



**Soil chemical and microbiological responses to irrigation with diluted winery  
wastewater in a Shiraz vineyard in Stellenbosch, Western Cape**

**By**

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## ABSTRACT

There is an increase in the shortage and demand for irrigation water in the farming system, especially in the Western Cape, South Africa. To reduce the pressure on the demand for clean water and meet the irrigation demand, the practice of supplementing available clean water with urban/industrial wastewater including winery wastewater (WWW) is becoming popular. Hence, a field study was conducted over three seasons (2018-2020) in a Shiraz/110 Richter vineyard at the Agricultural Research Council (ARC), Infruitec-Nietvoorbij, Stellenbosch, South Africa. The study assessed (i) the effect of diluted WWW and raw water on soil chemical parameters: pH, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), Carbon (C), Soluble-s, electrical conductivity (ECe), exchangeable potassium percentage (EPP') and exchangeable sodium percentage (ESP')) and soil enzyme activities ( $\beta$ -glucosidase, phosphatase and urease) where different cover crops and catch crops were cultivated (ii) the effect of the diluted WWW and raw water on the element content of the different catch and cover crops in the vineyard (iii) the performance of the summer growing catch crops and winter cover crops in the vineyard after irrigation with diluted WWW and raw water in the vineyard (iv) the effect of diluted WWW, raw water, summer catch crops and winter cover crops on the grapevine performance. Species cultivated as winter cover crops were oats (*Avena sativa* L.) and N-fixing whereas species cultivated as summer catch crops were pearl millet (*Pennisetum glaucum*), Dolichos beans (*Lablab purpureus*), chicory (*Cichorium intybus*) and a control (no cover crop). The experimental design was a randomized complete block design (RCBD) with 3 replications. Soils were sampled after irrigation with diluted WWW (after harvesting of catch crops) and after winter rainfall (after harvesting of cover crops) for soil chemical and soil enzyme activities. Catch and cover crops were sampled after irrigation with diluted WWW and after winter rainfall.

Throughout the study period, irrigation with either diluted WWW or raw water had no significant effect on soil  $\beta$ -glucosidase, phosphatase, and urease activities. After application of diluted WWW and raw water over three seasons, there was a significant difference in soil pH, P, EPP' and soluble in the 0-15 cm soil layer. In the 15-30 cm soil layer, the significant impact was on soil pH, P, K, ECe and EPP'. At 30-60 cm soil layer, only soil K differed significantly between the treatments and at 60-90 cm soil layer, soil P, K, Na, soluble-s and EPP' differed significantly between the treatments. After three seasons of irrigation with diluted WWW, summer catch crop, Dolichos bean produced higher Dry Matter Decomposition (DMP) compared to pearl millet while N-fixing winter cover crop produced higher DMP than oats. The chemical composition of winter cover crops (N, P, K, Ca, Mg and Na) differed significantly between the treatments in the 2018 and 2020 seasons after winter rainfall. Following dilution with winery

wastewater, the chemical composition of summer catch crops (N, P, K, Ca and Mg) differed significantly.

**Keywords:** Leaf blades, Leaf petioles, Macro-nutrients, Soil chemicals, Soil enzymes, Winery wastewater

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## GLOSSARY

### Abbreviations

Abbreviations	Description
%	Percentage
ARC	Agricultural Research Council
°C	grade Celsius
Ca	calcium
CEC	cation exchange capacity
COD	chemical oxygen demand
CO <sub>2</sub>	carbon dioxide
DWAF	department of Water Affairs
EC	electrical conductivity
EPP'	extractable potassium percentage
ESP'	extractable sodium percentage
Ha	hectare
K	potassium
Kg/ha	kilogram per hectare
KOH	potassium hydroxide
LSD	least significant difference
L	litre
m <sup>3</sup>	cubic meter
Mg	magnesium
Mg/L	milligram per litre
MgL	megaliters
MI	millilitre
Mm	millimetre
mS/m	milli Siemens per metre
Mql	thousands of quintals
N	nitrogen
Na	sodium
NaOH	sodium hydroxide
NS	not significant
O <sub>2</sub>	oxygen
PAR	potassium adsorption rate
P	phosphorus
Ph	alkali or acid range from 1 to 14
SAR	sodium adsorption rate

SO<sub>4</sub>  
t/ha  
WWW

sulphate  
tonnes per hectare  
Winery wastewater

# CHAPTER ONE

## 1.1 Introduction

The global climate change and continuous increase in world population are leading to water scarcity, growing demand for clean water and a decline in agricultural productivity (Faisal Anwar, 2011). This shift has further resulted in an increase in the shortage and demand for irrigation water in the farming system. To reduce the pressure on the demand for clean water and meet the irrigation demand, the practice of supplementing available clean water with untreated, treated, and urban/industrial wastewater is becoming popular (Mulidzi *et al.*, 2016).

Winery wastewater (WWW) can provide a valuable irrigation source especially in regions where water accessibility is problematic or sustainable disposal of waste is essential (Laurenson *et al.*, 2012). It is estimated that about 3 to 5 m<sup>3</sup> of WWW with high organic load and variable salinity and nutrient levels is produced per tonne of grapes crushed (Howell *et al.*, 2018). Using an industry assumption that every gallon of wine produced will generate seven gallons of WWW (Oakley, 2009), California potentially generated over 5 billion gallons WWW in 2014. The South African wine industry produces more than 980 000 m<sup>3</sup> volumes of WWW annually (Sawis, 2020). However, WWW is badly handled and deposited into freshwater sources, contributing to significant contamination in the environment (Odjadjare & Okoh, 2010). A potential solution to this issue is the reuse of this WWW for irrigation in agricultural soils. This resolution is being taken more seriously in various desert and semi-arid countries around the world where it is a popular activity where freshwater supplies are limited or not appropriate for irrigation (Levy *et al.*, 2014). From the world's total 301 million hectares of irrigated agricultural land, 1.5 to 6.6% have been estimated to be irrigated with WWW (Sato *et al.*, 2013).

Irrigation with WWW which is rich in nutrients (including potassium (K) and sodium (Na)) can be beneficial to the overall soil fertility as this can replace conventional fertilizers. However, the long-term application may alter soil physiochemical properties and increase the concentration of the salts associated with saline or sodic soils, which can be detrimental to the soil ecosystem and crop performance. Thus, it is imperative, that when WWW is used for irrigation, water conservation benefits are not compromised by a decline in soil health, plant productivity and environmental quality (Laurenson *et al.*, 2012). Irrigation with WWW needs to be optimized to minimize leaching while reducing nutrient removal by means of a catch crop. To reduce soil sodium adsorption ratio (SAR) and potassium adsorption rate (PAR), selected plants may be used for the removal of salts (Myburgh & Howell 2014). Previous studies have also shown that irrigation with WWW can affect soil quality properties such as microbial enzymes responsible for organic soil breakdown and mineralisation of nutrients (Bardgett *et al.*, 2005). Furthermore, WWW contains important plant nutrients that can increase crop

production (Chen *et al.*, 2013). This allows farmers not only to produce more income and increase local or regional economic activity for their households but also provide cities with fresh fruits and vegetables which would not be possible if farmers did not irrigate with WWW (Qadir *et al.*, 2007). There is less information on the effects of WWW on soil chemical and biological properties known to be reliable soil quality indicators. More information on this topic is crucial for broader understanding and proper management of WWW irrigation to minimise the negative impacts on the soil and environment and improve crop quality and yield while identifying knowledge gaps that deserve further research.

## **1.2 Objectives**

The aim of this study was to investigate the soil chemical parameters, soil enzymes activities, summer catch crops and winter cover crops where diluted Winery wastewater (WWW) is used for irrigation.

### **Specific Objectives**

1. To investigate the effect of diluted WWW and raw water on soil chemical parameters and soil enzyme activities where different catch/cover crops are cultivated.
2. To investigate the effect of the diluted WWW and raw water on the element content of the different catch and cover crops in the vineyard
3. To investigate the performance of the summer growing catch crops and winter cover crops in the vineyard after irrigation with diluted WWW and raw water in the vineyard.
4. To investigate the effect of diluted WWW, raw water, catch crops and winter cover crops on the grapevine performance

## **1.3 Hypothesis**

It was expected that vineyards irrigation using diluted WWW rather than raw water could change soil chemical and microbiological properties due to WWW having high levels of K and Na. Differences in production and nutrient content of the catch and cover crops are expected. This could affect leaf blade, petiole and juice characteristics.

#### **1.4 Problem Statement**

Soil degradation and water scarcity are among the key factors affecting agricultural productivity. The South African wine industry produces more than 980 000 m<sup>3</sup> volumes of wastewater annually. This has necessitated the need for the use of WWW as an alternative source of water for vineyard irrigation. However, WWW contains high concentrations of basic cations such as Na and K, associated with saline or sodic soils, which can be detrimental to the soil ecosystem and grapevine performance. Thus, there is a need to properly manage WWW irrigation to minimise the negative impacts on the environment and improve crop quality and yield.

## CHAPTER TWO

### LITERATURE REVIEW

#### **2.1 Statistics of the global wine industry**

Wine production plays a big role in the agricultural industry around the world. About 62 countries are considered to be noteworthy wine producers (Kierath & Wang, 2013). In 2020, the global production of wine amounted to a volume of  $258 \times 10^6$  hl (OIV, 2020). The top eight producing wine countries are Italy ( $47.2 \times 10^6$  hl), France ( $43.9 \times 10^6$  hl), Spain ( $37.5 \times 10^6$  hl), United States ( $24.7 \times 10^6$  hl), Argentina ( $10.8 \times 10^6$  hl), Australia ( $10.6 \times 10^6$  hl), South Africa ( $10.4 \times 10^6$  hl) and Chile ( $10.3 \times 10^6$  hl) (OIV, 2020).

#### **2.2 Origin/source and volume of winery wastewater produced**

Winery wastewater is generated from grapes being crushed at the cellar to the bottle of the final product (Devesa-Rey *et al.*, 2011; Hirzel *et al.*, 2017). Winemaking uses water in the different steps of the process (Vlyssides *et al.*, 2005; Brito *et al.*, 2007; Conradie, 2015) and gives rise to the production of WWW. It is estimated that for each litre of wine produced, 1-14 L of WWW is produced (Dominguez *et al.*, 2014; Ioannou *et al.*, 2015). The production of 1 L of wine has an associated production of 4 L of strong purplish and fruit smelling WWW (Silvana *et al.*, 2021). The volume and composition of WWW vary greatly based on the time of year, the size of the winery as well as the type of wine produced (Buelow *et al.*, 2015). Mosse *et al.* (2011) reported that about 3 to 5 m<sup>3</sup> of WWW is being produced per tonne of grapes crushed. At Berry Estate Winery in the Riverland region of South Australia, crushing 50 000 tonnes of grapes annually generates about 175 000 m<sup>3</sup> of WWW (Anonymous, 2010). It is estimated that 50%, that is 50 000 m<sup>3</sup>, of the raw water used by Lutzville Vineyard in South Africa winery ends up as WWW (Kriel, 2008).

#### **2.3 Characteristics of winery wastewater**

The characteristics of WWW vary substantially depending on the production stage, from low-strength wastewaters associated with activities such as the floor, barrel, and bottle washing, to high-strength wastewaters associated with grape harvesting, crushing, and racking (Buelow *et al.*, 2015; Lofrano & Meric, 2016). It is characterized by high organic content, such as alcohols, sugars, organic acids, polyphenols, lignins and tannins (Arienzo *et al.*, 2009; Solis *et al.*, 2017; Amor *et al.*, 2019). Consequently, WWW can present serious environmental problems for soil surface, and groundwater by affecting oxygen, pH, colour, temperature, turbidity, eutrophication, and addition of toxic products (Coetzee *et al.*, 2004; Ioannou *et al.*, 2015; Bolzonella *et al.*, 2019).

However, from an agricultural perspective (Prazeres *et al.*, 2017), WWW contains macro and micro-nutrients such as calcium (Ca), magnesium (Mg), K, phosphorus (P), copper (Cu), iron (Fe), and water that are essential for plant growth (Laurenson & Houlbrooke, 2011; Prazeres *et al.*, 2014; Conradie *et al.*, 2014). It also has a high chemical oxygen demand (COD), sodium adsorption ratio and pH (Conradie *et al.*, 2014). However, WWW has a high organic content, low pH, variable salinity, and nutrient levels, all of which indicate that WWW has the potential to pose an environmental threat (Mosse *et al.*, 2011; Laurenson *et al.*, 2012).

## **2.4 Effects of winery wastewater on soil chemical properties**

### **2.4.1 pH**

Irrigation with acidic WWW may result in a reduction of soil pH (Hoogendijk, 2019; Laurenson *et al.*, 2012). After diluted WWW application, soil pH<sub>s</sub> in the 0-90 cm and 90-180 cm soil layers tended to be lower than the baseline values (Howell *et al.*, 2018). The addition of organic acids from WWW could be associated with the decrease of soil pH<sub>s</sub> due to H<sup>+</sup> dissociation from carboxyl functional groups (Rukshana *et al.*, 2012). However, in some studies, application of WWW increased soil pH<sub>s</sub> from 4.6 to 5.0 in the topsoil and from 5.0 to 5.3 in the sub soil (Mosse *et al.*, 2012; Mulidzi *et al.*, 2015; Mulidzi *et al.*, 2019; Shilpi *et al.*, 2018). Similarly, in two case studies where pastures and a vineyard were irrigated with WWW, soil pH<sub>s</sub> also increased (Kumar *et al.*, 2014). In the Alexander Valley region and Napa Valley American region in California, there was no significant difference in pH<sub>s</sub> of soils treated with WWW and control in the 0 - 40 cm depths whereas in the 40-60 cm soil samples of the WWW, the block had a higher pH<sub>s</sub> (6.29) than the control soil samples pH (5.12) (Hirzel *et al.*, 2017). There was no change in soil pH<sub>s</sub> where WWW was used for the irrigation of soil with a clay content of 50% to 60% in a study by Quale *et al.* (2010).

### **2.4.2 Electrical conductivity of the saturated extract (EC<sub>e</sub>)**

In the Napa Valley American region and Alexander Valley region in California, WWW irrigated soils had higher EC<sub>e</sub> 1.42 to 2.06 dS/m and 1.38 to 1.72 dS/m than control soils of 0.343 to 0.611 dS/m and 0.384 to 0.485 dS/m in 20-40 cm and 40-60 cm soil layer (Hirzil *et al.*, 2017). Similarly, where woodlots were irrigated with WWW, soil EC<sub>e</sub> was higher compared to control (Kumar *et al.*, 2009). Hoogendijk (2019) reported that EC<sub>e</sub> of the topsoil increased marginally after one season of irrigation using fractionally applied WWW with raw water. Similar results were reported for a sandy alluvial vineyard soil in the Breede River region which was irrigated with diluted WWW for four seasons (Howell, 2016). After WWW irrigation, soil EC<sub>e</sub> increased from 0.14 dS/m at the surface to 0.4 dS/m at 60-90 cm soil layer (Mulidzi *et al.*, 2018; Wendy *et al.*, 2010). Compared to the river water, there were no clear trends in soil EC<sub>e</sub> that could be related to different dilution of WWW (Howell *et al.*, 2018). Application of WWW does not



appear to have a significant impact on soil EC<sub>e</sub> as the soil depth did not; as shown by this study.

#### **2.4.3 Phosphorus (P)**

Mulidzi *et al.* (2019) reported a high soil P which is more than 20 mg/kg (a norm for sandy soils) in all soil layers after irrigation with WWW. Howell *et al.* (2018) reported that after the first, second and third seasons of WWW application, the soil contained 114 mg/kg, 135 mg/kg, and 153 mg/kg P respectively in the 0-90 cm soil layer. While in the 90-180 cm soil layer, the soil P in the vine rows increased from 22 mg/kg to 26 mg/kg and 46 mg/kg from the first to second and third seasons, respectively, after WWW application (Howell *et al.*, 2018). However, same study showed that irrigation with WWW led to a substantial reduction in P level at soil depth 0-90cm. Irrigation with undiluted WWW increased soil P, but P fluctuated throughout the year in the different soil horizons (Mulidzi *et al.*, 2019; Hoogendijk, 2019). Note that the Bray II P in this study did not exceed the norm of 25 kg/mg P recommended for vineyard soils with a clay content between 6% and 15% (Conradie, 1994). Results from previous studies are inconsistent with regards to the impact of WWW application on soil P. Therefore, more studies are required in this aspect.

