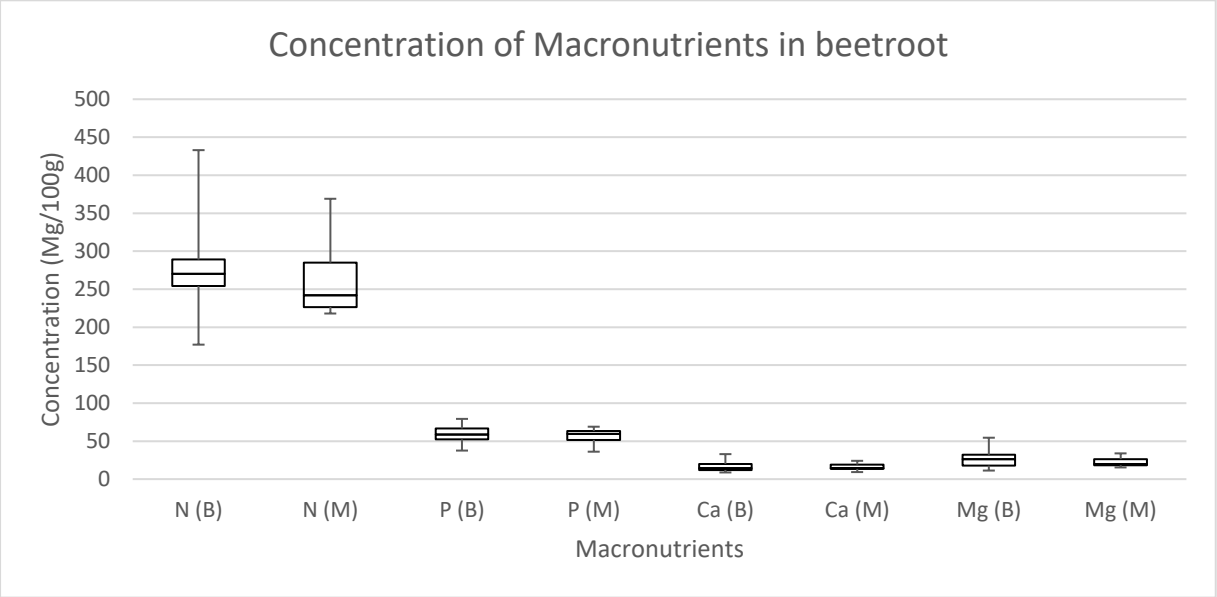

Figure 13: Box and whisker plots showing the concentration of available macronutrients in the beetroots of BTW and MTW fresh mass.

As shown by the median concentration, N (B) (270 mg/100 g) was comparatively high, and Mg (B) (26.6 mg/100 g) higher in beetroot compared to that in MTW, 265.5 mg/100 g and 22.5 mg/100 g respectively (Table B4). The ranges of N (B) (289-254 mg/100 g) and Mg (B) (242-226.5 mg/100 g) were subsequently higher than those measured in MTW-irrigated soil (Figure 24), suggesting that the measured values between the 25th and 75th percentiles were high. Rantao (2013) studied the response of beetroots to N fertilisation and found that the level of N in the soil did not significantly affect its accumulation in fruits. Consequently, the elevated N concentrations in BTW-irrigated soil may not be related to the accumulation in fruits as the transfer from water, soil, fertiliser to fruit was not accounted for in this study. However, the accumulation of nutrients in crops is a function of a combination of factors including soil type, soil physiochemistry, and prevailing environmental and climatic factors, among many Rantao (2013) added.

4.3.2. Micronutrients

Table 4: Guidelines for the concentration of micronutrients in vegetable crops adapted from Olayiwola *et al.* (2017).

Element	Laboratory normal range (mg/kg)		Nutrient sufficiency ranges in Campbell, 2000	WHO normal range in plant (mg/kg) (Olayiwola *et al.*, 2017)
	Lettuce	Spinach	Carrots	
Zn	70-25	75-50	60-20	100-20
Fe	200-130	250-200	30-20	500-400
Mn	300-60	60-30	60-30	-
Na	10000-0	4000	-	-

The ranges within which micronutrient concentrations in the vegetables are presented in Table 4. Therefore, the normal laboratory range is related to nutrient sufficiency ranges to determine deficiency and/or excess in the vegetables under study. Adapted from Olayiwola *et al.* (2017) are the WHO guidelines for the range of micronutrient concentrations in plants.

4.3.2.1. Lettuce

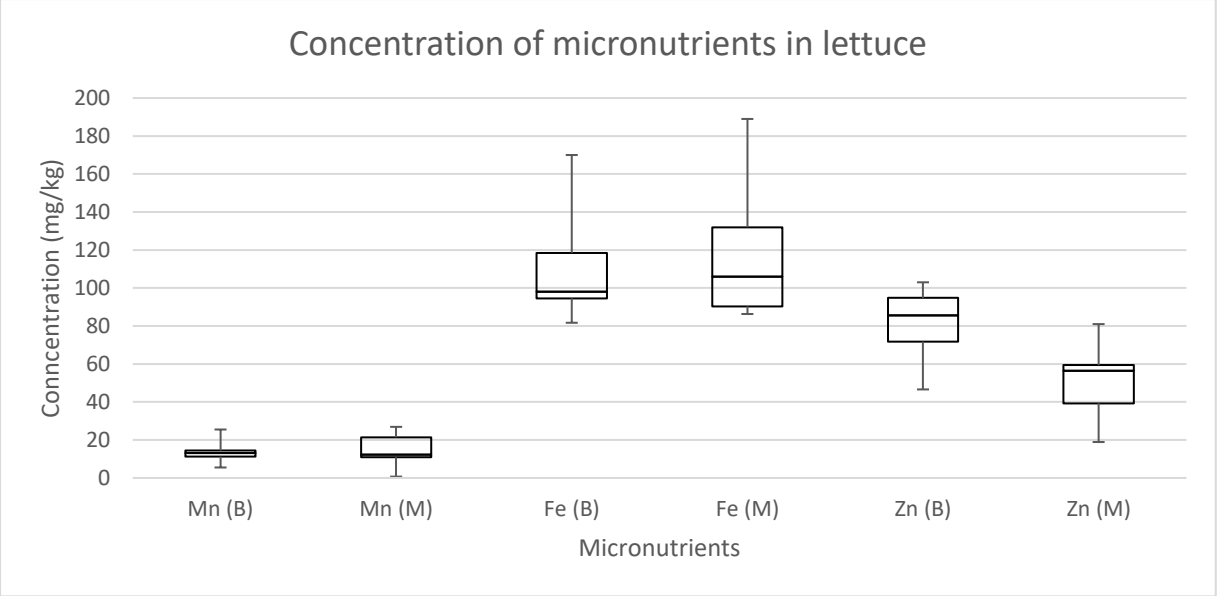

Figure 14: Box and whis*k*er plots showing the percentage of available micronutrients in lettuce from BTW and MTW.

Lettuce irrigated using BTW was comparatively low in Mn (13.2 mg/kg) and Fe (97.7 mg/kg) compared to that irrigated using MTW, 14.4 mg/kg, and 107 mg/kg, respectively), as shown by the median concentration (Table B5). Fe (B) displayed more pronounced asymmetry, indicating that most measured values were higher than the group median. Moreover, the range

between the 25th and 75th percentiles was relatively high, indicating that Fe (M) (132-90.3 mg/kg) was higher than Fe (B) (118.5-98.1 mg/kg) carrots (Figure 18). While Mañas *et al.* (2009) made a similar discovery for Mn while using treated wastewater, Mzini *et al*. (2015) found that irrigation with greywater increased the concentration of Fe in lettuce. The above finding may be explained by the relatively high concentration of Fe in soil irrigated with MTW (Figure 16), its high retention potential in the soil, ease of access to crops, and subsequent high uptake in lettuce. Leaching down the soil profile and the subsequent reduced concentration in the soil may also be attributed to the relatively low concentrations in lettuce irrigated with BTW.

