THE IMPACT OF WASTEWATER IRRIGATION BY WINERIES ON SOILS, CROP GROWTH AND PRODUCT QUALITY

Edited by

P.A. Myburgh and C.L. Howell

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ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa

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Water Research Commission
Private Bag X03
Gezina, 0031

orders@wrc.org.za or download from www.wrc.org.za

EXECUTIVE SUMMARY

Background

Wine production is an important industry in the Western Cape and the Lower Orange region in the Northern Cape. Wineries produce large volumes of low quality wastewater, particularly during the harvest period. In South Africa, historically most of the winery wastewater has been disposed of to land through irrigation of pastures. With regards to the National Water Act (NWA) (1956), if a person was unable to comply with the conditions of Section 21(1), the Minister could exempt such a person from compliance with either one or both the requirements of Section 21(1)(a) and 21(1)(b). South African wineries found compliance with the requirements of Sections 21(1)(a) and 21(1)(b) of the NWA (1956) technically and financially challenging. Therefore, most wineries applied for exemption from compliance with these requirements as they are based in the rural areas, where land is available for irrigation and the resulting grazing could be used. However, there are certain secondary environmental pollution risks associated with such irrigation, namely the potential occurrence of unpleasant odours and seepage as a result of over-irrigation which can result in saturated, anaerobic soils. Subsequently, many wineries were exempt from the requirements of Section 21(1), and there were certain conditions which limited the volume of wastewater that could be irrigated. However, many of the exemptions did not specify a minimum area that had to be irrigated nor a requirement for water quality analysis. In addition to this, very often there was no expiry date for the exemption. Consequently, over-irrigation and soil saturation occurred which led to an accumulation of organic material, as well as inorganic salts such as P, Na and K in the soil. This resulted in soil degradation and possible seepage of wastewater into underground resources at certain irrigation areas.

In 1997, DWAF published a Water Policy White Paper which repealed the NWA of 1956, and a new NWA (Act 36) was promulgated in 1998. This Act, in Section 22, did not make provision for exemption from compliance with the Act. The Act did define the irrigation of land with waste or water containing waste as a specific type of water use, in terms of section 37(1)(a). The controlled activity could be deemed a permissible water use activity. In 2004, DWAF, recognising that the lack of conditions requiring at least the monitoring of the quality and quantity of wastewater being irrigated did not allow for the responsible management of the irrigation practice, incorporated these conditions into all new authorizations under the NWA. These General Authorizations require not only the monitoring of wastewater quantity and quality, but also specifies limits for wastewater being irrigated based on the volume irrigated per day.

In 1999, Winetech initiated research projects into improving the understanding of the composition and handling of winery wastewater. In 2005, this researched culminated in guidelines for the management of winery wastewater that not only focussed on assisting wineries in developing wastewater management plans, but also advised wineries as to how they could improve the quality of the winery wastewater by employing cleaner production principles. Winetech also started to engage more pro-actively with DWAF and through their co-operation secured their input into the guideline development process to ensure that the industry was fully aware of the legal requirements of the NWA. They also actively engaged in securing compliance with these conditions. In 2004, there was a series of meetings in June and August between various role players to discuss a proposed General Authorization for the wine industry. DWAF indicated the need of the wine industry to move away from disposal irrigation of untreated wastewater.

In August 2004, a treatment and disposal methodology was agreed upon on which the proposed General Authorization could be based. Furthermore, treated winery wastewater, in combination with other water should be used for beneficial irrigation of agricultural crops such as vineyards. Furthermore, if winery wastewater could be used in a sustainable way, it would have the following benefits:

(i) Reducing the energy presently required for wastewater treatment, *e.g.* using pumps to aerate the water in ponds.
(ii) The presence of plant nutrients in the wastewater, *e.g.* N, P, and K, could also reduce cost of fertilization.
(iii) Where irrigation water is limited, the re-use of wastewater will have a positive impact on grape yields if additional irrigation could be applied.
(iv) If possible, the water saving and higher yields will contribute to the sustainability and economic viability of wine production.

Considering the foregoing, winery wastewater should be treated to specific quality standards, where after it could be stored in irrigation dams, and used for irrigation of crops. Until now, the impact of this practice has, however, not been studied comprehensively. Thus, to know the impact of irrigating with winery wastewater on the chemical composition and physical structure of the soil, grapevine performance, and wine quality, is indispensable. It is envisaged that irrigating vineyards with irrigation water with added winery wastewater might alter the wine character and overall quality, compared to vineyards irrigated with raw irrigation water. In this regard, a research project to investigate the possible use of augmented winery wastewater for vineyard irrigation was initiated and funded by the Water Research Commission of South Africa. The project was

co-funded by Winetech and the Agricultural Research Council. During a Workshop held on 15 May 2008 in Stellenbosch, the terms of reference for the project, including the range of COD levels to which the winery wastewater had to be augmented for the different treatments, were finalised.

Project objectives

The general objective of the project was to investigate the sustainable use of winery wastewater for irrigation of vineyards with respect to the effect on soils, vineyard performance and wine quality. The specific objectives of the project were:

(i) To determine the effect of winery wastewater augmented to different chemical oxygen demand (COD) levels with raw water on soil characteristics with reference to amongst others: physical, biological and chemical properties, whilst considering EC_e, pH, SAR, N, P and K.
(ii) To determine the effect of winery wastewater augmented to different COD levels with raw water on the response of vineyards, cover crops and weeds to irrigation with winery wastewater with reference to amongst others: vegetative growth, reproductive growth, grape composition, must and wine quality.
(iii) To organize field days for winemakers, farmers and technical advisors to demonstrate the effects of augmented winery wastewater on grapevine yield and wine quality and present the guidelines for the use of such water.
(iv) To publish popular and scientific articles regarding the use of augmented winery wastewater for vineyard irrigation in relevant journals and magazines.

Experiment layout

Based on the foregoing, the project was a multidisciplinary study which evaluated the impact of augmented winery wastewater on soils, vineyard performance and wine quality, if any, due to poor water quality as the main objective. The possibility of recycling winery wastewater for vineyard irrigation was investigated in a field trial near Rawsonville in the Breede River Valley. Wastewater obtained from a co-operative winery was augmented to levels of 100 mg/L, 250 mg/L, 500 mg/L, 1000 mg/L, 1500 mg/L, 2000 mg/L, 2500 mg/L and 3000 mg/L chemical oxygen demand (COD), respectively, using raw water obtained from the Holsloot River. The augmentation was carried out individually for each concentration in 15 m^3 tanks at the vineyard. Raw water from the river was used to irrigate the control grapevines. The irrigation treatments were applied to Cabernet Sauvignon grapevines planted in a sandy alluvial soil. Each treatment was replicated three times in a randomized block experiment layout. Determining the effect of augmented winery wastewater on the chemical properties of four different soils in a pot trial also formed part

of the project. These soils were (i) alluvial sand from Rawsonville, (ii) aeolic sand from Lutzville, (iii) shale-derived soil from Stellenbosch and (iv) granite-derived soil from Stellenbosch. Irrigation with wastewater augmented to *c.* 3000 mg/L COD was compared to irrigation with clean municipal water. The wastewater was obtained from the winery near Rawsonville. The pot experiment was carried out at the ARC Infruitec campus at Stellenbosch. During the 2010/11 season, an additional pot experiment was included to determine the effect of augmented winery wastewater on the hydraulic properties of the same four soils. However, these soils were subjected to different levels of wastewater augmentation in the vineyard where the field trial was carried out.

Soil chemical status

Field trial: Soil samples were collected in the work rows of selected treatments after the application of wastewater irrigations in May, and again from all treatments at bud break, *i.e.* following winter. Samples were taken over 30 cm increments to a depth of 1.8 m. Although there were no clear trends in soil $pH_{(KCl)}$, EC_e or acidity, EC_e was substantially higher after the seasonal wastewater irrigations compared to bud break. This was probably due to the higher salt content in the augmented wastewaters. There was a close correlation between P applied *via* the irrigation water and the P levels in the 0 to 30 cm soil layer in the work row. Under the prevailing conditions soil K (Bray II) increased with a decrease in the dilution of the wastewater during all four seasons. After four years, only the lowest level of augmentation, *i.e.* 3000 mg/L COD, maintained baseline K levels. Soil Ca and Mg did not show any consistent responses to the different levels of wastewater augmentation because there were no substantial differences to amounts of these particular elements applied *via* the irrigation water. Generally, soil Na increased with a decrease in the dilution of the wastewater. There were substantial differences in the amount of Na applied *via* the irrigation water. Although irrigation with winery wastewater had almost no other effects under the prevailing conditions, element accumulation, particularly with respect to K and Na, might be more prominent in heavier soils or in regions with low winter rainfall.

Pot experiments: Results obtained with the pot experiment showed that, irrespective of the clay content, irrigation with winery wastewater augmented to 3000 mg/L increased soil $pH_{(KCl)}$ by *c.* 2 units compared to clean municipal water, except for the Stellenbosch granitic soil where the increase was only *c.* 1 unit. Salinity levels in all four soils increased where winery wastewater was applied compared to clean water. In relation to the other exchangeable cations, soil Na_{ex} accumulated to the extent that the ESP threshold of 15% was exceeded, irrespective of the clay content. Irrigation with augmented winery

wastewater did not increase soil organic C compared to irrigation with clean water, irrespective of the clay content. This indicated that breakdown of organic material applied *via* the winery wastewater occurred between irrigations although the COD level was approximately hundred times higher in the winery wastewater. Since the pot experiment was carried out under a rain shelter, the foregoing reflects a worst case scenario, *i.e.* where no leaching due to rainfall occurred.

Results of the pot experiment carried out in the field trial showed that irrigation with augmented winery wastewater did not have any effect on the saturated hydraulic conductivity (K) of the soils during the first two years. In the case of the shale-derived soil, as well as the alluvial and aeolian sands, K decreased substantially with a decrease in the level of winery wastewater augmentation after three years. In the case of the granite-derived soil from Stellenbosch there was no effect on K after three years. At this stage there is no explanation for the behaviour of the granite-derived soil, except that its water permeability was generally low. It should be noted that the soils received no raw water irrigation which could have influenced the effect of the wastewater on K. In spite of this, the results indicated that severe reductions in K will occur in the long run if augmented winery wastewater is used for irrigation on these soils. Furthermore, the reduction in K might be aggravated if undiluted winery wastewater is used for irrigation of crops. Similar to the pot experiment under the rain shelter, the potted soils in the field trial were not exposed to rainfall or raw water irrigations. Therefore, the absence of leaching probably explains why the augmented water reduced K even on the sandy soils after only three years. The latter result is in contrast with the field trial where irrigation with augmented winery wastewater apparently did not have any effect on hydraulic properties of the sandy soil. This suggests that the cover crops probably masked the negative effect of the winery wastewater soil on K in the field trial.

Element uptake and removal by cover crops

Cover crops, *i.e. Avena sativa* L. cv. Pallinup (oats) and *Pennisetum glaucum* L. cv. Babala (pearl millet) were established in the work rows during winter and summer, respectively. The dry matter production (DMP) and element content of the above-ground growth of oats and pearl millet was determined over a period of four and three years, respectively. Oats tended to produce more dry matter when irrigated with augmented winery wastewater compared to raw water irrigation, if not preceded by pearl millet as a summer interception crop. Oats continuously produced acceptable amounts of fibre. The levels of Ca, Mg and K in the above-ground growth did not differ between treatments. Although differences occurred, no trends with respect to level of augmentation were observed for N and Na. However, the Na levels increased over time. Being sown on 10

January allowed the growth of pearl millet to peak while 91% of the augmented winery wastewater was applied. The latter improved the DMP of pearl millet. The augmented winery wastewater did not affect the levels of N, P, Ca and Mg in the above-ground growth, but increased the level of Na slightly over time. Although the levels of K differed between treatments, no trends were observed. Using both species, too much N, K, P, Mg and Ca was intercepted. However, the amounts of Na removed remained insignificant.

The fertiliser added (approximately R 2 800/ha/yr) to compensate for excess N and P intercepted by pearl millet, is much less than the R 15 000 to be made by selling the harvested crop for fodder. Employing only pearl millet as an interception crop could, therefore, be a sustainable practice if the COD level of the winery wastewater is between 1500 mg/L and 2500 mg/L. The use of species normally planted for grazing (especially N-fixers) as interception crops deserves investigation.

Soil microbial status

Soil microbial activity by enzyme analysis using a colorimetric assay was carried out in soils collected at different soil depth layers in grapevine rows over four seasons. This was supported by coarse-level comparisons of total heterotrophic and actinomycete populations by dilution plating on growth media, monitoring shifts in microbial communities using the ARISA method, as well as measuring soil glomalin by a simple Bradford assay. It was found that soil microbial enzyme activity was most sensitive to changes triggered in the top soil layers where it was highest in the 0 to 10 cm layer, and gradually decreased with increasing depth. Since this gradient in enzyme activity was observed, not only during pre- but also after-treatment assessments, it implies that irrigation with winery wastewater were of no negative consequence to organic matter breakdown processes in soil. In fact, the findings suggest that when irrigation was applied, easily decomposable organic matter would have been added to the soil, which, when assessed over the entire trial period, promoted soil enzyme activity, which coincided with an increase in organic loads, *i.e.* an increase in COD concentration.

Enzyme activity also seemed to have been stimulated over time as more irrigation was applied. When assessed over the entire trial period, microbial population sizes also decreased with depth, but the impact of irrigation with winery wastewater on general microbial counts was inconclusive. Likewise, the shifts in soil microbial communities were inconclusive, primarily due to inconsistent results. Glomalin content also decreased with an increase in soil depth, but did not respond to level of COD in the augmented wastewater. Given that both glomalin and soil microbial enzyme activity are considered good indicators of soil health, irrigation with winery wastewater should be of little to no

consequence to general soil health. Furthermore, soil fertility may even be improved given the marked positive effects of winery wastewater on soil microbial enzyme activity under the prevailing conditions of the current study. The foregoing findings should nevertheless be received with great caution as some of the findings should be substantiated with further research.

Grapevine responses

Vegetative growth and yield: Irrigation of grapevines using winery wastewater augmented up to a maximum COD level of 3000 mg/L did not affect vegetative growth or any of the yield components compared to the raw water control. Consequently, evapotranspiration (ET) and grapevine water status were not affected by the wastewater irrigation under the given conditions. Since organic carbon did not accumulate in the soil during the study period, it suggested that the soil was sufficiently aerated between irrigations to allow oxidation. The latter probably explains why the grapevines did not respond to level of COD *per se*. However, it is important to note that the salinity and sodicity levels in the augmented winery wastewater were below the thresholds for grapevines. This was confirmed by the lack of response in element content in the leaves and shoots. Therefore, the low salinity and sodicity levels in the augmented winery wastewater could be a further, and maybe more realistic, explanation why the grapevines did not respond to the wastewater irrigation. Since vegetative growth and yield of the grapevines were comparable to responses previously reported for Cabernet Sauvignon vineyards without a summer cover crop, the results suggested that the grapevines were not affected by the pearl millet growing in the work rows during summer. Visual observations revealed that the root system of this cover crop was shallow compared to that of the grapevines. Therefore, competition for water and nutrients was probably not strong enough to have induced negative effects on grapevine growth and yield. However, results indicated that a summer cover crop may increase the ET of vineyards substantially if growing conditions are favourable for the cover crop. The contribution of the slash and removal costs to already high production costs of vineyards is a further aspect that needs consideration.

Juice and wine characteristics: Under the prevailing conditions, irrigation of grapevines using winery wastewater augmented up to 3000 mg/L COD did not have any detrimental effects on juice ripeness parameters and ion content. Wine sensorial quality was also not affected. Under the conditions of the study, the high irrigation volumes were generally detrimental to wine quality. Since wine quality is an important aspect, particularly if wine needs to be exported, the poor overall quality is of great concern. However, there is ample evidence that less frequent irrigation, which allows higher levels of plant available water (PAW) depletion between irrigations, will enhance wine quality. This implies that the

winery wastewater will probably have to be applied over large areas to allow sufficient PAW depletion between irrigations. Distribution of winery wastewater over large areas will need additional infrastructure, which could be expensive. A pilot study carried out in the third season suggests that grapevine bunches exposed to direct contact with winery wastewater may decrease in spicy character, increase wine volatile acidity (VA) and cause a winery wastewater-like off-odour in wines. Furthermore, as the quality of the water decreases, these off-odours may increase. Therefore, even though wine colour and common sensory wine descriptors were not affected by the various treatments, any further increase in wine VA or wastewater off-odour may reduce wine quality. Although wastewater odours may differ from winery to winery, the risk for off-flavours cannot be excluded. The foregoing also clearly demonstrates that overhead sprinkler irrigation will not be suitable if winery wastewater is recycled for vineyard irrigation.

Recommendations

Based on the project results, the following criteria should be considered for possible amendments to the General Authorization for wineries when using augmented wastewater for irrigation of vineyards:

(i) The COD must be augmented to 3000 mg/L or less, preferably to less than 2000 mg/L to avoid unpleasant odours while irrigations are applied.

(ii) The EC_{iw} must be less than 0.75 dS/m.

(iii) The SAR_{iw} must be less than 10.

(iv) It should be a sandy soil with low CEC.

(v) The internal drainage in the root zone must be unrestricted.

(vi) The irrigation water must not percolate beyond the root depth.

(vii) Only micro-sprinklers should be used, since drippers have narrow flow paths and/or small orifices, and are more susceptible to clogging.

(viii) The irrigation must be applied with micro-sprinklers in such a way that the bunches are not wetted.

(ix) At least 50% PAW depletion should be allowed between irrigations to allow sufficient aeration for oxidation of organic material applied *via* the irrigation water.

(x) The irrigation frequency and volumes (schedule) should enhance, rather than negate, wine quality characteristics.

(xi) Due to the possibility that direct contact with winery wastewater may cause off-odours in the wine, overhead sprinkler irrigation is not recommended if winery wastewater is re-used for vineyard irrigation.

(xii) A summer interception crop, *e.g.* pearl millet, should be sown in early January to ensure that its growth peaks when the winery wastewater is applied, and that the species does not complete its life cycle too early.

(xiii) Since a summer interception crop may increase the ET of vineyards substantially if growing conditions are favourable, it could induce competition for water between grapevines and cover crop. However, this can be avoided by timely slashing of the interception crop, which will also minimise possible competition for N and P.

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Dr. A.J. Sanewe	Water Research Commission
Mr. J.H. Booysen	Winetech
Mrs. A. Andrag	Winetech
Dr. J.E. Hoffman	Stellenbosch University
Mr. H.M. du Plessis	Independent Consultant
Mr. J. Rossouw	Distell
Mr. D. Schietekat	Integrated Production of Wine (IPW)
Mr. H. Myburgh	Goudini Winery
Mrs. M. Lintnaar-Strauss	Department of Water Affairs and Sanitation (Western Cape)
Ms. A.D. Nefale	Department of Water Affairs and Sanitation (Head Office)
Mr. L. Van Schoor	Enviroscientific
Mrs. T. Nell	Secretary, ARC Infruitec-Nietvoorbij

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CHAPTER 1: MANAGEMENT OF WINERY WASTEWATER BY RE-USING IT FOR CROP IRRIGATION – BACKGROUND, PROJECT OBJECTIVES AND KNOWLEDGE REVIEW

P.A. Myburgh & C.L. Howell
The Fruit, Vine and Wine Institute of the Agricultural Research Council, Private Bag X5026, Stellenbosch, 7599

1.1. BACKGROUND

Wine production is an important industry in the Western Cape and the Lower Orange region in the Northern Cape. Wineries produce large volumes of low quality wastewater, particularly during the harvest period. In South Africa, historically most of the winery wastewater has been disposed of to land though irrigation of pastures (J. Rossouw, personal communication, 2014). With regards to the National Water Act (NWA) (1956), if a person was unable to comply with the conditions of Section 21(1), the Minister could exempt such a person from compliance with either one or both the requirements of Section 21(1)(a) and 21(1)(b). South African wineries found compliance with the requirements of Sections 21(1)(a) and 21(1)(b) of the NWA (1956) technically and financially challenging. Therefore, most wineries applied for exemption from compliance with these requirements as they are based in the rural areas, where land is available for irrigation and the resulting grazing could be used (J. Rossouw, personal communication, 2014). The use of untreated wastewater was also considered suitable for irrigation of kikuyu grass for grazing purposes as it consists mainly of biodegradable organic pollutants. The soil, and more particularly bacteria present in the soil, was found to be able to break down organic components in the wastewater under aerobic conditions. However, there are certain secondary environmental pollution risks associated with such irrigation, namely the potential occurrence of unpleasant odours and seepage as a result of over irrigation which can result in saturated, anaerobic soils. Subsequently, many wineries were exempt from the requirements of Section 21(1), and there were certain conditions which limited the volume of wastewater that could be irrigated (J. Rossouw, personal communication, 2014). However, many of the exemptions did not specify a minimum area that had to be irrigated nor a requirement for water quality analysis. In addition to this, very often there was no expiry date for the exemption. Consequently, over-irrigation and soil saturation occurred which led to an accumulation of organic material, as well as inorganic salts such as P, Na and K in the soil. This resulted in soil degradation and possible seepage of wastewater into underground resources at certain irrigation areas.

In 1997, DWAF published a Water Policy White Paper which repealed the NWA of 1956, and a new NWA (Act 36) was promulgated in 1998. This Act, in Section 22, did not make provision for exemption from compliance with the Act. The Act did define the irrigation of

land with waste or water containing waste as a specific type of water use, in terms of section 37(1)(a). The controlled activity could be deemed a permissible water use activity. In 2004, DWAF, recognising that the lack of conditions requiring at least the monitoring of the quality and quantity of wastewater being irrigated did not allow for the responsible management of the irrigation practice, incorporated these conditions into all new authorizations under the NWA (J. Rossouw, personal communication, 2014). These General Authorizations require not only the monitoring of wastewater quantity and quality, but also specifies limits for wastewater being irrigated based on the volume irrigated per day.

In 1999, Winetech initiated research projects into improving the understanding of the composition and handling of winery wastewater (J. Rossouw, personal communication, 2014). In 2005, this research culminated in guidelines for the management of winery wastewater that not only focussed on assisting wineries in developing wastewater management plans, but also advised wineries as to how they could improve the quality of the winery wastewater by employing cleaner production principles. Winetech also started to engage more pro-actively with DWAF and through their co-operation secured their input into the guideline development process to ensure that the industry was fully aware of the legal requirements of the NWA. They also actively engaged in securing compliance with these conditions. In 2004, there was a series of meetings in June and August between various role players to discuss a proposed General Authorization for the wine industry. DWAF indicated the need of the wine industry to move away from disposal irrigation of untreated wastewater.

In a meeting on 12 August 2004, a treatment and disposal methodology was agreed upon on which the proposed General Authorization could be based (L.H. Van Schoor, personal communication, 2014). Furthermore, treated winery wastewater, in combination with other water should be used for beneficial irrigation of agricultural crops such as vineyards. Therefore, winery wastewater should be treated to a specific quality standard, where after it could be stored in irrigation dams, and used for irrigation of other crops. Until now, the impact of this practice has, however, not been studied comprehensively. Adding winery wastewater to the current irrigation water (augmentation) may well become a necessity in the future with current water shortages becoming more and more an alarming reality. Thus, to know the impact of irrigating with winery wastewater on the chemical composition and physical structure of the soil, grapevine performance, and wine quality, is indispensable. It is envisaged that irrigating vineyards with irrigation water with added winery wastewater might alter the wine character and overall quality, compared to vineyards irrigated with raw irrigation water. In this regard, a research project to investigate the possible use of augmented winery wastewater for vineyard irrigation was initiated and funded by the Water Research Commission of South Africa. The project was co-funded by Winetech and the

Agricultural Research Council. During a Workshop held on 15 May 2008 in Stellenbosch, the terms of reference for the project, including the range of COD levels to which the winery wastewater had to be augmented for the different treatments, were finalized.

1.2. PROJECT OBJECTIVES

The general objective of the project was to investigate the sustainable use of winery wastewater for irrigation of vineyards with respect to the effect on soils, vineyard performance and wine quality.

The specific objectives of the project were:

- To determine the effect of winery wastewater augmented to different chemical oxygen demand (COD) levels with raw water on soil characteristics with reference to amongst others: Physical, biological and chemical properties, whilst considering EC_e, pH, SAR, N, P and K.
- To determine the effect winery wastewater augmented to different COD levels with raw water on the response of vineyards, cover crops and weeds to irrigation with winery wastewater with reference to amongst others: Vegetative growth, reproductive growth, grape composition, juice and wine quality.
- To organize field days for winemakers, farmers and technical advisors to demonstrate the effects of augmented winery wastewater on grapevine yield and wine quality and present the guidelines for the use of such water.
- To publish popular and scientific articles regarding the use of augmented winery wastewater for vineyard irrigation in relevant journals and magazines.

1.3. KNOWLEDGE REVIEW

1.3.1. INTRODUCTION

In South Africa, grapes are an important crop in regions such as the Western Cape and the Lower Orange river in the Northern Cape. The wine industry makes a significant contribution to the economy in these regions. In 2012 there were 3440 primary wine grape growers (SAWIS, 2013). Furthermore, the wine industry provides a large number of employment opportunities, particularly in the rural areas. In 2012, the vineyards planted for wine production in South Africa amounted to 100 093 hectares, of which *c.* 92% is considered as producing, *i.e.* four years and older (SAWIS, 2013). The number of wineries which crush grapes almost doubled from 1991 to 2002 (Table 1.1). Since 2005, the number of wineries appeared to be more or less stable. During this period, the industry produced around one billion litre of grape related products annually (Table 1.2).

Table 1.1. Growth trends in the South African wine industry (SAWIS, 2013).

Role player	Number							
	1991	2002	2005	2008	2009	2010	2011	2012
Wine cellars which crush grapes	212	427	581	504	527	593	505	509
Co-operatives	70	66	65	58	57	54	52	50
Wine producing wholesalers	6	11	21	23	23	26	25	23

Table 1.2. Wine production trends in the South African wine industry (SAWIS, 2013).

Product	Production (million litre)							
	2005	2006	2007	2008	2009	2010	2011	2012
Wine	628.5	709.7	730.4	780.2	739.0	779.8	831.2	870.9
Rebate	82.9	82.1	101.5	88.0	75.8	39.6	34.2	62.3
Juice	64.6	73.2	65.2	66.9	55.0	52.1	40.2	40.1
Distilling wine	129.2	147.9	146.4	157.9	152.2	113.3	107.2	121.8
Total	905.2	1012.9	1043.5	1093.0	1022.0	984.8	1012.8	1095.1

Using raw water is an integral part of the wine production processes. These processes generate wastewater of low quality that can be re-used for irrigation or disposed of in natural systems, subject to certain quality requirements. Winery wastewater can cause salinization and eutrophication of water resources, *i.e.* natural streams, rivers, dams, groundwater and wetlands (Van Schoor, 2005 and references therein, Laurenson *et al.*, 2012). Furthermore, wastewaters can cause soil sodicity, salinity, contamination with a wide range of chemicals, waterlogging and anaerobiosis, loss of soil structure and increased susceptibility to erosion. Where solid wastes are present, offensive odours may be generated and seepage may result in the contamination of soil and water resources that can inhibit vegetative performance (Van Schoor, 2005 and references therein).

1.3.2. VOLUME OF WATER INVOLVED IN THE WINEMAKING INDUSTRY

1.3.2.1. Water used for winemaking

Information on the actual amounts of water used by wineries is limited and appears to be inconsistent. A survey carried out in South Africa, which included wineries that crush up to 22 000 metric tons of grapes annually, showed that the volume of raw water increased significantly with the amount of grapes crushed (Sheridan *et al*., 2005). Although the variability between wineries was high, the slope of the relationship indicated that approximately 2 m^3 of water was required to crush one tonne of grapes. The Lutzville Vineyards' winery uses a measured average 100 000 m^3 of water to produce between 30 million and 40 million litre of wine annually (Kriel, 2008). Since this particular winery crushes

approximately 47 500 tons per year (G. Theron, personal communication), about 2.1 m^3 of raw water is required to process one ton of grapes. Although the amount of grapes crushed is substantially higher, the amount of water used by Lutzville Vineyards' winery agrees with the results of the survey carried out by Sheridan *et al*. (2005). According to Mosse *et al.* (2011), wineries in Australia generally require 3 m^3 to 5 m^3 water to crush a ton of grapes. The average annual grape production in South Africa was 1.33 million tonnes from 2010 until 2012 (SAWIS, 2013). If it is assumed that winemaking in South Africa requires approximately 2 m^3 of water to process one tonne of grapes, it can be roughly estimated that the wine industry is currently using 2.66 million litre of raw water annually.

1.3.2.2. Volume of wastewater generated during winemaking

Reports on the actual volumes of wastewater that are generated by wineries are also extremely limited. It is estimated that medium to large wineries generate more than 15 000 m^3 of wastewater annually, whereas small wineries generate less than 15 000 m^3 annually (Van Schoor, 2005 and references therein). Australian wineries generate about 5 m^3 of wastewater per tonne of grapes crushed (Chapman *et al.*, 1995). Crushing approximately 50 000 tonnes of grapes annually generate about 175 000 m^3 of wastewater at the Berri estates' winery in the Riverland region of South Australia (Anonymous, 2010). Hence, their wastewater generation amount to *ca.* 3.5 m^3 per tonne of grapes. Usually most of the raw water entering wineries end up as wastewater. In contrast, it is estimated that 50%, *i.e.* 50 000 m^3, of the raw water used by the Lutzville Vineyards' winery ends up as wastewater (Kriel, 2008). The other half of the water is presumably lost to evaporation under the warm, windy atmospheric conditions in the Lower Olifants River region. This means that this particular winery generates about 1.1 m^3 of wastewater per tonne of grapes crushed. In comparison, substantially lower volumes were reported by Bories and Sire (2010), who reported that 0.359 m^3 and 0.357 m^3 wastewater was generated per ton of grapes crushed in French cellars for off-skin white wine making, and rosé and thermo-vinification of red wines, respectively. Even lower values of 0.262 m^3 of wastewater generated per ton of grapes crushed, was reported for on-skin vinification of red wines (Bories & Sire, 2010).

1.3.3. ORIGIN OF WINERY WASTEWATER AND ASSOCIATED POLLUTANTS

1.3.3.1. Sources of pollutants

Wineries vary in size, operational procedures and management practices. They undertake similar, yet highly site-specific processes. The variations result in the production of different qualities and quantities of wastewater (Van Schoor, 2005). According to Bories and Sire (2010), wine making methods can have an impact on the quality of the wastewater generated. In off-skin wine making, wastewaters are produced which contain mainly sugars. In comparison, in cellars where classical red wine making methods are followed,

wastewaters are generated which have high ethanol levels. In South Africa, the typical wine production process can be divided into various stages (Table 1.3). Medium to large wineries with year-round operations generate approximately 50% of their wastewater during the vintage period, whereas small wineries may generate up to 80% of their wastewater during harvest (Van Schoor, 2005 and references therein). The major form of wastewater from wineries is water which is used for cleaning processes (Van Schoor, 2005). The primary winemaking processes related to winery wastewater generation and their associated contribution to wastewater quantity and quality, as well as possible effects on legal wastewater quality parameters are summarized in Table 1.4. The primary water quality parameters are chemical oxygen demand (COD), electrical conductivity (EC), sodium adsorption ratio (SAR) and pH.

Table 1.3. Typical stages of winery activities and their role in wastewater generation (after Van Schoor, 2005 and references therein).

Stage	Activities	Duration (weeks)
1. Pre-harvest	Bottling takes place and tanks are washed out with sodium or potassium hydroxide. Other equipment is also washed to prepare for the harvest period.	1 to 4
2. Early harvest	Wastewater generation increases drastically during this period and reaches 40% of the maximum weekly rate measured at peak. White wine production dominates harvest activities.	2 to 3
3. Peak harvest	Wastewater generation and harvest activities reach their peak.	3 to 14
4. Late harvest	Wastewater generation decreases to 40% of the maximum (peak) weekly flow and red wine production dominates harvest activities. Distillation of ethanol may take place.	2 to 6
5. Post-harvest	Pre-fermentation activities come to a close and maximum usage of hydroxide occurs.	6 to 12
6. None harvest	Wastewater volume is at its minimum (less than 30% of the peak weekly flow). Wastewater quality depends on daily activities.	10 to 20

Table 1.4. Major processes related to winery wastewater generation and their associated contribution to wastewater quantity and quality as well as possible effects on legal wastewater quality parameters (after Van Schoor, 2005).

Winery operation	Contribution to total wastewater quantity	Contribution to wastewater quality	Effect on legal wastewater quality parameters
Cleaning water			
Alkali washing (removal of K-bitartrate) and neutralization	Up to 33%	Increase in Na, K, COD and pH Decrease in pH	Increase in EC, SAR, COD Variation in pH
Rinse water (tanks, floors, transfer lines, bottles, barrels, etc.)	Up to 43%	Increase in Na, P, Cl, COD	Increase in EC, SAR, COD Variation in pH
Process water			
Filtration with filter aid	Up to 15%	Various contaminants	Increase COD and EC
Acidification and stabilization of wine	Up to 3%	H_2SO_4 or NaCl	Increase COD and EC Decrease in pH
Cooling tower waste	Up to 6%	Various salts	Increase COD and EC
Other sources			
Laboratory practices	Up to 5-10%	Various salts, variation in pH, etc.	Increase COD and EC

1.3.3.2. Quality of wastewater generated in wineries

Unlike the volumes of wastewater produced, there are many reports on the quality thereof, particularly in terms of COD or biological oxygen demand (BOD) (Chapman *et al*., 1995; Ryder, 1995; Deans, 2003; Jeison *et al.*, 2003; Sheridan *et al*., 2005; Baker & Hinze, 2007; Kriel, 2008; Matthews, 2008; Arienzo *et al*., 2009; Mulidzi *et al*., 2009a). The BOD is estimated as 66% of the COD (Van Schoor, 2005). Winery wastewaters also contain high levels of K and Na (Laurenson *et al.*, 2012). Although various parameters may be used to evaluate winery wastewater, COD, pH, SAR, EC, chloride, potassium, and sodium are considered to be important. A survey was carried out in 2000 to evaluate the winery wastewater generated by the South African industry in terms of these variables (Mulidzi *et al*., 2009a). Results of this survey showed that there is considerable variation in wastewater quality parameters between wineries, but there is also a strong seasonal variation at most wineries. A similar seasonal trend was reported for winery wastewater in Australia (Arienzo *et al*., 2009). These trends were confirmed where effluents of two wineries were monitored frequently (Sheridan *et al.*, 2011). Considering the legal requirements for irrigation water quality in South Africa (Table 1.5), results of the survey confirmed that the majority of South African wineries are not able to irrigate crops beneficially as part of the General Authorization with wastewater unless the water is first subjected to an effective form of pre-treatment, or unless there is relaxation of the General Authorization.

Table 1.5. General Authorizations for legislated limits for chemical oxygen demand (COD), faecal coliforms, pH, electrical conductivity (EC) and sodium adsorption ratio (SAR) for irrigation with wastewater in South Africa (DWA, 2013).

Parameter	Maximum irrigation volume allowed (m^3/day)		
	< 50	**< 500**	**< 2000**
COD (mg/L)	5 000	400	75
Faecal coliforms (per 100 ml)	1 000 000	100 000	1 000
pH	6-9	6-9	5.5-9.5
EC (mS/m)	200	200	70-150
SAR	<5	<5	Other criteria apply

Different winemaking processes also affect the composition of winery wastewater. In the case of off-skin winemaking, sugars are the main component of the organic load in the effluent water, whereas classical winemaking methods generate wastewaters containing high levels of ethers and ethanol (Bories & Sire, 2010). However, it is also possible that spikes of extremely low quality can be caused by process interruptions. Power failure, fire, flood, storms, over- or under-loading of wastewater treatment systems, temporary unavailability of wastewater holding dam capacity and the absence of trained operators

may cause process interruptions (Campos *et al*., 2000; Van Schoor, 2005; Baker & Hinze, 2007).

1.3.4. MANAGEMENT OF WINERY WASTEWATER

1.3.4.1 Wastewater treatment

Wastewater is usually collected in one or more sumps at the wineries. The first step in the treatment of winery wastewater is usually to remove the solids such as grape pips, skins and stems. This is obtained by passing the water through a screen filter. The latter is a simple, but effective step and helps to prevent other treatment machinery from getting clogged with solids (Mosse *et al.*, 2011). The wastewater is normally acidic and the pH can be less than 3. Therefore lime is added to the water in order to adjust the pH to between 6 and 8. The water is then pumped to sedimentation or maturation ponds to allow settling of the remaining solids. Depending on the quality of the wastewater at this stage, the water can be used to irrigate selected crops, such as Kikuyu grass, in specific soils. A further step could be to circulate and aerate the wastewater in dams using an aeration pump system. If these steps are managed correctly, the treatment of the wastewater can be fairly successful, particularly in reducing the COD levels (Tables 1.6 & 1.7 & Fig. 1.1).

Table 1.6. Mean winery wastewater quality during the crushing season and in aerated storage ponds in California's North Coast region (after Ryder, 1995).

Parameter	Crushing season	Treated ponds
COD[(1)] (mg/L)	3780	15
pH	4.1	7.7
Nitrogen (mg/L)	20	5
Phosphorus (mg/L)	10	2
Soluble solids (mg/L)	800	500

[(1)] Adjusted from biological oxygen demand (BOD) where BOD = 66% of COD.

Table 1.7. Variation of chemical oxygen demand (COD) and total suspended solids (TSS) in raw and treated winery effluent (after Baker & Hinze, 2007).

Sampling date	COD[(1)] (mg/L)		TSS (mg/L)	
	Wastewater	Final effluent	Wastewater	Final effluent
18 November 2005	9091	16	1700	92
19 December 2005	2727	28	265	66
13 February 2006	3788	8	280	16
23 March 2006	6621	788	940	1080
28 April 2006	644	72	319	683
08 June 2006	5788	64	245	460
18 January 2007	4848	14	400	53
28 March 2007	6712	379	1040	617

[(1)] Adjusted from biological oxygen demand (BOD) where BOD = 66% of COD.

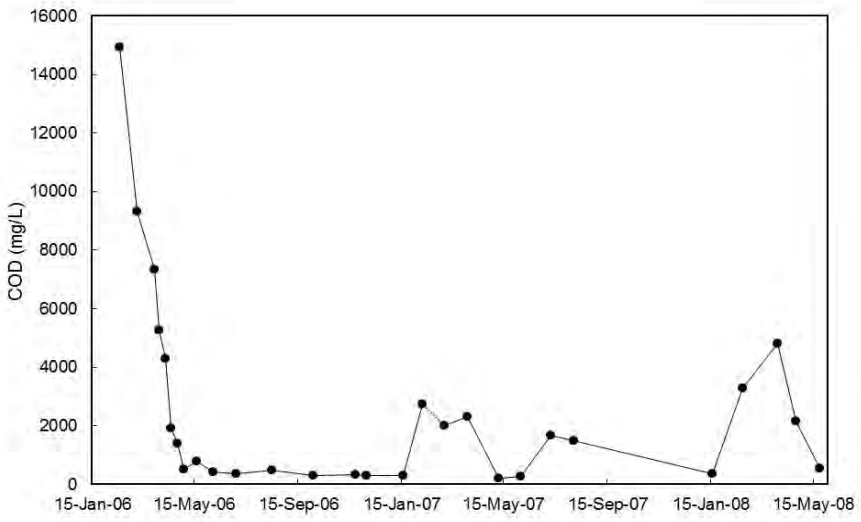

Figure 1.1. Seasonal variation in level of chemical oxygen demand (COD) in treated winery wastewater following aeration of the water which commenced in January 2006 (data supplied by the courtesy of the Botha winery).

Up-flow anaerobic sludge blanket (UASB) technology can also be used to treat winery wastewater (Matthews, 2008). This technology relies on anaerobic digestion, a biological process in which organic matter is converted to methane and carbon dioxide in the absence of air. The process involves a synergistic relationship between for different groups of bacteria, namely hydrolytic, fermentative-acidogenic and methanogenic. The bacteria cluster into granules which settle out to form a dense bed of sludge that is retained in the system. This is a distinct advantage over aerobic systems which produce masses of surplus sludge that must be disposed of. However, disadvantages are that nutrient removal is not feasible in anaerobic systems and trained staff is needed to operate UASB systems. Anaerobic digestion is often limited by the presence of refractory and toxic compounds in the wastewater, but ozone helps counter this effect. Pre-ozonation enhances the biodegradability of organic matter by converting these compounds into simpler molecules. Post-ozonation may be used as a “polishing” step. In addition, installation costs are relatively high (Mosse *et al.*, 2011).

Worldwide, most UASB plants have operational volumes of 100 000 litre to 10 million litre (Matthews, 2008). Only a few operate on less than 50 000 litre. A winery near Franschhoek operates a relatively small, fully automatic UASB system which can treat 25 000 litre per day. This particular wastewater treatment plant reduces the COD toc. 250 mg/L throughout the year. It was also shown that UASB technology can be used for the successful treatment of wastewater generated in the production of Chilean pisco, an aged drink distilled from grapes (Jeison *et al*., 2003). Expanded granular sludge bed (EGSB) technology was also tested in this study, but it was more difficult to operate and

required higher capital investment as well as operational costs compared to the UASB technology.

1.3.4.2. Disposal of winery wastewater

1.3.4.2.1. Return to natural resources

In terms of the General Authorizations published in Government Notice No. 665 (DWA, 2013) in terms of section 39 of the National Water Act (1998), untreated wastewater from wine cellars would rarely, if ever, qualify for discharge into natural water resources (Van Schoor, 2005). Given the quality of the treated water, most wastewaters would still not be suitable for discharge into natural water resources. Consequently, the latter practice is not really considered as a disposal option, unless the water is sufficiently treated.

1.3.4.2.2. Disposal ponds

Some wineries pump the treated wastewater into ponds or storage dams. If the water is not recycled for irrigation, it evaporates or seeps into deeper soil layers when the ponds or dams are unlined (Mulidzi *et al.*, 2009b). Multi-stage facultative aerobic ponds have been used successfully for some 30 years for treatment and storage of winery wastewater in California (Ryder, 1995). These ponds are lined to prevent seepage of water into underground water streams and are aerated sufficiently to prevent objectionable odour generation.

1.3.4.2.3. Irrigation with winery wastewater

In South Africa more than 93% of wine cellars dispose of their effluent by means of land application (Van Schoor, 1995). The majority of cellars currently dispose effluent by irrigation as the primary disposal option. Land application systems are ideally suited for the treatment of organic carbon contained in winery effluents because the water in the soil system transports the organic contaminants to the aerobic microbial populations. However, it is most important that waterlogging should be avoided. Consequently, it is essential to allow sufficient time between irrigations for the soil to become aerobic (Chapman, 1995).

1.3.4.2.3.1. Crops irrigated with winery wastewater

In most cases, the wastewater is used for the irrigation of small permanent pasture grazing paddocks close to the wineries. The pastures mainly consist of Kikuyu grass, but Fescue grass can also be irrigated with winery wastewater (Arienza *et al*., 2009). There are also cases in Australia where treelots, *e.g. Eucalyptus camadulensis*, are irrigated with winery wastewater (Chapman, 1995; Deans, 2003; Anonymous, 2010). Research results have also shown that lemon nursery trees could successfully be irrigated with wastewater generated by a pisco distillery after the water had been treated using UASB technology

(Jeison *et al*., 2003). The pisco distillery wastewater was also disposed of in a Eucalyptus tree lot on a commercial scale.

Winery wastewater stored in lined and aerated ponds is used for vineyard irrigation during the rain-free spring and summer in California (Ryder, 1995). At a winery in the Clare valley in Australia, treated wastewater of which the COD contents are presented in Table 1.7, is recycled into the raw irrigation water to be used for irrigation of grapevines (Baker & Hinze, 2007). In this particular case, the treated wastewater constituted only 10% of the annual irrigated volume. The actual wastewater applied was less than 10 mm. Although some vineyards have been irrigated using winery wastewater for long periods, the effect thereof on the soils and grapevines have not been reported. In one study, where grapevines were irrigated with simulated winery wastewater, it was reported that it had little, to no impact on the grapes after one season (Mosse *et al.*, 2013).

A range of leaf analyses has been carried out from representative areas in the Eucalyptus plantation where the Berri Estate's winery dispose the wastewater (Anonymous, 2010). The relatively low nutrient levels in the winery wastewater reflected in declining, but still acceptable, levels of nitrogen, phosphorus and potassium in the leaves. However, it was concluded that some nutrient addition might be necessary during the lifespan of the trees. During the first weeks after planting, leaves of Eucalyptus saplings that were irrigated with wastewater treated in a UASB reactor showed symptoms of sodium toxicity (Jeison *et al*., 2003). The lemon trees used in the experiments showed similar symptoms. The problem was caused by the NaOH required for pH control in the UASB reactor. Approximately 2 g/L NaOH had been applied during the first weeks after reactor start up. However, after a few weeks the biogas production provided a significant level of alkalinity by CO_2 dissolution. Consequently, NaOH application was reduced to less than 20% of its original level. The Eucalyptus saplings recovered without any permanent damage. Unfortunately, the Na concentrations in the treated wastewater were not reported. There are also no other reports on the effects of irrigation with winery wastewater on the performance of most of the different species mentioned above.

Recently, research has shown that potted fodder beet grown during summer in sandy soil collected from the field trial at Rawsonville, absorbed a substantial amount of Na applied *via* Na-enriched irrigation water (Myburgh & Howell, 2014). The concentration of Na applied was equal to that of the Na concentration in the irrigation water of the 3000 mg/L COD treatment in the field trial near Rawsonville. Furthermore, the fodder beet reduced the soil K_{ex} by 50%, thereby indicating that it could also absorb K applied *via* winery wastewater.

1.3.4.2.3.2. Irrigation systems used to dispose winery wastewater

High volume sprinklers are commonly used for applying irrigation to grazing paddocks. Full surface flood irrigation was used to dispose winery wasterwater onto Fescue grass (Arienzo *et al.*, 2009) and a Eucalyptus plantation, respectively (Anonymous, 2010). It must be noted that in the latter case, diatomaceaous earth solids entered the pipeline used to transport the winery wastewater to the plantation during the grape harvesting period. This required annual flushing and/or pigging to avoid blockages. Unfortunately, most other reports on the disposal of winery wastewater by means of irrigation did not mention the systems used to irrigated the different species. Since vineyards in Australia are almost invariably drip irrigated, it can be assumed that the winery in the Clare valley in Australia disposed the treated wastewater by means of a drip irrigation system (Baker & Hinze, 2007). Aboveground as well as subsurface drip irrigation was used in a field experiment in Israel to irrigate grapevines with water from sewerage waste stabilization ponds (Campos *et al*., 2000). The rationale for using drip irrigation, and particularly subsurface drip, was to minimise the risk of disease contamination by preventing direct contact between the wastewater and the edible part of crop.

1.3.4.2.3.3. Effects of winery wastewater on soil conditions

Soil chemical status: Land application of wastewaters can increase the levels of soluble and exchangeable forms of potassium (K & K_{ex}) more rapidly than with conventional inorganic fertilizers (Arienzo *et al.*, 2009). Furthermore, most of the K in wastewater is immediately available. The effects of excessively high K application on soils have not been extensively researched and are still unclear (Mosse *et al.*, 2011; Laurenson *et al.*, 2012). In addition, the fate of K in soils and on grapevines irrigated with winery wastewater has received limited attention (Laurenson *et al.*, 2012). Irrigation with K-rich wastewaters could be beneficial to overall soil fertility, although long-term application could affect soil chemical and physical properties (Laurenson *et al.*, 2011; Mosse *et al.*, 2011). A further advantage of using winery wastewater as a source of K over the use of conventional fertiliser is that it could be an efficient recycling practice in areas where the soil has low K. In addition to Na and K ions, winery wastewater can also contain Ca and Mg ions (Mosse *et al.*, 2011). Neither of these ions is harmful to soil structure and can ameliorate the impacts of sodium ion application indirectly *via* their role in reducing the sodium adsorption ratio (SAR). Wastewater with high levels of organic material can clog soil pores, which could decreases soil hydraulic conductivity. Due to the high K levels in winery wastewater, adsorption of Na, and exchangeable sodium percentage (ESP), will be less than in wastewaters of comparable Na concentration with no K (Laurenson *et al.*, 2011). In a study investigating the irrigation of an established vineyard with simulated winery

wastewater, grapevines were either irrigated with a raw water control, high K, high K plus wine, low K, and Na (Mosse *et al.*, 2013). All treatments caused an increase in soil K and Na. The accumulation of K was restricted to the 0 to 20 cm soil layer, with the exception of the treatment where wine was added to the irrigation water. The addition of wine enhanced K transport into the subsoil. Elevated Na levels were found in the 0 to 20 cm and 20 to 40 cm soil layer. Therefore, the presence of wine, *i.e.* organic material, facilitated the transport of the K within the profile. In a study investigating the long-term use of winery wastewater, *i.e.* 30 years, an accumulation of K, Na, Ca, Mg and B was reported (Mosse *et al.*, 2012). Furthermore, soil that had been irrigated with winery wastewater for 30 years showed a decrease in soil pH with depth. The increased concentrations of the cations were attributed to higher levels encountered in the wastewater.

A survey was carried out in South Africa to assess the soil chemical status where winery wastewater had been disposed over prolonged periods (Mulidzi *et al*., 2009b). Control soil samples were collected close to the area of land where the wastewater was disposed. Unfortunately, there was no history about the volumes of water or the quality of the wastewater that had been applied to the disposal sites. However, by comparing the disposal site to the control samples it was shown that the winery wastewater almost invariably induced negative effects, irrespective of soil type (Mulidzi *et al*., 2009b). Furthermore, it was concluded that (i) in general, effluent disposal is poorly planned and managed, and disposal sites rarely seem to have been selected, because their soil properties are inappropriate for effluent disposal. In particular, deep sandy soils are unsuitable for disposal by ponding, mainly because of their high infiltration rates, high permeability and low water storage capacity and (ii) many disposal sites are too limited in area to permit the large volumes of effluent to be absorbed without surface runoff. This problem invariably persists despite the presence of Kikuyu swards and sandy subsoil (Mulidzi *et al*., 2009b).

Soil physical status: Unfortunately, no references on the effect(s) of irrigation with winery wastewater on soil physical properties could be found in the literature. Soil chemical properties can be altered by wastewater irrigation (Vogeler, 2009; Lado & Ben-Hur, 2010), and this could influence soil structure and hydraulic properties (Sort & Alcaniz, 1999; Tarchitzky *et al.*, 1999). Dissolved and suspended solids, both organic and inorganic, may induce soil clogging through physical, chemical, and biological processes (Viviani & Iovino, 2004). Degradation of soil hydraulic properties due to physical clogging of the surface layer of soil is one of the expected risks involved in wastewater reuse for irrigation (Viviani & Iovino, 2004). Their investigations, conducted in soil columns, showed that the

reductions of saturated hydraulic conductivity (K_s) were only restricted to the 0 to 2 cm depth layer, and the lower part of the column was not affected by wastewater application.

Regular irrigation with olive mill wastewaters increased soil hydrophobicity and decreased drainable porosity, because of increasing organic matter content. Furthermore, soil hydraulic conductivity was reduced compared to a control site. After 15 years of application of such wastewater, the highest infiltration rate was observed because of the presence of large and deep shrinkage cracks (Mahmoud *et al.*, 2010). According to Barbera *et al.* (2013), irrigation with olive mill wastewaters can have a temporary positive effect on soil. However, in clay soils the accumulation of salts could lead to disintegration of the soil structure. Subsequently the hydraulic conductivity would decrease. Regarding the use of wastewater generated by oil production, research showed found that the use of such water created a sodicity problem, which had negative effects on soil physical properties such as infiltration rate, K_s and pore size distribution (Al-Haddabia *et al.*, 2004).

After four years of irrigation with secondary-treated municipal wastewater, saturated and near saturated hydraulic conductivity of a soil decreased from 567 and 40 mm/h to 56 and 3 mm/h, respectively (Sparling *et al.*, 2006). In a study on a sewage farm, to investigate the effects of long-term irrigation with sewage effluent on soil physical properties, bulk density was significantly lower compared to soil which was irrigated with well-water. Furthermore, the longer the irrigation with sewage water took place, the lower the bulk density became (Mathan, 1994). Subsequently, hydraulic conductivity increased. In a study to evaluate the long-term effect of wastewater application on soil physical properties, it was also found that this practice increased organic matter content and reduced bulk density. In addition to this, long-term wastewater irrigation resulted in a higher aggregate stability and saturated hydraulic conductivity (Vogelier, 2009). In contrast, leaching a loamy and a clay soil with treated sewage effluent reduced saturated hydraulic conductivity (Lado & Ben-Hur, 2009). This was attributed to the plugging of the pores with suspended solids. However, the saturated hydraulic conductivity of a sandy soil was not affected because of its large average pore size. In a non-calcareous, sandy soil, the higher sodicity enhanced seal formation, reduced infiltration, and increased runoff. However, there were no effects of the effluent on a calcareous soil under similar conditions. According to Tarchouna *et al*. (2010), irrigation with wastewater from a sludge treatment plant reduced both saturated and unsaturated hydraulic conductivity of a very sandy soil, but it was still high enough to allow water percolation.

The negative effects of high Na levels in irrigation water on the hydraulic properties of soils are well known. According to Levy and Van der Watt (1990), increasing the amount of K resulted in a decrease in hydraulic conductivity and infiltration rate of soils. There is a

broad spectrum of possible effect of K on infiltration, ranging from being similar to Na to being similar to Ca (Arienzo *et al.*, 2009). Furthermore, it was concluded that, relative to Ca_{ex} and Na_{ex}, K_{ex} had an intermediate effect on the soil hydraulic properties. Since winery wastewater can contain high Na and/or K concentrations, the effect of SAR and potassium adsorption ratios (PAR) on the soil hydraulic conductivity at a wastewater disposal site was investigated in a laboratory study (Arienzo *et al*., 2009). The results showed that the soil hydraulic conductivity was considerably reduced when the SAR or the PAR exceeded 20. These negative effects even occurred when the electrolyte concentrations in the soil was relatively high, *i.e.* > 40 meq/L. It was also shown that the negative effect of Na was more pronounced compared to K at the same electrolyte concentration.

Laurenson *et al*. (2012) used a combination of solutions with known SAR and PAR to investigate the binding of Na and K. Their conclusions were that ESP corresponding to a given SAR was increasingly lowered at higher potassium concentrations. Subsequently, if SAR in wastewater remains similar during vintage, reductions in ESP may occur because of increasing K and exchangeable potassium percentage (EPP). Changes in soil structure will therefore be less pronounced than if irrigated with wastewaters with comparable monovalent concentrations of only Na. Therefore, in the case of winery wastewater, replacing Na-based cleaners with K-based cleaners can contribute towards decreasing clay dispersion risks. Due to the high K content in winery wastewater, substitution of K-based cleaning agents with Na-based ones has been proposed (Arienzo *et al*., 2009).

Using Na-based cleaning agents might reduce the K, but in the long run increased Na levels in soil will certainly cause more structural damage compared to K. In addition, Na could reach toxic levels in soils. On the other hand, K accumulation in the soil could be reduced through uptake and removal by crops grown on winery wastewater disposal sites. Furthermore, it should be kept in mind that the cost of potassium hydroxide is substantially higher than sodium hydroxide (Mosse *et al.*, 2011).

1.3.4.2.3.4. Present guidelines if winery wastewater is recycled for irrigation

Wastewater quality standards were proposed for irrigation of vineyards with treated winery wastewater stored in aerated ponds in California (Ryder, 1995). The maximum COD, Faecal coliforms, pH, EC_e and SAR standards (Table 1.8) are more or less comparable to the legislated limits for irrigation with wastewater in South Africa, *i.e.* if less than 2 000 m^3 is irrigated per day (Table 1.5).

Table 1.8. Proposed reclaimed effluent water quality standards for vineyard re-use (after Ryder, 1995).

Parameter	Optimum value	Maximum values
pH	6.5-8.4	6.0-9.0
EC_e (dS/m)	< 0.75	< 1.50
TDS (mg/L)	< 500	< 1000
Alkalinity (mg/L $CaCO_3$)	< 150	< 250
Hardness (mg/L $CaCO_3$)	< 250	< 400
Ca (mg/L)	< 60	< 100
Mg (mg/L)	< 25	< 50
Na (mg/L)	< 65	< 100
K (mg/L)	< 5	< 10
Fe (mg/L)	< 5	< 5
Mn (mg/L)	< 0.2	< 0.5
Cu (mg/L)	< 0.01	< 0.05
Zn (mg/L)	< 2	< 5
Bicarbonate (mg/L)	< 200	< 300
Carbonate (mg/L)	< 5	< 10
Chloride (mg/L)	< 70	< 120
Sulfate (mg/L)	< 150	< 250
N (mg/L)	< 5	< 10
P (mg/L)	< 5	< 10
B (mg/L)	< 0.5	< 1
SAR	< 6	< 9
COD[(1)] (mg/L)	< 60	< 100
Coliforms (MPN/100ml)	< 23	< 230

[(1)] Adjusted from biological oxygen demand (BOD) where BOD = 66% of COD.

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CHAPTER 2: EXPERIMENT LAYOUT, INFRASTRUCTURE FOR AUGMENTING WINERY WASTEWATER AND PRELIMINARY MEASUREMENTS

P.A. Myburgh, E.L. Lategan & C.L. Howell
The Fruit, Vine and Wine Institute of the Agricultural Research Council, Private Bag X5026, Stellenbosch, 7599

2.1. INTRODUCTION

Following discussions with VINPRO consultants in the Breede river region, it was decided to carry out the field trial at the Botha winery between Worcester and Wolseley. A Shiraz vineyard, approximately one km from the winery, was selected. Permission to carry out the field trial in this particular vineyard was granted by the owners. The experimental plots were marked in June 2009 in order to measure cane mass that was to be used as a covariant in later statistical analyses, and to install neutron probe access tubes. However, heavy rain occurred during this period which totally saturated the sandy soil. There were indications that lateral water flow occurred through the vineyard which is on a slight slope. This created the danger of lateral flow between experiment plots, as well as contamination of the surrounding soils. Deep cut-off drains above and below the vineyard would have increased the cost of the field trial considerably. Once the experiment plots were marked, it turned out that the number of missing grapevines in some plots would be problematic in terms of the amount of grapes required to make wine from the grapes produced from each plot.

Based on the foregoing it was decided to move the field trial to the Goudini winery near Rawsonville, also in the Breede river valley. A Cabernet Sauvignon vineyard was identified approximately 500 m from the winery (Fig. 2.1). Although there appeared to be some variation in growth, this vineyard had almost no missing grapevines. A further advantage was that there were no stones in the soil which would make the regular soil sampling required for the project much easier. The absence of stones was quite a surprise since the project team were initially advised not to use a vineyard in this particular area due to the high stone content which is common to these alluvial soils. Furthermore, the vineyard is owned by the Goudini winery which would simplify administrative and logistic aspects.

Various materials, *e.g.* organic material, limestone as well as iron and manganese oxides, can be deposited on the inside of irrigation lines (Myburgh, 1989 and references therein). These deposits may cause clogging of drippers and micro-sprinklers, and in severe cases reduce water flow in irrigation lines. Given the poor quality of winery wastewater, particularly in terms of the high COD, it could be expected that foreign

material may cause problems if the water is recycled for irrigation of crops. Therefore, a further objective of this study was to determine the formation of foreign material deposits in irrigation lines where augmented winery wastewater is recycled for irrigation.

2.2. MATERIALS AND METHODS

2.2.1. Irrigation treatments

During a Workshop held on 15 May 2008 in Stellenbosch, the terms of reference for the project, including the range of COD levels to which the winery wastewater had to be augmented for the different treatments, were finalized (Table 2.1).

Table 2.1. Range of COD levels to which the winery wastewater was augmented for the different treatments.

Control	Target COD level in augmented winery wastewater (mg/L)							
T1	T2	T3	T4	T5	T6	T7	T8	T9
Control[(1)]	100	250	500	1000	1500	2000	2500	3000

[(1)] Raw water abstracted from the Holsloot River.

2.2.2. Vineyard characteristics

The field trial was carried out in an eight-year-old commercial Cabernet Sauvignon/99Richter vineyard near Rawsonville in the Breede River grape region of the Western Cape at 33° 41′ latitude. The region has a Mediterranean climate, and based on the growing degree days (GDD) from September until March (Winkler, 1962), the specific locality is in a class V climatic region (Le Roux, 1974). The vineyard is located on an alluvial flood plain of the Du Toit's Kloof mountains. The sandy soil belongs to the Fernwood form (Soil Classification Work Group, 1991). The soil was deep delved to 1.0 m before planting. Grapevines were planted 2.4 m x 1.2 m and trained onto a four strand lengthened Perold trellis (Booysen *et al*., 1992). Vertical shoot positioning was carried out to prevent the development of a sprawling canopy. The vineyard was previously irrigated by means of drippers. In addition to the standard viticultural practices, measures were taken to prevent erinose mite infestation in the vineyard. This consisted of a lime sulphur spray prior to bud break, as well as three additional sprays of MicroThiol™.

2.2.3. Experiment layout

All treatments were replicated three times in a randomised block design. The experiment plots were marked in July 2009. Experiment plots comprised one rows of six grapevines each, with two buffer grapevines at each end and a buffer row on each side. Each experiment plot covered 104 m^2.

2.2.4. Preliminary measurements

Grapevines in the experiment plots were pruned to two bud spurs. The baseline cane mass per plot was determined on 3 August 2009. A surface plot was created to obtain an indication of the growth vigour variation in the field trial part of the vineyard. To determine the baseline soil chemical status, soil samples were collected over 300 mm increments to a depth of 1.8 m in selected plots during August 2009. The sample sites represented the variation in vegetative growth. Visually, the growth of the existing cover crop showed the same variation as the grapevines. Therefore, samples of the cover crop were collected from each experiment plot during the first week of September 2009 to determine the dry matter content. The cover crop was still growing at that stage. These results will be presented and discussed in Chapter 5. Soil samples were also collected on 15 January 2010 to determine the baseline depth distribution of microbiological activity in this particular soil. These results will be presented and discussed in Chapter 6.

2.2.5. Installation and commissioning of infrastructure

2.2.5.1. Irrigation system in the experiment vineyard

The micro-sprinkler irrigation system, which allowed irrigation of the individual treatments, was installed in the vineyard during August 2009. Since the grapevine shoots could be damaged during installation of the irrigation system, it was essential to complete this task before bud break in September.

2.2.5.2. Wastewater mix and distribution plant

During the second week of August 2009, the Goudini winery installed a pipeline from a borehole near the experiment vineyard to their cooling plant at the winery. This provided an opportunity to install the 110 mm PVC pipeline required to pump the water from wastewater pit at the winery to the site proposed for the mix and distribution plant at the experiment vineyard (Fig. 2.1). The pipeline installation was completed by ARC Infruitec-Nietvoorbij farm personnel on 7 August. At the same time, four rows of an adjacent vineyard were pulled out to create space for the wastewater mix and distribution plant. Since the COD levels in winery wastewater can vary considerably over the course of a day, wastewater was first collected in a stock dam, from where it was pumped using a 30 m^3/h pump (Fig. 2.2) into the 15 m^3 plastic tanks at the mix and distribution plant (Fig. 2.3).

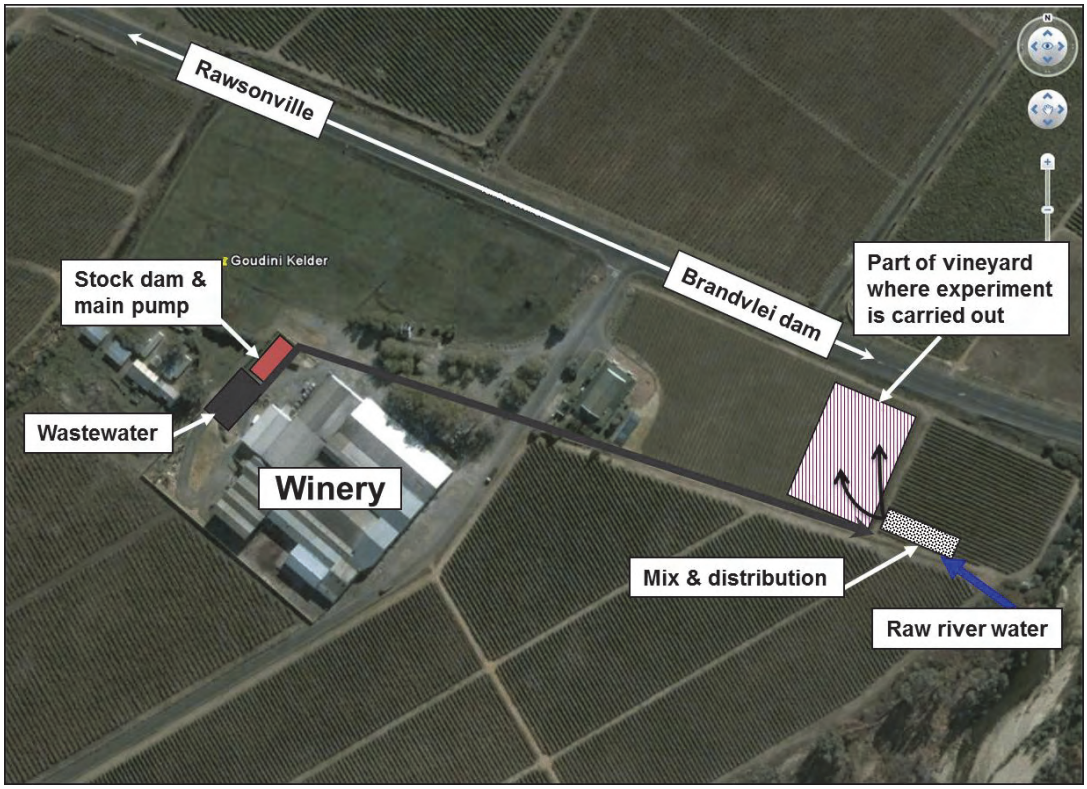

Figure 2.1. Layout of the infrastructure and experiment vineyard where irrigation with augmented winery wastewater was carried out.

Figure 2.2. Main pump and stock dam in which wasterwater from the winery was collected.

Due to the extent and complexity of the water mix and distribution plant, it was decided to allocate the work to a single contractor *via* a tender procedure. In order to get the appropriate equipment and specifications, the tender document was compiled which the assistance of engineers from the ARC Institute for Agricultural Engineering at Silverton. Two engineers visited the site on 6 August 2009 and the tender document was finalised two weeks later.

Figure 2.3. Tanks in which winery wastewater was augmented with raw river water before it was pumped to the different treatment plots.

Due to the high cost of the infrastructure, the Central Tender Committee at ARC head office in Pretoria had to approve the tender before it could be published. These steps slowed the process and the tender was eventually awarded to an approved contractor in November 2009. Construction of the water mix and distribution facility commenced on 15 January 2010. The works were completed on 9 February 2010 (Figs. 2.2 & 2.3). Commissioning of the water mix and distribution facility was completed on 12 February 2010.

2.2.5.3. Procedure for the augmentation of the winery wastewater

The wastewater coming from the winery is first screened to remove coarse particles (Fig. 2.4). During this process, lime is added to increase the pH of the water. The wastewater then flows through a sedimentation pond to allow settling of substances, *e.g.* tartaric acid (Fig. 2.5). The treated water is collected in a pit from where it is pumped onto a grass paddock. Wastewater for the experiment was abstracted from this pit. The augmentation, or mixing, procedure was carried out according to the following steps: (i) First, the 20 m^3 stock dam was filled with wastewater from the collection pit at the winery (Fig. 2.1). Following this, the chemical oxygen demand (COD) in the stock dam and the raw water from the Holsloot River, *c.* 80 m from the tanks was measured. These COD levels were used to calculate the volumes of winery wastewater and raw

water required to obtain the different COD levels. The COD in the samples was measured using a portable spectrophotometer (Aqualitic COD-reactor®, Dortmund) with the appropriate test kits (COD, CSB, 0-15000mg/L). This procedure requires a two hour oxidation time. The volume (m^3) of wastewater required from the stock dam (V_S) to obtain a certain target COD concentration (COD_T) was calculated as follows:

$$V_S = (COD_T - COD_R) \times V_T/(COD_S - COD_R) \quad \text{(Eq. 2.1)}$$

where COD_R and COD_S are the COD levels (mg/L) in the raw river water and the stock dam, respectively, and V_T is the tank volume (m^3).

Figure 2.4. Screening equipment used to remove coarse particles from the winery wastewater.

Figure 2.5. Sedimentation pond used to remove substances such as tartaric acid from the winery wastewater.

The required volume of winery wastewater for a specific treatment was first pumped into the designated tank. Following this, the tank was filled by pumping raw water from the river. Since the inlets were on near the bottom of the tanks, the raw water was forced to flow through the wastewater already inside the tanks. Furthermore, the inlets on the inside of the tanks were set at an angle of c. 45 degrees (Fig. 2.6), which created a swirling effect while water was flowing into the tank. The total volume pumped into the tanks was usually 13.5 m^3 to prevent spills. Once the tanks were filled, the water was pumped to the treatment plots (Fig. 2.6). Approximately one hour after the irrigation commenced, the COD in the augmented water was measured. The foregoing steps took almost three days to complete.