#### **2.4.4 Potassium (K)**

Where Kikuyu grass was irrigated with WWW, an increase in the K levels was observed in the 0-10 cm soil layer, and to some extent in the 10-20 cm soil layer, at the end of the harvest periods (Mulidzi *et al.*, 2019). Similarly, where WWW was used for irrigation in the South Eastern Australia Riverine plains for three years, soil surface K increased (Quale *et al.*, 2010). However, in the same study, there was no significant increase in K levels was observed at below 20 cm soil layer after three years (Mulidzi *et al.*, 2019). A study by Howell *et al.*, 2018 showed no clear trends with regard soil K in the 60-90 cm as well as in the 90-120 cm soil layers after application of diluted WWW. Potassium concentrations were higher in the WWW irrigated soils having 7.78 mg/kg soil at the surface compared to 1.25 mg/kg soil for the control irrigated soil in the Napa Valley American region (Hirzel *et al.*, 2017). It has also been shown that irrigation with WWW can increase the levels of soluble and exchangeable forms of K more rapidly than conventional or inorganic fertilisers (Arienzo *et al.*, 2009). Thus, the application of WWW in the cropping systems may lead to increases in K concentrations in the topsoil.

#### **2.4.5 Calcium (Ca)**

Calcium concentrations were higher in the WWW irrigated soils having 6.63 mg/kg at the 20-40 cm soil layer compared to 4.38 mg/kg for the control irrigated soil in the Napa Valley American region in Northern California (Hirzel *et al.*, 2017). In contrast, Mulidzi *et al.* (2018) reported that the application of WWW did not increase soil Ca over two and half years of the

study period. A previous study showed that continuous irrigation with WWW that is high in K and Na could cause the soil exchange sites to be dominated by monovalent ions, thereby pushing bivalent ions such as Ca out of the exchange complex (Mosse *et al.*, 2012). Howell *et al.* (2018) reported that irrigation with WWW diluted up to 3 000 mg COD/L had no effect on soil Ca due to low amounts present in the WWW. Pastures irrigated with WWW for over 100 years increased soil Ca levels substantially compared to controls (Kumar *et al.*, 2006). Soil Ca concentration of pastures irrigated with undiluted WWW for 15 to 20 years increased, yet these increases were not as substantial as in pastures that had been irrigated for 100 years (Kumar *et al.*, 2006). Irrigation with WWW increase soil Ca.

#### **2.4.6 Magnesium (Mg)**

Pastures irrigated with undiluted WWW for over 100 years increased soil Mg (Kumar *et al.*, 2006). In contrast, WWW irrigated soils had the lowest Mg at the surface and increased at each lower depth (Hirzel *et al.*, 2017). A previous study showed that continuous application of WWW high in K and Na could cause the soil exchange sites to be dominated by monovalent ions, thereby pushing bivalent ions such as Mg out of the exchange complex (Mosse *et al.*, 2012). Quale *et al.* (2010) reported that soil Mg concentrations decreased following four years of WWW irrigation. Mg concentrations were higher in the WWW irrigated soils having 9.10 mg/kg at the 20-40 cm soil layer compared to 4.90 mg/kg soil for the control irrigated soil in Napa Valley American region in Northern California (Hirzel *et al.*, 2017). Over a study period where Kikuyu grass was irrigated with WWW, Mg concentration in all layers showed only limited fluctuation (Mulidzi *et al.*, 2018). Howell *et al.* (2018) reported that where diluted WWW (3 000 mg/L COD) was used for the irrigation of a vineyard in a sandy, alluvial soil, due to their low levels in the diluted WWW, soil Mg did not respond to levels of dilution of the WWW.

#### **2.4.7 Sodium (Na)**

Irrigation with WWW increased the Na levels in the 0-10 cm and in the 10-20 cm soil layers (Mosse *et al.*, 2013; Mulidzi *et al.*, 2019). Similarly, where diluted WWW was used for the irrigation of a vineyard in a sandy, alluvial soil, soil Na increased linearly as the level of WWW dilution decreased, particularly in the topsoil (Howell *et al.*, 2018). Winery wastewater irrigated soils contained significantly higher concentrations of Na (from 48.7 to 72.6 mg/kg soil) than the control irrigated soils (from 7.52 to 16.1 mg/kg soil) across all depths in the Napa Valley American region (Hirzel *et al.*, 2017). A high concentration of Na in the soil due to WWW application can reduce soil aggregate stability (Laurenson & Houlbrooke, 2012). Sodium tended to increase in the first two soil layers compared to the levels at the beginning of the study however, at the end of the study period, there was no increase in Na deeper than 20 cm depth (Mulidzi *et al.*, 2019). A study done by Mulidzi *et al.* (2018) reported that irrigation of Kikuyu grass with WWW increased Na level in the 0-10 cm and decreased in the 10-20 cm

layers. Compared to a vineyard that was irrigated with river water, soil Na<sup>+</sup> level was high in the vineyard soils irrigated with WWW (Kumar *et al.*, 2006). Similarly, Mosse *et al.* (2012) reported that soil Na was greatly higher compared to soil where no WWW was applied. The application of WWW increase soil Na.

#### **2.4.8 Extractable potassium and sodium percentages**

Mulidzi *et al.* (2015) reported that after application of diluted WWW, soil extractable potassium percentage (EPP') in the 0-10 cm soil layer was marginally higher than in the 10-20 cm soil layer, irrespective of clay content. According to Arienzo *et al.* (2009), a higher amount of EPP' is retained by soils higher in clay content than soils low in clay content following WWW irrigation. Mulidzi *et al.* (2018) reported that with the exception of the 0-10 cm soil layer, the soil EPP' showed a steady increase over the study period with the steepest increase occurring in the 60-90 cm soil layer after WWW irrigation. Extractable potassium percentage was approximately 12 %, 14 % and 8% in the 0–10 cm, 10–30 cm and 30–60 cm soil layers, respectively and after irrigation with WWW EPP' increased in the 0–10 cm and 10–30cm layers from the beginning of the measurement, while a large increase in the 30–60 cm layer (Liang *et al.*, 2021). Winery wastewater application increases soil EPP'.

The study by Mulidzi *et al.* (2019) found that extractable sodium percentage (ESP') was relatively low, it would probably not have caused serious soil physical deterioration. However, high soil ESP' increases the risk of soil physical properties deteriorating through clay dispersion which will lead to structural breakdown and blockage of soil pores and reduced soil permeability (Bond, 1999). In the four differently textured soils, the degree of ESP' accumulation in the 0-10 cm soil layer was higher than that in the 10-20 cm soil layer after irrigation with diluted WWW (Mulidzi *et al.*, 2015). Irrigation with diluted WWW increased soil ESP' increase substantially over four seasons (Mulidzi *et al.*, 2015). Gray (2012) reported a substantial increase in soil ESP' in the 0-7.5 cm soil layer of soils irrigated with WWW. Although the soil ESP' levels remained similar to the baseline after the majority of irrigations were applied at two plots in the Lower Olifants river region, a slight increase occurred below 30 cm soil depth after the winter rainfall period (Hoogenjik, 2019). Irrigation with WWW increases soil ESP'.

#### **2.4.9 Carbon (C)**

Soils irrigated with WWW had higher C concentration than control soils in the 0-20 cm than in the 20-40 cm and 40-60 cm soil layers (Hirzel *et al.*, 2017). In the Alexander Valley region in California, WWW irrigated soils contained more than twice as much C as the control soil on the surface (Hirzel *et al.*, 2017). Soil C decreased by 9.5% and 6.8% at 0-90 cm and 90-180 cm soil layers, respectively after three years of irrigation with diluted WWW (Howell *et al.*,

2018). After the irrigation with WWW, total organic C in the 30 cm depth soil samples had declined to approximately 40% of its initial value probably through degradation (Wendy *et al.*, 2010). Soil organic C in the 0-10 and 10-20 cm soil layers was substantially higher than 2% after irrigation with WWW (Mulidzi *et al.*, 2019), which is relatively high for soils of the Western Cape wine regions (Conradie, 1994). Where a vineyard with sandy alluvial soil in the Breede River region in South Africa was irrigated with diluted WWW results showed inconsistent trends with regards to soil organic carbon (OC) as affected by WWW dilution (Howell *et al.*, 2018). The OC content in the 0-10 cm layers was significantly higher compared to the one in the deeper layers which during soil classification, visual observation revealed that this layer was rich in organic matter (Mulidzi *et al.*, 2018). More studies regarding irrigation with WWW on soil C is needed.

## **2.5 Effects of winery wastewater on soil enzyme activities.**

### **2.5.1 $\beta$ -glucosidase**

Glycosidases are a group of enzymes that catalyse the hydrolysis of glycosides (Martinez & Tabatabai, 1997).  $\beta$ -glucosidase (cellobiase) hydrolyses maltose and cellobiose (Utobo & Tewari, 2015).  $\beta$ -glucosidase activity is closely related to soil organic matter, C cycling and other soil ecosystem functions (Nannipieri *et al.*, 2012). The soil enzyme is sensitive to environmental changes, and it can provide an initial sign of management alterations long before they can be determined by other routine techniques (Lagomarsino *et al.*, 2009, Nannipieri *et al.*, 2012).  $\beta$ -glucosidase's involvement in C cycling has remarkably facilitated its adoption for soil quality testing (Adetunji *et al.*, 2017). The  $\beta$ -glucosidase activity was significantly greater in the 0-10 cm than the 10-20 cm soil layers after irrigation with diluted WWW (Mulidzi & Wooldridge, 2016).  $\beta$ -glucosidase activities were higher in the WWW than in the municipal water treatments (Mulidzi & Wooldridge, 2016). Furthermore,  $\beta$ -glucosidase activity increased remarkably in various soils amended with sewage sludge and irrigated with WWW rather than municipal water (Kizilkaya & Bayrakli, 2005, Mulidzi & Wooldridge, 2016). Proper management of WWW may increase  $\beta$ -glucosidase activity and carbon cycling thereby improving soil health.

### **2.5.2 Phosphatase**

Phosphatases are a group of enzymes that catalyze the hydrolysis of esters and anhydrides of phosphoric acid (Condrón *et al.*, 2005). Main sources of phosphatase enzymes in the soil are plants and microorganisms (Adetunji *et al.*, 2017). Phosphatase play a crucial role in P cycling (García-Ruiz *et al.*, 2008). They are highly sensitive to environmental factors and soil management changes (García-Ruiz *et al.*, 2008; Adetunji *et al.*, 2020). Additionally, phosphatase activity has been shown to respond to organic and inorganic N inputs under

various cropping systems (Lemanowicz, 2011; Maseko & Dakora, 2013). Mulidzi & Wooldridge, (2016) showed that soil depth had no effect on phosphatase activity in any of the soils after irrigation with diluted WWW. There are few results on the impact of WWW on soil Phosphatase. Therefore, more research is required regarding WWW application on soil phosphatase.

### **2.5.3 Urease**

Urease catalyzes the hydrolysis of dihydroxyurea, hydroxyurea, and semicarbazide, with nickel as a co-factor (Alef & Nannipieri, 1995). Urease performs a critical role in N cycling (García-Ruiz *et al.*, 2008). Urease activity increased in four different vineyard soils treated with WWW rather than municipal water (Mulidzi & Wooldridge, 2016). Urease activity was greater in season 3 than in season 4, although the effects of season on Rawsonville sand and Stellenbosch granite were not significant (Mulidzi & Wooldridge, 2016). Average activities of urease were significantly greater in the 0-10 than the 10-20 cm depth interval (Mulidzi & Wooldridge, 2016).

## **2.6. Catch crop/cover crop effects on the soil where winery wastewater is used for irrigation**

Cover crops improve soil physical properties, chemical properties, biological processes, weed suppression and pest control (Blanco-Canqui *et al.*, 2015). Cover crops such as *Pallinup* oats (Oats), could be utilized in a cropping system to absorb and recycle soil nutrients such as K due to the interception ability of certain winter growing annual cover crops (Fourie *et al.*, 2015). Previous studies with WWW treatment systems have included the removal of nutrients by plants like Kikuyu (*Pennisetum purpureum*), lucerne (*Medicago sativa*) and flanker grass/Italian ryegrass (*Lolium multiflorum*), saltbush (*Atriplex spp*), wetland plants and certain cover crops (Zingelwa, 2004; Zingelwa & Wooldridge, 2009; Zingelwa & Wooldridge, 2010). Some cover crops, they may also be ideal for the removal of extra nutrients from the soil, while decreasing leaching and excess run-off due to the interception ability (Fourie *et al.*, 2015). Bezuidenhout (2012) indicated that Oats have the potential to extract significant amounts of N and K from the soil. Using pearl millet (*Pennisetum glaucum*) and Oats to intercept the elements deposited *via* the diluted WWW resulted in more N, K, P, Mg and Ca being removed from the soil than was applied by means of the WWW and fertiliser (Fourie *et al.*, 2020). Similarly, WWW was used efficiently for the commercial production of pearl millet, WWW application enhanced plant growth and yield (Khan *et al.*, 2012). According to Fourie *et al.* (2020), *Eragrostis curvula* has the potential to be considered as a catch crop for the exclusion of excess Na from WWW irrigated soils. The fodder beet reduced soil EPP' by 50%, indicating that it could also absorb K applied via WWW (Myburgh & Howell, 2014). Compared to other

soil management practices, cover crops improve grapevine performance (Fourie *et al.*, 2006; Fourie *et al.*, 2007; Fourie, 2011).

### **2.7. Effects of winery wastewater on catch crops**

The effect of WWW on pearl millet was tested in a glasshouse study by Mosse *et al.* (2010) and the results showed that the WWW did not have a negative effect on Pearl millet. Pearl millet intercepted substantial amounts of K in a field study where Pearl millet (*Pennisetum glaucum*) and *Avena sativa* cv. Pallinup (Pallinup oats) were used as catch crops in a vineyard irrigated with diluted WWW (Fourie *et al.*, 2015). Pearl millet succeeds in well-drained loam soils (Fourie *et al.*, 2020). Pearl millet has the potential to generate an income of R45 per bale, which would amount to R19 485 per ha under the prevailing conditions as a fodder crop with an average production of 433 bales per hectare when irrigated with WWW, (Fourie *et al.*, 2015).

Myburgh & Howell, (2014) reported that Pearl millet irrigated with diluted WWW could be a sustainable fodder crop, as the diluted WWW did not affect the above-ground N, P, Ca and Mg concentrations. The average production of Saia oats was 2.92 t/ha from 1993/94 to 2002/03 seasons on sandy soil after irrigation with WWW (Fourie *et al.*, 2005). Whereas Ochse (2015) reported the highest yield of 5.75 t/ha on oats in Rawsonville which is comparable to the 7.07 ton/ha obtained in the Robertson area by Fourie (2006). Irrigation with WWW did not affect the growth of the Oats negatively for two seasons (Ochse, 2015). Sorghum could be used as an in effect accumulator of additional nutrients, common in WWW as the crop removed large amounts of P and K in manured sites (Fourie *et al.*, 2020). Where excess Na is applied *via* wastewater application, the use of fodder radish and beet as catch crops could be viable (Fourie *et al.*, 2020). Given the extensive accumulation of Na in the roots and stems, cowpeas in a catch/cover crop system inside the vineyard could potentially decrease the accumulation of Na added *via* application of WWW (Fourie *et al.*, 2020).

### **2.8. Grapevine responses to irrigation with winery wastewater**

There were no substantial differences in ripeness parameters, yield, and vegetative growth after one year where “simulated” WWW was used for vineyard irrigation (Mosse *et al.*, 2013). In a glasshouse study, in which WWW was applied either undiluted or diluted in different ratios to potted grapevines, petiole K<sup>+</sup> contents were below the recommended levels, irrespective of dilution level (Kumar *et al.*, 2014). There were also treatments in which solutions of different K<sup>+</sup> and Na<sup>+</sup> nutrient loads were used to irrigate grapevines in addition to the different levels of WWW dilution. Increasing K<sup>+</sup> concentrations increased petiole K<sup>+</sup> (Kumar *et al.*, 2014). In two paired field trials in which grapevines were irrigated with either raw water or WWW, there was no difference in the sensorial evaluation of the wines (Kumar *et al.*, 2014). Furthermore, where

grapevines were irrigated with WWW, wine Na<sup>+</sup> levels were still below 100 mg/L, whereas wine K<sup>+</sup> ranged from 1 220 mg/L to 1 400 mg/L and was within industry norms for red wines in Australia (Kumar *et al.*, 2014). The treated wastewater did not have a negative effect on the table grapes after six years of trial in Southern Israel (Netzer *et al.*, 2014).

