Sustainable Recycled Winery Water Irrigation based on Treatment Fit for Purpose Approach



FINAL REPORT to

GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

Project Number: CSL 1002

Principal Investigators: Anu Kumar and Pichu Rengasamy

Research Organisation: **CSIRO Land and Water**

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Principal Investigators: Anu Kumar and Pichu Rengasamy
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Abstract

A dramatic increase in wine production over the last two decades in Australia and other wine producing countries has led to the need for sustainable management of winery wastewater to meet environmental concerns. The end use of the winery wastewater is the most important factor to consider when deciding on treatment options. The best choice will include consideration of critical issues such as: chemical oxygen demand (COD), quality of the wastewater (including pH, Na and K levels) and Federal/State regulations, amongst others. Any treatment must be 'fit for purpose'. If wastewater could be rapidly used for irrigation, only minimal treatment (e.g. dilution of salts by shandying the winery wastewater) may be required. However, if it is necessary to reduce the BOD/COD to control odour a balance needs to be found between an adequate level of treatment and storage of wastewater (that may cause an odour nuisance) versus the cheaper option of rapid disposal on the land through irrigation. Guideline values for key indicators of recycled winery wastewater quality for on-site and off-site disposal were developed during this investigation

Currently, the sodium adsorption ratio (SAR) or exchangeable sodium percentage (ESP) are used as indices of sodicity effects on soil structure. We propose a new ratio called 'CROSS' (cation ratio of soil structural stability), which is analogous to SAR but incorporates the differential effects of Na and K in dispersing soil clays, and also the differential effects of Mg and Ca in flocculating soil clays, and is defined as:

$$CROSS = (Na + 0.56K) / [(Ca + 0.6Mg)/2]^{0.5}$$

where the concentrations of these ions are expressed in millimole charge/L.

The good correlations obtained between CROSS measured in soil solutions ($CROSS_{ss}$) and parameters like turbidity and Zeta potential support its use instead of SAR or PAR (potassium adsorption ratio) in identifying soil structural stability influenced by cations. The present study also indicated the influence of soil type, especially clay content and mineralogy, in changing the effects of potassium.

A number of companies in the wine industry have used various forms of recycled water, including winery wastewater, to supplement their vineyard irrigation requirements. Soil surveys had been undertaken at eight sites in South Australia over the last three years. The monitoring covered a cross section of locations, soils, crops, wastewater qualities and application methods. Salinity, sodicity and available potassium in soils, were noted to be elevated in the wastewater treated plots, especially woodlot and pasture sites at certain wineries. Wastewater irrigated soils had higher CROSS values which were related to the turbidity, a measure of clay dispersion.

Based on our laboratory experiments and soil monitoring at field sites, we have derived the soil structural risks associated with different CROSS values in conjunction with the EC values of irrigation water. These values may change according to individual soils based on their organic matter, clay mineralogy and pH. Gypsum application reduces clay dispersion and increases total porosity and pore connectivity – thereby improving soil structure. Application of gypsum as 'dissolved gypsum' is more efficient in reclamation than surface application of dry gypsum. Measuring clay dispersion potential in waste water irrigated soil will indicate soil structural degradation and can indicate the need for gypsum application

In the present study, the wastewater EC_{sw} was always below 3.5 dS/m and did not result in toxicity to grapevines during glasshouse and paired field trials. However, Na and K treatments above 40 mM and Na +K treatment at 20 mM each, were responsible for toxicity signs in the grapevines. These higher treatments were also prone to diseases. Based on the information available in literature and the field and glasshouse trials conducted in this study, we have established sodium and potassium thresholds (individually and in combinations) in grapevine irrigation water. Sodium levels less than 460 mg/L, potassium at 1250 mg/L and Na +K in combination at 625+ 1084 mg/L, respectively in winery wastewater will be safe for application on the grapevines. These thresholds listed are indicative values and are also dependent on the variety and the rootstock of grapevines used.

Based on ecotoxicological and microbiological assessments, catechin and gallic acid concentrations in the wastewater are safe at 1 mg/kg and coumaric acid at 0.3 mg/kg. Ethanol is highly toxic with a 0.1% or 1g/L threshold value in the wastewater.

This project also focussed on the opportunities presented by minimally treated wastewater which included:

- Reduced costs and environmental impact of wastewater treatment and management;
- Utilisation of wastewater organic carbon to enrich soils; and
- Utilisation of wastewater nutrients to benefit crop growth where possible.

Generation of winery wastewater (WWW) often occurs during the grapevine's dormant period, and thus its application to other cropping systems is a necessity to respond to future projections of limited water resources for irrigation. The ability to reuse winery wastewater would be of significant benefit to the wine industry, as it could potentially be a cost-effective method of wastewater management, whilst at the same time providing a valuable water resource. In the present study, 50% dilution of wastewater with fresh water would be required if WWW were to be used to irrigate beneficial crops such as turnips, kikuyu grass, pod peas and grape vines but this depends on the WWW quality (especially EC). This study highlighted that WWW with minimal treatment can be used for beneficial irrigation of turf grass at least on sandy soils. Best results for turf cultivation would be obtained by using a 1:1 (50%) mixture of tap water and WWW.

The actual disposal systems available have been summarised in the decision support tools as flow charts. These disposal systems include evaporation basins, disposal through a sewer system or other off-site management, treatment and storage and irrigation or rapid disposal to some receiving crop. Each of these systems requires infrastructure and has associated costs and potential benefits. It would be prudent to modify the quality of the wastewater so that it is optimal for the chosen method of ultimate disposal.

We estimate that minimally treated wastewater will reduce the operating costs of wastewater treatment by approximately 50%. The benefits of application to soils will also help reduce the cost of crop water and nutrient requirements. In the present study, COD of less than 2000 mg/L did not result in waterlogging conditions or compromised crop germination success and growth. The rapid disposal of WWW has two clear advantages in terms of carbon sequestration. In the first instance carbon compounds from the winery wastewater are retained in the soil. Secondly it is known that the treatment of winery waste is energy intensive and this typically will require carbon expenditure to produce the power. These two reasons suggests that if possible rapid usage of the winery wastewater will be more environmentally friendly than the alternative option of treat, store and then irrigate.

Executive summary

A dramatic increase in wine production over the last two decades in Australia and other wine producing countries has led to a corresponding increase in winery wastewater production. Sustainable management of winery wastewater has therefore become a priority to address environmental concerns. The end use of the wastewater is the most important factor to consider when deciding on treatment options. The best choice of management of the wastewater prior to disposal will depend on consideration of critical issues such as: chemical oxygen demand (COD), quality of the winery wastewater (including pH, Na, K levels) and Federal/State regulations, amongst others. Any treatment must be 'fit for purpose'. If wastewater could be rapidly used for irrigation, only minimal treatment may be required – that treatment may be filtration, pH adjustment and potentially shandying to dilute salt load. However if it is necessary to store the wastewater near an urban area, it may be necessary to reduce BOD/COD in order to control odour. Guideline values for key indicators of recycled winery wastewater quality for on-site and off-site disposal were developed during this investigation that focus on the balance between adequate treatment with its associated costs and rapid disposal through irrigation with its inherent risk of odour production.

Disposal of winery wastewater (whether treated or not) often poses a threat to soil structure. Currently, the sodium adsorption ratio (SAR) or exchangeable sodium percentage (ESP) are used as indices of sodicity effects on soil structure. The SAR model was originally developed on the basis of ratio law to predict the adsorption of cations on soil exchange sites in relation to the concentrations in soil solutions. SAR is estimated from the concentrations of Na, Mg and Ca in soil solutions and has been found to be highly correlated with ESP. Potassium is not considered in the SAR model. Traditionally, application of K to soils has been perceived as a benefit due to a large body of published research demonstrating the positive effects of K on soil fertility and crop yield. However, K has the potential to cause clay swelling and dispersion as well as increasing overall soil salt levels, and hence degrade soil quality and land productivity (Rengasamy and Marchuk, 2011). Elevated K concentrations could limit their disposal onto land. Further, Mg is treated as equal to Ca. However, the ionicity indices of Mg and Ca are different and hence, their flocculating powers. Similarly, the ionicity indices of Na and K are also different with different effects on clay dispersion. Based on these notions, Rengasamy and Marchuk (2011) proposed a new ratio called 'CROSS' (cation ratio of soil structural stability) which is analogous to SAR but incorporates the differential effects of Na and K in dispersing soil clays, and also the differential effects of Mg and Ca in flocculating soil clays, and is defined as:

$$CROSS = (Na + 0.56K) / [(Ca + 0.6Mg)/2]^{0.5}$$

where the concentrations of these ions are expressed in millimole charge/L.

The good correlations obtained between CROSS measured in soil solutions ($CROSS_{ss}$) and parameters like turbidity and zeta potential support its use instead of SAR or PAR in identifying soil structural stability influenced by cations. The present study also indicates the influence of soil type, especially clay content and mineralogy, in changing the effects of potassium.

A number of companies in the wine industry have used various forms of recycled water, including winery wastewater, to supplement their vineyard irrigation requirements. These same companies also actively monitor soil parameters from many vineyards and to a lesser extent other irrigation/disposal areas. Datasets from those wineries were used to investigate a possible relationship between variation in irrigation water quality from various sources and changes in soil properties. Several trends emerged from the historical data analyses and soil surveys conducted:

- Higher organic carbon content of the winery wastewater resulted in increased total organic carbon content in the soils irrigated with winery wastewater
- Available potassium increased in the winery irrigated soils at a rate of 9.6 mg/kg per year at the depth of 20 cm.

- Soils salinity, sodicity and available potassium in soils were elevated in the wastewater treated plots (especially woodlot and pasture sites) at certain wineries.
- Wastewater irrigated soils had higher CROSS values which were related to the turbidity, a measure of clay dispersion.

In addition, soil surveys had been undertaken at eight sites in South Australia over the last three years. The monitoring covered a cross section of locations, soils, crops, wastewater qualities and application methods. Salinity, sodicity and available potassium in soils, were elevated in the wastewater treated plots, again especially woodlot and pasture sites at certain wineries.

The following table depicting the soil structural risks associated with different CROSS values in conjunction with the EC values of irrigation water was derived from our laboratory experiments and soil monitoring at field sites. We have given the risk factors for two types of soils i.e. highly and poorly permeable. This table is tentative and the risk factors may change according to individual soils based on their organic matter, clay mineralogy and pH (refer Table 7.2 in Chapter 7).

Soil Structural risks due to CROSS values at different EC (salinity) levels in winery wastewaters

SOIL TYPE	WELL TO MODERATE DRAINING SOILS			SLOW DRAINING SOILS		
WATER SALINITY (dS/m)	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
<1.0	<3.0	3.0-6.0	>6.0	<2.5	2.5-5.0	>5.0
<1.0-1.5	<4.0	4.0-7.0	>7.0	<3.0	3.0-6.0	>6.0
1.5-2.0	<6.0	6.0-8.0	>8.0	<4.0	4.0-7.0	>7.0
2.0-3.0	<8.0	8.0-10.0	>10.0	<6.0	6.0-8.0	>8.0
3.0-4.0	<10.0	10.0-15.0	>15.0	<8.0	8.0-12.0	>12.0

Note: the criteria were derived on the basis of the principles presented in: Marchuk, A. and Rengasamy, P. (2012) Threshold electrolyte concentration and dispersive potential in relation to CROSS in dispersive soils. *Soil Research* **50**, 473-481

Because of the variations within a soil type, we recommend that each individual soil should be tested for clay dispersion and a decision on gypsum application should be based on this test. A detailed fact sheet on gypsum has been prepared. Gypsum application reduces clay dispersion and increases total porosity and pore connectivity – thereby improving soil structure. Application of gypsum as 'dissolved gypsum' is more efficient in reclamation than surface application of dry gypsum. Measuring clay dispersion in wastewater irrigated soil will indicate whether there has been soil structural degradation and can indicate the need for gypsum application.

Application of wastewater with high potassium levels was found to increase the overall level of soil fertility, but long-term application of such wastewater may cause the build-up of soil potassium and decrease the hydraulic conductivity of the receiving soils. In the present study, the wastewater EC_{sw} was always below 3.5 dS/m and did not result in toxicity to grapevines during glasshouse and paired field trials. However, Na and K treatments above 40 mM and Na +K treatment (at 20 mM each), produced toxicity symptoms in the grapevines. Vines receiving these higher treatments were also prone to diseases.

A reduction in vine growth generally occurs when the average root zone salinity over the growing period exceeds a designated threshold value. The link between soil-K and fruit-K is complex. Elevated berry K will influence the effect of other cations present and is thought to have impacts on fermentation properties and other wine sensory properties such as salty taste and bitterness, pH/sourness and the potential for some (additional) microbiologically influenced challenges. Based on the information available in literature and the field and glasshouse trials conducted in this study, we have established sodium and potassium thresholds (individually and in combination) in grapevine irrigation water. Sodium at levels less than 460 mg/L, potassium at 1250 mg/L and Na +K in combination at 625+ 1084 mg/L, respectively in winery wastewater will be safe for application on the grapevines. These thresholds listed are indicative values and are also dependent on the variety and the rootstock of grapevines used.

Based on ecotoxicological and microbiological assessments, catechin and gallic acid concentrations in the wastewater are safe at 1 mg/kg and coumaric acid at 0.3 mg/kg. Ethanol is highly toxic with a 0.1% or 1g/L threshold value in the wastewater.

This project also focussed on the opportunities presented by minimal treated wastewater which included:

- Reduced costs and environmental impact of wastewater treatment and management;
- Utilisation of wastewater organic carbon to enrich soils; and
- Utilisation of wastewater nutrients to benefit crop growth where possible.

Developing a wastewater program which produces wastewater that is fit for a particular disposal method will be an iterative process that involves the disposal methods and the production process within the winery. We suggest that the best place to start this process is to look at the options that are available for the ultimate disposal of the winery wastewater.

Generation of winery wastewater (WWW) often occurs during the grapevines' dormant period, and thus its application to other cropping systems is a necessity to respond to future projections of limited water resources for irrigation. The ability to reuse winery wastewater would be of significant benefit to the wine industry, as it could potentially be a cost-effective method of wastewater management, whilst at the same time providing a valuable water resource. In the present study 50% dilution of wastewater with fresh water would be required if WWW were to be used to irrigate beneficial crops such as kikuyu but this depends on the seasonal salinity of the wastewater. The use of WWW with dilution is therefore practically limited based on freshwater availability and cost, in conjunction with overall volumes of wastewater production.

This study highlighted that winery wastewater with minimal treatment can be used for beneficial irrigation of turf grass at least on sandy soils.Best results for turf cultivation would be obtained by using a 1:1 (50%) mixture of tap water and winery waste. Apparently some winery wastewater (up to at least 50%) enhanced the growth of kikuyu. Higher concentrations decreased plant growth. The lower biomass produced using 100% winery wastewater would not be commercially beneficial due to its limited nutrient removal potential. Later tests using another batch of WWW did not require any dilution as it had a lower EC.

Field peas were also grown successfully with a mixture of winery wastewater with maximum production again occurring with the shandy of tap water and winery waste water. The turnip crop produced only small tubers with each irrigation source with no apparent effect on growth due to the different mixtures present.

From the above, it is concluded that winery wastewater can be used effectively in the production of some commercial crops even with minimal treatment. The best choice of crop is still to be determined.

The crop choice will be determined by a range of factors including timing of the crop and the availability of the irrigation water. Ideally the crop should be in a state so that it can use the wastewater as soon as it is available. This could be done with annual crops, especially if they have already been established. Other alternatives are to use perennial crops such as lucerne and tree crops.

A further uncertainty arises with the variability of winery wastewater – both across the season and also between wineries. There may be opportunity to modify production such that the wastewater is more suited to use on beneficial crops. Such changes may include substitution of sodium with potassium as the potassium can be used (and is required by) the crops. A further consideration is the water usage – there may be little point in saving water in the production process if water is required for a shandy of the irrigation water if the wastewater is to be used within 24-48 hours of production.

The above considerations require further investigation before optimal management can be achieved. In the interim, it is suggested that:

- Winery wastewater should be utilised rather than wasted;
- The irrigated crop should be of commercial benefit;
- Rapid usage should be an aim to reduce the requirement for processing; and
- Where rapid disposal is an option, winery production should be matched with the disposal process.

The actual disposal systems available have been summarised in the decision support tools as flow charts. These disposal systems include evaporation basins, disposal through a sewer system or other off-site management, treatment and storage and irrigation, or rapid disposal to some receiving crop. Each of these systems requires infrastructure and has associated costs and potential benefits. It would be prudent to modify the quality of the wastewater so that it is optimal for the chosen method of ultimate disposal. This may require or allow certain processes to occur within the winery.

- Off-site disposal. A large component of the off-site disposal will be in transportation and potentially storage waiting for the disposal. In this situation the volume of the waste should be minimised using water recycling and segregation of waste streams.
- Evaporation basin. The limitation with the evaporation basin would also be the volume of water produced. The restrictions for evaporation basin disposal would therefore be similar to those required for off-site disposal.
- Treat and store. In this system not only is the volume of water important but also the quality because depending on the BOD, the amount of treatment will have to be modified. The quantity of water in this system may be limited by the storage available. Where storage is a limiting factor it is important to minimise the volume of water being produced. If storage is not limiting, and the wastewater requires shandying to reduce EC to a suitable level, minimising the volume of water being produced may be less important.
- Rapid disposal. Rapid disposal of winery wastewater requires an EC less than 3.5 dS/m. As this may require dilution of the wastewater stream with fresh water, the applicability of this method may depend on the location of the source of water that is available for shandying - if it is some distance from the winery then minimising water use in the production process would be beneficial. If however the water source is available at the winery the mixing process would naturally occur at the winery so there would be no advantage in using methods that are costly to save water within the wine production process.

With both 'treat and store' and 'rapid disposal' there will be an ongoing problem with sodium. Any modifications to processes within the winery that reduce the amount of sodium in the waste stream would therefore be beneficial regardless of the method of disposal. Similarly, controlling process spillages would be of benefit by reducing the BOD load entering the waste stream. When the wastewater will be used for the production of beneficial crops the inclusion of nutrients such as N, P and K may be advantageous so there is little need to remove them in the treatment process. If the wastewater can be rapidly used for irrigation, only minimal treatment is required – removal of COD would be just using energy to make CO_2. On the other hand, if it is necessary to store the wastewater near an urban area, it may be necessary to remove the COD to control odour.

Monitoring of the environment will be required for most disposal systems including evaporation basins, 'treat and store and irrigate' and 'rapid disposal'. We estimate that minimally treated wastewater will reduce operating costs of wastewater treatment by approximately 50%.

The benefits of application of WWW to soils will also help reduce the cost of crop water and nutrient requirements. The Margaret River wine region limits the irrigation rate according to maximum P (10-120 kg/ha/yr), Max available N (140 – 480 kg/ha/yr) and BOD (30 kg/ha/day). In the present study, COD of less than 2000 mg/L did not result in waterlogging conditions or compromise crop germination success and growth.

The rapid disposal of winery wastewater has two clear advantages in terms of carbon sequestration. In the first instance carbon compounds from the winery waste are retained in the soil. Secondly, it is known that the treatment of winery waste is energy intensive and this typically will require carbon expenditure to produce the power. These two reasons suggest that if possible, rapid usage of the winery wastewater will be more environmentally friendly than the alternative option of 'treat, store and then irrigate'.

Recommendations

The new knowledge generated by this project should be considered in the following terms:

1. No comparative costs were available to the study that would enable a comparison of the cost:benefit of rapid disposal versus the 'treat, store and irrigate' option. This should be further explored.
2. In the present study, two paired sites were established and monitored for three years. There is a need to continue the monitoring of these recently established field trials for another five years to establish the long-term effects of winery wastewater irrigation on vines. In addition, it would be useful for this approach to be extended to other wine regions with other varieties of vines, to assess the long-term impact of winery wastewater irrigation on petiole, grape juice and wine quality.
3. In the present study, Na and K effect on petiole and grape juice were evaluated over two years using glasshouse experiments. There is a need to establish mesocosm experiments in the real environment with different levels of Na and K irrigation regimes (individually and in combination) on several vine varieties over a period of at least five years to validate the Na and K thresholds established in this study.
4. The operating guidelines based on the new information generated through this project should be updated with a view to reducing the wine industry's industrial footprint, while not imposing an economic cost.

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Part I Background

1 Background

Recycled winery wastewater (quality) improvement for irrigation re-use is a keystone to the industry's environmental sustainability. The practices of irrigation of pastures, woodlots and grapevines with treated recycled winery water and land treatment of recycled winery water are becoming more common. It is important to recognise whether current irrigation practice is beneficial or detrimental to the particular soil and crop. The potential end use of the recycled winery water is the most important factor to consider when deciding on treatment options. The best choice will include consideration of critical issues, such as chemical oxygen demand (COD), flow rates and quality of the winery water, pH, Federal/ State regulations and location constraints, amongst others. The treatment must be 'fit for purpose'. A balance must be sought between the right level of treatment and storage of wastewater that may cause odour nuisance versus the suitability for its rapid disposal on the land through irrigation. Guideline values for key indicators of recycled winery wastewater quality for on-site and off-site disposal are required urgently.

A GWRDC Technical Industry Workshop held in May 2009 highlighted key areas of research for sustainable irrigation with recycled water. Our project objectives and research activities are addressing some of these issues.

1.1 Fit for purpose treatment and beneficial reuse opportunities

Most of the recycled winery wastewater applied on land typically results in the reduction of biochemical oxygen demand (BOD), at a significant energy cost and loss of potentially soil-sequesterable carbon. A 'fit for purpose treatment' option that maximises the carbon accumulation in soil has been demonstrated over several years and showed a parallel increase in soil microbial activity.

Disposal of the wastewater must be rapid to prevent the development of malodour. To do this the receiving soil must be able to accept the wastewater without creating water logging and the groundwater must be protected. Hence, research is required into finding ways to minimise methane and CO_2 production, to assess the economics of full treatment versus rapid disposal and the identification of appropriate receiving crops. If these challenges could be overcome, then a phased transition from full COD removal to rapid disposal may be feasible.

1.2 Use of CROSS as an index of structural stability over a range of soils containing varying quantities of Na, K, Mg and Ca

In the past, several soil scientists have questioned treating soil cations as equal, particularly in the calculation of the SAR soil stability index (Rengasamy and Sumner, 1998). The divalent cations, calcium and magnesium, aid flocculation but they are not equivalent in their flocculating effect. Similarly, the monovalent cations, sodium and potassium, do not have the same dispersive effect. Therefore, there was a need to derive and define a new ratio of these cations in place of SAR, which will indicate the effects of Na, K, Mg and Ca on soil structural stability. This will be achieved using a formula analogous to the SAR but which selectively incorporates the dispersive effects of Na and K on the one hand with the flocculating effects of Ca and Mg on the other.

Currently, the SAR or ESP are used as indices of sodicity effects on soil structure. The SAR model was originally developed on the basis of ratio law to predict the adsorption of cations on soil exchange sites in relation to the concentrations in soil solutions. The SAR is estimated from the concentrations of Na, Mg and Ca in soil solutions and has been found to be highly correlated to ESP. Potassium is not considered in the SAR model. Further, Mg is treated as equal to Ca. However, the ionicity indices of Mg and Ca are different and hence, also their flocculating powers. Similarly, the ionicity indices of Na and K are also different with

different effects on clay dispersion. Based on these notions, Rengasamy and Marchuk (2011) proposed a new ratio called 'CROSS' (cation ratio of soil structural stability) analogous to SAR but which incorporates the differential effects of Na and K in dispersing soil clays, and also the differential effects of Mg and Ca in flocculating soil clays, and is defined as:

$$CROSS = (Na + 0.56K) / [(Ca + 0.6Mg)/2]^{0.5}$$

where the concentrations of these ions are expressed in millimole charge/L.

The coefficient of K was based on the ratio of the dispersive powers (reciprocal of flocculating powers) of Na and K, and the coefficient of Mg was based on the ratio of flocculating powers of Ca and Mg. The flocculating powers of all of these cations have been derived theoretically and verified experimentally by Rengasamy and Sumner (1998).

CROSS is a new concept and its validity is not yet tested. To validate and make use of this concept as a parameter in soils and irrigation water, we have to research the following:

1. Applicability of CROSS as an index of structural stability and 'dispersive potential' over a range of soils containing varying quantities of Na, K, Mg and Ca.
2. Similar to the utility of SAR, the effects of CROSS on structural stability will depend largely on the total electrolyte concentration and soil texture. The influence of mineralogy, organic matter and pH are also important. By researching the various factors influencing the efficacy of CROSS, we can derive useful threshold values.

1.3 Manage plant uptake of K at high potassium soil levels

High potassium levels in recycled winery water have been identified by industry as an area of concern when reusing/disposing of wastewater by irrigation. Application of wastewater with high potassium levels was found to increase the overall level of soil fertility (Arienzo 2008, 2012), but long-term application of such wastewater may cause the build-up of soil potassium and decrease the hydraulic conductivity of the receiving soils. These potential impacts are uncertain and have been inadequately researched.

1.4 Impact of recycled winery water irrigation on wine chemistry and sensory properties

The link between soil-K and fruit-K is complex, but high must-K can precipitate tartrate salts and elevate pH, which require correction with (costly) tartaric acid to stabilise the must during vinification. Elevated berry K will influence the effect of other cations present and is thought to have impacts on fermentation properties and other wine sensory properties such as salty taste and bitterness, pH/sourness and the potential for some (additional) microbiologically influenced challenges. It is important to conduct paired field trials to adequately address this issue.

1.5 Beneficial reuse of recycled winery water

Trials, as part of GWRDC project CSL 05/02 (Kumar et al., 2009), showed that minimally treated winery wastewater can be used beneficially for the production of fodder crops (e.g. oats, triticale, vetch) through improved soil fertility in terms of available K and P and improved the organic content of the soil. The one problem highlighted in these data is the apparent leaching of Na through the profile. While this is a problem with untreated wastewater, it remains a problem even with treated wastewater. This stresses the need for the reduction of Na in the waste stream through cleaner production initiatives. Further work is required on the selection of a suitable crop to receive the wastewater irrigation and its management in vineyards. Ideally the crop should be able to utilise both the water application and the nutrients in the

wastewater. Limited work has been undertaken by the industry on the irrigation of perennial crops with wastewater. While wastewater is used on vines, the practice is not accepted by many winemakers. Hence, information on the effects of wastewater use on wine quality, vine growth, the uptake of cations and other nutrients, particularly K, and soil structure are needed.

It is recognised that the reuse of water and nutrients contained in the wastewater is generally a 'beneficial reuse'. However, the wastewater will only be considered as 'reuse' when evidence exists to demonstrate that the concentration of substances it may contain such as ethanol, phenolic compounds and tannins, provides a demonstrable benefit to the receiving land or, at least, has no deleterious impacts. When wastewater containing substances in concentrations in excess of those which benefit the land is applied, it is considered as an 'emission to land' for these substances and must be reported as such. There is currently a lack of information to prove that ethanol contained in wastewater is 'beneficial to reuse'

More and more wineries are considering irrigating woodlots, pastures and vines with treated wastewater for environmental, social and cost benefits. However, the irrigation methods and systems that are adopted are usually not scientifically tested and are under increasing attention from regulators. Thus, the industry needs to understand the long term impacts of wastewater management practices on soil function and crop quality. Reduced barriers to reuse (especially with regard to K), reduced costs and the sensible use of water in an era of declining water resources will be very timely initiatives for the wine industry.

1.6 Objectives

The overall aim of this project is to provide an integrated "systems approach" to sustainable recycled winery wastewater management that combines a comprehensive account of wastewater characteristics, cost-effective treatment strategies for nutrients, organic and chemical loads, and sustainable management options to meet desired environmental and crop specifications. Specific objectives are to:

1. Determine the effect of different chemical oxygen demand, (COD), levels (recycled winery water shandied with standard irrigation water) on soil characteristics and plant response. Explore opportunities to utilise carbon and nutrients in recycled winery water for beneficial crops.
2. Provide the wine industry with up to date knowledge of irrigation method(s), agronomic practices, juice and wine quality and of using recycled winery water to augment irrigation water for vines
3. Assess long-term consequences for soil structure in different soils (different pH, duplex / heavy clay) of long term irrigation with recycled winery water and evaluate the use of "cation ratio of structural stability", (CROSS), as an index of structural stability.
4. Develop strategies to minimise salt accumulation in soil from wastewater irrigation.
5. Monitor the impacts that recycled winery water irrigation (raw, shandied, timing, irrigation technique) has on soil microbiological health during crop and vine trials.
6. Develop threshold values for organic components in recycled winery water (ethanol and phenolic compounds) to gain confidence in the minimally treated water for irrigation.
7. Develop recommendations for sustainable recycled winery water irrigation based on "Treatment Fit for Purpose Approach".

1.7 Performance against planned outputs and performance targets

Planned outputs and performance targets of the project are given below (Table 1).

Table 1.1 Output and performance targets of the project

Outputs and Activities 2010-2011

YEAR 1	OUTPUT	ACTIVITIES
A	Establish program and select project advisory committee.	Identification of suitable participating wineries for historical data collection, field trials and sampling. Initial meeting of project steering group to outline project scope and responsibilities.

Outputs and Activities 2011-2012

YEAR 2	OUTPUT	ACTIVITIES
A	Establish ongoing soil monitoring program at existing winery- vineyard and/or other relevant field sites.	Establish field sites for conducting field sampling over two years.
B	Maintain soil monitoring program at winery-vineyard/field sites.	Collation of information from field sites based on first year of sampling.
C	Soil quality impacts determined from existing recycled winery water irrigation trials.	Collection of historical data on soil quality at participating wineries.
D	Establish a paired trial to determine impacts of recycled winery water irrigation on grape and wine quality.	Paired trial established and monitoring program in place for soil, petiole, grape and wine quality analyses.

Outputs and Activities 2012 - 2013

YEAR 3	OUTPUT	ACTIVITIES
A	Maintain soil monitoring program at winery-vineyard/field sites.	Collation of information from field sites based on second year of sampling collected at the existing field sites.
B	Publish a technical report: 1. describing protocols and techniques for assessing soil structure and function changes under the influence of recycle water irrigation, and 2. on the status of soil structure and function at recycled water irrigation sites especially in relation to sodium and potassium based on current practices.	Standardise, validate and compare methods for measuring changes in soil structure and function under the influence of recycled winery water irrigation. Analyses of historical soil quality data and collation and documentation of recycled water irrigation strategies. Interpretation of results and compilation into a technical report.
C	Develop threshold values of ethanol and phenolic compounds to promote safe and sustainable use of recycle water irrigation on vines and other crops.	Conduct microbiological assessment of soil irrigated with different levels of ethanol and phenolic compounds in the recycle winery water. Conduct ecotoxicological assessment of soil irrigated with different levels of ethanol and phenolic compounds in the recycle winery water using plants and earthworm as bioindicators.
D	A technical report and a journal article on the information on the long term consequences for soil structure in different soils (different pH, duplex/heavy clay) of long term irrigation with recycled winery water.	Analyses of vineyards, grasses, crops, and woodlots and date palm in relation to potassium uptake at existing field trials. Assess long-term consequences for soil structure in different soils (different pH, duplex/heavy clay) of long term irrigation with recycled winery water. Evaluate the use of the “cation ratio of structural stability”, CROSS, as an index of soil structural stability.

E	Refine the research strategies and promotion of research findings to wider wine industry.	Organise Advisory group meeting to discuss, review and revise information collected in the first year study and refine research strategies for the second year. Disseminate results to the wider Australian wine industry via an industry article.
F	Publish a journal article and magazine article on the on the impact of recycled winery water irrigation on the soil microbiological health.	Monitor the impacts that recycled winery water irrigation (raw, shandied, timing, irrigation technique) has on soil microbiological health during laboratory experiments. Monitor the impacts that recycled winery water irrigation (raw, shandied, timing, irrigation technique) has on soil microbiological health at field sites such as woodlots, pastures and vineyards.
G	Propose strategies to minimise salt accumulation in soil from recycled winery water irrigation based on laboratory and field trials. Develop a factsheet on gypsum application rates.	Conduct Gypsum trials using laboratory column experiments. Assess soil quality and health of vineyards applying gypsum to reduce the impact of high sodium in the recycled winery water. Assess other optional strategies such as mid row crop in vineyards for ameliorating sodicity impact due to recycled winery water application.

Outputs and Activities 2013 – 2014

YEAR 4	OUTPUT	ACTIVITIES
A	Publish a technical report on the wine quality from wineries producing wine from recycle water irrigated vines.	Collection of information on petiole analyses and wine quality from paired trial. Establishment of methods and protocols for assessing nutrient levels in petiole, grape juice and wine quality. Analyses of information on petioles, grape and wine quality.
B	Publish a technical report on the influence of the quality of recycled water in relation to juice quality and wine quality.	Compare nutrients in petioles and grapes irrigated with recycled winery water, bore water and shandied stormwater. Compare spectral analyses of wine produced from grapes irrigated with recycled winery water, bore water and shandied stormwater. Compare preliminary sensory evaluation of wines produced from grapes irrigated with recycled winery water, bore water and shandied stormwater.
C	A technical report on recycled water quality thresholds for various soil types under differing recycled winery water types and management actions.	Conduct soil column experiments with different soil types under different COD levels of recycled water. Analyse soluble and available sodium and potassium levels of the leachate and soil at different irrigation regimes.
D	Refine the research strategies and promotion of research findings to wider wine industry.	Organise Advisory group meeting to discuss, review and revise information collected in the second year of the project and refine research strategies for the final year of this project. Documentation of findings in a formal report to GWRDC. Disseminate results to the wider Australian wine industry via an industry article and road shows, regional workshops and technical conferences and papers - where appropriate.

E	Identify beneficial crops for harvesting nutrients based on laboratory pot trials and existing field trials.	Conduct pot trials involving selected plants (such as sugar beets, turnips, chickpeas, tomatoes, corn) at different salt levels. Conduct vine trials in glass house involving recycled water at different salt levels. Assess soil structure and function at winery sites using recycled water for growing beneficial crops. Assess soil structure and function at winery sites using recycled water for growing vines.
F	Develop recommendations for sustainable recycled Winery Water Irrigation based on Treatment Fit for Purpose Approach. Communication of research findings to wider wine industry.	Based on this study collate information on the effects of K (and other cations) and recycled winery water at different COD levels on vine physiology and grape attributes and other beneficial crops. Develop hazard guidelines related to the impact of recycled water use on soil degradation, vine nutritional properties and wine quality. Develop a framework and decision support tool for wineries to apply fit for purpose approach for recycling winery water. Organise Advisory group meeting to discuss, review and revise information collected in the third year of this project. Documentation of findings in a final report to GWRDC. Disseminate results to the wider Australian wine industry via road shows, regional workshops and technical conferences and papers.

1.8 References

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Part II Winery wastewater irrigation and soil structure

2 Winery wastewater irrigation and soil structure

2.1 Cation ratio of structural stability, CROSS, as an index of soil structural stability

2.1.1 INTRODUCTION

The quality of irrigation water can affect both soil properties and plant growth (Figure 2.1). In this section, soil structural change as influenced by winery wastewater (WWW) irrigation is discussed.

During irrigation or rain, soil aggregates can disintegrate into smaller particles as a result of water molecules interacting with soil particles, leading to unsatisfactory soil structure. As field soils become wet, clay particles may disperse from the aggregates, affecting the pore system and influencing a range of physical and mechanical properties that ultimately control plant growth.

When soil particles adsorb monovalent ions like sodium and potassium in higher proportion relative to the divalent cations, clay particles can swell due to wetting. When water content is above saturation, clay-to-clay assemblage separates over distances >7 nm. In calcium-saturated clay, these interactive forces limit the separation to a distance <7 nm. The separated clay particles revert to the position of zero repulsive energy when they flocculate as a result of the electro-osmotic forces induced by dissolved salts.

While many soil factors such as clay mineralogy, organic matter and other cementing agents can influence clay dispersion, the major factors are the proportion of adsorbed sodium (measured as either sodium adsorption ratio (SAR)or exchangeable sodium percentage (ESP)) and the total electrolyte concentration (salinity or electrical conductivity (EC)). The role of adsorbed potassium is receiving attention recently because of the occurrence of significant amounts of potassium in wastewater used for irrigation (Arienzo et al. 2008; 2012).

Potassium, being a monovalent cation can cause soil clay swelling and dispersion similar to sodium. The role of potassium in soil structure has been debated in literature, its effects being either equal to or less than that of sodium. Rengasamy and Sumner (1998) hypothesised that the ionicity of clay-cation bonds determines the dissociation of clay particles on interaction with polar water molecules and on this basis derived flocculating and dispersive powers of cations. Recently, Marchuk and Rengasamy (2011) derived ionicity indices of the cations Na^+, K^+, Mg^{2+} and Ca^{2+} using their ionisation potentials and charge. Their studies on homoionic pure and soil clays confirmed that clay behaviour such as dispersivity was regulated by the degree of ionicity of clay-cation bonds.

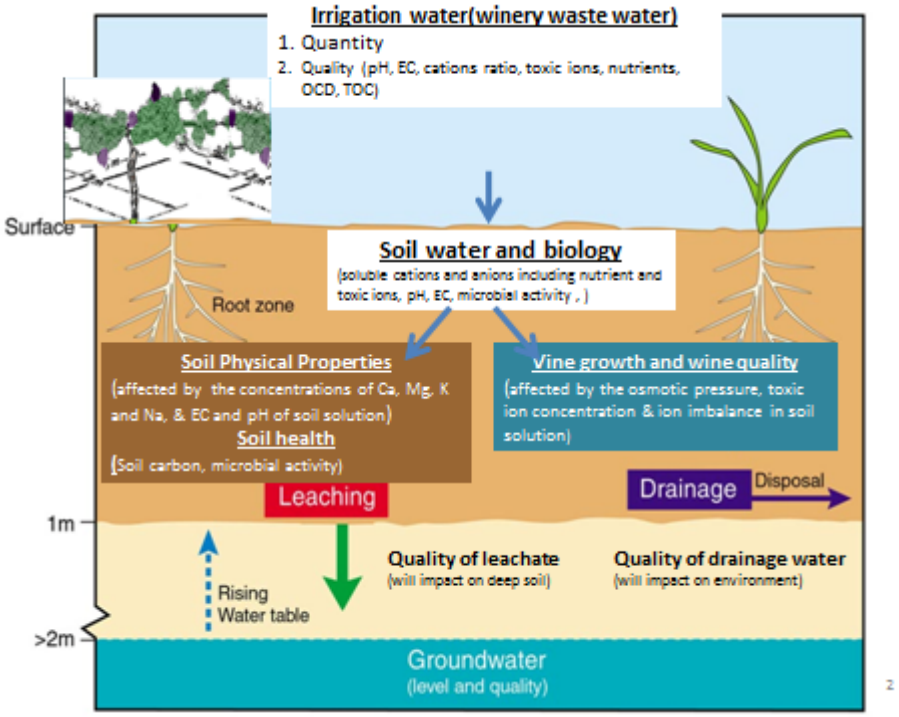


Figure 2.1 Factors to be considered when irrigating vineyards with winery wastewater

2.1.2 METHODS

Soil used

Three soils viz. Urrbrae, McLaren and Claremont were used in the present study. Their physicochemical properties and locations are presented in Table 2.1. The samples were taken by a hand auger, air-dried, sieved to 2 mm particle diameter and analysed for physical and chemical characteristics. Selection of these soils was based on the differences in their texture, clay mineralogy and pH.

Table 2.1 Selected physical and chemical properties, main clay minerals, classification and location of the soils used

SOIL PROPERTIES	UNITS	SOIL		
		URRBRAE	McLAREN	CLAREMONT
Depth	cm	15-40	15-40	15-40
pH (1:5 soil water solution)		6.7	7.3	8.3
EC (1:5 soil water solution)	dS/m	0.061	0.139	1.03
CROSS (Cation ratio of structural stability)	$mol^{05}\ m^{-1.5}$	0.24	0.59	0.32
Total carbon	%	1.49	0.76	4
Organic carbon		1.4	0.3	2.2
CEC_{eff}	$cmol\ kg^{-1}$	7-9	9-11	30-33
Zeta potential	mV	-38	-42	-19
Major clay minerals		30% illite	15% illite	Smectite reach illite-smectite
Taxonomic class Australian Soil Classification (Isbell, 2002)		Red Chromosol	Red-Brown Earth	Vertisol
Texture		Sandy loam	Clay -loam	Clay
Clay content (Gee and Bauder, 1986)	%	40	45	60
Location in South Australia		34°58'00.41" S 138°38'03.29" E Elevation: 109 m Waite Research Institute	35°15'04.31" S 138°33'44.9" E Elevation 89 m McLaren Vale vineyard	34°58'20.7" S 138°38'11.87" E Elevation 137 m Waite Research Institute

The soils were treated with different solutions containing varying amounts of potassium. The levels of Na, Can and Mg were kept constant. After equilibration, the soils were washed free of electrolytes and then SAR and CROSS were estimated in soil:water (1:5) extracts (Table 2.2).

Table 2.2 Cations and CROSS values of the treatment solutions and SAR and CROSS values after treatment

TREATMENT	CATIONS IN TREATMENT SOLUTIONS ($mmol_c/L$)				TCC (TOTAL CATION CONCENTRATION) ($mmol_c/L$)	SAR	CROSS
	K	Na	Ca	Mg			
CROSS 6	20	3.2	5.4	10	38.6	1.2	6.0
CROSS 8	30	3.2	5.4	10	48.6	1.2	8.4
CROSS 11	42	3.2	5.4	10	60.6	1.2	11.2
CROSS 15	60	3.2	5.4	10	78.6	1.2	15.4

Soil columns were prepared by packing approximately 100 cm^3 of soil into plastic cylinders (5 cm x 10 cm) to a bulk density of 1.5 mg/m^3. Initially the columns were wetted with the treatment solutions to saturation for 24 hours from the base by slow capillary rise and then the flow direction was reversed to continue the percolation from the top of the soil with a constant head of 4 cm (Klute and Diksen, 1986). Both column ends were fitted with nylon mesh screens with double disk of gauze mesh on the top of the soil to reduce surface disturbance. The columns were percolated with three wetting, draining and drying cycles using four CROSS treatment solutions for each soil. For each cycle, 1 L of one of the CROSS treatment solutions (Table 2.2) was percolated using inverted 1 L flasks mounted on the stand and then allowed to drain and dry for 1 week. Saturated hydraulic conductivity measurements were taken at the end of the last cycle with the deionised water passing through the columns to simulate the infiltration of the soil with rain water.

After hydraulic conductivity measurements, the soils from the first replicate were removed from the columns, air dried, crushed and passed through a 2-mm sieve. The final soils were then analysed for spontaneous dispersion, Zeta potential on separated clays and other selected properties such as $EC_{1:5}$, $pH_{1:5}$, CROSS and exchangeable cation ratio (ECR).

2.1.3 RESULTS AND DISCUSSION

The degree of ionicity of a clay-cation bond determines the ease with which water molecules break the bond. Marchuk and Rengasamy (2011) derived the ionicity indices of various cations involved in clay bonding from their ionisation potentials and ionic charge. The ionicity indices of Na, K, Mg and Ca were 0.89, 0.86, 0.73 and 0.67, respectively. Their studies comprehensively suggest that the dispersive effect of K is 0.56 times that of Na. Therefore, in explaining the soil structural deterioration caused by the combination of monovalent cations, the factor Na+0.56 K should be taken into account. Experiments reported in this study deal with the changes in soil structural parameters associated with increasing levels of K when the concentrations of Na, Mg and Ca were kept constant in the treatments.

The effect of the increased K concentrations in treatment solutions on the final soil solution compositions, exchange complex, turbidity, Zeta potential for all three soils used in this study are shown in Table 2.3. The statistical analysis performed on all the regressions discussed is summarised in Table 2.4.

Table 2.3 Relative hydraulic conductivity (K_{sr}), pH, EC, CROSS, and PAR of the soil solution, and ECR, turbidity and Zeta potential measured on the final leached soils

Treatment	pH (1:5)	EC (1:5) dS/m	$CROSS_{ss}$	PAR mol05 m-1.5	ECR %	Turbidity NTU	Zeta potential mV	K_{sr}
Urrbrae								
CROSS 6	7	0.286	2.9	3.6	46.4	500	-40	0.89
CROSS 8	7.1	0.291	3.7	4.7	49.2	960	-46	1.00
CROSS 11	7.3	0.320	4.7	6.2	54.3	1200	-48	0.80
CROSS 15	7.4	0.340	6.5	9.3	60.5	1551	-50	0.15
McLaren								
CROSS 6	7.4	0.290	3	2.9	35.7	410	-41.2	0.94
CROSS 8	7.5	0.304	3.6	3.6	40	650	-46.7	1.00
CROSS 11	7.6	0.354	5.3	4.8	46.5	1000	-49.3	0.43
CROSS 15	7.7	0.366	6.2	5.3	52.4	1363	-51.7	0.15
Claremont								
CROSS 6	8.6	0.536	1.7	1.5	32	184.0	-24	1.00
CROSS 8	8.7	0.513	2	1.8	34.6	229.0	-26	0.68
CROSS 11	8.8	0.587	2.1	2	40.7	240.0	-27	0.40
CROSS 15	8.9	0.600	2.3	2.5	42.1	260.0	-31	0.04

Adsorption of cations under these conditions resulted in their high concentrations in the CROSS of the treatment solutions. The use of de-ionised water to measure K_s can result in leaching, thus, changing the cationic composition of the soil solution. Hence, composition of the final soil solution was determined and $CROSS_{ss}$ values were calculated. Although values of $CROSS_{ss}$ were >50% lower than $CROSS_{tr}$, both these parameters were highly related. A positive correlation between $CROSS_{ss}$ and ECR% with an R^2 value of 0.73 was also noted (Figure 2.2), when all soils are pooled together. Electrolyte concentration of soil solution determines SAR-ESP relation. For example, ESP is approximately equal to SAR measured in saturation extracts while in dilute extracts (e.g. 1:5 extracts) ESP is more than twice the SAR. Similarly, the relation between $CROSS_{ss}$ and ECR % will depend on the electrolyte concentration of the soil solution.

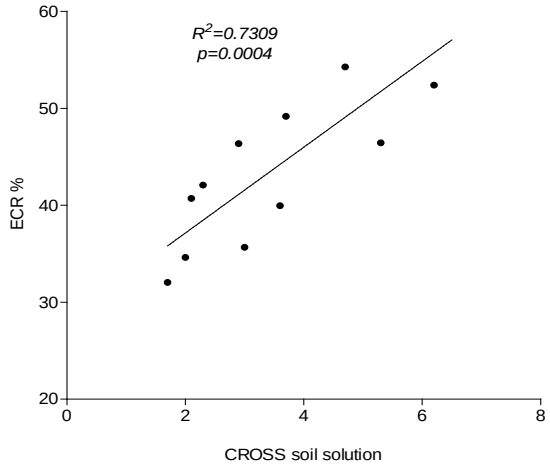


Figure 2.2 Relationships between the final soil cation ratio ($CROSS_{ss}$) and exchangeable cation ratio (ECR %) for all the three soils

Dispersible clay is considered as a measure of soil structural stability in water (Sumner, 1993; Rengasamy and Marchuk, 2011). The higher correlation between turbidity and $CROSS_{ss}$ (R^2 = 0.94) (Figure 2.3a) than the correlation between turbidity and PAR (Figure 2.3b) supports the use of $CROSS_{ss}$ instead of PAR in identifying soil structural deterioration in potassium dominated soils. The good relation between $CROSS_{ss}$ and ECR% validates the use of $CROSS_{ss}$ to represent exchangeable cations ratio and hence its effects on soil structure.

