# Effect of winery wastewater irrigation on selected soil chemical parameters and enzyme activities in three simulated irrigation seasons

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Dissertation accepted in fulfilment of the requirements for the degree
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**Graduation: May 2023**

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# DECLARATION

This dissertation is my original work and has not been presented for a degree at any other institution.

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# DECLARATION OF ORIGINALITY

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| Research tittle | **Effect of winery wastewater irrigation on selected soil chemical parameters and enzyme activities in three simulated irrigation seasons** |

DECLARATION

1. I understand what plagiarism is and I am aware of the University’s policy in this regard
2. I declare that this dissertation is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other peoples’ work or my own work has been used, this has properly been cited and referenced in accordance with the North-West University requirements.
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# Abstract

The climatic conditions in the Western Cape province in South African (SA) vary considerably and water shortages are inevitable; consequently, sustainable management of soil and water resources should be of increasing concern. Wine production is one of the major production industries in the Lower Orange Northern Cape and Western Cape and produces large volumes of low-quality wastewater. The aim of this study was to investigate the effect of winery wastewater (WW) as an irrigation source on selected soil chemical characteristics and enzyme activities.

Four soils (Stellenbosch clay loam, Stellenbosch sand, Lutzville sand and Robertson clay loam) were collected from the top 0-30 cm layer at different wine producing regions of the Western Cape and placed in plastic bags for transport and storage. After sieving, the soils were placed in 15.2 cm diameter and 20 cm height pots. The pots were irrigated with WW and municipal wastewater (MW) for three simulated irrigated seasons under a controlled environment, where municipal water was used as the control. Each simulated season contained six irrigations and soils were re-irrigated when water content reached 50-60% water deficit, which varied for each soil. Both water sources were analyzed for N, P, K, Ca, Mg, Na, $HCO_3^-$ and for pH, electrical conductivity (EC), and chemical oxygen demand (COD) at each irrigation event. At the end of each simulated irrigation season, the soils were analyzed for three enzymes (β-glucosidase, phosphatase and urease) and chemical parameters (total N, extractable $NO_3^-$, $NH_4^+$, P, K, Ca, Mg, and Na, pH, salinity ($EC_e$), organic carbon (OC)).

The WW used in this study had significantly higher amounts of almost all measured soil characteristics when compared to the MW. The WW had higher COD, pH, EC, sodium adsorption ratio (SAR) and potassium adsorption (PAR) and consequently their addition to soils significantly increased these soil chemical characteristics. Analysis of soils after irrigation showed that the initial (baseline) level of β-glucosidase and phosphatase in each of the four studied soils was higher than the levels measured after irrigation with either water source. However, irrigation with MW and WW during the three simulated irrigation seasons for the three study soils resulted in an increase in urease levels, with an exception in the Robertson clay loam.

The content in the WW was one order of magnitude greater for some chemicals (N, P, Ca, Mg, Na, $Cl^-$) and was two orders of magnitude greater for others (K, $HCO_3^-$). Most of these elements significantly increased in the soil after irrigation with WW except for N and Ca. The lack of increase in $NH_4^+$-N was possibly due to increased ammonia volatilization in soils with higher pH resulting from WW irrigation. The lack of increase in $NO_3^-$-N may be due to increased denitrification in soils with anaerobic conditions resulting from irrigation with WW with high COD levels. The lack of increase in soil exchangeable Ca may be caused by the formation of calcium carbonates, which is favoured by high pH, high carbonates added, and high Ca.

The increased soil $EC_e$ observed was up to four times the initial $EC_e$ value. However, the $EC_e$ values in the Robertson clay loam soil were similar to the baseline values for all simulated irrigation seasons mainly because the soil $EC_e$ values were initially high. The observed salts in the soil exceeded the threshold of 1.5 dS/m that is recommended for vineyards. Also, the increase in Na after WW addition resulted in an increased extractable sodium percentage (ExSP). However, this increase did not reach the threshold of 15 to cause soil physical permeability problems.

Winery wastewater contains essential elements and other ions which can have direct effects on soil properties. These effects include the increase in plant-available nutrients when essential elements are added in the irrigation water and the increase in possible enzyme activity if certain essential elements are added. The WW shows potential as irrigation water mainly because it can increase K, P, and urease activity. However, care is needed as the WW may increase salts and Na in the soil to detrimental levels and thus the WW should be monitored constantly. It is worth noting that these detrimental levels may not be reached in the field because this was a closed study and no leaching occurred. Therefore, in an open system, such as field conditions in a vineyard, leaching may remove some salts and Na.

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# Chapter 1 -Introduction

Wine production is one of the major agricultural production industries in the Northern Cape and Western Cape. Large volume of low-quality wastewater is produced in wineries (Mulidzi, 2016). Approximately 1 to 4 L of wastewater is produced per 1 L of wine produced, mainly during the harvest period (Myburgh & Howell, 2014). Distilleries and wineries generate their wastewater mainly from the washing of equipment (Mulidzi, 2016). Wine making involves many steps as describe by Vlyssides *et al.* (2005) (Figure 1.1). These steps include crushing, grape fermentation, seeds and skin straining, storage, clarification then followed by the young wine maturation .

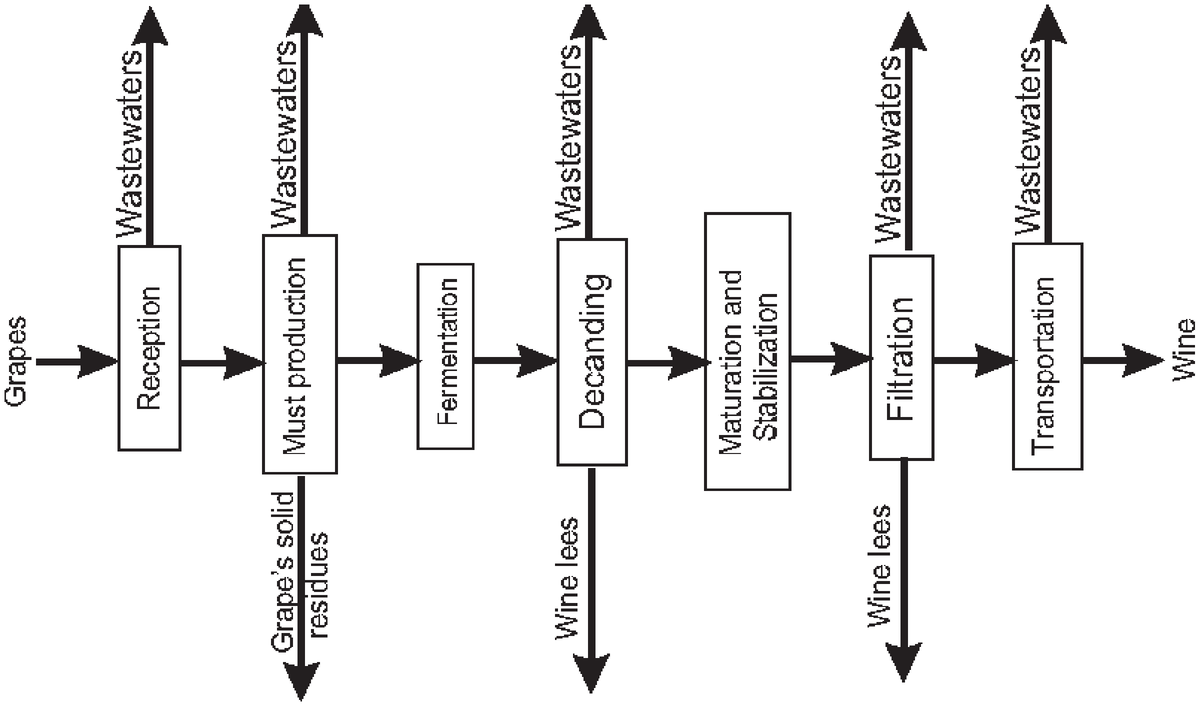

**Figure 1.1:** A schematic diagram representing the steps involved in the process of wine making in most wineries (Vlyssides *et al*., 2005).

The disposal of winery wastewater (WW) has been done in different ways but the most common disposal method is though pasture irrigation. However, such irrigation can be associated with pollution risks that could have a detrimental effect on the environment (Laurenson *et al*., 2011). These risks may include saturation of soils as a result of over-irrigation, occurrence of bad odours and anaerobic conditions could prevail (Myburgh & Howell, 2014). In addition, WW that is treated, whether by combing it with other water, should be used for the benefit of the agricultural sector through irrigation of crops mainly the vineyards.

Winery wastewater is often treated to an appropriate quality standard, so that it can be stored and used for irrigation of other crops (Mulidzi, 2016). The augmentation of irrigation water with WW may be used in conjunction with other resilient irrigation water saving techniques as solutions to supplement the water shortages faced in the world that are caused by climate-related droughts (Myburgh & Howell, 2014).

Using diluted WW for irrigation of crops has many advantages including the presence of essential plant nutrients and organic matter, which are necessary in improving crop productivity (Howell *et al*., 2018). The application of WW (Myburgh & Howell, 2014) and distillery wastewater (DW) may bring a positive effect in soils. Papini (2000) found that direct land use of stillage as irrigation water improved soil pH, while water and mineral salt retention characteristics increased as well as soil restoration. Land use of WW and DW seems easy, however, application on soils may have negative impacts such as contamination of groundwater and other natural resources (Bond, 1998). Potential adverse effects include excessive nitrate ($NO_3^-$) leaching to groundwater as well as increased soil sodium on current and future land use (Bond, 1998). Increased sodium (Na) contributes to poorly structured soils and adversely affects the soil's penetration rate and hydraulic conductivity (Chen *et al*., 2013). The soil quality can be degraded on deep sandy soils by leaching phosphorus (P) to groundwater (Papini, 2000).

## 1.1 Background to the research problem

The climatic conditions in the Western Cape province in South Africa (SA) range considerably. Sustainable management of soil and water resources is of increasing global concern. Irrigation of agricultural lands with wastewaters, following varying levels of treatment, is increasing around the world (Barkle *et al*., 2000; Arienzo *et al*., 2009; Papadopoulos *et al*., 2009). With projections of increased incidence and severity of drought (Portner *et al*., 2022, such practices are likely to become even more common. Examining the changes in climate, particularly rainfall volumes and distribution, may demonstrate that the use of WW for irrigation of the vineyard under field conditions is of paramount importance. The Western Cape province has different types of soils in addition to climatic variations. Different soil types can influence adsorption and accumulation of nutrient elements; therefore it is important to investigate the response of different soil types to irrigation with WW within the same climate zone.

The ARC Infruitec-Nietvoorbij has recently investigated the potential of WW for vineyard irrigation in various environments. This main ARC project had the objective of assessing the suitability of WW use for irrigation on different soil types with varying rainfall volumes and leaching levels on the yield of vineyards and wine quality. In this present pot study (which sat within the greater ARC project), WW was used to irrigate soils collected from three different regions of the wine grape producing areas of South Africa to determine its effect on the soil. These areas were Stellenbosch, Lutzville and Robertson.

Determining the impacts of irrigation with WW on crops and soils in field investigations requires specific infrastructure (Myburgh & Howell, 2014). Moreover, field experiments are ordinarily done with one explicit soil type. It has been observed that various soils react distinctively to WW irrigation (Mulidzi, 2001), therefore, it is fundamental to determine the impacts of WW on soils that vary pedogenically. It, however, would be advisable to conduct an experiment in a controlled environment to investigate the impact of wastewater in pedogenically different soils. Therefore, pot experiments appear to be the best choice to study impacts of wastewater on soil reactions. Given that WW can be stored in tanks, pot experiments can proceed consistently if the pots are protected from rain. This lessens the term of experiments contrasted with field trials. Furthermore, drainage and leaching of components can be avoided. Taking the above-mentioned into consideration, this study was a pot experiment to determine the effects of WW irrigation on soil chemical and biological responses of different soils and formed part of the above-mentioned project on the use of WW for vineyard irrigation, which was initiated, managed and funded by the Water Research Commission and co-funded by Winetech and the Agricultural Research Council.

Since it has been deduced that different conditions affect salt accumulation in the soil, it is anticipated that soil biological parameters are also affected. This could have important implications for soil aspects such as soil carbon. Soil enzymes play a critical role in catalyzing the organic matter (OM) decomposition and nutrient cycling reactions (Graham & Haynes, 2005). As such they can be linked to environmental quality, crop productivity, and energy transfer (Corbeels *et al.*, 2013). Management practices in plant production have numerous direct impacts on several soil enzymes (Muchakwana, 2011). Enzyme assays are often used as a way to provide an indication of soil quality with respect to some of the significant soil elements (Green *et al.*, 2007). Fluorescein diacetate (FDA) hydrolysis assay gives a manifestation of the whole microbial activity and is a great measure of soil organic matter (SOM) turnover (Green *et al.*, 2007). β-glucosidase is used to check on the turnover of cellulose whilst arylamidase and phosphatase are crucial in showing the prospective mineralization of organic nitrogen (N) and phosphorus (P), respectively (Green *et al.*, 2007). Furthermore, soil characteristics, plant variations and agricultural practices affect soil enzyme activities (Carson *et al.*, 2016).

Soil enzyme activities mediate soil biological processes, such as the breaking down of residues, recycling of nutrients, organic matter (OM) formation, soil structure and soil quality (Adetunji *et al*., 2017). Enzymatic activities are reported to usually decline with soil depth (Carson, 2016). Enzymes play a vital role in agriculture and in nutrient cycling, in particular, since they are constantly being synthesized, accumulated, inactivated and decomposed in the soil (Adetunji *et al.*, 2017). Soil enzyme activity also has to be investigated since enzyme activity in the soil ensures soil quality and hence could ensure yields of great quality in terms of vineyards. In agricultural soils, phosphatases play an important role in phosphorus cycles, and due to the fact that their activity is sensitive to management practices, they can be used as soil quality indicators (Makoi & Ndakidemi, 2008).

## 1.2 Problem statement

In recent years, the Western Cape province has faced a serious water challenge, so certain measures thus need to be put in place so as to mitigate the issue if it occurs again in the future. Adding to that, for every agricultural system, the use of water is very crucial in terms of soil quality and plant growth. Furthermore, farmers could be irrigating with the WW without having enough knowledge of the benefits that may be aligned with the use wastewater. Also, previous literature has focused more on the effect on the effect of winery wastewater on soil chemical parameters and hence there is little research done on the soil enzyme activities in relation to winery wastewater application. Winery wastewater irrigation can have advantages. However , winery wastewater irrigation also has its challenges as it often contains amounts of inorganic salts that can to increase soil salinity, which may not only negatively affect plant growth but also have unfourable effects on soil structure and other soil physical properties. Furthermore, groundwater contamination and further diminishing of fresh water reserves may result because of large applications of soluble salts (Hoogendijk, 2019).

## 1.3 Research question

Can the use of WW contribute to the mitigation of water crisis in the Western Cape province through its use as a source of water for irrigation and will the use of WW for irrigation have a positive or detrimental effect on soil quality in comparison to using MW?

1. Will the application of WW influence soil chemical parameters?
2. Can WW application influence the enzyme activity of soils sampled in different areas?

## 1.4 Aim and objectives

### 1.4.1 Main aim

The aim of this study is to investigate the effect of WW and MW irrigation on selected soil chemical characteristics and enzyme activities of different soils in a pot experiment over three simulated seasons of irrigation.

### 1.4.2 Specific objectives

1. To compare water quality between WW and MW.
2. To investigate the effect of WW application on total N, P, soil pH, exchangeable potassium (K), calcium (Ca) and magnesium (Mg), electrical conductivity of the saturated extract ($EC_e$) and organic carbon (OC) in different soils in comparison with MW.
3. To investigate the effect of WW application on β-glucosidase, phosphatase and urease enzyme activities in different soils compared to the effect of MW.

## 1.5 Hypotheses

**Null hypothesis:** Irrigation with WW will not affect soil levels of N, P, extractable bases (K, Na, Ca and Mg), pH, $EC_e$, OC, and soil enzyme levels (β-glucosidase, phosphatase and urease).

**Alternate hypothesis:** Irrigation with WW will affect soil levels of N, P, extractable bases (K, Na, Ca and Mg), pH, $EC_e$, organic carbon, and soil enzyme levels (β-glucosidase, phosphatase and urease).

## 1.6 Justification of the study

The use of WW as an alternative source of irrigation water focuses on many aspects. It is not only a socio-economic mitigation strategy, but also importantly preserving soil health. Sustainable use of the WW for the irrigation of crops could have a positive effect in many ways such as:

(i) minimizing the energy needed for the treatment of wastewater, such as the use of water aeration pumps in ponds;
(ii) the presence of nutrients in wastewater, such as N, P and K, could also minimize fertilization costs;
(iii) where irrigation water is limited, re-use of wastewater will have a positive effect on grape yields if additional irrigation can be used; and

(iv) water savings and higher yields will lead to the sustainability and economic viability of wine production.

# Chapter 2 – Literature Review

The use of municipal water is an essential part in the process of wine production. In this process, municipal water is used and a secondary product of low-quality wastewater is produced (Howell, 2016). Wastewater produced is potentially disposed through natural systems and/or re-used as irrigation water. The means of disposing the winery wastewater (WW) is subject to certain quality requirements (Mosse *et al.*, 2011). Improper WW disposal in environments can cause salinization and eutrophication in water sources (Van Schoor, 2005; Laurenson *et al.*, 2012; Myburgh & Howell, 2014). Furthermore, WW can cause various soil problems which includes soil chemical contamination, sodicity, salinity, loss of structure, waterlogging and anaerobiosis, and increased susceptibility to erosion. Additionally, WW that contains solid waste can facilitate the occurrence of bad odour (Laurenson *et al.*, 2012). Furthermore, WW seepage can also cause contamination of soil and water resources, which can lead to the inhibition of vegetative performance (Van Schoor, 2005; Myburgh & Howell, 2014).

## 2.1 Legislation on the use of winery wastewater

Winery wastewater is regarded as biodegradable commercial wastewater and its disposal and use as irrigation water, needs to follow guidelines set by the General Authorisations of the Department of Water and Sanitation (DWA, 2013). These General Authorisations first indicate that the maximum amount of wastewater that can be used as irrigation is 2000 $m^3$/day. Secondly, they indicate the acceptable levels of chemical and biological parameters for the use of a given volume of wastewater (Table 2.1). Notably, the acceptable levels are less than 5000 mg/L for COD, 6 - 9 for pH, 2 dS/m for EC, 5 for SAR when irrigating with 500 $m^3$ of wastewater per day (DWA, 2013).

**Table 2.1:** Water quality standards for the use of wastewater as an irrigation source (DWA, 2013).

| **Parameter** | **Irrigation volume ($m^3$)** | | |
|---|---|---|---|
| | **2000** | **500** | **50** |
| **COD (mg/L)** | 75 | <400 | <5000 |
| **EC (dS/m)** | 0.7-1.5 | 2.0 | 2.0 |
| **SAR** | Other criteria apply | 5 | 5 |
| **pH** | 5.5-9.5 | 6-9 | 6-9 |
| **Faecal coliforms (per 100 ml)** | 1000 | 100000 | 1000000 |
| **Nitrate/Nitrite (mg/L)** | 15 | - | - |
| **Ammonia (mg/L)** | 3 | - | - |

## 2.2 Biological effect of winery wastewater irrigation in soil

Soil enzyme activities can be used as a soil quality indicator (Adetunji *et al*., 2017) as they may reflect nutrient cycling potential, nitrification, oxidation, and different procedures that are basic to soil quality (Almeida *et al*., 2015). They are also sensitive to soil management and organic matter decomposition (Adetunji *et al*., 2017). However, determining soil fertility and plant yield using a single enzyme activity has been shown to be inaccurate (Tabatabai, 1994; Adetunji *et al*., 2017). Although the importance of the enzyme activity differences between different tillage treatments at different sampling dates was not comparable, the effects of different soil management practices on soil enzyme activity were reliably measurable in most seasonal samples (Jin *et al*., 2009; Bueis *et al*., 2018).