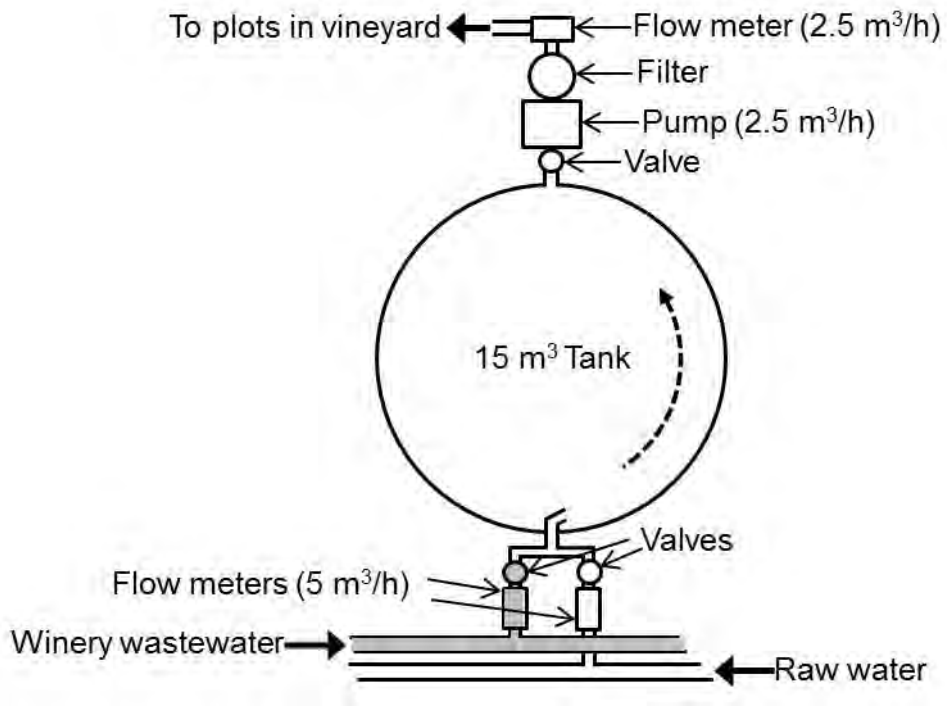

Figure 2.6. Schematic diagram to illustrate the components used to augment the winery wastewater in the tanks.

2.2.6. Monitoring deposits in irrigation lines

2.2.6.1. Non-destructive method

During the 2010/11, 2011/12 and 2012/13 seasons, foreign material accumulation on the inside surface of the irrigation lines was determined by means of non-destructive deposit detectors containing removable glass slides. The idea was to measure the deposits on the glass slides. The detectors were installed in the tubes which connected the micro-sprinklers to the lateral irrigation lines (Fig. 2.7). The deposit detectors were installed at the last micro-sprinkler in a lateral, *i.e.* where the highest level of deposits is to be expected. Before installation, the glass slide in each detector was weighed to the

nearest 0.001 mg using an electronic balance. Each season, the detectors were installed in November, *i.e.* before irrigation with raw water started. The detectors were only installed in the three replications of T1, T3, T5, T7 and T9. When the irrigation season ended in May, the glass slides were carefully removed from the detectors and placed in bottles for transportation to the laboratory. Following drying at 60°C for 24 hours in an oven, the slides were again weighed. The deposits were calculated as the difference between the initial mass and the mass recorded after drying on the both sides of the glass slide, and were expressed as mass of dry material per unit surface (mg/m^2).

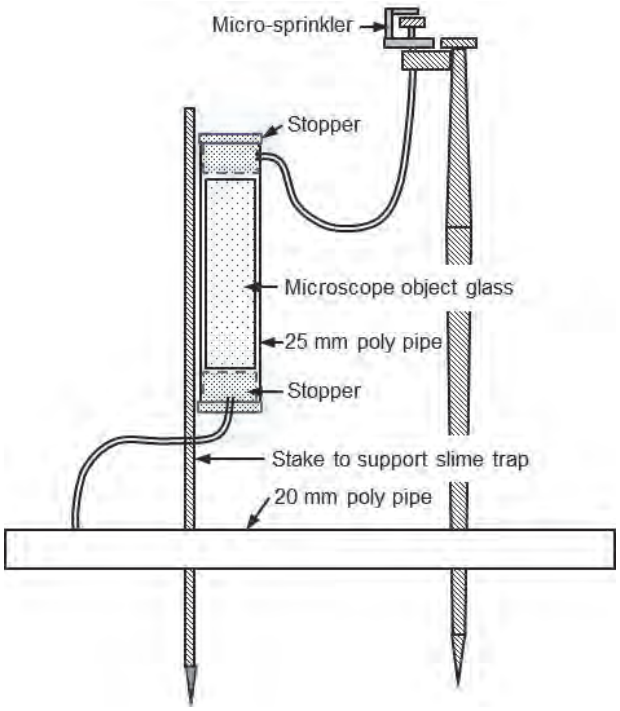

Figure 2.7. Schematic diagram illustrating the components of the deposit detectors.

2.2.6.2. Destructive method

Following the last wastewater irrigations in April 2013, 50 cm sections of poly pipe were removed from the irrigation laterals, and replaced with new pipe. Samples were collected from each experiment plot. The pipe samples were carefully cleaned on the outside, dried at 60°C for 24 hours and weighed. The pipe sections were then cleaned on the inside using a bottle brush. After rinsing under running water, the pipe samples were again dried at 60°C for 24 hours and weighed. The deposits accumulated over the four years on the inside surface of the pipes were calculated as the difference between the initial mass and the mass recorded after drying. The deposits were expressed as mass of dry material per unit surface (mg/m^2).

2.3. RESULTS AND DISCUSSION

2.3.1. Experiment layout

Before the wastewater treatments were applied, mean cane mass per experiment plot varied between 2.25 and 0.25 kg/grapevine (Fig. 2.8). The preliminary results indicated that the pruning mass variation between the replications was related to the P, K_{ex} and organic carbon contents in the soil (Tables 2.2 & 2.3). The role of organic carbon is not a direct nutritional effect, but serves as an indication of the N availability in the soil. The surface plot was used to determine the layout for the three replications required for reliable statistical analyses of the data as indicated in Figure 2.9. The ten treatments were randomly distributed within each replication.

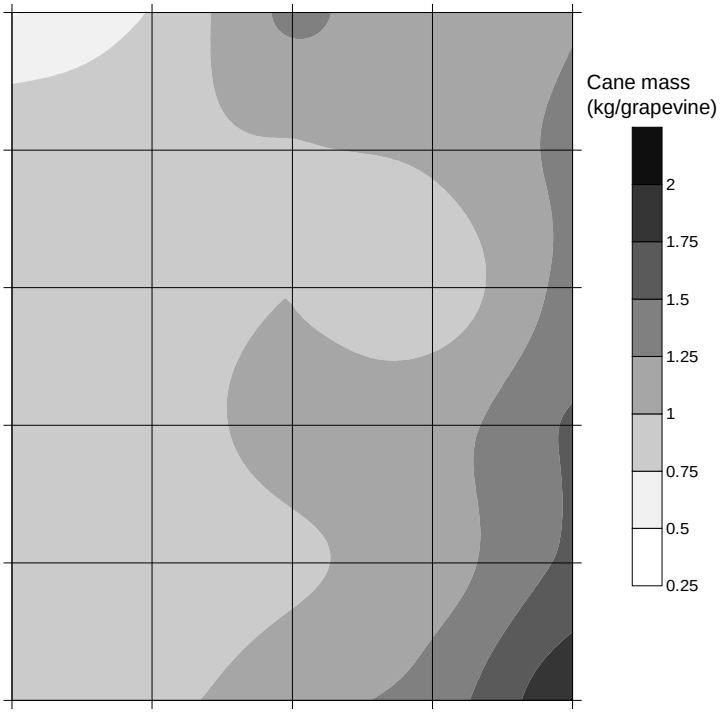

Figure 2.8. Surface plot indicating the variation in grapevine growth vigour in the part of the vineyard before the winery wastewater experiment commenced.

Table 2.2. Cane mass at pruning in August 2009, particle size analyses, estimated soil water retention (SWR) and exchangeable cation content in the sandy soil where the wastewater treatments were applied.

Replication	Cane mass	Clay	Silt	Sand (%)			SWR	P	Exchangeable cations (cmol(+)/kg)			
	(kg/grapevine	(%)	(%)	Fine	Medium	Coarse	(mm/m)	(mg/kg)	Na	K	Ca	Mg
1	0.66	1.7[(1)]	0.7	49.6	38.1	9.8	100	128	0.06	0.08	1.56	0.73
2	0.98	1.7	0.9	53.6	33.4	10.5	109	137	0.07	0.09	1.76	0.75
3	1.54	1.8	0.8	49.3	36.6	11.6	100	143	0.06	0.13	1.58	0.77

[(1)] Data are the means for the 0-1.8 m soil depth.

Table 2.3. Baseline organic carbon, micro nutrients and selected heavy metal contents in the sandy soil where the wastewater treatments were applied.

Replication	Organic carbon	Micro nutrients (mg/kg)					Heavy metals (mg/kg)		
	(%)	Cu	Zn	Mn	B	Fe	Cd	Pb	Hg
1	0.54[(1)]	2.66	1.38	2.32	0.06	14.75	0.06	0.12	0
2	0.61	1.99	1.57	2.87	.011	13.84	0.02	0.02	0
3	0.95	7.62	4.72	2.32	0.06	20.05	0.04	0.12	0

[(1)] Data are the means for the 0-1.8 m soil depth.

R1T10 Grower's control	**R1T4** COD = 500 mg/L	**R2T1** Raw water	**R2T2** COD = 100 mg/L	**R3T8** COD = 2500 mg/L
R1T8 COD = 2500 mg/L	**R1T5** COD = 1000 mg/L	**R2T6** COD = 1500 mg/L	**R2T3** COD = 250 mg/L	**R3T9** COD = 3000 mg/L
R1T9 COD = 3000 mg/L	**R1T3** COD = 250 mg/L	**R2T10** Grower's control	**R3T1** Raw water	**R3T5** COD = 1000 mg/L
R1T6 COD = 1500 mg/L	**R1T7** COD = 2000 mg/L	**R2T9** COD = 3000 mg/L	**R3T7** COD = 2000 mg/L	**R3T4** COD = 500 mg/L
R1T2 COD = 100 mg/L	**R2T5** COD = 1000 mg/L	**R2T4** COD = 500 mg/L	**R3T3** COD = 250 mg/L	**R3T2** COD = 100 mg/L
R1T1 Raw water	**R2T8** COD = 2500 mg/L	**R2T7** COD = 2000 mg/L	**R3T6** COD = 1500 mg/L	**R3T10** Grower's control

Figure 2.9. Experiment layout and treatments to determine the effects of augmented winery wastewater on soil properties, as well as grapevine yield and wine quality. (R = Replication and T = treatment; COD = target chemical oxygen demand level).

2.3.2. Efficacy of wastewater augmentation

The seasonal COD in the augmented winery wastewater was generally close to the target treatment values presented in Table 2.1. However, in some cases the COD in the augmented water differed from the target treatment values presented in Table 2.1. Therefore, the standard deviation from the mean was relatively large, particularly during the 2009/10 season (Fig 2.10). Possible reasons for the deviation from the target COD levels were as follows: The main pipeline was initially filled with raw water after the system had been completed. The COD concentration in the water in the pipeline was not accounted for, and therefore, probably had a diluting effect when the water was mixed. From the second irrigation onwards, the main pipeline was filled with winery wastewater. The volume of the water in the pipelines and the COD level was taken into consideration during the subsequent mixing procedures. The water meters used to monitor the volumes of water flowing into the tanks also presented problems, particularly when the flow rates increased when only one or two

tanks were being filled at a time. This problem was overcome by marking the required water levels on the outside of the translucent tanks.

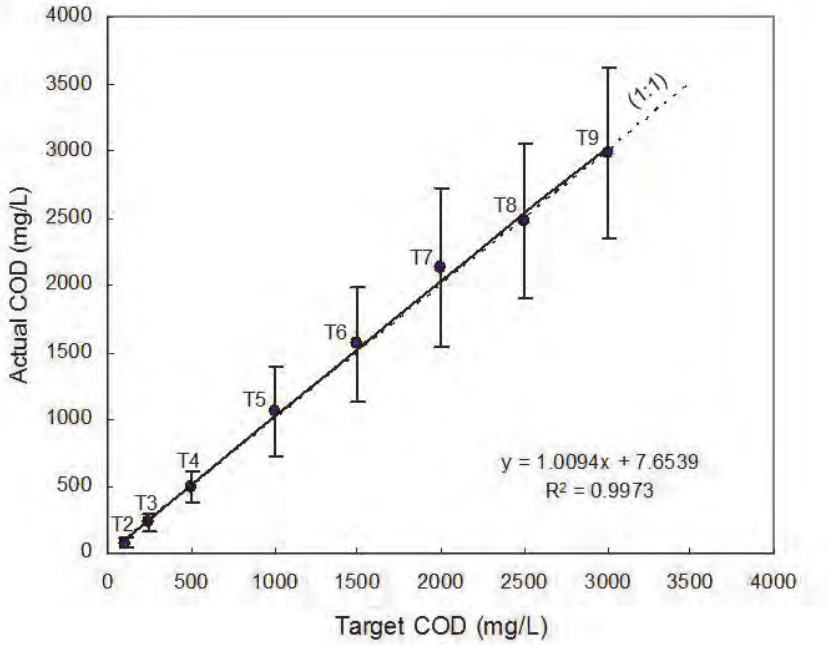

Figure 2.10. Relationship between mean actual chemical oxygen demand (COD) in augmented winery wastewater applied to grapevines and the target COD levels of treatments T2 to T9 during the 2009/10 season. Vertical bars indicate standard deviation (n = 3).

An operator error also occurred when the plunger of the mechanical pipette was pressed too deep when the water samples were transferred into the test kit vials. This caused an overestimation of the COD level in the stock dam, which in turn resulted in too low COD levels in the augmented water in the tanks, particularly when low COD in the water required sample volumes of 2 ml compared to the 0.2 ml required for the higher COD levels, *i.e.* > 500 mg/L. Since the augmentation and irrigation procedures took almost three days to complete, the project team investigated ways to save time. According to a technical advisor of a company that supply the test kits, one hour oxidation time would be adequate for most COD levels in water samples. If the oxidation time could be reduced, it would significantly reduce the time required to carry out the whole procedure as described above, particularly the two hour waiting period after the stock dam had been filled. Consequently, it was decided to reduce the oxidation time for the samples from the stock dam to one hour for the second and third treatment applications in 2009. Since the target COD levels after mixing were higher than the target levels in the second and third irrigations, the possibility that the one hour oxidation time could have underestimated the COD concentrations, particularly in the stock dam, was investigated. The COD in three

water samples containing different COD levels were measured after different oxidations times. These results showed that the COD reading became constant in less than one hour when the COD concentrations were below *ca.* 2000 mg/L (Fig. 2.11). However, in the case of the high concentrations, *e.g.* in the stock dam, the COD readings only reached a plateau after *ca.* 90 minutes. Based on these findings it was decided to standardize the oxidation time for all COD analyses to two hours.

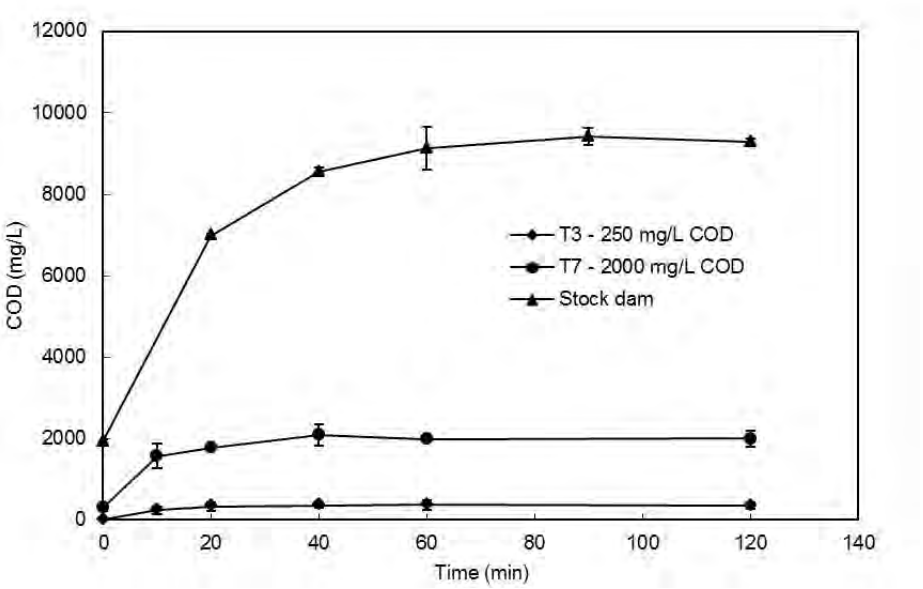

Figure 2.11. The effect of oxidation time on spectrophotometrically measured chemical oxygen demand (COD) in winery wastewater that was augmented to different COD levels.

As the project progressed, the abovementioned problems and possible causes for error were addressed and eliminated where possible. Due to this, the accuracy of the treatment application improved substantially in the subsequent seasons compared to 2009/10 (Figs. 2.12, 2.13 & 2.14).

Before the field work commenced, one of the major concerns was the efficiency of the mixing process in the tanks. On 12 April 2010, the variation in COD was measured as the irrigation progressed. The duration of the irrigations varied between 4.5 hours and 5 hours. Hence, water samples were collected one hour, 2.5 hours and 5 hours after the irrigations started. Water was only sampled at the T4 and T9 tanks. Analyses of the water showed that the COD levels remained reasonably constant as the irrigation progressed, irrespective of the COD concentration (Fig. 2.15). Furthermore, it indicated that the concentrations of the augmented wastewater within the tanks were fairly homogeneous, and that effective mixing occurred when the tanks were filled.

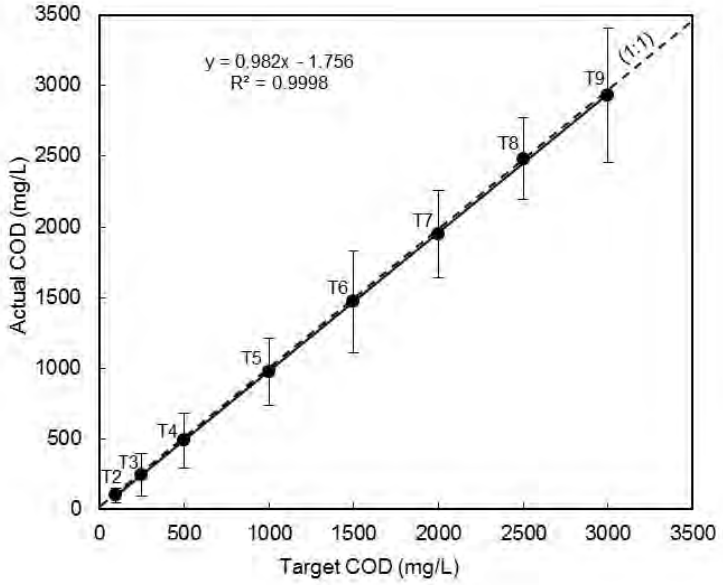

Figure 2.12. Relationship between mean actual chemical oxygen demand (COD) in augmented winery wastewater applied to grapevines and the target COD levels of treatments T2 to T9 during the 2010/11 season. Vertical bars indicate standard deviation (n = 6).

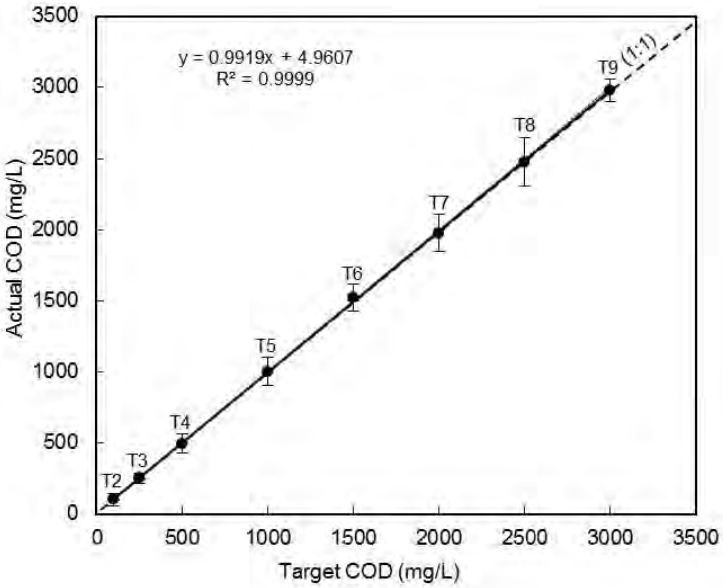

Figure 2.13. Relationship between mean actual chemical oxygen demand (COD) in augmented winery wastewater applied to grapevines and the target COD levels of treatments T2 to T9 during the 2010/11 season. Vertical bars indicate standard deviation (n = 6).

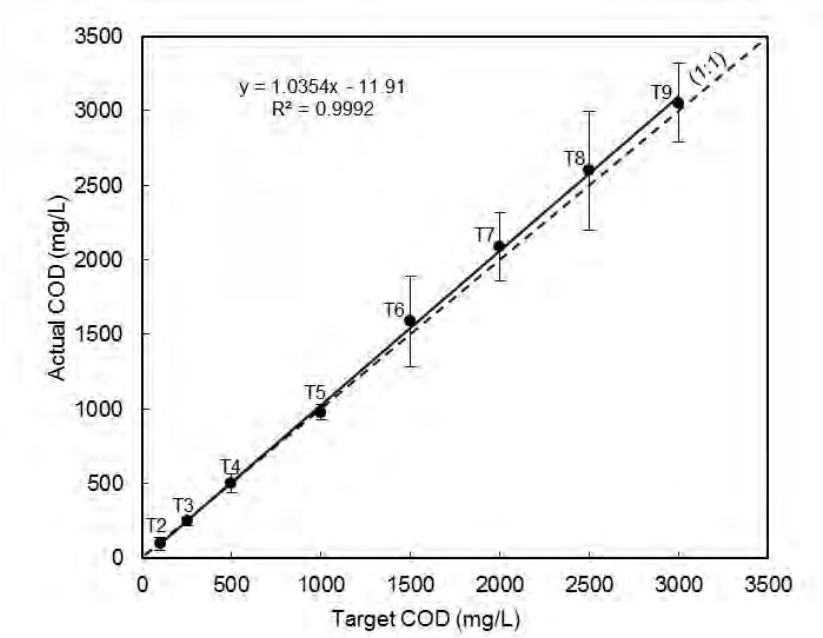

Figure 2.14. Relationship between mean actual chemical oxygen demand (COD) in augmented winery wastewater applied to grapevines and the target COD levels of treatments T2 to T9 during the 2012/13 season. Vertical bars indicate standard deviation (n = 6).

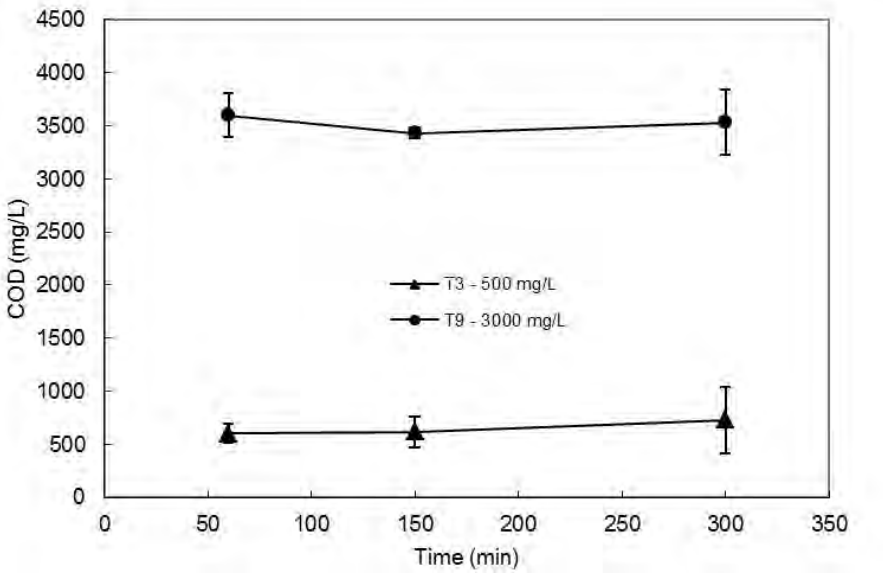

Figure 2.15. Temporal variation in chemical oxygen demand (COD) in augmented winery wastewater pumped from the mixing tanks for the irrigation of grapevines of two respective treatments, *i.e.* T4 & T9.

2.3.3. Deposits in irrigation lines

2.3.3.1. Non-destructive method

In the first season, material deposits increased with a decrease in level of augmentation of the wastewater (Fig. 2.16). However, this increase was non-linear, and the highest deposits occurred where the target COD concentration was 2000 mg/L (T7). The high standard deviation from the means was the result of variation between treatment replications. In the second and third seasons, this variability between treatment replications increased to the extent that the amount of deposits could not be related to level of COD (data not shown). A possible explanation for the inconsistency between treatment replications could be that the pipe containing the glass slides did not drain completely following irrigations. Between irrigations algae growth could have caused unnecessary deposits on the slides. The foregoing indicated that the methodology was not accurate enough to detect differences in the small amount of material on the glass slides.

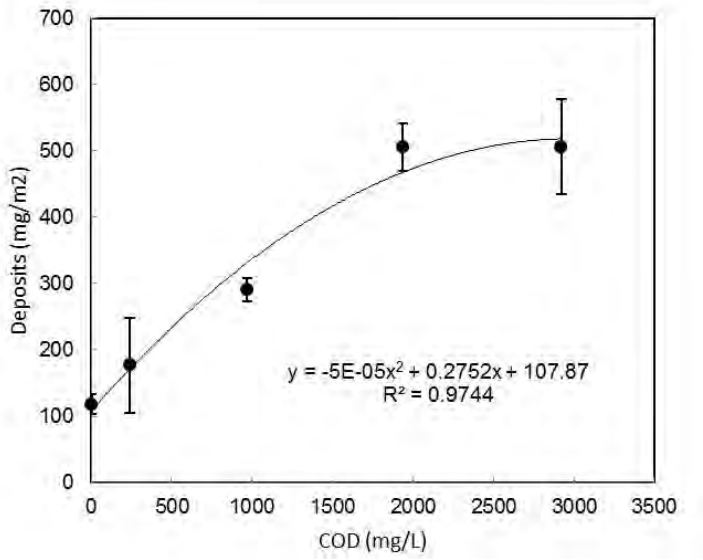

Figure 2.16. Effect of COD level in augmented winery wastewater on foreign material deposits accumulated in the 2010/11 season on the inside of the irrigation lines.

2.3.3.2. Destructive method

After four seasons, material deposits in the pipe samples increased with a decrease in the level of augmentation of the wastewater (Fig. 2.17). Similar to results obtained with the glass slides, the deposit increase was also non-linear. The non-linear increase of deposits to level of COD indicated that some constituent(s) in the augmented wastewater suppressed deposits in the irrigation lines over the three years. Iron and manganese oxides are known to form deposits which can cause clogging of irrigation systems. Perusal of the data revealed that the deposits increased with the mean Fe concentration in the augmented waters (Refer to Chapter 3, Table 3.9). Furthermore, visual observation revealed that the deposits on the insides of the irrigation lines had reddish, brown colour. However, the deposit increase with

increasing Fe was also non-linear (Fig. 2.18), which suggested that the contribution of Fe to the deposits was suppressed in the less diluted waters. Since the water pH decreased as the level of wastewater augmentation decreased (Refer to Chapter 3, Table 3.1), the foregoing suggested that the lower pH levels might have suppressed Fe-oxide formation.

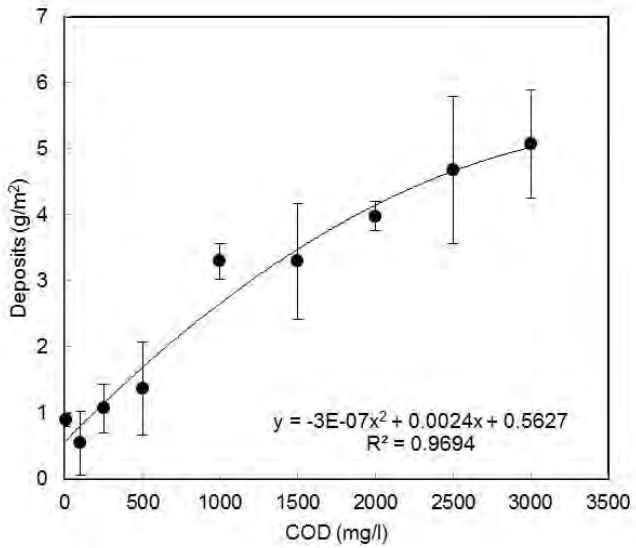

Figure 2.17. Effect of COD level in augmented winery wastewater on foreign material deposits accumulated over four seasons, *i.e.* 2009/19 to 2012/13 on the inside of the irrigation lines.

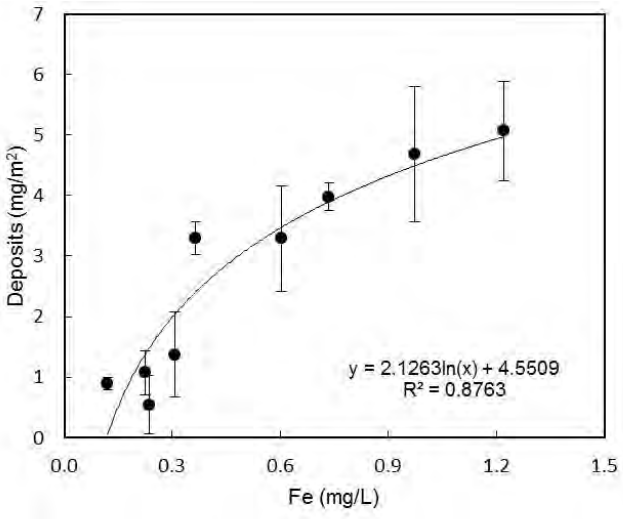

Figure 2.18. Effect of Fe concentration in augmented winery wastewater on foreign material deposits accumulated over four seasons, *i.e.* 2009/19 to 2012/13 on the inside of the irrigation lines.

2.4. CONCLUSIONS

Measuring cane mass at pruning on a per plot basis before the field work commenced indicated that the vegetative growth varied across the experiment vineyard. However, this allowed the treatment replications to be allocated accordingly. When doing COD

measurements, results will be more reliable if the oxidation time is standardized at two hours, irrespective of the level of COD in the water. Measuring COD concentrations while the irrigation water was being pumped from the tanks confirmed that the concentrations of augmented wastewater within the tanks were fairly homogeneous, and that effective mixing had taken place when the tanks were filled. After initial practical problems and sources of error were eliminated, treatment application in terms of COD concentrations was quite accurate, given the relative simplicity of the mix and distribution facility.

The formation of deposits on the inside of the irrigation lines appeared to be a function of the Fe and pH in the augmented winery wastewater. In practical terms, the deposited material formed a relatively thin layer, which implies that it could not have caused significant clogging of the irrigation lines or micro-sprinklers after three years. Therefore, negative effects on grapevine growth and/or yield due to increasing deposits would not be expected under the given conditions. Since drippers have narrow flow paths and/or small orifices, they are more susceptible to clogging than micro-sprinklers. Therefore, drip irrigation systems should be avoided where winery wastewater is to be recycled for irrigation of crops. The destructive method provided more reliable results than monitoring annual deposits with the non-destructive method. More reliable and accurate monitoring of deposit formation in irrigation lines by means of non-destructive methods requires further investigation.

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CHAPTER 3: WATER QUALITY, IRRIGATION VOLUMES AND AMOUNT OF ELEMENTS APPLIED *VIA* WASTEWATER IRRIGATION

C.L. Howell, P.A. Myburgh & E.L. Lategan
The Fruit, Vine and Wine Institute of the Agricultural Research Council, Private Bag X5026, Stellenbosch, 7599

3.1. INTRODUCTION

Wine grapes are an important crop in regions such as the Western Cape and the Lower Orange river in the Northern Cape. However, wineries produce large volumes of low quality wastewater, particularly during the harvest period. Reports on the actual volumes of wastewater that are generated by wineries are extremely limited. However, it is estimated that medium to large wineries generate more than 15 000 m^3 of wastewater annually, whereas small wineries generate less than 15 000 m^3 annually (Van Schoor, 2005 and references therein). Australian wineries generate about 5 m^3 of wastewater per tonne of grapes crushed (Chapman *et al.*, 1995). Crushing approximately 50 000 tonnes of grapes annually generate about 175 000 m^3 of wastewater at the Berri estates' winery in the Riverland region of South Australia (Anonymous, 2010). Hence, their wastewater generation amount to *ca.* 3.5 m^3 per tonne of grapes. It can be estimated that the Lutzville Vineyards' winery generates about 1.1 m^3 of wastewater per tonne of grapes crushed. However, this relatively low value is misleading, since 50% of the wastewater is presumably lost to evaporation (Kriel, 2008).

International requirements, as well as national legislation, are putting pressure on wine producers regarding the responsible management of this winery wastewater, which may have large-scale detrimental impact on the environment. In the Western Cape wine industry, most vineyards need irrigation and the ideal situation would be to implement a sustainable use of winery wastewater for wine grape irrigation by adding winery wastewater to existing irrigation water resources (augmentation). Until now, the impact of this practice has, however, not been studied comprehensively. Currently, the Department of Water Affairs (DWA) is drafting a new General Authorisation for wineries. Depending on the permitted water quality limits and volumes stipulated by the authorisations, adding winery wastewater to current irrigation water may well become a more viable practice in the future. Re-using winery wastewater in this way will be beneficial, particularly in situations where water shortages are becoming an alarming reality. However, the quality of underground water must still be monitored strictly, and pollution should be prevented at all cost. Thus, to know the impact of irrigating with winery wastewater on the chemical composition and physical structure of the soil, grapevine performance, and wine quality, is indispensable. In particular,

information pertaining to the water quality obtained by augmentation of winery wastewater to different chemical oxygen demand (COD) levels is necessary in order to determine what the quality of the water would be after augmentation In addition to this, the amount of extra elements applied *via* the irrigation water is important to quantify the additional nutrient load added to the vineyard.

3.2. MATERIALS AND METHODS

3.2.1. Irrigation volumes applied

Grapevines were irrigated with winery wastewater from mid-February when suitable wastewater became available from vintage processes in the Breede River valley. Control grapevines were irrigated at the same time with the same volume of raw river water. Since the irrigation procedure took approximately three days to complete, grapevines were generally irrigated at a fixed schedule every two weeks from the first winery wastewater irrigation. The application of irrigations was stopped either in mid-April or the beginning of May each year, when the winter rainfalls began and/or the COD of the wastewater was too low to make up all the tanks. Irrigation was applied by means of micro-sprinklers in order to apply larger volumes of water. Water meters were used to monitor the irrigation volumes applied to each treatment. Grapevines of all treatments received the same volume of water.

3.2.2. Water quality

Approximately one hour after wastewater irrigation started, a water sample of 500 mL was collected from each of the eight tanks to verify actual COD levels obtained. One 500 mL water sample of the Holsloot River was also collected at the same time. A sample of the stock dam, the river sample as well as the eight samples from the tanks were analysed by a commercial laboratory for pH, electrical conductivity (EC_{iw}), Na, K, Ca, Mg, Fe, Cl, CO_3, HCO_3, SO_4, B, Mn, Cu, Zn, P, NH_4-N, NO_3-N, F as well as heavy metals according to methods described by Clesceri *et al.* (1998).

3.2.3. Amount of elements applied

For the wastewater treatments, *i.e.* T2 to T9, the amount of wastewater applied was converted from mm into L per ha by means of the following. The element analysis of the wastewater from each tank per irrigation was used to calculate the amounts of additional elements added to the soil per unit area by means of wastewater irrigations, and expressed as kg/ha (1 mm = 10 m^3 per ha; 1 m^3 = 1000 L, therefore 10 m^3 = 10 000 L). In addition to this, the contribution of the elements deposited by the raw river water was taken into account. For T1, the same procedure was followed. The calculated amount of elements added through the irrigation water were expressed as amounts added pre- harvest, post-harvest and total

amounts applied per irrigation. Amount of elements applied per irrigation was calculated as follows:

$$M = I \times C_e / 100 \quad \text{(Eq. 3.1)}$$

where M is amount of element (kg/ha), I is the irrigation applied (mm) and C_e is the element concentration in the irrigation water. Amount of elements applied per irrigation season were summed to obtain the seasonal applications.

3.3. RESULTS AND DISCUSSION

3.3.1. Irrigation volumes applied

A full tank of augmented winery wastewater applied *c.* 41 mm on the three replication plots of each treatment. In general, treatments received the same mean volume of wastewater per irrigation (Table. 3.1) when a full tank of wastewater was applied to the plots. Due to low COD levels in the winery wastewater at the end of the 2010/11 season, T3 to T9 had to be augmented and applied in two irrigations on consecutive days in the middle of April. For the calculation of the mean volume, the volumes applied on these consecutive days were summed. Due to low COD levels in the winery wastewater as well as limited available water, only 1/3 of a tank of augmented water could be applied on 2 May In the 2011/12 season. This data was therefore not used for the calculation of the average mean volume of wastewater applied per irrigation. Grapevines of the augmented wastewater treatments received *c.* 25 mm, 14 mm and 8 mm of raw river water per irrigation in 2010/11, 2011/12, 2012/13, respectively (Table. 3.2). The amount of raw river water applied following wastewater irrigations was considerably less in 2012/13 than in previous years, because a continuous deficit strategy was followed to minimize drainage losses and to curtail excessive vegetative growth. Grapevines of all treatments received the same amount of irrigation water per irrigation (Table. 3.3). The total volumes of wastewater applied to augmented wastewater treatments amounted to 247 mm, 177 mm and 246 mm in 2010/11, 2011/12 and in 2012/13, respectively (Table. 3.4). The total volume of raw river water applied to grapevines of all treatments amounted to 152 mm in 2010/11, 70 mm in 2011/12 and 48 mm in 2012/13 (Table. 3.5). Subsequently, the total volumes of irrigation water applied to grapevines amounted to 398 mm in 2010/11, 247 mm in 2011/12 and 294 mm in 2012/13 (Table. 3.6).

Table 3.1. Mean amount of augmented winery wastewater applied per irrigation to Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Amount per irrigation (mm)			
	2009/10	2010/11[1]	2011/12[2]	2012/13
T1 - Control[3]	N/A	N/A	N/A	N/A
T2 - 100	38.8±4.6	41.1±0.3	40.8±0.1	41.0±0.2
T3 - 250	38.9±4.4	41.5±0.2	41.7±0.2	42.0±0.7
T4 - 500	39.1±4.5	41.1±0.3	40.8±0.2	40.9±0.5
T5 - 1000	39.4±4.1	41.3±0.4	41.4±0.1	40.9±1.2
T6 - 1500	39.4±4.0	41.8±0.2	42.0±0.2	41.7±0.2
T7 - 2000	39.2±3.7	41.2±0.7	40.8±0.6	40.8±0.4
T8 - 2500	38.7±4.2	40.7±0.2	40.7±0.2	40.0±1.3
T9 - 3000	38.2±4.5	40.8±0.2	40.7±0.1	40.7±0.4

(1) Due to low COD levels in the winery wastewater at the end of the irrigation season, T3 to T9 had to be augmented and applied in two irrigations on consecutive days in the middle of April. For the calculation of the mean volume, the volumes applied on these consecutive days were added together.
(2) Due to low COD levels in the winery wastewater as well as limited available water, only 1/3 of a tank of augmented water could be applied on 2 May. Therefore, this data was not used for the calculation of the averages.
(3) Raw water abstracted from the Holsloot River.

Table 3.2. Mean amount of raw river water used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Amount per irrigation (mm)			
	2009/10	2010/11	2011/12	2012/13
T1 - Control[1]	59.5±12.6	65.3±5.2	49.4±20.9	49.1±5.7
T2 - 100	15.4±3.4	25.1±0.6	14.3±13.8	8.1±5.6
T3 - 250	15.5±3.2	25.6±0.5	13.5±13.8	7.5±4.9
T4 - 500	15.8±3.4	25.4±0.7	14.3±13.8	8.1±5.2
T5 - 1000	16.4±3.4	25.3±0.4	13.7±13.9	7.9±5.5
T6 - 1500	16.2±3.1	25.8±0.4	13.3±13.7	7.9±5.2
T7 - 2000	14.5±3.3	25.2±0.4	14.3±13.5	8.1±5.8
T8 - 2500	15.3±2.9	24.9±0.5	14.3±14.0	8.3±5.7
T9 - 3000	15.0±2.9	25.1±0.7	14.7±13.8	8.3±5.8

(1) Raw water abstracted from the Holsloot River.

Table 3.3. Mean total amount of irrigation water applied per irrigation to Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Amount per irrigation (mm)			
	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	59.5±12.6	65.3.±5.2	49.4±20.9	49.1±5.7
T2 - 100	54.2±2.1	66.0±0.8	49.4±20.8	49.1±5.7
T3 - 250	54.4±1.9	67.1±0.5	49.4±20.9	49.5±5.5
T4 - 500	54.9±2.2	66.6±0.8	49.4±20.9	49.0±5.8
T5 - 1000	55.8±2.7	66.5±0.3	49.4±20.9	48.9±6.1
T6 - 1500	55.6±2.5	67.6±0.4	49.4±20.8	49.6±5.4
T7 - 2000	53.7±0.5	66.5±0.7	49.4±20.9	48.9±5.9
T8 - 2500	54.0±2.6	65.6±0.5	49.4±20.9	48.3±6.5
T9 - 3000	53.2±2.7	65.9±0.6	49.4±20.9	49.0±5.7

(1) Raw water abstracted from the Holsloot River.

Table 3.4. Total amount of augmented winery wastewater applied per season to Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Amount per season (mm)			
	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	N/A	N/A	N/A	N/A
T2 - 100	116.3	205.3	175.8	246.2
T3 - 250	116.7	249.2	179.5	251.7
T4 - 500	117.3	246.6	175.6	245.5
T5 - 1000	118.2	247.5	178.1	245.6
T6 - 1500	118.3	251.0	180.7	250.0
T7 - 2000	117.6	247.3	175.6	244.9
T8 - 2500	116.1	244.1	175.2	239.8
T9 - 3000	114.6	245.0	173.3	244.4

(1) Raw water abstracted from the Holsloot River.

Table 3.5. Total amount of raw water applied per season to Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Amount per season (mm)			
	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	178.5	391.6	246.9	294.7
T2 - 100	46.2	190.9	71.3	48.5
T3 - 250	46.5	153.5	67.4	45.1
T4 - 500	47.5	152.1	71.3	48.6
T5 - 1000	49.2	151.7	68.7	47.6
T6 - 1500	48.5	154.7	66.6	47.5
T7 - 2000	43.5	151.4	71.3	48.4
T8 - 2500	46.0	149.4	71.7	50.0
T9 - 3000	45.0	150.4	73.5	49.7

[(1)] Raw water abstracted from the Holsloot River.

Table 3.6. Total amount of irrigation water applied per season to Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Amount per season (mm)			
	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	178.5	391.6	246.9	294.7
T2 - 100	162.5	395.9	247.0	294.7
T3 - 250	163.2	402.8	246.9	297.0
T4 - 500	164.8	399.6	246.8	294.0
T5 - 1000	167.5	399.2	246.9	293.3
T6 - 1500	166.8	405.7	247.2	297.6
T7 - 2000	161.1	398.8	246.9	293.2
T8 - 2500	162.0	393.7	246.9	289.8
T9 - 3000	159.5	395.3	246.8	294.1

[(1)] Raw water abstracted from the Holsloot River.

3.3.2. Water quality

pH: For all four seasons, water pH tended to decrease with a decrease in the dilution of the wastewater, *i.e.* an increase in the COD level of the water (Table 3.7). The pH of the raw river water (T1) was generally the highest, whereas pH in the least diluted wastewater, *i.e.* T9, was comparable to the undiluted winery wastewater. In 2009/10 and 2011/12, pH of undiluted winery wastewater augmented to COD levels of 1500 mg/L and higher were comparable to the winery wastewater. In general, water pH levels tended to be below the recommended guideline for irrigation water pH, which ranges from 6.5 to 8.4 (DWAF, 1996; Howell & Myburgh, 2013a). With regards to the General Authorisations of 2013 (DWA, 2013),

up to 500 m^3 of wastewater may be irrigated on any given day provided that the pH is between 6 and 9. In general, pH of the wastewater fell outside the range of these norms.

Table 3.7. The pH in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	pH			
	2009/10	2010/11	2011/12	2012/13
Winery	5.4±0.3	4.3±0.8	4.7±0.7	4.4±0.5
T1 - Control(1)	6.6±0.5	5.8±0.5	6.1±0.2	5.5±0.5
T2 - 100	6.6±0.2	5.7±0.8	5.9±0.8	5.6±0.5
T3 - 250	6.4±0.2	5.5±0.9	5.3±0.6	5.1±0.5
T4 - 500	5.8±0.5	4.9±0.8	4.9±0.6	4.7±0.8
T5 - 1000	5.5±0.5	4.4±0.6	4.8±0.6	4.6±0.8
T6 - 1500	5.4±0.7	4.6±0.9	4.7±0.6	4.5±0.8
T7 - 2000	5.3±0.6	4.4±0.9	4.6±0.7	4.5±0.8
T8 - 2500	5.5±0.4	4.4±1.0	4.6±0.8	4.4±0.8
T9 - 3000	5.4±0.4	4.4±0.9	4.6±0.7	4.4±0.8

(1) Raw water abstracted from the Holsloot River.

EC_{iw}: As expected, EC_{iw} increased with a decrease in the level of augmentation of the wastewater, *i.e.* an increase in the COD level of the water (Table 3.8). This indicated that there was an increase in salt levels with a decrease in the dilution of the wastewater. Furthermore, the EC_{iw} in the least diluted water, *i.e.* wastewater augmented to a COD level of 3000 mg/L, was still substantially lower compared to the undiluted winery wastewater. This trend was consistent for all four seasons. On 12 April 2010, the EC_{iw} in the wastewaters augmented to COD levels of 2000 mg/L and higher, *i.e.* T7, T8 and T9, exceeded the critical value of 0.75 dS/m which is the salinity threshold for water used for grapevine irrigation (Van Zyl, 1981; Myburgh, 2012). This salinity threshold level was also exceeded in T9 on 3 May 2010. In 2010/11, the EC_{iw} in the T7, T8 and T9 waters, *i.e.* wastewaters augmented to COD levels of 2000 mg/L and higher, exceeded the critical value of 0.75 dS/m only on 14 April, which was the last wastewater irrigation of the season. On 2 May 2012, the EC_{iw} in the wastewaters augmented to COD levels of 2000 mg/L and higher, *i.e.* T7, T8 and T9, exceeded the critical value of 0.75 dS/m. This was the only date during this season on which this EC_{iw} threshold was exceeded. In 2012/13, the EC_{iw} did not exceed the critical value of 0.75 dS/m. With regards to the General Authorisations of 2013 (DWA, 2013), up to 500 m^3 of wastewater may be irrigated on any given day provided that the EC is less than 2 dS/m. In general, EC_{iw} were well below this norm.

Table 3.8. The electrical conductivity (EC_{iw}) in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	EC_{iw} (dS/m)			
	2009/10	2010/11	2011/12	2012/13
Winery	2.30±0.93	1.50±0.51	1.63±0.39	1.11±0.28
T1 - Control[(1)]	0.13±0.02	0.19±0.02	0.13±0.07	0.12±0.06
T2 - 100	0.16±0.02	0.20±0.03	0.16±0.07	0.13±0.06
T3 - 250	0.19±0.03	0.22±0.04	0.19±0.06	0.15±0.06
T4 - 500	0.26±0.04	0.27±0.05	0.25±0.03	0.19±0.06
T5 - 1000	0.42±0.13	0.36±0.08	0.34±0.06	0.24±0.07
T6 - 1500	0.53±0.20	0.43±0.10	0.42±0.11	0.27±0.07
T7 - 2000	0.64±0.26	0.53±0.17	0.50±0.20	0.32±0.09
T8 - 2500	0.74±0.29	0.59±0.21	0.61±0.25	0.37±0.10
T9 - 3000	0.87±0.36	0.69±0.30	0.66±0.24	0.42±0.11

[(1)] Raw water abstracted from the Holsloot River.

COD: In some cases in 2009/10, the COD in the augmented wastewaters differed substantially from the target values. There were a number of possible reasons for this, namely: (i) the main pipeline was initially filled with raw water after the system had been completed. The COD concentration in this water was not accounted for, and had a diluting effect when the water was mixed. From the second irrigation onwards, the main pipeline was filled with winery wastewater. The volume of the water in the pipelines and the COD level was taken into consideration during the subsequent mixing procedures. (ii) The water meters used to monitor the volumes of water flowing into the tanks also presented problems, particularly when the flow rates were high when only one or two tanks were being filled at a time. This problem was overcome by marking the required water levels on the outside of tanks. (iii) An operator error also occurred when the plunger of the mechanical pipette was pressed too deep when the water samples were transferred into the test kit vials. This caused an overestimation of the COD level in the stock dam, which in turn resulted in too low COD levels in the augmented water in the tanks, particularly when low COD in the water required sample volumes of 2 ml compared to the 0.2 ml required for the higher COD levels, *i.e.* > 1500 mg/L. (iv) Since the target COD levels after mixing were higher than the target in the second and third irrigations, the possibility that the one hour oxidation time could have underestimated the COD concentrations, particularly in the stock dam, was investigated. The COD in three water samples containing different COD levels were measured after different oxidations times. These results showed that the COD reading became constant in less than one hour when the COD concentrations were below *ca.* 2000 mg/L. However, in the case of the high concentration, *e.g.* in the stock dam, the COD readings only reached a plateau after *ca.* 90 minutes. Based on these findings it was decided to standardize the oxidation time for

all future COD analyses to two hours. The mean actual COD levels of the three irrigations applied in the post-harvest period were close to the target ones. On 2 March 2011, Eskom tripped. In general, the mean COD levels in the augmented winery wastewater were generally close to the target values (Table 3.9).

Table 3.9. The chemical oxygen demand (COD) in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	COD (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	7624±854	10098±4163	11990±9178	10560±1567
T1 - Control[(1)]	0±0	1±3	4±5	0±0
T2 - 100	81±45	100±117	100±59	96±45
T3 - 250	247±70	250±142	247±38	245±30
T4 - 500	545±83	499±181	492±102	499±61
T5 - 1000	1144±353	1004±233	978±225	976±52
T6 - 1500	1560±424	1486±378	1512±91	1587±498
T7 - 2000	2130±601	1994±408	1976±229	2087±273
T8 - 2500	2480±582	2520±610	2474±294	2597±503
T9 - 3000	2975±652	2997±641	3020±342	3053±295

[(1)] Raw water abstracted from the Holsloot River.

Potassium: The K levels in the augmented wastewater increased substantially with a decrease in augmentation, *i.e.* an increase in the COD level of the water (Table 3.10). In general, the K levels in the undiluted winery wastewater increased substantially from the first wastewater irrigation at the beginning of harvest in early February to the end of the peak harvesting season in March. The K levels in the augmented wastewater were still substantially lower than in the undiluted wastewater.

Sodium: As the field work progressed, it became evident that the Na content in the raw river water used for augmentation was consistently high. Sampling water at three places along the course of the river during the 2012/13 season (Table 3.11) revealed that the Na content increased substantially during summer along the floodplain up to the point where the water was abstracted near the Goudini winery. However, when the flow was high in winter, the Na concentrations were the same along the course of the river. Since no definite point of contamination could be identified, the Na probably became more concentrated under low flow conditions.

As expected, Na levels in the augmented wastewaters increased substantially with a decrease in dilution of the winery wastewater (Table 3.12). Since grapevines are considered

moderately sensitive to foliar injury from Na, a concentration of 115 mg/L is recommended as the upper threshold for overhead irrigation (Howell & Myburgh, 2013a). As the experiment vineyard was irrigated by means of micro-sprinklers, the leaves were not expected to become wet. The Na levels in the augmented wastewaters were such that even though leaves were wetted, no substantial damage to grapevines leaves would have occurred. The only occasion when the threshold was exceeded was during 2010/11 season, where the levels were 124 mg/L in irrigation water augmented to a COD level of 3000 mg/L (T9). In general, the levels of Na in the undiluted wastewater also did not exceed this level with the exception of 3 May 2010, 14 April 2011 and 15 Feb 2013.

Table 3.10. The potassium (K) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	K (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	621.2±264.6	241.6±109.0	313.7±93.0	189.1±58.2
T1 - Control[(1)]	2.9±1.4	2.0±1.0	2.3±1.6	2.0±1.0
T2 - 100	9.4±5.2	4.3±3.5	5.0±3.5	3.7±2.0
T3 - 250	17.7±10.6	11.3±9.5	11.2±3.8	8.5±3.6
T4 - 500	33.6±12.8	21.3±17.4	20.6±8.6	16.4±7.9
T5 - 1000	74.3±41.9	39.9±33.0	38.3±14.2	28.2±11.6
T6 - 1500	105.6±60.1	55.2±42.0	51.5±20.3	35.0±15.6
T7 - 2000	135.2±76.4	75.3±59.1	62.8±23.0	44.7±16.3
T8 - 2500	162.7±88.9	89.4±70.2	84.4±38.0	55.6±21.2
T9 - 3000	197.8±114.2	112.4±95.3	100.2±46.7	65.1±25.6

[(1)] Raw water abstracted from the Holsloot River.

Table 3.11. Temporal and spatial variation in Na content in water of the Holsloot River, a tributary of the Breede River.

Sampling date	Locality		
	In mountain	Beginning of floodplain	Near Goudini winery
4 December 2012	2.3	3.0	16.6
28 March 2013	1.8	2.2	11.6
30 September 2013	2.3	2.4	3.0

Table 3.12. The sodium (Na) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Na (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	120.6±49.8	92.8±43.8	90.8±11.4	86.0±48.4
T1 - Control[(1)]	10.2±0.6	12.8±0.9	11.7±6.0	10.1±3.2
T2 - 100	11.7±0.8	14.0±1.7	13.4±6.1	10.9±3.0
T3 - 250	13.4±1.2	17.7±5.8	15.0±4.6	12.5±3.2
T4 - 500	16.5±0.8	24.6±12.3	18.4±3.0	14.5±2.6
T5 - 1000	24.3±5.5	30.7±20.3	23.8±5.8	18.8±4.3
T6 - 1500	29.7±8.1	37.6±24.2	28.0±10.6	20.6±5.8
T7 - 2000	35.4±10.0	41.5±25.7	34.5±18.4	24.3±7.1
T8 - 2500	42.6±8.7	46.0±31.8	40.6±25.4	27.9±8.6
T9 - 3000	48.9±12.0	52.4±37.5	46.0±31.4	31.5±11.1

(1) Raw water abstracted from the Holsloot River.

Calcium: There are no guidelines for Ca levels in irrigation water (DWAF, 1996). However, it is important that the levels of these elements are determined so that the sodium adsorption ratio (SAR) of the water can be calculated (Myburgh, 2012). If the Ca to Mg ratio in the water is less than one, the potential negative effects of Na may be exacerbated (Ayers & Westcott, 1985). As expected, Ca levels increased with a decrease in the dilution of the winery wastewater (Table 3.13).

Table 3.13. The calcium (Ca) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Ca (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	40.4±20.4	24.9±5.0	47.7±17.3	29.3±11.7
T1 - Control[(1)]	5.4±0.8	7.7±0.6	5.2±3.3	6.5±2.4
T2 - 100	5.8±0.7	7.8±0.5	5.6±3.1	7.2±2.6
T3 - 250	6.3±0.5	7.7±0.9	6.8±2.4	7.6±2.6
T4 - 500	7.5±1.0	8.8±1.2	8.6±0.8	8.5±2.7
T5 - 1000	10.0±2.9	9.7±1.5	11.8±2.3	9.6±2.6
T6 - 1500	12.0±4.7	10.9±1.8	14.2±4.0	10.0±2.3
T7 - 2000	13.9±6.4	11.8±2.0	17.8±8.2	11.5±3.0
T8 - 2500	15.8±6.5	12.4±2.2	20.6±11.2	12.6±3.0
T9 - 3000	17.5±7.5	13.5±2.5	24.1±14.3	13.0±2.8

(1) Raw water abstracted from the Holsloot River.

Magnesium: As in the case of Ca, there are no guidelines for Mg levels in irrigation water (DWAF, 1996) but it is important to determine its level so that the SAR of the water can be calculated (Howell & Myburgh, 2013a). Furthermore, crops irrigated with water containing high levels of Mg may produce low yields due to a Mg-induced Ca deficiency (Ayers & Westcott, 1985). Although there is still insufficient data to make the Ca to Mg ratio an evaluation factor, should this ratio be less than one or the Ca to total cation ratio less than 0.15, a further evaluation of the water is required. Magnesium levels increased with a decrease in dilution of the winery wastewater (Table 3.14) but there were no substantial differences between treatments with regards to Mg levels. Under the conditions of this study, the Ca to Mg ratio was greater than one (data not shown).

Table 3.14. The magnesium (Mg) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Mg (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	8.5±1.9	9.8±2.5	11.0±4.9	9.9±5.3
T1 - Control(1)	3.5±0.6	4.9±0.2	3.4±2.2	3.3±1.5
T2 - 100	3.6±0.6	5.0±0.2	3.5±2.1	3.6±1.5
T3 - 250	3.7±0.5	5.0±0.2	3.8±2.1	3.7±1.5
T4 - 500	3.9±0.5	5.2±0.3	3.9±1.9	4.0±1.4
T5 - 1000	4.2±0.4	5.5±0.4	4.4±1.8	4.5±1.5
T6 - 1500	4.5±0.3	5.7±0.5	4.8±1.9	4.6±1.4
T7 - 2000	4.7±0.4	6.1±0.6	5.1±1.5	5.0±1.5
T8 - 2500	5.3±0.1	6.2±0.8	5.5±1.3	5.5±1.8
T9 - 3000	5.3±0.5	6.5±0.9	6.0±1.2	5.6±1.8

(1) Raw water abstracted from the Holsloot River.

Sodium adsorption ratio: Although Na as well as Ca and Mg increased with a decrease in level of augmentation of the wastewater, the SAR also increased (Table 3.15). This suggested that the increase in Ca and Mg could not counterbalance the effect of increased Na on the SAR. However, the SAR in the augmented winery wastewaters was still within acceptable limits for the irrigation of grapevines, *i.e*. < *ca.* 10 (Richards, 1954; Myburgh, 2012). The SAR in the augmented winery wastewater, as well as the undiluted winery wastewater, never exceeded the acceptable limits for the irrigation of grapevines. With regards to the General Authorisations of 2013 (DWA, 2013), up to 500 m^3 of wastewater may be irrigated on any given day provided that the SAR is less than 5. This norm was only exceeded on 14 April 2011 where the SAR of irrigation waters augmented to COD levels of 1500 mg and higher, *i.e.* T6, T7, T8 and T9 exceeded this value.

Table 3.15. Sodium adsorption ratio (SAR) in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	SAR			
	2009/10	2010/11	2011/12	2012/13
Winery	4.63±1.11	4.29±2.22	3.17±0.65	3.42±1.49
T1 - Control[(1)]	0.87±0.02	0.85±0.21	0.99±0.19	0.81±0.17
T2 - 100	0.97±0.06	0.92±0.26	1.10±0.19	0.84±0.14
T3 - 250	1.09±0.14	1.19±0.47	1.17±0.18	0.94±0.16
T4 - 500	1.25±0.08	1.40±0.78	1.32±0.20	1.05±0.18
T5 - 1000	1.67±0.27	1.84±1.24	1.51±0.40	1.28±0.32
T6 - 1500	1.90±0.32	2.17±1.45	1.63±0.56	1.37±0.41
T7 - 2000	2.12±0.31	2.35±1.44	1.81±0.73	1.52±0.44
T8 - 2500	2.47±0.22	2.51±1.75	2.00±0.89	1.66±0.49
T9 - 3000	2.70±0.28	2.90±1.90	2.08±1.01	1.84±0.58

(1) Raw water abstracted from the Holsloot River.

Iron: The Fe content increased with a decrease in the dilution of the wastewater, *i.e.* an increase in the COD level of the water, but the level in the least diluted water was still substantially lower compared to the undiluted winery wastewater (Table 3.16). Recommended maximum levels of Fe in irrigation water for continuous irrigation on all soils is 5 mg/L (Van Zyl, 1981). Furthermore, the Fe concentration becomes important in the case of drip irrigation where major clogging problems can be expected when Fe levels are higher than 1.5 mg/L (DWAF, 1996). The Fe content in the undiluted wastewater ranged from 0.79 mg/L to 9.89 mg/L. The levels of Fe in the augmented wastewaters never exceeded this value.

Chloride: Although the Cl contents in the augmented waters were lower compared to the undiluted winery wastewater, the range of dilutions did not cause any consistent trends (Table 3.17). Levels in both the augmented wastewaters and undiluted winery wastewater were substantially lower than the norm of 700 mg/L which is recommended for grapevines (Van Zyl, 1981) as well as the norm of 150 mg/L for overhead irrigation (Van Zyl, 1981).

Table 3.16. The iron (Fe) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Fe (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	4.23±1.90	3.30±1.74	5.63±2.45	2.09±1.58
T1 - Control[(1)]	0.03±0.06	0.32±0.75	0.10±0.05	0.03±0.04
T2 - 100	0.07±0.06	0.67±1.57	0.14±0.03	0.06±0.06
T3 - 250	0.10±0.10	0.48±1.16	0.21±0.07	0.11±0.07
T4 - 500	0.23±0.15	0.53±1.11	0.30±0.10	0.17±0.07
T5 - 1000	0.43±0.25	0.28±0.16	0.52±0.27	0.23±0.14
T6 - 1500	0.63±0.35	0.65±0.87	0.80±0.51	0.33±0.19
T7 - 2000	0.80±0.46	0.58±0.39	1.08±0.90	0.48±0.29
T8 - 2500	1.00±0.46	0.84±0.79	1.51±1.36	0.54±0.29
T9 - 3000	1.20±0.46	1.12±1.09	1.92±1.58	0.64±0.39

(1) Raw water abstracted from the Holsloot River.

Table 3.17. The chloride (Cl) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Cl (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	32.0±1.0	57.7±21.2	49.7±25.9	63.1±47.0
T1 - Control[(1)]	22.6±1.8	29.7±3.2	25.3±9.5	27.4±7.8
T2 - 100	22.0±4.1	29.1±3.7	27.0±9.4	25.6±8.5
T3 - 250	20.5±2.5	29.4±2.4	26.3±8.9	26.3±5.9
T4 - 500	19.5±4.2	28.7±2.2	25.9±6.3	27.6±8.6
T5 - 1000	21.7±4.4	29.1±3.3	25.0±8.6	29.0±10.6
T6 - 1500	23.8±1.8	31.1±2.8	25.6±11.6	30.9±9.1
T7 - 2000	23.4±5.2	32.9±4.0	23.1±7.0	28.4±9.6
T8 - 2500	21.1±2.3	31.7±2.2	30.7±9.8	27.7±7.6
T9 - 3000	22.9±3.5	34.6±3.5	31.4±9.7	36.6±13.9

(1) Raw water abstracted from the Holsloot River.

Bicarbonate: The HCO_3 increased with a decrease in the dilution of the wastewater, but the contents in the least diluted water (T9), *i.e.* wastewater augmented to a COD level of 3000 mg/L, was substantially lower compared to the winery wastewater (Table 3.18). On 9 February 2011 and 30 March 2011, no trends were evident for HCO_3 levels in the water. On 6 March 2012 and 19 March 2012, no trends were evident for HCO_3 levels in the water. No trends were evident for HCO_3 levels in the water with the exception of 30 April 2013. Although irrigation water with high HCO_3 can affect levels in plants, soils and irrigation

apparatus there are no recommended guidelines (DWAF, 1996; ANZECC, 2000; Howell & Myburgh, 2013b). However, irrigation waters that contain high levels of HCO_3 and CO_3 can increase HCO_3 in the soil solution. Consequently, Ca and Mg can precipitate as insoluble carbonates when the soil dries out.

Table 3.18. The bicarbonate (HCO_3) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	HCO_3 (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	1293.3±505.3	365.0±306.1	382.8±284.4	106.5±260.7
T1 - Control[(1)]	12.8±4.4	19.4±6.0	13.9±4.3	3.9±4.1
T2 - 100	33.7±9.3	20.7±3.6	16.8±5.2	6.1±4.5
T3 - 250	53.6±27.6	32.1±23.4	28.5±14.2	7.3±9.4
T4 - 500	91.6±48.5	43.9±35.7	43.2±33.1	7.6±15.9
T5 - 1000	164.9±108.1	65.5±71.7	65.9±57.1	10.2±21.4
T6 - 1500	221.0±163.9	110.1±87.1	90.6±78.7	15.5±38.0
T7 - 2000	296.0±194.5	127.3±156.4	124.0±143.0	19.1±46.8
T8 - 2500	373.1±185.3	145.0±173.9	139.0±163.2	25.0±61.1
T9 - 3000	451.7±235.0	190.3±212.4	176.1±185.0	28.0±65.7

[(1)] Raw water abstracted from the Holsloot River.

Sulphate: The SO_4 in the augmented irrigation waters generally increased with a decrease in the level of augmentation of the wastewater, but the contents in the least diluted water, *i.e.* wastewater augmented to a COD level of 3000 mg/L (T9), was substantially lower compared to the winery wastewater (Table 3.19).

Boron: The B levels in the augmented wastewater increased with a decrease in dilution, *i.e.* an increase in the COD level of the water (Table 3.20). Contents in the least diluted water (T9) were still substantially lower compared to the undiluted winery wastewater. Although B is essential for the growth of plants, it is toxic for plants at low concentrations. Grapevines have been classed as very sensitive (Van Zyl, 1981) to sensitive (Ayers &Westcott, 1985; DWAF, 1996, ANZECC, 2000 with regards to B toxicity. In general, B levels of 0.5 mg/L are considered ideal for vineyard irrigation (McCarthy *et al.*, 1988), whereas levels under 0.75 mg/L have been recommended by Ayers and Westcott (1985). In some cases the undiluted winery wastewater exceeded this limit. On 13 April 2011, T9 exceeded this limit and on 14 April, T8 and T9 exceeded this limit. On 2 May 2012, T6 to T9 exceeded this limit.

Table 3.19. The sulphate (SO_4) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	SO_4 (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	203.6±122.3	54.4±28.2	179.5±259.7	114.2±119.2
T1 - Control[(1)]	21.9±6.9	27.8±2.1	21.3±9.1	21.2±11.1
T2 - 100	20.0±4.0	27.8±3.2	17.6±10.0	20.0±8.5
T3 - 250	16.5±4.1	27.8±2.4	18.5±10.6	20.3±7.9
T4 - 500	21.9±6.5	31.5±9.2	19.6±7.6	28.7±20.1
T5 - 1000	29.8±11.8	28.2±1.6	45.9±39.2	24.3±10.0
T6 - 1500	32.8±12.1	25.4±6.9	43.9±36.3	34.7±32.6
T7 - 2000	23.0±9.2	29.4±2.8	107.5±132.1	43.3±49.5
T8 - 2500	89.6±37.8	26.1±8.9	141.3±234.7	41.7±39.6
T9 - 3000	65.5±42.2	38.7±22.7	153.5±290.1	40.7±37.0

(1) Raw water abstracted from the Holsloot River.

Table 3.20. The boron (B) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	B (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	1.03±1.11	0.90±0.55	0.92±1.01	0.35±0.14
T1 - Control[(1)]	0.06±0.06	0.02±0.01	0.01±0.01	0.01±0.01
T2 - 100	0.03±0.02	0.03±0.02	0.02±0.03	0.01±0.01
T3 - 250	0.04±0.02	0.05±0.04	0.05±0.08	0.02±0.01
T4 - 500	0.06±0.01	0.08±0.06	0.09±0.15	0.02±0.02
T5 - 1000	0.1±0.05	0.14±0.13	0.18±0.30	0.04±0.03
T6 - 1500	0.15±0.07	0.19±0.17	0.25±0.43	0.04±0.04
T7 - 2000	0.19±0.10	0.25±0.24	0.37±0.67	0.07±0.04
T8 - 2500	0.26±0.09	0.30±0.29	0.47±0.84	0.09±0.04
T9 - 3000	0.33±0.11	0.38±0.39	0.56±1.01	0.09±0.04

(1) Raw water abstracted from the Holsloot River.

Nitrogen: The fact that NO_3-N and NH_4-N were not determined in 2009/10 was identified as a shortcoming, and this was included in the future water analyses. In general, there were no consistent trends with regards to, NH_4-N, NO_3-N and total N, except the N levels were higher in the raw than in some of the augmented waters (Tables 3.21 to 3.23). At this stage there is no explanation for the latter trend. In 2011/12, the average NO_3-N and NH_4-N content in the winery wastewater was *ca.* 0.5 mg/L and 1.8 mg/L, respectively. In 2012/13, the average NO_3-N and NH_4-N content in the winery wastewater was *ca.* 0.86 mg/L and 0.61 mg/L, respectively.

Table 3.21. The ammonium nitrogen (NH_4-N) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2010/11, 2011/12 and 2012/13 seasons. The NH_4-N was not determined in the 2009/10 season.

Treatment no. & target COD (mg/L)	NH_4-N (mg/L)		
	2010/11	2011/12	2012/13
Winery	2.280±1.777	7.618±8.759	7.845±14.470
T1 - Control[(1)]	0.369±0.362	0.497±0.656	0.458±0.345
T2 - 100	0.159±0.078	0.135±0.106	0.255±0.213
T3 - 250	0.198±0.123	0.130±0.138	0.172±0.136
T4 - 500	0.172±0.057	0.741±0.932	0.182±0.153
T5 - 1000	0.214±0.056	1.191±1.984	0.420±0.724
T6 - 1500	0.517±0.713	2.168±3.608	0.733±1.216
T7 - 2000	0.298±0.095	3.005±4.854	1.147±1.798
T8 - 2500	0.337±0.172	3.720±6.251	1.453±2.474
T9 - 3000	0.430±0.265	4.579±8.157	0.695±0.635

[(1)] Raw water abstracted from the Holsloot River.

Table 3.22. The nitrate nitrogen (NO_3-N) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2010/11, 2011/12 and 2012/13 seasons. The NO_3-N was not determined in the 2009/10 season.