Further analysis revealed that the median concentration of Zn (85.3 mg/kg) in BTW-irrigated lettuce was higher than that in MTW, 51.9 mg/kg. However, the measured concentration of Zn (B) in lettuce was negatively skewed, with more pronounced asymmetry, indicating that most values were comparatively lower than the group median. As a result, the range of Zn (B) (94.8-71.8 mg/kg) measured for lettuce was relatively high compared to that of Zn (M) (59.4-39.3 mg/kg), indicating a high concentration of Zn (B). A similar finding was established by Mzini *et al.* (2015), wherein irrigation-diluted greywater increased the concentration of Zn compared with potable water. As a result, there may have been high Zn retention in the soil, high accessibility, and subsequently high uptake by carrots.

4.3.2.2. Spinach

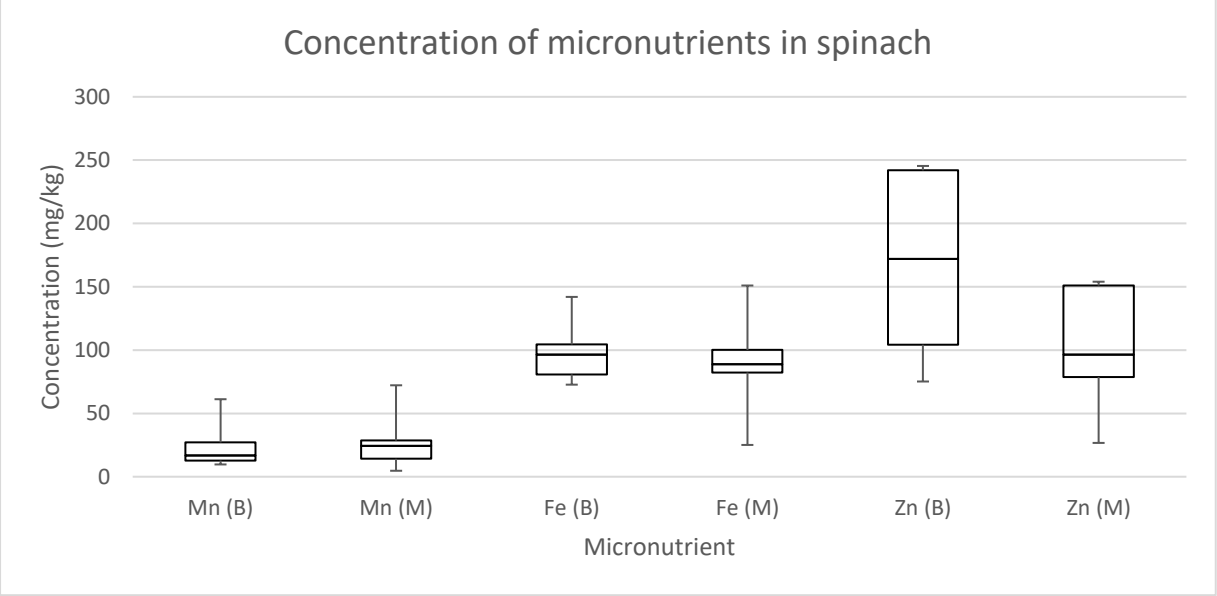

Figure 15: Box and whisker plots showing the concentration of available micronutrients in spinach from BTW and MTW.

The Zn concentration in spinach irrigated with BTW was higher than that in spinach irrigated with MTW. Consequently, because of the high absorptive capacity of Zn by spinach, relatively high soil concentration (Figure 13), and ease of access, its concentration was elevated compared with that of plants irrigated with MTW. As shown by the median values (Table B6), the concentration of Zn (B) (163 mg/kg) was higher than that of Zn (M) (96.3 mg/kg). Z (B) resembles less pronounced asymmetry and positive skewness. Therefore, the distribution of most measured values was clustered around the group median. Moreover, as shown by a relatively high range, the concentration of Zn (B) (242-104.3 mg/kg) was found to be higher in spinach leaves than in MTW, 151-78.7 mg/kg. In line with the findings of this study, wastewater-irrigated spinach displayed elevated leaf concentrations of Zn as opposed to tubewell-irrigated spinach (Khan *et al.*, 2015). Furthermore, following irrigation with treated wastewater, Kausar *et al*. (2017) found that the Zn concentration in spinach was higher than that in plants irrigated with groundwater. Contrary to the findings of this study, Hussain *et al.* (2019) found that irrigation with MTW led to an accumulation of Zn in spinach during a 60-day growing period compared to irrigation with treated municipal wastewater. According to Hussain *et al*. (2019), among other factors, the variation in the concentration of metals in crops depends on environmental conditions, adsorption capability of each metal by soil, absorption capability by crops, and the physicochemical nature of the soil.

4.3.2.3. Carrots

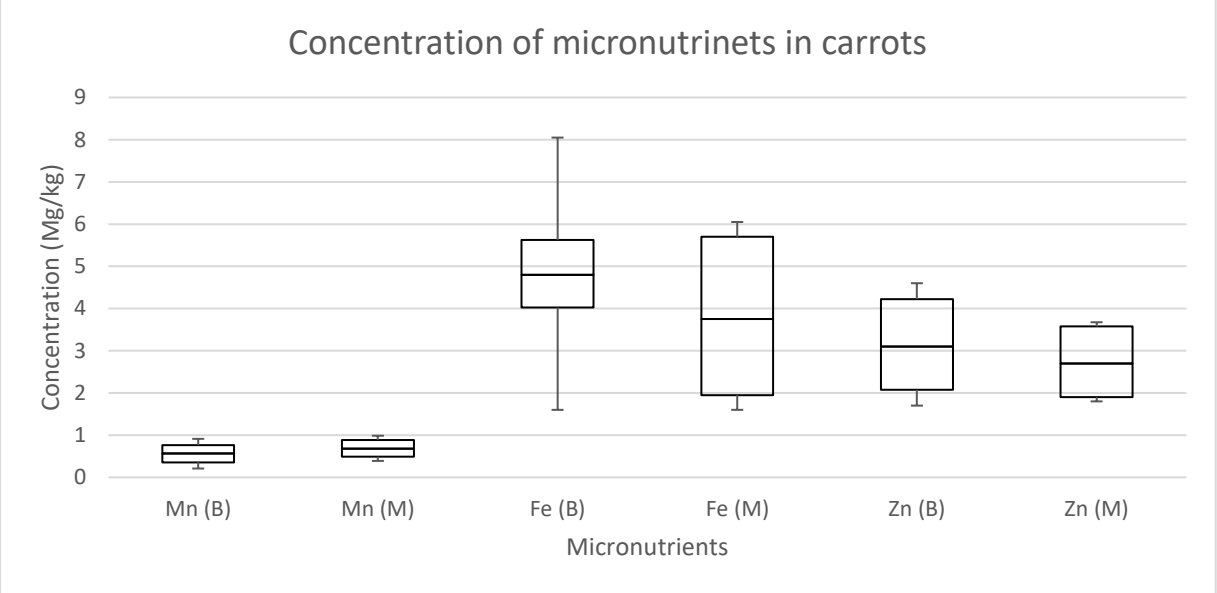

Figure 16: Box and whisker plots showing the concentration of available micronutrients in BTW and MTW carrots.