Additionally, a single enzyme can not determine the complete nutrient status of the soil because it is substrate specific (Nannipieri *et al*., 2012). Thus, the enzymes most widely used for evaluating the factors controlling plant matter decomposition and soil quality are those involved in the degradation of main material components and hydrolases, which are associated with the carbon (C) (β-glucosidase and β-galactosidase), nitrogen (N) (urease), phosphorus (P) (phosphatase) and sulphur (S) (arylsulphatase) cycle (Adetunji *et al.* 2017). Other soil enzymes may include amylase, amidase, phenol oxidase, cellulose, chitinase, de-hydrogenase and protease (Tabatabai, 1994; Adetunji *et al.* 2017;). Table 2.2 shows some of the common soil enzymes that can be used as biological soil quality indicators.

**Table 2.2:** Common soil enzymes that are used as biological soil quality indicators as adapted from Adetunji *et al.* (2017).

| **Soil enzyme** | **Enzyme reaction** | **Reaction catalyzed** | **Indicator of microbial activity** |
|---|---|---|---|
| Dehydrogenase | Electron transport System | $XH_2 + A \rightarrow X + AH_2$ | C-cycling |
| β-glucosidase | Cellobiose Hydrolysis | Glucoside + $H_2O$ → ROH + Glucose | C-cycling |
| Cellulase | Cellulose Hydrolysis | Hydrolysis of β-1, 4 – glucan Bonds | C-cycling |
| Phenol oxidase | Lignin hydrolysis | $A + H_2O_2 \rightarrow$ oxidized $A + H_2O$ | C-cycling |
| Urease | Urea hydrolysis | Urea → $2NH_3 + CO_2$ | N-cycling |
| Amidase | N-mineralization | Carboxylic acid amide + $H_2O$ → carboxylic acid + $NH_3$ | N-cycling |
| Protease | N-mineralization | Proteins → peptides and amino Acids | N-cycling |
| Phosphatase | Release of $PO_4^-$ | Phosphate ester + $H_2O$ → ROH Phosphate | P-cycling |
| Arylsulphatase | Release of $SO_4^-$ | $ROSO_3^- + H_2O \rightarrow ROH\ SO_4^{2-}$ | S-cycling |
| Other soil enzymes | Hydrolysis | Hydrolysis | General organic matter degradative enzyme activities |

One microbial enzyme in particular, β-glucosidase, is active in the cycling of carbon (Tabatabai, 1982), and is particularly sensitive to management-induced changes in the soil due to its strong relationship with soil organic matter content and quality (Masciandaro & Ceccanti, 1999). Enzyme activities are useful indicators of soil health because their activities reflect both short-term (Bandick & Dick, 1999) and long-term (Jin *et al.*, 2009) changes taking place in their respective pools of nutrients. However, irrigation that affects vertical distribution of roots, as of grapevines, are also likely to affect other functional microbial groups such as mycorrhizal fungi, since changes in quantity and quality in rhizo-deposition through root exudates, generally affects soil microbial community compositions (Smalla *et al.*, 2001). On a broader scale, the effect which irrigation with WW may have at microbial community levels is equally important to that at enzyme level. In fact, the levels of activities of enzymes in the soil can be seen as an expression by the soil microbial community to metabolic requirements and nutrient availability (Dick *et al.*, 1997; Badiane *et al.*, 2001; Moore-Kucera & Dick, 2008; Dotaniya *et al*., 2019). This implies that taking note of the diversity in microbial communities could be crucial in determining the effects that irrigation with WW may have on the general microbiology of the soil.

Several studies have shown a decrease in β-glucosidase activity with respect to soil depth (Adetunji *et al.*, 2017). This is because β-glucosidase activity greatly depends on substrate supply and the microorganisms that mainly produce this enzyme are active in the top soil. This implies that β-glucosidase activity can be used as an indication of the presence of higher simple sugars for microbial population in the soil surface layer. Bandick and Dick (1999) reported that β-glucosidase was lower in arable lands as compared to woodland and meadow soils. Most of the enzyme activities were higher in the vegetative growth stages as compared to the productive growth stages (Jin *et al.*, 2009). Although the value of differences in enzyme activities between different tillage treatments was not equal at different sampling dates, the effects of different soil management practices on soil enzyme activities were detectable in a consistent manner in samples from the majority of seasons (Jin *et al.*, 2009).

Acid and alkaline phosphatases are both known as phosphomonoesterases. There is general agreement that acid phosphatase is more dominant in acid soil and alkaline phosphatase in alkaline soils (Eivazi & Tabatabai, 1977). The production of alkaline phosphatase is enhanced by microorganisms while on the other hand, acid phosphatase is sometimes released by plant roots, particularly in soils with low P content as an in-built mechanism for enhancing P uptake from the same soils (Acosta-Martinez & Tabatabai, 2011; Kong *et al.*, 2014).

Studies of the impact of different soil treatments on soil phosphatase activity showed that air-drying increased acid phosphatase and phosphotriesterase activity decreased alkaline phosphatase activity but did not affect phosphodiesterase activity (Adetunji *et al*., 2017). The phosphatase activity in soils that were affected by forest fire was low but it increased over years as the burnt soil recovered (Staddon *et al*., 1998). The effect of drought also has a huge effect on phosphatase activity reduction when moisture was also reduced (Sardans & Penuelas, 2005). This implies that water availability also plays a very crucial role in soil enzyme activity. Phosphatase activity was also decreased by the presence of lead and other heavy metals in the soil (Kandeler *et al*., 1996).

The soil enzyme urease facilitates the hydrolysis of urea into $NH_3$ and $CO_2$, thereby increasing soil pH in the process (Cordero *et al*., 2019). The stability of urease depends on several factors, including soil moisture and temperature. Urease activity increases with increasing temperature, showing the effect of temperature on urease hydrolysis (Machuca *et al.*, 2015). Urease activity is also increased by the presence of metabolisable substrate and this was evident in a study by Mulidzi and Wooldridge (2016), where irrigation with WW enriched 0-10 cm of soils with metabolisable substrate and resulted in higher urease activity compared to the 10-20 cm. Microbially secreted soil enzymes, responsible for the decomposition of organic matter and cycling of nutrients in soil, are said to be stimulated through the supply of easily decomposable organic material in wastewater (Anissomova *et al*., 2014; van Huyssteen *et al*., 2020). In soil, enzymes typically bind to humus in organic matter through various mechanisms. Therefore, it is likely that urease activity response to various irrigation sources will differ between different soils. Mulidzi and Wooldridge (2016) found that urease activity in soils irrigated with WW decreased in the sequence: Stellenbosch shale > Stellenbosch granite, Rawsonville sand > Lutzville sand.

## 2.3 Chemical effect of winery wastewater in the soil

### 2.3.1 Soil pH

Soil pH plays an important role in influencing the biological, physical and chemical parameters in the soil. For example, it influences the rate at which phosphatase is synthesised, released, and its stability (Acosta-Martinez & Tabatabai, 2000). Irrigating soils with low pH WW will cause a decrease in soil pH (Laurenson *et al*., 2012). There is seasonal variation in pH of the WW and this can affect the pH of amended soils (Hoogendijk, 2019). Irrigation with WW produced during harvest caused lower soil pH (pH 4) compared to wastewater produced during post-harvest (pH 6) (Mahajan *et al*., 2009). On the other hand, a study by Hirzel *et al*. (2017) showed an increasing pH from 7.75 to 9.1 when WW was applied, while another study resulted in the increase of soil pH by one to two units after diluted WW was applied (Mulidzi, 2016).

### 2.3.2 Exchangeable K, Na, Ca and Mg

Winery wastewater from different wineries contain different levels of base cations (Table 2.3). Consequently, the effect of WW on soluble and exchangeable Ca, Mg, K and Na in the soil is dependent on the source and levels of WW applied. The levels of these cations in WW are determined by whether the wastewater is diluted or undiluted and also on the time it was produced, *e.g.* vintage (during harvest) and non-vintage.

**Table 2.3:** The levels of bases (K, Na, Ca and Mg) measured in various winery wastewaters used as an irrigation source.

| **Source** | **Levels of base cation (mg/L)** | | | | **Notes** |
|---|---|---|---|---|---|
| | **$K^+$** | **$Na^+$** | **$Ca^{2+}$** | **$Mg^{2+}$** | |
| Agustina *et al.*, 2008 | 201 | 204.0 | 286.0 | 33.0 | - |
| Arienzo *et al.*, 2009 | 250 | 130.0 | - | - | - |
| Laurenson *et al.*, 2011 | 179 | 120.2 | 21.4 | 3.6 | - |
| Laurenson *et al.*, 2010 | 42 | 112.6 | 6.6 | 3.8 | Obtained during the harvest period |
| Mulidzi, 2016 | 196 | 84.4 | 18.6 | 7.2 | Diluted winery wastewater |
| Quale *et al.*, 2010 | 595 | 95.9 | 9.7 | 48.2 | Diluted winery wastewater |
| Quale *et al.*, 2010 | 801 | 97.7 | 399.9 | 72.0 | Undiluted winery wastewater |
| | | | | | |

Adding wastewater to the soil can result in the quick accumulation of soluble and exchangeable potassium (K) as contrasted to the use of synthetic inorganic fertilizers (Arienzo *et al.*, 2009). Moreover, the vast majority of K in WW is promptly accessible. The high K contents of wastewaters used for irrigation could improve overall soil fertility, even though soil physical and chemical properties could be affected by long-term application (Laurenson & Houlsbrooke, 2011; Mosse *et al.*, 2011; Howell, 2016). Winery wastewater can be a source of K and an important recycling method in areas where the soil has low K. Research on the excessive irrigation with WW high in K has not been researched adequately (Mosse *et al.*, 2011; Laurenson *et al.*, 2012; Howell, 2016). Adding to that, the fate of K in soils and on grapevines that have received irrigation with WW as the source of the irrigation water has received little attention (Laurenson *et al.*, 2012; Howell, 2016). Winery wastewater with high levels of K can result in reduced Na adsorption and thus a lower exchangeable sodium percentage (ESP). Conversely, WW with high levels of Na and little to no K can result in higher soil Na and ESP (Laurenson & Houlsbrooke, 2011).

Sodium was found to be the most prevalent metallic species in the initial WW and wastewater produced during harvest because the cleaning agents used during the wine making season were sodium containing agents (Sheridan *et al.*, 2011). Sodicity is a major concern, where Na is applied through irrigation water. The Na concentration norm for irrigation water must not exceed 115 mg/L (DWA, 2013). In addition, high Na levels may cause sodic soils, which will result in extremely poor physical properties responsible for aggregate stability degradation and loss of macroporosity, which lead to a severe imbalance between air and water movement in the soil (Marchuk, 2013).

Winery wastewater can also contain $Ca^{2+}$ and $Mg^{2+}$ ions, which also serve as liming materials in acidic soils (Mosse *et al*., 2011). None of these ions (Ca and Mg) harm the soil structure and can indirectly contribute to mitigating the impact of Na application through their role in reducing SAR (Arienzo *et al*., 2009). In general, when Ca is compared with other elements, the levels of Ca present in WW is considerably less (Table 2.3). Irrigation with WW therefore rarely affects soil Ca levels. Mulidzi *et al*. (2015) and Howell *et al*. (2018) showed that irrigation with WW had little or no effect on soil Ca and they attributed this to low amounts of Ca in wastewater. Similarly, over four consecutive irrigation seasons with WW, Quale *et al*. (2010) recorded relatively constant levels of Ca soil for a clay soil. In contrast, other studies have shown an accumulation of Ca in the soil resulting from long-term irrigation of WW (Kumar *et al*., 2006; Mosse *et al*., 2012). Kumar *et al*. (2006) also reported that pastures irrigated for a century with WW had substantially higher levels of Ca compared with controls. There was also an accumulation of Ca in a silty clay loam soils that had been receiving WW for 30 years (Mosse *et al*., 2012).

Magnesium also occurs at low concentrations in WW and its impact on soil levels is often negligible (Laurenson, 2010; Gray, 2012; Mulidzi *et al*., 2015; Hirzel *et al*., 2017; Howell & Myburgh, 2018). The authors attributed the lack of Mg accumulation to high Na levels that caused the divalent cations to be displaced in the soil. By comparison, Kumar *et al*. (2006) recorded significantly increased soil Mg as a result of 100-years of irrigation with WW. Similar increases in soil Mg due to irrigation with Mg-rich WW were reported (Mosse *et al*., 2012; Mosse *et al*., 2013). Conversely, after four years of irrigation of WW, Quale *et al*. (2010) reported a reduction in soil Mg.

### 2.3.3 Nitrogen and phosphorus

There is limited research on the effect of WW on soil N. Nonetheless, Kumar *et al*. (2006) and Hirzel *et al*. (2017) recorded higher concentrations of soil N in a control soil compared to soils irrigated with WW. In a pot experiment performed in a glasshouse-controlled environment by irrigating crops with diluted WW, soil N was higher when the dilution of the WW was lower

(Shilpi *et al*., 2018). The levels of N in a soil that has been irrigated with WW is also affected by the seasonal variation of N levels in the WW. For instance, Mahajan *et al*. (2009) recorded marginally higher soil N during the harvest period than during the pre- or post-harvest era when soils were irrigated during the year with WW.

Information is scarce and inadequate regarding the effect of WW irrigation on soil P levels. According to Mulidzi *et al*. (2009), irrigation with undiluted WW increased soil P levels, but seasonal P variations in different soil horizons were observed.

### 2.3.4 Electrical conductivity

The high salt content of WW has been shown to increase the electrical conductivity of the saturated soil extract ($EC_e$) from irrigated soils (Kumar *et al*., 2006; Mahajan *et al*., 2009; Quale *et al*., 2010; Mosse *et al*., 2012; Hirzel *et al*., 2017; Shilpi *et al*., 2018). Conversely, over time, Kumar *et al*. (2014) reported a decrease in soil $EC_e$ in pasture and woodlot soils irrigated with WW. When field conditions were simulated in a pot experiment, soil $EC_e$ was unaffected by the type of irrigation water (winery wastewater, potable water and municipal water), irrespective of soil type (Laurenson, 2010).

### 2.3.5 Organic matter

Irrigating soil with C-rich WW may increase total organic carbon (TOC) (Kumar *et al*., 2006; Kumar *et al*., 2009). On the other hand, a study by Quale *et al*. (2010) showed that the irrigation of soil with WW had no significant effect on the soil's TOC, despite the high TOC in the wastewater. Their results showed inconsistent soil OC trends as being affected by the dilution of wastewater from wineries. This could be due to WW having inadequate amounts of OC to affect soil fertility or because the OM present in wastewater decomposed upon aeration between irrigation applications (Howell & Myburgh, 2018). Furthermore, Mulidzi (2001) reported that many highly permeable soils might not be suitable for irrigation with WW rich in OM because the OM will not be retained but will move with the percolate water and can lead to pollution of the natural water resources. The chemical, physical and biological fertility of soil is dependent on soil organic carbon (Mohammad *et al*., 2012).

### 2.3.6 Chloride, trace elements

Winery wastewater irrigation effects on soil Cl, trace elements and concentrations of heavy metals are not well known. Mosse *et al*. (2012) reported substantial increases in soil $B^{3+}$, $Cu^{2+}$, $Fe^{2+}$, and $Zn^{2+}$ as a consequence of long-term irrigation with WW. In contrast, Howell *et al*.

(2018) could not relate soil concentrations of $Cl^-$, $Cu^{2+}$, $Fe^{2+}$, and $Zn^{2+}$ to different levels at which WW was diluted in a field trial. However, after three and four seasons in which diluted WW was used for irrigation, soil levels of $B^{3+}$ were observed to increase up to a depth of 150 cm.

# Chapter 3 – Materials and Methods

## 3.1 Soil selection and collection

The study was conducted on four different soils, which were collected from the grape growing regions in the Western Cape province namely, Stellenbosch ,Lutzville Robertson The soils were collected from the plots of the greater ARC project(**Use of winery wastewater as a resource for irrigation of vineyards in different environments)**. The soils differed in their clay contents and OM. The locations of the four soils are shown in Figure 3.1. It should be noted that the soils were collected where no winery wastewater (WW) had previously been applied.

**Figure 3.1:** Map of selected sites where soils were taken for determining the effect of irrigation with winery wastewater on soil chemical and biological properties. Adapted from the ARC project.

Soil samples were collected from the top 0-30 cm layer at each area and placed in plastic bags for transport and storage. Each composite sample consisted of seven sampling points for each soil. The soils were sieved using a 6 mm mesh sieve to remove large fragments of organic matter and stone fractions. The soils were put in 15.2 cm diameter and 20 cm height pots after they had been sieved. The pot experiment was carried out under a fiberglass rain shelter at ARC Infruitec-Nietvoorbij.

## 3.2 Experimental study and design

The experimental design was a 4x2x3 factorial experiment fitted in a randomized complete block design (RCBD) with measurements taken over time. The four soils (factor one) differed in chemical parameters. The two water sources (factor two) were WW and municipal water (MW) (control). This was done over three simulated irrigation seasons (factor three). This resulted in twenty-four treatments.

There were four replicates of the twenty four treatments; each treatment was randomly assigned within each replicate. Three pots were fitted within each treatment for destructive soil sampling after each irrigation seasons. There were six irrigations in each season, which according to (Myburgh, 2013) is the number of irrigations a vineyard would require during the harvest period. A total of 18 irrigations were applied over the three irrigation seasons. The experimental layout is shown below in Figure 3.2.

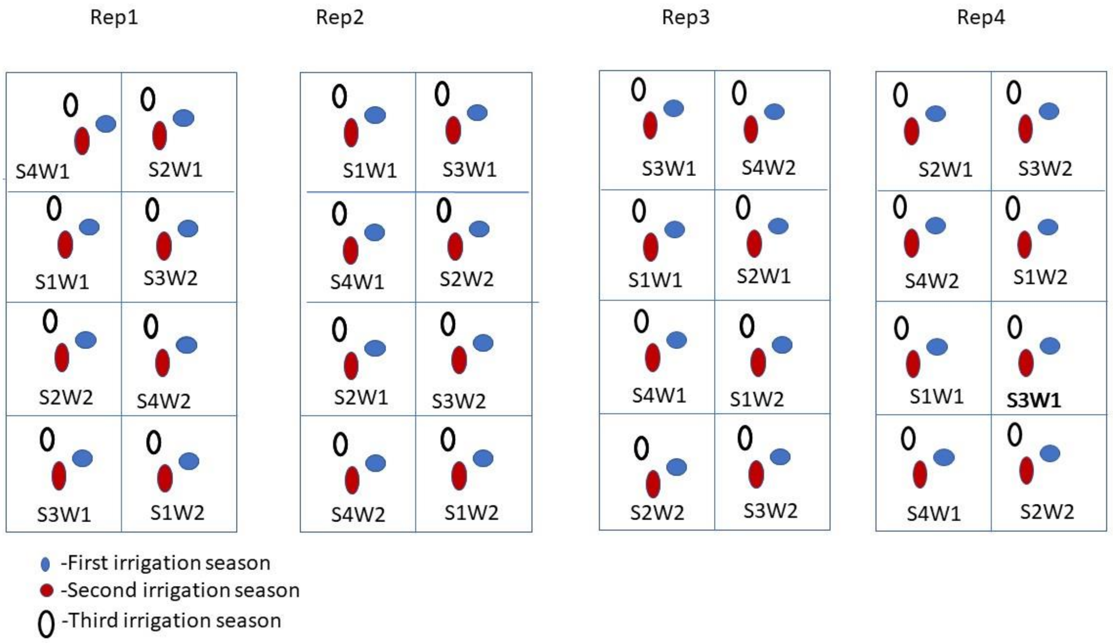

**Figure 3.2:** Experimental layout of the pot experiment that was carried out at ARC Infruitec-Nietvoorbij.

## 3.3. Irrigation

Municipal water for the control treatments was obtained from the ARC Infruitec-Nietvoorbij farm. The undiluted wastewater was collected from Bottelary wineries. Pumps were used to apply the MW and WW to the soil/water treatment combinations. Water was distributed through a network of pipes and applied to each pot using a 2 L/h pressure compensating button dripper.

### 3.3.1 Irrigation volumes

The field capacity (FC) of the soil was gravimetrically analysed before filling the pots with soil. The weight of the pot was recorded prior to filling it with the soil. The weight of the pot with the soil at FC (WMfc) was calculated using the formulae described in the work of Mulidzi (2016).

