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IMPACT OF WINERY WASTEWATER ON ECOSYSTEM HEALTH- AN INTRODUCTORY ASSESSMENT

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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Title:

IMPACT OF WINERY WASTEWATER ON ECOSYSTEM HEALTH

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EXECUTIVE SUMMARY

The wastewater generated by wineries has high biological oxygen demand (BOD), organic content, electrical conductivity and therefore needs to be managed carefully in the environment. Prior to this study there was a lack of information on the impact of winery wastewater on ecosystem health in aquatic and terrestrial environments. To effectively manage winery wastewater in the environment and as a potential resource for irrigation there is a need to correctly assess the environmental impact of winery wastewater on both aquatic and terrestrial ecosystems. Therefore, GWRDC and CSIRO initiated this project with the following objectives in mind.

The main objective of this project has been to assess the ecotoxicological impact of winery wastewater on aquatic and terrestrial ecosystems.

Specific objectives were to:

1. characterise the winery wastewater;
2. establish baseline levels of toxicity and variability of toxicity of the winery wastewater;
3. identify the classes of contaminants responsible for the toxicity of the winery wastewater;
4. assess the performance of artificial wetlands in decreasing pollution;
5. evaluate the toxicity of three commonly used polymers to selected aquatic fauna; and
6. assess the soil health of vineyards and woodlots during and after the application of wastewater.

Winery wastewater characterisation was carried out at the representative small, medium and large sized wineries in SA. For this purpose, five wineries were selected representing small-scale, moderate and large-scale wineries in the Barossa Valley and the McLaren Vale regions. These covered a range of treatment processes from almost no treatment to state of the art wastewater treatment available in the wine industry. Samples were collected from the selected five wineries on a weekly basis during the vintage seasons, and fortnightly in the non-vintage seasons. A total of 285 samples were collected over two years. A range of physicochemical characteristics of wastewater such as pH, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), TOC (Total Organic Carbon), salinity, electrical conductivity (EC), nutrients, heavy metal contaminants and total suspended solids were analysed following standard wastewater analyses methods.

Small and medium sized wineries showed highly variable data and poorer wastewater quality than the larger wineries sampled under this study. There were major differences in pH, EC, total organic carbon loading, SAR and BOD among wineries, due to the differences in wine processing and in treatment processes employed in these wineries. Winery wastewater produced during vintage always had higher biological oxygen demand, total nutrients, electrical conductivity and was found to be more acidic in nature. Canonical variate analyses revealed that during vintage and non-vintage periods, SAR was the dominant distinguishing variable among five wineries followed by EC and pH. These parameters are more likely to reflect the treatment process, e.g. pH adjustment.

Ecotoxicological investigations were conducted with waterflea (*Ceriodaphnia dubia)* and duckweed (*Spirodela* sp). Winery wastewater exhibited toxicity to the waterflea and duckweed, but its extent was highly variable. Consistently greater toxicity was observed during vintage season to both of the test organisms. It was also noted that wastewater from the winery representing small wineries tended to show greater toxicity to waterflea compared to those representing moderate and large scale wineries, during the vintage season. During non-vintage season, a clear trend did not emerge.

Toxicity Identification and Evaluation (TIE) procedures were followed to determine the sources of toxicity effects (e.g. class of contaminants) present the winery wastewater. The TIE process showed that manipulations such as solid phase extraction (which remove organics), EDTA chelation (which removes inorganics, especially heavy metals), aeration (which remove volatile organics), pH adjustment (mixed effect on range of components, e.g. on polymers) and filtration (which removes suspended solids) all reduced toxicity of the winery wastewater to some extent. In the non-vintage season, solid phase extraction treatment (removing organic compounds) completely removed toxicity from the winery wastewater, whereas in the vintage season it could only partially remove the toxicity, however, still making the biggest impact in toxicity reduction. This indicates that predominantly the organic compounds are likely to be the key source of toxicity of winery wastewater to aquatic organisms. Simple pH adjustment step was noted in some cases to result in dramatic decrease in toxicity, which appeared to be related to the polymer chemistry and toxicity response to pH.

To assess the efficiency of wetland system, four ponds and a dam in the wetland at winery B were sampled on a monthly basis. Bioassays with C. dubia for acute toxicity were performed on a series of dilutions of the winery wastewater. After 48 hour exposure, waterfleas alive at each dilution were used to calculate toxicity parameters, including no-observable effect

concentration (NOEC) and the lowest-observable effect concentration (LOEC) values for each wetland cell sample. In situ studies were also conducted by placing midges in the different ponds of the wetland. Their survival was monitored after 24-hour exposures.

During the 2003 vintage season, the health of the wetland at winery B was found to be highly impacted. Waterfleas died when exposed to the waters from different ponds of the wetland. Midges also did not survive in all the ponds even during a 24-hour exposure. In addition macroinvertebrate diversity in the wetland ponds was found to be highly impacted during the vintage season. This, however, significantly recovered in the non-vintage season. It became necessary to establish what was causing this effect. Toxicity identification evaluation (TIE) procedures were employed, which revealed that the high level of acidity was contributing to the toxicity of winery wastewater. On further follow-up with the winery, the polymer used was identified to be the contributing factor to the observed toxicity. The research that followed led to the identification and replacement of the toxic polymer by one that had lesser toxicity. Since this change (March 2004) the wetlands showed significant apparent recovery in aquatic health. The research highlighted the need for great caution in use of polymer to treat winery wastewater and especially in the selection of environmentally safer polymers. A significant knowledge gap on the appropriateness of available polymers was identified during the project. This has been and is being communicated to the industry via presentations at different fora.

On the impact of winery wastewater on the terrestrial ecosystem, both laboratory and field based approaches were employed. Sites were identified that have been receiving winery wastewater irrigation over a long-term (10-100 years). While these sites were not designed as experimental plots and had several limitations. However, since long term trials are not otherwise available, we saw significant value in investigating aspects of soil health at these sites, especially the soil biological health - the focus of this project. For the laboratory component, we carried out controlled experiments on soil columns packed with three different types of soils, as described below.

A range of indicators of soil microbial activity, i.e. respiration, nitrification and soil enzyme activities, were monitored at the sites receiving winery wastewater. These indicators covered overall soil microbiological activity (respiration) as well specialist functions (e.g. nitrification). Four different types of enzyme based assayed were used which allowed an assessment of activities of enzymes associated involved in carbon turn over and nutrient transformations.

Furthermore these were chosen to represent enzymes associated with bacteria, fungi and plant roots. These tools were used for both laboratory and field investigations.

Monitoring of the soil biological health was carried out in the vineyards, pastures and woodlots where wastewater has been used for irrigation purposes over a long term (10-20 years). Woodlot sites were selected at the two large wineries. The woodlot at winery F and Y had been receiving winery wastewater for 10 and 8 years, respectively. The woodlot at winery Y had received winery wastewater for the last eight years. The vineyards were selected at Winery Y which had received winery wastewater for the last 4-6 years. Pasture sites were receiving winery wastewater for approximately last 15-20 years, respectively. At a small winery the site had a very long history of winery wastewater application, possibly up to 100 years. Soils samples were collected from the treated areas as well as sites that were identified as those which can serve as control sites for these. The sampling strategy was designed keeping statistical consideration in mind and to gain a best possible handle on the non-experimental nature of sites.

A significant increase in the soil organic matter status of the soils receiving wastewater was observed. Results showed that biological activity (e.g. mineralisation of organic carbon) increased in irrigated pastures (which exhibit a build-up of organic carbon), compared to their respective controls. Substrate induced respiration (SIR) – which indicates overall microbial activity - in vineyard soils irrigated with winery wastewater did not differ significantly from that of control vineyards. Woodlot soils irrigated with winery wastewater exhibited increased SIR in comparison to the control woodlot soils. Nitrification (conversion of NH_4^+ to NO_3^-, the major source of nitrogen for plants) followed the same trend. Pasture soils irrigated with winery wastewater had higher substrate induced nitrification (SIN) in comparison to the control reference soils. In the vineyards, the control was not significantly different to the irrigated vineyards for these microbiological parameters. Woodlots soils irrigated with winery wastewater exhibited increased SIN than in comparison to the control woodlot soils. Microbial enzyme analyses also confirmed that winery wastewater irrigation of soils was not adversely impacting the microbial activity of soils. In summary, soil microbiological activity was not directly adversely affected in any of the wastewater treated plots. In fact, greater microbial activity was observed in wastewater treated plots, most likely due to the build-up of the organic carbon content in soils. However, salinity, sodicity and available potassium in soils, were noted to be elevated in the wastewater treated plots in comparison with the control plots. More than 100 years of application of winery wastewater at the pasture site of the small winery was responsible for the highest build-up of salts and organic carbon at this

site. Both of these properties can indirectly influence soil microbiological functions, e.g. prolonged waterlogged conditions creat anaerobic conditions adversely affecting aerobic microorganisms.

To investigate the tolerance of different soil types to winery wastewater in terms of adverse soil biological functions and or soil chemistry parameters, laboratory experiments were conducted on three different soil types (a loamy sand, a loam and clay soil) with contrasting physico-chemical characteristics. In these studies, repacked soil columns were irrigated with a high volume (up to 51 mm per week) of winery waste water (procured fortnightly from a local winery) for a period of 16 weeks. This was deliberately done, not to mimic possible real world scenarios, but more to push the system hard in a short term and make an assessment of response in terms of soil microbiological and chemical properties. The set of soil microbiological indicators (described above) were employed for the soil biological component. Build up of salt in soil was examined throughout the 20 cm long columns and chemical composition of the leachate was assessed during the experiment.

In terms of soil physical properties, it was observed that while the loam and loamy sand could take the irrigation of about 500 mm of water, the clay soil could not tolerate more than 200 mm by which time its pores got clogged leading to virtually zero infiltration and severe water logging. None of the soil microbiological parameters (SIR, SIN and four enzymes) showed any significant difference between the irrigated and control columns (irrigated with RO water). This was consistent with the field observations described above. However, the study did not allow commenting on indirect effects that may occur due to structural decline or salt build-up or waterlogging as a result of irrigation with wastewater. For example, if oxygen levels in soils are depleted due to continuous waterlogging then there is likely to be a significant adverse impact on soil microbiological activity.

In contrast to soil microbiological properties, marked effects of wastewater irrigation were noted on soil chemical properties. To examine the changes in soil chemical properties after irrigation with wastewater, soil samples from different depths in soil columns were analysed for a range of inorganic ions. The results showed that salt levels increased with irrigation in the soil columns. The increase was marked in the case of sodium and magnesium in soil and water extract, which showed about 40 and 90 fold increase respectively. Similarly calcium and potassium levels doubled during the experiment on loam soil, which received about 509 mm of wastewater. The leachate collected from the soil columns was also analysed for a range of elements. The salts started to leach through with 152 mm of irrigation water in 14 days. Similarly sodium levels in column leachate reached and exceeded the levels present in

wastewater within a period of 4 weeks (305 mm water). In contrast, however, potassium levels in leachate, although increased with time, did not reach the input concentration through wastewater irrigation even after 509 mm of wastewater irrigation. This is consistent with the data on soil accumulation of potassium, which nearly doubled during the experiment. Dissolved organic carbon (DOC) in leachate also increased initially with the increasing volume of wastewater used in irrigation but then stabilized, possibly due to enhanced microbial activity. Among nutrients, phosphorus levels in the leachate from wastewater irrigated columns of loam soil were almost the same as in the case of those irrigated with clean water. However, the nitrate and ammonium levels increased with time in the leachate from the loam soil column.

Overall conclusions:

The study provided some definitive data on winery wastewater characteristics, especially in relation to their ecotoxicological impacts on both aquatic and terrestrial ecosystems. For aquatic ecosystems, it became evident that during vintage the wastewater produced had higher toxicity and the toxicity was greatest in the case of small wineries not employing any proper treatment process. It was also noted however, that treatment processes were helping reduce the toxicity of wastewater for aquatic organisms but were leading to greater salt load in the wastewater which is of greater consequence to the terrestrial ecosystem.

The study identified that the bulk of the toxicity was associated with organic fraction of the wastewater which when removed rendered the wastewater suitable for sensitive aquatic organisms. Copper and zinc ions in winery wastewater could be of some concern. Use of toxic polymers was noted to cause a major effect on wetland ecosystems. Interventions such as pH adjustment were found very helpful in removing toxicity associated with the cationic polymers. Preliminary investigation on three commonly used polymers revealed that current cationic polymers used in the wineries can be classified as being highly to moderately toxic. This toxicity of cationic polymers to the aquatic organisms could be related to the surface membrane interactions. Polymer can bind to the surface of the integument and/ or to appendages on waterfleas inhibiting movement and the subsequent uptake of nutrients which could result in mortality of the exposed organisms. Greater care is needed in choosing non-toxic polymers such as anionic polymers for use in the treatment processes. In general, the species diversity and water quality were poorer at the treatment wetland system in comparison to any natural wetland system. However, the tested wetland system was able to improve the winery wastewater quality in the dam making it more suitable for irrigation purposes.

For terrestrial ecosystems, the data set shows that there was no adverse measurable impact on the soil microbiological activity or selected functions that were tested. On the other hand soil physical and chemical properties were significantly impacted by the use of winery wastewater. Indeed, the greatest impact of the winery wastewater irrigation on land is likely to be through the build up of salts, especially sodium and potassium. The build up of monovalent ions in the soil profile can result in dispersion of soil aggregates and deterioration of soil structure and impairing soil productivity. This aspect needs a thorough investigation to clearly establish the acceptable threshold for different soil types in tolerating winery wastewater.

Recommendations/Future work

1. Based on the extensive characterisation of winery wastewater in this study pH, EC, TOC and SAR can be recommended as four important key indicators of winery wastewater quality.
2. An integrated approach is needed to identify issues at source, treatment and reuse steps for better management of winery wastewater. Opportunities need to be exploited for minimizing the wastewater volume and the separation of product/waste streams (e.g. spills of juice, wine). This is being explored in a new GWRDC/CSIRO project on management of winery wastewater.
3. Simple treatment steps such as filtration, aeration and pH adjustment are desirable in improving the winery wastewater quality. However, some of the steps may lead to greater salt loading.
4. The pH of winery wastewater should be carefully adjusted if cationic polymers are being used as flocculants. Little ecotoxicological information is available on polymer toxicity to terrestrial organisms. Similarly there is a variety of chemicals such as cleaning agents and flocculants used across the wine industry, with little information on the toxicity of these chemicals. Further studies should focus on environmental rating of all cleaning agents and flocculants used.
5. Wetland systems adapt well to the wastewater fluctuations of wineries. Removal of solids from winery wastewater entering the wetland ponds, pH adjustment and aeration of first the pond are important management strategies for wetland systems receiving winery wastewater.
6. Based on the current investigation, the greatest impact of winery wastewater irrigation on land is likely to be through the build up of salts, especially sodium and potassium. The build-up of monovalent ions in the soil profile can result in deterioration of soil structure and consequently can adversely impact the soil productivity. This aspect needs a thorough investigation to clearly establish the threshold of different soil types in tolerating winery wastewater with respect to soil chemical, physical and biological perspectives
7. The treatment processes have historically been driven by an aquatic ecosystem as a receiving environment. The changed reuse pattern to an irrigation resource for vineyards or other land-use need to be considered. In particular the criteria such as BOD are of lesser importance than salt loading. There is a need to tailor the treatment steps to the chosen method of its reuse.

1. INTRODUCTION

Pollution pressures associated with urban, industrial and rural development threaten many ecosystems in Australia and worldwide. Increasing awareness of the effect of pollution has resulted in greater public pressure to assess, monitor and regulate polluting activities. The wastewater generated by wineries has become a significant concern to the environment in recent years due to its high biological oxygen demand (BOD), organic content, electrical conductivity and salinity.

Concerns have been expressed that very little information is available on the environmental impact of winery wastewater. It is noteworthy that the current policy requires guidelines for water quality to be based on ecotoxicological impacts rather than the contaminant concentrations. Therefore, it is essential to determine the ecological effects of winery wastewater on ecosystem health (both terrestrial and aquatic) in order to establish the safe limits for winery waste discharge. This is also necessary to maintain the “clean and green” image of the Australian grape and wine production systems.

1.1 Industry context

Winery wastewater is extremely variable in quality and discharge volume throughout the year depending on the winery operations underway at any particular time. Wastewater can cause salinisation and eutrophication of water resources. If high levels of BOD in winery wastewater are allowed to flow untreated to surface waters (streams, rivers, ponds and lakes), the dissolved oxygen in the receiving water may be quickly consumed. Aquatic and amphibious life forms could suffocate as the dissolved oxygen in the water is quickly depleted.

Winery wastewater is often used for irrigation of vineyards. Improper wastewater application can damage soil health by waterlogging, salinisation, chemical contamination, erosion and by affecting the diversity of soil micro-organisms. It is very important to maintain the diversity of soil micro-organisms as they help vines get the type of nutrition they need. A healthy soil microbe population is also essential for preventing plant diseases and pests from gaining an advantage in the field. There is not enough information on the impact of long-term application of winery wastewater on the soil microbe diversity in vineyards and woodlots.

The wine industry includes many small producers, whose effluent production quantity and financial resources may not be sufficient to warrant high-technology for the treatment of wastewater. As a result, effluent is often stored in evaporation ponds or sprayed over open land for evaporation. The impacts of concentrated, seasonal runoff of waters containing high

nutrient and organic loadings become increasingly well known to the environmental regulators. There is also a need to investigate the environmental impact of both large and small-scale wineries.

Public perception is also an important issue when it comes to waste management. Viticulture industry is promoting itself as “clean green’. Waste management can be a very critical issue particularly if waste contaminates the soil, groundwater or surface water. As the industry grows, the volume of winery wastewater is growing. This means that the problem could be growing and the risks of causing an incident which leads to a prosecution or some claim for damages are increasing. Little research has been directed towards on-site impacts and potential for downstream contamination. Therefore, there is an urgent need to determine the ecological effects of winery wastewater on ecosystem health, both terrestrial and aquatic in order to establish safe limits for winery wastewater discharge.

There is increasing pressure on suppliers to both the Australian and overseas markets to comply with the ISO14001 standard. At this stage, we don’t know the loads of wastewater a vineyard could tolerate without causing any harmful effects on the soil biota. Wetlands are constructed to deal with winery wastewater but at this stage we don’t have information on their efficiency.

1.2 Relevant R & D and guideline documents

A Gap analysis of priorities undertaken by GWRDC put wastewater management as the 4th highest combined priority in sustainable production. Analysing the wine maker’s priorities alone, wastewater was ranked as the highest priority together with lifecycle analysis to measure the ecological footprint of the whole industry and the need for guidelines for handling wine making products.

A winery wastewater handbook by Dr Jeanette Chapman provides an overview on the winery wastewater related issues (Chapman *et al.,* 2001). This book provides insight to sources of individual characteristics of winery wastewater and outlines the effects of the winery wastewater application on the soil and groundwater environment and its use for irrigation of vineyards and woodlot. Chapman and coworkers have extensively (1995) studied the removal of soluble carbon from synthetic winery wastewater by repeated application to soil cpolumns. To our knowledge, ecological effects associated with winery wastewater discharge and impact on soil microbes due to the use of winery wastewater for irrigation has not been investigated nationally or internationally.

Environmental Management Audit of wineries and distilleries in South Australia was carried out by EPA in 2001-2002 (SA EPA, 2002). The audit, commissioned by the EPA in cooperation with the SA Wine and Brandy Industry Association, involved sixty-three SA wineries each handling more than 500 tonnes of grapes a year. Environmental issues such as wastewater storage, treatment and disposal; solid waste management; noise, odour, contingency planning and stormwater management were discussed. Wastewater management related issues such as the lining of wastewater lagoons, disposal of wastewater to areas with steep slopes, as well as the combination of domestic and winery wastewater without necessary monitoring were highlighted in the Barossa region. In the Riverland, the main concern was wastewater disposal, with the sheer size of winery operations and the wastewater they generate being the key issues.

The Wine Industry Waste Management Forum, through a workshop in 2003 (Wightwick, 2003), identified the following types of wastes, listed here in the decreasing order of importance- grape marc; wastewater (including segregation of it); product waste; caustic cleaners; wastewater sludge; and filtration wastes (i.e. centrifuge sludge and diatomaceous earth). .

SA EPA (2004) has published guidelines to provide information that will assist wineries and distilleries to develop an environmental monitoring program to comply with the Environment Protection Act and relevant Environment Protection Policies.

The following publications refer to the issues and guidelines related to the winery wastewater management in Australia.

1.2.1 Guidelines

1. EPA SA (2004). EPA Guidelines for Wineries and Distilleries. Government SA and EPA SA. http://www.environment.sa.gov.au/epa/pdfs/guide_wineries.pdf
2. Guidelines for winery waste disposal (small wineries crushing less than 500 tonnes)' (Shire of Augusta-Margaret River). (http://www.amrsc.wa.gov.au/ppdfs/winewaste.pdf).
3. Commonwealth guidelines.

1.2.2 Books

1. Chapman J. (1996). 'Cleaner production for the wine industry'. (South Australian Wine and Brandy Industry Association. South Australia).

2. Chapman J, Baker P, Wills S. (2001). 'Winery wastewater handbook'. (Winetitles. South Australia).
3. PIRSA irrigation manual for re-use water.

1.2.3 Articles

1. Chapman J, Cass A. (1996). Winery wastewater management – towards 2000. *The Australian Grapegrower and Winemaker*. **390a**: 30 – 33.
2. Chapman J. A., Correll R.L, Ladd J.M. (1995). Removal of extractable organic carbon from winery and distillery wastewaters by application to soil. *The Australian Journal of Grape and Wine Research*. **1**: 30 –47.
3. Chapman. J. (1999). Winery wastewater management is it about changing 'dead' money into 'value added' money, environmental sustainability, or both? *The Australian Grapegrower and Winemaker*. **426a**: 32 – 35.
4. Chapman. J. A., Correll R.L, Ladd J.M. (1995). The removal of soluble organic carbon from synthetic winery wastewater by repeated application to soil. *The Australian Journal of Grape and Wine Research*. **1**: 76 – 85.
5. Goss P. (1995). Auditing water and wastewater systems. *The Australian Grape and Winemaker*. **378a**: 138 – 139.
6. Goss, P. (2003). Winery wastewater – the most important by-product of a winery. *The Australian and New Zealand Grapegrower and Winemaker,* February, 2003**,** 36-39.
7. Johansen, A. (2003). Winery liquid waste: lagoons and bioreactors. *The Australian and New Zealand Grapegrower and Winemaker,* April, 2003**,** 48-49.
8. Johansen, T. (2004). Evans and Tate adopt benchmark waste treatment technology. *The Australian and New Zealand Grapegrower and Winemaker,* Feb, 2004**,** 38-40.
9. Lehane R. (1995). 'Effluent irrigated plantations: Design and management'. CSIRO Divisions of Forestry and Soils Technical Paper No. 2.
10. Leske P. (1992). Cleaning and sanitation guidelines for wineries. *The Australian Grapegrower and Winemaker*.**347**: 47 - 49
11. Lyster, R. (2003). Latest trends in waste management at a state level: how they might affect your operations? *The Australian and New Zealand Grapegrower and Winemaker,* July, 2003**,** 43-45.
12. Lyster, R. (2003). Managing wastewater; are you compliant with the law? *The Australian and New Zealand Grapegrower and Winemaker,* December, 2003**,** 102-103.
13. Olden S. (2002). BRL Hardy's Berri Woodlot – a case study. *The Australian and New Zealand Grapegrower and Winemaker*. **467**: 62 – 65

14. Smith F. (2002). Winery waste water options. *The Australian and New Zealand Grapegrower and Winemaker*. **459**: 40 – 41.

1.3 Aims and Objectives

The main objective of this GWRDC funded project was to assess the ecotoxicological impact of winery wastewater in South Australia.

Specific objectives were:

1. characterisation of the winery wastewater;
2. establishment of baseline levels of toxicity and variability of toxicity of the winery wastewater;
3. assessment of the performance of artificial wetlands in decreasing pollution;
4. assessment of the soil health of vineyards and woodlots during and after the application of wastewater; and
5. assessment of water and sediment quality of the adjacent creeks and rivers receiving wastewater due to accidental spillage and subsurface flow of leachate.

Recognising the uncertainty associated with occurrence of spillage, as well sensitivity associated with such events (EPA involvement and litigation), the steering committee of the project had recommended (as reported in 2003-04) that this aspect of the project should be dropped and resources should be directed to other aspects.

The following additional sub-objectives were addressed in the project to replace Objective 5 of the original proposal submitted to GWRDC.

1. identification of the classes of contaminants responsible for toxicity in the winery wastewater following toxicity identification evaluation procedures; and
2. toxicity of three commonly used polymers to the selected aquatic fauna commonly found in the wetlands.

2. CHEMICAL CHARACTERISATION OF WINERY WASTEWATER

2.1 Introduction

Wineries produce 3-5 kL of wastewater per tonne of grapes crushed, which represents a few hundred ML per major wine producing district within Australia annually. Winery process wastewaters are among the most challenging to degrade biologically due to their extreme variability in duration, quantity and composition. Little information exists on the identities and relative concentrations of the dominant organic and inorganic components of the wastewaters.

The main aim of this component of the project was to characterise the winery wastewater quality for different wineries by assessing wastewater quality in terms of

- seasons (pre-vintage, vintage and post-vintage);
- size (Small, moderate and large wineries); and
- treatment processes used (no treatment versus combination of treatment processes such as pH adjustment, polymer treatment, settling ponds, aeration and sand filtration).

In addition, winery wastewater quality data submitted to EPA SA by 67 licensed wineries was also assessed.

2.2 Selection of wineries

Five wineries were selected representing small-scale, moderate and large-scale wineries in the Barossa Valley and the McLaren Vale regions. Key features of the wineries selected in this study are provided in Table 2.1. Based on an annual crush of greater than 20,000t, wineries Y, F and G were considered as the three large wineries. Winery B was chosen as a moderate size winery with an annual crush of 10,000t (Table 2.1). Winery K was a small winery.

Table 2.1 Wineries selected for the characterisation of wastewater

Winery	Wines produced	Region	Winery Size	Grapes crushed annually (tonnes)	Treatment processes used
B	Red/white	McLaren vale	Medium	10,000	1. Screening for solids 2. Polymer treatment 3. Constructed wetland for final polishing
F	Red/white	Barossa	Large	20,000	1. Polymer treatment 2. pH adjustment 3. Settling ponds 4. Aeration 5. Land irrigation
G	Only Red	Barossa	Large	30,000	1. Screening 2. Two Aeration tanks 3. Four dams for wastewater storage 4. Land irrigation
K	Red/white	McLaren vale	Small	400	1. No treatment before land disposal 2. Land disposal
Y	Red/white	Barossa	Large	24,000	1. Screening – 0.8mm 2. Equalisation & Neutralisation to pH 6-8 3. Clarification 4. Sand Filtration 5. Land irrigation

Winery B is exclusively a contract processing winery and crushes 10,000t grapes annually. This winery uses polymers to separate out lees and wastewater is passed through a series of ponds in a constructed wetland for final polishing. Wineries F, G and Y are classified as the large wineries in the Barossa valley region (Table 2.1). Winery G only makes red wines where as wineries F and Y are producing both red and white wines. Wine making processes involves polymer treatment, pH adjustment, settling ponds and aerobic ponds at F and G large wineries. At large winery Y, winery wastewater treatment involves screening – 0.8mm, equalisation & neutralisation to pH 6-8 by adding lime, clarification and sand filtration before it is used for irrigation of woodlots, pastures and vineyards.

Winery K is classified as a small winery, with a crush of 400 tones per year (Table 2.1). It is an old winery with the original fermentation tanks build in 1896 still in use. Waste from the winery is discharged into an open unlined earth drain, which discharges down a bank.

2.3 Methodology

2.3.1 EPA data analyses

The first stage of winery wastewater characterisation involved assessing the data that already exists with EPA. Data was analysed to pick up the trends in winery wastewater quality depending upon pre-vintage, vintage and post-vintage seasons.

2.3.2 Wastewater sampling

Winery wastewater varies in composition both within and between days. To account for the representative variation in the winery wastewater quality, samples were collected from the selected five wineries on a weekly basis during the vintage season. In the non-vintage season, sampling was conducted fortnightly. In total, 285 samples were collected from March 2003 to May 2004. This gave an opportunity for assessing winery waste water quality data from two vintage seasons.

At the large wineries G and F, winery wastewater was collected from the tanks and dams used for storing treated winery wastewater (winery wastewater) prior to irrigation. At wineries B and Y, winery wastewater was collected at four different time intervals within 24 hours and composite sample was used for further analyses and testing.

At winery K, there was no equipment to take a running sample. This small winery installed a 10 L sump at the outfall, and this gave some averaging effect of the winery wastewater. Samples were collected each Tuesday or alternate Tuesdays depending on the vintage or the non-vintage seasons. Each sample represented two 1 L bottles full of wastewater that was collected from the sump. The samples were stored overnight in a refrigerator and collected next day. Consideration was given to taking more frequent samples at this small winery and composting them to give a better approximation to continuous sampling. This idea was discounted because of

1. Sampling complexity – the probability of an error is increased by having a more elaborate scheme;
2. Comparability with other samples – the more continuous sampling would mean that some portion of the sample was up to a week old before the physico-chemical and toxicity testing was performed.

For these two reasons a simple once per week sample was preferred.

2.3.3 Physico-chemical analyses

Physicochemical characteristics of wastewater such as pH, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), TOC (Total Organic Carbon), salinity, electrical conductivity (EC), nutrients, heavy metal contaminants and total suspended solids were analyzed following standard wastewater analyses methods (APHA/AWWA, 1992). Acidity, alkalinity, sugar and phenol content of the wastewater samples were also determined.

Physico-chemical data was then compiled into the spreadsheets to further assess the spatial and temporal variation in the winery wastewater based on statistical analyses. Collected winery wastewater samples were divided into three groups representing three seasons of varying duration for each winery. Months of January and February were designated as the pre-vintage season. From early March to late May was classified as the vintage season. Period from June – December was assigned as the post-vintage season.

SAR calculation

The sodium adsorption ratio (SAR) of irrigation water is generally a good indicator of sodicity, or the sodium status that will occur in the soil. SAR is measured as:

$$SAR = \frac{[Na^+]}{\sqrt{\frac{1}{2}([Ca^{2+}]+[Mg^{2+}])}}$$

Na^+, Ca^{2+} and Mg^{2+} refer to sodium, calcium and magnesium and their ion concentrations are expressed as millimol/L. Concentrations in mg/L were converted into millimol/L by multiplying

Na^+: 0.0435

Ca^{2+}: 0.0500

$Mg^{2+:}$ 0.0833

2.4 Statistical methods

Mean and standard deviation was calculated for the key parameters of winery wastewater from each winery. Data were analysed for pre-vintage, vintage and post-vintage seasons for the five wineries selected in this study. In addition, Box plots and multivariate analyses were also conducted to identify key variations and trends in the winery wastewater quality data.

2.4.1 Boxplots

Boxplots were constructed using Splus as shown in Figure 2.1. Note that in these diagrams a logarithmic scale has been used. This technique indicated the median and interquartile range of the data, as well as showing the range of the data.

2.4.2 Multivariate analysis

The multivariate analysis was performed using two sets of parameters. The first set is shown in Table 2.2 and consisted of environmental parameters of potential concern to environmental managers while the second included the inorganic components of the wastewater.

Table 2.2 Variates used in multivariate analysis of indices of potential concern

Variable	Description
EC	Electrical conductance, which is a surrogate of salinity
pH	
SAR	Sodium adsorption ratio which is a measure of the ratio of monovalent to bivalent cations
DO	Dissolved oxygen (mg/L)
TOC	Total organic carbon (mg/L)

The second set of data consisted of the inorganic components (N, Ca.....Al, Zn)
The analyses were aimed at highlighting the differences between the wineries. This was achieved using canonical variate analysis. This technique forms weighted averages of the variables that maximises the differences between groups (wineries) while minimising the within group variability.

2.5 Results

2.5.1 EPA data

Winery wastewater data from EPA was assessed to determine the trends in the variation of winery wastewater quality. The data analyses revealed many gaps. The data on winery wastewater quality were not consistent among different wineries. The notable differences were related to frequency of sampling, units used to represent data, differences in protocols used for analyses by different laboratories and quantity and quality of data. Usually large wineries provided better information on winery wastewater quality as compared to the small-scale wineries.

2.5.2 Winery wastewater characteristics

pH varied significantly among wineries as shown in Figure 2.1. Winery wastewater of the small winery K showed the most variation in pH with values ranging from 3.2 – 9.5. Winery wastewater pH at the large winery Y was quite stable with mean value of 6.4 and 6.3 in the vintage and post-vintage season, respectively.

Winery wastewater EC varied from 900 – 3000 μS/cm (Figure 2.2). Winery K had the lowest EC during vintage, but this was less clear during the non-vintage season.

Total organic carbon (TOC) content of winery wastewater varied significantly for the wineries K and Y (Table 2.3 and Figure 2.3). Large wineries G and F exhibited winery wastewater with stable TOC during pre-vintage, vintage and post-vintage seasons.

Measure of sodium, calcium and magnesium ions in winery wastewater was used to calculate SAR. Winery G had higher SAR than the other four wineries (Figure 2.4). Moderate-scale winery B and the small winery K had the least SAR values.

Wastewater produced during vintage had higher BOD than in comparison to the pre-vintage and post-vintage seasons (Figure 2.5). In general, BOD of winery wastewater varied between 1000- 7000 mg/L in the vintage season and between 300 – 3500 mg/L in the non-vintage season. Wineries K and Y showed the highest variation in their BOD values. Red wine producing winery G had the least BOD values (Figure 2.5).