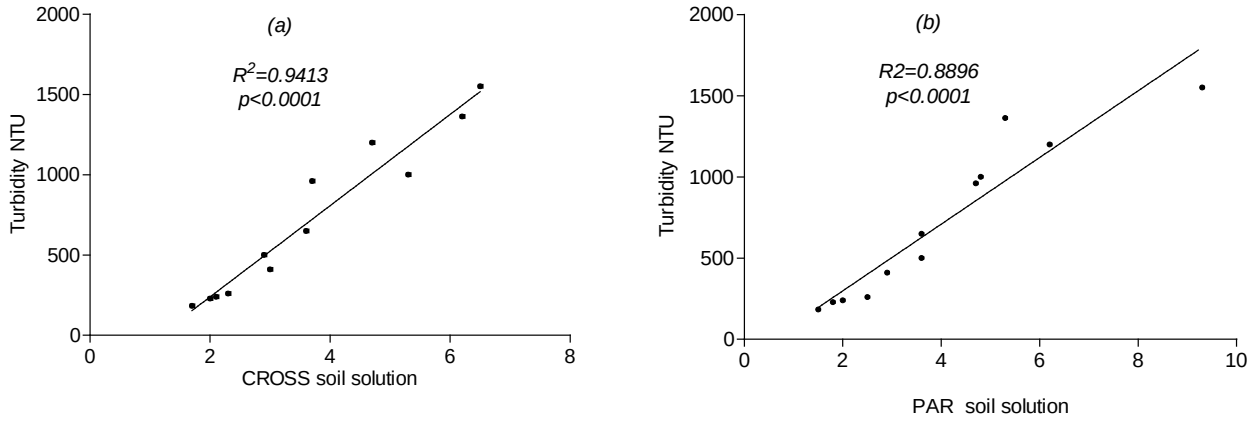


Figure 2.3 The relationships between CROSSss (a) and PARss (b) and the turbidity of final soil suspensions

Hydraulic conductivity in relation to $CROSS_{ss}$

Generally, as the concentration of K in soil solutions increases, the hydraulic conductivity decreases (Table 2.3). However, the correlations between $CROSS_{ss}$ and K_{sr}, and between ECR% and K_{sr} are significant only for individual soils (Figure 2.3a and 2.3b). The value of $CROSS_{ss}$ is low for each treatment because of the low EC of the final soils. Earlier reports (e.g. Rengasamy and Sumner 1998) have shown that the threshold electrolyte concentration for clay flocculation varies with soil types, particularly with clay content and composition. Therefore, as the measurements of hydraulic conductivity were done at different electrolyte concentrations for each soil (Table 2.3), the relationships between $CROSS_{ss}$ and K_{sr} highly depend on the soil type. Our future studies will focus on establishing the relationships between threshold electrolyte concentrations for flocculation and $CROSS_{ss}$ as influenced by soil type. Table 2.4 gives the summary of statistical results of linear regressions between $CROSS_{ss}$ and other parameters such as $CROSS_{tr}$, ECR%, turbidity and Zeta potential for the three individual soils.

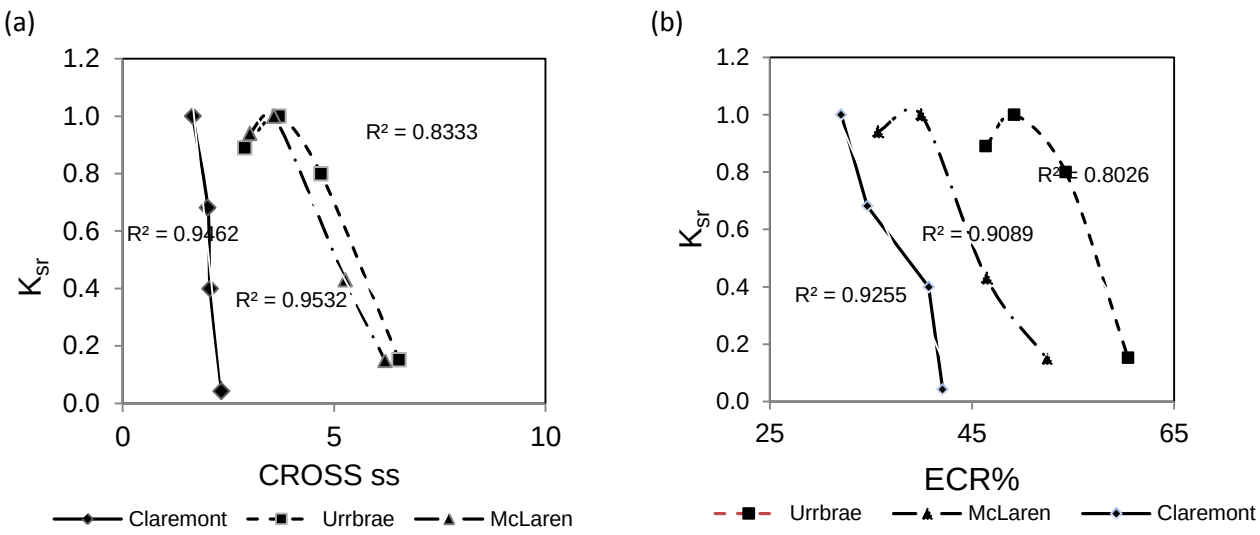


Figure 2.4 Relative hydraulic conductivity of each soil as a function of: $CROSS_{ss}$ (a) and ECR% (b)

Table 2.4 Correlations among CROSS, exchangeable cation ratio (ECR%), turbidity and Zeta potential

SOIL	X	Y	REGRESSION EQUATION	R^2
Urrbrae	$CROSS_{tr}$		2.517x-1.202	0.996
	ECR	CROSS ss	0.2499x-8.685	0.993
	Turbidity		0.00339x+0.873	0.932
	Zeta potential		0.3214x-10.34	0.800
McLaren	$CROSS_{tr}$		2.596x-1.746	0.965
	ECR	CROSS ss	0.2004x-4.216	0.986
	Turbidity		0.003528x+1.506	0.980
	Zeta potential		0.2946x-9.3695	0.853
Claremont	$CROSS_{tr}$		14.93x-20.24	0.909
	ECR	CROSS ss	0.04829x+0.2204	0.862
	Turbidity		0.007722x+0.2624	0.987
	Zeta potential		0.08077x-0.1558	0.904
SOIL	X	Y	REGRESSION EQUATION	R^2
Urrbrae	$CROSS_{tr}$		2.517x-1.202	0.996
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	Turbidity		0.007722x+0.2624	0.987
	Zeta potential		0.08077x-0.1558	0.904

2.1.4 CONCLUSIONS

Results obtained on hydraulic conductivity, turbidity and Zeta potential confirm that increasing levels of potassium in soil solutions affect soil structure. There is an indication that at low levels of potassium soil structure may not be affected. The good correlations obtained between CROSS measured in soil solutions ($CROSS_{ss}$) and the parameters such as turbidity and Zeta potential support its use instead of SAR or PAR in identifying soil structural stability influenced by cations. The present study also suggests the influence of soil type, especially clay content and mineralogy, in changing the effects of potassium.

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2.2 Long term consequences for soil structure in vineyards irrigated with recycled winery water over ten years

2.2.1 INTRODUCTION

Land based disposal is the main method of waste disposal currently used for many organic wastes. This is a major agricultural research priority in order to maintain the sustenance of soil productivity which is not only critical for production of food but also for the maintenance of environmental quality. The organic residues in winery waste can stimulate microbial activity. The use of winery waste water (WWW) containing high microbial activity for irrigation can result in reduced dependence on nitrogen, potassium and phosphorus-based fertilisers. In addition, repeated application of WWW can result in detrimental long-term effects on soil structure and potentially on soil dwelling organisms.

The main aim of wastewater irrigation by the wine industry is to dispose of WWW (Chapman *et al*., 2001). Rapid disposal would reduce storage time and prevent malodours. In response to the large amount of WWW produced, especially over vintage, this pressure can lead to over-irrigation. Over-irrigation can result in environmental impacts such as groundwater contamination through leaching of excess salts, nutrients and organics.

Irrigation could also cause waterlogging. This can result in filling large soil pores making the soil anoxic, which in turn can affect the developing plant roots and soil dwelling organisms. High BOD (biochemical oxygen demand) of wastewater rapidly decreases the oxygen supply in the soil resulting in anaerobic biochemical pathways leading to malodours and inefficient removal of organic contaminants from the soil.

Accumulation of salts in soils due to WWW application can reduce plant growth and productivity. High SAR or CROSS in WWW can adversely affect the soil structure. It is very important to maintain the diversity of soil micro-organisms as they help vines and other crops to obtain nutrients. A healthy soil microbe population is also essential for preventing plant diseases and pests from gaining an advantage in the field.

Chapman and co-workers (1995) extensively studied the removal of soluble carbon from synthetic WWW by its repeated application to soil in the laboratory. The main aim of this study was to assess the changes/impacts due to long-term application of WWW at selected field sites in South Australia. The soil monitoring program was designed to ensure that land treatment of WWW does not lead to adverse structure-loss, salinisation, waterlogging, or chemical contamination of the soil matrix. The soil structural aspects are discussed in this section.

As WWW generally has high levels of potassium and sodium there is a potential for the wastewater to harm the soil. Traditionally, application of K to soils has been perceived as a benefit due to a large body of published research demonstrating the positive effects of K on soil fertility and crop yield (Arienzo et al. 2008; 2012). However, K can cause clay swelling and dispersion and can increase soil salt levels, and hence degrade soil quality and land productivity (Rengasamy and Marchuk, 2011). Thus, elevated K concentrations in wastewater could limit its disposal onto land.

There are no field data cited in the literature that could be used to ascertain whether the application of WWW to land is affecting soil structure. Information is required to ascertain the long-term effect of disposal of WWW on soil structure. The aim of this study was to assess the long-term impacts of WWW irrigation at vineyards, pastures and woodlots.

2.2.2 METHODOLOGY

A number of companies in the wine industry have used various forms of recycled water, including WWW, to supplement their vineyard irrigation requirements. These companies also actively monitor soil parameters from many vineyards and to a lesser extent other irrigation/disposal areas. These data could be used to investigate a possible relationship between variation in irrigation water quality from various

sources and changes in soil properties. Current data from four participating wineries were collated and analysed as case studies.

The data were collected from different sources and the monitoring was done before the current project. Hence, the concept of CROSS and its relation to clay dispersion were not included. However, the trends in changes in exchangeable cations due to wastewater irrigation were identified.

The project team had good industry contacts in all South Australian wine regions. The data received by the team have limited statistical value for the following reasons:

1. Generally, irrigation water has been applied to complete vineyard blocks, so there is no effective field replication.
2. There was no effective reference site against which to compare the treated site.
3. Soil sampling protocols were not uniform.
4. It was difficult to quantify the volume and quality of irrigation water applied.

2.2.3 RESULTS

The results show the trends in soil chemistry with wastewater irrigation. The variation with seasons is due to the changes in rainfall and the quantity of water applied. The changes observed in vineyards compared to background information where available are described below.

The available C increased between 2002 and 2005, but the levels decreased after 2005 both in top soil and subsoil layers in irrigated soils. The EC levels fluctuated through the years (2002-2012), but the values were slightly higher than the background. The pH values of soils in the background data remained constant (pH 8.0) through the years. However in irrigated soils, the pH values fluctuated. After 2006, pH values increased from 8.4 to 8.6, suggesting accumulation of bicarbonate and carbonate ions (Figure 2.7). The SAR values fluctuated in both background and vineyards. During 2010-2012, SAR values in irrigated vineyards increased slightly compared to background. However, SAR values were comparatively lower in these years than in 2004 (Figure 2.5). There is an increasing trend in potassium levels in irrigated vineyards. The maximum level of K in background was 16,000 mg/kg. But in irrigated vineyards, the maximum level reached 35,000 mg/kg (Figure 2.6).

The observed increasing trend in K levels was a major concern. The current project therefore investigated the effects of K on soil structure.

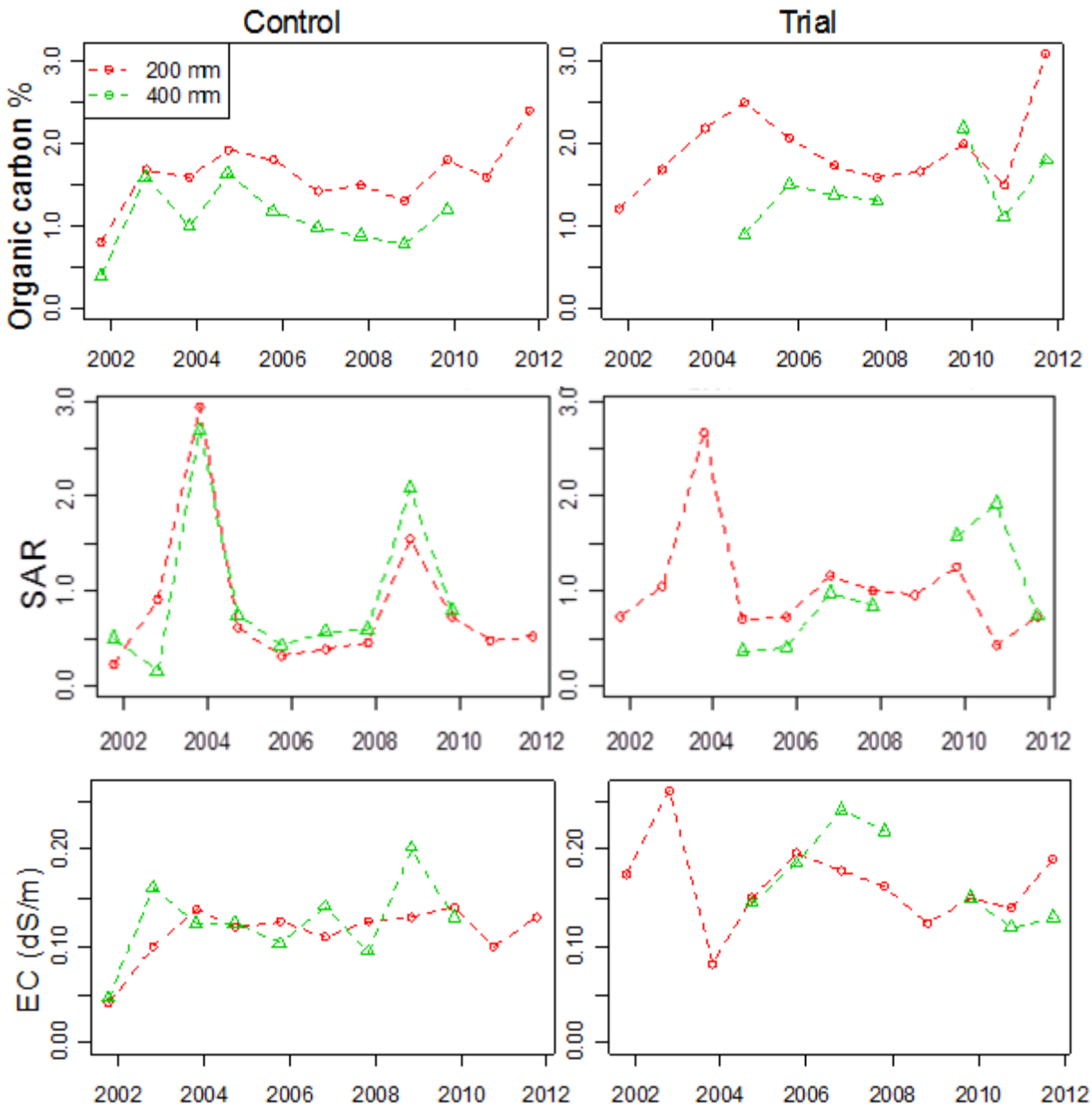


Figure 2.5 Changes in soil organic carbon, Electrical conductivity (EC) and SAR (sodium adsorption ratio) in the terra rossa control and winery wastewater irrigated vineyards

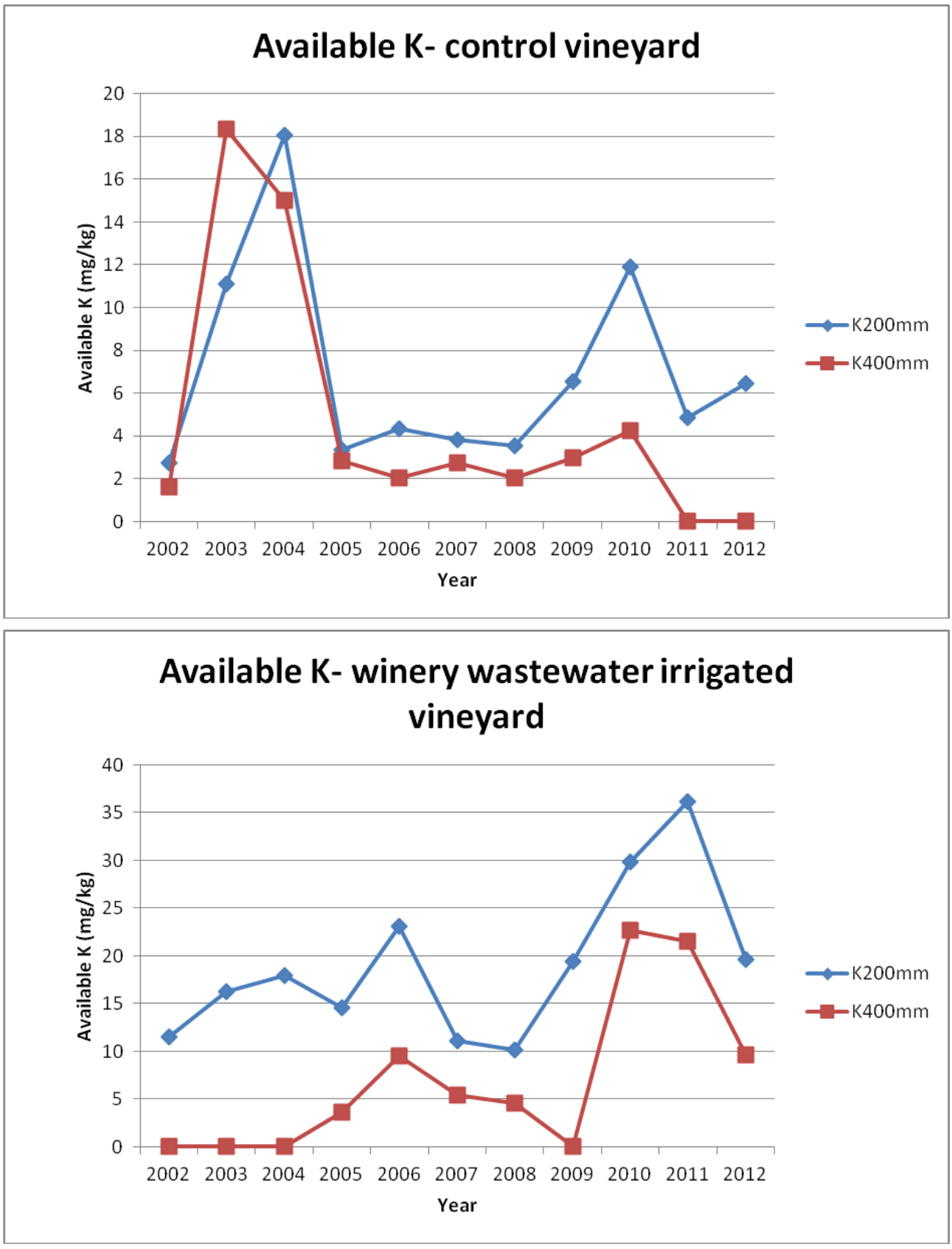


Figure 2.6 Changes in available potassium in the terra rossa control and winery wastewater irrigated vineyards.

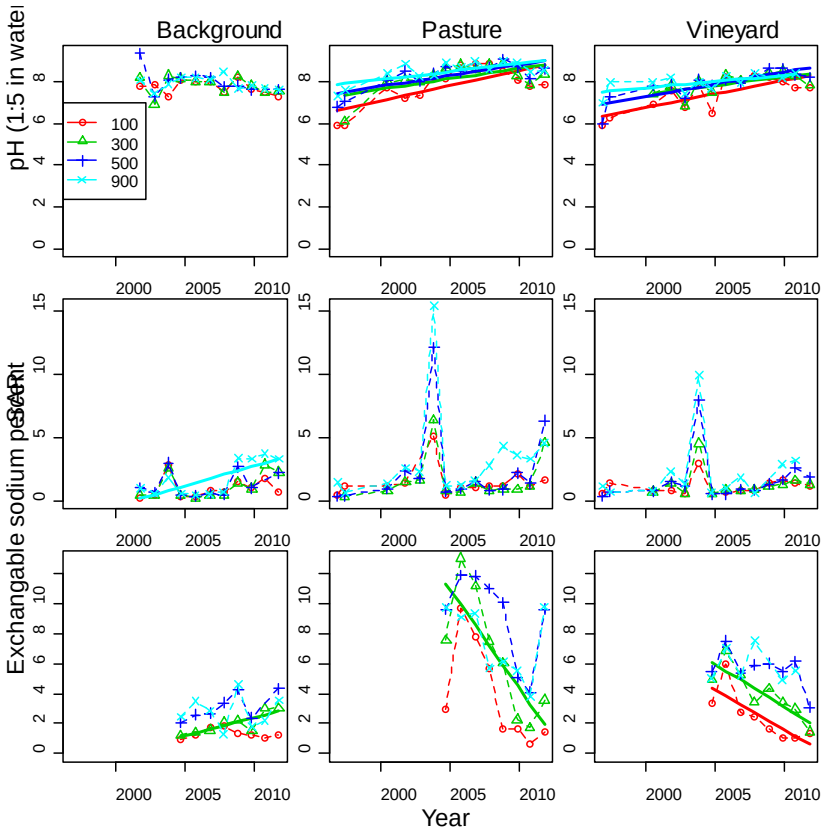


Figure 2.7 Changes in pH, SAR and ESP across time on the chromosol vineyard

For the second case-study, the data were available on adjacent areas that were irrigated vines (Riesling), irrigated pasture or an unirrigated (background) area. It was found that pH was significantly increased at all depths at both the pasture and the vineyard sites receiving wastewater. The ESP level was found to decrease significantly in both the pasture and vineyard sites, particularly at the depth of 300 mm, whereas the ESP in the background site increased at this depth. The initial ESP was very low in the background, intermediate in the vineyard and highest in the pasture (Figure 2.7).

Some of the case studies present parallel comparisons of monitoring data for impacted and control sites. Other data are a time series of chemical analyses of soil properties. There were some challenging sampling issues because of variability in size, land use, period of wastewater application and soil characteristics of the sites.

Several trends emerged from the historical data analyses and soil surveys conducted

- Increased TOC(total organic carbon) in the soil that had been irrigated with the wastewater
- Increased potassium levels in soil
- Lowering of soil pH
- Decreased levels of exchangeable sodium in the soil and possible grape juice.

The case studies discussed in this report considered only the effects of treated WWW. Treatment of wastewater is expensive, especially for small operations. One option is to rapidly dispose of the wastewater after filtration and pH adjustment. This option has several attractions, including increasing soil organic carbon, savings in energy and potentially avoiding the need for some capital expenditure. Data on the long-term effect of rapid disposal of WWW are lacking. This is an area that requires additional research. The potential effect on wine quality of irrigating vines with reclaimed wastewater is of great importance to the wine industry. Further data need to be collected and examined to assess any impact on the chemistry and other wine characteristics.

2.2.4 REFERENCES

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2.3 Monitoring of soil properties related to soil structural effects due to wastewater irrigation in a pasture and woodlot sites

2.3.1 SOILS AND METHODOLOGY

Wirra Wirra winery, McLaren Vale is one of many wineries in the Southern Vales region of South Australia which has looked at ways to utilise the wastewater from the production of wine. From 2005 Wirra Wirra started to adopt a new 'Environment Management Plan' for its vineyards. Their land management plan from 2010 has included extensive tree planting of native species and removal of exotic species. In line with their new management schemes, the approach to utilise winery waste also has changed. This included the creating of a composting area for marc and stalks and the reduction of sludge/lees by 70% from untreated wastewater.

Up until the 2010 vintage wastewater was applied to both selected vineyard blocks and a woodlot area. From 2010, the winery waste has been directed to an irrigated pasture block (1.9 Ha) and a woodlot area (0.5 Ha - see site map). Located just south of the irrigated pasture is the storage dam for the wastewater. For the area east of the dam (about 0.8 Ha), there has been no application of winery waste. In the area west of the dam (about 0.85 Ha), wastewater was applied during the 2010 and 2011 vintages, but not since.

As part of this current GWRDC project, we have been monitoring the sites at Wirra Wirra by looking at soil and plant data. Soils were sampled along five transects at a depth of 0-20 cm from the Woodlot area. Soil samples at depths of 0-20, 20-40 and 40-60 cm have been taken along two transects, running east to west, at three sampling points for each transect. In addition, soil samples were taken from either side of the wastewater storage dam at depths of 0-20 and 20-40 cm. Sampling times were in November 2011, July 2012, November 2012 and July 2013.

Table 2.5 Typical winery wastewater characteristics

PARAMETER	RANGE IN WINERY WASTEWATER
pH	[a]4-10
EC (dS/m)	[a]1.5-3.5
BOD (mg/L)	[b]1000 – 8000
SAR	[b]4-9
Sodium (mg/L)	[a]250-328
Potassium (mg/L)	[b]40-340
Ca (mg/L)	[b]13-45
Mg (mg/L)	[b]6-50

Source: [[a]Chapman et al. (2001). [b]ANZECC and ARMCANZ (1998)]

2.3.2 RESULTS AND DISCUSSION

Table 2.6 Changes in soil properties of a pasture irrigated with winery wastewater

NOV 2011	pH MEAN ± STD	EC MEAN ± STD	CROSS MEAN ± STD	SAR MEAN ± STD	ECR MEAN ± STD
Control 0-20	7.43 ± 0.72	0.09 ± 0.04	1.58 ± 1.10	0.65 ± 0.47	16.66 ± 8.15
Control 20-40	-	-	-	-	-
Control 40-60	-	-	-	-	-
Irrigated Pasture 0-20	7.76 ± 0.25	0.13 ± 0.04	2.47 ± 0.57	1.10 ± 0.41	18.22 ± 3.85
Irrigated Pasture 20-40	7.57 ± 0.22	0.09 ± 0.02	2.04 ± 0.42	1.13 ± 0.29	19.31 ± 2.56
Irrigated Pasture 40-60	7.67 ± 0.08	0.09 ± 0.01	1.68 ± 0.41	0.96 ± 0.30	16.35 ± 3.06
JULY 2012	**pH MEAN ± STD**	**EC MEAN ± STD**	**CROSS MEAN ± STD**	**SAR MEAN ± STD**	**ECR MEAN ± STD**
Control 0-20	6.85 ± 0.21	0.08 ± 0.01	1.46 ± 0.28	0.41 ± 0.12	15.50 ± 2.55
Control 20-40	6.90 ± 0.28	0.07 ± 0.03	1.23 ± 0.52	0.48 ± 0.08	17.85 ± 9.12
Control 40-60	6.95 ± 0.49	0.07 ± 0.02	1.23 ± 0.33	0.57 ± 0.00	15.45 ± 8.70
Irrigated Pasture 0-20	7.32 ± 0.22	0.07 ± 0.01	2.09 ± 0.95	1.07 ± 0.82	14.84 ± 2.11
Irrigated Pasture 20-40	7.37 ± 0.16	0.06 ± 0.01	2.03 ± 0.77	1.21 ± 0.60	15.87 ± 2.42
Irrigated Pasture 40-60	7.33 ± 0.23	0.06 ± 0.01	1.41 ± 0.21	0.82 ± 0.17	13.83 ± 1.47
NOV 2012	**pH MEAN ± STD**	**EC MEAN ± STD**	**CROSS MEAN ± STD**	**SAR MEAN ± STD**	**ECR MEAN ± STD**
Control 0-20	7.40 ± 0.27	0.09 ± 0.01	2.40 ± 0.84	0.63 ± 0.15	57.98 ± 38.54
Control 20-40	7.30 ± 0.59	0.06 ± 0.02	1.41 ± 0.62	0.56 ± 0.19	22.70 ± 11.73
Control 40-60	-	-	-	-	-
Irrigated Pasture 0-20	7.66 ± 0.30	0.08 ± 0.01	1.76 ± 0.16	0.95 ± 0.11	25.36 ± 6.46
Irrigated Pasture 20-40	7.58 ± 0.27	0.06 ± 0.01	1.41 ± 0.29	0.85 ± 0.25	27.62 ± 9.05
Irrigated Pasture 40-60	7.58 ± 0.36	0.05 ± 0.01	2.42 ± 0.16	1.31 ± 0.07	23.98 ± 7.48
JULY 2013	**pH MEAN ± STD**	**EC MEAN ± STD**	**CROSS MEAN ± STD**	**SAR MEAN ± STD**	**ECR MEAN ± STD**
Control 0-20	7.20 ± 0.66	0.10 ± 0.03	1.60 ± 0.50	0.60 ± 0.26	22.80 ± 6.04
Control 20-40	7.30 ± 0.70	0.10 ± 0.02	1.10 ± 0.33	0.60 ± 0.23	20.70 ± 5.75
Control 40-60	7.30 ± 0.59	0.10 ± 0.01	0.90 ± 0.13	0.40 ± 0.10	19.70 ± 6.80
Irrigated Pasture 0-20	8.10 ± 0.24	0.10 ± 0.01	2.30 ± 0.47	0.90 ± 0.19	34.40 ± 9.42
Irrigated Pasture 20-40	7.80 ± 0.35	0.10 ± 0.01	1.50 ± 0.30	0.80 ± 0.10	29.00 ± 7.73
Irrigated Pasture 40-60	7.10 ± 0.12	0.10 ± 0.03	1.60 ± 0.25	1.00 ± 0.21	16.00 ± 3.66
Control 40-60	7.30 ± 0.59	0.10 ± 0.01	0.90 ± 0.13	0.40 ± 0.10	19.70 ± 6.80
Irrigated Pasture 0-20	8.10 ± 0.24	0.10 ± 0.01	2.30 ± 0.47	0.90 ± 0.19	34.40 ± 9.42
Irrigated Pasture 20-40	7.80 ± 0.35	0.10 ± 0.01	1.50 ± 0.30	0.80 ± 0.10	29.00 ± 7.73
Irrigated Pasture 40-60	7.10 ± 0.12	0.10 ± 0.03	1.60 ± 0.25	1.00 ± 0.21	16.00 ± 3.66

Table 2.7 Changes in soil properties of a pasture irrigated with winery wastewater

NOV 2011	Na MEAN ± STD	K MEAN ± STD	ORG. C MEAN ± STD	TURBIDITY MEAN ± STD
Control 0-20	0.30 ± 0.13	1.02 ± 0.51	1.39 ± 0.18	610.00 ± 367.70
Control 20-40	-	-	-	-
Control 40-60	-	-	-	-
Irrigated Pasture 0-20	0.38 ± 0.10	1.12 ± 0.34	1.72 ± 1.01	671.60 ± 166.60
Irrigated Pasture 20-40	0.41 ± 0.06	0.69 ± 0.15	0.67 ± 0.19	1633.60 ± 464.96
Irrigated Pasture 40-60	0.50 ± 0.07	0.87 ± 0.15	0.78 ± 0.19	1015.00 ± 96.29
JULY 2012	**Na MEAN ± STD**	**K MEAN ± STD**	**ORG. C MEAN ± STD**	**TURBIDITY MEAN ± STD**
Control 0-20	0.96 ± 0.20	0.15 ± 0.05	1.15 ± 0.21	1405.00 ± 388.91
Control 20-40	0.94 ± 0.23	0.26 ± 0.16	0.75 ± 0.21	1660.00 ± 961.67
Control 40-60	1.65 ± 0.35	0.36 ± 0.04	0.65 ± 0.07	1115.00 ± 134.35
Irrigated Pasture 0-20	1.15 ± 0.26	0.21 ± 0.03	1.08 ± 0.20	1598.00 ± 513.00
Irrigated Pasture 20-40	1.13 ± 0.93	0.32 ± 0.22	0.63 ± 0.15	1773.33 ± 423.07
Irrigated Pasture 40-60	1.78 ± 1.09	0.46 ± 0.21	0.45 ± 0.14	1869.17 ± 399.08
NOV 2012	**Na MEAN ± STD**	**K MEAN ± STD**	**ORG. C MEAN ± STD**	**TURBIDITY MEAN ± STD**
Control 0-20	0.19 ± 0.08	1.57 ± 0.76	-	1167.50 ± 559.84
Control 20-40	0.11 ± 0.01	0.68 ± 0.27	-	910.00 ± 621.02
Control 40-60	-	-	-	-
Irrigated Pasture 0-20	0.22 ± 0.05	0.79 ± 0.20	-	1050.00 ± 364.36
Irrigated Pasture 20-40	0.20 ± 0.03	0.61 ± 0.13	-	993.33 ± 492.94
Irrigated Pasture 40-60	0.25 ± 0.10	0.49 ± 0.11	-	1788.00 ± 66.48
JULY 2013	**Na MEAN ± STD**	**K MEAN ± STD**	**ORG. C MEAN ± STD**	**TURBIDITY MEAN ± STD**
Control 0-20	0.10 ± 0.02	1.00 ± 0.35	1.20 ± 0.30	4063.75 ± 1488.933
Control 20-40	0.40 ± 0.18	0.70 ± 0.36	0.60 ± 0.20	4957 ± 1023.705
Control 40-60	0.30 ± 0.12	0.80 ± 0.35	0.60 ± 0.17	6934.5 ± 1349.864
Irrigated Pasture 0-20	0.20 ± 0.09	1.50 ± 0.48	1.50 ± 0.39	4171.333 ± 1026.235
Irrigated Pasture 20-40	0.40 ± 0.20	1.10 ± 0.25	0.90 ± 0.25	5870 ± 3804.234
Irrigated Pasture 40-60	1.00 ± 0.27	1.20 ± 0.24	0.80 ± 0.10	6372 ± 2353.251

Table 2.8 Changes in soil properties of a woodlot irrigated with winery wastewater

DEC 2011	pH MEAN ± STD	EC MEAN ± STD	ORG. C MEAN ± STD	TURBIDITY MEAN ± STD	CROSS MEAN ± STD
Control Woodlot, Transect, 0-20	7.2 ± 0.15	0.5 ± 0.11	5.0 ± 0.33	395.0 ± 52.26	2.5 ± 0.15
WWW Irrigated Woodlot Old, Transect, 0-20	7.4± 0.20	0.3 ± 0.06	5.9 ± 0.91	354.7 ± 32.33	2.0 ± 0.39
WWW Irrigated Woodlot New, Transect, 0-20	8.0 ± 0.17	0.3 ± 0.05	4.5 ± 0.61	453.3 ± 90.18	2.1 ± 0.35
JULY 2012	**pH MEAN ± STD**	**EC MEAN ± STD**	**ORG. C MEAN ± STD**	**TURBIDITY MEAN ± STD**	**CROSS MEAN ± STD**
Control Woodlot, Transect, 0-20	7.3 ± 0.12	1.093 ± 1.28	5.1 ± 0.21	351 ± 2.31	6.27 ± 6.70
WWW Irrigated Woodlot Old, Transect, 0-20	7.5 ± 0.00	0.245 ± 0.02	5.1 ± 0.35	352 ± 3.54	2.54 ± 0.10
WWW Irrigated Woodlot New, Transect, 0-20	8.0 ± 0.07	0.233 ± 0.07	3.8 ± 1.13	420 ± 56.57	2.42 ± 0.37
DEC 2012	**pH MEAN ± STD**	**EC MEAN ± STD**	**ORG. C MEAN ± STD**	**TURBIDITY MEAN ± STD**	**CROSS MEAN ± STD**
Control Woodlot, Transect, 0-20	7.5 ± 0.73	0.4 ± 0.21			4.4 ± 0.53
WWW Irrigated Woodlot Old, Transect, 0-20	7.3 ± 0.11	0.2 ± 0.08		886.7 ± 25.17	1.8 ± 0.09
WWW Irrigated Woodlot New, Transect, 0-20	7.8 ± 0.47	0.3 ± 0.19		826.7 ± 11.55	1.8 ± 0.12
JULY 2013	**pH MEAN ± STD**	**EC MEAN ± STD**	**ORG. C MEAN ± STD**	**TURBIDITY MEAN ± STD**	**CROSS MEAN ± STD**
Control Woodlot, Transect, 0-20	7.4 ± 0.13	0.2 ± 0.06	4.5 ± 0.42	6287.0 ± 1786.15	1.9 ± 0.08
WWW Irrigated Woodlot Old, Transect, 0-20	7.5 ± 0.03	0.3 ± 0.06	6.6 ± 0.99	5233.0 ± 2631.85	1.8 ± 0.14
WWW Irrigated Woodlot New, Transect, 0-20	8.0 ± 0.06	0.2 ± 0.01	3.7 ± 0.65	5229.0 ± 547.30	1.6 ± 0.09

In pasture sites, generally soil pH values slightly increased (from 7.0 to 7.6) at all depths between the 2011 and 2012 sampling. In July 2013, pH increased to 8.1 in the top soil layer (0-20 cm). However EC values decreased with time. Both CROSS values (including Na and K) and SAR values (includes only Na) significantly increased, particularly in top soil layers, suggesting the contribution of both Na and K in the wastewater used. Increasing levels of Na and K increased the clay dispersion (measured as turbidity), and hence affected the soil structure. When compared with the control, a decreasing trend in soil organic carbon due to irrigation was found.

In Woodlot sites, pH increased slightly up to 8.0, while EC values showed a decreasing trend in irrigated soils. Both SAR and CROSS values increased significantly in all soil layers during 2011 to 2013. As for the pasture sites, CROSS and SAR values showed an increasing trend. Particularly, K values were increasing more than Na values. The increase in clay dispersion (turbidity) was consistent with increasing K or CROSS. However, except in top soils at two sampling intervals (CROSS = 6.27 or 4.4), generally CROSS values have not reached the critical level of 3.0. Organic carbon values were higher in irrigated Woodlots compared to pasture sites.

As CROSS and K values show increasing trends, it is necessary to monitor soil chemistry frequently so that proper amendments can be applied to reduce the effects on soil structure.

2.3.4 REFERENCES

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2.4 Column experiments using gypsum

We completed the laboratory experiments using a winery soil (sandy loam). In these experiments we used the newly developed concept of CROSS (Rengasamy and Marchuk, 2011) instead of SAR. Use of CROSS takes into account the effects of both K and Na on soil structural deterioration. Further, the difference between the flocculating effects of Ca and Mg is also considered. Our results show that CROSS is highly correlated with clay dispersion from soils containing significant amounts of both K and Na. In order to quantify the effects of electrolytes (salts) on the influence of CROSS, we previously derived the 'threshold electrolyte concentration (TEC)' in relation to CROSS (Marchuk and Rengasamy 2012). If soil contains salts below TEC for a given CROSS, the soil will disperse with adverse effects on soil structure. The unit for TEC is dS/m and represents the electrical conductivity of the flocculated soil.

Dispersive soils having significant amounts of sodium and potassium can be improved by adding calcium and also organic matter. Usually calcium is added in the form of gypsum (calcium sulphate). Lime (calcium carbonate) can also be mixed with gypsum when the soil pH (water, 1:5) is low (less than 5.5), as lime will add calcium and also raise the soil pH to desired levels (pH 6-7). However, lime is not recommended for soils with a pH above 8, as it is almost insoluble under those conditions. The calcium amendments displace adsorbed sodium and potassium from the exchange complex improving the soil structural stability (Figure 2.8). This increases the moisture retention and hydraulic conductivity of soil layers leading to increased yields and profitability. Increased soil organic matter has a range of benefits including reduction of erosion, increased water holding capacity and improved biological and nutrient status of soils.

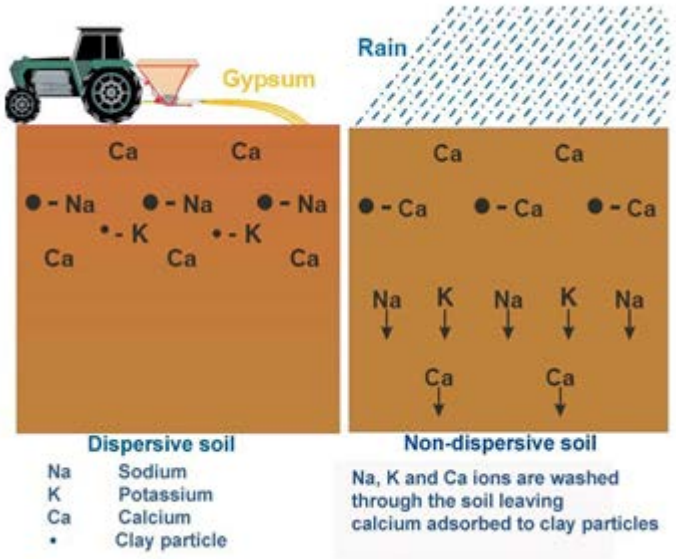


Figure 2.8 Mechanism of remediation of dispersive soils (containing Na and K) using gypsum

The aims of the column experiments were to determine:

1. How physico-chemical properties of a sandy loam soil change after leaching with wastewater
2. The effectiveness of gypsum application in reducing adsorbed Na and K and also in restoring soil structure
3. The relative effectiveness of application of gypsum as dry powder or as dissolved gypsum

2.4.1 EXPERIMENTAL DESIGN

A sandy loam soil of 500 g was packed in a plastic column (60 cm x 10 cm) to a bulk density of 1.3 g/cm^3. Three replicates of each treatment were used.

Wastewater treatments:

1. Wastewater obtained from the winery
2. Wastewater + 500 ppm of NaCl
3. Wastewater + 500 ppm of KCl

Gypsum treatments:

1. Dry gypsum spread on the surface soil in the column at 2.5 t/ha which equates to 7 g/column.
2. Dissolved gypsum leached through columns (10 mmol/L of Ca)

Table 2.9 Composition of waters used in the leaching of soil columns

NO.	TREATMENT	Ca	Mg	K	Na	SAR	CROSS	EC
1	WWW	1.77	0.57	6.41	1.81	1.68	5.26	0.87
2	WWW+Na	1.78	0.56	6.40	9.87	9.12	9.25	1.80
3	WWW+K	1.78	0.56	11.71	1.82	1.68	7.75	1.58

Note: [concentrations of cations are mmol$_c$/L; EC values in dS/m]

The changes in soil properties after leaching with treatment solutions are given in Table 2.10. From soil chemical analysis data, the following were calculated:

CROSS = (Na+ 0.56 K)/ [(Ca + 0.6 Mg)/2]$^{0.5}$ (cation concentrations in mmol charge/L)

SAR = Na / [(Ca + Mg)/2]$^{0.5}$ (cation concentrations in mmol charge/L)

EPP (exchangeable potassium percentage = 100 x (Exchangeable K / cation exchange capacity)

ESP (exchangeable sodium percentage) = 100 x (Exchangeable Na / cation exchange capacity)

ECR % (exchangeable cation ratio %) = 100 x (Exchangeable Na + K / cation exchange capacity)

Table 2.10 Soil properties after leaching the soil in columns

TREATMENT	SOIL SOLUTION (AFTER TREATMENT)		SOIL PROPERTIES (AFTER TREATMENT)		
	CROSS	SAR	EPP	ESP	ECR%
WWW	1.17	0.32	19.1	1.4	20.5
WWW + Na	1.90	0.80	17.7	6.7	25.4
WWW + K	2.20	0.45	23.5	1.5	26.3

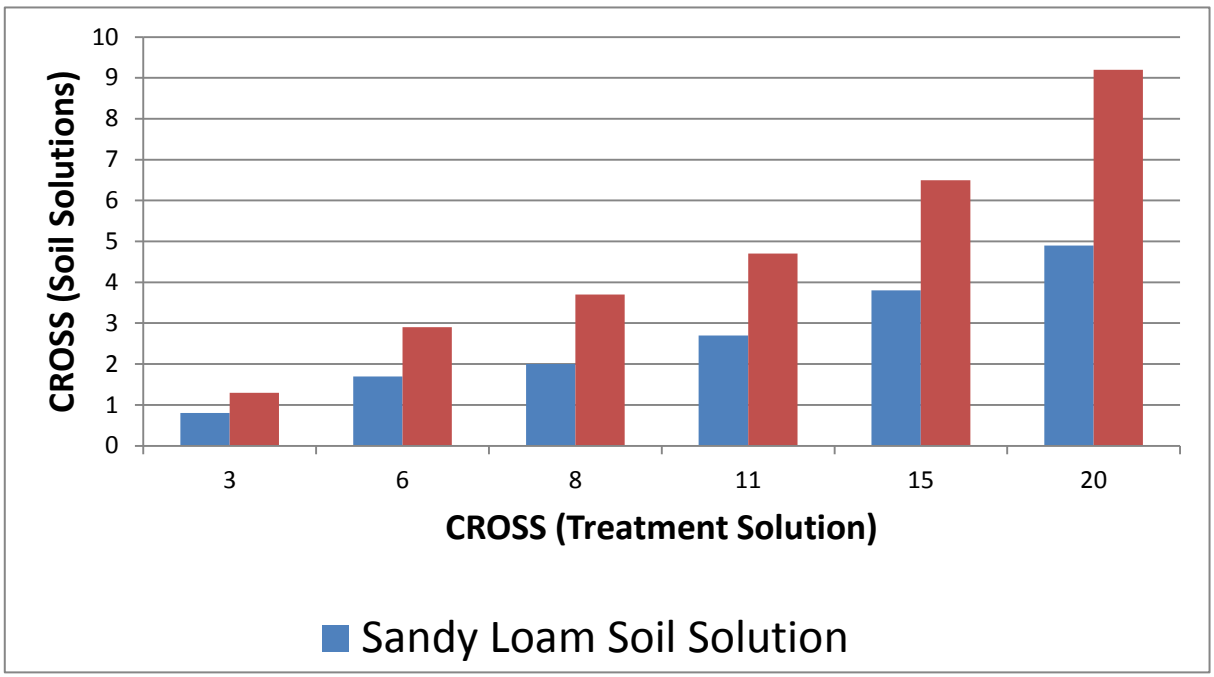


Figure 2.9 Adsorption of cations in relation to treatment solutions varies with clay content

2.4.2 RESULTS

The results showed that CROSS and SAR of soil solutions obtained after treatment were considerably lower than the values of treatment solutions (Table 2.11). Further lab studies showed that the clay content affects the adsorption of cations (Figure 2.9).This suggests that the chemical composition of soils irrigated with wastewater will not be equal to that of irrigation water and frequent soil tests are necessary. Increases in Na in the treatment water will reduce the adsorption of K (EPP, Table 2.12). Similarly, increases in K will reduce adsorption of Na (ESP). In spite of variations in Na and K, the exchangeable cation ratio (ECR %) (which includes the adsorption of both Na and K) and clay dispersion (Table 2.13) is related to CROSS of soil solution confirming our results published in Rengasamy and Marchuk (2011). Based on CT scans, pore connectivity and porosity were the highest in soluble gypsum treatments (Figures 2.10-2.12). This confirms that the application of gypsum as 'dissolved gypsum' is more efficient in reclamation than surface application of dry gypsum.