The results revealed that the concentrations of Fe and Zn in the carrots in BTW were higher than those in MTW. In line with the finding above, the median concentration of Fe (B) (4.8 mg/kg), and Zn (B) (3.1 mg/kg) in carrots was relatively higher than that in MTW, 3.75 mg/kg, and 2.7 mg/kg respectively (Table B7). The range of the measured concentration of Fe (B) (5.6-4.0 mg/kg), and that of Zn (B) (4.2-2.1 mg/kg) was high compared to that in MTW, 5.7-1.95 mg/kg, and 3.6-1.9 mg/kg respectively. Even though Fe was most pronounced in soils irrigated with MTW (Figure 16), relatively high concentrations accumulated in carrots grown in the BTW-irrigated soil. Therefore, it is probable that MTW-irrigated soil has a high adsorption capacity for Fe, limiting its accessibility to carrots with consequent low Fe concentrations. Similarly, Mzini *et al.* (2015) reported that Zn and Fe levels increase following greywater irrigation. While studying the effects of treated wastewater on vegetables, including carrots, Kausar *et al*. (2017) discovered an increase in the Zn concentration.

4.3.2.4. Beetroot

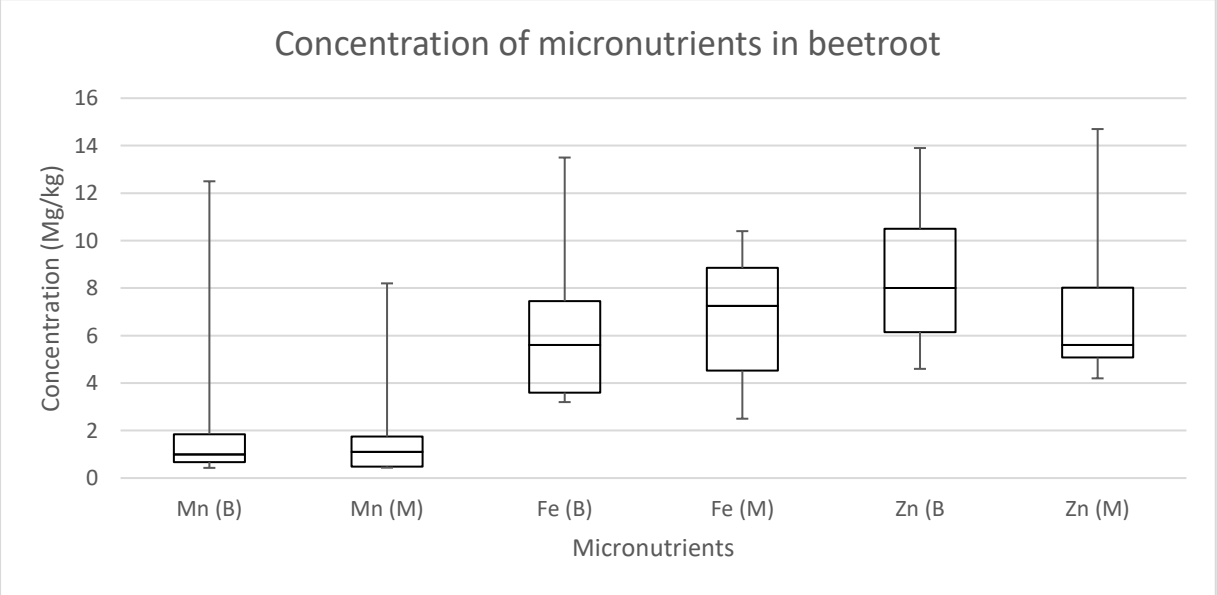

Figure 17: Box and whisker plots showing the concentration of available micronutrients in the beetroots of BTW and MTW.

Mzini *et al.* (2015) investigated the effects of irrigation with greywater on the chemical and nutrition levels of crops and reported significantly high concentrations of Fe and Zn in beetroots irrigated with greywater. Except for Fe (B) (6.4 mg/kg) having recorded lower concentration as opposed to that of Fe (M) (8 mg/kg), the above coincided with the finding of this study whereby the median concentration of Zn (B) (9 mg/kg) was higher than that of Zn (M) (6.6 mg/kg) (Table B8). Consequently, the range recorded for Zn (B) (10.5-6.15 mg/kg) was higher than that in MTW irrigated beetroot, 8.0-5.1 mg/kg. The distribution of the

measured concentration was positively skewed with less pronounced asymmetry signalling, and most measured values were low but comparable to the group median. Figure 13 shows that the concentration of Zn was most pronounced in BTW-irrigated soil. Consequently, the uptake of Zn (B) in beetroots was high, as was the contribution of BTW to the soil which compensated for subsequent losses to the crop. A similar interpretation may be advanced in reasoning for the high Fe concentrations observed in beetroots irrigated with MTW. However, owing to the competition among heavy metals for absorption and changes in the uptake mechanisms of certain ions, metal concentrations have demonstrated variability in their accessibility to plants (Chaoua *et al*., 2019).

4.3.2.5. Concentration of Na in lettuce and spinach

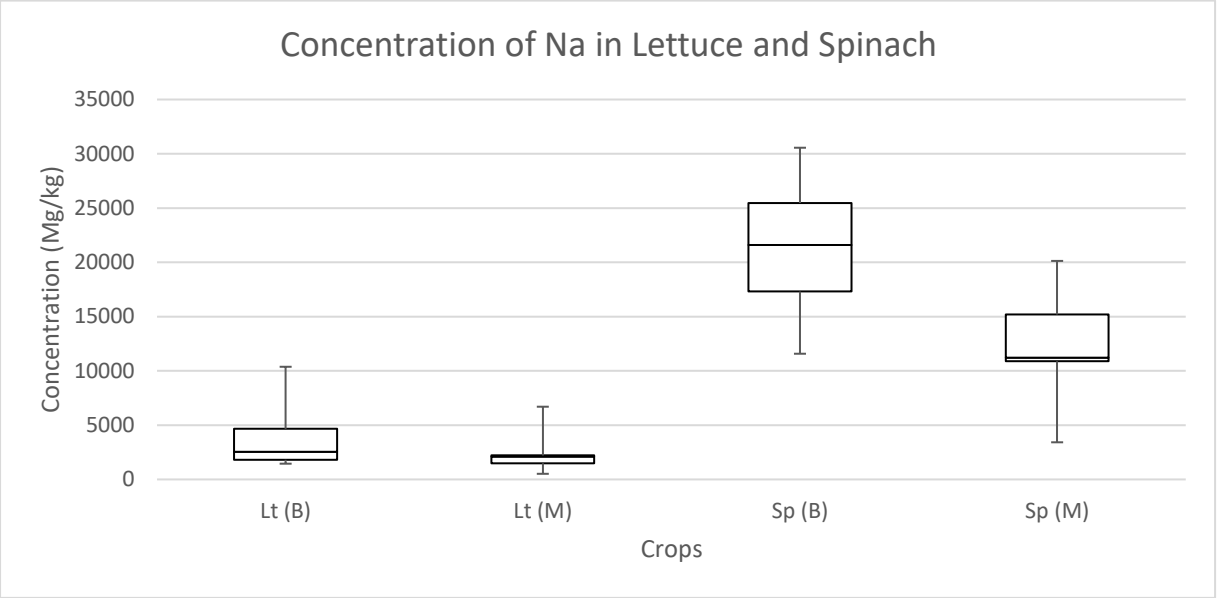

Figure 18: Box and whisker plots showing the concentration of Na in lettuce and spinach in BTW and MTW.