## **CHAPTER THREE**

### **RESEARCH DESIGN AND METHODOLOGY**

This study forms part of a large Agricultural Research Council (ARC) project titled “Evaluation of selected grass broadleaf crops suitable for fodder as interception crops where diluted WWW is re-used for irrigation”.

#### **3.1 Experimental layout**

The trial was carried out in a Shiraz/110 Richter vineyard established on a sandy loamy soil located at Nietvoorbij experimental farm in Stellenbosch (33° 55' 02", 18° 52' 04"). Grapevines were spaced 1.2 m in the row and 2.4 m between rows. The vineyard was divided into 104 m<sup>2</sup> plots, each containing 10 experimental vines, five in each of two adjacent rows. Eight treatments (six irrigated with diluted WWW and two irrigated with raw water) were applied (Table 3.1), each replicated randomly in three blocks (Table 3.2). Experimental grapevines in each plot were separated from those in the next plot by four buffer vines, with one buffer row between rows containing experimental vines.



**Table 3.1. Treatments applied in the micro-sprinkler irrigated Shiraz/110 Richter vineyard irrigated full surface at Nietvoorbij experiment farm**

<b>Treatment no.</b>	<b>Summer catch crops</b>	<b>Winter cover crops</b>	<b>Irrigation</b>
1	Pearl millet	Saia oats	Diluted winery wastewater
2	Pearl millet	Mixture of N-fixing species	Diluted winery wastewater
3	Dolichos beans	Saia oats	Diluted winery wastewater
4	Dolichos beans	Mixture of N-fixing species	Diluted winery wastewater
5	Chicory	Saia oats	Diluted winery wastewater
6	Chicory	Mixture of N-fixing species	Diluted winery wastewater
7	No cover crop	Saia oats	Raw water
8	No cover crop	Mixture of N-fixing species	Raw water

**Table 3.2 Experimental layout and catch crops/cover crops treatments irrigated with diluted winery wastewater and raw water. (R = replication, T = treatment)**

		<b>T8R2</b> No cover crops N-fixing		
		<b>T3R2</b> Dolichos beans Oats		
			<b>T4R2</b> Dolichos beans N-fixing	
		<b>T1R2</b> Pearl millet Oats		
<b>T5R1</b> Chicory Oats	<b>T7R1</b> No cover crops Oats	<b>T6R2</b> Chicory N-fixing	<b>T7R3</b> No cover crops Oats	<b>T5R3</b> Chicory Oats
<b>T8T1</b> No cover crops N-fixing	<b>T1R1</b> Pearl millet Oats	<b>T2R2</b> Pearl millet N-fixing	<b>T8R3</b> No cover crops N-fixing	<b>T2R3</b> Pearl millet N-fixing
<b>T6R1</b> Chicory N-fixing	<b>T2R1</b> Pearl millet N-fixing	<b>T7R2</b> No cover crops Oats	<b>T6R3</b> Chicory N-fixing	<b>T4R3</b> Dolichos beans N-fixing
<b>T3R1</b> Dolichos beans Oats	<b>T4R1</b> Dolichos beans N-fixing	<b>T5R2</b> Chicory Oats	<b>T3R3</b> Dolichos beans Oats	<b>T1R3</b> Pearl millet Oats

### 3.2. Application of irrigation

Micro-sprinklers were used as a type of irrigation. Winery wastewater was collected from the Leeuwenkuil winery. The vineyard is located on the ARC experimental farm in Stellenbosch and the farm planning committee gave permission for the project because of the use of the WWW for irrigation. The chemical oxygen demand (COD) and electrical conductivity (EC) of undiluted WWW was diluted to obtain COD and EC of less than 5 000 mg/L and 200 mS/m, respectively, to abide by the current laws specified by the General Authorization (Department of Water Affairs, 2013) on the quality of irrigation water. A water sample to determine the element content of the WWW was collected after dilution and prior to sprinkler irrigation. Irrigation of the vineyard commenced when there was available WWW. Due to the Level 5 lockdown in 2020, unfortunately, no WWW irrigations could be applied in April. In 2017/18, 2018/19 and 2019/20 seasons, eight, three and four irrigations with diluted WWW were applied, respectively (Table 3.3). The raw water treatments were irrigated with raw (clean) water from the local dam.

**Table 3.3. The time of application, volume applied, electrical conductivity of the irrigation water ( $EC_{iw}$ ) and chemical oxygen demand level (COD) of diluted winery wastewater irrigations applied to the Shiraz/110 Richter vineyard at Nietvoorbij, Stellenbosch in the 2017/18, 2018/19 and 2019/20 seasons**

Season	Time of irrigation	Volume applied (mm)	EC (mS/m)	COD (mg/L)
2017/18	28 February	15.31	226	2 915
	08 March	15.31	158	1 635
	15 March	15.31	149	2 755
	22 March	15.31	159	2 835
	27 March	15.31	151	3 525
	04 April	15.31	84	865
	10 April	15.31	231	3 160
	18 April	15.31	43	1 674
	<b>Average</b>	<b>15.31</b>	<b>150</b>	<b>2 545</b>
2018/19	19 March	15.31	205	2 171
	26 March	15.31	225	1 610
	02 April	15.31	213	820
	<b>Average</b>	<b>15.31</b>	<b>214</b>	<b>1 534</b>
2019/20	19 March	15.31	185	2 350
	11 May	15.31	198	2 950
	20 May	15.31	200	2 980
	26 May	15.31	162	2 000
	<b>Average</b>	<b>15.31</b>	<b>186</b>	<b>2 570</b>

### 3.3. Soil sampling and soil chemical analyses

Soil samples were collected from the 0-15 cm, 15-30, 30-60 and 60-90 cm soil layers inside the rows of the vineyards during May (after the harvesting of the catch crops) and September (after harvesting the winter growing cover crops) over the three seasons. Samples were air dried and passed through a 2 mm mesh sieve. Soils analyses were carried out by a commercial laboratory (Labserve). The samples were analysed to determine pH, EC<sub>e</sub>, P, K, Ca, Mg, Na, C, Soluble-S, EPP' and ESP'.

The pH was determined in a 1 M potassium chloride (KCL) suspension. The Ca, Mg, K and Na were extracted with 1 M ammonium acetate at pH 7. The cation concentrations in the extracts were determined by means of atomic emission using an optical emission spectrometer (Varian ICP-OES).

The EPP' was calculated as follows (Mulidzi, 2016):

$$EPP' = (K \div S) \times 100 \quad (\text{Eq. 1})$$

where K is the extractable K (cmol<sup>(+)</sup>/kg) and S is the sum of the basic cations (cmol<sup>(+)</sup>/kg). To get an indication of the sodicity status of the soil, the ESP' was calculated as follows:

$$ESP' = (Na \div S) \times 100 \quad (\text{Eq. 2})$$

where Na is the extractable Na (cmol<sup>(+)</sup>/kg) and S is the sum of the basic cations (cmol<sup>(+)</sup>/kg). Phosphorus was determined according to the Bray No. 2 method. That is extraction with 0.03 M NH<sub>4</sub>F (ammonium fluoride) in 0.01 M HCl (hydrochloric acid). The P concentration in the extract was determined by means of atomic emission as mentioned above. The soil cation exchange capacity (CEC) was determined using 0.2 M ammonium acetate (pH=7 as extractant of exchangeable cations) method as described by The Non-affiliated Soil Analyses Work Committee (1990).

### 3.4. Cover/Catch crop growth

The dry matter production (DMP) of the summer catch crops was determined in May 2018, 2019, and 2020 and the DMP of the winter cover crops were determined in September 2018, 2019, and 2020. This was done by using the method described by Fourie *et al.* (2001). Briefly, the above-ground vegetative growth was sampled in a 0.5 m<sup>2</sup> sub-plot randomly selected in each experimental plot. The DMP was measured as described by (Fourie & Theron, 2014). Plant samples were oven-dried for 48 hours at 65°C. Treatments were slashed and harvested two times, in July (summer catch crops) and September (winter cover crops). The DMP per hectare was calculated as follows (Fourie & Theron, 2014):

$$DMP = ODM \times 2 \div 100 \quad (\text{Eq. 3})$$

Where DMP is in t/ha, ODM is oven-dry mass sampled per plot (g/0.5 m<sup>2</sup>) and 100 is the conversion factor from g/m<sup>2</sup> to t/ha. As no harvester suitable to be utilized in a vineyard was available, the treatments were slashed full surface directly after sampling. Thereafter the

whole surface was raked, and the residues removed to prevent the elements absorbed by the above-ground growth from being returned to the soil through decomposition.

### **3.5. Chemical composition of the catch and cover crops**

A sample was collected by harvesting the above-ground growth in a 0.5 m<sup>2</sup> sub-plot randomly chosen in the experimental plot. These samples were analysed for macro-nutrients as described by Fourie & Theron (2014). The chemical composition of the summer catch crops was determined in May 2019 and 2020 whereas the winter cover crops were determined in September 2018, 2019, and 2020. After sampling, the leaf blades were washed with a Teepol® solution, rinsed with de-ionised water and dried overnight at 65°C in an oven. The dried leaves were then milled and ashed at 480°C, shaken up in a 50:50 hydrochloric acid (HCl) (32%) solution for extraction through filter paper (Campbell & Plank, 1998; Miller, 1998). The cation (N, P, K, Mg, Ca & Na) content of the extract was measured with a Varian ICP-OES optical emission spectrometer.

### **3.6. Amounts of the macro-elements intercepted by the catch crops and cover crops**

The amounts of the different macro-elements intercepted by the catch and cover crops were calculated by multiplying the DMP of the species with the concentration of the different elements (B) in the samples harvested for analyses. The amount of N, K, Mg, and Ca intercepted were calculated as follows (Fourie & Theron, 2014).

$$A = \text{DMP} \times B \times 10 \quad (\text{Eq. 4})$$

where A is the amount of element intercepted (kg/ha), DMP is the dry matter production in t/ha, B is the plant element concentration (%) and 10 is the conversion factor to obtain kg/ha. The amount of Na and micro-elements intercepted were calculated as follows:

$$A = \text{DMP} \times B \div 1\,000 \quad (\text{Eq. 5})$$

where A is the amount element intercepted (kg/ha), DMP is the dry matter production in t/ha, B is the plant element concentration (mg/kg) and 1000 is the conversion factor to obtain kg/ha.

### **3.7. Grapevine cane mass**

To quantify growth vigour, cane mass at pruning (July) was weighed per experiment plot using a hanging balance. Shoot mass per plot (kg) was converted to tons per hectare. Cane mass was determined in July 2018, 2019, and 2020.

### **3.8. Grape leaf and petiole analyses**

Leaf-blades and petiole samples were collected in November 2018, 2019, and 2020. Leaf samples were collected at flowering from locations directly opposite clusters. Leaves and petioles were separated immediately after sampling. The leaves and petioles were analysed as described by Howell, (2016).

### **3.9. Grapevine yield**

At harvest in March 2018, 2019, and 2020, all bunches of the experimental grapevines on each plot were picked and counted. Grapes were weighed using top loader mechanical balance to obtain the total mass per experiment plot. The number of bunches per grapevine was calculated by dividing the total number of bunches per plot by the number of experiment grapevines per plot. Grape mass per grapevine (kg/grapevine) was calculated and converted to yield (t/ha). To determine berry mass at harvest, ten randomly selected bunches were picked from each experiment plot for all the treatments. Ten berries were sampled from each of these bunches to obtain a sample of 100 berries. Berries mass was determined by weighing the samples using an electronic balance.

### **3.10. Grape characteristics**

Grape juice was analysed in March 2018, 2019, and 2020 after the harvest of the grapes. A representative sample (approximately one bunch per experimental vine) from each plot was crushed in a hydraulic press. The free-run juice was analysed for sugar content (temperature compensated Abbe refractometer), total titratable acid (50 ml juice titrated with 0.333 NaOH to pH 7.0 and expressed as g tartaric acid/L) and pH (654 Metrohm pH meter). Total juice N was determined using an automated colorimetric method (The Non-affiliated Soil Analysis Work Committee, 1990). Total P, K, Ca and Mg concentrations in the juice was determined by atomic absorption spectrophotometry, following digestion with nitric acid/perchloric acid.

### **3.11. Soil sampling and analysis for microbiological determination**

Soil sampling was done before irrigations commenced in March 2020, and after specific WWW irrigation applications in the 2019/20 season. Samples were also collected after the winter rainfall period. Soil samples were collected from 0-15 cm and 15-30 cm soil depths in all treatments using an auger that was sterilized in 70% ethanol to prevent contamination between samples. Soil samples were air-dried and sieved with a 2 mm mesh sieve. All microbiological analyses were carried out at the soil microbiology laboratory located in the Soil and Water Science division, ARC Infruitec-Nietvoorbij.

#### **3.11.1 Soil enzyme analyses**

The  $\beta$ -glucosidase activity was determined in field-moist soil in a reaction mixture containing 1.0 g soil, 0.25 mL toluene, 1.0 mL 25 mM p-nitrophenyl- $\beta$ -D-glucopyranoside (as substrate), and 4.0 mL Modified Universal Buffer (MUB) at pH 6.0 (Eivazi & Tabatabai, 1988). The mixture was incubated at 37°C for 60 min after which the reaction was terminated by adding 1.0 mL of 0.5 M CaCl<sub>2</sub> and 4.0 mL of 0.1 M, pH 12, tris (hydroxymethyl) aminomethane buffer. The amount of p-nitrophenol liberated during enzymatic hydrolysis was determined at 410 nm with a digital UV-Vis spectrophotometer by reference to a calibration curve corresponding to a p-nitrophenol standard (Sigma-Aldrich) incubated with each soil under the same conditions as

the samples, and after subtracting the absorbance values of the control. In the standard samples, the substrate was not added until after the reaction was stopped, immediately before filtration of the resulting soil suspension through Whatman no. 2V filter paper.

Acid phosphatase (EC 3.1.3.2) activity was determined by the method of Tabatabai & Bremner (1969) except that the reaction mixture consisted of 1.0 mL 25 mM p-nitrophenol phosphate as substrate, 4.0 mL MUB and 0.25 mL toluene, and that the released p-nitrophenol was extracted with 4.0 mL of 0.5 M NaOH at pH 6.5. Activities of  $\beta$ -glucosidase and of acid phosphatase were expressed as  $\mu\text{g}$  p-nitrophenol g/h.

Urease activity (EC 3.5.1.5) was determined by the unbuffered method of Kandeler & Gerber (1988). 2.5 mL of non-buffered urea solution (80 mM) were added to each 5.0 g field-moist soil sample which was then incubated for 2.0 h at 37°C. Controls received deionized water. The  $\text{NH}_4^+$  released by the action of the enzyme on its substrate was extracted with 50 mL KCl solution (1 N KCl and 0.01 N HCl). The solutions were shaken for 30 min on an orbital shaker. Determinations were based on the reaction of sodium salicylate with  $\text{NH}_4^+$  in the presence of sodium dichloroisocyanurate. Extinction was measured at 690 nm with a digital UV–Vis spectrophotometer against the reagent blank. The  $\text{NH}_4^+$  content was calculated by reference to a calibration curve obtained with standards containing 0, 1.0, 1.5, 2.0 and 2.5 mg  $\text{NH}_4^+$  per mL. Sodium nitroprusside was used as a catalyst. The activity was expressed as  $\mu\text{g}$  ammonium g/2 h. Two replicates and one control from each soil were analyzed for the  $\beta$ -glucosidase and acid phosphatase assays, and three replicates and one control for the urease determinations. Enzyme activities were expressed on a moisture-free basis. Soil moisture content was determined from the loss in weight after drying at 105°C for 24 h.

### **3.12. Statistical procedure**

The experimental design was a randomised complete block design with eight cover/catch crop treatments and three block replicates. The data were subjected to analysis of variance (ANOVA) using General Linear Models Procedure (PROC GLM) of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Shapiro-Wilk test was performed to verify the normality of standardized residuals (Shapiro and Wilk, 1965). Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott & Longnecker, 2010). A probability level of 5% was considered significant for all significance tests.