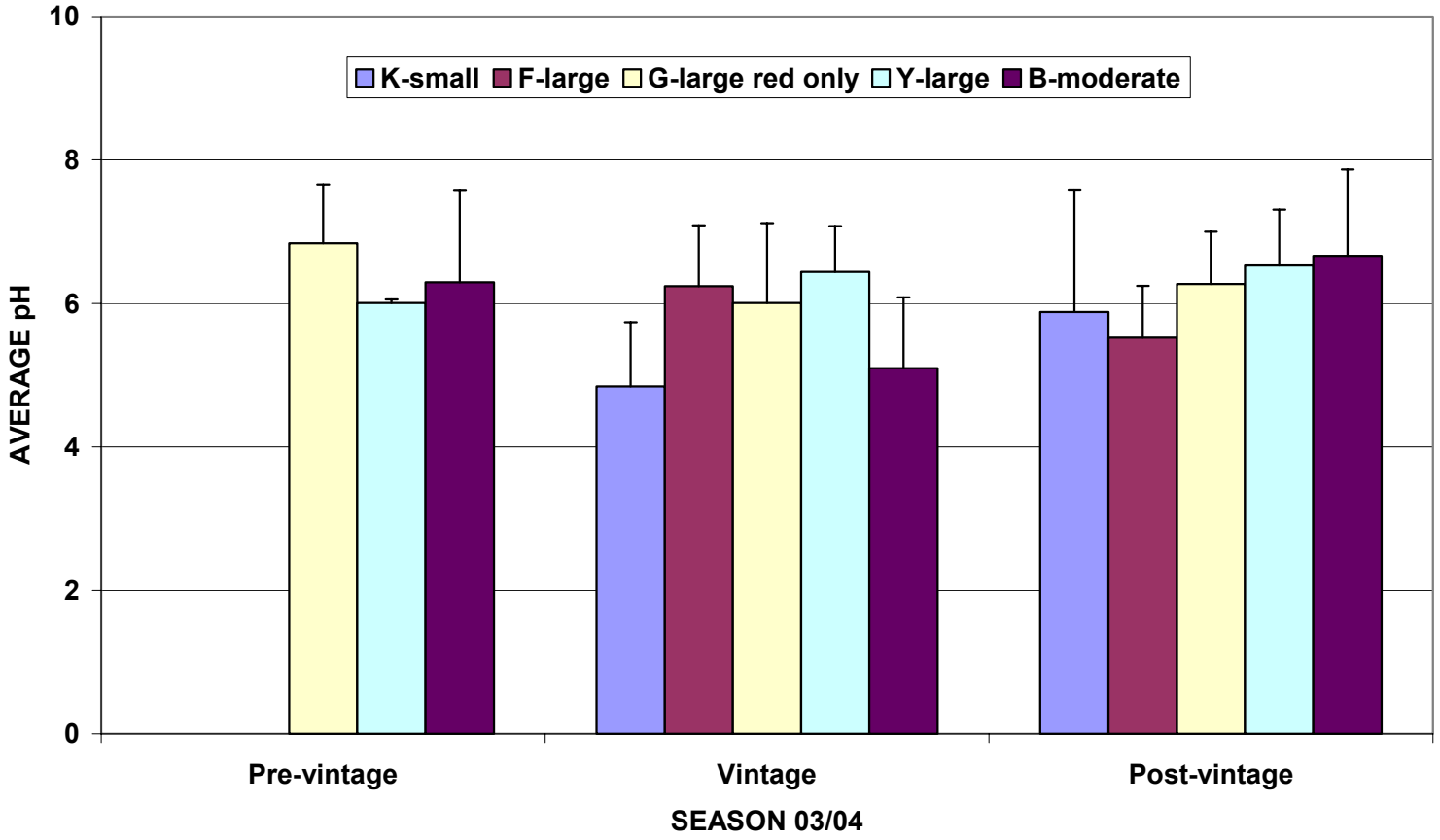


Figure 2.1 Seasonal variation of winery wastewater pH at five selected wineries

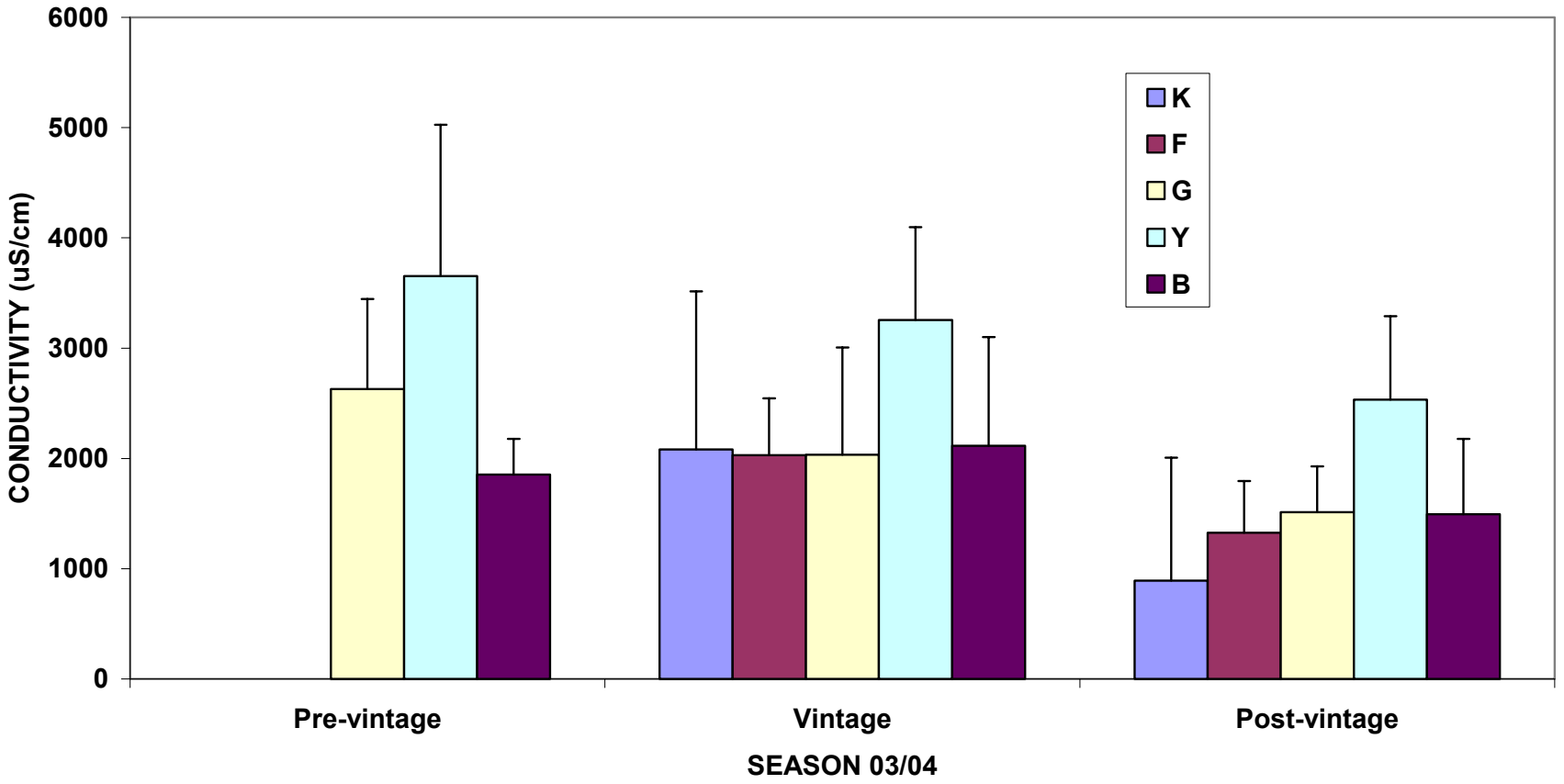


Figure 2.2 Seasonal variation of winery wastewater EC (electrical conductivity) at five selected wineries

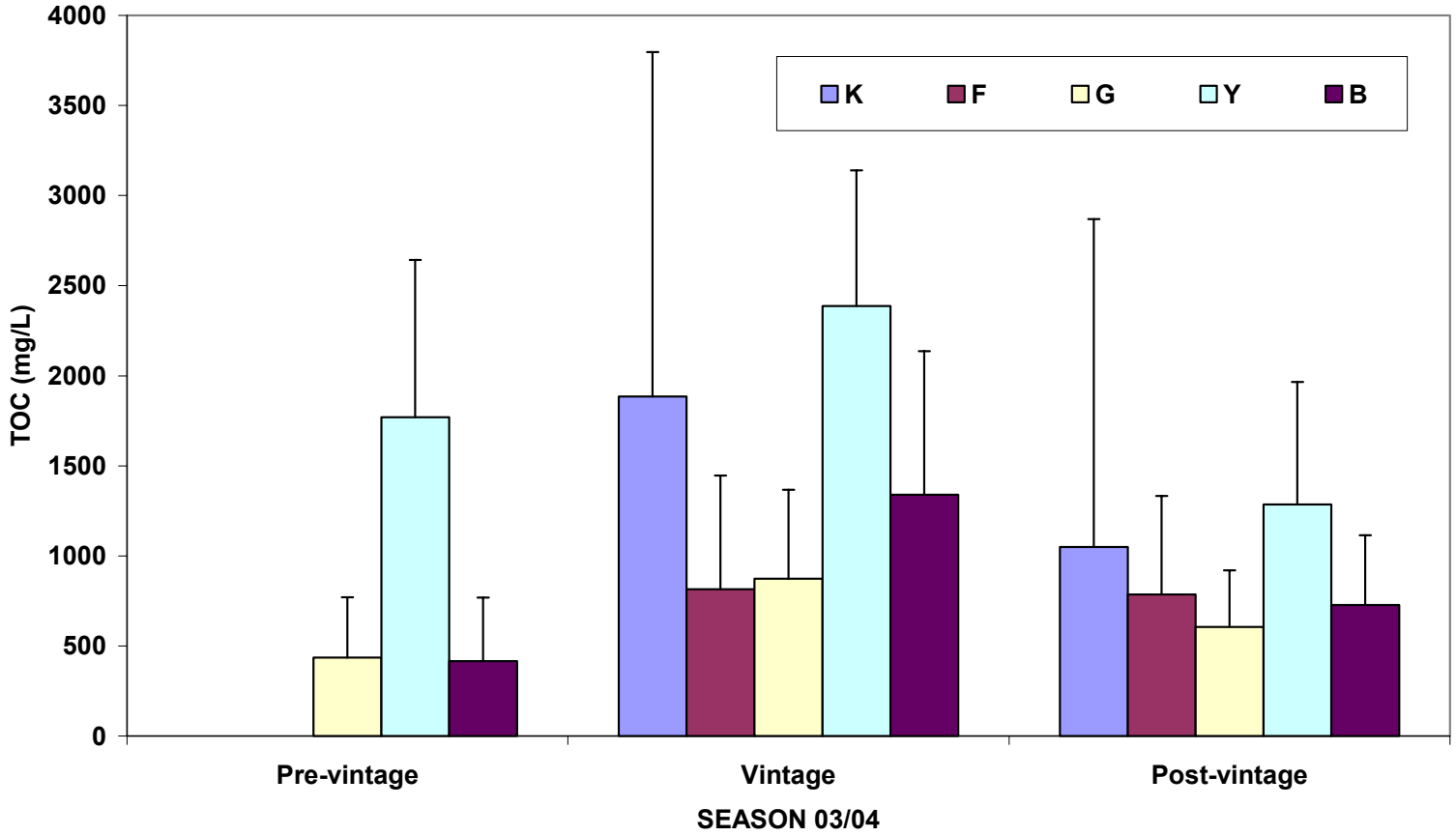


Figure 2.3 Seasonal variation in the total organic carbon (TOC) content of winery wastewater collected from five selected wineries

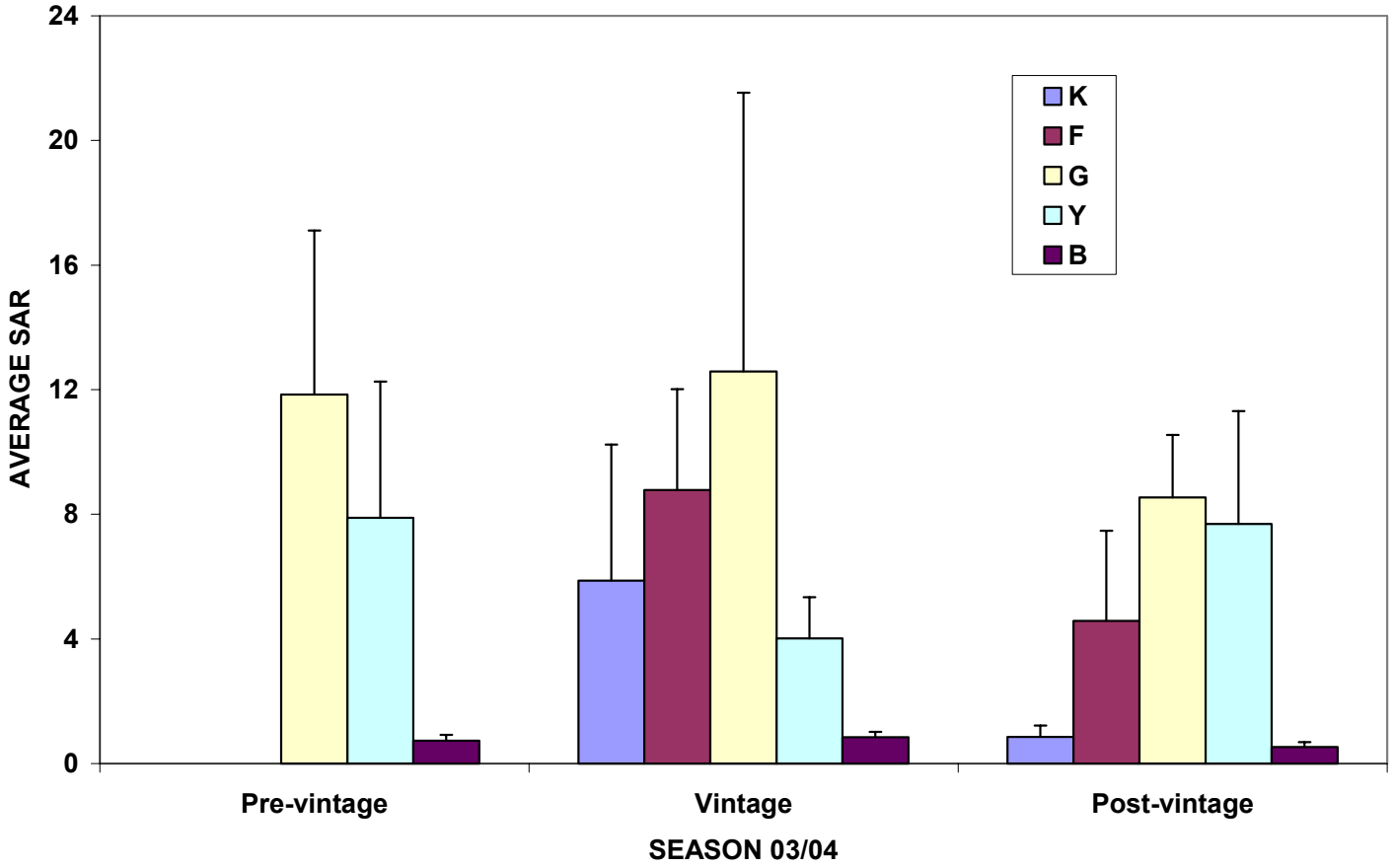


Figure 2.4 Seasonal variation in the Sodium Adsorption Ratio (SAR) of winery wastewater samples collected from five selected wineries

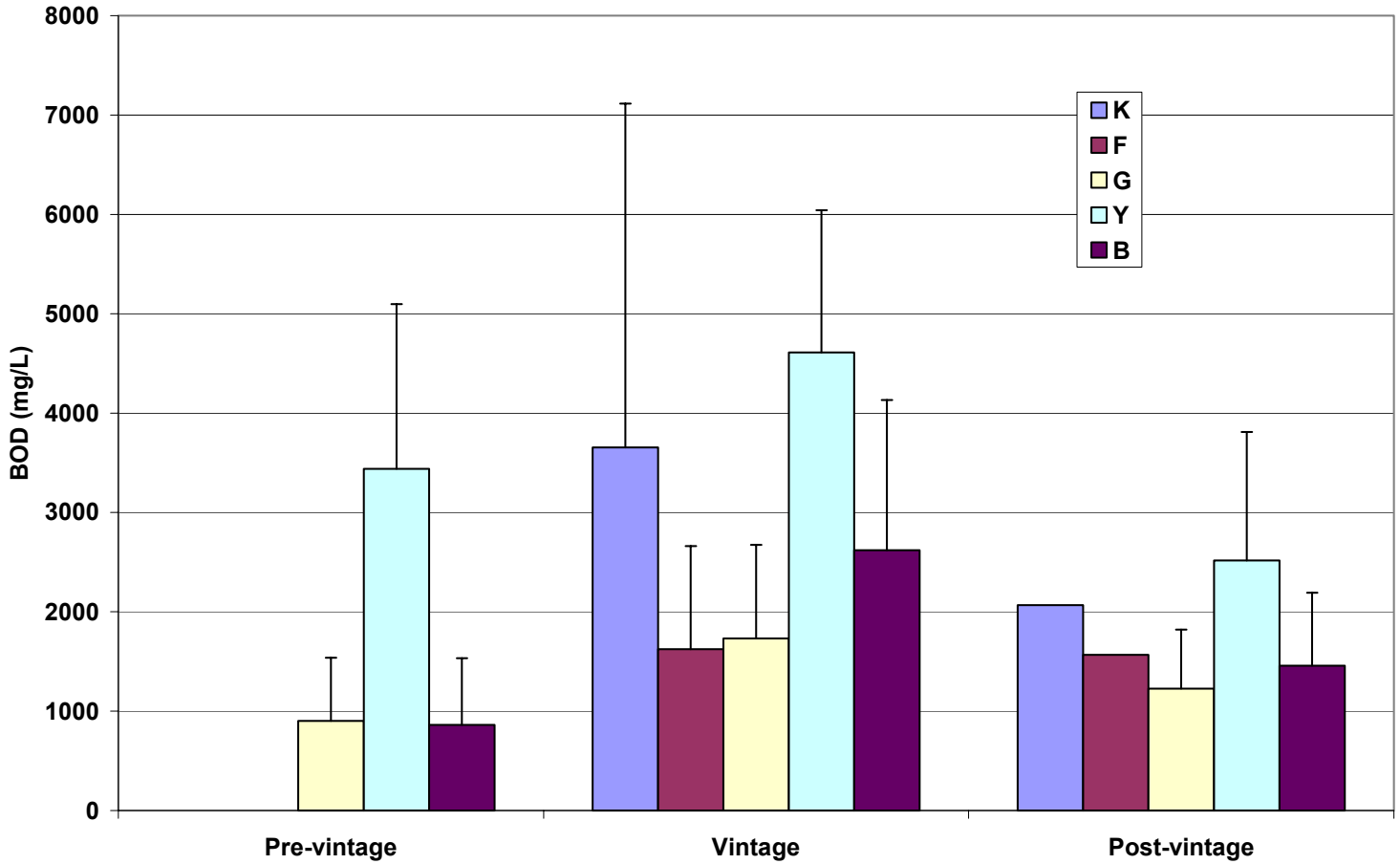


Figure 2.5 Seasonal variation in the Biological Oxygen Demand (BOD) of winery wastewater samples collected from five selected wineries

2.5.3 Multivariate analyses

2.5.3.1 Overall analyses

A graphical description of the multivariate analysis of indices of potential concern is shown in Figure 2.6. The centroids of the five wineries are indicated with large symbols. The arrows indicate the contribution of each parameter to the separation. There was a concentration of points from winery G to the right due to the high SAR whereas winery B had low SAR. Winery Y was concentrated near the top of the plot due to high EC as opposed to winery K that had lower EC. Winery F was intermediate on both components.

The first component accounted for 67% of the difference between the wineries and the second component included a further 18%. The first component was dominated by SAR and to a small extend this was contrasted with the EC. EC followed by pH were the most important variables in the second component.

2.5.3.2 Vintage analyses

The first component accounted for 65% of the difference between the wineries and the second component included a further 16%. The first component was dominated by SAR and to a small extend this was contrasted with the EC. EC and pH were the most important variables in the second component.

A graphical description of the multivariate analysis of the indices of potential concern is shown in Figure 2.7. The centroids of the five wineries are indicated with large symbols. The arrows indicate the contribution of each parameter to the separation. There was a concentration of points from winery G to the right due to the high SAR whereas winery B had low SAR. Winery Y was concentrated near the top of the plot due to high EC. Wineries F and K were intermediate on both components.

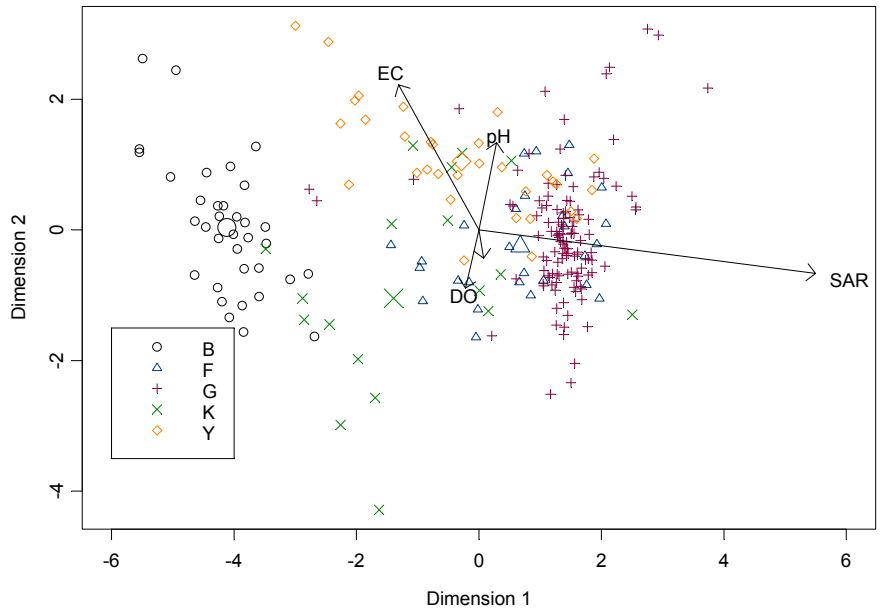


Figure 2.6 Canonical variate analysis on both vintage and non-vintage data using the parameters of potential concern

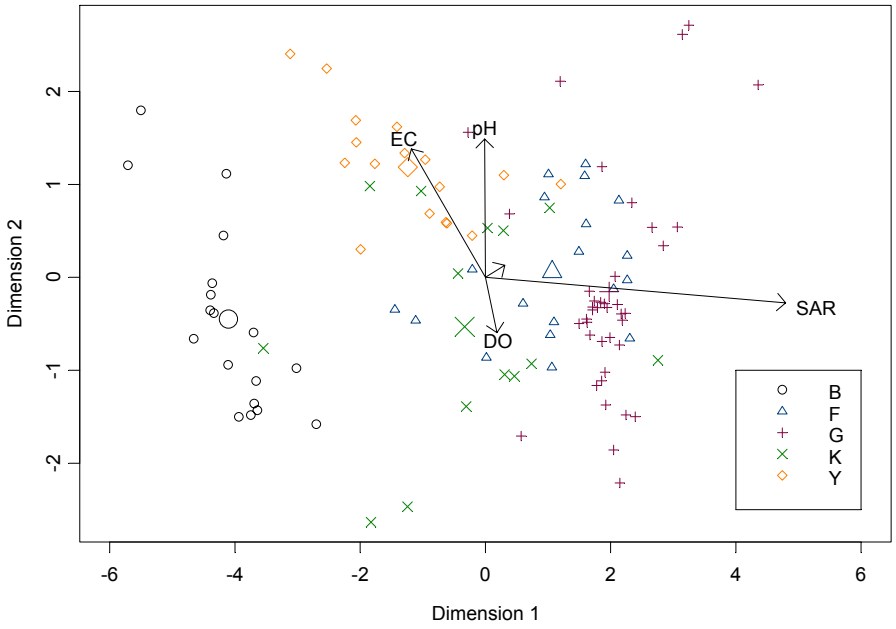


Figure 2.7 Canonical variate analysis on vintage data using the parameters of potential concern

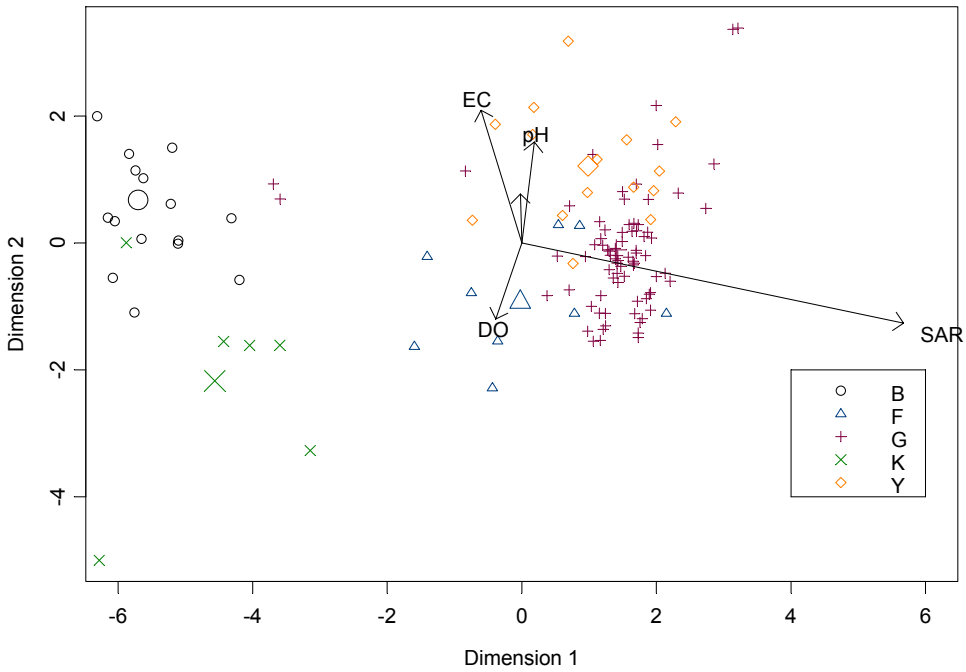


Figure 2.8 Canonical variate analysis on non-vintage data using the parameters of potential concern

2.5.3.3 Non-vintage analyses

The canonical variate analysis was dominated by the first component of 65% and the second 16%. The non-vintage analysis (Figure 2.8) was dominated by SAR as indicated by the SAR line. The samples in the G group were clustered on that line, indicating that the G winery had high SAR. By contrast winery B and to a lesser extent K had low SAR. Winery Y was shown to have high EC and to a lesser amount pH and low DO.

2.5.4 Univariate analyses of inorganic components

The chloride concentration at winery K was much more variable than at the other fours (Figure 2.9). Generally in post-vintage, there was little range of Cl concentrations. During vintage there was much more scatter from all the wineries.

The outstanding feature was the consistently high levels of calcium for winery Y, but this was a much more variable in the vintage season (Figure 2.9). Winery F had high Ca values in the vintage season. By contrast winery G had very low Ca levels especially in the post vintage season (Figure 2.9).

Copper levels were dominated by several very high concentrations especially at winery K and to a lesser extent at winery G. Fe data did not show very clear trends but was very variable(Figure 2.9).

Potassium levels were high for winery K in the non-vintage season, which contrasted with the low concentrations at winery G (Figure 2.9). Sodium levels were very low for some samples from winery K, particularly during the vintage season (Figure 2.10). Zinc levels were reported to be high for the wineries K and B (Figure 2.11).

2.5.5 Canonical variate analyses of inorganic data

2.5.5.1 Vintage season analyses

A plot of the canonical variate analysis for the vintage season is shown in Figure 2.12. The first two dimensions of this analysis accounted for 41% and 32% of the differences between the five wineries. The most outstanding feature of the analysis was the separation of winery Y along an axis that was dominated by Ca concentration. This can be seen by inspection of the univariate plots where winery Y had high Ca concentrations during vintage season.
There was secondary axis that contrasted high Zn, Cl and K levels against Na levels. Winery K had high Zn concentrations and low Na concentrations, hence it is placed on the top left corner of the plot. Winery G shows examples of low Cl, high Cu, low K, low Mg and high Na.

2.5.5.2 Non-Vintage season analyses

A plot of the canonical variate analysis for the non-vintage season is shown in Figure 2.13. The first two dimensions of this analysis accounted for 37% and 32% of the differences between the five wineries. The most outstanding feature of the analysis was again the separation of winery Y along an axis that was dominated by Ca concentration. This can be seen by inspection of the univariate plots where winery Y had high Ca concentrations during vintage season. A secondary axis was dominated by high Zn concentrations, which caused wineries K and B to be on the lower left corner.

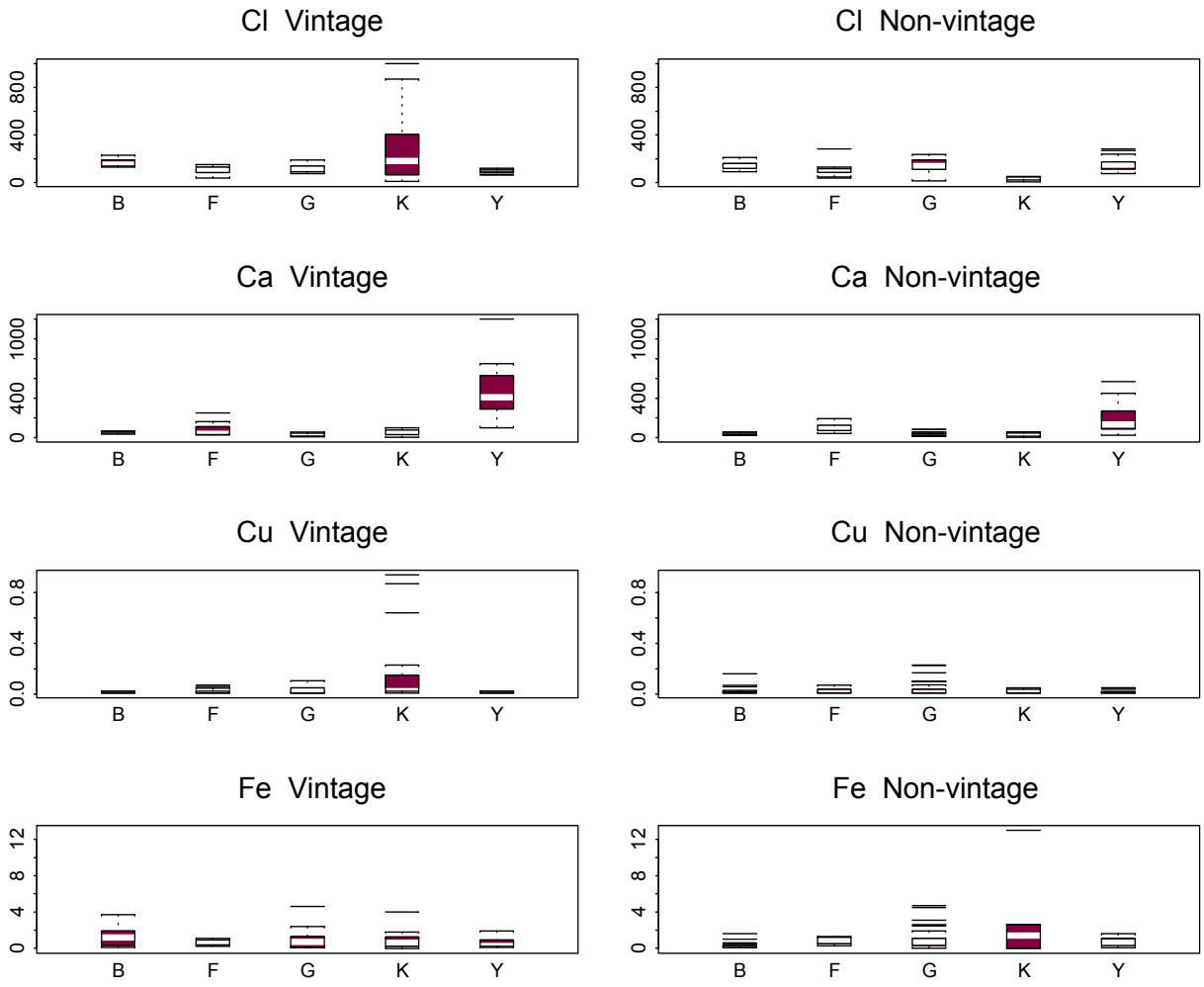


Figure 2.9 Chloride, calcium, copper and iron levels in the winery wastewater samples collected during vintage and post-vintage seasons

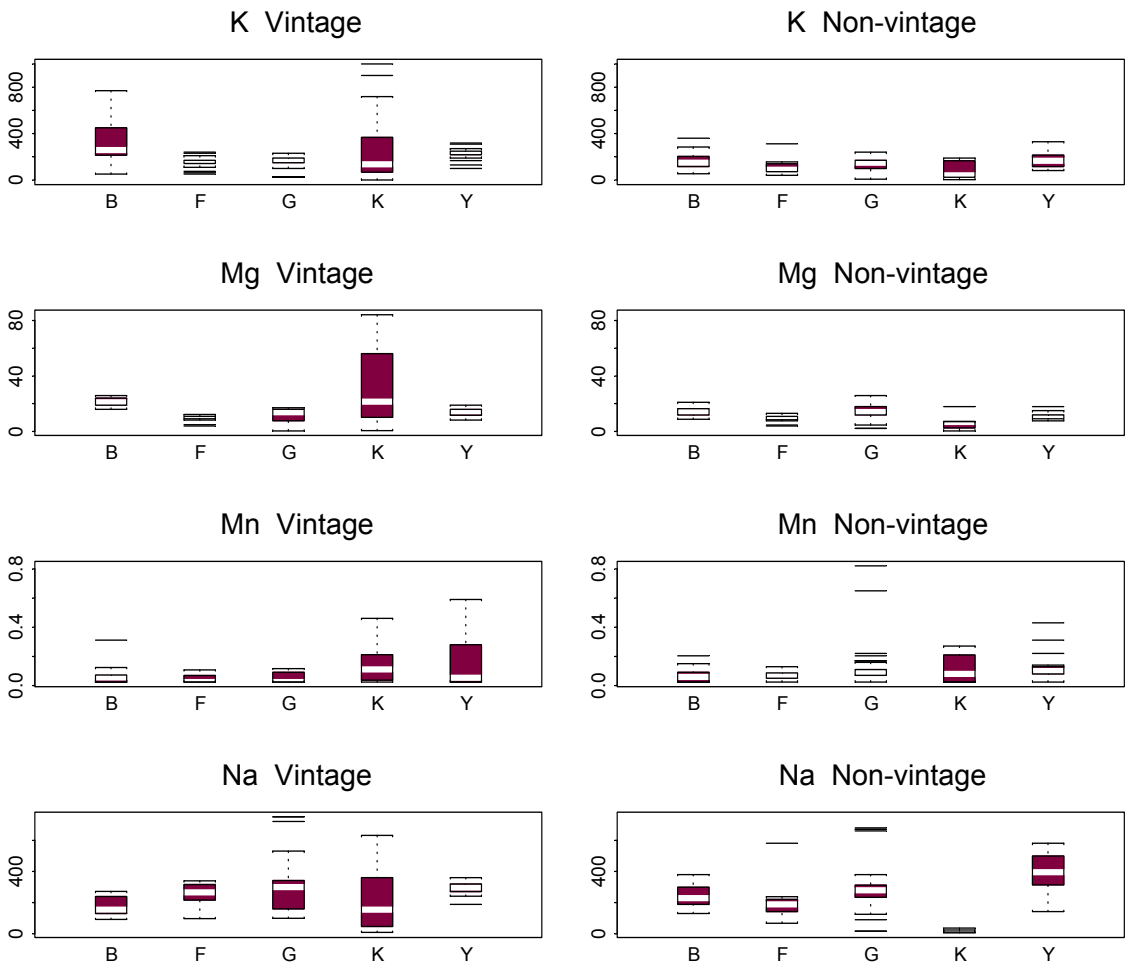


Figure 2.10 Potassium, magnesium, manganese and sodium levels in the winery wastewater samples collected during vintage and post-vintage seasons

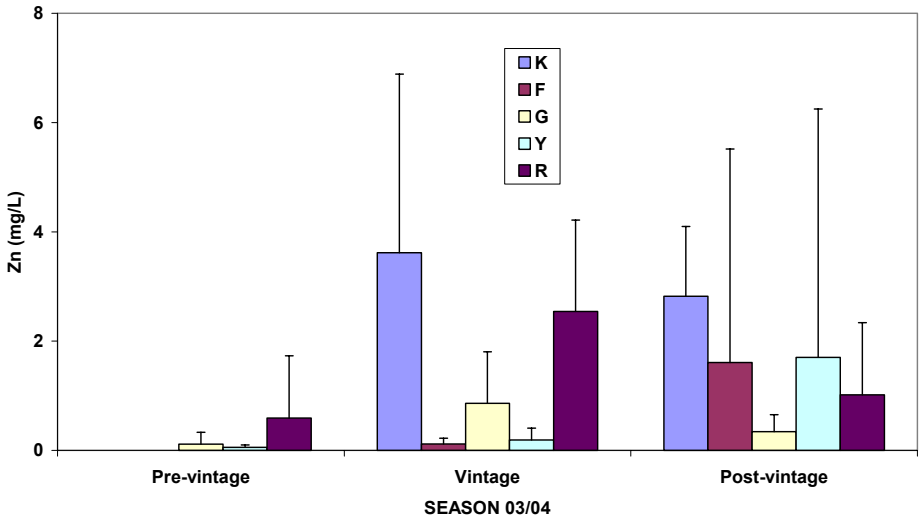


Figure 2.11 Zinc levels of the winery wastewater samples collected during vintage and post-vintage seasons

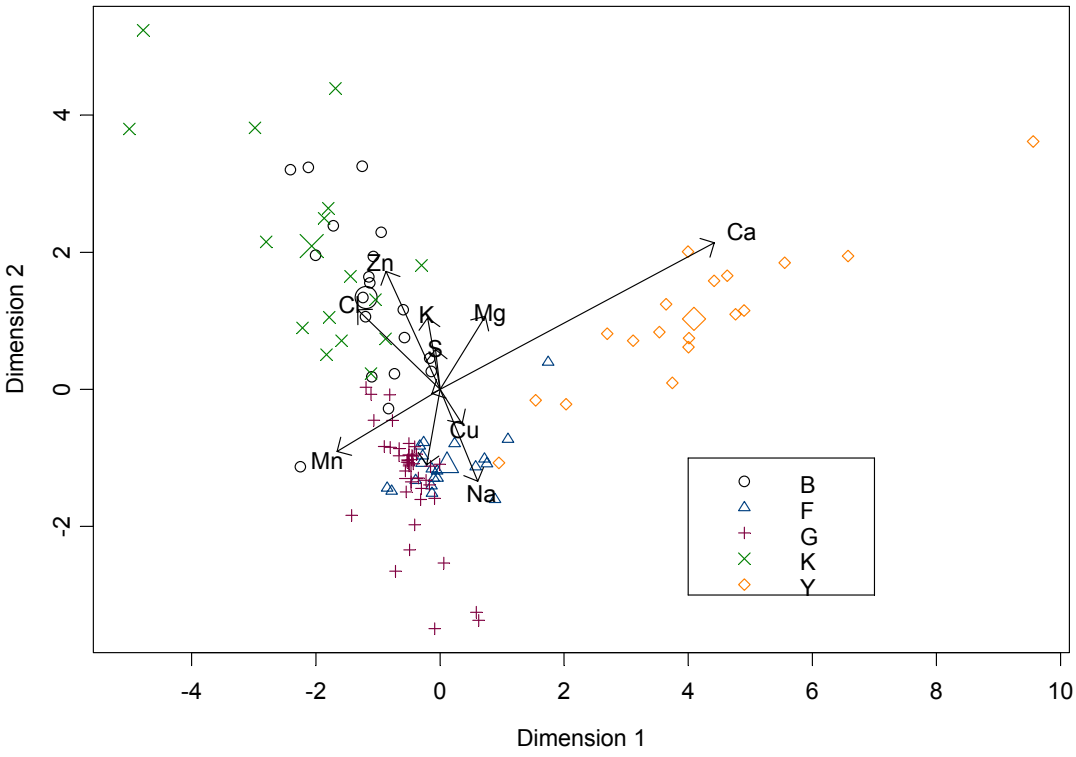


Figure 2.12 Canonical variate analysis of wineries classified by inorganic components in waste water - vintage, based on log transformed data

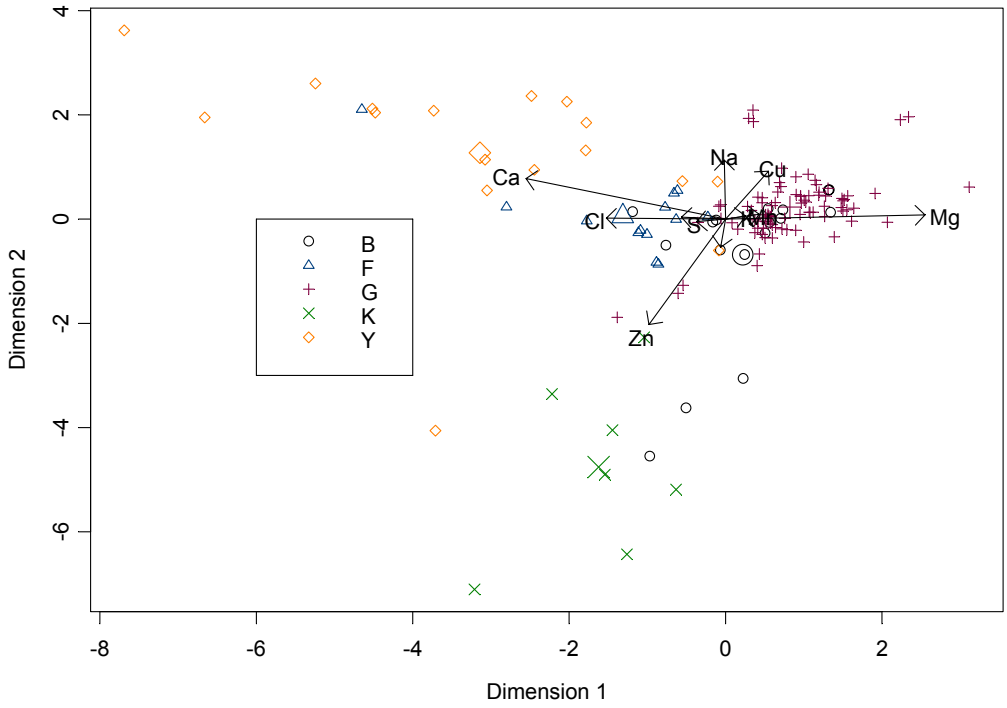


Figure 2.13 Canonical variate analysis of wineries classified by inorganic components in waste water – non-vintage, based on log transformed data

2.6 Conclusions

1. There was a wide variation in composition of winery wastewater, both spatially and temporally.
2. Winery wastewater produced during vintage always had higher biological oxygen demand, total nutrients and electrical conductivity.
3. Winery wastewater was more acidic in the vintage than in comparison to the winery wastewater produced during the non-vintage season.
4. Winery wastewater was generally alkaline and saline during non-vintage seasons.
5. Winery wastewater from winery G making only red wines had relatively lower BOD in the vintage season, phenolic materials such as red pigments and tannins could be contributing to this low BOD,
6. Copper and zinc ions were high in the winery wastewater at some wineries.
7. In Australia, a sodium adsorption (SAR) of 6 is considered the benchmark of sodicity. Winery wastewater was highly sodic in the vintage season with values ranging from 10-35 in the vintage season.
8. For large wineries, parameters like EC, SAR and BOD are important key indicators of winery wastewater quality.
9. For small wineries, pH, EC, SAR and BOD are important key indicators of winery wastewater quality.