Table 2.11 Soil solution SAR and CROSS after treating with dry gypsum or dissolved gypsum

TREATMENT	CONTROL		DRY GYPSUM		DISSOLVED GYPSUM	
	SAR	CROSS	SAR	CROSS	SAR	CROSS
WWW	0.32	1.17	0.31	1.05	0.30	0.85
WWW + Na	0.80	2.9	0.45	1.48	0.30	1.00
WWW + K	0.45	2.60	0.35	1.40	0.30	0.90

Table 2.12 Soil exchangeable cations after treating with dry gypsum or dissolved gypsum

TREATMENT	CONTROL			DRY GYPSUM			DISSOLVED GYPSUM		
	ESP	EPP	ECR%	ESP	EPP	ECR%	ESP	EPP	ECR%
WWW	1.4	19.1	20.5	0.9	13.8	14.1	0.7	10.8	11.7
WWW + Na	6.7	17.7	24.4	1.0	12.7	13.7	0.9	10.7	12.7
WWW + K	1.2	23.5	25.3	1.0	13.4	13.5	0.8	11.2	11.8

Table 2.13 Clay dispersion in water in relation to CROSS in soils with different treatments and after washing free of salts

TREATMENT	CONTROL		DRY GYPSUM		DISSOLVED GYPSUM	
	CROSS	CLAY %	CROSS	CLAY %	CROSS	CLAY %
Wastewater	1.17	0.22	1.05	0.16	0.85	0.07
Wastewater + Na	2.90	0.96	1.48	0.28	1.00	0.10
Wastewater + K	2.20	0.72	1.40	0.25	0.90	0.08

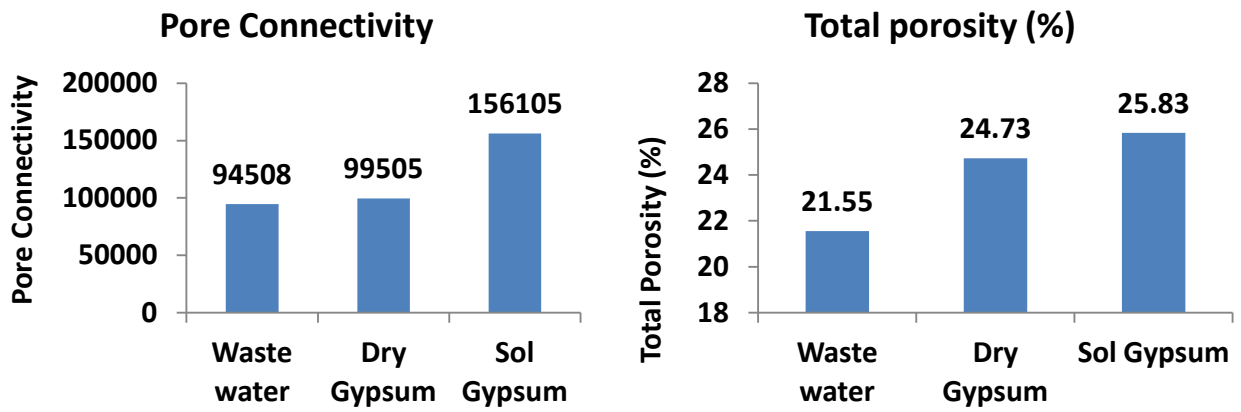


Figure 2.10 Soil pore architecture derived from CT-scans (Wastewater and gypsum)

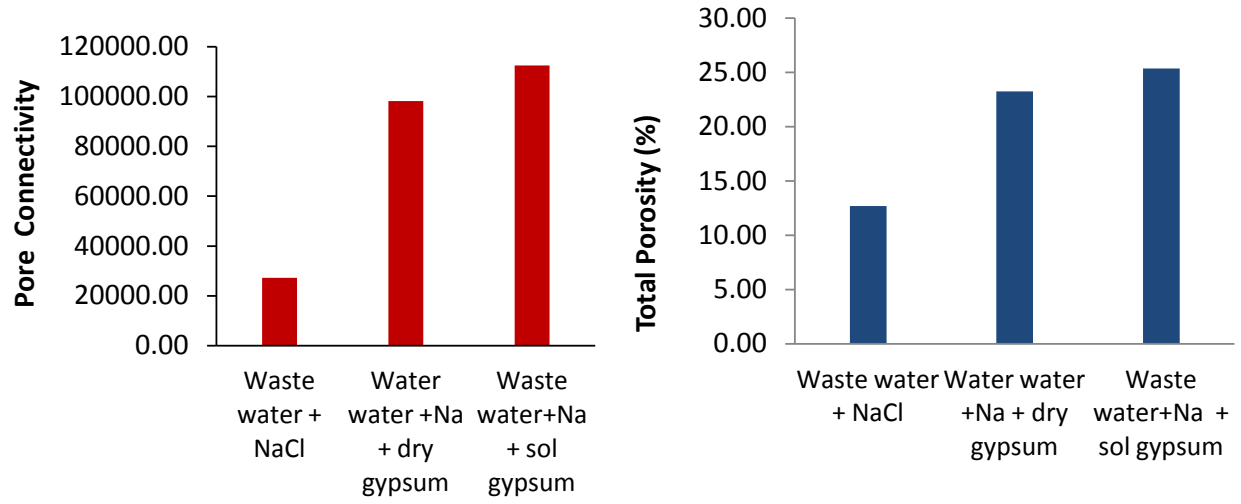


Figure 2.11 Soil pore architecture derived from CT-scans (Wastewater + Na and gypsum)

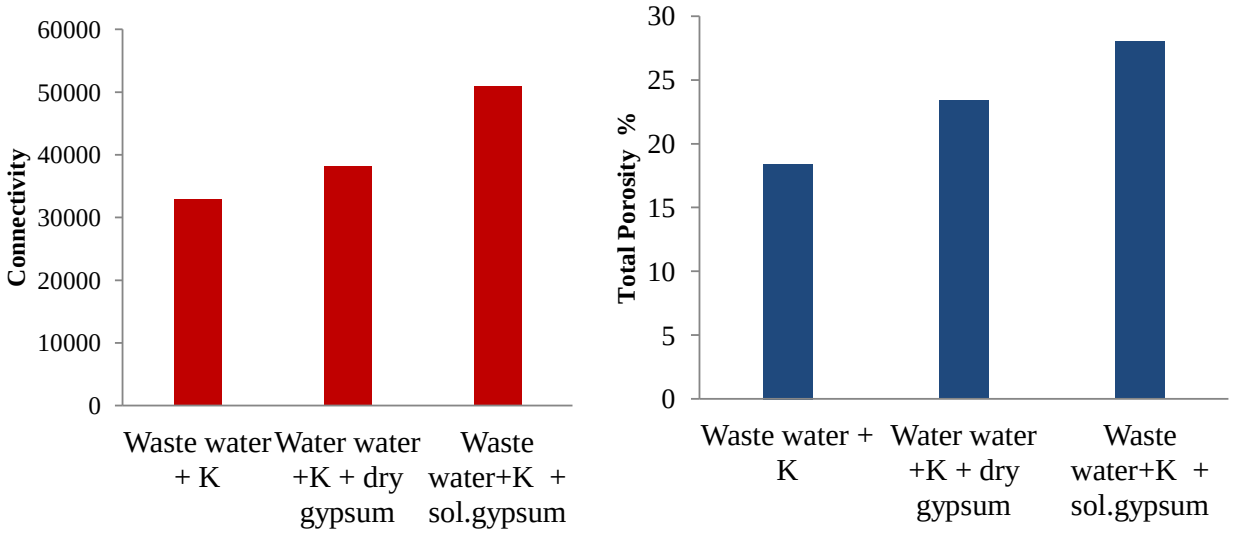


Figure 2.12 Soil pore architecture derived from CT-scans (Wastewater + K and gypsum)

2.4.3 CONCLUSIONS

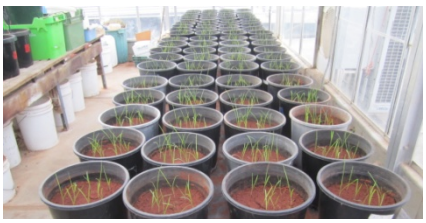
- Adsorption of K and Na in soils is proportional to the concentrations of these ions in the leaching (treatment) solutions. As Na and K increase in wastewater, soil ESP and EPP increase.
- The CROSS and SAR values of soil solutions (after treatment) are 3 to 4 times lower than the values of treatment solutions.
- The degree of adsorption of K and Na in soils from irrigation waters depend on the soil clay content.
- Gypsum application reduces clay dispersion and increases total porosity and pore connectivity, thereby, improving soil structure.
- Application of gypsum as 'dissolved gypsum' is more efficient in reclamation than surface application of dry gypsum.
- Measuring clay dispersion in wastewater irrigated soil will indicate soil structural degradation and can suggest the need for gypsum application

2.4.4 REFERENCES

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Part III Soil microbiological health

3 Soil microbiological health

3.1 Impact of recycled winery wastewater irrigation on soil microbiological health at field sites

3.1.1 INTRODUCTION

Wineries produce variable amounts of wastewater every year, ranging from 1.6–2.0 L/L wine (Mosse, 2010) depending on the winery, season or treatment process. The practice of using winery wastewater (WWW) for irrigation is an increasingly desirable choice for winemakers as it can be a valuable water resource for drought tolerant winegrowing regions, a cost effective disposal method, and a useful management practice for sustainability. A previous study has shown that the application of WWW can increase soil microbial activity and organic carbon in soil (Kumar et al, 2006). While increase in organic carbon can lead to increased soil fertility, it has also been shown to result in organic overloading which can block soil pores (Diacono et al. 2010). Therefore, the contaminants present in untreated or poorly treated WWW could potentially have an adverse impact on soil health and eventually, vine and grape quality or growth.

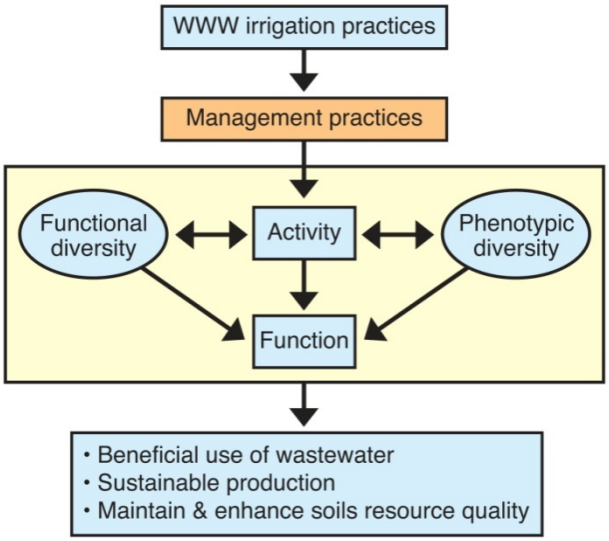


Figure 3.1 Role of microbes in maintaining soil health

Soil microbes are the most abundant and diverse group of organisms and play a vital role in maintaining the health of soils (Figure 3.1). In soil systems, they are primarily responsible for nutrient (N, S and C) and energy cycling and are crucial to the soil ecosystem health. It is therefore important that any management practices undertaken be done with minimal impact to the health of soil microbial communities. Soil microorganisms can be impacted by many environmental factors such as weather, plantation and land management practices. These factors can influence microbial population stability by affecting the resistance and resilience of the community (Mosse et al, 2011). Microorganisms are more sensitive than other higher life forms to chemical and physical changes in the environment. Therefore, their community structure and abundance can be used as an early detection system for soil disturbances. The impacts of WWW application on soil bacterial diversity have not been well documented. In this project, we implemented a high throughput, next generation sequencing (NGS) method to quantify and identify the key bacterial and archaeal phylum being affected by the application of WWW.

The NGS method has dramatically changed the field of microbial ecology research over the last 20 years. It allows for high throughput and in-depth investigation of environmental microbial community structure

using a small subunit ribosomal rRNA gene (16S) to assess the abundance and diversity of microbes in the environment. The 16S rRNA gene is universally present and highly conserved across bacterial species and used as a reliable marker to identify and classify the populations of soil microorganisms. With this method we are able to potentially detect early changes within the soil community that can possibly be detrimental to soil health and negatively impact wine quality.

3.1.2 METHODS

Site Information

To assess the impacts of WWW application on vineyard soil health from a microbiological perspective, we looked at three winery soils within South Australia and New South Wales. Site 1 consisted of irrigated WWW and non-irrigated (control) crops with a combination of vineyard, woodlot and pasture management practices and two different soil types: podzol and terra rossa (Table 3.1).

Table 3.1 Land use, irrigation and soil type information under use at winery site 1

LAND USE	IRRIGATION	SOIL TYPE
Vines	Bore	Terra rossa
		Podzol
	Winery wastewater	Terra rossa
		Podzol
Pasture	Winery wastewater	Podzol
Woodlot Eucalyptus	Winery wastewater	Podzol
Woodlot Pine	Winery wastewater	Podzol
	Rain	
Control	Rain	Terra rossa

The site 2 winery was a pasture site receiving wastewater over the last 15 years and was selected from the Griffith region. Site 3 was a woodlot site in South Australia, irrigated with winery wastewater over 20 years and was also selected for soil microbiological assessment.

Laboratory Methods

DNA extraction

Five replicate samples were collected from each site. The DNA was extracted from 0.25 g subsamples by the Australian Genome Research Facility (AGRF, Adelaide SA) using the PowerSoil DNA Isolation kit (MoBio). Quantification with PicoGreen (Life Technologies) was also performed by the AGRF and all DNA samples were diluted to 5 ng/µl for subsequent PCR analyses.

Pyrotag Sequencing

Amplicon pyrosequencing was performed on a 454 GS FLX Titanium system as outlined in Figure 3.2. Briefly, barcoded amplicons for multiplexing were prepared using the primers TX-9F (5'-GGATTAGAWACCCBGGTAGTC-3') and 1391R (5'-GACGGGCRGTGWGTRCA-3') extended with the respective A or B adapters, key sequence and multiplex identifiers (MID) as previously described by Dawson et al. (2012). Pyrotag PCR was performed in a Mastercycler gradient (Eppendorf) with the following cycling conditions: 20 cycles of denaturation (95°C, 10 s), annealing (55°C, 45 s) and elongation (70°C, 45 s). Each 25 µl PCR reaction contained 12.5 µL iProof HF mastermix (BioRad), 0.2 µm of each primer and 10 ng template DNA. The PCR products were cleaned up with ExoI and CIP (10U) and second round PCR was

performed as before but with 0.4 µM of each MID-primer (IDT DNA) instead of TX-9F. Amplicons were purified using Agencourt AMPure-XP beads (Beckman Coulter, Brea, CA) and pooled in equimolar ratios. Gel purification and sequencing were performed applying the GS FLX Titanium chemistry by Macrogen (Korea).

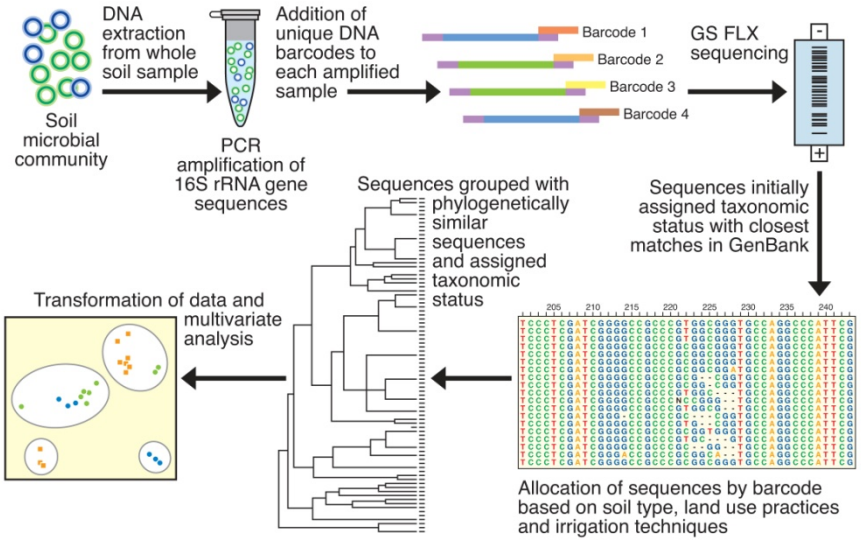


Figure 3.2 Steps involved in the amplicon pyrosequencing technique

3.1.3 RESULTS AND DISCUSSION

Winery wastewater application shifted the total bacterial abundance and structure in vineyard, pasture and woodlot soils (Figure 3.3) at winery site 1. Physicochemical data show that in control soils there is an effect of NH_4, whereas WWW irrigated soils are driven by EC and Na (Figures 3.4 and 3.5).

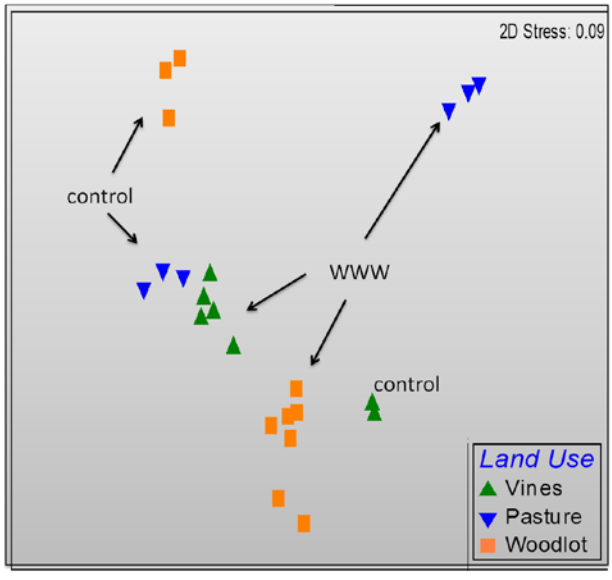


Figure 3.3 Non-metric dimensional scaling (nMDS) plot of bacterial communities analysed using 16S amplicon sequencing for winery site 1

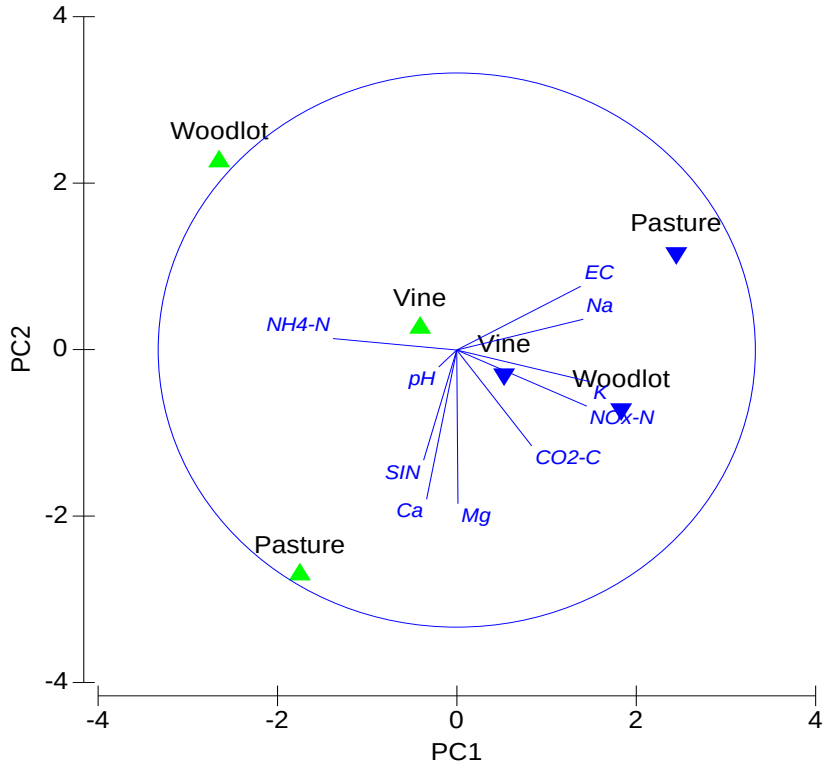


Figure 3.4 PCA plots of physicochemical properties at winery site 1

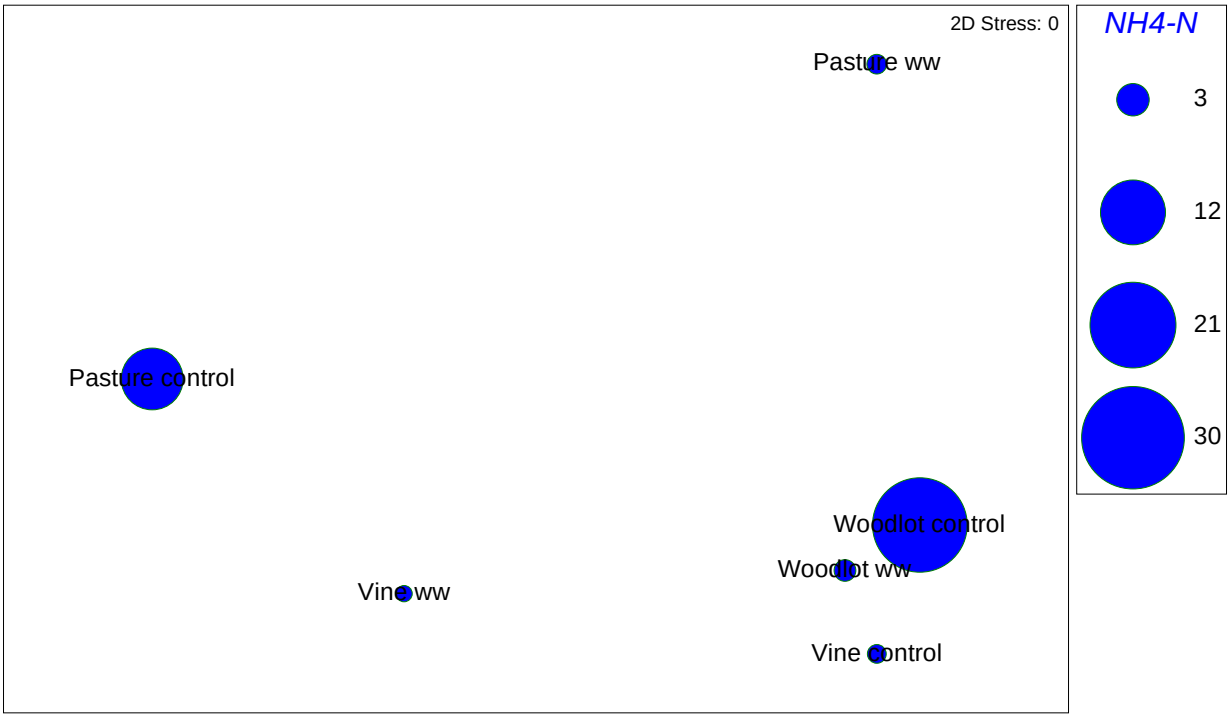


Figure 3.5 nMDS plots with bubble overlays showing difference in ammonia utilisation after 28 days of substrate addition, as determined by substrate induced nitrification for winery site 1. NH_4 (mg/kg) and Na (mg/L) account for the largest chemical differences between the samples

Pairwise analyses of similarity (ANOSIM) analysis showed that the primary drivers of the community shifts were the combination of irrigation and land use (R=0.831, p=0.001) and not soil type (Table 3.2).

Table 3.2 One and two-way crossed analyses of similarity (ANOSIM) tests comparing bacterial and fungal communities using amplicon pyrotag sequencing at winery site 1.

(A) GLOBAL TESTS		
		sequencing
Factor		16s
	R	P
Soil type	0.049	0.305
Crop type	0.426	0.001
Irrigation	0.203	0.056
(A) PAIRWISE TESTS		
Crop *vs.* irrigation	0.831	0.001
Soil *vs.* irrigation	0.162	0.157
Soil *vs.* crop	0.046	0.393

R>0.75 represents different groups
R>0.5 represents separated groups with some overlapping
R<0.25 represents no differences between groups (p = 0.05)

There was an overall trend for the diversity to increase with WWW application, as indicated by Shannon's diversity index. As shown in Figure 3.6, the effect WWW use on the microbial community in woodlot soils was minimal. Application of WWW on pasture soils showed an increase in actinobacteria and a decrease in firmicutes; and the application of WWW on vines showed an increase in firmicutes. A reduction in Crenarchaeota was observed after introducing WWW to both pasture and vines practices. Ammonia oxidation, the first step of nitrification is performed in soil systems by both ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA). The microbial species identified affiliated to Crenarchaeota were primarily AOA. It also counted as the second most abundant phyla present in the population at 26.1 %. Therefore a loss in this function could potentially have significant impacts on soil quality. The firmicutes that were affected by the WWW treatment were predominantly from the *Bacillus* genera. *Bacillus* is ubiquitous in nature and under adverse environmental conditions, such as a reduction in elements (N) many species will produce polyhydroxyalkanoates (PHAs) to store energy and carbon (Gomaa, 2014). An increase of *Bacillus* relative abundance could suggest a nutrient loss in soils, which could have undesirable effects on crop and/or grape production.

Actinobacteria were the most dominant bacterial phyla overall with a relative abundance of 26.5%. The species belonging to bacteria genus *Actinomycetes* and *Streptomyces* which are regarded as major contributors in biological buffering of soils and have roles in organic matter decomposition conducive to crop production, were largely affected by application of WWW. The bacteria classes Rubrobactor and Thermoleophilia species were also affected, however due to their ecological function being largely unknown, we could not conclude if these changes have effects on soil health.

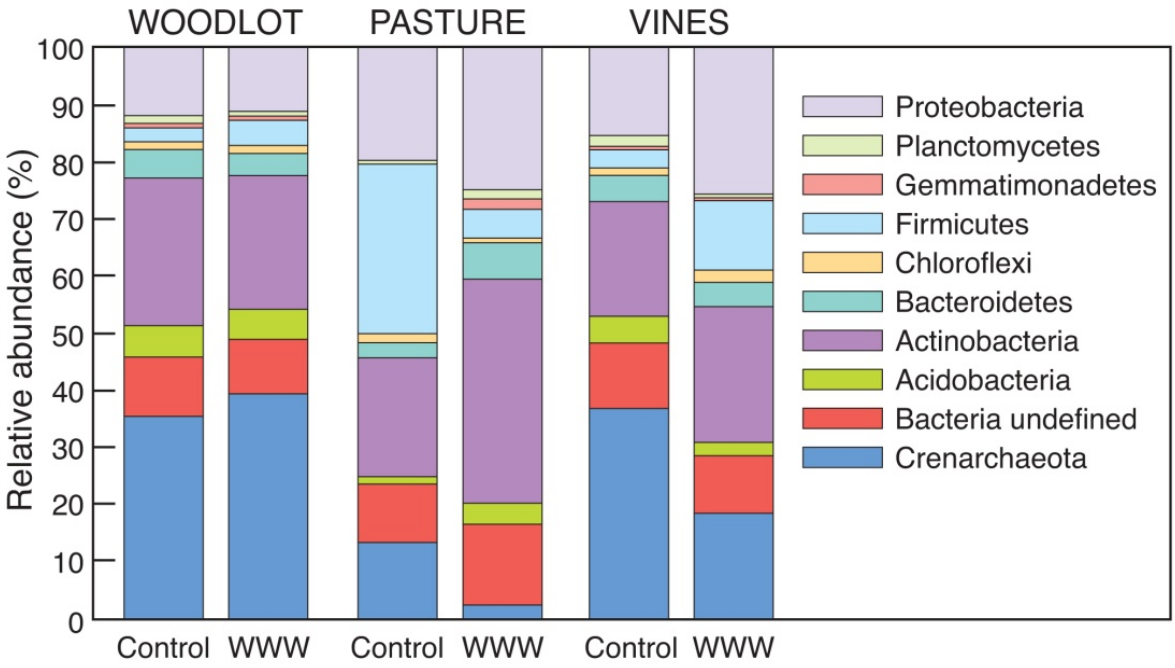


Figure 3.6 Percent contribution of dominant phyla within each land practice under winery wastewater irrigated *vs* control plots at winery site 1

There was no effect on the overall composition of the species at winery site 2 with a 20 year old irrigated woodlot (Figure 3.7). The reasons for this could be many ranging from the quality or amount of WWW used to soil conditions and climate providing more resilient microbial populations.

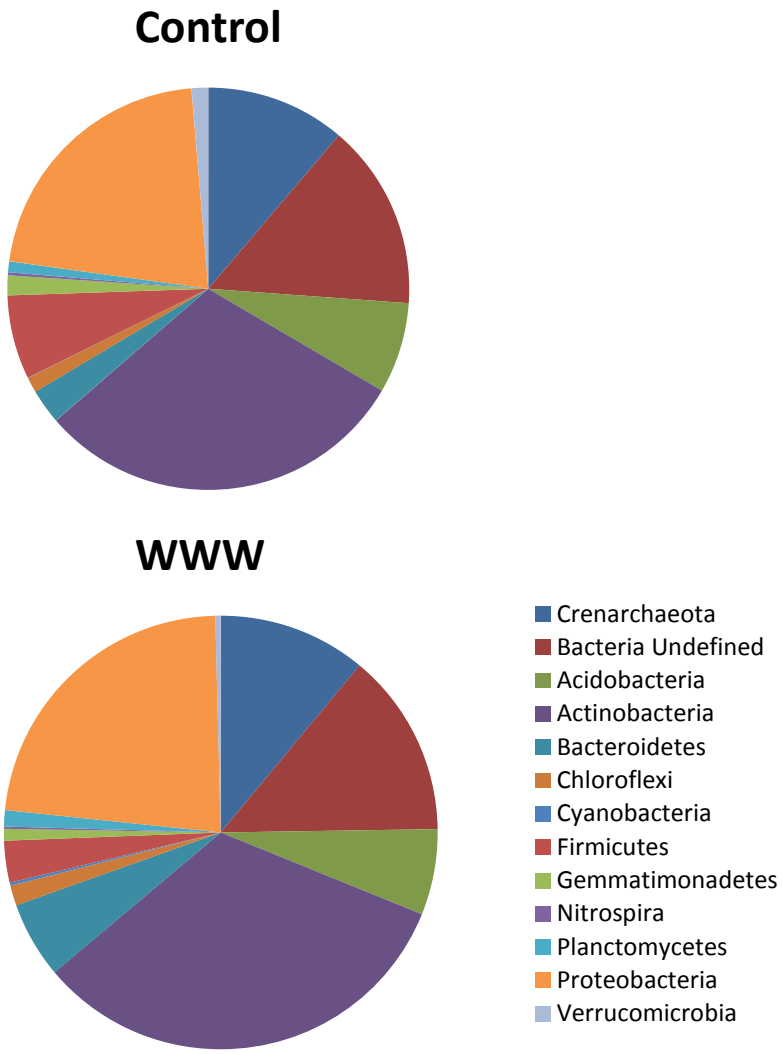


Figure 3.7 Percent contribution of dominant phyla under winery wastewater irrigated *vs* control plots at a woodlot site in South Australia

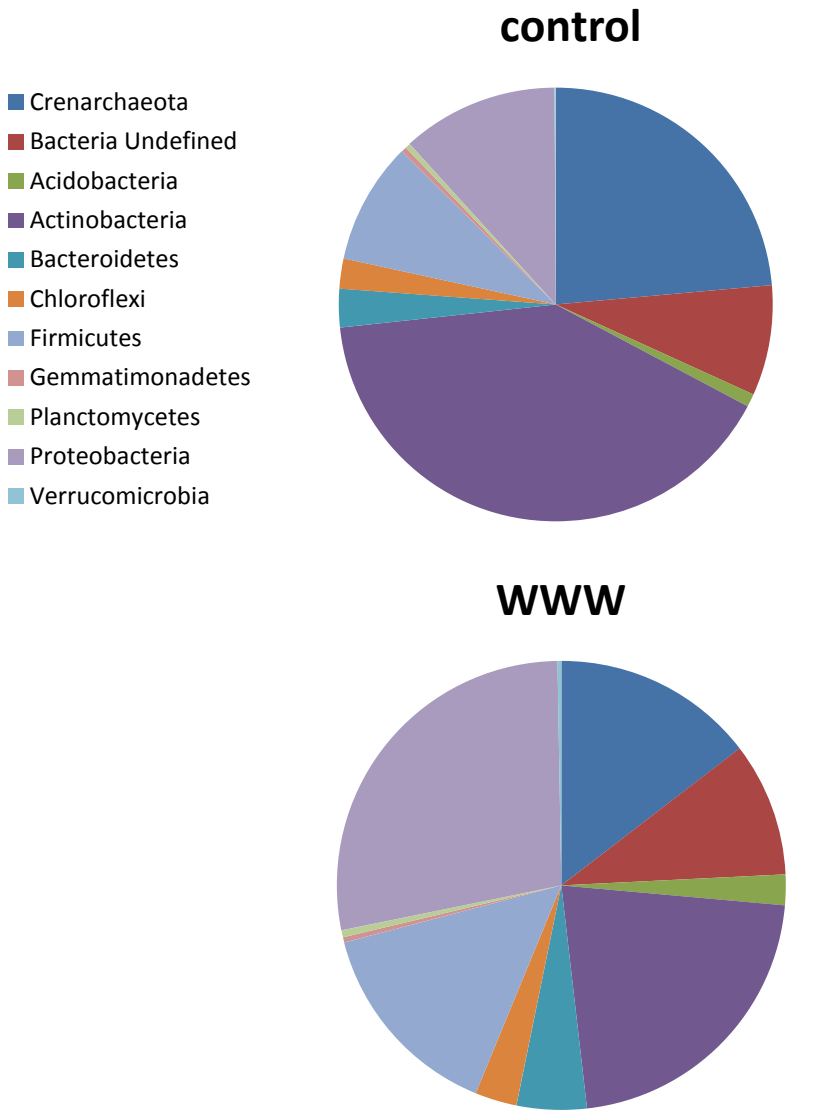


Figure 3.8 Percent contribution of dominant phyla under winery wastewater irrigated *vs* control plots at a pasture site in the Griffith region

Under pasture conditions in the Griffith site 3, there was an increase in the proteobacteria and decrease in archaea and actinobacteria in response to WWW application (Figure 3.8). The archaea and actinobacteria make up the majority of species at this site (24% and 41%, respectively) and as in site 1, a large decrease in the two dominant populations could lead to a decline in soil health.

The proteobacteria consist of six classes and contain a variety of beneficial and detrimental bacteria. Only four classes of proteobacteria were detected in soil. Figure 3.9 shows how they these classes of bacteria change after application of WWW. The most significant difference is the increase in gammaproteobacteria after WWW application. These bacteria contain a considerable amount of significant pathogens. An increase in these bacteria could result in unhealthy soil, susceptible to disease.

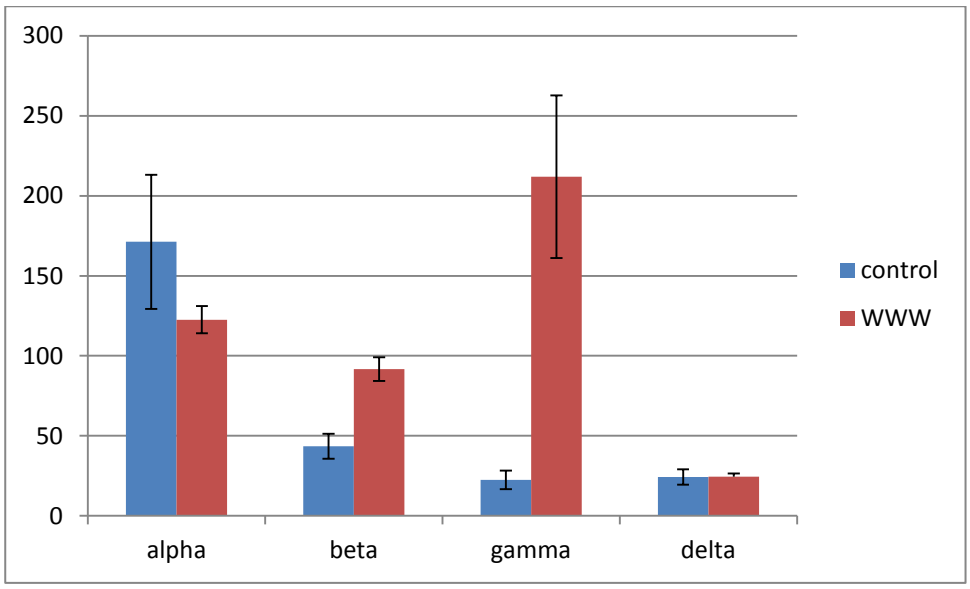


Figure 3.9 Distribution of proteobacteria at site 3

3.1.4 CONCLUSIONS

Using changes in the microbial community structure as an indicator of environmental changes has been an acceptable practice for accessing the health of ecosystems due to its sensitivity and absence of immediate resilience to disturbances. The structural shifts within microbial communities are often associated with changes in ecosystem processes and therefore ecosystem health. In this project, we have demonstrated that WWW application significantly altersthe soil microbiota associated with ecosystem functions, such as turnover of nitrogen. In addition, we identified these possible disruptive changes at an early stage that could potentially lead to detrimental effects on vine health, such as leaf and berry phenotypic changes.

The future for this research area is to include the ability to link phylogeny with function. This would allow better understanding of the functions to which each of the major taxa are contributing. We can already start to delve into this with commonly employed techniques such as quantitative PCR (qPCR) and microarrays of key functional genes responsible for specific processes such as nitrification. With the use of metagenomics, the latest in the NGS suite of technology we can get information about novel metabolic pathways, gene regulatory elements, genes of unknown function, and genes for pathogenesis, virulence and drug resistance (Torsvik et al, 2002). Soil microbiological health is a good indicator of sustainable irrigation practices in wineries and should be incorporated in winery wastewater management monitoring plans.

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3.2 Impacts of recycled winery wastewater irrigation on soil microbiological health during laboratory experiments

3.2.1 INTRODUCTION

Land disposal of winery wastewater (WWW) onto field crops, tree lots, pastures and vineyards is a widely used means of wastewater management. Wastewater reuse in agriculture has the potential to both treat a waste product at the same time as harnessing a valuable water source. Winery wastewater with appropriate treatment, can be a safe, affordable and sustainable source of irrigation water. This process is simple, low cost, and requires minimal technical expertise. However, there is limited understanding of the consequences of such a practice, and it has had variable success. Many wineries have used treated wastewater, or wastewater that has only undergone primary treatment, to produce a commercial crop. Numerous wineries are also considering using WWW for growing grasses, root crops or cereals, all of which have been trialled by some wineries. However, understanding the best management of WWW (timing, rate, application) so that it does not lead to deterioration of soil health (salinity, sodicity and microbiology) or crop health (e.g. phytotoxic shock, yield or crop quality) remains unclear.

Kikuyu grass (*Pennisetum clandestinum*) is a perennial yellowish-green grass that often forms turf. The aim of the present study was to assess the ability of this grass to utilise WWW applications over 18 weeks and to evaluate the soil microbiological health under controlled laboratory conditions using different ratios of tap water (tw) : WWW, (ranging from 0% to 100 % WWW).

3.2.2 METHODOLOGY

Pot trial set up

The study was conducted in both a controlled environment room and a glasshouse. A pot trial was conducted in a controlled environment, temperature range 15-25^{o}C, day/night cycle of 14 and 10 hours respectively.

The physic-chemical characteristics of the soil and wastewater used in this experimentation are listed in are Tables 3.3 and 3.4.

Table 3.3 Physicochemical characteristics of study soil

Soil	EC (dS/m)	pH	Cl (mg/kg)	Ca (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)	K (cmol/kg)	CEC (NH_4) (cmol/kg)
Berton Winery	0.029	6.2	0.55	1.5	0.34	0.11	0.08	11

Table 3.4 Wastewater Quality for Main Experiment, [Batch 2 from Berton Vineyards]

TREATMENT TW:WWW	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	K (mg/L)	COD (mg/L)	pH	EC (µS/cm)
0:100	28.7 ± 0.50	12.8 ± 0.25	48.5 ± 1.50	2.5 ± 0.04	<10	7.82 ± 0.01	495 ± 15
75:25	27.1 ± 0.29	12.3 ± 0.35	61.8 ±1.35	83.3 ± 4.6	1030 ± 230	5.19 ± 0.01	329 ± 15
50:50	25.7 ± 0.60	11.6 ± 0.80	76.6 ± 2.5	159 ± 4.2	2380 ± 130	5.02 ± 0.03	570 ± 8
25:75	24.1 ± 1.2	10.1 ± 0.08	89.3 ± 3.2	250 ± 3.2	3890 ± 160	5.11 ± 0.01	868 ± 5
0:100	22.5 ± 1.2	10.4 ± 0.13	102.9 ± 8.8	305 ± 23	5170 ± 320	5.16 ± 0.02	1124 ± 8

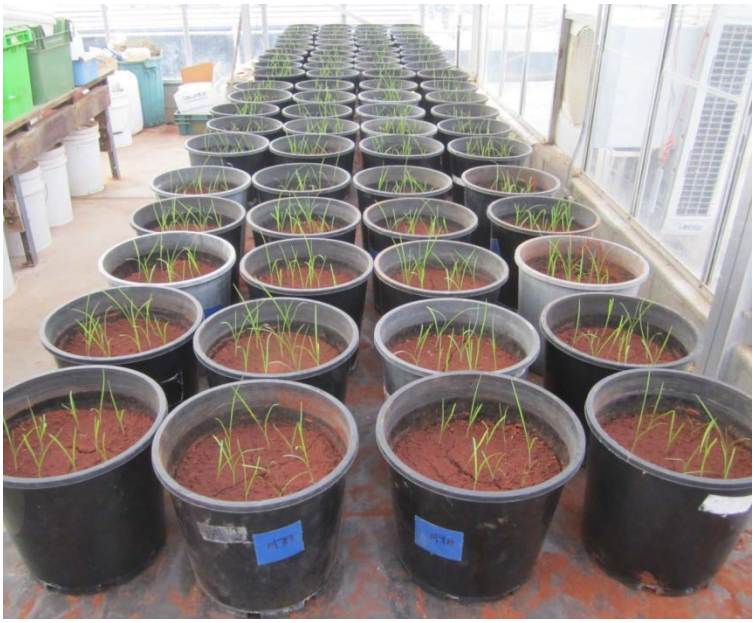


Figure 3.10 Experimental pots consisting of four replicates of 0%, 25%, 50%, 75% and 100% winery wastewater application, sown with Kikuyu grass

Soils were sampled from various treatments on week 10 and week 18 for microbiological assessment using the techniques and methods described below.

Soil DNA extraction

Total DNA was extracted from 0.25 g samples using the PowerSoil DNA Isolation kit (MoBio) with a mechanical disruption step of 5 m/s for 45 s using a FastPrep-24 tissue and cell homogeniser. The final elution volume was 100 µL.

The amount of total DNA extracted was quantified using Quant-it Picogreen dsDNA reagent (Invitrogen) and a MX3000P (Stratagene) real-time PCR instrument. The unknown samples were compared against a standard curve derived from known concentrations of lambda DNA. All samples were diluted to 5 ng/µL for PCR.

16S taqman PCR

Bacterial 16S genes were amplified in a Taqman qPCR assay as per Suzuki et al (2006).

The principles of the Taqman assay are that a fragment of DNA is amplified by specific primers and a dual-labelled probe is attached along the DNA sequence in between the two primers. As amplification occurs, the Taq polymerase cleaves (or degrades) the probe from the DNA causing an increase in fluorescence that can be directly measured by a real time PCR instrument. The rate of degradation of the probe is proportional to the amount of PCR product formed.

The PCR primers used were BACT1369F (5'CGGTGAATACGTTCYCGG 3') and PROK1492R (5' GGWTACCTTGTTACGACTT 3'); and probe TM1389F [5'(FAM) CTTGTACACACCGCCCGTC 3' (BHQ-1)]. Reactions were performed in a final volume of 25 µL containing 1.5 µM primer BACT1396F, 1 µMuM primer PROK1492R, 0.5 µM probe TM1389F and TaqMan Universal MasterMix (Life Technologies). The PCR amplification was performed in duplicate on the MX3000P Stratagene real time PCR instrument using the following program: 15min hotstart at 95°C followed by 40 cycles of 94°C for 15 s, 56°C for 1 min and 72°C for 1 min. A standard curve of cloned plasmid containing known copy numbers of 16s DNA was run in duplicate over a 6-order magnitude to quantify the PCR products. Data analysis was performed on the MxPro software (Stratagene) and the settings as determined by the software were maintained.

16S-DGGE

Denaturing gradient-gel electrophoresis (DGGE) was used to assess the changes in bacterial communities over time and WWW concentration. The PCR amplification of the V3 region of the 16S rRNA gene using primers F968-GC and R1401 (Duineveld et al. 1998)and separation was in an Ingeny Phor-U electrophoresis system as previously described (Wakelin et al 2009).

3.2.3 RESULTS AND DISCUSSION

There was a significant increase in bacteria numbers across all four wastewater treatments assessed by Taqman PCR (Figure 3.11) in comparison to the control pots.

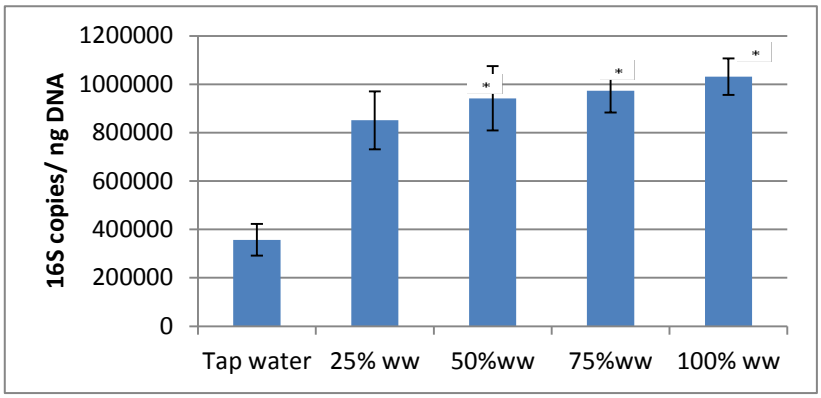


Figure 3.11 16S qPCR copy numbers g^{-1} of dry soil over the different treatments. Error bars indicate standard errors of the four replicates per treatment. Significantly different values ($p<0.001$) from the control (tap water) are shown by *

The DGGE profiles (Figure 3.12) confirm that the application of wastewater resulted in significant changes in the microbial community structure. Each band represents an operational taxonomic unit (OTU) and infers a bacterial species. The upper third of the gel shows an increase in the number of bands, representing the rise of a different set of bacteria when WWW is applied.

Several additional bands developed in the wastewater irrigated samples, and at 100% wastewater application the pots were dominated by completely different microbial communities. ANOSIM (Analysis of similarities, PRIMERv6) was used to assess the significance of these differences and showed a strong effect of wastewater irrigation on the bacterial community composition ($R=0.913$, $p=0.001$). A cluster dendogram and SIMPROF (similarity profiling based on structure) was applied to the data to look at the similarities between microbial groups. After ten weeks of experimental set-up, there was no significant difference between tap-water and the 25% treatment. Similar results were noted for 50 and 75% treatments. However, 100% treatments were significantly different from tap-water and 25% treatments. After 18 weeks, no significant difference was noted between different treatments. However, all were significantly different from the tap water control (Figure 3.13).

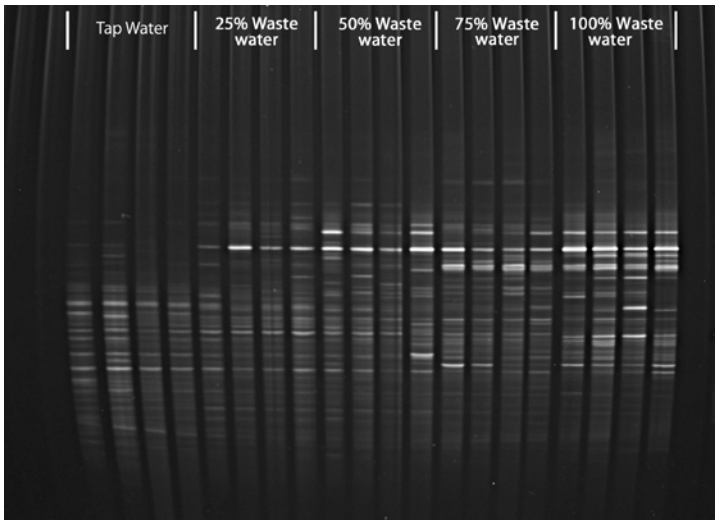
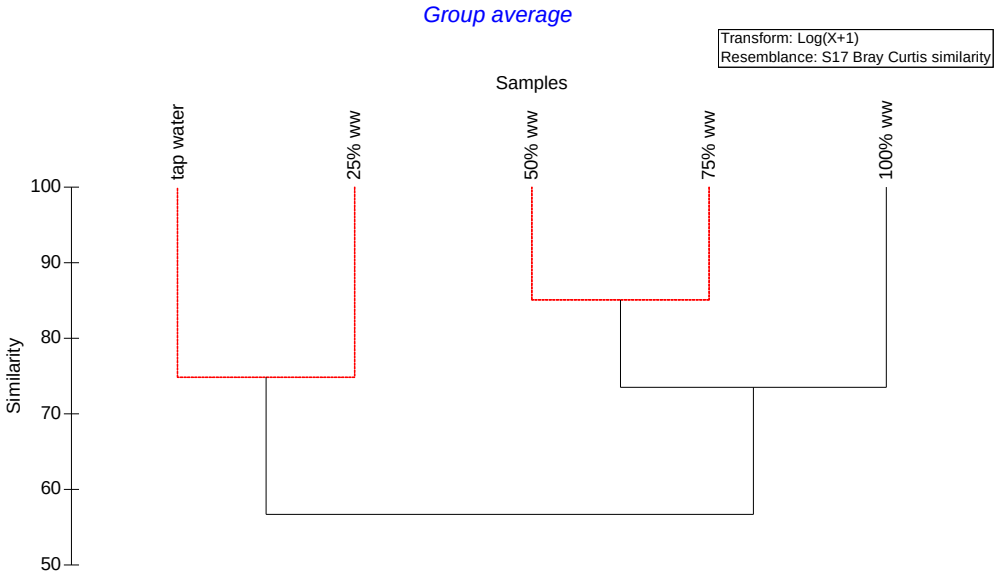


Figure 3.12 Denaturing gradient-gel electrophoresis (DGGE) targeting the bacterial 16S ribosomal RNA genes obtained from soils irrigated with different concentrations of wastewater. Each band on the gel corresponds to an operational taxonomic unit (OTU), representing a different bacterial species

3.2.4 CONCLUSIONS

By 18 weeks there was a significant increase in the relative abundance of bacteria and a change in the population dynamics compared to the tap water control irrigated pots. These changes occurred over several weeks, however once stabilised there was no significant difference detected between the different concentrations of wastewater applied to the soil (25-100 %). Future work to look at the recovery rate of the microbiology after a long-term application of wastewater is needed to determine the outcome of WWW irrigation.