Chlorosis and reduced plant water uptake are consequences of excess Na in crops (Mzini *et al.*, 2015). The concentration of Na in lettuce and spinach irrigated using BTW was relatively higher than that of MTW (Figure 21). Consequently, the median concentrations of Na in lettuce (3530 mg/kg) and spinach (23210 mg/kg) were higher than in those irrigated with MTW (1900 mg/kg and 11210 mg/kg, respectively) (Table B9). Even at high concentrations, a relatively high range in Lt (B) (4657.5-1807.5 mg/kg) and Sp (B) (24565-17307.5 mg/kg) indicates that, although there was high variability in the measured concentration, its concentration was also high compared to that of MTW, 2210-1490 mg/kg, and 15200-10880 mg/kg respectively. In a study by Rodda *et al.* (2011) on the effects of greywater irrigation from an informal settlement on crops and soil, an analysis of micronutrients revealed that spinach irrigated with greywater

exhibited high levels of Na, which were amplified with consecutive growth cycles on the same soil. A similar finding was reported by Mzini *et al.* (2015), where irrigation with greywater resulted in elevated concentrations of Na in lettuce compared with those irrigated with potable water. The current study revealed that lettuce and spinach irrigated with BTW exhibited higher concentrations of Na than those irrigated with MTW. Indeed, as shown in figure 9, there was an observed high accumulation of Na in the soils due to the contributions of BTW which would explain the observed excess accumulation by lettuce and spinach.

4.3.2.6. Concentration of Na in beetroot and carrot

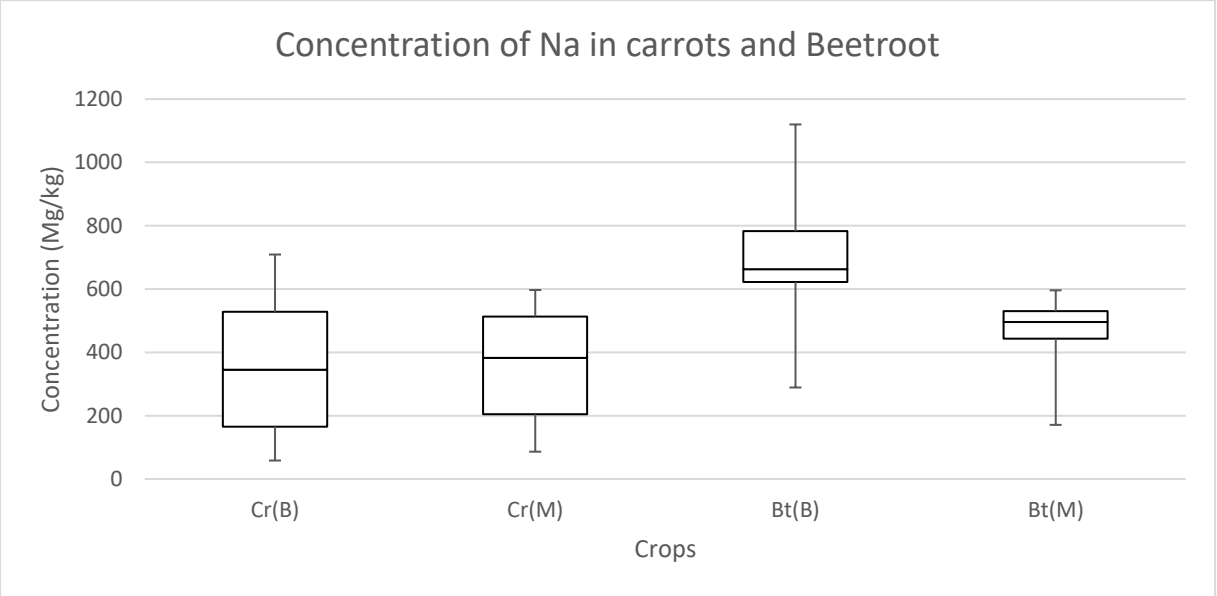

Figure 19: Box and whisker plots showing the concentrations of sodium carrots and beetroots in BTW and MTW.

According to Chaoua *et al*. (2019), the interplay between various heavy metals at the root surface and within the plant influences their absorption and transfer of heavy metals. Table B10 revealed that the median concentration of Na in Cr (B) (343.35 mg/kg) was comparable to that in Cr (M) (355.65 mg/kg). Moreover, Na + in Bt (B) (663 mg/kg) beetroot was more pronounced than in Bt (M) (495.5 mg/kg), as shown by the median concentration. The range within which the concentration in Bt (B) (783-622 mg/kg) was located indicated that most measured values were of a higher concentration compared to Bt (M) (530.25-495.5 mg/kg) (Figure 26). When investigating the effects of irrigation with secondary-treated municipal wastewater, Zavadil (2009) found that Na content in carrots increased significantly over a period of two years. There is a need to establish how long-term use of BTW to irrigate carrots

may influence the concentration of Na. Beetroots subjected to BTW irrigation showed elevated Na concentrations compared to those irrigated using MTW.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS.

This study focused on some aspects of the soil which are deemed most important in determining the soil quality and nutrient concentration of vegetables and revealed a notable difference in concentrations between the two irrigation conditions. By employing the principles of regenerative agriculture, the results of this study revealed that irrigation with BTW elevated the concentration of Zn, Na+, and CEC across all soil treatments (CsB, DwB, and BcB) compared with those irrigated with MTW. However, concentrations of Zn, Na+, and CEC was most pronounced in BcB irrigated with BTW. In addition, soils irrigated using BTW had the highest concentrations of Zn and Na+ signalling a notable contribution of salts from treated wastewater, and high retention by soil. Although limited data points made it difficult to produce a trend analysis, SOC improved from less than 2% to approximately 6% across all soil treatments and water irrigation conditions during the experimental period.

The concentrations of nutrients, including N, Na, and Zn, in vegetables irrigated using BTW were relatively high, whereas other nutrients were variable and comparable in concentration. The measured nutrients in the crops were within the guidelines prescribed by WHO, laboratory norms, and in Campbell (2000). However, the concentration of Na in BTW-irrigated spinach was higher than the laboratory norms which raises concerns over BTW use for spinach irrigation in the long term. However, the findings of this study do not exclusively indicate the quality of the soil as various aspects and determining factors of soil health were not examined, partly because of the limited funding, limited scope, and time constraints. Consequently, the study builds up on the initial research work by Winter *et al.* (2023) evaluating the use of BTW to irrigate safe to eat vegetables compared to that sold in the local markets.

Moreover, this study demonstrates the potential of using BTW to produce vegetables that conform to international regulations while contributing to soil sustainability. However, there are some key aspects that need further attention to give effect to the full potential of BTW for small-scale and subsistence food production.

Therefore, further research is required to cover the following:

- The transfer of nutrients from water to soil, and uptake by crops.
- The influence of rainfall and the rate of irrigation on the overall findings.
- To include all crucial soil quality determining factors such as nutrients and microbes.
- Provide Long-term changes in soil physicochemistry and vegetable nutrient status.
- Analysis of vegetable nutrition concentration from each soil treatment.

- Trend analysis of changes in soil physiochemistry and vegetable metal accumulation over time.
- Determination of the significance of the difference between soil physiochemistry and vegetable nutrition concentration in different soil media and water irrigation conditions.
- An analysis of the nutrient contribution of duckweed and biochar to soil.
- Analysis of the response of soil microbes to irrigation with BTW.

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APPENDICES

Appendix A: Descriptive statistics of measured soil physiochemistry.

Table A1: Descriptive statistics of SOC.