## CHAPTER FOUR

### RESULTS

#### **4.1. Response of soil chemical properties to diluted WWW and raw water where different cover crops and catch crops were cultivated over three seasons (2017 - 2020)** **2017/2018 season**

In 0-15 cm soil layer, the use of diluted WWW as a source of irrigation rather than raw water (May 2018) generally increased soil pH, K and soil EPP' (Appendix A). However, there were no significant differences in soil P, Ca, Mg, Na, C, S and EC<sub>e</sub> in the 0-15 cm soil layer. In the 15-30 cm layer, none of the soil chemical parameters responded to treatments except for ESP' where T4 was significantly higher than T1, T7 and T8. In the 30-60 cm soil layer, only soil P and Ca differed substantially between treatments compared to the other soil chemical parameters. In the 60-90 cm soil layer, treatments had no significant impact on all the soil chemical properties except on soil P where T7 irrigated with raw water was significantly higher than WWW treatments T1, T2 and T6 (Appendix A). In general, soil P somewhat decreased as the soil depths increased.

After winter rainfall (September 2018), soil pH in the 0-15 cm soil layer still differed significantly among treatments with T3, T4, T5 and T6 being significantly higher than T7 (Appendix B). Soil P content was significantly higher in T3 than T2, T6 and T8. Soil K also differed significantly among treatments with T3 being higher than T1, T2, T6, T7 and T8. The ESP' showed substantial differences between the treatments with T5 being significantly higher than all the other treatments except for T3. Compared to other soil chemical parameters in the 15-30 cm soil layer, only soil P and K responded to treatments. Soil P in T7 was significantly higher than T1, T2, T3, T4 and T8 and marginally higher than T6 and T5. The soil K in T3 was significantly higher than T1, T2, T5, T7 and T8 and marginally higher than T4 and T6. In the 30-60 cm soil layer, only soil P significantly responded to treatments where P was substantially higher in T3 than the other treatments. In the 60-90 cm soil layer, raw water and WWW had no significant effect on the tested soil chemical parameters except for soluble S where T1 was higher than T3, T4, T7 and T8. In general, out of the soil chemical properties examined in the 2017/2018 season, only soil P appeared to consistently respond to treatments across soil depth, except for 60 - 90cm. Soil P concentration also seems to generally reduce as the soil depths increase (Appendix B).



### **2018/2019 season**

In May 2019, irrigation with diluted WWW and raw water generally had no significant effect on soil chemical properties across all soil depth, except EPP at 15-30cm and 30-60cm (Appendix C). Soil K, Mg, Na and EPP' tended to be higher where diluted WWW was used for vineyard irrigation at 0 -15cm soil layer (Appendix C). A similar trend was observed for soil K and soil Na in the 15-30 cm soil layer. There was a significant difference in soil EPP' in the 15-30 cm soil layer with T1 (13.43%) being higher than T2 (7.06%), T6 (7.91%), T7 (5.8%) and T8 (5.65%). The EPP' of T1 was substantially higher than most of the other treatments in the 30-60 cm soil layer. In the 60-90 cm soil layer, soil K tended to be higher where diluted WWW was used for vineyard irrigation. The EPP' in samples collected after the cultivation of the winter cover crops and winter rainfall differed substantially between treatments in both the 0-15 cm and 15-30 cm soil layers (Appendix D). At 0-15 cm soil layer, T1 (11.21%) irrigated with diluted WWW was significantly higher than T7 (6.61%) irrigated with raw water and same trend at 15-30 cm soil layer where T1 (10.43%) was significantly higher than T7 (5.82%). There was no significant effect in the 30-60 cm and 60-90 cm soil layers after irrigation with either diluted WWW or raw water.

### **2019/2020 season**

In soil samples collected in May 2020 after the application of WWW, soil pH, P, EPP', and soluble S in the 0-15 cm soil layer differed significantly between treatments (Appendix E). In general, the use of diluted WWW as a source of irrigation water rather than raw water increased soil pH, K and EPP'. In the 15-30 cm soil layer, soil pH, P, K, EC<sub>e</sub> and EPP' differed significantly between treatments. Soil pH differed between treatments with T5 (5.97) irrigated with diluted WWW being significantly higher than T8 (5.17) irrigated with raw water. Similarly, soil K and EPP' differed between treatments with T1 irrigated with diluted WWW being significantly higher than T8 which was irrigated with raw water. However, irrigation with raw water (T8) showed significantly higher soil P and EC<sub>e</sub> compared to irrigation with diluted WWW (T5). There was no significant difference between soil Ca, Mg, Na, ESP', soluble s and C.

In the 30-60 cm soil layer, soil K ranged from 0.15 cmol<sup>(+)</sup>per kg for T7 to 0.51 cmol<sup>(+)</sup>per kg for T5. There was a significant difference in soil K with T5 being significantly higher than T7. In the 60-90 cm soil layer, soil K and Na differed significantly between treatments with T6 (0.37 cmol<sup>(+)</sup>) (0.30 cmol<sup>(+)</sup>) irrigated with diluted WWW being higher than T7 (0.16 cmol<sup>(+)</sup>) (0.12 cmol<sup>(+)</sup>) irrigated with raw water respectively. Soil P was significantly higher in T7 (22.67 mg/kg) than in T6 (5.30 mg/kg). Same results were found in soluble sulphur. There was a significant difference in soil P, K, Na, soluble S and EPP' in the 60-90 cm soil layer. Soil P differed significantly between treatments with T7 (22.67 mg/kg) irrigated with raw water being

higher than T6 irrigated with diluted WWW. However, soil K and Na differed significantly with T6 irrigated with diluted WWW was significantly higher than T7 irrigated with raw water. Similarly, soil soluble S and EPP' differed significantly between treatments with T5 irrigated with diluted WWW being higher than T7 irrigated with raw water. There was no significant difference in soil pH, Ca, Mg, C, ECe and ESP' in this soil layer.

#### **4.2. Response of soil enzyme activities to diluted WWW and raw water where different cover crops and catch crops were cultivated over one season (2020)**

In February 2020 prior to irrigation with either diluted WWW or raw water, there was no significant difference in soil enzyme activities ( $\beta$ -glucosidase, phosphatase and urease) at 0-15 cm and 15-30 cm soil layers (Appendix F). Diluted WWW, raw water and summer catch crops had no significant impact on soil enzyme activities in May 2020 (Appendix G). A similar trend happened after winter rainfall in September 2020 where winter rainfall and winter cover crops had no significant effect on soil enzyme activities (Appendix H). In general, irrigation with either diluted WWW, raw water and cultivation of catch/cover crops had no effect on soil enzyme activities (Appendix F).

#### **4.3. Diluted WWW and raw water effect on summer catch crop and winter cover crop growth over the three seasons (2018-2020)**

After irrigation of the vineyard with diluted WWW, T3 and T4 (Dolichos beans) produced the highest DMP compared to T1 and T2 (Pearl millet) summer catch crop (Table 4.1) in May of 2018 2019 and 2020. Unfortunately, the Chicory catch crop did not grow at all in the 2019/20 season. With regard to the winter cover crops, their DMP was substantially higher where N-fixing winter cover crops (i.e. T2, T4, T6 and T8) were cultivated compared to the oats winter cover crop (T1, T3, T5 and T7) in 2018 and 2019. However, T1, T3, T5 and T7 (Oats) winter cover crop had a higher DMP compared to T2, T4, T6, and T8 (N-fixing) winter cover crop in September 2020.

**Table 4.1. The dry matter production (DMP) of the catch- and cover crops harvested after irrigation with diluted WWW, raw water and after winter rain fall over the three seasons (2018-2020)**

Treatment no.	DMP (t/ha)					
	2018		2019		2020	
	May (Summer catch crop)	Sep (Winter cover crops)	May (Summer catch crop)	Sep (Winter cover crops)	May (Summer catch crop)	Sep (Winter cover crops)
1	0.00 b	0.63 c	0.30 bc	0.92 b	0.22 a	1.84 ab
2	0.00 b	4.18 ab	0.29 bc	3.71 a	0.37 a	0.34 c
3	0.52 a	0.62 c	1.07 ab	1.05 b	1.59 a	2.08 a
4	0.23 ab	2.95 b	1.22 a	3.23 a	1.68 a	0.77 bc
5	N/A	0.82 c	0.17 c	1.26 b	N/A	1.85 ab
6	N/A	4.74 a	0.06 c	3.18 a	N/A	0.61 c
7	N/A	1.14 c	N/A	0.80 b	N/A	1.45 abc
8	N/A	4.04 ab	N/A	3.01 a	N/A	0.52 c

Refer to Table 3.1 for details of treatments.

Values followed by the same letter within a column do not differ significantly ( $p \leq 0.05$ ).

N/A denotes No catch crop treatment.

#### **4.4. Chemical compositions of winter cover crops and summer catch crops in response to irrigation with diluted WWW in the vineyard over the three seasons (2018-2020)**

##### **4.4.1. Winter cover crops**

The chemical composition of the winter cover crops is given in Table 4.2. The N, P, K, Ca, Mg and Na levels in the winter cover crops differed significantly ( $p \leq 0.05$ ) between treatments in the 2018 and 2020 seasons after winter rainfall (Table 4.2). In contrast, there was no significant effect of WWW on N, P, K, Ca, Mg and Na levels in the 2019 season. Considering the effect of the winter cover crops in the 2018 and 2020 seasons, T2, T4, T6 and T8 (N - fixing) had significantly higher N, P, K, Ca, Mg and Na concentrations compared to T1, T3, T5 and T7 (Oats).

##### **4.4.2. Summer catch crops**

In May 2019 after irrigation with diluted WWW, chemical composition (N, P, K, Ca and Mg) of the summer catch crops differed significantly between treatments (Table 4.3). The N content in T3, T4 (Dolichos) was significantly higher than T5, T6 (Chicory) and T1, T2 (Pearl millet). T2 (Pearl millet) had significantly higher P and K content than T3 (Dolichos) and T6 (Chicory). In contrast, T6 (Chicory) had significantly higher Ca, Mg and Na content than T1 (Pearl millet) and T3 (Dolichos). In May 2020 after irrigation with diluted WWW, T1 (Pearl millet) had significantly higher K and Na content than T3 (Dolichos). In contrast, T1 had significantly lower Ca content than T3 (Dolichos). Irrigation with diluted WWW had no significant impact on the N, P and Mg chemical composition of the summer catch crops in May 2020. Note that in May 2020 there was no cultivation of Chicory.

**Table 4.2. The macro-nutrient of the winter cover crops determined in the vineyard after winter rainfall over the three seasons (2018 – 2020)**

Season	Treatment no.	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg/kg)
Sep 2018	1	1.84 b	0.28 b	2.30 b	0.35 b	0.10 c	2159 bc
	2	3.76 a	0.46 a	4.38 a	1.16 a	0.36 ab	3254 ab
	3	1.60 b	0.29 b	2.34 b	0.37 b	0.11 c	1964 c
	4	3.81 a	0.47 a	4.22 a	1.08 a	0.33 b	2361 bc
	5	1.58 b	0.29 b	2.46 b	0.34 b	0.10 c	1486 c
	6	3.62 a	0.41 a	4.09 a	1.10 a	0.32 b	2322 bc
	7	1.52 b	0.23 b	2.29 b	0.36 b	0.10 c	1903 c
	8	3.63 a	0.45 a	3.85 a	1.25 a	0.37 a	3585 a
Sep 2019	1	1.42 a	0.38 a	3.05 a	0.23 a	0.12 a	718 a
	2	3.15 a	0.31 a	3.17 a	1.07 a	0.30 a	1357 a
	3	1.41 a	0.39 a	3.12 a	0.26 a	0.13 a	626 a
	4	3.04 a	0.32 a	3.32 a	1.06 a	0.30 a	1560 a
	5	1.34 a	0.39 a	3.02 a	0.23 a	0.12 a	597 a
	6	3.06 a	0.30 a	2.88 a	1.18 a	0.31 a	1325 a
	7	1.41 a	0.39 a	2.93 a	0.26 a	0.14 a	808 a
	8	2.91 a	0.28 a	2.90 a	1.14 a	0.29 a	1560 a
Sep 2020	1	1.45 c	0.22 bc	1.96 cd	0.20 b	0.10 c	537 de
	2	3.12 a	0.29 ab	2.81 ab	0.94 a	0.24 b	1740 ab
	3	1.13 c	0.22 bc	1.95 cd	0.17 b	0.09 c	703 de
	4	2.40 b	0.30 a	2.41 bc	0.89 a	0.23 b	1109 cd
	5	1.35 c	0.19 c	1.80 d	0.18 b	0.09 c	445 e
	6	3.29 a	0.30 a	2.93 a	0.91 a	0.25 ab	1593 bc
	7	1.32 c	0.17 c	1.65 d	0.24 b	0.10 c	1043 cd
	8	3.13 a	0.28 ab	2.45 b	0.93 a	0.27 a	2190 a

Refer to Table 3.1 for details of treatments.

Values followed by the same letter within a column do not differ significantly ( $p \leq 0.05$ ).

**Table 4.3. The macro nutrients of the summer catch crops determined in the vineyard after irrigation with diluted WWT over the two seasons (2019 & 2020)**

Season	Treatment no.	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg/kg)
May 2019	1	1.55 d	0.41 ab	2.77 c	0.40 c	0.34 d	391 c
	2	1.87 c	0.43 a	4.52 a	0.42 c	0.44 c	647 c
	3	3.18 a	0.27 cd	2.56 cd	2.34 b	0.45 c	293 c
	4	2.85 b	0.29 bcd	2.19 d	2.80 ab	0.39 cd	326 c
	5	2.10 c	0.37 abc	2.94 c	2.24 b	0.58 b	5540 b
	6	2.70 b	0.17 d	3.99 b	3.04 a	0.69 a	1009 a
	7	N/A	N/A	N/A	N/A	N/A	N/A
	8	N/A	N/A	N/A	N/A	N/A	N/A
May 2020	1	2.40 a	0.22 a	2.66 a	0.48 b	0.33 a	738 a
	2	2.30 a	0.23 a	2.84 a	0.43 b	0.36 a	640 a
	3	2.77 a	0.22 a	1.30 b	1.65 a	0.26 a	227 b
	4	2.85 a	0.21 a	1.27 b	1.88 a	0.29 a	248 b
	5	N/A	N/A	N/A	N/A	N/A	N/A
	6	N/A	N/A	N/A	N/A	N/A	N/A
	7	N/A	N/A	N/A	N/A	N/A	N/A
	8	N/A	N/A	N/A	N/A	N/A	N/A

Refer to Table 3.1 for details of treatments.

Values followed by the same letter within a column do not differ significantly ( $p \leq 0.05$ ).

N/A denotes No catch crop treatment.

#### **4.5. Diluted WWW, raw water and winter rainfall effect on nutrient content of leaf petioles of the grapevine for two seasons (2018-2019)**

Diluted WWW and raw water had no significant effect on N, P, K and Ca content of grapevine leaf petioles in November 2018 (Appendix I). However, there was a significant impact on Mg and Na content of leaf petiole after irrigation with diluted WWW and raw water, (T7) (1.09%) irrigated with raw water had significantly higher Mg level than T4 (0.80%) irrigated with diluted WWW. Na level was significantly higher in T4 (579 mg/kg) than in T7 (478 mg/kg). There was no significant difference in N, P, Ca, Mg and Na of leaf petiole.

#### **4.6 Diluted WWW, raw water and winter rainfall effect on nutrient content of leaf blades of the grapevine for two seasons (2018-2019)**

Diluted WWW and raw water had no significant impact on N, P, K and Na content of leaf blades in November 2018 (Appendix J). T7 irrigated with raw water had significantly higher Ca and Mg content than T4 and T3 irrigated with diluted WWW. However, in 2019 there were no significant difference in N, P, K, Ca and Mg content of leaf blades. Na level was significantly higher in T2 than T8 and T3.

#### **4.7. Diluted WWW and raw water effect on the pH, total titratable acids (TTA) and sugar content of the juice for three seasons (2018-2020)**

There was no significant impact on the pH, total titratable acids (TTA) and sugar content of the juice after irrigation with either diluted WWW or raw water over the three seasons (Appendix K).

#### **4.8. Diluted WWW and raw water effect on the shoot mass, yield and berry mass of the grapevine for three seasons (2018-2020)**

Grapevine shoots and berry mass treatments did not differ significantly over the three seasons (Appendix L). At harvest in March 2018 and 2020, grapevine yield did not differ significantly between the treatments. However, there was a significant difference in grapevine yield between the treatments at harvest in March 2019. T4 and T5 showed significantly higher yields than T7, T8, T3 and T1.