(a) After 10 weeks of wastewater application



(b) After 18 weeks of wastewater application

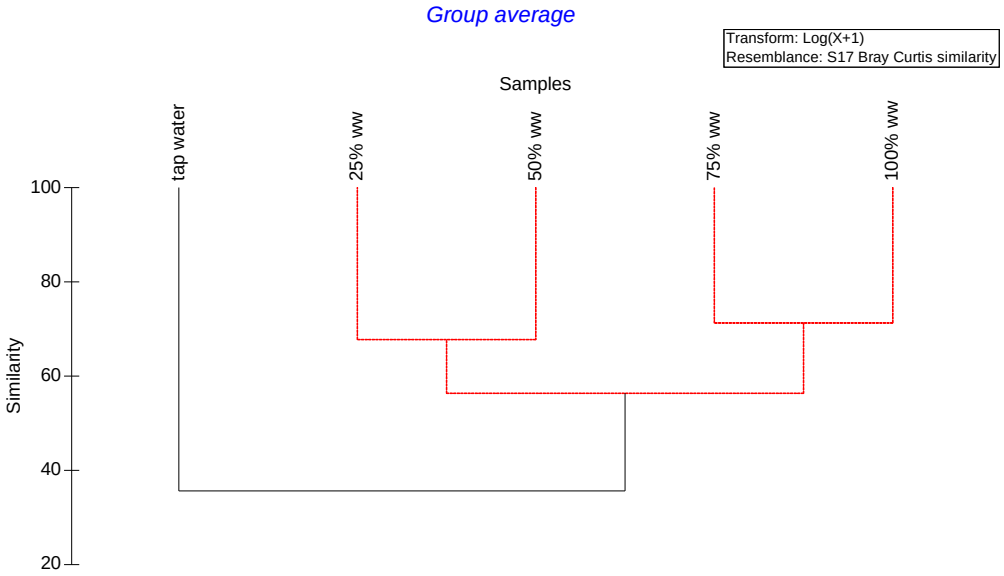


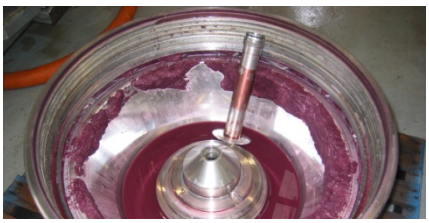
Figure 3.13 Cluster dendogram of 16S DGGE data. Solid black lines in the dendogram represent clusters significant at the 95% level using SIMPROF test; red lines represent clusters that are not significantly different at this level

3.2.5 REFERENCES

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Part IV Development of threshold values for ethanol and phenolic compounds

4 Development of threshold values for ethanol and phenolic compounds

4.1 Microbiological assessment of soil irrigated with different levels of ethanol and phenolic compounds in recycled winery wastewater

In the present study, a microbiological and ecotoxicological assessment of soil irrigated with different levels of ethanol and phenolic compounds in the recycled winery wastewater (WWW) was investigated. We used denaturing gradient-gel electrophoresis (DGGE) to study the soil microbiological response to applications of three phenolic compounds and ethanol and BioLog to look at the functional changes in response to ethanol addition.

4.1.1 METHODS

The total polyphenol content of WWW ranges from 10 – 1450 mg/L (Mosteo et al., 2008; Arienzo et al., 2009; Lucas et al., 2009; Mosse et al., 2011). Gallic acid, catechin and coumaric acid are the major phenolics in WWW. These compounds were, therefore, selected for the microbiological assessment (Table 4.1).

Table 4.1 Concentration of phenolics and ethanol selected for microbiological assessment

COMPOUND	CONCENTRATIONS SELECTED (MG/KG)
Catechin	0, 5, 10, 20, 40
Gallic Acid	0, 2.5, 5, 10, 20
Coumaric Acid	0, 1.5, 3, 6, 12
Ethanol	0, 0.5, 2, 4, 8, 16

Soil microcosm experiment set up

A sandy loam soil from McLaren Vale was collected in 2010 and stored at -20^{o}C. Prior to use, the sample was thawed and sieved through a 2 mm size sieve, adjusted to 16 % moisture content with sterile water and pre-incubated overnight at 24^{o}C. The microcosm experiment consisted of four concentrations of each chemical and a water control over a 14-day incubation time.

The second lowest concentration of each chemical represents the amount added in the field as determined by values reported in winery wastewater minus 10 %.The samples were divided into 20 g subsamples and the four treatments were applied. The samples were further subdivided into two to serve as replicates. The microcosm therefore consisted of 10 g in 50 mL Falcon tubes, which were incubated at 24±1^{o}C. Stock concentrations of 20 mg/mL were prepared in ethanol for the phenolic compounds and dilutions were performed in water. Ethanol concentrations were prepared by making a 25 % solution in water and performing serial dilutions.

DNA Extractions and DGGE

On day 14 DNA was extracted from 0.25 g of soil with PowerSoil DNA Isolation kit (MoBio) according to manufacturer's instructions with a mechanical disruption step of 5.5 m/s for 45 s on a FastPrep (MPBiomedicals) instrument. Isolated DNA was quantified with PicoGreen (Life Technologies) and diluted

to 5 ng/µL for PCR. Amplification of the 16S gene for DGGE analysis was performed in a Gradient Mastercycler (Eppendorf). Primer and program conditions have been previously described in section 3.

Community level physiological profiling

BioLog EcoPlates (Oxoid) were used to characterise the functional capacity of the microbial communities treated with ethanol by determining their ability to oxidise various carbon sources. For each sample 3 g of soil was suspended in 27 mL of phosphate-buffered saline (PBS; pH 6.8) and placed on a reciprocal shaker for 2 h. The tubes were left to stand for 3 min and 3 mL sub-samples were pipetted into fresh 27 mL PBS. One hundred and thirty microlitres of this dilution was loaded on an EcoPlate and incubated at 24^{o}C in the dark for 5 days. Each plate contains 31ecologically relevant C substrates in triplicate. Colour development in each well was determined at the start of the experiment using a BioLog microstation system (OD_{590}), and then at 24 hr intervals. The OD_{590} values for the control (water) samples were subtracted from the value for each substrate-containing well. At each sampling time, the well colour development was calculated relative to time zero values and the average value for each substrate calculated within each plate. Finally, a total plate average for colour development was determined (AWCD).

4.1.2 RESULTS AND DISCUSSION

MDS (multidimensional scaling) is a distance matrix used to visualise the similarity between points. Each point represents a sample at each concentration, and the closer the points the greater their similarity. Figure 4.1 shows the change in distribution from low to high chemical concentration for all four compounds. Each compound exhibits identical trends where the higher the concentration the more dissimilar the bacterial communities become in relation to the untreated control.

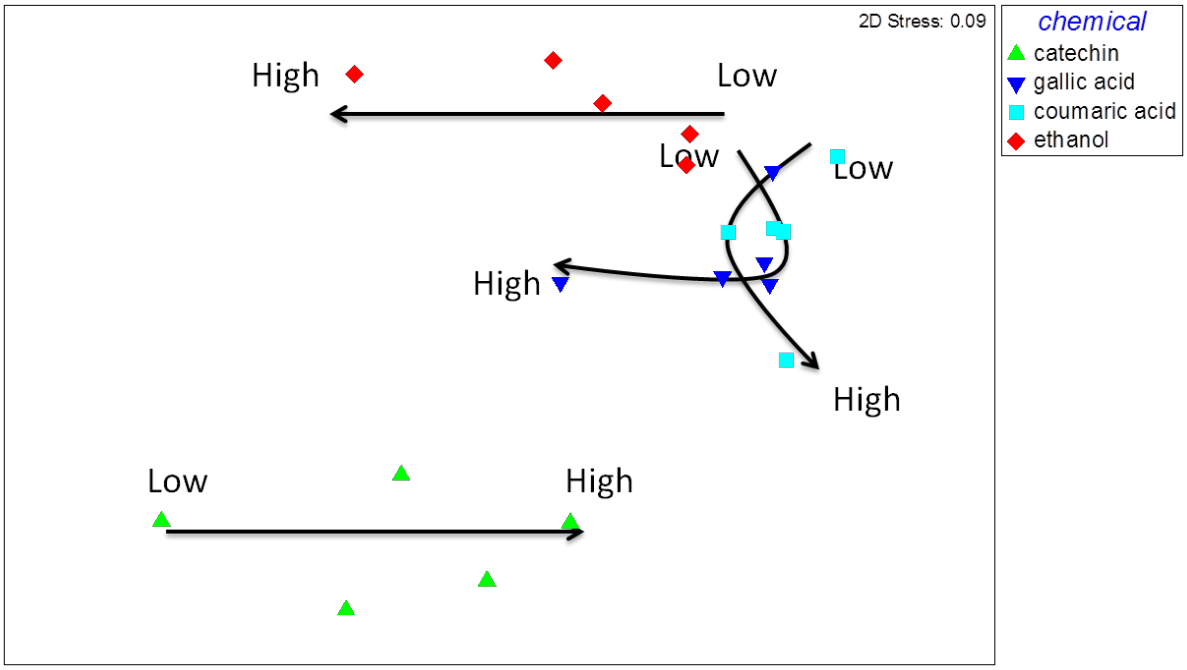


Figure 4.1 nMDS plot of phenolic and ethanol compound exposed soils after 14 days incubation

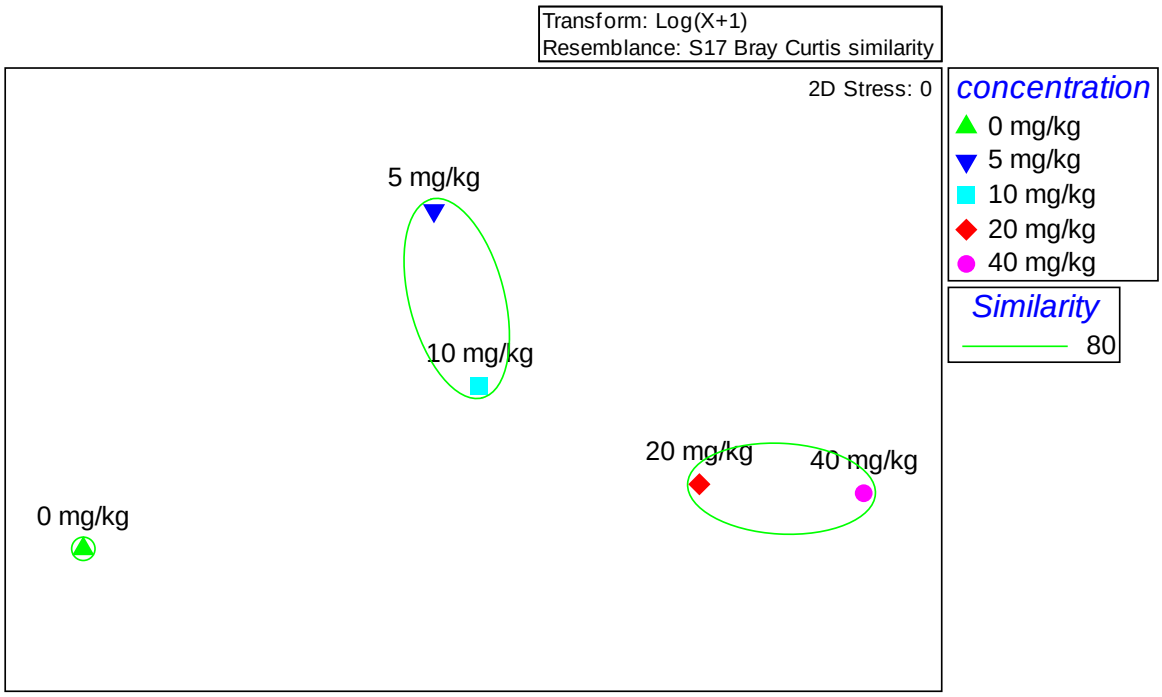


Figure 4.2 nMDS plot of DGGE profiles targeting the 16S bacteria in soils exposed to increasing concentrations of catechin (R=0.89 p=0.001). The groupings are based on SIMPROF testing (α=0.05) and indicate that they are significantly distinct from each other

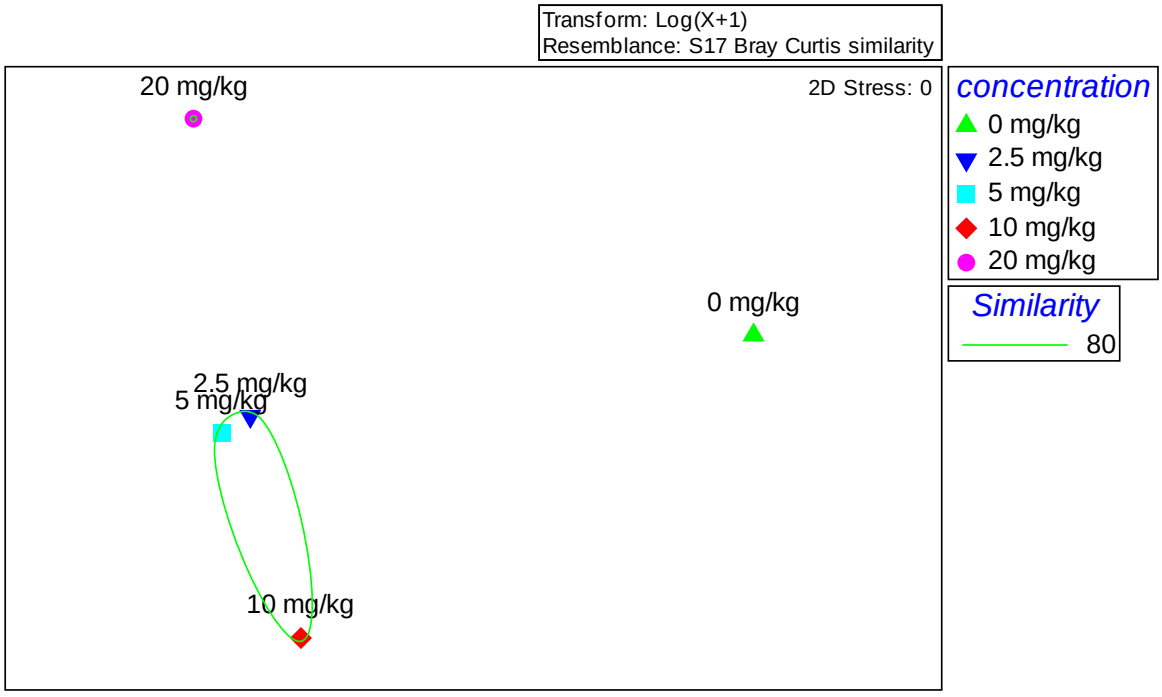


Figure 4.3 nMDS plot of DGGE profiles targeting the 16S bacteria in soils exposed to increasing concentrations of gallic acid (R=1 p=0.002).

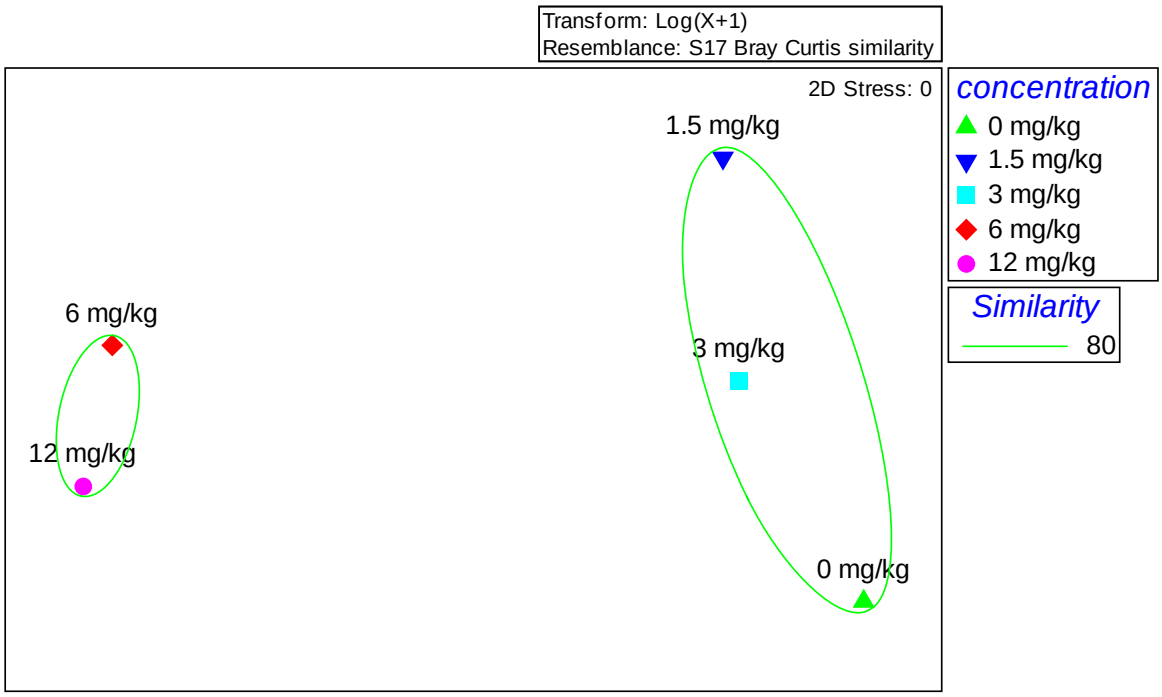


Figure 4.4 nMDS plot of DGGE profiles targeting the 16S bacteria in soils exposed to increasing concentrations of coumaric acid (R=0.72 p=0.015)

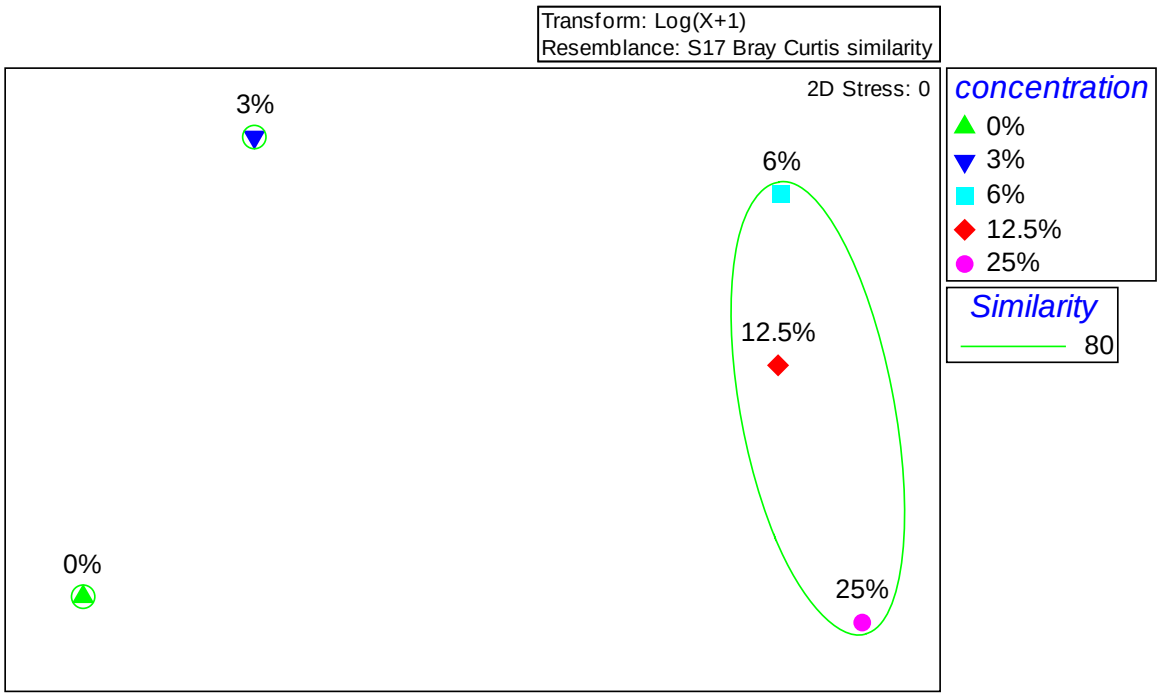


Figure 4.5 nMDS plot of DGGE profiles targeting the 16S bacteria in soils exposed to increasing concentrations of ethanol (R=0.68 p=0.003).

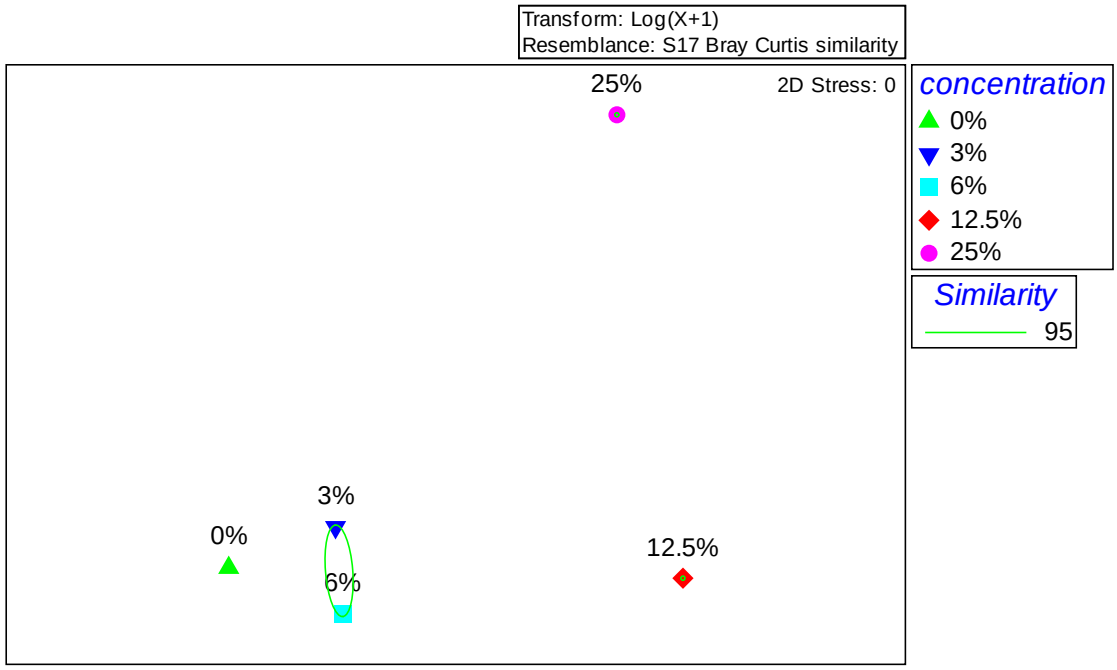


Figure 4.6 nMDS plot of BioLog AWCD values in soils exposed to increasing concentrations of ethanol. ANOSIM testing was not possible due to the replicate design of the plate

Figures 4.2-4.5 look at each of the compounds individually and show that increasing the compound concentration altered the profiles in all of the microcosms. Catechin and gallic acid resulted in three groupings with the control being distinctly different from the mid and high concentration groupings. Coumaric acid showed no significant difference between the three lowest concentrations and only the two highest were different. Ethanol DGGE profiles show a significant shift at 3 % but the higher concentrations showed no change (this could be an outlier). The BioLog profile (Figure 4.6), which estimates the functional capability of the soil, showed a grouping with the two lower concentrations, and each of the other concentrations all differ from each other. This could imply that although the population diversity is not changing there is a change in function of the community at differing concentrations.

Simpson's diversity index was applied on non-transformed DGGE data (Primer6) to assess the abundance and richness of bacterial species as a growth curve (Figure 4.7).

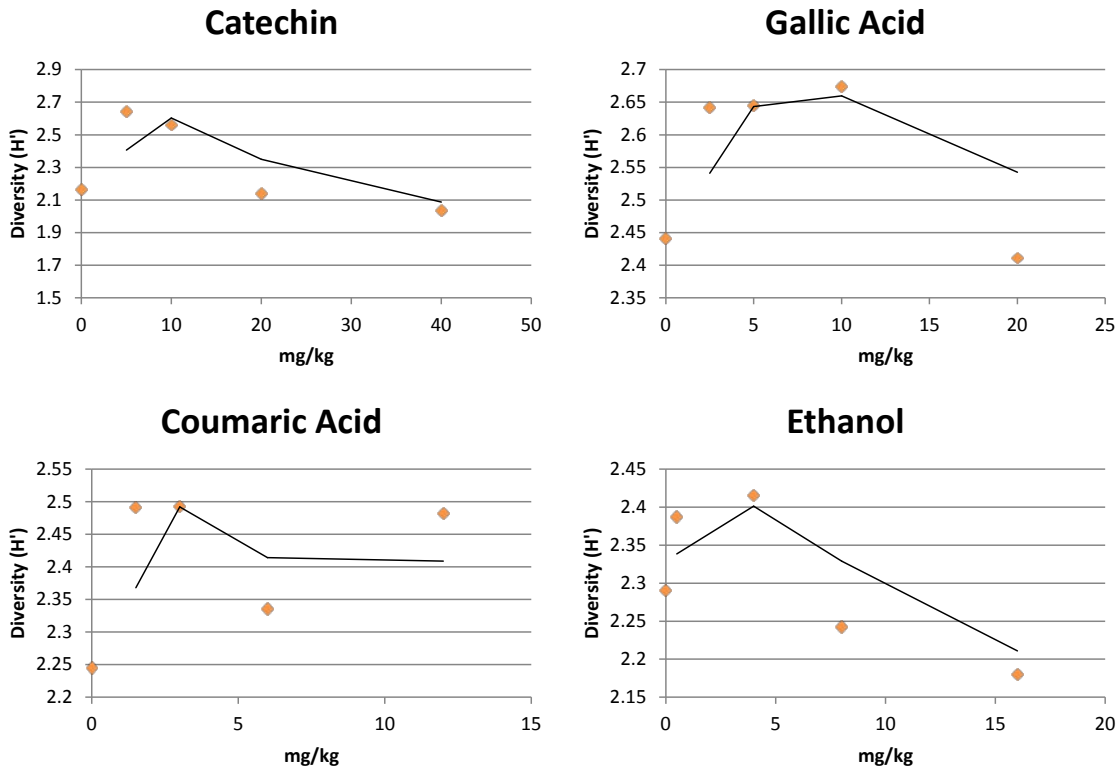


Figure 4.7 Simpson's diversity index to assess microbiological effects of phenolic compounds and ethanol

The diversity in all the treatments initially increased, until a 'critical' value is reached after which it decreased sharply. Even when the diversity returned to the level of control (0), the nMDS plots suggest that the population distribution or contribution was changed relative to the initial structure.

4.1.3 CONCLUSIONS

This study has shown that under laboratory conditions soil bacteria are affected by individual phenolic compounds commonly found in WWW. This needs to be further validated under field conditions.

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4.2 Development of threshold values for ethanol and phenolic compounds in the recycled winery wastewater using plants and earthworm as bioindicators

It is recognised that the reuse of water and nutrients contained in winery wastewater is generally a 'beneficial reuse'. However, the wastewater will only be considered as 'reuse' when evidence exists to demonstrate that the concentration of substances it may contain, such as ethanol, phenolic compounds and tannins, provides a demonstrable benefit to the receiving land. When wastewater containing substances in concentrations in excess of those which benefit the land is applied, it is considered an 'emission to land' for these substances and must be reported as such. There is currently a lack of information to prove that ethanol contained in wastewater is 'beneficial to reuse'. In the present study, ecotoxicological assessment of three phenolic compounds and ethanol was conducted using standard protocols for testing earthworm survival over 14 days and plant seed germination test.

4.2.1 METHODOLOGY

The total polyphenol content of winery wastewater ranges from 10 – 1450 mg/L (Mosteo et al., 2008; Arienzo et al., 2009; Lucas et al., 2009; Mosse et al., 2011). Gallic acid, catechin and coumaric acid are the major phenolics in winery wastewater. These compounds were, therefore, selected as three phenolic compounds for the ecotoxicological assessment. The concentration range of various compounds used for testing is given in Table 4.2. Ethanol was tested as a component of wine and as 99 % analytical grade reagent.

Table 4.2 Concentration of phenolics and ethanol selected for ecotoxicological assessment

COMPOUND	CONCENTRATIONS SELECTED (MG/KG)
Catechin	0, 5, 10, 20, 40, 80
Gallic Acid	0, 2.5, 5, 10, 20, 40
Coumaric Acid	0, 1.5, 3, 6, 12, 24
Ethanol (%)	0, 0.75, 1.5, 3, 6

Toxicity testing protocol

The effect of phenolic compounds and ethanol on plant growth was assessed in a glasshouse study. Ten turnip seeds were sown into separate plastic, free-draining pots containing 660 g of a coarse textured seed raising mix comprising sand and pine bark, with no additional nutrients. Four replicate pots for each treatment were watered daily. Plants were grown in a glasshouse where the temperature range was maintained between 15 and 25^{o}C. The pots were arranged in a randomised complete block design. Plants were destructively harvested 11 days after sowing. At the time of harvesting, plants were removed from the pots, and the soil was gently washed away from the roots and rinsed with RO water. The roots and shoots were separated, and fresh weights determined.

The invertebrate toxicity testing was conducted to comply with the OECD Guidelines for the testing of chemicals 208: Earthworm Reproduction Test (OECD, 2004) and was conducted using the earthworm *Eisenia fetida* as the test species. Following spiking and preparation of the soils, 500 g replicates were removed and hydrated to 70% of water holding capacity. This hydration level in the soil was maintained throughout the test on a weekly basis by weight. At the commencement of the test, ten adult worms that had been cleaned with ultrapure water were weighed and added to each test container. The test containers were then placed in a growth cabinet which was maintained at a constant temperature of 22°C (± 2°C) and a light: dark cycle of 12:12 h.

The earthworm survival and earthworm growth endpoints were determined from the adult worms after 14 days. Four replicates containing ten worms in each were used. Earthworm survival was presented as the percentage of worms present after 14 days relative to the initial number of worms (i.e. 10 worms). The tests were deemed valid if there was ≥ 90% survival in the control soils. The growth of the worms was presented as a relative weight change over the initial 14 days of the test. This was done by calculating the average weight per worm in each of the test containers at day zero and day 14 and then determining the ratio of these two values (i.e. d_{14}/d_0).

The data collected throughout the invertebrate toxicity test were used to evaluate two endpoints, namely, earthworm survival and earthworm growth.

4.2.2 RESULTS

Coumaric acid, catechin and gallic acid did not significantly affect the germination of turnip seeds (Figure 4.8). There was no difference in the germination success on day 5 and day 11. The plant height was also not significantly affected at the highest concentration tested for these phenolic compounds. In contrast, ethanol exposure significantly affected seed germination at 1.5 % concentration (Figure 4.9). Ethanol in the wine sample was less toxic to turnip seedlings in comparison to the neat ethanol exposure (Figure 4.9). The other beneficial compounds present in the wine could be providing additional nutrients to the growing seedlings and hence promoting growth.

Ethanol exposure at 2 % concentration did not result in earthworm mortality where as 4% and 8% ethanol exposure caused significant earthworm mortality during 14 day exposures (30% and 100%, respectively). Exposure to coumaric, gallic acid and catechin did not result in earthworm mortality at the highest concentration tested (12, 20, 40 mg/kg, respectively).

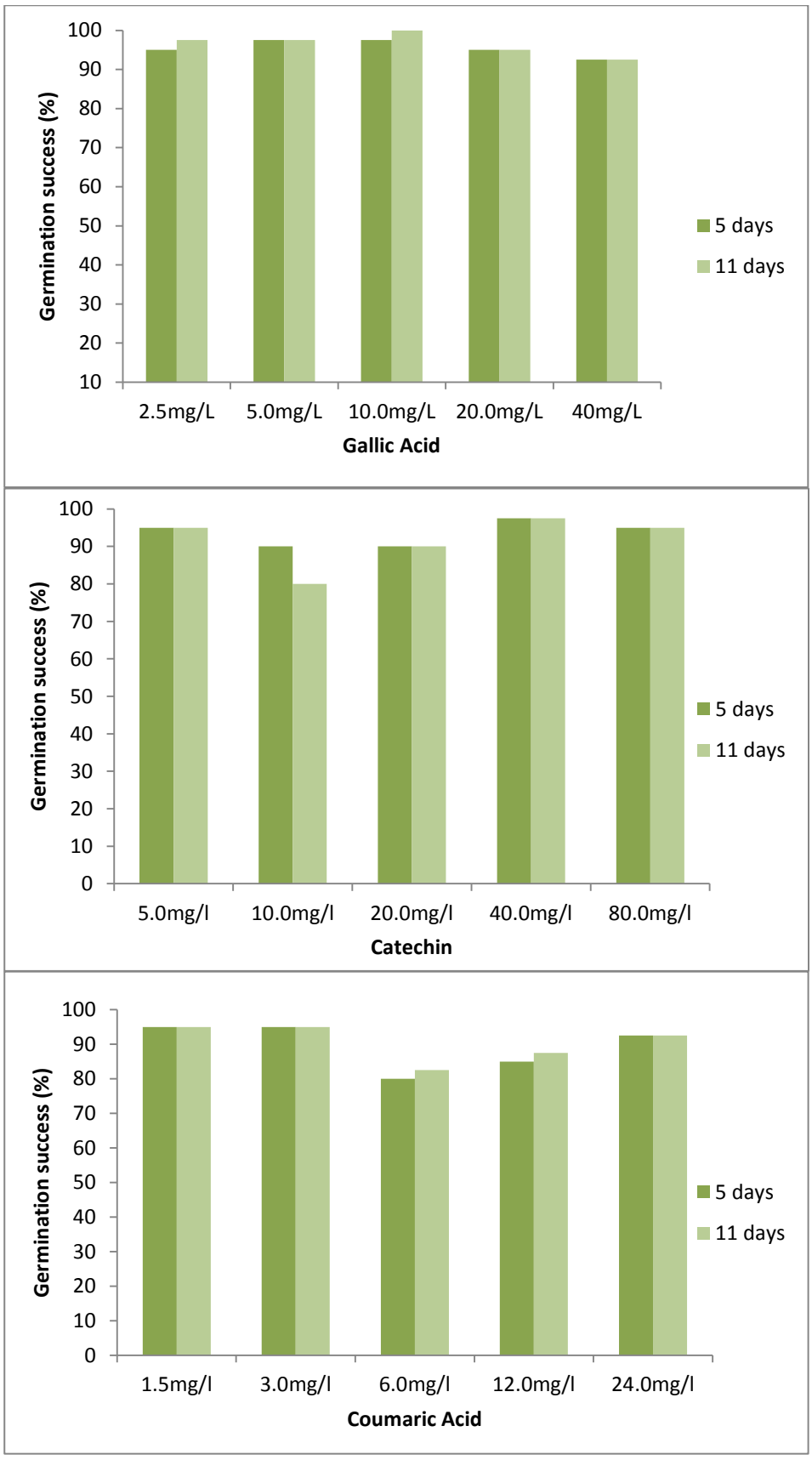


Figure 4.8 Turnip seed germination success at different concentrations of phenolic compounds

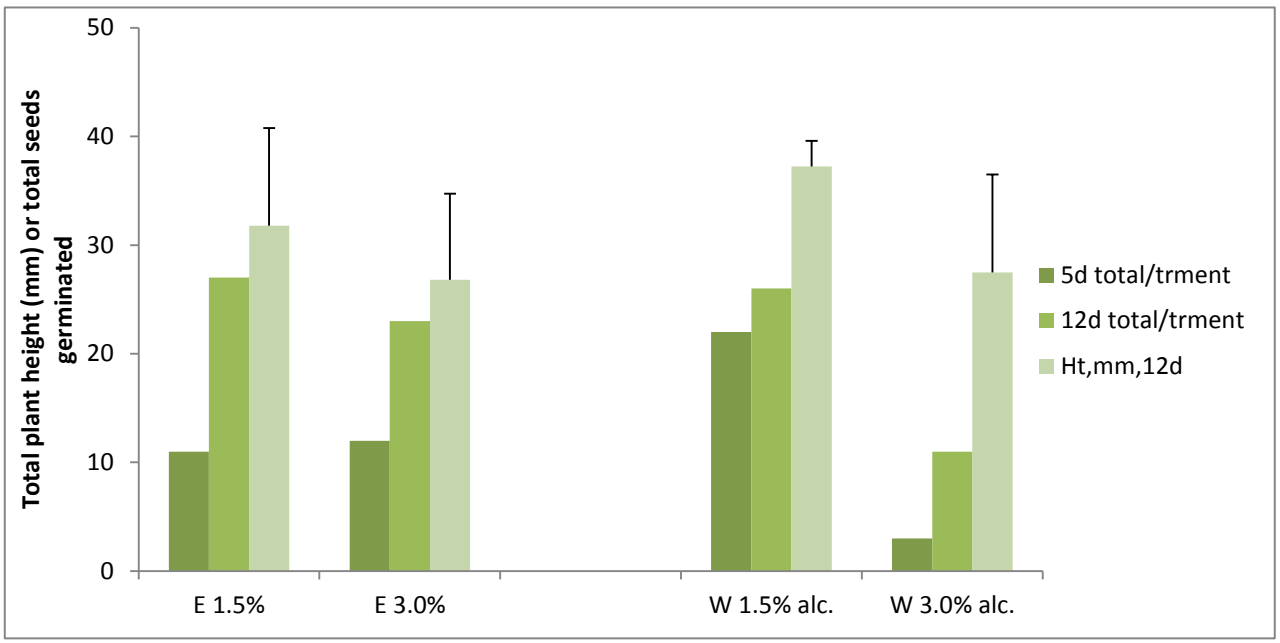


Figure 4.9 Seed germination success and plant height during different ethanol treatments

4.2.3 CONCLUSIONS

Based on the ecotoxicological and microbiological assessments, the lowest observed effect concentration (LOEC) was calculated from each bioassay. The lowest LOEC value was considered for the derivation of threshold values. Based on the limited data-set and lack of studies reported in the literature, a safety factor of 10 was applied to the lowest No-observed-effect concentration (NOEC) observed in this study for deriving threshold values (Table 4.3). The NOEC is the highest tested dose or concentration of a substance at which no such adverse effect is found in exposed test organisms where higher doses or concentrations resulted in an adverse effect. The LOEC is the lowest concentration or amount of a substance (i.e. a drug or chemical), found by experiment or observation that causes an adverse alteration of morphology, function, capacity, growth, development, or lifespan of a target organism distinguished from normal organisms of the same species under defined conditions of exposure. The regulatory bodies use LOEC and NOEC data to develop guideline/threshold values.

Table 4.3 No-observed effect concentration based on microbiological and ecotoxicological assessment

CHEMICAL	NOEC (EARTHWORM SURVIVAL)	NOEC (MICROBIOLOGY)	NOEC (TURNIP GERMINATION)	LOWEST NOEC	THRESHOLD VALUES
Catechin	10 mg/kg	10 mg/kg	>40 mg/L	10 mg/kg	1 mg/kg
Gallic acid	>20 mg/kg	10 mg/kg	>80 mg/L	10 mg/kg	1 mg/kg
Coumaric acid	>12 mg/kg	3 mg/kg	>24 mg/L	3 mg/kg	0.3 mg/kg
Ethanol	2%	4%	07.5%	0.75%	0.1%

Microbiological end-point was a sensitive indicator of toxicity due to phenolic compounds resulting in the lowest LOEC values. The plant germination test was highly sensitive to ethanol and the least sensitive to phenolic compounds. Based on this study, catechin and gallic acid concentrations are safe at 1 mg/kg in wastewater (Table 4.3). Ethanol is highly toxic with a 0.1% or 1g/L threshold value in wastewater (Table 4.3).

4.2.4 REFERENCES

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Part V Interactions between soil, petiole, grape juice and wine quality

5 Interactions between soil, petiole, grape juice and wine quality

5.1 Effect(s) of growing vines, [*Shiraz tahbilk*] using winery wastewater and Sodium and Potassium treatments in a glasshouse environment

5.1.1 INTRODUCTION

A vine trial was established in a glasshouse at the CSIRO, Land and Water campus, Urrbrae using a *Shiraz tahbilk* vine. The aim was to test under 'controlled' conditions both the effects of the application of winery wastewater and high Na and K nutrient loads. Initially the study consisted of two parts, and was carried out in 2012-2013 and 2013-2014.

In 2012-13, winery wastewater, both pre- and post-treatment was applied to the vines, with vine leaf and petiole samples taken. This was followed by applying a range of Na and K treatments to the same vines, during a 'second' vintage period (2013-14). Petioles and grape berries were sampled.

5.1.2 METHODS

Twelve month old vine cuttings [*Shiraz tahbilk*] were planted into free draining 30 cm pots. The total volume of soil in each pot was 15 kg. After initial trials, a premium commercial potting mix was selected over sandy loam soil. This was based on the potting mix having an 'open' structure that would be more likely retain its 'free draining' characteristics for the anticipated 2-3 year duration of the study.

Winery wastewater was supplied from Yalumba winery, Angaston. Two types were used, pre-treatment wastewater involving primary settlement only and post treatment wastewater after lime addition (to adjust to between pH 8-8.5) and filtration. Winery wastewater treatments used in this experiment included

1. tap water
2. 25% WWW (pre and post)
3. 50% WWW (pre and post); and
4. 100% WWW

Twenty five litres of the treatment solution were added to each of the pots. Additional volumes of tap water were added on days when the temperature of the glasshouse exceeded 25°C for more than about 3 hours. Wastewater characteristics and total Na and K added from the wastewater treatments are listed in Tables 5.1 and 5.2.

Table 5.1 Physicochemical data for Yalumba winery wastewater

	EC (µS/cm)			COD (mg/L)		
	25%	50%	100%	25%	50%	100%
Pre-treatment	446	821	1601	585	1353	2773
Post-treatment	751	1272	2446	444	1294	2888

Table 5.2 Sodium (Na) and potassium (K) levels in Yalumba winery wastewater

	25% WWW		50% WWW		100% WWW	
	Na	K	Na	K	Na	K
Pre-treatment (mg/L)	20	87	40	173	78	235
Total load (mg)	500	2175	1000	4325	1950	5875
Post-Treatment (mg/L)	26	149	40	266	77	510
Total load (mg)	650	3725	1000	6650	1925	12750

Na and K treatments were set-up at concentrations of 20, 40, 60 mM for both plus a combination of Na+K at 20 mM concentration. A total of 24 litres was added to each treatment pot. The total quantity (mg) of Na added per pot was 11.04, 22.08, 33.12 mg for the 20, 40, 60 mM Na treatments, respectively. Total K added per pot was 18.72, 37.44, 56.16 mg for the 20, 40, 60 mM K treatments, respectively.

Petioles and vine leaves were collected in the 2012-2013 vintage. Petioles and grape berries were collected in the 2013-14 vintage.

5.1.3 RESULTS

The measurement of salt levels in grapevine tissue offers the most direct method to assess the presence of problematic saline conditions. The industry standards for petiole and grape juice sodium and chloride concentrations are given in Figure 5.9. Petiole analyses at flowering with greater than 0.5 % sodium and more than 1-1.5% chloride are considered to be toxic to vine health and are sign of a salinity problem (Robinson 1992). Recommended petiole potassium levels for grapevines at flowering range from 1.8-3% (Robinson et al., 1990). In the present study, the potassium level in the petioles was twice the levels recommended for 20, 40 and 60 mM treatments in both the 2012-13 and 2013-14 vintage seasons (Figures 5.1 and 5.2). However, the potassium concentrations in all wastewater treatments were below the recommended levels (Figures 5.1 and 5.2).

In the present study, 20 and 40 mM potassium treatments exhibited high chloride and nitrogen content in petioles (Figures 5.3 and 5.4). The wastewater application to the vines at 100-25% concentration exhibited chloride in the petioles at concentrations three times below the thresholds recommended (Figures 3 and 5). In 2014, the nitrogen levels in petioles for all potassium treatments and potassium and sodium combination (20mM each) were above the recommended 0.8-1.1% level.

Berry weights were significantly increased in the potassium treatment at 60 mM (Figure 5.6) leading to significantly higher potassium in the berry juice at this treatment (Figure 5.7). The rate of uptake of chloride into fruit when soil salinity is high between flowering and veraison is double that when salinity is high before or after this growth stage In the present study, chloride in the berry juice for 2013-2014 vintage was below the recommended threshold level of 200 mg/L for all Na and K treatments (Figure 5.8).