Chemical analyses usually are insufficient to provide insight into the potential ecological risk due to pollution. They do not allow for an integration of the combined effects of the mixture of all contaminants, including their bioavailability. Bioassays integrate these effects and are therefore recommended for the ecological risk assessments. Ecotoxicological assessment of winery wastewater sampled during this study is provided in the following sections.

3. ECOTOXICOLOGICAL ASSESSMENT OF WINERY WASTEWATER

3.1 Introduction

Studying the response of aquatic organisms through toxicity testing of effluents can complement chemical analyses and significantly enhance the predictive capabilities of monitoring programs. Toxicity tests or bioassays are particularly useful in assessing mixtures of contaminants since the response of the organisms serves to integrate the effects of all components. At its most basic, a bioassay involves exposing organisms to a range of concentrations of the material being tested and measuring some defined response, most often under controlled laboratory conditions. Acute bioassays commonly last 48 to 96 hours and focus on mortality, while longer term chronic tests may last weeks or months depending on the species being used. Chronic tests usually focus on sub lethal responses such as impairment of growth or reproduction.

The objective of Toxicity Identification and Evaluation (TIE) was to identify the toxic components in complex mixtures such as wastewater. There are three phases in a full TIE test; Phase I is used to identify the general classes of toxic components in the mixture. This is accomplished by a combination of chemical and physical manipulations of the sample followed by additional toxicity testing to determine whether effects have been eliminated. Phase II subjects the mixture to specific chemical analyses to further identify potential toxicants and Phase III uses a series of follow-up toxicity tests on pure samples of chemicals to confirm the suspected toxicants in the original complex sample (USEPA, 1991).

The main objective of this study was:

1. to assess toxicity of winery wastewater from selected wineries; and
2. to use Phase 1 of toxicity identification evaluation methods for identifying causes of toxicity and recommending treatment methods for reducing winery wastewater toxicity.

3.2 Methodology

3.2.1 Test organisms selected:

Waterflea- *Ceriodaphnia dubia* and Duckweed- *Spirodela sp* were chosen as test species to assess the toxicity of winery wastewater.

Figure 3.1 Waterflea - *Ceriodaphnia dubia*

The choice of the waterflea, *C. dubia,* as the species to use in toxicity tests was based on a number of points. Waterfleas are common invertebrates in freshwater systems such as wetlands and slow moving streams and provide a food source for fish and juvenile stages of some amphibians (Figure 3.1). They have also been used extensively in toxicity testing with standardised procedures available for maintaining populations under laboratory conditions and conducting toxicity tests. The use of a standardised testing protocol (USEPA, 1993) means that other laboratories can carry out the bioassays in an identical manner.

Duckweed (Figure 3.2) plays an important role in oxygen production, nutrient cycling, controlling water quality, sediment stabilisation and providing habitat and shelter for aquatic organisms. A chemical which decreases the growth of duckweed may therefore affect organisms higher up the food chain. The test protocol was based on the OECD Test Guideline 221 (2002).

Figure 3.2 Duckweed- *Spirodela sp*

The objective of toxicity testing was to determine:

- The No Observed Effect Concentration (NOEC), where no statistical difference ($P \leq 0.05$) was found between exposed and unexposed (or control) specimens.
- The Lowest Observed Effect Concentration (LOEC), where the smallest statistical difference ($P \leq 0.05$) was found between exposed and unexposed (or control) specimens.
- The median effect concentration (LC50/EC50), was the concentration of the winery wastewater that was estimated to be effective in producing mortality (in case of waterfleas and tadpoles), growth (for duckweed bioassay) in 50% of test organisms.

Statistical significance was determined at $\alpha = 0.05$. Data were tested for normality and homogeneity of variance using Toxstat (1994). An analysis of variance (ANOVA) with Bonferroni (unequal replicates) or Dunnett tests (equal replicates) was used to determine significant differences in various treatments. This information was used for the estimation of the LOEC and NOEC.

3.2.1 Initial toxicity screening of winery wastewater

Mass cultures of *C. dubia* were maintained in 1L beakers in a constant temperature room (23 $\pm$ 1°C) with a 16 h light: 8 h dark photoperiod using cool white fluorescent lamps at the CSIRO Adelaide laboratories. 48-hour acute bioassays with *C. dubia* were performed according to USEPA (1993). Dilutions of the wastewater were prepared by addition of formulated moderately hard water (MHW). A 0.5 dilution factor was used to produce test concentrations of 100, 50, 25, 12.5, 6.25% (v/v) wastewater (Figure 3.3). Tests were initiated by randomly distributing daphnid neonates (<24hr old) in each of the four replicate beakers per concentration until each container had five animals. Toxicity tests were conducted under the same controlled environmental conditions as culturing. Conductivity, temperature, pH,

and dissolved oxygen were measured in each dilution at the start of the tests using a TPS model 90-FL water quality meter. After 48 hour exposure, waterfleas alive at each dilution were recorded and on the basis of this information LC50, LOEC and NOEC values were calculated for each winery wastewater sample.

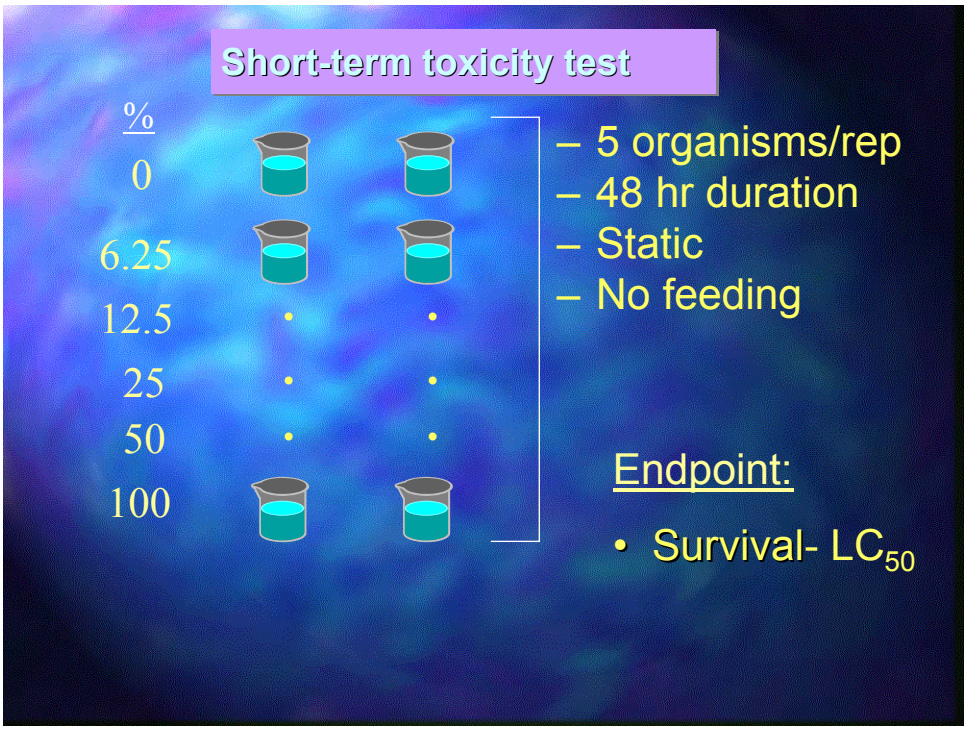


Figure 3.3 Experimental design for conducting waterflea test

3.2.2 Duckweed bioassay

The primary objective of the duckweed bioassay was to determine the inhibition of growth of duckweed, *Sprirodela* sp after 7 day exposures to winery wastewater. The test protocol was based on the OECD Test Guideline 221 (2002). Test solutions were prepared by diluting the winery wastewater samples with culture media to promote good duckweed growth. Colonies consisting of 2 to 4 visible fronds were transferred from the inoculum culture and randomly assigned to the test vessels under aseptic conditions. A total 12 fronds were added in each test vessel. Test vessels were placed randomly in the incubator to minimise the influence of spatial differences in light intensity or temperature. The test was terminated after seven days exposure and total numbers of fronds were counted in each test vessel. Test solution were renewed on day 3 and 5 to make sure that test conditions remained constant during the test.

3.2.3 Toxicity Identification Evaluation

In this study we only followed Phase I investigations. The following manipulations were performed (Figure 3.4). Positive controls were run concurrently with each manipulation to check for any toxicity from the reagents and cartridge resins used.

3.2.3.1 Aeration test

Aeration can drive off volatile or oxidisable compounds. Winery wastewater samples were aerated for one hour using an aquarium aeration pump prior to starting the toxicity test.

3.2.3.2 EDTA chelation test

The EDTA (Ethylenediaminetetraacetic acid) chelation test was designed to detect toxicity caused by cationic metals such as lead, copper, zinc and cadmium. EDTA was added to the winery wastewater at a concentration of 25 mg/L and left for one hour before testing the solution.

3.2.3.3 Graduated pH test

This test was applied to determine whether winery wastewater toxicity could be attributed to pH changes. The pH of the winery wastewater sample was raised to 10 and lowered to 3 with four exposure concentrations at each pH. Toxicity tests were compared at initial pH, and at pH 3 and 10.

3.2.3.4 C18 Solid Phase Extraction (SPE) test

The SPE treatment removes non-polar organic compounds, some metals and surfactants. Winery wastewater samples were first filtered through a 0.45 μm membrane and then 50 mL of this filtrate was passed through an activated Sep-Pak C18 cartridge. The eluate from the column was then used for toxicity testing.

3.2.3.5 Activated Carbon test

Activated carbon is very commonly used to reduce organic pollutant loads associated with the agricultural and industrial wastewaters. Winery wastewater samples were passed through the activated carbon packed column to check its efficiency in reducing the winery water toxicity.

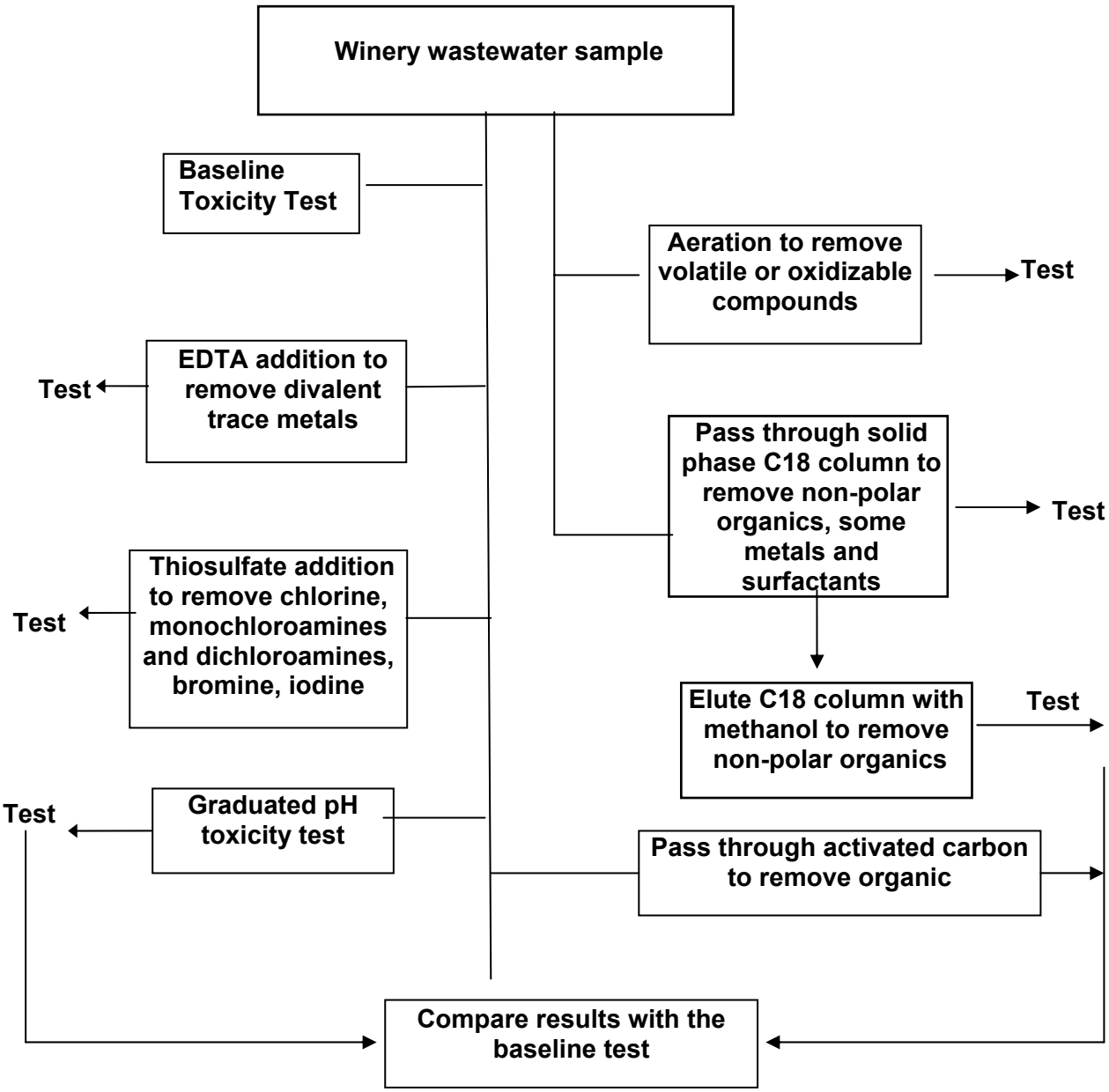


Figure 3.4 Flow chart of Phase 1 TIE manipulations applied to winery wastewater samples. Baseline test represents the initial toxicity test of the winery wastewater sample without any manipulation. Positive controls were run concurrently with each manipulation.

3.3 Results

3.3.1 Toxicity to waterfleas

Winery wastewater was highly toxic to the waterfles, *C. dubia*. The lower NOEC value corresponds to the greater toxicity. In the vintage season, severe toxicity was observed to the waterfleas, when exposed to the winery wastewater from the small winery K and the moderate-size winery B than in comparison to the winery wastewater from the large winery G (Figure 3.5). However, there was no general trend in toxicity to waterfleas in the post-vintage season with NOEC ranging from 1 - 50% for the small winery K, 1 - 100% for the moderate size winery B and 3 -50% for the large winery G (Figure 3.6).

Among, large wineries, the red wine facility showed no significant differences in the toxicity to waterfleas for the vintage season 2003 and 2004 (Figure 3.7). In contrast, for the white wine facility, the two vintage seasons demonstrated variable toxicity to waterfleas with the mean NOEC of 7% in the year 2003 and 16% in the year 2004 (Figure 3.8). In the post-vintage season, the white winery facility had lower NOEC (25%) in comparison with the red wine facility (NOEC40%, Figures 3.7 and Figure 3.8)

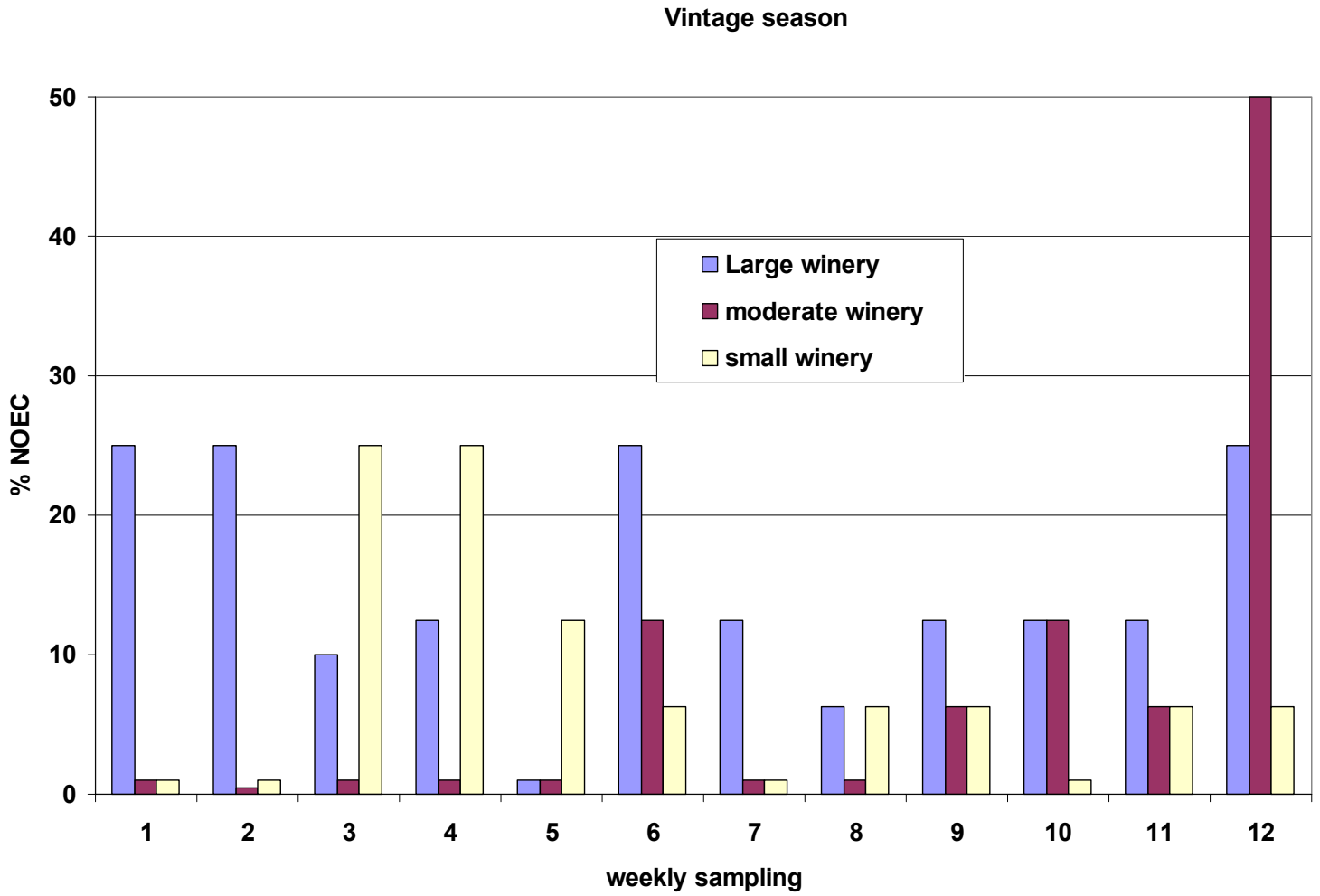
Winery wastewater in the post-vintage season, for both small and moderate size wineries, exhibited lower toxicity than in comparison to the vintage season winery wastewater (Figures 3.9 and 3.10).

For the moderate size winery B, toxicity to waterfleas was more severe in the vintage 2003 (NOEC 2%) than in comparison to that of vintage 2004 (NOEC 20%, Figure 3.9). However, for the small winery there were no significant differences in the toxicity to watefleas during the vintage seasons 2003 and 2004 (mean NOEC 10%).

3.3.2 Toxicity to duckweed

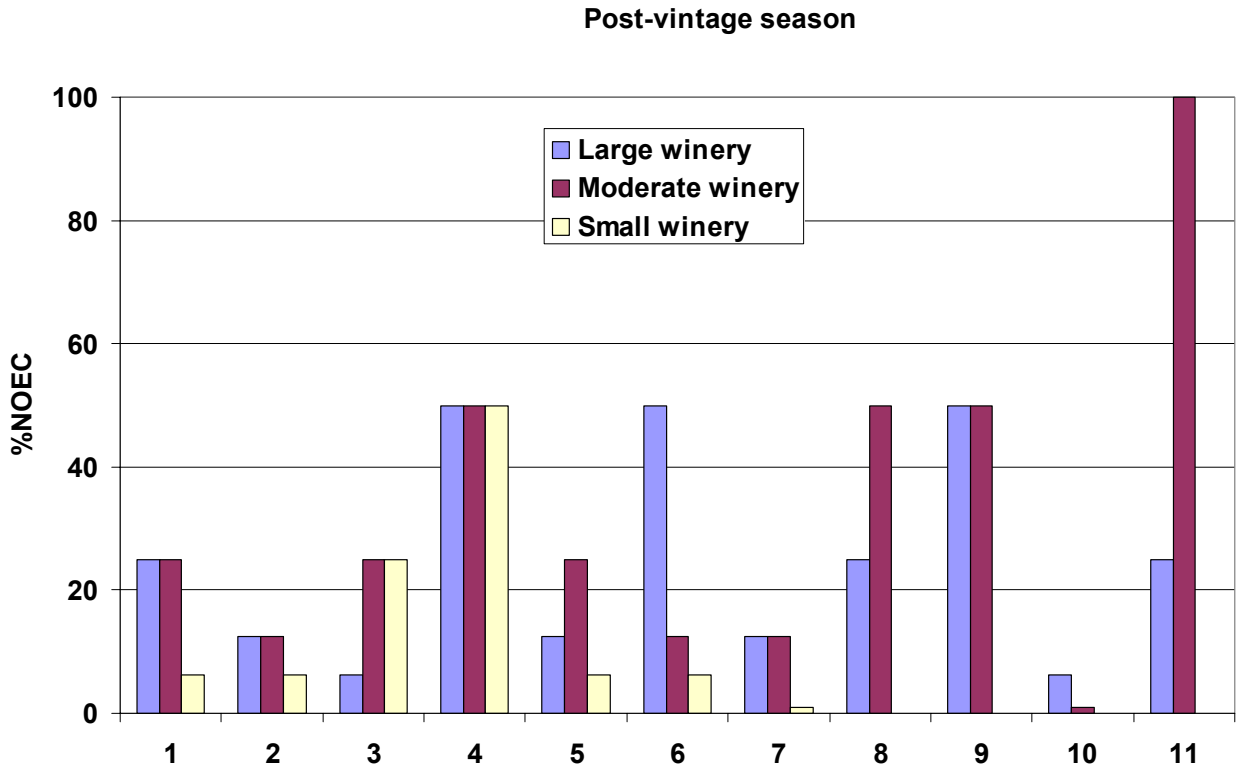
Duckweed, *Spirodela* sp was exposed to winery wastewater in serial dilutions. After seven days exposure, increase in frond numbers was measured as an endpoint. Based on the NOEC and EC50 values, duckweed showed more severe toxic response to winery wastewater exposure in the vintage season than in comparison to the post-vintage season (Figures 3.11 – 3.13). The only exception was the red wine facility which did not exhibit any significant changes in the toxicity to duckweed during both, vintage and non-vintage seasons (Figure 3.12). Winery wastewater from the large winery exhibited lesser toxicity to duckweed

than in comparison to the winery wastewater from the small and medium sized wineries. The mean NOEC value for winery wastewater exposure to duckweed in the vintage season ranged from 2 – 4% at the small and moderate size wineries (Figures 3.11 – 3.12). In contrast, duckweed had less toxicity to winery wastewater exposures at the large winery Y during the vintage season, with mean NOEC of 20% (Figure 3.11).



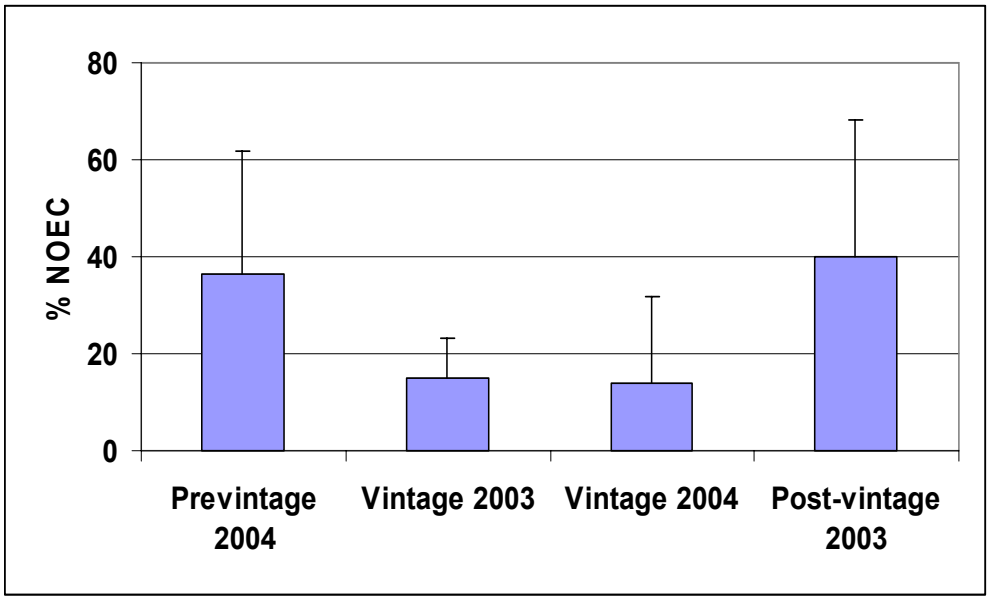
NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 3.5 Weekly variation in toxicity of winery wastewater in the vintage season based on the *Ceriodaphnia dubia* acute bioassays



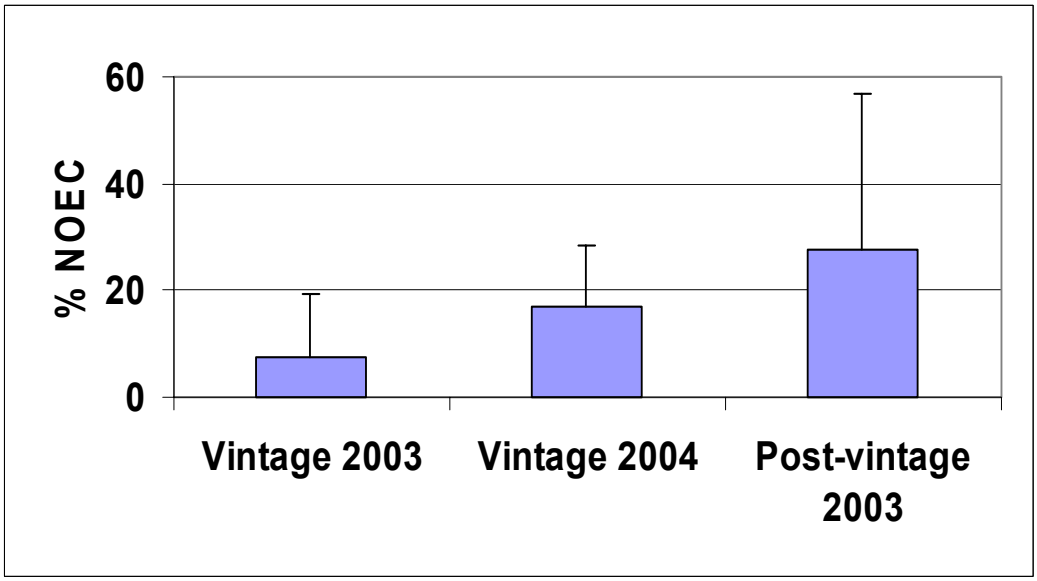
NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 3.6 Fortnightly variation in the toxicity of winery wastewater in the post-vintage season based on *Ceriodaphnia dubia* acute bioassays



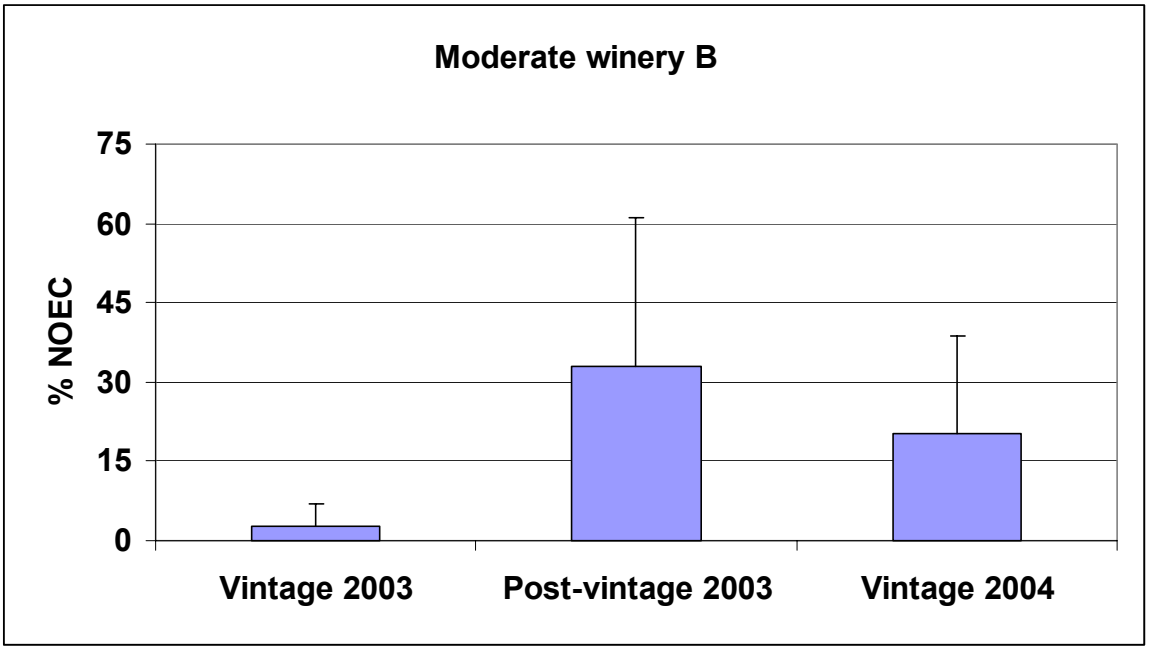
NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 3.7 NOECs based on *Ceriodaphnia* bioassays during exposures to winery wastewater from a large red wine facility



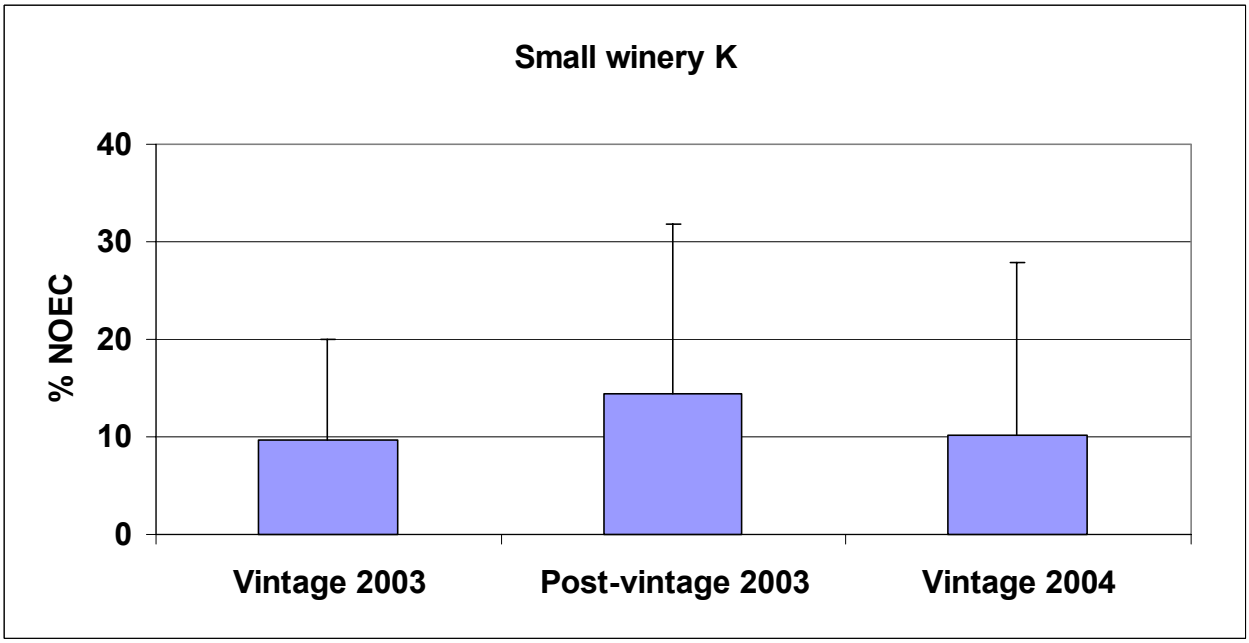
NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 3.8 NOECs based on *Ceriodaphnia* bioassays during exposures to winery wastewater from a large white wine facility



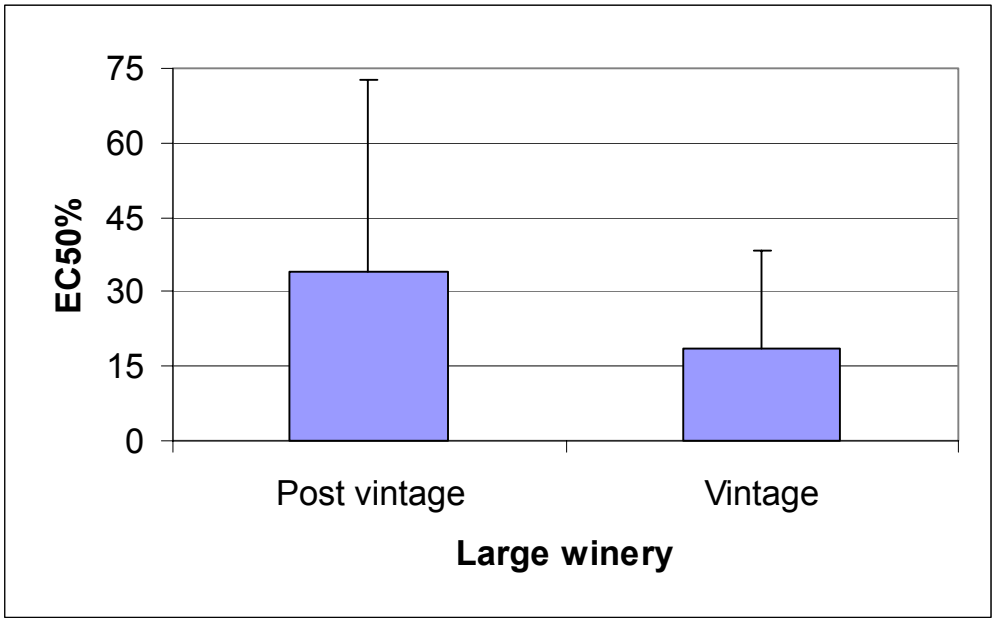
NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 3.9 NOECs based on *Ceriodaphnia* bioassays during exposures to winery wastewater from a moderate size winery



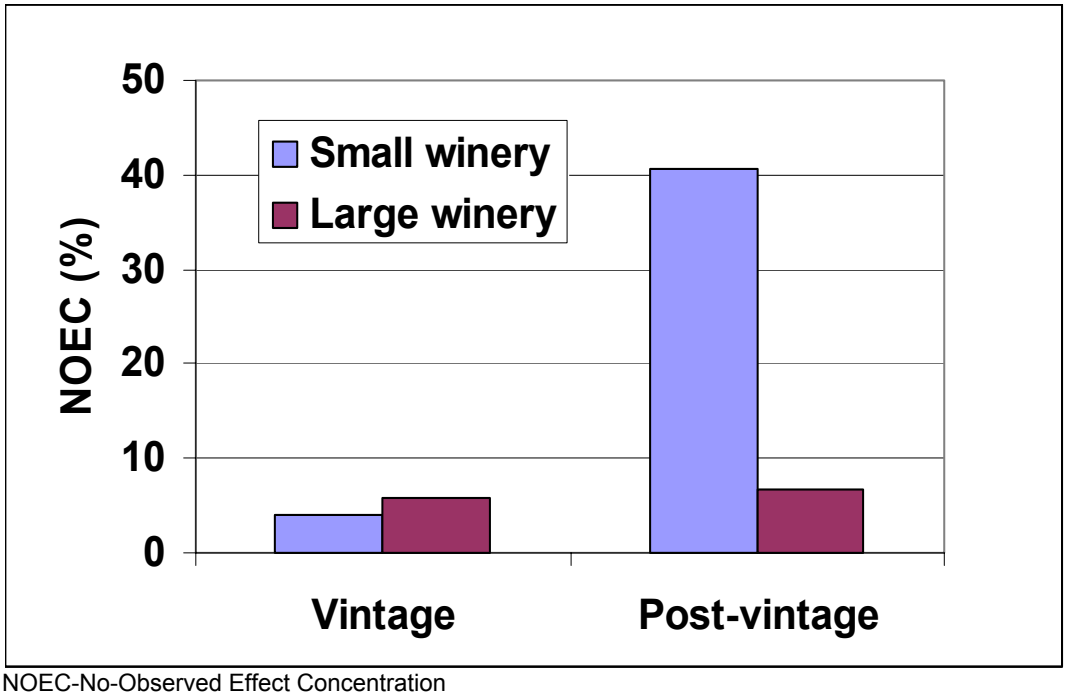
NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 3.10 NOECs based on *Ceriodaphnia* bioassays during exposures to winery wastewater from a small size winery