#### **4.9. Macro nutrient content of the juice after irrigation with diluted WWW and raw water for three seasons (2018-2020)**

Treatments did not differ significantly after irrigation with diluted WWW or raw water at harvest in February 2018 (Appendix M). At harvest in March 2019, treatments affected juice K and Mg. It appeared that juice K and Mg were higher where diluted WWW was used for irrigation rather than where raw water was used in February 2020. In February 2020, treatments

affected juice Na. In 2020, it appeared that juice Na was higher where diluted WWW was used for irrigation rather than raw water.



## CHAPTER FIVE

### DISCUSSION

#### **5.1. Response of soil chemical parameters to diluted WWW and raw water where different cover crops and catch crops were cultivated over the three seasons (2018-2020)**

Results of this study showed that after three years of diluted winery wastewater (WWW) application in the vineyard, soil pH increased in the 0-15 cm and 15-30 cm soil layers. This is consistent with other studies where the application of WWW increased soil pH from 4.6 to 5.0 in the top-soil and from 5.0 to 5.3 in the sub-soil (Shilpi *et al.* 2018; Mulidzi *et al.*, 2019). Mulidzi *et al.* (2015) reported that soil pH increased when irrigated with WWW, regardless of the types of the soil (Mulidzi *et al.*, 2015). Similarly, in two case studies where pastures and a vineyard were irrigated with WWW, soil pH increased (Kumar *et al.*, 2014). In contrast, Howell, (2016) reported that after irrigation with WWW, there were no clear trends in soil pH that could be related to the different levels of WWW dilution compared to the river water control. In the current study, the trend for higher soil pH where diluted WWW was used for vineyard irrigation was still observed after winter rainfall. Increase in soil pH better nutrient balance for plant growth. Given that irrigation using WWW is likely to increase soil K and Na, soil pH will consequently increase via alkaline hydrolyses (Howell, 2016; Mulidzi, 2016). Thus, after wastewater application, excessive soil K together with relatively high winter rainfall in Stellenbosch induced alkaline hydrolysis. Therefore, soil pH still showed differences with regard to WWW irrigations after winter rainfall.

In the current study, irrigation with diluted WWW led to an increase in soil K in the 15-90cm soil layers. This was to be expected given that WWW contains high levels of K. It is highly likely that high soil K could lead to an increase in K uptake by grapevines, which could have negative effects such as grape musts with high pH, malate concentrations and poor colour (Mpelasoka *et al.*, 2003; Kodur, 2011). A number of other studies have also reported increased K due to irrigation with WWW (Quale *et al.*, 2010; Hirzel *et al.*, 2017; Mulidzi *et al.*, 2019). Soil surface K increased where WWW was used for irrigation of soil typical of the South Eastern Australia Riverine plains for three years (Quale *et al.*, 2010). Mulidzi (2016) reported that irrigation with diluted WWW increased K substantially in the 0-10 cm layer of four different soils over four simulated seasons in a pot study. Despite the cultivation of Kikuyu grass where a plot of land was irrigated with WWW, K levels increased in the 0-10 cm soil layer, and to some extent in the 10-20 cm soil layer, at the end of the harvest periods (Mulidzi *et al.*, 2019). In the current study, after winter rainfall, there were still treatment differences about the application of either raw or diluted WWW. This indicated that the winter rainfall was not enough to leach all the K from the soil. Howell, (2016) reported that when winter rainfall at Rawsonville was higher than the average of 300 mm, soil K after winter rainfall was much lower than after

irrigation with WWW. When winter rainfall was much lower, soil K levels were similar to levels after WWW irrigation. In one of the seasons of the study, there was an accumulation of soil K after winter rainfall bud break in some soil layers. Although there was no likely explanation for this trend, it could be possible that the roots of the catch crops absorbed K during WWW application. Due to favourable dry winter conditions these roots mineralized, releasing K but the rainfall was too low to leach away the K. This indicated less leaching under the prevailing conditions. Note that the quantification of interception crop root mineralization was beyond the scope of the current study.

For ECe, there were no consistent trends relating to the type of water used for irrigation or catch/cover crop treatments. Likewise, compared to the river water/control, there were no clear trends in soil ECe that could be related to the different levels of dilution in a study by Howell, (2016). However, in the Napa Valley American and Alexander Valley regions in California, WWW irrigated soils had higher ECe in the 20-40 cm and 40-60 cm soil layers than the control (Hirzil *et al.*, 2017). Similarly, soil ECe was higher where woodlots were irrigated with WWW compared to a control (Kumar *et al.*, 2009).

After WWW application, the soil EPP' in the 0-15 cm and 15-30 cm soil layers was consistently higher for treatments where WWW was used for irrigation rather than where raw water was used for irrigation. Liang *et al.* (2021) reported that after irrigation with WWW, soil EPP' increased in the 0-10 cm and 10-30 cm soil layers from the beginning of the measurement, whereas there was substantial increase in the 30-60 cm layer. Another study showed that EPP' tended to be lower after WWW application followed by an increase during winter (Mulidzi *et al.*, 2019). Similarly, Mulidzi *et al.* (2015) reported that after diluted WWW application, soil EPP' in the 0-10 cm soil layer was marginally higher than in the 10-20 cm soil layer, irrespective of clay content. According to Arienzo *et al.* (2009), a higher amount of EPP' is retained by soils that are higher in clay content than soils that are low in clay content following WWW irrigation. Mulidzi *et al.* (2018) reported that with the exception of the 0-10 cm soil layer, the soil EPP' showed a steady increase over the study period with the steepest increase occurring in the 60-90 cm soil layer after WWW irrigation. Therefore, irrigation with diluted WWW increases soil EPP which make the soil to be sodic resulting in dispersion of clay and silt particles in the soil collapsing the soil structure and blocking soil pores.

In the 60-90 cm soil layer, soil Na was higher where diluted WWW was used for irrigation compared to where raw water was used in the 2019/20 season. Similarly, compared to a vineyard that was irrigated with river water, soil Na in 60-90 cm soil layer were higher in the vineyard soils irrigated with WWW (Kumar *et al.*, 2006). Mosse *et al.* (2012) also reported that

soil Na was substantially higher in WWW irrigated soil compared to soil where no WWW was applied. Furthermore, Howell (2016) reported that soil Na in the 0-30 cm and 60-90 cm soil layers increased linearly with an increase in the COD level of the irrigation water. Mulidzi (2016) reported that irrigation with WWW increased Na substantially. High Na levels cause soil dispersion and suspend silt in the soil water solution.

### **5.2. Response of soil enzyme activities to diluted WWW and raw water where different cover crops and catch crops were cultivated over one season (2020)**

Results from this study showed that irrigation with either diluted WWW or raw water had no significant impact on the activities of  $\beta$ -glucosidase, phosphatase, and urease, after one season. In contrast to results from the current study, the  $\beta$ -glucosidase and urease activities were substantially greater in the 0-10 cm compared to the 10-20 cm soil layers after irrigation with diluted WWW in a study by Mulidzi & Wooldridge, 2016. Furthermore, the application of WWW irrigation had a significant effect on  $\beta$ -glucosidase activity in a field study at Rawsonville (Meyer, 2014), where activity was more pronounced in the topsoil than in the sub-soils and the  $\beta$ -glucosidase activity also increased as the COD concentration in WWW increased. Therefore, an increase in  $\beta$ -glucosidase reflects the soil's ability to break down organic matter and improve the availability of nutrients.

### **5.3. Diluted WWW and raw water effect on summer catch crop and winter cover crop growth**

Under the prevailing conditions, i.e. sandy loam soil with high winter rainfall, only the Dolichos beans catch crop produced foliage for the duration of the study. The DMP of the Pearl millet catch crop was very low. In comparison, where diluted WWW was used for vineyard irrigation at Rawsonville, a Pearl millet catch crop cultivated on a sandy soil produced  $10.4\pm 0.8$ ,  $6.0\pm 1.0$  and  $6.4\pm 0.9$  t/ha dry matter for 2010/11, 2011/12 and 2012/13 seasons, respectively (Howell, 2016). The chicory catch crop grew so poorly under the prevailing conditions that it did not produce DMP in May 2018 and 2020. It was evident in the first two years of the study that the N-fixing winter cover crops produced more DMP than the Saia oats cover crop. The growth of the Saia oats cover crop was lower than that reported for a standard winter cover crop of *Avena sativa L. cv. Pallinup* (oats) cultivated in the Rawsonville area which produced  $5.4\pm 0.3$ ,  $4.7\pm 1.0$ ,  $6.7\pm 1.2$  and  $7.5\pm 1.1$  t/ha DMP for 2009/10, 2010/11, 2011/12 and 2012/13 seasons, respectively (Fourie & Theron, 2014).

It has been reported that irrigation of WWW did not affect the growth of oats negatively and perhaps, even stimulated the growth of oats in the 2012 season (Ochse, 2015). In an irrigation trial that was done with treated wastewater irrigation in the Loess area of China, the growth production of irrigated cover crops was higher and no negative effect on the growth of wheat was observed (Wang *et al.*, 2007). In the current study, the highest yield of oats was obtained

in September 2020 and was 1.84 t/ha. This value is substantially lower than the yield of 7.07 t/ha obtained for oats cultivated in the Robertson area (Fourie, 2006) and 5.57 t/ha in Rawsonville (Ochse, 2015). Visual observations revealed that the low DMP of the N-fixing cover crop was probably due to the excessive growth of Ramnas weed.

#### **5.4. Summer catch crop and winter cover crop chemical composition response to irrigation with diluted WWW and raw water**

After winter rainfall, N in the oats did not differ significantly over the three seasons. Similar results were reported by Ochse (2015), where N in the oats did not differ significantly among treatments over the three seasons. In the current study, the concentration of N in the pearl millet was double, and oats were almost doubled off the 1.08% concentration of barley over the three seasons (Rusan *et al.*, 2007). The irrigated pearl millet with WWW had higher amounts of P than the irrigated pearl millet with raw water according to Ozores-Hampton (2012).

#### **5.5. The amounts of nutrients removed by summer catch crops after irrigation with diluted WWW and raw water and amounts removed by winter cover crops after winter rainfall**

Results showed that higher amounts of N, P, K, Mg and Na were extracted by the Dolichos beans used as a catch crop in summer and the N-fixing cover crop used in winter. Ochse, (2015) reported that higher amounts of N were removed by Pearl millet in the second harvest than in the first harvest. Arienzo *et al.* (2009) reported that wheat (*Triticum aestivum*) that produced a DMP of 2 t/ha could take up 149 kg/ha K. Taking this into consideration, the N-fixing cover crop showed a similar potential uptake reported in wheat. Mohammed & Ayadi (2004) also observed high uptake of K with secondary treated wastewater irrigated corn (*Zea mays*) for two seasons and vetch (*Vicia sativa*) for one season. Cover/catch crops reduce nutrient losses by holding the soil in place and taking up excess nitrogen from the soil during the winter months. The more water that enters the soil profile, the less runoff that flows over the field and less total risk of erosion.

#### **5. 6. Diluted WWW, raw water and winter rainfall effect on element content of leaf petioles and leaf blades of the grapevines**

The application of diluted WWW increased leaf petiole K only in November 2019 and only tended to affect leaf blade K at that time. According to the norms for grapevine nutrient levels in leaves (Conradie, 1994), i.e. 1.6% to 2.7% for N, 0.14% to 0.55% for P, 0.65% to 1.3% for K, 1.2% to 2.2% for Ca, and 0.16% to 0.55% for Mg, leaf analyses were well within these norms. This showed that the additional amounts of elements applied via the diluted WWW, in particular K<sup>+</sup> and Na<sup>+</sup>, were not taken up by the grapevine to such an extent that negative effects were obtained. In a glasshouse study in which WWW was applied either undiluted or

diluted in different ratios to potted Shiraz grapevines, petiole K contents were below the recommended levels, irrespective of dilution level (Kumar *et al.*, 2014). In addition to the different levels of WWW dilution, there were also treatments in which solutions of different K and Na nutrient loads were used to irrigate grapevines. Increasing K concentrations increased petiole K in a study by Kumar *et al.* (2014).

#### **5.7. Diluted WWW and raw water effect on the shoot mass, yield, berry mass, and the sugar content, TTA and pH of the juice.**

Though there was a significant difference in grapevine yield between the treatments at harvest in March 2019, this could not be related to either water quality or catch/cover crop treatments and there is no clear explanation for the differences. Where “simulated” WWW was used for vineyard irrigation, there were no substantial differences in ripeness parameters, yield, and vegetative growth after one year (Mosse *et al.*, 2013). Compared to the river water control, irrigation using diluted WWW did not affect grapevine yield (Howell, 2016). The shoot mass, berry mass, sugar content, TTA and pH of the juice were not significantly affected after irrigation with diluted WWW. Similarly, irrigation with diluted WWW had no effect on berry mass at harvest compared to the river water control (Howell, 2016).

#### **5.8. Macro nutrient content of the juice after irrigation with diluted WWW and raw water.**

Diluted WWW did not have a negative effect on the macronutrient content of the juice. Furthermore, where grapevines were irrigated with WWW, wine Na levels were still below 100 mg/L, whereas wine K ranged from 1 220 mg/L to 1 400 mg/L and was within industry norms for red wines in Australia (Kumar *et al.*, 2014). Treated wastewater was irrigated on table grapes in a six-year trial on table grapes *V. vinifera* cv. Superior Seedless (also called ‘Sugraone’) in Southern Israel (Netzer *et al.*, 2014). The outcome of the study was that the treated wastewater did not have a negative effect on the superior table grapes (‘Sugraone’) after six years. Therefore, application of diluted WWW is beneficial to the juice quality as it does not give a negative effect.

## CHAPTER SIX

### CONCLUSION AND RECOMMENDATIONS

#### 6.1 Conclusion

Water scarcity and nutrient depletion are limitations to optimal fruit and crop production. The objective of this study was to investigate the soil chemical parameters, soil enzyme activities, the effect of the different catch and cover crops on the element content of the soil, the performance of the summer growing catch crops and winter cover crops and the effect of the different treatments on the grapevine performance in the vineyard where diluted WWW and raw water was used as the source of irrigation.

The field experiment showed that the application of diluted WWW responded differently to soil chemical parameters and catch/cover crops. However, the use of diluted WWW as a source of irrigation water rather than raw water increased soil K, pH, P, Na, EPP', C, Ca and solubles in the vineyard. Under the prevailing conditions, the Dolichos beans produced the highest DMP and thus extracted the most elements from the soil. The winter N fixing cover crop produced substantially more DMP in the first two seasons of the study but not in the last season of the study. Consequently, N fixing winter cover crops removed the substantially higher amounts of soil elements. Despite the increase in soil pH and K as a result of WWW application, neither the water quality nor the different catch/cover crop combinations affected vineyard performance negatively in terms of yield, berry mass, shoot mass and leaf petiole, blade and juice element composition. Irrigation with diluted WWW rather than raw water does not have a negative effect on soil enzyme activities ( $\beta$ -glucosidase, phosphatase, and urease). Therefore, it is advisable to use diluted WWW as a source of irrigation instead of raw water.

#### 6.2 Recommendation

Wineries produce large volumes of poor-quality wastewater, particularly during harvest. Results of the study show that using diluted WWW as another source of irrigation water can be used to produce grapes successfully. This re-use of WWW for vineyard irrigation could have many potential benefits for the wine industry. Since water is becoming increasingly scarce, the use of WWW as an alternative source of irrigation water for vineyards could reduce the pressure on water resources. It should be noted that in heavier textured soils or in regions with less winter rainfall, less effective leaching is more likely to result in more salt accumulation. Under the prevailing conditions, Dolichos beans showed the most potential to be cultivated as a catch crop in summer to intercept excessive salts, particularly K, where diluted WWW is used for vineyard irrigation. Given that this catch crop intercepted substantially more K than Na, it is also recommended that cellars consider using K-based cleaning agents rather than Na-based ones. Despite the poor growth in the last season of the

study, results indicated that cultivating a N-fixing cover crop should be considered in winter. The use of such crops in the disposal of WWW is a financially viable option, the use of cover crops and catch crops with spreading habit as interception crops in vineyard irrigated with WWW should be researched more. More studies regarding irrigation of diluted WWW on soil enzyme activities is needed.