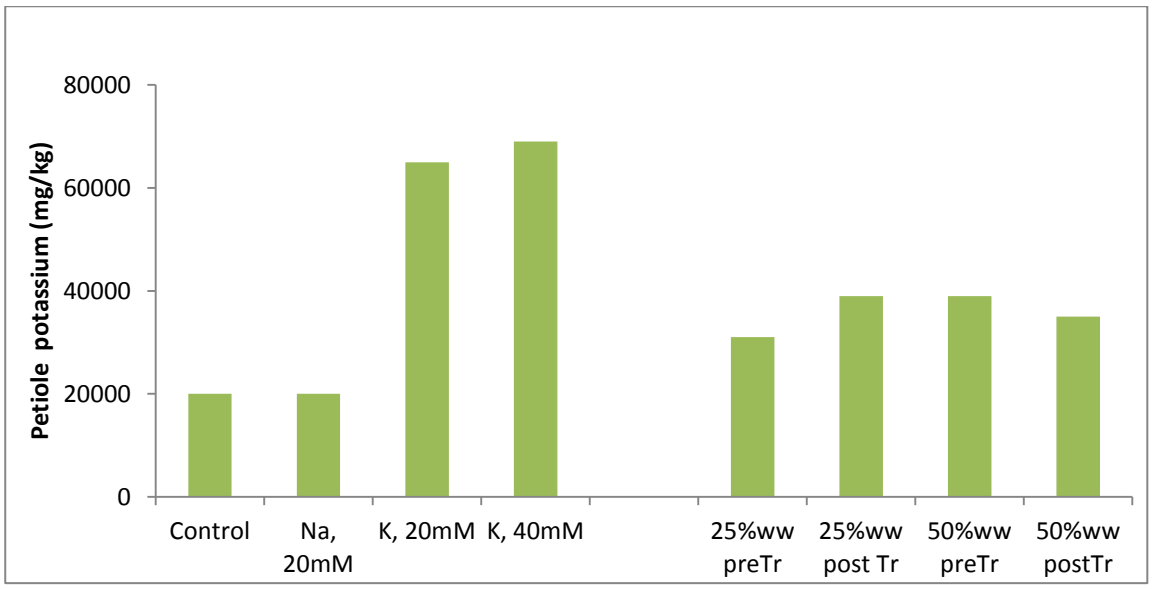


Figure 5.1 Potassium (K) in petioles during 2012-13 vintage (May 2013)

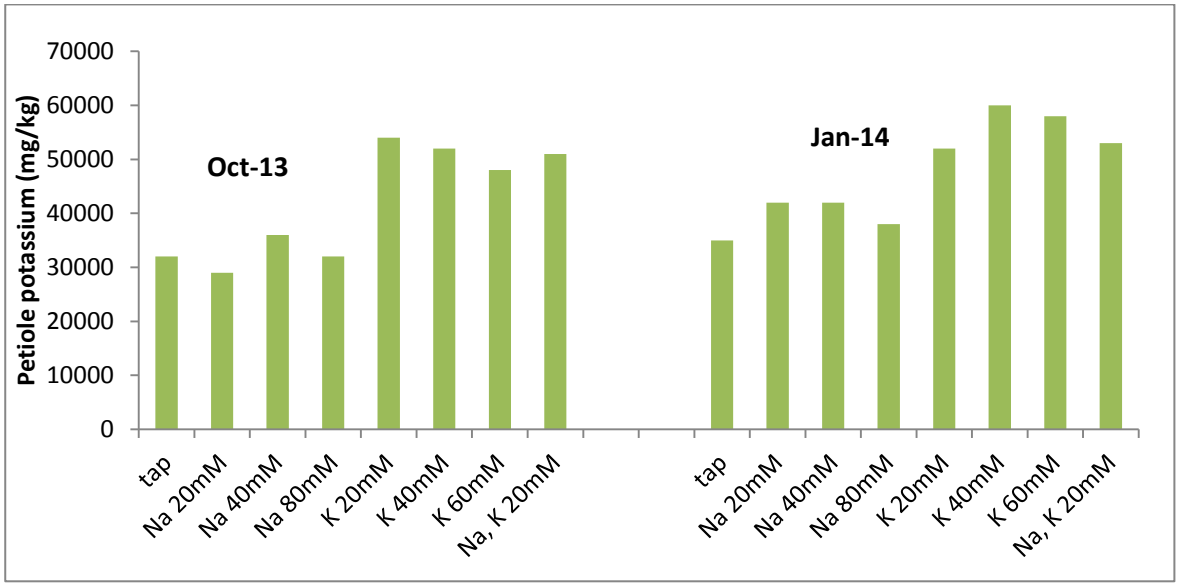


Figure 5.2 Potassium (K) in petioles during 2013-14 vintage

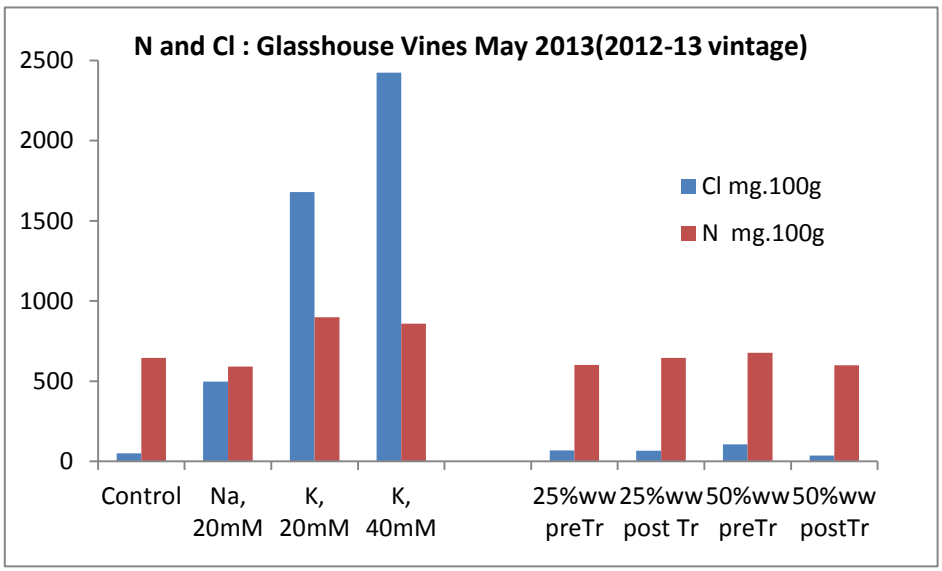


Figure 5.3 Nitrogen (N) and chloride (Cl) in glasshouse vines, 2012-2013 vintage

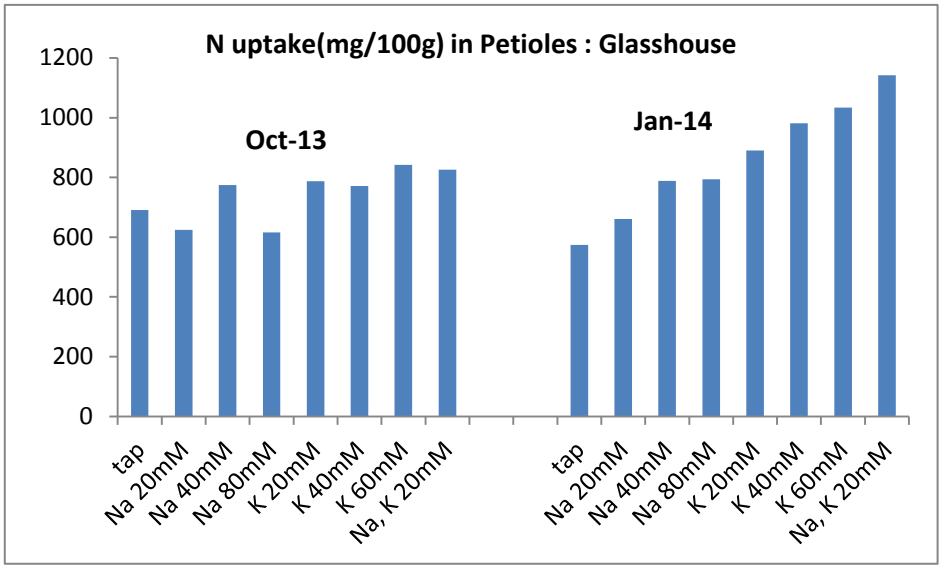


Figure 5.4 Nitrogen (N) in petioles during 2013-14 vintage

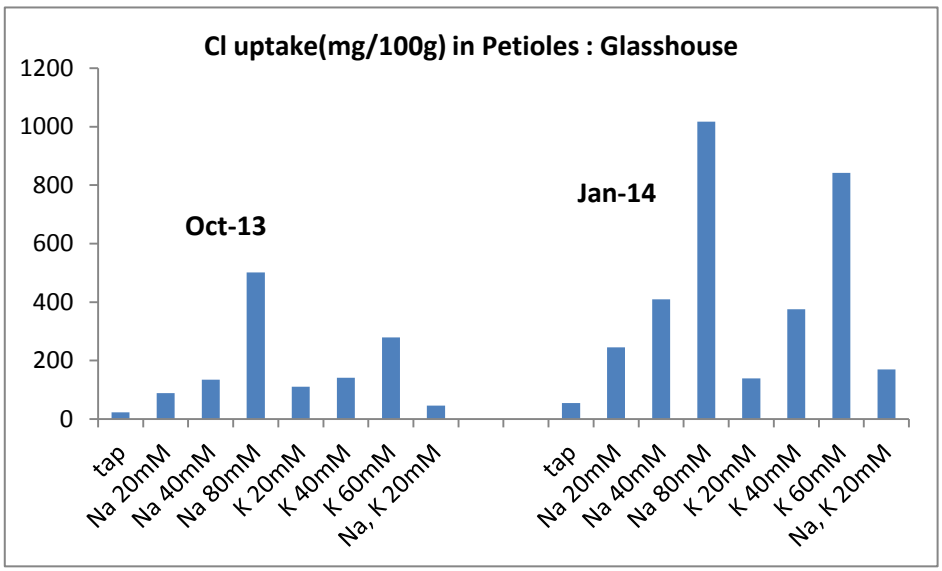


Figure 5.5 Chloride (Cl) in petioles during 2013-2014 vintage

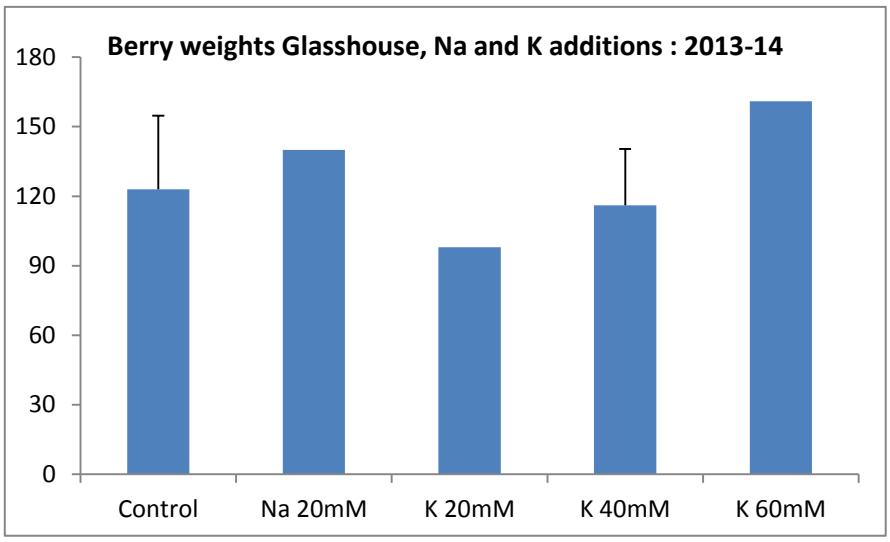


Figure 5.6 Berry weights for sodium (Na) and potassium (K) treatments, 2013-14 vintage

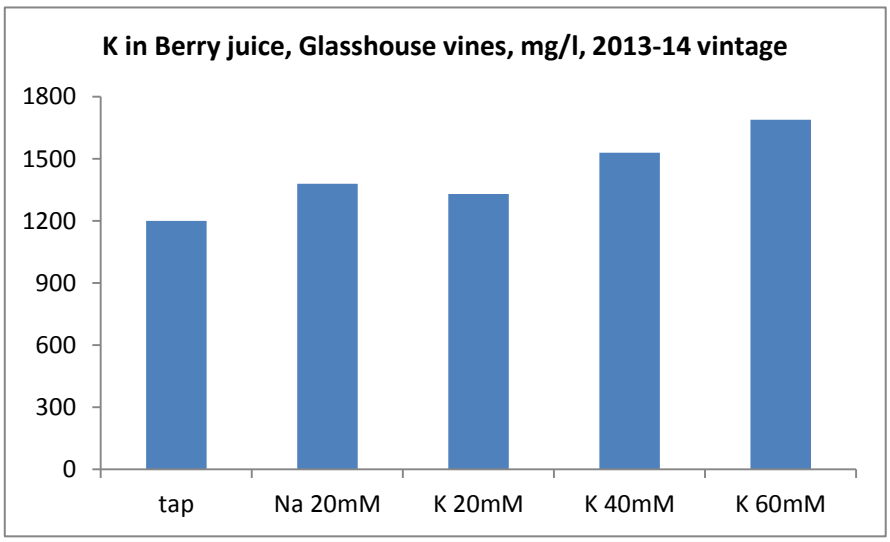


Figure 5.7 Potassium in berry Juice during sodium (Na) and potassium (K) treatments, 2013-14 vintage

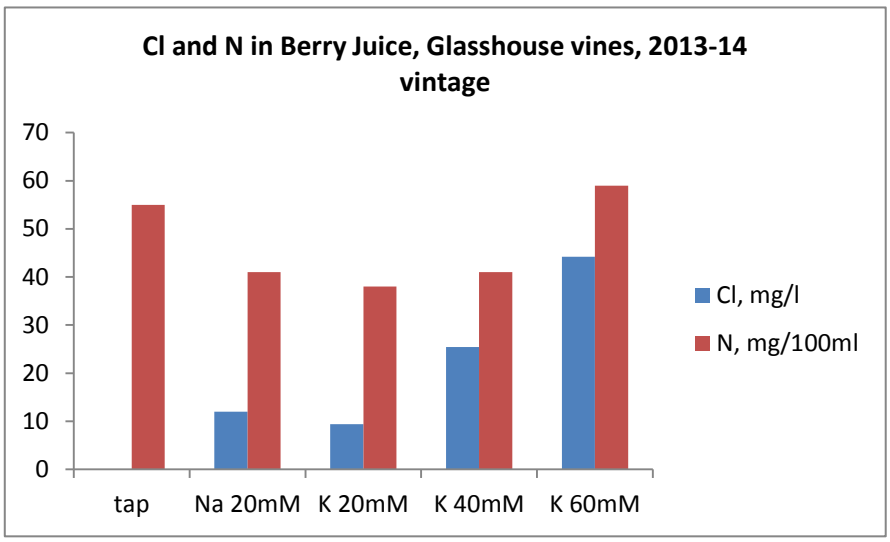


Figure 5.8 Chloride (Cl) and nitrogen (N) in Berry Juice, (Glasshouse), 2013-14 vintage

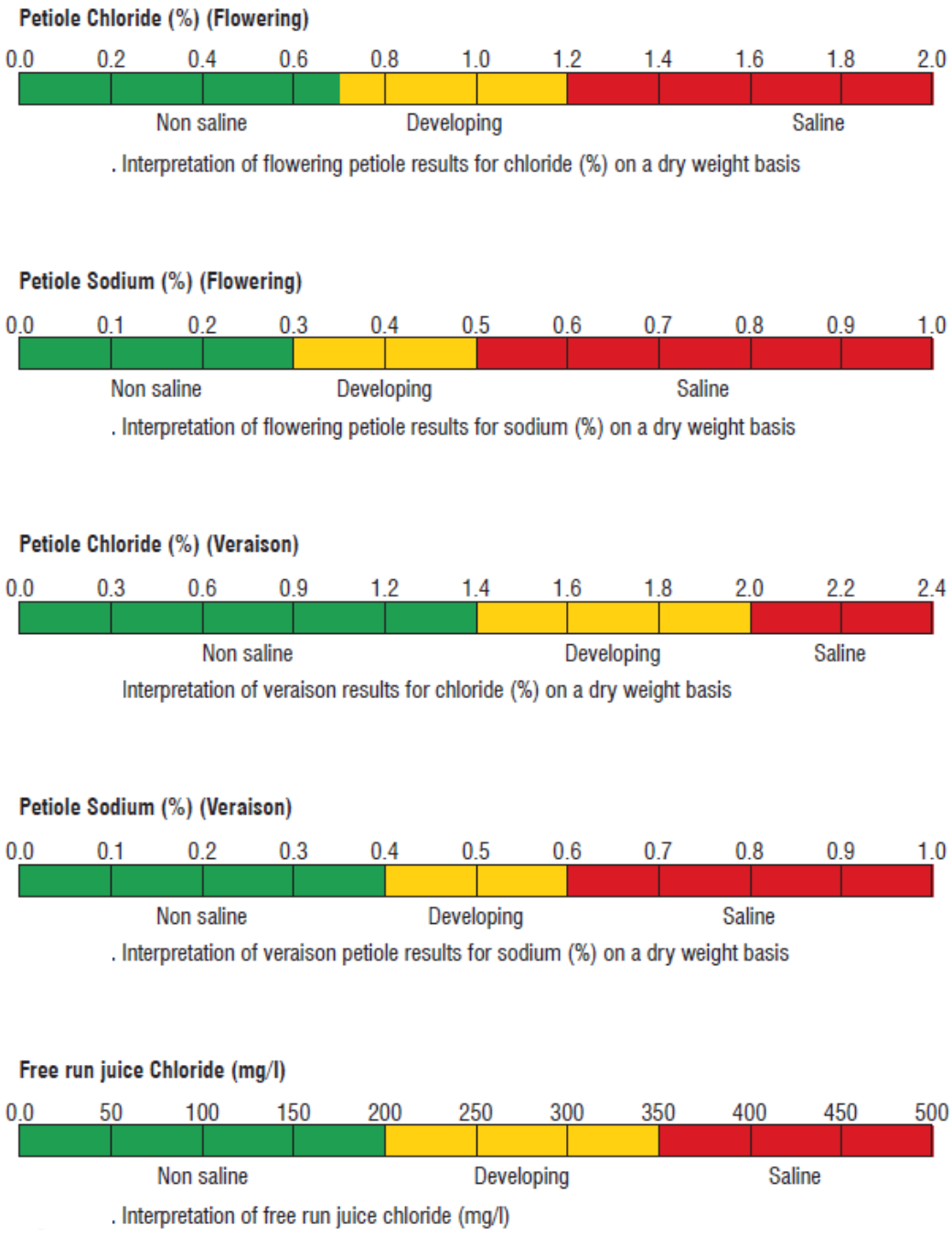


Figure 5.9 Recommended sodium, chloride levels in petiole and grape juice (adapted from Robinson 1992 and Lanyon 2011)

Based on the recommendations by (Lanyon 2011), for own rooted grapevines, yield decline begins at soil salinity EC_e above 2.1 dS/m. At EC_{sw} of less than 3.5 dS/m, no value of EC_e was above the threshold for salinity damage of 2.1 dS/m. Thus any measure below 3.5 dS/m suggests acceptable soil salinity. For extracts with salinity between 3.5 and 7 dS/m, the soil salinity could be either excessive or below the level of concern, and, for values in this range, a definitive assessment of vineyard salinity status could only be obtained by application of other methods. Values of EC_{sw} above 7 dS/m indicate an EC_e greater than 2.1 dS/m, and an

unacceptably high level of soil salinity. In the current study, EC for pre and post-treatment wastewater at 100% concentration was below 3.5 dS/m (maximum up to 2.5 dS/m) suggesting very low risk to the vines.

5.1.4 CONCLUSIONS

Treating the Shiraz vines with 25 and 50% WWW showed similar values for K, N and Cl in their petioles. The vines grew well and showed minimal effects of disease or insect infestation. Treating the vines with up to 10 and 16 times the amounts of K and Na, respectively (when compared to the winery wastewater applications), affected the overall vine vigour and the plants were noticeably more prone to insect damage. However, these effects did not translate into a marked difference in berry weights when we compared the weights from the glasshouse trial to those from the Yalumba Winery paired sites at Angaston and Oxford Landing. Average weights were 128 g (glasshouse) and 134 g (paired sites) per 150 berries over the same vintage period.

Using a shandied winery wastewater for vine irrigation can be an acceptable practice. Monitoring of Na and K, as well as EC, would be required prior to application to ensure that industry (maximum) recommendations are not exceeded.

5.1.5 REFERENCES

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5.2 Effect(s) of growing vines, (*Shiraz tahbilk*) using winery wastewater: two paired site trials

5.2.1 INTRODUCTION

Winemakers are reluctant to utilise winery wastewater (WWW) for irrigating vines due to its high sodium and potassium concentrations. Elevated berry potassium due to WWW application may influence the effect of other cations present and is thought to have impacts on fermentation properties and other wine sensory properties such as salty taste and bitterness, pH/sourness and has potential for some (additional) microbiologically influenced challenges. In the present study, two paired field sites were established in Barossa to assess the impact of winery wastewater irrigation on the growing vines.

5.2.2 METHODS

Two paired field trials were established in collaboration with Yalumba Winery, one located at Angaston and a second at Yalumba's Oxford Landing block at Moppa, Nuriootpa. At each of the sites the vineyard block was divided into two sections where irrigation water was supplied as bore water or mains water and winery wastewater. Soil, petiole and grape berry samples were collected over a three year period.

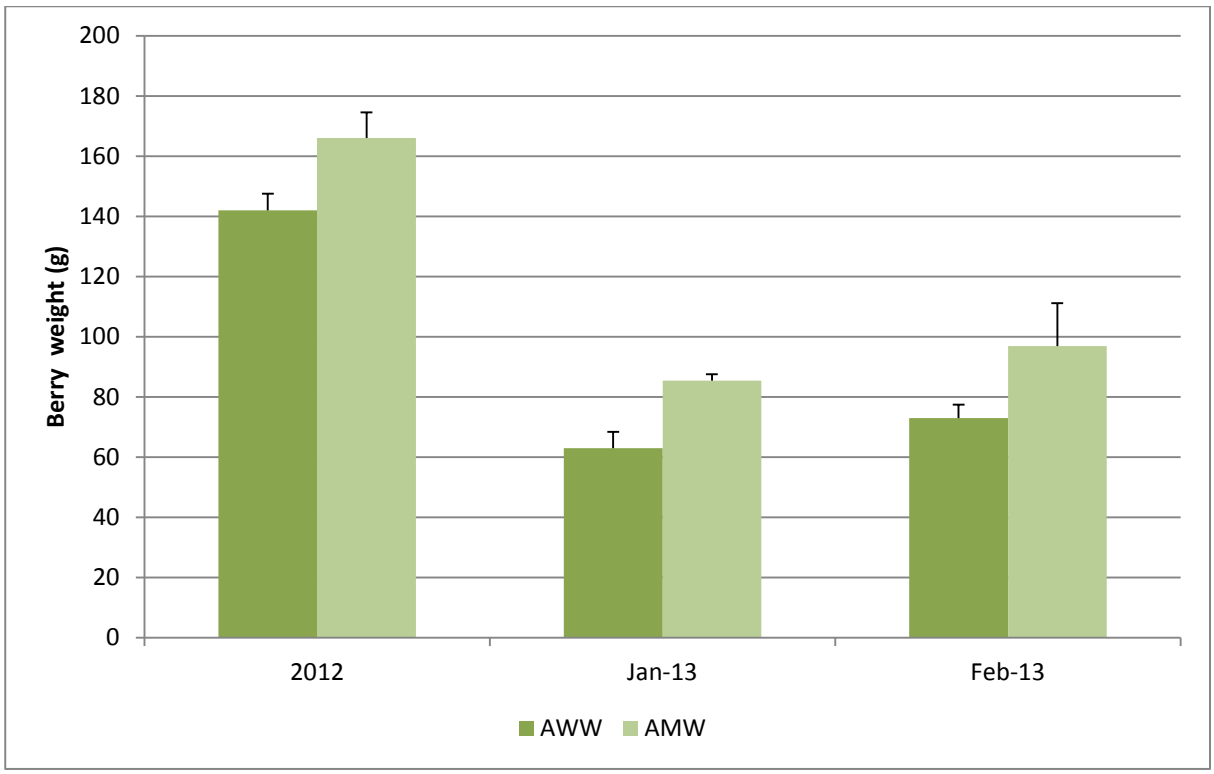
5.2.3 RESULTS

All results are summarised in Figures 5.10-5.19



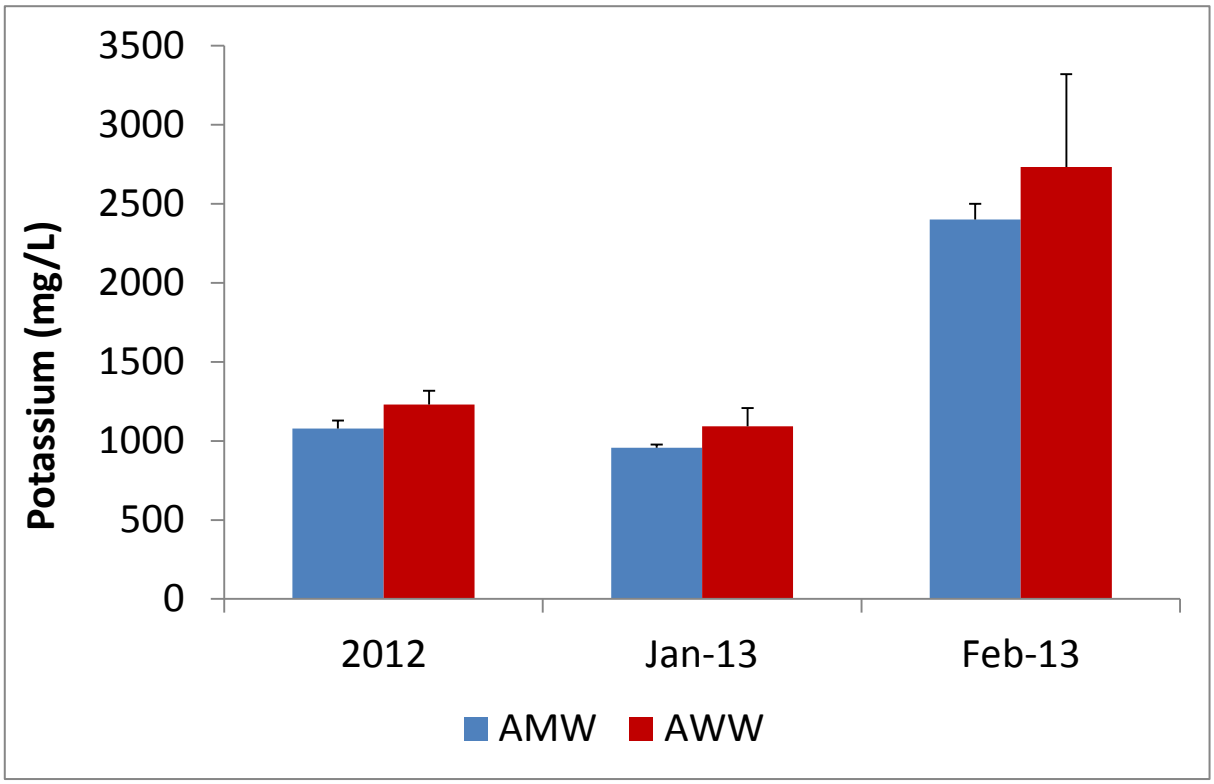
WWW- winery wastewater

Figure 5.10 Total irrigation volume at Angaston site



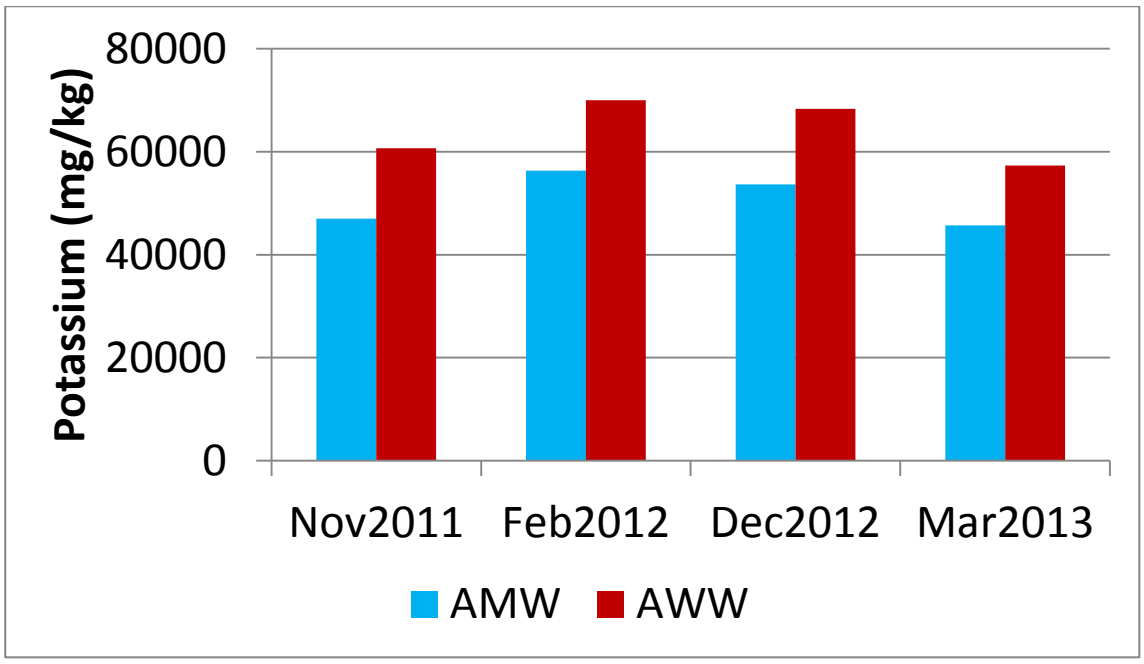
AWW: Angaston winery wastewater; AMW: Angaston mains water

Figure 5.11 Berry weights at Angaston site



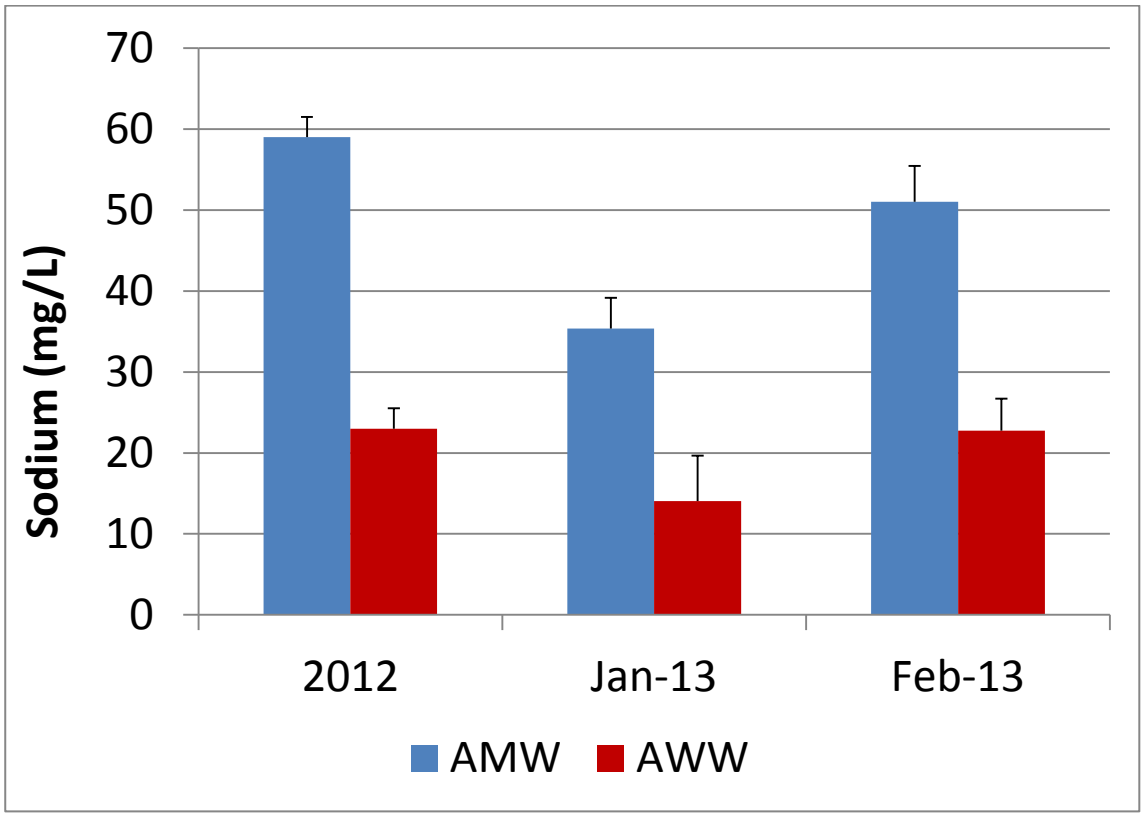
AWW: Angaston winery wastewater; AMW: Angaston mains water

Figure 5.12 Potassium in grape juice at Angaston site



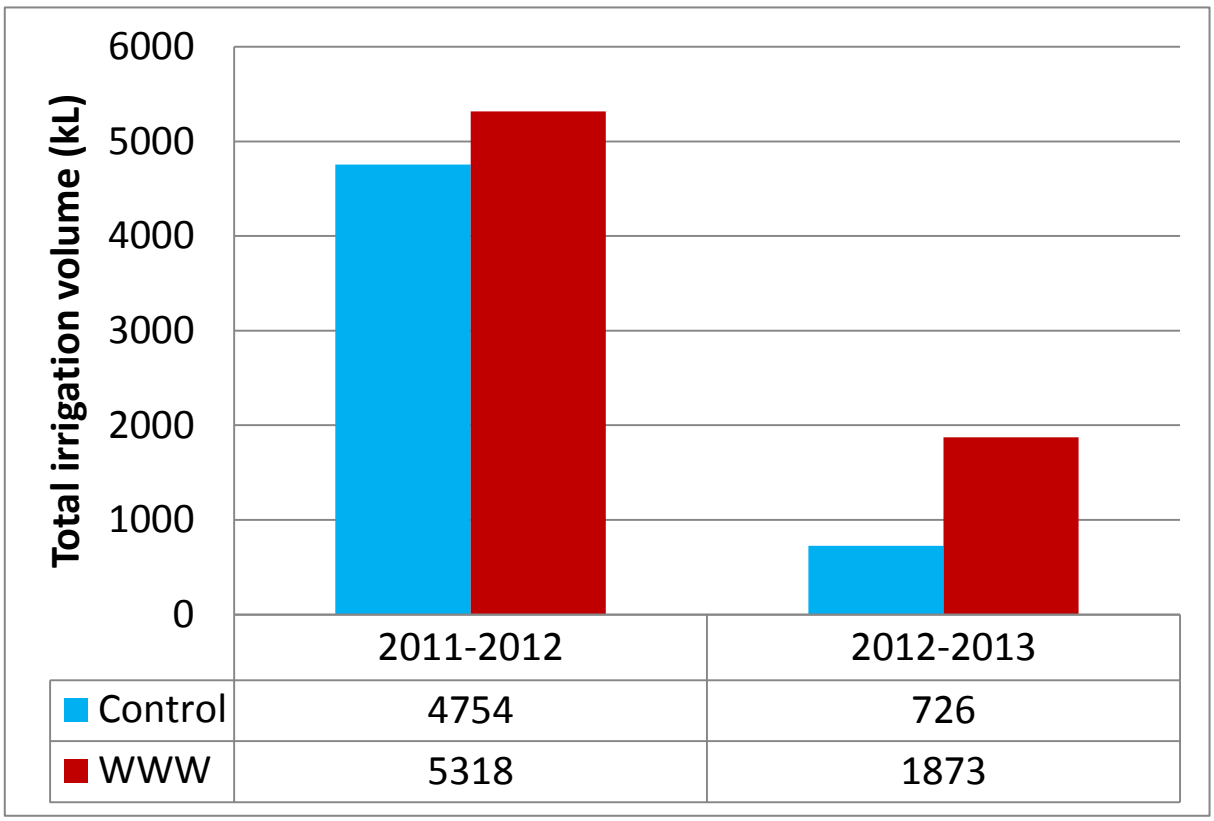
AWW: Angaston winery wastewater; AMW: Angaston mains water

Figure 5.13 Potassium in petioles at Angaston site



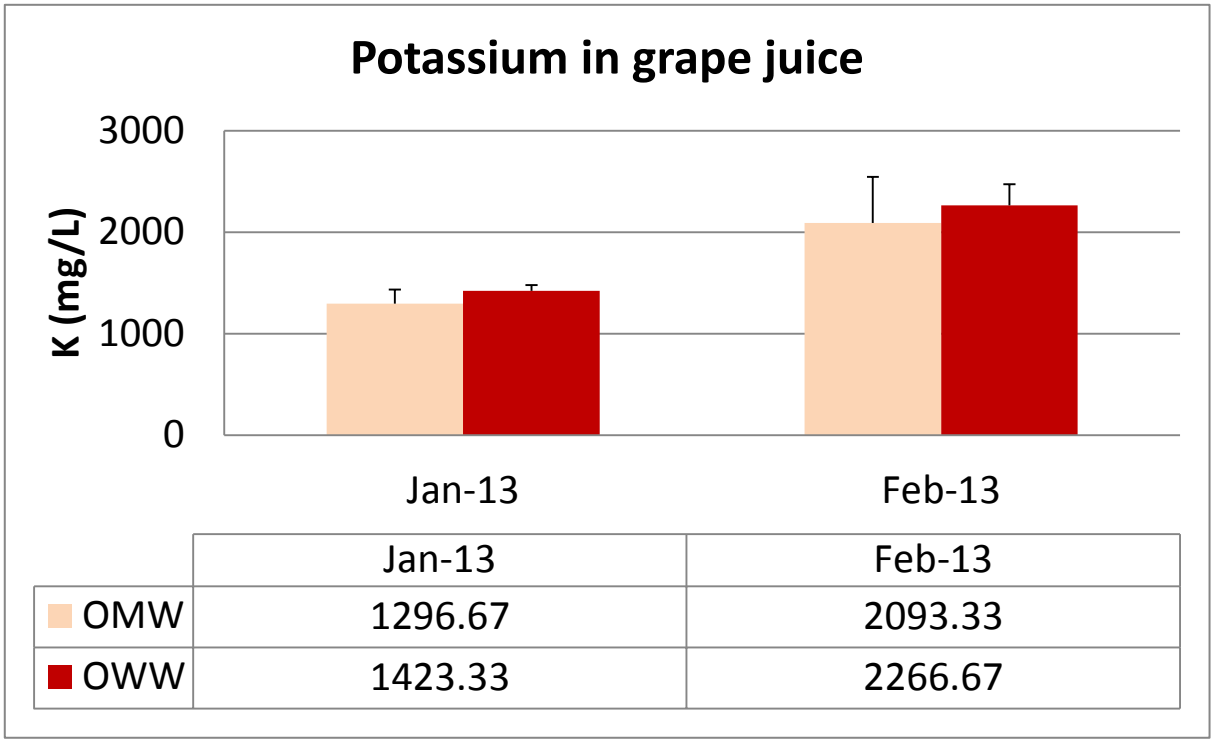
AWW: Angaston winery wastewater; AMW: Angaston mains water

Figure 5.14 Sodium in petioles at Angaston site



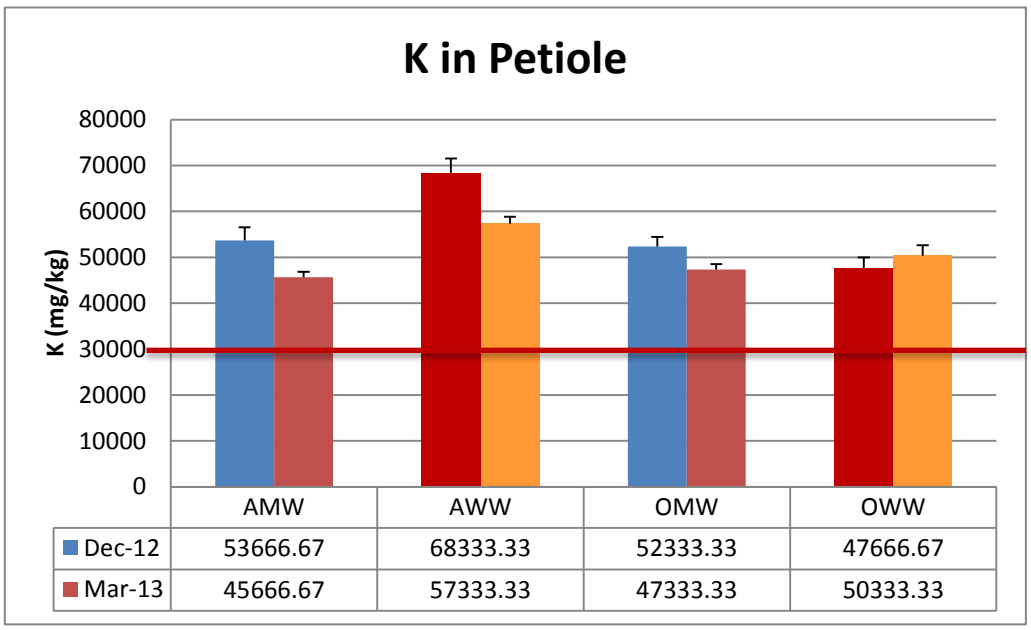
WWW- winery wastewater

Figure 5.15 Total irrigation volumes at Oxford Landing site



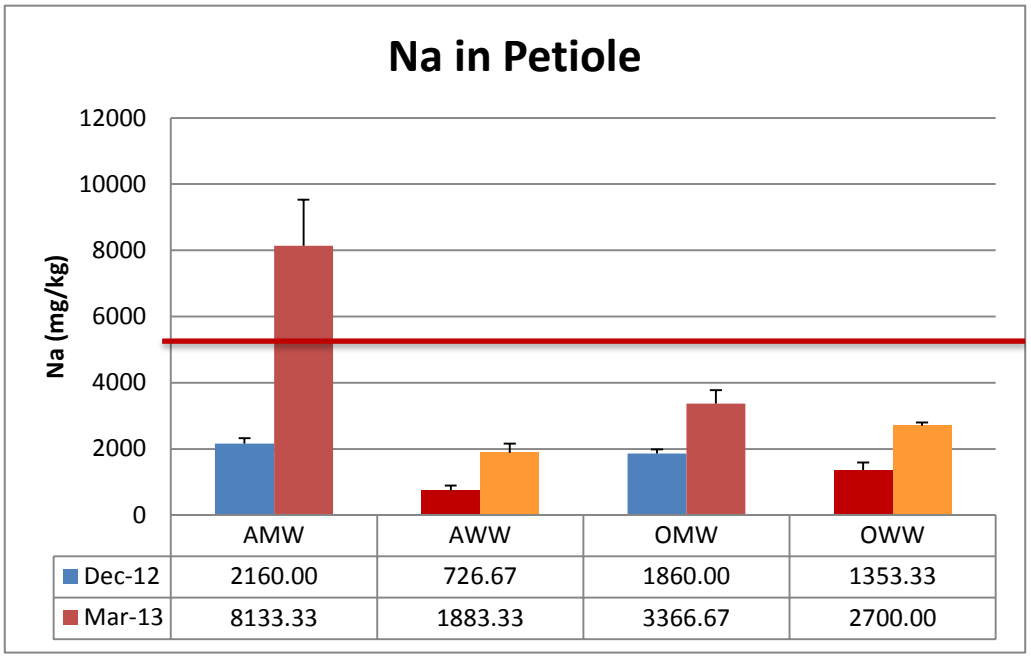
OMW: Oxford Landing mains water; OWW: Oxford Landing wastewater

Figure 5.16 Potassium in grape juice at Oxford Landing site



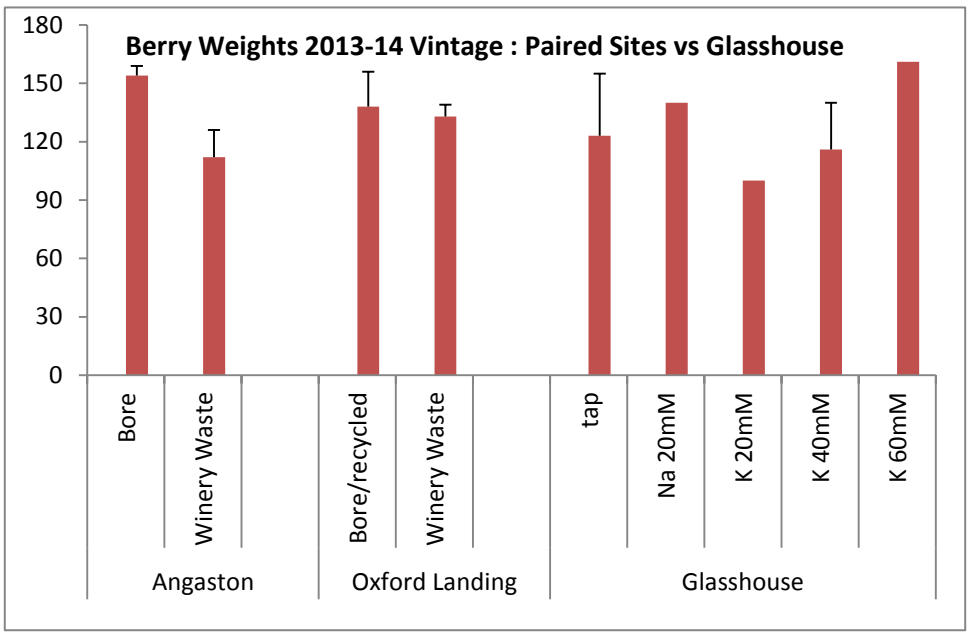
AWW: Angaston winery wastewater; AMW: Angaston mains water
OMW: Oxford Landing mains water; OWW: Oxford Landing wastewater

Figure 5.17 Potassium in petioles at Oxford Landing site



AWW: Angaston winery wastewater; AMW: Angaston mains water
OMW: Oxford Landing mains water; OWW: Oxford Landing wastewater

Figure 5.18 Sodium in petioles at Oxford Landing site



AWW: Angaston winery wastewater; AMW: Angaston mains water
OMW: Oxford Landing mains water; OWW: Oxford Landing wastewater

Figure 5.19 Berry weights, 2013-14 vintage, Paired Sites vs Glasshouse vines

5.2.4 RECOMMENDATIONS BASED ON LABORATORY AND FIELD TRIALS

Hird et al. (1996) have established limitations for wastewater application based on topographical and soil limitations (Table 5.3). If sodium is present in high amounts in poor quality irrigation water, it may replace calcium attached to clay particles. Soil then becomes sodic causing soil structural decline and is more prone to water logging and setting hard when dry. Stevens and Pitt (2012) evaluated soil salinity levels for own rooted grapevines, and stated that yield decline begins at a soil salinity EC_e above 2.1 dS/m. At EC_{sw} of less than 3.5 dS/m, no value of EC_e was above the threshold for salinity damage of 2.1 dS/m. Therefore, a salinity of 3.5 dS/m in the WWW can be considered as a safe level for grapevine irrigation. In the present study, the wastewater EC_{sw} was always below 3.5 dS/m and did not result in toxicity to grapevines during glasshouse and paired field trials. However, Na and K treatments above 40 mM and Na +K treatment at 20 mM each, were responsible for toxicity signs in the grapevines. These higher treatments were also prone to diseases.

A reduction in vine growth generally occurs when the average root zone salinity over the growing period exceeds a designated threshold value. Soil salinity thresholds have been developed for a range of grapevine varieties and rootstocks (Tables 5.4-5.6 and Figure 5.20). Based on the information available in the literature and the field and glasshouse trials conducted in this study, we have established sodium and potassium thresholds (individually and in combination, Table 5.7). Na +K in combination at 625+ 1084 mg/L, respectively, in winery wastewater will be safe for application on grapevines. These thresholds listed are indicative values and are also dependent on the variety and the rootstock used.

Table 5.3 Limitations for wastewater effluent application based on topographic and soil limitations

PROPERTY	LIMITATION			
	Slight	Moderate	Severe	Comment
Topographic Limitations				
Slope % (Sprinklers)	<6	6-12	>12	
Flooding	None-rare	Occasional-frequent		Flooding
Landform	Hillcrests, convex slopes and plains	Concave slideslopes and footslopes	Drainage plains and incised channels	Erosion and seasonal waterlogging risk
Surface rock outcrop	Nil	0-5%	>5%	Interferes with cultivation, risk of runoff
Soil limitations				
ESP	0-5	5-10	>10 (>40cm)	Structural degradation
EC (dS/m)	<4	4-8	>8	Excess salt restricts plant growth
Depth to high water table (cm)	>90	45-90	<45	Wetness, risk to groundwater
Depth to bedrock or hardpan (cm)	>90	45-90	<45	Restricts plant growth
Hydraulic conductivity (mm/hr, 0-100 cm)	20-80	2-20	<5	Excess runoff, waterlogging, acts as a poor filter
Available water capacity (mm/m)	>200	<200		Little plant available water
Bulk density (g/cm^3)				Restricts root growth
sandy loam	<1.4	>1.4		
loam and clay loam	<1.6	>1.6		
clay	<1.8	>1.8		
Surface Soil pH ($CaCl_2$)	>6.0	<6.0		Reduces optimum plant growth
CEC (cmol(+)/kg) for 0-40 cm	>15	<15		Unable to “hold” plant nutrient
P sorption index	>4	<4		Unable to fix phosphorus

Source: [From Hird *et al.* (1996)]

Table 5.4 A guide to salt tolerance of a range of varieties and rootstocks.

SALT-TOLERANCE CLASSIFICATION	SOIL SALINITY THRESHOLD (EC_e) FOR % YEILD LOSS			GRAPEVINE VARIETY OR ROOTSTOCK
	0%	25%	50%	
Sensitive	1.8 dS/m	4.1 dS/m	6.7 dS/m	**Own Roost**: Sultana, Shiraz, Chardonnay, Pinot Noir, Riesling, Semillon, Merlot, Cabernet Franc, Cabernet Sauvignon, Grenache **Rootstocks**: 3309, 1202C, K51-40
Moderately sensitive	2.5 dS/m	4.8 dS/m	7.4 dS/m	**Own Roots**: Colombard **Rootstocks**: Kober 5BB, 5C Teleki, Richter 110, Richter 99, K51-32, S04
Moderately tolerant	3.3 dS/m	5.6 dS/m	8.2 dS/m	**Rootstocks**: Rupestris St. George, Ruggeri 140, Schwarzmann, 101-14, Ramsey
Tolerant	5.6 dS/m	7.9 dS/m	10.5 dS/m	**Rootstocks**: 1103 Paulsen

Source: [Walker et al. 2002, Tee et al. 2003, Zhang et al. 2002 and Adams et al. 2006]

Note: These values are based on field trials over a 4-6 year period, More recent studies suggest that longer term exposure to saline conditions results in greater and more sustained yield losses at lower salinity levels.