WMfc = Mfc - Mod **(Eq. 1)**

Mfc represented the mean pot weight, including the weight of the soil at field capacity in grams. While Mod represented the pot weight plus the weight of oven-dried soil in grams.

In order to irrigate, the soil moisture content was allowed to drop to 50% FC, the 50% FC weight, which was soil- and pot-based weight was calculated as follows:

Mdepl = Mod + (WMfc x 50 ÷ 100) **(Eq. 2)**

where, the pot plus soil mass at that 50% FC were represented by Mdepl.

Mdepl = Mod + (WMfc x P ÷ 100)

The moisture content was evaluated in four representative pots per soil/water treatment for each replicate. The pots were weighed every two days, using an electronic balance, until 50% FC was reached for irrigation. The braces bearing the micro-tubes were removed before weighing these pots. Assuming that the water density is 1 g/cm$^3$, the irrigation volume required per pot was calculated as follows:

Virr = WMfc - Mact **(Eq. 3)**

where Virr represented the volume of water required to bring each pot to FC (mL) and Mact represented the actual pot weight plus the soil weight before irrigation (g). The time (t) required to apply the water (Virr) was calculated as follows:

t = Virr ÷ Qdrip **(Eq. 4)**

where, Time was in minutes, and Qdrip was in mL / min, reflecting the total water flow rate through the four microtubes.

The soils were irrigated at 50-60 % of their P. This high depletion level was designed to ensure sufficient soil aeration between irrigations. When pots were removed for soil chemical analysis, their irrigation water was stored and discarded in 500 mL glass beakers. The same irrigation system flow rate was maintained throughout the experiment.

### 3.3.2 Irrigation water quality

A 500 mL sample of irrigation water was taken for analysis from each water source (WW and MW) during their application. Water chemical measurements were as follows:

The samples were analysed for ammonium nitrogen ($NH_4^+$-N), nitrate nitrogen ($NO_3^-$-N), phosphorus (P), calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), $K^+$, $Na^+$, pH, EC, bicarbonate ($HCO_3^-$) by Labserve. The chemical oxygen demand (COD) in both irrigation waters were measured using a portable spectrophotometer (Aqualitic COD-reactor®, Dortmund) with the appropriate test kits (COD, CSB, 0-15000 mg/L).

The potassium adsorption ratio (PAR) was calculated as follows:

$$PAR = K^+ \div [(Ca^{2+} + Mg^{2+}) \div 2]^{0.5} \quad \textbf{(Eq. 5)}$$

where $K^+$, $Ca^{2+}$ and $Mg^{2+}$ represented the concentration of potassium, calcium and magnesium, respectively, divided by their molecular mass. Similarly, the sodium adsorption ratio (SAR) was calculated as follows:

$$SAR = Na^+ \div [(Ca^{2+} + Mg^{2+}) \div 2]^{0.5} \quad \textbf{(Eq. 6)}$$

where $Na^+$ is the sodium concentration (mg/L) divided by the molecular mass. The $NH_4^+$-N and $NO_3^-$-N concentrations were summed to obtain the total nitrogen (Total-N).

### 3.3.3 Amount of essential elements added *via* irrigation

The volume of wastewater added was converted from mm to L per ha as follows: V = I for $10^4$ **(Eq. 7)** where, I is the amount of irrigation applied (mm) and $10^4$ is the factor used to translate water depth (mm) to volume (L) per hectare (1 mm = 10 $m^3$ per hectare = $10^4$ L per hectare).

For each irrigation, the concentrations of elements in the undiluted wastewater were used to measure the quantities of elements added to the soil by irrigation per hectare as follows:

$$m = V - Ce \quad \textbf{(Eq. 8)}$$

where m is the amount of element (mg / ha), V is the amount of water applied per hectare (L / ha) and Ce is the concentration of elements (mg / L) in the irrigation water.

Therefore, after the same process, the contribution of the elements deposited by the municipal water was considered. The quantity of element in milligram per hectare was translated to kilogram per hectare (M) as follows:

$M= m \div 10^6$ **(Eq. 9)**

The quantity of elements added per irrigation for seasonal applications was summed up.

### 3.4 Soil sampling and analyses

Following each simulated irrigation season, a pot for each treatment within each replicate was destructively sampled. Soil samples were collected from the 0-18 cm layer in the pots of all the replications. Core (1.5 cm diameter) sampling was used as a sampling technique. Soil samples were air dried and passed through a 2 mm mesh sieve.

#### 3.4.1 Soil chemical analyses

All analyses were carried out by a commercial laboratory (Labserve Mycro, Stellenbosch). Triplicate samples were collected from the composited soils. Standard baseline soil chemical analysis was conducted on the collected soils samples before the application of WW. At the end of the study, samples were collected for the analyses of treatment effects on the soil. The chemical analyses of the four study soils are shown in Table 3.1.

The Ca, Mg, K and Na were extracted with 1 M ammonium acetate buffered at pH 7 and their concentrations in the extract were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 7300 DV, Waltham, Massachusetts).

Most South African laboratories obtain an estimated CEC by summing up the extractable cations and refer to this estimate as the S-value, mainly because the process of determining CEC is tedious (Conradie, 1994; Mulidzi, 2016). Given the above-mentioned, the exchangeable potassium percentage (EPP) and exchangeable sodium percentage (ESP) of the soil could not be calculated. However, the extractable potassium percentage (ExPP) was calculated as follows:

$\text{ExPP} = (K \div S) \times 100$ **(Eq 10)**

where K is the extractable potassium ($\text{cmol}^{(+)}/\text{kg}$) and S is the S-value ($\text{cmol}^{(+)}/\text{kg}$), *i.e.* the sum of extractable Ca, Mg, K and Na.

**Table 3.1:** Chemical analytical methods for the four study soils.

| **Soil characteristics** | **Method** |
|---|---|
| Total N | Kjeldahl method (AgriLASA, 2004). |
| Available P | Bray No. 2 process. (Bray & Kurtz, 1945) |
| Extractable K, Ca, Mg, Na | 1 M ammonium acetate at pH 7 AgriLASA (2004) |
| Soil C | Walkey-Black method: AgriLASA (2004) |
| Soil pH | KCl method (Thomas, 1996) |
| Soil $EC_e$ | Longenecker and Lyerly (1964) |
| Extractable nitrate and ammonium using 1 M potassium chloride extraction | (Kachurina *et al*. 2000) |

In addition, the extractable sodium percentage (ExSP) was calculated as follows:

$$\text{ExSP} = (\text{Na} \div \text{S}) \times 100 \qquad \textbf{(Eq 11)}$$

where Na is the extractable sodium (cmol$^{(+)}$/kg) and S is the S-value (cmol$^{(+)}$/kg), *i.e.* the sum of extractable Ca, Mg, Kand Na.

The designation ExPP includes both the adsorbed K and K in solution; therefore, it should not be confused with EPP. Similarly, the designation ExSP includes both the adsorbed $Na^+$ and $Na^+$ in solution; therefore, it should not be confused with ESP.

### 3.4.2 Soil enzyme analyses

Three soil enzymes were analyzed. β-glucosidase was measured by a simple assay performed in the laboratory according to the Eivazi and Tabatabai (1988) process. Phosphatase was analyzed by the procedure used by Icoz and Stotzky (2008). Urease activity (EC 3.5.1.5) was analyzed by a 2-h incubation of a reaction mixture of 5.0 g of the field-moist soils and 2.5 mL of 80 mM urea solution at 37 °C (Kandeler & Gerber, 1988).

The Alteration Index 3 **(AI3)** values were calculated to determine the combined effect on the three enzymes of concern, as follows:

$$\text{AI3} = (7.87 \times \beta\text{-glucosidase}) - (8.22 \times \text{phosphatase}) - (0.49 \times \text{urease})$$

where enzyme activities were expressed in micromoles of, respectively, p-nitrophenyl-β-D-glucoside and p-nitrophenylphosphate per gram of soil per hour, and micrograms of urea per gram of soil per hour.

### 3.5 Statistical analyses

The data was subjected to analyses of variance (ANOVA) for each season separately, as well as with season as sub-plot factor, using SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC, USA, 2000). Tukey's HSD was calculated at the 5% level to compare treatment means. Relationships between variables were calculated by means of linear regression using Microsoft Office Excel 365 version.

# Chapter 4: Results

## 4.1 Chemical composition and amount of elements applied of the MW and WW

Only the first irrigation of simulated season 1 using municipal water (MW) and winery wastewater (WW) was the same for the four study soils. All subsequent irrigations in season 1, season 2 and season 3 varied for the four study soils depending on when each soil reached the 50-60% water deficit point. Therefore, the comparison of the chemical composition of MW and WW used in this study is presented for each study soil separately.

### 4.1.1 Stellenbosch clay loam soil

A comparison of the various chemical parameters of MW and WW applied to the Stellenbosch clay loam over three simulated irrigation seasons is given in Tables 4.1 to 4.4.

#### 4.1.1.1 Plant primary nutrients (N, P, K) – Table 4.1

The levels of $NO_3^-$, $NH_4^+$, total-N, P, and K were significantly higher in the WW than in the MW for all three simulated irrigation seasons, except for nitrate in seasons 1 and 3, $NH_4^+$ and total-N in season 3. There was no significance difference in levels of $NO_3^-$, $NH_4^+$, total-N, P, and K in the MW for all three simulated irrigation seasons, except for K, which was significantly lower in season 1. However, in the WW, $NO_3^-$ levels were significantly higher for season 2 and total N was significantly lower for season 3.

**Table 4.1:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (N, P, K) applied over three simulated irrigation seasons to a Stellenbosch clay loam.

| **Chemical parameter** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **$NO_3^-$ (mg/L)** | 0.2a,x* | 0.1a,y | 0.1a,x | 8.6b,x | 0.1a,x | 3.5a,xy | |
| **$NH_4^+$ (mg/L)** | 2.3a,x | 20.3b,x | 1.4a,x | 20.4b,x | 0.9a,x | 5.1a,x | |
| **Total N (mg/L)** | 2.5a,x | 20.5b,xy | 1.5a,x | 28.9b,x | 1a,x | 8.6a,y | 0.5 |
| **P (mg/L)** | 0.1a,x | 4.3b,x | 0.1a,x | 4.4b,x | 0.1a,x | 4.4b,x | 0-2 |
| **K (mg/L)** | 1.6a,y | 931.8b,x | 3.8a,x | 928.7b,x | 5.2a,x | 945.3b,x | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.1.2 Bases and anions – Table 4.2

**Bases (Ca, Mg, Na):** The Ca, Mg and Na levels were significantly higher in the WW than in MW for all three irrigation seasons. The Ca and Mg levels in MW showed no significant differences during the three simulated irrigation seasons. The Na levels in the MW were higher during season 3 as compared to season 2. The Ca, Mg and Na levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3. There are no guidelines for levels of Ca and Mg in irrigation water (DWAF, 1996).

**Anions ($HCO_3^-$, $Cl^-$, $F^-$):** The $HCO_3^-$, $Cl^-$ and $F^-$in the WW used for irrigation were significantly higher than in MW for all the three simulated irrigation seasons. There was no significant differences in $Cl^-$ and $F^-$ levels for MW during the three simulated irrigation seasons. The $HCO_3^-$ levels in the MW significantly higher during seasons 2 as compared to seasons 1. The $HCO_3^-$ and $Cl^-$ levels in the WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3. The $F^-$ levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3.

**Table 4.2:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (Ca, Mg, Na, $HCO_3^-$ $Cl^-$, $F^-$) applied over three irrigation seasons to a Stellenbosch clay loam.

| **Chemical parameter** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **Ca (mg/L)** | 2.8a,x* | 186.3b,x | 4.6a,x | 46.3b,y | 10a,x | 33b,y | |
| **Mg (mg/L)** | 0.8a,x | 25.8b,x | 0.9a,x | 21.7b,y | 1.18a,x | 20b,y | |
| **Na (mg/L)** | 8.5a,xy | 174b,x | 6.5a,y | 134.5b,y | 8.1a,x | 140.7b,y | 115 |
| **$HCO_3^-$ (mg/L)** | 7.7a,y | 1685.5b,x | 20.2a,x | 1382.3b,y | 15.5a,xy | 1270.8b,y | |
| **$Cl^-$ (mg/L)** | 9a,x | 190.9b,x | 8.6a,x | 114.8b,y | 9.3a,x | 119b,y | 150 |
| **$F^-$ (mg/L)** | 0.1a,x | 0.8b,x | 0.1a,x | 0.4b,y | 0.1a,x | 0.4b,y | 1 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.1.3 Measurable parameters (pH, EC, SAR, PAR, COD) – Table 4.3

The pH, EC, SAR and PAR in WW used for irrigation was significantly higher than in MW for all the three simulated irrigation seasons. The COD in WW used for irrigation was significantly higher than in MW for seasons 2 and 3. The pH levels in MW were significantly higher in the simulated irrigation season 3 when compared to seasons 1 and 2. There was no significant difference in EC, SAR, PAR and COD levels in MW during the three simulated irrigation seasons. The pH levels in WW were significantly higher in seasons 2 and 3 when compared to season 1. The range of pH means for MW was lower than 8.40 that is the maximum threshold for irrigation (DWAF, 1996). The EC levels in WW were significantly higher in the simulated irrigation seasons 2 and 3 when compared to season 1. The SAR and PAR levels in WW were significantly higher in the simulated irrigation season 3 when compared to season 2. Similarly, season 2 was significantly higher than season 1. The COD levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3.

**Table 4.3:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (pH, EC, SAR, PAR, COD) applied over three simulated irrigation seasons to a Stellenbosch clay loam.

| **Chemical Parameters** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **pH** | 7.4a,y* | 7.8b,y | 7.3a,y | 8.6b,x | 7.7a,x | 8.8b,x | 6-9 |
| **EC (dS/m)** | 0.1a,x | 4.9b,x | 0.11a,x | 3.6b,y | 0.1a,x | 3.8b,y | 2 |
| **SAR** | 1a,x | 3.3b,z | 0.7a,x | 4.1b,y | 0.8a,x | 4.8b,x | 13 |
| **PAR** | 0.13a,x | 10.3b,z | 0.2a,x | 16.6b,y | 0.3a,x | 19.1b,x | |
| **COD (mg/L)** | 0a,x | 3146a,x | 0a,x | 271.8b,y | 0a,x | 274.8b,y | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

#### 4.1.1.4 Trace elements (B, Mn, Cu, Zn, Fe) – Table 4.4

The B in WW used for irrigation was significantly higher than in MW for the three simulated irrigation seasons. While Mn was highly significant in season, Cu in season 2 and Fe was highly significant in seasons 1 and 2. There was no significant differences in B, Mn and Fe levels in MW during the three simulated irrigation seasons. There was no significant differences Cu and Zn levels for MW and WW during the three simulated irrigation seasons. The B, Mn and Fe levels in WW were significantly higher in the simulated irrigation seasons 1 when compared to seasons 2 and 3. The mean Zn values were less than the maximum concentration of 2 mg/L, which is recommended for grapevines under continuous irrigation (Van Zyl, 1981).

**Table 4.4:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (B, Mn, Cu, Zn, Fe) applied over three irrigation seasons to a Stellenbosch clay loam.

| **Chemical Parameters** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **B (mg/L)** | 0.4a,x* | 0.9b,x | 0.1a,x | 0.6b,y | 0.1a,x | 0.6b,y | |
| **Mn (mg/L)** | 0.01a,x | 0.1b,x | 0.01a,x | 0.01a,y | 0.02a,x | 0.01a,y | 0.2 |
| **Cu (mg/L)** | 0.03a,x | 0.02a,x | 0.1a,x | 0.02b,x | 0.04a,x | 0.03b,x | |
| **Zn (mg/L)** | 0.3a,x | 0.03a,x | 0.1a,x | 0.02a,x | 0.2a,x | 0.1a,x | |
| **Fe (mg/L)** | 0.1a,x | 3.9b,x | 0.1a,x | 0.2b,y | 0.1a,x | 0.14a,y | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.2 Stellenbosch sandy soil

A comparison of the various chemical parameters of the MW and WW applied to the Stellenbosch sand over three simulated irrigation seasons is given in Tables 4.6, 4.7, 4.8 and 4.9.

#### 4.1.2.1 Plant primary nutrients (N, P, K) – Table 4.5

The levels of $NH_4^+$, total-N, P, and K were significantly higher in the WW than in the MW for all the three simulated irrigation seasons, except for $NO_3^-$ in seasons 1 and 3, $NH_4^+$ and total-N in season 3. There was no significant difference in levels of $NO_3^-$, $NH_4^+$, total-N, P, and K in MW for all the three simulated irrigation seasons. However, in the WW, $NH_4^+$ levels were significantly higher for season 3 and total N was significantly lower for seasons 1 and 3.

**Table 4.5:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (N, P, K) applied over three simulated irrigation seasons to a Stellenbosch sand.

| **Chemical parameter** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **$NO_3^-$ (mg/L)** | 0.11a,x* | 0.2a,x | 0.2a,x | 6.4a,x | 0.1a,x | 5.2a,x | |
| **$NH_4^+$ (mg/L)** | 1.8a,x | 18.7b,xy | 1.9a,x | 26.2b,x | 0.9a,x | 7b,y | |
| **Total N (mg/L)** | 1.9a,x | 18.8b,y | 2.1a,x | 32.6b,x | 1.02a,x | 12.2b,y | 0.5 |
| **P (mg/L)** | 0.1a,x | 4.4b,x | 0.1a,x | 4.4b,x | 0.1a,x | 4.4b,x | 0-2 |
| **K (mg/L)** | 1.7a,x | 426.5b,y | 4.8a,x | 918.5b,x | 5.5a,x | 940.2b,x | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.2.2 Bases and anions – Table 4.6

**Bases (Ca, Mg, Na):** The Ca, Mg and Na levels were significantly higher in the WW than in MW for all the three irrigation seasons. There was no significant difference in Ca, Mg and Na levels in MW during the three simulated irrigation seasons. The Ca, Mg and Na levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3. There are currently no guidelines for levels of Ca and Mg in irrigation water (DWAF, 1996).

**Table 4.6:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (Ca, Mg, Na, $HCO_3^-$ $Cl^-$, $F^-$) applied over three simulated irrigation seasons to a Stellenbosch sand.

| **Chemical parameter** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **Ca (mg/L)** | 2.9a,x* | 203.2b,x | 5a,x | 47b,y | 13.5a,x | 39b,y | |
| **Mg (mg/L)** | 0.9a,x | 26.7b,x | 1a,x | 21.5b,y | 1.2a,x | 20b,y | |
| **Na (mg/L)** | 4.5a,x | 178.3b,x | 6.9a,x | 136.2b,y | 8a,x | 140b,y | 115 |
| **$HCO_3^-$ (mg/L)** | 7.7a,y | 1689.3b,x | 17.7a,x | 1434.8b,y | 16.5a,x | 1300.3b,y | |
| **$Cl^-$ (mg/L)** | 8.8a,x | 195.4b,x | 9.2a,x | 122.7b,y | 11.03a,x | 118.3b,y | 150 |
| **$F^-$ (mg/L)** | 0.1a,x | 0.8b,x | 0.1a,x | 0.4b,y | 0.1a,x | 0.4b,y | 1 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season while different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

**Anions ($HCO_3^-$, $Cl^-$, $F^-$)-** The $HCO_3^-$, $Cl^-$ and $F^-$ in WW used for irrigation was significantly higher than in MW for all the three simulated irrigation seasons. There $HCO_3^-$ levels in MW was significantly higher during seasons 2 and 3 as compared to seasons 1. There was no significant difference in $Cl^-$ and $F^-$ levels in MW during the three simulated irrigation seasons. The $HCO_3^-$ , $Cl^-$ and $F^-$ levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3. The $Cl^-$ levels in the WW exceeded the permissible maximum norm of 150 mg/L for continuous irrigation of grapevines (Howell & Myburgh, 2013 and references therein). The irrigation water sources had $F^-$ concentrations that were below the maximum concentration of 1 mg/L recommended by Ayers and Westcot (1985).