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## Appendices

**Appendix A. The chemical status of the soil in the 0-15, 15-30, 30-60 and 60-90 cm soil layers of the micro-sprinkler irrigated Shiraz/110 Richter vineyard as determined in May 2018 after irrigation with diluted WWW and raw water**

Treatment no.	pH	P (Bray II) (mg/kg)	Exchangeable cations (cmol <sup>(+)</sup> /kg)				C (%)	Soluble S (mg/kg)	EC <sub>e</sub> (mS/m)	EPP' (%)	ESP' (%)
			K	Ca	Mg	Na					
<b>0-15 cm</b>											
1	5.70 bcd	76.4 a	0.19 d	2.68 a	0.90 a	0.15 a	1.02 a	8.69 a	19.08 a	4.80 d	3.75 a
2	5.83 abc	40.82 a	0.37 b	2.44 a	0.85 a	0.16 a	0.78 a	9.01 a	18.38 a	9.83 b	4.29 a
3	5.83 abc	27.63 a	0.40 b	2.76 a	0.98 a	0.18 a	0.93 a	8.43 a	10.70 a	9.12 bc	4.26 a
4	6.10 a	80.77 a	0.59 a	2.75 a	0.88 a	0.21 a	1.02 a	7.70 a	16.81 a	13.34 a	4.63 a
5	6.03 ab	39.89 a	0.50 ab	2.69 a	0.90 a	0.16 a	0.96 a	8.64 a	15.92 a	11.95 ab	3.79 a
6	6.03 ab	25.17 a	0.36 bc	2.60 a	0.82 a	0.17 a	0.84 a	9.90 a	18.52 a	9.14 b	4.37 a
7	5.36 d	33.86 a	0.21 d	2.36 a	0.77 a	0.09 a	0.81 a	8.86 a	15.50 a	6.09 cd	2.51 a
8	5.57 cd	26.71 a	0.23 cd	2.54 a	0.91 a	0.14 a	0.59 a	7.72 a	23.65 a	5.96 d	3.58 a
<b>15-30 cm</b>											
1	5.60 a	15.45 a	0.15 a	2.56 a	0.67 a	0.14 a	0.74 a	9.28 a	20.78 a	4.49 a	4.19 bcd
2	5.53 a	15.50 a	0.19 a	2.34 a	0.66 a	0.17 a	0.62 a	7.83 a	20.78 a	5.76 a	5.18 abcd
3	5.40 a	11.10 a	0.24 a	2.02 a	0.66 a	0.17 a	0.64 a	7.99 a	19.89 a	7.52 a	5.45 abc
4	5.57 a	16.21 a	0.26 a	1.85 a	0.64 a	0.21 a	0.77 a	9.72 a	18.16 a	9.01 a	7.05 a
5	5.63 a	13.07 a	0.24 a	2.37 a	0.72 a	0.18 a	0.76 a	9.79 a	17.01 a	7.10 a	5.32 abcd
6	5.67 a	16.16 a	0.21 a	2.10 a	0.65 a	0.18 a	0.93 a	10.68 a	21.06 a	6.56 a	5.78 ab
7	5.47 a	23.95 a	0.17 a	2.27 a	0.66 a	0.11 a	0.75 a	7.96 a	15.63 a	5.22 a	3.39 d
8	5.57 a	10.04 a	0.16 a	2.31 a	0.68 a	0.12 a	0.89 a	8.63 a	21.06 a	4.87 a	3.69 cd
<b>30-60 cm</b>											
1	5.53 a	12.78 b	0.16 a	2.21 a	0.64 a	0.11 a	0.71 a	7.52 a	14.12 a	5.08 a	3.65 a
2	5.43 a	8.70 b	0.15 a	1.75 b	0.58 a	0.10 a	0.48 a	7.06 a	16.37 a	5.85 a	3.90 a
3	5.43 a	13.17 b	0.20 a	2.10 a	0.60 a	0.14 a	0.83 a	9.39 a	16.93 a	6.60 a	4.56 a
4	5.30 a	12.39 b	0.20 a	1.69 b	0.56 a	0.14 a	0.54 a	7.81 a	18.42 a	7.74 a	5.38 a
5	5.47 a	8.90 b	0.18 a	1.96 ab	0.63 a	0.15 a	0.66 a	10.22 a	15.95 a	6.24 a	5.21 a
6	5.5 a	10.61 b	0.13 a	2.08 a	0.62 a	0.14 a	0.64 a	7.72 a	15.50 a	4.35 a	4.55 a
7	5.47 a	22.26 a	0.16 a	1.91 ab	0.60 a	0.09 a	0.73 a	5.91 a	15.40 a	5.91 a	3.10 a
8	5.53 a	10.32 b	0.15 a	1.93 ab	0.57 a	0.15 a	0.64 a	6.80 a	14.77 a	5.28 a	5.43 a
<b>60-90 cm</b>											
1	5.53 a	2.86 c	0.14 a	1.98 a	0.66 a	0.09 a	0.73 a	10.81 a	11.10 a	4.92 a	3.21 a
2	5.50 a	3.09 c	0.14 a	1.60 a	0.55 a	0.08 a	0.45 a	7.77 a	13.55 a	5.73 a	3.10 a
3	5.30 a	8.97 ab	0.13 a	1.96 a	0.47 a	0.09 a	0.67 a	6.72 a	13.76 a	4.92 a	3.39 a
4	5.63 a	5.69 abc	0.23 a	1.98 a	0.55 a	0.10 a	0.63 a	7.67 a	14.73 a	7.76 a	3.54 a
5	5.40 a	4.80 abc	0.20 a	1.90 a	0.63 a	0.12 a	0.75 a	11.97 a	17.57 a	6.85 a	4.19 a
6	5.60 a	4.23 c	0.12 a	2.34 a	0.65 a	0.12 a	0.58 a	9.22 a	17.48 a	3.65 a	3.63 a
7	5.60 a	9.60 a	0.15 a	1.87 a	0.56 a	0.07 a	0.53 a	6.46 a	12.42 a	5.65 a	2.84 a
8	5.67 a	5.10 abc	0.14 a	1.92 a	0.58 a	0.11 a	0.47 a	7.96 a	13.11 a	5.05 a	4.05 a

Refer to Table 3.1 for details of treatments.

Values designated by the same letters within a column do not differ significantly ( $p \leq 0.05$ ).



**Appendix B. The chemical status of the soil in the 0-15, 15-30, 30-60 and 60-90 cm soil layers of the micro-sprinkler irrigated Shiraz/110 Richter vineyard as determined in September 2018 after winter rainfall**

Treatment no.	pH	P (Bray II) (mg/kg)	Exchangeable cations (cmol <sup>(+)</sup> /kg)				C (%)	Soluble S (mg/kg)	EC <sub>e</sub> (mS/m)	EPP' (%)	ESP' (%)
			K	Ca	Mg	Na					
<b>0-15 cm</b>											
1	5.40 a	36.64 ab	0.19 c	2.54 a	0.92 a	0.10 a	2.54 a	6.92 a	10.07 a	2.67 a	5.00 d
2	5.37 ab	27.30 bc	0.23 bc	2.36 a	0.87 a	0.10 a	2.36 a	6.75 a	9.92 a	2.79 a	6.43 cd
3	5.67 a	40.42 a	0.42 a	2.75 a	1.04 a	0.10 a	2.75 a	7.19 a	12.09 a	2.26 a	9.77 ab
4	5.60 a	36.64 ab	0.33 ab	2.71 a	0.95 a	0.09 a	2.71 a	6.66 a	11.78 a	2.30 a	8.21 bc
5	5.63 a	32.21 abc	0.42 a	2.35 a	0.87 a	0.10 a	2.35 a	6.18 a	9.80 a	2.63 a	11.21 a
6	5.60 a	23.08 c	0.25 bc	2.61 a	0.97 a	0.10 a	2.61 a	5.67 a	10.47 a	2.48 a	6.30 cd
7	5.03 b	30.31 abc	0.19 c	2.11 a	0.72 a	0.09 a	2.11 a	5.85 a	7.99 a	2.84 a	6.02 cd
8	5.33 ab	26.24 bc	0.18 c	2.72 a	1.10 a	0.09 a	2.72 a	6.64 a	12.84 a	2.28 a	4.42 d
<b>15-30 cm</b>											
1	5.43 a	10.01 bc	0.14 b	2.44 a	0.73 a	0.15 a	0.63 a	8.11 a	8.51 a	4.45 a	4.44 a
2	5.30 a	10.78 bc	0.20 b	1.88 a	0.69 a	0.12 a	0.71 a	7.54 a	8.89 a	6.75 a	4.41 a
3	5.33 a	10.53 bc	0.32 a	1.96 a	0.73 a	0.15 a	0.62 a	8.01 a	9.51 a	10.13 a	4.53 a
4	5.17 a	9.07 c	0.24 ab	1.96 a	0.78 a	0.16 a	0.47 a	8.32 a	10.84 a	7.60 a	5.29 a
5	5.50 a	13.49 ab	0.17 b	1.82 a	0.60 a	0.18 a	0.60 a	7.02 a	8.75 a	6.19 a	6.28 a
6	5.47 a	12.40 abc	0.20 ab	2.29 a	0.77 a	0.22 a	0.76 a	7.38 a	9.93 a	5.96 a	6.17 a
7	5.33 a	17.50 a	0.14 b	1.94 a	0.60 a	0.12 a	0.60 a	7.04 a	8.33 a	5.05 a	4.33 a
8	5.37 a	9.45 bc	0.17 b	2.20 a	0.79 a	0.13 a	0.67 a	7.84 a	9.10 a	5.26 a	4.42 a
<b>30-60 cm</b>											
1	5.33 a	7.38 c	0.11 a	2.11 a	0.68 a	0.18 a	0.57 a	10.88 a	10.19 a	3.94 a	5.80 a
2	5.13 a	8.02 bc	0.18 a	1.86 a	0.71 a	0.17 a	0.55 a	8.25 a	9.88 a	6.18 a	5.85 a
3	5.40 a	13.00 a	0.21 a	1.93 a	0.57 a	0.15 a	0.53 a	9.81 a	9.93 a	7.62 a	5.27 a
4	5.13 a	11.89 ab	0.14 a	1.54 a	0.57 a	0.16 a	0.57 a	8.05 a	9.37 a	5.67 a	6.49 a
5	5.27 a	6.03 c	0.16 a	1.7 a	0.62 a	0.22 a	0.51 a	9.62 a	11.56 a	5.91 a	8.03 a
6	5.43 a	5.39 c	0.13 a	2.1 a	0.69 a	0.25 a	0.65 a	10.96 a	10.61 a	4.17 a	7.69 a
7	5.40 a	5.84 c	0.15 a	1.88 a	0.61 a	0.15 a	0.45 a	6.53 a	9.63 a	5.49 a	5.17 a
8	5.43 a	6.58 c	0.21 a	2.35 a	0.8 a	0.15 a	0.68 a	7.73 a	10.40a	6.14 a	4.66 a
<b>60-90 cm</b>											
1	5.37 a	2.33 a	0.12 a	1.78 a	0.64 a	0.17 a	0.41 a	14.91 a	8.94 a	4.53 a	6.39 a
2	5.30 a	7.11 a	0.16 a	1.75 a	0.58 a	0.13 a	0.45 a	11.07 ab	9.54 a	6.22 a	5.07 a
3	5.37 a	8.98 a	0.22 a	1.94 a	0.61 a	0.12 a	0.43 a	9.66 b	8.62 a	7.84 a	4.22 a
4	5.20 a	8.44 a	0.15 a	1.72 a	0.57 a	0.14 a	0.47 a	9.41 b	11.87 a	5.66 a	5.48 a
5	5.47 a	4.08 a	0.12 a	1.89 a	0.60 a	0.19 a	0.43 a	13.79 ab	11.87 a	4.28 a	7.17 a
6	5.53 a	8.17 a	0.15 a	2.29 a	0.69 a	0.19 a	0.73 a	10.87 ab	12.77 a	4.43 a	5.82 a
7	5.63 a	4.26 a	0.12 a	1.94 a	0.60 a	0.21 a	0.52 a	9.71 b	15.63 a	4.07 a	7.39 a
8	5.53 a	5.56 a	0.19 a	2.19 a	0.80 a	0.13 a	0.61 a	9.29 b	10.26 a	5.66 a	4.31 a

**Appendix C. The chemical status of the soil in the 0-15, 15-30, 30-60 and 60-90 cm soil layers of the micro-sprinkler irrigated Shiraz/110 Richter vineyard as determined in May 2019 after irrigation with diluted WWW and raw water**

Treatment no.	pH	P (Bray II) (mg/kg)	Exchangeable cations (cmol <sup>(+)</sup> /kg)				C (%)	Soluble S (mg/kg)	EC <sub>e</sub> (mS/m)	EPP' (%)	ESP' (%)
			K	Ca	Mg	Na					
<b>0-15 cm</b>											
1	6.03 a	29.47 a	0.77 a	3.36 a	1.08 a	0.19 a	0.82 a	6.02 a	36 a	14.45 a	3.38 a
2	5.89 a	13.88 a	0.35 a	2.36 a	0.90 a	0.19 a	0.62 a	4.02 a	30 a	9.41 a	4.85 a
3	6.00 a	13.79 a	0.43 a	3.09 a	1.06 a	0.18 a	0.83 a	4.70 a	30 a	9.15 a	3.70 a
4	6.03 a	9.74 a	0.55 a	2.89 a	0.90 a	0.19 a	0.70 a	4.92 a	39 a	12.35 a	4.20 a
5	6.17 a	9.95 a	0.58 a	3.13 a	1.08 a	0.27 a	0.75 a	4.32 a	28 a	11.23 a	5.24 a
6	5.95 a	14.04 a	0.40 a	2.59 a	0.92 a	0.16 a	0.62 a	3.90 a	25 a	9.86 a	4.00 a
7	5.76 a	12.52 a	0.28 a	2.32 a	0.85 a	0.10 a	0.65 a	3.89 a	18 a	7.94 a	2.70 a
8	5.60 a	8.52 a	0.23 a	2.60 a	0.87 a	0.11 a	0.74 a	3.91 a	27 a	5.98 a	2.86 a
<b>15-30 cm</b>											
1	5.97 a	31.61 a	0.62 a	2.99 a	0.91 a	0.17 a	0.62 a	4.74 a	27 a	13.34 a	3.53 a
2	5.76 a	3.71 a	0.21 a	1.98 a	0.68 a	0.14 a	0.43 a	2.83 a	22 a	7.06 c	4.60 a
3	5.83 a	3.88 a	0.38 a	2.55 a	0.89 a	0.12 a	0.57 a	4.57 a	21 a	9.64 abc	3.19 a
4	5.87 a	2.00 a	0.37 a	2.15 a	0.66 a	0.13 a	0.50 a	3.94 a	19 a	11.34 ab	3.95 a
5	6.02 a	3.01 a	0.47 a	2.36 a	0.84 a	0.23 a	0.50 a	5.29 a	25 a	12.04 ab	5.66 a
6	5.83 a	3.12 a	0.26 a	2.21 a	0.75 a	0.15 a	0.45 a	3.65 a	19 a	7.91 bc	4.40 a
7	5.73 a	2.00 a	0.18 a	2.09 a	0.70 a	0.11 a	0.47 a	3.54 a	13 a	5.8 c	3.48 a
8	5.71 a	2.00 a	0.18 a	2.13 a	0.72 a	0.14 a	0.49 a	3.76 a	23 a	5.65 c	4.40 a
<b>30-60 cm</b>											
1	5.91 a	3.57 a	0.70 a	3.07 a	0.90 a	0.17 a	0.66 a	5.96 a	27 a	14.85 a	3.61 a
2	5.69 a	3.75 a	0.18 a	1.88 a	0.59 a	0.13 a	0.41 a	3.44 a	16 a	6.47 b	4.67 a
3	5.80 a	2.00 a	0.31 a	2.17 a	0.77 a	0.14 a	0.51 a	4.14 a	18 a	9.10 b	4.40 a
4	5.86 a	2.00 a	0.37 a	2.25 a	0.69 a	0.15 a	0.47 a	5.33 a	17 a	10.74 ab	4.70 a
5	5.93 a	2.00 a	0.38 a	1.96 a	0.63 a	0.20 a	0.37 a	5.12 a	20 a	10.93 ab	6.40 a
6	5.80 a	2.54 a	0.15 a	1.95 a	0.65 a	0.17 a	0.36 a	4.24 a	17 a	6.08 b	5.66 a
7	5.71 a	2.00 a	0.15 a	1.88 a	0.62 a	0.12 a	0.39 a	5.31 a	14 a	5.46 b	4.49 a
8	5.79 a	2.00 a	0.15 a	1.97 a	0.65 a	0.11 a	0.43 a	4.39 a	14 a	5.35 b	3.99 a
<b>60-90 cm</b>											
1	5.90 a	2.96 a	0.62 a	2.85 a	0.81 a	0.15 a	0.63 a	5.05 a	20 a	14.37 a	3.43 a
2	5.89 a	3.68 a	0.24 a	1.99 a	0.70 a	0.13 a	0.44 a	4.77 a	18 a	7.90 a	4.16 a
3	5.73 a	2.00 a	0.25 a	1.77 a	0.58 a	0.12 a	0.37 a	4.19 a	14 a	9.05 a	4.56 a
4	5.96 a	2.00 a	0.34 a	3.19 a	0.67 a	0.14 a	0.52 a	6.63 a	20 a	8.58 a	3.85 a
5	5.98 a	2.00 a	0.38 a	1.92 a	0.61 a	0.22 a	0.35 a	5.83 a	20 a	11.07 a	7.38 a
6	5.83 a	2.00 a	0.12 a	1.94 a	0.60 a	0.15 a	0.30 a	4.64 a	16 a	4.15 a	5.42 a
7	5.85 a	2.00 a	0.16 a	1.85 a	0.56 a	0.14 a	0.38 a	10.51 a	15 a	6.03 a	5.26 a
8	5.86 a	2.00 a	0.12 a	1.88 a	0.58 a	0.13 a	0.57 a	5.27 a	15 a	4.55 a	4.89 a