	CsB	**CsM**	**DwB**	**DwM**	**BcB**	**BcM**
Mean	2.97	3.46	2.66	3.19	3.87	4.23
Standard error	0.65951	0.80376	0.49538	0.5309	0.63311	0.24076
Median	3.46	3.49	2.97	3.07	3.91	3.97
Standard deviation	1.47472	1.96881	1.1077	1.0618	1.41567	0.53835
Skewness	-1.27447	0.04411	-1.54022	0.52857	-0.84161	0.99951
Range	3.79	5.74	2.88	2.43	3.86	1.24
IQR	1,2	1,90	0,63	1,25	0,78	0,71
Minimum	0.6	0.62	0.81	2.1	1.67	3.79
Maximum	4.39	6.36	3.69	4.53	5.53	5.03

Table A2: Descriptive statistics of soil Ca2+.

	CsB	CsM	DwB	DwM	BcB	BcM
Mean	14.7	13.57	10.36	10.35	16.94	16.5
Standard error	1.877	2.049	0.514	0.397	0.6013	1.249
Median	14.5	12.7	10.4	10.25	17.2	15.7
Standard deviation	4.198	5.020	1.150	0.794	1.345	2.794
Skewness	0.234	1.769	-0.369	0.416	-0.321	1.797
Range	11.8	14.7	2.8	1.7	2.9	7.2
IQR	1,2	1,545	1,6	1,1	2,4	1
Minimum	9	8.5	8.8	9.6	15.3	14.1
Maximum	20.8	23.2	11.6	11.3	18.2	21.3

Table A3: Descriptive statistics for soil Na+.

	CsB	CsM	DwB	DwM	BcB	BcM
Mean	0.24	0.15	0.19	0.11	0.26	0.13
Standard error	0.054	0.058	0.034	0.034	0.048	0.027
Median	0.22	0.11	0.18	0.085	0.27	0.13
Standard deviation	0.121	0.143	0.077	0.068	0.11	0.0597
Skewness	1.598	2.335	1.27	1.795	0.149	0.927
Range	0.31	0.37	0.21	0.15	0.27	0.15
IQR	0,07	0,04	0,02	0,045	0,13	0,06
Minimum	0.13	0.07	0.11	0.06	0.13	0.07
Maximum	0.44	0.44	0.32	0.21	0.4	0.22

Table A4: Descriptive statistics of soil CEC.

	CsB	CsM	DwB	DwM	BcB	BcM
Mean	18.29	17.73	12.28	12.3	21.92	21.48
Standard error	2.5897	3.6365	0.5291	0.38795	0.90239	2.58254
Median	17.43	15.4	12.28	12.46	21.85	19.5
Standard deviation	5.79075	8.90764	1.18306	0.7759	2.01781	5.77474
Skewness	0.77038	2.15822	0.20639	-0.21184	1.29519	2.13887
Range	15.92	24.75	3.11	1.53	5.35	13.82
IQR	2.92	2.32	1.22	1.2075	1.04	1.3
Minimum	11.29	10.69	10.79	11.52	19.86	17.9
Maximum	27.21	35.44	13.9	13.05	25.21	31.72

Table A5: Descriptive statistics for Zn in soil.

	CsB	CsM	DwB	DwM	BcB	BcM
Mean	133	68.67	106.47	50.37	110.03	68.4
Standard error	1.732	1.567	14.914	4.134	11.563	3.707
Median	133	67.1	105	46.9	108	67.3
Standard deviation	3	2.714	25.831	7.1598	20.028	6.421
Skewness	0	1.732	0.255	1.668	0.452	0.748
IQR	1.5	2.4	25.8	6.5	19.95	6.4
Range	6	4.7	51.6	13	39.9	12.7
Minimum	130	67.1	81.4	45.6	91.1	62.6
Maximum	136	71.8	133	58.6	131	75.3

Table A6: Descriptive statistics of soil Fe.

	CsB	CsM	DwB	DwM	BcB	BcM
Mean	151.33	221	274	247.67	160	170
Standard error	13.482	20.664	7.767	18.4421	8.963	38.214
Median	156	232	270	235	159	181
Standard deviation	23.352	35.791	13.454	31.942	15.524	66.189
Skewness	-0.863	-1.252	1.2196	1.504	0.289	-0.727
IQR	23	34.5	13	30	15.5	65.5
Range	46	69	26	60	31	131
Minimum	126	181	263	224	145	99
Maximum	172	250	289	284	176	230

Appendix B: Descriptive statistics of measured leaf and bulb macro and micronutrient concentration.

Table B1: Descriptive statistics of macronutrient concentrations in lettuce *from* BTW and MTW.

	N(B)	N(M)	P(B)	P(M)	Ca(B)	Ca(M)	Mg(B)	Mg(M)
Mean	3.757	2.749	0.49	0.486	0.87	0.811	0.372	0.321
Standard error	0.292	0.393	0.044	0.048	0.081	0.047	0.039	0.026
Median	4.02	2.9	0.53	0.49	0.97	0.87	0.41	0.34
Standard deviation	0.875	1.039	0.131	0.136	0.242	0.133	0.118	0.074
Skewness	-0.312	-0.578	-0.666	-1.202	-0.249	-1.427	-0.045	-1.563
Range	2.5	3.12	0.47	0.41	0.63	0.37	0.41	0.25
Minimum	2.36	0.95	0.23	0.21	0.56	0.55	0.17	0.16
Maximum	4.86	4.07	0.7	0.62	1.19	0.92	0.58	0.41

Table B2: Descriptive statistics of the concentrations of macronutrients in spinach of BTW and MTW.

	N(B)	N(M)	P(B)	P(M)	Ca(B)	Ca(M)	Mg(B)	Mg(M)
Mean	4.1933	3.847	0.892	0.983	0.956	1.087	0.781	0.869
Standard error	0.326	0.302	0.134	0.218	0.083	0.150	0.028	0.090
Median	4.3	4.15	0.68	0.74	0.9	1.1	0.8	0.9
Standard deviation	0.978	0.907	0.402	0.653	0.248	0.450	0.085	0.270
Skewness	-1.030	-0.378	1.809	0.730	0.639	-0.305	0.013	-1.272
Range	3.03	2.2	1.22	0.15	0.76	1.53	0.28	0.93
Minimum	2.26	2.65	1.82	2.11	0.65	0.28	0.64	0.28
Maximum	5.29	4.85	1.82	2.11	1.41	1.81	0.92	1.21

Table 3B: Descriptive statistics of macronutrient concentrations in BTW and MTW carrots.

	N(B)	N(M)	P(B)	P(M)	Ca(B)	Ca(M)	Mg(B)	Mg(M)
Mean	165.75	146.13	44.092	41.186	28.72	27	12.083	12.16
Standard error	11.306	19.641	4.251	4.360	1.571	2.465	1.079	1.244
Median	165	142	42.4	38.05	28.75	26.55	11.85	11.4
Standard deviation	39.167	55.56	14.726	12.332	5.442	6.973	3.738	3.518
Skewness	0.084	0.197	0.245	0.479	0.265	0.210	0.647	1.046
Range	142	172	45.6	37.3	17.1	20.9	11.4	11.1
Minimum	97	61	22.3	24	21.2	16.8	7.6	8
Maximum	239	233	67.9	61.3	38.3	37.7	19	19.1

Table B4: Descriptive statistics of the concentrations of macronutrients in beetroots of BTW and MTW.

	P(B)	P(M)	N(B)	N(M)	Ca(B)	Ca(M)	Mg(B)	Mg(M)
Mean	57.56	55.35	286.2	276.7	17.67	16.67	29.01	24.52
Standard error	4.674	5.195	19.499	23.333	2.715	2.317	4.056	2.479
Median	58.5	58.45	270	265.5	15.8	16.5	26.6	22.5
Standard deviation	14.023	12.725	58.497	57.155	8.146	5.675	12.168	6.073
Skewness	-0.053	-0.626	2.360	0.819	0.844	0.041	1.114	0.751
Range	41.7	32.9	198	151	24.1	14.8	28	14.8
Minimum	37.6	36.1	235	218	8.8	9.3	16.5	19.1
Maximum	79.3	69	433	369	32.9	24.1	54.5	33.9

Table B5: Descriptive statistics of micronutrient concentrations in lettuce from BTW and MTW.