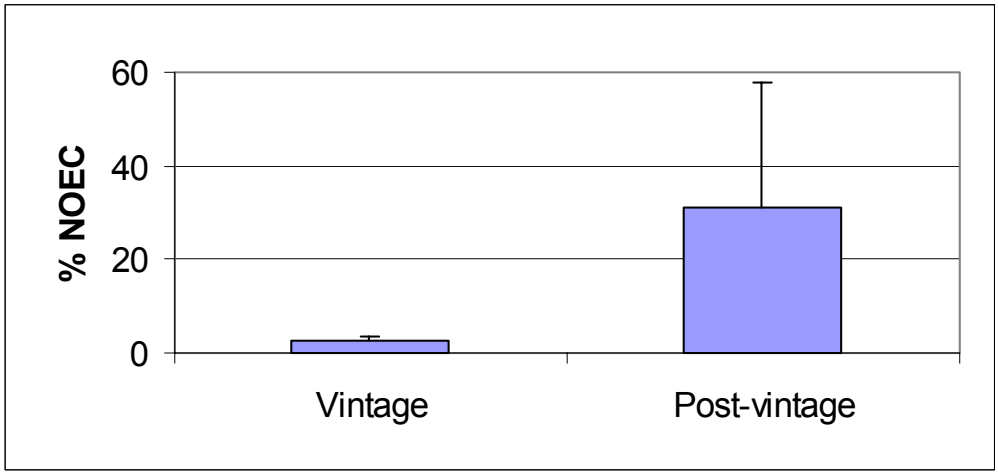
EC50- Median Effect Concentration
Lower EC50 value corresponds to greater toxicity

Figure 3.11 EC50 (%) winery wastewater from the large winery Y based on duckweed bioassays



NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 3.12 NOECs based on duckweed bioassays during exposures to winery wastewater from the large winery G and small sized winery K

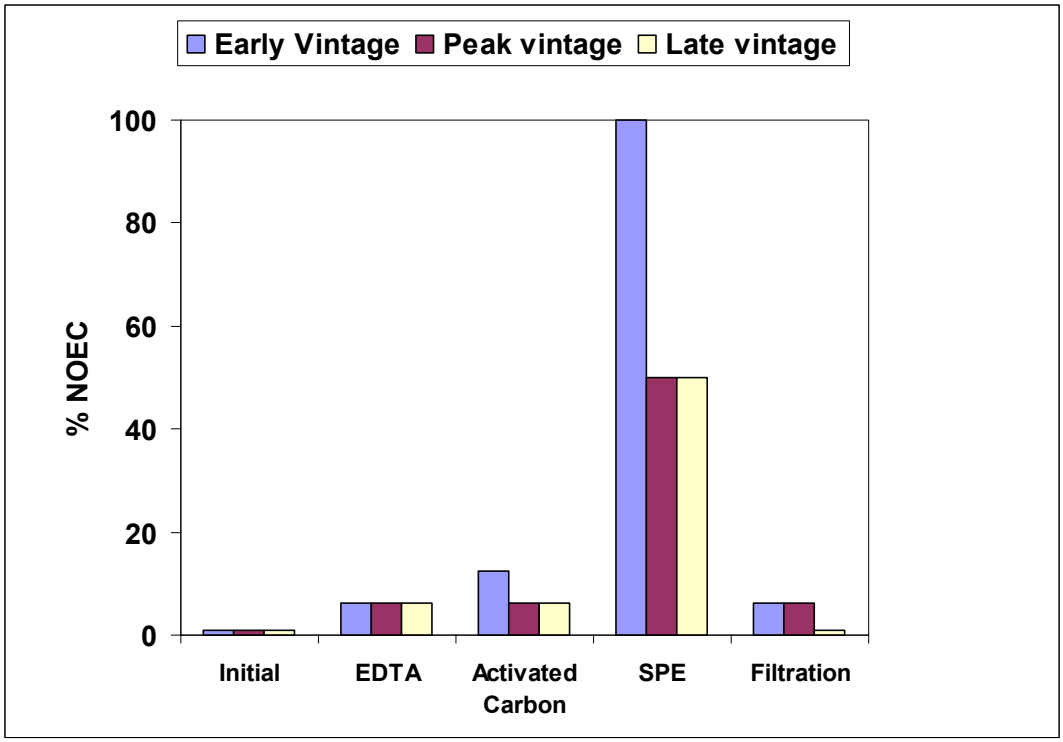


NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 3.13 NOECs based on duckweed bioassays during exposures to winery wastewater from the moderate-size winery B

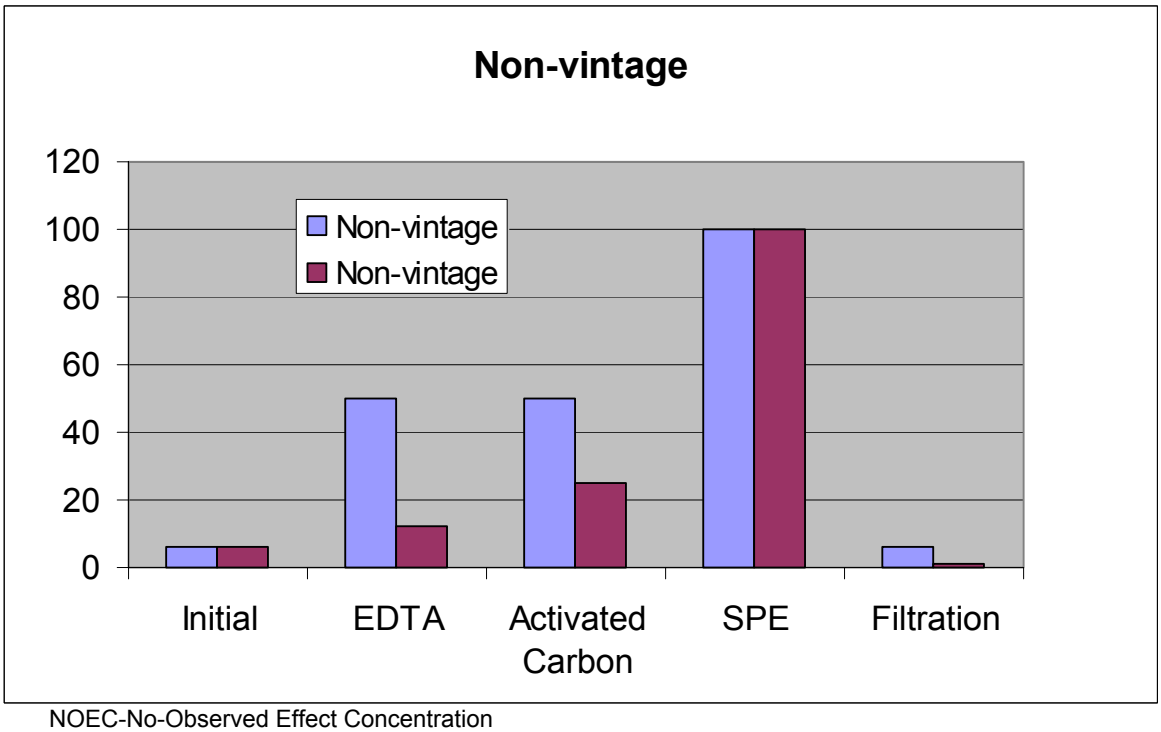
3.3.3 Toxicity Identification Evaluation, Phase 1

TIE manipulations such as EDTA addition, passing winery wastewater through C18 cartridge significantly reduced toxicity of the winery wastewater samples. The NOECs of TIE manipulations were higher than the baseline toxicity NOECs (Figures 3.14 - 3.15). In the non-vintage season, passing effluent through C18 completely removed toxicity. NOEC value of initial winery wastewater sample was 6.25% and after C18 manipulation the NOEC was 100% (Figure 3.15). Based on TIE manipulations it can be concluded that heavy metals and organic contaminants were the major toxicants of concern in winery wastewater. Zinc and copper levels were high in winery wastewater samples and they could be contributing to `winery wastewater toxicity. Further TIE work can help in confirming this.



NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

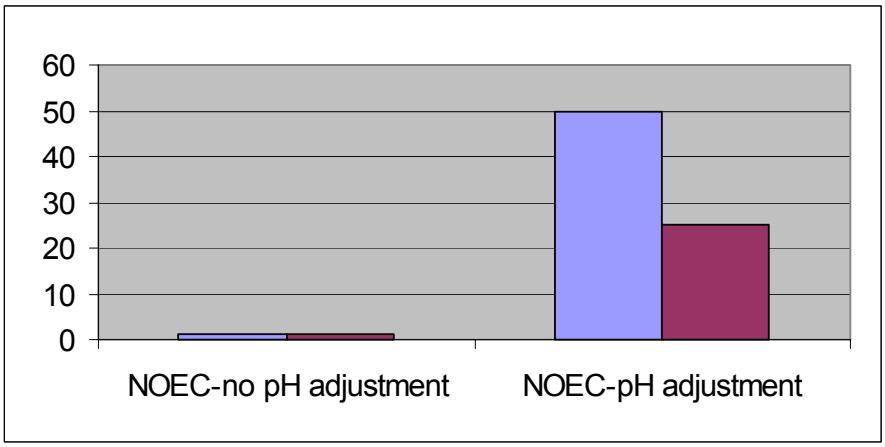
Figure 3.14 TIE manipulations of the winery wastewater samples in the vintage season



NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 3.15 TIE manipulations of the winery wastewater samples in the non-vintage season

Winery wastewater sample from winery B showed significant difference in toxicity after pH adjustments. Winery wastewater had pH of 3 and by increasing its pH to 9, there was significant decrease in its toxicity (Figure 3.16). Further testing confirmed that this toxicity was related to the polymer which was being used at moderate winery B. After pH adjustment, the polymer precipitated out of the winery wastewater. It is postulated that pH adjustment of winery wastewater to 9 resulted in better flocculation of solids and also polymer was stripped out with the solids. Further analytical work is required to confirm this hypothesis.



NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 3.16 Winery wastewater sample before and after pH adjustment

TIE manipulations confirm that the major source of winery wastewater toxicity was organic in nature. Heavy metals were also contributing to the winery wastewater toxicity but in a smaller fraction.

Based on the literature, the organic loading in winery wastewater typically originates from:

1. product loss – i.e., juice, wine and lees;
2. residues in caustic soda/citric acid cleaning waste;
3. residues in diatomaceous earth filter waste;
4. solids reaching wastewater drains..

The wastewater stream also contain waste sodium hydroxide from wine tank cleaning activities, sulphuric acid from the ion-exchange process and occasionally traces of other chemicals such as sulphur dioxide, chlorine and ammonia. All these components could be contributing to the winery wastewater toxicity. It is difficult to comment on the relative contribution of each to the overall toxicity based on this investigation as the treatment processes and wastewater management strategies vary significantly across the wineries.

3.4 Conclusions

1. *Ceriodaphnia* and duckweed showed toxic responses during winery wastewater exposures.
2. There was spatial and temporal variation evident in terms of winery wastewater toxicity. These differences could be related to the size of the different wineries selected during this study, treatment processes involved and the seasonal activities carried out at each winery.
3. In general, large wineries had less toxic winery wastewater than in comparison to the small and moderate size wineries selected in this study. This could be related to better treatment processes in place at large wineries such as screening of solids, sand filtration, pH adjustment and aeration of winery wastewater resulting in the reduced toxicity of winery wastewater. In comparison, moderate and small scale wineries have very low to no treatment resulting in high strength wastewater which is more toxic than the winery wastewater from the large scale wineries.
4. Vintage season winery wastewater samples were more toxic to duckweed and *Ceriodaphnia* than in comparison to the winery wastewater samples from the post-vintage season. Toxicity was so severe during the vintage season that in some cases 99% dilution of winery wastewater was required to prevent the acute toxic responses in *Ceriodaphnia.* Combination of factors such as higher BOD due to higher organic load, high SAR and acidic quality of winery wastewater could be contributing to the severe toxicity observed in the vintage season.
5. Based on the toxicity identification evaluation (TIE). It was confirmed that the most toxic component of the winery wastewater was organic in nature. To some extent TIE manipulations such as EDTA treatment, aeration were also able to reduce the winery wastewater toxicity confirming volatiles and heavy metals such as copper and zinc were also contributing to the overall toxicity of winery wastewater.
6. The bioassay approach was successful in assessing toxicity of winery wastewater based on variables such as size, treatment processes and season.
7. Filtration, aeration, pH adjustment are some of the commonly used manipulations that could reduce the toxicity of winery wastewater.
8. This study only assessed the acute toxicity of winery wastewater to waterfleas. Long-term exposures of winery wastewater may affect growth and reproduction in the organisms. Such chronic and sub-lethal toxicity of the winery wastewater needs to be established.
9. There is a variety of chemicals such as cleaning agents and flocculants used across the wine industry. Currently there is a lack of information on the toxicity of these

chemicals. Thus, further studies should focus on benchmarking the toxicity of all cleaning agents and flocculants

10. There is a lack of information on the contribution of organic acids, tannins and resin acids towards toxicity. A Toxicity profile of such components of winery wastewater is also required for the sustainable management of winery wastewater. .
11. Comprehensive use of TIE approach to identify toxic components of winery wastewater will help in better management of winery waste water.

4. TOXICITY ASSESSMENT OF POLYMERS USED IN THE WINERY INDUSTRY

4.1 Introduction

- The majority of polymers used at wineries are cationic.
- Cationic polymers are known to be highly toxic to aquatic organisms.
- Currently no guideline values are available to regulate their use.
- Polymers persist in the environment and are of environmental concern.

Correct choice of polymer is necessary if the winery wastewater has to be passed through a wetland system. Currently, polymer use is not being regulated by EPAs. There is a need to audit the use of polymers in the wine industry to suggest environmentally friendly polymers that are less toxic.

The main objectives of this study were:

1. to assess toxicity of three polymers used at the wineries; and
2. to assess toxicity of wastewater from a winery, before and after polymer treatment.

4.2 Methodology

Polymer usage was evaluated at a large and a medium scale winery. Three commonly used polymers samples were collected and tested using ecotoxicological approaches.

Toxicity testing was undertaken on three polymers using organisms from a range of trophic levels, since different species can respond differently to toxicants. The following three polymers were selected for bioassays:

1. Jetfloc HP3 Cationic Powder flocculant
2. Ciba Zetag7689 flocculant
3. Zetag 7635 flocculant

Test species were selected on the basis of their ecological relevance and on the availability of standard tests with known sensitivity and reproducibility. The use of a standardised testing protocol means that other laboratories can carry out the bioassays in an identical manner. It is also possible that the above indicator organisms respond differently to different type of contaminants. Together these assays can provide a comprehensive environmental risk assessment of toxicants such as polymers.

4.2.1 Test organisms selected

1. Waterflea- *Ceriodaphnia dubia*
2. Duckweed- *Spirodela sp*
3. Tadpoles, Spotted-marsh frog- *Limnodynastes tasmaniensis*
4. Midge*, Chironomus tepperi*

Chironomids (midge) are test organisms commonly used to assess toxicity of sediments and bioaccumulation of sediment-bound contaminants (USEPA, 1994). Their intimate contact with bottom sediments and interstitial and overlying waters for extended periods of their life cycle increases the likelihood of adverse effects in the presence of contaminated sediments (Burton, 1992).Tadpoles of grass spotted frog were also chosen for ecotoxicological work because wetlands are getting popular with the wineries and amphibians are often a dominant member of wetland fauna. Winery wastewater toxicity assessment to tadpoles will assist in maintaining ecosystem health of these wetlands.

The objective of toxicity testing was to determine for each polymer:

- The No Observed Effect Concentration (NOEC), where no statistical difference ($P \leq 0.05$) was found between exposed and unexposed (or control) specimens.
- The Lowest Observed Effect Concentration (LOEC), where the smallest statistical difference ($P \leq 0.05$) was found between exposed and unexposed (or control) specimens.
- The median effect concentration (LC50/EC50) was the concentration of the polymer that was estimated to be effective in producing mortality (in case of waterfleas and tadpoles), growth (for duckweed bioassay) in 50% of test organisms.

Statistical significance was determined at $\alpha = 0.05$. Data were tested for normality and homogeneity of variance using Toxstat (1994). An analysis of variance (ANOVA) with Bonferroni (unequal replicates) or Dunnett tests (equal replicates) was used to determine significant differences in various treatments. This information was used for the estimation of the LOEC and NOEC.

4.2.2 Before and after polymer treatment

Four winery wastewater samples were collected from winery F. These samples represented winery wastewater samples before polymer treatment and after polymer treatment on a given day. Toxicity testing was conducted using *Ceriodaphnia* to compare differences in the toxicity of these winery wastewater samples.

4.3 Results

4.3.1 Polymer toxicity

On the basis of acute *Ceriodaphnia* testing the 48-h LC50 values for the three polymers varied between 0.7 – 1.4 mg/L (Table 1). The lower the LC50 value, the greater the toxicity. Jetfloc HP3 was the most toxic polymer to *Ceriodaphnia* followed by Zetag 7635 and then Zetag 7689.

Table 4.1 Polymer toxicity to *Ceriodaphnia dubia*

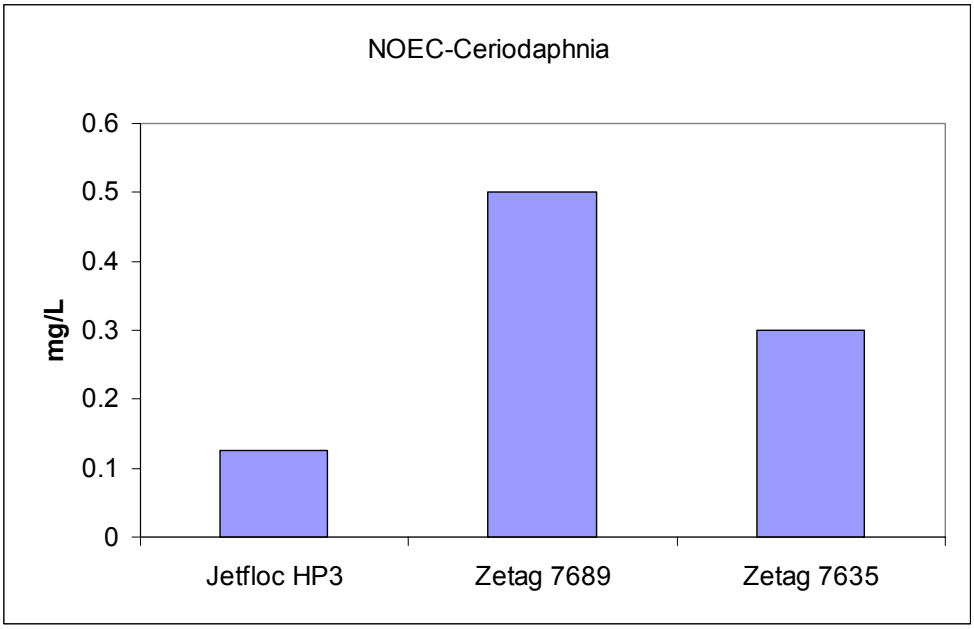
Polymer	48-h LC50
Jetfloc HP3 Cationic Powder flocculant	0.72 mg/L
Ciba Zetag7689 flocculant	1.40 mg/L
Zetag 7635 flocculant	1.20 mg/L

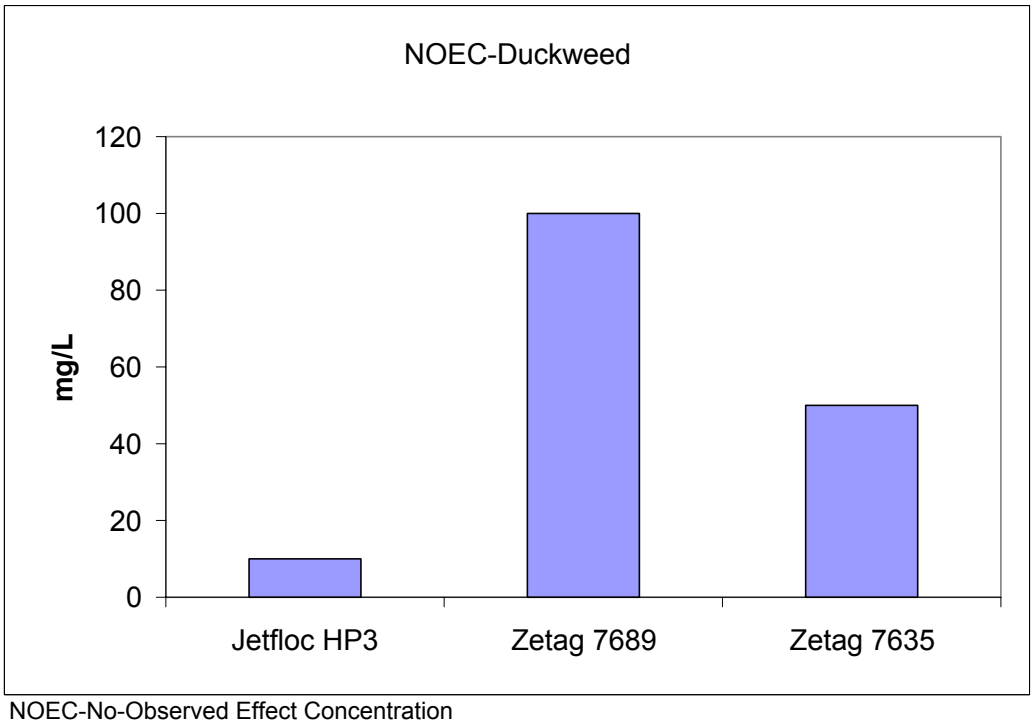
Toxicity of the three polymers to duckweed was also investigated. Duckweed showed lesser sensitivity to polymer exposures in comparison to the waterfleas. NOEC values for duckweed were higher in comparison to the *Ceriodaphnia* NOECS (Figure 4.1)

The three polymers also exhibited toxicity to the tadpoles during 48 hour exposures with LC50 values ranging from 22- 48 mg/L (Table 4.2). Jetfloc HP3 was the most toxic polymer to spotted-marsh frog tadpoles followed by Zetag 7689 and then Zetag 7635.

Table 4.2 Polymer toxicity to tadpoles of the spotted marsh frog*, Limnodynastes tasmaniensis*

Polymer	48-h LC50
Jetfloc HP3 Cationic Powder flocculant	22.08 mg/L
Ciba Zetag7689 flocculant	26.57 mg/L
Zetag 7635 flocculant from Orlando	48.47 mg/L





NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 4.1 Comparison of No-Observed-Effect Concentration (NOEC) for the three polymers

Chemicals can be categorised based on their toxicity data. The framework used by US EPA to categorise is given in Table 4.3.

Table 4.3 Toxicity categories for aquatic organisms based on US EPA

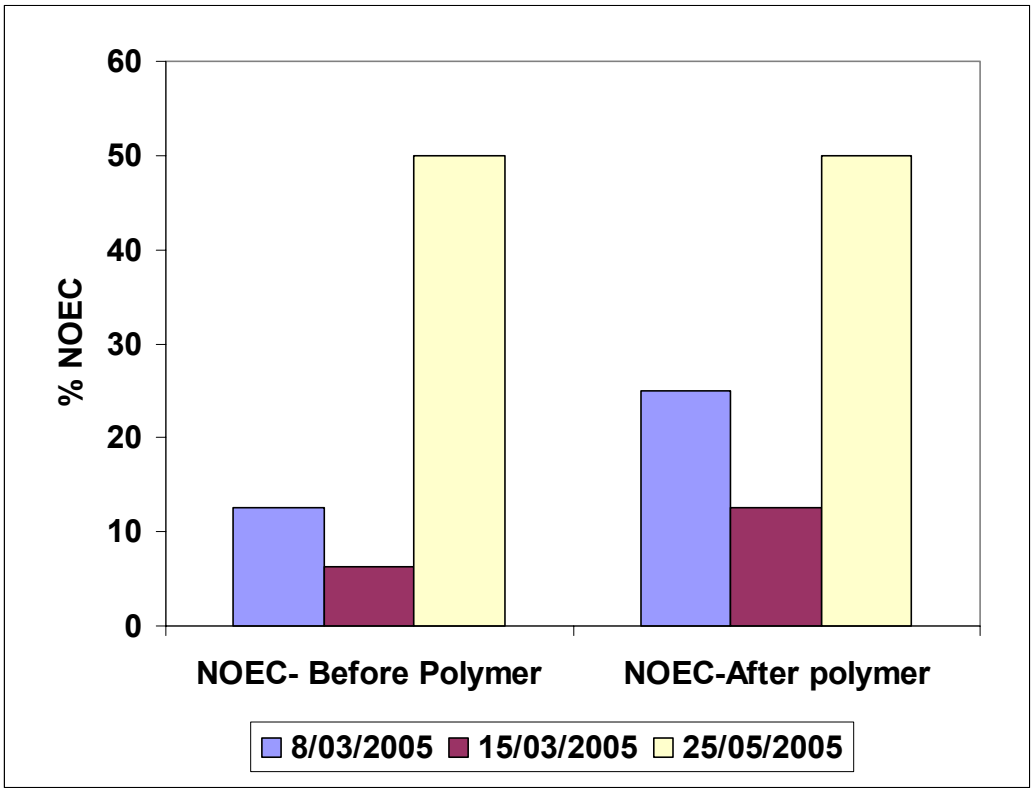
Concentration (mg/L)	Toxicity Category
<0.1	very highly toxic
0.1 - 1	highly toxic
>1 - 10	moderately toxic
>10 - 100	slightly toxic
>100	practically non-toxic

As *Ceriodaphnia* was the most sensitive species to the polymer exposures, *Ceriodaphnia* toxicity data were used to rank the three polymers. Based on the ranking given by USEPA, Jet floc can be considered as highly toxic whereas the two Zetag polymers can be considered to be moderately toxic. Ecotoxicological data on polymers used in the winery industry is highly limited. Further chronic/sub-lethal toxicity testing is required to recommend the safe concentration of these polymers in the environment.

4.3.2 TIE phase 1 manipulations

To follow-up polymer toxicity work, winery wastewater samples were collected from the large winery G. These winery wastewater samples represented samples before and after polymer treatment. *Ceriodaphnia* toxicity testing was conducted on these samples. There was not much difference between the two winery wastewater samples in terms of their toxicity (Figure 4.2). Physico-chemical analyses confirmed that pH of these samples were similar, around 8. In addition, the polymer used by winery G was less toxic than the polymer used by the other winery. Therefore, this further confirms that polymer toxicity in the winery wastewater samples can be reduced by choosing less toxic polymer and also by adjusting pH of the winery wastewater to approximately 9. Although polymers play a great role in reducing winery wastewater toxicity by flocculating solids, addition of toxic polymers or their use under unstable conditions could increase the toxicity of winery wastewater. Further work is required

to understand the fate and toxicity of different chemical classes or nature of the polymers used in the winery industry.



NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity

Figure 4.2 Winery wastewater sample toxicity before and after polymer treatment

4.4 Conclusions

1. Based on the preliminary investigation, current polymers used in the wineries can be classified as being highly to moderately toxic.
2. Jetfloc HP3 Cationic Powder flocculant was more toxic in comparison to the other two polymers tested under this investigation. This could be related to the differences in the chemical nature of these polymers.
3. Ciba Zetag7689 flocculant and Zetag 7635 flocculant exhibited similar level of toxicity to all the organisms selected in this study.
4. The order of sensitivity of different organisms to polymers was:

   Waterfleas > tadpoles > midges > duckweed
5. Polymer toxicity was reduced at pH 9 and above. It is recommended to adjust winery wastewater pH to 9, if cationic polymers are being used as flocculant. In general, cationic polymers perform efficiently under alkaline conditions while their efficiency is significantly reduced under acidic conditions.
6. Currently, a large number of cationic polymers are available for use and there is lack of information on their fate, mobility and toxicity. This study only investigated three polymers in terms of their toxicity. Risk ranking of all polymers used by the wine industry is urgently needed.
7. Chronic and sub-lethal toxicity of the polymers needs to be known, especially for the wineries using wetland systems for treating/polishing their wastewaters.
8. There is a lack of information available on the polymer toxicity to terrestrial organisms. This is important when land disposal of winery wastewater is a common practice.
9. Information on the fate and persistence of polymers in the aquatic and terrestrial environments is required for better management of their use in treating winery wastewater.

5. EFFICIENCY OF WETLANDS TO TREAT WINERY WASTEWATER

5.1 Introduction

As the wine industry expands and attracts tighter scrutiny from regulators, many wineries are not finding conventional pond or lagoon systems sufficient to meet waste discharge requirements and limits. In addition, insufficient treatment of wastewater used for irrigation purposes can cause degradation in the quality of grapes produced and in groundwater quality. Wineries have reacted to these problems by upgrading their pond systems or, in a few cases, adding bioreactors or treatment wetland systems to their treatment processes.

Treatment wetlands are constructed wastewater systems that rely on physical, chemical, and biological processes typically found in natural wetlands to treat wastewater. A treatment wetland replicates the processes occurring in natural wetlands, but uses wastewater or stormwater as the water source. Although the primary purpose of treatment wetlands is to treat various kinds of wastewater, these wetlands can also function as wildlife habitat. If properly built, maintained and operated, treatment wetlands can effectively remove many pollutants--including suspended solids, pathogens, nitrogen, phosphorus, BOD (biochemical oxygen demand), hydrocarbons and some metals--without compromising habitat value.

Research trends in the environmental assessment of wetlands and natural water bodies have demonstrated the need for an integrated approach which combines chemical characterisation with biological effects evaluation, both in laboratory (toxicity bioassays) and *in situ* (macroinvertebrate community structure analysis). Three lines of evidence involving chemistry, ecotoxicology and benthic ecology were used to assess the efficiency of a wetland system receiving winery wastewater.

5.2 Methodology

The wetland selected under this study was a constructed wetland at the medium sized winery B. Solids were removed from the winery wastewater by treatment with polymer before it entered the first cell of the wetland. Aerators were working continuously in the first cell of the wetland. After passing through a series of four cells the wastewater was stored in a dam (Figures 5.1 and 5.2). The polished water from the dam was then used for irrigation purposes.

5.2.1 Sampling regime

Sampling was conducted once a month from December 2002 until June 2004. The sampling involved collection of water samples from the four wetland cells and a dam (Figure 5.3). Each sampling period involved the following three components

5.2.1.1 Chemical characterisation of water from a series of wetland cells

Physicochemical characteristics of wastewater such as pH, BOD, COD, TOC (Total Organic Carbon), salinity, electrical
conductivity (EC), nutrients, heavy metal contaminants and total suspended solids were analyzed following standard wastewater analyses methods (APHA/AFFA, 1995).

5.2.1.2 Toxicity of water from a series of wetland cells (p1-p4) to waterflea, Ceriodaphnia dubia

48-hour acute bioassays with *C. dubia* were performed according to USEPA (1993). Dilutions of the water from different wetland cells were prepared by addition of formulated moderately hard water. A 0.5 dilution factor was used to produce test concentrations of 100, 50, 25, 12.5, 6.25% (v/v). After 48 hour exposure, waterfleas alive at each dilution were recorded and on the based of this information LC50, LOEC and NOEC values were calculated for each wetland cell sample.

5.2.1.3 In-situ midge toxicity

In situ studies were conducted in 2003-2004 by placing midges (in cages) in the different ponds of the wetland. Every month survival after 24 h exposure was monitored.

5.2.1.4 Macroinvertebrate sampling and data analyses

To obtain data on macroinvertebrate abundancy and diversity, quantitative samples were taken using a hand-held D framed dip net with 320×250mm opening and with 250μm mesh (Figure 5.4). Each dip net sample was inverted with the contents washed into a plastic jar, which was filled with 70% ethanol. Taxa were identified using a dissecting microscope to family level. Samples were collected on a monthly basis from all four cells of the wetland system and the dam receiving final polished water.

Analysis of Diversity Data was based on the Shannon Index (Shannon and Weaver, 1949).

This index assumes that all species are represented in the sample and are randomly sampled (Krebs, 1989) and increasing values corresponded to increasing diversity.

Figure 5.1 First cell of the wetland system

Figure 5.2 Dam receiving winery wastewater after passing it through a series of four cells.

Figure 5.3 A water sample collection from cell 4 for further laboratory analyses.

Figure 5.4 Macroinvertebrate sampling at cell 4 of the wetland system.

5.3 Results

5.3.1 Chemical characterisation of water from a series of wetland cells

Winery wastewater quality was significantly improved during its residence time in the different cells of the wetland system. pH significantly increased from being as low 4 in cell 1 to 6 - 7.5 in the dam. Similarly, dissolved oxygen of water in cell 4 of wetland increased to 5-7 mg/L while it was as low as 0.5 mg/L in the first cell of the wetland system. Total organic carbon content was lower in the dam (50 – 150 mg/L) in comparison to that of cell 1 of the wetland system (300 – 900 mg/L).

5.3.2 Toxicity of water from a series of wetland cells (p1-p4) to waterflea, Ceriodaphnia

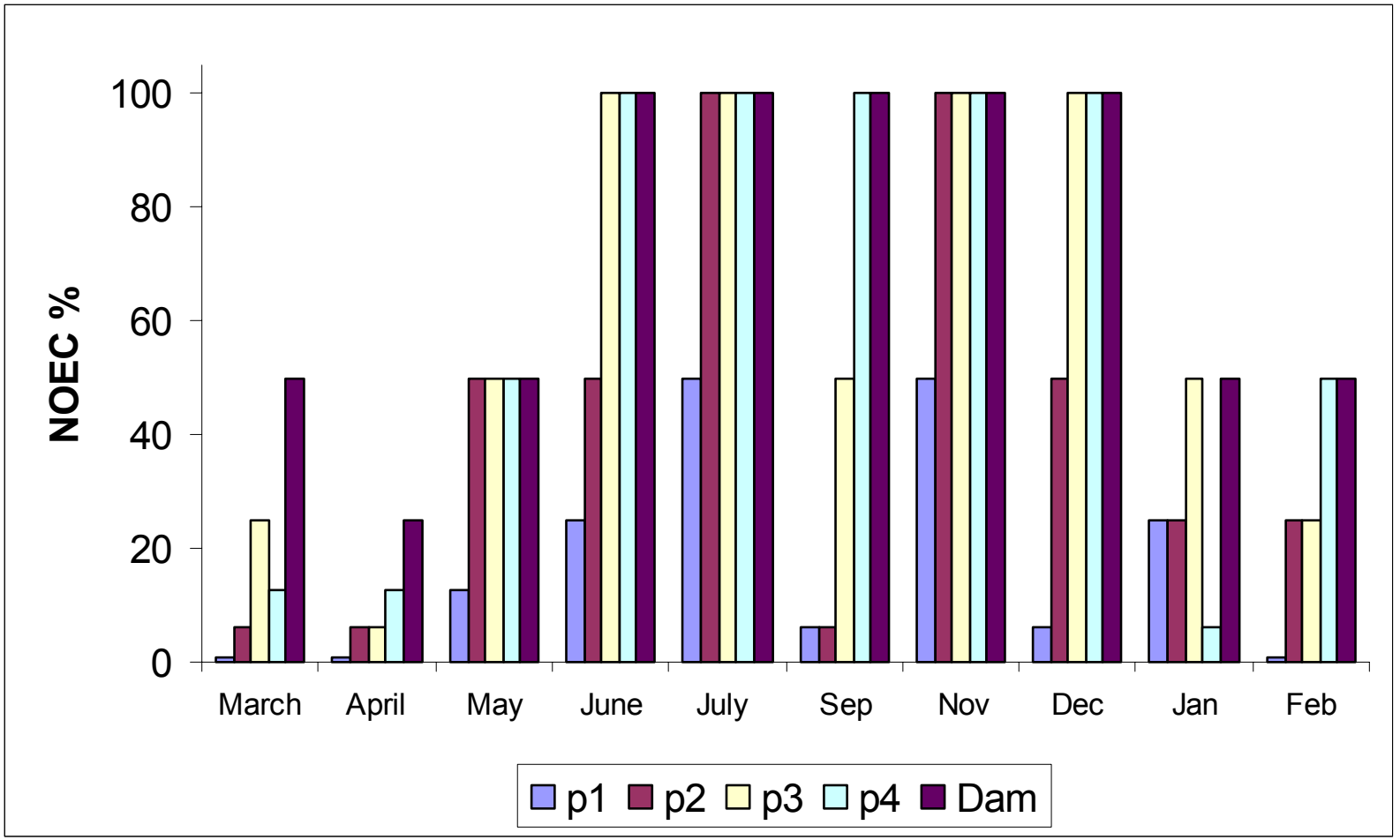
The wetland tested in this study was found to be highly impacted during the vintage season but showed significant recovery in the non-vintage season. 100% *Ceriodaphnia* survival was observed in cells 2, 3 and 4 of the wetland during the post-vintage season (Figure 5.5).

5.3.3 In-situ midge toxicity

During the vintage season, only 0-10% midge survived in all the wetland cells (Figure 5.6). In the post-vintage season, cells 3 and 4 were working efficiently in reducing the toxicity of wastewater, indicated by survival of midges increasing to 70-100% in cells 3 and 4. Cells 1 and 2 were still impacted by wastewater input with reduced survival (0-10%). In the non-vintage season, the wetland system recovered significantly, with 40-60% survival in the first cell and 70-100% survival in cells 2, 3 and 4 (Figure 5.6).