Treatment no. & target COD (mg/L)	NO_3-N (mg/L)		
	2010/11	2011/12	2012/13
Winery	2.683±4.737	0.092±0.198	8.608±15.473
T1 - Control[(1)]	0.970±0.362	1.038±1.158	1.493±2.446
T2 - 100	0.492±0.371	0.249±0.227	0.337±0.412
T3 - 250	0.267±0.304	0.166±0.185	0.108±0.185
T4 - 500	0.067±0.151	0.220±0.223	0.162±0.328
T5 - 1000	0.162±0.286	0.224±0.309	0.343±0.747
T6 - 1500	0.282±0.599	0.214±0.304	0.630±1.222
T7 - 2000	0.684±1.286	0.337±0.483	1.008±1.503
T8 - 2500	0.663±1.585	1.090±1.572	1.673±2.558
T9 - 3000	0.850±1.843	1.027±1.927	1.985±3.099

[(1)] Raw water abstracted from the Holsloot River.

Table 3.23. The total nitrogen (Total-N) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2010/11, 2011/12 and 2012/13 seasons. The total-N was not determined in the 2009/10 season.

Treatment no. & target COD (mg/L)	Total-N (mg/L)		
	2010/11	2011/12	2012/13
Winery	4.963±5.611	7.710±8.672	16.453±18.224
T1 - Control[(1)]	1.339±0.621	1.535±1.010	1.952±2.494
T2 - 100	0.651±0.440	0.383±0.325	0.592±0.583
T3 - 250	0.465±0.410	0.296±0.296	0.280±0.254
T4 - 500	0.239±0.157	0.961±0.877	0.343±0.378
T5 - 1000	0.376±0.258	1.415±1.875	0.763±0.978
T6 - 1500	0.800±1.303	2.381±3.502	1.363±1.551
T7 - 2000	0.982±1.235	3.342±4.694	2.155±2.098
T8 - 2500	1.000±1.493	4.810±5.722	3.127±3.136
T9 - 3000	1.281±1.717	5.606±7.661	2.680±3.219

[(1)] Raw water abstracted from the Holsloot River.

Phosphorus: The P was diluted to such an extent that detectable levels only occurred in the least diluted waters (Table 3.24). Although there are no guidelines for P levels in irrigation water, there is a long term critical value recommended by ANZECC (2000) of 0.05 mg/L. This norm has been established to minimise the risk of algal blooms developing in storage facilities and to reduce the likelihood of bio-fouling (biological fouling) in irrigation equipment. Levels in augmented wastewaters, as well as undiluted winery wastewater generally exceeded this norm.

Table 3.24. The phosphorus (P) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	P (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	7.43±8.37	22.11±13.03	18.80±8.63	10.82±4.61
T1 - Control[(1)]	0.00±0.00	0.02±0.04	0.03±0.03	0.24±0.26
T2 - 100	0.00±0.00	0.02±0.04	0.17±0.19	0.23±0.24
T3 - 250	0.00±0.00	0.18±0.22	0.45±0.39	0.28±0.28
T4 - 500	0.20±0.35	0.64±0.44	1.02±0.92	0.54±0.50
T5 - 1000	0.67±1.15	1.64±0.90	2.12±1.74	1.12±0.77
T6 - 1500	1.20±1.82	2.65±1.37	3.11±2.44	1.53±1.04
T7 - 2000	1.67±2.39	3.87±1.81	4.08±3.91	2.08±1.21
T8 - 2500	2.60±2.76	4.85±2.10	5.27±4.91	2.55±1.47
T9 - 3000	3.30±3.40	6.26±3.01	6.40±5.92	3.04±1.58

[(1)] Raw water abstracted from the Holsloot River.

Heavy metals: In most cases, the heavy metal contents in the irrigation water tended to be lower compared to the undiluted winery wastewater (Table 3.25 to 3.27). However, the selected heavy metals did not show any consistent trends with respect to the different levels of wastewater augmentation. Since there had been no consistent trends with respect to levels of wastewater augmentation and heavy metal contents in the irrigation water in the 2009/10, 2010/11 and 2011/12 seasons, heavy metals were not determined in 2012/13.

Table 3.25. The cadmium (Cd) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Cd (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	0.001±0.001	0.001±0.001	0.003±0.003	0.002±0.002
T1 - Control[(1)]	0.001±0.001	0.000±0.001	0.002±0.002	[(2)]
T2 - 100	0.001±0.001	0.001±0.001	0.001±0.001	[(2)]
T3 - 250	0.001±0.001	0.000±0.001	0.001±0.001	[(2)]
T4 - 500	0.001±0.001	0.000±0.000	0.001±0.001	[(2)]
T5 - 1000	0.001±0.001	0.001±0.001	0.002±0.001	[(2)]
T6 - 1500	0.001±0.001	0.001±0.002	0.003±0.003	[(2)]
T7 - 2000	0.000±0.001	0.001±0.002	0.001±0.001	[(2)]
T8 - 2500	0.001±0.001	0.001±0.002	0.001±0.001	[(2)]
T9 - 3000	0.001±0.001	0.001±0.001	0.001±0.001	

[(1)] Raw water abstracted from the Holsloot River.
[(2)] Not determined.

Table 3.26. The chromium (Cr) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Cr (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	0.015±0.009	0.038±0.049	0.009±0.009	0.022±0.016
T1 - Control[(1)]	0.012±0.007	0.023±0.018	0.004±0.007	[(2)]
T2 - 100	0.010±0.009	0.032±0.025	0.003±0.005	[(2)]
T3 - 250	0.013±0.006	0.024±0.026	0.004±0.004	[(2)]
T4 - 500	0.012±0.005	0.019±0.014	0.003±0.004	[(2)]
T5 - 1000	0.018±0.002	0.023±0.014	0.003±0.003	[(2)]
T6 - 1500	0.013±0.007	0.025±0.020	0.004±0.004	[(2)]
T7 - 2000	0.015±0.009	0.016±0.010	0.005±0.005	[(2)]
T8 - 2500	0.017±0.010	0.021±0.018	0.005±0.004	[(2)]
T9 - 3000	0.015±0.006	0.019±0.013	0.003±0.006	[(2)]

[(1)] Raw water abstracted from the Holsloot River.
[(2)] Not determined.

Table 3.27. The arsenic (As) content in winery wastewater, raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	As (mg/L)			
	2009/10	2010/11	2011/12	2012/13
Winery	0.007±0.008	0.001±0.001	0.006±0.009	0.099±0.149
T1 - Control[(1)]	0.003±0.002	0.001±0.001	0.003±0.006	[(2)]
T2 - 100	0.002±0.004	0.000±0.001	0.003±0.006	[(2)]
T3 - 250	0.000±0.000	0.000±0.000	0.001±0.002	[(2)]
T4 - 500	0.002±0.004	0.002±0.003	0.006±0.010	[(2)]
T5 - 1000	0.003±0.005	0.000±0.000	0.003±0.005	[(2)]
T6 - 1500	0.003±0.003	0.000±0.001	0.000±0.001	[(2)]
T7 - 2000	0.005±0.004	0.000±0.000	0.003±0.004	[(2)]
T8 - 2500	0.001±0.001	0.001±0.001	0.001±0.002	[(2)]
T9 - 3000	0.007±0.006	0.001±0.001	0.000±0.001	[(2)]

[(1)] Raw water abstracted from the Holsloot River.
[(2)] Not determined.

3.3.3. Amount of elements applied

Potassium: The amounts of K applied per hectare increased substantially with a decrease in the dilution of the winery wastewater (Table 3.28). In 2010/11 and 2012/13 similar amounts were applied in the pre- and post-harvest periods. Since there was only one irrigation applied in the post-harvest period of 2011/12, amounts of K added *via* the irrigation water were substantially less than during the pre-harvest period. The total amounts of K added *via* the irrigation water for the highest COD level, *i.e*. 3000 mg/L, ranged from 158 kg/ha in 2011/12 to 215 kg/ha in 2010/11.

Sodium: The amounts of Na applied per hectare also increased substantially with a decrease in level of augmentation of the winery wastewater (Table 3.29). In 2010/11 and 2012/13 similar amounts were applied in the pre- and post-harvest periods. As there was only one irrigation in the 2011/12 post-harvest period, amounts of Na added *via* the irrigation water were substantially less than during the pre-harvest period. The total amounts of Na added *via* the water for the highest COD level, *i.e.* 3000 mg/L, ranged from 74 kg/ha in 2011/12 to 122 kg/ha in 2010/11.

Calcium and magnesium: The amounts of Ca and Mg applied per hectare increased with a decrease in level of dilution of the winery wastewater (Tables 3.30 & 3.31) but the differences between the highest level of augmentation, *i.e.* T1, and the lowest level of augmentation*, i.e*. T9, were not as substantial as in the case of K and Na.

Table 3.28. The calculated amounts of potassium (K) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, irrigations were only applied in the post-harvest period.

Treatment no. & target COD (mg/L)	K (kg/ha)										
	Pre-harvest			Post-harvest				Total			
	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	5.99	6.37	2.29	4.93	1.47	0.19	3.42	4.93	7.46	6.56	5.71
T2 - 100	12.14	10.32	3.36	12.89	1.38	0.69	6.48	12.89	13.52	11.01	9.84
T3 - 250	16.34	19.81	9.59	23.42	8.80	1.77	12.58	23.42	25.14	21.58	22.17
T4 - 500	24.86	32.15	18.50	43.10	19.00	3.71	22.55	43.10	43.86	35.86	41.05
T5 - 1000	41.05	57.89	29.81	92.84	37.99	7.24	40.84	92.84	79.04	65.13	70.65
T6 - 1500	61.96	77.47	33.84	132.86	50.87	9.85	54.42	132.86	112.83	87.32	88.26
T7 - 2000	78.48	92.37	46.66	166.97	70.31	11.58	63.87	166.97	148.79	103.95	110.53
T8 - 2500	92.61	116.78	57.84	200.08	80.80	17.68	77.53	200.08	173.41	134.46	135.37
T9 - 3000	106.18	140.28	67.56	243.63	108.66	17.39	91.94	243.63	214.84	157.67	159.50

(1) Raw water abstracted from the Holsloot River.

Table 3.29. The calculated amounts of sodium (Na) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, irrigations were only applied in the post-harvest period.

Treatment no. & target COD (mg/L)	Na (kg/ha)										
	Pre-harvest			Post-harvest				Total			
	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	31.02	31.90	17.98	18.35	19.34	0.90	11.54	18.35	50.36	32.80	29.52
T2 - 100	35.44	34.25	18.89	19.04	18.15	1.25	12.56	19.04	53.59	35.50	31.45
T3 - 250	38.16	35.67	21.07	21.36	21.67	1.86	14.74	21.36	59.83	37.53	35.81
T4 - 500	48.47	39.74	22.32	25.24	24.58	2.68	17.97	25.24	73.05	42.42	40.29
T5 - 1000	49.72	44.82	26.41	34.99	31.45	4.64	24.30	34.99	81.17	49.46	50.71
T6 - 1500	61.23	48.75	27.65	41.90	36.07	6.15	28.31	41.90	97.30	54.90	55.96
T7 - 2000	64.42	52.74	32.40	47.83	40.23	8.71	31.84	47.83	104.65	61.45	64.24
T8 - 2500	66.99	58.05	35.68	56.85	43.13	11.02	36.01	56.85	110.12	69.07	71.69
T9 - 3000	70.72	62.78	40.27	64.51	51.61	11.19	41.30	64.51	122.33	73.97	81.57

(1) Raw water abstracted from the Holsloot River.

Table 3.30. The calculated amounts of calcium (Ca) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, irrigations were only applied in the post-harvest period.

Treatment no. & target COD (mg/L)	Ca (kg/ha)										
	Pre-harvest			Post-harvest				Total			
	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	19.01	14.67	12.37	9.76	11.01	0.27	6.70	9.76	30.02	14.94	19.07
T2 - 100	20.62	15.05	13.57	9.55	10.12	0.38	7.27	9.55	30.74	15.43	20.84
T3 - 250	20.30	16.90	14.26	10.29	10.49	0.61	7.85	10.29	30.79	17.51	22.11
T4 - 500	21.65	18.98	15.30	11.87	11.25	1.07	8.59	11.87	32.90	20.05	23.89
T5 - 1000	22.68	23.06	16.26	14.91	12.24	1.89	10.27	14.91	34.92	24.95	26.53
T6 - 1500	24.31	26.04	16.90	17.58	14.20	2.53	11.19	17.58	38.51	28.57	28.09
T7 - 2000	25.64	28.75	19.22	19.30	14.30	3.74	12.00	19.30	39.94	32.49	31.22
T8 - 2500	25.77	31.84	20.32	21.74	15.02	4.61	13.03	21.74	40.79	36.45	33.35
T9 - 3000	27.50	35.88	21.03	23.89	15.63	4.81	13.99	23.89	43.13	40.69	35.02

(1) Raw water abstracted from the Holsloot River.

Table 3.31. The calculated amounts of magnesium (Mg) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, irrigations were only applied in the post-harvest period.

Treatment no. & target COD (mg/L)	Mg (kg/ha)										
	Pre-harvest			Post-harvest				Total			
	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	12.25	9.51	5.69	6.44	6.83	0.15	4.13	6.44	19.08	9.66	9.82
T2 - 100	13.28	9.67	6.11	6.05	6.31	0.19	4.50	6.05	19.59	9.86	10.61
T3 - 250	13.32	10.19	6.18	6.22	6.51	0.21	4.84	6.22	19.83	10.40	11.02
T4 - 500	13.46	10.32	6.39	6.55	6.75	0.25	5.14	6.55	20.21	10.57	11.53
T5 - 1000	13.59	11.00	6.67	6.92	7.12	0.34	6.03	6.92	20.71	11.34	12.70
T6 - 1500	14.16	11.79	6.84	7.31	7.48	0.40	6.43	7.31	21.64	12.19	13.27
T7 - 2000	14.51	11.92	7.39	7.28	7.72	0.52	6.70	7.28	22.23	12.44	14.09
T8 - 2500	14.17	12.19	7.62	8.11	7.88	0.63	7.30	8.11	22.05	12.82	14.92
T9 - 3000	14.82	13.02	7.62	8.13	8.09	0.65	7.89	8.13	22.92	13.67	15.51

[(1)] Raw water abstracted from the Holsloot River.

Iron: The amounts of Fe applied per hectare increased with a decrease in level of dilution of the winery wastewater (Table 3.32). Total amounts applied for highest concentration was less than 3.30 kg/ha.

Chloride: Although amounts of Cl added *via* the irrigation water were higher for T9 compared to the raw river water control (T1) (Table 3.33), increases across the COD levels were inconsistent. It should be noted that the river water also contained high levels of Cl.

Bicarbonate: In 2010/11 and 2011/12, there was a substantial increase in HCO_3 applied per ha with decrease in dilution of the wastewater (Table 3.34). In the last season of the study, *i.e.* 2012/13, very low HCO_3 concentrations were observed for the first five irrigations. Levels were also inconsistent with Increasing COD levels. During the last irrigation, high levels of HCO_3 were measured. Much lower levels were measured in the last season.

Sulphate: Although levels of SO_4 were higher for treatments with low levels of dilutions, and levels applied to T1 consistently lower than T9, increases were inconsistent with regard to COD levels (Table 3.35).

Boron: Despite low levels of B being applied *via* the irrigation water, amounts increased with increase in COD level (Table 3.36).

Nitrogen: In terms of NH_4-N, NO_3-N and total-N amounts added *via* the irrigation water were generally higher for T9 compared to the raw river water control (T1) but trends across the range of COD levels were inconsistent (Tables 3.37 to 3.39).

Phosphorus: Phosphorus increased with a decrease in dilution of the wastewater (Table 3.40).

Heavy metals: Since there were such low concentrations of Cd, Cr and As in the undiluted winery wastewater, there were no trends with regards to COD levels (data not shown).

Table 3.32. The calculated amounts of iron (Fe) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, irrigations were only applied in the post-harvest period.

Treatment no. & target COD (mg/L)	Fe (kg/ha)										
	Pre-harvest			Post-harvest				Total			
	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	0.04	0.18	0.02	0.06	1.25	0.03	0.05	0.06	1.29	0.21	0.07
T2 - 100	0.08	0.25	0.04	0.10	2.05	0.04	0.11	0.10	2.13	0.29	0.15
T3 - 250	0.10	0.37	0.14	0.14	1.78	0.05	0.15	0.14	1.88	0.42	0.29
T4 - 500	0.21	0.48	0.25	0.31	1.74	0.07	0.18	0.31	1.95	0.55	0.43
T5 - 1000	0.37	0.75	0.40	0.55	0.80	0.13	0.16	0.55	1.17	0.88	0.56
T6 - 1500	0.53	1.04	0.56	0.81	1.71	0.22	0.27	0.81	2.24	1.26	0.83
T7 - 2000	0.73	1.19	0.85	1.00	1.22	0.34	0.32	1.00	1.95	1.53	1.17
T8 - 2500	0.94	1.56	0.93	1.24	1.70	0.50	0.36	1.24	2.64	2.06	1.29
T9 - 3000	1.11	2.10	1.15	1.49	2.18	0.51	0.44	1.49	3.29	2.61	1.59

[(1)] Raw water abstracted from the Holsloot River.

Table 3.33. The calculated amounts of chloride (Cl) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, irrigations were only applied in the post-harvest period.

Treatment no. & target COD (mg/L)	Cl (kg/ha)										
	Pre-harvest			Post-harvest				Total			
	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	73.31	64.38	48.94	40.81	42.93	3.01	31.49	40.81	116.24	67.39	80.43
T2 - 100	78.15	66.88	48.43	37.49	37.98	3.33	27.54	37.49	116.13	70.21	75.97
T3 - 250	78.20	65.08	47.11	35.95	39.99	3.42	31.07	35.95	118.19	68.50	78.18
T4 - 500	76.81	63.97	50.73	34.92	38.36	3.52	29.99	34.92	115.17	67.49	80.72
T5 - 1000	80.36	63.26	53.55	37.95	37.02	3.19	30.31	37.95	117.38	66.45	83.86
T6 - 1500	79.79	65.81	55.96	40.56	42.76	2.79	34.09	40.56	122.55	68.60	90.05
T7 - 2000	80.62	60.37	51.41	38.45	43.51	2.86	31.02	38.45	124.13	63.23	82.43
T8 - 2500	79.67	75.12	49.23	36.27	41.96	3.09	30.29	36.27	121.63	78.21	79.52
T9 - 3000	82.92	75.46	52.29	38.26	44.83	3.35	50.20	38.26	127.75	78.81	102.49

(1) Raw water abstracted from the Holsloot River.

Table 3.34. The calculated amounts of bicarbonate (HCO_3) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, irrigations were only applied in the post-harvest period.

Treatment no. & target COD (mg/L)	HCO_3 (kg/ha)										
	Pre-harvest			Post-harvest				Total			
	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	52.13	33.70	11.57	21.72	23.97	2.38	0.29	21.72	76.10	36.08	11.86
T2 - 100	54.72	38.06	10.37	47.30	25.11	2.89	6.75	47.30	79.83	40.95	17.12
T3 - 250	57.35	46.41	6.44	72.44	35.77	7.76	13.71	72.44	93.12	54.17	20.15
T4 - 500	70.72	57.35	4.62	119.65	42.93	13.35	16.17	119.65	113.65	70.70	20.79
T5 - 1000	85.92	79.30	5.06	208.94	58.34	21.40	22.18	208.94	144.26	100.70	27.24
T6 - 1500	172.40	109.45	2.12	281.46	80.83	27.92	38.95	281.46	253.23	137.37	41.07
T7 - 2000	139.36	115.08	2.22	368.33	101.07	45.81	47.27	368.33	240.43	160.89	49.49
T8 - 2500	150.92	124.84	2.19	461.73	116.92	52.75	59.66	461.73	267.84	177.59	61.85
T9 - 3000	198.62	175.18	4.75	559.12	156.79	52.09	65.41	559.12	355.41	227.27	70.16

[(1)] Raw water abstracted from the Holsloot River.

Table 3.35. The calculated amounts of sulphate (SO_4) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, irrigations were only applied in the post-harvest period.

Treatment no. & target COD (mg/L)	SO_4 (kg/ha)										
	Pre-harvest			Post-harvest				Total			
	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	69.53	50.40	45.89	38.28	38.93	4.72	17.58	38.28	108.46	55.12	63.47
T2 - 100	72.81	49.15	41.37	34.27	37.29	2.86	19.22	34.27	110.10	52.01	60.59
T3 - 250	75.49	51.10	41.38	30.19	37.35	2.75	20.24	30.19	112.84	53.85	61.62
T4 - 500	83.70	49.84	60.87	37.01	37.53	3.87	21.17	37.01	121.23	53.71	82.04
T5 - 1000	75.51	96.65	48.02	47.10	35.88	6.15	22.70	47.10	111.39	102.80	70.72
T6 - 1500	66.79	61.78	73.57	51.03	38.29	15.64	24.42	51.03	105.08	77.42	97.99
T7 - 2000	76.27	99.11	93.21	37.35	37.71	43.25	24.06	37.35	113.98	142.36	117.27
T8 - 2500	64.85	76.07	84.38	119.47	38.17	72.05	27.65	119.47	103.02	148.12	112.03
T9 - 3000	99.47	55.07	82.50	89.88	39.03	74.43	28.30	89.88	138.50	129.50	110.80

[(1)] Raw water abstracted from the Holsloot River.

Table 3.36. The calculated amounts of boron (B) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, irrigations were only applied in the post-harvest period.

Treatment no. & target COD (mg/L)	B (kg/ha)										
	Pre-harvest			Post-harvest				Total			
	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	0.05	0.02	0.03	0.12	0.03	0.01	0.01	0.12	0.08	0.03	0.04
T2 - 100	0.07	0.02	0.03	0.06	0.03	0.01	0.01	0.06	0.10	0.03	0.04
T3 - 250	0.09	0.03	0.03	0.07	0.05	0.03	0.01	0.07	0.14	0.06	0.04
T4 - 500	0.11	0.05	0.05	0.10	0.07	0.05	0.01	0.10	0.18	0.10	0.06
T5 - 1000	0.16	0.08	0.07	0.18	0.12	0.09	0.02	0.18	0.28	0.17	0.09
T6 - 1500	0.22	0.11	0.09	0.22	0.18	0.13	0.03	0.22	0.40	0.24	0.12
T7 - 2000	0.27	0.13	0.12	0.26	0.22	0.20	0.05	0.26	0.49	0.33	0.17
T8 - 2500	0.32	0.16	0.14	0.35	0.26	0.25	0.07	0.35	0.58	0.41	0.21
T9 - 3000	0.37	0.19	0.15	0.44	0.33	0.26	0.08	0.44	0.70	0.45	0.23

[(1)] Raw water abstracted from the Holsloot River.

Table 3.37. The calculated amounts of ammonium nitrogen (NH_4-N) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, NH_4-N was not determined.

Treatment no. & target COD (mg/L)	NH_4-N (kg/ha)								
	Pre-harvest			Post-harvest			Total		
	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13
T1 - Control[(1)]	0.96	0.44	0.48	0.53	0.35	0.90	1.49	0.79	1.38
T2 - 100	0.57	0.38	0.38	0.37	0.14	0.50	0.94	0.53	0.88
T3 - 250	0.70	0.37	0.21	0.34	0.14	0.45	1.04	0.51	0.66
T4 - 500	0.61	0.72	0.21	0.34	0.41	0.48	0.96	1.14	0.69
T5 - 1000	0.69	0.61	0.19	0.37	0.74	1.11	1.06	1.35	1.29
T6 - 1500	0.72	1.05	0.38	1.24	1.22	1.70	1.95	2.27	2.08
T7 - 2000	0.78	1.52	0.65	0.49	1.58	2.43	1.27	3.11	3.08
T8 - 2500	0.86	1.67	0.30	0.48	1.99	3.44	1.35	3.66	3.74
T9 - 3000	0.77	1.68	0.46	0.77	2.21	1.49	1.54	3.89	1.95

[(1)] Raw water abstracted from the Holsloot River.

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Table 3.38. The calculated amounts of nitrate nitrogen (NO_3-N) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, NO_3-N was not determined.

Treatment no. & target COD (mg/L)	NO_3-N (kg/ha)								
	Pre-harvest			Post-harvest			Total		
	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13
T1 - Control[(1)]	2.65	2.52	4.64	1.16	0.01	0.43	3.81	2.53	5.07
T2 - 100	1.58	0.93	1.76	1.09	0.01	0.44	2.67	0.94	2.02
T3 - 250	1.64	0.73	1.25	0.60	0.01	0.25	2.24	0.74	1.50
T4 - 500	1.24	0.88	1.33	0.43	0.01	0.40	1.67	0.89	1.72
T5 - 1000	1.51	0.86	1.37	0.44	0.01	0.83	1.95	0.87	2.20
T6 - 1500	1.24	0.81	1.54	1.10	0.01	1.35	2.34	0.82	2.89
T7 - 2000	2.67	1.14	2.39	0.82	0.01	1.49	3.49	1.15	3.88
T8 - 2500	2.90	2.64	3.62	0.45	0.01	1.86	3.35	2.65	5.48
T9 - 3000	3.20	2.51	4.23	0.68	0.01	2.02	3.89	2.52	6.25

[(1)] Raw water abstracted from the Holsloot River.

Table 3.39. The calculated amounts of total nitrogen (N) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, total N was not determined.

Treatment no. & target COD (mg/L)	Total N (kg/ha)								
	Pre-harvest			Post-harvest			Total		
	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13
T1 - Control[(1)]	3.61	2.96	5.12	1.69	0.36	1.33	5.30	3.32	6.45
T2 - 100	2.15	1.31	2.14	1.46	0.15	0.94	3.61	1.46	3.08
T3 - 250	2.34	1.10	1.46	0.94	0.15	0.70	3.28	1.25	2.16
T4 - 500	1.85	1.60	1.54	0.77	0.42	0.88	2.62	2.03	2.42
T5 - 1000	2.20	1.47	1.56	0.81	0.75	1.94	3.01	2.21	3.50
T6 - 1500	1.96	1.86	1.92	2.34	1.23	3.05	4.30	3.09	4.97
T7 - 2000	3.45	2.66	3.04	1.31	1.59	3.92	4.76	4.25	6.95
T8 - 2500	3.76	4.31	3.92	0.93	2.00	5.30	4.69	6.31	9.22
T9 - 3000	3.97	4.19	4.69	1.45	2.22	3.51	5.42	6.41	8.20

[(1)] Raw water abstracted from the Holsloot River.

Table 3.40. The calculated amounts of phosphorus (P) applied *via* raw river water and augmented winery wastewater used for irrigation of Cabernet Sauvignon/99R during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons. In 2009/10, irrigations were only applied in the post-harvest period.

Treatment no. & target COD (mg/L)	P (kg/ha)										
	Pre-harvest			Post-harvest				Total			
	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13	2009/10	2010/11	2011/12	2012/13
T1 - Control[(1)]	0.00	0.06	0.28	0.00	0.09	0.01	0.42	0.00	0.09	0.07	0.70
T2 - 100	0.04	0.16	0.29	0.00	0.04	0.06	0.37	0.00	0.08	0.22	0.66
T3 - 250	0.22	0.49	0.29	0.00	0.17	0.15	0.52	0.00	0.39	0.64	0.81
T4 - 500	0.72	1.04	0.47	0.25	0.47	0.33	0.95	0.25	1.19	1.37	1.42
T5 - 1000	2.11	2.30	1.17	0.81	1.07	0.66	1.69	0.81	3.18	2.96	2.86
T6 - 1500	3.67	3.51	1.35	1.49	1.68	0.92	2.59	1.49	5.35	4.43	3.94
T7 - 2000	6.01	3.87	1.96	2.04	2.45	1.37	3.26	2.04	8.46	5.24	5.22
T8 - 2500	7.43	5.08	2.20	3.18	2.95	1.75	4.04	3.18	10.38	6.83	6.24
T9 - 3000	9.40	6.22	2.88	4.04	4.06	1.80	4.64	4.04	13.46	8.02	7.52

[(1)] Raw water abstracted from the Holsloot River.

3.4. CONCLUSIONS

There were substantial increases in the K and Na levels in the augmented wastewater, and these levels could be related to the different levels of augmentation of the undiluted winery wastewater. There were also increases in Ca, Mg, B and P levels of the irrigation water which could be related to different levels of augmentation. With regards to anions, there were substantial increases in the HCO_3 and SO_4 levels of the water, and these levels could be related to the different levels of augmentation of the undiluted winery wastewater. Subsequently, the differences in the water quality were reflected in the amounts of additional elements applied to the vineyard *via* the irrigation water.

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CHAPTER 4: EFFECT OF IRRIGATION WITH AUGMENTED WINERY WASTEWATER ON SOIL CHEMICAL STATUS

C.L. Howell & P.A. Myburgh
The Fruit, Vine and Wine Institute of the Agricultural Research Council, Private Bag X5026, Stellenbosch, 7599

4.1. INTRODUCTION

Although there is extensive literature available regarding the effect of irrigation with wastewaters of various origins on soil chemical properties (Hulugalle *et al.*, 2006; Walker & Lin, 2008; Duan *et al.,* 2010; Rana *et al*., 2010; Lado *et al*., 2011; Moraetis *et al*., 2011; Blum *et al*., 2012; Chavez *et al.*, 2012; Barbera *et al.,* 2013*;* Di Bene *et al,.* 2013; Thapliyal *et al.,* 2013), very little is known about the effects of irrigation using augmented winery wastewater on soil chemical status. In Australia, it is estimated that approximately 3 to 5 m^3 of winery wastewater, with high organic load and variable salinity and nutrient levels, is generally produced when a ton of grapes is crushed (Mosse *et al*., 2011). This means that this particular winery generates about 1.1 m^3 of wastewater per ton of grapes crushed. The effects of high concentrations of potassium (K) ions application on soils have not been extensively researched and are still unclear (Mosse *et al.*, 2011; Laurenson *et al*., 2012). On the other hand, limited irrigation water supplies could be restricted further in future allocations of irrigation water (Van Zyl & Weber, 1981; Petrie *et al.*, 2004). If winery wastewater could be used to irrigate vineyards, with no detrimental impacts on soil chemical status, it could be a possible viable alternative to using either raw river or recycled municipal water.

According to Arienzo *et al.* (2009), land application of wastewaters can increase the levels of soluble and exchangeable forms of K more rapidly than with conventional inorganic fertilizers. Furthermore, most of the K in wastewater is immediately available. Irrigation with K-rich wastewaters could be beneficial to overall soil fertility, although long-term application could affect soil chemical and physical properties (Laurenson *et al*., 2011; Mosse *et al.*, 2011). A further advantage of using winery wastewater as a source of K over the use of conventional fertiliser is that it could be an efficient recycling practice in areas where the soil has low K. In addition to sodium (Na) and K ions, winery wastewater can also contain calcium (Ca) and magnesium (Mg) ions (Mosse *et al*., 2011). Neither of these ions is harmful to soil structure and can ameliorate the impacts of Na *via* their role in reducing the sodium adsorption ratio (SAR).

The aim of this study was to determine the effect of vineyard irrigation with augmented winery wastewater on the chemical status of a sandy, alluvial soil over a four year period. In addition

to this, guidelines as to what quality of augmented wastewater, *i.e.* chemical oxygen demand (COD) level, could be applied to a sandy, alluvial soil without irreversible detrimental effects.

4.2. MATERIALS AND METHODS

Soil samples were collected with a Thompson auger in August 2009 before the trial commenced in order to quantify the chemical status before treatments were applied, *i.e*. the baseline soil chemical status. After the first season of applying wastewater irrigation in 2009/2010, the second set of soil samples was collected in the work rows of all the experiment plots in May 2010. Soil from each of the three replications of each treatment was pooled for analysis, therefore there were 54 soil samples in total. Soil samples were also collected at bud break during October 2010, September 2011 and 2012 from the work rows in all experimental plots. Soil from each of the three replications of each treatment was pooled for analysis. In April 2011, soil samples were collected from each of the three replications of T1, T3, T5, T7 and T9 *c.* one week after the end of the application of wastewater irrigations. These particular treatments were selected so as to obtain a wide range of COD levels. In order to investigate the changes in the soil chemical properties within the vineyard as a result of the water distribution pattern of the irrigation system, samples were taken in the work row as well as the grapevine row. In contrast to soil samples taken in early May 2010, no rain occurred in the period preceding the collection of these samples. The same procedure was followed for samples collected after the application of winery wastewater irrigations had stopped in May 2012 and 2013. All the soil samples were taken at 30 cm increments to a depth of 1.8 m. At the end of the trial in September 2013, soil samples were collected in the work rows of all the experiment plots at 30 cm increments from the soil surface until 3.0 m, where possible. Soil which was collected deeper than 1.8 m was sampled using a modified soil auger (Fig. 4.1). All samples were analysed by a commercial laboratory for $pH_{(KCl)}$, electrical conductivity (EC_e), acidity, phosphorus (P) (Bray II) and K (Bray II), exchangeable Ca, Mg, K, Na and C. In additions, samples analysed after the application of wastewater irrigations were also analysed for trace elements and chloride (Cl). Soil pH was measured in 1M KCl and EC_e in a US Bureau of Standards cup). The P and K (Bray II), Na, K, Ca, Mg, Cu, Zn, Mn, and B were analysed using an inductively coupled plasma atomic emission spectrometer (PerkinElmer Optima 7300 DV, Waltham, Massachusetts). Total organic C contents were determined using the method described by Walkley and Black (1934). Soil Cl was determined volumetrically through titration of a soil extract (0.1M KNO_3) with 0.043 M $AgNO_3$ using potassium dichromate (K_2CrO_4) as an indicator (Chapman & Pratt, 1961).

Figure 4.1. A modified soil auger, which was used to collect soil samples to 3 m depth.

The percentage exchangeable cations, *i.e.* Ca, Mg, K and Na, were reported by the laboratory and was calculated as follows:

Percentage exchangeable cation = [Cation] ÷ T value X 100 (Eq. 4.1)

where [Cation] is the specific cation concentration expressed in cmol(+)/kg and the T value is the sum of the exchangeable Ca, Mg, Na, K and H^+ concentrations, expressed in cmol(+)/kg. As the water soluble cations of the soil were not determined, it was not possible to calculate the sodium adsorption ratio (SAR). The SAR was estimated from the exchangeable sodium percentage (ESP), according to the equation of Richards (1954), where:

ESP = [Na] ÷ ([Ca] + [Mg] + [K] + [Na]) X 100 (Eq. 4.2)

where the cations are concentrations expressed in cmol(+)/kg and

SAR = (1.26+ESP X 0.9874) ÷ (1.475-0.01475 X ESP) (Eq. 4.3)

where ESP was calculated according to Eq. 4.2.

4.3. RESULTS AND DISCUSSION

4.3.1. $pH_{(KCl)}$, EC_e and acidity

In general, there were no clear trends in soil $pH_{(KCl)}$ that could be related to the different wastewater treatments, *i.e.* different levels of augmentation of winery wastewater, compared to the raw water control. However, in a study investigating the long-term use of winery

wastewater over 30 years, there was a decrease in soil pH with depth (Mosse *et al*., 2012). There has also been conflicting reports of either an increase or decrease in soil pH (Laurenson *et al.*, 2012 and references in), and soil pH changes can be related to the characteristics of the wastewater. The baseline values for $pH_{(KCl)}$ were 5.3 and 4.7 for the 0 to 90 cm and 90 to 180 cm soil layer, respectively. Soil $pH_{(KCl)}$ levels remained similar to baseline values until the end of the trial in September 2013 (Tables 4.1 & 4.2) when the soil $pH_{(KCl)}$ in both the 0 to 90 cm and 90 to 180 cm layers tended to be lower than the baseline values. In particular, the $pH_{(KCl)}$ in the 90 to 180 cm layer tended to be below the norm of 5.0 to 7.5 recommended by Saayman (1981) for optimal grapevine growth.

Although there were no consistent trends with regards to $EC_{e,}$ in April 2011, EC_e was more than double that in May 2010. This difference can be attributed to the heavy rain that precipitated in May 2010 before the soil could be sampled. There were no clear trends in soil EC_e that could be related to the different levels of augmentation compared to T1. However, it was evident that EC_e was considerably higher after the application of wastewater irrigations compared to bud break. This trend suggested an accumulation of salts during the grapevine growing season partly due to irrigation with augmented winery wastewater which contains salts (Laurenson *et al.*, 2012). At the end of the trial in September 2013, EC_e in the 0 to 90 cm layer was similar to the baseline levels whereas the EC_e in the 90 to 180 cm layer was slightly higher than the baseline levels (Tables 4.1 & 4.2). This indicated that under the prevailing conditions, irrigation with augmented wastewater did not lead to a long-term accumulation of salts in the soil. However, this does not rule the possibility that winter rainfall could have leached salts beyond the measured depth. This confirms the importance of only irrigating where the roots occur, *i.e.* within the root zone. In heavier soils, less effective leaching is more likely to result in salt accumulation.

In general, irrigation with winery wastewater augmented to different COD levels did not affect H^+. The baseline values for H^+ were 0.60 and 0.85 cmol(+)/kg, for the 0 to 90 cm and 90 to 180 cm soil layer, respectively. At the end of the trial in September 2013, H^+ in the 0 to 90 cm layer was similar to the baseline value whereas the H^+ in the 90 to 180 cm layer was slightly higher than the baseline value (Tables 4.1 & 4.2).

4.3.2. Phosphorus (Bray II)

In 2012 and 2013, there was a close correlation between P applied *via* the irrigation water and the soil P levels in the 0 to 30 cm layer in the work row. However, there did not seem to be any further relationships between the P applied *via* the irrigation water and P in the subsoil layers. In October 2010, the P concentration in the work rows tended to be higher than in August 2009 and May 2010, irrespective of the level of wastewater augmentation (Table 4.1). These

increases suggested that the winter cover crop probably did not fully absorb the P fertilisers applied to enhance its growth. Since the vineyard was previously irrigated by means of drippers, fertilisers were most probably band placed on the grapevine rows.

As a result, the nutrient status was initially probably relatively low in the work rows compared to the grapevine rows. In April 2011, the P concentrations in the work rows up to a depth of 90 cm tended to be substantially lower than in August 2009 and October 2010. This decrease suggested that the grapevines, as well as the interception crop, absorbed a significant amount of P. However, the P levels in the 90 cm to 180 cm layer were similar to levels determined at the start of the trial. At bud break in September 2011, *i.e.* after two seasons, it was evident that there was an accumulation of soil P where grapevines were irrigated with winery wastewater augmented to a COD level of 3000 mg/L (T9) compared to T1. In May 2012, P levels in the work rows up to a depth of 90 cm tended to be lower than in August 2009. This decrease suggested that grapevines, as well as the interception crop, absorbed a substantial amount of P. Furthermore, the P concentrations in the 90 cm to 180 cm layer were similar to levels at the start of the trial. In May 2013, P concentrations in the work rows of T1, T3, T5 and T7 up to a depth of 90 cm tended to be lower than in August 2009. This decrease suggested that grapevines, as well as the interception crop, absorbed a significant amount of P. Furthermore, the P concentrations in the 90 to 180 cm layer were similar to levels at the start of the trial. The baseline values for P were 153 and 29 mg/kg, for the 0 to 90 cm and 90 to 180 cm soil layer, respectively. At the end of the trial in September 2013, P in the 0 to 90 cm layer was substantially lower than this whereas P levels in the 90 to 180 cm layer was similar to the baseline value (Tables 4.1 & 4.2). Proposed P norms by Conradie (1994) are based on P (Bray II) for soil with a pH of 5.5. Soils with a clay content ranging from 0 to 6% have a P norm of 20 mg/kg. In general, under the prevailing conditions, levels up to a depth of 90 cm were substantially higher than this norm.

Table 4.1. Mean values for selected soil chemical parameters as measured in the work rows for the duration of the trial. Data are means for all treatments on a specific date.

Soil parameter	May 2010	Oct. 2010	April 2011	Sep. 2011	May 2012	Sep. 2012	May 2013	Sep. 2013
				0-90 cm				
$pH_{(KCl)}$	5.1±0.5	6.4±0.9	5.1±0.6	5.1±0.4	5.1±0.5	5.2±0.5	5.1±0.4	4.8±0.5
EC_e (dS/m)	0.068±0.014	0.059±0.010	0.150±0.050	0.042±0.011	0.200±0.009	0.080±0.020	0.110±0.080	0.050±0.010
H^+ (cmol/kg)	0.69±0.22	0.73±0.24	0.48±0.23	0.67±0.19	0.76±0.34	0.61±0.24	0.43±0.20	0.63±0.32
P (mg/kg)	116±74	193±116	110±82	133±72	137±91	116±87	124±91	99±86
Ca (cmol(+)/kg)	1.58±0.76	1.52±0.70	1.78±1.08	1.66±0.68	1.73±0.98	1.78±0.90	1.66±0.85	1.55±0.95
Mg (cmol(+)/kg)	0.66±0.30	0.74±0.27	0.67±0.35	0.72±0.27	0.73±0.33	0.73±0.33	0.74±0.28	0.73±0.35
Cu (mg/kg)	2.78±2.09	(1)	3.20±2.81	(1)	4.11±3.15	(1)	4.02±2.26	3.14±2.81
Zn (mg/kg)	1.4±1.2	(1)	1.4±1.4	(1)	1.7±1.5	(1)	1.6±1.2	1.1±1.2
B (mg/kg)	0.09±0.04	(1)	0.09±0.04	(1)	0.09±0.04	(1)	0.11±0.03	0.10±0.09
Fe (mg/kg)	125.09±88.00	(1)	93.50±70.26	(1)	77.93±35.63	(1)	45.76±22.14	61.00±29.15
C (%)	0.66±0.17	0.74±0.13	0.66±0.17	0.72±0.22	0.66±0.17	0.63±0.20	0.67±0.27	0.67±0.32
Cl (mg/kg)	(1)	(1)	23.77±8.49	(1)	27.15±10.86	(1)	37.50±10.65	(1)
				90-180 cm				
$pH_{(KCl)}$	4.5±0.1	5.9±0.9	4.4±0.1	4.4±0.3	4.3±0.1	4.4±0.1	4.4±0.2	4.1±0.2
EC_e (dS/m)	0.067±0.015	0.072±0.022	0.170±0.090	0.064±0.026	0.150±0.050	0.130±0.050	0.110±0.080	0.070±0.050
H^+ (cmol/kg)	0.93±0.36	0.98±0.31	0.75±0.34	1.01±0.29	1.17±0.38	0.97±0.33	0.78±0.39	1.08±0.51
P (mg/kg)	27±6	46±32	25±8	47±36	29±13	22±4	35±41	23±10
Ca (cmol(+)/kg)	0.37±0.11	0.33±0.31	0.34±0.16	0.52±0.41	0.21±0.17	0.31±0.09	0.44±0.45	0.23±0.13
Mg (cmol(+)/kg)	0.15±0.04	0.20±0.13	0.14±0.06	0.24±0.15	0.15±0.10	0.15±0.04	0.24±0.22	0.15±0.11
Cu (mg/kg)	0.26±0.13	(1)	0.23±0.09	(1)	0.49±0.35	(1)	1.45±0.92	0.18±0.23
Zn (mg/kg)	0.3±0.1	(1)	0.2±0.1	(1)	0.3±0.3	(1)	0.6±0.6	0.1±0.1
B (mg/kg)	0.09±0.04	(1)	0.06±0.01	(1)	0.12±0.06	(1)	0.08±0.02	0.04±0.02
Fe (mg/kg)	173.97±188.87	(1)	86.23±108.40	(1)	50.40±110.01	(1)	25.09±17.80	40.89±64.58
C (%)	0.35±0.18	0.47±0.16	0.44±0.20	0.53±0.16	0.56±0.24	0.42±0.19	0.50±0.27	0.41±0.24
Cl (mg/kg)	(1)	(1)	31.35±14.72	(1)	26.86±11.42	(1)	40.97±14.57	(1)

(1) Not determined.

Table 4.2. Mean values for selected soil chemical parameters as measured on the grapevine rows for the duration of the trial. Data are means for all treatments on a specific date.

Soil parameter	April 2011	May 2012	May 2013
		0-90 cm	
$pH_{(KCl)}$	5.2±0.5	5.3±0.6	5.1±0.4
EC_e (dS/m)	0.100±0.040	0.110±0.030	0.060±0.030
H^+ (cmol/kg)	0.59±0.31	0.74±0.37	0.44±0.26
P (mg/kg)	114±61	135±77	153±89
Ca (cmol(+)/kg)	2.00±1.04	2.01±0.99	1.88±0.92
Mg (cmol(+)/kg)	0.84±0.41	0.76±0.34	0.80±0.30
Cu (mg/kg)	3.94±2.80	4.65±3.04	4.96±2.30
Zn (mg/kg)	1.5±1.6	2.2±2.2	2.2±1.8
B (mg/kg)	0.20±0.06	0.21±0.05	0.11±0.04
Fe (mg/kg)	87.53±47.23	80.49±25.27	47.50±14.85
C (%)	0.71±0.26	0.85±0.26	0.74±0.26
Cl (mg/kg)	28.35±12.52	15.19±7.69	33.25±6.20
		90-180 cm	
$pH_{(KCl)}$	4.6±0.4	4.6±03	4.6±0.3
EC_e (dS/m)	0.070±0.020	0.100±0.040	0.060±0.030
H^+ (cmol/kg)	0.77±0.31	0.97±0.29	0.57±0.27
P (mg/kg)	22±8	26±9	46±44
Ca (cmol(+)/kg)	0.36±0.31	0.42±0.19	0.50±0.50
Mg (cmol(+)/kg)	0.17±0.18	0.15±0.09	0.25±0.22
Cu (mg/kg)	0.24±0.15	0.50±0.13	2.06±1.30
Zn (mg/kg)	0.2±0.1	0.2±0.1	0.7±1.0
B (mg/kg)	0.15±0.02	0.17±0.10	0.08±0.03
Fe (mg/kg)	86.87±165.60	41.71±59.48	21.16±16.30
C (%)	0.38±0.21	0.49±0.16	0.36±0.22
Cl (mg/kg)	25.12±9.73	19.74±17.59	34.67±8.97

4.3.3. Potassium (Bray II)

In general, soil K (Bray II) increased with a decrease in the dilution of the wastewater, *i.e.* an increase in the COD level of the irrigation water (Fig. 4.2). In 2010, soil K was the only element that increased with a decrease in the level of augmentation of the irrigation water as the actual amounts applied to the soil were relatively large compared to the other elements. In April 2011, there was a narrow correlation between the amount of K applied *via* the irrigation water and soil K levels in the 0 to 30 cm layer. However, there did not seem to be any relationship between the K in the irrigation water and K in the subsoil layers.

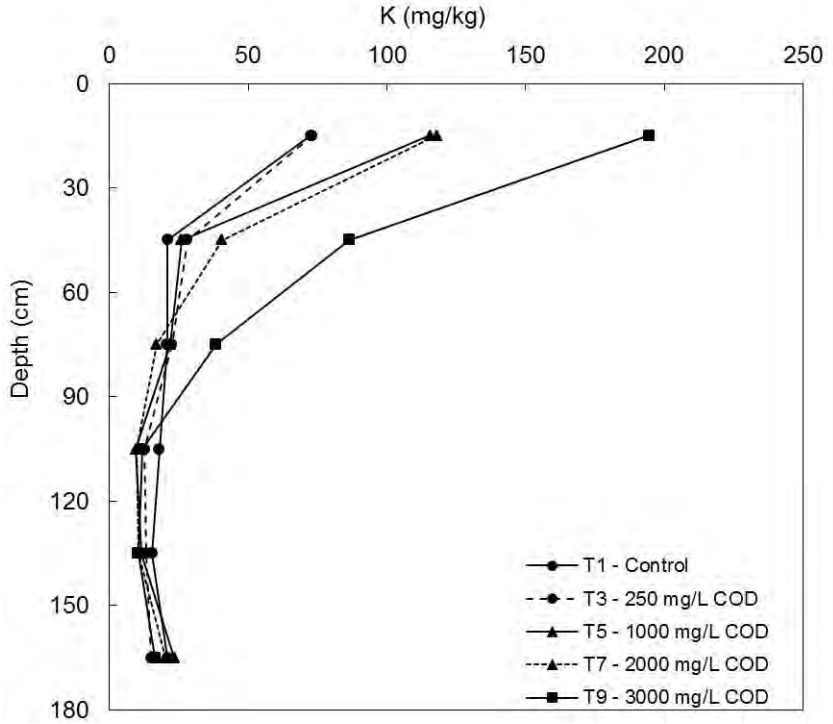

Figure 4.2. Soil potassium (K) contents in May 2013 after grapevines were irrigated with raw water and augmented winery wastewater.

Similarly, in 2012 there was a close correlation between the amount of K applied *via* the irrigation water and the soil K levels for all layers up to a depth of 90 cm. However, in the 90 cm to 120 cm layer in the work row, there was a negative relationship between the amounts of K applied *via* the water and the soil K levels, whereas the opposite trend occurred in the grapevine row. In May 2012 and 2013, there was a close correlation between the amount of K applied *via* the water and the soil K levels in the vineyard row up to a depth of 60 cm. In a study investigating the long-term use of winery wastewater over 30 years, there was also an accumulation of K (Mosse *et al*., 2012). Similar results were reported where grapevines were irrigated with simulated winery wastewater (Mosse *et al*., 2013). In their study, the addition of wine, *i.e*. organic material, to the simulated wastewater enhanced K movement to the subsoil. According to Laurenson *et al.* (2012), the fate of K in soils and on grapevines irrigated with winery wastewater has received limited attention. Land application of wastewaters can increase the levels of soluble and exchangeable forms of K more rapidly than with conventional inorganic fertilizers (Arienzo *et al.*, 2009). It is almost certain that a high availability of K in the soil could lead to an increase in K uptake by grapevines. This may have negative consequences on grapevine responses (Mpelasoka *et al.*, 2004).

In comparison to the baseline K levels in August 2009, K content in May 2010 in the top 30 cm soil of T1 decreased substantially, whereas the K levels in subsoil layers deeper than 60 cm tended to increase (Fig. 4.3). However, in the case of T9, K increased in the topsoil and in most of the subsoil layers compared to baseline levels. In general, the seasonal K loss in the

topsoil decreased as the amount of K applied *via* the irrigation water increased, and in the case of T9 there seemed to be a net gain in topsoil K. The foregoing suggested that the K from the topsoil, as well K applied *via* the irrigation water had leached into the deeper soil layers. Since there was no deep percolation when the post-harvest irrigations were applied, and no significant rainfall occurred after irrigation with the augmented waters started, the 85 mm rainfall on top of the last irrigation in May must have leached the K from the topsoil into the deeper layers.

In October 2010, K levels in the work rows tended to be higher than in August 2009 and May 2010, irrespective of the level of wastewater augmentation. These increases suggested that the winter cover crop probably did not fully absorb the fertilisers applied to enhance its growth. Since the vineyard was previously irrigated by means of drippers, fertilizers were most probably band placed on the grapevine rows. As a result, the nutrient status was initially low in the work rows compared to the grapevine rows. Furthermore, the different levels of wastewater augmentation, or element removal by the winter cover crop, had no effect on the mean soil chemical status over 1.8 m depth compared to the raw water control. Perusal of the data revealed that there were also no differences at specific 30 cm depth increments. This suggested that the differences between the element gains or losses were too small to have reflected in soil chemical status. However, it could also be that leaching of the elements, particularly the more soluble ones, by winter rainfall had nullified possible differences between treatments. In April 2011, K levels in the topsoil of the selected treatments, *i.e.* T1, T3, T5, T7 and T9, were similar to levels in May 2010. This indicated that, although there was an accumulation of K in the soil as a result of wastewater irrigation, this accumulation may not be a potential problem. In comparison to the baseline K soil profile in August 2009, K content in the top 30 cm of T1 and T3 soils decreased substantially, whereas K levels in the subsoil layers deeper than 60 cm tended to increase. The levels of K in the topsoil of T5, T7 and T9 were higher in April 2011 when compared to the baseline levels in August 2009, but levels in the deeper layers were similar to the baseline levels. In September 2011, it was evident that there had been an accumulation of K in the deeper soil layers of T7 and T9. However, the reason for this increase was unclear.

In May 2012, soil K levels of the selected treatments differed substantially. In addition, the levels of K in the topsoil of T5, T7 and T9 were higher in May 2012 when compared to the baseline levels in August 2009, but levels in the deeper layers were similar to the baseline levels. It appeared that soil K levels in T7 and T9 increased up to a depth of 90 cm, despite no application of K fertilizer during the growing season and the cultivation of an interception crop. Furthermore, soil K levels tended to be higher than for previous years.

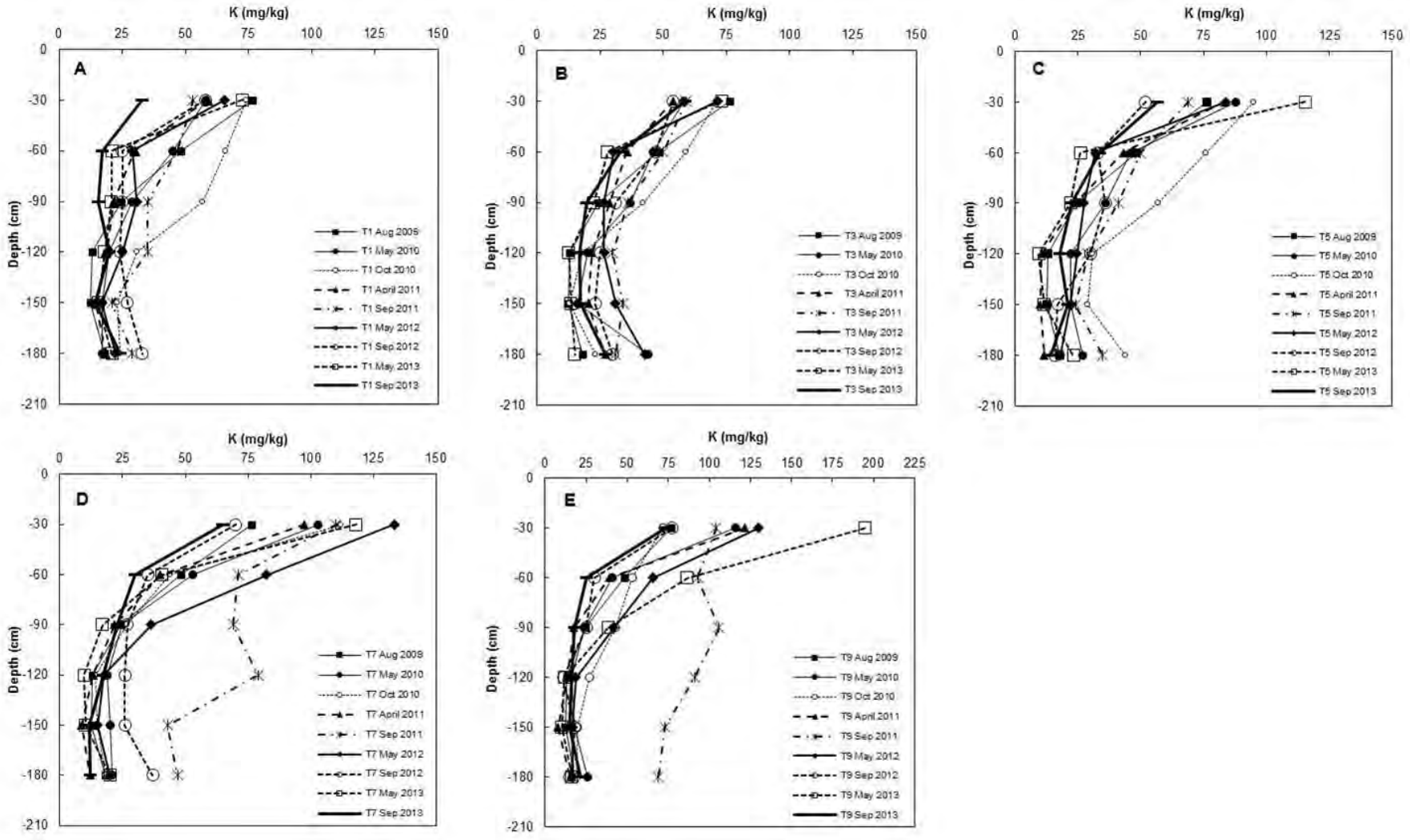

Figure 4.3. Temporal variation in work row soil potassium (K) contents where grapevines were irrigated with (A) raw river water and winery wastewater augmented to (B) 250 mg/L COD, (C)1000 mg/L COD, (D) 2000 mg/L and (E) 3000 mg/L COD respectively.

At bud break in September 2012, *i.e.* after three seasons, it was evident that the heavy winter rainfall had negated treatment differences with respect to K. Subsequently, all treatments were fertilized in December 2012 with 30 kg K per ha. In contrast to September 2011, it was evident that there was no accumulation of K in the deeper soil layers of T7 and T9. This implied that the heavy winter rainfall probably leached K from the soil profile. The K levels in the 0 to 30 cm layers of T1 and T3 were also similar to levels observed in September 2011. In contrast, lower levels of K in the 0 to 30 cm layer were observed for T5, T7 and T9 when compared to levels in September 2011. Furthermore, K levels of T1, T3 and T5 were much lower than the baseline levels quantified in August 2009. It appeared that T7 and T9 maintained their baseline K levels.

In May 2013, levels of soil K of T1, T3, T5, T7 and T9 differed substantially and it was evident that soil K levels tended to be higher than for previous years. Soil K levels in T9 increased up to a depth of 90 cm, despite only one application of K fertiliser during the growing season and the cultivation of an interception crop. Furthermore, K contents in all layers of T1 and T3 soils were similar to the baseline K soil profile with the exception of the 30 to 60 cm layer, which was lower. In the case of T5 and T7, K levels in the 0 to 30 cm layer were higher when compared to the baseline levels in August 2009, whereas levels in the layers deeper than 60 cm were similar to the baseline levels. Soil K levels in both the 0 to 30 cm and 30 to 60 cm layers of T9 were substantially higher than baseline levels, whereas the deeper layers tended to be similar.

At bud break in September 2013, it was evident that the heavy winter rainfall had negated treatment differences with respect to soil elements. This implied that the heavy winter rainfall probably leached K from the soil profile, and this was substantiated by mineral analysis of soil samples collected deeper than 180 cm with a modified soil auger (Fig. 4.4). At the end of the trial in September 2013, K levels of T1, T3, T5 and T7 were lower than the baseline levels, whereas T9 had maintained its baseline K levels.

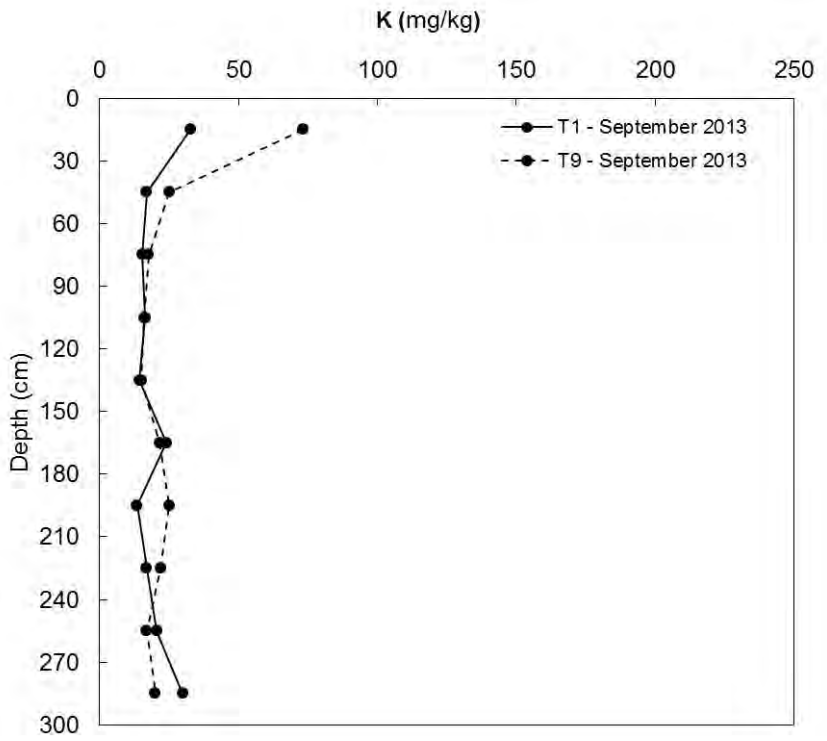

Figure 4.4. Soil potassium (K) contents over 3 m depth measured near Rawsonville in September 2013.

4.3.4. Calcium

In general, soil Ca did not show any consistent trends with respect to the different levels of wastewater augmentation. This was expected since there were no substantial differences with regards to the amounts of Ca applied *via* the irrigation water. At bud break in October 2010, the different levels of wastewater augmentation, or element removal by the winter cover crop, had no effect on the mean soil chemical status over 1.8 m depth compared to the raw water control. Perusal of the data revealed that there were also no differences at specific 30 cm depth increments. This suggested that the differences between the element gains or losses were too small to have reflected in soil chemical status. However, it could also be that leaching of the elements, particularly the more soluble ones, by winter rainfall had nullified possible differences between treatments. In May 2011, soil Ca levels were comparable to baseline levels determined in August 2009. In September 2011, it was evident that an accumulation of Ca in the 90 to 180 cm soil layer of T7 and T9 had occurred. However, this trend was not evident when soil samples were collected in September 2012. The baseline values for Ca were 1.72 and 0.30 cmol(+)/kg, for the 0 to 90 cm and 90 to 180 cm soil layer, respectively. Levels of Ca in the soil at the end of the trial in September 2013 were similar to these baseline values (Tables 4.1 & 4.2). According to Conradie (1994), in the Western Cape fruit industry, it is recommended that the ratio of

exchangeable Ca forms 70 to 80% of the cation exchange capacity. Under the prevailing conditions, the exchangeable Ca percentage fell below this norm.

4.3.5. Magnesium

In general, soil Mg did not show any consistent trends with respect to the different levels of wastewater augmentation. This was expected since there were only slight differences with regards to the amounts of Mg applied *via* the irrigation water. At bud break in October 2010, the different levels of wastewater augmentation, or element removal by the winter cover crop, had no effect on the mean soil chemical status over 1.8 m depth compared to the raw water control. In May 2011, Mg levels were comparable to baseline levels determined in August 2009. In September 2011, it was evident that an accumulation of Mg in the 90 cm to 180 cm soil layer of T7 and T9 had occurred. However, this trend was not evident when soil samples were collected in September 2012. The baseline values for Mg were 0.80 and 0.17 cmol(+)/kg, for the 0 to 90 cm and 90 to 180 cm soil layer, respectively. Levels of Mg in the soil at the end of the trial in September 2013 were similar to these baseline values. According to Conradie (1994), in the Western Cape fruit industry, it is recommended that the ratio of exchangeable Mg forms 10 to 15% of the cation exchange capacity. In general, exchangeable Mg in the 0 to 90 cm layer exceeded this norm.

4.3.6. Potassium

Similar trends to K (Bray II) were observed. In the Western Cape fruit industry, the recommended ratio of exchangeable K is 3 to 4% of the cation exchange capacity (Conradie, 1994). After wastewater irrigations were applied in 2010, soil from the 0 to 30 cm layer of T7, T8 and T9 exceeded this norm. In 2011, 2012 and 2013, this norm was exceeded consistently in soil collected from the 0 to 30 cm layers of treatments irrigated with wastewater containing 1000 mg/L COD and higher, *i.e.* T5, T9 and T9.

4.3.7. Sodium

In 2010, the different amounts of Na applied *via* the irrigation water did not reflect in the soil chemical status. However, in April 2011, soil Na increased with a decrease in the dilution of the wastewater, *i.e.* an increase in the COD level of the irrigation water (Fig. 4.5). There were substantial differences in the amount of Na applied *via* the irrigation water during this season.

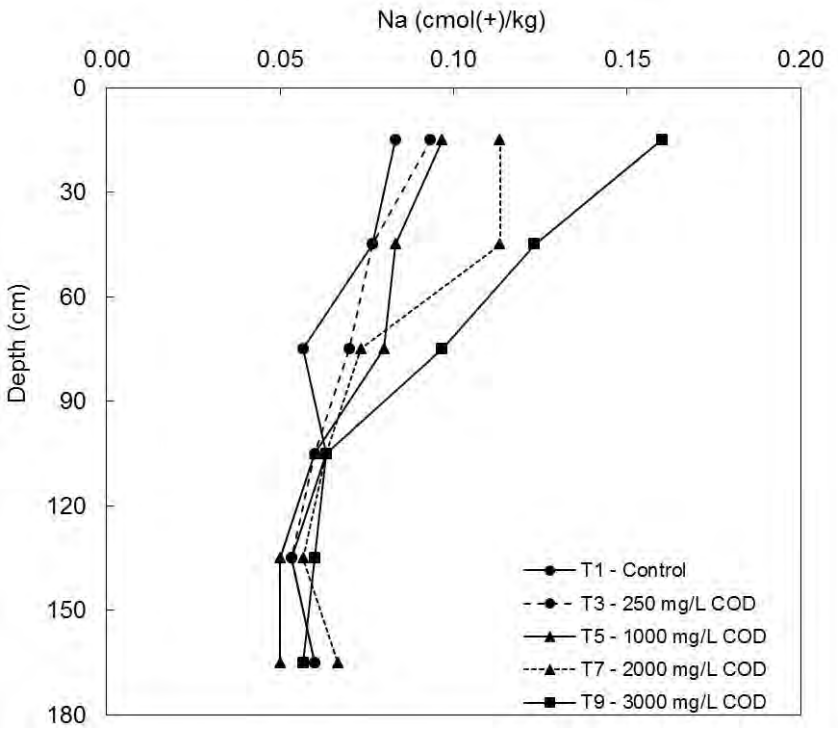

Figure 4.5. Soil sodium (Na) contents in April 2011 after grapevines were irrigated with raw water and augmented winery wastewater.

Several studies have reported increases of Na due to the use of wastewater irrigation. In a study investigating the long-term use of winery wastewater, *i.e.* 30 years, an accumulation of Na was reported (Mosse *et al*., 2012). In a field study where grapevines were irrigated with simulated winery wastewater, elevated Na levels in the 0 to 20 cm, as well as the 20 to 40 cm soil layer were reported (Mosse *et al*., 2013). In 2012, there was not such a clear response with regards to Na. This was probably due to lower Na levels in the wastewater compared to 2010/11. Furthermore, there is probably a maximum level of Na which a specific soil can absorb before leaching occurs. In the case of Na, there was no agreement between Na applied *via* the irrigation water and Na levels in both the 0 to 30 cm and 30 cm to 60 cm soil layers. Presumably, Na in the 0 to 30 cm and 60 cm to 90 cm layers had reached the maximum level that could be absorbed. However, for the 60 cm to 90 cm layer, there was a close correlation between soil Na levels and irrigation water applied. In May 2013, there was a close correlation between Na applied *via* the irrigation water and Na levels in both the 0 to 30 cm and 60 to 90 cm soil layers.

At bud break in October 2010, the different levels of wastewater augmentation, or element removal by the winter cover crop, had no effect on the mean soil chemical status over 1.8 m depth compared to the raw water control (Fig. 4.6). Perusal of the data revealed that there were also no differences at specific 30 cm depth increments. This suggested that the differences between the element gains or losses were too small to have reflected in soil chemical status. However, it could also be that leaching of the elements, particularly the

more soluble ones, by winter rainfall had nullified possible differences between treatments. In contrast to soil K where similar levels were observed for May 2010 and April 2011, it was evident that from August 2009 there was an accumulation of Na in the topsoil, particularly where more Na was applied *via* the irrigation water. However, in the layers deeper than 60 cm there was no clear trend. In September 2011, there was an accumulation and redistribution of Na from the 0 to 30 cm layer to deeper layers in the case of T7 and T9. In contrast to soil K where similar levels were observed for May 2010 and April 2011, it was evident that from August 2009 there was an accumulation of Na in the topsoil, particularly where more Na was applied *via* the irrigation water. Although levels of Na were lower in September 2012 compared to May 2012, Na levels of all treatments increased from baseline levels. The accumulation and redistribution of Na from the 0 to 30 cm layer to deeper layers of all treatments was of particular concern. In May 2013, Na levels of T1 and T3 were similar to baseline levels in August 2009 whereas for T5, T7, and T9, Na levels up to a depth of approximately 90 cm were higher than the baseline levels. After the heavy rainfall of winter 2013, Na levels of T1, T3 and T5 were lower than baseline levels. This implied that the heavy winter rainfall probably leached Na from the soil profile (Fig. 4.7).

4.3.8. ESP and SAR

In general, the ESP did not exceed the norm of 15% proposed for most soils by Seilsepour *et al. (*2009). Although soil SAR responses were generally inconsistent with regards to level of augmentation of the wastewater, it appeared that SAR was lower where the levels of augmentation was higher, and higher where the wastewater was less diluted. At the end of the trial in September 2013, SAR was generally below 3.4 in the 0 to 90 cm layer and below 8 in the 90 to 180 cm soil layer.