### 4.1.2.3 Measurable parameters (pH, EC, SAR, PAR, COD) – Table 4.7

The pH, EC, SAR, PAR, COD in the WW used for irrigation was significantly higher than in the MW for all the simulated irrigation seasons. The pH levels in MW were significantly higher in the simulated irrigation season 3 when compared to seasons 1 and 2. There was no significant differences in EC, SAR, PAR and COD levels in MW during the three simulated irrigation seasons. The pH, SAR and PAR levels in WW was significantly higher in seasons 2 and 3 when compared to season 1. The EC and COD levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3.

**Table 4.7:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (pH, EC, SAR, PAR, COD) applied over three simulated irrigation seasons to a Stellenbosch sand.

| **Chemical parameters** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **pH** | 7.3a,y* | 7.9b,y | 7.3a,y | 8.5b,x | 7.6a,x | 8.8b,x | 6-9 |
| **EC (dS/m)** | 0.1a,x | 4.9b,x | 0.1a,x | 4.4b,y | 0.1a,x | 3.78b,y | 2 |
| **SAR** | 1.01a,x | 3.2b,y | 0.8a,x | 4.2b,x | 0.7a,x | 4.7b,x | 13 |
| **PAR** | 0.13a,x | 9.8b,y | 0.3a,x | 16.6b,x | 0.28a,x | 18.4b,x | |
| **COD (mg/L)** | 0a,x | 3760.3b,x | 0a,x | 290b,y | 0a,x | 283.2b,y | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season while different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

#### 4.1.2.4 Trace elements (B, Mn, Cu, Zn, Fe) – Table 4.8

The B in WW used for irrigation was significantly higher than in MW for the three simulated irrigation seasons. While Mn was highly significant in season 1 and Fe was highly significant in seasons 1 and 2. There was no significant difference in B, Mn and Fe levels in MW during the three simulated irrigation seasons. There was no significant difference in Cu and Zn levels in MW and WW during the three simulated irrigation seasons. The B, Mn and Fe levels in WW were significantly higher in the simulated irrigation seasons 1 when compared to seasons 2 and 3.

**Table 4.8:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (B, Mn, Cu, Zn, Fe) applied over three simulated irrigation seasons to a Stellenbosch sand.

| **Chemical parameters** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **B (mg/L)** | 0.4a,x* | 0.9b,x | 0.1a,x | 0.6b,y | 0.1a,x | 0.6b,y | |
| **Mn (mg/L)** | 0.01a,x | 0.1b,x | 0.02a,x | 0.02a,y | 0.02a,x | 0.01a,y | 0.2 |
| **Cu (mg/L)** | 0.04a,x | 0.02a,x | 0.1a,x | 0.02b,x | 0.04a,x | 0.02b,x | |
| **Zn (mg/L)** | 0.4a,x | 0.02a,x | 0.04a,x | 0.02a,x | 0.2a,x | 0.1a,x | |
| **Fe (mg/L)** | 0.1a,x | 2.3a,x | 0.1a,x | 0.3a,y | 0.1a,x | 0.2a,y | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.3 Lutzville sandy soil

A comparison of the various chemical parameters of MW and WW applied to the Lutzville sand over the three irrigation seasons is given in Tables 4.9 to 4.12.

#### 4.1.3.1 Plant primary nutrients (N, P, K) – Table 4.9

The levels of $NH_4^+$, total-N, P, and K were significantly higher in the WW than in the MW for all three simulated irrigation seasons, except for nitrate in seasons 1 and 3, $NH_4^+$ and total-N in season 3. There was no significance difference in levels of $NO_3^-$, $NH_4^+$, total-N, P, and K in MW for all the three simulated irrigation seasons. However, in WW, $NH_4^+$ levels were significantly higher for season 3 and the total N was significantly lower for seasons 1 and 3.

**Table 4:9:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (N, P, K) applied over three simulated irrigation seasons to a Lutzville sand.

| **Chemical parameter** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **$NO_3$- (mg/L)** | 0.1a,x* | 0.2a,x | 0.2a,x | 6.4a,x | 0.1a,x | 5.2a,x | |
| **$NH_4^+$ (mg/L)** | 1.8a,x | 18.7b,xy | 1.9a,x | 26.2b,x | 0.9a,x | 7b,y | |
| **Total N (mg/L)** | 1.9a,x | 18.8b,y | 2.1a,x | 32.6b,x | 1.02a,x | 12.2b,y | 0.5 |
| **P (mg/L)** | 0.1a,x | 4.4b,x | 0.1a,x | 4.4b,x | 0.1a,x | 4.4b,x | 0-2 |
| **K (mg/L)** | 1.7a,x | 426.5b,y | 4.8a,x | 918.5b,x | 5.5a,x | 940.2b,x | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.3.2 Bases and anions – Table 4.10

**Bases (Ca, Mg, Na):** The Ca, Mg and Na levels were significantly higher in the WW than in MW for all the three irrigation seasons. The Ca, Mg and Na levels in MW showed no significant differences during the three simulated irrigation seasons. The Ca, Mg and Na levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3. There are currently no guidelines for levels of Ca and Mg in irrigation water (DWAF, 1996).

**Table 4.10:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (Ca, Mg, Na, $HCO_3^-$ $Cl^-$, $F^-$) applied over three simulated irrigation seasons to a Lutzville sand.

| **Chemical Parameter** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **Ca (mg/L)** | 2.9a,x* | 203.2b,x | 5a,x | 47b,y | 13.5a,x | 39b,y | |
| **Mg (mg/L)** | 0.9a,x | 26.7b,x | 1a,x | 21.5b,y | 1.2a,x | 20b,y | |
| **Na (mg/L)** | 4.5a,x | 178.3b,x | 6.9a,x | 136.2b,y | 8a,x | 140b,y | 115 |
| **$HCO_3^-$ (mg/L)** | 7.7a,y | 1689.3b,x | 17.7a,x | 1434.8b,y | 16.5a,x | 1300.3b,y | |
| **$Cl^-$ (mg/L)** | 8.8a,x | 195.4b,x | 9.2a,x | 122.7b,y | 11.03a,x | 118.3b,y | 150 |
| **$F^-$ (mg/L)** | 0.1a,x | 0.8b,x | 0.1a,x | 0.4b,y | 0.1a,x | 0.4b,y | 1 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

**Anions ($HCO_3^-$, $Cl^-$ $F^-$)**: The $HCO_3^-$, $Cl^-$ and $F^-$ in WW used for irrigation was significantly higher than in MW for all the three simulated irrigation seasons. The $HCO_3^-$ levels in MW were significantly higher during seasons 2 and 3 as compared to season 1. There was no significant difference in $Cl^-$ and $F^-$ levels in MW during the three simulated irrigation seasons. The $HCO_3^-$ , $Cl^-$ and $F^-$ levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3. The $Cl^-$ levels in the WW exceeded the permissible maximum norm of 150 mg/L for continuous irrigation of grapevines (Howell & Myburgh, 2013 and references therein). The irrigation water sources had $F^-$ concentrations that were below the maximum concentration of 1 mg/L recommended by Ayers and Westcot (1985).

### 4.1.3.3 Measurable parameters (pH, EC, SAR, PAR, COD) – Table 4.11

The pH, EC, SAR, PAR and COD in WW used for irrigation was significantly higher than in MW for all the simulated irrigation seasons. The pH levels in MW were significantly higher in the simulated irrigation season 3 when compared to seasons 1 and 2. There was no significant difference in EC, SAR, PAR and COD levels in MW during the three simulated irrigation seasons. The pH, SAR and PAR levels in WW were significantly higher in seasons 2 and 3 when compared to season 1. The EC and COD levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3.

**Table 4.11:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (pH, EC, SAR, PAR, COD) applied over three irrigation seasons to a Lutzville sand.

| **Chemical Parameters** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **pH** | 7.3a,y* | 7.9b,y | 7.3a,y | 8.5b,x | 7.6a,x | 8.8b,x | 6-9 |
| **EC (dS/m)** | 0.1a,x | 4.9b,x | 0.1a,x | 4.4b,y | 0.1a,x | 3.8b,y | 2 |
| **SAR** | 1.01a,x | 3.2b,y | 0.8a,x | 4.2b,x | 0.7a,x | 4.7b,x | 13 |
| **PAR** | 0.13a,x | 9.8b,y | 0.3a,x | 16.6b,x | 0.3a,x | 18.4b,x | |
| **COD (mg/L)** | 0a,x | 3760.3b,x | 0a,x | 290b,y | 0a,x | 283.2b,y | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.3.4 Trace elements (B, Mn, Cu, Zn, Fe) – Table 4.12

The B in WW used for irrigation was significantly higher than in MW for the three simulated irrigation seasons, while Mn was highly significant in season and Fe was highly sig/nificant in seasons 1 and 2. There was no significant difference in B, Mn and Fe levels in MW during the three simulated irrigation seasons. There was no significant difference in Cu and Zn levels in MW and WW during the three simulated irrigation seasons. The B, Mn and Fe levels in WW were significantly higher in simulated irrigation seasons 1 when compared to season 2 and season 3.

**Table 4.12:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (B, Mn, Cu, Zn, Fe) applied over three simulated irrigation seasons to a Lutzville sand.

| **Chemical parameters** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **B (mg/L)** | 0.4a,x* | 0.9b,x | 0.1a,x | 0.6b,y | 0.1a,x | 0.6b,y | |
| **Mn (mg/L)** | 0.01a,x | 0.1b,x | 0.02a,x | 0.02a,y | 0.02a,x | 0.01a,y | |
| **Cu (mg/L)** | 0.04a,x | 0.02a,x | 0.1a,x | 0.02b,x | 0.04a,x | 0.02b,x | |
| **Zn (mg/L)** | 0.4a,x | 0.02a,x | 0.04a,x | 0.02a,x | 0.2a,x | 0.1a,x | |
| **Fe (mg/L)** | 0.1a,x | 2.3a,x | 0.1a,x | 0.3a,y | 0.1a,x | 0.2a,y | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.4 Robertson clay loam soil

A comparison of the various chemical parameters of municipal water and winery wastewater applied to the Robertson clay-loam soil over three irrigation seasons is given in Tables 4.13 to 4.16.

#### 4.1.4.1 Plant primary nutrients (N, P, K) – Table 4.13

The levels of $NH_4^+$, total-N, P, and K were significantly higher in the WW than in the MW for all the three simulated irrigation seasons, except for $NO_3^-$ in seasons 1 and 3, $NH_4^+$ and total-N in season 3, except for P, which was significanlty lower in season 1. There was no significance difference in levels of $NO_3^-$, $NH_4^+$, total-N, P, and K in MW for all the three simulated irrigation seasons. However, in the WW, $NH_4^+$ levels were significantly higher for season 3 and total N was significaly lower for seasons 1 and 3.

**Table 4.13:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (N, P, K) applied over three simulated irrigation seasons to a Robertson clay loam.

| Chemical parameter | Season 1 | | Season 2 | | Season 3 | | Threshold |
|---|---|---|---|---|---|---|---|
| | MW | WW | MW | WW | MW | WW | |
| $NO_3^-$ (mg/L) | 0.1a,x* | 0.1a,x | 0.14a,x | 6.4a,x | 0.1a,x | 5.2a,x | |
| $NH_4^+$ (mg/L) | 1.3a,x | 16b,xy | 1.8a,x | 26.3b,x | 0.9a,x | 7b,y | |
| Total N (mg/L) | 1.5a,x | 16.1b,y | 2.1a,x | 32.7b,x | 1.02a,x | 12.2b,y | 0.5 |
| P (mg/L) | 0.1a,y | 4.4b,x | 0.1a,xy | 4.4b,x | 0.1a,x | 4.4b,x | 0-2 |
| K (mg/L) | 1.7a,x | 426.5b,x | 4.8a,x | 918.5b,x | 5.5a,x | 940.2b,x | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.4.2 Bases and anions – Table 4.14

**Bases (Ca, Mg, Na):** The Ca, Mg and Na levels were significantly higher in WW than in MW for all the three irrigation seasons. The Ca and Mg levels in MW showed no significant differences during the three simulated irrigation seasons. The sodium levels in MW were significantly higher during seasons 1 and 3 as compared to season 2. The Ca and Mg levels in WW were significantly higher in the simulated irrigation seasons 1 and 2 when compared to season 3. The Na levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3. There are no guidelines for levels of Ca and Mg in irrigation water (DWAF. 1996).

**Anions ($HCO_3^-$, $Cl^-$, $F^-$)-** The $HCO_3^-$, $Cl^-$ and $F^-$ in WW used for irrigation was significantly higher than in MW for all the three simulated irrigation seasons. The $HCO_3^-$ levels in the MW showed higher significant differences during seasons 2 and 3 as compared to seasons 1. There was no significant differences in $Cl^-$ and $F^-$ levels in MW during the three simulated irrigation seasons. The $HCO_3^-$ ,$Cl^-$ and $F^-$ levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3. The $Cl^-$ levels in the WW exceeded the permissible maximum norm of 150 mg/L for continuous irrigation of grapevines (Howell & Myburgh, 2013 and references therein). The irrigation water sources had $F^-$ concentrations that were below the maximum concentration of 1 mg/L recommended by Ayers and Westcot (1985).

**Table 4.14:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (Ca, Mg, Na, $HCO_3^-$ $Cl^-$, $F^-$) applied over three simulated irrigation seasons to a Robertson clay loam.

| **Chemical Parameter** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **Ca (mg/L)** | 2.7a,x* | 213.8b,x | 4.7a,x | 53.3b,y | 13.5a,x | 39b,y | |
| **Mg (mg/L)** | 0.9a,x | 28b,x | 0.9a,x | 21.8b,y | 1.2a,x | 20b,y | |
| **Na (mg/L)** | 7.8a,x | 186.7b,x | 6.5a,y | 135.5b,y | 8a,x | 140b,y | 115 |
| **$HCO_3^-$ (mg/L)** | 6.2a,y | 1689b,x | 12.8a,xy | 1465b,y | 16.5a,x | 1300b,y | |
| **$Cl^-$ (mg/L)** | 8.6a,x | 199.8b,x | 9.1a,x | 115.8b,y | 11a,x | 118b,y | 150 |
| **$F^-$ (mg/L)** | 0.1a,x | 0.8b,x | 0.1a,x | 0.5b,y | 0.1a,x | 0.4b,y | 1 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.4.3 Measurable parameters (pH, EC, SAR, PAR, COD) – Table 4.15

The pH, EC, SAR, PAR and COD in the WW used for irrigation was significantly higher than in MW. The pH levels in MW were significantly higher in the simulated irrigation season 3 when compared to season 2. The SAR levels in MW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3. There was no significant difference in EC, PAR and COD levels in MW during the three simulated irrigation seasons. The pH levels in WW were significantly higher in season 3 when compared to seasons 2 and 1 respectively. The EC and COD levels in WW were significantly higher in the simulated irrigation season 1 when compared to seasons 2 and 3. The SAR levels in WW were significantly higher in the simulated irrigation season 3 when compared to season 1. The PAR levels in WW were significantly higher in the simulated irrigation seasons 2 and 3 when compared to season 1.

**Table 4.15:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (pH, EC, SAR, PAR, COD) applied over three simulated irrigation seasons to a Robertson clay loam.

| **Chemical parameters** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **pH** | 7.4a,xy* | 7.8b,z | 7.3a,y | 8.4b,y | 7.6a,x | 8.8b,x | 6-9 |
| **EC (dS/m)** | 0.1a,x | 4.9b,x | 0.1a,x | 3.8b,y | 0.1a,x | 3.8b,y | 2 |
| **SAR** | 1.1a,x | 3.3b,y | 0.8a,y | 4b,xy | 0.7a,y | 4.7b,x | 13 |
| **PAR** | 0.15a,x | 9.7b,y | 0.2a,x | 16.2b,x | 0.3a,x | 18.4b,x | |
| **COD (mg/L)** | 0a,x | 4002.8b,x | 0a,x | 281b,y | 0a,x | 283.2b,y | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.4.4 Trace elements (B, Mn, Cu, Zn, Fe) – Table 4.16

The B, Zn and Fe in WW used for irrigation was significantly higher than in MW in all the three simulated irrigation seasons, while Mn was highly significant in season 1 and Cu highly significant in seasons 2 and 3. There was no significant difference in Mn levels in MW during the three simulated irrigation seasons. The Cu levels in MW were significantly higher in the simulated irrigation seasons 2 when compared to season 1. There was no significant difference in Zn and Fe levels in MW and WW during the three simulated irrigation seasons. The B and Mn levels in WW were significantly higher in the simulated irrigation seasons 1 when compared to seasons 2 and 3. The Cu levels in WW showed no significant differences during the three simulated irrigation seasons.

**Table 4.16:** Comparison of municipal water (MW) and winery wastewater (WW) chemical characteristics (B, Mn, Cu, Zn, Fe) applied over three simulated irrigation seasons to a Robertson clay loam.

| **Chemical parameters** | **Season 1** | | **Season 2** | | **Season 3** | | **Threshold** |
|---|---|---|---|---|---|---|---|
| | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | |
| **B (mg/L)** | 0.4a,x* | 1b,x | 0.1a,y | 0.6b,y | 0.07a,y | 0.6b,y | |
| **Mn (mg/L)** | 0.01a,x | 0.1b,x | 0.02a,x | 0.02a,y | 0.02a,x | 0.01a,y | 0.2 |
| **Cu (mg/L)** | 0.03a,y | 0.02a,x | 0.1a,x | 0.02b,x | 0.04a,xy | 0.02b,x | |
| **Zn (mg/L)** | 0.3a,x | 0.02a,x | 0.1a,x | 0.02a,x | 0.2a,x | 0.05a,x | |
| **Fe (mg/L)** | 0.1a,x | 2.99a,x | 0.1a,x | 0.4a,x | 0.1a,x | 0.2a,x | |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.1.5 Overall comparison of chemical characteristics of the MW and WW used throughout this study

#### 4.1.5.1 Comparison of the total amount of elements/compounds in the applied irrigation water (MW and WW) during the three simulation irrigation seasons to the four study soils

As seen in Section 4.1.1 to 4.1.4 above, the comparison of cation and anion concentrations in the applied water was significantly higher in the WW rather than in the MW during the three irrigation seasons, while trace element levels in the two irrigation sources were very small and relatively the same. Additionally, COD level of the WW was substantially higher in the WW compared to the MW. Given the higher levels of cations and anions in the WW, the total amount of cations and anions added to the four study soils *via* the two water sources (MW and WW) during the three simulated irrigation seasons was calculated. This information is shown in Table 4.17 for cations and Table 4.18 for anions and COD.

Results for total cations added show that irrigation with WW added much higher amounts of all the cations to the four soils in comparison to the amounts added with MW irrigation. For some elements (N, P, Ca, Mg, Na), the increased amount added in the WW was one order of magnitude greater. For other elements (K), the increased amount was two orders of magnitude greater. Potassium was added in the largest amount in the WW followed by Na, then Ca, then Mg and N, and finally, P was added in the least amount.