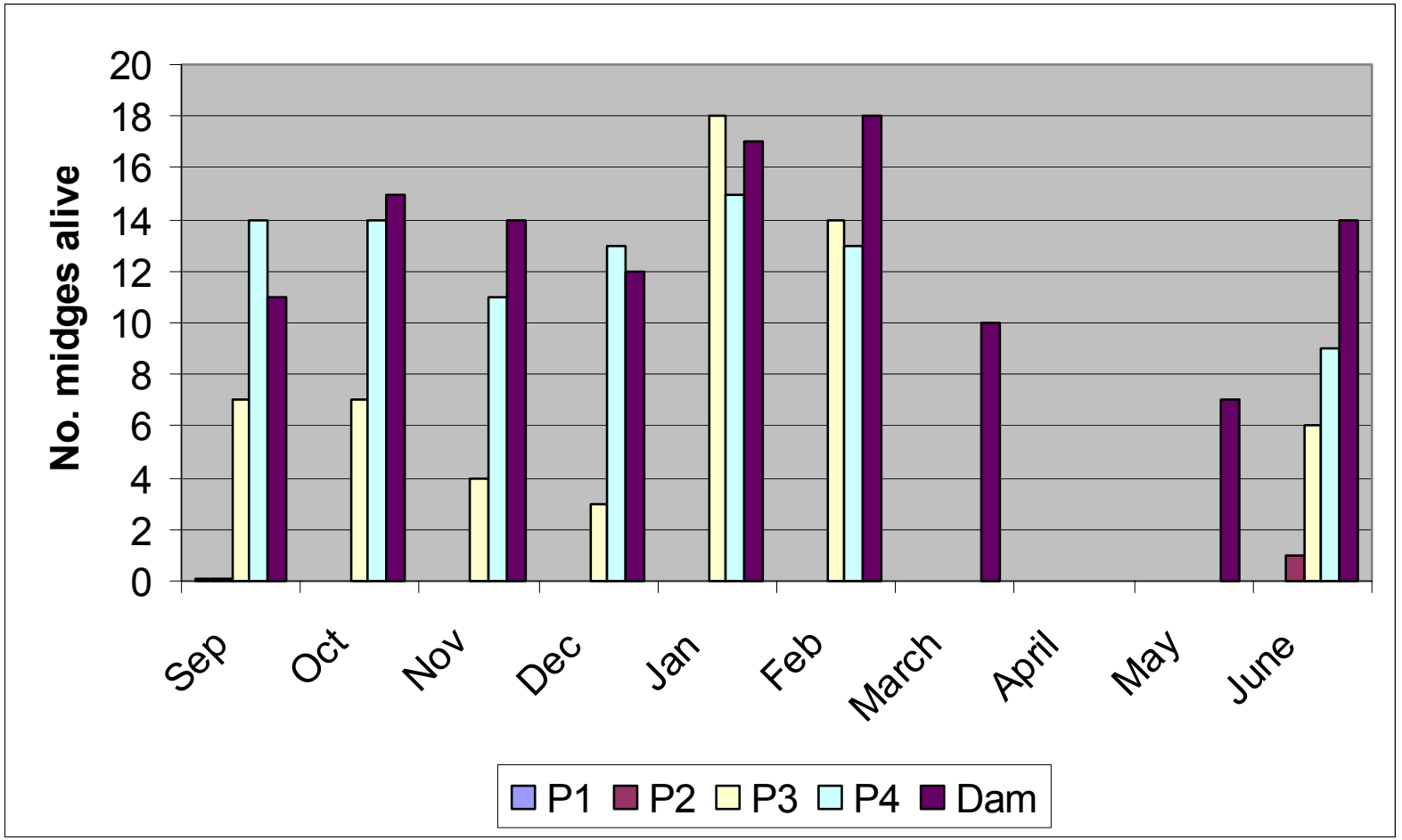
5.3.4 Macroinvertebrate diversity

Species abundance data indicates that the non-vintage and post-vintage seasons had the highest species abundance than in comparison to the vintage season (Figure 5.7). The Shannon-Weaver diversity indices revealed the lowest diversity indices for cell 1 and the highest for the cell 4 and the dam (Figure 5.8 – 5.12). Species diversity was always low in the vintage season (Figure 5.9) compared to the pre-vintage season (Figure 5.8). Cells 1 and 2 were highly impacted during the vintage season, as macroinvertebrates were absent from these two cells (Figure 5.9). The wetland system recovered during the post-vintage and non-vintage seasons with higher species diversity (Figures 5.10 and 5.11).



NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity
P1- P4: a series of ponds in a wetland system under investigation

Figure 5.5 Variation in toxicity to *Ceriodaphnia* exposed to waters from different ponds in a wetland system



NOEC-No-Observed Effect Concentration
Lower NOEC value corresponds to greater toxicity
P1- P4: a series of ponds in a wetland system under investigation
Results based on 20 midges at beginning of exposure

Figure 5.6 Midge toxicity in different ponds of the wetland during 24 hour in-situ exposures

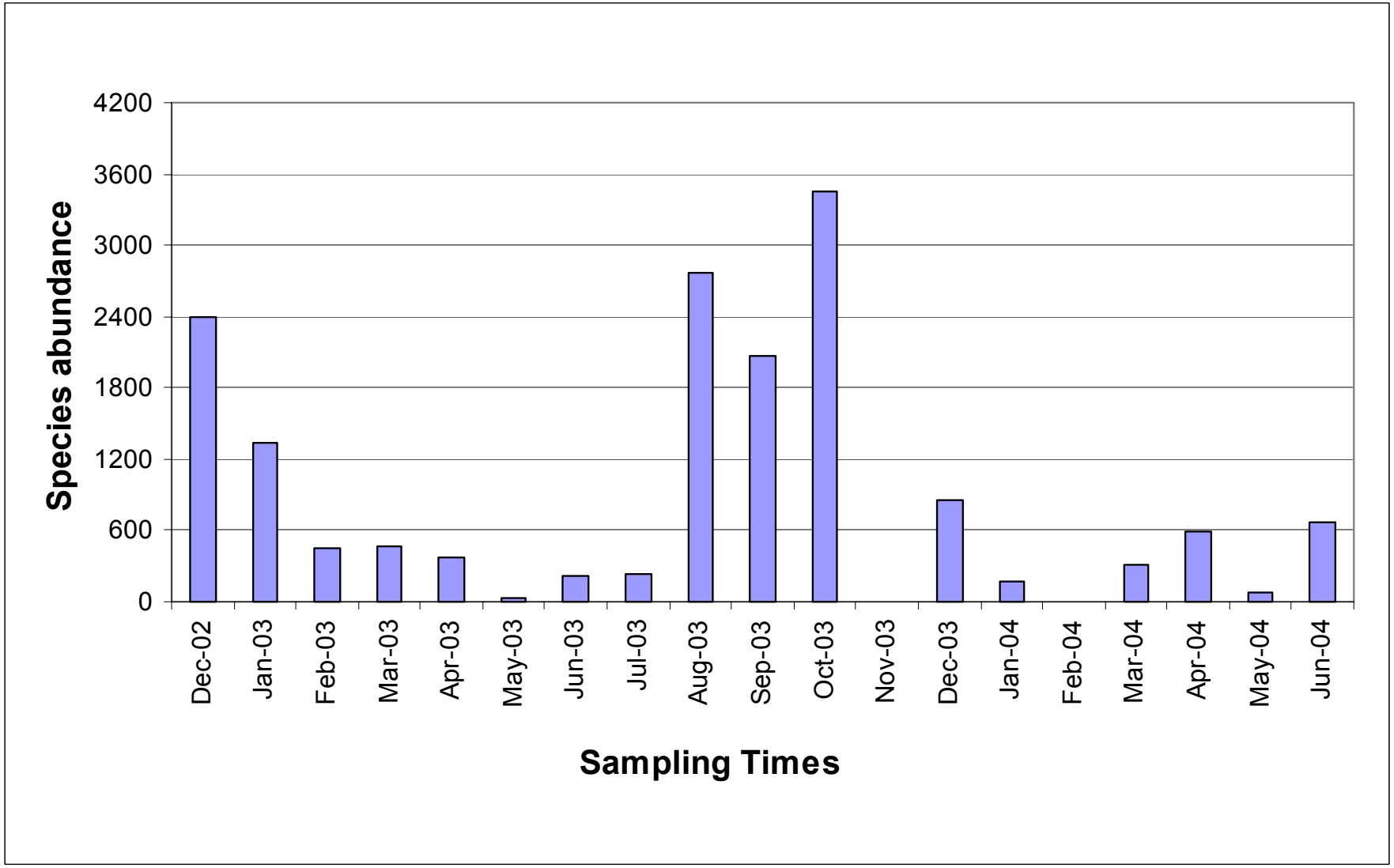


Figure 5.7 Species abundance in the wetland system during vintage and non-vintage seasons

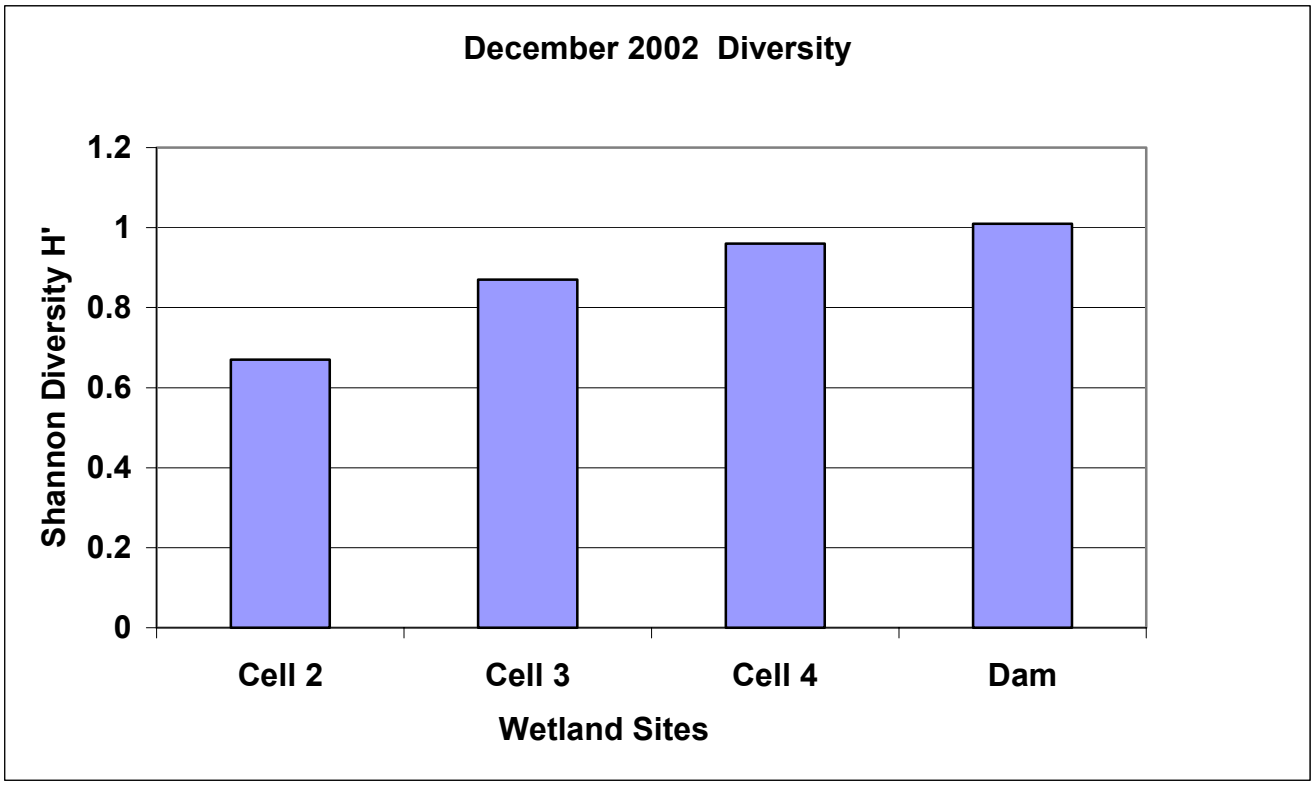


Figure 5.8 Macroinvertebrate diversity in the wetland system during Decemebr 2002 (pre-vintage season) sampling

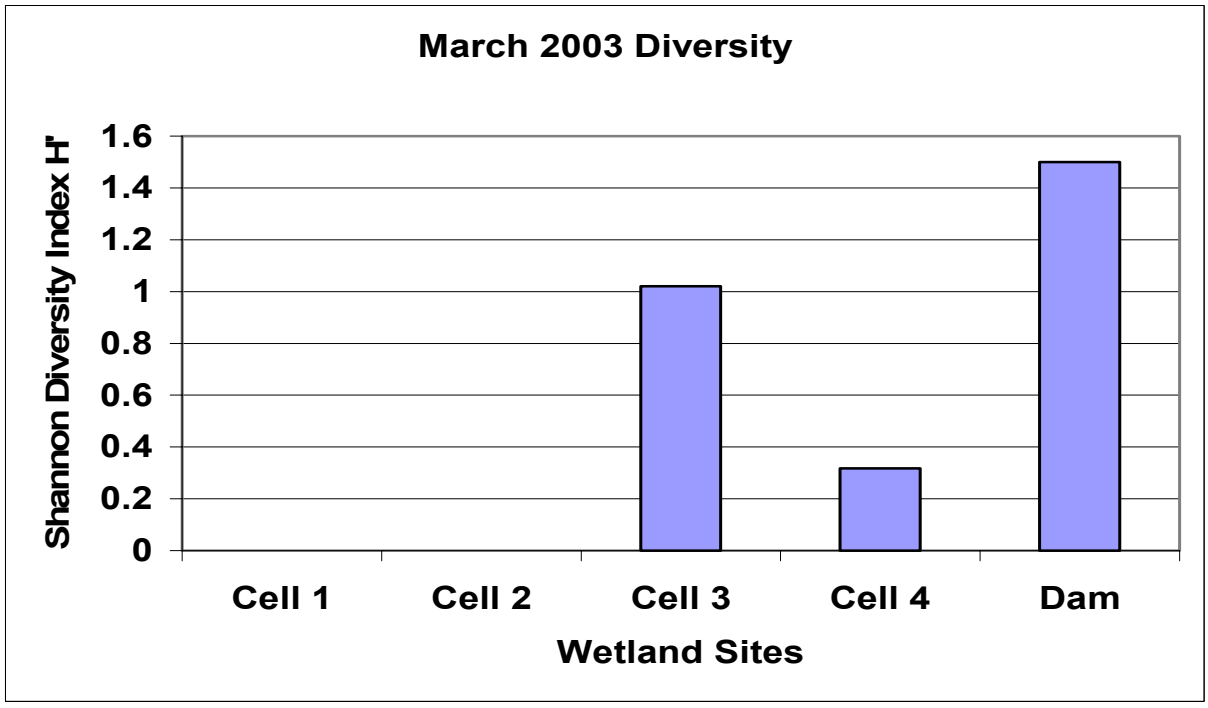


Figure 5.9 Macroinvertebrate diversity in the wetland system during March 2003 (vintage season) sampling

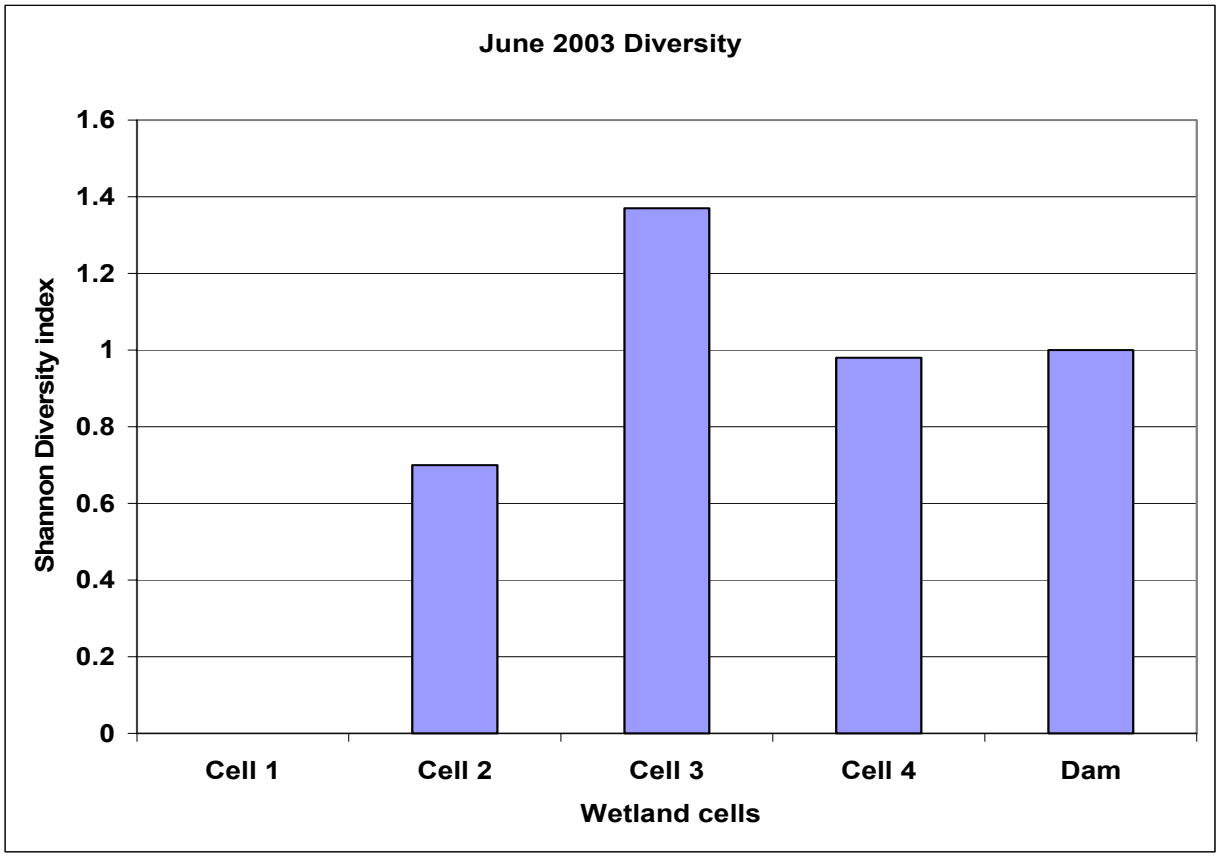


Figure 5.10 Macroinvertebrate diversity in the wetland system during June 2003 (post-vintage season) sampling

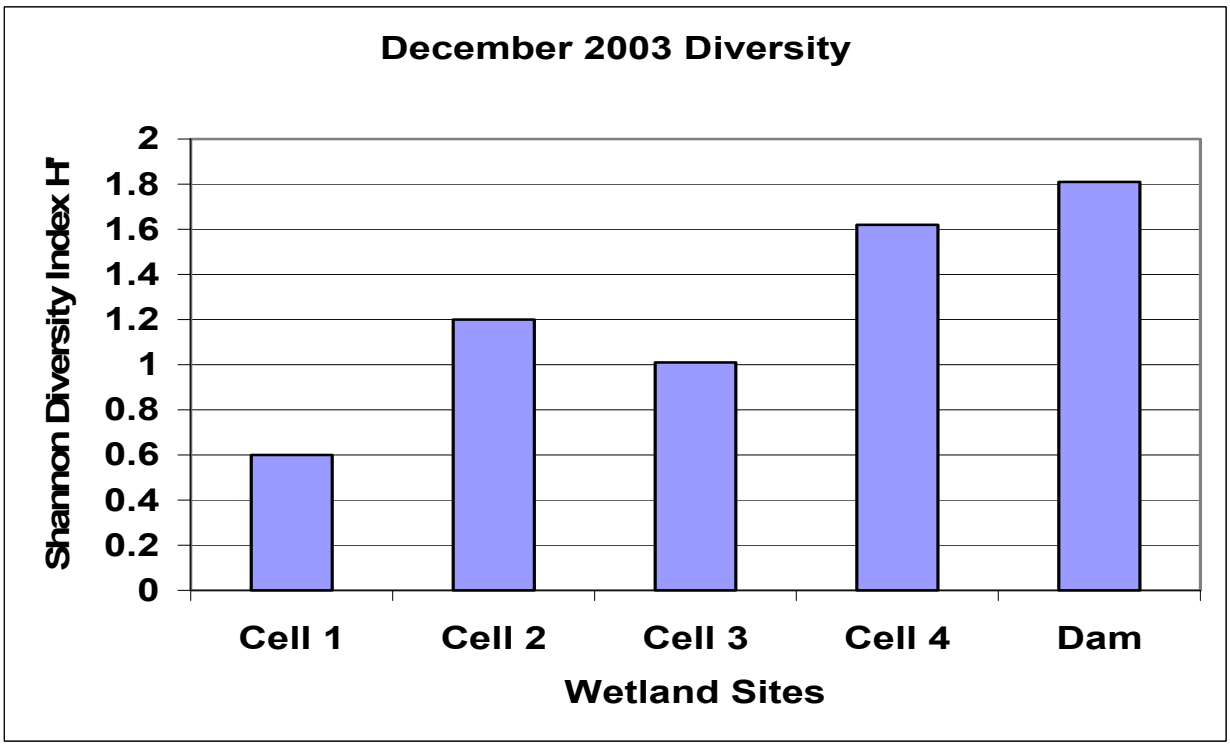


Figure 5.11 Macroinvertebrate diversity in the wetland system during Decemebr 2003 (non-vintage season) sampling

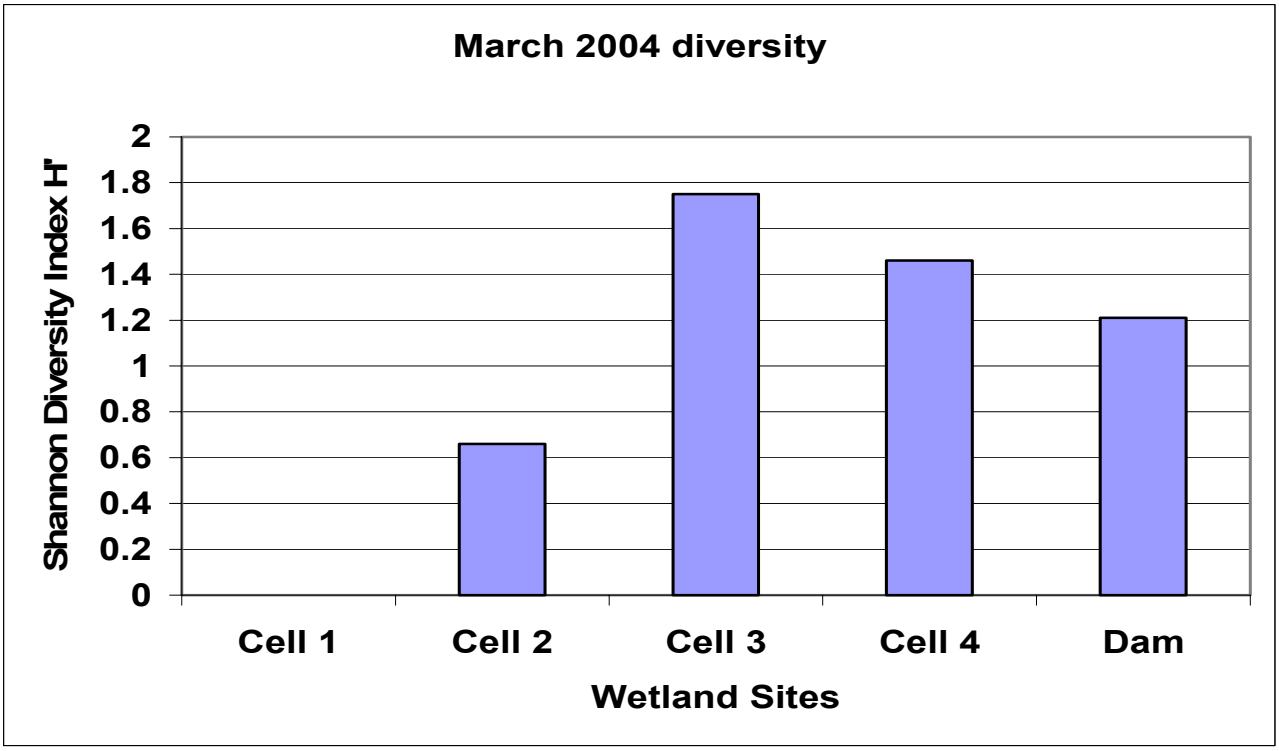


Figure 5.12 Macroinvertebrate diversity in the wetland system during March 2004 (vintage season) sampling

5.3.5 Seasonal variation in the efficiency of the wetland

During 2003 vintage season, the health of wetland was highly impacted. Midges did not survive in all the ponds during 24-hour exposures. Waterfleas also died when exposed to the waters from different ponds of the wetland. In addition, macroinvertebrate diversity in the wetland ponds was found to be highly impacted during the vintage season but showed significant recovery in the non-vintage season. Toxicity identification evaluation (TIE) procedures revealed that low pH was contributing to the toxicity of winery wastewater. On further follow-up with the winery, polymer use was identified to be a contributing factor to the observed toxicity. The research carried out in this project has made a major contribution, as it led to the identification and replacement of the toxic polymer by a less toxic one. The wetlands have shown significant apparent recovery in aquatic health since this change (March 2004). Thus the polymer used to treat winery wastewater can cause direct toxicity to organisms. Caution is needed in choosing environmentally safer polymers.

5.4 Conclusions

The wetland tested in this study was found to be highly impacted during the vintage season but showed significant recovery in the non-vintage season based on the measurement of pyhico-chemical parameters, bioassays and macroinvertebrate assessment and in-situ assessment. In general, the species diversity was low at this treatment wetland system in comparison to any natural wetland system. However, this wetland system was able to improve winery wastewater quality in the dam making it suitable for irrigation purposes.

As the wine industry continues to expand in Australia, treatment wetlands provide a low-energy, low maintenance, aesthetically pleasing advanced wastewater treatment option for wineries with both small and large amounts of wastewater. Wetland systems adapt well to the wastewater fluctuations of wineries Removal of solids from winery wastewater entering wetland ponds, pH adjustment and aeration of first pond are important management strategies for wetland systems receiving winery wastewater.

6. IMPACTS OF WINERY WASTEWATER DISCHARGE ON SOIL HEALTH

6.1 Introduction

Land based disposal is the main method of waste disposal currently used for many organic wastes The organic residues in the winery waste may stimulate microbial activity and the possible use of this kind of organic waste for irrigation may help to cut down the use of nitrogen and phosphorus based fertilisers. However, repeated application of winery wastewater could result in long-term effects on soil properties and soil dwelling organisms. . Research in this area is therefore a major priority both in terms of soil sustenance and maintenance of environmental quality.

The main aim of winery wastewater irrigation by the wine industry is to dispose of winery wastewater (Chapman *et al*., 2001) as this would reduce its storage time and prevent malodours. In response to the large volume of winery wastewater production, this pressure may lead to over-irrigation. Over-irrigation can lead to detrimental environmental impacts e.g. an impact on groundwater and leaching of excess salts from the soil irrigated with winery wastewater. Water logging could also result from over-irrigation. This can result in filling large soil pores which in turn can affect the developing plant roots and soil dwelling organisms. High BOD of wastewaters can cause imbalance in the oxygen supply in soil or could shift the biochemical pathways leading to malodours and inefficient removal of organic contaminants from the soil (pers comm. Dr Cecil Camilleri). Build up of salts in soils due to winery wastewater application to land can reduce plant growth and productivity. High SAR of winery wastewater could also affect the soil structure. It is very important to maintain the diversity of soil micro-organisms as they help vines obtain the type of nutrition they need. A healthy soil microbe population is also essential for preventing plant diseases and pests from gaining an advantage in the field. Chapman and coworkers have extensively (1995) studied the removal of soluble carbon from synthetic winery wastewater by repeated application to soil. To our knowledge, ecological effects associated with winery wastewater discharge and its impact on soil microbes due to the use of winery wastewater for irrigation has not been investigated nationally or internationally.

The main aim of this study was to assess the changes/impacts due to long--term application of winery wastewater at selected sites in the Barossa and McLaren Vale regions. The soil monitoring program was designed to ensure that land treatment of winery wastewater does not lead to structure loss, salinisation, waterlogging, or chemical contamination of the soil matrix. In addition, soil microbial health was also assessed at these sites.

6.2 Methodology

In order to investigate the impact of winery wastewater on soil status in terms of microbial health and general soil properties (e.g. physico-chemical characteristics), surveys were undertaken from several sites. Site selection for field monitoring was done in consultation with various stakeholders (Figure 6.1). Soils were sampled in April 2003, November 2003 and June 2004 on different sites, (pastures, woodlots and vineyards), which had received different volumes of winery wastewater. Sampled sites included those irrigated for more than 20 years and controls (never received any waste water). Details of the sampling sites and their history of winery wastewater irrigation is provided in Table 6.1.

Figure 6.1 Selection of the sites for field monitoring

Woodlot sites were selected at the two large wineries. The woodlot at winery F (Figure 6.2) had received winery wastewater for nearly 10 years. The woodlot at winery Y (Figure 6.3) had received winery wastewater for the last eight years. The vineyards were selected at Winery Y (Figure 6.4), all these vineyards had received winery wastewater for the last 4-6 years. Pasture sites included natural reserve (NR, Figure 6.5) and an irrigated pasture. Both these sites had received winery wastewater for approximately last 15-20 years, respectively. The pasture site selected at small winery K had a very long history of winery wastewater application, probably more than 100 years.

Table 6.1 Sites selected for the soil health monitoring

Land use	Sites where winery wastewater has been applied	Period of winery wastewater irrigation	Control sites
Vineyard	1. MV at winery Y 2. TH at winery Y 3. TB ay winery Y	10 years 4 years Approx 18 years	Control vineyard at winery Y
Woodlot	1. Main woodlot at winery Y 2. Woodlot at winery F	30 years 10 years	Road verge as control woodlot site near winery Y
Pasture	1. Wildlife reserve at winery Y 2. Irrigated pasture near woodlot at winery Y 3. Long-term irrigated pasture at small winery K	6 years 30 years More than 100 years	Control pasture at winery Y Adjacent area with same soil type as a control site at winery K

Figure 6.2 Woodlot site at winery F, has been irrigated with winery wastewater for last ten years

Figure 6.3 Woodlot site at winery Y, has been irrigated with winery wastewater for last thirty years

Figure 6.4 Vineyard site at winery Y, has been irrigated with winery wastewater for last ten years

Figure 6.5 Wildlife reserve site at winery Y, has been irrigated with winery wastewater for last six years

6.2.1 Soil sample collection and replication

There were some challenging sampling issues due to variability in the sites due to size, land use, period of winery wastewater application and soil characteristics. Finding an appropriate reference site for different land uses was also considered during soil sampling. In general, it was recommended that 5 soil samples be used to represent each site. Each sample was to be chosen to be a composite of 5 cores. Typically the cores were taken on a 5"x 5" grid, and the compositing was done along the axis with the largest environmental gradient (Figure 6.6). This scheme ensured that there was maximum variation within a composite, which guaranteed minimum variation between the composite samples.

There was an issue in the sampling of vineyards, because of local effects of drippers. There may be dry patches in the vine row between drippers while at the same time there was water flowing from the row to the inter row area. It was imperative to obtain good data on this. For surface samples, it was recommended that again five cores be used to form a single replicate, and that five composite samples be taken to represent the 'under dripper', 'between dripper' and 'between row' areas. It was anticipated that there would be more variation under the drippers, and less between rows. However, the area represented between rows was much larger, so that the increased precision for that component was considered warranted.

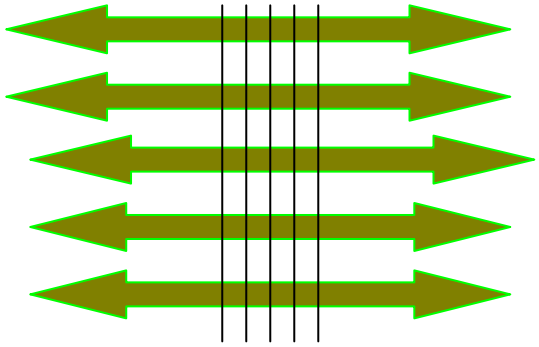


Figure 6.6 Selection of sampling points at a vineyard
Each green area indicates area of a composite sample, vertical rows represent wine rows.

At the small winery K, the area affected by the winery waste was indicated by the presence of green grass. This was sampled using 5 composite samples as shown in Figure 6.7. Each composite sample consisted of five samples taken along a transect that follows the contours and intersects the drain. The cores within a composite sample differed markedly but there should be less variation between the composite samples.

There was a comparably formed area to the south of the affected area that was sampled as a control site. A similar sampling scheme was used on the control site. A comparison between the treated site and the control site was made using a paired '*t*' test. The contamination from the sampling site at winery K was basically a point source. This contrasts with the effluent disposal at the large winery Y where the waste was spread (i.e. diffuse source). There was a question as to how the affected area should be defined. A smaller area would have a higher level of contamination. The pragmatic choice was to use all the area that is apparently affected by the wastewater. A further investigation could be carried out to investigate how rapidly the effect of the wastewater is dissipated. However, that question was outside the scope of this study.

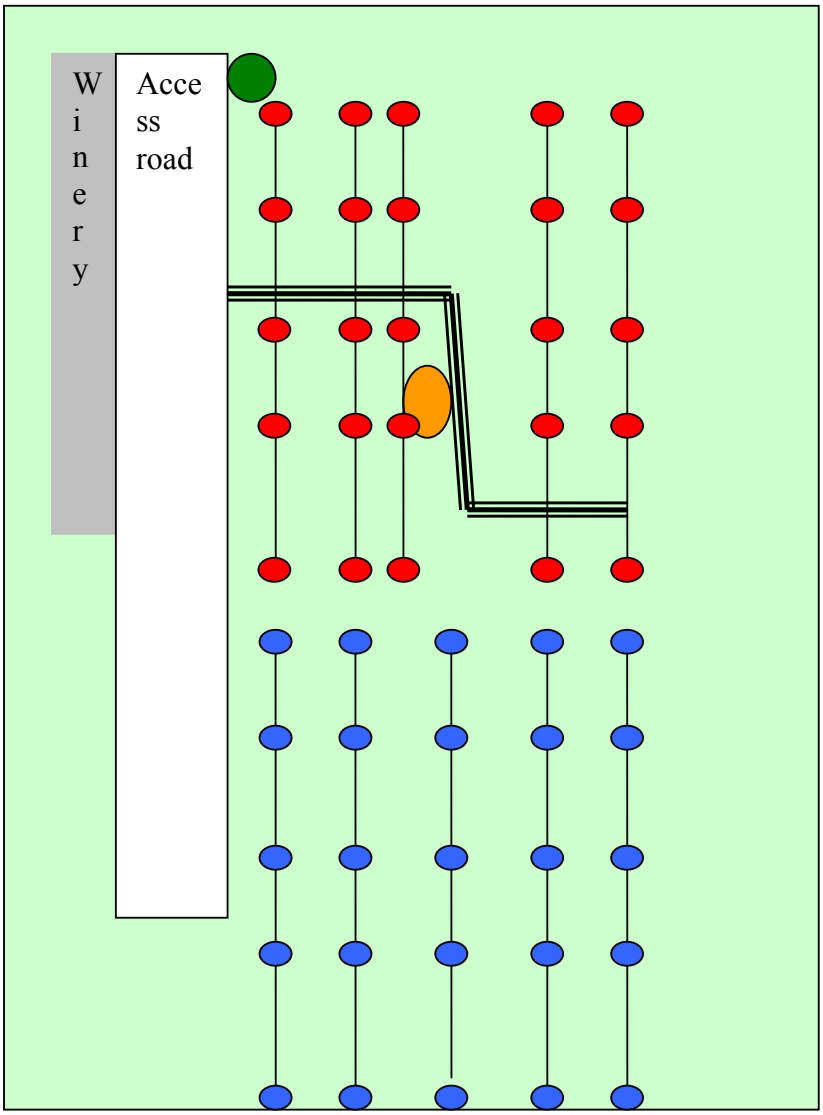


Figure 6.7 Diagram of study area at Winery K. Cores affected by the waste water are indicated as red and the controls are shown in blue. Green and orange areas denote water storage and rain tanks on the property.

6.2.2 Soil sample preparation

Samples collected in the field were processed in the laboratory for further analyses. The first step involved air-drying all samples at 40°C in the aluminium foil trays. This was followed by grinding and sieving of the dried soil samples. The samples were then thoroughly mixed, sub-sampled into containers and thereafter stored for further microbiological and physico-chemical analyses.

6.2.2 Microbial assessment of soils collected

Microbial activity in soil has a direct influence on ecosystem stability and fertility since microbes play a fundamental role in biogeochemical cycling (nutrient cycling). Soil enzymatic activities have been used as indicators of soil fertility since they integrate the effects of climate, agronomic practices, soil properties and organic amendments. While there are limitations associated with different types of indicators of soil health, the suite of enzyme assays covering a range of processes together with substrate induced respiration and nitrification provided comprehensive microbiological assessment. Soil microbial assays conducted under this study are shown in Table 6.2.

Table 6.2 Soil microbiological assays used in the study and their significance

Indicator	Significance
Substrate Induced Respiration (SIR)	This is a measure of the organic carbon mineralisation after the addition of glucose (organic substrate) to the soil. This is a potential metabolic activity and can also be used to quantify the pool of active microorganisms in soils. Therefore it is an assessment of overall microbial activity in soils.
Substrate Induced Nitrification (SIN)	This is another potential metabolic activity, but concerning nitrification this time (and not carbon mineralisation unlike SIR). Nitrification being the transformation of NH4+ into NO3-. Sometimes while the overall activity is not affected the specialists such as nitrifiers are affected.
Acid Phosphatase	Is the measure of phosphorus cycling in the soil by enzymes released by plant root systems.
Alkaline Phosphatase	Measures the phosphorus cycling in the soil by micro organisms.
B Glucosidase	Measures the carbon cycling in the soil by micro organisms.
Chitinase	Looks at the fungal activity in the soil. Nutrient cycling is slower by fungal enzymes as they tend to attack more complex organic compounds.

6.2.3 Physico-chemical analyses of soils collected

The following physico-chemical characteristics parameters were measured:

1. TOC (%)
2. EC (dS/m)
3. Calcium (mg/kg; 1:5 soil : water for the determination of Sodium Adsorption Ratio (SAR))
4. pH (pH units; 1:5 soil : water)
5. Total available phosphorus (mg/kg ; Bicarbonate extraction method)
6. Magnesium (mg/kg, 1:5 soil : water for the determination of SAR)
7. Total Kjeldahl nitrogen (TKN, mg/kg)
8. Potassium (mg/kg, Bicarbonate extraction method)
9. Sodium (mg/kg, 1:5 soil : water for the determination of SAR)

6.3 Results

6.3.1 Microbiological response

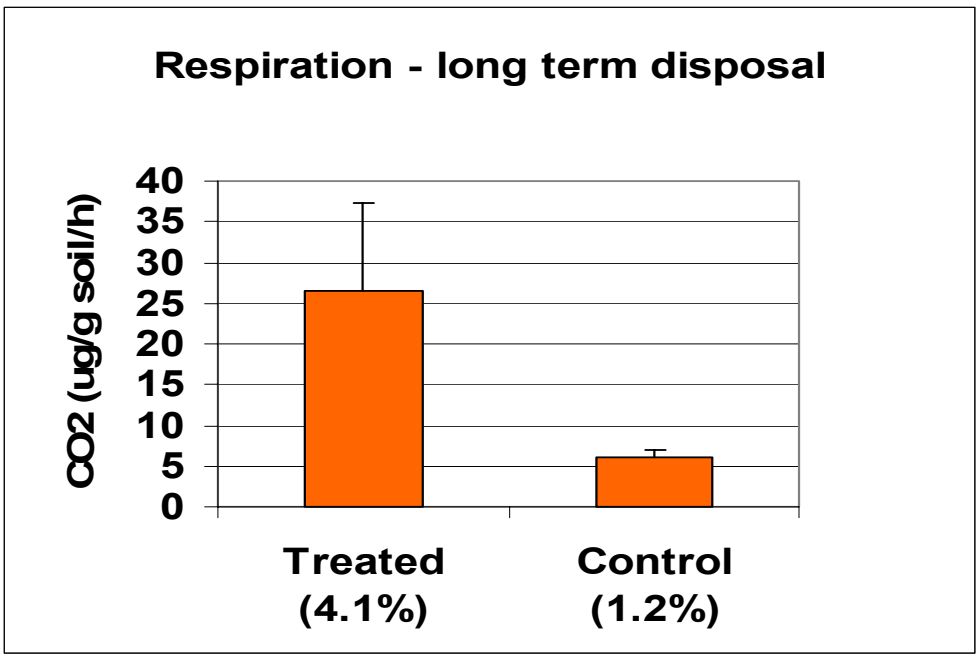
From a biological health point of view, two indicators were used. One, an indicator of overall microbial activity (substrate induced respiration) and two, specific functions performed by micro-organisms (substrate induced nitrification and enzymes).