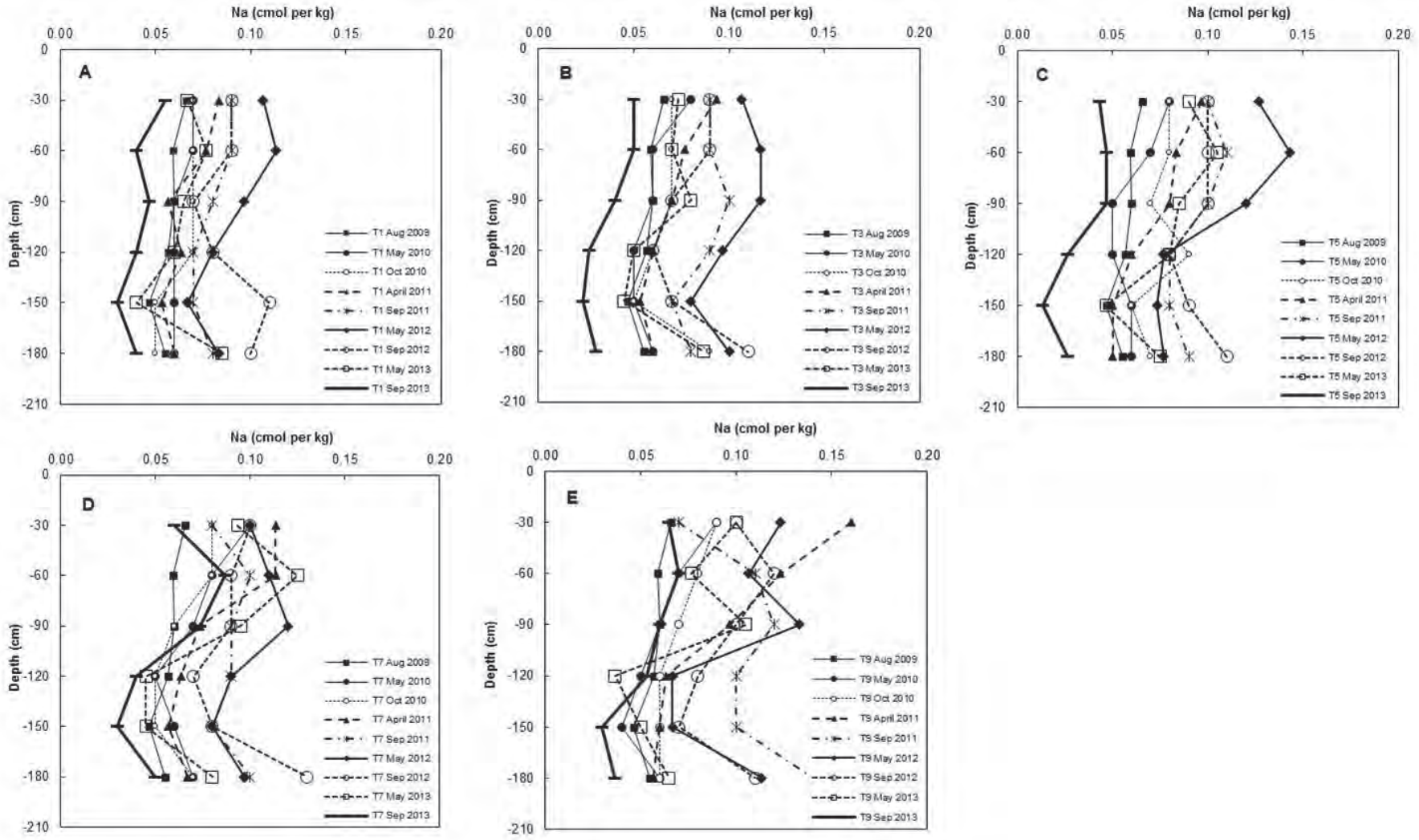

Figure 4.6. Temporal variation in work row soil sodium (Na) contents where grapevines were irrigated with (A) raw river water and winery wastewater augmented to (B) 250 mg/L COD, (C)1000 mg/L COD, (D) 2000 mg/L and (E) 3000 mg/L COD respectively.

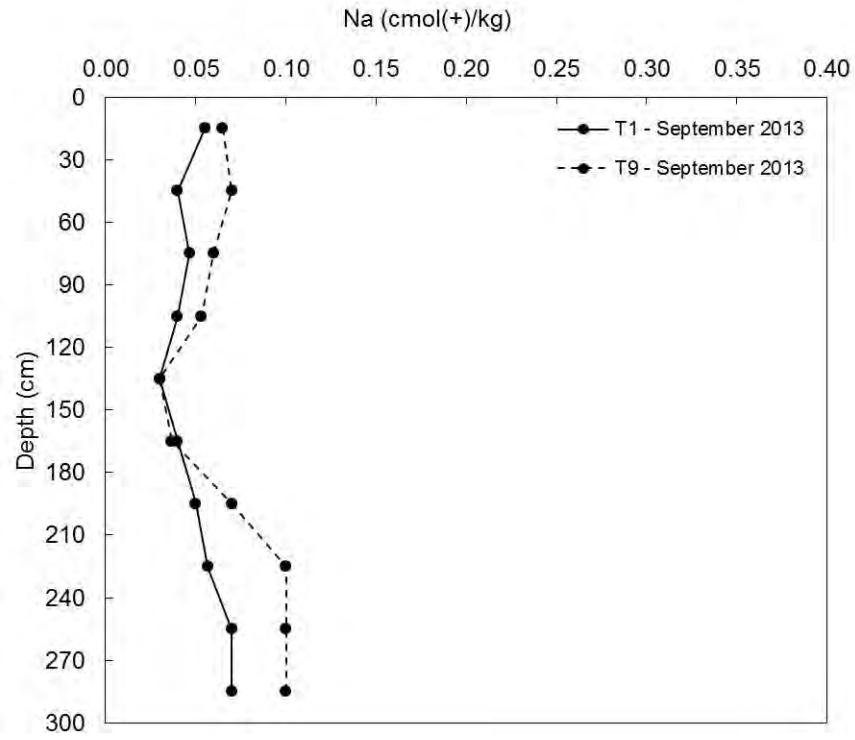

Figure 4.7. Soil sodium (Na) contents over 3 m depth measured near Rawsonville in September 2013.

4.3.9. Organic carbon and trace elements

There were no consistent trends with regards to soil organic C, which indicated that the organic C content in the wastewater was still too low to have had a positive effect on soil fertility. It is also possible that organic material in the wastewater, which could have led to an accumulation of organic soil C, decomposed when the soil was aerated between irrigations. In April 2011, levels were comparable to those in August 2009 (Tables 4.1 & 4.2), whereas levels of soil organic C contents in May 2012 were all higher. This indicated towards some organic matter accumulation over time, particularly in the 90 cm to 180 cm layer. Levels of soil organic C contents in May 2013 in the 90 to 180 cm layer were higher compared to those in August 2009.

In general, soil Fe, Zn and Cu did not show any trends with regards to the different levels of augmentation when compared to T1. Furthermore, it was evident that the soil Fe content was fairly heterogeneous.

Although soil B levels in April 2011 could not be correlated to the amounts of the element applied *via* the irrigation water, it was clear that there was an increase in the soil B in the 90 cm to 180 cm layer for all of the treatments. This suggested that B is relatively mobile within the soil profile under the prevailing conditions. In May 2012 and 2013, there was a non-linear increase in soil B levels with increasing amounts of B applied *via* the irrigation

water, and soil B levels up to a depth of 150 cm in the work rows was well correlated to the amounts of B applied *via* the water. The baseline values for B were 0.13 and 0.03 mg/kg, for the 0 to 90 cm and 90 to 180 cm soil layer, respectively. Levels of B in the soil at the end of the trial in September 2013 were similar to these baseline values. Boron levels in this sandy, alluvial soil were well below the toxicity threshold of 3.8 mg/kg, proposed by Van Schoor *et al.* (2000).

4.3.10. Chloride

In general, soil Cl did not show any trends with respect to the different wastewater treatments compared to the raw water control. Furthermore, it was evident that the soil Cl content was fairly heterogeneous.

4.4. CONCLUSIONS

Under the prevailing conditions, the element concentrations in the soil did not respond to irrigation with augmented winery wastewater, except for K and Na. This was probably due the low levels of the other elements applied *via* the irrigation in relation to K and Na. In each of the four seasons, there was an increase in soil K level after wastewater irrigations were applied. The increase in soil K could also be related to the level of augmentation of the winery wastewater. These K increases could have a negative impact on wine colour stability should it be taken up by the grapevine in sufficient quantities, particularly. The level of soil Na also tended to increase as the level of augmentation decreased, although responses were more inconsistent than K. However, in heavier soils or in regions with low winter rainfall Na could accumulate to levels where it could impact negatively on soil physical conditions or grapevine growth and yield.

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CHAPTER 5: EFFECT OF IRRIGATION WITH WINERY WASTEWATER AUGMENTED TO DIFFERENT COD LEVELS ON THE PERFORMANCE OF TWO GRASS COVER CROP SPECIES AND THEIR ABILITY TO FUNCTION AS AN INTERCEPTION CROP

J.C. Fourie[(1)] & H. Theron[(2)]

[(1)] The Fruit, Vine and Wine Institute of the Agricultural Research Council, Private Bag X5026, Stellenbosch, 7599

[(2)] Cape Peninsula University of Technology, Private Bag X8, Wellington, 7654

5.1. INTRODUCTION

Untreated winery wastewater does not qualify for disposal in natural water resources (Van Schoor, 2001). The South African wine grape industry produced approximately1 095 million litres of wine during 2012 (SAWIS, 2013). For every litre of wine produced, between two and fourteen litres of effluent is produced (Lagoudi *et al*., 2004). Therefore, the cellars in the local industry probably generate between 2 190 million and 15 330 million litres of effluent per annum. Currently wine cellars in South Africa are treating their effluent by means of aerated ponds, aerobic facultative lagoons and high rate system bioreactors (Lagoudi *et al*., 2004). More recently constructed wetlands were also considered (Mulidzi, 2005), the latter being the most biologically active natural ecosystems on earth (Shepherd & Grismer, 1997). Although the cost of constructed wetlands are low in comparison to other treatment systems, the construction of a 56.7 m^3 pore volume wetland still amounts to approximately R 51 000, excluding VAT and labour (Mulidzi, 2008).

According to Lagoudi *et al*. (2004), land surface applications such as vineyard and field irrigation are also disposal methods to be considered. Irrigation with winery wastewater is a practice increasingly applied by grape growers (Kumar & Christen, 2009). Wastewater from agricultural sources can function as a source of organic matter and nutrients (Cameron *et al*., 1996). In general, it provides a variety of nutrients that may enhance crop growth and yield (Al-Jaloud *et al*., 1995; Vasquez-Montiel *et al*., 1996). However, it may also increase soil salinity, result in an undesirable soil pH, cause excessive leaching of nutrients and heavy metals and adversely affect the physical properties of the soil (Shainberg & Oster, 1978; Cameron *et al*., 1996; Wang *et al*., 2007). When using winery wastewater for irrigation, an application strategy that will minimize leaching from the crop root zone needs to be developed (Arienzo *et al*., 2009a). This can be done by optimizing wastewater applications whilst maximizing nutrient removal. The application of wastewater should therefore be synchronized with crop uptake. However, irrigation with winery wastewater caused a decline in the nutrient status of grapevines (Neilsen *et al.*, 1989; McCarthy, 2010). A progressive increase in soil salinity resulted in nutrient deficiencies (McCarthy, 1981; Paranychianakis

et al., 2006) and may reduce shoot and root growth, result in a decline in bunch number and berry weight, as well as cause lower photosynthesis activity, poor nutrient utilisation and osmotic stress (McCarthy, 1981; Prior *et al*., 1992; Leske *et al*., 1997; Stevens & Walker, 2002; Paranychianakis *et al*., 2006).

The characteristics of winery wastewater fluctuate considerably and are determined by the size of the winery, treatment processes, as well as the pre-vintage, vintage and post-vintage activities in the cellar. The high potassium (K) content of grape juice (2000 to 3000 mg/L) and cleaning products (potassium hydroxide) used in the cellar may cause the K concentration in winery wastewater to be as high as 1000 mg/L during the vintage season (Arienzo *et al*., 2009a), which is classified by Mulidzi *et al*. (2009) as being high (above 200 mg/L). However, Arienzo *et al*. (2009b) suggested that sodium (Na)-based cleaning agents should be replaced by K-based cleaning agents, due to the greater potential for K uptake and removal by crops grown on land application sites. Most of the K in wastewater is in mineral form and therefore immediately available for plant uptake (Mengel *et al*., 2001).

Grasses have a high K removal potential (Arienzo *et al.*, 2009a). *Lolium* species (ryegrass) have the potential to remove between 484 and 708 kg/ha K from the soil (Gamroth & Moore, 1995). However the species have developed resistance to two non-selective broad-spectrum herbicides, namely paraquat and glyphosate (Busi & Powles, 2011). It should, therefore, not be considered as a suitable crop for the interception of excess nutrients applied by means of irrigation with winery wastewater. According to Pederson *et al.* (2002), *Avena sativa* L. (oats) has the potential to be used as an interception crop, with hay production and sale off-farm as a viable method for removing excess nutrients. *Pennisetum glaucum* L. (pearl millet) is also widely used as fodder in developing countries (Blümmel *et al*., 2003; Al-Suhaibani, 2011; Khan *et al*., 2012). It can also be an important summer cereal crop, which does not require a lot of irrigation due to its short growing season (Khan *et al.*, 2012). Al-Jaloud *et al*. (1995) indicated that the irrigation of sorghum plants with wastewater did not increase the mineral concentrations in the plants, while Albassam (2001) observed that the negative effect that high concentrations of Na had on the growth of pearl millet could be partially restored by the application of nitrogen (N). In general, pearl millet is considered to be fairly saline tolerant (Krishnamurthy *et al*., 2007), which can be associated with a reduced N content in the shoots and an increase in the K and Na content.

The aim of this study was (1) to determine whether irrigation with winery wastewater augmented to different COD levels affected the performance and element content of oats and pearl millet, and (2) to determine the ability of these two species to intercept sufficient amounts of Na and K on a sandy soil near Rawsonville.

5.2. MATERIALS AND METHODS

5.2.1. Seeding density, seeding date and seedbed preparation

Avena sativa L. cv. Pallinup (oats) was sown at a seeding density of 100 kg/ha (Fourie *et al.*, 2001). The species was sown on 14 April 2010 and 7 April 2011, as suggested by Fourie *et al.* (2006). Seedbed preparation was done with a disc harrow (Fig. 5.1) approximately six weeks before the seeding date to allow for the breakdown of any mulch material on the soil surface that may affect seed germination and the growth of the young seedlings negatively. During 2012 and 2013 the oats had to be sown during the last week of April, as the second (final) harvest of *Pennisetum glaucum* L. cv. Babala (pearl millet) occurred on 23 April 2012 and 22 April 2013. This did not allow for any seedbed preparation. The pearl millet residues were, however, incorporated into the soil just before the seeds were sown. After sowing by hand, the seeds were covered with soil using a disc harrow. Pearl millet was sown at a seeding density of 30 kg/ha. The species was sown on 23 November 2010 as suggested by Agricol, to ensure effective germination and sufficient growth during the 2011 harvest. However, the species grew proliferous under the conditions prevalent in the trial and completed its life cycle on 24 February when only the second irrigation with augmented cellar effluent was applied during the 2011 harvest period. Therefore, the species was sown approximately six weeks later during 2012 and 2013, namely 10 January.

Figure 5.1. Seedbed preparation with disc harrow.

5.2.2. Treatments applied

The nine treatments applied consisted of one treatment in which raw water was applied throughout the season (T1) and eight treatments (T2 to T9) that were irrigated with winery wastewater augmented to different chemical oxygen demand (COD) levels from February to May (Refer to Chapter 3). Irrigation volumes, water quality and amount of elements applied *via* the irrigation water during the 2009/10, 2010/11, 2011/12 and 2012/13 seasons are also presented in Chapter 3.

5.2.3. Fertilizer applied

The amounts of fertilizer applied to supply in the need of the two cover crop species (interception crops), are shown in Table 5.1. Phosphate (P) was applied just before seedbed preparation on two occasions, namely March 2010 and end of November 2011 for oats and pearl millet, respectively. This was done to ensure a sufficient supply of P to the cover crops in the top soil layer. Both species received N during the two to four leaf stages as proposed by Van Huyssteen and Van Zyl (1984). In contrast to the 2010 and 2011 seasons an additional 28 kg of N was applied just after the oats was sown during the fourth week of April 2012 to prevent an N-deficit from developing in the top soil due to the incorporation of the pearl millet residues just before the oats was sown, as well as to compensate for the significant amounts of N removed by the pearl millet. To avoid excessive vegetative growth of the grapevines, this was not repeated during April 2013. Both the P and N were broadcasted by hand.

Table 5.1. The amounts of fertilizer applied to supply in the need of the two cover crop species (interception crops).

Date	Fertiliser applied (kg/ha)
2009/10 harvest season:	
30 March 2010	40.5 P
17 June 2010	28.0 N
2010/11 harvest season:	
7 December 2010	28.0 N
12 May 2011	28.0 N
2011/12 harvest season:	
30 November 2011	40.5 P
10 January 2012	28.0 N
24 April 2012	28.0 N
6 June 2012	28.0 N
2012/13 harvest season:	
5 February 2013	28.0 N
31 May 2013	28.0 N

5.2.4. Weed control

Full surface post-emergence weed control was achieved end of September (after the oats has been slashed and harvested) by applying glyphosate at a rate of 1.44 kg/ha by means of a covered sprayer mounted behind a quadbike (Fig. 5.2). During the grapevine growing season, post-emergence weed control was achieved in the vine row by applying paraquat at a rate of 1.25 kg/ha with knapsack sprayers as soon as the weeds reached a height of approximately 300 mm.

Figure 5.2. Chemical control of the harvested *Avena strigosa* cv. Saia (oats) with a systemic post-emergence herbicide (glyphosate) applied with a covered sprayer drawn behind a quad bike.

5.2.5. Measurements

5.2.5.1. Cover crop growth

Dry matter production (DMP) by the cover crops was estimated by sampling the above-ground vegetative growth in a 0.5 m^2 sub-plot randomly chosen in the experimental plot (Fig. 5.3). Samples were oven-dried for 48 hours at 105ºC. During 2010 the oats was slashed and harvested two times, namely 29 July and 29 September, whereas in the following three years the species was harvested only once, *i.e*. during September. As far as the pearl millet is concerned, the species was harvested twice in a season throughout the duration of the study. The DMP per hectare was calculated as follows:

$$DMP = ODM \times 2 \div 100 \qquad \text{(Eq. 5.1)}$$

where DMP is in t/ha, ODM is oven dry mass sampled per plot (g/0.5 m^2) and 100 is the conversion factor from g/m^2 to t/ha. As no harvester suitable to be utilized in a vineyard was available, the cover crops were slashed full surface directly after sampling (Fig. 5.4).

Thereafter the whole surface was raked (Fig. 5.5) and the residues removed to prevent the elements absorbed by the above-ground growth from being returned to the soil through decomposition.

Figure 5.3. The above-ground vegetative growth of the cover crops was estimated from a sample harvested in a 0.5 m^2 sub-plot randomly chosen in the experimental plot.

5.2.5.2. Element content of the cover crops

A sample was collected by harvesting the above-ground growth in a 0.5 m^2 sub-plot randomly chosen in the experimental plot. The samples were analysed for macro- and micro-elements throughout the study and for heavy metals during the 2010/11 season. After sampling, the leaf blades were washed with a Teepol® solution, rinsed with de-ionised water and dried over night at 70^{o}C in an oven. The dried leaves were then milled and ashed at 480^{o}C, shaken up in a 50:50 hydrochloric acid (HCl) (32%) solution for extraction through filter paper (Campbell & Plank, 1998; Miller, 1998). The cation (K, magnesium (Mg), calcium (Ca) and Na) and micro-nutrient (boron (B), iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn)) content of the extract was measured with a Varian ICP-OES optical emission spectrometer.

Figure 5.4. The cover crops being slashed directly after sampling.

Figure 5.5. The cover crop residues being removed from the vineyards to prevent the elements absorbed by the above-ground growth from being returned to the soil through decomposition.

Total N content of the ground leaves was determined through total combustion in a Leco N-analyser. For heavy metal (cadmium (Cd), chrome (Cr), lead (Pb), arsenic (As) and mercury (Hg) analyses, 5 g of the dried and milled leaves were digested with 20 ml HNO_3 (55%) and

5 ml hydrogen peroxide (30%) on a heated sandbed (180^{o}C) until the solution turned clear, where after it was filtered using Whatman filter paper. The heavy metal content of the extract was measured with a Varian ICP-OES optical emission spectrometer at different wavelengths for the different metals, *i.e.* Cd: 214.4 nm; Cr: 267.7 nm; Pb: 220 4 nm; As: 193.7 nm; and 184.9 nm for Hg, as described in Chapman and Pratt (1961).

5.2.5.3. Amounts of the macro-elements intercepted by pearl millet and oats

The amounts of the different macro- and micro-elements intercepted by the cover crops were estimated by multiplying the DMP of the species with the concentration of the different elements (B) in the samples harvested for analyses and the percentage surface area covered by the interception crops (estimated to be 0.8). The amount of N, K, Mg, and Ca intercepted were calculated as follows:

$$A = DMP \times B \times 0.8 \times 10 \quad \text{(Eq. 5.2)}$$

where A is the amount 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:

$$A = DMP \times B \times 0.8 \div 1\,000 \quad \text{(Eq. 5.3)}$$

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.

5.3. RESULTS AND DISCUSSION

5.3.1. Oats

5.3.1.1. Yield

The irrigations applied from March to May 2010 with winery wastewater augmented to different COD levels, had no effect on the vegetative growth of the oats from 14 April to 29 September 2010 (Table 5.2, Fig. 5.6). However the winery enriched water applied from 9 February 2011 (one month before seedbed preparation) to 14 April 2011 (one week after the oats was sown) improved the performance of the oats in T4, T6 and T7 compared to that of T1. This supports the observations of Rusan *et al.* (2007) in a trial where barley was irrigated with municipal wastewater. The DMP of the oats in the other treatments irrigated with winery enriched water tended to be higher than that of T1 as well. This could be attributed to the winery wastewater supplying additional K and N to the oats during the first six weeks of growth. During both seasons, the DMP of oats was similar to that reported for a sandy soil near Lutzville (31º35ˈS, 18º52ˈE) (Fourie *et al*., 2005), a sandy loam soil near Stellenbosch

(33º55ˈS, 18º52ˈE) (Fourie *et al*., 2006a) and a sandy clay loam near Robertson (33º50ˈS, 19º54ˈE) (Fourie *et al*., 2006b).

Despite the fact that the oats was established approximately three weeks later during 2012 (26 April 2012 vs. 7 April 2011) and controlled approximately three weeks earlier (4 September 2012 vs. 26 September 2011), the oats produced on average 42% more in 2012 than in 2011 (Table 5.2). This was attributed to the additional 28 kg/ha N applied on 24 April 2012 just after the oats was sown. In contrast to 2011, the winery wastewater applied from March to May 2012 did not improve the performance of the oats in the treatments irrigated with augmented winery wastewater (T2-T9) in comparison with the treatment irrigated with raw water (T1). Although the DMP did not differ significantly between treatments, the DMP in T2 and T9 was approximately 41% less than that measured in T1. The reason for this is not clear. Similar to 2012, the DMP of oats measured 11 September 2013 did not differ between treatments, although no additional N was applied when the cover crop was sown.

Table 5.2. Dry matter production (DMP) of the winter growing cover crop, *Avena sativa* L. cv. Pallinup (oats), established on a sandy soil near Rawsonville and irrigated with cellar effluent augmented to different COD levels, as measured during 2010, 2011, 2012 and 2013.

Treatment no & target COD (mg/L)	DMP (t/ha)					
	2010			2011	2012	2013
	29 July	29 Sept	Total	22 Sept	4 Sept	11 Sept
T1 - Control[1]	4.08 a[2]	1.35 a	5.43 a	2.75 b	8.27 a	8.59 a
T2 - 100	3.30 a	2.45 a	5.75 a	4.01 ab	4.76 a	7.27 a
T3 - 250	3.24 a	1.98 a	5.22 a	4.68 ab	7.08 a	7.36 a
T4 - 500	4.04 a	1.71 a	5.75 a	6.26 a	7.72 a	7.29 a
T5 - 1000	3.44 a	1.97 a	5.41 a	4.42 ab	6.78 a	7.58 a
T6 - 1500	3.62 a	1.92 a	5.54 a	5.64 a	7.21 a	8.30 a
T7 - 2000	2.56 a	2.62 a	5.18 a	5.36 a	7.26 a	9.12 a
T8 - 2500	2.70 a	2.03 a	4.73 a	4.46 ab	6.05 a	6.02 a
T9 - 3000	3.35 a	2.02 a	5.37 a	4.45 ab	4.84 a	5.99 a

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Figure 5.6. The vegetative growth of *Avena strigosa* cv. Saia (oats) was not affected by the winery wastewater applied from March to May 2010.

5.3.1.2. Chemical composition

Throughout the study, the levels of K in the above-ground growth did not differ between treatments (Table 5.3) and was similar to the level reported by Whitehead (2000) to be typical of grasses. During the 2010/11 season, the levels of K measured in all the treatments after 106 days of growth (29 July 2010) was higher than that observed for the 29 September harvest. This is an indication that oats take up more K during the early growing stages, which supports the results of Palazzo (1981). The percentage K present in the leaves 106 days after being sown was similar to that reported by Bezuidenhout (2012).

The Na levels in the above-ground growth did not differ between treatments during the 2010/11 and 2011/12 seasons (Table 5.3). Despite the significant differences observed between treatments during the 2012/13 and 2013/14 seasons, no definite trends were detected. However, the level of Na in the leaves of all the treatments during September increased over time, especially from 2011 to 2012. The Na content of the oats in September 2012 was on average 4.5 times higher than that of the previous season. This trend was attributed to the increasing levels of Na in the soil (Figure 4.6). Although the level of Na declined from 2012 to 2013, it remained higher than the values reported for 2011. In this study, the levels of Na observed 106 days after emergence (29 July 2010) were similar to

that reported by Bezuidenhout (2012), but the levels measured during September each year were higher. This is an indication that oats accumulate more Na during the later growing stages. Bezuidenhout (2012) reported a higher uptake during the first seven weeks after emergence. These trends indicate that the uptake of Na by oats during the different growing stages needs clarification.

The N content of the oats did differ between treatments during the 2010 (29 July), 2011 and 2013 seasons (Table 5.4). However, no definite trends were observed. The percentage N measured in the leaves of oats throughout this study (Table 5.4) was between 16% and 70% lower than that reported by Bezuidenhout (2012). The N applied just after sowing during 2012 did not affect the N levels in the leaves compared to the other seasons (Table 5.4).The levels of P did not differ between treatments or seasons throughout the study, despite the fact that additional P was applied during March 2010 and November 2011. The results were similar to the levels reported by Bezuidenhout (2012). Both the Ca and Mg content of the oats did not differ between treatments throughout the study (Table 5.5) and were less than 50% and 40% of that reported by Bezuidenhout (2012), respectively. The levels of Ca and Mg in crops growing in the Western Cape tend to be lower than that reported for other regions, due to the fact that the Ca and Mg content of soils in the Western Cape tend to be lower (W.J. Conradie, personal communication, 2014). No specific trends were observed in the present study, as far as the micro-elements were concerned (data not shown).

Table 5.3. Effect of irrigation using augmented winery wastewater on the potassium (K) and sodium (Na) content of *Avena sativa* L. cv. Pallinup (oats), established on a sandy soil near Rawsonville, as measured during 2010, 2011, 2012 and 2013.

Treatment no & target COD (mg/L)	K (%)					Na (mg/kg)				
	2010		2011	2012	2013	2010		2011	2012	2013
	29 July	29 Sept	22 Sept	4 Sept	11 Sept	29 July	29 Sept	22 Sept	4 Sept	11 Sept
T1 - Control[1]	2.65 a[2]	1.06 a	1.25 a	1.64 a	1.30 a	172 a	321 a	373 a	2286 abc	1567 a
T2 - 100	2.64 a	1.59 a	1.56 a	1.73 a	1.45 a	213 a	332 a	461 a	2622 ab	861 b
T3 - 250	2.91 a	1.71 a	1.14 a	1.91 a	1.21 a	247 a	549 a	467 a	982 e	2211 a
T4 - 500	2.83 a	1.59 a	1.34 a	1.91 a	1.46 a	256 a	239 a	438 a	1582 cde	1711 a
T5 - 1000	2.31 a	1.55 a	1.38 a	1.64 a	1.44 a	118 a	296 a	492 a	2080 bcd	523 b
T6 - 1500	2.64 a	1.73 a	1.27 a	2.13 a	1.45 a	200 a	334 a	404 a	1386 cde	762 b
T7 - 2000	3.11 a	1.58 a	1.73 a	2.51 a	1.69 a	158 a	203 a	366 a	2058 bcd	798 b
T8 - 2500	2.71 a	1.78 a	1.80 a	2.00 a	1.54 a	174 a	168 a	375 a	1270 de	689 b
T9 - 3000	3.08 a	1.68 a	1.73 a	2.54 a	1.65 a	145 a	308 a	358 a	3199 a	580 b

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Table 5.4. Effect of irrigation using augmented winery wastewater on the nitrogen (N) and phosphate (P) content of *Avena sativa* L. cv. Pallinup (oats), established on a sandy soil near Rawsonville, as measured during 2010, 2011, 2012 and 2013.

Treatment no & target COD (mg/L)	N (%)					P (%)				
	2010		2011	2012	2013	2010		2011	2012	2013
	29 July	29 Sept	22 Sept	4 Sept	11 Sept	29 July	29 Sept	22 Sept	4 Sept	11 Sept
T1 - Control[1]	1.83 bc[2]	1.21 a	1.34 bc	1.57 a	1.12 bcd	0.40 a	0.31 a	0.33 a	0.29 a	0.23 a
T2 - 100	2.16 bc	1.26 a	1.26 bc	1.65 a	1.01 cd	0.40 a	0.34 a	0.28 a	0.29 a	0.24 a
T3 - 250	2.65 a	1.21 a	1.33 bc	1.19 a	0.92 d	0.43 a	0.35 a	0.33 a	0.25 a	0.23 a
T4 - 500	1.77 c	1.28 a	1.41 bc	1.66 a	1.14 abcd	0.42 a	0.31 a	0.34 a	0.29 a	0.27 a
T5 - 1000	1.74 c	1.14 a	1.27 bc	1.30 a	1.27 ab	0.37 a	0.30 a	0.32 a	0.29 a	0.24 a
T6 - 1500	2.15 bc	1.73 a	1.23 c	1.36 a	1.18 abc	0.37 a	0.32 a	0.31 a	0.29 a	0.23 a
T7 - 2000	2.04 bc	1.30 a	1.61 ab	1.84 a	1.03 bcd	0.43 a	0.34 a	0.32 a	0.32 a	0.25 a
T8 - 2500	1.84 bc	1.41 a	1.91 a	1.53 a	1.16 abcd	0.42 a	0.47 a	0.39 a	0.28 a	0.26 a
T9 - 3000	2.25 ab	1.38 a	1.61 ab	1.87 a	1.38 a	0.44 a	0.40 a	0.43 a	0.36 a	0.25 a

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Table 5.5. Effect of irrigation using augmented winery wastewater on the calcium (Ca) and magnesium (Mg) content of *Avena sativa* L. cv. Pallinup (oats), established on a sandy soil near Rawsonville, as measured during 2010, 2011, 2012 and 2013.

Treatment no & target COD (mg/L)	Ca (%)					Mg (%)				
	2010		2011	2012	2013	2010		2011	2012	2013
	29 July	29 Sept	22 Sept	4 Sept	11 Sept	29 July	29 Sept	22 Sept	4 Sept	11 Sept
T1 - Control[1]	0.32 a [2]	0.14 a	0.18 a	0.17 a	0.19 a	0.18 a	0.15 a	0.22 a	0.18 a	0.15 a
T2 - 100	0.34 a	0.16 a	0.17 a	0.24 a	0.16 a	0.19 a	0.15 a	0.21 a	0.20 a	0.13 a
T3 - 250	0.37 a	0.21 a	0.16 a	0.15 a	0.16 a	0.20 a	0.19 a	0.22 a	0.13 a	0.12 a
T4 - 500	0.32 a	0.16 a	0.17 a	0.14 a	0.18 a	0.20 a	0.13 a	0.22 a	0.14 a	0.13 a
T5 - 1000	0.30 a	0.18 a	0.15 a	0.15 a	0.18 a	0.16 a	0.14 a	0.22 a	0.13 a	0.13 a
T6 - 1500	0.29 a	0.20 a	0.14 a	0.14 a	0.18 a	0.16 a	0.18 a	0.21 a	0.13 a	0.13 a
T7 - 2000	0.31 a	0.15 a	0.16 a	0.17 a	0.17 a	0.18 a	0.14 a	0.22 a	0.17 a	0.13 a
T8 - 2500	0.32 a	0.16 a	0.15 a	0.15 a	0.18 a	0.16 a	0.14 a	0.23 a	0.14 a	0.14 a
T9 - 3000	0.29 a	0.17 a	0.14 a	0.16 a	0.15 a	0.17 a	0.15 a	0.22 a	0.17 a	0.12 a

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

5.3.2. Pearl millet

5.3.2.1. Yield

The winery wastewater applied in February 2011 did not affect the growth of pearl millet negatively (Table 5.6). The species grew proliferous and, despite being slashed and harvested on 12 January 2011, went into the reproductive phase earlier than anticipated, namely mid-February 2011. During the first 50 days of growth, between 1.47 t/ha and 2.76 t/ha fibre was produced, whilst more than 7 t/ha was produced during the following 43 days. Although the DMP achieved in this study was much lower than that reported by Ayoub *et al*. (2009), it was still sufficient to allow pearl millet to function as an interception crop. To prevent the species from completing its life cycle too early, as well as minimise competition with the grapevines for nutrients, it was sown on 10 January (six weeks later) during the following two seasons (2011/12 & 2012/13). Although pearl millet was established later, the first harvest (1 March 2012) still occurred one week before the first irrigation with winery wastewater could be applied. This harvest was executed to ensure that pearl millet remained in the vegetative phase for a longer period of time, as well as to prevent the upright growing interception crop from hampering the picking of the grapes. Another reason was that the grapes in the region ripened later compared to the previous season, resulting in sufficient amounts of winery wastewater only becoming available one month later.

Similar to 2011, the DMP did not differ between treatments (Table 5.6), indicating that the winery wastewater had no effect on the performance of pearl millet (Fig. 5.7). The DMP of the second harvest (after a re-growth period of 54 days) was higher than that of the first harvest (after a growing period of 49 days), irrespective of the treatments applied. This supports the trend observed in the previous season. During 2012, the average growth tempo of pearl millet up to the first harvest was 31.7 kg dry matter/ha/day, compared to that between the first and second harvest being 81.8 kg dry matter/ha/day. Despite the fact that seasonal variation makes it extremely difficult to determine the optimal seeding date for the summer growing interception crop, the pearl millet remained in the vegetative phase, with the peak growth period (Table 5.6) occurring while 91% of the winery wastewater was applied, namely 8 March to 18 April 2012. Although the trends were similar during both seasons, the average DMP of pearl millet declined from 10.42 t/ha in the 2010/11 season to 5.97 t/ha in the 2011/12 season (Table 5.6). This decline occurred despite the fact that the growth period was 10 days longer in 2011/12 than that of 2010/11. The species being sown 48 days later than the generally accepted seeding date could have caused this decline.

Table 5.6. Dry matter production (DMP) of the summer growing crop, *Pennisetum glaucum* L. hybrid Babala (pearl millet), on a sandy soil near Rawsonville and irrigated with cellar effluent augmented to different COD levels, as measured during 2011, 2012 and 2013.

Treatment no & target COD (mg/L)	DMP (t/ha)								
	2011			2012			2013		
	12 January	24 February	Total	1 March	23 April	Total	5 March	22 April	Total
T1 - Control[1]	2.76 a[2]	7.40 a[2]	10.16 a[2]	1.07 a[2]	3.81 a[2]	4.88 a[2]	2.22 ab[2]	2.88 c[2]	5.10 e[3]
T2 - 100	1.76 a	7.63 a	9.39 a	2.01 a	3.70 a	5.71 a	2.74 ab	3.20 bc	5.94 bcde
T3 - 250	1.69 a	7.92 a	9.61 a	2.04 a	6.25 a	8.29 a	1.74 b	3.78 bc	5.52 cde
T4 - 500	2.63 a	8.06 a	10.69 a	1.13 a	5.14 a	6.27 a	3.27 a	4.61 a	7.88 a
T5 - 1000	1.47 a	8.61 a	10.08 a	1.20 a	3.55 a	4.75 a	2.38 ab	4.47 b	6.85 abc
T6 - 1500	2.53 a	7.27 a	9.80 a	2.03 a	4.07 a	6.10 a	3.27 a	3.85 bc	7.12 ab
T7 - 2000	1.70 a	10.17 a	11.88 a	1.53 a	4.96 a	6.49 a	2.48 ab	4.15 bc	6.63 abcd
T8 - 2500	1.85 a	8.98 a	10.84 a	1.94 a	3.62 a	5.56 a	2.46 ab	4.46 b	6.92 abc
T9 - 3000	2.50 a	8.90 a	11.29 a	1.03 a	4.64 a	5.67 a	2.01 b	3.27 bc	5.28 de

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).
[3] Values followed by the same letter within a column do not differ significantly ($p \leq 0.10$).

Figure 5.7. The vegetative growth of *Pennisetum glaucum* L. hybrid Babala (pearl millet) was not affected by the winery wastewater.

The dry matter produced by pearl millet during 2013 was, with the exception of T3 and T9 more than that of the previous season (Table 5.6). The augmented winery wastewater applied on 15 and 27 February in treatments T2 to T9 did not affect the performance of the pearl millet negatively or positively, compared to that of T1 (first harvest 5 March 2013). The fertiliser N applied on 5 February helped to ensure acceptable cover crop growth in all the treatments. The cover crop dry matter produced in T2, T3, T6, T7 and T9 from 5 March to 23 April 2013 exceeded that of T1, whilst in the case of T4, T5 and T8 it was significantly higher. The total seasonal growth of pearl millet showed a similar trend, with the dry matter produced in T4, T5, T6, T7 and T8 being significantly higher than that of T1. This is an indication that the pearl millet did benefit from the amounts of macro- and micro-elements applied by means of the augmented winery wastewater used for irrigations from February to April 2013 on this sandy soil. This supports the observations of Rusan *et al.* (2007) in a trial where barley was irrigated with municipal wastewater.

5.3.2.2. Chemical composition

With the exception of the harvest that occurred on 24 February 2011, the percentage K in the above-ground growth of pearl millet did not differ between treatments (Table 5.7). Despite the differences observed for the 24 February 2011 harvest, no definite trends could be detected. Throughout the study, the concentration of K in all treatments tended to be

higher during the first harvest than during the second harvest (Table 5.7), which supports the results of Palazzo (1981). The levels of K measured during the second harvest (Table 5.7) corresponded with that reported by Whitehead (2000), while that measured during the first harvest (Table 5.7) was higher than the range reported for species in general (Whitehead, 2000). This is an indication that pearl millet is a heavy K-feeder during its early growing stages.

The Na levels differed between treatments during the harvests of 24 February 2011 and 5 March 2013 (Table 5.7). During both seasons the level of Na in T9 was higher than that of T1. The percentage Na in all the treatments irrigated with augmented winery wastewater tended to be higher than that of T1 during the 24 February 2011 and 22 April 2013 harvests as well. With the exception of T1 during 2011 and 2012, as well as T2, T7 and T9 during 2012, the Na levels tended to increase from the first to the second harvest. These results indicate that the augmented winery wastewater did affect Na uptake by pearl millet, albeit very slightly.

Throughout the study, irrigation with augmented winery wastewater did not affect the N status of pearl millet (Table 5.8). With the exception of 1 March 2012, this was true for P as well. The percentage P in T5, T6, T7 and T9 treatments was lower than that of T1, as measured for the pearl millet harvested 1 March 2012. This occurred despite the fact that 40.5 kg of fertiliser P was broadcasted in all the treatments on 30 November 2011, which ensured a sufficient supply of P to the interception crop (Al-Suhaibani, 2011). The reason for the observed differences is not clear.

The levels of Ca measured on 12 January 2011 and 1 March 2012 differed between treatments (Table 5.9). However, in both cases the seasonal application of augmented winery wastewater has not occurred yet. Therefore, the differences were not attributed to irrigation with winery wastewater. This was also true for the Mg levels present in the pearl millet harvested on 12 January 2011. The levels of Mg detected in the interception crop sampled in T8 on 22 April 2013, was lower than that of T1. Despite the discussed differences, no trends were observed between treatments. This supports the results of Al-Jaloud *et al*. (1995) which indicated that wastewater irrigation did not increase mineral concentrations of macro- and micro-elements in sorghum plants.

Table 5.7. Effect of irrigation using augmented winery wastewater on the potassium (K) and sodium (Na) content of *Pennisetum glaucum* L. hybrid Babala (pearl millet), established on a sandy soil near Rawsonville, as measured during 2011, 2012 and 2013.

Treatment no & target COD (mg/L)	K (%)						Na (mg/kg)					
	2011		2012		2013		2011		2012		2013	
	12 Jan	24 Feb	1 March	23 April	5 March	22 April	12 Jan	24 Feb	1 March	23 April	5 March	22 April
T1 - Control[1]	5.41 a[2]	1.80 b	3.50 a	1.64 a	2.47 a	2.06 a	218 a	212 b	211 a	211 a	178 b	193 a
T2 - 100	5.66 a	2.53 ab	4.26 a	0.90 a	2.51 a	2.37 a	229 a	305 ab	242 a	189 a	166 b	211 a
T3 - 250	5.02 a	2.65 ab	3.40 a	2.28 a	2.49 a	1.94 a	221 a	267 ab	167 a	202 a	147 b	226 a
T4 - 500	5.29 a	2.17 ab	3.29 a	2.08 a	2.45 a	2.27 a	213 a	262 ab	174 a	220 a	177 b	265 a
T5 - 1000	5.25 a	3.12 ab	3.56 a	1.67 a	2.64 a	2.11 a	215 a	290 ab	184 a	220 a	183 b	273 a
T6 - 1500	5.15 a	2.72 ab	2.78 a	2.03 a	2.57 a	2.49 a	215 a	258 ab	177 a	212 a	149 b	328 a
T7 - 2000	5.31 a	1.62 b	3.75 a	2.00 a	2.62 a	1.86 a	224 a	217 b	205 a	191 a	161 b	248 a
T8 - 2500	5.60 a	3.66 a	3.68 a	2.16 a	2.91 a	2.00 a	216 a	328 ab	212 a	242 a	171 b	268 a
T9 - 3000	5.25 a	2.92 ab	3.49 a	1.72 a	2.86 a	2.06 a	221 a	385 a	201 a	200 a	225 a	311 a

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Table 5.8. Effect of irrigation using augmented winery wastewater on the nitrogen (N) and phosphate (P) content of *Pennisetum glaucum* L. hybrid Babala (pearl millet), established on a sandy soil near Rawsonville, as measured during 2011, 2012 and 2013.

Treatment no & target COD (mg/L)	N (%)						P (%)					
	2011		2012		2013		2011		2012		2013	
	12 Jan	24 Feb	1 Mar	23 Apr	5 Mar	22 Apr	12 Jan	24 Feb	1 Mar	23 Apr	5 Mar	22 Apr
T1 - Control[1]	2.94 a[2]	1.36 a	2.63 a	1.27 a	3.73 a	2.67 a	0.70 bcd	0.36 a	0.44 a	0.38 a	0.29 a	0.27 a
T2 - 100	2.98 ab	0.76 a	2.63 a	1.63 a	3.13 a	2.29 a	0.60 d	0.36 a	0.39 abc	0.32 a	0.25 a	0.27 a
T3 - 250	3.16 a	0.86 a	2.27 a	1.84 a	3.19 a	1.78 a	0.74 bc	0.49 a	0.33 abcd	0.48 a	0.26 a	0.26 a
T4 - 500	3.16 a	0.99 a	3.31 a	1.44 a	3.73 a	2.83 a	0.65 cd	0.32 a	0.32 abcde	0.29 a	0.25 a	0.25 a
T5 - 1000	3.46 a	1.16 a	3.40 a	1.26 a	3.45 a	2.55 a	0.61 d	0.44 a	0.19 e	0.29 a	0.28 a	0.24 a
T6 - 1500	3.16 a	1.00 a	2.66 a	1.54 a	3.42 a	2.19 a	0.95 a	0.42 a	0.25 de	0.28 a	0.27 a	0.32 a
T7 - 2000	3.11 a	1.16 a	2.82 a	1.68 a	3.66 a	2.01 a	0.61 d	0.37 a	0.27 cde	0.38 a	0.23 a	0.18 a
T8 - 2500	2.43 b	1.01 a	2.62 a	1.63 a	3.06 a	1.72 a	0.76 b	0.50 a	0.41 ab	0.38 a	0.29 a	0.20 a
T9 - 3000	2.93 ab	0.85 a	3.49 a	1.77 a	3.21 a	2.15 a	0.70 bcd	0.41 a	0.28 bcde	0.31 a	0.28 a	0.23 a

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Table 5.9. Effect of irrigation using augmented winery wastewater on the calcium (Ca) and magnesium (Mg) content of *Pennisetum glaucum* L. hybrid Babala (pearl millet), on a sandy soil near Rawsonville, as measured during 2011, 2012 and 2013.

Treatment no & target COD (mg/L)	Ca (%)						Mg (%)					
	2011		2012		2013		2011		2012		2013	
	12 Jan	24 Feb	1 Mar	23 Apr	5 Mar	22 Apr	12 Jan	24 Feb	1 Mar	23 Apr	5 Mar	22 Apr
T1 - Control[1]	0.63 bc[2]	0.38 a	0.74 a	0.40 a	0.83 a	0.61 a	0.44 ab	0.34 a	0.49 a	0.40 a	0.61 a	0.62 ab
T2 - 100	0.63 bc	0.38 a	0.57 bc	0.39 a	0.66 a	0.62 a	0.39 ab	0.37 a	0.43 a	0.42 a	0.58 a	0.59 bc
T3 - 250	0.74 b	0.41 a	0.72 a	0.36 a	0.77 a	0.48 a	0.41 ab	0.38 a	0.39 a	0.37 a	0.57 a	0.47 bc
T4 - 500	0.70 bc	0.34 a	0.69 ab	0.32 a	0.82 a	0.52 a	0.47 a	0.32 a	0.43 a	0.38 a	0.62 a	0.78 a
T5 - 1000	0.72 bc	0.50 a	0.82 a	0.41 a	0.79 a	0.50 a	0.44 ab	0.42 a	0.50 a	0.41 a	0.49 a	0.63 ab
T6 - 1500	0.93 a	0.43 a	0.54 cd	0.38 a	0.69 a	0.66 a	0.36 b	0.40 a	0.42 a	0.45 a	0.52 a	0.63 ab
T7 - 2000	0.68 bc	0.35 a	0.35 e	0.34 a	0.65 a	0.49 a	0.40 ab	0.31 a	0.32 a	0.40 a	0.46 a	0.55 bc
T8 - 2500	0.62 c	0.51 a	0.41 de	0.44 a	0.64 a	0.45 a	0.36 ab	0.46 a	0.40 a	0.52 a	0.60 a	0.42 c
T9 - 3000	0.65 bc	0.35 a	0.53 cd	0.44 a	0.66 a	0.47 a	0.35 b	0.35 a	0.40 a	0.44 a	0.51 a	0.50 bc

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

5.3.3. Calculated amounts of elements removed by interception crops

5.3.3.1. April to September 2010

The amounts of N removed by oats up to 29 July (106 days after being sown) did not differ between treatments (Table 5.10). From 29 July to 29 September the oats removed more N in T6 and T7 than in T1. This was attributed to a tendency to produce more dry matter (Table 5.2) and the fact that the concentration of N in the above-ground growth of the first-mentioned two treatments tended to be higher than that of T1 as well (Table 5.6). The amounts of K, P, Ca, Mg intercepted by oats did not differ between treatments (Table 5.10). A similar trend was observed for Na up to the first harvest. From the first to the second harvest, however, more Na was intercepted in T3 than in T1. Although not significant, this trend was also observed for T2 and T5 to T9.

The average rate at which N was intercepted by oats from the seeding date to the first harvest amounted to 526 g/ha/day compared to 348 g/ha/day (34% less) from the first harvest to the second harvest (Table 5.10). This was attributed to the 28 kg/ha N applied 42 days after the oats was sown, making more N available to the cover crop. Although not as pronounced, a similar downward trend was observed in the average rate at which P was intercepted. In contrast to N and P, the average rate at which Na was intercepted increased from 4.72 g/ha/day (from the seeding date to first harvest) to 20.81 g/ha/day (from the first to second harvest). Despite this 440% increase in the rate at which Na was intercepted, the amounts of Na removed by oats were still insignificant.

5.3.3.2. October 2010 to September 2011

The application of winery wastewater augmented to different COD levels did not have an effect on the amounts of N, K, Na, P, Mg, and Ca that were removed by pearl millet and oats (Tables 5.11 & 5.12). However, the amounts removed by oats in T2 to T9 tended to be higher than that of T1. With the exception of K in T7 (Table 5.13) and Ca in T2 (Table 5.14), a similar trend was observed for pearl millet as far as N, K, Na and Ca were concerned (Tables 5.13 & 5.14). These trends were less prominent than that detected for oats. This was attributed to the species receiving only two irrigations with augmented winery wastewater 16 days and 6 days before the end of its growing season, *i.e.* on 9 and 18 February 2011.

The two crops combined intercepted too much N, K, P, Mg and Ca, but too little Na (Tables 5.11 & 5.12). The large amounts of N and K removed by pearl millet were attributed to the vigorous growth of the species (producing on average 163 kg of dry matter per hectare per day). Both species removed too much N. Pearl millet removed too much K, while the amounts that oats intercepted were inadequate. It seemed, however, that pearl millet

removed acceptable amounts of Mg and Ca, while oats removed acceptable amounts of P in T9.

5.3.3.3. October 2011 to September 2012

Similar to the previous season, the augmented winery wastewater had no effect on the amounts of N, K, Na, P, Mg, and Ca removed by oats (Tables 5.13 & 5.14). In contrast to the previous season, however, the amounts of Na, Mg and Ca removed by oats in T2 to T9 tended to be lower than that of T1. This was also observed for P, to a lesser extent Table 5.14). No trends were detected for N and K (Table 5.13).

As far as pearl millet is concerned, no trends were observed between treatments for P and Ca, although differences did occur between treatments as far as P was concerned (Table 5.14). More N was removed by pearl millet from T2 to T9 than from T1, with the exception of T5 (Table 5.13). Although not significant, the same trend was observed for Na and Mg (Tables 5.13 & 5.14). More K was removed from T3, T4, T6, T7 and T8 than from T1. The K removed by pearl millet from T2, T5 and T9 also tended to be higher than that removed from T1.

The pearl millet grew less vigorous during the 2011/12 season than during the 2010/11 season (Table 5.6). The trend observed for oats was just the opposite (Table 5.2). This resulted in pearl millet removing less of the macro-nutrients whilst oats removed more, compared to the previous season (Tables 5.11 to 5.14). Therefore, similar to the trend observed for the previous season, the two crops combined again intercepted too much N, K, P, Mg and Ca, but too little Na. With the 84 kg of fertilizer N applied to promote cover crop growth during the 2011/12 season, the amounts of N added to the soil were similar to that removed by pearl millet in T2, T4, T6, T8 and T9 (Table 5.13). In the case of K, the best balance was achieved in T7 and T8. Excluding the fertiliser P applied, the best balance was achieved in T9, whilst in the case of Ca, the amount extracted by pearl millet and the amount applied by means of the augmented winery wastewater was approximately the same for T5, T6 and T7 (Table 5.14). The oats removed approximately the same amount of Mg that was applied *via* irrigation in all the treatments, while the interception by pearl millet was excessive. The above-mentioned results indicate that the exclusion of oats as interception crop (not harvesting and removing it from the vineyard), whilst employing only pearl millet as an interception crop could be a sustainable practice where winery wastewater augmented to COD's of between 1500 (T6) and 2500 (T8) is used for irrigating grapevines.

Table 5.10. Calculated amount of macro-elements removed by *Avena sativa* L. cv. Pallinup (oats) from 14 April to 29 September 2010.

Treatment no & target COD (mg/L)	Calculated amount (kg/ha)											
	N		K		P		Ca		Mg		Na	
	29 July	29 Sept	29 July	29 Sept	29 July	29 Sept	29 July	29 Sept	29 July	29 Sept	29 July	29 Sept
T1 - Control[1]	60 a[2]	15 b	87 a	33 a	12.9 a	3.9 a	10.3 a	4.3 a	6.0 a	4.6 a	0.55 a	0.99 bc
T2 - 100	58 a	25 ab	72 a	85 a	10.7 a	6.7 a	9.0 a	8.5 a	5.1 a	8.0 a	0.54 a	1.19 bc
T3 - 250	69 a	19 ab	76 a	76 a	10.9 a	5.5 a	9.7 a	9.3 a	5.2 a	8.4 a	0.63 a	2.43 a
T4 - 500	58 a	18 ab	95 a	54 a	13.3 a	4.3 a	10.7 a	5.4 a	6.6 a	4.4 a	0.90 a	0.81 c
T5 - 1000	51 a	18 ab	63 a	59 a	9.5 a	4.7 a	8.3 a	6.8 a	4.6 a	5.3 a	0.30 a	1.12 bc
T6 - 1500	63 a	27 a	78 a	68 a	10.8 a	4.9 a	8.7 a	7.9 a	4.8 a	7.1 a	0.60 a	1.32 bc
T7 - 2000	42 a	27 a	64 a	90 a	8.9 a	7.1 a	6.3 a	8.6 a	3.7 a	7.9 a	0.31 a	1.15 bc
T8 - 2500	40 a	23 ab	59 a	109 a	9.2 a	7.6 a	7.0 a	9.8 a	3.5 a	8.5 a	0.32 a	1.03 bc
T9 - 3000	61 a	22 ab	70 a	87 a	12.0 a	6.5 a	8.0 a	8.8 a	4.7 a	7.8 a	0.39 a	1.59 b

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Table 5.11. Calculated amount of nitrogen (N), potassium (K) and sodium (Na) removed from a sandy soil near Rawsonville by *Pennisetum glaucum* L. hybrid Babala (pearl millet) and *Avena sativa* L. cv. Pallinup (oats) and the calculated balance as affected by fertiliser and winery wastewater additions from October 2010 to September 2011.

Treatment no & target COD (mg/L)	Calculated N (kg/ha)					Calculated K (kg/ha)				Calculated Na (kg/ha)			
	Removed		Applied		Balance	Removed		Applied	Balance	Removed		Applied	Balance
	Pearl millet	Oats	Irrigation	Fertiliser		Pearl millet	Oats	Irrigation		Pearl millet	Oats	Irrigation	
T1 - Control[1]	145 a[2]	29 a	5.3	56	-112.7	226 a	28 a	7.6	-246.4	1.74 a	0.82 a	50.36	47.80
T2 - 100	88 a	40 a	3.6	56	-68.4	234 a	50 a	13.5	-270.5	2.18 a	1.48 a	53.59	50.34
T3 - 250	97 a	50 a	3.3	56	-87.7	236 a	43 a	25.1	-253.9	1.99 a	1.75 a	59.83	56.26
T4 - 500	130 a	71 a	2.6	56	-142.4	251 a	67 a	43.9	-274.1	2.18 a	2.19 a	73.05	68.68
T5 - 1000	121 a	45 a	3.0	56	-107.0	277 a	49 a	79.0	-247.0	2.25 a	1.74 a	81.17	77.18
T6 - 1500	122 a	55 a	4.3	56	-116.7	262 a	57 a	112.8	-206.2	1.94 a	1.82 a	97.30	93.54
T7 - 2000	137 a	69 a	4.8	56	-145.2	204 a	74 a	148.8	-129.2	2.07 a	1.57 a	105.65	102.01
T8 - 2500	109 a	68 a	4.7	56	-116.3	346 a	64 a	173.4	-236.6	2.68 a	1.34 a	110.12	106.10
T9 - 3000	119 a	57 a	5.4	56	-114.6	313 a	62 a	214.8	-160.2	3.18 a	1.27 a	122.33	117.88

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Table 5.12. Calculated amount of phosphate (P), magnesium (Mg) and calcium (Ca) removed from a sandy soil near Rawsonville by *Pennisetum glaucum* L. hybrid Babala (pearl millet) and *Avena sativa* L. cv. Pallinup (oats) and the calculated balance as affected by the fertiliser and effluent water additions for the period October 2010 to September 2011.

Treatment no & target COD (mg/L)	Calculated P (kg/ha)				Calculated Mg (kg/ha)				Calculated Ca (kg/ha)			
	Removed		Applied		Removed		Applied		Removed		Applied	
	Pearl millet	Oats	Irrigation	Balance	Pearl millet	Oats	Irrigation	Balance	Pearl millet	Oats	Irrigation	Balance
T1 - Control[1]	37 a[2]	7 a	0.1	-43.9	30 a	5 a	19.1	-15.9	36 a	4 a	30.0	-10.0
T2 - 100	30 a	9 a	0.1	-38.9	28 a	7 a	19.6	-15.4	32 a	5 a	30.7	-6.3
T3 - 250	41 a	12 a	0.4	-52.6	30 a	8 a	19.8	-18.2	36 a	6 a	30.8	-11.2
T4 - 500	34 a	17 a	1.2	-49.8	31 a	11 a	20.2	-21.8	37 a	9 a	32.9	-13.1
T5 - 1000	37 a	11 a	3.2	-44.8	34 a	8 a	20.7	-21.3	43 a	5 a	34.9	-13.1
T6 - 1500	44 a	14 a	5.4	-52.6	31 a	9 a	21.6	-18.4	44 a	6 a	38.5	-11.5
T7 - 2000	38 a	14 a	8.5	-43.5	31 a	9 a	22.2	-17.8	38 a	7 a	39.9	-5.1
T8 - 2500	47 a	14 a	10.4	-50.6	38 a	8 a	22.1	-23.9	46 a	5 a	40.8	-10.2
T9 - 3000	43 a	15 a	13.5	-44.5	32 a	8 a	22.9	-17.1	38 a	5 a	43.1	0.1

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Table 5.13. Calculated amount of nitrogen (N), potassium (K) and sodium (Na) removed from a sandy soil near Rawsonville by *Pennisetum glaucum* L. hybrid Babala (Pearl millet) and *Avena sativa* L. cv. Pallinup (oats) and the calculated balance as affected by effluent water additions for the period October 2011 to September 2012.

Treatment no & target COD (mg/L)	Calculated N (kg/ha)					Calculated K (kg/ha)				Calculated Na (kg/ha)			
	Removed		Applied			Removed		Applied		Removed		Applied	
	Pearl millet	Oats	Irrigation	Fertiliser	Balance	Pearl millet	Oats	Irrigation	Balance	Pearl millet	Oats	Irrigation	Balance
T1 - Control[1]	61 c[2]	104 a	3.3	84	-77.7	80 e	109 a	6.6	-182.0	0.82 a	15.12 a	32.80	16.86
T2 - 100	91 b	63 a	1.4	84	-68.5	95 cde	66 a	11.0	-150.0	0.95 a	9.98 a	35.50	24.57
T3 - 250	129 a	67 a	1.3	84	-110.7	169 a	108 a	21.6	-255.4	1.28 a	5.56 a	37.53	30.69
T4 - 500	89 b	103 a	2.0	84	-106.0	115 bcd	118 a	35.9	-197.1	1.06 a	9. 77 a	42.42	31.59
T5 - 1000	68 c	71 a	2.2	84	-52.8	82 e	89 a	65.0	-106.0	0.80 a	11.28 a	49.46	37.38
T6 - 1500	93 b	78 a	3.1	84	-83.9	111 bcd	123 a	87.0	-147.0	0.98 a	7.99 a	54.90	45.93
T7 - 2000	101 b	107 a	4.3	84	-119.7	125 b	146 a	104.0	-167.0	1.01 a	11.95 a	61.45	48.49
T8 - 2500	88 b	74 a	6.3	84	-71.7	120 bc	97 a	134.5	-82.5	1.03 a	6.15 a	69.07	61.89
T9 - 3000	94 b	72 a	6.4	84	-75.6	93 de	98 a	157.7	-33.3	0.91 a	12.39 a	73.97	60.67

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Table 5.14. Calculated amount of phosphate (P), magnesium (Mg) and calcium (Ca) removed from a sandy soil near Rawsonville by *Pennisetum glaucum* L. hybrid Babala (pearl millet) and *Avena sativa* L. cv. Pallinup (oats) and the calculated balance as affected by the fertiliser and effluent water additions for the period October 2011 to September 2012.

Treatment no & target COD (mg/L)	Calculated P (kg/ha)					Calculated Mg (kg/ha)				Calculated Ca (kg/ha)			
	Removed		Applied		Balance	Removed		Applied	Balance	Removed		Applied	Balance
	Pearl millet	Oats	Irrigation	Fertiliser		Pearl millet	Oats	Irrigation		Pearl millet	Oats	Irrigation	
T1 - Control[1]	15 cde[2]	19 a	0.1	40.5	6.6	16 a	12 a	9.7	-18.3	19 a	11 a	14.9	-15.1
T2 - 100	16 bcd	11 a	0.2	40.5	13.7	19 a	8 a	9.9	-17.1	21 a	9 a	15.4	-14.6
T3 - 250	29 a	14 a	0.6	40.5	-1.9	25 a	7 a	10.4	-21.6	30 a	8 a	17.5	-20.5
T4 - 500	15 cde	18 a	1.4	40.5	8.9	20 a	9 a	10.6	-18.4	19 a	9 a	20.1	-7.9
T5 - 1000	10 f	16 a	3.0	40.5	17.5	16 a	7 a	11.3	-11.7	20 a	8 a	25.0	-3.0
T6 - 1500	13e	17 a	4.4	40.5	14.9	21 a	7 a	12.2	-15.8	21 a	8 a	28.6	-0.4
T7 - 2000	18 b	19 a	5.2	40.5	8.7	20 a	10 a	12.4	-17.6	18 a	10 a	32.5	4.5
T8 - 2500	17 bc	14 a	6.8	40.5	16.3	21 a	7 a	12.8	-15.2	19 a	7 a	36.5	10.5
T9 - 3000	14 de	14 a	8.0	40.5	20.5	20 a	7 a	13.7	-13.3	21 a	6 a	40.7	13.7

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

5.3.3.4. October 2012 to September 2013

Similar to the 2010/11 and 2011/12 seasons, augmented winery wastewater had no effect on the amounts of N, K, Na, P, Mg, and Ca removed by oats (Tables 5.15 & 5.16). The amount of N, Mg, Ca and Na intercepted by oats from treatments T2 to7 T9 tended to be lower than that of T1, with the exception of N in T5 and T6 and Na in T3. In the case of Mg, Ca and Na, it supported the trends observed during the 2011/12 season (Tables 5.13 & 5.14). Similar to the other four macro-elements, amounts of K and P removed by oats in the treatments irrigated with augmented winery wastewater (T2 to T9) tended to be lower than that removed from T1, with the exception of T6 and T7 for K, as well as T4 and T7 for P.

As far as pearl millet is concerned, no trends were observed between treatments for P and Ca (Table 5.16). This supports the trends observed during the 2011/12 season. The trends observed for K and Na (Table 5.16) were also similar to that reported for the 2011/12 season (Table 5.13). In contrast to the previous two seasons, no definite pattern could be observed for the interception of N by pearl millet (Table 5.15). Pearl millet intercepted more Mg in T4 than T1 and tended to remove more Mg from T2, T5, T6, T7, and T8 as well (Table 5.16). The trend observed for Mg supports the results of the previous season (Table 5.14).

Similar to the trend observed for the previous two seasons, the two cover crops combined intercepted too much N, K, P, Mg and Ca, but too little Na (Tables 5.15 & 5.16). With the 56 kg of fertilizer N applied to promote cover crop growth, the amounts of N added to the soil were similar to that removed by oats in T2, T3, T8 and T9 (Table 5.15). To neutralise the N removed by pearl millet, an average of 120 kg of fertiliser N needs to be applied. This will increase the input cost by R 2 311/ha. The amounts of K removed by pearl millet in T7 and T8 were similar to the amounts added to the soil by irrigating with augmented winery wastewater (Table 5.15). As in the case of N, the deficits created by the extraction of excess P by pearl millet, can be corrected by fertilising with approximately 9.6 kg/ha P at a cost of R 504/ha. However, with an average of 433 bales per hectare being produced by pearl millet, an income of as much as R 45 per bale will amount to R 19 485. This will enable cellars to treat its effluent, whilst making a profit of approximately R 15 000 per hectare per year.

Table 5.15. Calculated amount of nitrogen (N), potassium (K) and sodium (Na) removed from a sandy soil near Rawsonville by *Pennisetum glaucum* L. hybrid Babala (pearl millet) and *Avena sativa* L. cv. Pallinup (oats) and the calculated balance as affected by the fertiliser and effluent water additions for the period October 2012 to September 2013.

Treatment no & target COD (mg/L)	Calculated N (kg/ha)					Calculated K (kg/ha)				Calculated Na (kg/ha)			
	Removed		Applied		Balance	Removed		Applied	Balance	Removed		Applied	Balance
	Pearl millet	Oats	Irrigation	Fertiliser		Pearl millet	Oats	Irrigation		Pearl millet	Oats	Irrigation	
T1 - Control[1]	128 cd[2]	77 a	6.5	56	-142.5	91 a	89 a	5.7	-174.3	0.76 a	10.77 a	29	17.47
T2 - 100	127 cd	59 a	3.1	56	-127.9	116 a	84 a	9.8	-190.2	0.90 a	5.01 b	28	22.09
T3 - 250	98 e	54 a	2.1	56	-93.3	93 a	71 a	22.2	-141.8	0.89 a	13.02 a	33	19.09
T4 - 500	202 a	66 a	2.4	56	-209.6	148 a	85 a	41.0	-192.0	1.44 a	9.98 a	37	25.58
T5 - 1000	157 b	77 a	3.5	56	-174.5	126 a	87 a	70.7	-142.3	1.32 a	3.14 b	48	43.54
T6 - 1500	157 b	78 a	5.0	56	-174.0	144 a	96 a	88.3	-151.7	1.40 a	5.06 b	53	46.54
T7 - 2000	139 bc	75 a	7.0	56	151.0	114 a	123 a	110.5	-126.5	1.14 a	5.82 b	61	54.04
T8 - 2500	122 cde	56 a	9.2	56	-112.8	129 a	74 a	135.4	-67.6	1.29 a	3.32 b	69	64.39
T9 - 3000	108 de	66 a	8.2	56	-109.8	100 a	79 a	159.5	-19.5	1.18 a	2.78 b	78	74.04

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Table 5.16. Calculated amount of phosphate (P), magnesium (Mg) and calcium (Ca) removed from a sandy soil near Rawsonville by *Pennisetum glaucum* L. hybrid Babala (pearl millet) and *Avena sativa* L. cv. Pallinup (oats) and the calculated balance as affected by the fertiliser and effluent water additions for the period October 2012 to September 2013.

Treatment no & target COD (mg/L)	Calculated P (kg/ha)				Calculated Mg (kg/ha)				Calculated Ca (kg/ha)			
	Removed		Applied		Removed		Applied		Removed		Applied	
	Pearl millet	Oats	Irrigation	Balance	Pearl millet	Oats	Irrigation	Balance	Pearl millet	Oats	Irrigation	Balance
T1 - Control[1]	11 a[1]	16 a	0.7	-26.3	25 b	10 a	9.8	-25.2	29 a	13 a	19.0	-23.0
T2 - 100	12 a	14 a	0.7	-25.3	28 b	8 a	10.6	-25.4	30 a	9 a	20.8	-18.2
T3 - 250	11 a	14 a	0.8	-24.2	22 b	7 a	11.0	-18.0	25 a	9 a	22.1	-11.9
T4 - 500	16 a	16 a	1.4	-30.6	45 a	8 a	11.5	-41.5	41 a	10 a	23.9	-27.1
T5 - 1000	14 a	15 a	2.9	-26.2	32 ab	8 a	12.7	-27.3	33 a	11 a	26.5	-17.5
T6 - 1500	17 a	15 a	3.9	-28.1	33 ab	9 a	13.3	-28.7	38 a	12 a	28.1	-21.9
T7 - 2000	11 a	18 a	5.2	-23.8	27 b	9 a	14.1	-21.9	29 a	12 a	31.2	-9.8
T8 - 2500	13 a	13 a	6.2	-19.8	27 b	7 a	14.9	-19.1	29 a	9 a	33.4	-4.6
T9 - 3000	11 a	12 a	7.5	-15.5	21 b	6 a	15.5	-11.5	23 a	7 a	35.0	5.0

[1] Raw water abstracted from the Holsloot River.
[2] Values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

5.4. CONCLUSIONS

In general, the DMP, element content and the amounts of the elements intercepted by both oats and pearl millet were not affected by the application of winery wastewater. Using both species to intercept the elements deposited by the winery wastewater resulted in too much N, K, P, Mg and Ca being removed from the soil. However, the amounts of Na removed were insignificant. The wine grape industry should, therefore, consider using K based cleaning agents rather than Na based cleaning agents, as especially pearl millet showed the ability to intercept large amounts of K.

Managing oats as a cover crop (not harvesting and removing it from the vineyard), whilst employing only pearl millet as an interception crop during the period when winery wastewater is likely to be applied in the vineyard, could be a sustainable practice. It seems, however, that the COD level of the winery wastewater should preferably be between 1500 and 2500 mg/L. The growing season of the oats being shortened by as much as six weeks due to the pearl millet being grown during harvest, did not affect the vegetative growth of the species negatively. The oats should, therefore, produce enough fibre to create quality summer mulches for pre-vèraison weed control.

As interception crop in grapevines, pearl millet should be sown early January to help ensure that its growth peaks when the winery wastewater needs to be applied and that the species does not complete its life cycle too early. In doing so, competition between pearl millet and the grapevines for N and P will also be minimised. The additional expense for fertiliser (approximately R 2800 per hectare per year) to compensate for the excess N and P intercepted by pearl millet, is much less than the profit to be made by selling the harvested crop for fodder (approximately R 15 000 per hectare per year).

As the use of an interception crop in the disposal of winery wastewater is a financially viable option, the use of N-fixers with a spreading habit as interception crops in vineyards irrigated with winery wastewater should be researched. The possibility of using species normally planted for grazing should also be investigated.

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CHAPTER 6: EFFECT OF IRRIGATION WITH AUGMENTED WINERY WASTEWATER ON SOIL MICROBIAL STATUS

A.H. Meyer
The Fruit, Vine and Wine Institute of the Agricultural Research Council, Private Bag X5026, Stellenbosch, 7599

6.1. INTRODUCTION

Each year wineries generate large volumes of low quality effluent during the various stages of wine production (Maria Gomez-Bradon *et al.*, 2011). These by-products pose serious disposal challenges. The irrigation of vineyards with adequately pre-treated wastewater could improve vineyard soil quality by supplying mineral nutrients. Irrigation of vineyards with pre-treated wastewater water represents a potential overall reduction in water use in water-poor winegrowing regions of the world, including South Africa, and may also offer benefits in terms of reduced liquid waste disposal costs in wineries.