**Appendix D. The chemical status of the soil in the 0-15, 15-30, 30-60 and 60-90 cm soil layers of the micro-sprinkler irrigated Shiraz/110 Richter vineyard as determined in September 2019 after irrigation with diluted WWW and raw water**

Treatment no.	pH	P (Bray II) (mg/kg)	Exchangeable cations (cmol <sup>(+)</sup> /kg)				C (%)	Soluble S (mg/kg)	EC <sub>e</sub> (mS/m)	EPP' (%)	ESP' (%)
			K	Ca	Mg	Na					
<b>0-15 cm</b>											
1	6.16 a	28.9 a	0.64 a	4.00 a	0.96 a	0.11 a	0.69 a	4.33 a	19.00 a	11.21 a	1.93 a
2	6.08 a	19.73 a	0.44 a	3.22 a	0.87 a	0.10 a	0.67 a	4.33 a	21.33 a	9.63 ab	2.26 a
3	6.21 a	28.07 a	0.51 a	4.07 a	1.08 a	0.11 a	0.76 a	4.00 a	20.33 a	8.64 bc	1.95 a
4	6.08 a	24.07 a	0.44 a	3.72 a	0.89 a	0.07 a	0.66 a	4.00 a	22.00 a	8.70 bc	1.68 a
5	6.18 a	25.2 a	0.49 a	3.70 a	1.00 a	0.12 a	0.67 a	3.00 a	17.67 a	9.23 ab	2.25 a
6	6.08 a	22.20 a	0.37 a	3.44 a	0.89 a	0.10 a	0.67 a	3.67 a	18.33 a	7.83 bc	2.01 a
7	5.98 a	29.00 a	0.31 a	3.44 a	0.84 a	0.09 a	0.66 a	4.00 a	17.67 a	6.61 c	1.94 a
8	5.70 a	18.93 a	0.35 a	3.07 a	0.86 a	0.10 a	0.62 a	4.67 a	19.33 a	7.92 bc	2.29 a
<b>15-30 cm</b>											
1	6.06 a	8.23 a	0.44 a	3.17 a	0.68 a	0.15 a	0.45 a	6.67 a	19.67 a	10.43 ab	3.64 a
2	5.95 a	10.47 a	0.23 a	2.58 a	0.66 a	0.16 a	0.46 a	6.33 a	21.67 a	6.33 c	4.45 a
3	5.95 a	11.83 a	0.54 a	3.12 a	0.85 a	0.13 a	0.48 a	4.00 a	19.00 a	11.60 a	2.90 a
4	5.95 a	11.87 a	0.46 a	2.66 a	0.82 a	0.17 a	0.32 a	3.67 a	21.00 a	11.39 a	4.07 a
5	6.06 a	9.70 a	0.46 a	2.70 a	0.84 a	0.20 a	0.43 a	5.33 a	21.33 a	10.74 a	4.89 a
6	5.93 a	9.33 a	0.31 a	2.82 a	0.71 a	0.14 a	0.38 a	3.00 a	16.00 a	7.76 abc	3.47 a
7	5.94 a	15.20 a	0.20 a	2.57 a	0.63 a	0.10 a	0.39 a	3.67 a	15.67 a	5.82 c	2.95 a
8	5.70 a	11.67 a	0.15 a	2.81 a	0.80 a	0.11 a	0.50 a	3.67 a	17.00 a	3.77 c	2.95 a
<b>30-60 cm</b>											
1	6.02 a	7.3 a	0.34 a	2.62 a	0.70 a	0.18 a	0.32 a	6.0 a	20.00 a	9.03 a	4.83 a
2	5.94 a	6.7 a	0.23 a	2.38 a	0.72 a	0.20 a	0.35 a	7.0 a	19.33 a	6.42 a	5.66 a
3	6.00 a	8.7 a	0.52 a	2.33 a	0.56 a	0.12 a	0.35 a	4.0 a	16.33 a	17.35 a	3.61 a
4	5.87 a	9.7 a	0.29 a	2.20 a	0.65 a	0.16 a	0.30 a	3.7 a	16.33 a	8.81 a	4.98 a
5	6.01 a	7.7 a	0.34 a	2.47 a	0.69 a	0.17 a	0.32 a	4.3 a	16.33 a	9.20 a	4.49 a
6	5.96 a	5.0 a	0.18 a	2.52 a	0.65 a	0.18 a	0.25 a	4.3 a	16.00 a	5.15 a	5.15 a
7	6.01 a	6.7 a	0.21 a	2.81 a	0.67 a	0.13 a	0.35 a	4.7 a	19.33 a	5.18 a	3.44 a
8	5.96 a	5.7 a	0.20 a	2.51 a	0.70 a	0.13 a	0.49 a	5.3 a	17.67 a	5.77 a	3.61 a
<b>60-90 cm</b>											
1	5.85 a	3.3 a	0.27 a	2.81 a	0.71 a	0.18 a	0.34 a	8.7 a	24.33 a	6.98 a	4.45 a
2	5.99 a	5.3 a	0.15 a	2.00 a	0.57 a	0.15 a	0.24 a	5.3 a	17.00 a	5.26 a	5.30 a
3	6.10 a	5.3 a	0.33 a	2.37 a	0.57 a	0.15 a	0.32 a	5.7 a	17.33 a	9.41 a	4.33 a
4	5.96 a	4.0 a	0.25 a	2.26 a	0.59 a	0.18 a	0.29 a	3.7 a	18.00 a	7.55 a	5.45 a
5	5.94 a	5.3 a	0.30 a	2.13 a	0.66 a	0.14 a	0.28 a	5.3 a	15.33 a	9.31 a	4.19 a
6	5.97 a	3.3 a	0.14 a	2.57 a	0.61 a	0.17 a	0.23 a	4.3 a	17.00 a	3.96 a	4.90 a
7	6.14 a	4.3 a	0.21 a	2.56 a	0.64 a	0.16 a	0.24 a	6.0 a	17.33 a	5.84 a	4.36 a
8	6.02 a	4.0 a	0.21 a	2.50 a	0.64 a	0.12 a	0.32 a	5.7 a	19.33 a	5.97 a	3.41 a

**Appendix E. The chemical status of the soil in the 0-15, 15-30, 30-60 and 60-90 cm soil layers of the micro-sprinkler irrigated Shiraz/110 Richter vineyard as determined in May 2020 after irrigation with diluted WWW and raw water**

Treatment no.	pH	P (Bray II) (mg/kg)	Exchangeable cations (cmol <sup>(+)</sup> /kg)				C (%)	Soluble S (mg/kg)	EC <sub>e</sub> (mS/m)	EPP' (%)	ESP' (%)
			K	Ca	Mg	Na					
<b>0-15 cm</b>											
1	5.87 a	33.43 ab	0.50 a	2.57 a	0.75 a	0.18 a	1.42 a	7.50 b	22.00 a	12.41 a	4.38 a
2	5.93 a	23.40 bc	0.50 a	2.60 a	0.80 a	0.17 a	1.34 a	7.67 b	18.33 a	12.41 a	4.11 a
3	5.83 a	22.97 bc	0.51 a	2.53 a	0.78 a	0.18 a	1.18 a	8.83 b	23.50 a	12.46 a	4.55 a
4	5.77 a	42.73 a	0.50 a	2.67 a	0.72 a	0.20 a	1.36 a	10.20 b	28.33 a	12.42 a	5.18 a
5	6.10 a	26.13 bc	0.42 a	2.70 a	0.76 a	0.23 a	1.31 a	11.23 b	22.00 a	10.18 ab	5.65 a
6	6.10 a	18.43 c	0.50 a	2.70 a	0.81 a	0.18 a	1.06 a	9.07 b	20.67 a	11.72 a	4.43 a
7	5.20 b	31.67 ab	0.25 a	2.47 a	0.69 a	0.13 a	1.20 a	8.67 b	21.33 a	7.00 b	3.65 a
8	5.10 b	32.90 ab	0.32 a	3.03 a	0.90 a	0.18 a	1.49 a	16.80 a	28.00 a	7.14 b	4.07 a
<b>15-30 cm</b>											
1	5.77 ab	14.13 c	0.53 a	2.17 a	0.64 a	0.18 a	0.86 b	7.40 a	16.00 b	15.05 a	4.99 a
2	5.73 abc	11.70 c	0.41 bc	1.77 a	0.56 a	0.18 a	0.62 b	11.67 a	21.00 b	14.08 a	6.28 a
3	5.53 bc	8.83 d	0.37 abc	1.87 a	0.62 a	0.16 a	0.65 b	9.20 a	20.67 b	12.10 a	5.21 a
4	5.73 abc	28.87 c	0.46 a	2.53 a	0.73 a	0.18 a	1.37 a	8.40 a	22.33 b	12.41 a	4.88 a
5	5.97 a	9.57 d	0.41 ab	2.20 a	0.68 a	0.19 a	0.78 b	10.30 a	19.00 b	11.77 a	5.59 a
6	5.83 ab	14.27 c	0.36 abc	2.30 a	0.69 a	0.20 a	0.61 b	8.30 a	20.67 b	10.19 ab	5.50 a
7	5.40 cd	24.30 b	0.20 c	2.30 a	0.65 a	0.13 a	0.93 b	6.07 a	18.67 b	6.26 b	4.06 a
8	5.17 d	27.87 a	0.25 bc	2.47 a	0.76 a	0.13 a	1.00ab	9.63 a	36.00 a	6.88 b	3.54 a
<b>30-60 cm</b>											
1	5.70 a	34.97	0.23 cd	2.27 a	0.63 a	0.21 a	0.75 a	8.43 a	19.67 a	7.23 a	6.25 a
2	5.57 a	20.89 a	0.19 cd	0.73 a	0.52 a	0.21 a	0.57 a	7.17 a	25.00 a	7.35 a	7.76 a
3	5.47 a	16.88 a	0.27 bcd	1.97 a	0.55 a	0.18 a	0.60 a	10.27 a	16.00 a	9.33 a	5.99 a
4	5.63 a	23.56 a	0.38 ab	2.00 a	0.60 a	0.15 a	0.96 a	8.00 a	17.67 a	12.42 a	5.00 a
5	5.80 a	50.65 a	0.51 a	2.13 a	0.69 a	0.19 a	0.57 a	9.87 a	17.00 a	14.48 a	5.36 a
6	5.67 a	42.35 a	0.31 bc	2.47 a	0.74 a	0.24 a	0.78 a	9.70 a	17.67 a	8.10 a	6.42 a
7	5.47 a	17.63 a	0.15 d	2.30 a	0.57 a	0.13 a	0.87 a	6.37 a	15.33 a	4.65 a	4.28 a
8	5.30 a	22.31 a	0.20 cd	2.17 a	0.67 a	0.12 a	0.67 a	9.07 a	15.67 a	6.14 a	3.93 a
<b>60-90 cm</b>											
1	5.60 a	4.73 c	0.14 b	1.83 a	0.58 a	0.21 ab	0.60 a	13.83 ab	18.33 a	5.13 b	7.69 a
2	5.63 a	7.70 c	0.15 b	1.73 a	0.48 a	0.17 b	0.57 a	8.03 c	14.00 a	6.02 b	6.70 a
3	5.70 a	14.60 b	0.42 a	2.03 a	0.63 a	0.19 b	0.85 a	8.57 c	16.67 a	12.07 a	5.94 a
4	5.63 a	6.60 c	0.31 ab	2.03 a	0.57 a	0.16 b	0.70 a	8.23 c	14.67 a	10.39 a	5.34 a
5	5.77 a	5.63 c	0.37 a	1.93 a	0.62 a	0.21 ab	0.58 a	14.13 a	20.00 a	11.88 a	6.63 a
6	5.67 a	5.30 c	0.37 a	2.47 a	0.71 a	0.30 a	0.49 a	8.97 bc	16.00 a	8.87 ab	7.73 a
7	5.50 a	22.67 a	0.16 b	2.10 a	0.51 a	0.12 b	0.70 a	6.03 c	11.67 a	5.49 b	4.29 a
8	5.40 a	6.47 c	0.15 b	2.17 a	0.63 a	0.12 b	1.20 a	8.40 c	13.67 a	5.74 b	4.00 a

**Appendix F. B-glucosidase, phosphatase and urease activity determined in the soil in the 0-15 cm and 15-30 cm soil layers in February 2020 before the application of diluted WWW and raw water**

Treatment no	B-glucosidase ( $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$ )	Phosphatase ( $\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$ )	Urease ( $\mu\text{g NH}_4^+ \text{ g}^{-1} \text{ soil 2 h}^{-1}$ )
<b>0-15 cm</b>			
1	105.66 a	205.28 a	27.81 a
2	93.80 a	360.17 a	32.44 a
3	141.91 a	293.69 a	39.07 a
4	107.02 a	249.79 a	37.50 a
5	132.56 a	261.03 a	39.52 a
6	85.54 a	286.30 a	26.38 a
7	79.29 a	258.55 a	15.49 a
8	92.80 a	257.88 a	18.06 a
<b>15-30 cm</b>			
1	107.16 a	191.48 a	25.87 a
2	53.60 a	151.37 a	14.28 a
3	95.79 a	355.45 a	19.60 a
4	70.24 a	208.52 a	21.52 a
5	39.44 a	163.24 a	29.11 a
6	38.29 a	112.73 a	18.92 a
7	83.29 a	286.29 a	13.03 a
8	89.73 a	246.66 a	24.57 a

Refer to Table 3.1 for details of treatments.

Values designated by the same letters within a column do not differ significantly ( $p \leq 0.05$ ).

**Appendix G. B-glucosidase, phosphatase and urease determined in the soil in the 0-15 cm and 15-30 cm soil layers in May 2020 after irrigation with diluted WWW and raw water**

Treatment no	B-glucosidase ( $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$ )	Phosphatase ( $\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$ )	Urease ( $\mu\text{g NH}_4^+ \text{ g}^{-1} \text{ soil 2 h}^{-1}$ )
<b>0-15 cm</b>			
1	151.30 a	327.32 a	45.48 a
2	145.02 a	322.95 a	49.03 a
3	165.04 a	341.48 a	56.74 a
4	112.87 a	287.82 a	35.86 a
5	122.08 a	291.11 a	34.61 a
6	127.53 a	290.18 a	44.09 a
7	126.99 a	284.60 a	38.54 a
8	156.82 a	379.02 a	40.80 a
<b>15-30 cm</b>			
1	37.41 a	117.51 a	15.84 a
2	34.57 a	116.89 a	12.53 a
3	30.94 a	109.71 a	15.34 a
4	34.71 a	126.60 a	13.29 a
5	45.76 a	133.57 a	13.42 a
6	24.98 a	92.42 a	13.01 a
7	36.23 a	106.23 a	12.02 a
8	36.36 a	121.66 a	12.26 a

Refer to Table 3.1 for details of treatments.

Values designated by the same letters within a column do not differ significantly ( $p \leq 0.05$ ).