Results showed that biological activity (e.g. mineralisation of organic carbon) increased in irrigated pastures (which exhibit a build-up of organic carbon), compared to their respective controls (Figures 6.8 and 6.9). Substrate induced respiration (SIR) in vineyard soils irrigated with winery wastewater did not differ significantly from that of control vineyards (Figure 6.10). Woodlots soils irrigated with winery wastewater exhibited increased SIR in comparison to the control woodlot soils (Figure 6.11). Nitrification (conversion of NH_4^+ in NO_3^-, the major source of nitrogen for plants) followed the same trend. Pasture soils irrigated with winery wastewater had higher substrate induced nitrification (SIN) in comparison to the control reference soils (Figures 6.12 and 6.13). In the vineyards, the control was not significantly different to the irrigated vineyards for these microbiological parameters, but this was not surprising, as the vineyards have been irrigated for a shorter time (Figure 6.14). Woodlots soils irrigated with winery wastewater exhibited increased SIN in comparison to the control woodlot soils (Figure 6.15).

Microbial enzyme analyses also confirmed that the winery wastewater irrigation of soils was not impacting the microbial activity of soils. Vineyards irrigated with winery wastewater such as the MV site showed increased activity of alkaline phosphatase, acid phophatase and β-glucosidase during the 2003 sampling (Figure 6.16). However, these differences were not

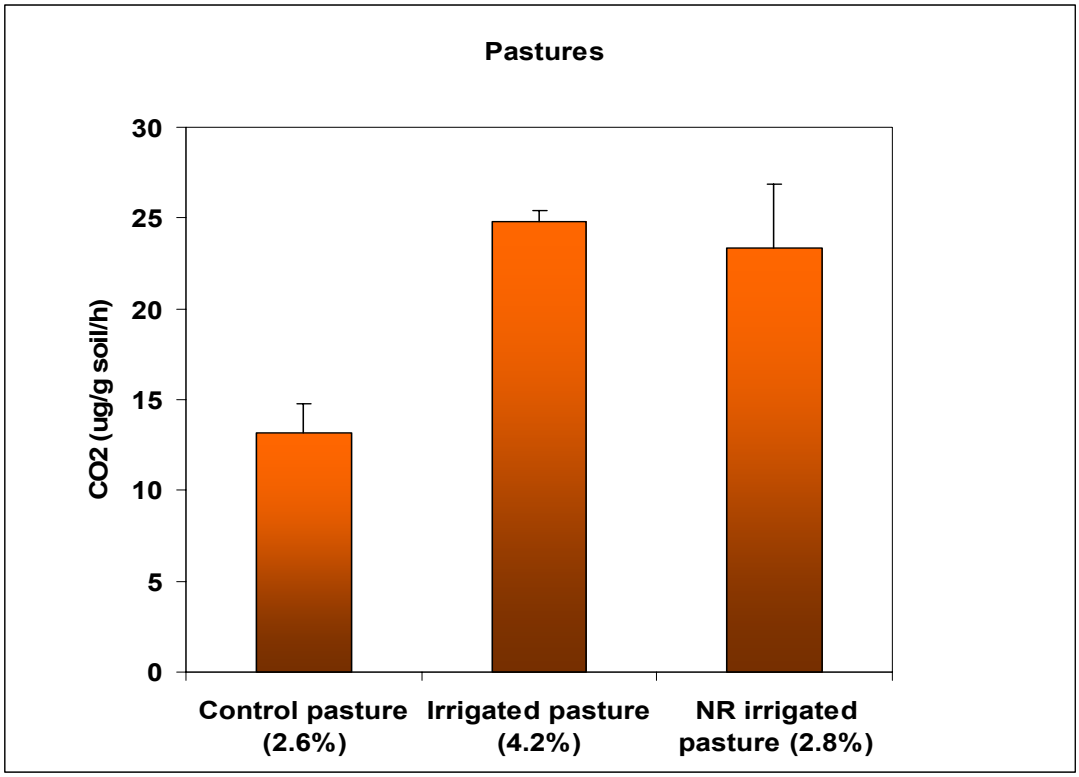
significant during the sampling for MV and TH vineyard soils in 2004 (Figure 6.17). Chitinase activity was not significantly affected by winery wastewater irrigation of the vineyard soils during both sampling periods. Pasture soil that had been irrigated with winery wastewater over 100 years exhibited increased microbial activity as measured by the four microbial enzymes (Figure 6.18). Pastures that have been irrigated with winery wastewater for the last 15-20 years did not show such marked difference in their microbial enzyme activity when compared to the control pasture soils (Figures 6.19 and 6.20). Microbial enzyme activity was also markedly increased at the woodlot sites irrigated with the winery wastewater (Figure 6.21).

Therefore, based on the microbial health assessment of soils receiving winery wastewater for some time, it can be concluded that soil microbiological activity of woodlots, vineyards and pastures was not adversely affected due to winery wastewater irrigation. In fact, greater microbial activity was observed in the wastewater treated plots, most likely due to the build up of organic carbon content. However, waterlogging due to over irrigation could impact the microbial activity of soils receiving winery wastewater. In the current investigation, all sites selected for soil monitoring were well drained and waterlogging was not observed during our sampling.



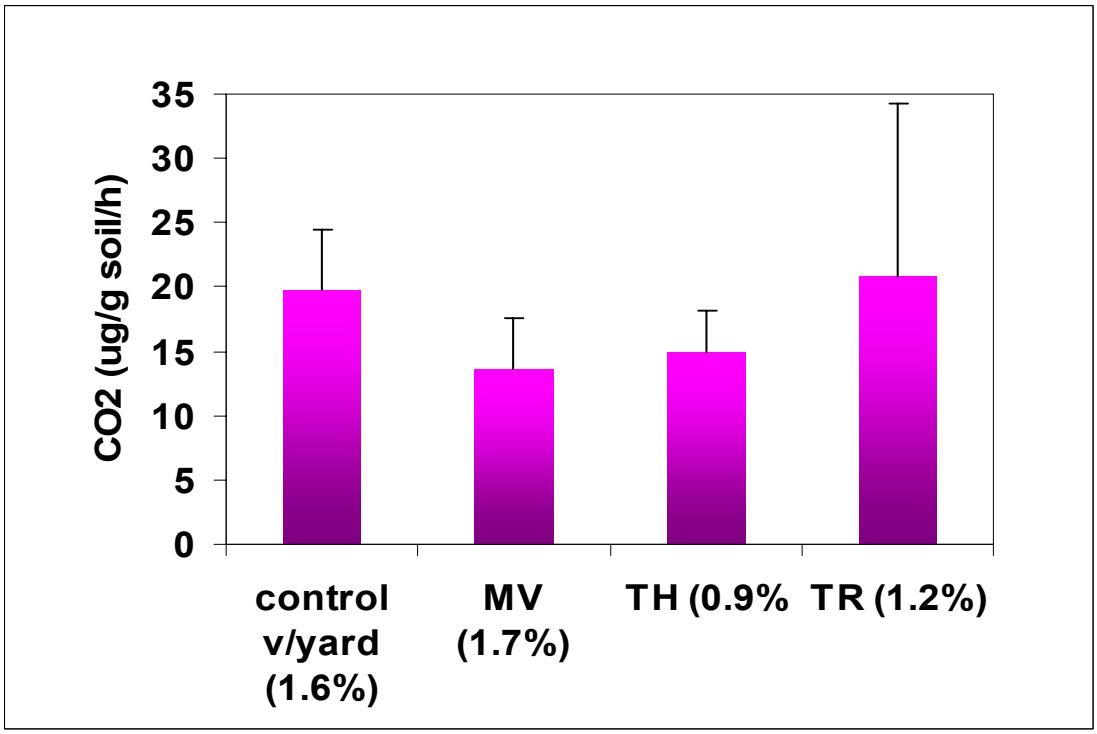
Figures in parenthesis represent TOC (%)

Figure 6.8 Substrate induced respiration in the pasture soils irrigated long-term (100 years) with winery wastewater



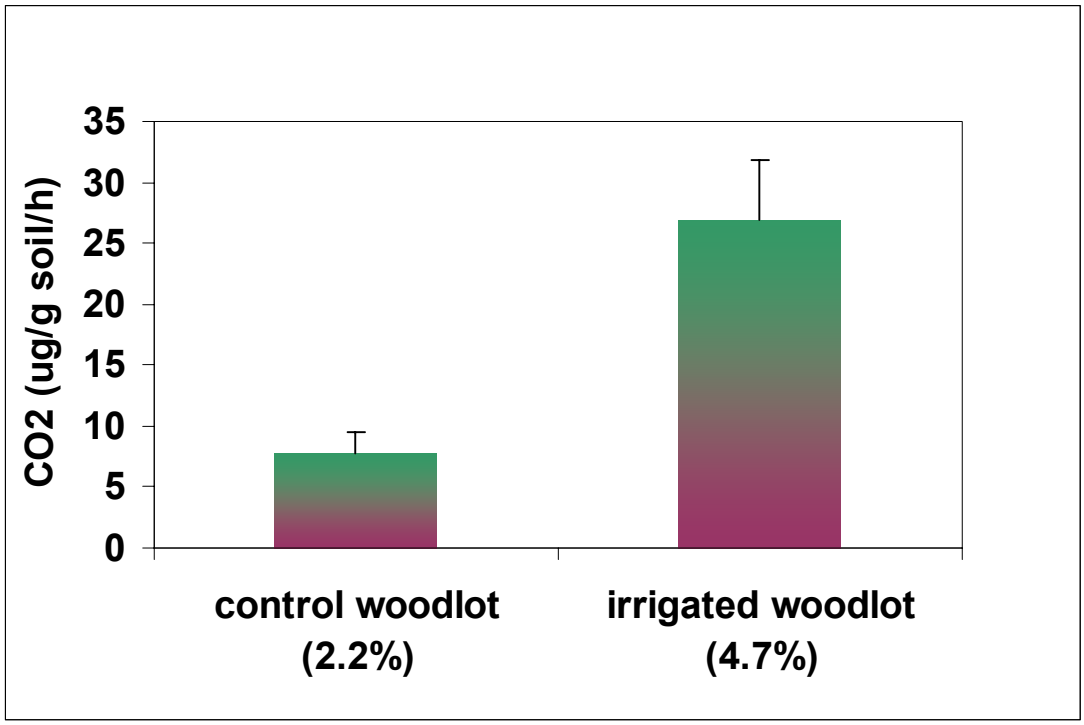
Figures in parenthesis represent TOC (%)

Figure 6.9 Substrate induced respiration in the pasture soils irrigated with winery wastewater



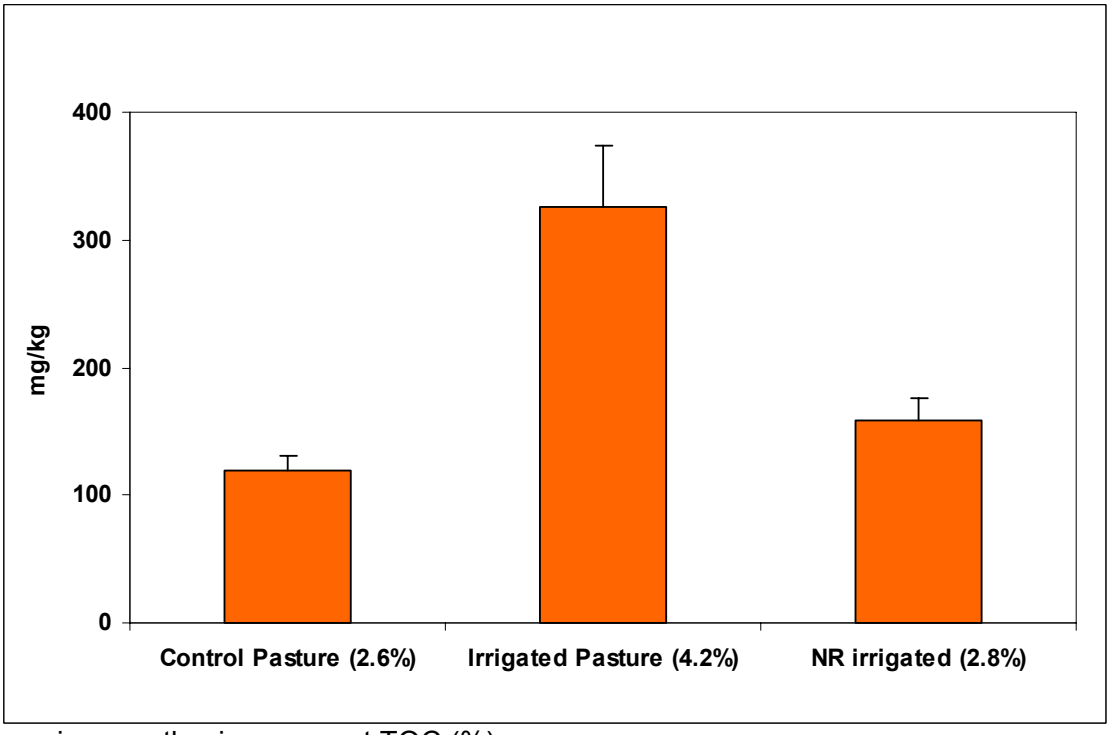
Figures in parenthesis represent TOC (%)

Figure 6.10 Substrate induced respiration in the vineyard soils irrigated with winery wastewater



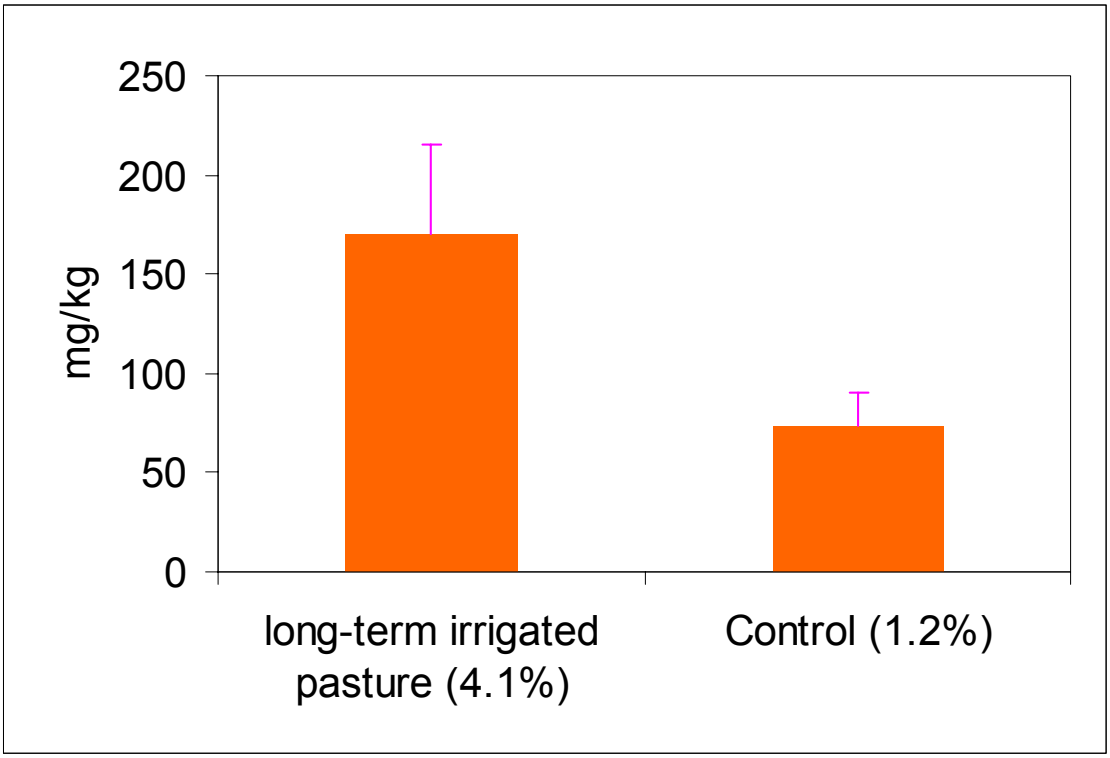
Figures in parenthesis represent TOC (%)

Figure 6.11 Substrate induced respiration in the woodlot soils irrigated with winery wastewater



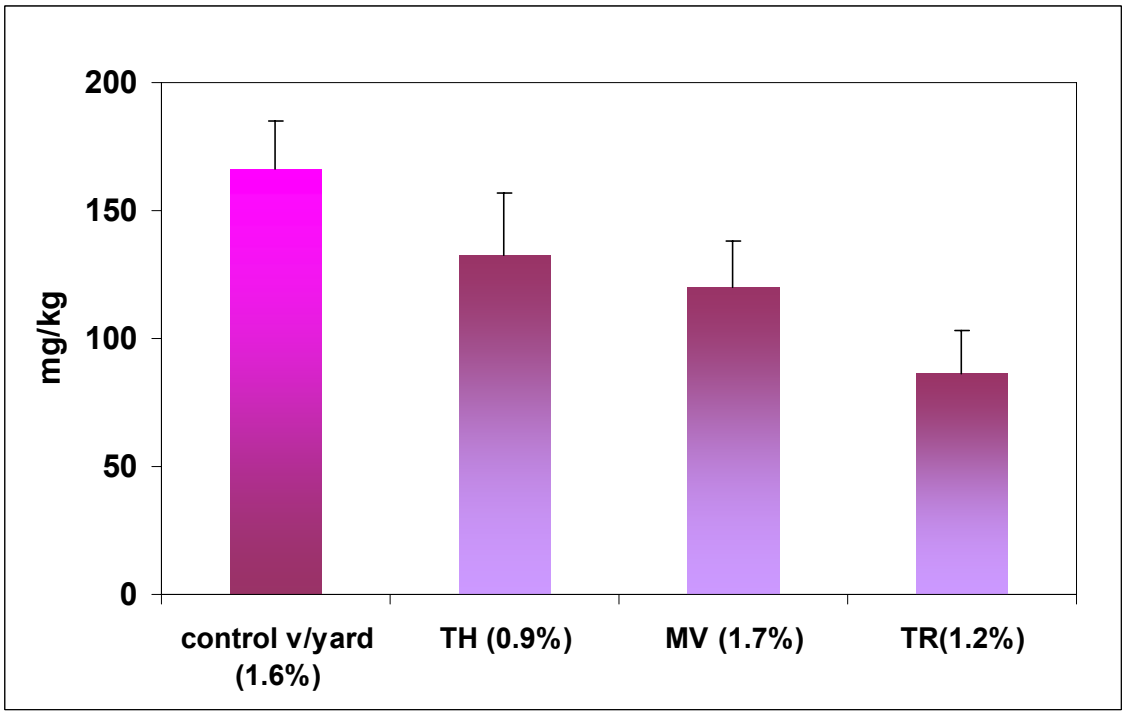
Figures in parenthesis represent TOC (%)

Figure 6.12 Substrate induced nitrification in the pasture soils irrigated with winery wastewater



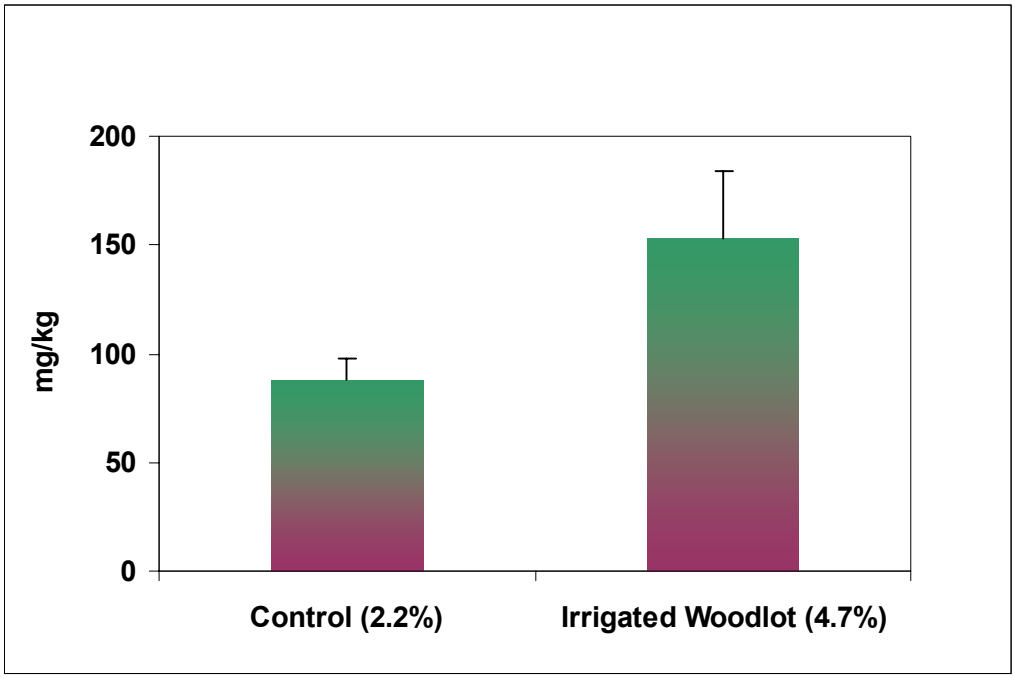
Figures in parenthesis represent TOC (%)

Figure 6.13 Substrate induced nitrification in the pasture soils irrigated long-term (100 years) with winery wastewater



Figures in parenthesis represent TOC (%)

Figure 6.14 Substrate induced nitrification in the vineyard soils irrigated with winery wastewater



Figures in parenthesis represent TOC (%)

Figure 6.15 Substrate induced nitrification in the woodlot soils irrigated with winery wastewater

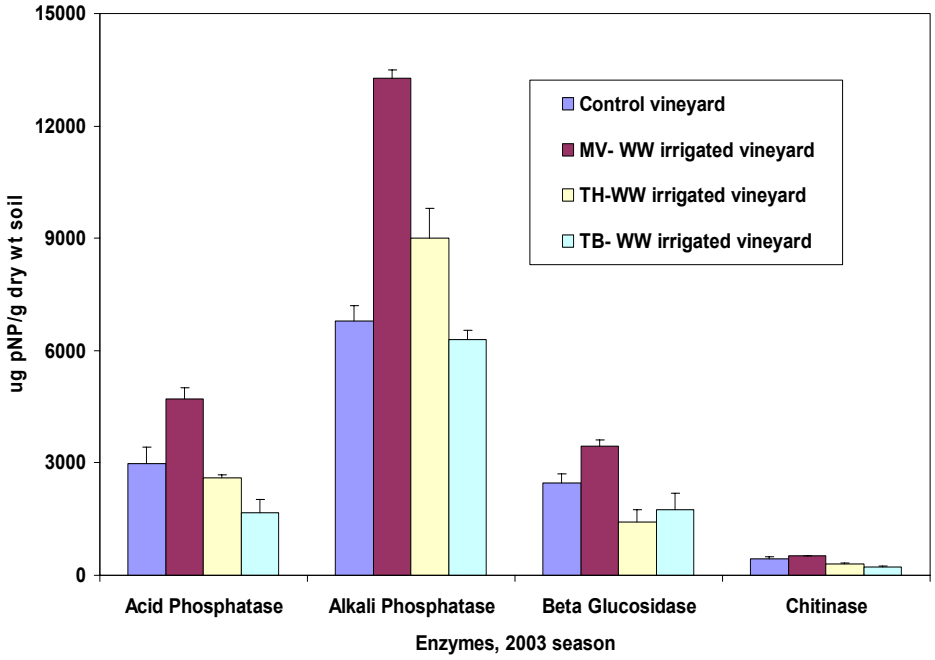


Figure 6.16 Response of microbial enzymes in winery wastewater irrigated vineyard soils sampled in 2003

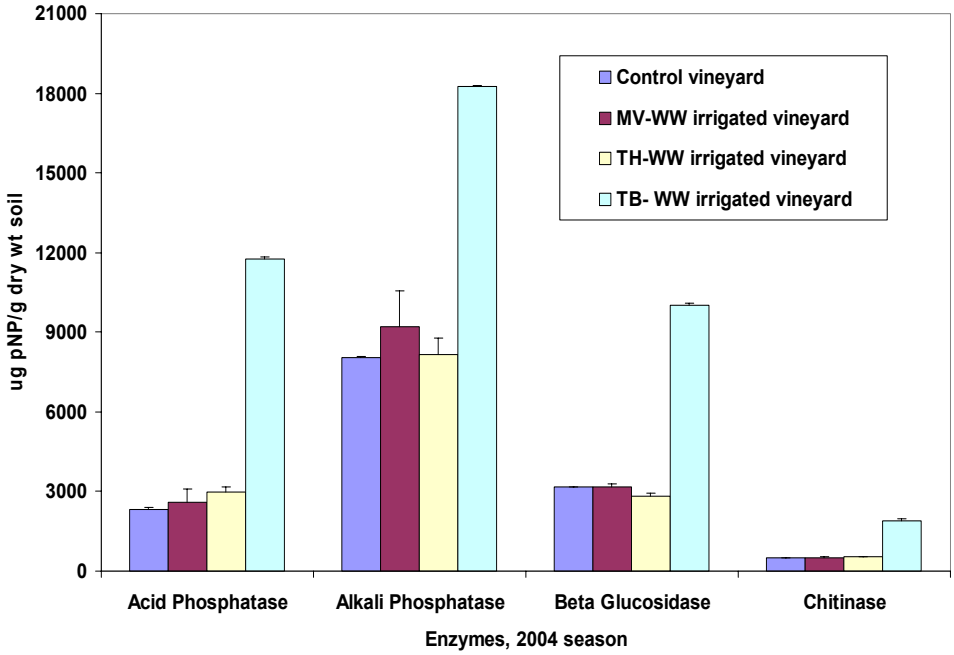


Figure 6.17 response of microbial enzymes in winery wastewater irrigated vineyard soils sampled in 2004

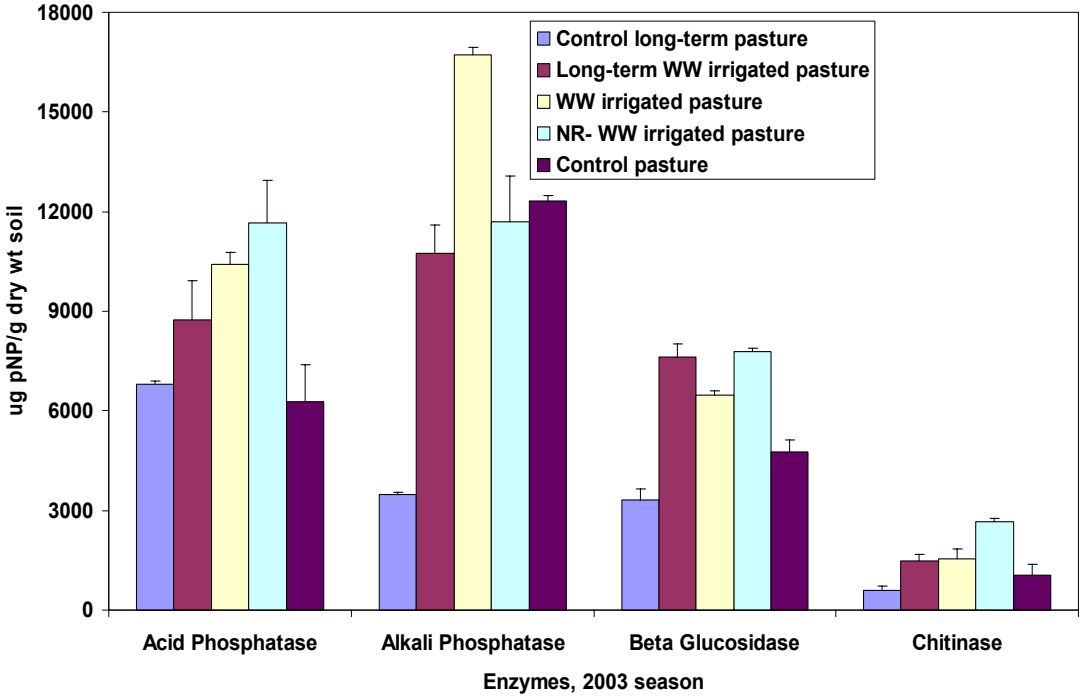


Figure 6.18 Response of microbial enzymes in irrigated pasture soils sampled in 2003

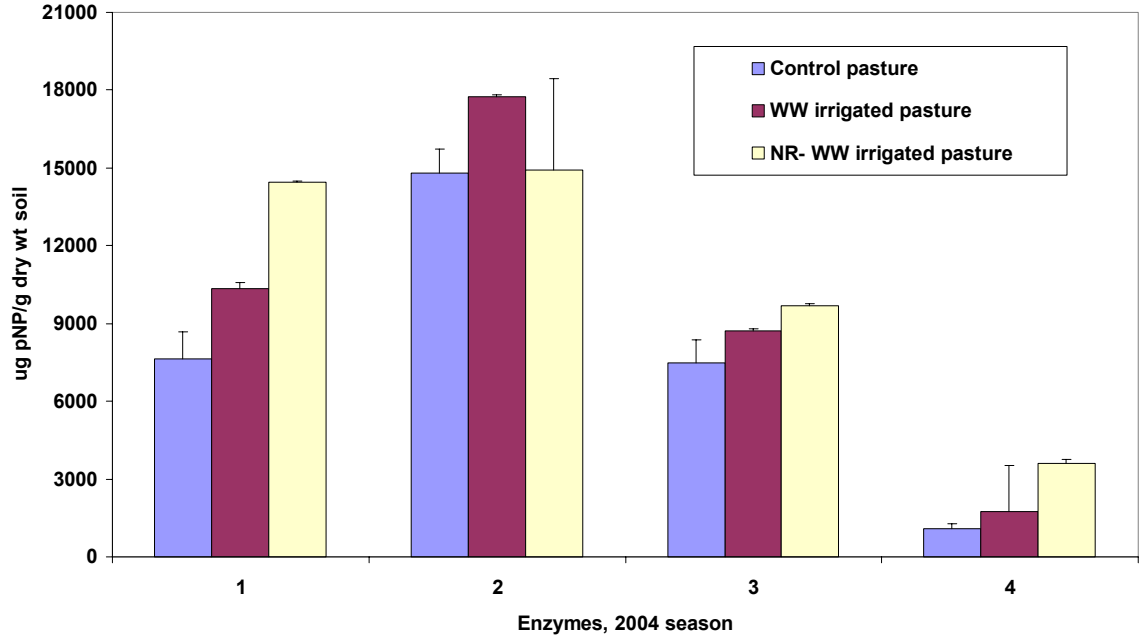


Figure 6.19 Response of the microbial enzymes in irrigated pasture soils sampled in 2004

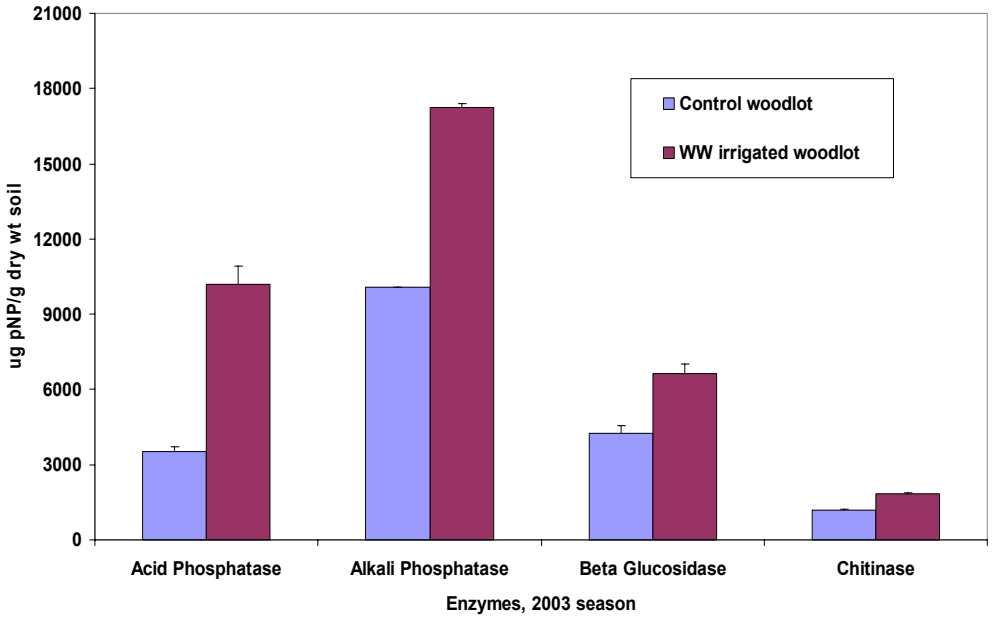


Figure 6.20 Response of the microbial enzymes in irrigated woodlot soils sampled in 2003

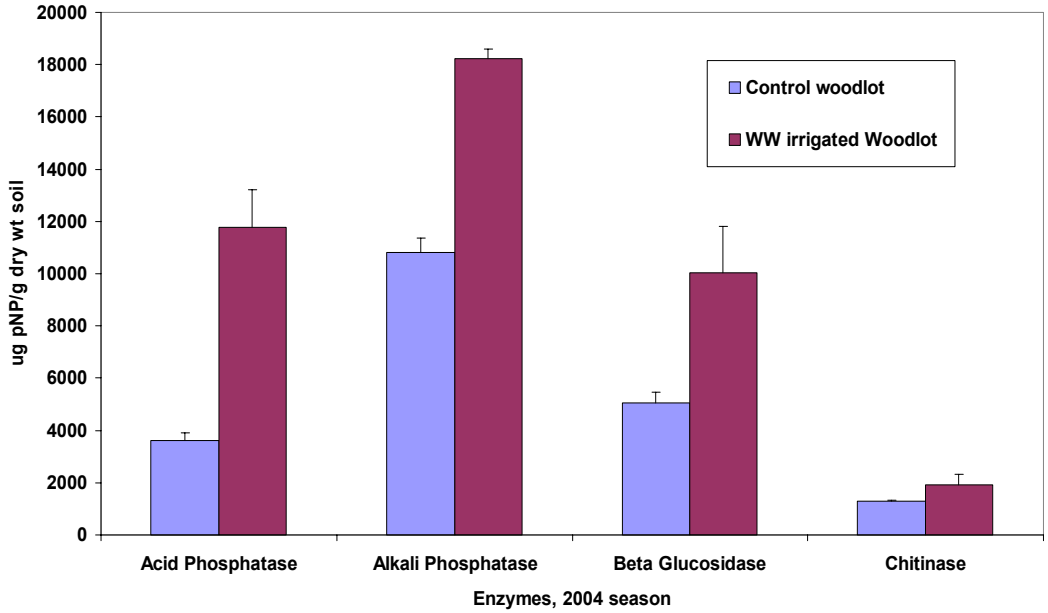


Figure 6.21 Response of the microbial enzymes in irrigated woodlot soils sampled in 2004

6.3.2 Trend of soil physico-chemical properties on sites irrigated with wastewater

In pastures receiving winery wastewater for more than 100 years, there was a significant increase in levels of total organic carbon and nitrogen, compared to the corresponding controls (Figures 6.22 and 6.23). There was progressive increase in levels of specific ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) at the long-term irrigated pastures. The pastures that had received winery wastewater for only 15-20 years did show a subtle increase in the available ions but the increase was not as evident as for 100 years irrigated pastures (Figures 6.24 – 6.25). An elevated SAR was also noted in the winery wastewater irrigated pastures as compared to the control plots (Figure 6.26).

Organic carbon content and total nitrogen in the soils from vineyard sites irrigated with winery wastewater did not differ from that of control vineyards during 2003 and 2004 sampling periods (Figures 6.27 - 6.28). Elevated SAR was only noted in winery wastewater irrigated pastures during 2003 sampling (Figure 6.29). Available Ca^{2+} and Mg^{2+} ions also did not differ significantly from that of control vineyards. However, available sodium and potassium ions were found to be significantly higher at TH, MV and TB vineyard sites than in comparison to the levels measured in the control vineyard soils during 2003 sampling (Figures 6.30) In

2004, the levels of available Na^+ and K^+ were not found do be as high as observed during the 2003 sampling for TH and TB vineyard sites (Figure 6.31).

Woodlots at winery Y has over the years received probably the greatest volume of wastewater, which was reflected in its properties, especially available Na^+ and K^+ levels (Table 6.3). However, SAR values did not show any significant increase over the years (Table 6.3).