The potential of achieving soil fertility *via* the use of winery wastewater is premised upon the known ingredients of this water, which is rich in easily-decomposable organic matter and nutrients (Friedel *et al.*, 2000; Chen *et al.*, 2008). The current impact of these wastewaters to vineyard soil is uncertain, but owing to the immense financial and environmental benefits thereof, it is imperative for it to be investigated in detail. Rather surprisingly, the vast majority of studies on the effect of organic matter input on soil quality have almost exclusively been limited to using chemical parameters as indicators of soil health (Pagliai & Vittori Antisari, 1993). However, significant changes to soil organic matter content can often occur slowly and several years may pass before anything is spotted (Sparling, 1992). Soil microbiological parameters have also been shown to be sensitive to long (Powlson *et al.*, 1987; Sparling 1992) and short (Friedel *et al.*, 2000; Caldwell, 2005; Wakelin *et al.*, 2008) term trends in fertility and soil quality and therefore indicative of potentially unsustainable practices. Studies of microbial communities residing not only in the top soil, but throughout the entire soil profile are required (Rumpel & Kögel-Knabner, 2011; Kramer *et al.*, 2013) since up to 50% of the worldwide carbon pools are believed to be located beneath the region where tillage normally takes place (Jobbagy & Jackson, 2000). Nevertheless, surface soil layers are more exposed to mechanical, chemical or vegetation changes, and thus, more pronounced effects on the physiology of the soil microbial communities are usually expected in the top- than in the subsoil. Soil microorganisms and the functions they fulfill are affected either by being stimulated or inhibited by such changes in the soil environment, depending on the agricultural activity. Breakdown and mineralization of organic matter is a specialized role bestowed to microorganisms (Bardgett *et al.*, 2005). The quantity and quality of mineralizable carbon

substrates decrease as the depth of the soil column increase (Blume *et al.*, 2002; Bausenwein *et al.*, 2008; Gelsomino & Azzellino, 2011), implying that soil organic matter is less degradable with depth (Richter & Markewitz, 1995; Ajwa *et al.*, 1998; Trumbore, 2000). Changes in the assemblages of microorganisms downwards the soil profile (Zelles *et al.*, 1995; Bossio & Scow, 1998; Griffiths *et al.*, 1999; Schütz *et al.*, 2009) are also likely to be dependable on the gradients in available mineralizable substrates across the soil profile layers, given that the spreading of soil microorganisms through soil usually reflects patchiness of available nutrients (Schütz *et al.*, 2009). However, insofar as monitoring microorganisms are concerned, it is not possible to evaluate the ecological significance of a microorganism simply by determining their numbers. It is of greater importance to obtain information on their activity (Alexander, 1977). This view was reiterated by Naseby and Lynch (1997), who considered enzymatic determinations more useful than microbial measures, since they can be made with higher precision. In fact, all functions and processes which microorganism perform or mediate, can only proceed in the presence of microbially-secreted enzymes (Dick, 1994; Paul & Clark, 1996), the activities of which have been shown to generally decrease with increasing depth (Kramer *et al.*, 2013), a trend also noticed in vineyard soils by the South Australian Research and Development Institute (Rawnsley, 2007). Therefore, enzyme activity, which forms the basis of the decomposition process of the soil organic fraction, is a key aspect of the current research, which is supported by coarse level comparison of sizes of functional microbial groups.

One microbial enzyme in particular, *β*-glucosidase, is active in the cycling of principle nutrient, carbon (C) (Tabatabai, 1982), and is particularly sensitive to management-induced changes in the soil due to its strong relationship with soil organic matter content and quality (Garcia *et al.* 1994; Masciandaro & Ceccanti 1999; Caravaca *et al.* 2002). Enzyme activities are useful indicators of soil health because their activities reflect both short (Bandick & Dick, 1999) and long-term (Jin *et al.*, 2009) changes taking place in their respective pools of nutrients. However, winery wastewater irrigation that affect vertical distribution of roots, as of grapevines, are also likely to affect other functional microbial groups, as mycorrhizal fungi, since changes in quantity and quality in rhizo-deposition through root exudates generally affects soil microbial community compositions (Smalla *et al.*, 2001; Yao *et al.*, 2005), including mycorrhizal fungi; their growth and development are triggered by the constant supply of the root exudates. In this regard, the effect that wastewater irrigation may have on the glomalin contents of vineyard soil is largely unexplored. Glomalin is a glycoproteinaceous substance, produced by arbuscular mycorrhizal (AM) fungi, and represents a potentially valuable pool of C in the soil (Rillig *et al.*, 2001). Glomalin is also generally considered to be an indicator of soil health (Treseder & Turner, 2007). At a broader scale, the effect which irrigation with winery wastewater may have at microbial community level is equally important

to that at enzyme level; in fact, the levels of activities of enzymes in the soil can be seen as an expression by the soil microbial community to metabolic requirements and nutrient availability (Dick *et al.*, 1996; Badiane *et al.*, 2001; Moore-Kucera & Dick, 2008). Therefore, assessing the diversity in microbial communities could be helpful in determining the effects that irrigation with winery wastewater may have on the general microbiology of the soil.

Wastewater quantity and quality on the soil microbiology across a transect of top- and subsoil layers may also be reliant upon environmental conditions in the soil. For example, both soil temperature (Zogg *et al.*, 1997) and moisture (Kieft *et al.*, 1993; Lundquist *et al.*, 1999; Schimel *et al.*, 1999) have been shown to affect soil microbial community composition. Generally, the outer soil surfaces undergo temperature and moisture fluctuates that are more extensive during the day than soils found at greater depths (Brady & Weil, 2002). That soil moisture and temperature become less variable with depth, suggests that the effects of irrigation with winery wastewater on soil moisture and temperature could be smallest in sub-soils, which means that the effects on microbial populations and their enzyme constituents, would also be littlest. Furthermore, since the intervals between irrigation cycles imply periods of varying moisture and temperature conditions, it is likely that temporal effects of irrigation with winery wastewater on the microbiology of the soil, can be anticipated.

The results described in this report are part of a much larger multi-disciplinary study. The objective of the present study was to examine the depth-wise and temporal changes in the microbiology of the soil as affected by irrigation with winery wastewater, augmented to different concentrations of COD, by (i) measuring microbial enzyme activity (*β*-glucosidase activity), (ii) enumerating important functional groups of micro-organisms (heterotrophic microbes and actinomycetes), (iii) measuring soil glomalin content, and (iv) monitoring shifts in microbial populations (microbial diversity).

6.2. MATERIALS AND METHODS

6.2.1. Pre-irrigation assessment

The reason for this assessment was to obtain a baseline figure of the biochemical and microbial properties before each irrigation cycle commenced, *i.e.* early and late (as indicated by different months) during each year of the trial. The trial started off by randomly collecting samples once in January 2010, thereafter samples were collected randomly in February and April 2011, March and May 2012, as well as February and April 2013, from in soil layers at 0 to 10 cm, 10 to 20 cm, 20 to 30 cm and 30 to 60 cm layers (to establish the degree of microbiological activity in the soil profile), using an auger that was sterilized in 70% ethanol to prevent contamination between samples. Samples from each depth within a replication were pooled. In 2012, additional baseline samples were taken at deeper layers in the soil profile at depths 60 to 90 cm, 90 to 120 cm, 120 to 150 cm and 150 to 180 cm. The samples were

transported to the lab and examined to obtain a baseline assessment of the *β*-glucosidase activity, supported by course level comparisons of population sizes of microbial functional groups, *i.e.* actinomycete and heterotroph populations, and glomalin content of the soil. The above biochemical and microbiological parameters were determined according to methods described under sections 6.2.3. to 6.2.6.

6.2.2. Post-irrigation assessment

Samples were taken at one, three, seven and 14 days after winery wastewater irrigations were applied in the respective soil layers as described above. After the samples were transported to the lab, *β*-glucosidase activity was determined, and actinomycetes and heterotrophic bacteria were counted. The glomalin content of the soil and shifts in microbial populations were also determined, once every two years, glomalin in January 2010, as well as in March and May 2012, three days after treatment application; microbial shifts in April 2011, May 2012 and April 2013. In March and May 2012, samples were also taken at deeper layers, on day three, at depths 60 to 90 cm, 90 to 120 cm, 120 to 150 cm and 150 to 180 cm. The above measurements were carried out in accordance with to the methods described under sections 6.2.3. to 6.2.6.

6.2.3. Microbial enzyme activity

The *β*-glucosidase is measured by a simple assay performed in the laboratory. The *β*-glucosidase activity (EC 3.2.1.21) according to the method of Eivazi and Tabatabai (1988) was determined in field moist soil in a reaction mixture containing 1.0 g soil, 0.25 ml toluene, 1.0 ml 25mM p-nitrophenyl-*β*-D-glucopyranoside (PNG) as substrate, and 4.0 ml modified universal buffer (MUB) at pH 6.0.

6.2.4. Microbial counts

Generally, actinomycete and heterotrophic plate counts are performed in a Petri dish in selective and non-selective growth medium, respectively (Pepper & Gerba, 2005). In the present study, similar procedures were used but Plate Count Agar (Sigma-Aldrich) and Actinomycete Isolation Agar (Sigma-Aldrich) were used to isolate total heterotrophic bacteria and actinomycetes, respectively, from soil, each by standard plate count method, by surface spread plate procedure. The Plate Count Agar and Actinomycete Isolation Agar growth mediums were prepared according to the instructions of the manufacturers. Enumeration was carried out on air-dried samples using the standard dilution plate procedure, *i.e.* using 1 g of soil (air dry weight equivalent), agitated in a 10-fold dilution of 0.15 M NaCl (w/v) with a vortex for 10 seconds. One ml of the soil suspension was then diluted serially and serial dilutions of 10-4 and 10-5 plated. Each dilution was plated (0.1 ml aliquots) in triplicate. The

inoculated plates were incubated in an inverted position at 26°C for 5 days for total heterotrophs and 30°C for 3 days to permit growth of actinomycetes.

6.2.5. Microbial shifts

Microbial shifts were monitored using Automated Ribosomal Intergenic Spacer analysis (ARISA) following the same procedure described by Caleb (2010). ARISA was used to characterise fungal (F-ARISA) and bacterial (B-ARISA) communities from soil samples. The intergenic spacer (ITS) region from the fungal rRNA operon was amplified using ITS4 and fluorescently FAM (6 carboxylfluorescein) labelled ITS5 primers. Similarly, the 16S-23S intergenic spacer region from the bacterial rRNA operon was amplified using ITSR and FAM-labelled ITSF primers. The sensitivity of the technique allowed discrimination between soil microbial communities of the different treatments.

6.2.6. Glomalin contents

Glomalin extractions from sub-samples were carried out as described by Wright and Upadhyaya (1998). Easily-extractable glomalin (EEG) was extracted with 20 mm citrate, pH 7.0 at 121 °C for 30 min.

6.3. RESULTS AND DISCUSSION

6.3.1. Pre-irrigation assessment

6.3.1.1. Depth variation in enzyme activity, microbial counts and glomalin content

According to these data collected shortly before each irrigation cycle of each year (February and April 2011, March and May 2012 and February and April 2013) (data of January 2010 not shown), the *β*-glucosidase activity was significantly higher in the topsoil layer (0 to 10 cm) and decreased as the depth of the soil column increased (Table 6.1). This finding fits the generalized view that enzyme activity decreases with increasing depth (Acosta-Martínez & Tabatabai, 2000, Kramer *et al.*, 2013). This gradient in enzyme activity was observed in soil horizons as deep as 180 cm during monitoring in March and May 2012 (Table 6.2). This change in enzyme activity is usually consistent with changes in available mineralizable substrates, the quantity and quality of which usually decrease as the depth of the soil column increases (Blume *et al.*, 2002; Bausenwein *et al.*, 2008; Gelsomino & Azzellino, 2011). Similar results were obtained with regards to the soil glomalin contents in March and May 2012 (Table 6.3). This decrease in the concentration of glomalin, a product of mycorrhizhal

Table 6.1. Depth variation in mean *β*-glucosidase activity (µg/g soil/h) before winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville early and late during the 2011, 2012, and 2013 seasons.

Soil depth	2011		2012		2013		Mean	
(cm)	Early season	Late season	Early season	Late season	Early season	Late season	Early season	Late season
0-10	9.89 a[(1)]	-	128.21 a	137.04 a	116.98 a	69.99 a	85.03 a	103.51 a
10-20	3.42 a	-	72.44 ab	56.81 b	51.50 b	38.06 b	42.45 b	47.44 b
20-30	1.72 a	-	57.74 b	31.22 b	22.97 b	25.76 bc	27.48 bc	28.49 bc
30-60	3.53 a	-	24.97 b	26.59 b	25.84 b	8.48 c	18.11 c	17.53 c

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

Table 6.2. Depth variation in mean *β*-glucosidase activity (µg/g soil/h) before winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville as determined in March and May 2012.

Soil depth (cm)	March 2012	May 2012
0-10	128.20 a[(1)]	137.04 a
10-20	72.43 b	6.82 b
20-30	57.74 bc	31.22 c
30-60	24.97 bcd	26.59 cd
60-90	19.26 cd	16.57 cd
90-120	18.64 cd	8.33 cd
120-150	10.26 cd	6.38 d
150-180	5.92 d	4.72 d

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

Table 6.3. Depth variation in the mean glomalin concentration (mg/g soil) as determined in January 2010, as well as in April and May 2012 before winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	2010	2012		Mean
	January	April	May	
0-10	0.77667 a[(1)]	0.56667 a	0.55000 a	0.63111 a
10-20	0.93000 b	0.48667 ab	0.42000 b	0.61222 a
20-30	0.76000 a	0.48667 ab	0.35667 bc	0.53444 b
30-60	0.87667 ab	0.39000 b	0.29667 c	0.52111 b

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

fungi, with an increase in depth, could be as a result of a vertical decrease in root density since growth and development of mycorrhizal fungi is triggered by the supply of the root exudates. Contrary to the baseline enzyme activity and glomalin data, actinomycete and heterotrophic plate counts did not display the same degree of sensitivity and consistency across the soil depth layers, neither when the first four soil layers were examined up to 60 cm depth (Table 6.4) nor when layers up to 180 cm depth were examined (Table 6.5), during March and May 2012. The question at this stage was whether actinomycete and heterotroph populations would react differently if exposed to irrigation with winery effluent, and whether the same degree of sensitivity in enzyme activity and glomalin concentration, as with the baseline assessment, would be observed in soils after irrigation.

6.3.2. Post-irrigation assessment

6.3.2.1. Depth variation in enzyme activity and microbial counts

As for the pre-irrigation assessment, *β*-glucosidase activity was again significantly higher in the topsoil layer (0 to 10 cm) after irrigation with winery wastewater, and decreased as the

Table 6.4. Depth variation in the mean actinomycete plate count (APC) and heterotrophic plate count (HPC) expressed as colony forming units (CFU) per gram soil (CFU/g soil) as determined in January 2010, February 2011, March and May 2012, as well as February and April 2013 before winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	2010		2011		2012				2013			
	January		February		March		May		February		April	
	APC	HPC	APC	HPC	APC	HPC	APC	HPC	APC	HPC	APC	HPC
0-10	1.37.E+07 a[(1)]	5.47.E+06 a	2.94.E+07 a	1.22.E+07 a	1.94.E+07 a	1.84.E+07 a	8.22.E+06 ab	1.74.E+07 a	5.56.E+06 a	7.22.E+06 a	2.49.E+07 a	2.36.E+07 a
10-20	1.49.E+07 a	4.84.E+06 a	1.62.E+07 a	1.28.E+07 a	1.16.E+07 a	1.22.E+07 a	1.24.E+07 ab	2.51.E+07 a	3.22.E+06 a	5.44.E+06 a	3.22.E+06 a	1.68.E+07 a
20-30	1.64.E+07 a	5.00.E+06 a	2.17.E+07 a	1.16.E+07 a	1.31.E+07 a	1.54.E+07 a	1.61.E+07 a	2.33.E+07 a	5.00.E+06 a	7.22.E+06 a	4.56.E+06 a	1.27.E+07 a
30-60	1.74.E+07 a	6.87.E+06 a	2.68.E+07 a	1.29.E+07 a	1.12.E+07 a	1.99.E+07 a	4.67.E+06 b	1.50.E+07 a	1.89.E+06 a	5.00.E+06 a	8.11.E+06 a	7.56.E+06 a

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

Table 6.5. Depth variation in the mean actinomycete plate count (APC) and heterotrophic plate count (HPC) expressed as colony forming units (CFU) per gram soil (CFU/g soil) as determined in March and May 2012 before winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	March		May	
	APC	HPC	APC	HPC
0-10	1.94.E+07 a[(1)]	1.84.E+07 ab	8.22.E+06 abc	1.74.E+07 ab
10-20	1.16.E+07 a	1.22.E+07 ab	1.24.E+07 ab	2.51.E+07 a
20-30	1.31.E+07 a	1.54.E+07 ab	1.61.E+07 a	2.33.E+07 a
30-60	1.12.E+07 a	1.99.E+07 b	4.67.E+06 bc	1.50.E+07 abc
60-90	6.67.E+06 a	1.43.E+07 ab	3.89.E+06 c	1.16.E+07 abc
90-120	8.89.E+06 a	1.17.E+07 ab	4.67.E+06 bc	8.11.E+06 bc
120-150	1.86.E+07 a	1.88.E+07 ab	2.00.E+06 c	6.44.E+06 bc
150-180	4.67.E+06 a	5.67.E+06 a	2.78.E+06 c	2.22.E+06 c

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

depth of the soil column increased (Tables 6.6 to 6.8). These decreases in enzyme activity were consistently observed throughout the trial at the respective dates of sampling (number of days after irrigation) after each irrigation cycle of each year (February & April 2011, March & May 2012, as well as February & April 2013). Interestingly, this decrease in *β*-glucosidase activity with soil depth was similar to those reported by the South Australian Research and Development Institute (Rawnsley, 2007). However, in the latter case only raw water was used to irrigate vineyards. Perhaps *β*-glucosidase activity decrease as the depth of the soil volume increase because of its active involvement in the cycling of principle nutrient C, and because it is particular sensitive to management-induced changes in the soil due to its strong relationship with soil organic matter content and quality (Garcia *et al.* 1994; Masciandaro & Ceccanti 1999; Caravaca *et al.* 2002). If *β*-glucosidase activity is affected by C-substrate availability (Blume *et al.*, 2002; Bausenwein *et al.*, 2008; Gelsomino & Azzellino, 2011), and irrigation with winery wastewater implies adding easily-decomposable organic matter to the soil (Friedel *et al.*, 2000; Chen *et al.*, 2008), it perhaps may explain why the gradient in enzyme activity across soil layers was observed.

Nonetheless, since soil profiles often reach vertical distances well below the plough zone, it was important to establish whether the decrease trend in enzyme activity would persistent deeper down the soil profile or whether irrigation with winery wastewater would ultimately lead to slow build-up of organic solids in deeper soil horizons over time. So, as progressively deeper inner sub-layers of the soil profile were being examined three days after irrigation was applied in March and May 2012 (at depths 60 to 90 cm, 90 to 120 cm, 120 to 150 cm & 150 to 180 cm), the gradient in enzyme activity was still found to be visible beyond the 60 cm zone, dropping sharply thereafter to reach a maximum low at a depth of 180 cm (Table 6.9). That soil organic matter is less degradable at depth (Richter & Markewitz, 1995; Ajwa *et al.*, 1998; Trumbore, 2000) perhaps may explain this finding. It further reject the thought that organic solids settled and accumulated at the bottom layers of the soil profile as a result of high organic loads (COD concentrations) in winery wastewater. In the event that this would occur, it would usually lead to ‘’soil anaerobiosis’’, which evidently was not the case in the present study.

The degree to which *β*-glucosidase activity was affected by irrigation with winery effluent, implies that organic matter in the irrigation water can be decomposed easily by microorganisms residing in the various soil layers (Friedel *et al.*, 2000; Chen *et al.*, 2008), especially those inhabiting the surface layers of the soil profile. The fact that soil enzyme activities are considered the direct expression of soil microorganisms to metabolic requirements and available nutrients (Dick *et al.*, 1996; Badiane *et al.*, 2001), support this supposition. However, depth variation in microbial abundance, both actinomycetes and

Table 6.6. Depth variation (up to 60 cm) in mean *β*-glucosidase activity (µg/g soil/h) as determined in February and April 2011 after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	February				April		
	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7
0-10	29.65 a[(1)]	38.09 a	29.43 a	22.25 a	31.66 a	67.19 a	52.56 a
10-20	8.48 b	8.32 b	10.47 b	10.29 b	13.25 b	26.74 b	22.18 b
20-30	9.56 b	11.00 b	11.37 a	4.02 b	13.19 b	26.63 b	19.93 b
30-60	4.96 b	2.59 b	1.97 b	3.64 a	12.33 b	22.76 b	19.57 a

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

Table 6.7. Depth variation (up to 60 cm) in mean *β*-glucosidase activity (µg/g soil/h) as determined in March and May 2012 after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	March				May			
	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7	Day 14
0-10	117.25 a[(1)]	109.54 a	124.17 a	113.35 a	116.34 a	123.95 a	104.42 a	89.08 a
10-20	57.72 b	51.97 b	65.61 b	63.18 b	50.69 b	39.07 b	48.69 b	55.87 b
20-30	43.65 bc	26.25 c	29.06 c	40.98 c	32.57 c	29.88 bc	31.96 bc	29.98 c
30-60	32.69 c	15.35 c	15.32 c	28.61 c	24.29 c	23.66 c	23.48 c	24.30 c

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

Table 6.8. Depth variation (up to 60 cm) in mean β-glucosidase activity (µg/g soil/h) as determined February and April 2013 after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	February				April		
	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7
0-10	127.56 a[(1)]	112.51 a	116.31 a	107.28 a	133.21 a	121.22 a	110.07 a
10-20	50.67 b	49.33 b	64.93 b	55.90 b	78.31 b	57.88 b	41.68 b
20-30	30.79 c	29.26 c	32.80 c	33.87 c	44.30 c	38.48 bc	33.07 bc
30-60	22.64 c	21.19 c	23.05 c	26.31 c	34.26 c	23.01 c	23.30 c

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

Table 6.9. Depth variation in mean β-glucosidase activity (µg/g soil/h) as determined in March and May 2012 three days after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	March	May
0-10	109.54 a[(1)]	123.95 a
10-20	51.97 b	39.61 b
20-30	26.26 c	29.34 bc
30-60	15.35 d	23.66 c
60-90	8.76 de	7.96 d
90-120	4.24 e	6.63 d
120-150	2.51 e	5.22 d
150-180	3.07 e	5.56 d

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

heterotrophs, was not equally affected by wastewater irrigation treatments as enzyme activity was, neither when soil layers up to 60 cm were examined (Tables 6.10 to 6.15) nor when layers up to 180 cm were examined (Table 6.16). The absences of consistent differences were observed throughout the trial at the respective dates of sampling (number of days after irrigation) after each irrigation cycle in January 2010, February and April 2011, March and May 2012, as well as February and April 2013. This resonates with the statement by Alexander (1977) that it is not always possible to evaluate the ecological significance of a microorganism simply by knowing its number, but that it is more important to obtain information on its activity. This was reiterated by Naseby and Lynch (1997), who considered enzymatic determinations more useful than microbial measures, since they can be made with higher precision.

Table 6.10. Depth variation in the mean actinomycete plate count (APC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in February 2011 after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville. Data for April not available.

Soil depth (cm)	APC		
	Day 1	Day 3	Day 7
0-10	4.79.E+07 a[(1)]	1.45.E+08 a	7.52.E+07 a
10-20	1.18.E+08 b	1.41.E+08 a	6.21.E+07 a
20-30	8.96.E+07 ab	1.02.E+08 a	1.17.E+07 a
30-60	4.60.E+07 a	5.58.E+07 a	3.45.E+07 a

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

Table 6.11. Depth variation in the mean heterotrophic plate count (HPC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in February and April 2011 after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	HPC						
	February			April			
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7	Day 14
0-10	4.66.E+07 a[(1)]	1.13.E+08 a	5.79.E+07 a	1.69.E+07 a	3.82.E+07 a	1.93.E+07 a	9.22.E+07 a
10-20	1.34.E+08 b	1.49.E+08 a	3.86.E+07 a	2.83.E+07 a	3.46.E+07 a	1.16.E+07 b	3.25.E+07 a
20-30	1.07.E+08 ab	1.92.E+08 a	7.36.E+07 a	2.85.E+07 a	4.12.E+07 a	8.56.E+06 b	9.09.E+07 a
30-60	6.11.E+07 ab	1.57.E+08 a	3.17.E+07 a	1.61.E+07 a	3.83.E+07 a	1.05.E+07 b	2.44.E+07 a

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

Table 6.12. Depth variation in the mean actinomycete plate count (APC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in March and May 2012 after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	APC							
	March				May			
	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7	Day 14
0-10	1.71.E+07 a[(1)]	2.02.E+07 a	3.07.E+07 a	2.49.E+07 a	1.93.E+07 a	5.38.E+07 a	1.23.E+07 ab	1.21.E+07 a
10-20	9.62.E+06 a	2.13.E+07 a	6.05.E+07 a	2.63.E+07 a	4.80.E+07 b	4.44.E+07 a	6.45.E+06 a	1.20.E+07 a
20-30	1.06.E+07 a	1.63.E+07 a	1.88.E+07 a	1.65.E+07 b	3.03.E+07 ab	1.48.E+07 a	9.13.E+06 a	1.09.E+07 a
30-60	1.08.E+07 a	2.34.E+07 a	1.63.E+07 a	1.29.E+07 b	1.85.E+07 a	1.16.E+07 a	1.53.E+07 b	1.49.E+07 a

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

Table 6.13. Depth variation in the mean heterotrophic plate count (HPC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in March and May 2012 after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	HPC							
	March				May			
	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7	Day 14
0-10	1.36.E+07 a[(1)]	2.37.E+07 a	5.22.E+07 a	2.89.E+07 a	3.78.E+07 a	9.54.E+07 a	9.51.E+06 a	1.22.E+07 a
10-20	1.32.E+07 a	2.16.E+07 a	6.95.E+07 a	2.71.E+07 ab	1.62.E+08 b	8.03.E+07 a	8.36.E+06 a	1.18.E+07 a
20-30	1.52.E+07 a	1.92.E+07 a	5.73.E+07 a	2.41.E+07 ab	8.05.E+07 ab	2.83.E+07 a	8.00.E+06 a	1.18.E+07 a
30-60	1.55.E+07 a	2.63.E+07 a	5.98.E+07 a	2.11.E+07 b	3.62.E+07 b	1.73.E+07 a	8.87.E+06 a	1.18.E+07 a

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

Table 6.14. Depth variation in the mean actinomycete plate count (APC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in February and April 2013 after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	APC					
	February			April		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
0-10	8.93.E+06 a[(1)]	1.12.E+07 a	2.02.E+07 a	4.73.E+07 a	3.55.E+07 a	3.98.E+07 a
10-20	7.64.E+06 a	1.29.E+07 ab	1.97.E+07 a	3.57.E+07 a	4.26.E+07 a	2.65.E+07 a
20-30	7.07.E+06 a	1.48.E+07 b	2.11.E+07 a	4.14.E+07 a	2.69.E+07 a	2.46.E+07 a
30-60	7.42.E+06 a	1.07.E+07 a	1.91.E+07 a	4.06.E+07 a	3.49.E+07 a	5.67.E+07 a

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

Table 6.15. Depth variation in the mean heterotrophic plate count (HPC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in February and April 2013 after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	HPC					
	February			April		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
0-10	9.93.E+06 a[(1)]	1.44.E+07 a	2.30.E+07 a	2.19.E+07 a	8.25.E+07 a	5.52.E+07 a
10-20	1.10.E+07 a	1.40.E+07 a	1.94.E+07 a	1.14.E+07 b	8.65.E+07 a	6.29.E+07 a
20-30	8.69.E+06 a	1.42.E+07 a	2.35.E+07 a	2.01.E+07 ab	7.49.E+07 a	8.19.E+07 a
30-60	1.05.E+07 a	1.26.E+07 a	2.17.E+07 a	1.36.E+07 ab	8.11.E+07 a	5.31.E+07 a

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

Table 6.16. Depth variation in the mean actinomycete plate count (APC) and heterotrophic plate count (HPC) expressed as colony forming units (CFU) per gram soil in March and May 2012 on day three after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	March		May	
	APC	HPC	APC	HPC
0-10	2.02.E+07 abc[(1)]	2.37.E+07 a	5.19.E+07 a	6.43.E+07 a
10-20	2.13.E+07 ab	2.16.E+07 ab	1.76.E+07 a	2.85.E+07 a
20-30	1.63.E+07 abc	1.92.E+07 abc	4.24.E+07 a	8.09.E+07 a
30-60	2.34.E+07 a	2.63.E+07 a	1.24.E+07 a	5.11.E+07 a
60-90	1.06.E+07 bc	1.64.E+07 abc	3.39.E+07 a	3.28.E+07 a
90-120	1.30.E+07 abc	1.31.E+07 bc	8.78.E+06 a	1.71.E+07 a
120-150	1.33.E+07 abc	1.05.E+07 c	1.83.E+07 a	1.72.E+07 a
150-180	9.97.E+06 c	1.07.E+07 c	7.82.E+06 a	1.35.E+07 a

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

Glomalin was measured only in the 0-10 cm soil layer after irrigation treatments were applied, and hence, depth variation was not examined (only during pre-irrigation assessment, as discussed above under section 6.3.1.1).

6.3.2.2. Temporal variation in “depth effect” on enzyme activity and microbial counts

There seems to be a lack of consistent changes in enzyme activity over time with depth when the various sampling days after irrigation (days 1, 3, 7 & 14) were considered (Tables 6.6 to 6.8). Similar findings were made with respect to the microbial abundance (Tables 6.10 to 6.15). However, when the data of the different years (2011, 2012 & 2013) were pooled, independent of days after treatment or irrigation cycles, and of treatment (T1, T4, T5, T7 & T9), variation over time upon ‘’depth effect’’ was noticeable across a soil layer transect between 0-30 cm and 30-60 cm, in which case the activity was clearly higher in the top soil layer (0-30 cm) than in the bottom layer (30-60 cm) and increased over time in the sequence 2011>2012> 2013 at the respective depths (Table 6.17). Similar observations were made with respect to a decrease in microbial count with depth (Table 6.18) during each year (2011, 2012 & 2013).

Table 6.17. Depth variation in mean *β*-glucosidase activity (µg/g soil/h) in the 0-30 cm and 30-60 cm soil layers as determined in 2011, 2012 and 2013 seasons (independent of irrigation cycle) after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	2011	2012	2013
0-30	21.98 a[(1)]	66.33 a	69.98 a
30-60	10.36 b	23.46 b	24.83 b

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

Since glomalin was measured only in the 0-10 cm soil layer after treatment application, as earlier mentioned (under section 6.3.2.1), temporal variation on ‘’depth effect’’ could not be determined.

6.3.2.3. Treatment effects and temporal variation in “treatment effect” on enzyme activity, microbial counts and glomalin content

A lack of consistency in enzyme activity over time upon ‘’treatment effect’’ was evident when the various sampling days after irrigation with winery wastewater (days 1, 3, 7 & 14) were individually considered (Tables 6.19 to 6.21), although activity tend to increase with an increase in COD level from time to time. Consistency and sensitivity in data were, all the more so, less pronounced with respect to microbial abundance (Tables 6.22 to 6.27) and the glomalin data (Table 6.28). However, when the different years were compared (2011, 2012 & 2013, independent of days after treatment or irrigation cycle, and of

Table 6.18. Depth variation in the mean actinomycete plate count (APC) and heterotrophic plate count (HPC) expressed as colony forming units (CFU) per gram soil in the 0-30 cm and 30-60 cm soil layers, as determined in the 2011, 2012 and 2013 seasons (independent of irrigation cycle) after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

Soil depth (cm)	2011		2012		2013	
	APC	HPC	APC	HPC	APC	HPC
0-30	1.00.E+08 a[1]	6.54.E+07 a	2.25.E+07 a	3.76.E+07 a	2.46.E+07 a	6.21.E+07 a
30-60	4.57.E+07 b	4.84.E+07 b	1.53.E+07 b	2.46.E+07 b	2.82.E+07 a	5.25.E+07 a

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

Table 6.19. The effect of irrigation using different levels of augmented winery wastewater on β-glucosidase activity (µg/g soil/h), as determined in February and April 2011 in a sandy soil near Rawsonville.

Treatment no. & target COD (mg/L)	February				April		
	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7
T1 - Control[1]	12.68 a[2]	8.85 a	8.69 ab	6.82 a	11.31 a	33.89 a	19.79 a
T4 - 500	7.77 a	7.35 a	5.67 a	4.75 a	11.86 a	26.28 a	19.41 a
T9 - 3000	13.54 a	9.23 a	11.49 b	7.06 a	14.03 a	29.02 a	28.84 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 6.20. The effect of irrigation using different levels of augmented winery wastewater on *β*-glucosidase activity (µg/g soil/h), as determined in March and May 2012 in a sandy soil near Rawsonville.

Treatment no. & target COD (mg/L)	March				May			
	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7	Day 14
T1 - Control[1]	70.19 a[2]	38.25 a	47.32 a	40.54 a	41.48 a	48.23 a	57.36 a	50.29 a
T4 - 500	77.94 a	50.33 a	54.02 a	53.35 a	30.15 a	55.56 a	59.44 a	40.00 a
T9 - 3000	53.92 a	52.17 a	66.70 a	83.42 b	100.42 b	60.75 a	53.54 a	49.68 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 6.21. The effect of irrigation using different levels of augmented winery wastewater on *β*-glucosidase activity (µg/g soil/h), as determined in February and April 2013 in a sandy soil near Rawsonville.

Treatment no. & target COD (mg/L)	February				April		
	Day 1	Day 3	Day 7	Day 12	Day 1	Day 3	Day 7
T1 - Control[1]	44.43 a[2]	40.11 a	42.99 a	62.51 a	56.90 a	34.49 a	59.91 a
T4 - 500	51.51 ab	49.18 a	54.16 a	52.86 a	73.49 a	43.25 a	39.80 a
T9 - 3000	67.33 b	60.18 a	55.56 a	62.25 a	105.37 a	81.45 a	57.39 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 6.22. The effect of irrigation using different levels of augmented winery wastewater on the mean actinomycete plate count (APC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in February 2011 in a vineyard in a sandy soil near Rawsonville.

Treatment no. & target COD (mg/L)	APC		
	Day 1	Day 3	Day 7
T1 - Control[1]	3.26.E+07 a[2]	1.41.E+08 ab	9.46.E+07 a
T4 - 500	1.29.E+08 a	1.95.E+08 a	4.54.E+07 a
T9 - 3000	4.38.E+07 a	6.38.E+07 b	6.89.E+07 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

depth (0-30 cm & 30-60 cm), variation in enzyme activity over time upon ''treatment effect'' was clearly noticeable across the various treatments (T1, T4, T5, T7 & T9), in which case the activity increased from the lowest to the highest concentration in COD, *i.e.* in the trend T1>T4>T5>T7>T9, at least in as far as the last two seasons are concerned, 2012 and 2013, and also increased over time in the sequence 2011> 2012> 2013 in correspondence to the respective treatments (Table 6.29). In as far as microbial abundance data are concerned, a lack of correlation between actinomycetes and heterotrophs with COD concentrations was evident (data not shown). Findings regarding enzyme data seem to be in agreement with other reports on irrigation with urban wastewater (Chen *et al.*, 2008; Adrover *et al.*, 2012) and with winery wastewater (Mosse *et al.*, 2010), agreeing with the generalized view that irrigation over time lead to enhanced microbial activity.

6.3.3. Soil microbial shifts

The findings regarding the shifts in soil microbial populations are as follows:

2011 season: Bacterial communities showed little differences in their diversity between different treatments (Fig. 6.1). However, results suggest that there was a significant difference between T2 and T4, with T2 having a considerably lower diversity. Considering the structure of the community, there was a statistical difference between T1, T2 and T3, as well as between these treatments and the rest of the treatments (Table 6.30). This would suggest that T1, T2 and T3 would have an impact on the composition and structure of the bacterial communities, with a reduction in diversity of T2. Fungal communities showed a different picture. In this case there was no significant difference in the diversity of the communities (Fig. 6.2), although there were significant differences between the community structures of some of the irrigation treatments (Table 6.31). This would suggest that the treatments had an effect on the composition of the fungal communities, although the diversity was similar.

Table 6.23 The effect of irrigation using different levels of augmented winery wastewater on the mean heterotrophic plate count (HPC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in February and April 2011 in a vineyard in a sandy soil near Rawsonville.

Treatment no. & target COD (mg/L)	HPC						
	February			April			
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7	Day 14
T1 - Control[1]	3.87.E+07 a[2]	1.11.E+08 a	8.77.E+07 a	1.03.E+07 a	6.86.E+07 a	1.09.E+07 a	2.31.E+07 a
T4 - 500	1.64.E+08 a	2.69.E+08 a	3.84.E+07 a	9.25.E+06 a	5.17.E+07 a	8.83.E+06 a	3.01.E+07 a
T9 - 3000	3.90.E+07 a	9.73.E+07 a	4.14.E+07 a	2.88.E+07 a	1.91.E+07 a	1.79.E+07 a	1.71.E+07 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 6.24 The effect of irrigation using different levels of augmented winery wastewater on the mean actinomycete plate count (APC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in March and May 2012 in a vineyard in a sandy soil near Rawsonville.

Treatment no. & target COD (mg/L)	APC							
	March				April			
	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7	Day 14
T1 - Control[1]	1.35.E+07 a[2]	1.96.E+07 a	1.93.E+07 a	1.72.E+07 a	2.99.E+07 a	1.31.E+07 a	2.09.E+07 a	6.22.E+06 a
T4 - 500	1.18.E+07 a	2.13.E+07 a	1.81.E+07 a	1.77.E+07 a	4.18.E+07 a	2.11.E+07 a	6.52.E+06 b	6.31.E+06 a
T9 - 3000	6.33.E+06 a	2.87.E+07 a	2.88.E+07 a	1.70.E+07 a	2.25.E+07 a	1.99.E+07 a	8.25.E+06 b	1.86.E+07 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 6.25 The effect of irrigation using different levels of augmented winery wastewater on the mean heterotrophic plate count (HPC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in March and May 2012 in a vineyard in a sandy soil near Rawsonville.

Treatment no. & target COD mg/L)	HPC							
	March				May			
	Day 1	Day 3	Day 7	Day 14	Day 1	Day 3	Day 7	Day 14
T1 - Control[1]	1.72.E+07 a[2]	2.33.E+07 a	1.94.E+07 a	2.03.E+07 a	7.16.E+07 a	1.63.E+07 a	1.35.E+07 a	5.75.E+06 a
T4 - 500	1.38.E+07 ab	2.63.E+07 a	2.26.E+07 a	2.29.E+07 a	1.64.E+08 b	2.59.E+07 a	5.89.E+06 a	9.44.E+06 ab
T9 - 3000	9.24.E+06 b	1.59.E+07 a	7.49.E+07 a	2.39.E+07 a	2.28.E+07 a	7.99.E+07 a	7.58.E+06 a	1.84.E+07 b

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 6.26. The effect of irrigation using different levels of augmented winery wastewater on the mean actinomycete plate count (APC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in February and April 2013 in a vineyard in a sandy soil near Rawsonville.

Treatment no. & target COD (mg/L)	APC					
	February			April		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
T1 - Control[1]	3.89.E+06 a[2]	8.28.E+06 a	3.07.E+07 a	4.51.E+07 a	3.35.E+07 a	7.01.E+07 a
T4 - 500	7.33.E+06 a	1.56.E+07 b	1.81.E+07 b	5.62.E+07 a	1.68.E+07 a	1.95.E+07 a
T9 - 3000	1.77.E+07 b	1.06.E+07 a	1.45.E+07 b	3.27.E+07 a	6.28.E+07 b	3.93.E+07 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 6.27. The effect of irrigation using different levels of augmented winery wastewater on the mean heterotrophic plate count (HPC) expressed as colony forming units (CFU) per gram soil (CFU/g soil), as determined in February and April 2013 in a vineyard in a sandy soil near Rawsonville.

Treatment no. & target COD (mg/L)	HPC					
	February			April		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
T1 - Control[1]	5.83.E+06 a[2]	1.02.E+07 a	3.85.E+07 a	1.02.E+08 a	7.93.E+07 a	8.93.E+07 a
T4 - 500	6.61.E+06 a	1.47.E+07 a	2.21.E+07 ab	2.53.E+08 b	5.29.E+07 a	7.08.E+07 a
T9 - 3000	2.29.E+07 b	1.09.E+07 a	1.38.E+07 b	1.09.E+08 a	1.26.E+08 b	7.06.E+07 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 6.28. The effect of irrigation using different levels of augmented winery wastewater on the mean glomalin concentration (mg/g soil) of the soil as determined in January 2010, as well as March and May 2012 in a vineyard in a sandy soil near Rawsonville.

Treatment no. & target COD (mg/L)	2010	2012		Mean
	January	March	May	
T1 - Control[1]	1.1533 a[2]	0.4800 a	0.49333 a	0.70889 a
T4 - 500	0.9267 a	0.6567 a	0.51333 a	0.69889 a
T5 - 1000	1.0767 a	0.5333 a	0.55000 a	0.72000 a
T7 - 2000	0.8267 a	0.4067 a	0.41667 a	0.55000 b
T9 - 3000	1.0633 a	0.6100 a	0.42333 a	0.69889 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 6.29. The effect of irrigation using different levels of augmented winery wastewater on mean *β*-glucosidase activity (µg/g soil/h), as determined in 2011, 2012 and 2013 (independent of irrigation cycle or depth) in a sandy soil near Rawsonville.

Treatment no. & target COD (mg/L)	2011	2012	2013
T1 - Control[1]	11.33 a[2]	39.26 a	39.49 a
T4 - 500	9.66 a	43.89 ab	42.06 ab
T5 - 1000	25.96 b	42.09 ab	51.34 ab
T7 - 2000	20.63 ab	46.38 ab	48.65 ab
T9 - 3000	13.27 a	52.85 b	55.45 b

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

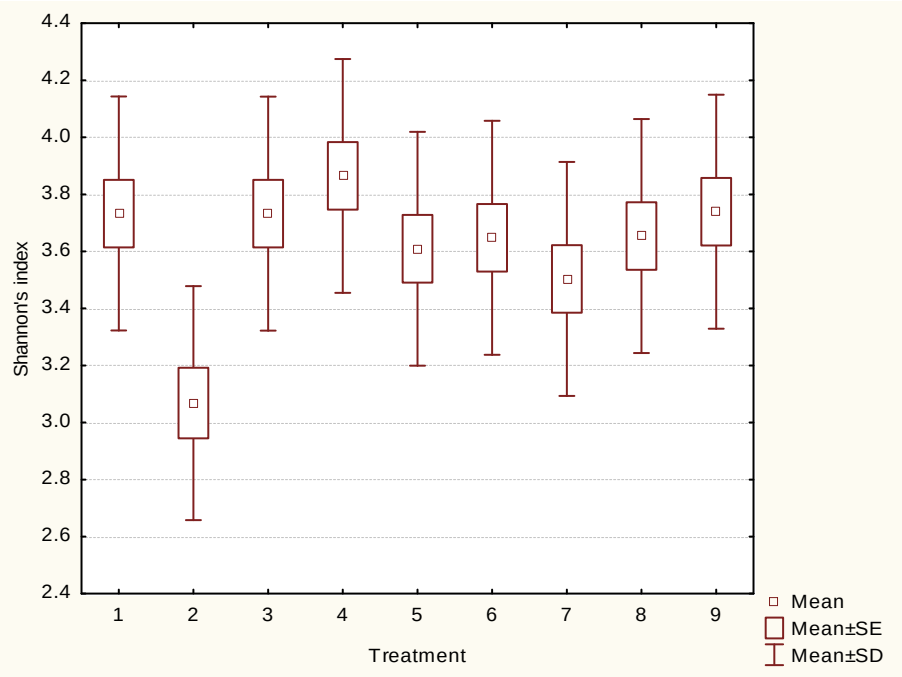

Figure 6.1. Box and whiskers plot of Shannon's diversity index for bacteria associated with different levels of winery wastewater augmentation as determined in February and April 2011 after wastewater irrigations were applied to a vineyard in a sandy soil near Rawsonville. The Kruskal-Wallis test indicates significant differences between treatments.

Table 6.30. Analysis of similarity (ANOSIM) of bacterial ARISA (based on Bray- Curtis similarity values) associated with treatments 1 to 9 as determined in February and April 2011 (highlighted values are those comparisons with p < 0.05).

Treatment no. & target COD (mg/L)	T1 - Control[(1)]	T2 - 100	T3 - 250	T4 - 500	T5 - 1000	T6 - 1500	T7 - 2000	T8 - 2500	T9 - 3000
T1 - Control[(1)]	**********	0.594290	0.495897	0.538931	0.506839	0.509365	0.335648	0.568182	0.512837
T2 - 100	0.594290	**********	0.269346	0.381543	0.377410	0.411095	0.273479	0.297396	0.414225
T3 - 250	0.495897	0.269346	**********	0.101115	0.152252	0.095960	0.154251	0.136364	0.187500
T4 - 500	0.538931	0.381543	0.101115	**********	0.000421	0.008628	0.005577	0	0.051136
T5 - 1000	0.506839	0.377410	0.152252	0.000421	**********	-0.03914	-0.00284	0.180976	0.038400
T6 - 1500	0.509365	0.411095	0.095960	0.008628	-0.03914	**********	-0.0343	0	-0.04167
T7 - 2000	0.335648	0.273479	0.154251	0.005577	-0.00284	-0.0343	**********	-0.0101	-0.0081
T8 - 2500	0.568182	0.297396	0.136364	0	0.180976	0	-0.0101	**********	-0.02283
T9 - 3000	0.512837	0.414225	0.187500	0.051136	0.038400	-0.04167	-0.0081	-0.02283	**********

[(1)] Raw water abstracted from the Holsloot River.

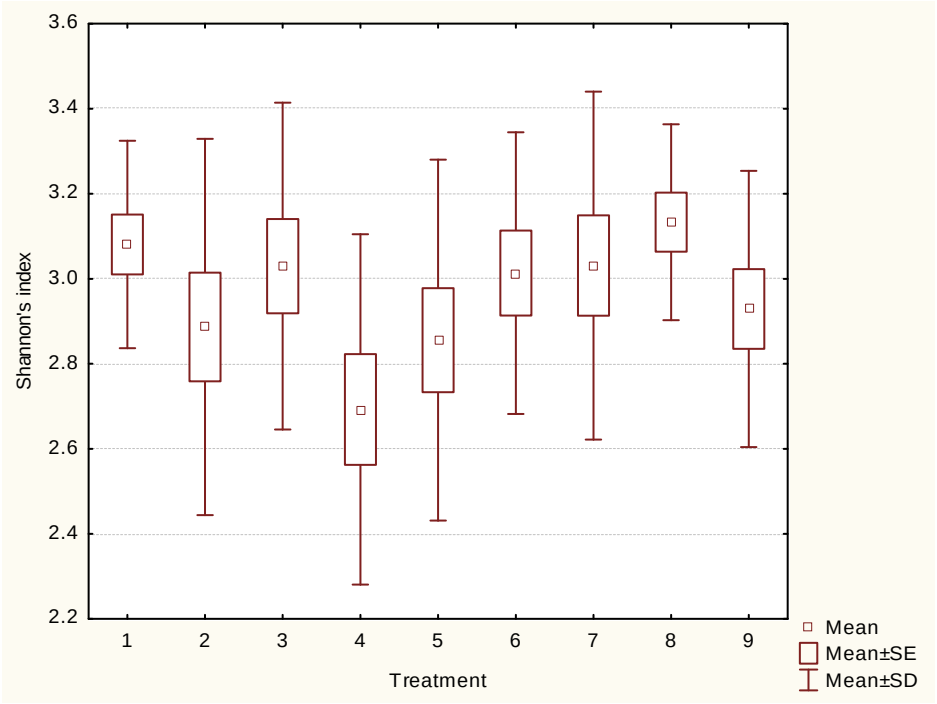

Figure 6.2. Box and whiskers plot of Shannon's diversity index for fungi associated with different levels of winery wastewater augmentation as determined in February and April 2011 wastewater irrigations were applied to a vineyard in a sandy soil near Rawsonville. The Kruskal-Wallis test showed no significant differences between treatments.

2012 season: The bacterial and fungal diversity of the soil community was not significantly affected by the application of 500 mg/L COD (T4), 1000 mg/L COD (T5), 2000 mg/L COD (T7) and 3000 mg/L COD (T9) (Figs. 6.3 & 6.4). The bacterial and fungal community structure of soil community was also not significantly affected by the application of 500 mg/L COD (T4), 1000 mg/L COD (T5), 2000 mg/L COD (T7) and 3000 mg/L COD (T9) (data not shown). Thus, the community structure did not change due to the added organic compounds in the soil within the specific time-frame. However, the community structure of the fungal community from the March 2012 sampling differed significantly from the May 2012 sampling cycle.

2013 season: Analysis showed no significant differences in the fungal or bacterial species diversity. The structure of the microbial communities was, however, distinct. There also seem to be no discernible pattern in the microbial community structure of the different treatments, and it would appear that the treatment had no effect on the microbial community compositions of a sample (data no shown).

Table 6.31. Analysis of similarity (ANOSIM) of fungal ARISA (based on Bray- Curtis similarity values) associated with treatments 1 to 9 as determined in February and April 2011 (highlighted values are those comparisons with p < 0.05).

Treatment no. & target COD (mg/L)	T1 - Control[(1)]	T2 - 100	T3 - 250	T4 - 500	T5 - 1000	T6 - 1500	T7 - 2000	T8 - 2500	T9 - 3000
T1 - Control[(1)]	**********	0.081966	0.044400	0.222200	0.181818	0.033050	0.227483	0.170799	0.143624
T2 - 100	0.081966	**********	-0.045770	0.037538	0.071549	0.068244	0.140678	0.067368	0.054924
T3 - 250	0.044400	-0.04577	**********	0.045190	0.062395	0.071625	0.131629	0.088405	0.049663
T4 - 500	0.222200	0.037538	0.045190	**********	-0.012	0.152909	0.117117	0.184182	0.060060
T5 - 1000	0.181818	0.071549	0.062395	-0.012	**********	0.094916	0.098695	0.120586	0.037668
T6 - 1500	0.033050	0.068244	0.071625	0.152909	0.094916	**********	0.059479	0.086401	-0.0083
T7 - 2000	0.227483	0.140678	0.131629	0.117117	0.098695	0.059479	**********	0.021913	-0.03125
T8 - 2500	0.170799	0.067368	0.088405	0.184182	0.120586	0.086401	0.021913	**********	-0.02079
T9 - 3000	0.143624	0.054924	0.049663	0.060060	0.037668	-0.0083	-0.03125	-0.02079	**********

[(1)] Raw water abstracted from the Holsloot River.

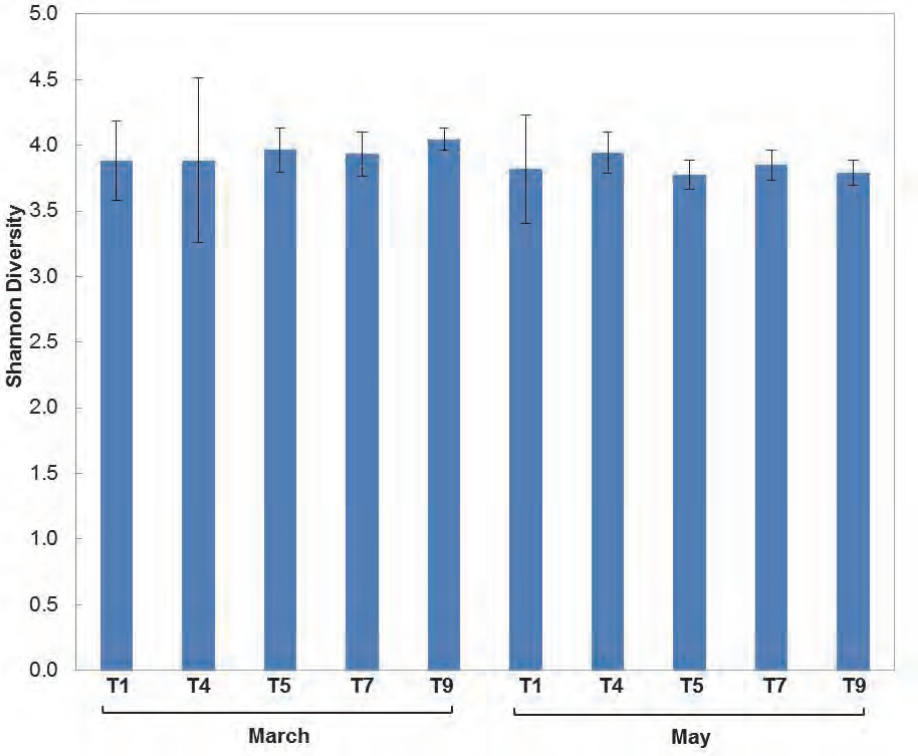

Figure 6.3. Bacterial diversity associated with treatments (=T) 1, 4, 5, 7 and 9 as determined in March and May 2012 after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

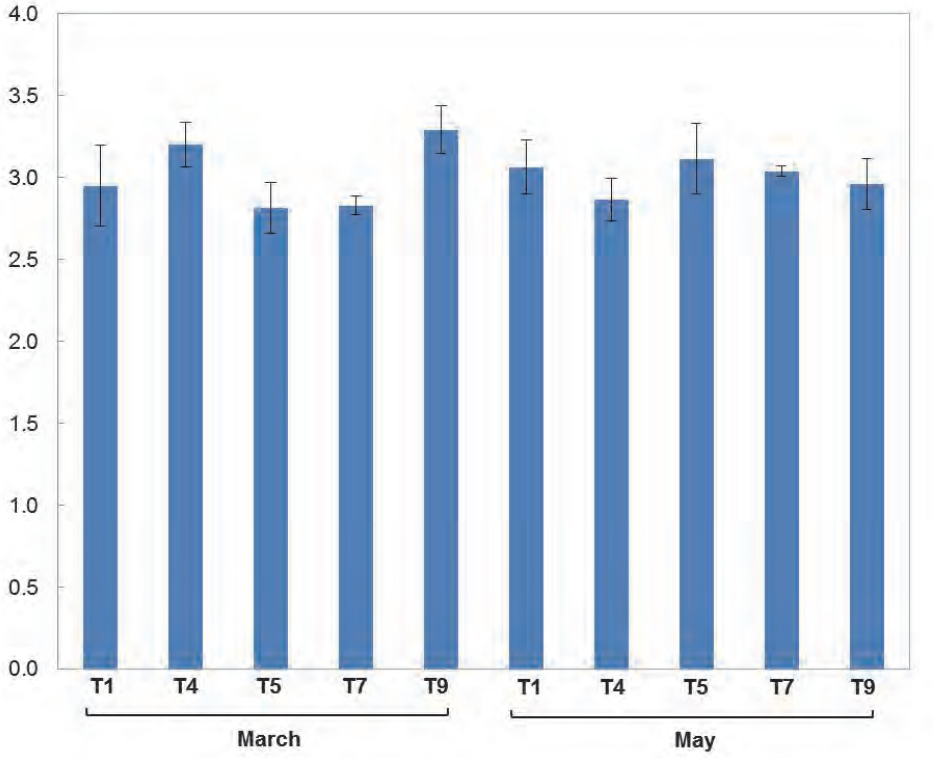

Figure 6.4. Fungal diversity associated with treatments (=T) 1, 4, 5, 7 and 9 as determined in March and May 2012 after winery wastewater treatments were applied to a vineyard in a sandy soil near Rawsonville.

In brief, the above results from seasons 2011, 2012 and 2013, jointly indicate that irrigation with winery wastewater did not have a remarkable impact on the diversity of the soil microbial community, neither the COD concentrations nor the interval between sampling times, mainly due to the lack of consistency between treatments and sampling times. Since it is possible for soil microbial communities to adapt to environmental conditions (Nielsen & Winding, 2002), change or adaption in the microbial community to winery wastewater was therefore, not detectable in the present study. It would nevertheless be informative to see which organisms in the microbial community were dominant and which were not, as microorganisms that are best adapted will usually be most dominant, and this may also be useful in determining responses to winery wastewater treatments in the microbial community. In this regard, identification of species for example, using denaturing gradient gel electrophoresis (DGGE), could be helpful in future research on this topic.

6.4. CONCLUSIONS

Winery wastewater irrigation had an effect on *β*-glucosidase activity, which was more pronounced in the top- than in the sub-soils since the activity decreased as the depth of the soil column increased. The *β*-glucosidase activity also increased as the COD concentration in winery wastewater increased, probably due to increases in organic loading, as well as due the strong relationship that *β*-glucosidase activity has with soil organic matter content and fertility. Enzyme activity also seemed to have been stimulated over time as more irrigation was applied. When assessed over the entire trial period, microbial population sizes decreased with soil depth, but the effect of different COD concentrations on microbial population sizes was inconclusive, so also the shifts in soil microbial communities, primarily due to inconsistent results. Glomalin content decreased with an increase in soil depth, but it remained unchanged in response to different COD treatments. Given that both glomalin and soil microbial enzyme activity are considered indicators of soil health, irrigation with winery wastewater should be of little to no consequence to general soil heath or that soil fertility may even be improved given the marked positive effects on soil microbial enzyme activity under the prevailing conditions in the present study.

The above findings should nevertheless be received with great caution as some of the findings should be substantiated with further research. The composition of winery wastewater is potentially quite different from cellar to cellar, as the conditions can be quite complex and unique from one vineyard to another. As such, the benefits and drawbacks with respect to the use of winery wastewater for irrigation purposes of vineyards should be carefully considered in terms of soil fertility, soil and plant health. It should be weighed up against clearly defined objectives, in recognition of the potential interactions between cultivar, unique site-specific characteristics, including climate, soil type, and the nutrient and

microbiology status of the soil. Further research is required to substantiate some of the findings presented in this report.

6.5. REFERENCES

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CHAPTER 7: EFFECT OF IRRIGATION WITH AUGMENTED WINERY WASTEWATER ON GRAPEVINE WATER STATUS, GROWTH, YIELD AND EVAPOTRANSPIRATON

C.L. Howell, P.A. Myburgh & E.L. Lategan
The Fruit, Vine and Wine Institute of the Agricultural Research Council, Private Bag X5026, Stellenbosch, 7599

7.1. INTRODUCTION

Although there is extensive literature available regarding the irrigation of grapevines with saline water (Walker *et al*., 1997; Stevens *et al*., 1999; Ben-Asher *et al*., 2006; Stevens *et al.*, 2011), very little is known about the effects of irrigation using augmented winery wastewater on grapevine, juice and wine responses. Recent studies have shown that approximately 3 to 5 m^3 of winery wastewater, with high organic load and variable salinity and nutrient levels, is generally produced when a ton of grapes is crushed (Mosse *et al.*, 2011). On the other hand, limited irrigation water supplies could be restricted further in future allocations of irrigation water (Van Zyl & Weber, 1981; Petrie *et al.*, 2004). If winery wastewater could be used to irrigate vineyards, with no detrimental impacts on either grapevines or subsequent wine quality and chemical composition, it could be a possible viable alternative to abstracting raw water from natural resources. In addition to this, guidelines as to what limits of quality criteria, *e.g.* level of COD, EC or SAR, of augmented winery wastewater, would be allow reuse with no negative consequences on grapevine, juice and yield responses.

Therefore, the objective of this study was to determine the effect of irrigation with augmented winery wastewater on grapevine water status, growth, yield and evapotranspiration (ET).

7.2. MATERIALS AND METHODS

7.2.1. Soil water content

The soil water content in the experiment vineyard was measured extensively using the neutron scattering technique. Access tubes were installed in the grapevine row in all plots. Refer to Chapter 2 for details of the experiment layout. Soil water content was measured over 30 cm increments to a depth of 1.8 m. In addition to this, soil water content for grapevines irrigated with wastewaters augmented to 250 mg/L COD and 3000 mg/L COD was measured to a depth of 2.1 m. A field calibration was carried out to convert neutron counts to volumetric soil water content. Soil water content was measured weekly from October, as well as before and after irrigations. After irrigations stopped in either April or May, soil water content was measured every two weeks.

7.2.2. Grapevine water status

Grapevine water status was quantified by measuring grapevine water potential in mature, unscathed leaves on primary shoots by means of the pressure chamber technique (Scholander *et al.*, 1965), according to the protocol described by Myburgh (2010). Predawn (Ψ_{PD}) and midday (Ψ_L) leaf water potentials, as well as midday stem (Ψ_S) water potential were measured in one leaf per plot. For Ψ_L measurements, leaves were covered in aluminium bags (Choné *et al.*, 2001; Myburgh, 2010) for at least one hour before Ψ_S measurements were carried out. Since the augmented wastewater irrigations only commenced after harvest in 2010, grapevine water status was not determined in the 2009/10 season. During the 2010/11 season, Ψ_{PD}, Ψ_L, and Ψ_S were measured during berry development (December) berry ripening (March). During the 2011/12 and 2012/13 growing seasons, Ψ_L and Ψ_S were only measured on selected days during berry ripening.

7.2.3. Vegetative growth

7.2.3.1. 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.

7.2.3.2. Leaf and shoot chemical status.

In order to allow maximum exposure to the wastewater *via* the irrigation, leaf samples were collected prior to harvest in the 2010/11 to 2012/13 seasons. Thirty mature, unscathed leaves opposite a bunch on the second spur were collected per plot. Shoot samples consisting of four primary canes per plot were collected at pruning in July. Leaf and shoot N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B contents were determined by a commercial laboratory (BEMLAB, Strand).

7.2.4. Yield and its components

In order to monitor berry development and ripening, berries were sampled from selected treatments, namely the raw water control (T1) and winery wastewater augmented to COD levels of 1000 mg/L (T5), 2000 mg/L (T7) and 3000 mg/L (T9), respectively. One hundred berries per plot were sampled approximately every ten days from middle December until harvest by collecting ten berries from each of the ten experiment grapevines therein. The mass of the berries was determined by weighing the samples in the laboratory at Stellenbosch using an electronic balance. The berry mass development measurements were only carried out in 2010/11 and 2011/12.

To determine berry mass at harvest, ten randomly selected bunches were picked from each experiment plot for all the treatments. Twenty berries were sampled from each of these

bunches in order to obtain a sample of 200 berries. Berries mass was determined by weighing the samples using an electronic balance. At harvest, all bunches of the experiment 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).

7.2.5. Evapotranspiration

The ET was determined by calculating a soil water balance on a weekly basis as described by Myburgh and Howell (2007). Monitoring soil water content to 1.8 m showed that almost no deep percolation occurred during the irrigation season. Consequently, drainage losses were not accounted for in the soil water balance equation. Daily ET was used to calculate mean monthly values.

7.2.6. Statistical analyses

The data were subjected to an analysis of variance. Least significant difference (LSD) values were calculated to facilitate comparison between treatment means. Means that differed at $p \leq 0.05$ were considered to be significantly different. STATGRAPHICS® was used for the analyses of variance.

7.3 RESULTS AND DISCUSSION

7.3.1 Soil water content

During the four seasons, irrigation using augmented winery wastewater had no effect on the soil water status compared raw river water. Therefore, only trends in the mean soil water content for each season will be presented and discussed.

2009/10 season: The experiment vineyard was drip irrigated once a week for 12 hours from the end of November until the first irrigation with the micro-sprinkler system to be used in the field trial was applied in February. Since the grower applied the drip irrigation according to a continuous deficit strategy, the soil was relatively dry at that stage (Fig. 7.1). Initially, one of the major concerns was the efficiency of the mixing process in the tanks. Consequently, the objective of the first micro-sprinkler irrigation was to wet the total soil volume thoroughly using only raw water from the river. Since the infrastructure was only completed at the end of January 2010, irrigation using augmented winery wastewater only commenced after the grapes had been picked.

Under the prevailing conditions, three irrigations could be applied during the post-harvest period. Due to late ripening of the 2010 harvest in general, the winery was still crushing

grapes when the first two irrigations were applied. The relatively high soil water content indicated that most layers were still saturated when the soil water content was measured shortly after the irrigation was stopped (Fig. 7.1). However, the soil water content in the 150 cm to 180 cm layer only showed an increase six days later. This indicated that percolation from the saturated shallower soil layers into the deep layer must have occurred in the first few days following the irrigation. Smaller irrigations, *i.e.* approximately 55 mm, were applied when the wastewater treatments commenced. These irrigations only wetted the soil to a depth of *c.* 90 cm and the soil water content measurements showed that no percolation into the deeper layers occurred (Fig. 7.1). As a result, the soil water content in the deepest layers remained fairly constant in the period following the first irrigation. The day after the third irrigation was applied in May, 85 mm rainfall was recorded at the field trial. The combined effect of the irrigation and the rainfall saturated the upper soil layers to such an extent that deep percolation substantially increased the soil water content in the deepest layers.

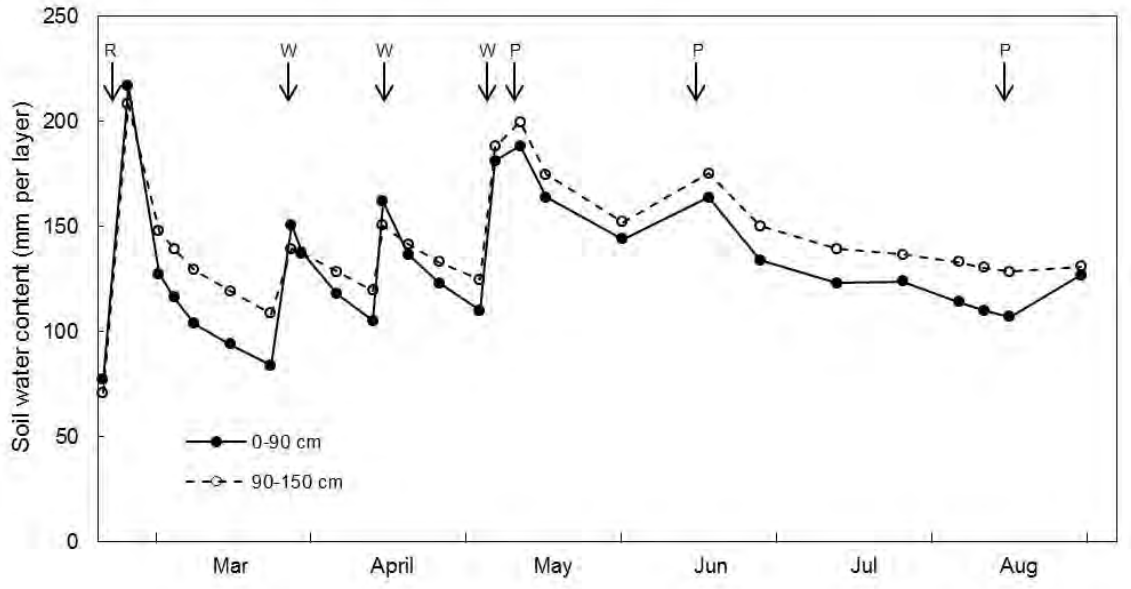

Figure 7.1. Variation in soil water content during the 2009/10 season where augmented winery wastewater was used to irrigate Cabernet Sauvignon grapevines in a sandy soil near Rawsonville. (P = precipitation, R = raw water irrigation and W = wastewater irrigation).

2010/11 season: Due to the relatively low winter rainfall in 2010, the soil was relatively dry at bud break in September (Fig. 7.2). Despite the relatively dry soil conditions, grapevine vegetative growth did not show any visual signs of water constraints, and the first irrigation was only applied in December 2010. The first of the six wastewater irrigations was applied on 9 February 2011. The wastewater irrigations were applied at *c.* 14 day intervals. The different wastewater irrigation treatments did not have any effect on the soil water status

compared to the raw water control (data not shown). The objective was (i) to apply irrigation only within the grapevine root zone, *i.e.* < 90 cm, in order to prevent leaching to the deeper layers and (ii) to allow element uptake by the pearl millet interception crop. This was achieved until the interception crop was removed, but once this crop was removed the irrigation water percolated into the deeper soil layers (Fig. 7.2). Rainfall in May (94 mm), June (150 mm) and July (56 mm) also caused percolation into the deeper layers.

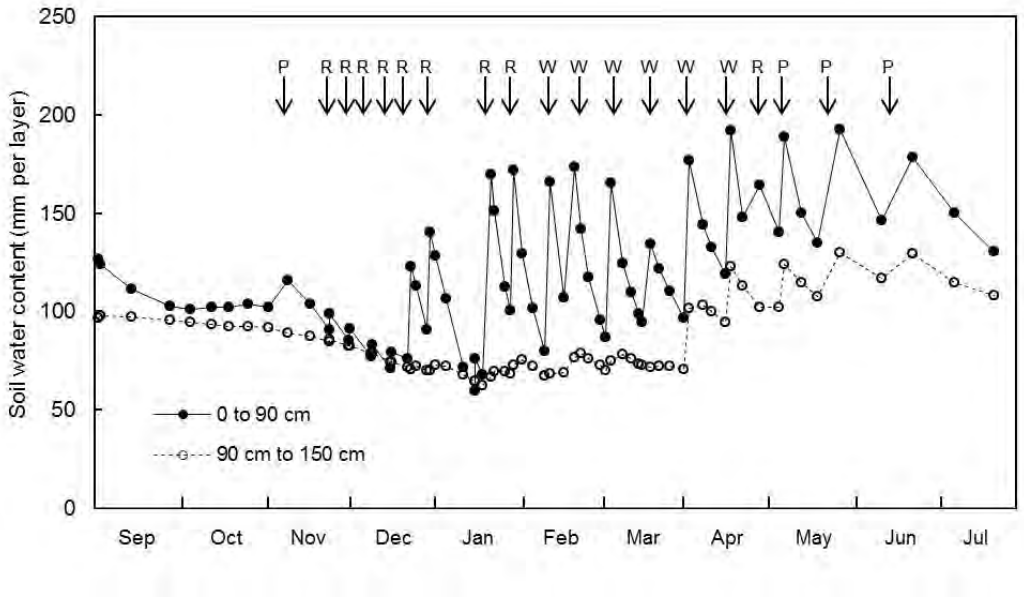

Figure 7.2. Variation in soil water content during the 2010/11 season where augmented winery wastewater was used to irrigate Cabernet Sauvignon grapevines in a sandy soil near Rawsonville. (P = precipitation, R = raw water irrigation and W = wastewater irrigation).