**Table 4.17:** The total amount of cations (kg/ha) in irrigation water (MW and WW) applied per simulated irrigation season to four different soils.

| **Element** | **Season** | **Amount applied (kg/ha)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | | **Stellenbosch sand** | | **Lutzville sand** | | **Robertson clay loam** | |
| | | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** |
| N | 1 | 4.3 | 36.7 | 3.3 | 32.4 | 3.3 | 32.4 | 2.7 | 29.9 |
| | 2 | 3.2 | 59.9 | 4.4 | 68.0 | 4.4 | 44.1 | 4.2 | 68.4 |
| | 3 | 2.2 | 18.1 | 2.3 | 26.6 | 2.3 | 26.6 | 2.3 | 26.6 |
| | **Total** | **9.7** | **114.7** | **10.0** | **127.0** | **10.0** | **103.1** | **9.2** | **124.9** |
| P | 1 | 0.2 | 7.8 | 0.2 | 8.2 | 0.2 | 8.3 | 0.2 | 8.3 |
| | 2 | 0.2 | 8.9 | 0.2 | 9.1 | 0.2 | 9.5 | 0.2 | 9.5 |
| | 3 | 0.2 | 9.9 | 0.2 | 9.8 | 0.2 | 9.8 | 0.2 | 9.8 |
| | **Total** | **0.6** | **26.6** | **0.6** | **27.1** | **0.6** | **27.6** | **0.6** | **27.6** |
| K | 1 | 2.9 | 1671.0 | 3.0 | 790.0 | 3.0 | 790.0 | 3.4 | 1683.5 |
| | 2 | 8.3 | 1943.6 | 10.5 | 1918.9 | 10.5 | 1918.9 | 6.7 | 2025.0 |
| | 3 | 11.5 | 2135.6 | 12.4 | 2123.9 | 12.4 | 2123.9 | 12.4 | 2123.9 |
| | **Total** | **22.7** | **5750.2** | **25.9** | **4832.8** | **25.9** | **4832.8** | **22.5** | **5832.4** |
| Ca | 1 | 5.1 | 336.8 | 5.0 | 372.5 | 5.0 | 372.5 | 4.9 | 383.1 |
| | 2 | 10.4 | 101.2 | 10.8 | 97.3 | 10.8 | 97.3 | 10.2 | 111.4 |
| | 3 | 22.1 | 74.0 | 29.8 | 85.4 | 29.8 | 85.4 | 29.8 | 85.4 |
| | **Total** | **37.6** | **512.0** | **45.6** | **555.2** | **45.6** | **555.2** | **44.9** | **579.9** |
| Mg | 1 | 1.5 | 46.5 | 1.5 | 48.1 | 1.5 | 48.1 | 1.6 | 50.2 |
| | 2 | 2.0 | 44.9 | 2.1 | 44.5 | 2.1 | 44.5 | 2.0 | 45.3 |
| | 3 | 2.7 | 45.2 | 2.7 | 45.2 | 2.7 | 45.2 | 2.7 | 45.2 |
| | **Total** | **6.2** | **136.6** | **6.3** | **137.8** | **6.3** | **137.8** | **6.3** | **140.7** |
| Na | 1 | 13.2 | 313.3 | 13.3 | 321.3 | 13.3 | 321.3 | 14.0 | 334.1 |
| | 2 | 14.1 | 284.2 | 14.5 | 285.5 | 14.5 | 285.5 | 13.8 | 284.1 |
| | 3 | 18.3 | 317.9 | 18.0 | 316.3 | 12.4 | 316.3 | 18.0 | 316.3 |
| | **Total** | **45.6** | **915.4** | **45.8** | **923.1** | **40.2** | **923.1** | **45.8** | **934.5** |

**Table 4.18:** The total amount of anions in irrigation water (MW and WW) applied per simulated irrigation season to four different soils.

| **Compound or Element** | **Season** | **Amount applied (kg/ha)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | | **Stellenbosch sand** | | **Lutzville sand** | | **Robertson clay loam** | |
| | | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** |
| $HCO3^-$ | 1 | 1.40 | 302.81 | 1.35 | 302.84 | 1.35 | 302.84 | 1.10 | 302.61 |
| | 2 | 4.30 | 287.97 | 3.70 | 301.40 | 3.70 | 301.40 | 2.63 | 308.30 |
| | 3 | 3.50 | 285.70 | 3.77 | 293.23 | 3.77 | 293.23 | 3.77 | 293.23 |
| | **Total** | **9.20** | **876.48** | **8.82** | **897.47** | **8.82** | **897.47** | **7.50** | **904.14** |
| $Cl^-$ | 1 | 1.61 | 34.30 | 1.56 | 35.10 | 1.56 | 35.10 | 1.54 | 35.87 |
| | 2 | 1.77 | 24.06 | 1.94 | 25.69 | 1.94 | 25.69 | 1.93 | 24.29 |
| | 3 | 2.06 | 26.92 | 2.49 | 26.76 | 2.49 | 26.76 | 2.49 | 26.76 |
| | **Total** | **5.44** | **85.28** | **5.99** | **87.55** | **5.99** | **87.55** | **5.96** | **86.92** |
| $F^-$ | 1 | 0.02 | 0.15 | 0.02 | 0.14 | 0.01 | 0.14 | 0.02 | 0.15 |
| | 2 | 0.02 | 0.09 | 0.02 | 0.09 | 0.02 | 0.09 | 0.02 | 0.10 |
| | 3 | 0.02 | 0.09 | 0.02 | 0.10 | 0.02 | 0.10 | 0.02 | 0.10 |
| | **Total** | **0.06** | **0.33** | **0.06** | **0.33** | **0.05** | **0.33** | **0.06** | **0.35** |

Results for total anions added show that WW added much higher amounts of all the anions to the four soils in comparison to the amounts added with MW. For some elements/compounds ($HCO_3^-$, $Cl^-$, $F^-$), the increased amount added in the WW was one order of magnitude greater. $HCO_3^-$ was added in the largest amount in the WW, followed by $Cl^-$, and finally, $F^-$ was added in the least amount.

### 4.1.5.2 Overall comparison of the pH and EC of the MW and WW used in this study

The average values of pH and EC of the MW and WW for the three simulated irrigations in the four study soils used are given in Table 4.19.

Results for pH consistently showed a pH one unit higher in WW used for irrigation for all the three simulated seasons on the four study soils. In addition, the EC was an order of magnitude higher for WW used for irrigation for all three simulated seasons for the four study soils.

**Table 4.19:** The pH and EC of the MW and WW used for irrigation during three simulated seasons in the four study soils.

| | **Season** | **pH or EC (dS/m) values** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | | **Stellenbosch sand** | | **Lutzville sand** | | **Robertson clay loam** | |
| | | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** | **MW** | **WW** |
| pH | 1 | 7.40 | 7.86 | 7.34 | 7.87 | 7.34 | 7.87 | 7.41 | 7.84 |
| | 2 | 7.29 | 8.55 | 7.30 | 8.45 | 7.30 | 8.45 | 7.32 | 8.40 |
| | 3 | 7.65 | 8.83 | 7.61 | 8.79 | 7.61 | 8.79 | 7.62 | 8.79 |
| | **Ave.** | **7.45** | **8.41** | **7.42** | **8.37** | **7.42** | **8.37** | **7.62** | **8.79** |
| EC | 1 | 0.75 | 49.15 | 0.73 | 49.18 | 0.73 | 49.18 | 0.77 | 49.22 |
| | 2 | 1.13 | 36.30 | 0.90 | 38.18 | 0.90 | 38.18 | 0.87 | 38.28 |
| | 3 | 1.02 | 37.93 | 1.03 | 37.78 | 1.03 | 37.78 | 1.03 | 37.78 |
| | **Ave.** | **0.97** | **41.13** | **0.89** | **41.71** | **0.89** | **41.71** | **0.89** | **41.76** |

## 4.2 The effect of three simulated seasons of irrigation using municipal water and winery wastewater on soil chemical characteristics

### 4.2.1 Nitrogen

The effect of using MW and WW during the three simulated seasons on soil N in the four study soils is shown in Table 4.20. The comparison of the two irrigation sources (MW and WW) showed no significant differences in soil N for all the three simulated irrigation seasons in the Stellenbosch clay loam, Stellenbosch sand, and Lutzville sand. However, the soil N in the Robertson clay loam was significantly higher during the irrigation season 2 and lower during season 3 when WW was used.

The comparison of soil N during the three simulated irrigation seasons showed no significant differences for both irrigation waters (MW and WW) in the Stellenbosch clay loam and the Stellenbosch sand. However, both the MW and WW use resulted in higher soil N levels during season 3 for the Lutzville sand. Similarly, the use of both MW and WW in the Robertson clay loam resulted in the highest soil N for the season 3. The lowest soil N in this soil was found with the use of MW in season 2.

The linear regression analysis of applied N in the two sources of irrigation water (MW and WW) versus soil N, showed no correlation (Table 4.20).

**Table 4.20:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil nitrogen (mg/kg) levels for the four study soils, including the correlation coefficient ($R^2$) of the linear relationship between applied N and soil N.

| **Simulated season** | **Irrigation water** | **Soil N (mg/kg)** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **1** | **MW** | 1032.6 a,x* | 715.5 a,x | 543.4 a,xy | 1807.2 a,y |
| | **WW** | 958.4 a,x | 692.8 a,x | 585.8 a,y | 1520.1 a,y |
| **2** | **MW** | 854.8 a,x | 633.3 a,x | 498.6 a,y | 1406.1 b,z |
| | **WW** | 990.9 a,x | 733.6 a,x | 544.4 a,y | 1558.3 a,xy |
| **3** | **MW** | 933.3 a,x | 655.4 a,x | 815.8 a,x | 2280.1 a,x |
| | **WW** | 977.6 a,x | 610.9 a,x | 997.4 a,x | 1920.6 b,x |
| **Correlation coeff. ($R^2$)** | | +0.10 | -0.20 | +0.30 | 0.00 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.2.2 Phosphorus

The effect of using MW and WW during the three simulated seasons on soil P in the four study soils is shown in Table 4.21. The comparison of the two irrigation sources (MW and WW) showed higher significant differences in soil P for all the three simulated irrigation seasons in the Stellenbosch sand and Lutzville sand when WW was used. However, the soil P in the Stellenbosch clay loam and Robertson clay loam showed no significant different during the irrigation season 1 and was significantly higher during seasons 2 and 3 when WW was used.

The comparison of soil P during the three simulated irrigation seasons showed significant differences for both irrigation waters (MM and WW) in the Stellenbosch clay loam and the Stellenbosch sand. However, MW use resulted in higher soil P levels during season 1 for the Stellenbosch clay loam and Stellenbosch sand and Lutzville sand. However, for the Robertson clay loam, the use of MW showed no significant different for the simulated irrigation. The use of WW showed no significant difference in the three simulated irrigation seasons for the Stellenbosch clay loam, Stellenbosch sand and Lutzville sand. However, the use of WW was significantly higher in season 3 for the Robertson clay loam.

The linear regression analysis of applied P in the two sources of irrigation water (MW and WW) versus soil P showed moderate positive correlation in the Stellenbosch sand, strong positive correlation in the Lutzville sand and very strong positive correlation in the Stellenbosch clay loam and Robertson clay loam (Table 4.21).

**Table 4.21:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil phosphorus (mg/kg) levels for the four study soils, including the correlation coefficient ($R^2$) of the linear relationship between applied P and soil P.

| Simulated season | Irrigation water | Soil P (mg/kg) | | | |
|---|---|---|---|---|---|
| | | Stellenbosch clay loam | Stellenbosch sand | Lutzville sand | Robertson clay loam |
| Baseline | | 65.1 | 55.5 | 32.0 | 99.9 |
| 1 | MW | 64.0a,x* | 63.1b,x | 38.8b,x | 104.3a,x |
| | WW | 74.8a,x | 85.9a,x | 56.1a,x | 112.2a.y |
| 2 | MW | 53.9b,y | 51.1b,y | 27.7b,y | 97.7b,x |
| | WW | 84.8a,x | 80.2a,x | 49.4a,x | 116.5a,y |
| 3 | MW | 52.6b,y | 53.0a,y | 33.4b,xy | 103.6b,x |
| | WW | 84.9a,x | 81.1a,x | 54.2a,x | 133.4a,x |
| Correlation coeff. ($R^2$) | | +0.80 | +0.56 | +0.68 | +0.94 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.2.3 Potassium

The effect of using MW and WW during the three simulated seasons on soil K in the four study soils is shown in Table 4.22. The comparison of the two irrigation sources (MW and WW) showed higher significant differences in soil K for all the three simulated irrigation seasons in the four soils when WW was used.

The soil K during the three simulated irrigation seasons showed no significant differences for MW in the Stellenbosch clay loam, Stellenbosch sand and Robertson clay loam. However, both the MW and WW use resulted in higher soil K levels during season 3 for the Lutzville sand. Similarly, the use of both MW and WW in the Robertson clay loam resulted in the highest soil K for season 3. The lowest soil K in this soil was found with the use of MW in season 1.

The linear regression analysis of applied K in the two sources of irrigation water (MW and WW) versus soil K showed very strong positive correlation in the four study soils (Table 4.22). The rate of increase in soil K with an increase in applied K (soil K/K applied per ha) differed between the soils and could be related to the clay content thereof (Figure. 4.1).

**Table 4.22:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil potassium (cmol/kg) levels for the four study soils, including the correlation coefficient ($R^2$) of the linear relationship between applied K and soil K.

| **Simulated season** | **Irrigation water** | **Soil K (cmol/kg)** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 0.6 | 0.2 | 0.6 | 1.1 |
| **1** | **MW** | 0.8b,x* | 0.3b,x | 0.4b,y | 1.2b,x |
| | **WW** | 2.7a,y | 3.3a,y | 1.7a,y | 2.9a,y |
| **2** | **MW** | 0.7b,x | 0.3b,x | 0.7b,x | 1.4b,x |
| | **WW** | 5.3a,x | 4.3a,xy | 5.1a,x | 4.7a,xy |
| **3** | **MW** | 0.6b,x | 0.2b,x | 0.8b,x | 1.6b,x |
| | **WW** | 5.6a,x | 6.1a,x | 6.3a,x | 5.3a,x |
| **Correlation coeff. ($R^2$)** | | +0.94 | +0.89 | +0.96 | +0.96 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

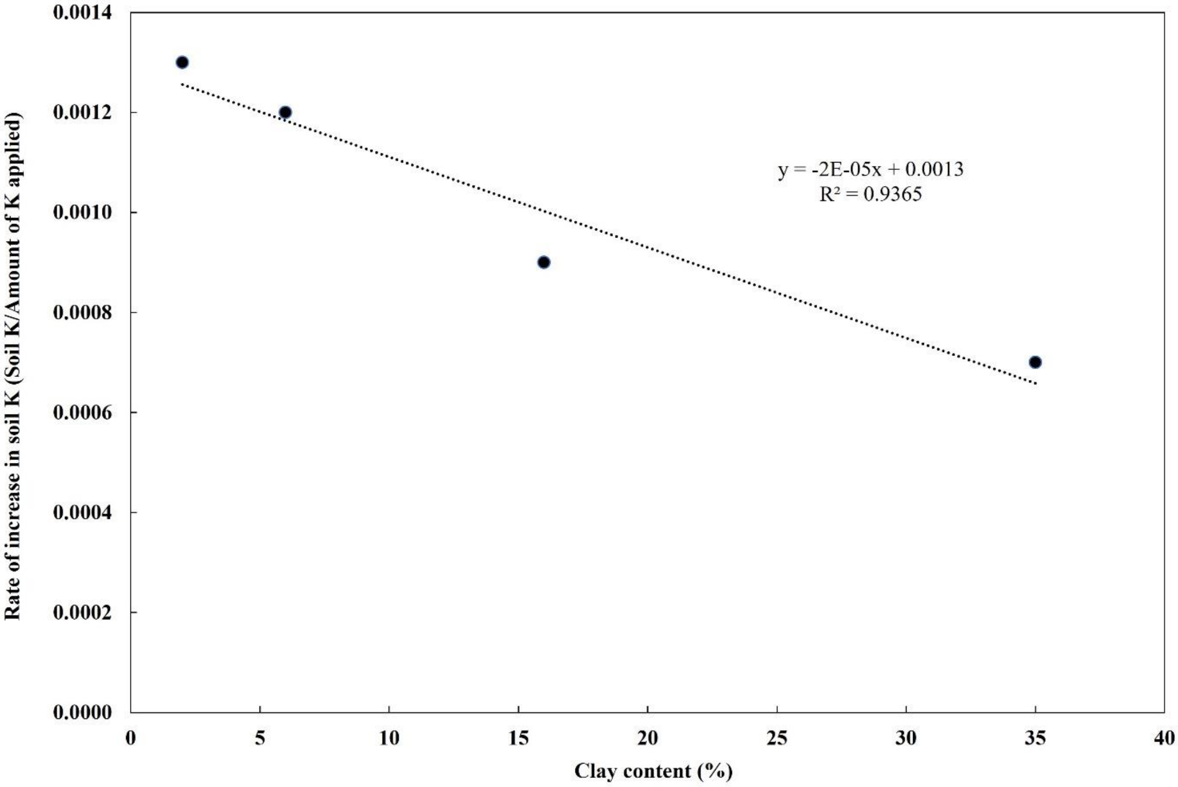

**Figure 4.1:** Relationship between the ratio of soil K to applied K per ha and clay content for the four different soils.

### 4.2.4 Calcium

The effect of using MW and WW during the three simulated seasons on soil Ca in the four study soils is shown in Table 4.23. The comparison of the two irrigation sources (MW and WW) showed no significant differences in soil Ca for all the three simulated irrigation seasons in the Robertson clay loam. However, the soil Ca in the Stellenbosch clay loam, Stellenbosch sand and Lutzville sand was significantly higher during irrigation season 2 when WW was used.

The comparison of soil Ca during the three simulated irrigation seasons showed no significant differences for both irrigation waters (MW and WW) in the Stellenbosch sand and the Lutzville sand. However, both the MW and WW use resulted in higher soil Ca levels during seasons 2 and 3 for the Stellenbosch clay loam. Similarly, the use of both MW and WW in the Robertson clay loam resulted in the highest soil Ca for the season 3. The lowest soil Ca in this soil was found with the use of MW in season 2.

The linear regression analysis of applied Ca in the two sources of irrigation water (MW and WW) versus soil Ca showed very strong positive correlation in the Stellenbosch clay loam and

Stellenbosch sand, weak positive correlation in the Lutzville sand and moderate positive correlation in the Robertson clay loam (Table 4.23).

**Table 4.23:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil calcium (cmol/kg) levels for the four study soils, including the correlation coefficient ($R^2$) of the linear relationship between applied Ca and soil Ca.

| **Simulated season** | **Irrigation water** | **Soil Ca (cmol/kg)** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 5.4 | 3.3 | 3.5 | 10.7 |
| **1** | **MW** | 4.9a,x* | 3.7a,x | 4.4a,x | 10.6a,x |
| | **WW** | 5.6a,y | 4.3a,x | 5.3a,x | 10.4a,y |
| **2** | **MW** | 5.0b,x | 3.3b,x | 3.1b,x | 10.3a,x |
| | **WW** | 6.7a,x | 4.8a,x | 4.3a,x | 11.3a,xy |
| **3** | **MW** | 5.5a,x | 3.4a,x | 3.9a,x | 11.2a,x |
| | **WW** | 6.4a,x | 4.7a,x | 4.3a,x | 12.6a,x |
| **Correlation coeff. ($R^2$)** | | +0.81 | +0.92 | +0.26 | +0.44 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.2.5 Magnesium

The effect of using MW and WW during the three simulated seasons on soil Mg in the four study soils is shown in Table 4.24. The comparison of the two irrigation sources (MW and WW) showed no significant differences in soil Mg for seasons 1 and 3 in the Stellenbosch clay loam, Lutzville sand and Robertson clay loam. However, the soil Mg in the Stellenbosch sand was significantly higher during the three simulated irrigation seasons when WW was compared to MW use.

The comparison of soil Mg during the three simulated irrigation seasons showed high significant differences for MW in the Stellenbosch clay loam. Similarly, both the MW and WW use resulted in higher soil Mg levels during seasons 2 and 3 for the Stellenbosch sand. Similarly, the use of both MW and WW in the Lutzville sand resulted in the highest soil Mg for the season 3. The lowest soil Mg in this soil was found with the use of MW and WW in season 1.

The linear regression analysis of applied Mg in the two sources of irrigation water (MW and WW) versus soil Mg showed weak positive correlation in the Stellenbosch clay loam, very strong positive correlation in the Stellenbosch sand, moderate negative correlation in the Lutzville sand and very weak positive correlation in the Robertson clay loam (Table 4.24).