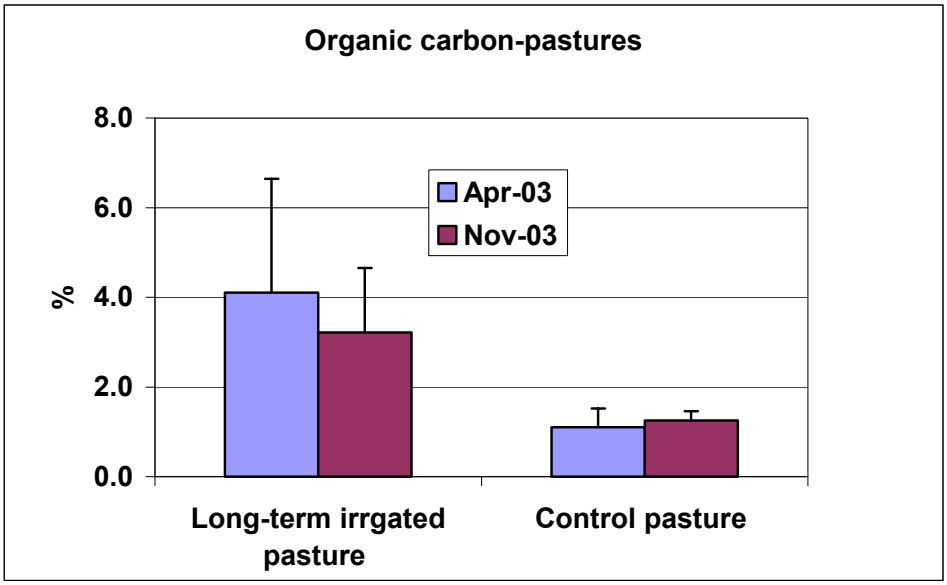


Figure 6.22 Total organic carbon content in the long-term winery wastewater irrigated pasture soils

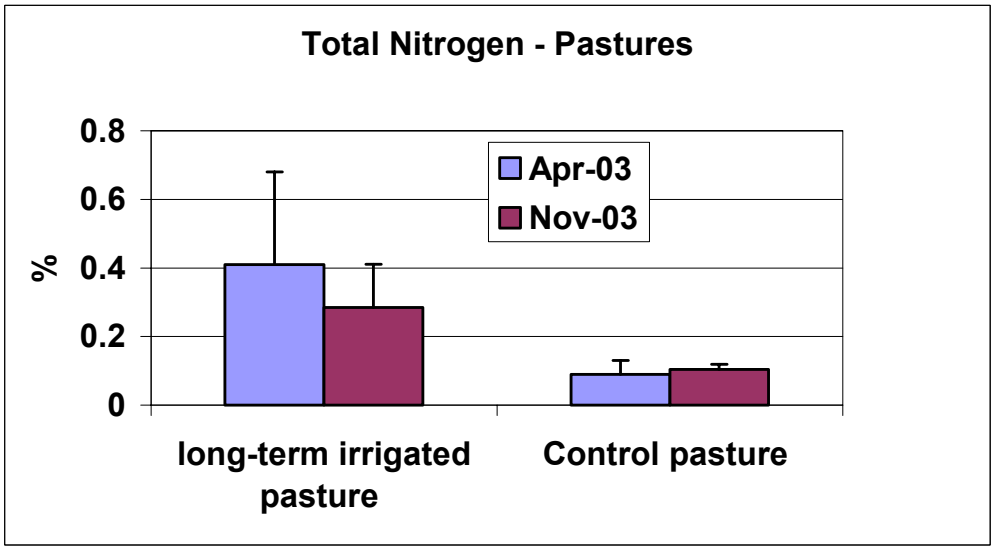


Figure 6.23 Total nitrogen content in the long-term winery wastewater irrigated pasture soils

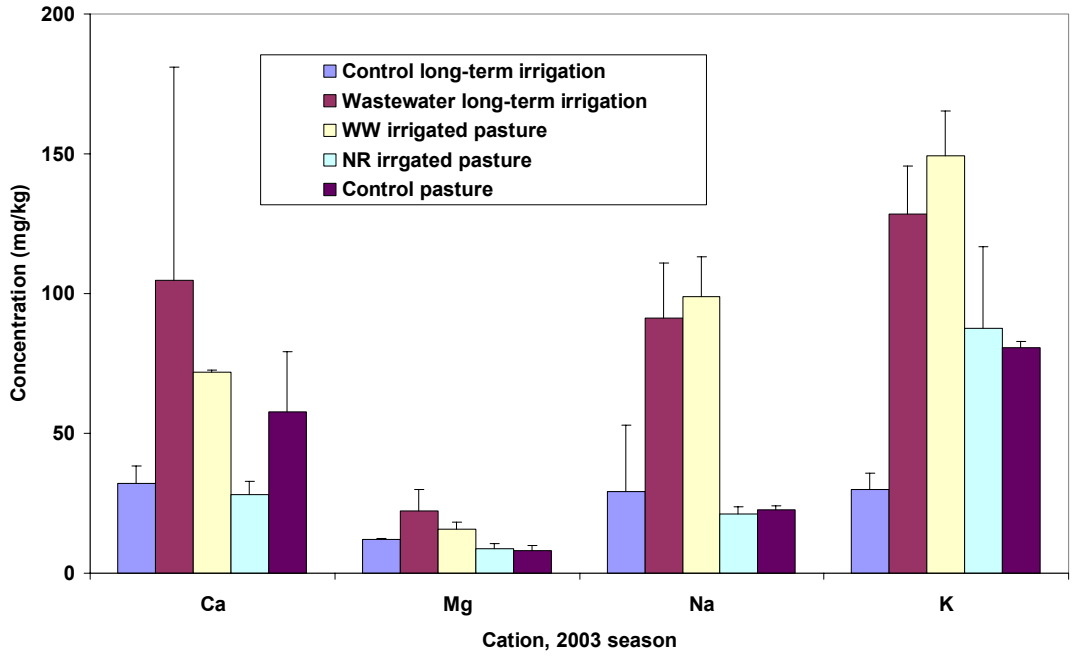


Figure 6.24 Available calcium, magnesium, sodium and potassium in pasture soils irrigated with winery wastewater during 2003 monitoring

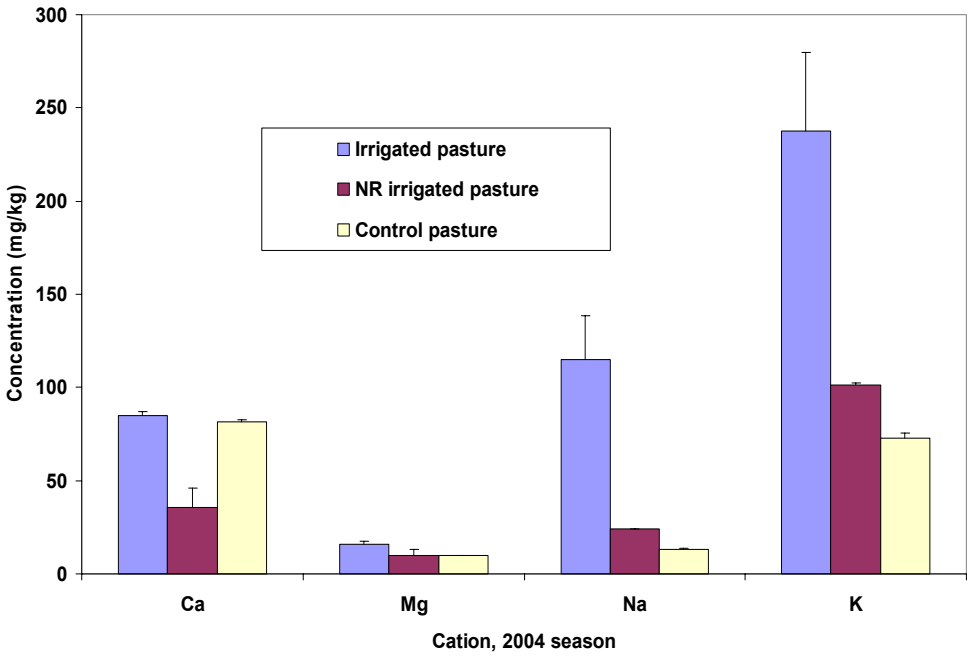


Figure 6.25 Available calcium, magnesium, sodium and potassium in pasture soils irrigated with winery wastewater during 2004 monitoring

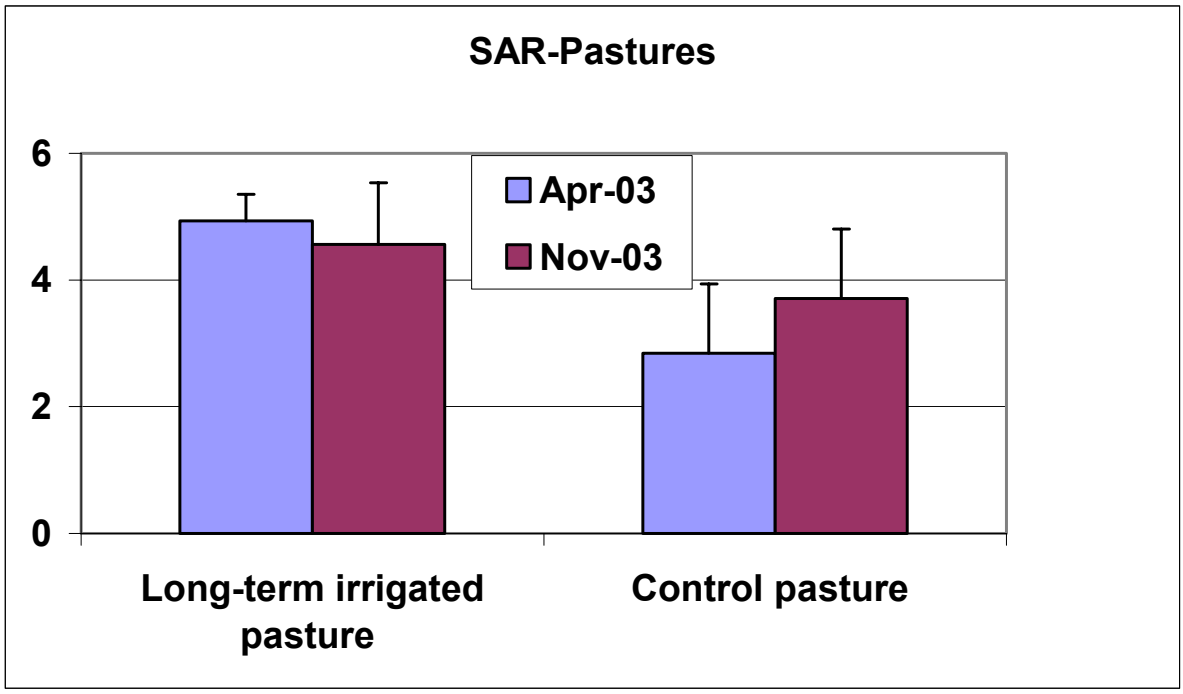


Figure 6.26 Calculated sodium adsorption ratio (SAR) in long-term winery wastewater irrigated pasture soils

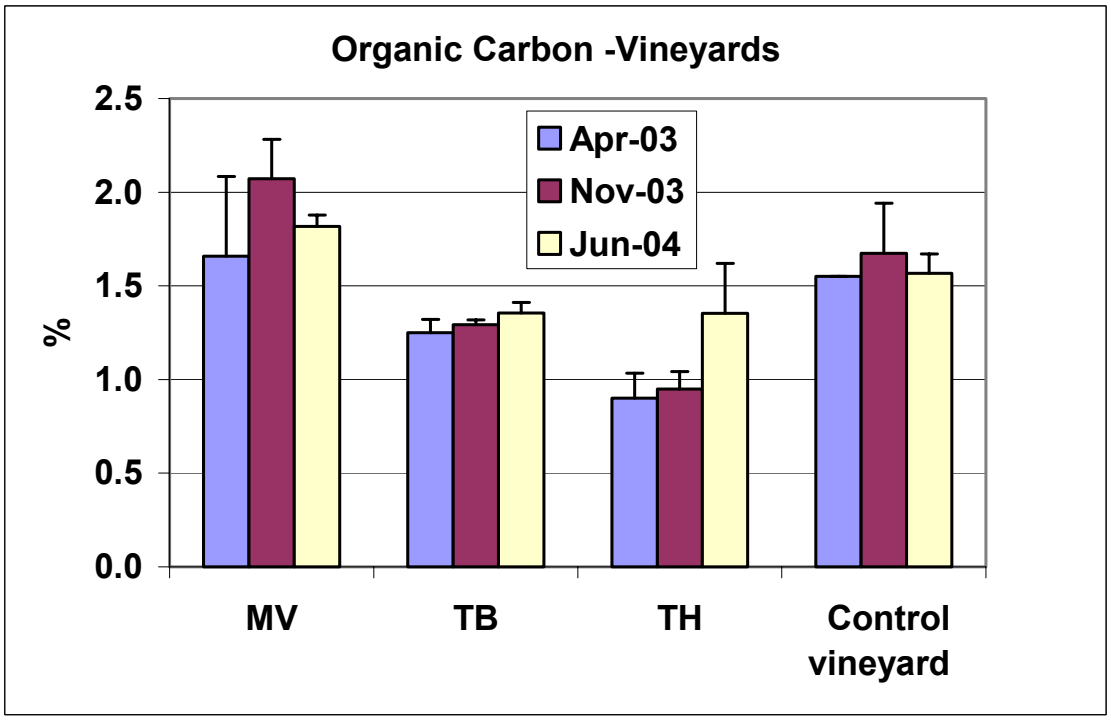


Figure 6.27 Total organic carbon content in vineyard soils irrigated with winery wastewater

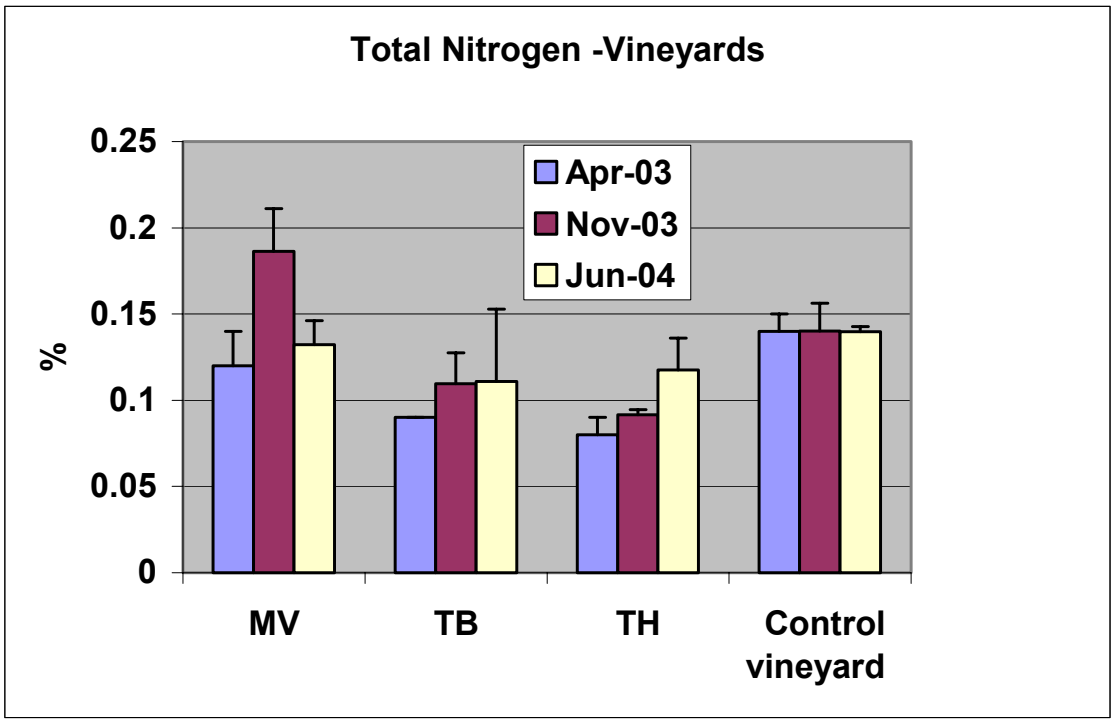


Figure 6.28 Total nitrogen content in vineyard soils irrigated with winery wastewater

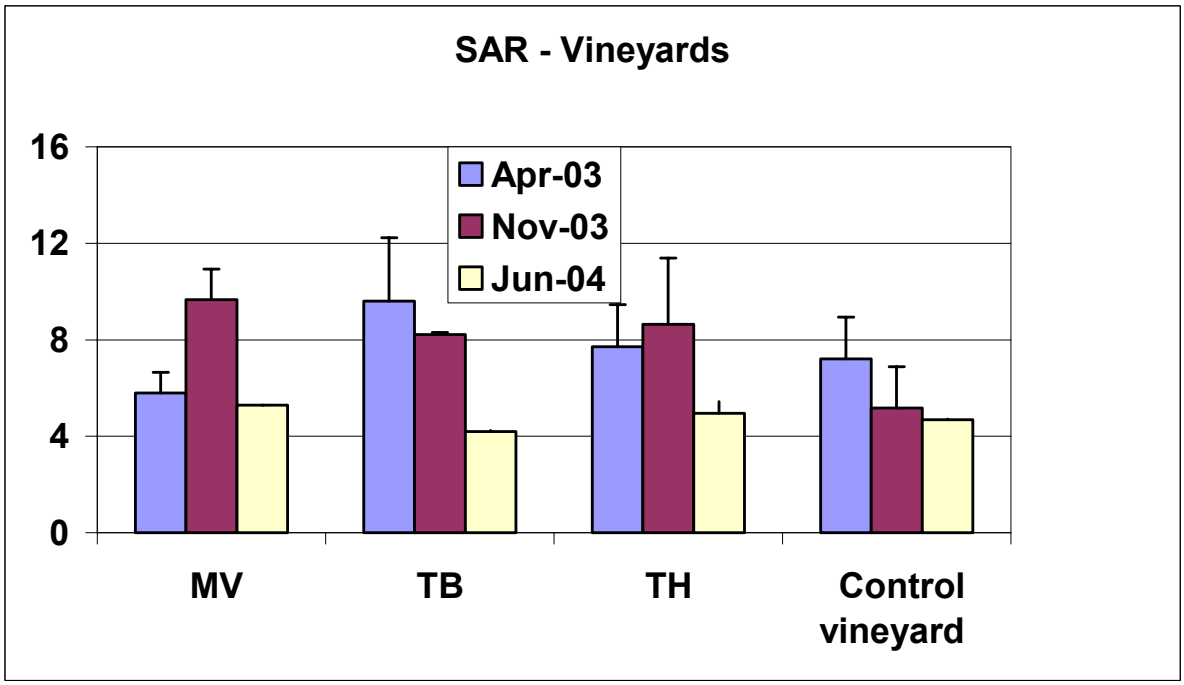


Figure 6.29 Calculated sodium adsorption ratio (SAR) in vineyard soils irrigated with winery wastewater

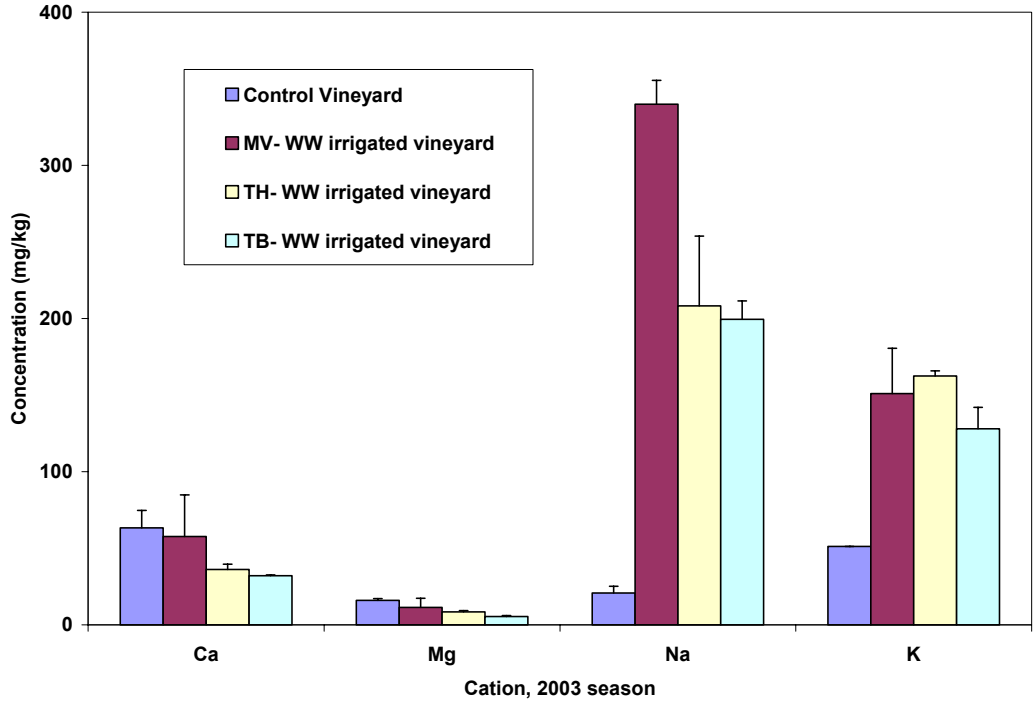


Figure 6.30 Available calcium, magnesium, sodium and potassium in vineyard soils irrigated with winery wastewater during the year 2003 monitoring

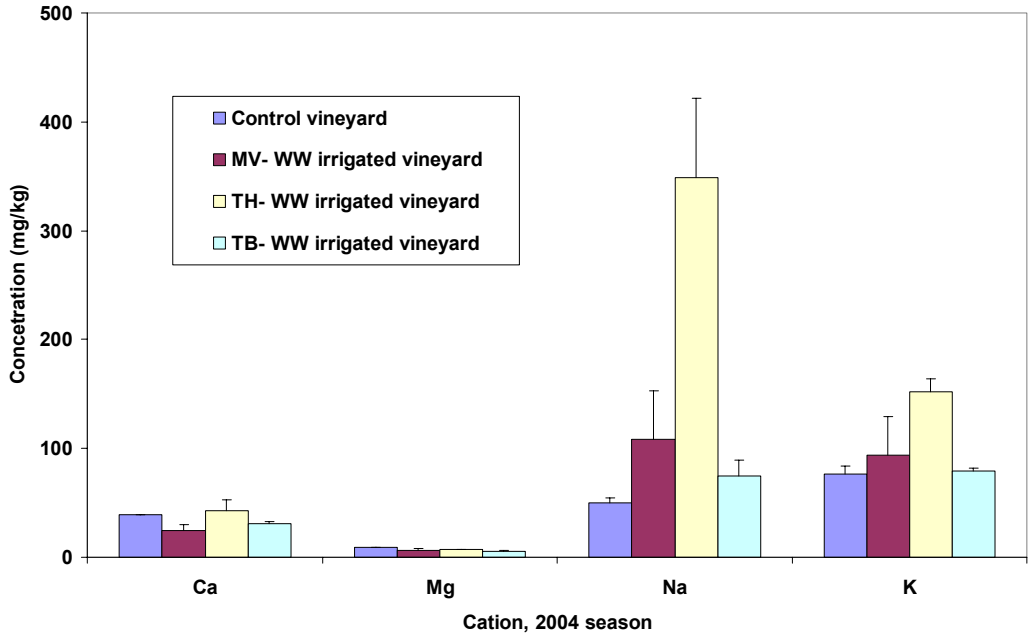


Figure 6.31 Available calcium, magnesium, sodium and potassium in vineyard soils irrigated with winery wastewater during the year 2004 monitoring.

Table 6.3 Comparison of soil chemistry data for woodlot receiving wastewater at winery Y

Woodlot at winery Y	Depth	EC 1:5	pH	Avail P	Avail K	Org C	Soluble Na	Soluble K	SAR	Dispersion Index
Sampling year	Cm	mS/cm	1:5 H2O	mg/kg	mg/kg	%	mg/kg	mg/kg	%	
May 2003	0-30	NA	NA	NA	NA	NA	NA	NA	NA	NA
	30-45	0.38	8.5	171	1,466	2.41	NA	NA	2.6	NA
	45-60	0.60	8.4	76	1,547	1.38	NA	NA	3.2	NA
	60-90	0.61	8.6	35	675	0.93	NA	NA	3.8	NA
Nov 2003	0-30	0.41	8.83	184	1,395	3.03	276	275	2.1	10
	30-60	0.43	9.09	114	1,780	1.51	416	502	2.0	15
	60-90	0.88	8.85	59	1,765	1.10	952	267	4.4	16
July 2004	0-30	0.36	8.9	168	1,495	2.64	325	275	1.9	7
	30-60	0.48	9.0	119	1,690	1.48	508	520	2.1	6
	60-90	1.01	8.6	50	1,490	0.99	914	138	6.7	13

NA: Data not available

6.3.3 Salt build-up at various sites due to winery wastewater irrigation

More than 100 years of application of winery wastewater at the pasture site of winery K was responsible for the highest build-up of salts and organic carbon at this site. Being a small winery, data are not available on the amount of winery wastewater applied over the years at this site. Currently SA EPA recommends monitoring of soil quality when the wastewater application rate exceeds 100 millimetres per year.

Based on the irrigation volume records available from winery Y, the total volume of wastewater discharged was highest (484 – 614 mm/year) in the woodlot at winery Y (*Eucalyptus* sp.) followed by vineyards (MV and TH). The higher application of wastewater per unit area and time in the woodlot and some vineyards (MV and TB) had relatively greater impact on the soil properties in these blocks. Obviously, not only the volume but also the quality of wastewater applied is an important factor. As shown in Table 6.4, the winery wastewater quality used for irrigation had higher BOD, COD and TOC in the year 2003 than in 2004. This could be responsible for higher build-up of salts at some sites in 2003.

In the case of the woodlot (*Eucalyptus* sp.), the months during which the amounts of water applied were in excess of water requirements (surplus) were April to August. For most vineyards, the surplus was generally much smaller but for longer period, from April to October. This has implications for salt leaching as well as a potential impact on groundwater.

Table 6.4 Summary of wastewater quality data during 2002-03 and 2003-04 seasons (post sand filter)

	2002-03	2002-03	2003-04	2003-04
Parameter	**Median**	**S.D.**	**Median**	**S.D.**
pH	6.0	0.50	6.5	0.78
EC (uS/cm)	2950	609	3400	1046
BOD (mg/L)	4450	1863	3665	1355
COD (mg/L)	7045	2512	5900	2044
TOC (mg/L)	2030	977	1740	475
SAR	8.3	4.27	8.42	2.76

6.4 Conclusions

1. Higher organic carbon content of the winery wastewater resulted in increased total organic carbon content in the soils irrigated with winery wastewater.
2. Soil microbiological activity was not adversely affected in the wastewater treated plots. Greater microbial activity was observed in wastewater treated plots, most likely due to the build up of organic carbon content. However, indirect effects due to excessive water application leading to structural decline and prolonged waterlogging conditions were not tested.
3. Salinity, sodicity and available potassium in soils, were noted to be elevated in the wastewater treated plots, especially woodlot and pasture sites, in comparison with the control plots.
4. Currently, very little information exists on the loads of salts that different soil types can tolerate before ecological effects could be observed. Therefore, information on the tolerance of different soil types to winery wastewater in terms of adverse soil biological functions and/or soil chemistry parameters is urgently required.
5. There is very limited information available on the toxicity of winery wastewater components to terrestrial organisms. It is important to obtain this information, when land disposal of winery wastewater is becoming a more common practice.

7. IMPACT OF WINERY WASTEWATER IRRIGATION ON SOIL MICROBIOLOGICAL HEALTH AND SOIL CHEMISTRY: LABORATORY EXPERIMENTS WITH DIFFERENT SOIL TYPES

7.1 Introduction

As discussed earlier in this report, data on indicators of soil microbiological health from field sites, that had received wastewater irrigation for varying time periods, showed little adverse impact. However, there was some evidence of salt accumulation in some cases and a build up of potassium in the soil profile.

To investigate the tolerance of different soil types to winery wastewater in terms of adverse soil biological functions and or soil chemistry parameters, laboratory experiments were conducted on three different soil types with contrasting physico-chemical characteristics.

The specific objectives of these experiments were to:

1. assess the response of soil microbiological indicators to varying volumes of wastewater irrigation to different soil types;
2. assess the build up of inorganic ions in soil and leaching through the soil columns; and
3. observe the deterioration of infiltration behaviour of soil (if any) after application of varying volumes of wastewater.

To achieve these objectives, soil column experiments were conducted under laboratory conditions on three contrasting soils collected from wine growing regions in South Australia. All three soils are currently receiving irrigations with winery wastewater.

The intention of these experiments was not to represent a particular site with a soil type but more importantly to see if different soil types (texture and not structure; as well as other soil properties) would respond differently to wastewater irrigation in terms of soil biological or chemical indicators. Therefore, in these studies, repacked soil columns were used instead of intact soil cores. Soil heterogeneity and spot specific soil structural variations make it difficult to represent a particular soil type with an intact core.

It should also be noted that deliberately higher volumes of wastewater were applied to assess the response of soils to wastewater in a shorter time period. Clearly the irrigation regimes used here do not necessarily represent common practices.

7.2 Materials and Methods

7.2.1 Soils

Three soils of contrasting texture (a loamy sand, a loam and a clayey soil) and other properties were collected from the areas where winery wastewater is currently being used for irrigation of woodlots, pastures or vineyards. Composite samples of soil from surface layers (0-15 cm) were collected from the Barossa and McLaren Vale regions. The soils were air dried ground and sieved through a 2 mm sieve before use in the experiment. The physico-chemical properties of the three soils prepared in this way are presented in Table 7.1.

Table 7.1 Physico-chemical properties of the three soils used in column experiments

Soil	pH[1]	EC[1] dS/m	Clay (%)	Sand (%)	OC (%)	K[2] (mg/kg)	NH_4-N[3] (mg/kg)	NO_3-N[3] (mg/kg)	CO_3 as $CaCO_3$ %
Loamy sand	5.83	0.14	8	85	1.98	209	2.1	14.9	<0.5
Clay Loam	8.04	0.19	27	54	2.5	732	7.7	4.1	9.6
Clay	8.89	0.35	51	36	2.45	665	4.3	7.9	2.3

[1] 1:5 water extraction
[2] Bicarbonate extraction method
[3] KCl extraction method

7.2.2 Columns set-up

Thirty six PVC columns of 30 cm length and 5 cm diameter were used for each soil. The bases of columns were capped with a perforated cap and a nylon mesh was placed at the bottom of column to stop particle loss through percolating water. The columns were placed so that drainage water through the columns could be collected into plastic containers. Soil was packed into each column to a 20 cm thickness to desired bulk density (sand 1.5, loam 1.4 and clay 1.2 g/cm3), leaving a 10 cm section on top to receive winery wastewater irrigation. Containers with a perforated base were placed on top of the columns to deliver the irrigation water slowly.

A schematic diagram of the column set up and photos showing set-up are provided in Figures 7.1 and 7.2.

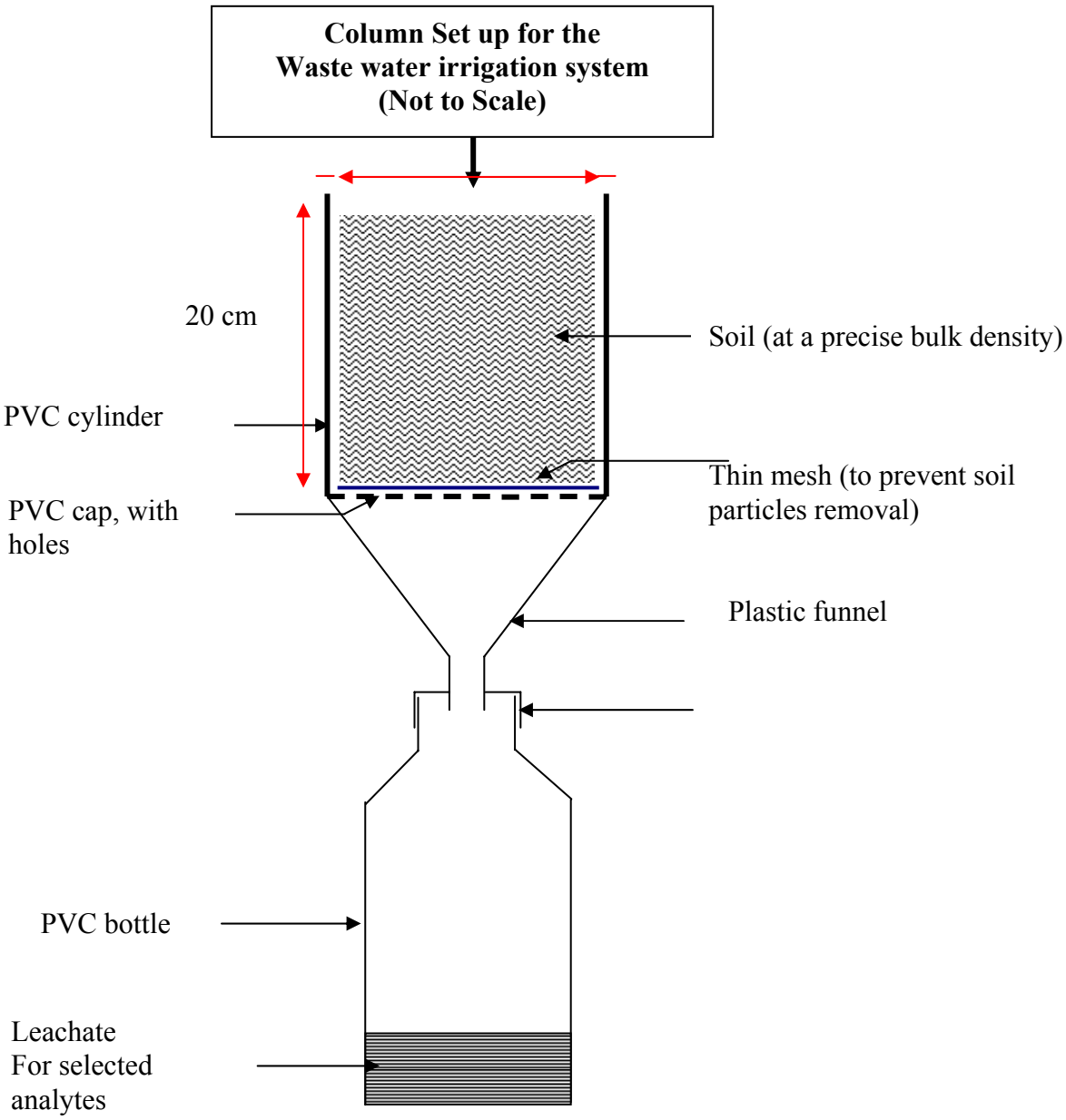


Figure 7.1 Schematic diagram showing the column set-up used in the wastewater irrigation experiment

Figure 7.2 Photos of the column experiment in the laboratory.

7.2.3 Irrigation Regime

Winery wastewater was obtained from a local winery F on a fortnightly basis, stored in a cold room and used for irrigation of soils in the columns. Twice a week irrigations were applied, with a volume equivalent to about 50 mm of water. This continued every week for 16 weeks or as long as the soil allowed infiltration of the applied amount. In the case of the clay soil this could not continue due to poor infiltration. For control columns, the soil was irrigated with water which had undergone reverse osmosis.

The experiment was run in triplicate. Every sampling time (0, 3, 14, 28, 56 and 112 days after treatment) three control and three treated columns were sacrificed. The soils were analysed for soil biological and chemical properties. The leachate was collected and analysed at certain times.

Table 7.2 Volume of wastewater used with time in the soil columns

Days after treatment commenced	Amount of wastewater applied to Loam and Loamy sand (mm)	Amount of wastewater applied to clay soil (mm)
3	51	51
14	152	127
28	305	178
56	458	229
112	509	-

7.2.4 Measurements of soil properties:

7.2.4.1 Soil microbiological indicators

To assess soil biological health, several indicator assays were carried out after each treatment. Table 6.2 shows what soil biological function each indicator represented.

Soils were incubated at fixed moisture content for a week to revive the biological activity in soil and to remove any temporal variations in moisture content. Thus the soil samples collected at different times from the field had similar conditions prior to microbiological assessment.

7.2.4.2 Soil chemical properties

To assess the changes in soil chemical properties due to wastewater irrigation, the soils were analysed for multi-elemental species by using ICP-MS. The drainage water was also analysed at certain times using the same technique. These analyses allowed an assessment of build up of salts or changes of other chemical properties.

The measurements allowed calculation of Sodium Adsorption Ratio (SAR), available K, total dissolved organic carbon and general elemental composition.

7.2.4.3 Soil physical properties

Only soil water infiltration to the column was examined during the study by measuring the rate at which the applied amount infiltrated into the soil column. For this the water level in the soil column was logged against time to calculate the infiltration rate. This was not a thorough test of soil hydraulic properties but merely an indicative parameter for soil permeability.

7.3 Results and Discussion

7.3.1 Characteristics of wastewater used for irrigation of soil columns

The characteristics of wastewater were determined as the batches of wastewaters were received and were noted to show significant temporal variations. The average characteristics of the wastewater used are provided in Table 7.3, together with the standard deviation. The largest variation was noted in pH (varying from 4.5 to 7.2), total carbon (449-748 mg/L) and dissolved organic carbon (DOC) content (360-740 mg/L).

Table 7.3 Characteristics of winery wastewater used for irrigation. Average values with standard deviation are shown for each parameter.

Parameter	Values (Mean ± STD)
EC	1.6 ± 0.4 dS/m
pH	5.9 ± 1.4
NH_4 – N	1.9 ± 2.0 mg/L
NO_3-N	<0.02 ± 0.0 mg/L
Total C	583 ± 170 mg/L
DOC	532 ± 198 mg/L
K^+	128 ± 13 mg/L
Na^+	238 ± 62 mg/L
Ca^{++}	78 ± 32 mg/L
Mg^{++}	14 ± 4.2 mg/L
Cu^{++}	0.1 ± 0.04 mg/L
Zn^{++}	0.4 ± 0.23 mg/L

7.3.2 Soil infiltration

The sand and loam soils continued to take winery wastewater for the project duration (Table 7.2). However, in the case of clay soil, the infiltration was very slow and the irrigation regime had to be reduced (Table 7.2). In 8 weeks only 229 mm of water could be applied. After this period the columns were not irrigated and the experiment on clay soil was terminated.

The infiltration rates of wastewater observed for clay and loamy sand soil during the experiment are shown in Table 7.4. As is evident from the data, the clay soils had inherently low permeability which deteriorated significantly with application of wastewater. The decrease in infiltration rate was also observed for the control treatment with RO water. The decrease in soil permeability with the RO water was even greater, due to lower ionic strength of RO water facilitating dispersion of soil aggregates and thus clogging of pores. The repacked nature of columns may have also exacerbated the infiltration problem, due to destruction of structure, especially in clay soil. The sandy soil as expected had a much greater infiltration rate allowing ready intake of wastewater during the experiment.