2011/12 season: Due to the winter rains during 2011, the soil was relatively wet at bud break in September (Fig. 7.3). The first raw water irrigation to supply the grapevine's water requirements was only applied in middle December 2011. The second raw water irrigation was required early January 2012, which was followed by three weekly raw water irrigations of 16 mm each to supply the water requirements of the pearl millet summer cover crop. The grapevines were irrigated twice during February 2012 with raw river water. Since inadequate volumes of suitable winery wastewater produced in February, the first of the five wastewater irrigations could only be applied on 6 March. The wastewater irrigations were applied at *c.* 14 day intervals. The different wastewater irrigation treatments did not have any effect on the soil water status compared to the raw water control (data not shown). The objective was to apply irrigation only within the grapevine root zone, *i.e.* 0 to 90 cm depth, in order to prevent leaching of elements into the deeper layers. When established in November 2010, the pearl millet interception crop increased the vineyard evapotranspiration (ET) from November until February compared to the same period in the 2009/10 season. Since the pearl millet was only established in January 2012, ET for

January and February was lower compared to the previous season. These results confirmed that a summer interception crop established earlier in the season, *e.g.* in November, will increase the ET of vineyards substantially compared to clean cultivated or mulched soil surfaces.

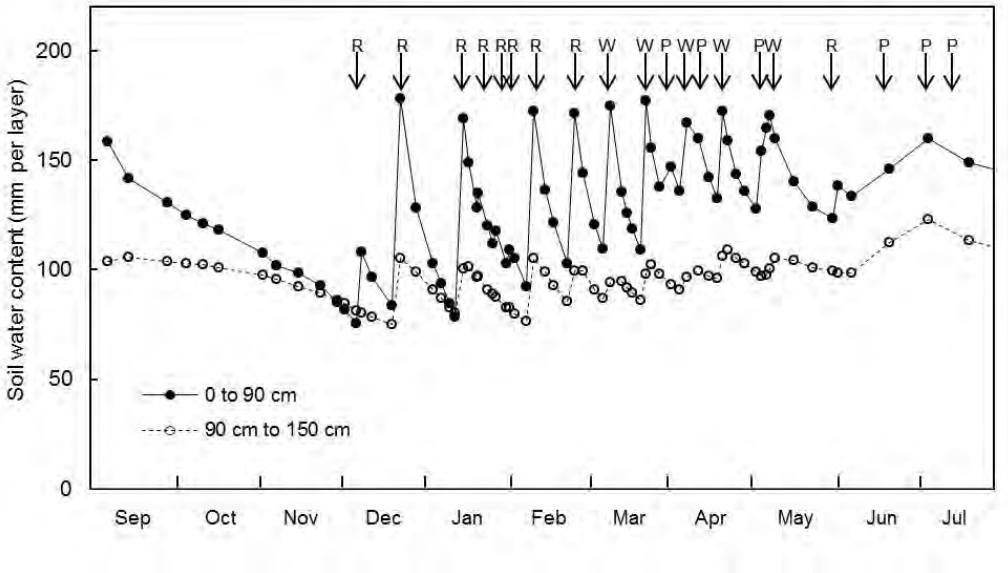

Figure 7.3. Variation in soil water content during the 2011/12 season where augmented winery wastewater was used to irrigate Cabernet Sauvignon grapevines in a sandy soil near Rawsonville. (P = precipitation, R = raw water irrigation and W = wastewater irrigation).

2012/13 season: Due to the winter rains during 2012, the soil was relatively wet at bud break in September (Fig. 7.4). The first raw water irrigation to supply the grapevine's water requirements was only applied towards the end of December 2012. In early January 2013, raw water irrigation was applied to facilitate the cultivation of the soil for the planting of the pearl millet summer crop. This was followed by three weekly raw water irrigations of 16 mm each to supply the water requirements of this crop. The second raw water irrigation for the vineyard was applied in early February 2013. The first of the six wastewater irrigations was applied on 14 February, and these irrigations were applied at *ca.* 14 day intervals until the middle of March. However, on 25 March the COD level of the wastewater in the stock dam was too low (< 5000 mg/L) to have sufficient wastewater to make up all the 15 m^3 tanks. On 26 March, the COD levels were higher than 30 000 mg/L. It was therefore decided to postpone the irrigation for a week to obtain more suitable water. On 2 April, the COD level of the stock dam was *ca*. 9750 mg/L and wastewater irrigations were applied every two weeks until the end of April. In May 2013, river water irrigation was applied to the oats cover crop. The different wastewater irrigation treatments did not have any effect on the soil water status compared to the raw water control (data not shown). Irrigation was applied only to the upper soil layers, *i.e.* 0 to 60 cm depth, to prevent leaching of elements into the deeper

layers. In addition, such a continuous deficit irrigation strategy would reduce excessive growth and enhance ripening. However, a rainfall event of 67 mm after the wastewater irrigation in mid-April probably leached elements into the deeper layers. It was evident that the continuous deficit irrigation strategy also reduced ET in January 2013 compared to January 2012. Furthermore, the pearl millet interception crop did not increase ET substantially during the ripening period.

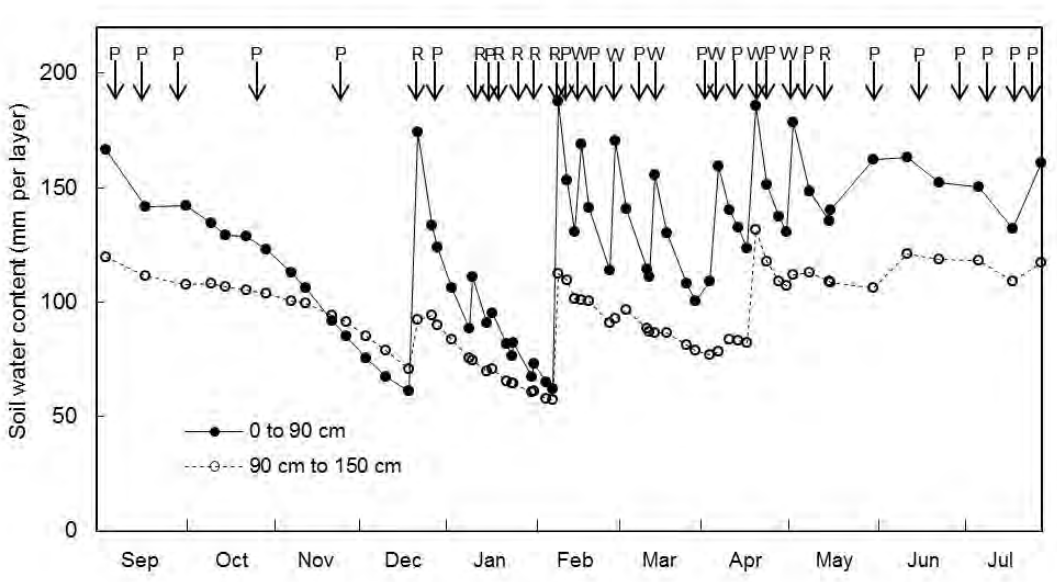

Figure 7.4. Seasonal variation in soil water content during the 2012/13 season where augmented winery wastewater was used to irrigate Cabernet Sauvignon grapevines in a sandy soil near Rawsonville. (P = precipitation, R = raw water irrigation and W = wastewater irrigation).

7.3.2. Grapevine water status

Measurements, carried out in the 2010/11 season showed that Ψ_{PD} was around -0.2 MPa (Table 7.1) which is the lower threshold for no water constraints (Deloire *et al.*, 2004). This confirmed that the water status in the grapevines was able to fully recover during the night under the given conditions. During daytime, the grapevines only experienced low water constraints, *i.e.* Ψ_L ranged between -1.0 MPa and -1.4 MPa, *i.e.* the Ψ_L thresholds according to Greenspan (2005). The low daytime water constraints were substantiated by ranging between -0.4 MPa and -1.0 MPa, *i.e.* the Ψ_S thresholds proposed by Van Leeuwen *et al.* (2009). The foregoing indicated that grapevine only experienced low water constraints, irrespective of the grapevine's phenological stage. Furthermore, irrigation with the augmented wastewaters clearly had no effect on the grapevine water status compared to the raw water control.

In the 2011/12 season, midday Ψ_L and Ψ_S values again ranged between the low constraints thresholds as mentioned above (Tables 7.2 & 7.3). When deficit irrigation was applied in the

2012/13 season, midday Ψ_L and Ψ_S also remained between the thresholds for low water constraints (data not shown). These results confirmed that that the grapevines experienced low water constraints throughout the study period, and that the different augmented winery wastewater treatments clearly had no effect on the daytime grapevine water status compared to the raw water control. Therefore, it can be assumed that the functioning of other physiological processes would not have been negatively affected by water deficits.

Table 7.1. Predawn (Ψ_{PD}), as well as midday leaf (Ψ_L) and stem (Ψ_S) water potential in Cabernet Sauvignon/99R irrigated using augmented winery wastewater during the 2010/11 season.

Treatment no. & target COD (mg/L)	Ψ_{PD} (MPa)		Ψ_L (MPa)		Ψ_S (MPa)	
	08 Dec	02 Mar	08 Dec	15 Mar	08 Dec	15 Mar
T1 - Control[1]	-0.20 a[2]	-0.24 a	-1.23 a	-1.33 a	-0.81 a	-0.68 a
T2 - 100	-0.18 a	-0.20 a	-1.13 a	-1.15 a	-0.70 a	-0.54 a
T3 - 250	-0.19 a	-0.23 a	-1.24 a	-1.15 a	-0.79 a	-0.65 a
T4 - 500	-0.23 a	-0.25 a	-1.11 a	-1.17 a	-0.74 a	-0.63 a
T5 - 1000	-0.18 a	-0.23 a	-1.22 a	-1.14 a	-0.76 a	-0.68 a
T6 - 1500	-0.18 a	-0.23 a	-1.25 a	-1.18 a	-0.78 a	-0.60 a
T7 - 2000	-0.20 a	-0.23 a	-1.26 a	-1.18 a	-0.73 a	-0.68 a
T8 - 2500	-0.21 a	-0.25 a	-1.08 a	-1.18 a	-0.68 a	-0.64 a
T9 - 3000	-0.21 a	-0.25 a	-1.18 a	-1.23 a	-0.75 a	-0.60 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 7.2. Midday leaf water potential (Ψ_L) in Cabernet Sauvignon/99R irrigated using augmented winery wastewater during the 2011/12 season.

Treatment no. & target COD (mg/L)	Ψ_L (MPa)				
	08 March	19 March	21 March	02 April	18 April
T1 - Control[1]	-1.03 a[2]	-1.03 a	-1.10 a	-0.92 a	-1.20 a
T2 - 100	-0.90 a	-0.91 a	-1.14 a	-0.99 a	-1.13 a
T3 - 250	-0.88 a	-0.83 a	-1.21 a	-1.03 a	-1.11 a
T4 - 500	-0.91 a	-1.02 a	-1.18 a	-0.94 a	-1.05 a
T5 - 1000	-1.01 a	-1.03 a	-1.20 a	-0.94 a	-1.25 a
T6 - 1500	-0.90 a	-1.16 a	-1.15 a	-0.88 a	-1.25 a
T7 - 2000	-0.85 a	-0.83 a	-1.14 a	-1.03 a	-1.10 a
T8 - 2500	-0.87 a	-1.24 a	-1.15 a	-0.98 a	-1.17 a
T9 - 3000	-0.89 a	-1.02 a	-1.23 a	-1.03 a	-1.10 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 7.3. Midday stem (Ψ_S) water potential in Cabernet Sauvignon/99R irrigated using augmented winery wastewater during the 2011/12 season.

Treatment no. & target COD (mg/L)	Ψ_S (MPa)				
	08 March	19 March	21 March	02 April	18 April
T1 - Control[(1)]	-0.61 a	-0.58 a	-0.56 a	-0.51 a	-0.64 a
T2 - 100	-0.53 a	-0.43 a	-0.51 a	-0.45 a	-0.51 a
T3 - 250	-0.54 a	-0.55 a	-0.53 a	-0.49 a	-0.60 a
T4 - 500	-0.48 a	-0.53 a	-0.57 a	-0.59 a	-0.55 a
T5 - 1000	-0.55 a	-0.56 a	-0.55 a	-0.58 a	-0.67 a
T6 - 1500	-0.49 a	-0.53 a	-0.53 a	-0.47 a	-0.66 a
T7 - 2000	-0.52 a	-0.55 a	-0.50 a	-0.50 a	-0.57 a
T8 - 2500	-0.52 a	-0.56 a	-0.55 a	-0.55 a	-0.68 a
T9 - 3000	-0.58 a	-0.63 a	-0.53 a	-0.58 a	-0.63 a

[(1)] Raw water abstracted from the Holsloot River.
[(2)] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

7.3.3. Vegetative growth

7.3.3.1. Leaf and shoot chemical status

Due to high costs of chemical analyses, only leaf and shoot samples of Replication 2 was analysed. Samples of the other two replications were dried and stored, only to be analysed if Replication 2 indicated that the leaf and/or shoot chemical status consistently responded to the level augmentation of the winery wastewater. Therefore, only the standard deviation from the mean for a data set is presented in Tables 7.4 to 7.6. With the exception of Ca, the augmented winery wastewater irrigation treatments did not affect the chemical composition of the leaf blades and shoots compared to the raw water control. In the 2011/12 season, leaf Ca tended to decrease as the level of augmentation of the wastewater decreased, *i.e.* as level of COD increased (Table 7.5). Shoot Ca did not show such a prominent trend. In the 2012/13 season, leaf blade and shoot Ca also tended to decrease as the level of COD in the augmented winery wastewater increased (Table 7.6). At this stage, there is no explanation for the Ca trend. According to norms for grapevine nutrient levels in leaves (Conradie, 1994), *i.e.* 1.6-2.7% for N, 0.14-0.55% for P, 0.65-1.3% for K, 1.2-2.2% for Ca, and 0.16-0.55% for Mg, none of the macro elements were at deficient levels during any of the seasons, except for low K in 2012/13. The latter was probably due a competition effect of the pearl millet (*Pennisetum glaucum* L.) in summer. Otherwise, the pearl millet and oats (*Avena sativa* L. cv. Pallinup) combination in winter did not seem to have any negative effects on grapevine nutrient status under the given conditions. The leaf Na content was well below 0.25%, *i.e.* the maximum for grapevines (Conradie, 1994), thereby reflecting the low sodicity risk of the augmented winery wastewater under the given conditions.

Table 7.4. Nutrient status of Cabernet Sauvignon leaf blades sampled prior to harvest in March and shoots sampled in the winter of 2011.

Treatment no. & target COD (mg/L)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	B (mg/kg)
Leaf blades											
T1 - Control[1]	2.04±0.08[2]	0.18±0.02	0.78±0.08	2.17±0.3	0.54±0.03	187±19	160±26	376±60	281±58	84±13	36±4
T2 - 100	1.82±0.08	0.16±0.02	0.77±0.08	1.82±0.3	0.51±0.03	202±19	95±26	271±60	142±58	49±13	46±4
T3 - 250	1.82±0.08	0.17±0.02	0.75±0.08	1.68±0.3	0.48±0.03	193±19	89±26	206±60	138±58	49±13	45±4
T4 - 500	1.85±0.08	0.16±0.02	0.67±0.08	2.09±0.3	0.55±0.03	225±19	84±26	223±60	151±58	47±13	39±4
T5 - 1000	1.90±0.08	0.15±0.02	0.70±0.08	1.90±0.3	0.51±0.03	184±19	101±26	205±60	105±58	53±13	34±4
T6 - 1500	1.95±0.08	0.16±0.02	0.75±0.08	1.89±0.3	0.49±0.03	172±19	92±26	220±60	116±58	47±13	41±4
T7 - 2000	1.84±0.08	0.14±0.02	0.69±0.08	1.42±0.3	0.46±0.03	172±19	75±26	189±60	97±58	43±13	47±4
T8 - 2500	1.82±0.08	0.13±0.02	0.55±0.08	1.43±0.3	0.49±0.03	163±19	75±26	177±60	91±58	43±13	40±4
T9 - 3000	1.95±0.08	0.17±0.02	0.82±0.08	1.78±0.3	0.51±0.03	178±19	96±26	223±60	114±58	52±13	42±4
Shoots											
T1 - Control[1]	2.11±0.05	0.13±0.01	0.52±0.05	0.42±0.05	0.15±0.02	242±23	13±0.9	55±15	4±0.5	26±10	13±2
T2 - 100	1.96±0.05	0.14±0.01	0.49±0.05	0.41±0.05	0.15±0.02	189±23	12±0.9	56±15	4±0.5	30±10	11±2
T3 - 250	2.07±0.05	0.15±0.01	0.51±0.05	0.41±0.05	0.16±0.02	190±23	11±0.9	47±15	3±0.5	42±10	11±2
T4 - 500	2.00±0.05	0.14±0.01	0.51±0.05	0.41±0.05	0.16±0.02	197±23	13±0.9	45±15	4±0.5	48±10	11±2
T5 - 1000	1.98±0.05	0.12±0.01	0.45±0.05	0.37±0.05	0.13±0.02	204±23	14±0.9	37±15	3±0.5	47±10	10±2
T6 - 1500	2.01±0.05	0.12±0.01	0.47±0.05	0.34±0.05	0.11±0.02	170±23	12±0.9	22±15	3±0.5	21±10	9±2
T7 - 2000	1.99±0.05	0.13±0.01	0.40±0.05	0.31±0.05	0.13±0.02	171±23	12±0.9	16±15	3±0.5	37±10	8±2
T8 - 2500	1.99±0.05	0.12±0.01	0.37±0.05	0.31±0.05	0.13±0.02	165±23	12±0.9	19±15	3±0.5	29±10	8±2
T9 - 3000	2.02±0.05	0.11±0.01	0.42±0.05	0.34±0.05	0.12±0.02	187±23	12±0.9	28±15	3±0.5	29±10	9±2

[1] Raw water abstracted from the Holsloot River.

Table 7.5. Nutrient status of Cabernet Sauvignon leaf blades sampled prior to harvest in March and shoots sampled in the winter of 2012.

Treatment no. & target COD (mg/L)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	B (mg/kg)
Leaf blades											
T1 - Control[1]	1.73±0.05[2]	0.15±0.03	0.76±0.08	2.39±0.19	0.68±0.04	262±23	116±17	332±51	28±3.4	30±3.2	45±2
T2 - 100	1.80±0.05	0.22±0.03	0.72±0.08	2.28±0.19	0.64±0.04	227±23	106±17	360±51	27±3.4	32±3.2	47±2
T3 - 250	1.70±0.05	0.13±0.03	0.59±0.08	2.02±0.19	0.63±0.04	191±23	91±17	204±51	23±3.4	32±3.2	43±2
T4 - 500	1.74±0.05	0.14±0.03	0.56±0.08	2.14±0.19	0.66±0.04	238±23	83±17	223±51	25±3.4	32±3.2	46±2
T5 - 1000	1.68±0.05	0.13±0.03	0.58±0.08	2.24±0.19	0.62±0.04	206±23	137±17	238±51	33±3.4	33±3.2	44±2
T6 - 1500	1.77±0.05	0.15±0.03	0.62±0.08	2.15±0.19	0.60±0.04	202±23	88±17	276±51	32±3.4	28±3.2	42±2
T7 - 2000	1.77±0.05	0.12±0.03	0.50±0.08	1.93±0.19	0.63±0.04	192±23	93±17	241±51	25±3.4	23±3.2	44±2
T8 - 2500	1.75±0.05	0.13±0.03	0.62±0.08	1.83±0.19	0.61±0.04	209±23	105±17	251±51	30±3.4	33±3.2	42±2
T9 - 3000	1.84±0.05	0.17±0.03	0.71±0.08	1.90±0.19	0.54±0.04	212±23	94±17	254±51	26±3.4	31±3.2	39±2
Shoots											
T1 - Control[1]	0.83±0.05	0.13±0.01	0.61±0.05	0.35±0.19	0.12±0.02	115±35	16±3	54±11	5±0.5	18±8	12±2
T2 - 100	0.84±0.05	0.13±0.01	0.59±0.05	0.36±0.19	0.14±0.02	162±35	9±3	37±11	5±0.5	28±8	11±2
T3 - 250	0.77±0.05	0.12±0.01	0.50±0.05	0.38±0.19	0.14±0.02	156±35	6±3	26±11	5±0.5	23±8	10±2
T4 - 500	0.81±0.05	0.12±0.01	0.47±0.05	0.36±0.19	0.13±0.02	150±35	13±3	24±11	5±0.5	24±8	10±2
T5 - 1000	0.73±0.05	0.11±0.01	0.49±0.05	0.39±0.19	0.12±0.02	130±35	15±3	23±11	5±0.5	20±8	9±2
T6 - 1500	0.84±0.05	0.13±0.01	0.53±0.05	0.35±0.19	0.14±0.02	158±35	13±3	27±11	6±0.5	30±8	10±2
T7 - 2000	0.78±0.05	0.12±0.01	0.51±0.05	0.38±0.19	0.16±0.02	177±35	12±3	37±11	6±0.5	29±8	17±2
T8 - 2500	0.87±0.05	0.12±0.01	0.54±0.05	0.37±0.19	0.17±0.02	220±35	11±3	22±11	6±0.5	43±8	13±2
T9 - 3000	0.88±0.05	0.13±0.01	0.57±0.05	0.44±0.19	0.16±0.02	213±35	14±3	22±11	6±0.5	38±8	13±2

[1] Raw water abstracted from the Holsloot River.

Table 7.6. Nutrient status of Cabernet Sauvignon leaf blades sampled prior to harvest in March and shoots sampled in the winter of 2013.

Treatment no. & target COD (mg/L)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	B (mg/kg)
Leaf blades											
T1 - Control[1]	1.91±0.09[2]	0.22±0.03	0.45±0.04	2.88±0.25	1.01±0.10	144±21	51±17	258±50	39±6.1	25±1.9	44±4.4
T2 - 100	2.18±0.09	0.25±0.03	0.49±0.04	2.87±0.25	0.88±0.10	139±21	46±17	424±50	43±6.1	22±1.9	49±4.4
T3 - 250	2.20±0.09	0.23±0.03	0.43±0.04	2.48±0.25	0.80±0.10	158±21	45±17	282±50	40±6.1	26±1.9	44±4.4
T4 - 500	2.15±0.09	0.19±0.03	0.46±0.04	2.47±0.25	0.75±0.10	191±21	45±17	365±50	45±6.1	27±1.9	45±4.4
T5 - 1000	2.03±0.09	0.16±0.03	0.51±0.04	2.32±0.25	0.68±0.10	175±21	62±17	321±50	53±6.1	25±1.9	41±4.4
T6 - 1500	2.03±0.09	0.19±0.03	0.49±0.04	2.48±0.25	0.79±0.10	135±21	46±17	301±50	37±6.1	23±1.9	55±4.4
T7 - 2000	2.07±0.09	0.18±0.03	0.53±0.04	2.30±0.25	0.77±0.10	146±21	48±17	344±50	45±6.1	25±1.9	52±4.4
T8 - 2500	2.08±0.09	0.17±0.03	0.49±0.04	2.19±0.25	0.79±0.10	187±21	54±17	361±50	55±6.1	28±1.9	49±4.4
T9 - 3000	2.11±0.09	0.19±0.03	0.56±0.04	2.30±0.25	0.71±0.10	156±21	47±17	342±50	48±6.1	27±1.9	49±4.4
Shoots											
T1 - Control[1]	1.09±0.09	0.12±0.01	0.34±0.04	0.38±0.06	0.19±0.03	288±41	16±4	84±57	6±0.7	27±10	11±1.2
T2 - 100	0.90±0.09	0.08±0.01	0.19±0.04	0.27±0.06	0.12±0.03	169±41	6±4	49±57	4±0.7	13±10	7±1.2
T3 - 250	0.96±0.09	0.10±0.01	0.25±0.04	0.37±0.06	0.16±0.03	180±41	16±4	149±57	5±0.7	25±10	10±1.2
T4 - 500	1.05±0.09	0.11±0.01	0.28±0.04	0.40±0.06	0.18±0.03	226±41	18±4	180±57	5±0.7	44±10	11±1.2
T5 - 1000	1.05±0.09	0.12±0.01	0.24±0.04	0.37±0.06	0.21±0.03	238±41	17±4	115±57	5±0.7	38±10	10±1.2
T6 - 1500	0.88±0.09	0.09±0.01	0.25±0.04	0.30±0.06	0.14±0.03	224±41	9±4	52±57	4±0.7	16±10	10±1.2
T7 - 2000	0.83±0.09	0.09±0.01	0.28±0.04	0.27±0.06	0.15±0.03	178±41	10±4	48±57	6±0.7	25±10	10±1.2
T8 - 2500	0.96±0.09	0.10±0.01	0.25±0.04	0.31±0.06	0.20±0.03	228±41	11±4	29±57	5±0.7	34±10	9±1.2
T9 - 3000	0.92±0.09	0.09±0.01	0.25±0.04	0.24±0.06	0.13±0.03	167±41	12±4	12±57	5±0.7	27±10	9±1.2

[1] Raw water abstracted from the Holsloot River.

7.3.3.2. Cane mass

The various augmented wastewater irrigation treatments consistently had no effect on vegetative growth of the grapevines compared to the raw water control (Table 7.7). The lack of differences was to be expected, since the wastewater irrigation had no effect on grapevine water status, or the chemical status of the leaves and shoots as discussed above. The cane mass was slightly higher in the 2011/12 season compared to the 2010/11 season, but comparable to the 2009/10 season. In 2013, the cane mass was slightly higher compared to the 2010/11 season, but comparable to the values reported for the 2009/10 and 2011/12 seasons. Generally, the cane mass was comparable to the values reported for frequently drip irrigated Cabernet Sauvignon grapevines in sandy soils in the Lower Olifants River Valley (Bruwer, 2010). However, the vegetative growth vigour was substantially higher compared to non-irrigated grapevines in the Swartland region (Mehmel, 2010). The foregoing suggested that the cover crop combination of pearl millet (*Pennisetum glaucum* L.) in summer and oats (*Avena sativa* L. cv. Pallinup) in winter did not have a pronounced negative effect on the vegetative growth of the grapevines under the given conditions.

Table 7.7. Effect of irrigation using augmented winery wastewater on cane mass at pruning in July of Cabernet Sauvignon/99R measured during four seasons near Rawsonville.

Treatment no. & target COD (mg/L)	Cane mass (t/ha)			
	2009/10	2010/11	2011/12	2012/13
T1 - Control[1]	2.9 a[2]	2.3 a	2.7 a	2.8 a
T2 - 100	3.1 a	2.5 a	2.8 a	2.8 a
T3 - 250	2.8 a	2.4 a	2.7 a	2.7 a
T4 - 500	3.0 a	2.4 a	2.8 a	2.7 a
T5 - 1000	2.9 a	2.2 a	2.6 a	2.7 a
T6 - 1500	2.6 a	1.9 a	2.5 a	2.6 a
T7 - 2000	2.9 a	2.0 a	2.4 a	2.3 a
T8 - 2500	2.8 a	2.3 a	2.5 a	2.7 a
T9 - 3000	3.0 a	2.2 a	2.8 a	2.8 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

7.3.4. Yield and its components

Bunches per grapevine: Low grapevine fertility occurred throughout the Breede River region in the 2010/11 season. This was probably caused by unfavourable atmospheric conditions during bunch initiation in the preceding year. The various augmented wastewater irrigation treatments had no effect on grapevine fertility quantified in terms of the number of bunches

per grapevine, except in the 2010/11 season (Table 7.8). In this particular season, the bunch numbers of T7 grapevines were lower compared to some of the other treatments. This result was co-incidental, and probably occurred since many grapevines had to be pruned severely in July 2010 to obtain a more correct grapevine structure.

Table 7.8. Effect of irrigation using augmented winery wastewater on number of bunches per grapevine of Cabernet Sauvignon/99R measured during four seasons near Rawsonville.

Treatment no. & target COD (mg/L)	Bunches per grapevine			
	2009/10	2010/11	2011/12	2012/13
T1 - Control[1]	28 a[2]	23 ab	33 a	29 a
T2 - 100	26 a	24 a	31 a	28 a
T3 - 250	27 a	23 ab	32 a	30 a
T4 - 500	28 a	22 ab	32 a	29 a
T5 - 1000	30 a	24 a	33 a	31 a
T6 - 1500	29 a	23 ab	30 a	27 a
T7 - 2000	28 a	19 b	32 a	28 a
T8 - 2500	29 a	24 a	31 a	30 a
T9 - 3000	26 a	23 ab	31 a	28 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Berry size: In the 2010/11 season, berry development of the selected treatments showed the typical double sigmoid curve expected for grapes (Fig. 7.5). In the 2011/12 season berry development showed the same trend (data not shown). However, the various augmented wastewater irrigation treatments had no effect on berry size development during both seasons. This was probably due to lack of differences in grapevine water status between the various wastewater treatments and the control during berry development (Table 7.1). Over the four seasons, irrigation with augmented winery wastewater had no effect on berry mass at harvest compared to the raw water control (Table 7.9). Mean berry at harvest mass ranged between 1.2 and 1.5 g/berry, which was comparable to values reported for drip irrigated Cabernet Sauvignon in the Breede River valley (Roux, 2005). Where Cabernet is subjected severe water constraints, *i.e.* Ψ_L below 1.6 MPa, berry mass is expected to be *c.* 1 g/berry (Bruwer, 2010; Mehmel, 2010). The foregoing confirmed that the grapevines experienced low levels of water constraints. Since there were no differences in grapevine water status, there is no explanation for the smaller berries of T9 grapevines compared to T2 grapevines as determined at harvest in March 2011 (Table 7.9).

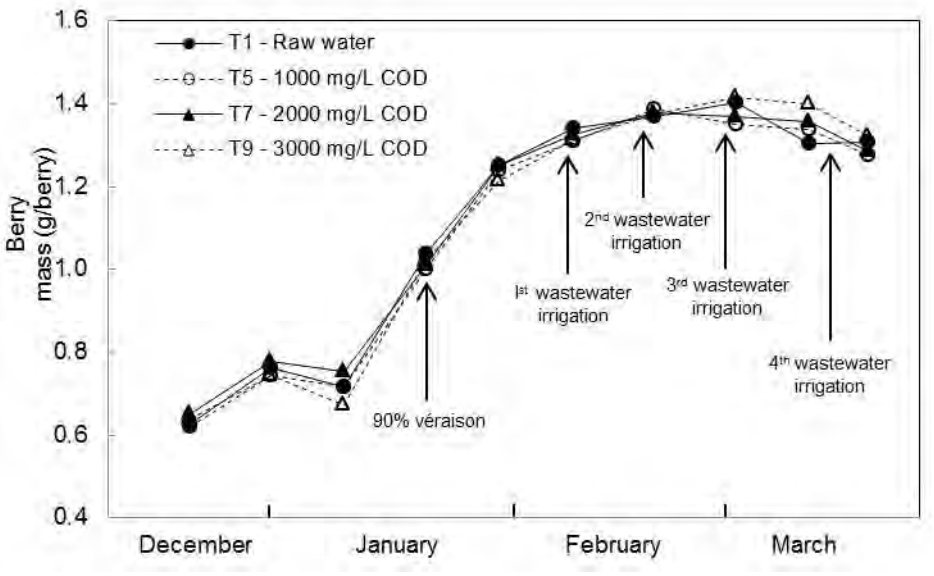

Figure 7.5. Effect of irrigation using augmented winery wastewater on berry mass development of Cabernet Sauvignon/99R measured during the 2010/11 season near Rawsonville.

Table 7.9. Effect of irrigation using augmented winery wastewater on berry mass of Cabernet Sauvignon/99R measured at harvest during four seasons near Rawsonville.

Treatment no. & target COD (mg/L)	Berry mass (g)			
	2009/10	2010/11	2011/12	2012/13
T1 - Control[1]	1.19 a[2]	1.31 ab	1.36 a	1.38 a
T2 - 100	1.28 a	1.38 a	1.38 a	1.48 a
T3 - 250	1.22 a	1.34 ab	1.31 a	1.38 a
T4 - 500	1.25 a	1.31 ab	1.34 a	1.34 a
T5 - 1000	1.19 a	1.28 ab	1.33 a	1.32 a
T6 - 1500	1.25 a	1.36 ab	1.36 a	1.41 a
T7 - 2000	1.23 a	1.32 ab	1.36 a	1.30 a
T8 - 2500	1.28 a	1.36 ab	1.41 a	1.42 a
T9 - 3000	1.24 a	1.27 b	1.35 a	1.27 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Bunch mass: However, the lower berry mass reflected in smaller bunches (Table 7.10) and lower yield (Table 7.11) compared to some of the other treatments. In the case of the T7 grapevines, the low bunch number (Table 7.9) seemed to have caused the lower yield compared to most of the other treatments. Since yield showed no trend with respect to level augmentation in this particular season, the lower yield was definitely not the result of irrigation with augmented winery wastewater.

Table 7.10. Effect of irrigation using augmented winery wastewater on bunch mass of Cabernet Sauvignon/99R measured during four seasons near Rawsonville.

Treatment no. & target COD (mg/L)	Bunch mass (g)			
	2009/10	2010/11	2011/12	2012/13
T1 - Control[1]	148 a[2]	126 ab	153 a	174 a
T2 - 100	156 a	135 ab	156 a	167 a
T3 - 250	142 a	139 a	155 a	164 a
T4 - 500	153 a	139 a	163 a	167 a
T5 - 1000	149 a	126 ab	152 a	173 a
T6 - 1500	137 a	136 ab	165 a	169 a
T7 - 2000	146 a	123 ab	150 a	154 a
T8 - 2500	148 a	134 ab	173 a	169 a
T9 - 3000	149 a	119 b	142 a	164 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Yield: The various wastewater irrigation treatments had no effect on grapevine yield compared to the raw water control, except in the 2010/11 (Table 7.7). However, the observed differences in the 2010/11 season were not related to level of wastewater augmentation. It was probably a result of the severe pruning applied in July 2010 to obtain more correct grapevine structures. mean yield was lower compared to 2009/10 (Table 7.11). Lower grapevine fertility in the region, as well as the severe pruning probably caused the generally lower yields in the 2010/11 season. During the other seasons, mean yields were comparable to that of frequently drip irrigated Cabernet Sauvignon grapevines in the Olifants River region (Bruwer, 2010). However, yields were substantially higher compared to that of non-irrigated Cabernet Sauvignon grapevines in the Swartland region (Mehmel, 2010). This serves as confirmation that the cover crop combination of pearl millet (*Pennisetum glaucum* L.) in summer and oats (*Avena sativa* L. cv. Pallinup) in winter did not seem to have any negative effects on grapevine yield under the given conditions.

Table 7.11. Effect of irrigation using augmented winery wastewater on yield of Cabernet Sauvignon/99R measured during four seasons near Rawsonville.

Treatment no. & target COD (mg/L)	Yield (t/ha)			
	2009/10	2010/11	2011/12	2012/13
T1 - Control[1]	13.9 a[2]	9.8 abc	17.2 a	17.6 a
T2 - 100	14.1 a	11.2 a	17.0 a	16.1 a
T3 - 250	13.4 a	11.0 ab	17.4 a	17.2 a
T4 - 500	14.9 a	10.6 ab	18.0 a	18.1 a
T5 - 1000	15.6 a	10.6 ab	17.5 a	18.3 a
T6 - 1500	13.7 a	10.6 ab	16.9 a	15.7 a
T7 - 2000	13.9 a	8.2 c	16.6 a	15.0 a
T8 - 2500	14.6 a	11.2 a	18.6 a	18.9 a
T9 - 3000	13.6 a	9.4 bc	16.5 a	16.2 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

7.3.5. Evapotranspiration

Since irrigation using augmented winery wastewater did not affect soil water status compared to raw water irrigation, there were no differences in vineyard ET between treatments (Data not shown). Under the given conditions, vineyard ET was comparable to that of micro-sprinkler irrigated Pinotage near Robertson in the Breede River valley (Myburgh, 2011), except in January and February 2011 (Table 7.12). Following sowing in November 2010, vegetative growth of the pearl millet (*Pennisetum glaucum* L.) interception cover crop was extremely vigorous. At full canopy cover, the pearl millet was almost as tall as the grapevine canopies (Fig 7. 6). This indicated that the interception crop increased the vineyard ET from November until February compared to the same period in the other seasons (Table 7.12). The ET declined considerably in March 2011, *i.e.* after the interception crop had been cut and removed. In the 2011/12 season, when the pearl millet was sown later, *i.e.* in January 2012, ET during January and February was lower compared to the 2010/11 season. When the augmented wastewater was applied in the 2012/13 season, when the continuous deficit irrigation strategy was followed, vineyard ET was slightly lower in 2013 compared to the other years (Table 7.12). It must be noted that the continuous deficit irrigation did not have any negative effects on grapevine yield under micro-sprinklers compared to the other seasons. This was in contrast with yield reductions where continuous deficit irrigation was applied to drip irrigated Shiraz grapevines in the Breede River valley (Lategan, 2011).

Table 7.12. Mean monthly daily evapotranspiration of Cabernet Sauvignon grapevines in a sandy soil near Rawsonville during four seasons.

Season	Evapotranspiration (mm/day)											
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
2009/10	(1)	0.7	0.9	2.1	5.2	7.2	6.1	3.0	5.3	3.3	0.7	0.9
2010/11	0.9	0.3	1.7	3.5	8.1	9.4	5.7	5.5	5.1	3.4	1.4	0.9
2011/12	1.4	0.8	1.2	3.8	5.9	6.5	6.8	4.4	2.2	0.9	1.0	0.8
2012/13	1.8	0.9	1.8	4.2	4.2	5.8	5.0	3.2	2.4	1.2	1.2	1.3

(1) Not determined.

Figure 7.6. Stand of the pearl millet (*Pennisetum glaucum* L.) interception crop during February 2011.

7.4. CONCLUSIONS

Irrigation of grapevines using winery wastewater augmented up to a maximum COD level of 3000 mg/L did not affect vegetative growth or any of the yield components compared to the raw water control. Consequently, the water use and water status of the grapevines was not affected by the wastewater irrigation under the given conditions. Since organic carbon did not accumulate in the soil during the study period, it suggested that the soil was sufficiently aerated between irrigation to allow oxidation. The latter probably explains why the grapevines did not respond to level of COD *per se*. However, it is important to note that the salinity and sodicity levels in the augmented winery wastewater were below the thresholds for grapevines. This was confirmed by the lack of response in element content in the leaves and shoots. Therefore, the low salinity and sodicity levels in the augmented winery wastewater could be a further, but maybe more realistic explanation why the grapevines did not respond to the wastewater irrigation.

Since vegetative growth and yield of the grapevine were comparable to responses previously reported for vineyards without a summer cover crop, the results suggested that the grapevines were not affected by the pearl millet growing in the work rows during summer. Visual observations revealed that the root system of this cover was shallow compared to that of the grapevines. Therefore, the competition for water and nutrients was probably not strong enough to have induced negative effects on grapevine growth and yield. However, results indicated that a summer cover crop may increase the ET of vineyards substantially if growing conditions are favourable for the particular crop. The contribution of the slash and removal costs to already high production costs of vineyards is a further aspect that needs consideration.

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CHAPTER 8: EFFECT OF IRRIGATION WITH AUGMENTED WINERY WASTEWATER ON JUICE AND WINE CHARACTERISTICS WITH SPECIAL REFERENCE TO OFF-FLAVOURS

C.L. Howell[(1)], C.S. Schoeman[(2)] & P.A. Myburgh[(1)]

[(1)] The Fruit, Vine and Wine Institute of the Agricultural Research Council, Private Bag X5026, Stellenbosch, 7599

[(2)] Department of Viticulture and Oenology, Stellenbosch University, Private Bag X1, Matieland, 7602

8.1. INTRODUCTION

Although there is extensive literature available regarding the irrigation of grapevines with saline water very little is known about the effects of re-using winery wastewater on juice and wine quality characteristics. Winery wastewater contains high numbers of microorganisms, ranging from 105 to 108 colony forming units per millilitre (Jourjon *et al*., 2005). The dominant yeast species are *Saccharomyces cerevisiae*, *Candida intermedia, Hanseniaspora uvarum* and *Pichia membranaefaciens* (Malandra *et al.,* 2003). Winery wastewater also contains high lactic acid bacteria (LAB) and acetic acid bacteria (AAB) populations (Jourjon *et al*., 2005). Therefore, if contact is made between winery wastewater and grapes during irrigation, some microbes may survive on grape berries and end up in grape must and wine. If certain unfavourable microbes are transferred from the wastewater into the juice and wine, wine composition and quality may be detrimentally affected. Winery wastewater has a foul smell due to the conversion of organic compounds to, among others, methane under anaerobic conditions (McCarty, 1964). If these off-odours are transferred onto or into berries and the resulting wines, it may result in tainted wines.

If winery wastewater irrigation is applied, for example through overhead irrigation, contact between irrigation water and bunches would be inevitable. A study by Kennison *et al.* (2009) showed that, when grapevines were exposed to smoke between véraison and harvest it caused a ‘smoke taint’ in the resulting wines. Similarly, wines made from grapevines which are situated nearby *Eucalyptus* tree plantations, has been found to obtain higher *Eucalyptus*-like or minty characters, which may be obtained from the trees (Novak, 2002; Van Leeuwen *et al*., 2007). If these odours are transferred from the atmosphere onto or into grapes and the resulting wines, the sharp, foul odour of winery wastewater may quite possibly be transferred onto or into grapes and wine if direct contact is made between wastewater and berries. To our knowledge the possible effects of direct contact between grapevine bunches and winery wastewater on wine sensory characteristics have not been investigated. Therefore, the primary purpose of this study was to determine whether direct contact between grapevine

bunches and winery wastewater affected wine sensorial quality in any manner. In addition, the sensorial wine-aroma analysis would primarily investigate whether off-odours excreted by winery wastewater can adhere to, or be absorbed by grapes and transferred into the produced wines, resulting in faulty wines.

If winery wastewater could be used to irrigate vineyards, with no detrimental impacts on either grapevines or subsequent wine quality and chemical composition, it could be a possible viable alternative to using raw river or municipal water. Therefore, the objective of this study was to determine the effect of irrigation using augmented winery wastewater on grapevine, juice and wine quality characteristics.

8.2. MATERIALS AND METHODS

8.2.1. Juice characteristics

Refer to Chapter 2 for details of the experiment layout. Berry volume, juice total soluble solids (TSS), titratable acidity (TTA) and pH was determined for selected treatments using the berry samples collected to monitor berry development as described in Chapter 7. Juice TSS, TTA and pH were determined according to standard procedures of the winery at the Infruitec-Nietvoorbji Institute of the Agricultural Research Council (ARC) near Stellenbosch. Berry volume was determined by means of water displacement. Berry volume was used to calculate sugar content per berry (mg/berry) according to the method described by Deloire (2011). Grape samples were also collected at harvest from all experiment plots, and analysed for TSS, TTA and pH. Juice ion composition was analysed according to methods described by Clesceri *et al.* (1998) by a commercial laboratory (BEMLAB, Strand).

8.2.2. Wine quality

8.2.2.1. Sensorial and chemical characteristics in the main study

Grapes were harvested when they reached the target sugar content of 24°B. In 2010/11 and 2011/12, four wastewater irrigations were applied prior to harvest whereas in 2012/13, three wastewater irrigations were applied prior to harvest. Wines were made from the grapes (*ca.* 40 kg) of each experiment plot according to the standard red winemaking procedure used by the experiment winery at ARC Infruitec-Nietvoorbij as described by Myburgh (2011). In order to determine whether the wines were safe for tasting, *i.e.* free of harmful bacteria, the wine samples were analysed for the presence of total bacteria, coliforms and *E. Coli* during all three seasons. After six months, the wines were evaluated sensorially by a panel of at least 12 industry experts. Wines were evaluated on an unmarked line scale of 100 mm for wine colour, overall intensity, vegetative character, berry character, spicy character, acidity, body, astringency and overall quality. The panel was also asked to give an indication of the occurrence of off-flavours (off-odours and off-tastes) and any other a-typical red wine characteristics. Following tasting, the alcohol, extract, residual sugar, glucose, fructose,

volatile acidity, tartaric acid, malic acid, total acidity and pH of the wines were analysed by a commercial laboratory (Koelenhof winery, Stellenbosch). The ion composition of the wine was analysed according to methods described by Clesceri *et al.* (1998) by a commercial laboratory (BEMLAB, Strand). Colour of grape berry skins prior to harvest was also quantified.

8.2.2.2. Effect of direct wastewater contact in the preliminary study

Treatments comprised spraying grapes directly with raw river water (control) and winery wastewater containing two levels of chemical oxygen demand (COD). The two wastewater treatments comprised augmented winery wastewater (3000 mg/L COD) and undiluted winery wastewater (6000 mg/L COD). Grapevine bunches were sprayed using water spray bottles. The COD is a measure of the total organic content in water in terms of the amount of oxygen needed for its total breakdown *via* oxidation. In this study, the level of COD in the irrigation water is a direct indication of water quality, the two being indirectly proportional. Furthermore, the foul odour intensity of the water treatments increases as the level of COD increase. Each treatment was applied to three experimental grapevines, receiving four spray treatments within three weeks prior to harvest. Treatments were applied approximately once every five days by wetting only the bunch zone. Furthermore, treatments were applied on warm, sunny days at noon. Each grapevine was considered as a treatment replication, thus each of the three treatments were replicated three times. Treatment application entailed complete water coverage of all bunches with the various water quality treatments.

Wines were made in the 2012 vintage. Grapes, harvested at *c.* 24˚B, from each experimental grapevine were crushed separately by hand and transferred to 2 L plastic containers. At least one hour skin contact was allowed before inoculation with rehydrated pure wine yeast (VIN 13, Anchor Yeast, Cape Town, South Africa) at 30 g/hL. Di-ammonium phosphate (DAP) was added immediately after inoculation at 50 g/hL as yeast nutrition. Alcoholic fermentation (AF) was conducted on the skins at 25 ˚C, during which the skin caps were punched down two times a day to ensure sufficient skin contact and extraction. Must was fermented to between 0°B and 2°B, after which skins were separated and pressed by using a handmade steel press. Press and free run wines were combined, after which it was fermented to dryness at 25°C. After completion of AF, 50 mg/L sulphur dioxide (SO_2) was added to the wines. Wines were stored at 14°C until sensory evaluation in June 2012.

Wine sensorial evaluation was performed by a panel consisting of 11 wine experts. Only the visual appearance and sensorial aroma of the wines were evaluated. Wines were scored on the occurrence of off-odours and the presence of atypical colour, overall intensity, vegetative character, berry character, spicy character and overall quality. Off-odours were further evaluated within three sub categories, *i.e.* volatile acidity, wastewater odour and other off-

odours. Wines were scored by means of a 100 mm unmarked line scale. Each score mark was measured and averages determined. Average length was used to determine the degree of presence/liking/disliking by the judges. The average score for each treatment was calculated as an average score of the triplicates. Wines were not tasted, as its safety could not be guaranteed.

8.2.3. Statistical analyses

The data were subjected to an analysis of variance. Least significant difference (LSD) values were calculated to facilitate comparison between treatment means. Means that differed at $p \leq 0.05$ were considered to be significantly different. STATGRAPHICS® was used for the analyses of variance.

8.3. RESULTS AND DISCUSSION

8.3.1. Juice characteristics

In the 2009/10 season, the grapevines were not subjected to augmented winery wastewater irrigation before harvest. In this particular season, mean juice TSS, TTA and pH were 22.4±0.4°B, 4.5±0.3g/L and 3.7±0.1, respectively when the grapes were harvested. In the 2010/11 and 2011/12 seasons, the selected augmented winery wastewater irrigation treatments had no effect on berry size development during ripening compared to the raw water control (Fig. 8.1A). The rate of sugar loading into the berries during ripening was not affected by the wastewater irrigation compared to the raw water control (Fig. 8.1B). Similarly, the wastewater irrigation had no effect on acid breakdown (Fig. 8.1C). The fact that ripening was comparable to the control indicated that the winery wastewater had no effect on the physiological functioning of the grapevines, irrespective of the level of augmentation. Consequently, there were no differences in the sugar concentration and TTA in the juice at harvest in the 2011, 2012 or 2013 seasons (Table 8.1). Due to berry ripening being considerably slower in the 2011/12 season compared to the previous ones, grapes were harvested five weeks later than in 2010/11. In the 2011/12 season, juice pH in T9 grapes was higher than for control grapevines, as well as grapevines that were irrigated with winery wastewater containing COD levels lower than 1 000 mg/L (Table 8.1). In general, juice pH tended to increase with a decrease in level of augmentation with the exception of T1 and T2 in 2012/13 and juice pH increased linearly with increasing amounts of K applied *via* the irrigation water.

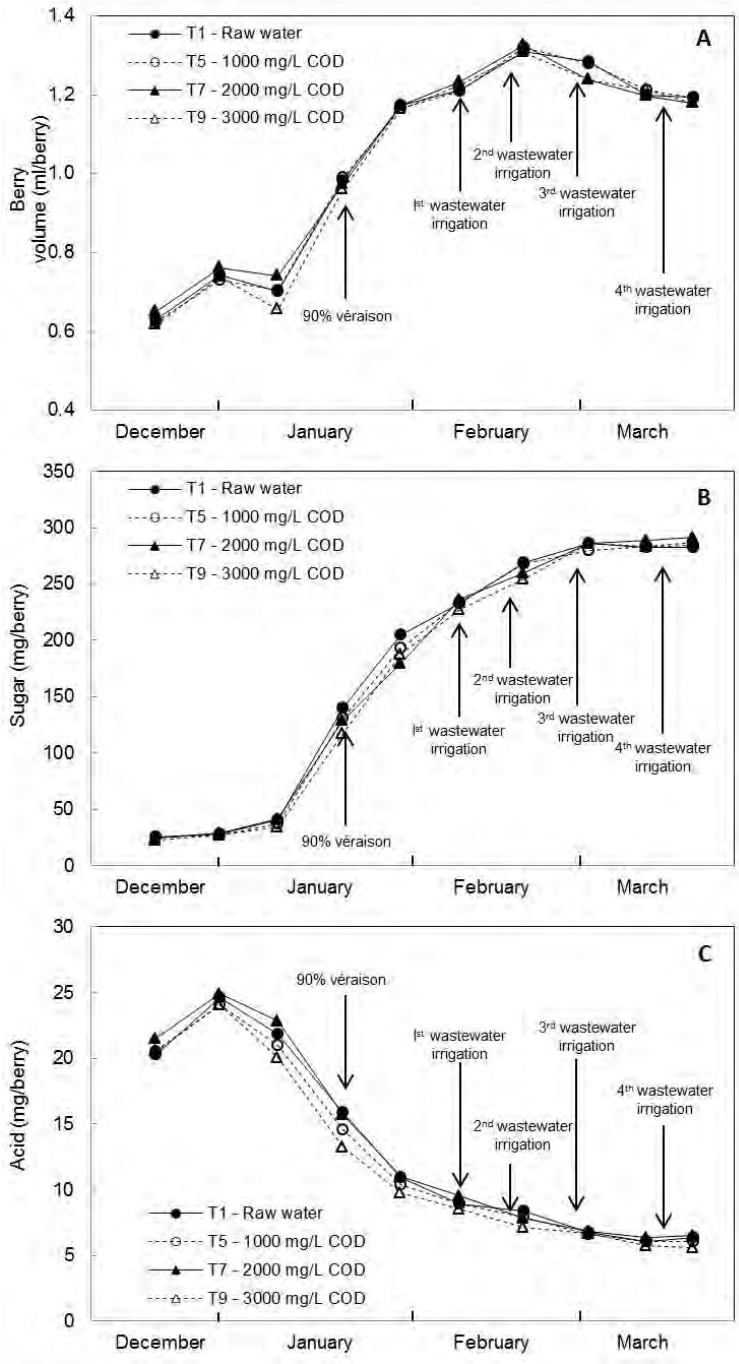

Figure 8.1. The effect of irrigation using augmented winery wastewater on (A) berry volume, as well (B) sugar and (C) total titratable acid per berry during ripening in the 2010/11 season.

In the 2009/10 season, mean juice N, P, K, Ca, Mg and Na were 337.9±86.5 mg/L, 95.8±15.5 mg/L, 761.5±149.4 mg/L, 26.5±4.0 mg/L, 47.9±4.8 mg/L and 4.5±0.8 mg/L, respectively when the grapes were harvested. In the subsequent seasons, irrigation with augmented winery wastewater generally did not affect juice N, P, K, Ca, Mg and Na compared to the raw water control (Tables 8.2 & 8.3).

Table 8.1. Total soluble solids (TSS), total titratable acidity (TTA) and pH in juice of Cabernet Sauvignon/99R irrigated using augmented winery wastewater during the 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	TSS (°B)			TTA (g/L)			pH		
	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13
T1 - Control[1]	23.6 a[2]	23.2 a	22.9 a	5.33 a	5.38 a	4.93 a	3.49 a	3.68 bc	3.60 a
T2 - 100	23.5 a	22.5 a	22.8 a	5.45 a	5.43 a	5.33 a	3.47 a	3.67 bc	3.63 a
T3 - 250	23.6 a	22.9 a	22.8 a	5.47 a	4.90 a	4.67 a	3.45 a	3.62 c	3.57 a
T4 - 500	24.3 a	23.0 a	22.8 a	5.20 a	4.98 a	4.73 a	3.52 a	3.70 bc	3.58 a
T5 - 1000	24.1 a	24.2 a	23.0 a	5.17 a	4.17 a	4.93 a	3.49 a	3.76 ab	3.64 a
T6 - 1500	23.4 a	22.9 a	23.1 a	5.47 a	4.75 a	4.87 a	3.47 a	3.75 ab	3.66 a
T7 - 2000	23.9 a	22.6 a	22.5 a	5.37 a	6.03 a	5.12 a	3.53 a	3.76 ab	3.61 a
T8 - 2500	23.6 a	22.7 a	23.1 a	5.47 a	4.77 a	5.17 a	3.52 a	3.77 ab	3.65 a
T9 - 3000	24.7 a	24.1 a	24.0 a	4.82 a	4.97 a	4.70 a	3.57 a	3.85 a	3.70 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.2. Nitrogen, phosphorus and potassium in juice of Cabernet Sauvignon/99R irrigated using augmented winery wastewater during the 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	N (mg/L)			P (mg/L)			K (mg/L)		
	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13
T1 - Control[1]	202 bc[2]	239 a	1940 a	136 a	182 a	513 a	1626 a	1794 a	2779 a
T2 - 100	148 ab	246 a	1768 a	141 a	202 a	445 a	1780 a	1822 a	3038 a
T3 - 250	147 ab	240 a	2520 a	130 a	187 a	497 a	1675 a	1479 a	2413 a
T4 - 500	127 a	235 a	1688 a	131 a	182 a	480 a	1852 a	1677 a	2532 a
T5 - 1000	123 a	223 a	1573 a	131 a	192 a	471 a	1905 a	1685 a	2789 a
T6 - 1500	136 a	214 a	1582 a	124 a	188 a	487 a	1759 a	1717 a	2996 a
T7 - 2000	135 a	222 a	1630 a	146 a	214 a	435 a	1894 a	1852 a	2931 a
T8 - 2500	137 a	213 a	1830 a	136 a	204 a	503 a	1926 a	1686 a	2863 a
T9 - 3000	221 c	281 a	1913 a	137 a	215 a	382 a	1939 a	1916 a	3179 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.3. Calcium, magnesium and sodium in juice of Cabernet Sauvignon/99R irrigated using augmented winery wastewater during the 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Ca (mg/L)			Mg (mg/L)			Na (mg/L)		
	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13
T1 - Control[1]	47.2 de[2]	41.1 a	86.6 a	97.0 a	113.4 a	165.1 a	7.6 ab	22.2 a	25.3 a
T2 - 100	49.4 e	46.2 a	86.7 a	102.0 a	124.8 a	170.1 a	7.7 ab	22.6 a	25.1 a
T3 - 250	43.8 cd	44.4 a	83.3 a	94.0 a	114.6 a	159.1 a	7.1 a	21.2 a	23.2 a
T4 - 500	42.1 bc	45.4 a	87.1 a	102.0 a	116.8 a	171.2 a	8.0 ab	23.4 a	25.7 a
T5 - 1000	38.2 ab	46.9 a	91.7 a	97.0 a	116.0 a	177.1 a	8.8 bc	22.6 a	25.4 a
T6 - 1500	34.3 a	44.8 a	80.1 a	92.0 a	113.2 a	169.6 a	7.9 ab	22.9 a	24.1 a
T7 - 2000	35.7 a	48.8 a	82.0 a	100.0 a	121.4 a	172.8 a	8.4 bc	22.5 a	23.0 a
T8 - 2500	37.6 ab	48.1 a	82.6 a	96.0 a	115.4 a	172.0 a	8.5 bc	22.5 a	27.4 a
T9 - 3000	34.5 a	47.1 a	68.3 a	96.0 a	119.8 a	144.9 a	9.6 c	22.3 a	22.4 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

However, the following exceptions were observed. In the 2010/11 season, there were significant differences with regards to juice N, but there were no trends that could be related to the augmented wastewater irrigation (Table 8.2). Furthermore, juice Na increased with an increase in the COD level of the augmented winery wastewater, whereas juice Ca decreased (Table 8.3). It was previously reported that sodic soil conditions could cause high concentrations of Na in grapevine tissue and concomitantly reduce Ca concentrations (Stevens *et al*., 2011 and references therein). Although there were differences in juice Cr between treatments, they could not be related to the level of augmentation (data not shown). The various irrigation treatments had no effect on Cd in the juice. No arsenic or other heavy metals (Pb & Hg) were detected in the grape juice (data not shown). This result was to be expected since these elements were not present in the raw or augmented water.

8.3.2. Wine quality

8.3.2.1. Sensorial and chemical characteristics in the main study

Throughout the study, none of the wines contained any bacteria, coliforms and *E. Coli* and could therefore be considered as safe for the sensorial evaluation (data not shown). The application of wastewater irrigation, regardless of level of augmentation, consistently had no effect on the sensorial wine characteristics compared to the raw water control during the three seasons (Tables 8.4 to 8.6). All the wines tended to have a stronger berry-like character than vegetative or spicy characters, consistent with Cabernet Sauvignon wine made from ripe berries from warmer localities such as Rawsonville. Generally, the wines were generally of low quality (< 40% is low and > 70% is excellent quality). This trend was to be expected, since grapevines of all treatments did not experience any water constraints during the season (refer to Chapter 7). However, it is important to note that the off-flavours were almost undetectable by the panel, and that there was no trend with respect to off-flavours that could be related to the level of wastewater augmentation. This confirmed that no wastewater associated off-odours and/or off-tastes were transferred into wines or produced as a response to wastewater irrigation under the given conditions. Perusal of the scorecards also revealed that members of the tasting panel were highly inconsistent with respect to their perception of off-tastes. The observed off-odours and off-tastes were all related to frequently occurring off-odours and off-tastes in wines such as volatile acidity and bitterness.

The level of winery wastewater augmentation did not affect berry skin colour and the total phenolic compounds when the grapes were harvested compared to the raw water control during any of the seasons (data not shown). These similarities indicated that grapevine canopy microclimate, as well as grapevine functioning with regards to colour pigment and phenolic compound accumulation was not affected by the various wastewater irrigation treatments during ripening. In general, irrigation using augmented winery wastewater did not

Table 8.4. Effect of irrigation with different levels of augmented winery wastewater on sensorial characteristics of Cabernet Sauvignon wine determined in September 2011.

Treatment no. and target COD (mg/L)	Overall intensity (%)	Vegetative (%)	Berry (%)	Spicy (%)	Off-flavours (%)	Acidity (%)	Body (%)	Astringency (%)	Off-tastes (%)	Overall quality (%)
T1 - Control[1]	48.4 a[2]	31.0 a	40.6 a	21.8 a	8.1 a	37.1 a	37.6 a	24.7 a	7.5 a	43.4 a
T2 - 100	46.9 a	25.0 a	30.2 a	12.4 a	16.7 a	41.4 a	31.9 a	25.3 a	10.8 a	33.2 a
T3 - 250	44.4 a	21.7 a	40.2 a	18.5 a	4.8 a	39.0 a	36.1 a	29.4 a	9.0 a	38.4 a
T4 - 500	48.4 a	24.4 a	42.8 a	22.8 a	7.1 a	38.9 a	37.3 a	28.7 a	8.4 a	41.2 a
T5 - 1000	50.7 a	29.3 a	45.9 a	19.8 a	4.6 a	38.3 a	41.1 a	26.6 a	5.2 a	43.9 a
T6 - 1500	40.4 a	31.4 a	30.9 a	14.6 a	4.7 a	37.9 a	31.8 a	23.9 a	5.2 a	32.4 a
T7 - 2000	47.6 a	32.3 a	37.3 a	18.3 a	4.6 a	43.0 a	38.7 a	28.8 a	6.0 a	43.5 a
T8 - 2500	45.8 a	29.3 a	34.3 a	17.7 a	11.8 a	40.9 a	36.5 a	27.0 a	8.7 a	38.0 a
T9 - 3000	52.0 a	25.0 a	45.9 a	17.7 a	12.9 a	40.5 a	40.8 a	30.5 a	11.5 a	44.1 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.5. Effect of irrigation with different levels of augmented winery wastewater on sensorial characteristics of Cabernet Sauvignon wine determined in September 2012.

Treatment no. and target COD (mg/L)	Overall intensity (%)	Vegetative (%)	Berry (%)	Spicy (%)	Off-flavours (%)	Acidity (%)	Body (%)	Astringency (%)	Off-tastes (%)	Overall quality (%)
T1 - Control[1]	50.9 a[2]	25.6 a	39.2 a	17.0 a	5.9 a	33.9 a	34.8 a	33.9 a	9.9 a	37.8 a
T2 - 100	44.9 a	23.3 a	31.5 a	17.0 a	7.1 a	31.4 a	31.6 a	28.9 a	8.5 a	34.7 a
T3 - 250	45.9 a	23.8 a	34.3 a	17.5 a	5.1 a	33.7 a	35.2 a	32.5 a	9.5 a	39.0 a
T4 - 500	47.9 a	26.7 a	34.9 a	17.1 a	6.4 a	33.4 a	32.8 a	27.3 a	9.6 a	34.6 a
T5 - 1000	50.9 a	22.9 a	41.6 a	18.1 a	4.4 a	34.8 a	37.5 a	30.4 a	8.8 a	39.3 a
T6 - 1500	47.4 a	18.8 a	38.0 a	13.5 a	4.0 a	31.5 a	31.5 a	26.8 a	7.0 a	37.0 a
T7 - 2000	42.5 a	19.5 a	31.4 a	13.6 a	3.9 a	30.6 a	32.1 a	26.7 a	6.6 a	36.0 a
T8 - 2500	46.6 a	21.3 a	37.2 a	14.9 a	4.1 a	31.4 a	33.0 a	27.5 a	7.0 a	37.0 a
T9 - 3000	48.5 a	24.6 a	39.0 a	16.9 a	2.9 a	33.3 a	35.2 a	31.6 a	4.6 a	37.7 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.6. Effect of irrigation with different levels of augmented winery wastewater on sensorial characteristics of Cabernet Sauvignon wine determined in August 2013.

Treatment no. and target COD (mg/L)	Overall intensity (%)	Vegetative (%)	Berry (%)	Spicy (%)	Off-flavours (%)	Acidity (%)	Body (%)	Astringency (%)	Off-tastes (%)	Overall quality (%)
T1 - Control[1]	40.2 a[2]	24.8 a	34.2 a	16.8 a	4.7 a	38.1 a	30.3 a	28.7 a	4.8 a	31.7 a
T2 - 100	39.9 a	24.5 a	35.4 a	18.5 a	4.7 a	39.5 a	32.3 a	32.2 a	5.4 a	31.5 a
T3 - 250	34.1 a	19.8 a	29.8 a	17.0 a	5.9 a	40.9 a	31.2 a	30.2 a	7.7 a	29.9 a
T4 - 500	37.8 a	22.4 a	34.4 a	16.2 a	3.4 a	42.7 a	32.2 a	30.8 a	3.2 a	35.4 a
T5 - 1000	41.2 a	25.0 a	35.8 a	16.2 a	3.8 a	38.4 a	29.2 a	29.2 a	5.1 a	34.1 a
T6 - 1500	37.6 a	19.4 a	34.2 a	14.7 a	6.8 a	39.8 a	30.0 a	30.1 a	7.4 a	31.6 a
T7 - 2000	43.3 a	22.4 a	40.9 a	17.9 a	2.2 a	38.6 a	31.1 a	28.4 a	4.2 a	34.2 a
T8 - 2500	37.6 a	21.1 a	31.8 a	15.9 a	4.6 a	39.0 a	30.1 a	30.3 a	7.7 a	30.7 a
T9 - 3000	39.6 a	22.9 a	38.3 a	17.5 a	3.5 a	43.0 a	37.3 a	33.1 a	4.2 a	38.7 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

affect wine chemical properties compared to the raw water control (Tables 8.7 to 8.12). However, in the 2011 and 2012 seasons, wine pH increased with a decrease in level of augmentation, *i.e.* an increase in COD level. In addition to this, tartaric acid in wines of the 2012/13 season decreased with a decrease in level of augmentation. At this stage there is no plausible explanation for these trends.

With regards to wine ion composition, no consistent trends could be observed (Tables 8.13 & 8.14). However, in wines from the 2011/12 growing season SO_4 increased with a decrease in level of augmentation, whereas in 2012/13 wine K increased with an increase in of COD level of the irrigation water.

8.3.2.2. Effect of direct wastewater contact in the preliminary study

Direct contact between grapevine bunches and augmented winery wastewater had no significant effect on wine colour, overall intensity, vegetative character, berry character, spicy character or overall wine quality statistically significant (Table 8.15). A clear tendency of decreased spicy character was, however, observed with an increase in the level of COD in the treatment water. This is largely inexplicable, but may have been the result of conflicting odours masking or changing the commonly known spicy nuances. Even though wine volatile acidity (VA) was not significantly increased, it showed a trend to increase as the level of COD in the winery wastewater increased. Winery wastewater contains large populations of acetic acid bacteria (Jourjon *et al*., 2005). If these bacteria are transferred into grape juice and wine, it may cause an increase in wine VA. Juice and wine microbial populations were, however, not monitored and could not be related to these findings. Winery wastewater odour was increased in the wines as the COD concentration in the winery wastewater increased. This increase indicates that off-odours, present in winery wastewater, may possibly adhere to and/or be absorbed by berries when contact is made between wastewater and berries *via* irrigation or by other means.

Although overall wine quality was not reduced by treating the grapes with wastewater in this study, the results indicate that there may be a risk that wines may be spoiled if contact of grapes and wine with winery wastewater is not avoided. As the winery wastewater off-odour was recognisable in the wine aroma, it is possible that it may also have affected the palate. As wines were not tasted, this aspect could not be investigated further. Any other wine off-odours were, however, not affected when direct contact between grapevine bunches and winery wastewater was made.

Table 8.7. Effect of irrigation with different levels of augmented winery wastewater on selected chemical properties of Cabernet Sauvignon wine measured six months after harvest in 2011.

Treatment no. and target COD (mg/L)	Alcohol (%)	Extract	Residual sugar (g/L)	Glucose (g/L)	Fructose (g/L)	Volatile acidity (g/L)	Tartaric acid (g/L)	Malic acid (g/L)	Total acidity (g/L)	pH
T1 - Control[1]	12.11 a[2]	26.77 a	1.89 a	0.06 a	0.07 a	0.52 a	1.20 a	0.01 a	4.01 a	4.14 a
T2 - 100	11.91 a	26.57 a	1.25 a	0.06 a	0.06 a	0.60 a	1.18 a	0.03 a	4.43 a	4.17 a
T3 - 250	12.65 a	27.60 a	1.10 a	0.05 a	0.08 a	0.66 a	1.17 a	0.02 a	3.93 a	4.12 a
T4 - 500	12.36 a	26.43 a	1.96 a	0.04 a	0.06 a	0.70 a	1.24 a	0.14 a	4.21 a	4.22 ab
T5 - 1000	12.33 a	25.80 a	1.77 a	0.16 a	0.07 a	0.61 a	1.28 a	0.00 a	3.71 a	4.34 ab
T6 - 1500	12.80 a	26.00 a	1.22 a	0.09 a	0.09 a	0.55 a	1.30 a	0.02 a	3.63 a	4.36 ab
T7 - 2000	12.68 a	27.53 a	1.72 a	0.06 a	0.07 a	0.63 a	1.34 a	0.07 a	3.77 a	4.38 c
T8 - 2500	12.27 a	28.57 a	1.12 a	0.05 a	0.07 a	0.64 a	1.29 a	0.02 a	3.80 a	4.33 ab
T9 - 3000	13.00 a	28.90 a	1.29 a	0.05 a	0.08 a	0.55 a	1.29 a	0.02 a	3.68 a	4.34 ab

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.8. Effect of irrigation with different levels of augmented winery wastewater on selected chemical properties of Cabernet Sauvignon wine measured six months after harvest in 2012.