	Mn(B)	Mn(M)	Fe(B)	Fe(M)	Zn(B)	Zn(M)

Mean	13.878	14.762	108.64	121.08	79.489	51.763
Standard error	1.717	3.304	8.854	12.693	6.461	7.186
Median	13.2	14.45	97.7	107	85.3	51.9
Standard deviation	5.152	9.347	26.563	35.902	19.379	20.323
Skewness	1.144	-0.232	1.343	1.131	-0.821	-0.039
IQR	3,275	10,5	23,975	41,7	23	20,1
Range	20	26.2	73.3	102.7	54.4	62.1
Minimum	5.5	0.7	81.7	86.3	46.6	18.9
Maximum	25.5	26.9	155	189	101	81

Table B6: Descriptive statistics of micronutrient concentrations in the spinach of BTW and MTW.

	Mn(B)	Mn(M)	Fe(B)	Fe(M)	Zn(B)	Zn(M)
Mean	25.44	26.144	98.94	92.5	176.02	142.5
Standard error	5.486	6.362	7.037	11.836	27.771	48.97
Median	23.4	24.4	97.3	97.6	163	96.3
Standard deviation	16.456	19.085	21.112	35.508	83.314	146.92
Skewness	1.362	1.935	0.850	-0.307	0.114	2.572
Range	51.5	67.5	69.3	125.9	206.8	492.3
Minimum	9.7	4.7	72.2	25.1	75.2	26.7
Maximum	61.2	72.2	142	151	282	519

Table B7: Descriptive statistics of micronutrient concentrations in the carrots of BTW and MTW.

	Mn(B)	Mn(M)	Fe(B)	Fe(M)	Zn(B)	Zn(M)
Mean	0.6	0.7	4.64	3.99	3.28	3.18

Standard error	0.101	0.091	0.476	0.816	0.387	0.578
Median	0.57	0.68	4.8	3.75	3.1	2.7
Standard deviation	0.351	0.256	1.648	2.309	1.339	1.635
Skewness	0.886	0.291	-0.608	0.211	0.495	1.204
IQR	0,4075	0,3975	1,6	3,75	2,15	1,675
Range	1.09	0.71	5.3	5.6	4.1	4.3
Minimum	0.21	0.39	1.6	1.6	1.7	1.8
Maximum	1.3	1.1	6.9	7.2	5.8	6.1

Table B8: Descriptive statistics of the micronutrient concentrations in beetroots of BTW and MTW.

	Mn(B)	Mn(M)	Fe(B)	Fe(M)	Zn(B)	Zn(M)
Mean	2.55	2.51	7.04	7.77	8.64	8
Standard error	1.267	1.181	1.154	0.953	1.127	1.484
Median	1.2	1.3	6.4	8	9	6.6
Standard deviation	3.800	2.892	3.461	2.333	3.380	3.636
Skewness	2.798	2.089	0.931	-1.059	0.252	1.581
IQR	1,175	1,27	3,85	4,325	4,35	2,95
Range	11.87	7.72	10.3	6.7	9.3	9.5
Minimum	0.63	0.48	3.2	3.7	4.6	5.2
Maximum	12.5	8.2	13.5	10.4	13.9	14.7

Table B9: Descriptive statistics of sodium in lettuce and spinach of BTW and MTW.

	Lt(B)	Lt(M)	Sp(B)	Sp(M)
Mean	4455.78	1722.25	22072.2	12443.3
Standard error	1057.75	269.595	1942.44	1562.74

Median	3530	1900	23210	11210
Standard deviation	3173.26	762.53	5827.33	4688.22
Skewness	0.967	-0.4355	-0.101	-0.291
IQR	2850	720	8157,5	4320
Range	8930	2282	17780	16710
Minimum	1450	518	30560	3420
Maximum	10380	2800	30560	20130

Table B10: Descriptive statistics of sodium in carrots and beetroots of BTW and MTW.

	Cr(B)	Cr(M)	Bt(B)	Bt(M)
Mean	357.21	354.8	696.22	456.17
Standard error	69.418	62.534	79.425	60.162
Median	343.34	355,65	663	495.5
Standard deviation	230.234	187.602	238.274	147.365
Skewness	0.286557	-0.168017	0.09503	-1.8767
IQR	362,75	309	161	87,25
Range	650.7	510.8	831	425
Minimum	58.3	86.2	289	171
Maximum	709	597	1120	596

Appendix C: Box and whisker plots of additional parameters measured for soil physiochemistry and crops nutrient concentration.

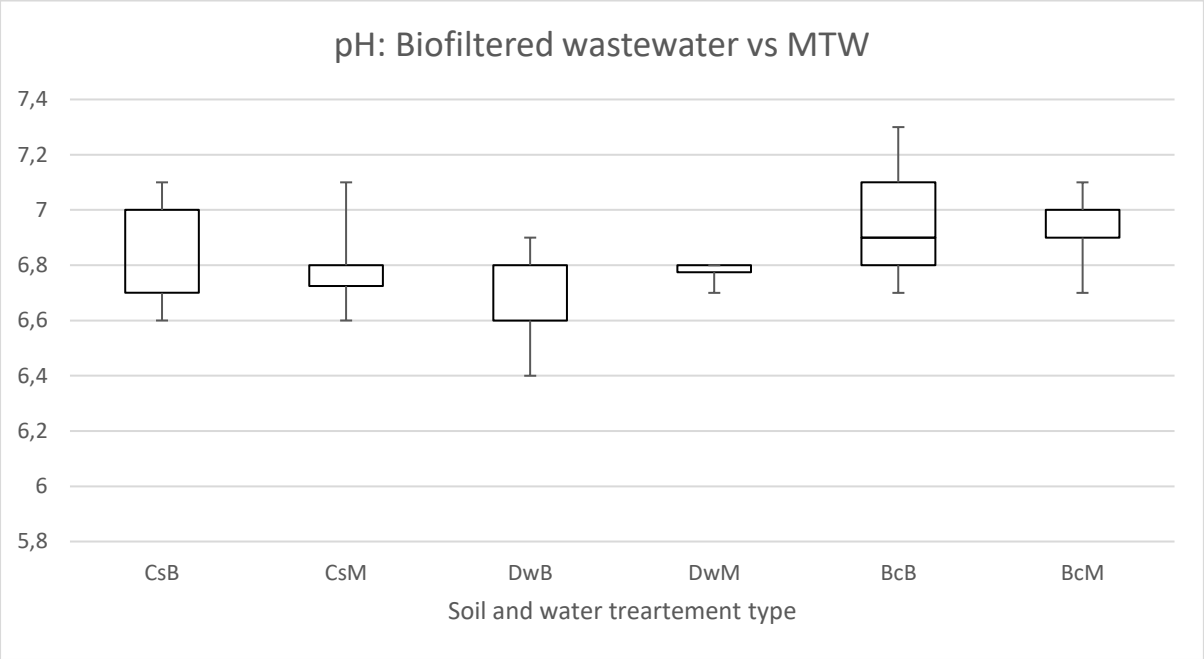

Figure B1: Box and whisker plots of soil pH.