Na and K levels in irrigation water also affect the amount of ions that accumulate in the vine, grape and ultimately wine. Our understanding of ion accumulation dynamics in grapes is not comprehensive but is dependent on variety and what rootstock is used. The Australian Food Standards Code (P4) (www.foodstandards.gov.au) specifies an upper limit of 1000 mg/L soluble chlorides expressed as sodium chloride (606 mg/L of Cl). While there is no standard for sodium in Australia, there are some potential export destinations including Canada, Switzerland and Poland that do specify maxima for sodium which range from 60 to 500 mg/L Na. In the two field trials conducted in this study, the sodium levels in wines produced from the grapevines irrigated with winery wastewater were below 100 mg/L confirming the safe use of winery wastewater for grapevine irrigation. In addition, the potassium level varied between 1220-1400 mg/L in the wines produced from wastewater irrigated grapevines and was within the range of potassium levels reported for red wines in Australia (up to 2700 mg/L).

Table 5.5 Guide to root-zone salinity threshold for grapevines when measured in SoluSAMPLER™ solution.

CROP SENSITIVITY	VARIETIES	EC_{sw} AT WHICH YIELD DECLINE STARTS
Sensitive to moderately sensitive	Own roots (Vitis vinifera): e.g. Sultana, Shiraz, Chardonnay. Rootstocks: 1202C, Kober 5BB, Teleki 5C, S04	3.6 (dS/m)
Moderately tolerant to tolerant	Rootstocks: e.g. Ramsey, 1103 Paulsen, Ruggeri 140, Schwarzmann, 101-14, Rupestris St. George.	6.6 (dS/m)

Source: [Modified from Zhang et al.2002. Australian Journal of Grape and Wine Research 8: 150-156 and R Walker, CSIRO Merbein, personal communication 2007.

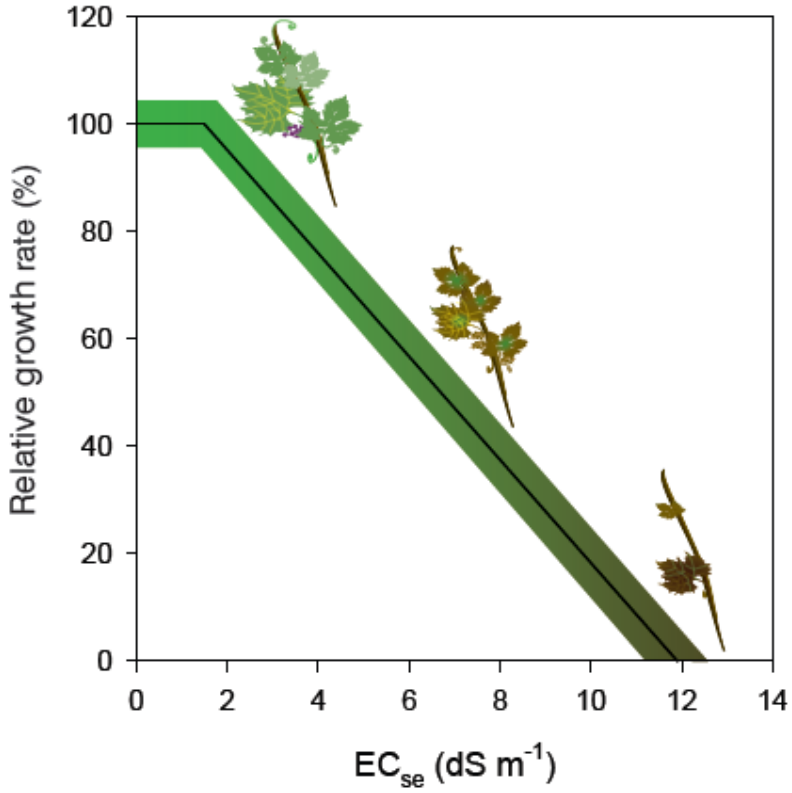


Figure 5.20 The relative response of vine growth to soil salinity (EC_{se}) where EC_{se} is the electrical conductivity of the saturated extract (source Mass and Hoffman, 1977)

Table 5.6 Salinity hazards for vines

SALINITY HAZARD	MEASURED AS SATURATION EXTRACT EC_{SE}	EFFECT ON VINES
Non-saline	<2	None
Slightly saline	2-4	Own rooted affected
Moderately saline	4-8	Some rootstocks tolerate
Very saline	8-16	No vines grow
Highly saline	>16	Vines die

Source: [Cass A., (1998) Measuring and managing chemical impediments to growth. Australian Grape Grower and Winemaker, July 1998, pp13-16.]

Table 5.7 Guidelines for interpreting laboratory data on water suitability for grapes. "Severe" in this table reflects an expected 25% reduction in productivity.

POTENTIAL IRRIGATION PROBLEM	UNITS	NONE	DEGREE OF RESTRICTION ON USE SLIGHT TO MODERATE	SEVERE
Osmotic effects[1]				
EC_w	ds/m	<1	1.0-2.7	>2.7
Toxicity effects				
Sodium (Na^+)[2]	mg/l or ppm	<460	-	-
Chloride (Cl^-)[2]	mg/l or ppm	<140	140 - 530	>530
Boron (B)	mg/l or ppm	<1	1 – 3	>3
Nitrate-nitrogen (NO_3-N)	mg/l or ppm	<5	5 - 30	>30
*Potassium	mg/L or ppm	<1250		
*Sodium + Potassium	mg/L or ppm	<625 + 1084 (20mM and 20 mM)		

Source: (Ayers and Westcot 1994; Nicholas 2004)
*This study
[1]Assumes that rainfall and extra water applied owing to inefficiencies of normal irrigation will supply the crop needs plus about 15 percent extra for salinity control.
[2]With overhead sprinkler irrigation, sodium or chloride in excess of 3mg/L under extreme drying conditions may result in excessive leaf absorption, leaf burn and crop damage. If overhead sprinklers are used for cooling by frequent on-off cycling, damage may occur even at lower concentration.

5.2.5 REFERENCES

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Walker RR, Blackmore CH, Clingeleffer PR, Correll RL (2002) Rootstock effects on salt tolerance of irrigated field-grown grapevines (Vitis Vinifera L. cv. Sultana). 1. Yield and vigour inter-relationships. Aust. J. Grape Wine Res., 8:3-14.

Tee E, Burrows D, Boland AM and Putland S 2003, Best Irrigation Management Practices for Viticulture in the Murray Darling Basin, Cooperative Research Centre for Viticulture, Adelaide.

Zhang X, Walker RR, Stevens RM and Prior LD (2002) Yield salinity relationships of grapevine (Vitis Vinifera L.) on own roots and a range of rootstocks, Aust. J. Grape Wine Res., 8:150-156.

5.3 Impact of irrigation wastewater on Shiraz wines

Two paired sites were established at Yalumba to determine whether there was a significant effect on the chemical and sensory properties of Barossa Shiraz wines made from grapes grown on vines irrigated with mains water and wines made from grapes grown on vines irrigated with WWW.

5.3.1 METHODS

Chemical analysis

Samples of each wine were submitted for the following:

- Basic wine attributes: % Alcohol, Titratable acidity at pH7.0 and 8.2, Volatile acidity as acetic acid, Glucose + fructose, specific gravity
- Colour attributes: % pigmented tannin, chemical age 1 and 2, colour density, free anthocyanins, hue, pigmented tannin, tannin, total phenolics, total pigment

All chemical analyses were performed by AWRI Commercial Services' NATA accredited (ISO 17025 certified) wine laboratory by trained staff in accordance with NATA accredited quality assurance measures. Standards, blanks, duplicates and control samples were included. The quality control measures were required to meet established criteria before acceptance of the analytical data.

Sensory analysis

The balanced reference triangle test was applied, following the Australian Standard: Sensory analysis of foods - Specific methods – Triangle Test AS 2542.2.2. The triangle test is a forced choice test and is not valid for determining preferences, nor the character or degree of any perceived difference.

In both investigations control and treatment wines were supplied as single wines only, with no replication. Details of the wines provided for each test are outlined in Table 5.8. Three bottles of each wine were supplied and bottles within each wine sample were blended before pouring.

Table 5.8 Treatment details for test 1 and 2

TEST	VINEYARD	TREATMENT DETAILS
1	Moppa Vineyard	Control (Mains water)
	Moppa Vineyard	Recycled water
2	Vintage Vignerons Site	Control (Mains water)
	Vintage Vignerons Site	Recycled water

Thirty experienced, screened and qualified assessors were asked to evaluate the wines for aroma and palate, selecting the sample in the set that was different from the other two. Panellists were also asked to comment on any differences between the wines, if possible. There was a forced rest of 60 s between sets.

Samples were presented to panellists in 30 mL aliquots in 3-digit-coded, covered ISO standard wine glasses at 22 – 24°C, in isolated booths under sodium lighting, with randomised presentation order across judges. Data was collected using a computerised data acquisition system (Fizz 2.47B, Biosystemes, France). Data analysis to determine statistical significance was carried out using the binomial model.

5.3.2 RESULTS AND DISCUSSION

Chemical analysis

Results from the analysis of basic wine attributes and colour attributes are presented below in Table 5.9.

Table 5.9 Chemical analysis of Moppa vineyard and Vintage Vignerons wines

ATTRIBUTE	MOPPA (MAINS)	MOPPA (RECYCLED WASTEWATER)	VINTAGE VIGNERONS (MAINS)	VINTAGE VIGNERONS (RECYCLED WASTEWATER)
% Pigmented Tannin	13.7	15.6	10.6	11.1
Alcohol (% v/v)	14.7	14.8	14.6	13.9
Chemical Age 1	0.38	0.40	0.31	0.34
Chemical Age 2	0.14	0.16	0.11	0.11
Colour Density (a.u.)	20.2	24.3	17.7	14.3
Free Anthocyanins (mg/L)	540	585	538	428
Glucose + Fructose (g/l)	0.4	0.5	0.5	<0.3
Hue	0.59	0.59	0.59	0.67
pH	3.85	3.84	3.7	3.75
Pigmented Tannin (a.u.)	4.8	6.1	3.5	2.9
Specific gravity	0.994	0.995	0.994	0.994
Tannin (wine) (g/L)	2.54	3.08	2.20	2.00
Titratable acid pH 7.0 (g/L)	5.1	5.2	5.5	5.6
Titratable acid pH 8.2 (g/L)	5.7	5.8	6.2	6.2
Total Phenolics (a.u.)	81	92	73	68
Total Pigment (a.u.)	35.0	39.5	32.7	26.3
Volatile Acidity as Acetic Acid (g/L)	0.58	0.72	0.57	0.58

The main differences between the wines in each pair appeared to relate to the colour attributes. With the Moppa samples, the wine made from fruit irrigated with recycled wastewater shows higher levels of colour and tannin than the one made from fruit irrigated with mains water. With the Vintage Vignerons samples however, this trend is reversed, with the wine made from fruit irrigated with recycled wastewater exhibiting lower levels of colour and tannin.

Sensory analysis

Table 5.10 below provides the results from the sensory triangle tests for both pairs of wines.

Table 5.10 Results of triangle tests for main water vs recycled water irrigation

COMPARISON	CORRECT ANSWERS	SIGNIFICANCE
Moppa Vineyard Mains vs Recycled wastewater	10	ns
Vintage Vignerons Site Mains vs Recycled wastewater	12	ns

ns – not significant at 5% ($P < 0.05$) level

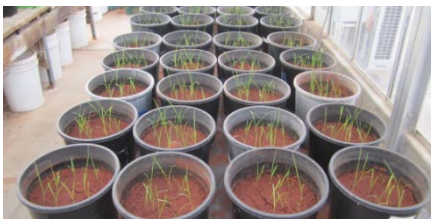
There was no significant difference between the control and treatment wines for either pair of wines (Table 5.10). There were a number of comments from the assessors regarding bitterness and astringency; however, there was no pattern in the comments, so they did not yield any additional information about any perceived differences between treatments.

The test provides good statistical power, as calculated after the test, of greater than 0.97 and greater than 0.89 respectively in tests 1 and 2, of finding a difference if one existed for the treatment, given 25% of

correct answers above chance. In other words, if 25% of assessors could perceive an effect of the treatment, the tests would have had greater than 97% and 89% likelihood of finding the difference.

5.3.3 CONCLUSIONS

Chemical and sensory analysis carried out on the two pairs of wines, Moppa Vineyard and Vintage Vignerons Shiraz, shows small differences between the wines in each pair, predominantly due to colour and tannin attributes. These could be related to the sites chosen for fruit sampling and/or the impact of winemaking techniques. The direct impact of the irrigation water type used can only be confirmed or refuted with a higher degree of sample and winemaking replication. Based on the present study, sensory evaluation indicated that there was no significant difference between the control and treatment wines for either pair of wines.

Part VI Beneficial crops

6 Beneficial crops

6.1 Effect of winery wastewater on plant germination

6.1.1 INTRODUCTION

Land disposal of winery wastewater onto field crops, tree lots, pastures and vineyards is a widely used means of wastewater management. Wastewater reuse in agriculture has the potential to both treat a waste product at the same time as harnessing a valuable water resource. Winery wastewater (WWW), with appropriate treatment, can be a safe, affordable and sustainable resource of irrigation water. This process is simple, low cost, and requires minimal technical expertise; however there is limited understanding of the consequences of such a practice, and it has had variable success. Many wineries have used treated wastewater, or wastewater that has only undergone primary treatment, to produce a commercial crop. Numerous wineries are also considering using WWW for growing grasses, root crops or cereals, all of which have been trialled by some wineries. However, understanding the best management of WWW (timing, rate, application) so that it does not lead to deterioration of soil (salinity, sodicity and pH) or crop health (phytotoxic shock, yield, crop quality) remains unclear.

The aim of the current study was to assess the use of winery wastewater irrigation for germination of following crops:

1. kikuyu grass
2. turnip
3. field pea
4. cucumber
5. silver beet
6. zucchini

The crops selected represent a range of grasses, legumes and vegetables. The water requirement of vegetable crops such as turnip, zucchini and cucumber is generally high and hence the removal of Na and K from such crops during WWW irrigation would be at maximum level. Kikuyu grass is known to be salt tolerant and has potential for commercial use.

6.1.2 METHODS

The study was conducted in both a controlled environment room and a glasshouse. Germination tests were initially conducted in a controlled environment, temperature range 15-25^{o}C, day/night cycle of 14 and 10 h respectively. The main body of the experimental work was in a glasshouse with temperature only being adjusted with an air conditioning system if 25°C was exceeded.

A range of tap water (TW) through to WWW shandies in the ratios 100:0, 90:10, 75:25, 50:50, 25:75 and 0:100 were used in this study.

Two hundred grams of air dried, <2 mm soil, was weighed into 250 mL plastic pots, (no drainage holes). Each pot was adjusted to 40 % water holding capacity (WHC) by using the appropriate treatment solution. The moistened soil was allowed to ‘equilibrate’ for three days prior to sowing with twenty (20) seeds. After sowing pots were maintained at the set WHC on a daily basis, using the treatment solutions. There were four replicates per treatment and the germination study was conducted over 18 days. Physical characteristics of the soil are given in Table 6.1 and the wastewater characteristics are listed in Table 6.2.

Table 6.1 Physicochemical characteristics of study soil

Soil	EC (dS/m)	pH	Cl (mg/kg)	Ca (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)	K (cmol/kg)	CEC (NH_4) (cmol/kg)
Berton Winery	0.029	6.2	0.55	1.5	0.34	0.11	0.08	11

Table 6.2 Wastewater Quality for Germination Experiment

TREATMENT TW:WWW	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	K (mg/L)	COD (mg/L)	pH	EC (mS/cm)
0:100	28.7 ± 0.5	12.80 ± 0.25	41.50 ± 1.5	3.2 ± 0.08	>10	7.78 ± 0.02	0.50 ± 0.15
75:25	26.6 ± 0.39	12.08 ± 0.38	56.5 ±0.75	171.3 ± 3.6	840 ± 130	6.51 ± 0.01	1.50 ±0. 15
50:50	28.5 ± 0.65	11.26 ± 0.29	72.5 ± 2.3	344.4 ± 4.2	2400 ± 100	6.42 ± 0.03	2.51 ±0.28
25:75	29.9 ± 0.59	10.05 ± 0.06	86.8 ± 2.3	502.8 ± 3.2	3980 ± 140	6.12 ± 0.01	3.46 ± 0.25
0:100	28.52 ± 2.19	9.56 ± 0.23	102.3 ± 8.5	651± 18	5200 ± 222	6.00 ± 0.02	4.42 ±0.38

6.1.3 RESULTS

Germination of kikuyu grass

A generalised linear model found no statistically significant effect on seed germination of the proportion of winery wastewater in the shandy.

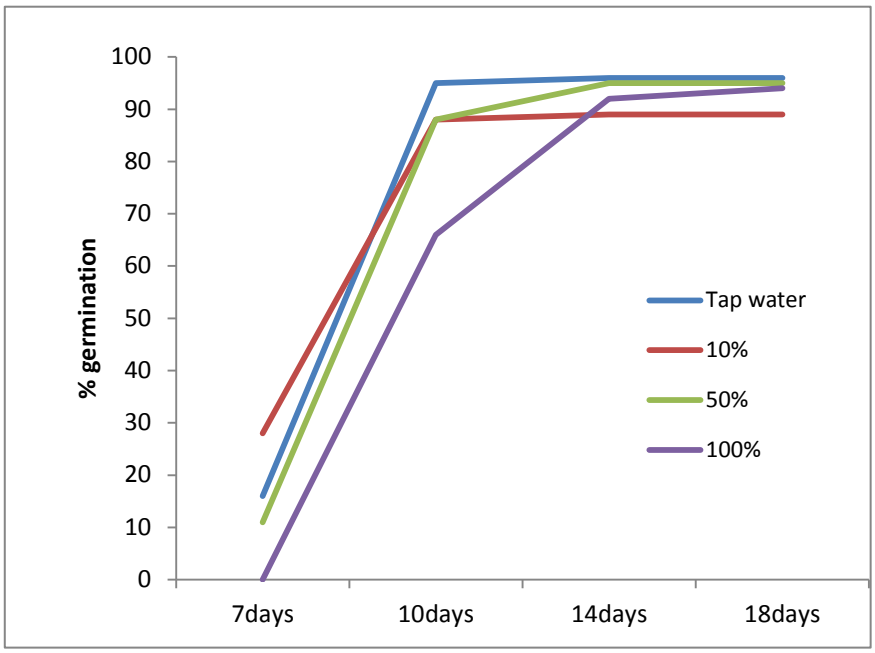


Figure 6.1 Effect of proportion of winery wastewater on germination percentage of kikuyu seed

There was a similar increase in the germination time of turnip seeds. A summary of these effects are given in Table 5. The effect on germination percentage is shown in Figure 6.1 and the effect on time to

germination in Figure 6.2. Although the effect on germination time was very significant ($p < 0.001$), the actual increase was only approximately a day (or 10% increase) from tap water to winery wastewater.

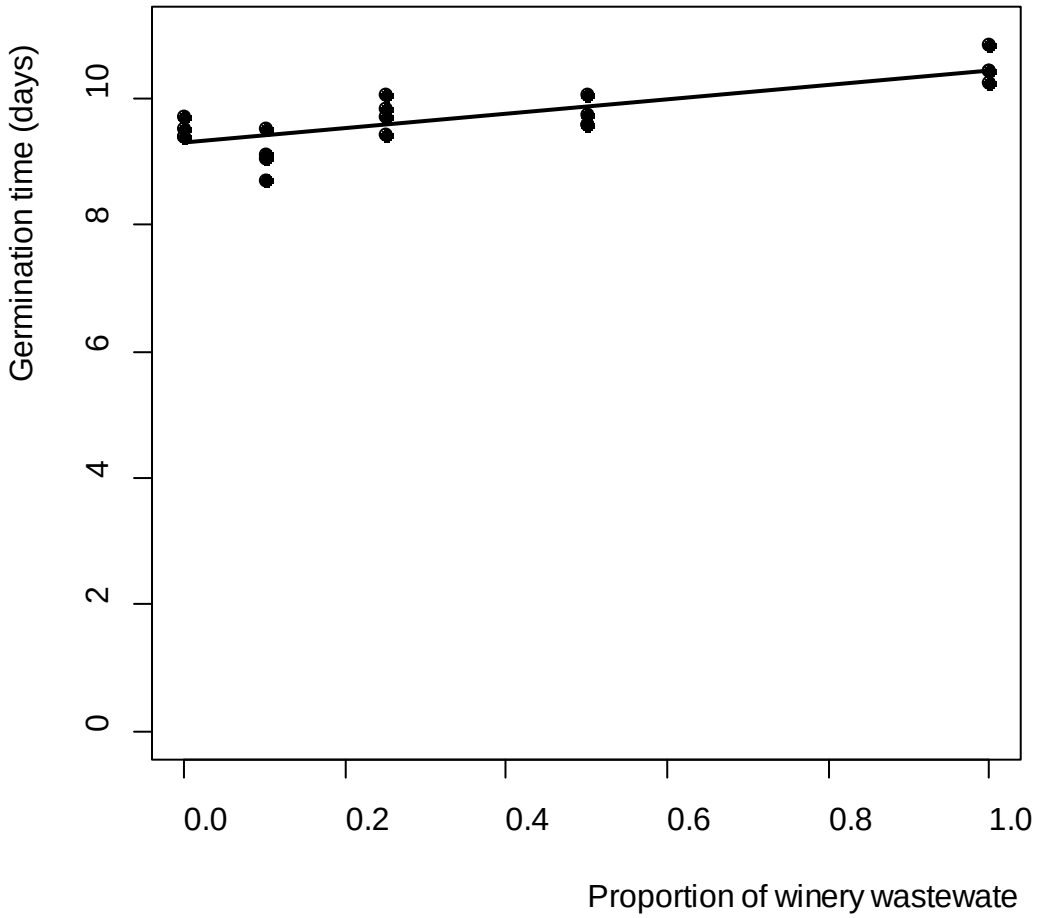


Figure 6.2 Effect of proportion of winery wastewater on germination time of kikuyu seeds

Field peas

Mean germination time for the field peas was eight days. There was no effect of increasing the proportion of WWW on the germination percentage (Figure 6.3) or on the time to germination (Figure 6.4).

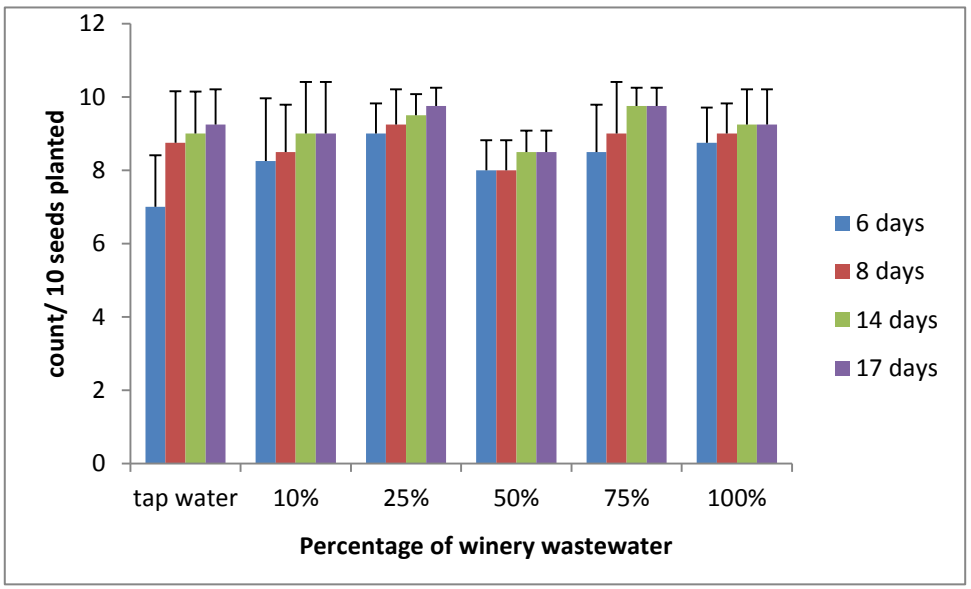


Figure 6.3 Effect of proportion of winery wastewater on number of pea seeds (out of 10) germinated

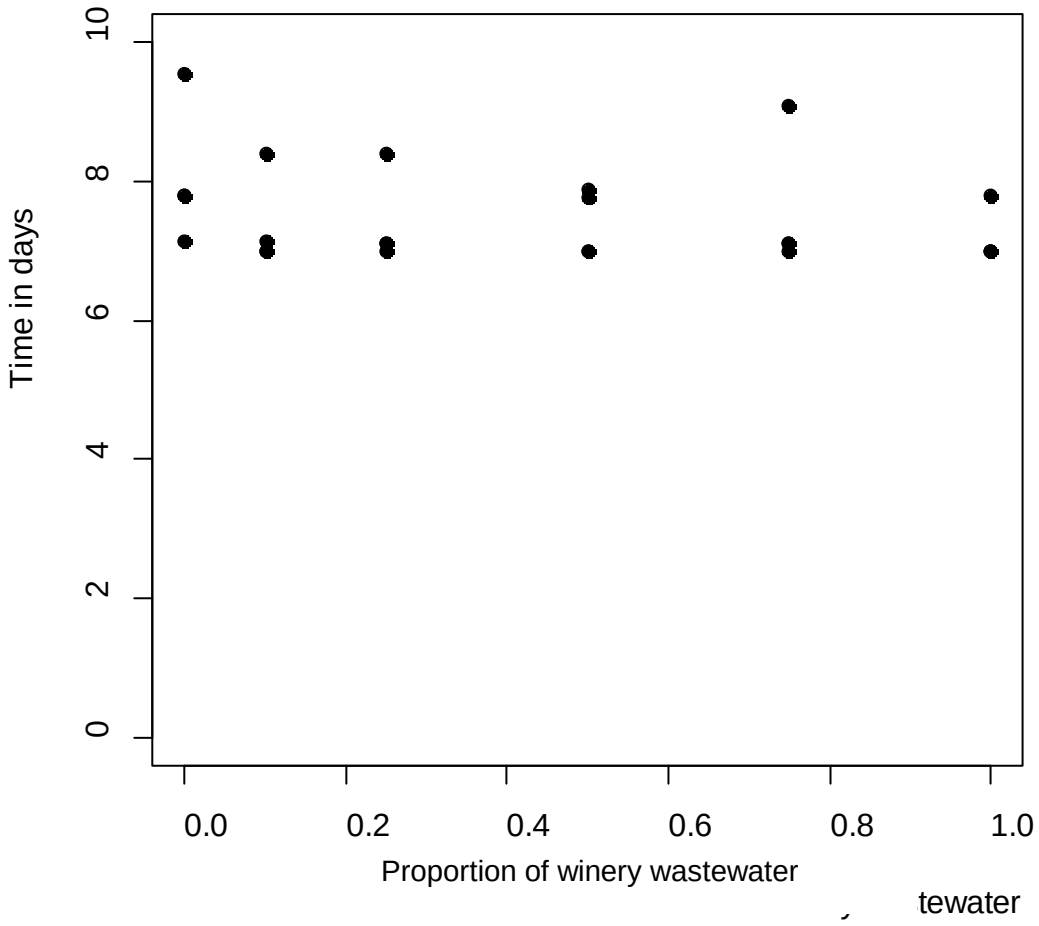


Figure 6.4 Effect of proportion of winery wastewater on germination time of field pea seeds

Cucumber

There was no effect of increasing the proportion of winery wastewater on the germination percentage (Figure 6.5) or on the time to germination (Figure 6.6).

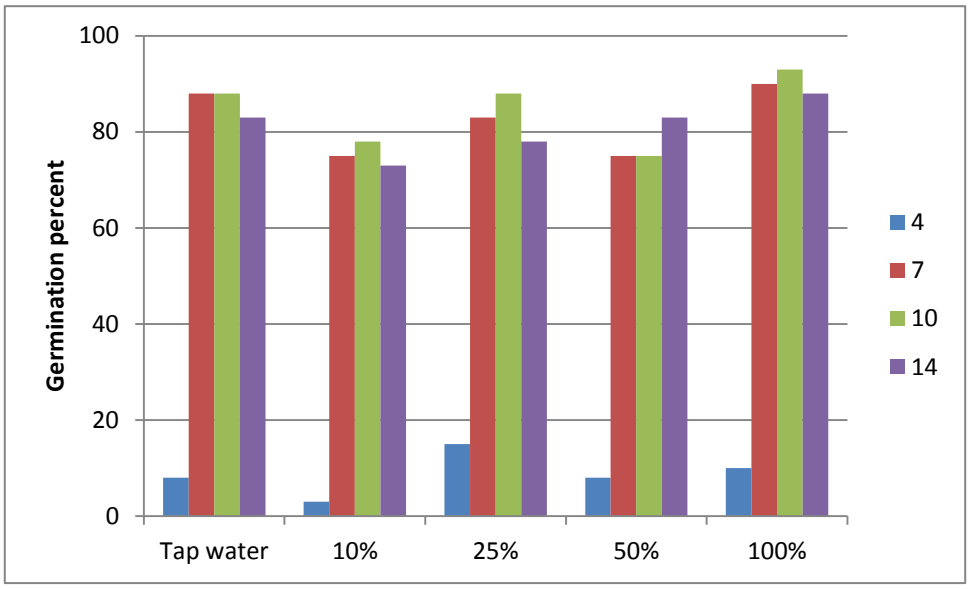


Figure 6.5 Effect of proportion of winery wastewater on germination percentage of cucumber seed

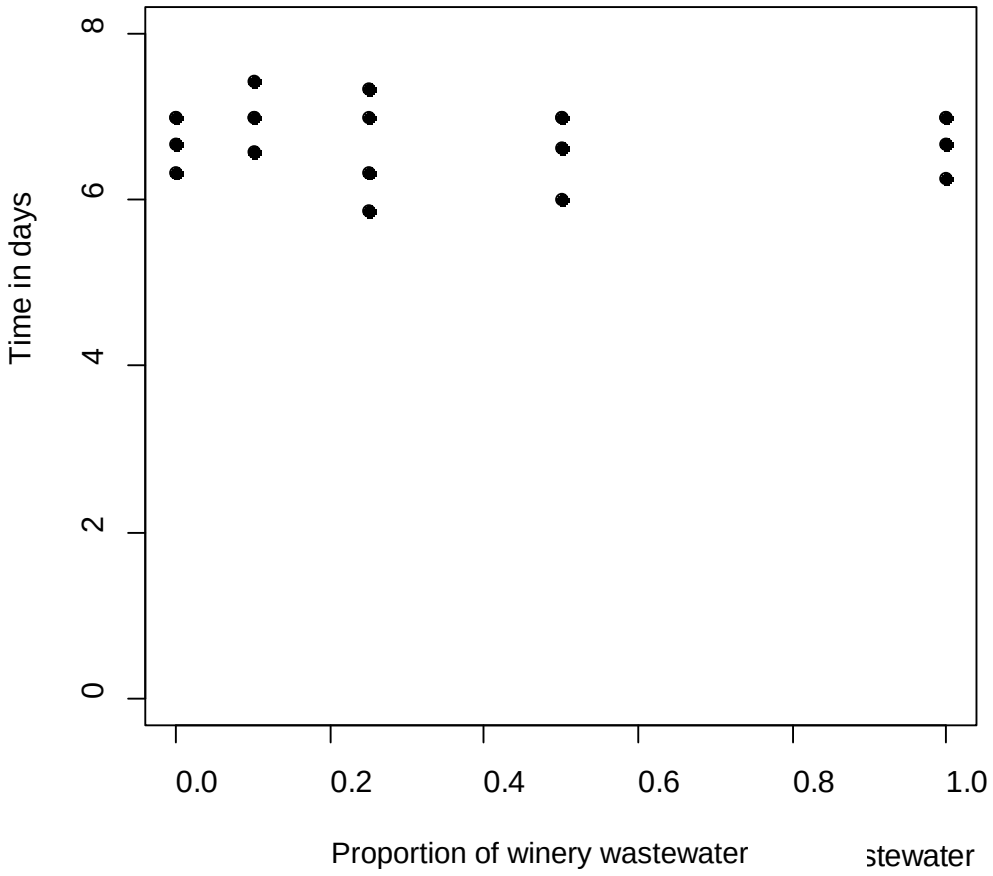


Figure 6.6 Effect of proportion of winery wastewater on germination time of cucumber seeds

Zucchini

There was a positive effect of winery wastewater on the germination percentage of zucchini (Figure 6.7). This was confirmed using a generalised linear model (with binomial errors) that indicated statistical significance at the $p < 0.01$ level. A linear regression indicated that the proportion of winery wastewater had no effect on germination time (Figure 6.8).

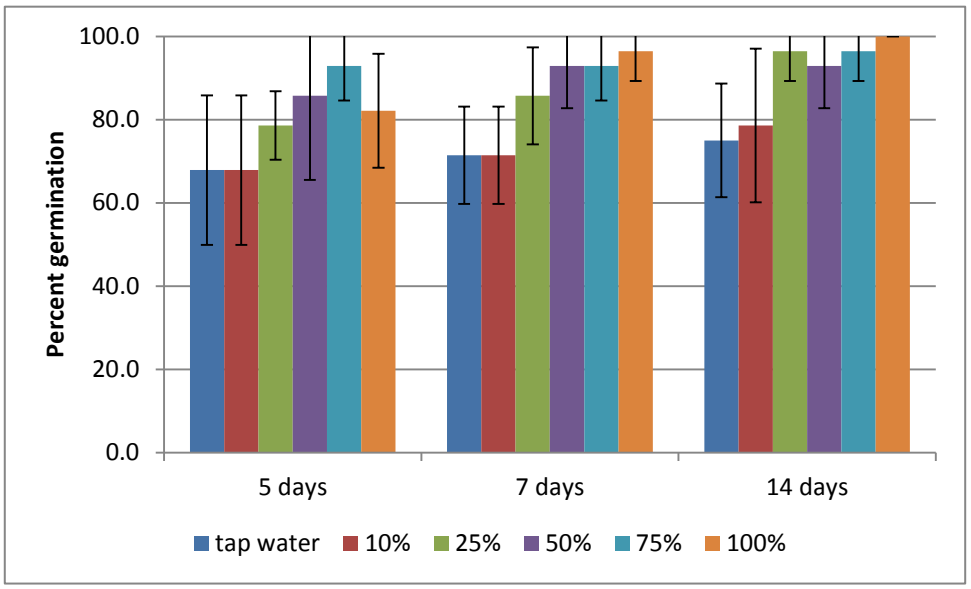


Figure 6.7 Effect of concentration of winery wastewater on germination percentage of zucchini

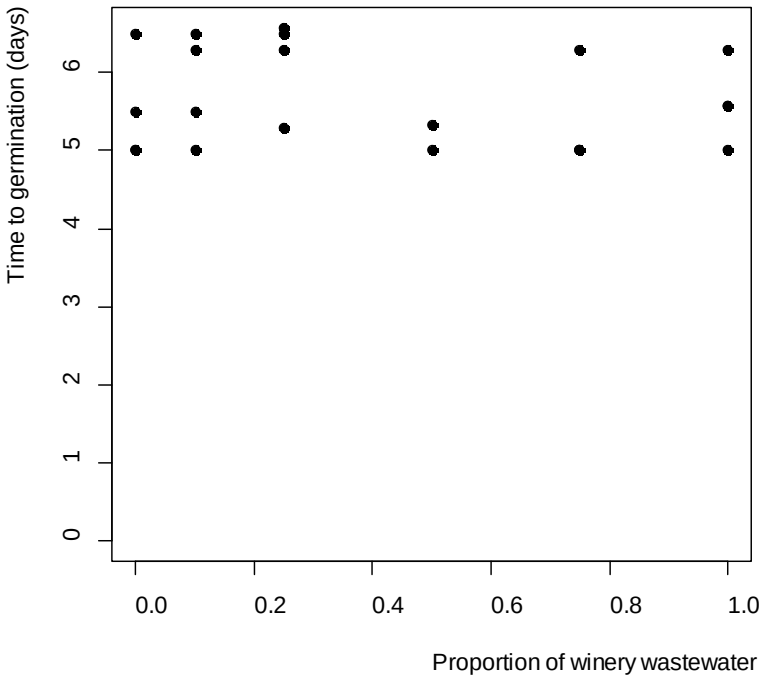


Figure 6.8 Effect of proportion of winery wastewater on germination time of zucchini

Turnip

The final germination percentage was not affected by the proportion of WWW (Figure 6.9). There was however a marked increase in germination time, being five days for concentrations up to 50%, but then increasing to eight days. A linear regression indicated that the effect was statistically significant ($p < 0.05$), but the data were clearly not linear (Figure 6.10).

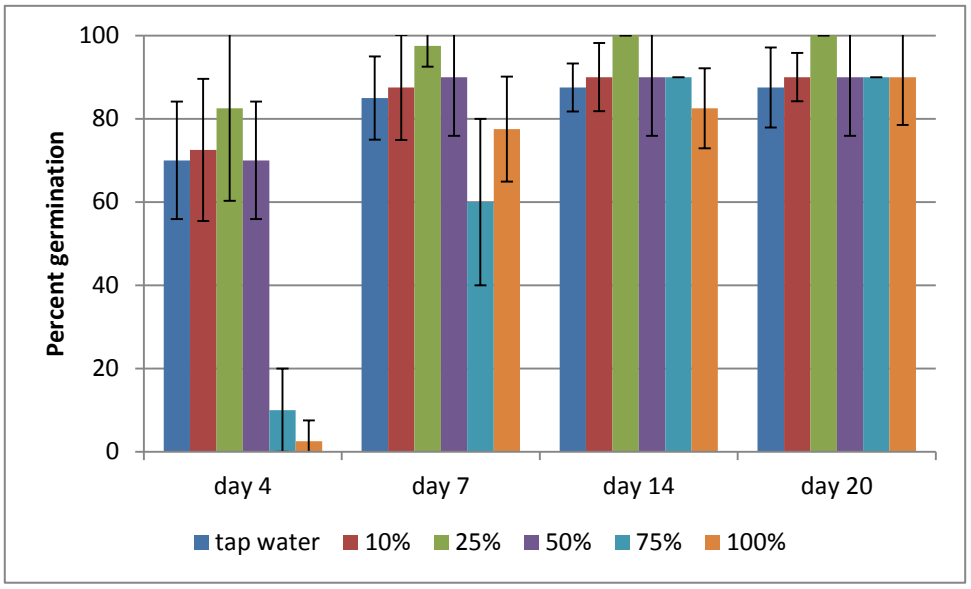


Figure 6.9 Effect of concentration of winery wastewater on germination percentage of turnip

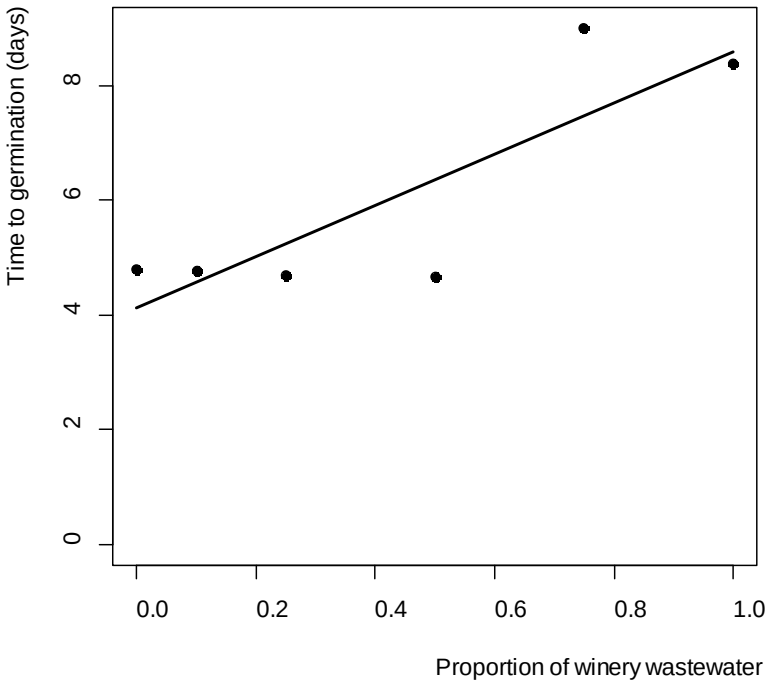


Figure 6.10 Effect of proportion of winery wastewater on germination time of turnip

Silver beet

Winery wastewater decreased germination from 90% to 70% ($p < 0.05$) as can be seen in Figure 6.11. A linear regression showed no effect on germination time – those data are shown in Figure 6.12.

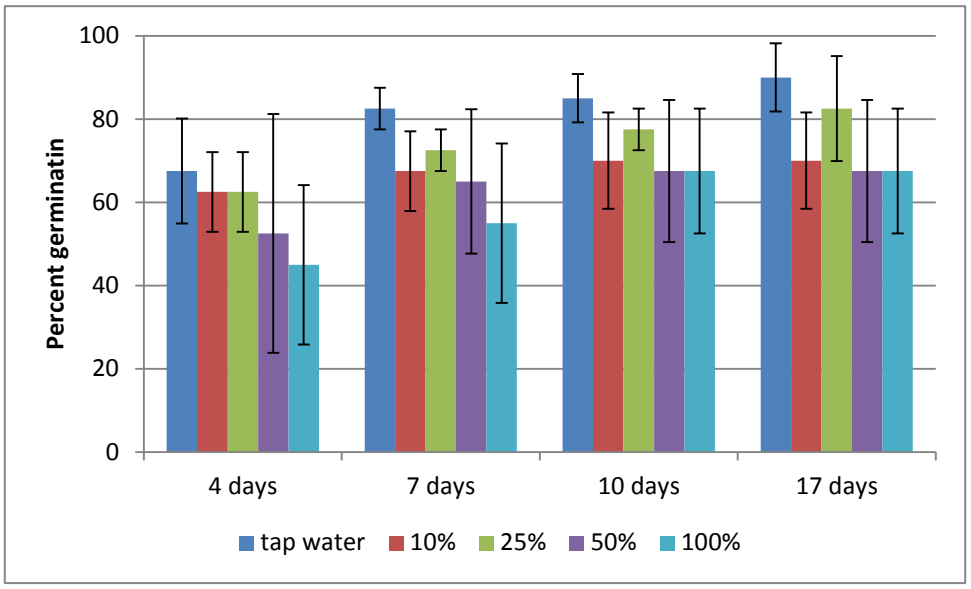


Figure 6.11 Effect of concentration of winery wastewater on germination percentage of silver beet

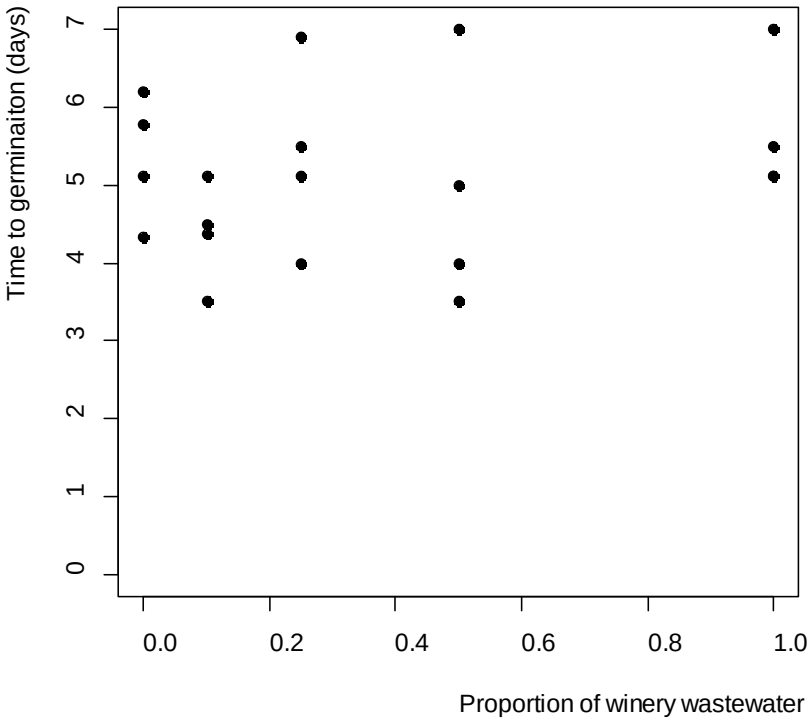


Figure 6.12 Effect of proportion of winery wastewater on germination time of silver beet

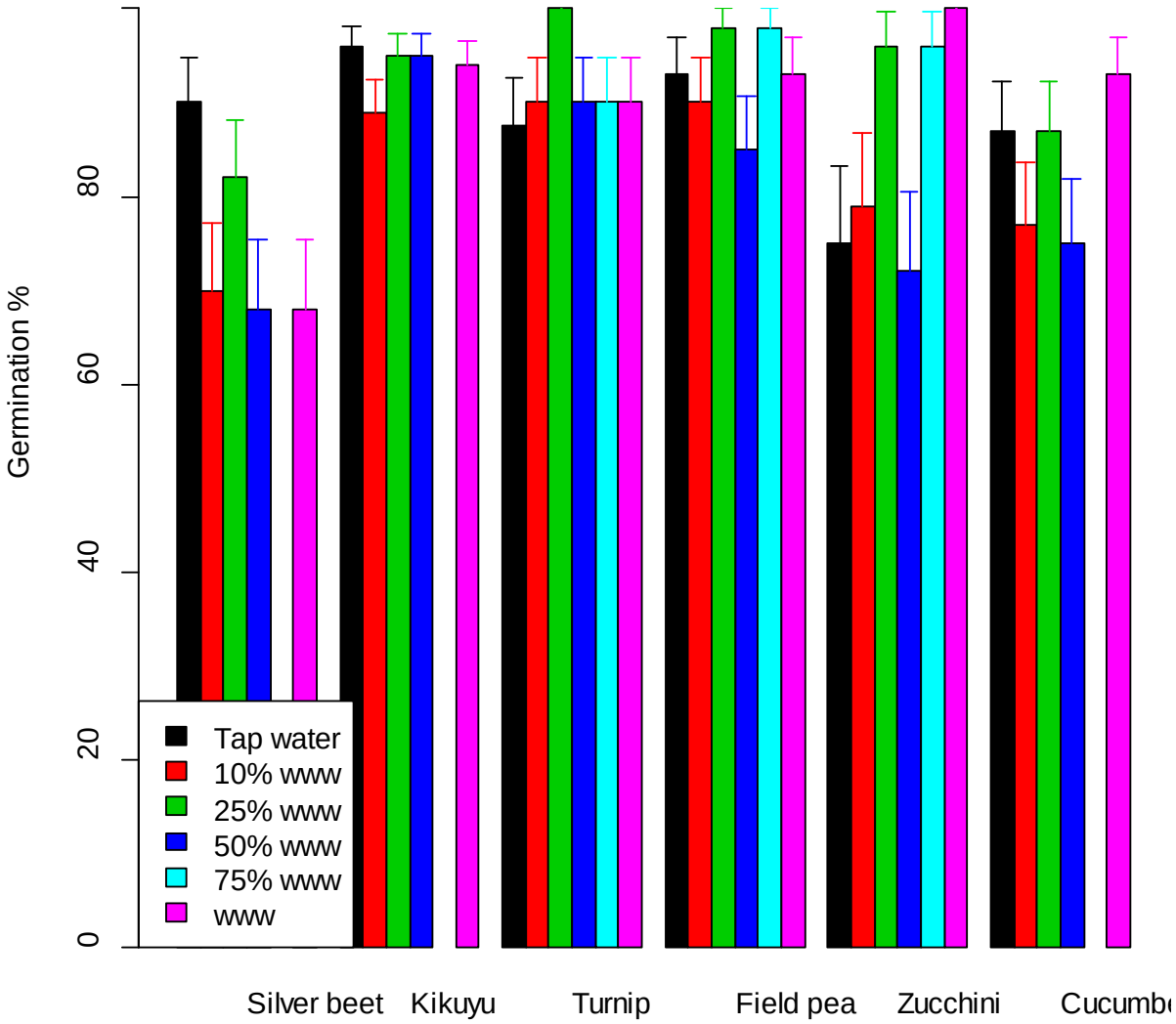


Figure 6.13 Effect of increasing concentration of WWW on percentage and mean percentage germination of kikuyu

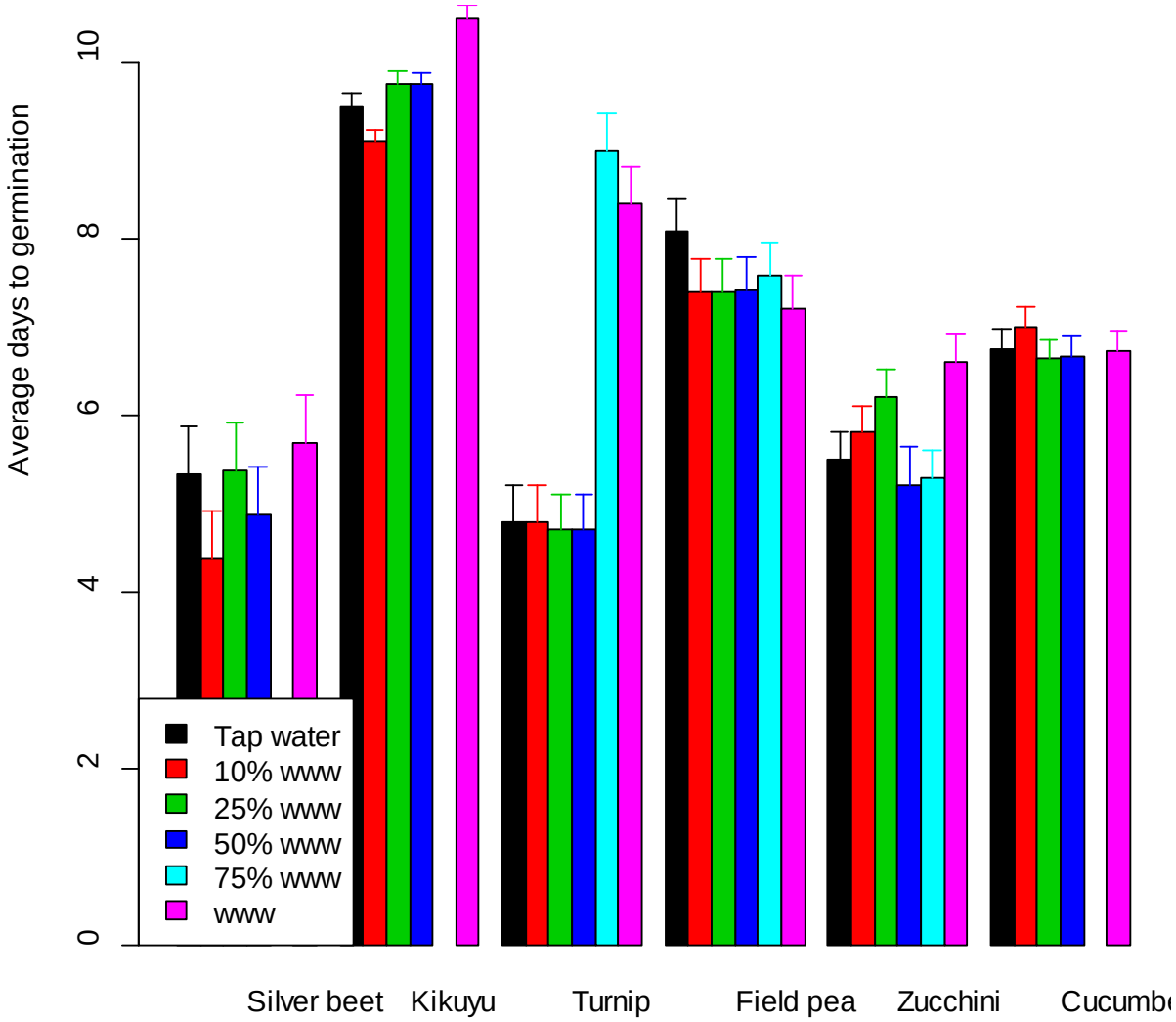


Figure 6.14 Days to germination for six species of plant that received a range of WWW concentrations. The error bars are standard errors derived from analyses of variance

6.1.4 DISCUSSION AND CONCLUSION

The addition of WWW in general had little effect on the germination percentage. Germination in silver beet was affected ($p < 0.05$). In general, a decrease in the germination rate with increased WWW addition was observed in zucchini ($p < 0.05$). There was no significant effect observed in the other four species. Overall the data suggest that the addition of WWW had little effect over the range of species tested.