**Appendix H. B-glucosidase, phosphatase and urease determined in the soil in the 0-15 cm and 15-30 cm soil layers in September 2020 after winter rainfall**

Treatment no.	B-glucosidase ( $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$ )	Phosphatase ( $\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$ )	Urease ( $\mu\text{g NH}_4^+ \text{ g}^{-1} \text{ soil 2 h}^{-1}$ )
<b>0-15 cm</b>			
1	213.34 a	660.40 a	40.17 a
2	248.72 a	611.38 a	27.74 a
3	162.89 a	518.52 a	39.39 a
4	187.70 a	505.89 a	20.34 a
5	224.33 a	493.72 a	33.42 a
6	204.06 a	553.01 a	30.81 a
7	197.42 a	560.37 a	27.74 a
8	150.46 a	553.39 a	24.04 a
<b>15-30 cm</b>			
1	77.65 a	300.34 a	14.46 a
2	73.36 a	371.29 a	9.22 a
3	94.05 a	372.08 a	16.59 a
4	61.41 a	388.85 a	3.03 a
5	79.21 a	350.87 a	13.36 a
6	140.99 a	362.01 a	19.70 a
7	111.05 a	477.54 a	11.37 a
8	75.85 a	404.86 a	6.40 a

Refer to Table 3.1 for details of treatments.

Values designated by the same letters within a column do not differ significantly ( $p \leq 0.05$ ).

**Appendix I. Nutrient content of leaf petiole of grapevine after irrigation with diluted WWW and raw water over the two seasons (2018-2019)**

Season	Treatment no.	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg/kg)
Nov 2018	1	1.74 a <sup>2</sup>	0.32 a	2.11	1.68 a	1.04 a	593 a
	2	1.56 a	0.36 a	2.43 a	1.59 a	1.02 ab	589 a
	3	1.59 a	0.25 a	2.54 a	1.66 a	0.90 c	486 bc
	4	1.57 a	0.28 a	2.41 a	1.42 a	0.89 c	579 a
	5	1.56 a	0.24 a	2.10 a	1.64 a	0.92 bc	578 a
	6	1.72 a	0.2 a	2.32 a	1.61 a	1.01 abc	534 ab
	7	1.70 a	0.32 a	2.11 a	1.88 a	1.09 a	478 bc
	8	1.71 a	0.25 a	2.40 a	1.62 a	0.91 bc	418 c
Nov 2019	1	1.51 a <sup>2</sup>	0.16 a	3.07 a	1.06 a	0.60 a	971 a
	2	1.51 a	0.18 a	2.97 ab	1.06 a	0.63 a	1005 a
	3	1.40 a	0.16 a	3.23 a	1.10 a	0.61 a	1084 a
	4	1.52 a	0.17 a	3.04 a	1.05 a	0.59 a	954 a
	5	1.40 a	0.19 a	2.62 ab	1.09 a	0.61 a	1007a
	6	1.46 a	0.14 a	2.74 ab	1.16 a	0.64 a	908 a
	7	1.43 a	0.17 a	2.33 b	1.21 a	0.68 a	845 a
	8	1.52 a	0.13 a	2.36 b	1.03 a	0.66 a	750 a

Refer to Table 3.1 for details of treatments.

Values designated by the same letters within a column do not differ significantly ( $p \leq 0.05$ ).

**Appendix J. Nutrient content of leaf blades of grapevine after irrigation with diluted WWW and raw water over the two seasons (2018-2019)**

Season	Treatment no.	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg/kg)
Nov 2018	1	3.20 a	0.23 a	0.97 a	1.74 ab	0.38 ab	402 ab
	2	3.08 a	0.24 a	0.94 a	1.64 abc	0.37 ab	431 a
	3	3.11 a	0.23 a	0.95 a	1.73 ab	0.35 bc	377 bc
	4	3.11 a	0.23 a	0.99 a	1.42 c	0.32 c	388 ab
	5	3.14 a	0.22 a	0.87 a	1.80 a	0.38 ab	388 ab
	6	3.15 a	0.21 a	0.93 a	1.72 ab	0.37 ab	381 abc
	7	3.36 a	0.23 a	0.80 a	1.88 a	0.40 a	413 ab
	8	3.19 a	0.22 a	0.93 a	1.51 bc	0.34 bc	331 c
Nov 2019	1	2.67 a	0.20 a	1.17 a	1.19 a	0.28 a	611 a
	2	2.68 a	0.19 a	1.15 a	1.18 a	0.28 a	628 a
	3	2.56 a	0.19 a	1.21 a	1.26 a	0.29 a	574 a
	4	2.55 a	0.19 a	1.23 a	1.11 a	0.29 a	599 a
	5	2.56 a	0.20 a	1.10 a	1.09 a	0.27 a	564 a
	6	2.61 a	0.19 a	1.14 a	1.17 a	0.29 a	589 a
	7	2.66 a	0.19 a	1.07 a	1.24 a	0.30 a	554 a
	8	2.62 a	0.18 a	1.01 a	1.06 a	0.27 a	546 a

Refer to Table 3.1 for details of treatments.

Values designated by the same letters within a column do not differ significantly ( $p \leq 0.05$ ).



**Appendix K. The sugar content, TTA and pH of the Shiraz/110 Richter vines after irrigation with diluted WWW and raw water for three seasons (2018-2020)**

Treatment no.	Juice								
	Sugar content (°B)			TTA (mg/L)			pH		
	2018	2019	2020	2018	2019	2020	2018	2019	2020
1	25.87 a	24.57 a	26.23 a	5.15 a	5.29 a	5.20 a	3.86 a	3.87 a	3.57 a
2	25.53 a	24.30 a	25.57 a	6.46 a	5.32 a	5.75 a	3.86 a	3.86 a	3.53 a
3	25.33 a	24.70 a	26.97 a	6.46 a	5.24 a	5.76 a	3.91 a	3.87 a	3.58 a
4	25.60 a	24.37 a	25.73 a	5.78 a	5.15 a	5.60 a	3.89 a	3.86 a	3.57 a
5	25.73 a	24.53 a	25.27 a	5.69 a	5.37 a	5.53 a	3.92 a	3.84 a	3.56 a
6	25.47 a	24.70 a	26.97 a	5.50 a	5.24 a	5.50 a	3.90 a	3.87 a	3.53 a
7	25.97 a	24.93 a	26.17 a	5.03 a	4.99 a	5.20 a	3.94 a	3.80 a	3.50 a
8	24.20 a	24.40 a	25.40 a	6.02 a	5.09 a	5.74 a	3.77 a	3.81 a	3.52 a

Refer to Table 3.1 for details of treatments.

Values designated by the same letters within a column do not differ significantly ( $p \leq 0.05$ ).

**Appendix L. The shoot mass, harvest mass and berry mass, of the Shiraz/110 Richter vines after irrigation with diluted WWW and raw water for three seasons (2018-2020)**

Treatment no.	Shoot mass (t/ha)			Yield (t/ha)			Berry mass (g/berry)		
	2018	2019	2020	2018	2019	2020	2018	2019	2020
1	3.93 a	4.2 a	4.44 a	9.97 a	12.0 b	14.8 a	0,79 a	1.41 a	1.57 a
2	4.43 a	4.0 a	4.97 a	11.47 a	14.8 ab	17.0 a	0,81 a	1.36 a	1.68 a
3	3.64 a	4.2 a	4.24 a	9.05 a	12.2 b	14.3 a	0,78 a	1.46 a	1.64 a
4	4.89 a	5.6 a	5.45 a	11.33 a	17.7 a	18.0 a	0,85 a	1.40 a	1.70 a
5	4.21 a	3.8 a	4.57 a	11.28 a	16.9 a	17.0 a	0,81 a	1.49 a	1.65 a
6	3.83 a	4.4 a	4.28 a	9.82 a	13.9 ab	16.7 a	0,82 a	1.50 a	1.66 a
7	4.42 a	5.0 a	4.88 a	11.34 a	11.6 b	16.2 a	0,81 a	1.53 a	1.76 a
8	4.00 a	5.0 a	5.05 a	8.65 a	11.4 b	17.4 a	0,87 a	1.53 a	1.73 a

Refer to Table 3.1 for details of treatments.

Values followed by the same letter within a column do not differ significantly ( $p \leq 0.05$ ).

**Appendix M. Macro nutrient status of juice after irrigation with diluted WWW and raw water over the three seasons (2018-2020)**

Season	Treatment no.	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)
Feb 2018	1	25.14 a	1215 a	138.69 a	303.87 a	8.62 a
	2	28.19 a	933 a	144.43 a	319.48 a	10.04 a
	3	26.66 a	1132 a	141.31 a	297.44 a	7.44 a
	4	28.69 a	854 a	145.47 a	333.87 a	7.84 a
	5	32.52 a	1022 a	146.04 a	344.50 a	8.79 a
	6	27.13 a	1189 a	123.45 a	301.61 a	7.61 a
	7	28.58 a	874 a	139.09 a	293.49 a	7.26 a
	8	25.92 a	750 a	122.18 a	293.61 a	7.09 a
Feb 2019	1	133 a	1043 ab	50 a	72 abc	10 a
	2	160 a	1098 a	50 a	88 a	12 a
	3	152 a	1110 a	46 a	77 ab	11 a
	4	110 a	947 ab	41 a	77 ab	10 a
	5	97 a	797 c	34 a	58 bc	9 a
	6	103 a	870 c	35 a	63 bc	9 a
	7	107 a	773 c	36 a	58 c	8 a
	8	97 a	770 c	37 a	65 bc	7 a
Feb 2020	1	0.25 a	5.39 a	0.17 a	0.25 a	655 a
	2	0.24 a	5.13 a	0.16 a	0.24 a	589 a
	3	0.21 a	4.73 a	0.14 a	0.21 a	458 a
	4	0.26 a	5.88 a	0.16 a	0.26 a	512 a
	5	0.30 a	7.58 a	0.17 a	0.25 a	536 a
	6	0.26 a	6.07 a	0.15 a	0.24 a	428 a
	7	0.24 a	6.08 a	0.15 a	0.22 a	449 b
	8	0.28 a	5.66 a	0.16 a	0.24 a	437 b

Refer to Table 3.1 for details of treatments.

Values designated by the same letters within a column do not differ significantly ( $p \leq 0.05$ ).

**Appendix N. The amount of nutrients removed from soil by winter cover crops after winter rainfall in the vineyard over the three seasons (2018-2020)**

<b>Season</b>	<b>Treatment no.</b>	<b>N (%)</b>	<b>P (%)</b>	<b>K (%)</b>	<b>Ca (%)</b>	<b>Mg (%)</b>	<b>Na (mg/kg)</b>
Sep 2018	1	11.59	1.76	14.49	2.21	0.63	1.36
	2	157.17	19.23	183.08	48.49	15.05	13.60
	3	9.92	1.80	14.51	2.29	0.68	1.22
	4	112.40	13.87	124.49	31.86	9.74	6.96
	5	12.96	2.38	20.17	2.79	0.82	1.22
	6	171.59	19.43	193.87	52.14	15.17	11.01
	7	N/A	N/A	N/A	N/A	N/A	N/A
	8	N/A	N/A	N/A	N/A	N/A	N/A
Sep 2019	1	13.06	3.50	28.06	2.12	1.10	0.66
	2	116.87	11.50	117.61	39.70	11.13	5.03
	3	14.81	4.10	32.76	2.73	1.37	0.66
	4	98.19	10.34	107.24	34.24	9.69	5.04
	5	16.88	4.91	38.05	2.90	1.51	0.75
	6	97.31	9.54	91.58	37.52	9.86	4.21
	7	N/A	N/A	N/A	N/A	N/A	N/A
	8	N/A	N/A	N/A	N/A	N/A	N/A
Sep 2020	1	26.68	4.05	36.06	3.68	1.84	0.99
	2	10.61	0.99	9.55	3.20	0.82	0.59
	3	23.50	4.58	40.56	3.54	1.87	1.46
	4	18.48	2.31	18.56	6.85	1.77	0.85
	5	24.98	3.52	33.30	3.33	1.67	0.82
	6	20.07	1.83	17.87	5.55	1.53	0.97
	7	N/A	N/A	N/A	N/A	N/A	N/A
	8	N/A	N/A	N/A	N/A	N/A	N/A

Refer to Table 3.1 for details of treatments.  
N/A denotes No catch crop treatment.

**Appendix O. The amount of nutrients removed by summer catch crops after irrigation with diluted WWW and raw water in the vineyard over the two seasons (2019-2020)**

<b>Season</b>	<b>Treatment no.</b>	<b>N (%)</b>	<b>P (%)</b>	<b>K (%)</b>	<b>Ca (%)</b>	<b>Mg (%)</b>	<b>Na (mg/kg)</b>
May 2019	1	4.65	1.23	8.31	1.20	1.02	0.12
	2	5.42	1.25	13.11	1.22	1.28	0.19
	3	34.03	2.89	27.39	25.04	4.82	0.31
	4	34.77	3.54	26.72	34.16	4.76	0.40
	5	3.57	0.63	5.00	3.81	0.99	0.94
	6	1.62	0.10	2.39	1.82	0.41	0.06
	7	N/A <sup>2</sup>	N/A	N/A	N/A	N/A	N/A
	8	N/A	N/A	N/A	N/A	N/A	N/A
May 2020	1	5.28	0.48	5.85	1.06	0.73	0.16
	2	8.51	0.85	10.51	1.59	1.33	0.24
	3	44.04	3.50	20.67	26.24	4.13	0.36
	4	47.88	3.53	31.58	31.86	4.87	0.42
	5	N/A	N/A	N/A	N/A	N/A	N/A
	6	N/A	N/A	N/A	N/A	N/A	N/A
	7	N/A	N/A	N/A	N/A	N/A	N/A
	8	N/A	N/A	N/A	N/A	N/A	N/A

Refer to Table 3.1 for details of treatments.  
N/A denotes No catch crop treatment.

**Appendix P. The amount of nutrients removed from a loamy sandy soil by winter cover crops in the vineyard and calculated balance as affected by diluted WWW additions over three seasons (2017/18-2019/20)**

Season	Treatment no.	K			Ca			Mg			Na		
		Removed by winter cover crop (kg/ha)	Added via waste water irrigation (kg/ha)	Balance (kg/ha)	Removed by winter cover crop (kg/ha)	Added via wastewater irrigation (kg/ha)	Balance (kg/ha)	Removed by winter cover crop (kg/ha)	Added via wastewater irrigation (kg/ha)	Balance (kg/ha)	Removed by winter cover crop (kg/ha)	Added via wastewater irrigation (kg/ha)	Balance (kg/ha)
2017/18	1	14.49	190.00	175.51	2.21	133.04	130.83	0.63	21.28	20.65	1.36	115.44	114.08
	2	183.08	190.00	6.92	48.49	133.04	84.55	15.05	21.28	6.23	13.60	115.44	101.84
	3	14.51	190.00	175.51	2.29	133.04	130.75	0.68	21.28	20.60	1.22	115.44	114.22
	4	124.49	190.00	65.51	31.89	133.04	101.18	9.74	21.28	11.54	6.96	115.44	108.48
	5	20.17	190.00	169.83	2.79	133.04	130.25	0.82	21.28	20.46	1.22	115.44	114.22
	6	193.87	190.00	-3.87	52.14	133.04	80.90	15.17	21.28	6.11	11.01	115.44	104.43
2018/19	1	28.06	238.22	210.16	2.12	43.15	41.03	1.10	10.40	9.30	0.66	67.78	67.12
	2	117.61	238.22	120.61	39.70	43.15	3.45	11.13	10.40	- 0.73	5.03	67.78	62.75
	3	32.76	238.22	205.46	2.37	43.15	40.42	1.37	10.40	9.04	0.66	67.78	67.12
	4	107.24	238.22	130.98	34.24	43.15	8.91	9.69	10.40	0.71	5.04	67.78	62.74
	5	38.05	238.22	200.17	2.90	43.15	40.25	1.51	10.40	8.89	0.75	67.78	67.03
	6	91.58	238.22	146.64	37.52	43.15	5.63	9.86	10.40	0.54	4.21	67.78	63.57
2019/20	1	36.06	324.67	288.61	3.68	94.99	90.81	1.84	14.90	13.06	0.99	79.01	78.02
	2	9.55	324.67	315.12	3.20	94.99	91.29	0.82	14.90	14.08	0.59	79.01	78.42
	3	40.56	324.67	284.11	3.54	94.99	90.95	1.87	14.90	13.03	1.46	79.01	77.55
	4	18.56	324.67	306.11	6.85	94.99	87.64	1.77	14.90	13.13	0.85	79.01	78.16
	5	33.30	324.67	291.37	3.33	94.99	91.16	1.67	14.90	13.24	0.82	79.01	78.19
	6	17.87	324.67	306.80	5.55	94.99	88.94	1.53	14.90	13.38	0.97	79.01	78.04

Refer to Table 3.1 for details of treatments.