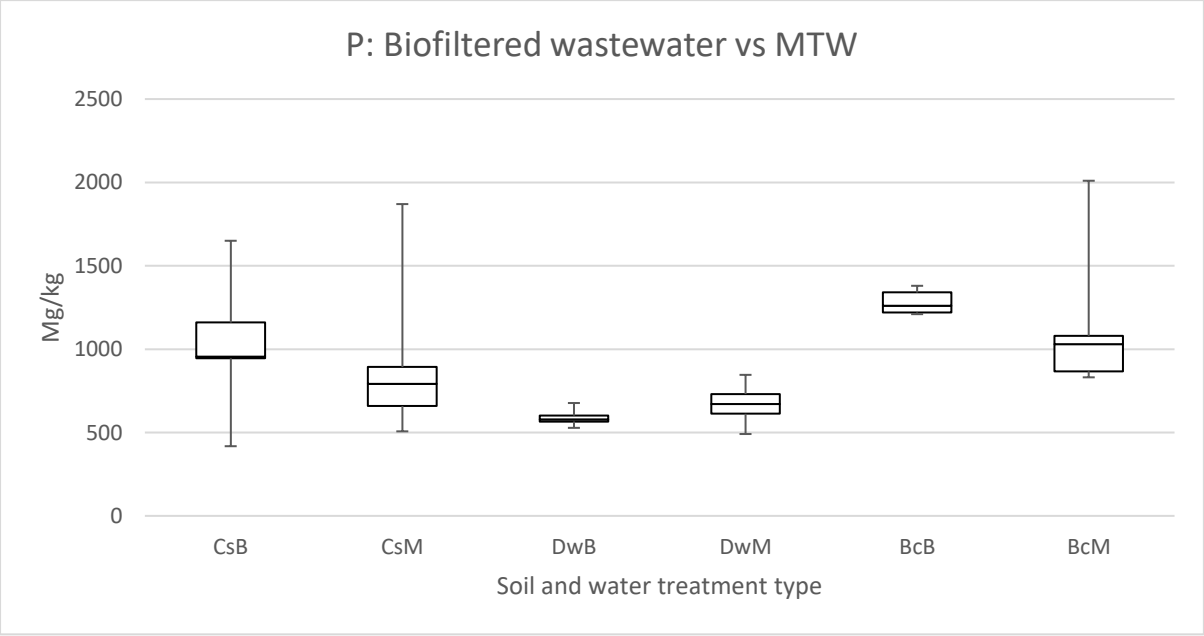

Figure B2: Box and whisker plots of soil P.

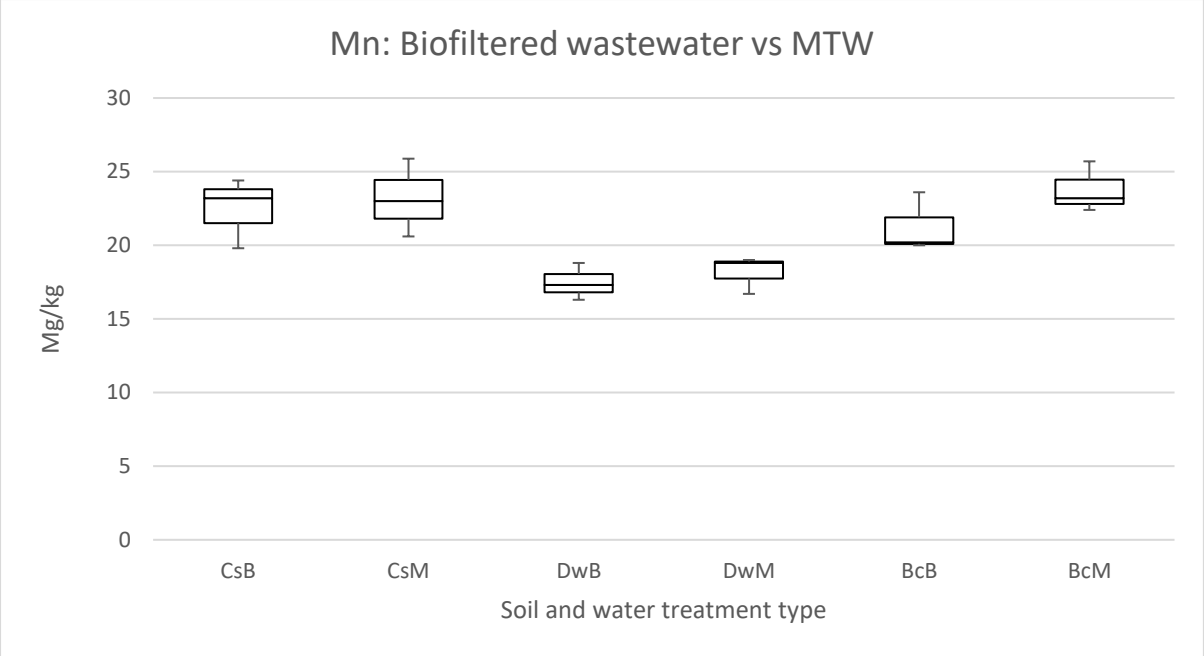

Figure B3: Box and whisker plots of soil Mn.

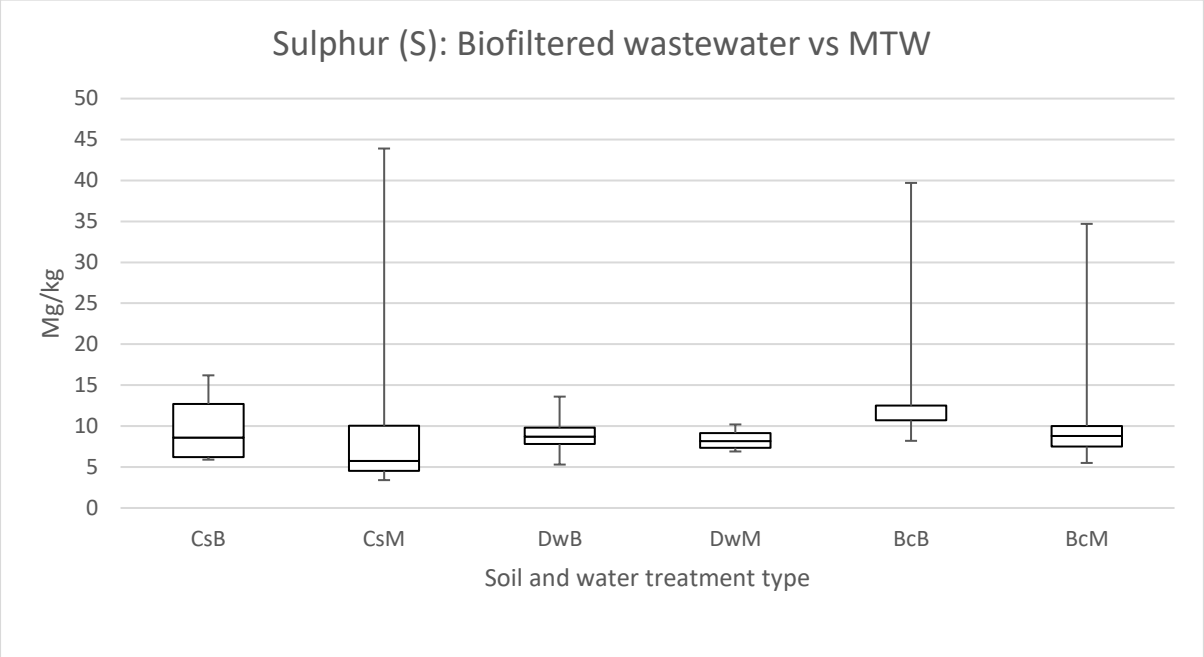

Figure B4: Box and whisker plots for soil S.