There was a general increase in germination time for kikuyu grass. There were significant differences among the groups ($p = < 0.001$) germination time with increasing WWW concentration, ranging from 9.5 days with tap water to 10.5 days with 100% WWW. Over 80% of the difference among dilution treatments could be explained by a linear trend.

Table 6.3 Summary of effects of increasing winery wastewater concentration on germination percentage time to germination

TAXON	EFFECT ON GERMINATION %	EFFECT ON TIME TO GERMINATION
Kikuyu	No significant effect	9.5 increasing to 10.5 days
Peas	No significant effect	No significant effect
Cucumbers	No significant effect	No significant effect
Zucchini	Increase ($p<0.01$) from 80% to 100%	No significant effect
Turnip	No significant effect	Increase 4.7 to 8.7 days
Silver beet	Decrease ($p < 0.05$) 82% decreased to 65%.	No significant effect

6.2 Effect of winery wastewater on plant growth

6.2.1 INTRODUCTION

Kikuyu grass (*Pennisetum clandestinum*) is a perennial yellowish-green grass that often forms turf. This grass spreads by numerous large, fleshy, creeping stolons and rhizomes that sometimes reach two metres or more in length and depth. Its stolons root readily at each node, producing stout leafy branches 60 cm in length. Thus, it is considered an excellent coloniser and soil stabiliser. Kikuyu grass is able to recover rapidly from wear and divot injury and competes well with weed invasion. It can tolerate a wide range of water supply and soil pH between 5.5 and 8.0, persisting under annual rainfall of at least 600–700 mm and developing well from sea level to 3500 m (Skerman and Riveros, 1990). The optimum temperature for kikuyu grass growth is said to be in the range 18–30^{o}C (Russel and Webb, 1976) but it retains active growth and colour at lower (10^{o}C) and higher (38^{o}C) temperatures. The attention of researchers to this grass is due to its high growth rate and well-developed root system and to its high nutritive properties as pasture.

In addition, this grass has good drought, heat and salinity tolerance. This allows it to grow under irrigated and rain-fed conditions. It is noted that both of these types of agriculture are threatened by salinisation. Thus turf grass *P. clandestinum* may be an important candidate plant for utilisation and reclamation of salinised areas to be used as a potential ground cover and as fodder grass. The aim of the current study was to assess the use of winery wastewater irrigation for growing kikuyu grass. Growth data are also presented for turnip and field peas.

6.2.2 METHODS

The study was conducted in both a controlled environment room and a glasshouse. Germination tests were initially conducted in a controlled environment, temperature range 15-25^{o}C, day/night cycle of 14 and 10 h, respectively. The main body of the experimental work was in a glasshouse with temperature only being adjusted with an air conditioning system if 25^{o}C was exceeded.

18-week Kikuyu trial

We used the WWW:tap water ratios, 0:100, 25:75, 50:50, 75:25 and 100:0. The test medium was 3.8 kg of the air dried, <5mm soil supplied by Berton Vineyards, which was weighed into 25 cm diameter plastic (free draining) pots, with an additional 200 g <2 mm weighed on top of the < 5mm material, in order to have a suitable soil medium in which to sow the seeds. Pots were watered to 40% WHC (water holding capacity) using the appropriate WWW:TW ratios and then left to equilibrate prior to sowing ten (10) kikuyu seeds. Pots were maintained at the set WHC on a daily basis, (100 mL) using the treatment solutions. If the environmental conditions meant the temperature of the glasshouse was about 25^{o}C (or greater) for most of the daylight hours, an additional volume (50 mL) of deionised water was applied. Two batches of wastewater supplied by Berton Vineyards were used for the 18 week experiment. Batch 1 was applied during the germination phase (two weeks), then for an additional 10 weeks of the experiment. Batch 2 was used for the final six weeks of the trial. The wastewater quality characteristics are listed in Tables 6.5 and 6.6.

Table 6.4 Physicochemical characteristics of study soil

Soil	EC (dS/m)	pH	Cl (mg/kg)	Ca (cmol/kg)	Mg (cmol/kg)	Na (cmol/kg)	K (cmol/kg)	CEC (NH_4) (cmol/kg)
Berton Winery	0.029	6.2	0.55	1.5	0.34	0.11	0.08	11

Table 6.5 Wastewater Quality for plant growth trial [Batch 1 from Berton Vineyards]

TREATMENT TW:WWW	Ca (mg/L)	Mg (Mg/L)	Na (mg/L)	K (mg/L)	COD (mg/L)	pH	EC (µS/CM)
0:100	28.7 ± 0.5	12.80 ± 0.25	41.50 ± 1.5	3.2 ± 0.08	>10	7.78 ± 0.02	0.50 ± 0.15
75:25	26.6 ± 0.39	12.08 ± 0.38	56.5 ±0.75	171.3 ± 3.6	840 ± 130	6.51 ± 0.01	1.50 ±0. 15
50:50	28.5 ± 0.65	11.26 ± 0.29	72.5 ± 2.3	344.4 ± 4.2	2400 ± 100	6.42 ± 0.03	2.51 ±0.28
25:75	29.9 ± 0.59	10.05 ± 0.06	86.8 ± 2.3	502.8 ± 3.2	3980 ± 140	6.12 ± 0.01	3.46 ± 0.25
0:100	28.52 ± 2.19	9.56 ± 0.23	102.3 ± 8.5	651± 18	5200 ± 222	6.00 ± 0.02	4.42 ±0.38

Table 6.6 Wastewater Quality for plant growth trial , [Batch 2 from Berton Vineyards]

TREATMENT TW:WWW	Ca (mg/L)	Mg (Mg/L)	Na (mg/L)	K (mg/L)	COD (mg/L)	pH	EC (µS/CM)
0:100	28.7 ± 0.50	12.8 ± 0.25	48.5 ± 1.50	2.5 ± 0.04	<10	7.82 ± 0.01	495 ± 15
75:25	27.1 ± 0.29	12.3 ± 0.35	61.8 ±1.35	83.3 ± 4.6	1030 ± 230	5.19 ± 0.01	329 ± 15
50:50	25.7 ± 0.60	11.6 ± 0.80	76.6 ± 2.5	159 ± 4.2	2380 ± 130	5.02 ± 0.03	570 ± 8
25:75	24.1 ± 1.2	10.1 ± 0.08	89.3 ± 3.2	250 ± 3.2	3890 ± 160	5.11 ± 0.01	868 ± 5
0:100	22.5 ± 1.2	10.4 ± 0.13	102.9 ± 8.8	305 ± 23	5170 ± 320	5.16 ± 0.02	1124 ± 8

Plant material was washed then dried at 60°C prior to grinding and analysis for nutrient concentrations. A soil subsample was taken from the treated pots at intervals during the experiment, dried at 40°C and ground to pass <2 mm prior to analysis for EC, pH and nutrient concentrations.

For each treatment plant height and biomass were measured at 8, 10, 13, 15 and 16 weeks from germination. For plants cut at eight weeks, an additional cut (height) was made five weeks later, then after another three weeks. For plants cut at ten weeks, cuts were made at +3 and then +4 weeks. For the 13 and 15 week harvests a second cut was made at +5 and +3 weeks respectively.

A series of five crops (or harvests) of kikuyu were grown, and in all but the fifth crop multiple cuts were taken. A summary of the harvest intervals plus the total irrigation applied are listed in Table 6.7.

Table 6.7 Harvest times and irrigation applied for the Kikuyu plant growth experiment using winery wastewater

SAMPLE ID	TIME PERIOD	DATES	IRRIGATION VOLUME/DAY/POT (mL)	TOTAL IRRIGATION VOLUME/POT (mL)
Harvest 1 - Cut 1	Week 8	27-Sep	100	5600
Harvest 1 - Cut 2	+5 weeks= 13 weeks	1-Nov	100	3500
Harvest 1 - Cut 3	+5 weeks = 18 weeks	27-Nov	100	2800
Harvest 2 - Cut 1	Week 10	11-Oct	100	7000
Harvest 2 - Cut 2	+3 weeks= 13 weeks	1-Nov	100	2100
Harvest 2 - Cut 3	+4 weeks=17 weeks	28-Nov	100	2800
Harvest 3 - Cut 1	Week 13	1-Nov	100	9100
Harvest 3 - Cut 2	+ 5 weeks= 18 weeks	5-Dec	100	3500
Harvest 4 - Cut 1	Week 15	15-Nov	100	10500

Harvest 4 - Cut 2	+ 3 weeks= 18 weeks	5-Dec	100	2100
Harvest 5 - Cut 1	Week 16	2-Nov	100	11200

Turnip growth

The turnips were grown in a glasshouse. At 17 days plant height was measured and some plants were removed and weighed. Growth then continued to 90 days when plant height was measured.

Field pea growth

The field peas were grown in a glasshouse. At 17 days plant height was measured and some plants were removed and weighed. Growth then continued to 90 days when plant height and pod weight were measured.

6.2.3 RESULTS

Kikuyu grass

The biomass was strongly affected by the concentration of wastewater ($p < 0.001$) as shown in Figure 6.15. Biomass production could be described by a cubic polynomial – the optimal concentration of WWW was then located by identifying where the differential was zero. The optimal concentrations are shown in Table 6.8.

Table 6.8 Concentrations of www that produced maximum biomass

HARVEST	1	2	3	4	5	TOTAL
% WWW	61%	66%	72%	69%	43%	65%

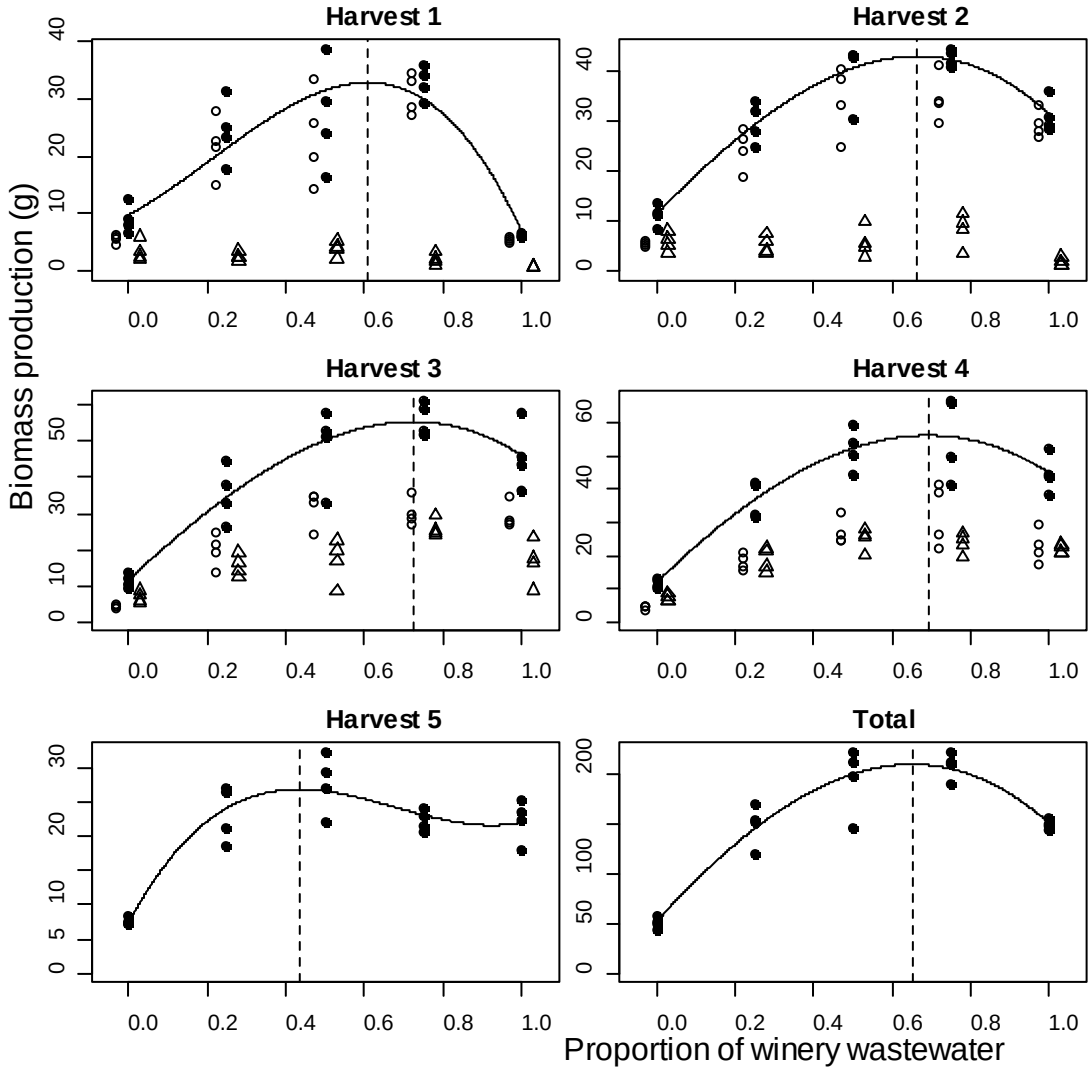


Figure 6.15 Effect of concentration of winery wastewater in irrigation water. The solid lines are a cubic fit and the dashed lines indicate the concentration that gave maximum biomass production

Plant height was affected by the fraction of WWW and showed a similar pattern to that of biomass. Details of the first four harvests are shown in Figure 6.16. There was only a single cut taken for harvest 5 – those data are shown in Figure 6.17.

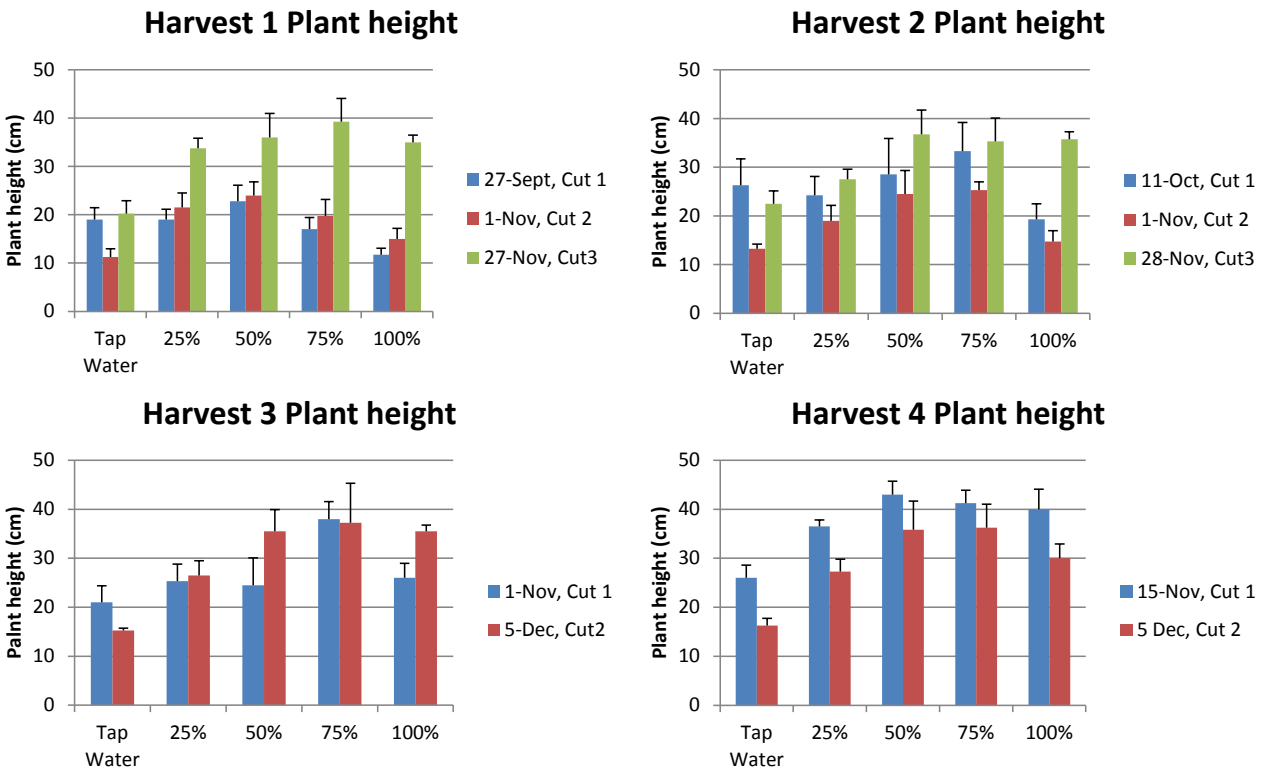


Figure 6.16 Plant height at different harvests and cuts during 18-week winery wastewater trial of kikuyu growth

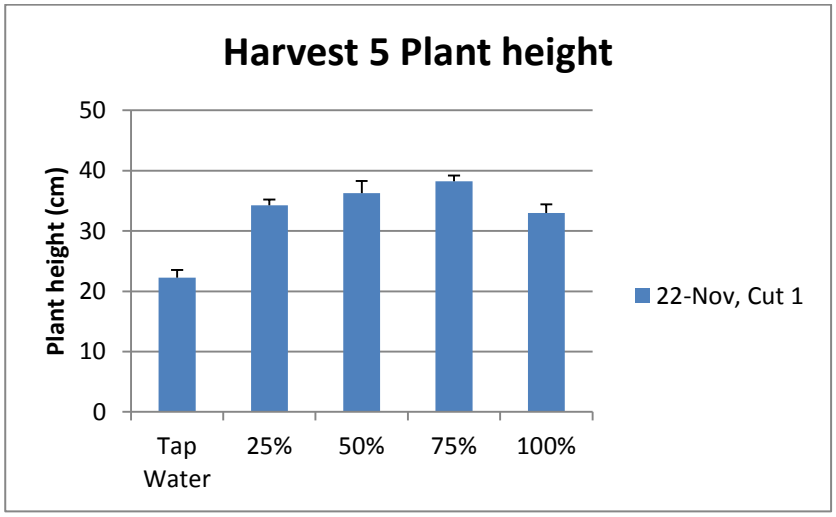


Figure 6.17 Plant heights at 5th harvest after 18-week winery wastewater trial

The 50 and 75% treated plants consistently showed a higher biomass and plant height over the 18 week period of this study (Figures 6.17-6.20). It should also be noted that during the month of November there was accelerated growth generally for all treatments as the average temperature of the glasshouse rose because of the outside rise in ambient temperature. However, the trend of increased growth for the 50% and 75% treatments remained the same. Height and biomass were higher for the second and third cuts (Figure 3) compared to cut one, once again linked to temperature.

Plants grown with just tap water and 100 % WWW were visually not as robust. In addition as the experiment neared the 15-18 week time period, 'white' deposits from the evaporated 100 % applied solution were also quite visible (Figure 6.18).



Figure 6.18 100% WWW treated pot at 15 weeks

Turnip growth

Table 6.9 Effect of irrigation type on turnip growth (2nd experiment)

IRRIGATION TYPE	TUBER LENGTH (MM)		TUBER DIAMETER (mm)		TUBER FRESH WEIGHT (g)		SHOOT FRESH WEIGHT (g)	
	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
Tap water	25.8	2.7	15.5	2.9	7.4	2.7	31.0	3.5
50%:50%	25.9	3.1	15.1	3.3	6.8	3.1	32.2	4.0
Winery wastewater	25.6	3.1	18.4	3.3	10.4	3.1	38.6	4.0

Calculations based on plot means

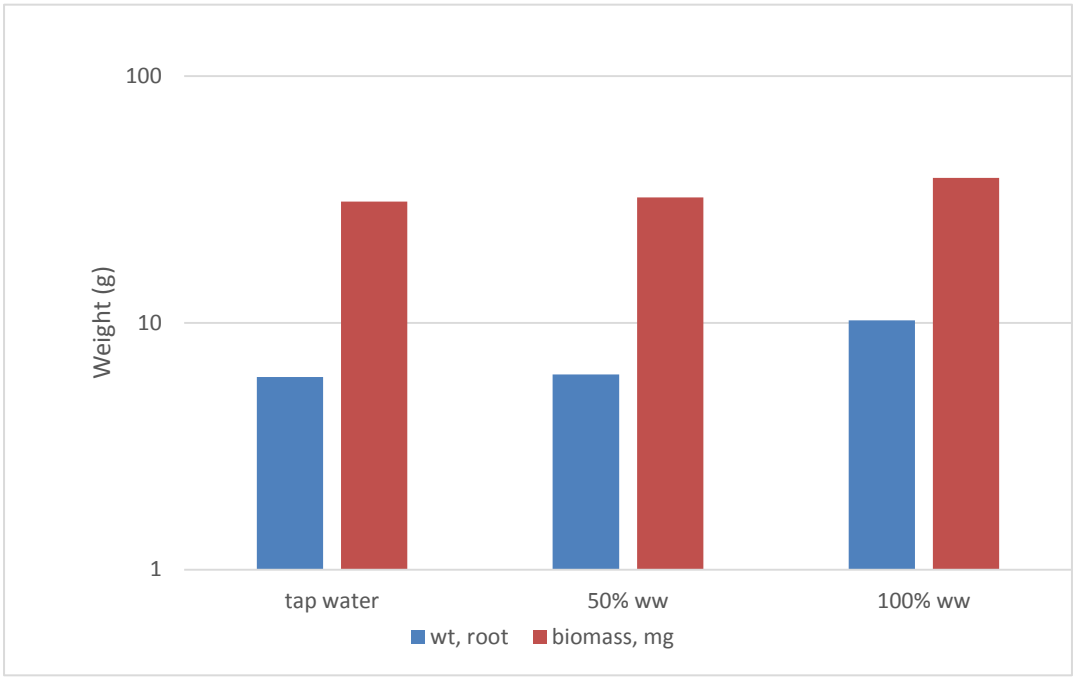


Figure 6.19 Effect of winery wastewater on turnip biomass (90 day data from Experiment 2)

Field pea growth

Table 6.10 Field Pea properties after 90 day treatment with mixtures of winery wastewater

IRRIGATION TYPE	POD WEIGHT (g)	PLANT HEIGHT (cm)	Na (mg)	K (mg)
Tap water	6.01	76	275	17
50%:50%	7.88	82	265	920
100% WWW	3.55	89	575	1800

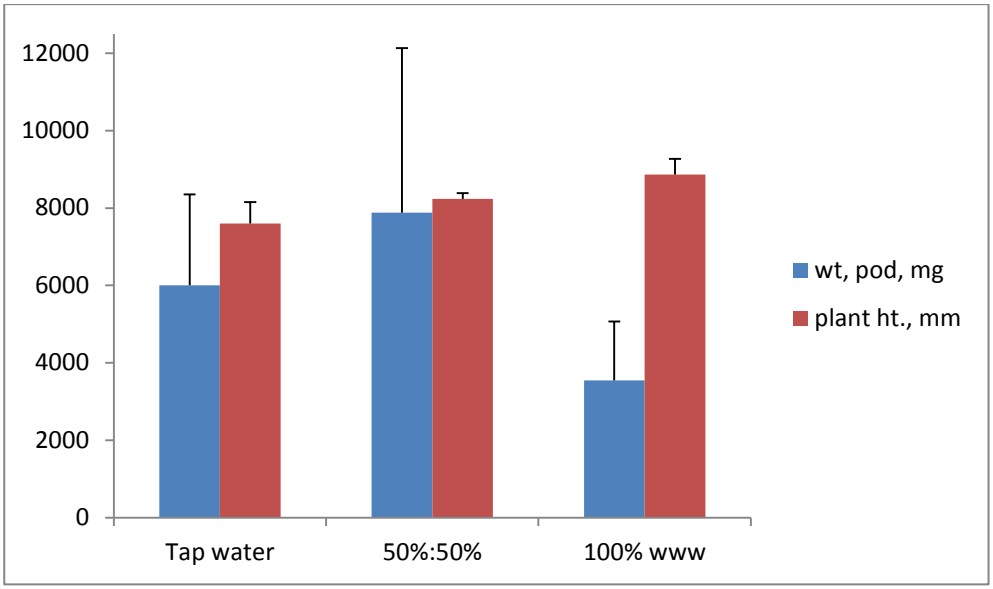


Figure 6.20 Field Pea pod weight and plant height after 90 days of treatment with winery waste water

6.2.4 EFFECT OF MIXTURE OF SODIUM AND POTASSIUM ON PLANT GROWTH

The effect of adding sodium, potassium and a mixture of sodium and potassium to the growing medium is illustrated in Figure 6.20 for peas and Figure 6.21 and 6.22 for turnips. It was found that 20 mM of sodium reduced growth to approximately 50 % and 40 mM to approximately 40% for turnip and 25 % for peas. The addition of 20 or 40 mM of K had little effect on growth. Mixtures of 10 mM Na + 10 mM K and 20 mM Na + 20 mM K had less detrimental effect than the corresponding 20 mM of Na and 40 mM of Na thus suggesting that the inhibition is not purely due to osmotic effects as the 20 mM Na + 20 mM K would have the equivalent osmotic pressure of 40 mM Na.

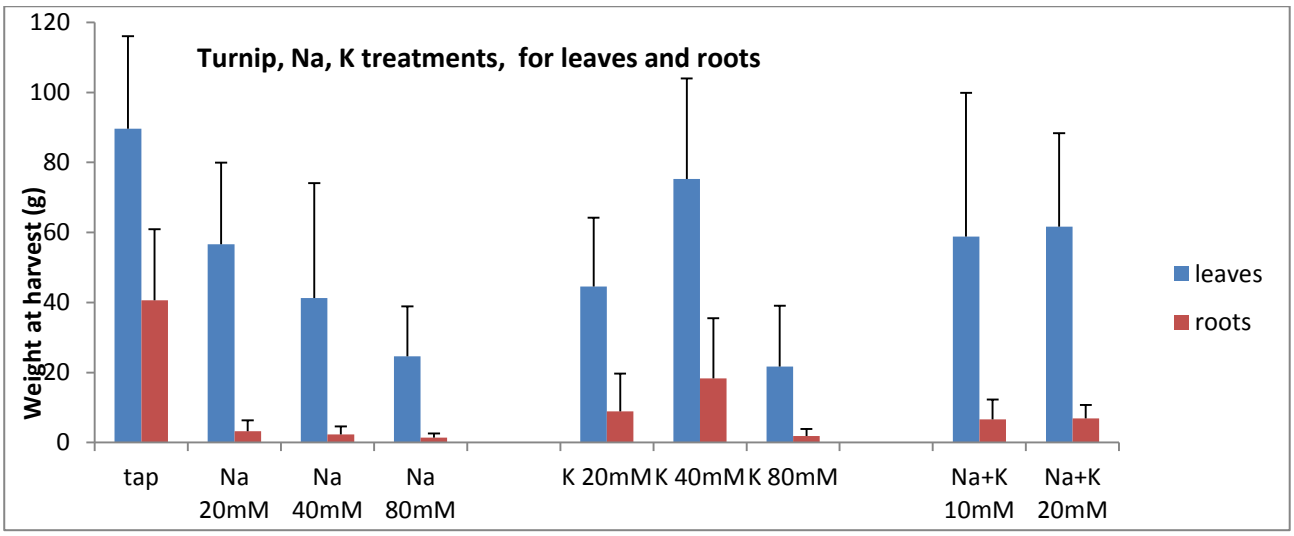


Figure 6.21 Effect of sodium and potassium on turnip growth

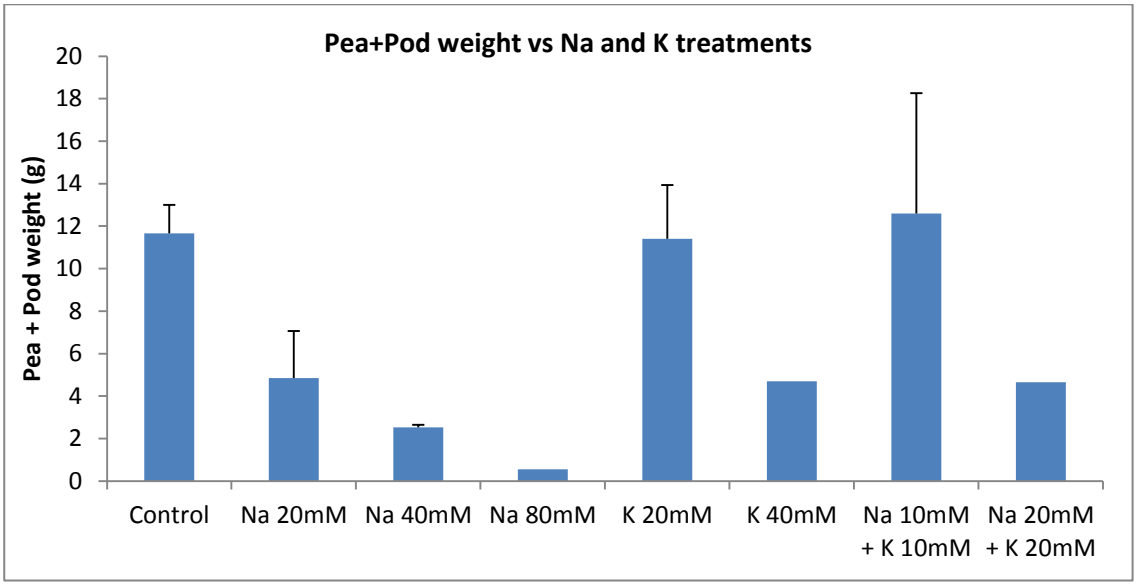


Figure 6.22 Effect of sodium and potassium on turnip growth

6.2.5 CONCLUSION

This study highlighted that WWW with minimal treatment can be used for beneficial irrigation of turf grass at least on sandy soils.

Best results for turf cultivation would be obtained by using a 1:1 (50 %) mixture of tap water and winery wastewater. Apparently some winery wastewater (up to at least 50 %) enhanced the growth of kikuyu. Higher concentrations decreased plant growth. The use of 100 % WWW would be less commercially attractive for production and less effective in terms of nutrient removal.

Field peas were also grown successfully with a mixture of WWW with maximum production again occurring with the shandy of tap water and winery wastewater. The turnip crop produced only small tubers with each irrigation source with no apparent effect on growth due to the different mixtures present.

From the above, it is concluded that WWW can be used effectively in the production of some commercial crops even with minimal treatment. The best choice of crop is still to be determined.

The crop choice will be determined by a range of factors including timing of the crop and the availability of the irrigation water. Ideally the crop should be in a state so that it can use the wastewater as soon as it is

available. This could be done with annual crops, especially if they have already been established. Other alternatives are to use perennial crops –options may include lucerne and tree crops.

A further uncertainty arises with the variability of WWW – both across the season and also between wineries. There are opportunities to use cleaner production practices within the wineries to reduce the salt load (both Na and K) in the WWW used for irrigation (Kumar et al., 2009; Forsyth et al., 2013). A further consideration is to segregate the high strength WWW streams from the main wastewater collection sump to enhance its potential for sustainable irrigation. The reduction of water usage in winery processes resulting in high Na and K in the WWW requires shandying to make it suitable for irrigation.

The above considerations require further investigation before optimal management can be achieved. In the interim, it is suggested that:

- Winery wastewater should be utilised rather than wasted.
- The irrigated crop should be of commercial benefit.
- Rapid usage should be an aim to reduce the requirement for processing.
- Where rapid disposal is an option, winery production should be matched with the disposal process.

6.2.6 REFERENCES

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Part VII Fit for purpose approach based on decision support tools

7 Decision support tools based on fit for purpose approach

The management of winery wastewater (WWW) is a complex problem with no single best solution. There are a large number of variables affecting wastewater volume, quality and appropriate management. Some of these are product style/mix, winery layout, winery management, land availability, location, climate and financial situation. As such, each winery needs to develop its own particular solutions for sustainable recycled WWW irrigation practices. The various disposal/reuse options have very different fitness-for-purpose (quality and quantity) requirements. Thus water disposed of onto tree lots would have different fitness-for-purpose than that disposed of onto grapevines to that disposed of into a municipal sewer. However, the fitness-for-purpose of wastewater for each disposal method will also vary depending upon the local conditions – land availability and use, soil health, climate, proximity of neighbours etc. In applying the reduce-reuse-recycle and fit-for-purpose concepts it is important to acknowledge that practices in the winery, wastewater treatment, reuse/disposal and soil health are all linked for sustainable winery wastewater irrigation, Figure 7.1.

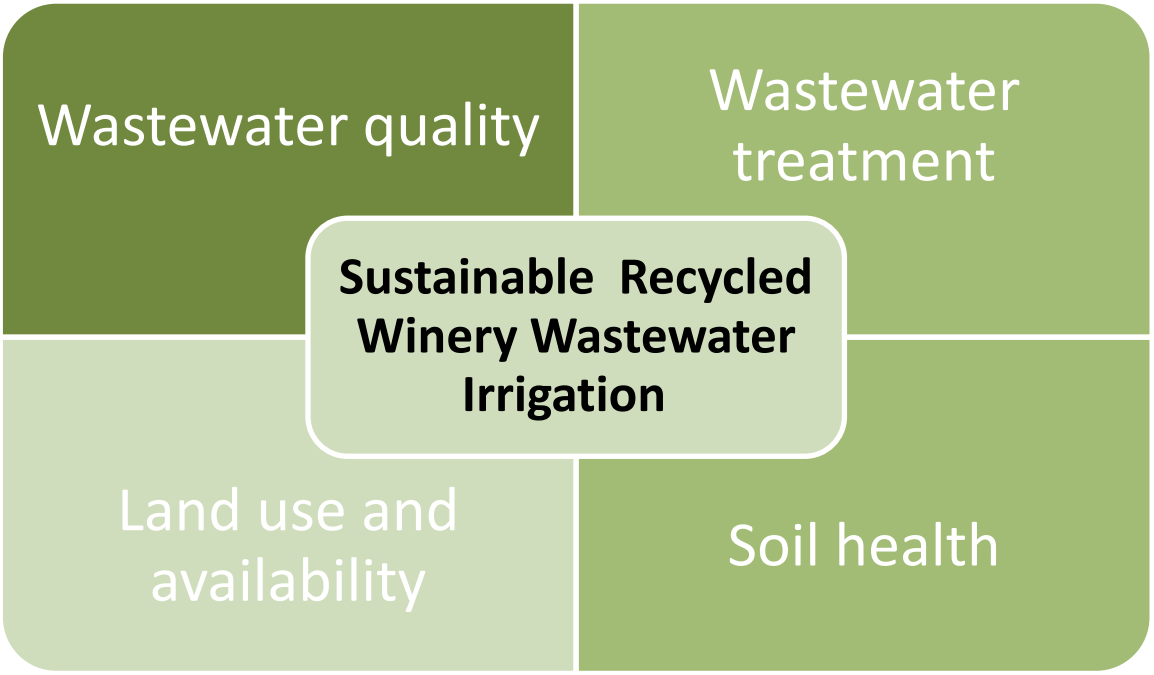


Figure 7.1 Decision support tools based on fit for purpose approach

The decision support system depends on a definition of the type of decision

1. Short term management decision – typically a few years
2. Long term investment strategy – over decades.

The following guidelines cover both of the above and are illustrated in Figures 7.2 and 7.3.

7.1 Short term management decision

Short term decisions must be determined using existing infrastructure. The decisions could be made to

- Minimise cost
- Maximise environmental sustainability

At least to some extent, these two criteria are similar. This is further discussed below.

The key environmental drivers for the decision making are odour, salinity, nutrients, soil health and groundwater quality. The choice of management strategies is driven by these criteria.

7.1.1 USE OF EVAPORATION BASINS AS A SHORT TERM OPTION

Once salinity (as measured by EC > 3.5 dS/m) is excessive, the mechanisms are evaporation or reverse osmosis (RO). If no RO facility is available, any wastewater stream with EC exceeding 3.5 dS/m should eventually be directed to an evaporation basin.

Another driver is odour – if the evaporation basin is deeper than 50 mm, and the BOD/COD exceeds 2000 mg/L and malodour could affect residents or commerce, there would be a need to treat the wastewater prior to its release to the evaporation basin such that the COD in the effluent is less than 2000 mg/L.

Some wineries have the facilities to isolate saline (EC > 3.5 dS/m) waste streams. In some cases these waste streams may have properties (such as low pH or high pH) that may be useful in adjusting pH of other components of the liquid waste. In that case it may be beneficial to store that component. In other cases, where it is not practical to store saline wastewater it must be sent to an evaporation basin. If there is saline wastewater (EC > 3.5 dS/m) and no means of disposal (i.e. no evaporation basin and shandying is not feasible) the system is unsustainable.

7.1.2 RAPID DISPOSAL

Research has indicated that application of wastewater that contains organic carbon (as measured by BOD or COD) to soil is beneficial, not detrimental (Kumar and Kookana, 2006). This is in contrast to BOD load being applied to surface water. This finding affects the recommendations as to the best method of disposing of winery wastewater.

The cheapest method (if given infrastructure is available) to dispose of wastewater is to irrigate as soon as possible, provided that the salinity is less than 3.5 dS/m. Some primary treatment will be required before the irrigation involving removal of solids of size greater than a given diameter to minimise blockages, pH and SAR adjustment. The size of the particles that should be retained is determined by the sprinkler system – large sprinklers tolerate larger particles than small under-tree sprinklers.

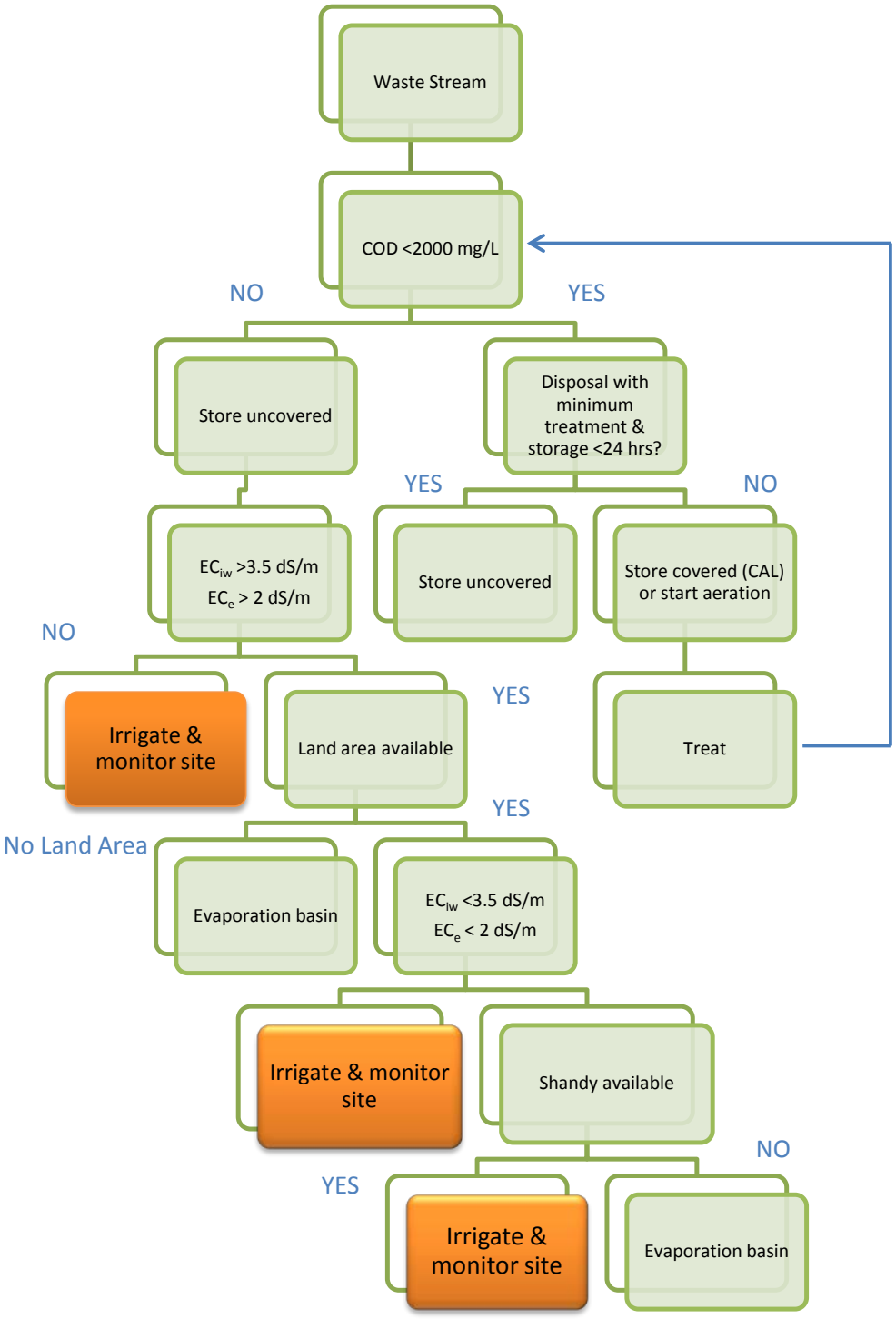


Figure 7.2 A decision support tool for winery wastewater management based on the Electrical Conductivity (EC) and chemical oxygen demand (COD) of the wastewater stream

The treatment of the wastewater may require shandying with better quality water. Shandying is sometimes used to reduce salinity so that the wastewater can be disposed of to an irrigation lot – the merit of this practice as opposed to diverting the saline waste stream to an evaporation basin needs careful consideration. For example, the use of shandying may require a larger leaching fraction than that used with fresh water – for this reason the benefits from shandying may be of limited value to the area being irrigated.

The irrigation infrastructure (including the area irrigated) determines the maximum rate of rapid disposal of the effluent.

Further limitations on the rate of rapid disposal are posed by soil properties, including infiltration rate and soil capacity. The infiltration rate will be much higher in sand than in a heavier soil; it may also vary depending on the moisture content of the soil. Infiltration rates are also affected by soil structure – soil with a poor structure will have a lower infiltration rate than one with good structure so it is advantageous to ensure that the soil structure is maintained.

Soil has only a limited capacity to absorb water. If that capacity is exceeded there is likely to be runoff and drainage. While some drainage (leaching fraction) is essential for sustainability, this must be controlled to ensure that there is no significant harm to the groundwater.

Rapid disposal of wastewater by irrigation should be maximised being limited by

- Irrigation infrastructure; or
- Ability of the soil to accept water (land area × infiltration rate); or
- Capacity of soil to accept water (land area × [field capacity of soil – current moisture content]).
- Local regulations that currently impose limits on the rate of application of BOD (e.g. the Shire of Augusta, WA, limits the application of BOD to 30 kg/ha/day).

There is a range of potential crops available for irrigation. The choice of crops should take into account

- Value obtained from the crop (e.g. firewood, fodder, other produce);
- Cost of maintaining that crop (e.g. pruning, fire control);
- Ability of the crop to use water at the critical time;
- Ability to survive between irrigations without water; and
- Ability to absorb nutrients (especially potassium and sodium).

A wide range of crops is being used, ranging from woodlots (eucalypts, casuarinas and acacias), flower gardens, beets, pasture, vines and dates. Insufficient data are available at this stage to give useful guidance on which crops are the most effective.

7.1.3 TREATMENT TO REMOVE BOD WHEN RAPID DISPOSAL IS INADEQUATE

When the rate of generation of wastewater exceeds the potential rate of rapid disposal, the wastewater should be treated to prevent malodours. The treatment process should reduce the level of BOD to an acceptable level so that there will be no odour issue.

The rate of treatment of the wastewater will be determined by its BOD level and the size of the facility – typically in a moderate to large plant this will also be a function of the size and effectiveness of aerators. This will not always be the case because there may be some removal of BOD by an anaerobic process.

There are a range of treatment options available; for details of current options see Kumar *et al.* (2009).

7.1.4 WASTEWATER STORAGE

Typically there is a need for two types of storage

- Storage of untreated wastewater
- Storage of treated wastewater

If the rate of wastewater production exceeds the combined capacity of the irrigation system used for rapid disposal and the ability to treat the wastewater, the excess wastewater must be stored. Because that water has not been treated and is therefore likely to have a high BOD load, it presents an odour problem. The odour problem is typically managed by holding the effluent in a covered lagoon.

Once wastewater has been treated, it can be stored in an open facility and used to give maximum benefit. The storage facility must be empty before the next peak load is anticipated, which may have practical limitations.