Treatment no. and target COD (mg/L)	Alcohol (%)	Extract	Residual sugar (g/L)	Glucose (g/L)	Fructose (g/L)	Volatile acidity (g/L)	Tartaric acid (g/L)	Malic acid (g/L)	Total acidity (g/L)	pH
T1 - Control[1]	12.65 a[2]	25.63 a	1.66 a	0.08 a	0.07 a	0.29 a	0.73 a	0.10 a	4.37 a	3.96 a
T2 - 100	11.93 a	24.67 a	1.70 a	0.05 a	0.04 a	0.29 a	0.63 a	0.00 a	3.98 a	4.20 a
T3 - 250	11.96 a	25.03 a	1.76 a	0.06 a	0.03 a	0.34 a	0.69 a	0.00 a	4.24 a	4.05 a
T4 - 500	12.34 a	26.60 a	1.61 a	0.07 a	0.06 a	0.31 a	0.70 a	0.25 a	4.26 a	4.06 a
T5 - 1000	13.23 a	28.47 a	1.83 a	0.06 a	0.04 a	0.31 a	0.67 a	0.05 a	4.26 a	4.09 a
T6 - 1500	12.19 a	25.83 a	1.58 a	0.07 a	0.05 a	0.37 a	0.81 a	0.21 a	4.15 a	4.16 a
T7 - 2000	12.13 a	25.80 a	1.41 a	0.07 a	0.04 a	0.39 a	0.64 a	0.06 a	3.99 a	4.18 a
T8 - 2500	12.19 a	25.97 a	1.48 a	0.05 a	0.04 a	0.35 a	0.66 a	0.05 a	4.00 a	4.24 a
T9 - 3000	13.00 a	27.10 a	1.94 a	0.06 a	0.06 a	0.30 a	0.75 a	0.08 a	4.03 a	4.24 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.9. Effect of irrigation with different levels of augmented winery wastewater on selected chemical properties of Cabernet Sauvignon wine measured six months after harvest in 2013.

Treatment no. and target COD (mg/L)	Alcohol (%)	Extract	Residual sugar (g/L)	Glucose (g/L)	Fructose (g/L)	Volatile acidity (g/L)	Tartaric acid (g/L)	Malic acid (g/L)	Total acidity (g/L)	pH
T1 - Control[1]	12.7 ab[2]	24.7 a	1.08 a	0.11 a	0.08 a	0.40 a	1.19 e	0.57 a	4.69 a	3.88 a
T2 - 100	12.6 a	24.2 a	1.14 a	0.09 a	0.07 a	0.38 a	1.06 bcde	0.78 a	4.33 a	4.01 a
T3 - 250	12.7 ab	23.8 a	1.14 a	0.11 a	0.06 a	0.39 a	1.06 bcde	0.63 a	4.54 a	3.88 a
T4 - 500	12.8 ab	25.6 a	1.16 a	0.09 a	0.06 a	0.32 a	1.09 cde	0.95 a	5.17 a	3.82 a
T5 - 1000	13.1 bc	26.5 a	1.28 a	0.09 a	0.07 a	0.39 a	1.10 de	0.65 a	4.71 a	3.92 a
T6 - 1500	13.0 ab	26.7 a	1.17 a	0.12 a	0.07 a	0.40 a	1.01 bcd	1.06 a	4.51 a	4.00 a
T7 - 2000	12.7 ab	26.0 a	1.28 a	0.09 a	0.08 a	0.37 a	0.87 a	1.07 a	4.89 a	3.89 a
T8 - 2500	13.0 ab	24.1 a	1.25 a	0.08 a	0.07 a	0.37 a	0.95 ab	1.00 a	4.64 a	3.96 a
T9 - 3000	13.5 c	25.2 a	1.07 a	0.10 a	0.09 a	0.34 a	0.96 abc	1.16 a	5.01 a	3.95 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.10. Effect of irrigation with different levels of augmented winery wastewater on selected chemical properties of Cabernet Sauvignon wine measured six months after harvest in 2011.

Treatment no. and target COD (mg/L)	Free amino nitrogen (mg/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Brown colour (420 nm)	Red colour (520 nm)	Total tannins (510 nm)	Total phenols (280 nm)
T1 - Control[1]	116.67 a[2]	40.67 a	87.00 a	2.01 a	1.92 a	0.82 a	34.44 a
T2 - 100	122.27 a	39.33 a	84.00 a	1.76 a	1.68 a	0.47 a	33.67 a
T3 - 250	112.93 a	42.00 a	83.67 a	1.95 a	1.79 a	0.61 a	34.68 a
T4 - 500	121.33 a	51.00 a	88.00 a	1.94 a	1.74 a	1.10 a	36.80 a
T5 - 1000	117.60 a	43.67 a	87.00 a	2.22 a	1.98 a	1.09 a	34.75 a
T6 - 1500	112.00 a	51.67 a	80.00 a	2.07 a	1.71 a	0.84 a	34.72 a
T7 - 2000	116.67 a	42.00 a	83.67 a	2.25 a	2.14 a	1.13 a	36.91 a
T8 - 2500	115.73 a	51.33 a	83.33 a	2.00 a	1.79 a	1.39 a	33.98 a
T9 - 3000	114.80 a	33.00 a	84.00 a	2.53 a	2.45 a	1.02 a	36.84 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.11. Effect of irrigation with different levels of augmented winery wastewater on selected chemical properties of Cabernet Sauvignon wine measured six months after harvest in 2012.

Treatment no. and target COD (mg/L)	Free amino nitrogen (mg/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Brown colour (420 nm)	Red colour (520 nm)	Total tannins (510 nm)	Total phenols (280 nm)
T1 - Control[1]	119.5 a[2]	40.00 a	80.33 a	0.34 a	0.45 a	0.47 a	36.10 a
T2 - 100	113.9 a	37.33 a	78.33 a	0.26 a	0.28 a	0.81 a	31.79 a
T3 - 250	115.7 a	38.00 a	78.00 a	0.27 a	0.38 a	0.86 a	34.62 a
T4 - 500	118.5 a	39.00 a	78.67 a	0.35 a	0.47 a	0.67 a	34.62 a
T5 - 1000	117.6 a	35.33 a	75.00 a	0.50 a	0.53 a	1.00 a	36.33 a
T6 - 1500	116.7 a	39.00 a	80.67 a	0.32 a	0.38 a	0.72 a	34.68 a
T7 - 2000	115.7 a	37.00 a	77.33 a	0.26 a	0.30 a	0.94 a	35.52 a
T8 - 2500	118.5 a	40.00 a	80.67 a	0.28 a	0.24 a	0.92 a	33.91 a
T9 - 3000	115.7 a	37.00 a	74.33 a	0.37 a	0.45 a	0.51 a	35.42 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.12. Effect of irrigation with different levels of augmented winery wastewater on selected chemical properties of Cabernet Sauvignon wine measured six months after harvest in 2013.

Treatment no. and target COD (mg/L)	Free amino nitrogen (mg/L)	Free SO_2 (mg/L)	Total SO_2 (mg/L)	Brown colour (420 nm)	Red colour (520 nm)	Total tannins (510 nm)	Total phenols (280 nm)
T1 - Control[1]	136.3 a[2]	40.7 a	83.0 a	0.92 a	1.10 a	0.52 a	29.9 a
T2 - 100	136.3 a	38.0 a	80.0 a	1.03 a	1.08 a	0.32 a	31.1 a
T3 - 250	128.8 a	35.7 a	78.3 a	0.87 a	1.02 a	0.43 a	29.4 a
T4 - 500	137.2 a	37.7 a	78.7 a	1.15 a	1.45 a	0.48 a	32.7 a
T5 - 1000	136.3 a	38.3 a	80.3 a	1.25 a	1.40 a	0.47 a	33.7 a
T6 - 1500	126.0 a	35.7 a	77.3 a	1.06 a	1.13 a	0.60 a	31.5 a
T7 - 2000	135.3 a	37.3 a	78.0 a	1.08 a	1.25 a	0.22 a	31.9 a
T8 - 2500	135.3 a	40.3 a	77.7 a	0.95 a	1.07 a	0.22 a	30.6 a
T9 - 3000	142.8 a	38.3 a	78.3 a	1.35 a	1.64 a	0.32 a	34.3 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.13. Phosphorus, potassium and sodium content in wine of Cabernet Sauvignon/99R irrigated using augmented winery wastewater during the 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	P (mg/L)			K (mg/L)			Na (mg/L)		
	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13
T1 - Control[1]	412 a[2]	175 a	3034 ab	1740 a	982 a	833 a	32.8 a	19.5 a	14.4 a
T2 - 100	423 a	184 a	3416 a	1971 a	1008 a	971 ab	31.7 a	18.7 a	15.2 a
T3 - 250	419 a	170 a	2498 c	1662 a	845 a	997 ab	28.9 a	17.6 a	13.2 a
T4 - 500	411 a	173 a	3153 ab	1709 a	836 a	1019 ab	30.4 a	17.5 a	14.8 a
T5 - 1000	384 a	183 a	3099 ab	1826 a	949 a	1022 ab	29.9 a	19.7 a	16.0 a
T6 - 1500	374 a	167 a	2906 bc	1879 a	927 a	1162 bc	28.9 a	18.7 a	14.3 a
T7 - 2000	411 a	171 a	3018 ab	2023 a	953 a	1111 bc	28.0 a	17.6 a	14.0 a
T8 - 2500	403 a	168 a	3096 ab	1933 a	987 a	1220 c	28.6 a	18.2 a	15.4 a
T9 - 3000	405 a	183 a	3088 ab	1929 a	1078 a	1224 c	31.8 a	19.1 a	15.6 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.14. Chlorine and sulphate content in wine of Cabernet Sauvignon/99R irrigated using augmented winery wastewater during the 2010/11, 2011/12 and 2012/13 seasons.

Treatment no. & target COD (mg/L)	Cl (mg/L)			SO_4 (mg/L)		
	2010/11	2011/12	2012/13	2010/11	2011/12	2012/13
T1 - Control[1]	35.5 a[2]	38.6 a	50.4 a	587 a	108 ab	5311 a
T2 - 100	41.4 a	47.5 a	80.1 a	543 a	98 a	5144 a
T3 - 250	26.6 a	47.5 a	80.1 a	538 a	108 ab	4812 a
T4 - 500	35.5 a	44.5 a	65.3 a	565 a	108 ab	5040 a
T5 - 1000	23.7 a	44.5 a	68.2 a	556 a	126 bc	5022 a
T6 - 1500	35.5 a	38.6 a	86.0 a	549 a	127 bc	5138 a
T7 - 2000	38.5 a	38.6 a	50.4a	550 a	131 c	4920 a
T8 - 2500	32.6 a	44.5 a	86.0 a	566 a	137 cd	5191 a
T9 - 3000	35.5 a	56.4 a	47.5 a	547 a	153 d	5004 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 8.15. Aroma analysis of wines made from Cabernet Sauvignon/99R grapes sprayed with winery wastewater during the 2011/12 season.

Water source	Colour	Overall intensity	Vegetative	Berry	Spicy	Volatile acidity	Waste-water odour	Other off odours
T1 - Control[1]	55.9 a[2]	47.7 a	11.9 a	41.1 a	14.8 a	4.8 a	2.9 a	3.7 a
T2 - 3 g/L COD	72.7 a	47.7 a	12.8 a	39.3 a	12.8 a	7.5 a	5.7 ab	6.8 a
T3 - Stock dam	56.6 a	49.1 a	14.5 a	40.0 a	5.7 a	7.7 a	6.9 b	2.2 a

[1] Raw water abstracted from the Holsloot River.
[2] Values designated by the same letters within a column do not differ significantly ($p \leq 0.1$).

8.4. CONCLUSIONS

Main study: Results of the main study showed that irrigation of grapevines using winery wastewater augmented up to 3000 mg/L COD did not have detrimental effects on juice characteristics with regards to ripeness parameters and ion content under the prevailing conditions. Wine sensorial quality was not affected. Under the conditions of the main study, the relatively large irrigation volumes applied during berry ripening were definitely detrimental to wine quality. Since wine quality is an important aspect, particularly if wine needs to be exported, the generally poor quality is of great concern. However, there is ample evidence that less frequent irrigation, which allows higher levels of plant available water (PAW) depletion between irrigations, will enhance wine quality. This implies that the winery wastewater will probably have to be applied over large areas to obtain sufficient PAW depletion between irrigations. Distribution of winery wastewater over large areas will need additional infrastructure, which could be expensive.

Preliminary study: Results of the preliminary study, where grapes were sprayed with wastewater, indicated that direct contact between grapevine bunches and winery wastewater may cause a decrease in spicy character, increase in wine VA and the presence of a winery wastewater-like off-odour in wines. Furthermore, as the quality of the water decreases, these off-odours may increase. Therefore, even though the main study showed that wine colour and common sensory wine descriptors were not affected by the various wastewater irrigation treatments, any further increase in wine VA or wastewater off-odour due to direct contact may reduce wine quality. Wastewater odours may also differ from winery to winery, and the risk for off-flavours can therefore not be excluded.

8.5. REFERENCES

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CHAPTER 9: EFFECT OF IRRIGATION WITH AUGMENTED WINERY WASTEWATER ON THE CHEMICAL PROPERTIES OF FOUR DIFFERENT SOILS

A.R. Mulidzi & P.A. Myburgh
The Fruit, Vine and Wine Institute of the Agricultural Research Council, Private Bag X5026, Stellenbosch, 7599

9.1. INTRODUCTION

The rapid growth in wine production during the last decade and the intensification of land use in most countries need to be matched with greater emphasis on reducing the impact of operations as winery wastewater is the most important aspect in wine cellars that could be detrimental to the environment (Chapman *et al*., 1995; Gajdos, 1998). The increases in production of wine in South Africa over the past decade have put more pressure on the natural resources such as vegetation, water and soil. This initiated the development of guidelines for the management of wastewater and solid waste at wineries (Van Schoor, 2005).

The demand for high quality water together with water challenges and shortages in arid and semi-arid regions had increased the water challenges (Oron *et al.,* 1999). The deterioration of water quality due to the disposal of wastewater into water bodies has resulted in the treatment of wastewater through land application all over the world (Cook *et al.*, 1994). Water is the most valuable resource, and if not handled with care, arid and semi-arid regions may face enormous shortages of good quality water in the coming decades especially due to global climate change (Faisal Anwar, 2011). In most African and Asian cities, the growth in population has outpaced the sanitation and wastewater infrastructure, making it difficult to manage urban wastewater (Qadir *et al*., 2010). The shortage of good quality water leads to an increasing demand to irrigate with water contaminated with salts such as saline groundwater, drainage water and treated wastewater (Jalali *et al.*, 2008). Irrigation in arid and semi-arid regions throughout the world has been linked with an increase in the concentration of salts in the soil. The increase of sodium (Na) content in the soil could still happen even with the use of normal irrigation water (Walker & Lin, 2008).

The effects of using industrial and municipal wastewater for agricultural irrigation and their impact on the soil have been widely documented. However, the environmental impact of irrigating with treated wastewater has not been widely investigated, yet the demand or need to use reclaimed sewage effluent due to water shortages is ever increasing (Walker & Lin, 2008).

Due to the intensification of environmental legislation, wine growers are expected to find solutions for the treatment or recycling of winery wastewater (Van Schoor, 2001). According to Lieffering and McLay (1996), soil pH and exchangeable Na tended to increase when wastewater with high pH and Na concentrations was used for irrigation. The accumulation of monovalent cations (such as Na) on the exchange sites has the ability to affect soil structure through clay dispersion and deflocculation processes (Mosse *et al.*, 2011). The presence of hydroxide solutions in wastewater has the ability to increase the soil cation exchange capacity (CEC). The high concentration of grey water contributes to a higher electrical conductivity of the saturated paste (EC_e) due to the presence of salts in cleaning detergents (Faisal Anwar, 2011).

Land application of winery wastewater results in the accumulation of potassium (K) in the soil and leaching of calcium (Ca) and magnesium (Mg), which could lead to the long term instability of the soil structure. The replacement of bivalent ions such as Ca and Mg by the monovalent ones such as K and Na during continuous or long term repeated irrigation can potentially lead to the breakdown of the soil structure, thereby affecting the hydraulic conductivity of the soil. Long term application of winery wastewater on pastures resulted in the build-up of available K levels that had the potential to leach into the groundwater and other water sources (Christen *et al.*, 2010). Although the effects of having high K ions applied in the soil have not been researched extensively, it has been suggested that irrigating with K-rich wastewater could be advantageous to overall soil fertility, but long term application could result to the alteration of the physicochemical soil properties (Mosse *et al.*, 2011).

The improvement of wastewater as well as its treatment is needed in order to minimize health and environmental risks associated with wastewater irrigation. The effective disposal of wastewater through reuse depends on the soil properties as well as the irrigation technology (Oron *et al.*, 1999). Disposal of winery wastewater through land application has been practiced for many years. Although it seems convenient, this practice may have a negative impact on the natural resources including the soil (Bond, 1998). It was previously reported that direct land application of stillage for irrigation and as fertilizer had positive effects, *e.g.* increase in soil pH, water and mineral salt retaining characteristics, as well as restoration and maintenance of soil micro flora (Papini, 2000). However, according to Bond (1998) the application of winery wastewater resulted in excessive nitrate leaching to the groundwater, increasing soil sodicity of arable land and long term detrimental effects on soil physical and chemical properties.

Where wineries use Na based cleaning agents, *i.e.* sodium hydroxide (NaOH), Na accumulation in the receiving environment can be problematic, more especially when the wastewater is discharged onto arable land (Mosse *et al.*, 2011). Disposal of wastewater through land application on poorly drained soils can lead to salinisation and water logging, thereby affecting the long-term sustainability of the site at which it is applied (Christen *et al.*, 2010). Land application of organic wastes stimulates the soil microbial pool through the addition of nutrients and carbon substrate (Chapman, 1994). The wastes themselves can also contribute a considerable number of microorganisms to the soil microbial pool (Cameron *et al.*, 1997). Therefore, wastewater disposal by land application may result in a variable effect on the infiltration rate and hydraulic conductivity of the soil. The presence of clay, organic matter and high CEC in the soil affects the adsorption and protection of heavy metals such as zinc (Zn) but in soils with low CEC, the heavy metals are mostly absorbed by plants (Bahmanyar, 2008). Soil microorganisms play an integral part of sustainable ecosystems therefore, any changes to microbial population as a result of irrigation with winery wastewater need to be considered (Mosse *et al.*, 2011).

For the past decade, the South African wine industry has co-funded projects that sought to develop technology that contributes to responsible management of winery wastewater, notably where such wastewater is utilized for crop irrigation. The South African Department of Water Affairs is currently amending the General Authorization for winery wastewater to allow its use for beneficial crop irrigation. Different soil types will react differently to winery wastewater irrigation due to their textural differences. Therefore, the objective of this study is to determine the effects of irrigation with augmented winery wastewater on the chemical properties of soils commonly found in the Western Cape Province.

9.2. MATERIALS AND METHODS

9.2.1. Experiment layout

The study was carried out under a 20 m x 40 m translucent fiberglass rain shelter at the ARC Infruitec-Nietvoorbij near Stellenbosch. Four pedogenetically different soils were used in the study (Table 9.1). For the purpose of this study, the soils will be referred to as Rawsonville sand (S1), Lutzville sand (S2), Stellenbosch shale (S3) and Stellenbosch granitic soil (S4). Each soil was irrigated with clean water (control) and winery wastewater. Municipal drinking water from Stellenbosch was used as the control. For the wastewater treatment, water was collected from the wastewater pit at a winery near Rawsonville in the Breede River valley. This water was then augmented to a chemical oxygen demand (COD) level of 3000 mg/L.

The clean water and wastewater irrigations were applied over four simulated seasons, which consisted of six irrigations each. The latter was estimated as the number of irrigations a

micro-sprinkler irrigated vineyard would require during the harvest period, *i.e.* when the highest volumes of wastewater are produced. Hence, a total of 24 irrigations were applied over the four simulated seasons. Each soil/water treatment was replicated four times in a complete randomised design. Since the soil sampling was destructive, each replication consisted of four pots (Fig. 9.1). Following each season, one of the four pots was removed for sampling, *i.e.* after six (T1), twelve (T2), 18 (T3) and 24 (T4) irrigations.

Table 9.1. Origin, description and co-ordinates for the four soils used to determine the effect of augmented winery wastewater on selected soil chemical properties.

Soil number	Origin	Description	Co-ordinates
S1	Rawsonville	Alluvial sand, from a vineyard	-33.693698°, 19.322569°
S2	Lutzville	Aeolian sand, from open land	-31.558906°, 18.353115°
S3	Stellenbosch	Shale derived, from open land	-33.911717°, 18.871152°
S4	Stellenbosch	Granite derived, from open land	-33.917296°, 18.864484°

9.2.2. Packing of soils to a predetermined bulk density

Soils were packed into 4.5 dm^3 PVC pots. The pots were 20 cm high with an inner diameter of 15 cm. A 10 mm hole was drilled in the bottom of each pot to allow drainage. A piece of 1.5 mm plastic mesh was placed on the bottom of each pot to prevent the soil from flowing through the drainage hole. All pots were cleaned and weighed before being filled with the soil. The day before the pots were filled up, the bulked soils were moistened using clean water to enhance compaction.

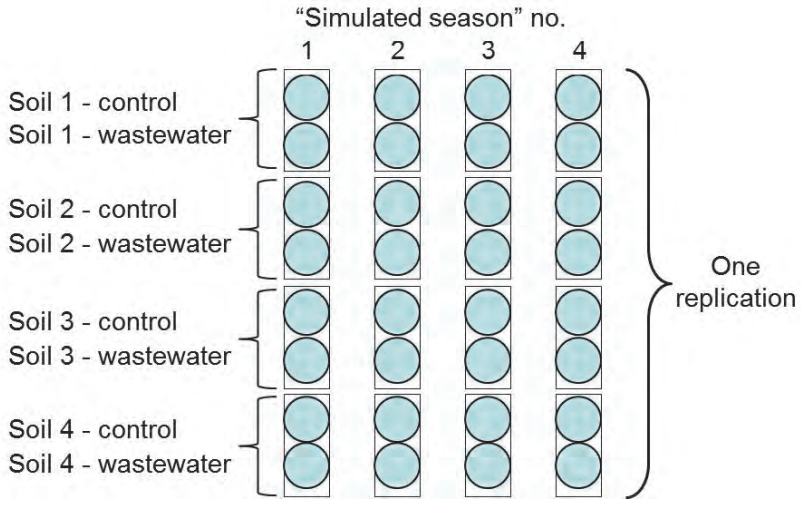

Figure 9.1. Schematic illustration of the layout of one replication of the treatment pots. In the actual trial, the pot positions were randomised.

The soils were then mixed thoroughly, and covered using plastic sheets to minimize water loss during the night. Following the mixing of the soils, gravimetric soil water contents of each soil were determined in triplicate. For this, samples of the moist soil were collected in

metal cans and weighed, and then oven dried at 105^0C for 16 hours. Thereafter, samples were allowed to cool in a desiccator and weighed. Gravimetric soil water content (Θ_m) was calculated as follows:

$$\Theta_m = (M_w - M_d) \div (M_d - M_c) \quad \text{(Eq. 9.1)}$$

where M_w is the mass of the wet soil, M_d is the oven-dried mass of the soil and M_c being the mass of the soil sample can. Mass percentage soil water content (SWC_m) was calculated as follows:

$$SWC_m = \Theta_m \times 100 \quad \text{(Eq. 9.2)}$$

The mass of moist soil required to obtain a bulk density (ρ_b) of 1.5 g/cm^3 was calculated as follows:

$$M_p = (\rho_b \times V_p) \times (1 + SWC_m \div 100) \quad \text{(Eq. 9.3)}$$

where M_p is the mass of the moist soil that needs to be packed onto the pot (g), ρ_b is the required bulk density (g/cm^3), V_p is the soil volume in cm^3 and SWC_m is the water content of the bulked soil (%). A mechanical press was used to compact the soils to obtain the required ρ_b. The soil in each pot received 1 mm of municipal water after filling.

9.2.3. Application of clean and wastewater to the soils

Water was applied to the potted soil by means of 2 L/h button drippers (Netafim, Kraaifontein). To distribute the irrigation water uniformly over the surface, a cross made from galvanised steel plate was placed over each pot (Fig.9.2). A four-way manifold was connected to each dripper (Fig. 9.3). The water was applied *via* four micro-tubes pushed through holes drilled in the metal cross. To enable even water distribution through the four micro-tubes, an arrow dripper (Netafim, Kraaifontein) was inserted in the open end of each tube to create some back pressure. The volumetric soil water content at field capacity for each soil was determined before the pot trial commenced. Pots were weighed every second day. Irrigation was applied when *c.* 50% of the water had evaporated. The latter was considered to be the recommended level of depletion if vineyards are irrigated with augmented winery wastewater.

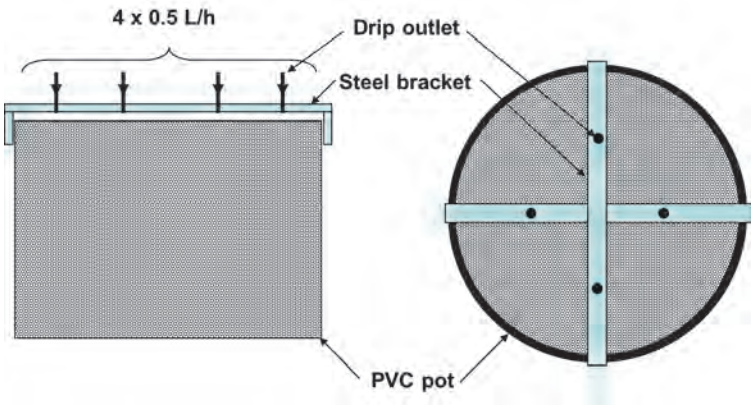

Figure 9.2. Schematic illustration of PVC pot with steel bracket.

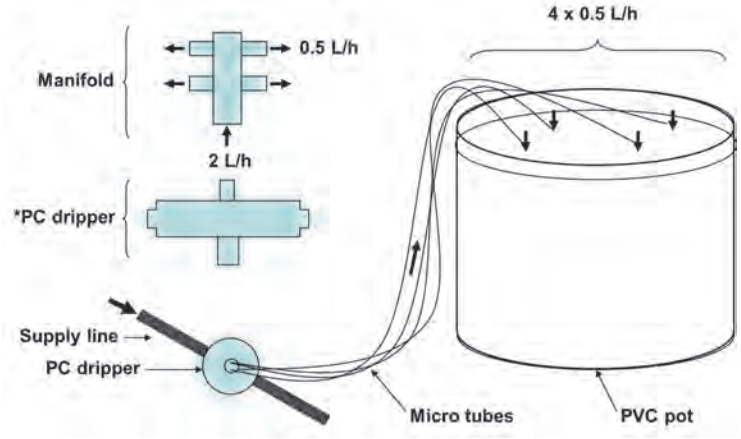

Figure 9.3. Schematic illustration of manifold and micro tubes to obtain more even water supply to the pots (*PC = pressure compensated).

9.2.4. Water analyses

Samples were collected from the clean water and wastewater tanks prior to each of the irrigations. The pH, electrical conductivity (EC), Na, K, Ca, Mg, Fe, Cl, HCO_3, SO_4, B and COD in the water was determined at a commercial laboratory (BEMLAB, Strand). The sodium adsorption ratio (SAR) of the water was calculated using the Na, Ca and Mg concentrations (mg/L) as follows:

$$SAR = Na \div [(Ca + Mg) \div 2]^{½} \quad \text{(Eq. 9.4)}$$

9.2.5. Irrigation amounts applied

The volumes of clean water and augmented wastewater applied to each soil were measured using water meters. Water volumes were converted to depth of water as follows:

$$D = V \div A \quad \text{(Eq. 9.5)}$$

where D is depth of water (mm), V is the volume of water applied per irrigation (L) and A is the surface area of the soil in each pot (m^2).

9.2.6. Soil sampling and analyses

Soil samples were collected from the 0 to 100 mm and 100 to 200 mm depth layers. All analyses were carried out at a commercial laboratory (BEMLAB, Strand). The pH (1M KCl) and EC_e (in a US Bureau of Standards cup) were determined. The P and K (Bray II), Na, K, Ca, Mg, Cu, Zn, Mn, and B were analysed using an inductively coupled plasma atomic emission spectrometer (PerkinElmer Optima 7300 DV, Waltham, Massachusetts). Exchangeable, as well as water soluble Na, K, Ca and Mg were determined. Total organic C contents were determined using the method described by Walkley and Black (1934). The water soluble Na, Ca and Mg were used to calculate the soil SAR according to Equation 9.4. The potassium adsorption ratio (PAR) was calculated from the water soluble cations as follows:

$$PAR = K \div [(Ca + Mg) \div 2]^{½} \quad \text{(Eq. 9.6)}$$

The exchangeable Na, K, Ca, and Mg were used to calculate the exchangeable sodium percentage (ESP) as follows:

$$ESP = [Na \div (Ca + Mg + K + Na)] \times 100 \quad \text{(Eq. 9.7)}$$

9.2.7. Statistical procedures

The experiment layout was a randomized block design. The four soils (S1, Rawsonville; S2, Lutzville; S3, Stellenbosch shale and S4 Stellenbosch granitic) were randomly allocated within each block. The treatment design was a split-plot. The main plot factor was soil type and the sub-plot factor was soil depth, *i.e.* the 0 to 100 mm and 100 to 200 mm soil layers.
Analyses of variance were performed separately for each season using SAS version 9.2 (SAS, 2008). The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). Student's t-least significant difference (LSD) was calculated at the 5% significance level to facilitate comparison between treatment means (Ott, 1998).

9.3. RESULTS AND DISCUSSION

9.3.1. Soil texture and water holding capacity

The clay content of the four soils ranged between 2.3% and 12% (Table 9.2). The soils from Rawsonville and Lutzville were of a sandy texture, the shale derived soil from Stellenbosch was of a sandy loam texture, whereas the gravelly, granite derived soil from Stellenbosch was of a loamy sand texture. With the exception of the granitic soil from Stellenbosch, fine sand dominated the sand fraction (Table 9.2).

Table 9.2. Particle size distribution of the four soils used to determine the effect of augmented winery wastewater on selected soil chemical properties.

Origin	Particle size (%)				
	Clay (<0.002 mm)	Silt (0.002-0.02 mm)	Fine sand (0.02-0.2 mm)	Medium sand (0.2-0.5 mm)	Coarse sand (0.5-2 mm)
S1 - Rawsonville	2.3	1.0	59.6	29.4	7.7
S2 - Lutzville	2.3	1.0	68.5	26.4	1.8
S3 - STB[(1)] (shale)	12.0	13.0	60.7	8.0	6.3
S4 - STB (granitic)	4.0	10.3	11.9	34.9	6.0

[(1)] STB = Stellenbosch.

Table 9.3. Water holding capacity between 10 kPa and 100 kPa matric potential of the four soils used to determine the effect of augmented winery wastewater on selected soil chemical properties.

Soil	Soil water content (%)		Water holding capacity (mm/m)
	10 kPa	100 kPa	
S1 - Rawsonville	19.0	6.9	121.5
S2 - Lutzville	21.0	7.4	136.6
S3 - STB[(1)] (shale)	31.3	16.0	153.3
S4 - STB (granitic)	26.9	14.3	125.9

[(1)] STB = Stellenbosch.

9.3.2. Irrigation amounts applied

The irrigation amounts applied to Rawsonville sand (S1), the Lutzville sand (S2), and Stellenbosch shale soil (S3) soils were comparable (Tables 9.4 to 9.7). However, the Stellenbosch granitic soil (S4) received substantially less water compared to the other soils. After four simulated seasons, *i.e.* 24 irrigations, the S1, S2, S3 and S4 received a total of 1156 mm, 1126 mm, 986 mm and 728 mm, respectively.

Table 9.4. Irrigation amounts (mm) of clean or winery wastewater applied to four different soils during simulated season No. 1 in a pot experiment at Stellenbosch.

Soil	Irrigation number						Total
	1	2	3	4	5	6	
S1 - Rawsonville	49	48	48	49	48	49	291
S2 - Lutzville	48	47	46	46	47	47	281
S3 - STB[(1)] (shale)	42	41	40	41	41	41	246
S4 - STB (granitic)	31	30	30	30	30	30	181

[(1)] STB = Stellenbosch.

Table 9.5. Irrigation amounts (mm) of clean or winery wastewater applied to four different soils during simulated season No. 2 in a pot experiment at Stellenbosch.

Soil	Irrigation number						Total
	1	2	3	4	5	6	
S1 - Rawsonville	49	49	48	46	48	49	289
S2 - Lutzville	47	47	47	47	47	47	282
S3 - STB[(1)] (shale)	42	42	42	41	42	41	250
S4 - STB (granitic)	30	30	31	30	30	29	180

[(1)] STB = Stellenbosch.

Table 9.6. Irrigation amounts (mm) of clean or winery wastewater applied to four different soils during simulated season No. 3 in a pot experiment at Stellenbosch.

Soil	Irrigation number						Total
	1	2	3	4	5	6	
S1 - Rawsonville	48	48	48	48	48	47	287
S2 - Lutzville	47	47	46	47	47	48	282
S3 - STB[(1)] (shale)	41	41	41	41	41	41	246
S4 - STB (granitic)	31	30	31	31	30	31	184

[(1)] STB = Stellenbosch.

Table 9.7. Irrigation amounts (mm) of clean or winery wastewater applied to four different soils during simulated season No. 4 in a pot experiment at Stellenbosch.

Soil	Irrigation number						Total
	1	2	3	4	5	6	
S1 - Rawsonville	48	48	48	49	48	48	289
S2 - Lutzville	47	47	47	47	47	47	281
S3 - STB[(1)] (shale)	41	40	41	41	41	41	245
S4 - STB (granitic)	30	31	30	31	30	30	183

[(1)] STB = Stellenbosch.

9.3.3. Chemical composition of the irrigation waters

The overall quality variables for the clean municipal water were in line with the national legislation for drinking water during the four simulated seasons (Tables 9.8 to 9.11). With the exception of pH, the winery wastewater quality chemical parameters were considerably higher than those of the clean water. On most irrigation dates, the winery wastewater was more acidic than the clean water. It must be noted that the average SAR of the winery wastewater was close to 5 (Tables 9.12 to 9.15) which is the upper limit for irrigation with wastewater according to the South African legislation, *i.e.* if the irrigation volume does not exceed 500 m^3 per day (DWA, 2013).

Table 9.8. Variation in quality of Stellenbosch municipal water used for irrigation of four different soils in a pot experiment during simulated season No. 1.

Water quality variables	Date					
	23/03/10	13/04/10	7/5/2010	8/6/2010	6/7/2010	3/8/2010
pH	7.2	8.0	8.3	7.4	7.4	7.9
EC_e (mS/m)	11.2	10.3	10.9	5.0	5.6	6.8
Na (mg/L)	9.3	9.5	9.7	5.0	4.7	5.9
K (mg/L)	1.4	0.9	1.2	0.2	0.7	0.7
Ca (mg/L)	8.3	7.8	8.0	4.8	4.2	4.7
Mg (mg/L)	2.1	2.1	1.9	0.3	0.6	0.7
Fe (mg/L)	0.1	0.0	0.1	0.0	0.1	0.1
Cl (mg/L)	21.2	22.0	17.6	9.7	8.8	14.1
HCO_3 (mg/L)	36.7	53.6	6.1	30.6	15.3	53.5
SO_4 (mg/L)	3.2	2.7	2.6	3.0	1.3	2.7
B (mg/L)	0.0	0.0	0.0	0.0	0.0	0.0
SAR	0.7	0.8	0.8	0.6	0.6	0.7
COD (mg/L)	45	29	37	40	38	22

Table 9.9. Variation in quality of Stellenbosch municipal water used for irrigation of four different soils in a pot experiment during simulated season No. 2.

Water quality variables	Date					
	26/08/10	30/09/10	27/10/10	9/11/2010	24/11/10	7/1/2011
pH	7.2	7.0	7.5	7.8	7.4	7.8
EC_e (mS/m)	5.8	5.2	5.7	11.2	7.8	7.6
Na (mg/L)	4.6	7.4	4.0	10.4	8.5	8.4
K (mg/L)	0.0	0.5	0.7	1.3	0.8	1.0
Ca (mg/L)	4.8	5.2	5.5	8.9	6.3	5.4
Mg (mg/L)	0.6	0.6	0.9	2.0	1.5	1.0
Fe (mg/L)	0.0	0.2	0.0	0.1	0.0	0.0
Cl (mg/L)	12.4	13.2	11.5	21.2	12.3	13.2
HCO_3 (mg/L)	30.6	13.8	15.3	26.0	23.0	26.0
SO_4 (mg/L)	1.2	0.0	0.0	10.8	13.5	1.3
B (mg/L)	0.0	0.2	0.0	0.0	0.0	0.0
SAR	0.5	0.8	0.4	0.8	0.8	0.9
COD (mg/L)	33	17	19	29	24	25

Table 9.10. Variation in quality of Stellenbosch municipal water used for irrigation of four different soils in a pot experiment during simulated season No. 3.

Water quality variables	Date					
	14/2/2011	7/3/2011	5/4/2011	9/5/2011	12/7/2011	1/9/2011
pH	7.4	8.1	7.4	7.8	7.8	7.6
EC_e (mS/m)	12.3	14.1	10.0	9.0	5.0	6.4
Na (mg/L)	10.3	10.6	9.9	6.5	5.8	5.2
K (mg/L)	1.0	1.3	1.0	0.5	1.4	0.2
Ca (mg/L)	7.4	8.4	6.8	5.1	4.4	4.6
Mg (mg/L)	2.3	2.3	1.9	1.1	0.7	0.7
Fe (mg/L)	0.1	0.0	0.1	0.1	0.0	0.1
Cl (mg/L)	23.8	17.6	20.3	16.7	16.6	15.9
HCO_3 (mg/L)	30.6	13.8	30.6	15.3	4.6	15.3
SO_4 (mg/L)	0.1	2.0	8.0	2.0	2.1	1.9
B (mg/L)	0.0	0.0	0.0	0.0	0.0	0.01
SAR	0.9	0.8	0.9	0.7	0.7	0.6
COD (mg/L)	19	37	25	23	18	31

Table 9.11. Variation in quality of Stellenbosch municipal water used for irrigation of four different soils in a pot experiment during simulated season No. 4.

Water quality variables	Date					
	31/1/2012	22/2/2012	27/3/2012	23/4/2012	28/5/2012	15/8/2012
pH	7.7	6.8	6.9	6.3	6.4	7.2
EC_e (mS/m)	10	9.0	14.6	9.0	7.0	6.3
Na (mg/L)	7.8	11.5	12.3	8.4	6.9	4.1
K (mg/L)	2.3	1.0	1.6	1.4	3.3	0.3
Ca (mg/L)	4.7	5.8	5.7	5.0	3.6	7.2
Mg (mg/L)	1.5	3.0	2.9	1.4	1.0	1.1
Fe (mg/L)	0.1	0.6	0.1	0.1	0.1	0.1
Cl (mg/L)	26.0	20.3	22.0	20.3	18.0	14.5
HCO_3 (mg/L)	31.0	24.5	31.0	28.0	23.0	19.8
SO_4 (mg/L)	1.0	11.7	5.0	2.0	9.3	5.3
B (mg/L)	0.0	0.0	0.0	0.0	0.0	0.0
SAR	0.8	1.0	1.1	0.9	0.8	0.4
COD (mg/L)	18	28	31	33	29	19

Table 9.12. Quality variation of winery wastewater augmented to 3000 mg/L COD and used for irrigation of four different soils in a pot experiment during simulated season No. 1.

Quality variables	Date					
	23/03/10	13/04/10	7/5/2010	8/6/2010	6/7/2010	3/8/2010
pH	4.6	4.7	4.9	6.9	5.4	5.1
EC_e (mS/m)	49.9	119.3	143.2	78.0	69.0	105.5
Na (mg/L)	47.0	72.7	87.1	100.3	67.3	78.9
K (mg/L)	91.7	294.8	359.2	119.4	137.6	174.2
Ca (mg/L)	10.5	17.6	17.5	11.5	12.0	15.2
Mg (mg/L)	3.6	7.6	7.8	2.5	2.9	5.1
Fe (mg/L)	0.4	1.7	2.2	0.7	1.6	2.0
Cl (mg/L)	26.5	38.8	31.7	12.4	22.1	12.3
HCO_3 (mg/L)	137.5	447.1	718.1	522.1	405.7	837.4
SO_4 (mg/L)	10.0	170.1	425.2	14.0	49.7	61.4
B (mg/L)	0.3	0.5	0.6	0.1	0.2	0.2
SAR	3.2	3.7	4.4	7.0	4.5	4.5
COD (mg/L)	2950	3220	3155	2940	3260	3370

Table 9.13. Quality variation of winery wastewater augmented to 3000 mg/L COD and used for irrigation of four different soils in a pot experiment during simulated season No. 2.

Quality variables	Date					
	26/08/10	30/09/10	27/10/10	9/11/2010	24/11/10	7/1/2011
pH	5.4	5.0	5.4	6.2	6.9	7.2
EC_e (mS/m)	103.1	104.4	106.2	77.8	93.6	173.7
Na (mg/L)	97.3	113.0	98.7	113.1	93.8	173.4
K (mg/L)	208.2	189.7	230.7	123.2	124.7	243.2
Ca (mg/L)	17.7	18.1	19.0	14.9	18.3	19.9
Mg (mg/L)	5.5	8.9	9.4	6.8	8.0	11.8
Fe (mg/L)	2.4	2.5	3.4	2.7	2.7	2.8
Cl (mg/L)	17.7	18.5	23.0	26.5	48.5	60.9
HCO_3 (mg/L)	620.1	470.1	664.7	487.5	581.8	1106.3
SO_4 (mg/L)	180.7	8.1	190.3	125.5	81.5	149.0
B (mg/L)	0.2	1.0	0.9	0.6	0.7	1.2
SAR	5.2	5.5	4.7	6.1	4.6	7.7
COD (mg/L)	3380	3000	3430	3180	3420	3130

Table 9.14. Quality variation of winery wastewater augmented to 3000 mg/L COD and used for irrigation of four different soils in a pot experiment during simulated season No. 3.

Quality variables	Date					
	14/2/11	7/3/2011	5/4/2011	9/5/2011	12/7/2011	1/9/2011
pH	4.8	4.5	4.9	6.3	3.8	4.9
EC_e (mS/m)	64.4	75.6	78.0	113.0	88.0	148.7
Na (mg/L)	53.4	41.9	85.1	113.7	48.3	129.5
K (mg/L)	81.5	129	213.3	277.8	225.1	302.5
Ca (mg/L)	21.9	20.2	27.3	18.3	16.9	15.4
Mg (mg/L)	6.5	5.9	10.9	6.6	4.4	4.6
Fe (mg/L)	4.1	3.6	4.1	1.7	1.9	4.5
Cl (mg/L)	39.7	22.9	47.7	30.8	43.3	30.9
HCO_3 (mg/L)	267.9	286.3	362.9	672.3	288.1	751.7
SO_4 (mg/L)	17.6	18.8	161.0	7.0	13	42.1
B (mg/L)	0.3	0.3	0.6	0.6	0.2	0.3
SAR	2.6	2.1	3.5	5.8	2.7	7.5
COD (mg/L)	3190	3240	3460	3010	3420	3140

Table 9.15. Quality variation of winery wastewater augmented to 3000 mg/L COD and used for irrigation of four different soils in a pot experiment during simulated season No. 4.

Quality variables	Date					
	31/1/2012	22/2/2012	27/3/2012	23/4/2012	28/5/2012	15/8/2012
pH	8.0	7.8	4.5	4.5	4.9	3.7
EC_e (mS/m)	197	212	49	51	109	97
Na (mg/L)	125	123	27	31	86	20
K (mg/L)	358	356	67	99	175	123
Ca (mg/L)	6.4	6.3	10.4	14.9	13.2	26
Mg (mg/L)	6.9	6.9	5.1	5.9	10.3	19.5
Fe (mg/L)	1.7	1.3	2.0	3.2	2.1	4.2
Cl (mg/L)	57	46	29.1	34.4	35.3	35
HCO_3 (mg/L)	1062	1284	146	217	473	136
SO_4 (mg/L)	175	21	14	22	64	114
B (mg/L)	0.3	0.4	0.1	0.1	0.2	0.2
SAR	8.2	8.1	1.7	1.7	4.3	0.7
COD (mg/L)	3000	3190	3490	2920	3080	3460

9.3.4. Amount of elements applied

Considerably more cations, anions and boron were applied *via* the augmented winery wastewater compared to the clean municipal water (Table 9.16). Since K is an essential plant nutrient, the large amount of K in winery wastewater can be beneficial to crops. The high amount of Na applied *via* the augmented wastewater which was applied to the soil could be problematic, since Na is not an essential nutrient, and could be detrimental to the physical status of the soil. Since Na concentrations are likely to be higher in undiluted winery wastewater, the probability of soil damage will be higher if the wastewater is not augmented.

Table 9.16. Amount of cations, anions and boron applied *via* clean water and augmented winery wastewater to four different soils (S1 to S4) over four simulated seasons (T1 to T4) in a pot experiment near Stellenbosch.

Element	Season	Amounts applied (kg/ha)							
		S1		S2		S3		S4	
		Clean	Waste	Clean	Waste	Clean	Waste	Clean	Waste
Na	T1	100	1315	97	1276	85	1115	62	818
	T2	125	1514	121	1477	107	1303	78	948
	T3	139	1358	136	1329	119	1165	89	865
	T4	147	1189	143	1156	125	1008	93	753
K	T1	11	3414	11	3312	10	2895	7	2124
	T2	12	2535	12	2472	10	2181	7	1587
	T3	16	3538	15	3463	13	3034	10	2253
	T4	29	3406	28	3312	24	2887	18	2157
Ca	T1	86	245	84	237	73	207	54	152
	T2	104	270	101	263	89	232	65	169
	T3	106	345	103	338	91	296	67	220
	T4	92	388	90	378	78	329	59	246
Mg	T1	17	85	16	83	14	72	10	53
	T2	19	114	18	112	16	98	12	72
	T3	26	112	25	110	22	96	17	71
	T4	32	158	31	153	27	134	20	100
Cl	T1	220	417	214	405	187	354	137	260
	T2	242	426	236	415	208	366	152	266
	T3	319	620	312	607	274	531	203	395
	T4	348	686	338	667	295	581	220	434
HCO_3	T1	550	8900	534	8636	467	7547	342	5538
	T2	389	8234	379	8029	335	7085	244	5156
	T3	317	7567	310	7407	272	6489	202	4820
	T4	452	9587	439	9322	383	8128	286	6071
SO_4	T1	37	2118	36	2056	32	1796	23	1318
	T2	77	1697	75	1654	67	1460	48	1062
	T3	46	747	45	731	40	640	30	476
	T4	99	1186	96	1154	84	1006	63	751
B	T1	0	5	0	5	0	5	0	3
	T2	1	10	1	10	1	8	0	6
	T3	0	7	0	7	0	6	0	4
	T4	0	4	0	4	0	3	0	2

9.3.5. Soil chemical status

9.3.5.1. Rawsonville sand (S1)

$pH_{(KCl)}$: Soil pH for soils irrigated with augmented wastewater was higher for four consecutive seasons when compared to soils irrigated with clean water. For season one and three, wastewater treatment showed higher pH on 0 to 100 mm compared to 100 to 200mm depth interval. For season two and four, there was no pH difference between soil depths for the wastewater treatment (Table 9.17). The pH increase for wastewater treatment could be attributed to high concentration of bicarbonate in the winery wastewater (Laurenson *et al*., 2012). The average winery wastewater bicarbonate concentrations for the four seasons ranges between 438-655 mg/L (Tables 9.12 to 9.15), while bicarbonate concentrations in the clean water for the four seasons ranges from 18.4-32.6 mg/L (Tables 9.8 to 9.11). This foregoing indicated that continuous winery wastewater irrigation has the potential to increase soil salinity. Lieffering and McLay (1996) reported that soil pH and exchangeable Na has the tendency to increase when wastewater with high pH and high Na concentration is used for irrigation.

Table 9.17. Effects of irrigation with clean municipal water and augmented winery wastewater on $pH_{(KCl)}$, EC_e, organic C, P (Bray II) and K (Bray II) in a sandy soil from Rawsonville (S1) over four simulated seasons.

Variable	Water	Depth (mm)	Season				
			Initial[1]	1	2	3	4
$pH_{(KCl)}$	Clean	0-100	5.7	5.9c[2]	6.3b	5.9c	5.9b
		100-200	5.7	6.0c	6.2b	5.9c	5.9b
	Wastewater	0-100	5.7	6.7a	6.7a	7.5a	7.7a
		100-200	5.7	6.6b	6.6a	7.2b	7.5a
EC_e (dS/m)	Clean	0-100	0.3	0.3a	0.5ab	0.8b	0.9a
		100-200	0.3	0.2b	0.2b	0.3c	0.7a
	Wastewater	0-100	0.3	0.3a	0.7a	0.9a	1.2a
		100-200	0.3	0.1b	0.2b	0.3c	1.1a
Org C (%)	Clean	0-100	1.0	0.9a	1.0a	2.4a	0.7a
		100-200	1.0	1.0a	1.0a	2.1a	0.7a
	Wastewater	0-100	1.0	1.0a	1.0a	2.9a	0.6ab
		100-200	1.0	1.0a	1.0a	3.0a	0.5b
P (mg/kg)	Clean	0-100	217	257a	246a	167a	248a
		100-200	217	275a	261a	165a	244a
	Wastewater	0-100	217	271a	228a	45b	42b
		100-200	217	274a	254a	38b	34b
K (mg/kg)	Clean	0-100	87	77c	92c	71c	83b
		100-200	87	72c	74c	59c	83b
	Wastewater	0-100	87	332a	852a	709a	887a
		100-200	87	152b	303b	367b	726a

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

EC_e: There was no difference in soil EC_e between clean and wastewater treatments during season one, two and four (Table 9.17). During season one and three, a difference was observed between the 0 to 100 mm and 100 to 200 mm soil layers.

Since the EC_e also increased where clean water was applied, it suggested that the soil pH increase for wastewater treatments was not caused by an increase in the Na, K, Ca and Mg salts.

Organic carbon: Irrigation with augmented winery wastewater had no effect on soil organic carbon after four seasons compared to the clean municipal water treatment, with the exception of the 100 to 200 mm soil layer in season four (Table 9.17). The results indicate that the organic carbon content in the wastewater was too low to have increased the soil organic material. Furthermore, the organic material could have decomposed when the soil was aerated between irrigations. These findings are contrary to increased soil organic carbon content resulting from winery wastewater irrigation (Papini, 2000).

Phosphorus (Bray II): Regardless of the water used for irrigation, soil P levels were the same in season one and two (Table 9.17). During season three and four however, the level of P in the treatment irrigated with municipal water was higher than that of the wastewater treatments. The initial soil P levels were more than seven-fold the maximum of 30 mg/kg recommended for grapevines (Conradie, 1994). This was probably the result of P fertilizers applied to the grapevines before the bulk soil sample was collected. During seasons three and four, irrigation with augmented winery wastewater reduced soil P compared to clean water irrigation. At this stage there is no clear explanation for the unexpected P reduction. Generally winery wastewater irrigation results in P increase in the soil (Papini, 2000). However, it could be that a complex with P was formed by the constituents in the wastewater. If soluble, the P complex would probably have been leached out. Alternatively, a stable P complex may have formed that was neither leached out, nor susceptible to breakdown and P release by the Bray II reagent. In both cases, the overall effect of the wastewater was to reduce Bray II soil P levels from highly excessive to slightly excessive levels. The P reducing effect of wastewater may be beneficial from a plant production viewpoint, but involves wastage of a soil mineral resource, *i.e.* either by fixation or leaching into the groundwater. In the latter case P could become a pollutant.

Potassium (Bray II): The initial level of K in the soil was within the norms proposed for grapevines (Conradie, 1994). Irrigation with augmented winery wastewater increased soil K substantially compared to clean water irrigation (Table 9.17). The higher K levels were more pronounced in the 0 to 100 mm soil layer during the first three seasons. In the fourth season, K in the 0 to 100 mm and 100 to 200 soil layers were comparably high. This suggested a rapid accumulation of K from the wastewater in the 0 to 100 mm layer, and a slower K increase in the 100 to 200 mm soil layer. This implies that the soil has a limited ability to adsorb K from the wastewater, with excess K moving progressively deeper as the more superficial soil material becomes saturated.

Where winery wastewater is recycled for irrigation, accumulation of K in the soil and the replacement of bivalent cations, *e.g.* Ca and Mg, by monovalent K or Na has the potential for soil structural breakdown, if not carefully managed (Christen *et al.*, 2010).

Exchangeable cations:

$Na_{ex:}$ Irrigation with clean water had almost no effect on the soil Na_{ex}, but the augmented winery wastewater resulted in an increase over the four seasons (Table 9.18).

Table 9.18. Effects of irrigation with clean municipal water and augmented winery wastewater on exchangeable cations, sodium adsorption ratio (SAR), potassium adsorption ratio (PAR) and exchangeable sodium percentage (ESP) in a sandy soil from Rawsonville (S1) over four simulated seasons.

Variable	Water	Depth	Season				
		(mm)	Initial[1]	1	2	3	4
Na_{ex} ($cmol^{(+)}$/kg)	Clean	0-100	0.1	0.1c[2]	0.2bc	0.1c	0.2a
		100-200	0.1	0.1c	0.1c	0.1c	0.2a
	Wastewater	0-100	0.1	0.5a	1.6a	1.1a	1.2a
		100-200	0.1	0.2b	0.4b	0.4b	1.0a
K_{ex} ($cmol^{(+)}$/kg)	Clean	0-100	0.2	0.2c	0.2c	0.2c	0.2b
		100-200	0.2	0.2c	0.2c	0.2c	0.2b
	Wastewater	0-100	0.2	0.9a	2.2a	1.8a	2.3a
		100-200	0.2	0.4b	0.8b	0.9b	1.9a
Ca_{ex} ($cmol^{(+)}$/kg)	Clean	0-100	3.5	3.1b	3.6a	3.1a	3.5a
		100-200	3.5	3.2ab	3.3b	3.2a	3.5a
	Wastewater	0-100	3.5	3.0b	3.0c	2.8b	3.1b
		100-200	3.5	3.3a	3.3b	3.1a	3.1b
Mg_{ex} ($cmol^{(+)}$/kg)	Clean	0-100	1.6	1.4b	1.6a	1.2a	1.3a
		100-200	1.6	1.3c	1.4c	1.1b	1.4a
	Wastewater	0-100	1.6	1.2d	1.3c	0.9c	1.2a
		100-200	1.6	1.5a	1.5b	1.1ab	1.2a
SAR	Clean	0-100	0.1	0.1c	0.1b	0.1bc	0.1a
		100-200	0.1	0.1c	0.1b	0.0c	0.1a
	Wastewater	0-100	0.1	0.3a	1.0a	0.8a	0.8a
		100-200	0.1	0.2b	0.3b	0.3b	0.7a
PAR	Clean	0-100	0.1	0.1c	0.1c	0.1c	0.1b
		100-200	0.1	0.1c	0.1c	0.1c	0.1b
	Wastewater	0-100	0.1	0.6a	1.5a	1.3a	1.6a
		100-200	0.1	0.3b	0.5b	0.6b	1.3a
ESP %	Clean	0-100	1.7	2.7c	3.5c	2.7c	3.1b
		100-200	1.7	1.8c	2.3c	1.4c	2.9b
	Wastewater	0-100	1.7	8.9a	19.3a	16.5a	15.2a
		100-200	1.7	4.5b	7.4b	8.0b	12.9a

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

This effect was more pronounced in the 0 to 100 mm soil layer during the first three seasons. After the fourth season, Na_{ex} was comparably high in both soil layers. It was reported that winery wastewater containing high Na levels, due to sodium hydroxide being used as cleaning detergent, has the potential to increase Na_{ex}, and reduce the hydraulic conductivity of the soil when applied (Laurenson *et al*., 2012).

K_{ex}: Irrigation with augmented winery wastewater increased the K_{ex} compared to clean water in both soil layers over four simulated seasons (Table 9.18). The wastewater treatment showed higher values in the 0 to 100mm when compared to the 100 to 200 mm soil layers during the first three seasons. After four simulated seasons, soils irrigated with augmented winery wastewater increased the K_{ex} from 0.2 to 2.3 $cmol^{(+)}/kg$ in the 0 to100mm soil layer with the K_{ex} in both soil levels being similar (Table 9.18).

Ca_{ex}: Irrigating with augmented winery wastewater lowered the Ca_{ex} in the 100 to 200 mm soil layer for four consecutive seasons (Table 9.18). After four simulated seasons, soils irrigated with clean water had higher Ca_{ex} values compared to those irrigated with wastewater. Irrigating with augmented winery wastewater resulted in reduction of Ca_{ex} from 3.5 to 3.1$cmol^{(+)}/kg$,while the clean water treatment remained at 3.5$cmol^{(+)}/kg$ after four simulated seasons. The reduction in Ca_{ex} after wastewater irrigation is a cause for concern as Ca is an important cation responsible for soil structure stability.

Mg_{ex}: The treatments had an effect on Mg_{ex} of both soil layers during season one (Table 9.18). The Mg_{ex} was lowest in the 0 to 100 mm and 100 to 200 mm soil layer of the treatments irrigated with augmented winery wastewater and clean water respectively. This trend was observed for seasons two and three as well, although it weakened over time. After four simulated seasons, there was no difference between treatments (Table 9.18).

SAR: Irrigation with augmented winery wastewater increased the SAR of the soil compared to clean water over the four seasons (Table 9.18). This trend was more pronounced in the 0 to 100 mm soil layer during the first three seasons. During the fourth season, the SAR was comparable in both soil layers. However, these results indicated that prolonged irrigation with winery wastewater could be detrimental to the physical properties of this particular soil if the Na increases substantially compared to Ca and Mg.

PAR: Irrigation with augmented winery wastewater increased the PAR of this particular soil compared to clean water over four seasons (Table 9.18). The PAR increase was more pronounced in the 0 to 100 mm soil layer. The source of higher PAR in the soil was the result of higher amount of K from the augmented winery wastewater applied to the soil (Tables 9.12 to 9.15).

ESP: Irrigation with augmented winery wastewater increased the ESP of the soil substantially compared to clean water irrigation over the four seasons (Table 9.18). This trend was also more pronounced in the 0 to 100 mm soil layer. The ESP threshold proposed for most soils is 15% (Seilsepour *et al.,* 2009). Therefore, wastewater irrigation increased the ESP in the 0 to 100 mm soil layer to above the threshold value which can lead to clay dispersion and structure instability.

Copper: Irrigation with augmented winery wastewater increased Cu in the soil compared to clean water (Table 9.19). This suggested that the wastewater contained higher Cu concentrations than the clean water. However these Cu levels were less than 10mg/kg which is the maximum for grapevine cultivation (Van Schoor *et al*., 2000).

Zinc: Irrigation with augmented winery wastewater increased Zn in the soil compared to clean water (Table 9.19). This suggested that the wastewater contained higher Zn levels than the clean water. The Zn threshold levels for grapevine cultivation are 5 mg/kg (Van Schoor *et al.,* 2000). Continuous irrigation with winery wastewater will result in build-up of Zn in the soil.

Manganese: Irrigation with augmented winery wastewater increased Mn substantially in S1 compared to clean water (Table 9.19). This suggested the wastewater contained more Mn than the clean water. For simulated season three, Mn values at 100 to 200 mm interval was above the threshold value of 20 mg/kg for grapevine (Van Schoor *et al.,* 2000).

Boron: Irrigation with augmented winery wastewater increased B in the 0 to 100 mm soil layer to levels (Table 9.19) exceeding the 4 mg/kg threshold for grapevine cultivation (Van Schoor *et al*., 2000). This trend was observed in the 100 to 200 mm soil layer from the third simulated season onwards as well (Table 9.19). After four simulated seasons, there was a good relationship between the amount of B applied *via* the water and the levels in the 0 to 100 mm and 100 to 200 mm depth layers (Table 9.16). The high concentration of B in the winery wastewater was due to its presence in the washing detergents (Kot *et al.*, 2012).

Iron: Although Fe decreased in the four seasons, decrease was more prominent in the treatment where municipal clean water was used thus indicating that the higher Fe levels in the wastewater (Tables 9.12 to 9.15) were reflected in the soil (Table 9.19).

Sulphur: Irrigation with augmented winery wastewater increased S levels in the 0 to 100 mm soil layer compared to clean water during seasons two and three (Table 9.19).

Table 9.19. Effects of irrigation with clean municipal water and augmented winery wastewater on Cu, Zn, Mn, B, Fe and S in a sandy soil from Rawsonville (S1) over four simulated seasons.

Variable	Water	Depth (mm)	Season				
			Initial[1]	1	2	3	4
Cu (mg/kg)	Clean	0-100	7.3	7.6a[2]	7.3b	6.9b	7.6b
		100-200	7.3	7.5a	7.3b	6.9b	7.4b
	Wastewater	0-100	7.3	7.9a	8.7a	8.7a	9.5a
		100-200	7.3	7.8a	8.2ab	8.1a	9.1a
Zn (mg/kg)	Clean	0-100	3.0	3.4b	5.5b	4.2b	4.9a
		100-200	3.0	2.7c	3.6c	2.6c	3.7a
	Wastewater	0-100	3.0	4.9a	7.6a	6.9a	7.3a
		100-200	3.0	2.9c	5.0b	3.3bc	5.6a
Mn (mg/kg)	Clean	0-100	7.9	6.4b	6.5b	7.7b	4.5b
		100-200	7.9	7.3b	7.0b	9.2b	5.3b
	Wastewater	0-100	7.9	10.6ab	15.7a	19.7a	17.6a
		100-200	7.9	13.0a	15.9a	21.6a	14.9a
B (mg/kg)	Clean	0-100	0.2	0.3bc	0.1c	0.1c	0.1a
		100-200	0.2	0.2c	0.1c	0.1c	0.1a
	Wastewater	0-100	0.2	0.5a	0.7a	0.7a	0.9a
		100-200	0.2	0.4ab	0.4b	0.5b	0.7a
Fe (mg/kg)	Clean	0-100	188	85a	104a	80b	83c
		100-200	188	76a	101a	85ab	93bc
	Wastewater	0-100	188	85a	145a	126ab	138ab
		100-200	188	93a	136a	132a	150a
S (mg/kg)	Clean	0-100	10	12a	17b	16b	12a
		100-200	10	7ab	9c	11b	13a
	Wastewater	0-100	10	9ab	50a	48a	33a
		100-200	10	7b	9c	17b	22a

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

9.3.5.2. Lutzville sand (S2)

$pH_{(KCl)}$: During the last three seasons, irrigation with augmented winery wastewater increased the soil pH compared to clean water (Table 9.20). Similarly to S1, this trend was probably due to the relatively high bicarbonate concentration in the wastewater.

EC_e: Irrigation with augmented winery wastewater increased the EC_e in the 0 to 100 mm soil layers compared to clean water during seasons two and four (Table 9.20).

Organic carbon: Similarly to the S1 soil, irrigation water quality had no effect on soil organic carbon (Table 9.20).

Table 9.20. Effects of irrigation with clean municipal water and augmented winery wastewater on $pH_{(KCl)}$, EC_e, organic C, P (Bray II) and K (Bray II) in a sandy soil from Lutzville (S2) over four simulated seasons.

Variable	Water	Depth	Season				
		(mm)	Initial[1]	1	2	3	4
$pH_{(KCl)}$	Clean	0-100	6.7	7.9ab[2]	7.9c	7.5c	7.7c
		100-200	6.7	7.7b	7.7d	7.3d	7.1d
	Wastewater	0-100	6.7	8.2ab	8.9a	9.2a	9.2a
		100-200	6.7	8.4a	8.5b	8.7b	8.9b
EC_e (dS/m)	Clean	0-100	0.5	0.4a	0.4b	0.5a	1.5b
		100-200	0.5	0.1a	0.1c	0.1a	0.2c
	Wastewater	0-100	0.5	0.3a	0.6a	0.7a	3.0a
		100-200	0.5	0.4a	0.3b	0.6a	0.9bc
Org C (%)	Clean	0-100	0.2	0.2a	0.1b	0.2a	0.1ba
		100-200	0.2	0.2a	0.2ab	0.2a	0.1ba
	Wastewater	0-100	0.2	0.3a	0.2a	0.2a	0.1a
		100-200	0.2	0.3a	0.2ba	0.2a	0.04b
P (mg/kg)	Clean	0-100	6.0	3a	4b	2c	3c
		100-200	6.0	4a	4b	2c	4c
	Wastewater	0-100	6.0	4a	30a	26a	42a
		100-200	6.0	6a	8b	10b	18b
K (mg/kg)	Clean	0-100	183	201a	193c	172c	187c
		100-200	183	163a	147d	126c	142d
	Wastewater	0-100	183	309a	867a	710a	1174a
		100-200	183	438a	419b	460b	761b

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Phosphorus (Bray II): The augmented winery wastewater increased the P levels in the 0 to 100 mm soil layer compared to clean water irrigation from the second season onwards. A similar trend was observed in the 100 to 200 mm soil layer (Table 9.20). P in clean water decreased as number of season increased while the wastewater values showed the opposite trend (Table 9.20).This indicated that the higher level of P in the winery wastewater compared to clean water (Refer to Chapter 3) reflected positively in the P level of this particular soil.

Potassium (Bray II): There were no significant differences between treatments during the first season (Table 9.20). However, from the second season onwards, irrigation with winery wastewater resulted in higher soil K accumulation in both soil layers (Table 9.20). Therefore after four simulated seasons, the amount of K applied *via* the waste and clean waters (Tables 9.12 to 9.15) clearly reflected in both soil layers of this soil.

Exchangeable cations:

Na_{ex} : Irrigation with clean water did not affect the soil Na_{ex} in 100 to 200 mm soil layer in all four seasons, while irrigation with augmented winery wastewater resulted in increases in Na_{ex} over four seasons (Table 9.21). The increases were more prominent in the 0 to 100 mm soil layer in season two and four. The increase indicated that the soil had the ability to adsorb the Na_{ex} up to a certain level thereafter leaching could take place. With proper winery wastewater irrigation strategies and better management such as measuring amount of wastewater applied and using Na free detergents during cleaning of wine cellars, the leaching of Na_{ex} to the subsoil can be minimized.

K_{ex}: Irrigation with the augmented winery wastewater increased the K_{ex} in the soil compared clean water irrigations from the second season onwards (Table 9.21). Although not significant, this trend was observed in the first season as well. The clean water irrigation in the 100 to 200 mm soil layer reduced the K_{ex} to below the initial values.

Ca_{ex}: In seasons one, two and four there were no differences between treatments (Table 9.21). While In season three there was a difference between the 0 to 100 mm and 100 to 200 mm soil layers irrigated with clean water. According to Christen *et al*. (2010), repeated irrigation with winery wastewater lead to leaching of Ca due to exchange sites being occupied by the monovalent cations such as Na and K.

Mg_{ex}: Results showed no significant difference between treatments only in season one, while season two and three there was difference between treatments as well as between different soil layers. In season four soils irrigated with clean water tended to be higher compared to those irrigated with winery wastewater. There was also a significant difference between clean and wastewater treatments at both 0 to 100 mm and 100 to 200 mm soil layer intervals (Table 9.21).

SAR: Irrigation with augmented winery wastewater increased the SAR of the soil compared to clean water with exception of season one (Table 9.21). This trend was more pronounced in the 0 to 100 mm soil layer. However, these results indicated that prolonged irrigation with winery wastewater could be detrimental to the physical properties of this particular soil if the Na increases substantially compared to Ca and Mg.

PAR: Irrigation with augmented winery wastewater increased the PAR of the soil compared to clean water, except for season one (Table 9.21). This trend was also more pronounced in the 0 to 100 mm soil layer of the wastewater treatment (Table 9.21).

ESP: Irrigation with augmented winery wastewater increased the ESP of the soil substantially compared to clean water over three seasons (Table 9.21). Similar to SAR, this trend was more pronounced in the 0 to 100 mm soil layer of the wastewater treatment.

Furthermore, wastewater irrigation increased the ESP above the threshold value of 15% (Seilsepour *et al.,* 2009).

Table 9.21. Effects of irrigation with clean municipal water and augmented winery wastewater on exchangeable cations, sodium adsorption ratio (SAR), potassium adsorption ratio (PAR) and exchangeable sodium percentage (ESP) in a sandy soil from Lutzville (S2) over four simulated seasons.

Variable	Water	Depth	Season				
		(mm)	Initial[1]	1	2	3	4
Na_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	0.1	0.2a[2]	0.2c	0.2c	0.3c
		100-200	0.1	0.1a	0.1d	0.1d	0.1d
	Wastewater	0-100	0.1	0.4a	1.1a	0.9a	1.3a
		100-200	0.1	0.6a	0.6b	0.6b	0.6b
K_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	0.5	0.5a	0.5c	0.4c	0.5c
		100-200	0.5	0.4a	0.4d	0.3c	0.4d
	Wastewater	0-100	0.5	0.8a	2.2a	1.8a	3.0a
		100-200	0.5	1.1a	1.1b	1.2b	2.0b
Ca_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	2.4	2.4a	2.8a	2.6a	2.7a
		100-200	2.4	2.1a	2.7a	2.2b	3.1a
	Wastewater	0-100	2.4	2.4a	2.4a	2.3b	2.9a
		100-200	2.4	2.2a	2.7a	2.3b	2.7a
Mg_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	0.8	0.8a	0.8a	0.7a	0.8a
		100-200	0.8	0.7a	0.7b	0.6b	0.7b
	Wastewater	0-100	0.8	0.8a	0.6c	0.5c	0.6c
		100-200	0.8	0.7a	0.8a	0.5c	0.5d
SAR	Clean	0-100	0.1	0.2a	0.2c	0.2c	0.2c
		100-200	0.1	0.1a	0.1d	0.0d	0.1d
	Wastewater	0-100	0.1	0.3a	0.9a	0.7a	1.0a
		100-200	0.1	0.5a	0.4b	0.5b	0.4b
PAR	Clean	0-100	0.4	0.4a	0.4c	0.3c	0.4c
		100-200	0.4	0.3a	0.3c	0.3c	0.3d
	Wastewater	0-100	0.4	0.6a	1.7a	1.6a	2.3a
		100-200	0.4	0.9a	0.8b	1.0b	1.5b
ESP %	Clean	0-100	3.5	6.1a	5.1c	5.5c	6.3c
		100-200	3.5	2.9a	2.7d	1.8d	1.8d
	Wastewater	0-100	3.5	8.4a	17.9a	16.1a	17.2a
		100-200	3.5	11.8a	11.3b	12.5b	9.8b

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Copper: Irrigating with both clean and winery wastewater tended to increase soil Cu concentration over time (Table 9.22). The Cu in the 0 to 100 mm soil layer was higher where wastewater was applied compared to clean water during season one to four.