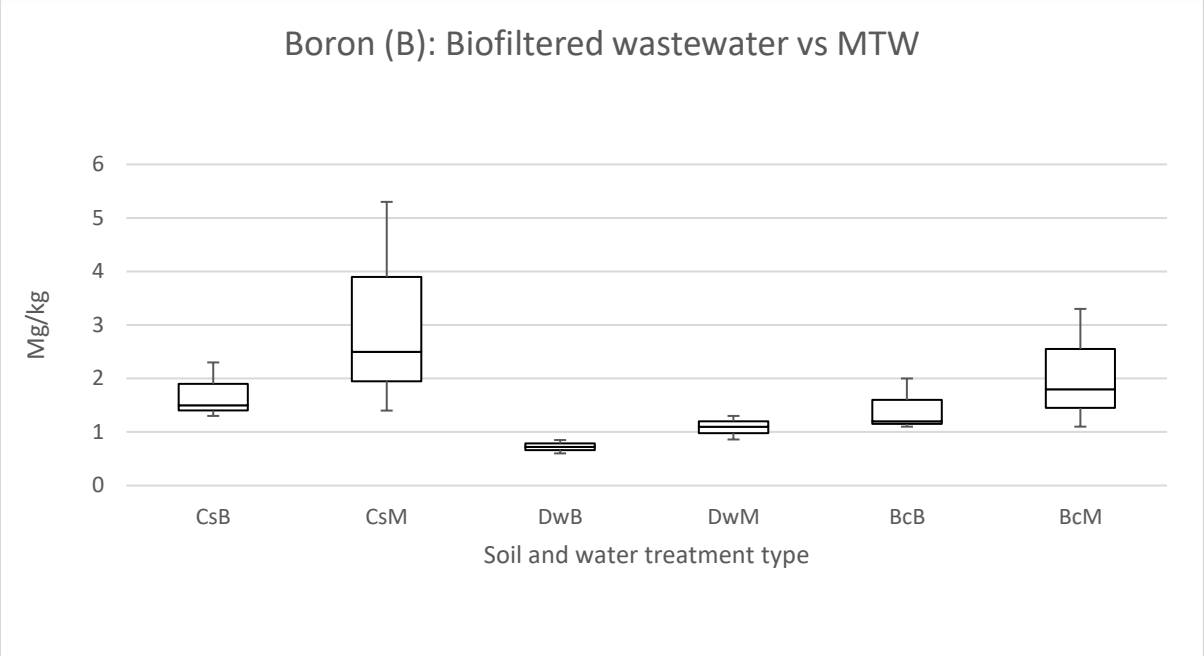

Figure B5: Box and whisker plots of Soil B.

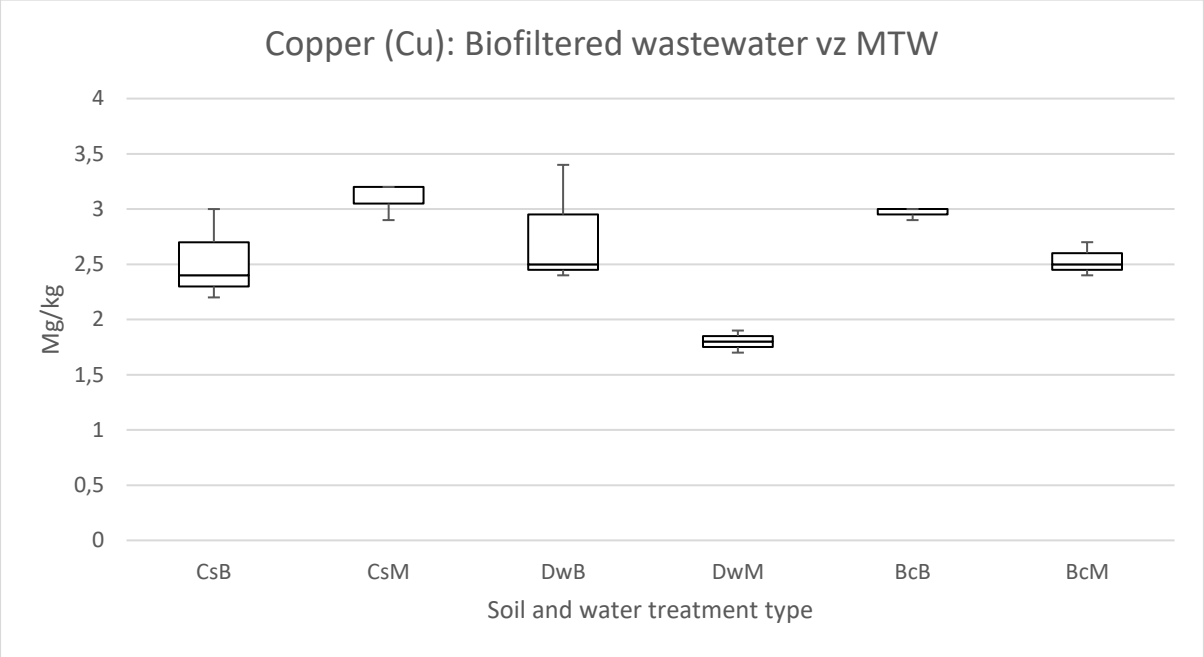

Figure B6: Box and whisker plots of soil Cu.

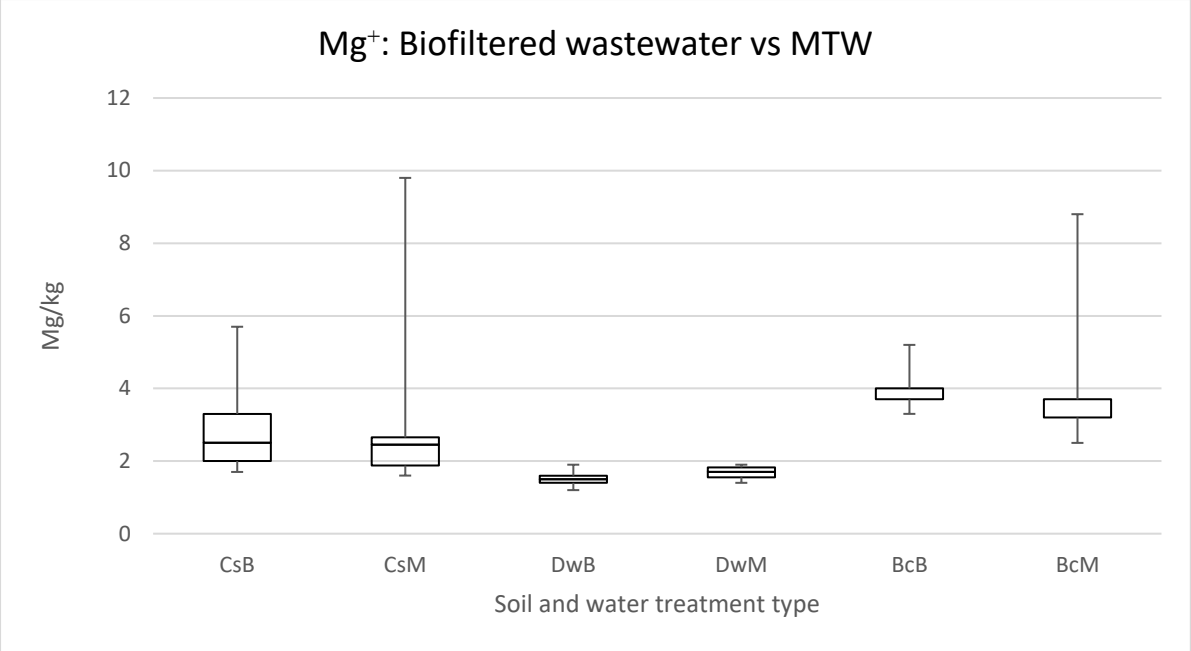

Figure B7: Box and whisker plots of soil Mg+ .