7.1.5 REFERENCES

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7.2 Long term investment strategy

7.2.1 MINIMISING LOAD

One option for investment in wastewater management is in waste prevention. If the disposal mechanism is rapid disposal, the key criterion would be the volume of wastewater produced. Strategies that reduce water use are therefore important.

Reduction of BOD load by minimising product loss will save both in terms of increased production of wine and decreased treatment cost.

While there are simple mechanisms available for dealing with the water and BOD components of the waste stream, potassium and sodium loads are persistent. Reduction in the net use of caustic is therefore important.

7.2.2 EVAPORATION BASINS AS A LONG TERM OPTION

Evaporation basins may be a cost effective method of disposing of highly saline wastewater streams. Such a facility requires an area of flat land where the basin can be constructed. The installation of piezometers to monitor groundwater may be required to be included in the cost.

7.2.3 INVESTING IN RAPID DISPOSAL INFRASTRUCTURE

Infrastructure is required for the rapid disposal of wastewater. This infrastructure would include pumps, main pipes, EC and SAR measurement and adjustment, land on which to spread the water, monitoring facilities (for soil moisture, infiltration rates and possibly groundwater) and potentially a perennial crop.

The benefits of the rapid disposal facility are potentially a useful crop and decreased requirement on wastewater treatment and covered storage.

The exact amount of water that can be disposed of in this manner varies depending on the site, climate, current rainfall and crop. A working figure might be 2 mm per day, but this figure requires further consideration. Using that figure, together with the infrastructure cost (and hence a running cost) of rapid disposal can be estimated.

7.2.4 INVESTING IN TREATMENT PLANT AND STORAGE

The infrastructure and running cost of a treatment plant for a various size is known (see Kumar *et al*. 2009 and Table 7.1), as is the running cost of that option. The cost of a covered storage facility can also be obtained. Given these data, it would be feasible to derive an optimum allocation of resources to treatment, storage and rapid disposal.

Table 7.1 Wastewater system operating and capital cost by winery size on a per kL basis

EQUIVALENT CRUSH (TONNE / ANNUM)	NUMBER OF WINERIES[1]	OPERATING COST ($/KL)		CAPITAL COST ($/KL)	
		AVERAGE	RANGE	AVERAGE	RANGE
<2,500	10 – 11	6.6[2]	1.6 – 17	120	29 – 285
2,500 – 10,000	6 – 7	2.8	0.7 – 7.7	26	7.1 – 47
> 10,000	9 – 10	2.4	0.4– 4.7	24	2.0 – 53
TOTAL	25 – 28/45	2.95 ± 0.52	-	38.5 ± 9.4	-

Typically the rates of wastewater production, efficiency of wastewater treatment and the maximum feasible rate of wastewater disposal will vary from season to season. This poses a design capacity problem as it would be wasteful to build a system that would cope with the worst possible case. Some contingency plan is therefore required for the extreme case. Possible contingency plans may be limiting production, overloading the disposal area or carting waste off-site.

7.2.5 INVESTMENT IN SHANDYING FACILITIES

In some cases an effective means of disposing of wastewater is to mix it with water of better quality before it is used for irrigation. Shandying will require a supply of better quality water and facilities for monitoring water quality both of the waste stream and the shandy.

Typically the degree of shandying will be determined by salinity (as measured by EC), which can be monitored continually.

7.2.6 OPTIMISING OFF-SITE INVESTMENT

Estimating the cost of managing WWW is complex and includes all the components described above. The costing must include the cost of infrastructure investment (interest on capital invested, maintenance and obsolescence), electricity and staff costs. Good record keeping is essential to enable estimates of treatment efficiency, wastewater volume and loading over the year, soil monitoring and evaporation rates. In addition there are stochastic components that include rainfall, evaporation and year to year changes in the wastewater stream.

Changes to the wastewater management system should also be costed. Comparisons could be made of the infrastructure for rapid disposal or for ‘treat and store’ or even off-site disposal. It may be feasible to model the cost involved and then choose an option that minimises costs.

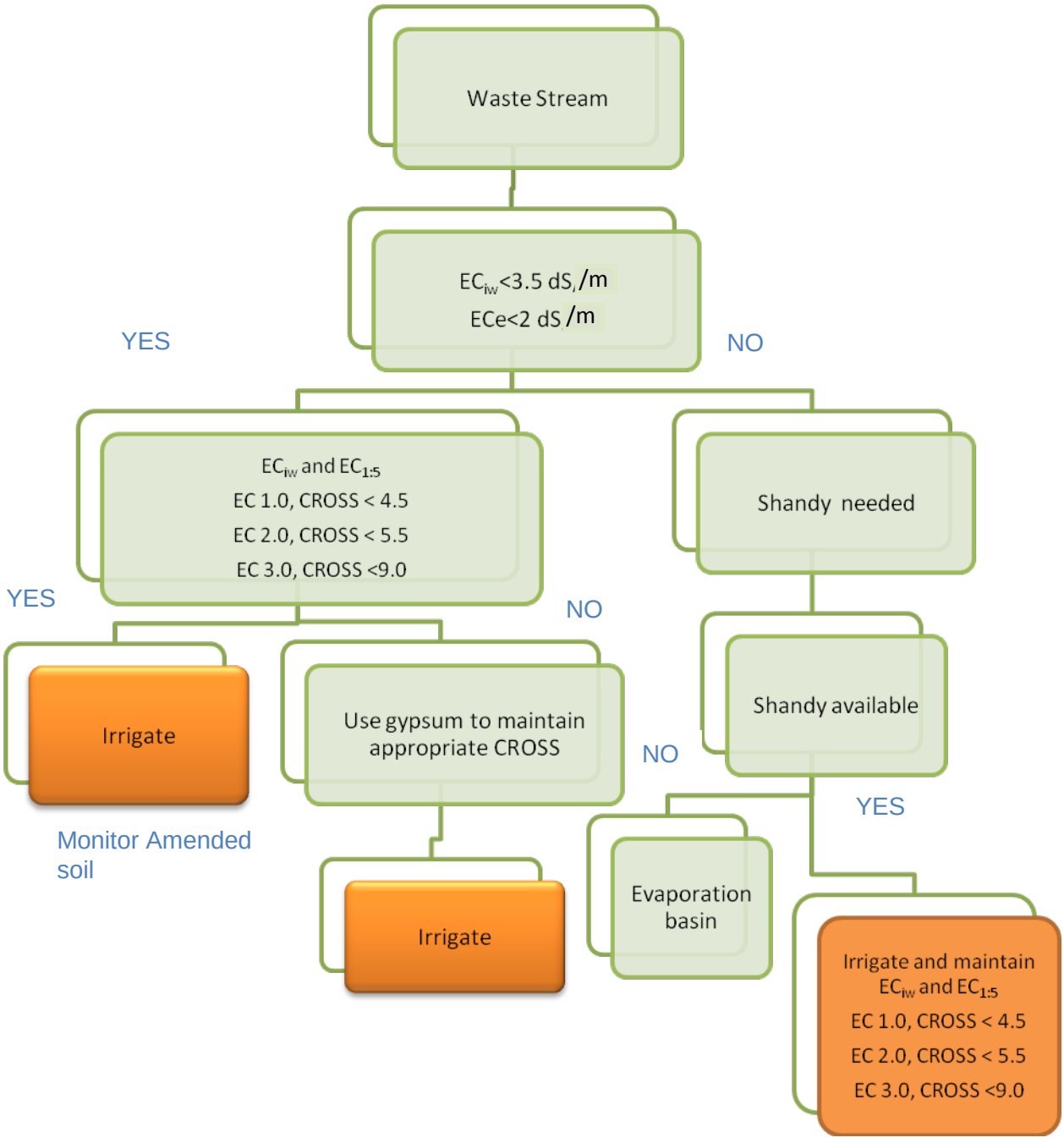
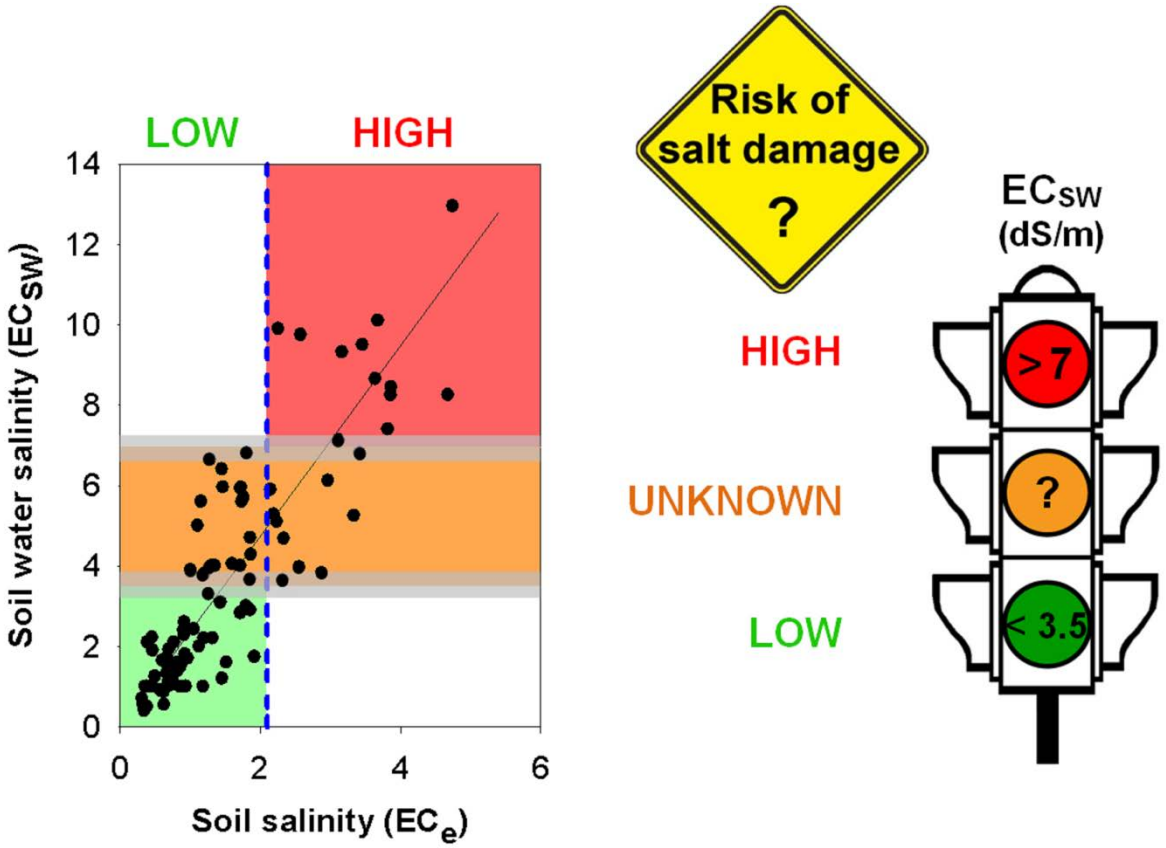


Figure 7.3 Decision tree for handling winery wastewater as determined by water quality

7.3 Recycled winery wastewater thresholds for various soil types



Based on our laboratory experiments and soil monitoring at field sites, we have derived the following data depicting the soil structural risks associated with different CROSS values in conjunction with the EC values of irrigation water. We have given the risk factors for two types of soils, viz. highly permeable (leaching fraction >25%) and poorly permeable (leaching fraction < 25%). This table is tentative and the risk factors may change according to individual soils based on their organic matter, clay mineralogy and pH (Table 7.2).

Table 7.2 Soil Structural risks due to CROSS values at different EC (salinity) levels in winery wastewater

SOIL TYPE	WELL TO MODERATE DRAINING SOILS (LEACHING FRACTION < 25%)			SLOW DRAINING SOILS (LEACHING FRACTION > 25%)		
WATER SALINITY (dS/m)	LOW	MEDIUM	HIGH	LOW	MEDIUM	HIGH
<1.0	<3.0	3.0-6.0	>6.0	<2.5	2.5-5.0	>5.0
<1.0-1.5	<4.0	4.0-7.0	>7.0	<3.0	3.0-6.0	>6.0
1.5-2.0	<6.0	6.0-8.0	>8.0	<4.0	4.0-7.0	>7.0
2.0-3.0	<8.0	8.0-10.0	>10.0	<6.0	6.0-8.0	>8.0
3.0-4.0	<10.0	10.0-15.0	>15.0	<8.0	8.0-12.0	>12.0

Note: the criteria were derived on the basis of the principles presented in: Marchuk, A. and Rengasamy, P. (2012) Threshold electrolyte concentration and dispersive potential in relation to CROSS in dispersive soils. *Soil Research* **50**, 473-481

Because of the variations within a soil type, we recommend that each individual soil should be tested for clay dispersion, as given in the following flow chart and decision on gypsum application should be based on this test. A detailed fact sheet on gypsum has been prepared.

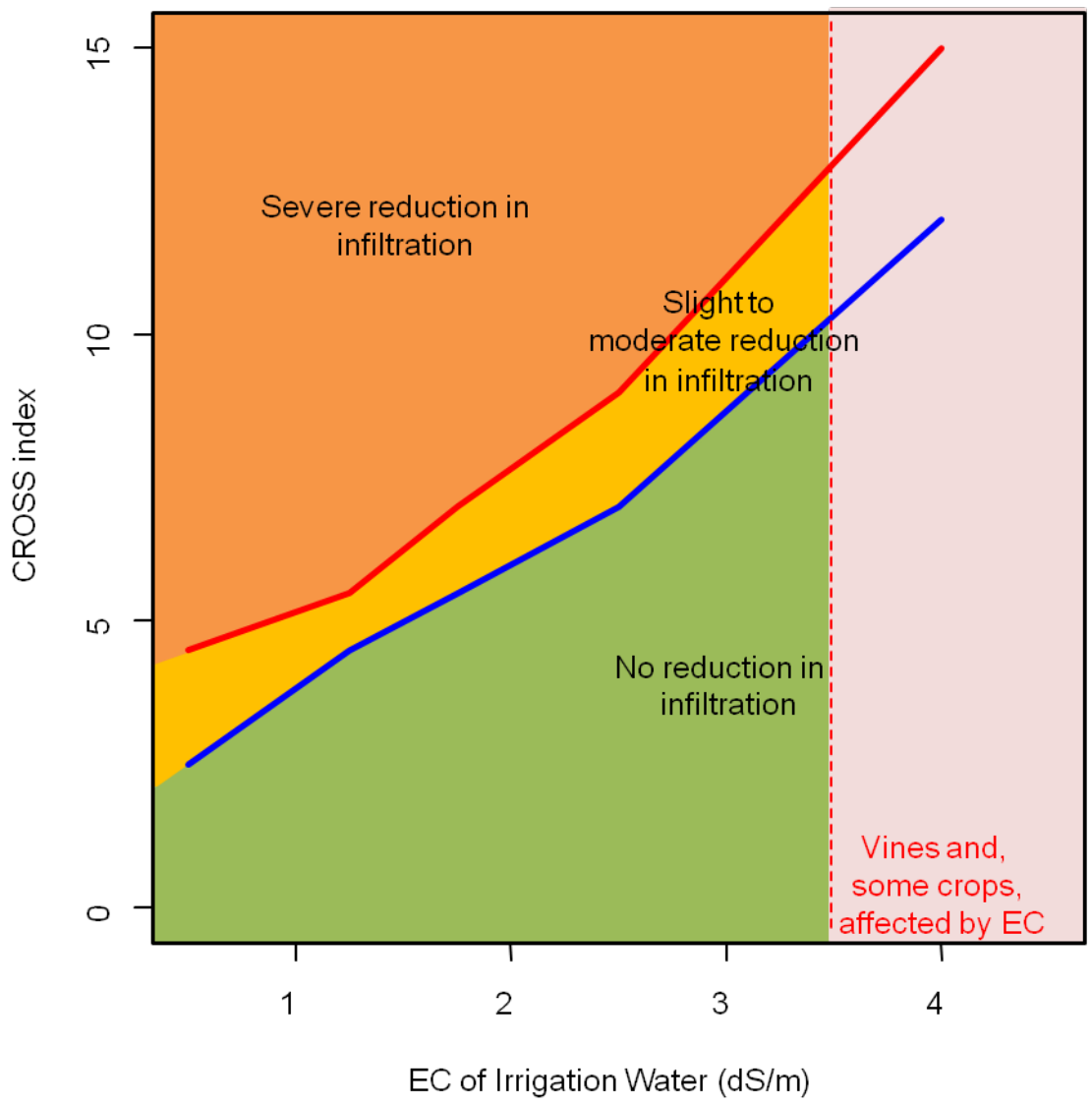


Figure 7.4 Estimation of risk of damage to soil structure based on irrigation water quality

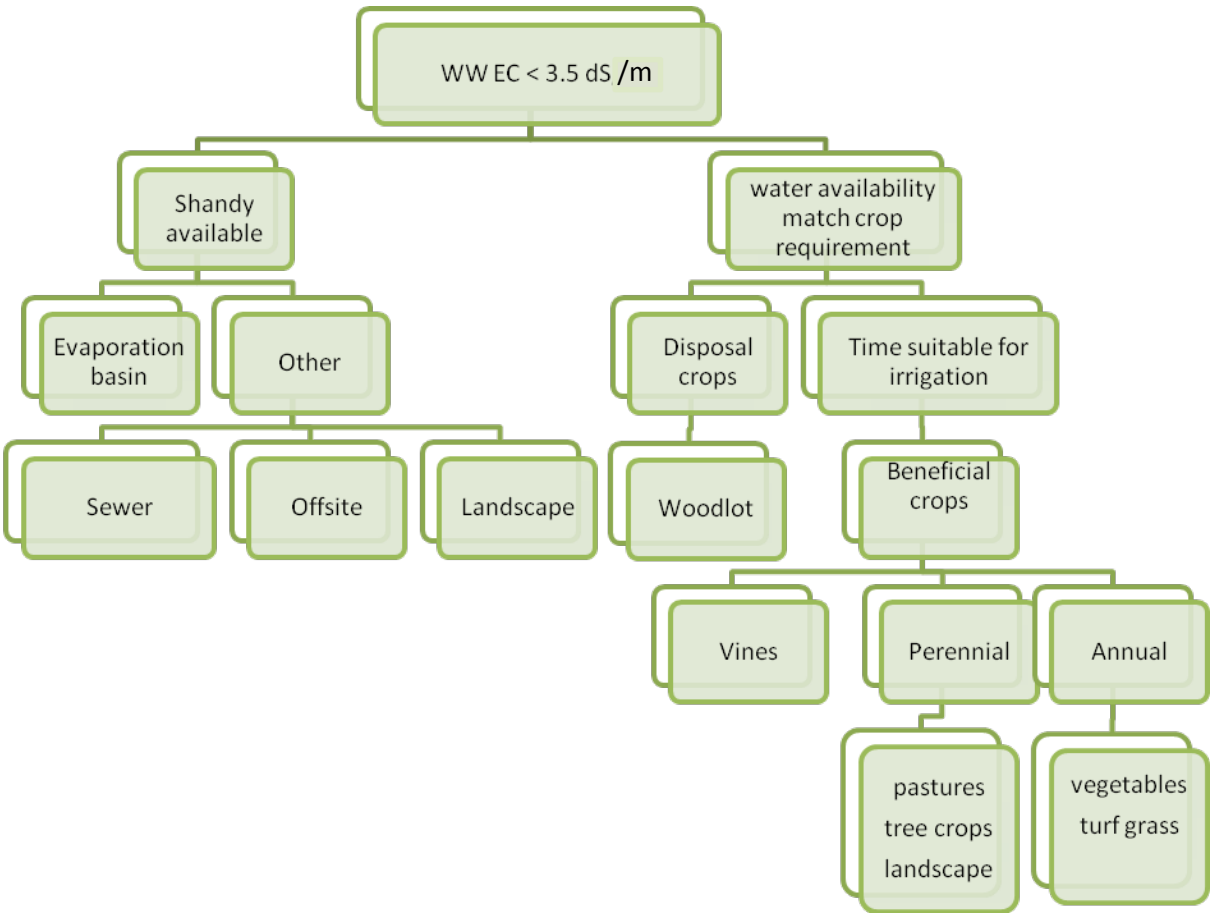


Figure 7.5 Decision tree for use of winery wastewater incorporating beneficial crops

7.4 Wastewater fit for purpose

Developing a wastewater program that produces wastewater that is fit for a particular disposal method will be an iterative process that involves the disposal methods and the production process within the winery. We suggest that the best place to start this process is to look at the options that are available for the ultimate disposal of the winery wastewater. In this section we consider some of the interactions that would be involved in such a process.

The actual disposal systems available have been summarised in the decision trees supplied above. These disposal systems include evaporation basins, disposal through a sewer system or other site off-site management, treatment and storage and irrigation or rapid disposal to some receiving crop. Each of these systems requires infrastructure and has associated costs and potential benefits. It would be prudent to modify the quality of the wastewater so that it is optimal for the chosen method of ultimate disposal. This may require or allow certain processes to occur within the winery.

- Off-site disposal. A large component of the off-site disposal will be in transportation and potentially storage prior to the disposal. In this situation the volume of wastewater should be minimised using water recycling and segregation of waste streams.

- Evaporation basin. The limitation with the evaporation basin would also be the volume of water produced. The restrictions for evaporation basin disposal would therefore be similar to those required for off-site disposal.
- Treat and store. In this system not only is the volume of water important but also the quality because depending on the BOD of the water, the amount of treatment will have to be modified. The quantity of water in this system may be limited by the storage available. Where storage is a limiting factor it is important to minimise the volume of water being produced. If storage is not limiting, and shandying is required to reduce EC to a suitable level, minimising the volume of water being produced may be less important.
- Rapid disposal. The rapid disposal of WWW requires an EC less than 3.5 dS/m. There is little point in trying to save water in the winery if water has to be added later during the shandying process. This may depend on the location of the source of water that is available for shandying - if it is some distance from the winery then minimising water use in the production process would be beneficial. If however the water source is available at the winery, the mixing process would naturally occur at the winery so there would be no benefit in using methods that are costly to save water within the winery production process.

With both 'treat and store' and 'rapid disposal' there will be an ongoing problem with sodium. Any modifications to processes within the winery that reduce the amount of sodium in the waste stream would therefore be beneficial regardless of the method of disposal. Similarly, controlling process spillages would be of benefit by reducing the BOD load entering the waste stream. When the wastewater is to be used for the production of beneficial crops the inclusion of nutrients such as N, P and K may be advantageous so there is little need to remove them in the treatment process.

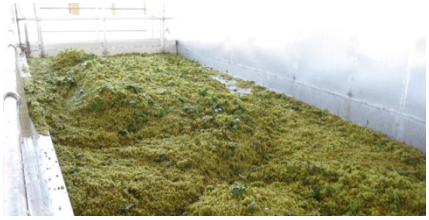
Monitoring of the environment will be required for most disposal systems including evaporation basins, 'treat and store and irrigate' and rapid disposal. The type of monitoring required is well described in the SA EPA (2004) publication. The monitoring data should be used to determine how the production, treatment and disposal processes should be modified to minimise environmental impact in an economically feasible manner.

7.5 References

EPA (2004) *EPA Guidelines for Wineries and Distilleries* Environmental Protection Authority GPO Box 2607 Adelaide 5001.

Kumar A and Kookana R (2006) Impact of winery wastewater on ecosystem health – an introductory assessment Final Report to Grape and Wine Research and Development Corporation, Project CSL 02/03

Kumar A, Arienzo M, Quayle W, Christen E, Grocke S, Fattore S, Doan H, Gonzago H, Zandonna R, Bartrop K, Smith L, Correll R and Kookana R (2009) *Developing a systematic approach to winery wastewater management. CSL 05/02.* CSIRO. Adelaide SA.www.gwrdc.com.au/webdata/resources/project/CSL_05-02.pdf





Part VIII Overall conclusions and recommendations

8 Overall conclusions and recommendations

8.1 Choice of beneficial crops

This project has demonstrated that it is feasible to use beneficial crops in association with disposal of winery wastewater (WWW). In this study only six crops were assessed in glasshouse conditions (kikuyu grass, turnip, field pea, cucumber, zucchini and silver beet. In addition, data were also collected on the effect of WWW on grapevines grown in the glasshouse. Quantitative data on both were only obtained for kikuyu grass, field peas and turnip. The study leaves open the question as to which crops have the best potential to utilise the wastewater.

Some criteria that can be used to assess which crops to use should include

- The availability of the crop to accept wastewater within 24 h of its production. This may favour the use of a perennial crop rather than an annual crop like turnips or silver beet. Some possibilities that may be considered would include pasture (e.g. lucerne), ornamental crops or tree crops.
- The crops should have some economic benefit for example the production of food or timber or flowers.
- A perennial crop should be drought tolerant so that it can survive between irrigations using rainfall, noting that both the supply of wastewater and rainfall are both variable.
- Depending on the quality of the wastewater, the crop may need to be salt tolerant.

One obvious choice of a beneficial crop would be the local grapevines. They are always present near wineries and the potassium content of the wastewater may be beneficial to the vines. However care must be taken to ensure that irrigation with that water does not lead to adverse effects on crops or the environment. Adverse effects may include higher ion concentration in grape juice (and consequently wine) or the accumulation of harmful salts or organic contaminants in the soil.

Another crop that is worthy of consideration is date palms. They are perennial but drought tolerant and are being successfully grown using wastewater in some areas. No data on the benefit of date palms were available at the time of writing this report. It is also challenging to conduct experiments on a crop like date palms as they require many years of monitoring.

8.2 Opportunities and Limitations

The data from plant growth shows that application of WWW enhanced the growth of kikuyu grass. However at higher concentrations of WWW, the growth was inhibited due to high EC (4.2 dS/m). No significant effects on growth were noted in peas and turnips. In the trial where sodium and potassium levels varied independently there was inhibition of growth of turnips at 80 mM Na or K. Turnips were found to be less responsive than peas.

In the second trial, it was demonstrated that growth inhibition was directly related to the EC in solution. It was also noted that the EC of the 40 mM potassium solution was lower than the EC of the 40 mM sodium solution. The data also suggested correlations between EC and plant growth.

The data from the present study suggested that sodium affected peas and turnips to a greater extent than potassium. This can be related to EC and potentially to osmotic effects. Turnips and peas behaved differently and this presents a challenge when selecting crops that may benefit from application of WWW.

One of the problems from irrigation using WWW is the salinity of the wastewater (EC 4.2 dS/m). It is challenging to remove salt from the wastewater in an economical manner. Unlike organic matter, salt does not break down with time and therefore has to be carefully managed. The accumulation of salt in the soil profile must be managed by including a leaching fraction in the irrigation plan (see for example Skewes and Meissner 2007). The presence of salt in the soil creates salinity problems which affect plants directly through osmotic effects. There is also a threat to soil structure.

8.2.1 SOIL STRUCTURE PROBLEMS

Soil structure can be affected by the ionic imbalance especially on the clay surfaces. There are several ways of assessing the effect of the ionic imbalances on soil structure. Of importance is the ratio of monovalent to divalent ions. A common measure of the threat to soil structure is the SAR which considers the effects of sodium, calcium and magnesium. While this ratio has provided useful information in the past it has been shown to be less than ideal partly because it ignores the effects of potassium on soil structure and also gives equal weighting to each of the ions present. Those inefficiencies have been rectified in the new CROSS index which is discussed in Part 2 of this report.

Both SAR and CROSS are largely driven by the sodium concentration in the waste stream. One of the ways to reduce the impact of sodium on soil has been the replacement of sodium hydroxide with potassium hydroxide for cleaning wineries. The consequence of this is the increased amount of potassium hydroxide for cleaning in wineries and an increased level of K in WWW. Recycling of caustic cleaners also has potential for reducing load in the waste stream. Both of these measures are expected to have beneficial impact on soil structure in wastewater receiving areas.

8.2.2 GROUNDWATER

The requirement for a leaching fraction immediately implies that there will be leaching through the soil profile and that the leachate will eventually reach the groundwater. The effects on the groundwater could be quite diverse ranging from a beneficiary recharge through to an elevation of salinity problems and contamination from both inorganic and organic sources. The effects of wastewater application on the groundwater are beyond the scope of this report. All methods of disposal of wastewater should consider the leaching into groundwater. Irrigation using untreated wastewater will result in application of organic matter to the soil. Earlier studies (Chapman et al., 1995) suggested that organic matter breaks down rapidly, making leaching to groundwater an unlikely event. Although the threat to groundwater from dissolved organics is small, it still requires monitoring.

8.2.3 MONITORING

An ongoing monitoring system will be required for any system of land disposal of wastewater whether it is treated or untreated. The amount and the type of monitoring require careful consideration and a good guide has been provided by EPA SA (2007).

8.2.4 MODELLING

The necessity of monitoring will be ongoing and the extent of the monitoring will invariably be limited by the resources available. Monitoring will also not be able to make forward predictions especially in new developments where there is a short history of the application of wastewater. In those situations monitoring can be augmented by some modelling of the system. The modelling may be very simple such as a simple mass balance of the salt being applied or may take the form of some other more complex monitoring. In general more complex matter models require more data than usually available and require more assumptions than simple models.

A simple model that should be considered is that of Clarke 2003 which considers the effect of spreading nutrient rich wastes on agricultural land. A more sophisticated model is Hutson (2003) which has been widely used in South Australia but it requires details of soil structure and daily rainfall and irrigation. *Manual for spreading nutrient–rich wastes on agricultural land* (Clark, 2003) provide a model which concentrates primarily on nutrient element chemistry.

8.2.5 CURRENT REGULATIONS

The Margaret River region limits irrigation rate by maximum P (10-120 kg/ha/yr), maximum available N (140 – 480 kg/ha/yr) and BOD (30 kg/ha/day). In the current study, the WWW used for irrigating beneficial crops was within the regulatory limits (BOD equivalent to <15 kg/ha/day).

8.2.6 AREA REQUIRED FOR LAND DISPERSAL

The area required for the disposle of winery wastewater can be estimated from the quality of the wastewater and the volume available. A template for estimating this has been developed by the Shire of Augusta, Margaret River. This template works on the load of dissolved material in the wastewater. Some contaminants such as sodium are not reduced by any conventional treatment system and can be concentrated. The area required for disposal of a given amount of wastewater might, therefore, not be affected by treatment of wastewater when the equations are based on Na.

A difference between the approach taken in this report and that of the Shire of Augusta is in the organic loading of the wastewater. Data from this report and other previous reports (Kumar and Kookana, 2006 and Kumar et al., 2009) suggests that the organics in wastewater are not harmful to the soil. They enhance the quality by increasing the organic content. One of the driving factors in treating the wastewater is odour control. Usage of wastewater within 24 hours eliminates this problem. The area required for disposal of the WWW would be similar if it is treated or untreated. The critical difference is in the timing.

8.2.7 COSTS –BENEFIT

No comparative costs were available to the study that would enable a comparison of the cost:benefit of rapid disposal and the ‘treat, store and irrigate’ option. Some data on the cost of treating WWW were obtained in a previous project (Kumar et al., 2009). The data suggest that the average cost for treating one kilolitre of WWW generated by a small winery was $6.60 per kL. This cost should be added to a capital cost of $120 per kilolitre though the actual cost of the treatment is much greater than the $6.60 per kL which includes operating cost.

Both the rapid disposal and 'treat, store, irrigate' options require facilities for moving the wastewater away from the winery to the disposal site. The capacity of that system would probably have to be greater in the rapid disposal system because the 'treat and store' option can use the water more or less on demand. As discussed above the total area required for the disposal of wastewater is similar for both treated and untreated wastewater.

Applying untreated wastewater has potential for creating malodours especially if there is a delay in disposal (perhaps due to a local rainfall event) or if there is some other technical problem. This threat may affect where the disposal of the wastewater can occur and the mitigation of that risk may involve additional financial cost.

8.2.8 CARBON SEQUESTRATION

Rapid disposal of WWW has two clear advantages in terms of carbon sequestration. In the first instance carbon compounds from wastewater are retained in the soil. Secondly it is known that treatment is energy intensive and this typically will require carbon expenditure to produce the power. These two reasons suggest that if possible, rapid usage of the wastewater will be more environmentally friendly than the alternative option of 'treat, store and then irrigate'.

8.2.9 PRODUCTION-DISPOSAL INTERACTION

It may be possible to modify wine production in a manner that makes the treatment of the WWW more efficient. One method is to minimise the amount of waste going into the waste stream and this will be beneficial for all methods of wastewater handling. Other changes may require a reduction of cleaning chemicals within the winery.

In some wineries the use of caustic soda for cleaning has been replaced by potassium hydroxide. Potassium as a nutrient enhances the growth of beneficial crops and is much less likely to harm the groundwater than any sodium in the system. This change could apply to both rapid disposal of winery wastewater and also disposal after treatment and storage. However, based on long term soil monitoring conducted during the present study, we have shown that potassium affects soil structure equivalent to half of the effects of sodium.

A further consideration is the amount of water that is used within the winery. Disposal of the water may require shandying for both treated and untreated wastewater. If the wastewater is going to be used for irrigation immediately there is little point in being very economical with water within the winery if water has to be added as shandy at the time of irrigation. However, if the disposal system requires storage of WWW prior to irrigation, the reduction of WWW volume may be very important as storage could be a limiting factor.

8.3 Application of wastewater on vineyards

High potassium levels were identified by industry as an area of concern when reusing/disposing of WWW by irrigation. Application of wastewater with high potassium levels was found to increase the overall level of soil fertility, but long-term application may cause the build-up of soil potassium and decrease the hydraulic conductivity of the receiving soils. In the glasshouse trial conducted in the present study, treating the Shiraz vines with 25 % and 50% WWW showed similar values for K, N and Cl in their petioles. The vines grew well and showed minimal effects to disease or insect infestation. Treating the vines with up to 10 and 16 times the amounts of K and Na respectively (when compared to the WWW applications) affects vine vigour and the plants were more noticeably prone to insect damage. Using a shandied WWW for vine irrigation can be an acceptable practice. Monitoring of Na and K, as well as EC, would be required prior to application to ensure Industry (maximum) recommendations are not exceeded.

In the present study, the wastewater EC_{sw} was always below 3.5 dS/m and did not result in toxicity to grapevines during glasshouse and paired field trials. However, Na and K treatments above 40 mM and Na +K treatment (at 20 mM each) were responsible for toxicity signs in the grapevines. These higher treatments were also prone to diseases.

A reduction in vine growth generally occurs when the average root zone salinity over the growing period exceeds a designated threshold value. The link between soil-K and fruit-K is complex, but high must-K can precipitate tartrate salts and elevate pH, which require correction with (costly) tartaric acid to stabilise the must during vinification. Elevated berry K will influence the effect of other cations present and is thought to have impacts on fermentation properties and other wine sensory properties such as salty taste and bitterness, pH/sourness and the potential for some (additional) microbiologically influenced challenges. Based on the information available in the literature and the field and glasshouse trials conducted in this study, we have established sodium and potassium thresholds (individually and in combination) in grapevine irrigation water. Sodium at levels less than 460 mg/L, potassium at 1250 mg/L and Na +K in combination at 625+ 1084 mg/L, respectively in winery wastewater will be safe for application on the grapevines. These thresholds listed are indicative values and are also dependent on the grapevine variety and the rootstock used.

The sensory evaluation of the wines produced from the two paired-site field trials indicated no significant differences in the wines produced from mains water and the winery wastewater irrigated grapevines.

8.4 Recommendations

The new knowledge generated by this project should be considered in the following terms:

1. No comparative costs were available to the study that would enable a comparison of the cost:benefit of rapid disposal versus the 'treat, store and irrigate' option. This should be further explored.
2. In the present study, two paired sites were established and monitored for three years. There is a need to continue the monitoring of these recently established field trials for another five years to establish the long-term effects of winery wastewater irrigation on vines. In addition, it would be useful for this approach to be extended to other wine regions with other varieties of vines to assess the long-term impact of winery wastewater irrigation on petiole, grape juice and wine quality.
3. In the present study, Na and K effects on petiole and grape juice were evaluated over two years using glasshouse experiments. There is a need to establish mesocosm experiments in the real environment with different levels of Na and K irrigation regimes (individually and in combination) on several vine varieties over a period of at least five years to validate the Na and K thresholds established in this study.
4. The operating guidelines based on the new information generated through this project should be updated with a view to reducing the wine industry's industrial footprint, while not imposing an economic cost.

8.5 References

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Appendix A Communication

The project team has been involved in the communication of project results through a number of mechanisms.

A.1 Presentations at conferences and workshops

1. Findings were presented as part of a workshop at the Australian Wine Environment Conference, Adelaide Showgrounds on the 19th of October 2011. On 22nd November 2012 at Australian Society of Viticulture and Oenology (ASVO) 2012, Anu Kumar gave a presentation on sustainable irrigation of winery wastewater at this forum.
2. Lester Smith and Anu Kumar (2013). Beneficial crop options using winery wastewater. Proceedings of Thirteenth Australian Wine Industry Technical Conference 13-18th July 2013, Sydney.
3. Pichu Rengasamy, Alla Marchuk and Anu Kumar (2013). High potassium in winery wastewater can contribute to soil structure degradation. Proceedings of Thirteenth Australian Wine Industry Technical Conference 13-18th July 2013, Sydney.
4. Anu Kumar, Pichu Rengasamy, Lester Smith, Alla Marchuk and Ray Correll (2013). Long-term impact of winery wastewater irrigation on soils. Proceedings of Thirteenth Australian Wine Industry Technical Conference 13-18th July 2013, Sydney.
5. Adrienne Gregg and Anu Kumar (SETAC 2013).Winery wastewater irrigation reduces beneficial archaeal nitrifiers at a South Australian winery. Proceedings of the Australasian Society of Environmental Toxicology and Chemistry Conference. October 2013, Melbourne.
6. Sustainable irrigation of winery wastewater; Invited presentation at the Sustainability conference in the Barossa on 24 September 2014.
7. Sustainable irrigation of winery wastewater, Invited presentation at OCE Cutting Edge Science Symposium “Critical Zone Science: Challenges and Opportunities” from 16 to 18 September 2014 in McLaren Vale, SA.

A.2 Posters

8. Lester Smith and Anu Kumar (2013). Beneficial crop options using winery wastewater. Thirteenth Australian Wine Industry Technical Conference 13-18th July 2013, Sydney.
9. Pichu Rengasamy, Alla Marchuk and Anu Kumar (2013). High potassium in winery wastewater can contribute to soil structure degradation. Thirteenth Australian Wine Industry Technical Conference 13-18th July 2013, Sydney.
10. Anu Kumar, Pichu Rengasamy, Lester Smith, Alla Marchuk and Ray Correll (2013). Long-term impact of winery wastewater irrigation on soils. Thirteenth Australian Wine Industry Technical Conference 13-18th July 2013, Sydney.
11. Adrienne Gregg and Anu Kumar (SETAC 2013).Winery wastewater irrigation reduces beneficial archaeal nitrifiers at a South Australian winery. Australasian Society of Environmental Toxicology and Chemistry Conference. October 2013, Melbourne.

A.3 Manuscripts

1. Arienzo M , Christen EW, Jayawardane NS and Quayle WC (2012) The relative effects of sodium and potassium on soil hydraulic conductivity and implications for winery wastewater management. Geoderma 173-174, 303–310.
2. Marchuk A, Rengasamy P, McNeill A, and Kumar A (2012) Nature of the clay–cation bond affects soil structure as verified by X-ray computed tomography. Soil Research, volume: 50, issue: 8, pages: 638-644.
3. Marchuk A and Rengasamy P (2013) Threshold electrolyte concentration and dispersive potential in relation to CROSS in dispersive soils. Soil Research, volume: 50, issue: 6, pages: 473-481.
4. Pichu Rengasamy, Anu Kumar and Alla Marchuk. Cation ratio of soil structural stability (CROSS) is an appropriate measure for assessing soil structure degradation under the influence of winery wastewater irrigation

A.4 Fact sheets

1. Pichu Rengasamy and Anu Kumar. Fact Sheet on CROSS.
2. Pichu Rengasamy and Anu Kumar. Diagnosis of Dispersive Soils in the Vineyard for Gypsum Application.

A.5 Technical reports

1. Kumar A, Correll R, Lanyon D, Smith L, and Rengasamy P (2013) Impact of wastewater on soil and vine properties: three case studies.
2. Evaluation of soil chemistry data from the Hudson Site.

A.6 Wider communication

The project team has contributed significantly in the development of the tools and resources on the winery wastewater management and recycling.

Appendix B Performance against planned outputs and performance targets

Planned outputs and performance targets of the project are given below (Table B1). We were able to achieve all listed performance targets.

B.1 Output and performance targets of the project

Apx Table B.1 Output and performance targets of the project

Outputs and Activities 2010-2011

YEAR 1	OUTPUT	ACTIVITIES	
A	Establish program and select project advisory committee.	Identification of suitable participating wineries for historical data collection, field trials and sampling.	Completed
		Initial meeting of project steering group to outline project scope and responsibilities.	Completed

Outputs and Activities 2011-2012

YEAR 2	OUTPUT	ACTIVITIES	
A	Establish ongoing soil monitoring program at existing winery- vineyard and/or other relevant field sites.	Establish field sites for conducting field sampling over two years.	Completed
B	Maintain soil monitoring program at winery-vineyard/field sites.	Collation of information from field sites based on first year of sampling.	Completed
C	Soil quality impacts determined from existing recycled winery water irrigation trials.	Collection of historical data on soil quality at participating wineries.	Completed
D	Establish a paired trial to determine impacts of recycled winery water irrigation on grape and wine quality.	Paired trial established and monitoring program in place for soil, petiole, grape and wine quality analyses.	Completed

Outputs and Activities 2012 - 2013

YEAR 3	OUTPUT	ACTIVITIES	
A	Maintain soil monitoring program at winery-vineyard/field sites.	Collation of information from field sites based on second year of sampling collected at the existing field sites.	Completed
B	Publish a technical report: 1. describing protocols and	Standardise, validate and compare methods for measuring changes in soil structure and function under the	Completed Technical report 1

	techniques for assessing soil structure and function changes under the influence of recycle water irrigation, and 2. on the status of soil structure and function at recycled water irrigation sites especially in relation to sodium and potassium based on current practices.	influence of recycled winery water irrigation. Analyses of historical soil quality data and collation and documentation of recycled water irrigation strategies. Interpretation of results and compilation into a technical report.	Article 1 Fact sheet 1
C	Develop threshold values of ethanol and phenolic compounds to promote safe and sustainable use of recycle water irrigation on vines and other crops.	Conduct microbiological assessment of soil irrigated with different levels of ethanol and phenolic compounds in the recycle winery water. Conduct ecotoxicological assessment of soil irrigated with different levels of ethanol and phenolic compounds in the recycle winery water using plants and earthworm as bioindicators.	Completed Part 4 of the Annual report Completed Part 3 of the Annual report
D	A technical report and a journal article on the information on the long term consequences for soil structure in different soils (different pH, duplex/heavy clay) of long term irrigation with recycled winery water.	Analyses of vineyards, grasses, crops, and woodlots and date palm in relation to potassium uptake at existing field trials. Assess long-term consequences for soil structure in different soils (different pH, duplex/heavy clay) of long term irrigation with recycled winery water. Evaluate the use of the "cation ratio of structural stability", CROSS, as an index of soil structural stability.	Completed Part 2 of the Annual report Technical report 1 Fact sheet Article 1
E	Refine the research strategies and promotion of research findings to wider wine industry.	Organise Advisory group meeting to discuss, review and revise information collected in the first year study and refine research strategies for the second year. Disseminate results to the wider Australian wine industry via an industry article.	Completed Advisory Group meeting Presentations at AWTC 2013 and SETAC 2013 Copies provided to GWRDC as additional material
F	Publish a journal article and magazine article on the on the impact of recycled winery water irrigation on the soil microbiological health.	Monitor the impacts that recycled winery water irrigation (raw, shandied, timing, irrigation technique) has on soil microbiological health during laboratory experiments. Monitor the impacts that recycled winery water irrigation (raw, shandied, timing, irrigation technique) has on soil microbiological health at field sites such as woodlots, pastures and vineyards.	Completed Part 3 of the Annual report Article 2
G	Propose strategies to minimise salt accumulation in soil from recycled winery water irrigation based on laboratory and field trials. Develop a factsheet on gypsum application rates.	Conduct Gypsum trials using laboratory column experiments. Assess soil quality and health of vineyards applying gypsum to reduce the impact of high sodium in the recycled winery water.	Completed Part 2 of the Annual report Fact sheet 2

		Assess other optional strategies such as mid row crop in vineyards for ameliorating sodicity impact due to recycled winery water application.	

Outputs and Activities 2013 – 2014

YEAR 4	OUTPUT	ACTIVITIES	
A	Publish a technical report on the wine quality from wineries producing wine from recycle water irrigated vines.	Collection of information on petiole analyses and wine quality from paired trial. Establishment of methods and protocols for assessing nutrient levels in petiole, grape juice and wine quality. Analyses of information on petioles, grape and wine quality.	Completed Part 5 of the Annual report
B	Publish a technical report on the influence of the quality of recycled water in relation to juice quality and wine quality.	Compare nutrients in petioles and grapes irrigated with recycled winery water, bore water and shandied stormwater. Compare spectral analyses of wine produced from grapes irrigated with recycled winery water, bore water and shandied stormwater. Compare preliminary sensory evaluation of wines produced from grapes irrigated with recycled winery water, bore water and shandied stormwater.	Completed Part 5 of the Annual report
C	A technical report on recycled water quality thresholds for various soil types under differing recycled winery water types and management actions.	Conduct soil column experiments with different soil types under different COD levels of recycled water. Analyse soluble and available sodium and potassium levels of the leachate and soil at different irrigation regimes.	Completed Part 2 of the Annual report Fact sheet 2
D	Refine the research strategies and promotion of research findings to wider wine industry.	Organise Advisory group meeting to discuss, review and revise information collected in the second year of the project and refine research strategies for the final year of this project. Documentation of findings in a formal report to GWRDC. Disseminate results to the wider Australian wine industry via an industry article and road shows, regional workshops and technical conferences and papers - where appropriate.	Completed
E	Identify beneficial crops for harvesting nutrients based on laboratory pot trials and existing field trials.	Conduct pot trials involving selected plants (such as sugar beets, turnips, chickpeas, tomatoes, corn) at different salt levels. Conduct vine trials in glass house involving recycled water at different salt levels.	Completed Part 6 of the Annual report Part 5 of the Annual report

		Assess soil structure and function at winery sites using recycled water for growing beneficial crops. Assess soil structure and function at winery sites using recycled water for growing vines.	
F	Develop recommendations for sustainable recycled Winery Water Irrigation based on Treatment Fit for Purpose Approach. Communication of research findings to wider wine industry.	Based on this study collate information on the effects of K (and other cations) and recycled winery water at different COD levels on vine physiology and grape attributes and other beneficial crops. Develop hazard guidelines related to the impact of recycled water use on soil degradation, vine nutritional properties and wine quality. Develop a framework and decision support tool for wineries to apply fit for purpose approach for recycling winery water. Organise Advisory group meeting to discuss, review and revise information collected in the third year of this project. Documentation of findings in a final report to GWRDC. Disseminate results to the wider Australian wine industry via road shows, regional.	Completed Part 7 of the Annual report

Appendix C Staff

ORGANISATION	STAFF NAME	POSITION	ROLE
CSIRO	Dr Anu Kumar	Principal Research Scientist	Project Leader
Adelaide University	Dr Pichu Rengasamy	Principal Research Scientist	Chief investigator and soil scientist
Adelaide University	Dr Alla Marchuk	PhD student	Soil analyst
CSIRO	Mr Lester Smith	Research Support officer	Experimental scientist and soil analyst
CSIRO	Dr Ray Correll	Statistician	Reviewer and experimental planner
CSIRO	Ms Adrienne Gregg	Research Support officer	Microbiologist
CSIRO	Mr Hai Doan	Research Support officer	Experimental scientist
CSIRO	Miss Debra Gonzago	Research Support officer	Wastewater analyst
CSIRO	Dr Wendy Quayle *	Research scientist	Manuscript on soil effects
ARRIS	Dr. Dean Lanyon**	Research scientist	Historical data collation and viticulturist
CSIRO	Supriya Lath and Donald Oats	Casuals	Assistance with glass house experiments

*involved only in first year of the project

** Involved only in the first half of the project

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FOR FURTHER INFORMATION

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