**Table 4.24:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil magnesium (cmol/kg) levels for the four study soils, including the correlation coefficient ($R^2$) of the linear relationship between applied Mg and soil Mg.

| **Simulated season** | **Irrigation water** | **Soil Mg (cmol/kg)** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 0.9 | 0.4 | 1.5 | 2.6 |
| **1** | **MW** | 0.7a,y* | 0.3b,y | 1.1a,y | 2.3a,x |
| | **WW** | 0.7a,y | 0.4a,y | 1.0a,x | 2.3a,y |
| **2** | **MW** | 0.9b,x | 0.4b,x | 1.3a,xy | 2.9a,x |
| | **WW** | 1.1a,x | 0.7a,x | 1.0b,x | 2.6b,xy |
| **3** | **MW** | 0.8a,x | 0.4b,x | 1.6a,x | 2.9a,x |
| | **WW** | 0.9a,y | 0.7a,x | 1.0a,x | 2.8a,x |
| **Correlation coeff. ($R^2$)** | | +0.28 | +0.85 | -0.40 | 0.00 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.2.6 Sodium

The effect of using MW and WW during the three simulated seasons on soil Na in the four study soils is shown in Table 4.25. The comparison of the two irrigation sources (MW and WW) showed higher significant differences in soil Na for all the three simulated irrigation seasons in the four soils when WW was used.

The soil Na during season 3 showed higher significant differences for both the MW and WW in the four study soils. Similarly, MW use resulted in higher soil Na levels during season 1 for the Stellenbosch clay loam, Stellenbosch sand and Robertson clay loam. Similarly, the use of WW in the Robertson clay loam, resulted in the highest soil Na for season 3. The lowest soil Na in this soil was found with the use of WW in season 1.

The linear regression analysis of applied Na in the two sources of irrigation water (MW and WW) versus soil Na showed very strong correlation in all the study soils (Table 4.25). However, the rate of increase in soil Na with an increase in applied Na (soil Na/Na applied per ha) did not differ between the soils and could not related to the clay content thereof (Figure. 4.2).

**Table 4.25:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil sodium (cmol/kg) levels for the four study soils, including the correlation coefficient ($R^2$) of the linear relationship between applied Na and soil Na.

| **Simulated season** | **Irrigation water** | **Soil Na (cmol/kg)** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 0.4 | 0.2 | 0.5 | 0.4 |
| **1** | **MW** | 0.3b,x* | 0.2b,x | 0.2b,y | 0.5b,x |
| | **WW** | 0.7a,y | 0.8a,y | 0.4a,y | 1.0a,y |
| **2** | **MW** | 0.2b,y | 0.2b,x | 0.3b,y | 0.4b,x |
| | **WW** | 1.1a,x | 1a,xy | 1a,x | 1.3a,xy |
| **3** | **MW** | 0.3b,x | 0.2b,x | 0.4b,x | 0.5b,x |
| | **WW** | 1.2a,x | 1.5a,x | 1.5a,x | 1.6a,x |
| **Correlation coeff. ($R^2$)** | | +0.94 | +0.98 | +0.94 | +0.98 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

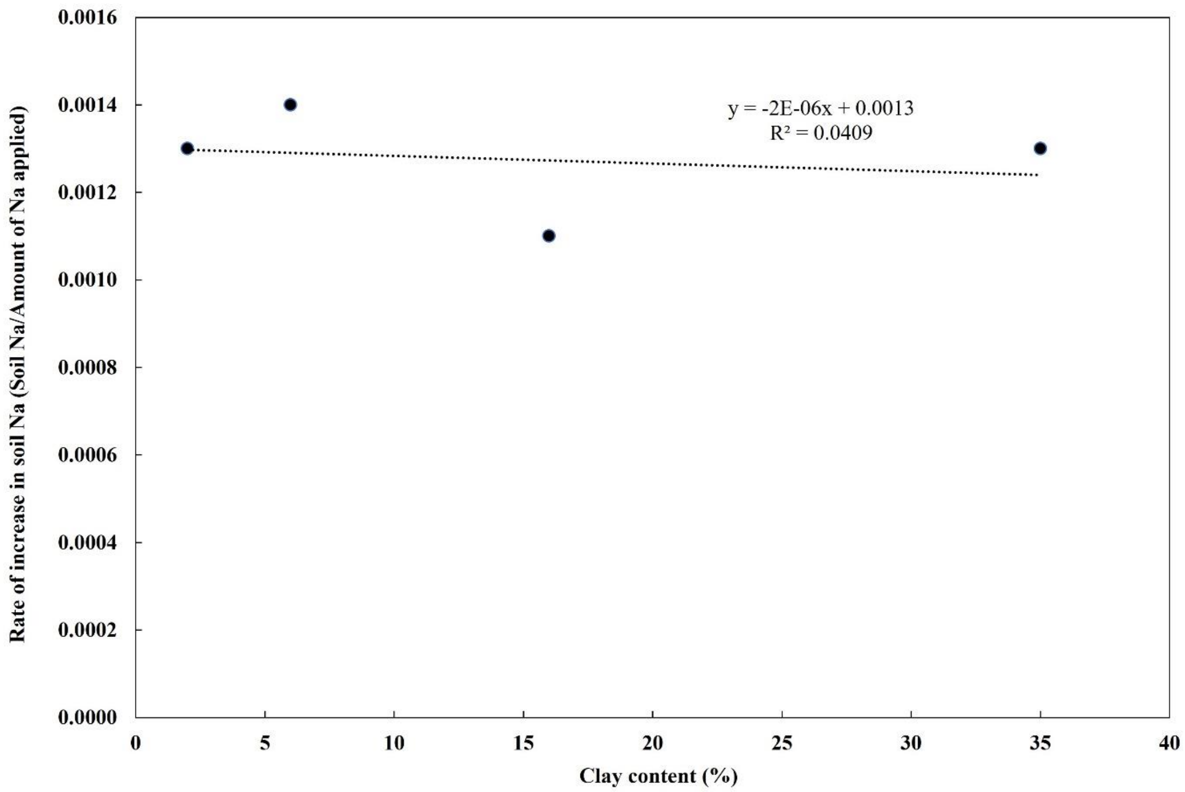

**Figure 4.2:** Relationship between the ratio of soil Na to applied Na per ha and clay content for the four different soils.

### 4.2.7 pH$_{(KCl)}$

The effect of using MW and WW during the three simulated seasons on soil pH$_{(KCl)}$ in the four study soils is shown in Table 4.26. The comparison of the two irrigation sources (MW and WW) showed higher significant differences in soil pH$_{(KCl)}$ for all the three simulated irrigation seasons in the four soils when WW was used.

The soil pH$_{(KCl)}$ showed no significant differences for MW in the Stellenbosch clay loam in seasons 2 and 3, Stellenbosch sand and Robertson clay loam in season 1 for both the MW and WW use. However, MW use resulted in higher soil pH$_{(KCl)}$ levels during season 1 for the Lutzville sand. The lowest soil pH in this soil was found with the use of MW in season 3. Similarly, the use of WW in the Lutzville sand resulted in the highest soil pH$_{(KCl)}$ for season 3. The lowest soil pH$_{(KCl)}$ in this soil was found with the use of WW in season 1.

The relationship between the soil pH$_{(KCl)}$, and soil extractable bases and applied alkalinity were analyzed using linear regression to determine any correlation between the variables. The correlation coefficients of these regression analyses given in Table 4.26. The linear regression analysis of applied alkalinity in the two sources of irrigation water (MW and WW) versus soil

$pH_{(KCl)}$ showed very strong correlation in all the Stellenbosch clay loam and Lutzville sand, a strong positive correlation for the Stellenbosch sand and a moderate positive correlation for the Robertson clay loam. Strong positive correlation between the soil $pH_{(KCl)}$ and soil K and Na were observed for all four study soils with correlation coefficients ranging from 0.67 to 0.98 for soil $pH_{(KCl)}$ against soil K, and 0.64 to 0.98 for soil $pH_{(KCl)}$ against soil Na. A strong relationship between soil pH against soil Ca was only observed for the Stellenbosch clay loam with a correlation coefficient of 0.89. Strong negative relationship between soil $pH_{(KCl)}$ and soil Mg was observed for the Lutzville sand with a correlation coefficient of -0.61 while a moderate positive relationship was observed for the Stellenbosch sand with correlation coefficient of 0.59.

**Table 4.26:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil $pH_{(KCl)}$ levels for the four study soils, including the correlation coefficient ($R^2$) of the linear relationship between soil pH against soil Na, K, Ca, Mg and applied alkalinity against soil $pH_{(KCl)}$.

| **Simulated season** | **Irrigation water** | **Soil $pH_{(KCl)}$** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 5.9 | 6.2 | 7.9 | 6.9 |
| **1** | **MW** | 5.9b,x* | 6.4b,x | 7.3b,x | 7.1b,x |
| | **WW** | 7.5a,y | 9.5a,x | 9.4a,z | 7.8a,x |
| **2** | **MW** | 5.9b,x | 6.3b,y | 7.1b,y | 7.3b,x |
| | **WW** | 8.9a,x | 9.7a,x | 9.7a,y | 8.2a,x |
| **3** | **MW** | 5.9b,x | 6.2b,y | 7.0b,z | 6.1a,y |
| | **WW** | 8.8a,x | 9.9a,x | 10.1a,x | 8.3a,x |
| **Correlation coeff. ($R^2$) – Na** | | +0.98 | +0.87 | +0.65 | +0.69 |
| **Correlation coeff. ($R^2$)- K** | | +0.99 | +0.91 | +0.76 | +0.67 |
| **Correlation coeff. ($R^2$) – Ca** | | +0.89 | +0.37 | +0.34 | +0.15 |
| **Correlation coeff. ($R^2$) – Mg** | | +0.35 | +0.59 | -0.61 | +0.06 |
| **Correlation coeff. ($R^2$) - Applied alkalinity** | | +0.91 | +0.78 | +0.89 | +0.53 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.2.8 Electrical conductivity of the saturated soil extract ($EC_e$)

The initial (baseline) level of $EC_e$ in the three study soils was lower than the levels measured after irrigation with either water source, with the exception of the Robertson clay loam. The increased $EC_e$ observed was up to four times the initial $EC_e$ value. However, the $EC_e$ values after irrigation for all three seasons was similar to the baseline for the Robertson clay loam mainly because the soil $EC_e$ values were initially high.

The effect of using MW and WW during the three simulated seasons on soil $EC_e$ in the four study soils is shown in Table 4.27. The comparison of the two irrigation sources (MW and WW) showed no significant differences in soil $EC_e$ for all the three simulated irrigation seasons in the Robertson clay loam. However, the soil $EC_e$ in the Stellenbosch clay loam, Stellenbosch sand and Lutzville sand was significantly higher during irrigation season 2 when WW was used.

The comparison of soil $EC_e$ during the three simulated irrigation seasons showed no significant differences for both irrigation waters (MW and WW) in the Stellenbosch sand and Robertson clay loam. The use of MW for irrigation of the pots resulted in no significant difference of soil $EC_e$ levels during the three simulated irrigation seasons for the Stellenbosch clay loam and Lutzville sand. However, the use of WW for the irrigation of the Stellenbosch clay loam and Lutzville sand resulted in the highest soil $EC_e$ for season 3.

The linear regression analysis of applied salinity (EC) in the two sources of irrigation water (MW and WW) versus soil EC, showed strong positive correlation in the Stellenbosch clay loam, Stellenbosch sand and Lutzville sand, and weak negative correlation in the Robertson clay loam (Table 4.27).

**Table 4.27**: The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil electrical conductivity (dS/m) levels for the four study soils, including the correlation coefficient ($R^2$) of the linear relationship between applied salinity (EC) and soil $EC_e$.

| Simulated season | Irrigation water | Soil $EC_e$ (dS/m) | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 0.7 | 0.3 | 0.5 | 2.4 |
| **1** | **MW** | 0.5b,x* | 0.3b,x | 0.5b,x | 2.5a,x |
| | **WW** | 0.9a,y | 1.1a,x | 1.3a,y | 1.9a,x |
| **2** | **MW** | 0.5b,x | 0.3b,x | 0.5b,x | 3.0a,x |
| | **WW** | 1.8a,x | 2.1a,x | 1.9a,xy | 2.1a,x |
| **3** | **MW** | 0.3b,y | 0.3b,x | 0.5b,x | 1.8a,x |
| | **WW** | 1.6a,x | 2.0a,x | 2.4a,x | 2.2a,x |
| **Correlation coeff. ($R^2$)** | | +0.72 | +0.68 | +0.68 | -0.24 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.2.9 Soil organic carbon

The effect of using MW and WW during the three simulated seasons on soil OC in the four study soils is shown in Table 4.28. The comparison of the two irrigation sources (MW and WW) showed no significant differences in soil OC for all the three simulated irrigation seasons in the Stellenbosch clay loam, Stellenbosch sand, and Robertson clay loam. However, the soil OC in the Lutzville sand was significantly higher during irrigation season 1 and lower during season 3 when WW was used.

The comparison of soil OC during the three simulated irrigation seasons showed no significant differences for both irrigation waters (MW and WW) in the Stellenbosch clay loam and Stellenbosch sand. However, both the MW and WW use resulted in higher soil OC levels during season 1 for the Lutzville sand. Similarly, the use of both MW and WW in the Robertson clay loam, resulted in the highest soil OC for the season 3. The lowest soil OC in this soil was found with the use of MW in season 2. The linear regression analysis of applied $HCO_3^-$ in the two sources of irrigation water (MW and WW) versus soil OC showed very weak correlation in all the four irrigation soils (Table 4.28).

**Table 4.28:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil organic carbon (%) levels for the four study soils, including the correlation coefficient ($R^2$) of the linear relationship between applied $HCO_3^-$ and soil OC.

| **Simulated season** | **Irrigation water** | **Soil OC (%)** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 0.7 | 0.4 | 0.3 | 0.7 |
| **1** | **MW** | 0.6a,x* | 0.4a,x | 0.2a,x | 0.7a,x |
| | **WW** | 0.6a,x | 0.4a,x | 0.2a,x | 0.8a,xy |
| **2** | **MW** | 0.6a,x | 0.3a,x | 0.2a,xy | 0.6a,x |
| | **WW** | 0.6a,x | 0.3a,x | 0.16a,x | 0.6a,y |
| **3** | **MW** | 0.6a,x | 0.4a,x | 0.1b,y | 0.8a,x |
| | **WW** | 0.6a,x | 0.5a,x | 0.2a,x | 0.9a,x |
| **Correlation Coeff. ($R^2$)** | | 0.00 | +0.05 | +0.07 | +0.08 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

### 4.2.10 ExPP

The effect of using MW and WW during the three simulated seasons on soil ExPP in the four study soils is shown in Table 4.29 (please refer to Section 3.4 for the definition of ExPP). A comparison of the two irrigation sources (MW and WW) showed higher significant differences in soil ExPP for all the three simulated irrigation seasons in the four soils when WW was used.

The soil ExPP during the three simulated irrigation seasons showed no significant differences for MW in the Stellenbosch clay loam, Stellenbosch sand and the Robertson clay loam. However, both the MW and WW use showed no significant difference in soil ExPP levels for the Stellenbosch sand. Similarly, the use of WW in the Stellenbosch clay loam and Lutzville sand, resulted in the highest soil ExPP for season 3.

The linear regression analysis of applied K in the two sources of irrigation water (MW and WW) versus soil ExPP, showed very strong positive correlation in the Stellenbosch clay loam, Lutzville sand and Robertson clay loam and strong positive correlation in the Stellenbosch sand (Table 4.29). The rate of increase in soil ExPP with an increase in applied K (soil ExPP/K applied per ha) differed between the soils and could be related to the clay content thereof (Figure 4.3).

**Table 4.29:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil extractable potassium percentage (%) levels for the four study soils, including the correlation coefficient ($R^2$) of the linear relationship between applied K and soil ExPP.

| **Simulated Season** | **Irrigation water** | **Soil ExPP (%)** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 8.2 | 4.9 | 10.4 | 7.6 |
| **1** | **MW** | 11.8b,x* | 6.5b,x | 7.0b,z | 8.1b,x |
| | **WW** | 28.3a,y | 37.4a,x | 20.5a,y | 17.5a,y |
| **2** | **MW** | 10.0b,xy | 6.2b,x | 13.8b,x | 9.0b,x |
| | **WW** | 37.2a,x | 40.5a,x | 44.5a,x | 23.6a,xy |
| **3** | **MW** | 8.3b,y | 5.5b,x | 11.4b,y | 9.8b,x |
| | **WW** | 39.7a,x | 44.3a,x | 48.3a,x | 25.4a,x |
| **Correlation coeff. ($R^2$)** | | +0.89 | +0.69 | +0.91 | +0.93 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

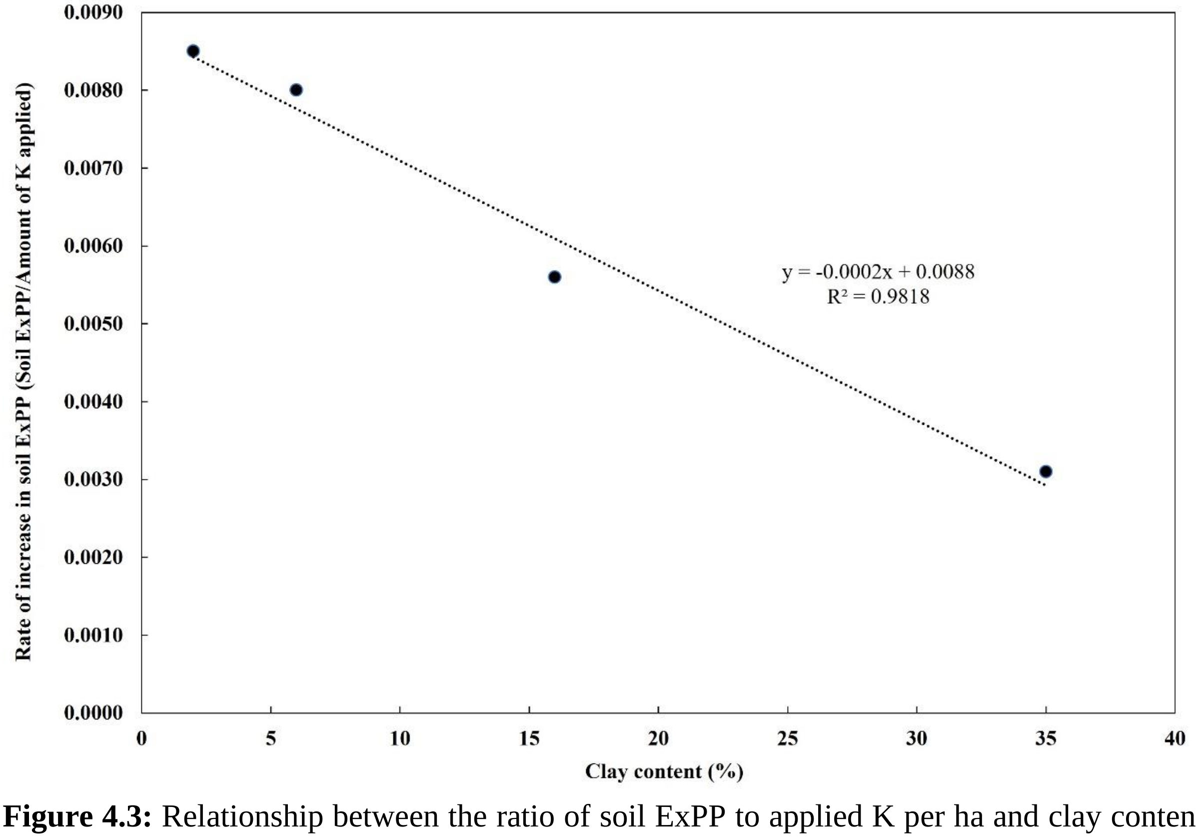

**Figure 4.3:** Relationship between the ratio of soil ExPP to applied K per ha and clay content for the four different soils.

### 4.2.11 ExSP

The effect of using MW and WW during the three simulated seasons on soil ExSP in the four study soils is shown in Table 4.30 (please refer to Section 3 for the definition of ExSP). The comparison of the two irrigation sources (MW and WW) showed higher significant differences in soil ExSP for all the three simulated irrigation seasons in the four soils when WW was used.

The comparison of soil N during the three simulated irrigation seasons showed no significant differences for both irrigation waters (MW and WW) in the Robertson clay loam. However, WW use resulted in no significant difference in soil ExSP levels during the three simulated irrigation seasons for the Stellenbosch clay loam and Stellenbosch sand. On the other hand, the use of MW in the Stellenbosch clay loam resulted in the highest soil ExSP for season 3. The lowest soil ExSP in this soil was found with the use of MW in season 2.