After four simulated seasons, the Cu concentration in the 100 to 200mm soil layer where wastewater was used had increased from the initial value of 0.3 to 2.4 mg/kg. However the values were still below the threshold value of 4 mg/kg for grapevine (Van Schoor *et al.,* 2000).

Zinc: The Zn concentration tended to increase over time (Table 9.22). From season two to four in the 0 to 100 mm soil layer and 100 to 200 mm soil layer in season four, Zn levels for wastewater irrigated soils were above the threshold value of 5mg/kg for grapevine cultivation (Van Schoor *et al*., 2000).

Table 9.22. Effects of irrigation with clean municipal water and augmented winery wastewater on Cu, Zn, Mn, B, Fe and S in a sandy soil from Lutzville (S2) over four simulated seasons.

Variable	Water	Depth	Season				
		(mm)	Initial[1]	1	2	3	4
Cu (mg/kg)	Clean	0-100	0.3	0.5ab[2]	0.4b	0.7c	1.3a
		100-200	0.3	0.3b	0.4b	0.7c	1.4a
	Wastewater	0-100	0.3	0.6a	0.7a	1.2a	1.7a
		100-200	0.3	0.6a	0.5b	0.9b	2.4a
Zn (mg/kg)	Clean	0-100	1.0	1.4a	1.9b	2.2b	2.4ab
		100-200	1.0	0.7a	0.8c	0.9c	0.8b
	Wastewater	0-100	1.0	2.4a	6.3a	6.0a	6.4a
		100-200	1.0	5.0a	1.1bc	1.3c	6.1ab
Mn (mg/kg)	Clean	0-100	15	19b	19a	23b	17b
		100-200	15	21a	20a	29a	19a
	Wastewater	0-100	15	20ab	20a	25ab	16bc
		100-200	15	20ab	20a	28ab	15c
B (mg/kg)	Clean	0-100	0.4	0.5a	0.3c	0.3c	0.2b
		100-200	0.4	0.5a	0.3c	0.3c	0.3b
	Wastewater	0-100	0.4	0.6a	0.7a	0.7a	1.1b
		100-200	0.4	0.7a	0.4b	0.6b	2.2a
Fe (mg/kg)	Clean	0-100	50	138a	85a	72bc	66a
		100-200	50	157a	102a	113a	84a
	Wastewater	0-100	50	185a	106a	56c	65a
		100-200	50	169a	119a	103ab	80a
S (mg/kg)	Clean	0-100	6	8a	12ab	9b	12b
		100-200	6	8a	4c	5b	5c
	Wastewater	0-100	6	7a	16a	18a	21a
		100-200	6	13a	10b	8b	7c

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Manganese: Irrigation with clean water resulted in higher levels of Mn in the 100 to 200 mm soil layer than in the 0 to 100 mm soil layer (Table 9.22). The Mn values frequently exceeded the 20 mg/kg threshold reported by (Van Schoor *et al*., 2000).

Boron: Irrigation with augmented winery wastewater tended to increase B concentration in the soil over three consecutive seasons when compared to the clean water (Table 9.22). Although wastewater B concentration increased from initial value of 0.4 to 2.2 mg/kg in the 100-200mm soil layer, it was still below the threshold value of 4 mg/kg for grapevine cultivation (Van Schoor *et al.,* 2000).

Iron: Except in the third season, irrigation with augmented winery wastewater had no effect on the Fe concentration in the soil compared to clean water (Table 9.22).

Sulphur: The S concentration in the 0 to 100 mm soil layer of the soil tended to increase over the four seasons, irrespective of water type (Table 9.22). However, the trend was more pronounced where augmented wastewater was applied.

9.3.5.3. Stellenbosch shale soil (S3)

$pH_{(KCl)}$: Soil pH tended to increase over time regardless of the water type, but the augmented winery wastewater treatment resulted in a substantial increase in both soil layers (Table 9.23). Furthermore, wastewater irrigated soils had higher soil pH values than the clean water ones. Lieffering and McLay (1996) reported that soil pH and exchangeable Na has the tendency to increase when wastewater with high pH and high Na concentration is used for irrigation.

EC_e: In general, the EC_e in the 0 to 100 mm soil layer in the soil tended to increase from seasons two to four, irrespective of water quality (Table 9.23). However, the trend was more pronounced where augmented wastewater was applied.

Organic carbon: Irrigation with augmented winery wastewater had no effect on the organic C content in the soil compared to clean water (Table 9.23).

Phosphorus (Bray II): Irrigation with augmented winery wastewater tended to increase P in the 0 to 100 mm soil layer over four simulated seasons and exceeded that of the treatment in which clean water was applied (Table 9.23). Furthermore, soil P was higher in the 100 to 200 mm soil layer where wastewater was used compared to where clean water was used during seasons two and four.

Potassium (Bray II): Augmented winery wastewater resulted in a substantial increase of K in both soil layers over four simulated seasons (Table 9.23).

Table 9.23. Effects of irrigation with clean municipal water and augmented winery wastewater on $pH_{(KCl)}$, EC_e, organic C, P (Bray II) and K (Bray II) in a shale soil from Stellenbosch (S3) over four simulated seasons.

Variable	Water	Depth	Season				
		(mm)	Initial[1]	1	2	3	4
$pH_{(KCl)}$	Clean	0-100	4.2	4.5c[2]	4.5b	4.6c	4.6c
		100-200	4.2	4.5c	4.5b	4.5d	4.4d
	Wastewater	0-100	4.2	5.1a	5.7a	5.8a	6.6a
		100-200	4.2	4.9b	5.6a	5.7b	6.2b
EC_e (dS/m)	Clean	0-100	0.1	0.1ab	0.5b	0.4b	0.6b
		100-200	0.1	0.1ab	0.2c	0.2c	0.2c
	Wastewater	0-100	0.1	0.2a	0.8a	1.4a	1.9a
		100-200	0.1	0.7b	0.1c	0.1c	0.2c
Org C (%)	Clean	0-100	1.2	1.0a	1.1a	1.4a	0.9a
		100-200	1.2	1.0a	1.3a	1.2a	0.9a
	Wastewater	0-100	1.2	1.1a	1.4a	1.3a	0.9a
		100-200	1.2	1.0a	1.2a	1.0a	0.8a
P (mg/kg)	Clean	0-100	8.0	5b	7c	5b	7b
		100-200	8.0	5b	6c	6b	7b
	Wastewater	0-100	8.0	9a	15a	16a	20a
		100-200	8.0	5b	13b	11ab	18a
K (mg/kg)	Clean	0-100	137	175c	153c	123c	135c
		100-200	137	155c	141c	115c	142c
	Wastewater	0-100	137	491a	761a	741a	1093a
		100-200	137	259b	434b	464b	719b

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Exchangeable cations: Soil Na_{ex} and K_{ex} of the treatment irrigated with augmented winery wastewater increased substantially over the four simulated seasons, particularly in the 0 to 100 mm soil layer (Table 9.24). This supports Laurenson *et al*. (2012) who indicated that winery wastewater, containing high Na levels due to sodium hydroxide being used as cleaning detergents, has the potential to increase Na_{ex}, and reduce the hydraulic conductivity of the soil when applied.

After the third simulated season, visual observation revealed that the augmented wastewater started to pond on the surface of the soil, indicating possible clogging of the soil pores. Although no scientific explanations can be provided, it could be that the clay of this particular soil was highly dispersible when irrigated with the wastewater. Most of the shale derived soils in South Africa are often unstable/highly dispersible (M.C. Laker, Personal communication, 2013).

Table 9.24. Effects of irrigation with clean municipal water and augmented winery wastewater on exchangeable cations, sodium adsorption ratio (SAR), potassium adsorption ratio (PAR) and exchangeable sodium percentage (ESP) in a shale soil from Stellenbosch (S3) over four simulated seasons.

Variable	Water	Depth	Season				
		(mm)	Initial[1]	1	2	3	4
Na_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	0.1	0.3b[2]	0.3c	0.2c	0.2c
		100-200	0.1	0.3b	0.2c	0.1c	0.1c
	Wastewater	0-100	0.1	0.8a	1.4a	1.7a	2.1a
		100-200	0.1	0.4b	0.5b	0.3b	0.5b
K_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	0.4	0.5c	0.4c	0.3c	0.4c
		100-200	0.4	0.4c	0.4c	0.3c	0.4c
	Wastewater	0-100	0.4	1.3a	2.0a	1.9a	2.8a
		100-200	0.4	0.7b	1.1b	1.2b	1.8b
Ca_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	1.6	1.8a	2.0a	1.7a	1.9a
		100-200	1.6	1.6b	1.8b	1.4c	1.7a
	Wastewater	0-100	1.6	1.6b	1.8b	1.6b	2.0a
		100-200	1.6	1.7ab	1.9ab	1.7a	1.9a
Mg_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	0.8	0.9a	1.0a	0.7a	0.8a
		100-200	0.8	0.9a	0.8b	0.6b	0.7b
	Wastewater	0-100	0.8	0.9a	0.9a	0.7a	0.9a
		100-200	0.8	0.9a	1.0a	0.8a	0.9a
SAR	Clean	0-100	0.1	0.3b	0.2c	0.2c	0.2c
		100-200	0.1	0.2b	0.1c	0.1c	0.1c
	Wastewater	0-100	0.1	0.7a	1.2a	1.5a	1.8a
		100-200	0.1	0.3b	0.4b	0.3b	0.5b
PAR	Clean	0-100	0.3	0.4b	0.3c	0.3c	0.3c
		100-200	0.3	0.4b	0.3c	0.3c	0.3c
	Wastewater	0-100	0.3	1.2a	1.7a	1.8a	2.3a
		100-200	0.3	0.6b	0.9b	1.1b	1.6b
ESP %	Clean	0-100	4.2	8.8b	6.8c	5.7c	6.2c
		100-200	4.2	8.0b	4.8d	3.4d	4.1c
	Wastewater	0-100	4.2	17.9a	23.5a	28.4a	27.0a
		100-200	4.2	10.4b	10.7b	8.6b	10.2b

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Ca_{ex}: Results showed no significant difference between soils irrigated with augmented winery wastewater compared to those irrigated with clean water in season four (Table 9.24). Although differences did occur in seasons one to three, no trends were observed.

Mg_{ex}: In seasons two to four the level of Mg_{ex} in the 100 to 200 mm of the clean water treatment was the lowest and significantly lower than that of the other soil layer and the treatment irrigated with augmented winery wastewater (Table 9.24).

SAR: Irrigation with augmented winery wastewater increased the SAR of the soil compared to clean water during seasons two to four (Table 9.24). This was more pronounced in the 0 to 100 mm soil layer. However, these results indicated that prolonged irrigation with winery wastewater could be detrimental to the physical properties of this particular soil if the Na increases substantially compared to Ca and Mg.

PAR: Similar to SAR, Irrigation with augmented winery wastewater tended to increase soil PAR from seasons two to four in all soil layers but also the 0 to 100 mm soil layer in season one, compared to clean water (Table 9.24). The trend was more pronounced in the 0 to 100 mm soil layer. The source of higher PAR in the soil was the result of higher amount of K from the augmented winery wastewater applied to the soil (Tables 9.12 to 9.15).

ESP: Irrigation with augmented winery wastewater tended to increase the ESP of the soil substantially compared to clean water in season's two to four (Table 9.24). Similarly to SAR, this trend was also more pronounced in the 0 to 100 mm soil layer. Furthermore wastewater irrigation increased the ESP above the threshold value of 15% (Seilsepour *et al.,* 2009). It could be speculated that high ESP value of above 15% from season two until season four in wastewater treatment could be linked with poor water filtration and clogging observed after third season.

Copper: In general the Cu concentration in the clean, as well as wastewater irrigated S3 soil tended to increase over time (Table 9.25). This trend was more pronounced in the 0 to 100 mm soil layer with the level of Cu being higher than that of the 100 to 200 mm soil layer in season two. In season one, this trend was observed for the wastewater treatment only. The Cu concentration was below the threshold value of 10mg/kg for grapevine cultivation (Van Schoor *et al.,* 2000).

Zinc: The Zn level in the clean, as well as wastewater irrigated soil tended to increase over time (Table 9.25). This trend was more pronounced in the 0 to 100 mm layer. The Zn levels were higher in the 0 to 100 mm soil layer of both treatments during the second and third seasons compared to the 100 to 200 mm soil layer. During season one, this trend was observed for the wastewater treatment only. In season two and four, the Zn levels in the 0 to 100 mm soil layer of the wastewater irrigated soil were slightly above 5 mg/kg which is the threshold value for grapevine cultivation (Van Schoor *et al.,* 2000).

Manganese: Irrigation with augmented winery wastewater and clean water had no effect on soil Mn concentration in the soil (Table 9.25). The initial Mn soil concentration was already above threshold concentration of 20 mg/kg for grapevine cultivation (Van Schoor *et al*., 2000).

Boron: Irrigation with augmented winery wastewater increased the soil B levels of the soil compared to clean water over four seasons with the exception of the 100 to 200 mm soil layer in season four (Table 9.25). This trend was more pronounced in the 0 to 100 mm soil layer. Although B levels were lower than 4 mg/kg which is the threshold for grapevine cultivation (Van Schoor *et al*., 2000), there is an indication that B accumulated over time in the soil irrigated with winery wastewater (Table 9.25).

Iron: Irrigation with augmented winery wastewater increased the soil Fe levels substantially compared to clean water in seasons one and two (Table 9.25). Although not as prominent, this trend was also observed during season four.

Sulphur: Irrigation with augmented winery wastewater increased the soil S levels in the 0 to 100 mm layer compared to clean water over the four seasons (Table 9.25).

Table 9.25. Effects of irrigation with clean municipal water and augmented winery wastewater on Cu, Zn, Mn, B, Fe and S in a shale soil from Stellenbosch (S3) over four simulated seasons.

Variable	Water	Depth	Season				
		(mm)	Initial[1]	1	2	3	4
Cu (mg/kg)	Clean	0-100	1.8	1.9ab[2]	1.8b	2.2a	2.5a
		100-200	1.8	1.9ab	1.7c	2.4a	2.8a
	Wastewater	0-100	1.8	1.9a	2.1a	2.9a	4.1a
		100-200	1.8	1.8b	1.9b	2.7a	2.7a
Zn (mg/kg)	Clean	0-100	1.1	1.4b	3.2b	2.4b	3.2a
		100-200	1.1	1.3b	1.7c	1.3c	3.6a
	Wastewater	0-100	1.1	3.9a	5.1a	4.9a	5.6a
		100-200	1.1	1.2b	2.2c	2.3b	3.9a
Mn (mg/kg)	Clean	0-100	69	77a	73a	66b	63a
		100-200	69	90a	75a	77a	47a
	Wastewater	0-100	69	74a	66b	61b	54a
		100-200	69	73a	71ab	76a	73a
B (mg/kg)	Clean	0-100	0.3	0.2c	0.2c	0.2c	0.2b
		100-200	0.3	0.2c	0.2c	0.2c	0.3b
	Wastewater	0-100	0.3	0.4a	0.7a	0.9a	3.5a
		100-200	0.3	0.3b	0.4b	0.5b	1.1b
Fe (mg/kg)	Clean	0-100	68	97c	48c	276a	92ab
		100-200	68	90c	65bc	430a	51b
	Wastewater	0-100	68	205a	95a	294a	417a
		100-200	68	161ab	72b	271a	207ab
S (mg/kg)	Clean	0-100	8.1	9.9b	14.2b	14.1b	15.2b
		100-200	8.1	9.2b	13.8b	9.5b	14.7b
	Wastewater	0-100	8.1	24.4a	56.8a	82.2a	96.2a
		100-200	8.1	6.5b	7.5b	7.8b	7.2b

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

9.3.5.4. Stellenbosch granitic soil (S4)

$pH_{(KCL)}$: Irrigation with augmented wastewater increased the pH of the soil compared to clean water during seasons two to four (Table 9.26). Furthermore irrigation with the wastewater resulted in a more favorable pH for both acidic soils.

EC_e: The EC_e of the soil increased over time in the 0-100 mm layer regardless of water quality (Table 9.26). The increase in EC_e could be attributed to the amount of salts present in both the clean and augmented winery wastewater.

Table 9.26. Effects of irrigation with clean municipal water and augmented winery wastewater on $pH_{(KCl)}$, EC_e, organic C, P (Bray II) and K (Bray II) in a granitic soil from Stellenbosch (S4) over four simulated seasons.

Variable	Water	Depth	Season				
		(mm)	Initial[1]	1	2	3	4
$pH_{(KCl)}$	Clean	0-100	4.4	4.6a[2]	4.7c	4.6c	4.6b
		100-200	4.4	4.7a	4.6d	4.6c	4.5b
	Wastewater	0-100	4.4	5.0a	5.2a	5.4a	5.5a
		100-200	4.4	4.7a	5.0b	5.3b	5.6a
EC_e (dS/m)	Clean	0-100	0.2	0.3a	0.5a	0.6a	2.1a
		100-200	0.2	0.1a	0.1a	0.2b	0.1b
	Wastewater	0-100	0.2	0.3a	0.6a	0.6a	2.1a
		100-200	0.2	0.2a	0.2a	0.2b	0.2b
Org C (%)	Clean	0-100	1.2	1.3a	1.4a	1.3a	1.1a
		100-200	1.2	1.3a	1.6a	1.3a	1.2a
	Wastewater	0-100	1.2	1.4a	1.5a	1.3a	1.0a
		100-200	1.2	1.4a	1.5a	1.3a	0.8a
P (mg/kg)	Clean	0-100	15	9.7a	12b	22a	13c
		100-200	15	9.0a	11b	17b	13c
	Wastewater	0-100	15	9.7a	17a	11c	28b
		100-200	15	9.3a	12b	10c	32a
K (mg/kg)	Clean	0-100	126	148b	132c	126c	159c
		100-200	126	123b	87d	82d	82d
	Wastewater	0-100	126	276a	393a	332a	661a
		100-200	126	242ab	229b	251b	483b

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

Organic carbon: Irrigation with augmented winery wastewater had no effect on the organic C content in the soil compared to clean water (Table 9.26).

Phosphorus (Bray II): Although the treatments affected the soil P level, no definite trends were observed (Table 9.26).

Potassium (Bray II): Irrigation with augmented winery wastewater resulted in a substantial increase in both soil layers during four seasons, with the levels of K in this treatment being higher than that of the treatment irrigated with clean water from seasons two to four (Table 9.26). This trend was observed in the 0 to 100 mm soil layer only during the first season. After four simulated seasons, there was a direct relationship between the amount of K applied *via* the water and the K levels in 0 to 100 mm and 100 to 200 mm layers (Tables 9.12 to 9.15).

Exchangeable cations:

Na_{ex}: Irrigation with clean and augmented winery wastewater increased the Na_{ex} of the soil for four simulated seasons in the 0-100 mm soil layer, while in the 100 to 200mm soil layer, irrigation with clean water result in the stabilization of Na_{ex} at a lower level for four seasons. Augmented winery wastewater increased the Na_{ex} in the 100 to 200 mm soil layer slightly for three seasons while in season two it remain the same (Table 9.27). The soil that was irrigated with the augmented winery wastewater showed complete structural breakdown after the third simulated season. The wastewater irrigated soil started to develop large cracks, whereas the soil irrigated with clean water remained intact.

K_{ex}: Irrigation with augmented winery wastewater increased the K_{ex} in the soil throughout four simulated seasons while clean water irrigation did increase the K_{ex} only in season one and four in the 0 to 100 mm soil layers. The effect of irrigation with wastewater was more pronounced in the 0 to 100 mm soil layer throughout four seasons. The clean water irrigation in the 100 to 200 mm soil layer reduces the values even below the initial value in seasons two and four (Table 9.27).

Ca_{ex}: Results showed no significant difference between treatments in season one, two and four while in season three; there was a difference at soil layers between treatments. The cause for Ca_{ex} reduction in season three in the 100 to 200 mm soil layer is unknown (Table 9.27).

$Mg_{ex:}$ Mg levels in the 0 to 100 mm increased in all four seasons regardless of water quality while in the 100 to 200 mm, there was a decrease in season three and four for both clean and wastewater irrigated soils (Table 9.27).

SAR: Irrigation with augmented winery wastewater increased the SAR of the 0 to 100 mm soil layer compared to clean water over the last three simulated seasons (Table 9.27). This trend was also observed for the 100 to 200 mm soil layer.

Table 9.27. Effects of irrigation with clean municipal water and augmented winery wastewater on exchangeable cations, sodium adsorption ratio (SAR), potassium adsorption ratio (PAR) and exchangeable sodium percentage (ESP) in a granitic soil from Stellenbosch (S4) over four simulated seasons.

Variable	Water	Depth	Season				
		(mm)	Initial[1]	1	2	3	4
Na_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	0.2	0.3a[2]	0.3b	0.4b	0.4b
		100-200	0.2	0.1a	0.1c	0.1c	0.1d
	Wastewater	0-100	0.2	0.4a	0.7a	1.1a	1.4a
		100-200	0.2	0.3a	0.2b	0.3b	0.3c
K_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	0.3	0.4b	0.3c	0.3c	0.4c
		100-200	0.3	0.3b	0.2d	0.2d	0.2d
	Wastewater	0-100	0.3	0.7a	1.0a	0.9a	1.7a
		100-200	0.3	0.6ab	0.6b	0.6b	1.2b
Ca_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	1.8	1.9a	2.2a	2.0a	2.4a
		100-200	1.8	1.7a	1.9a	1.7bc	2.2a
	Wastewater	0-100	1.8	1.8a	2.2a	1.8ab	2.1a
		100-200	1.8	1.8a	2.0a	1.6c	1.9a
Mg_{ex} ($cmol^{(+)}/kg$)	Clean	0-100	0.8	1.0a	1.1a	0.9a	1.0a
		100-200	0.8	0.8b	0.8b	0.7b	0.5d
	Wastewater	0-100	0.8	0.9ab	1.1a	0.9a	0.9b
		100-200	0.8	0.9ab	0.8b	0.6c	0.7c
SAR	Clean	0-100	0.1	0.3a	0.2b	0.3b	0.3b
		100-200	0.1	0.1a	0.1c	0.1c	0.0d
	Wastewater	0-100	0.1	0.3a	0.5a	0.9a	1.2a
		100-200	0.1	0.3a	0.2b	0.3b	0.2c
PAR	Clean	0-100	0.3	0.3b	0.3c	0.3c	0.3c
		100-200	0.3	0.3b	0.2c	0.2d	0.2d
	Wastewater	0-100	0.3	0.6a	0.8a	0.7a	1.4a
		100-200	0.3	0.5ab	0.5b	0.6b	1.1b
ESP %	Clean	0-100	4.8	8.3a	6.7b	9.2b	9.1b
		100-200	4.8	3.0a	2.6d	9.9b	2.1d
	Wastewater	0-100	4.8	9.6a	13.5a	10.4a	23.3a
		100-200	4.8	7.9a	5.7c	3.8c	6.5c

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

However, these results indicated that prolonged irrigation with winery wastewater could be detrimental to the physical properties of this particular soil if the Na increases substantially compared to Ca and Mg.

PAR: Irrigation with augmented winery wastewater increased the PAR of the soil compared to clean water during the last three seasons (Table 9.27). During season one only the 0 to 100 mm soil layer of the wastewater irrigated soil had higher PAR than the clean water irrigated one. This trend was more pronounced in the 0 to 100 mm soil layer.

ESP: Comparing the two soil layers independently, Irrigation with augmented winery wastewater increased the ESP substantially compared to clean water over the last three seasons (Table 9.27). During season one, this trend was observed in the 0 to 100 mm soil layer only. It must be noted that the wastewater irrigation increased the ESP in season four above the threshold value of 15% (Seilsepour *et al.,* 2009).

Copper: The Cu concentration in the soil tended to increase during the fourth season, irrespective of water quality (Table 9.28) but still lower than the Cu concentration threshold for grapevine cultivation *i.e.*10 mg/kg (Van Schoor *et al*., 2000). Furthermore, during seasons two and three, inconsistent trends were observed with Cu levels being lower and higher.

Zinc: Results showed no significant difference between clean and wastewater irrigated soils in seasons one and four (Table 9.28). In season two and three, the levels of Zn in the soil irrigated with wastewater were higher in the 0 to 100 mm soil layer compared to that irrigated with clean water. The Zn threshold levels for grapevine cultivation are 5 mg/kg (Van Schoor *et al.,* 2000) and were exceeded in the 0 to 100 mm soil layer of the treatment irrigated with augmented winery wastewater during season three.

Manganese: In contrast to most of the other elements, Mn concentration tended to decrease over time, irrespective of the water quality (Table 9.28).

Boron: Although significant differences occurred between treatments, no significant trends were observed (Table 9.28), as the recorded values remained far below the B threshold for grapevine cultivation, namely 4 mg/kg (Van Schoor *et al.,* 2000).

Iron: Fe concentration in the soil was inconsistent with respect to water quality and soil layer. Irrigation with augmented winery wastewater increased the Fe concentration compared to clean water only in the 0 to 100 mm layer during the fourth season (Table 9.28).

Sulphur: Irrigation with clean and augmented winery wastewater increased the S concentration of the soil substantially in the 0 to 100 mm layer over the four seasons (Table 9.28).

Table 9.28. Effects of irrigation with clean municipal water and augmented winery wastewater on Cu, Zn, Mn, B, Fe and S in a granitic soil from Stellenbosch (S4) over four simulated seasons.

Variable	Water	Depth	Season				
		(mm)	Initial[1]	1	2	3	4
Cu (mg/kg)	Clean	0-100	0.4	0.6a[2]	0.4a	0.6a	1.9a
		100-200	0.4	0.6a	0.3b	0.6a	1.3a
	Wastewater	0-100	0.4	0.6a	0.4a	0.4b	1.7a
		100-200	0.4	0.6a	0.4a	0.4b	1.3a
Zn (mg/kg)	Clean	0-100	1.0	2.7a	2.2b	3.2b	3.6a
		100-200	1.0	1.8a	1.7b	1.6c	1.9a
	Wastewater	0-100	1.0	5.0a	4.4a	6.2a	3.8a
		100-200	1.0	3.8a	2.0b	2.9b	3.0a
Mn (mg/kg)	Clean	0-100	33	26a	16b	14c	21a
		100-200	33	28a	18a	19a	14a
	Wastewater	0-100	33	24a	14c	17b	11a
		100-200	33	25a	18a	17b	12a
B (mg/kg)	Clean	0-100	0.2	0.2a	0.1c	0.4a	0.2ab
		100-200	0.2	0.1b	0.1c	0.4a	0.1b
	Wastewater	0-100	0.2	0.2a	0.4a	0.2b	0.9a
		100-200	0.2	0.2a	0.2b	0.2b	0.6ab
Fe (mg/kg)	Clean	0-100	118	81b	52b	83b	76a
		100-200	118	104a	74a	116a	87a
	Wastewater	0-100	118	90ab	61b	65c	129a
		100-200	118	91ab	81a	92b	85a
S (mg/kg)	Clean	0-100	11	18a	22a	24b	30a
		100-200	11	4a	6b	8c	9b
	Wastewater	0-100	11	13a	24a	33a	43a
		100-200	11	9a	6b	6c	6b

[1] Initial values were determined in the bulk soil before it was packed into the pots.
[2] For each variable, values followed by the same letter within a column do not differ significantly ($p \leq 0.05$).

9.4. CONCLUSIONS

Irrigation with augmented winery wastewater increased soil $pH_{(KCl)}$ and the salinity levels in all four soils. Although the salinity levels were relatively low, irrigation with winery wastewater is likely to create saline condition over time. Soil sodicity increased where winery wastewater was applied. In relation to the other exchangeable cations, soil Na_{ex} accumulated to the extent that the ESP threshold of 15% was exceeded, irrespective of the clay content. The high ESP probably contributed to the observed structural breakdown of the granitic soil from Stellenbosch and poor water infiltration in the case of the shale soil from Stellenbosch.

The Rawsonville and Lutzville sands did not show any of these signs where the augmented wastewater was applied over the four simulated seasons. Therefore, soils containing less than 4% clay will probably be more suitable for recycling of winery wastewater with COD concentrations of 3000 mg/L *via* irrigation than soils with higher clay contents. Although soils with more than 4% clay seem unsuitable for winery wastewater irrigation with COD concentration of 3000 mg/L, it must be noted that the results exclude any interaction between soil and the plants after wastewater irrigation as no crop was planted on the soil.

Irrigation with augmented winery wastewater had limited effects on soil Cu, Zn, Mn, Fe and S compared to clean municipal water. However, these effects were inconsistent with respect to water quality, soil type and depth. In contrast, soil B increased where winery wastewater was applied compared to clean municipal water which was probably caused by the use of B containing cleaning agents.

Soil organic C was not affected, irrespective of the clay content. This indicated that breakdown of organic material applied *via* the winery wastewater occurred between irrigations although the COD level was approximately hundred times higher in the winery wastewater. Irrigation with augmented winery wastewater increased soil P compared to clean municipal water, except for the Rawsonville sandy soil. For some inexplicable reason, the latter soil initially contained exceptionally high P levels. In general, re-using winery wastewater will reduce the need for P fertilizers, *i.e.* depending of the crop requirements and soil type. Likewise, re-using winery wastewater could have a positive effect on soil K. However, if soil K accumulates to the extent that it is luxuriously adsorbed by grapevines, it could increase juice pH, and subsequently cause poor wine colour stability. The current study showed that trends may vary between soils. It is also possible that in a scenario where crops are grown on these soils, the trends observed may be slightly change or be less severe. It is recommended that future studies should investigate why winery wastewater irrigation did not increase soil organic carbon. The reduction of P levels in the Rawsonville sandy soil after wastewater irrigation should be investigated.

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CHAPTER 10: EFFECT OF IRRIGATION WITH AUGMENTED WINERY WASTEWATER ON THE HYDRAULIC PROPERTIES OF FOUR SELECTED SOILS

C.L. Howell & P.A. Myburgh
The Fruit, Vine and Wine Institute of the Agricultural Research Council, Private Bag X5026, Stellenbosch, 7599

10.1. INTRODUCTION

In the South African wine industry, most vineyards require irrigation. However, water is becoming an increasingly scarce resource and will become even more so should climate change realise. Wastewater is a potential important source of water for irrigation (Viviani & Iovino, 2004, Arienzo *et al.*, 2009) and in the wine industry, it would be ideal to use winery wastewater for vineyard irrigation by adding winery wastewater to existing irrigation water resources. The availability of such wastewater, in conjunction with the nutrients it could contain, makes it an attractive source of irrigation water (Arienzo *et al.*, 2009).

In areas where grapevines may not require much irrigation, but winery wastewater is being generated, it could potentially be used for irrigation of other crops. The composition of winery wastewater fluctuates with time of season *i.e.* pre-vintage, vintage and post-vintage (Arienzo *et al*., 2009; Bories & Sire, 2010) and K (Arienzo *et al*., 2009) and Na concentrations (Samaras *et al*., 2009) are generally high. Wastewater also contains high concentrations of organic and inorganic suspended and dissolved solids (Tarchitzky *et al*., 1999). Soil chemical properties can be altered by wastewater irrigation (Vogeler, 2009; Lado & Ben-Hur, 2010) and this could influence soil structure (Sparling *et al*., 2006) and hydraulic properties (Mathan, 1994; Sort & Alcaniz, 1999; Tarchitzky *et al*., 1999; Al-Haddabia *et al*., 2004; Coppola et al., 2004; Viviani & Iovino, 2004; Bagarello et al., 2006; Hawke & Summers, 2006; Bhardwaj *et al.*, 2007; Gonçalves *et al.*, 2007; Arienzo *et al*., 2009; Vogeler, 2009). Dissolved and suspended solids, both organic and inorganic, may induce soil clogging through physical, chemical, and biological processes (Viviani & Iovino, 2004).

The effects of wastewater irrigation are closely related to both wastewater and soil properties. Although augmentation of winery wastewater has already been performed by a few wineries, and is believed to have little or no effect on grapevine yield and quality, the impact of this practice on soil physical properties is unknown. This could be due to the fact that changes in soil physical properties are difficult to quantify because they tend to occur only over the long term and soil physical properties are greatly variable (Hawke & Summers, 2006). Since soil is an irreplaceable resource, guidelines for the judicious use of such water, particularly in terms of physical properties, are vital for the wine industry. Therefore, the aim

of this study was to determine the effect of winery wastewater, augmented to five different COD levels, on the hydraulic properties of four selected soils.

10.2. MATERIALS AND METHODS

Since inherent soil properties could influence the soil physical changes when irrigated with winery wastewater, different soils other than the Rawsonville sand were included in the study. The three additional soils were red, aeolic sand from the Lower Olifants River region and two soils from Stellenbosch which were derived from granite and shale parent material, respectively. Further details on the localities where the soils were collected, as well as the textural components presented in Chapter 9. The soil from both Lutzville and Rawsonville were classified as sand, whereas the shale and granite soil from Nietvoorbij were classified as sandy loam and sandy clay loam, respectively. The four different soils were collected and packed in PVC pots to a bulk density of 1.5 g/cm^3 with the exception of the granite soil which was packed to a bulk density of 1.55 g/cm^3 as described in Chapter 9.

One pot per replication per treatment was used. In total, there were therefore 18 pots per soil. The pots were installed at the field experiment at Rawsonville in all three replications of the raw water control (T1) and where wastewater was augmented to COD levels of 100 mg/L (T2), 500 mg/L (T4), 1000 mg/L (T5), 2000 mg/L (T7) and 3000 mg/L (T9), respectively. These treatments were selected in order to obtain a range of COD levels. The pots were installed in hole, 30 cm wide, 60 cm long 20 cm deep. The soil at the bottom of the hole was leveled and thereafter coarse material was added to the bottom of the hole. The pots were placed onto the coarse material so that at their top ends protruded above the level of the soil surface and their identification number faced the front (Fig. 10.1). A plastic lid was placed on the pot during the installation process to prevent soil and coarse material contaminating the soil surface. The hole was then filled with soil from the vineyard (Figure 10.2). The level of the soil surface in the pots was the same as the surrounding soil. The pots remained covered until wastewater treatment application commenced in either early February or March. The lids were only taken off during the application of the wastewater irrigations. Before raw river water was applied to grapevines after the wastewater irrigations, the lids were also placed back onto the pots. After the completion of irrigations, the lids were taken off and the pots were allowed to dry out.

Figure 10.1. The PVC pots were covered with lids and placed onto a layer of coarse sand.

Figure 10.2. Soil from the vineyard was used to fill up the hole.

Pots were subjected to the same wastewater irrigations that the grapevines were subjected to in 2010/11, 2011/12 and 2013. Further details regarding the amount of irrigation water applied, as well as the water quality and elements applied *via* the irrigation water can be found in Chapter 3. After the end of the application of the wastewater irrigations for each

season, the pots were removed from the soil and transported to the laboratory at the ARC Nietvoorbij campus.

Mini disk infiltrometers (Decagon Devices, Pullman, WA) were used to quantify the hydraulic conductivity (K) of the four different soils (Fig. 10.3). In order to perform measurements on each of the three replications per treatment concurrently, three of these mini disk infiltrometers were used. Mini disk infiltrometer measurements were replicated five times in each pot. Measurements of the Nietvoorbij shale and granitic soils were carried out at a suction head of 2 cm, whereas the measurements of the Rawsonville and Lutzville sands were carried out at a suction head of 4 cm. The K values (mm/h) were calculated by means of the following equation:

$$K = \{[(V_{I.} - V_E) \div 1000] \div 0.001521\} \text{ X } 60 \div \Delta t_{.} \qquad \text{(Eq.10.1)}$$

where $V_{I.}$ is initial volume reading (mL) at the beginning of the measurement, V_E is volume reading (mL) at the end of the measurement, 0.001521 is the area of the ceramic plate at the bottom of the infiltrometer in m^2 and $\Delta t_{.}$ is the time between measurements (min). It should be noted that data presented is the median value of the five average values for K.

Figure 10.3. Using a mini disk infiltrometer to measure K in the laboratory at the ARC Nietvoorbij campus.

Raw water collected from the Holsloot River during the winter was used for the infiltration studies. Samples of this raw river water were sent to a commercial laboratory for chemical analysis according to the methods of Clesceri *et al.* (1998). As expected, the water from the Holsloot River was of good quality and levels of elements were generally low (Table 10.1).

Table 10.1. Chemical composition of raw water from Holsloot River used for the infiltration studies.

Time of sampling	pH	EC (dS/m)	Element (mg/L)								
			Na	K	Ca	Mg	Fe	Cl	HCO_3	SO_4	P
July 2011	7.6	0.04	5.8	4.0	6.7	1.1	0.10	9.8	10.4	3.0	0.10
July 2012	7.0	0.03	2.3	0.2	0.6	0.7	0.12	9.2	20.4	1.5	0.04
July 2013	5.6	0.02	4.2	1.0	5.2	1. 7	0.02	8.9	1.5	21.5	0.04

10.3. RESULTS AND DISCUSSION

10.3.1. Shale-derived soil

The K values were generally within the range expected for fine sandy loam soils (Klute & Dirksen, 1986). During the first two seasons, *i.e.* 2010/11 and 2011/12, the augmented winery wastewater had no effect on K of the fine sandy loam shale derived soil from Stellenbosch (data not shown). However, after the third season, *i.e.* 2012/13, K became lower with a decrease in the level of augmentation (Fig. 10.4).

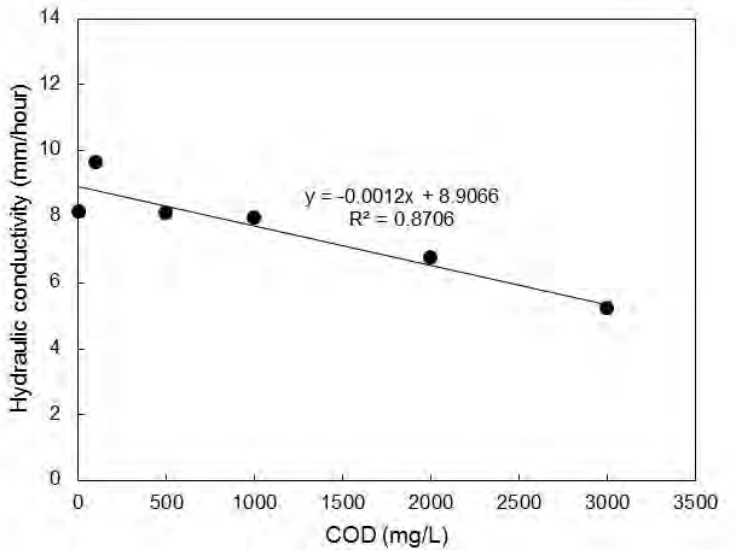

Figure 10.4. Effect of irrigation with augmented winery wastewater on the hydraulic conductivity of shale soil from Stellenbosch in 2013, *i.e.* after three years.

Visual observations revealed that the shale soil had a salt-like precipitation on the surface where the winery wastewater was augmented to 3000 mg/L (T9) (Figure 10.5). This precipitation was present on all three replications, and could have been caused by chemical clogging. The latter includes swelling and dispersion of clay particles induced by Na concentrations higher than that of fresh water. High Na levels in irrigation water combined

with low soil-water electrical conductivity can lower soil's permeability, and decrease its infiltration capacity through the swelling and dispersion of clays and slaking of aggregates (Al-Haddabia *et al*., 2004). Swelling of clays can narrow the conducting pores of soils, which could have caused the reduction in K with an increase in the level of winery wastewater augmentation.

Figure 10.5. Salt-like precipitation on the surface of the fine sandy loam soil where winery wastewater augmented to 3000 mg/L were applied.

10.3.2. Granite-derived soil

There were no clear trends in K for the granitic coarse sand clay loam soil from Stellenbosch after three years (Fig. 10.6). Although K of this soil was the lowest of the four soils, it was within the range reported for sandy clay soil (Klute & Dirksen, 1986). The K values were almost similar to 2.32 mm/h reported previously for this soil (Louw & Bennie, 1991).

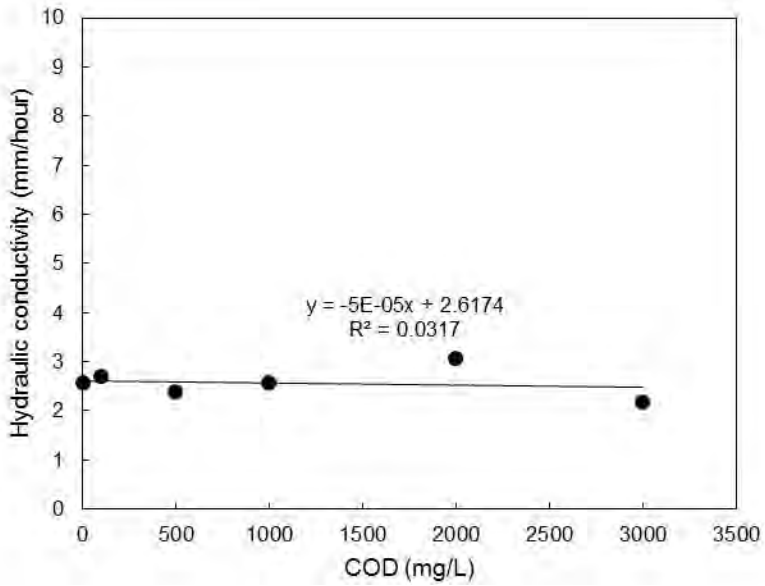

Figure 10.6. The effect of irrigation with augmented winery wastewater on the hydraulic conductivity of granitic soil from Stellenbosch in 2013, *i.e.* after three years.

10.3.3. Alluvial sand

The K values were generally within the range expected for sandy soils (Hillel, 1980; Klute & Dirksen, 1986). In 2010/11 and 2011/12, no clear trends that could be related to level of wastewater augmentation could be observed in K for the fine sandy soil from Rawsonville (data not shown). However, similar to the shale derived soil, K of this fine sandy soil showed a clear reduction with a decrease in level of augmentation of the winery wastewater after the third year of irrigation (Fig. 10.7). In 2012/13, the K of the soil irrigated with winery wastewater augmented to a COD level of 3000 mg/L (T9) was 40% less than that of the raw river water control (T1).

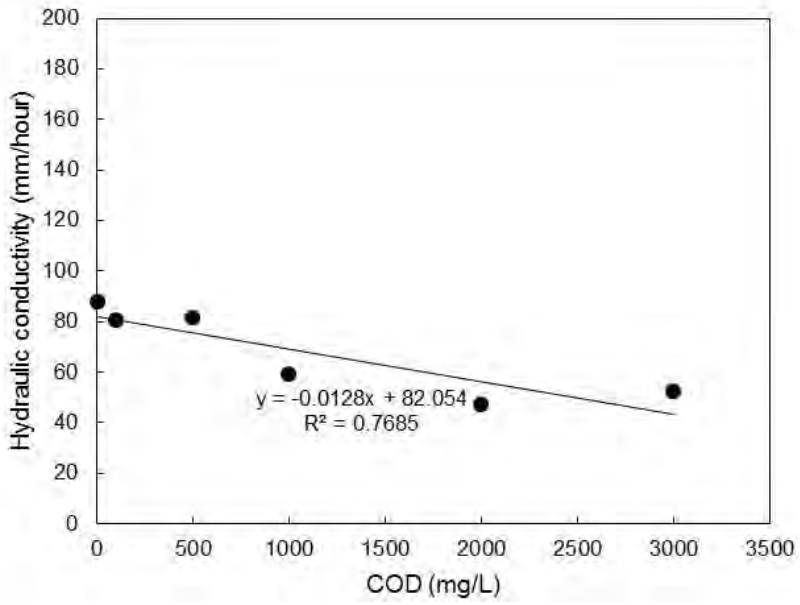

Figure 10.7. The effect of irrigation with augmented winery wastewater on the hydraulic conductivity of sandy soil from Rawsonville in 2013, *i.e.* after three years.

10.3.4. Aeolian sand

Generally, K was higher than expected for this fine sandy soil from Lutzville, and was more comparable to K values reported for coarse sand (Klute & Dirksen, 1986). During the first two seasons, *i.e.* 2010/11 and 2011/12, the augmented winery wastewater had no effect on K of the fine sandy soil (data not shown). However, after the third season, *i.e.* 2012/13, K showed a substantial decrease with a decrease in the level of augmentation (Fig. 10.8). As in the case of the alluvial Rawsonville soil, K was 60% where winery wastewater augmented to 3000 mg/L was applied (T9) compared to the raw river water control (T1).

10.4. CONCLUSIONS AND RECOMMENDATIONS

During the first two years, irrigation with augmented winery wastewater did not seem to have had any effect on K of the soils, except for the granite-derived soil from Stellenbosch where there was no effect after three years. At this stage there is no explanation for the behaviour

of the granite-derived soil, except that its water permeability is generally low. In the case of the shale-derived soil, as well as the alluvial and aeolian sands, K decreased

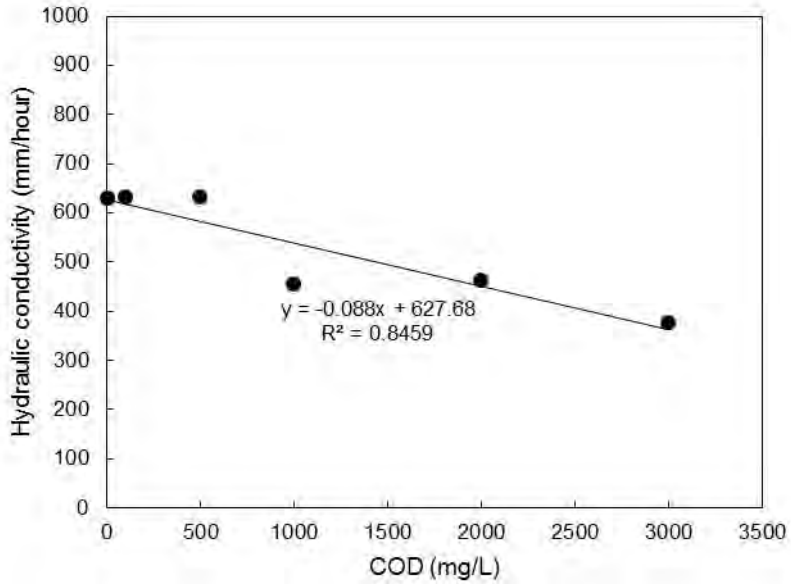

Figure 10.8. The effect of irrigation with augmented winery wastewater on the hydraulic conductivity of sandy soil from Lutzville in 2013, *i.e.* after three years.

substantially with a decrease in the level of augmentation after three years. It should be noted that the soils received no raw water irrigation which could have influenced the effect of the wastewater on K. In spite of this, the results indicated that severe reductions in K will occur in the long run if augmented winery wastewater is used for irrigation on these soils. Furthermore, the reduction in K might be worse if undiluted winery wastewater is used for irrigation of crops. Since Na and K can influence the soil physical properties, the use of Na should be minimised, and chemical practices should be applied judiciously. In addition, the use of water within the winery should be minimised. Subsequently, reduced volumes of water containing lesser chemicals would be produced.

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CHAPTER 11: RESULTS, RECOMMENDATIONS AND THE WAY FORWARD

The objectives of the project did not include aspects concerning water use and management of chemicals within wineries. However, water use and the contamination thereof by wineries have been investigated by previous research studies. Recommendations regarding practices to reduce the negative effects of winemaking processes on water quality are well documented.

11.1. RESULTS

Since treatment application in terms of COD concentrations was quite accurate, *i.e.* after initial practical problems and sources of error were eliminated, the relatively simple mix and distribution infrastructure can be used where irrigation treatments require a series of water qualities, or nutrient levels in field experiments. However, due to the poor water quality, the tanks will have to be cleaned on the inside from time to time. This will be a costly operation that needs to be budgeted for in future projects of this kind. Results have shown that, although the deposit formation on the inside of the irrigation lines was low, it increased as the level of augmentation decreased.

Managing oats as a cover crop, *i.e.* not harvesting and removing it from the vineyard, whilst employing only pearl millet as an interception crop during the period when winery wastewater is likely to be applied in the vineyard, proved to be a sustainable practice under the prevailing conditions. It seems, however, that the COD level of the augmented winery wastewater should preferably be between 1500 mg/L and 2500 mg/L. The growing season of the oats being shortened by as much as six weeks due to the pearl millet being grown during harvest, did not affect the vegetative growth of the species negatively. The oats should, therefore, produce enough fibre to create quality summer mulches for pre-vèraison weed control. The present study showed that the pearl millet cover crop grown in summer had almost no effect on grapevine performance, but did not remove the Na applied *via* the irrigation water. Recently, research has shown that potted fodder beet grown during summer in sandy soil collected from the field trial at Rawsonville, absorbed a substantial amount of Na applied *via* Na-enriched irrigation water. The concentration of Na applied was equal to that of the Na concentration in the irrigation water of the 3000 mg/L COD treatment in the field trial near Rawsonville. Furthermore, the fodder beet reduced the soil K_{ex} by 50%, thereby indicating that it could also absorb K applied *via* winery wastewater.

The pot experiments have shown that irrigation with winery wastewater augmented to 3000 mg/L COD, has negative effects on the soil chemical and hydraulic properties, under no rainfall conditions. The negative effects appeared to be more pronounced in the heavier

soils. Irrigation with augmented winery wastewater did not have any consistent effect on the soil microbial status. In fact, there were indications that soil health may even be improved. Due to temporal variability under the prevailing conditions, these findings should be received with great caution.

Irrigation of grapevines using augmented winery wastewater did not affect vegetative growth or yield of grapevines compared to the raw water control under the given conditions. Since organic carbon did not accumulate in the soil during the study period, it suggested that the soil was sufficiently aerated between irrigation to allow oxidation. The latter probably explains why the grapevines did not respond to level of COD *per se*. However, it is important to note that the salinity and sodicity levels in the augmented winery wastewater were below the thresholds for grapevines. Therefore, the low salinity and sodicity levels in the augmented winery wastewater could be a further, but maybe more realistic explanation why the grapevines did not respond to the wastewater irrigation. Since vegetative growth and yield of the grapevine were comparable to responses previously reported for vineyards without a summer cover crop, the results suggested that the grapevines were not affected by the pearl millet growing in the work rows during summer. Visual observations revealed that the root system of this cover crop was shallow compared to that of the grapevines. Therefore, the competition for water and nutrients was probably not strong enough to have induced negative effects on grapevine growth and yield.

Results of the main study showed that irrigation of grapevines using winery wastewater augmented up to 3000 mg/L COD did not have detrimental effects on juice characteristics with regards to ripeness parameters and ion content under the prevailing conditions. Wine sensorial quality was also not affected. The relatively large irrigation volumes applied during berry ripening were definitely detrimental to wine quality. However, although the main study showed that wine colour and common sensory wine descriptors were not affected by the various wastewater irrigation treatments, off-odours due to direct contact with wastewater may reduce wine quality.

11.2. RECOMMENDATIONS

Based on the project results, the following criteria should be considered for possible amendments to the General Authorization for wineries when using augmented wastewater for irrigation of vineyards:

(i) The COD must be augmented to 3000 mg/L or less, preferably to less than 2000 mg/L to avoid unpleasant odours while irrigations are applied.

(ii) The EC_{iw} must be less than 0.75 dS/m.

(iii) The SAR_{iw} must be less than 10.

(iv) It should be a sandy soil with low CEC.

(v) The internal drainage in the root zone must be unrestricted.

(vi) The irrigation water must not percolate beyond the root depth.

(vii) Only micro-sprinklers should be used, since drippers have narrow flow paths and/or small orifices, and are more susceptible to clogging.

(viii) The irrigation must be applied with micro-sprinklers in such a way that the bunches are not wetted.

(ix) At least 50% PAW depletion should be allowed between irrigations to allow sufficient aeration for oxidation of organic material applied *via* the irrigation water.

(x) The irrigation frequency and volumes (schedule) should enhance, rather than negate, wine quality characteristics.

(xi) Due to the possibility that direct contact with winery wastewater may cause off-odours in the wine, overhead sprinkler irrigation is not recommended if winery wastewater is re-used for vineyard irrigation.

(xii) A summer interception crop, *e.g.* pearl millet, should be sown in early January to ensure that its growth peaks when the winery wastewater is applied, and that the species does not complete its life cycle too early.

(xiii) Since a summer interception crop may increase the ET of vineyards substantially if growing conditions are favourable, it could induce competition for water between grapevines

(xiv) and cover crop. However, this can be avoided by timely slashing of the interception crop, which will also minimise possible competition for N and P.

11.3. THE WAY FORWARD

The following are more general recommendations and suggestions that need to be considered if augmented winery wastewater is used for vineyard irrigation.

- Since the organic matter in the wastewater seemed to have oxidized between irrigations, it might be more appropriate to evaluate the water quality for vineyard irrigation in terms of pH, EC and SAR rather than COD.

- Furthermore, it must be determined if the existing COD norms could be relaxed for winery wastewaters, since the organic material in the latter could break down more readily upon aeration between irrigations compared to organic material in wastewaters produced by other processes, *e.g.* dairies and cheese factories.

- If the industry moves towards K based cleaning agents, further research should be done to determine acceptable PAR norms to avoid excessive K application and accumulation in soils, and subsequently in grapevines.

- As the use of an interception crop in the disposal of winery wastewater appears to be a financially viable option, the use of N-fixers with a spreading habit as interception crops in vineyards irrigated with winery wastewater should be investigated. The possibility of using species normally planted for grazing should also be studied. The contribution of the slash and removal costs of interception and cover crops to already high production costs of vineyards is a further aspect that needs consideration.
- Augmentation of winery wastewater to a specific COD level for re-use *via* irrigation proved to be a tedious process that requires expertise and specialized equipment. Due to these constraints, and the need for large storage dams, it will be impractical to pre-augment wastewater on a commercial scale, particularly in the case of larger wineries. In the case of smaller wineries, the wastewater could be augmented by mixing it into existing dams or reservoirs, *i.e.* given that the water quality remains within the statutory quality norms.
- For larger wineries, injecting the wastewater directly into existing irrigation systems on soils and grapevines might be an alternative. However, injecting wastewater directly into irrigation systems will require continuous monitoring of the wastewater quality in order to adjust the injection rate into the irrigation water as the quality of the wastewater changes. Since the quality of winery wastewater changes almost hourly, it will complicate the injection process. Therefore, it might be more viable to inject the wastewater at a fixed dilution ratio, *i.e.* volume per volume. For a specific winery, the ideal dilution ratio will depend on the wastewater quality, as well as the temporal variability thereof. Since spikes of extremely poor water quality could occur within a given dilution ratio, the sustainability of the fixed dilution concept needs to be investigated with respect to soil and grapevine responses in a field trial.
- Given the importance of wine quality, particularly if the wine needs to be exported, the generally poor wine quality obtained under the prevailing conditions is of great concern. Since less frequent irrigation allows higher levels of plant available water depletion between irrigations, the winery wastewater will probably have to be applied over large areas under grapevines to obtain sufficient PAW depletion between irrigations. The latter approach will require careful planning and design, as well as expensive infrastructure that will inevitably increase the cost of water management.
- Although soil Na and K did not accumulate to excessively high levels in the sandy soil of the field trial, they were reduced to the baseline concentrations following heavy winter rain. This result is of great concern, since it suggests that these elements leached into natural water resources. Leaching due to heavy rainfall also serves as further motivation

to investigate the possibility of biological interception of mineral elements. In contrast to pearl millet where the roots remain in the soil, halophytes such as fodder beet have the advantage that almost the entire plant is removed at harvest. Similar to fodder beet, halophytic grasses could be grown to absorb Na from soils. To ensure optimum element uptake from the soil, the halophytes must be able to grow during the period when wineries produce the highest volumes of wastewater. Irrigation of vineyards in heavier soils with augmented winery wastewater cannot be recommended, unless Na and K can be successfully intercepted in a biological way with halophytes. If Na could be intercepted effectively, wineries could continue to use Na hydroxide in a judicious way, which will more efficient and cheaper than K containing cleaning agents. Therefore, the efficacy of selected halophytes, such as fodder beet and grasses, to absorb Na from different textured soils, and the suitability of the halophytes to different soil and climatic conditions in the wine producing regions of South Africa needs to be investigated.

CHAPTER 12: CAPACITY BUILDING

The following students, namely Mr. A.R. Mulidzi, Ms. C.L. Howell, Mr. C.S. Schoeman and Mr. C.H. Ochse, made valuable contributions to the project as part of their post-graduate studies. Mr. Mulidzi worked on a pot study at the ARC Infruitec campus, as well as a field study to determine seasonal soil chemistry dynamics. The other three students, namely Ms. Howell, Messrs. Schoeman and Ochse worked at the main field trial at Rawsonville. Mr. Schoeman received his M.Sc. Agric (Oenology) degree in 2012 from the Stellenbosch University. The studies of Ms. Howell, Mr. Mulidzi, and Mr. Ochse are still in progress, and will be completed as soon as possible.

A.R. Mulidzi

Mr. Mulidzi was responsible for the investigation into the impact of augmented winery wastewater on the chemical status of different soil types. He is registered at the Stellenbosch University to obtain a Ph.D. Agric (Soil Science) degree. His study leaders are Dr. C. Clarke and Prof. A. Roychoudhury. Mr. Mulidzi completed four seasons' irrigations on all four soils included in the pot experiment at the ARC Infruitec campus at Stellenbosch, as well as measurements in two field plots. The objectives of the latter measurements were to determine the seasonal soil chemistry dynamics in (i) an existing grazing paddock at the Goudini winery where wastewater is being used over many years for irrigation and (ii) a new paddock at the Koelenhof winery near Stellenbosch. Mr. Mulidzi plans to submit his thesis for examination in 2014. The findings of the pot experiment are presented in Chapter 9 of this Report. The field measurements still need to be completed, and did not form part of the project.

C.L. Howell

Miss Howell investigated the effect on irrigation using augmented winery wastewater on *in situ* soil chemical status, as well as grapevine responses. In addition to this, she was also responsible for a pot trial investigating the effect of irrigation using augmented winery wastewater on the hydraulic properties of four selected soils. She is registered at the Stellenbosch University to obtain a Ph.D. Agric (Soil Science) degree. Her study leaders are Dr. J.E. Hoffman and Dr. P.A. Myburgh. Miss Howell will submit her thesis for examination in 2014. The findings regarding the soil chemical status are presented in Chapter 4 of this Report, whereas findings on the effect of irrigation with augmented winery wastewater on grapevine responses are presented in Chapters 7 and 8. Results of the pot experiment are presented in Chapter 10.

C.S. Schoeman

Mr. Schoeman was responsible for investigating the effect of irrigation with augmented winery wastewater on wine quality aspects. In 2011 and 2012, he was registered at the Stellenbosch University to obtain a M.Sc. Agric (Oenology) degree. His study leaders were Prof. M. Du Toit and Prof. J.J. Hunter. Mr. Schoeman has completed his dissertation “Grape and wine quality of *V. vinifera* L. cv. Cabernet Sauvignon/99R in response to irrigation using winery wastewater” and was awarded his M.Sc. Agric degree Cum Laude. His dissertation is summarised below.

Grapevine performance and wine quality are influenced by various factors, two of the most important being the availability and quality of irrigation water. In relatively dry countries such as South Africa the conservation and effective use of water is of utmost importance. Expected increases in temperature and decreases in rainfall in the future due to climate change impacts highlights the importance of water conservation. This inspired investigations into possible alternative irrigation water sources and therefore the possibility of vineyard irrigation using winery wastewater is of utmost importance for the sustainability of the wine industry.

Winery wastewater contains higher concentrations of certain elements other than water generally used for vineyard irrigation, the most important differences being Na and K levels. Furthermore, winery wastewater contains larger populations of microorganisms such as yeasts, lactic acid bacteria and acetic acid bacteria, typical associated with wine production. If irrigation using winery wastewater affects the uptake of certain elements or alters grapevine water status, it may affect grapevine growth, juice and wine composition. Furthermore, if juice and wine composition is affected wine composition and sensorial quality may be affected.

Cabernet Sauvignon/99R grapevines, growing in a sandy soil in the Breede River Valley, were subjected to eight irrigation treatments using augmented winery wastewater in addition to irrigation using raw river water as control. The study was carried out during the 2010/11 and 2011/12 seasons. The various wastewater irrigation treatments were made up by augmenting winery wastewater with raw river water to obtain a target chemical oxygen demand (COD) concentration. In this study, the level of COD in the irrigation water is a direct indication of water quality, the two being indirectly proportional. The eight wastewater irrigation treatments ranged from 100 mg/L COD up to 3000 mg/L COD.

The first objective of the study was to determine the effect of irrigation using augmented winery wastewater on grapevine response, with regards to vegetative growth, berry development and berry composition. The various wastewater irrigation treatments did not

affect grapevine vegetative growth or reproductive growth, including yield, throughout berry development up to harvest. Berry sugar accumulation and evolution in acid concentrations were also not affected. An increase in berry juice pH was observed with an increase in the level of COD in the augmented winery wastewater only in the second season. The amount of elements, ions and heavy metals in juice was not affected by wastewater irrigation, indicating that there was no absorption by the grapevines. Berry skin thickness, colour and phenolic content as well as yield and its associated components were not affected by irrigation using augmented winery wastewater.

The second objective of the study was to determine the effect of irrigation using augmented winery wastewater on wine microbial and chemical composition, fermentation performance and wine sensorial characteristics. The natural yeast and bacteria flora of juice was not affected by the various wastewater irrigation treatments. In addition, the ability of the inoculated yeast and lactic acid bacteria strains to conduct their respective fermentation processes were not affected. With the exception of total titratable acidity (TTA) and pH, irrigation using augmented winery wastewater did not affect wine chemical composition with regards to basic wine parameters as well as colour, phenolic and tannin composition. Similar to juice, phosphorus and selected ions were not affected. None of the measured wine sensorial characteristics were affected by irrigation using augmented winery wastewater.

The third objective of the study was to investigate the effect of direct contact between berries and winery wastewater on wine sensorial characteristics. The study focussed on the transference of off-flavours from the wastewater into the wine and the occurrence of off-flavours as a response to contact with winery wastewater. Wine colour and general sensory wine descriptives were not affected by direct contact with winery wastewater. The presence of a winery wastewater-like off-odour and volatile acidity was, however, more detectable in wines made from berries that were in contact with the most concentrated wastewater. Therefore, it may be possible for off-odours to be transferred from the winery wastewater into the wines, or that off-odours are formed as a direct or indirect result of contact with winery wastewater.

Under the given conditions, results obtained in this two seasons of the study suggest that irrigation using augmented winery wastewater does not affect grapevine performance or wine quality substantially. The major impact that was observed was an increase in wine pH and a decreasing trend in TTA. Both these parameters could be rectified by simply adding acid to the wines. Therefore, irrigation using augmented winery wastewater may be considered as a possible future alternative source for vineyard irrigation. It is, however, important to remember that some of the effects of wastewater irrigation may be cumulative

and could possibly arise only after several years. Furthermore, different field conditions and cultivars may respond differently.

C.H. Ochse

Mr. Ochse was involved in the study to investigate the possibility of using cover crops to prevent element accumulation, particularly potassium and sodium in vineyard soils that are being irrigated using augmented winery wastewater. He is registered at the Cape Peninsula University of Technology to obtain an M.Tech. degree. Mr. Ochse plans to submit his dissertation for examination in 2014. The findings regarding the effects of cover crops on element uptake and removal are presented in Chapter 5 of this Report.

CHAPTER 13: TECHNOLOGY TRANSFER AND PUBLICATIONS

13.1. TECHNOLOGY TRANSFER

The information generated by the Project was disseminated to the different stakeholders *via* information sessions, *i.e.* producers' meetings and Winetech meetings, as well as scientific oral and poster presentations at national and overseas conferences as listed below.

2011

Myburgh, P.A., Project introduction and preliminary results. Presented to members of the Goudini Winery, Annual General Meeting, 25 May 2011, Rawsonville.

2012

Howell, C.L., Myburgh, P.A. & Lategan, E.L., A study to assess the feasibility of recycling winery wastewater for vineyard irrigation. Presented to industry and researchers at SASEV Spring day, 21 September 2012, Stellenbosch.

Howell, C.L., Myburgh, P.A. & Lategan, E.L., A study to assess the feasibility of recycling winery wastewater for vineyard irrigation. Presented to researchers attending IBSA meeting, 4 October 2012, Stellenbosch.

Howell, C.L., Myburgh, P.A. & Lategan, E.L., A study to assess the feasibility of recycling winery wastewater for vineyard irrigation. Presented to visiting University of the North agriculture students, 11 October 2012, Stellenbosch.

Mulidzi, R.A., Clarke, C., Myburgh, P.A. & Roychoudhury, A., The effects of winery wastewater irrigation on the chemical properties of four different soils. Paper presented at SASEV Conference, 14-16 November 2012, Franschhoek.

Meyer, A. & Myburgh, P.A., Soil depth and temporal variation in microbial enzyme activity in a vineyard soil irrigated with winery wastewater. Poster presented at SASEV Conference, 14-16 November 2012, Franschhoek.

Schoeman, C., Hunter, J.J., Myburgh, P.A., Strever, A.E. & Du Toit, M., Grapevine response to irrigation with winery wastewater: yield, grape and wine composition and sensory quality. Poster presented at SASEV Conference, 14-16 November 2012, Franschhoek.

Ochse, C.H., Fourie, J.C., Theron, H. & Myburgh, P.A., Effect of irrigation with augmented winery wastewater on the performance of *Avena sativa* L and *Pennisetum glaucum* used as interception crops in a vineyard near Rawsonville. Poster presented at SASEV Conference, 14-16 November 2012, Franschhoek.

Myburgh, P.A., Howell, C.L. & Lategan, E.L., Preliminary results on the responses of soil and grapevines to irrigation using recycled winery wastewater. Paper presented at the 7th CIGR International Technical Symposium, 28 November 2012, Stellenbosch.

2013

Mulidzi, R.A., Clarke, C., Myburgh, P.A. & Roychoudhury, A., The effects of winery wastewater irrigation on the chemical properties of four different soils. Paper presented at 6th IWA Specialized Conference, Winery 2013: Viticulture and winery wastes, 26-30 May 2013, Narbonne, France.

Howell, C.L., Myburgh, P.A. & Lategan, E.L., A study to assess the feasibility of recycling winery wastewater for vineyard irrigation – soil and grapevine responses. Paper presented at 6th IWA Specialized Conference, Winery 2013: Viticulture and winery wastes, 26-30 May 2013, Narbonne, France.

Howell, C.L., Myburgh, P.A. & Lategan, E.L., Assessing the feasibility of recycling winery wastewater for vineyard irrigation – soil, grapevine and wine responses. Poster presented at 15th AWITC Conference, 13-18 July 2013, Sydney, Australia.

Myburgh, P.A. & Howell, C.L., Recycling winery wastewater for vineyard irrigation – Experiment layout, evapotranspiration and grapevine water status. Paper presented at SASEV Congress, 13-15 November 2013, Somerset West.

Howell, C.L. & Myburgh, P.A., Recycling winery wastewater for vineyard irrigation – Soil chemistry, plant nutrient status and grapevine responses. Paper presented at SASEV Congress, 13-15 November 2013, Somerset West.

Mulidzi, R., Feedback on the 6th IWA International Specialized Conference on Sustainable Viticulture – "Winery 2013". Paper presented at SASEV Congress, 13-15 November 2013, Somerset West.

2014

Myburgh, P.A., Recycling winery wastewater for vineyard irrigation – Experiment layout, irrigation and evapotranspiration. Presented at WRC Information Day, 28 January 2014, Rawsonville.

Howell, C.L., Effect of winery wastewater on the soil chemical status. Presented at WRC Information Day, 28 January 2014, Rawsonville.

Fourie, J., Using cover crops to restrict accumulation of elements such as K and Na in the soil. Presented at WRC Information Day, 28 January 2014, Rawsonville.

Meyer, A., Effect of winery wastewater on the soil microbial status. Presented at WRC Information Day, 28 January 2014, Rawsonville.

Howell, C.L., What was the effect of winery wastewater on grapevine growth, yield and quality? Presented at WRC Information Day, 28 January 2014, Rawsonville.

13.2. PUBLICATIONS

The information was also disseminated through the following publications:

Posthumus, C., 2014. Recycling winery wastewater for vineyard irrigation. What are the soil & grapevine responses? SABI Magazine, Vol. 6, Issue 2, 8-9.

Schoeman, C.S., 2012. Grape and wine quality of *V. vinifera* L. cv. Cabernet Sauvignon/99R in response to irrigation using winery wastewater. Dissertation, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa.

The following articles are planned:

- Developing the infrastructure to irrigate crops with augmented winery wastewater in field experiments.
- Effect of irrigation with augmented winery wastewater on the chemical status of a sandy vineyard soil.
- Effect of irrigation with augmented winery wastewater on the chemical status of different textured soils.
- Effect of irrigation with augmented winery wastewater on the water infiltration of different textured soils.
- Effect of irrigation with augmented winery wastewater on grapevine and wine responses.
- Efficacy of cover crop practises to intercept and remove mineral elements applied through irrigation with augmented winery wastewater.
- Effect of irrigation with augmented winery wastewater on the microbial status of a sandy soil.
- Re-using winery waste for irrigation of crops – A review.

13.3. DATA AVAILABILITY

The raw, unprocessed data are available on compact disk from ARC Infruitec-Nietvoorbij. Direct enquiries with a short motivation to:

The Programme Manager
Soil and Water Science
ARC Infruitec-Nietvoorbij
Private Bag X5026
Stellenbosch
7599
South Africa
Telephone: +27 21 809 3100
Fax: +27 21 809 3002