The linear regression analysis of applied Na in the two sources of irrigation water (MW and WW) versus soil ExSP showed very strong positive correlation in the Stellenbosch clay loam and Lutzville sand and strong positive correlation in the Stellenbosch sand and Robertson clay loam (Table 4.30). The rate of increase in soil ExSP with an increase in applied Na (soil ExSP/Na applied per ha) differed between the soils and could be related to the clay content thereof (Figure. 4.4).

**Table 4.30:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil extractable sodium percentage (%) levels for the four study soils, including the correlation coefficient ($R^2$) of the linear relationship between applied Na and soil ExSP.

| **Simulated season** | **Irrigation water** | **Soil ExSP (%)** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 5.6 | 4.3 | 8.8 | 2.7 |
| **1** | **MW** | 4.8b,x* | 3.4a,y | 3.2a,y | 3.7b,x |
| | **WW** | 7.0a,x | 9.6a,x | 4.9a,z | 6.2a,x |
| **2** | **MW** | 2.7b,z | 3.8b,xy | 5.0b,x | 2.9b,x |
| | **WW** | 7.8a,x | 9.33a,x | 8.8a,y | 6.6a,x |
| **3** | **MW** | 3.8b,y | 5.8b,x | 5.8b,x | 3.2b,x |
| | **WW** | 8.6a,x | 10.7a,x | 11.3a,x | 6.6a,x |
| **Correlation coeff. ($R^2$)** | | +0.83 | +0.79 | +0.86 | +0.78 |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each irrigation source (MW or WW).

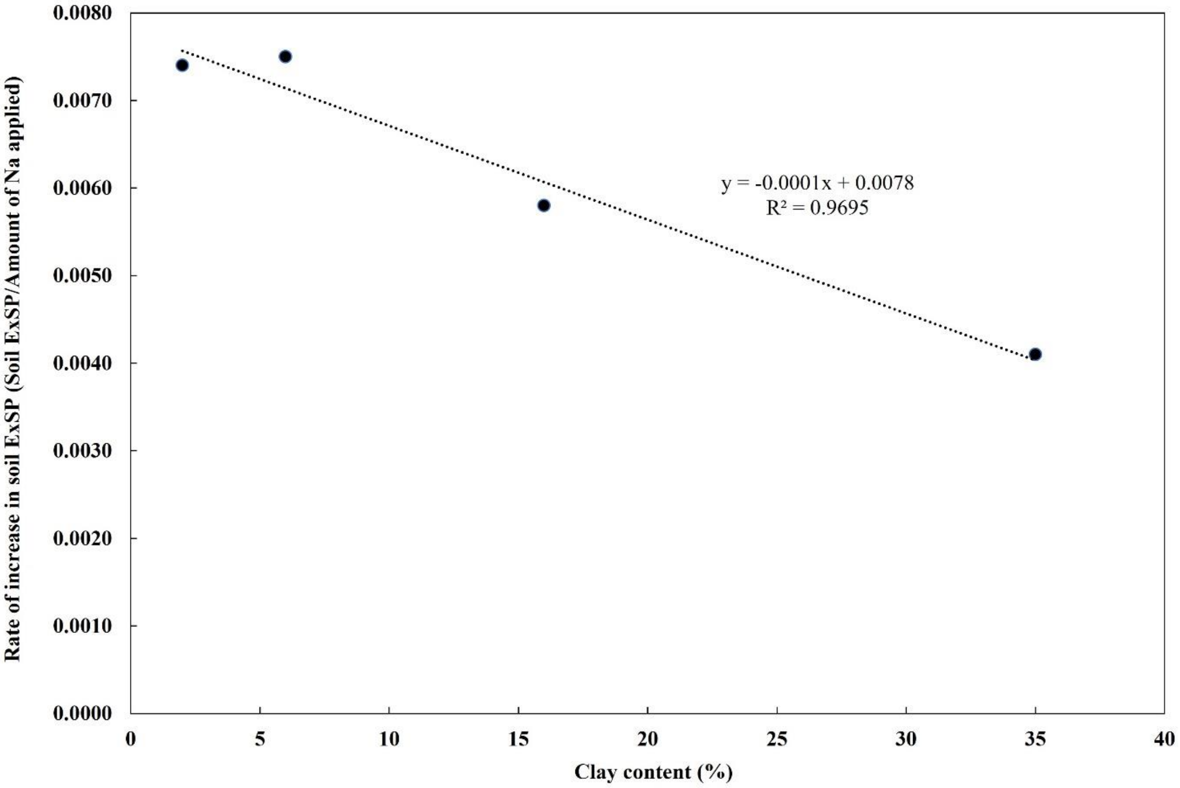

**Figure 4.4:** Relationship between the ratio of soil ExSP to applied Na per ha and clay content for the four different soils.

## 4.3: Effect of three simulated seasons of irrigation using municipal water and winery wastewater on soil enzymes

### 4.3.1: β-Glucosidase

The levels of β-glucosidase in the four study soils after three simulated irrigation seasons using two irrigation waters, either WW or WW, are given in Table 4.31. The initial (baseline) level of β-glucosidase in each of the four study soils was higher than the levels measured after irrigation with either water source.

The β-glucosidase in the Stellenbosch clay loam was significantly higher in the simulated irrigation season 2 when MW was used in comparison to WW. There was no significant difference in β-glucosidase in the simulated irrigation seasons 1 and 3 for MW or WW. There was a significant decrease in β-glucosidase during season 3 for the MW usage. There was no significant difference in β-glucosidase levels during the three simulated irrigation seasons for WW.

The β-glucosidase in the Stellenbosch sand was significantly higher in the simulated irrigation seasons 1 and 2 when MW was used in comparison to WW, but not significantly different in

Season 3. There was no significant difference in β-glucosidase levels during the three simulated irrigation seasons for WW. There was significant higher β-glucosidase in the simulated irrigation Season 1 when compared to Season 3 for MW.

The β-glucosidase in the Lutzville sand was significantly higher in the simulated irrigation Seasons 1 and 2 when MW was used in comparison to WW. There was no significant difference in β-glucosidase levels during the three simulated irrigation seasons for WW. There was a significantly higher β-glucosidase in the simulated irrigation season 1 when compared to season 3 for MW.

The β-glucosidase in the Robertson clay loam showed no significant difference in the three simulated irrigation seasons when MW was used in comparison to WW. There was no significant difference in β-glucosidase in simulated irrigation seasons 1, 2 and 3 for the use of MW or WW.

**Table 4.31:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil β-glucosidase levels (μg p-nitrophenol $g^{-1}$ $h^{-1}$) for the four study soils.

| **Simulated season** | **Water** | **Soil** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 175.9 | 199.1 | 100.6 | 163.0 |
| **Season 1** | MW | 114.1a,x* | 150.9a,x | 60.4a,x | 100.5a,x |
| | WW | 110.6a,x | 102.9b,x | 47.0 b,x | 96.9a,x |
| **Season 2** | MW | 122.7a,x | 134.1a,xy | 26.5a,xy | 78.0 a,x |
| | WW | 97.0b,x | 64.1b,x | 45.4b,x | 88.2a,x |
| **Season 3** | MW | 91.6a,y | 84.0 a,y | 35.5a,y | 74.3a,x |
| | WW | 95.3a,x | 92.0 a,x | 34.1a,x | 85.2a,x |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicates significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each water source (MW or WW).

### 4.3.2: Acid phosphatase

The levels of acid phosphatase in the four study soils after three simulated irrigation seasons using two irrigation waters, WW and WW are given in Table 4.32. The initial (baseline) level of acid phosphatase in each of the four study soils was higher than the levels measured after irrigation with either water source.

The acid phosphatase in the Stellenbosch clay loam was significantly higher in the simulated irrigation season 1 when MW was used in comparison to WW. There was no significant difference in acid phosphatase in the simulated irrigation seasons 2 and 3 for MW or WW. There was no significant difference in acid phosphatase levels during the four simulated irrigation seasons for WW and MW.

The acid phosphatase in the Stellenbosch clay loam was significantly higher in the simulated irrigation season 1 and season 2 when MW was used in comparison to WW. There was no significant difference in acid phosphatase in the simulated irrigation season 3 for MW or WW. There was no significant difference in acid phosphatase levels during the three simulated irrigation seasons for WW and MW.

The acid phosphatase in the Lutzville sand was significantly higher in the simulated irrigation season 2 when MW was used in comparison to WW, but not significantly different in seasons 1 and 3. There was a significant difference in acid phosphatase in season 1 as compared to season 2 in the simulated irrigation seasons for WW. There was significant higher acid phosphatase in the simulated irrigation season 1 when compared to season 3 for MW.

The acid phosphatase in the Robertson clay loam showed no significant difference in the three simulated irrigation seasons when MW was used in comparison to WW. There was no significant difference in acid phosphatase in the simulated irrigation seasons 1, 2 and 3 for the use of MW or WW.

**Table 4.32:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil acid phosphatase levels (µg p-nitrophenol $g^{-1}$ $h^{-1}$) for the four study soils.

| **Simulated season** | **Water** | **Soil** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 594.4 | 413.1 | 277.9 | 167.7 |
| **Season 1** | MW | 204.6a,x* | 209.2a,x | 46.0 a,x | 93.5a,x |
| | WW | 110.1b,x | 49.0b,x | 42.0a,x | 69.9a,x |
| **Season 2** | MW | 148.9a ,x | 179.0a,x | 35.1a,xy | 45.5a,x |
| | WW | 66.6b,x | 53.6b,x | 22.7b,y | 62.6a,x |
| **Season 3** | MW | 125.5a,x | 113.5a,x | 25.7a,y | 47.0a,x |
| | WW | 90.3a,x | 111.4a,x | 32.2a,xy | 77.4a,x |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each water source (MW or WW).

### 4.3.3Urease

The levels of urease in the four study soils after three simulated irrigation seasons using two irrigation waters, WW and WW are given in Table 4.33. The initial (baseline) level of urease in the Robertson clay loam was higher than the levels measured after irrigation with either water source. However, irrigation with MW and WW during the three simulated irrigation seasons for the remaining three study soils resulted in urease levels that were either higher or lower than the baseline.

The urease levels in the Stellenbosch clay loam showed no significant difference in the three simulated irrigation seasons when MW was used in comparison to WW. There was no significant difference in urease levels during the three simulated irrigation seasons for WW. There was a significantly higher urease in seasons 2 and 3 than season 1 for MW usage. The use of WW in the simulated irrigation seasons 2 and 3 showed an average urease level higher than the baseline, while the other treatments showed averages below the baseline.

The urease levels in the Stellenbosch sand showed no significant difference in the simulated irrigation seasons 2 and 3 when MW was used in comparison to WW while there was a significant difference in urease levels during season 1. There was no significant difference in urease levels in the simulated irrigation seasons 1, 2 and 3 for the use of MW or WW. The use of WW and MW in simulated irrigation Season 2 showed an average urease level higher than the baseline, while the other treatments showed averages below the baseline.

The urease in the Lutzville sand showed no significant difference in the simulated irrigation seasons 1, 2 and 3 when MW was used in comparison to WW. There was no significant difference in urease in the simulated irrigation seasons 1, 2 and 3 for the use of MW or WW. The use of WW and MW in the simulated irrigation seasons 2 and 3 showed an average urease level higher than the baseline, while the other treatments showed averages below the baseline.

The urease in the Robertson clay loam showed no significant difference in the simulated irrigation seasons 1, 2 and 3 when MW was used in comparison to WW. There was no significant difference in urease in the simulated irrigation seasons 1, 2 and 3 for the use of MW or WW. The use of WW and MW in three simulated irrigation seasons showed an average urease level lower than the baseline.

**Table 4.33:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil urease levels (µg $NH_4^+$ $g^{-1}$ 2 $h^{-1}$) for the four study soils.

| **Simulated season** | **Water** | **Soil** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | 23.8 | 16.0 | 19.1 | 16.6 |
| **Season 1** | MW | 5.8a,y* | 15.5a,x | 8.7a,x | 6.1a,x |
| | WW | 5.5a,x | 13.9b,x | 14.5a,x | 12.3a,x |
| **Season 2** | MW | 15.7a,x | 17.7a,x | 14.3a,x | 7.4a,x |
| | WW | 25.3a,x | 18.9a,x | 41.4a,x | 11.2a,x |
| **Season 3** | MW | 12.4a,x | 29.4a,x | 13.1a,x | 9.0 a,x |
| | WW | 31.7a,x | 7.0a,x | 42.6a,x | 9.6a,x |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each water source (MW or WW).

### 4.3.4Alteration index 3 (AI3)

The levels of AI3 in the four study soils after three simulated irrigation seasons using two irrigation waters, WW and WW are given in Table 4.34. The initial (baseline) level of AI3 in each of the four study soils was lower than the levels measured after irrigation with either water source.

The AI3 in the Stellenbosch clay loam was significantly lower in the simulated irrigation season 1 when MW was used in comparison to WW, but not significantly different in seasons 2 and 3. There was no significant difference in AI3 in the simulated irrigation seasons 1, 2 and 3 for the use of MW or WW. The AI3 in the Stellenbosch sand was significantly lower in the simulated irrigation season 1 when MW was used in comparison to WW, but not significantly different in seasons 2 and 3. There was no significant difference in AI3 in the simulated irrigation seasons 1, 2 and 3 for the use of MW or WW. The AI3 in the Lutzville sand was significantly higher in the simulated irrigation season 1 when MW was used in comparison to WW, but not significantly different in seasons 2 and 3. There was no significant difference in AI3 in the simulated irrigation seasons 1, 2 and 3 for the use of MW or WW. The AI3 in the Robertson clay loam showed no significant difference in the simulated irrigation Seasons 1, 2 and 3 when MW was used in comparison to WW. There was no significant difference in AI3 in the simulated irrigation seasons 1, 2 and 3 for the use of MW or WW.

**Table 4.34:** The effect of three simulated seasons of irrigation using municipal water (MW) and winery wastewater (WW) on soil alteration index 3 (AI3) levels for the four study soils.

| **Simulated season** | **Water** | **Soil** | | | |
|---|---|---|---|---|---|
| | | **Stellenbosch clay loam** | **Stellenbosch sand** | **Lutzville sand** | **Robertson clay loam** |
| **Baseline** | | -31.0 | -17.1 | -15.4 | -4.8 |
| **Season 1** | MW | -7.1b,x* | -7.6b,x | -1.4a,x | -1.3a,x |
| | WW | -1.6a,x | -0.5a,x | -3.4b,x | -1.7a |
| **Season 2** | MW | -4.8a,x | -6.9a,x | -9.7a,x | -1.1a,x |
| | WW | -4.6a,x | -4.2a,x | -3.3a,x | -1.4a |
| **Season 3** | MW | -5.3a,x | -9.2a,x | -2.7a,x | -0.8a,x |
| | WW | -7.7a,x | -3.1a,x | -10.4a,x | -2.1a,x |

*Tukey's HSD test was used to determine significant differences. Different letters (a.b) indicate a significant difference (0.05) between MW and WW values within each season. While different letters (x.y.z) indicate a significant difference (0.05) between seasons 1, 2 and 3 values within each water source (MW or WW).

### 4.3.5 Linear regression analysis of applied total elements (kg/ha) in irrigation water, soil pH and soil $EC_e$ (dS/m) versus soil enzymes for the four study soils.

The relationship between the three soil enzymes and various soil chemical properties were analyzed using linear regression to determine any correlation between the variables. The correlation coefficients of these regression analyses in given in Table 4.35.

The strength of correlation coefficients were adopted from Schober *et al*., (2018), who indicated that values of 0.80 - 1.0; 0.60 - 0.79; 0.40 - 0.59; 0.20 - 0.39; and 0.00 - 0.19 corresponds to very strong; strong; moderate; weak; and very weak relationships, respectively.

#### 4.3.5.1 Linear regression correlation of β-glucosidase with soil factors

Correlations between β-glucosidase and all other soil factors were mainly weak or moderate negative for the Stellenbosch clay loam and Stellenbosch sand. However, added N, Ca, and salinity (EC) showed a strong negative correlation β-glucosidase.. All the correlations for the Lutzville sand and Robertson clay loam had very weak positive and negative relationship.

### 4.3.5.2 Linear regression correlation of phosphatase with soil factors

There was a moderate to very strong negative correlation between phosphatase and all other soil factors for the Stellenbosch clay loam. The correlations in the Stellenbosch sand were mainly weak negative except for added Ca, soil pH and electrical conductivity (EC), which showed a moderate negative correlation. All the correlations for the Lutzville sand and Robertson clay loam had very weak positive and negative relationship.

### 4.3.5.3 Linear regression correlation of urease with soil factors

Very strong, strong and moderate positive correlations were shown between urease and all other soil factors for the Stellenbosch clay loam and Lutzville sand. Soil OC was the exception. In the Robertson clay loam, the correlations ranged from moderately negative to moderately positive However, all the correlations for the Stellenbosch sand were negative in the range of weak to moderate negative.

**Table 4.35:** Correlation coefficients ($R^2$) for linear regression analysis of total elements applied via the irrigation water (kg/ha), soil $pH_{(KCl)}$ and soil $EC_e$ (dS/m) versus soil enzymes (β-glucosidase, phosphatase, urease) for the four study soils.

| **Total element applied** | Linear Regression Correlation Coefficient ($R^2$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **β –glucosidase** | | | | **Phosphatase** | | | | **Urease** | | | |
| | **Soil A** | **Soil B** | **Soil C** | **Soil D** | **Soil A** | **Soil B** | **Soil C** | **Soil D** | **Soil A** | **Soil B** | **Soil C** | **Soil D** |
| **N** | -0.32 | -0.41 | -0.03 | +0.02 | -0.64 | -0.28 | -0.19 | +0.04 | +0.74 | -0.30 | +0.93 | +0.24 |
| **P** | -0.28 | -0.34 | -0.02 | +0.02 | -0.55 | -0.27 | -0.13 | +0.07 | +0.70 | -0.40 | +0.88 | +0.26 |
| **Ca** | -0.24 | -0.44 | -0.00 | -0.01 | -0.69 | -0.55 | -0.10 | +0.05 | +0.50 | -0.34 | +0.75 | +0.56 |
| **Mg** | -0.29 | -0.37 | -0.02 | +0.00 | -0.59 | -0.31 | -0.13 | +0.06 | +0.69 | -0.39 | +0.88 | +0.31 |
| **K** | -0.30 | -0.30 | -0.03 | +0.01 | -0.55 | -0.18 | -0.13 | +0.07 | +0.70 | -0.40 | +0.90 | +0.30 |
| **Na** | -0.30 | -0.36 | -0.02 | +0.00 | -0.58 | -0.31 | -0.12 | +0.06 | +0.68 | -0.39 | +0.87 | +0.30 |
| **Soil $pH_{(KCl)}$** | -0.25 | -0.36 | +0.00 | +0.03 | -0.69 | -0.59 | -0.04 | +0.13 | +0.53 | -0.37 | +0.66 | +0.23 |
| **Soil $EC_e$** | -0.20 | -0.50 | -0.01 | +0.11 | -0.61 | -0.46 | -0.11 | -0.00 | +0.60 | -0.48 | +0.86 | -0.46 |
| **Soil OC** | 0.00 | 0.00 | +0.01 | -0.06 | -0.85 | -0.46 | -0.28 | 0.00 | 0.00 | -0.21 | -0.06 | +0.06 |

Soil A = Stellenbosch clay loam, Soil B = Stellenbosch sand, Soil C = Lutzville sand, Soil D = Robertson clay loam. The + or – of the correlation coeffient ($R^2$) indicates if the relationship was positive or negative. The correlation of the two data sets was done at 0.05 confidence level.

# Chapter 5 – Discussion and Conclusions

## 5.1 Use of MW and WW irrigation waters on soil chemical characteristics

The element content in the WW was one order of magnitude greater for some chemical characteristics (N, P, Ca, Mg, Na, $Cl^-$) and was two orders of magnitude greater for other characteristics (K, $HCO_3^-$) compared to the MW. Similarly, Howell (2016), Mulidzi (2016) and Hoogendijk (2019) also reported that WW contained substantially higher levels of the plant nutrients. It was expected that adding these larger amounts during the three simulated irrigation seasons would increase soil levels of each of the chemical characteristics.

### 5.1.1 Primary nutrients (N, P, K)

As mentioned above, the WW used for irrigation had significantly higher concentrations of primary elements (N, P, K) when compared to the composition of the MW. As expected, the use of WW in comparison to MW, resulted in an increase in soil P and soil K for each irrigation season and at the end of the study (Tables 4.21 and 4.22). The substantial amounts of P and K applied *via* irrigation with WW was a primary reason for the increased levels of soil P and K observed in this study. This was confirmed with correlation coefficients ranging from 0.56 to 0.94 and 0.89 to 0.96 for the regression analysis of applied P versus soil P and applied K versus soil K, respectively, for the four soils Soil P and soil K levels were significantly higher for almost all of the three irrigation seasons for all the four study soils. In fact, soil K was an order of magnitude higher when WW was used for irrigation. The application of WW also increased soil K in the 0-10 cm and 10-20 cm soil layers where WW was used for the irrigation of a grazing paddock near Rawsonville (Mulidzi, 2016; Mulidzi *et al.*, 2019). It was observed that the soil K increased over three years. There was an increase in soil K where vineyards in clay loam soil at Robertson as well as shallow and deep sandy soil at Spruitdrift and Lutzville, respectively, were irrigated with the in-field fractional use (augmentation) of WW with raw water for one season (Hoogendijk, 2019). In that particular study, 1 000 kg/ha K and 1 200 kg/ha K was applied *via* the irrigation water at the Spruitdrift and Lutzville plots, respectively. In a field study where diluted WW was used for the irrigation of a sandy soil near Rawsonville, soil K increased linearly with a decrease in wastewater dilution (Howell, 2016; Howell *et al.*, 2018). It should be noted that in that particular study, the additional K applied *via* the diluted winery wastewater ranged from 7 kg/ha/year for a control to 177 kg/ha/year where winery wastewater was diluted to 3 000 mg/L. In a pot study, irrigation with diluted WW increased K substantially over four seasons (Mulidzi, 2016). Furthermore, soil K in the 0-10 cm soil layer was slightly higher compared to the 10-20 cm layer, irrespective of clay content. This could be due to soils being less leached into deeper levels. Contrary to the latter, deeper soils can provide more water and nutrients as compared to shallow soils (Rajakaruna *et al*., 2008).

The WW used for irrigation resulted in significantly higher soil ExPP when compared to the use of MW for almost all the three irrigation seasons for all four study soils. The increase in soil ExPP was a direct result of the higher amounts of K added *via* the irrigation with WW with a concurrent non increase in Ca and Mg (see Section 5.2.3 below). This was confirmed with positive correlation coefficients ranging from +0.69 to +0.93 for the regression analysis of applied K versus soil ExPP for the four study soils. The increasing ExPP values over the three simulated irrigation seasons for WW, far exceeded the threshold value of 15 (Levy & Torrento, 1995) and might cause problems of deteriorating soil structural stability and reduced hydraulic properties. A previous study found that if K was not taken by plants but instead adsorbed to soil particles, the concentration of K in soil where winery wastewater irrigation was applied might be substantial, lowering the risk of leaching (Arienzo *et al*., 2009). However, the impact of high K levels in soil on soil structure stability owing to WWW irrigation can lead to long term instability of soil structure (Mulidzi *et al*., 2019). Mulidzi *et al.* (2018) deduced that following an initial increase in K in the 0 to 10 cm layer of Kroonstad soil near Stellenbosch soil that had not previously been irrigated with WW, K remained relatively constant.

The ExPP in the 0-30 cm soil layer could consistently be related to the amount of K applied *via* diluted WW where a vineyard was irrigated with diluted WW (Howell, 2016). Irrigation with diluted WW in a pot study increased ExPP over four seasons (Mulidzi, 2016). Similarly, there was a substantial increase in ExPP where a shallow and deep sandy soil at Spruitdrift and Lutzville, respectively, were irrigated with the in-field fractional use (augmentation) of WW with raw water for one season (Hoogendijk, 2019). The increases were solely attributed to the substantial amounts of K that were applied *via* the irrigation water.

In relation to soil N, there was no significant difference in soil N levels when either WW or MW was used for almost all the three irrigations in the four soils (Table 4.20), even though the total N added in the WW was an order of magnitude greater. The total application of N with both irrigation water sources (WW and MW) did not result in an increase of soil N found in the four study soils. This is confirmed by regression analysis of applied N versus soil N with resultant correlation coefficients ranging from -0.2 to +0.3.

This unexpected result may be explained by looking at the two forms of nitrogen added in the irrigation waters: ammonium and nitrate. First, there may have been soil N loss through ammonia volatilization due to the effect of WW on soil pH (Table 4.26). The use of WW resulted in a significant increase in soil pH to the range of 8.8 to 10.1 by the end of season 3 irrigation. This very high soil pH is more conducive to soil nitrogen losses through ammonia volatilization (Zhenghu & Honglang, 2000). Secondly, a potential loss of nitrogen from the soil can occur from denitrification, which is favoured by anaerobic conditions (Zheng *et al*., 2018). The WW had a high COD level, ranging from 285 to 4002 mg/L, which may result in anaerobic conditions and thus promote denitrification (Reddy *et al*., 1975; Zheng *et al*., 2018).

### 5.1.2 Bases (Ca, Mg, Na)

The WW used for irrigation had significantly higher amounts of Ca and Mg when compared to MW; however, this did not result in significant increases in soil Ca and Mg levels when WW was used for the three irrigations in the four soils (Tables 4.23 and 4.24). Also, the total addition of Ca and Mg in the irrigation waters (WW and WW) did not result in an increase in soil Ca and soil Mg found in three of the four study soils. This was confirmed with correlation coefficients ranging from 0.26 to 0.44 for the regression analysis of applied Ca versus soil Ca for these three study soils, and negative to positive correlation coefficients ranging from -0.4 to 0.28 for the regression of applied Mg versus soil Mg.

The reason for not having increases in soil Ca and Mg was probably because the measured soil Ca and Mg were exchangeable and solution Ca and Mg as determined by the ammonium acetate method. If the applied Ca and Mg in the WW formed complexes with the carbonates also added with this water, then this would not be measured as soil Ca and soil Mg using the ammonium acetate method. The high soil pH (8.3 to 10.1) of the four study soils at the conclusion of this study are conducive for the formation of such Ca and Mg carbonates. In addition, a previous study deduced that the application of WW is unlikely to have any benefits of Ca and Mg supply to plants, due to the fact that the wastewater contained only small quantities of this element (Mulidzi *et al.,* 2018).

The WW used for irrigation had significantly higher amounts of Na when compared to MW. As expected, the use of WW in comparison to MW, resulted in an increase in soil Na for each irrigation season and at the end of the study (Tables 4.25). Also, the total application of Na with both irrigation water sources (WW and MW) was a primary reason for the increased levels of soil Na found in this study, as confirmed with the positive correlation coefficients ranging from

0.94 to 0.98 for the regression analysis of applied Na versus soil Na for the four study soils. The application of WW also increased soil Na in the 0-10 cm and 10-20 cm soil layers where WW was used for the irrigation of a grazing paddock near Rawsonville (Mulidzi, 2016; Mulidzi *et al.*, 2019). It was observed that the soil Na increased over the duration of the study. Similarly, soil Na in the 0-30 cm as well as the 60-90 cm soil layers increased linearly with a decrease in WW dilution where diluted WW was used for vineyard irrigation (Howell, 2016; Howell, 2018). It should be noted that in this particular study, the additional Na applied *via* the WW ranged from 38 kg/ha/year for the control to 93 kg/ha/year for the lowest level of dilution. Irrigation with diluted WW in a pot study increased soil Na over four seasons (Mulidzi, 2016). In contrast, there was no increase in soil Na where vineyards in sand and clay soils at Stellenbosch were irrigated with the in-field fractional use (augmentation) of WW with raw water for one season (Hoogendijk, 2019). In that particular study, 40 kg/ha Na was applied *via* the irrigation water at those specific plots. Hoogendijk (2019) also reported that there was no increase in soil Na where vineyards at Robertson, Vredendal and Lutzville were irrigated with the in-field fractional use (augmentation) of WW with raw water for one season (Hoogendijk, 2019).

The WW used for irrigation had significantly higher amounts of soil ExSP when compared to the composition of the MW (Table 4.30). Also, the total addition of Na in the irrigation waters (WW and WW) resulted in an increase soil in ExSP found in all three irrigations for the four study soils. This was confirmed with positive correlation coefficients ranging from +0.78 to +0.86 for the regression of applied Na versus soil ExSP. The ExSP is a function of the amount of exchangeable Na in relation to exchangeable Ca and Mg in the soil. This study showed that exchangeable Na increased significantly with the use of WW while exchangeable Ca and Mg remained unchanged. This combination of results helps explain why ExSP increased with the use of WW. High levels of Na in winery wastewater can reduce soil permeability and may cause infiltration issues (Van Schoor, 2005). However, the current study showed that although soil ExSP significantly increased with the use of WW, it did not reach the threshold of 15 to cause soil physical permeability problems. A previous study alluded that except for an initial increase in May 2011, soil Na tended to decline steadily throughout the study period, particularly in the 0 to 10 cm and 10 to 20 cm layers (Mulidzi *et al.*, 2018).

### 5.1.3 Measurable parameters (Soil $pH_{(KCl)}$ and $EC_e$)

The WW used for irrigation had significantly higher pH when compared to MW. As expected, the use of WW in comparison to MW resulted in an increase in soil pH for each irrigation season and at the end of the study (Tables 4.26). Also, the total application alkalinity with both irrigation water sources (WW and MW) was a primary reason for the increased soil pH found in this study, as confirmed with the positive correlation coefficients ranging from 0.53 to 0.91 for the regression analysis of applied alkalinity versus soil pH for the 4 study soils.

The effect of irrigation water source on soil pH as described above resulted in an increase in soil K and Na, and to a lesser extent, soil Ca and Mg. This was supported by the correlation coefficients of soil pH against each soil base (Table 4.26). The reduced effect of soil pH on soil Ca and Mg may be due to greater precipitation of each of these bases with added carbonates in the irrigation sources, especially the WW. In contract to the findings, a previous study found that irrigation using in-field fractionally applied WW with MW did not have a substantial effect on soil pH(KCl) (Hoogendijk ,2019).

The WW used for irrigation had significantly higher salts when compared to MW. As expected, the use of WW resulted in an increase in soil $EC_e$ for each irrigation season and at the end of the study (Tables 4.27) in the Stellenbosch clay loam, Stellenbosch sand and Lutzville sand. Also, the total application of salts with both irrigation water sources (WW and MW) was a primary reason for the increased levels of salinity found in this study, as confirmed with correlation coefficients for these three soils, ranging from 0.68 to 0.72 for the regression analysis of applied salinity ($EC_e$) versus soil salinity (EC). The lone exception to this trend was in the Robertson clay loam, which showed no significant effect due to irrigation water, also confirmed by a correlation coefficient of -0.24. Robertson clay loam initially had more salt as compared to other three soils. Excess salts inhibit plant growth by disrupting the soil-water equilibrium. The salts in the soil exceeded the threshold of 1.5 dS/m (Table 4.27) for vines (Suarez *et al.*, 2019) and may thus inhibit their growth. However, these levels might not be reached in the field mainly because this study was a closed system, whereby no leaching of salts occurred and thus salts accumulated to high and detrimental levels. Previous studies deduced that winery wastewater has been proven to increase the EC of irrigated soils due to its high salt content (Hirzel *et al.*, 2017; Howell *et al.*, 2018 and references therein). Also, the accumulation of high and detrimental levels of salts may be mitigated by diluting the WW before it is used for irrigation.

## 5.2 Use of MW and WW irrigation waters on soil enzymes

Soil enzyme activity is a good measure of soil health as an indicator of soil organic matter breakdown and subsequent carbon, nitrogen and phosphorus cycling in soil systems. In this regard, three important soil enzymes are β-glucosidase, which is important in the general breakdown of carbohydrates, phosphatase, which is important in hydrolyzing organic phosphorus compounds into inorganic polyphosphates, and urease, which converts organic soil nitrogen into inorganic forms of nitrogen. These three soil enzyme levels are affected by various edaphic factors such as carbon availability, soil pH, soil EC and nutrient availability. Also, the rhizosphere of growing plants has shown to enhance soil enzyme activities (Sarapatka, 2003). However, this study had no growing plants, thus this specific effect on increased overall and specific enzyme activity would not be expected.

In this study, the application of WW and MW as irrigation water both resulted in a reduction of β-glucosidase for all the four study soils. A closer look shows that the reduction in β-glucosidase appeared to be greater from irrigation season 1 to season 3, although this reduction was not shown to be significant. A reduction of β-glucosidase activity might suggest that the irrigation water could have been a cause of this reduction over time. However, regression analysis of total elements applied (N, P, Ca, Mg, K, Na) in irrigation waters with soil β-glucosidase showed negative moderate to no correlations with two soils and no correlation with two soils (Table 4.31). Thus, although both irrigation waters resulted in a decrease in β-glucosidase activity, there was nothing in the irrigation waters that could be attributed to this possible decrease.

Additionally, the soil characteristics (EC, pH and soil OC) similarly showed poor correlation with β-glucosidase in the four study soils. Soil pH and OC may explain the possible decrease in β-glucosidase despite poor correlation between these soil characteristics and β-glucosidase. Firstly, the soil pH measured for all seasons after irrigating with WW varied between 7.5 and 10.1 and these are higher than the pH optima of 6 for β-glucosidase (Wade *et al.*, 2021). Secondly, the measured soil OC, in this study, was most likely a more stable soil OC after decomposition, thus no easily decomposable organic matter was present in the soils, allowing an increased β-glucosidase activity. Previous studies have shown that β-glucosidase activity is linked to easily decomposable organic matter emanating from crop residues and litter accrued on the soil surface in areas under zero tillage (Mukumbareza *et al.*, 2015; Adetunji *et al.*, 2020).

Similar results to the β-glucosidase activity were observed for phosphatase activity in this study. The application of both WW and MW as irrigation water resulted in a reduction of phosphatase for all four study soils with also an apparent, but non-significant reduction from season 1 to season 3. Once again, no correlation was found between total elements applied (N, P, Ca, Mg, K, Na) and soil $EC_e$, soil pH, and soil OC. Omenda *et al*. (2019) deduced that higher the soil organic carbon can result in a higher level of soil phosphatase. In this study, the percentage soil OC was relatively low (between 0.1 and 0.9) and most likely consisted of more stable forms of OC. The phosphatase activity was low during the course of the study because the pH values were high and favoured the alkaline phosphatase more whereas acidic phosphatase was measured in the current study. In their studies, Herbien and Neal (1990) and Wade *et al*. (2021) deduced that at the measured soil pH of 6.5, acidic phosphatase activity should be at its maximum.

An opposite trend was observed for urease, where its activity increased over the seasons except for the Stellenbosch sand (Table 4.33). This might be due to the stronger influence that N has on urease activity compared to β-glucosidase and phosphatase activities. This can be supported by strong to very strong positive correlation coefficients of +0.74 and +0.93 for the regression analysis of applied N versus soil urease for the Stellenbosch clay loam and Lutzville sand. Soil pH could have had played a role in the increase in urease activity as evidenced by moderate to strong correlation coefficients of +0.53 and +0.66 for the regression analysis of applied N versus soil urease for the Stellenbosch clay loam and Lutzville sand. However, the relationship between soil pH and urease is still not well understood (Fisher *et al*., 2017).

AI3 generates numerical scores from the activities of urease, phosphatase and β-glucosidase. These scores enable differences in treatment-induced alteration states between applied treatment combinations to be quantified and compared. The AI3 is therefore, an aid or adjunct to interpretation rather than an alternative to enzyme analysis. In this role, it has potential for use in the monitoring and management of enzymatic activity in vineyard soils (Meyer *et al*., 2014; Adetunji *et al*., 2019). The more negative the index, the better is the soil in terms of quality.

On average, in terms of AI3 (Table 4.34), irrigation with MW had better soil quality as compared to WW in the Stellenbosch clay loam (-5.7 versus -4.6) and Stellenbosch sand (-7.9 versus -2.6). However, irrigation with WW had better soil quality as compared to MW in the Lutzville sand (-5.7 versus -4.6) and Robertson clay loam (-1.7 versus -1.1). This variability in AI3 is probably due to the different responses of the three enzymes to irrigation sources in the four study soils.

## 5.3 Conclusions

The use of MW and WW affected the soil in two ways. First, irrigation water contains essential elements and other ions, which can have direct effects on soil properties. These effects include the increase in plant-available nutrients when essential elements are added in the irrigation water and the increase in possible enzyme activity if certain essential elements are added. Also, the soil pH and EC are directly influenced by the alkalinity of the added water and the salinity level in the added irrigation water, respectively. Second, the irrigation water can have indirect effects on soil properties and enzyme levels. These occur when the irrigation water changes the soil pH, which subsequently affects plant-availability of certain essential elements or affect enzyme activity. As well, changes in the soil EC and aerobic status of the soil by the irrigation water can affect nutrient availability or enzyme activity.

Results showed that both the direct and indirect effects of the use of MW and WW were observed in this study. However, the effects were greater with the WW since this irrigation source had higher levels of many of the essential elements and other ions.

The effect of irrigation with WW varied amongst the three measured enzyme activities. The β-glucosidase and phosphates decreased while urease activity increased after WW application. The urease activities were affected directly by the added N through the irrigation water whereas β-glucosidase and phosphatase were indirectly affected by the composition of WW, which resulted in an increase in soil pH. These two enzymes have optimal pH around 6 - 6.5 and irrigation with WW increased the soil pH to 7.5 - 10.1. The nature of SOC present in the soils also played a role in the reduced β-glucosidase because the SOC obtained in the four soils was probably the well decomposed SOC while on the other hand, β-glucosidase is enhanced by the easily decomposable SOC.

In this study, the direct effects of the added WW were most apparent with the observed increase in soil K, P and Na. High levels of K, P and Na in the WW increased plant-available levels of each of them in the soil. Consequently, high ExPP and ExSP levels were also observed in the soils. This is beneficial for K and P since both are essential for plant growth, but this would be problematic for Na as it is not essential and may eventually result in the formation of a sodic soils. The increase in soil K and Na might be less pronounced if diluted WW is used for irrigation of crops. However, the same result was not seen with Ca, Mg and N even though high levels of each of these were observed in the WW. This is most likely due to the indirect effects mentioned above. In the case of Ca and Mg, this is likely caused by the high levels of $HCO_3^-$

in the WW, which resulted in the precipitation of Ca and Mg carbonates into unavailable forms. In the case of N, it appears that the application of WW may have had an indirect effect in reducing plant-available N through volatilization of N at the high soil pH, which resulted from the WW application, and from possible denitrification losses under the anaerobic conditions resulting from high COD levels of the WW used for irrigation of the pots.

In summary, WW shows a huge potential as irrigation water, mainly because it can increase K, P, and urease activity. Since one of the incentives for using WW is that it could serve as a possible nutrient source, it is important to take note of the nutrient requirement of the crop that would be irrigated with such water. It is highly likely that the use of WW for crop irrigation would supply substantially more K than what the plant would require., Therefore, K contents of WW as well as the PAR should be considered as a water quality parameter when using WW for crop irrigation.

However, care is needed as it may increase salts and Na to detrimental levels and thus it should be monitored constantly. The Na would mostly be detrimental to clay soils than sandy soils. It is thus worth noting that the results of these detrimental levels may not be reached in the field because this was a closed study and no leaching occurred. Therefore, in an open system, like field conditions, leaching may remove some salts and Na. If WW is to be used for irrigation of crops, there should be routine analysis of the water, the soils that are being irrigated as well as the plant tissues of the crops that are being irrigated with the WW. Crop performance should also be monitored. It is therefore recommended to use an integrated fertiliser management program by adjusting fertiliser applications according to the amounts of nutrients present in the wastewater.

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