Table 7.4 Infiltration rates observed in clay and loamy sand soils during the column experiments (after 51 mm of irrigation water)

Soil / condition	Infiltration rate (mm/day)
Loamy sand - wastewater	4500
Loamy sand - RO water	3600
Clay loam - wastewater	15.00
Clay loam - RO water	12.71
Clay soil - wastewater	5.45
Clay soil - RO water	1.67

7.3.3 Soil Microbiological Tests

The impact of winery wastewater on soil microbiological health was assessed by using indicators described in Table 6.2. These included a general microbial

parameter such as respiration as well as specialist functions such as nitrification. A range of other enzymes indicate activities of bacterial and fungal activities. These are discussed below.

The data for two soils after 8 and 16 weeks of irrigation are presented in Figure 7.3. No significant difference in the respiration parameter was found between the control and wastewater treatments. This indicates that wastewater irrigation did not influence the overall microbial activities in the two soils. Similarly the data on nitrification presented in Figure 7.4 also did not show any significant difference between soils irrigated with wastewater and RO water in both sand and clay soils. However, it was noted that with time the overall nitrification activity decreased in both wastewater and control treatment. The reasons for this are unclear and it may be related to continuous irrigation of columns resulting in unfavourable conditions for specialist microorganisms.

The enzyme data for four different enzymes tested are presented in Figures 7.5 and 7.6, for two depths of loam soil. Once again, in general, none of the four enzymes showed any significantly different activities in soil irrigated with wastewater or with clean water. This was true for the surface (0-5 cm) as well as subsurface layers that were tested in this case.

None of the indicators for soil microbiological activities and functions indicated any adverse effect that might be directly attributable to irrigation with wastewater. However, the study did not assess any indirect effects that may occur due to the structural decline or salt build up or waterlogging as a result of irrigation with wastewater. For example, if oxygen levels in soils are depleted due to continuous waterlogging then one can expect significant adverse impact on aerobic microorganisms in soils and gradual shift towards anaerobes.

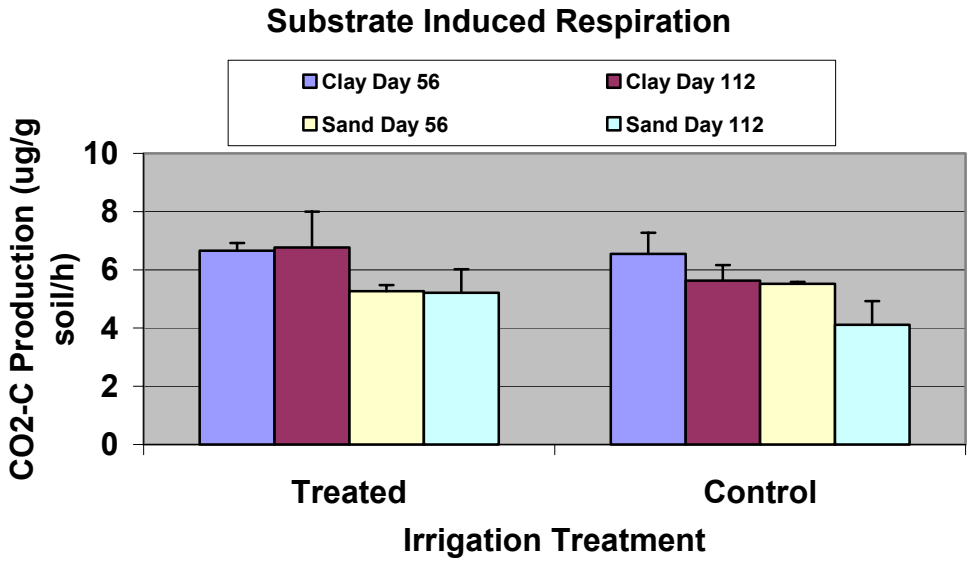


Figure 7.3 Activity of nitrifying bacteria in 0-5 cm layer of loam soil after irrigation with different amounts of winery wastewater. (For amount of wastewater used on different soils, please refer to Table 7.2)

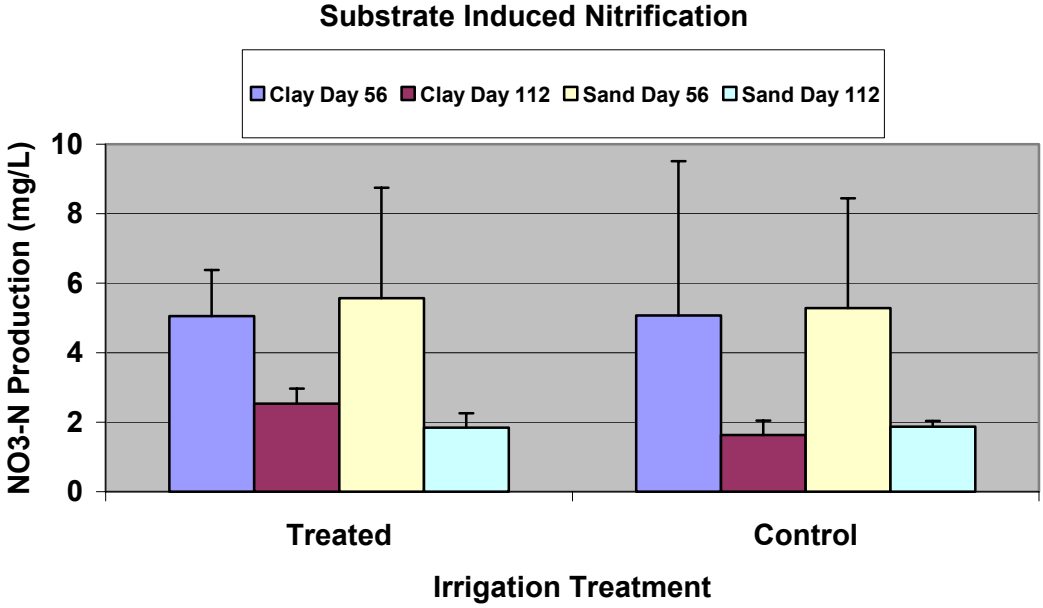


Figure 7.4 Activity of nitrifying bacteria in 0-5 cm layer of loam soil after irrigation with different amounts of winery wastewater. (For amount of wastewater used on different soils, please refer to Table 7.2)

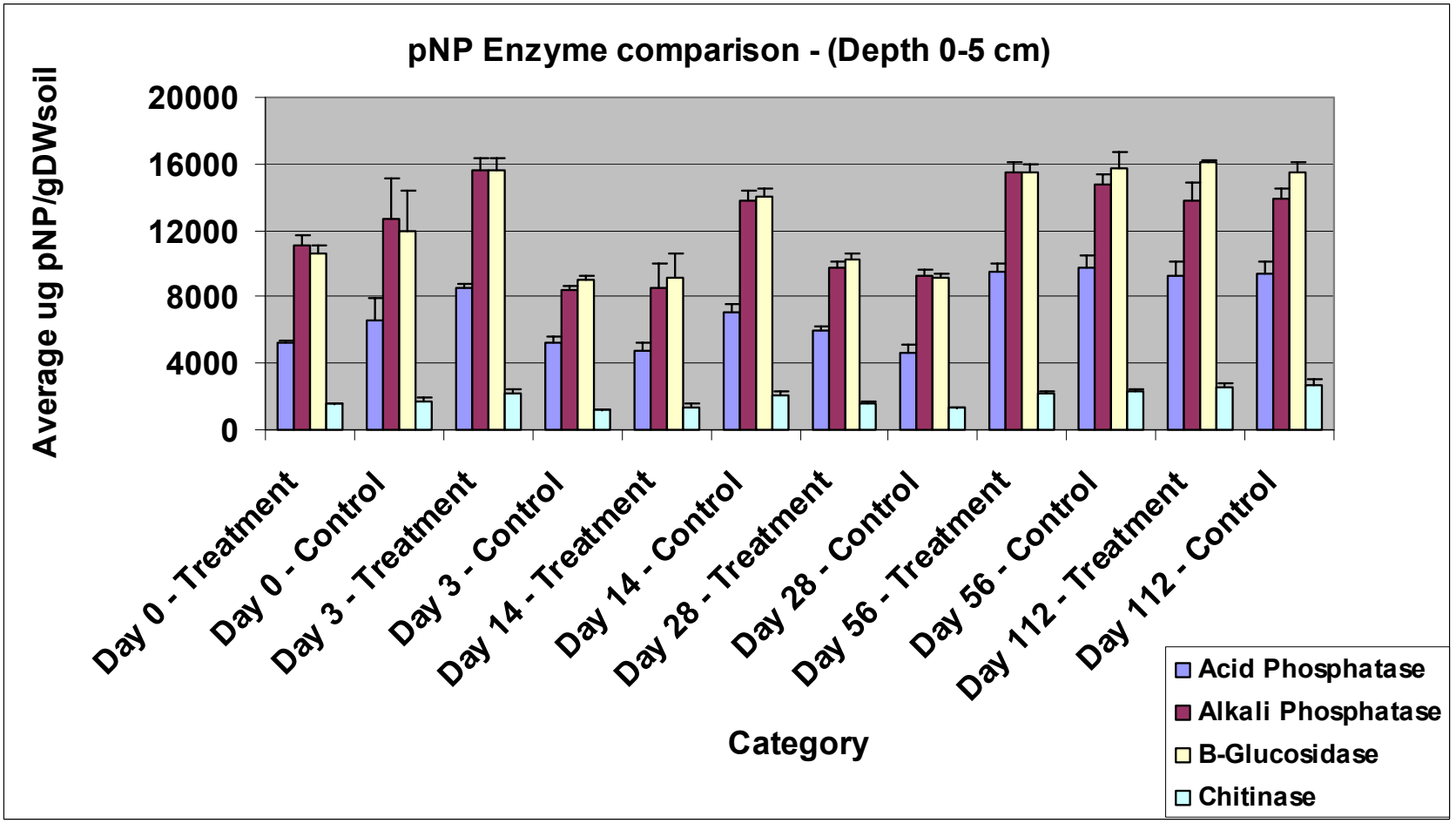


Figure 7.5 Response of four enzymes to different amount of winery wastewater in surface 0-5 cm layer of loam soil

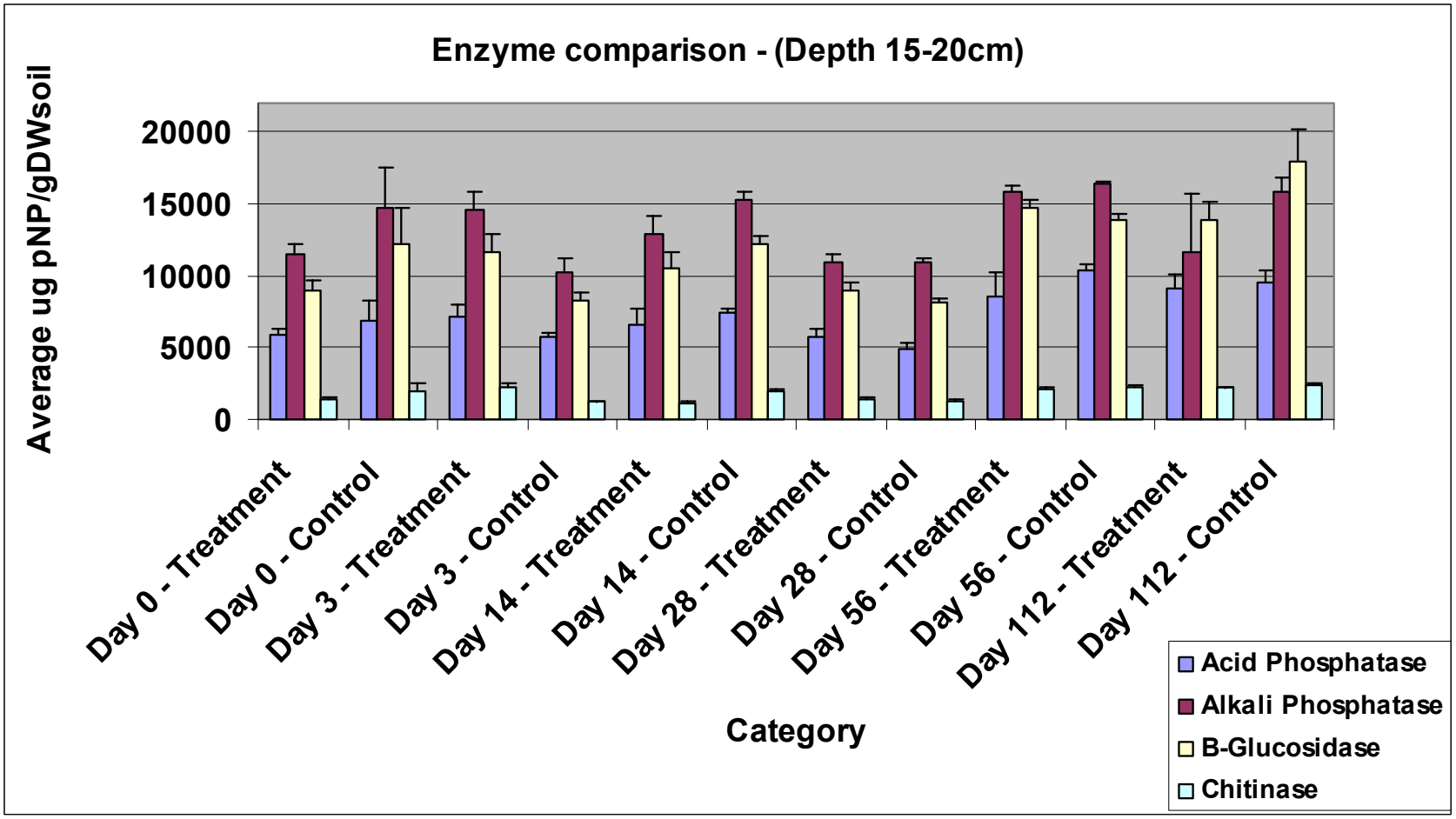


Figure 7.6 Response of four enzymes to different amount of winery wastewater in surface 15-20 cm layer of loam soil

7.3.4 Build up of salts in soil profiles

To examine the changes in soil chemical properties after irrigation with wastewater, soil samples from different depths in soil columns were analysed for a range of inorganic ions. The data on electrical conductivity, soluble salts (measured in 1:5 soil: water extract) such as sodium, calcium, sodium adsorption ratio (measure of relative dominance of sodium over divalent cations i.e. calcium + magnesium) for the entire profile (0-20cm) are presented in Figures 7.7 to 7.11.

The data in these figures show that salt levels increased with irrigation in the soil profile, especially sodium and magnesium in soil and water extract, which showed about a 40 and 90 fold increase respectively. Similarly calcium and potassium levels doubled during the experiment. Data in Table 7.5 show the accumulation of salts in the top (0-5 cm) and bottom (15-20 cm) layers of the profile. It is evident that salt levels increased throughout the profile and were somewhat higher in the surface layer compared to the last 5 cm layer of the soil column.

The data set shows that the greatest impact of winery wastewater irrigation on land is likely to be through the build up of salts, especially sodium and potassium. The build up of monovalent ions in the soil profile can result in deterioration of soil structure and consequently can adversely impact soil productivity. This aspect needs a thorough investigation to clearly establish the threshold of different soil types in tolerating the winery wastewater.

Table 7.5 Changes in salt levels in surface and subsurface layers of loam soils at the end of the irrigation experiment

	Untreated soil 0-5 cm (Day 0)	Untreated soil 15-20 cm (Day 0)	Irrigated soil 0-5 cm (Day 112)	Irrigated soil 15 -20 cm (Day 112)
EC (dS/cm)	0.18	0.18	0.33	0.25
pH	8.07	8.09	8.24	8.45
Na^{+} (mg/L)*	5.2	5.0	204.4	150.4
Ca^{++} (mg/L)*	22.6	22.1	43.3	42.0
Mg^{++} (mg/L)*	2.0	2.1	194.6	144.6
K^{+} (mg/L)*	17.1	17.2	32.2	22.8
Available K^{+} (mg/kg)#	735	697	1308	1139

*in 1:5, soil:water extract
Bicarbonate extract

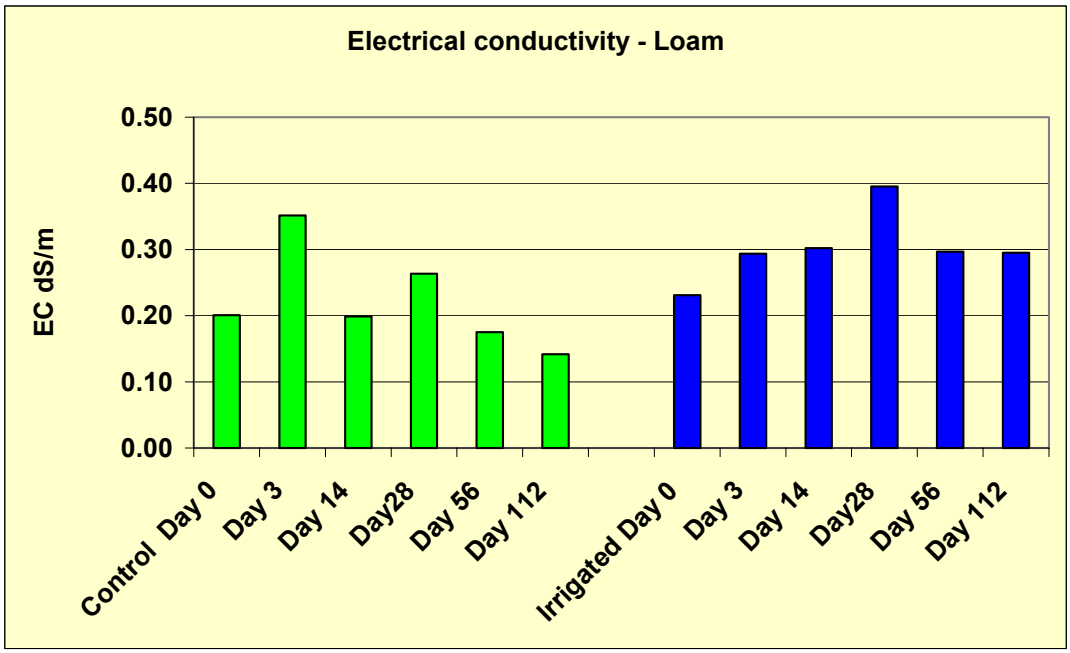


Figure 7.7 Electrical conductivity in loam soil (average value for the whole 20 cm profile) in relation to increasing volume of wastewater irrigation (please refer to table 7.2)

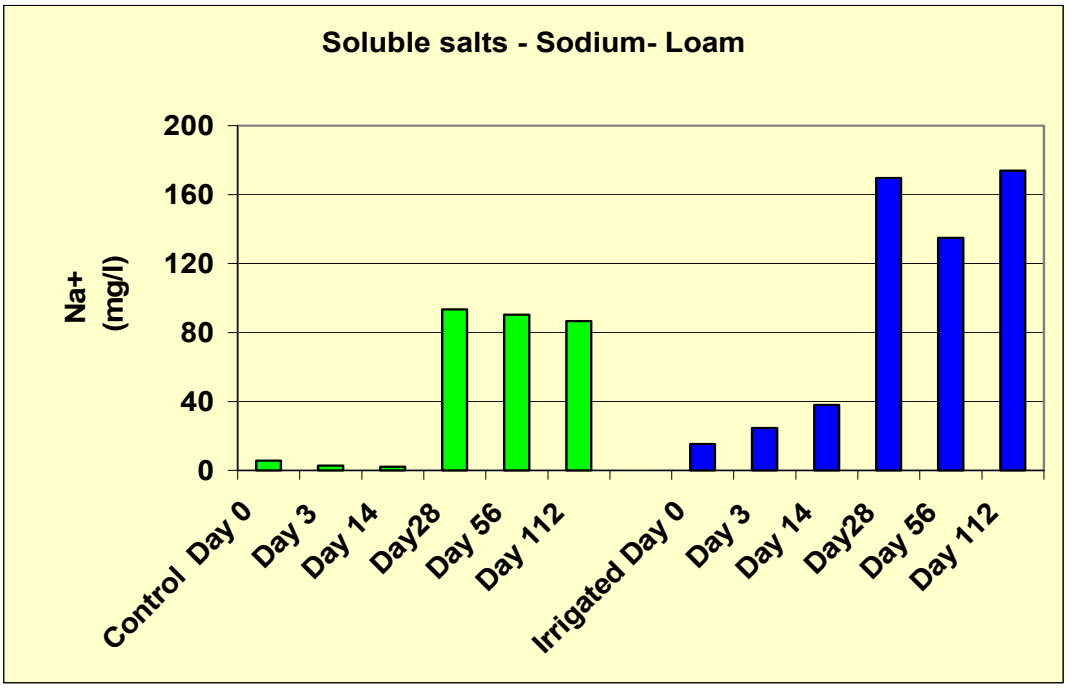


Figure 7.8 Soluble sodium in loam soil (average value in 1:5, soil: water extract for the whole 20 cm profile) in relation to increasing volume of wastewater irrigation (please refer to Table 7.2)

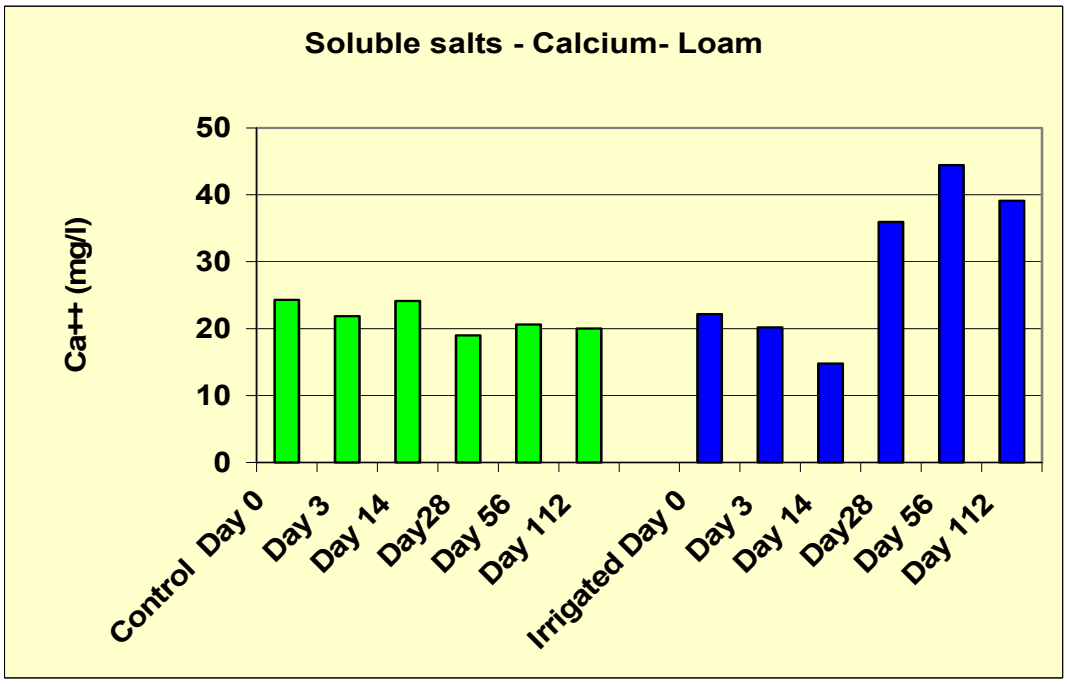


Figure 7.9 Soluble Calcium in loam soil (average value in 1:5, soil: water extract for the whole 20 cm profile) in relation to increasing volume of wastewater irrigation (please refer to Table 7.2)

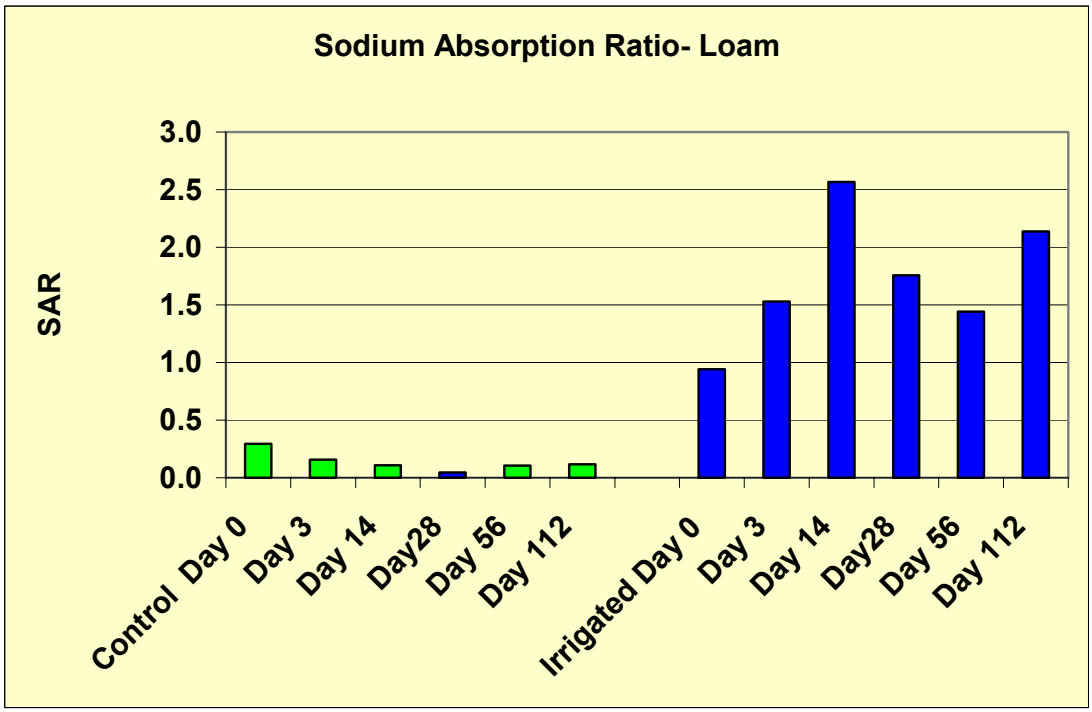


Figure 7.10 Sodium Absorption Ratio- SAR (average value in 1:5, soil: water extract for the whole 20 cm profile) in relation to increasing volume of wastewater irrigation (please refer to Table 7.2)

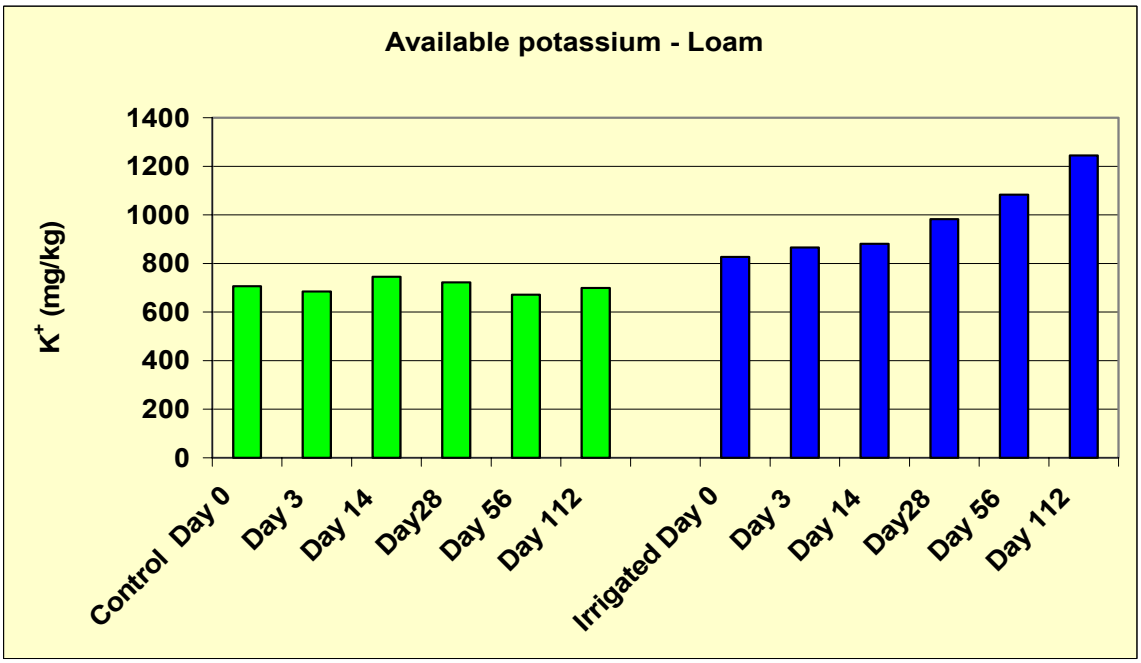


Figure 7.11 Bicarbonate extractable potassium in loam soil (average value for the whole 20 cm profile) in relation to increasing volume of wastewater irrigation (please refer to table 7.2)

7.3.5 Salts and dissolved organic carbon leaching through the columns

The leachate collected from the soil column was analysed for a range of elements and some of these are presented in Figures 7.12 to 7.16. The data presented in these figures show that through the 20 cm column and under reasonably heavy irrigation schedule, the salts started to leach through within 2 weeks (152 mm irrigation in 14 days). The EC of leachate was as high as the wastewater within this period (Figure 7.12) and similarly sodium levels in column leachate reached and exceeded the levels present in wastewater within a period of 4 weeks (Figure 7.13). In contrast, however, potassium levels in leachate, although increased with time, did not reach the input concentration through wastewater irrigation even after 16 weeks. This is consistent with the data on soil accumulation of potassium, which nearly doubled during the 112 day experiment (as shown in Figure 7.14 and Table 7.5).

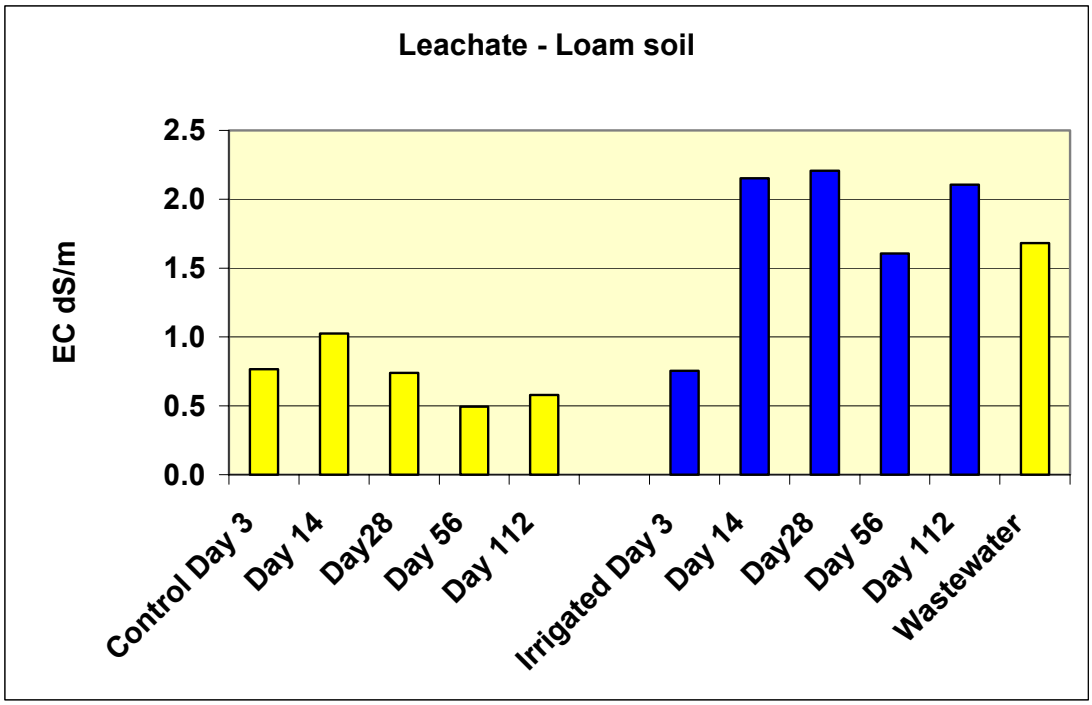


Figure 7.12 Electrical Conductivity (EC) in leachate from 20 cm columns of loam soil with increasing volume of wastewater irrigation (please refer to Table 7.2)

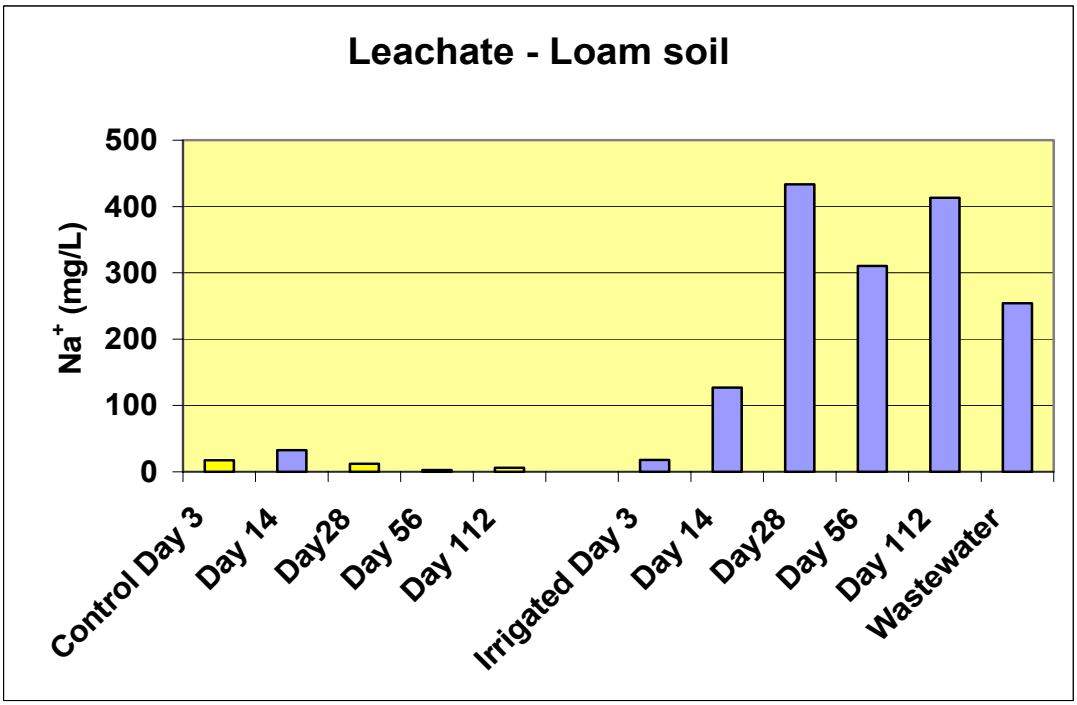


Figure 7.13 Sodium concentrations in leachate from 20 cm columns of loam soil with increasing volume of wastewater irrigation (please refer to Table 7.2)

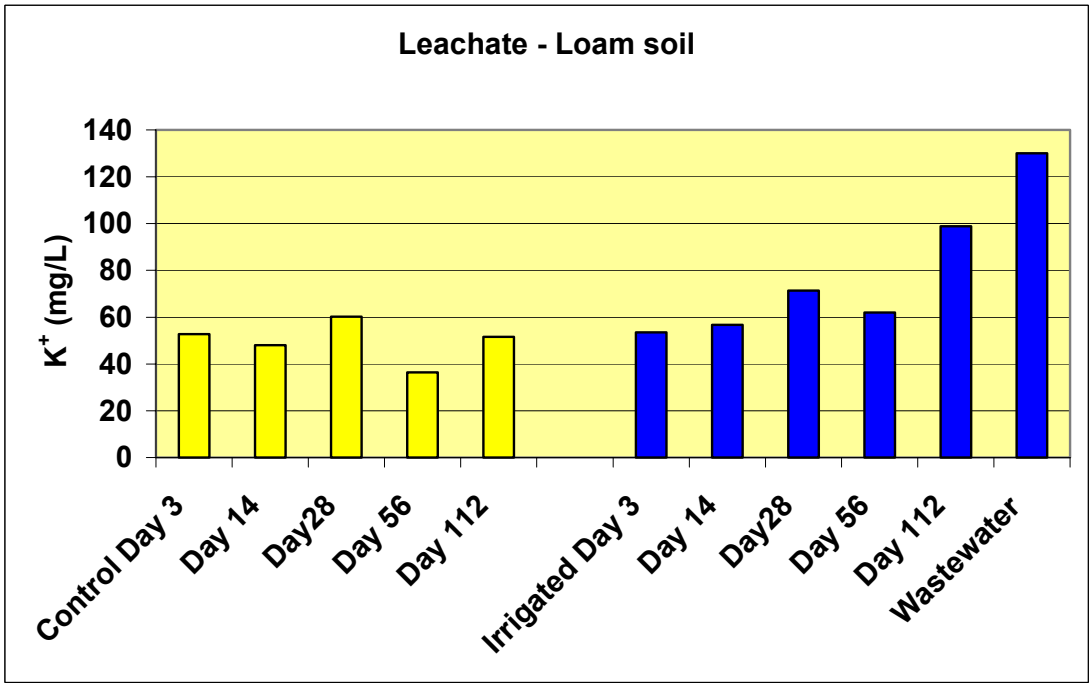


Figure 7.14 Potassium concentrations in leachate from 20 cm columns of loam soil with increasing volume of wastewater irrigation (please refer to Table 7.2)

7.3.6 Dissolved organic carbon and nutrients leaching through the columns

Dissolve organic carbon (DOC) in leachate also increased initially with the increasing volume of wastewater used in irrigation but then stabilised (Figure 7.15). Even by the end of the experiment the DOC leaching through the column did not reach to the level present in wastewater. It appears that initially more DOC leached through the column due to possible lack of microbial activity in soil column. Possibly the carbon input in soil enhanced the microbial activity in first couple of weeks and as a result the DOC started to get consumed by the soil microbes and as a result less DOC was available to leach through the column.

Among nutrients, ammonium, nitrate and phosphorus were measured in the leachate. Nitrate levels in the leachate remained below the detection limit (<0.2 mg/L) for up to 56 days and then broke through the columns (Figure 7.17). In the case of ammonium (Figure 7.16), an increase in the leachate was noted in both irrigation treatments (with clean water as well as with wastewater). After 56 days of irrigation (some 458 mm of water) the ammonium levels reached or exceeded the levels present in wastewater in both treatments. The average concentration in wastewater was noted to be 2.3 ± 2.0 mg/L.

The phosphorus levels in leachate remained within the range (< 0.5 mg/L) that was observed in the soil columns treated with clean water. Only after 112 days the P level in leachate through the 20 cm long column increased to 1.55 mg/L. The average input of P through wastewater irrigation was 7.7 ± 1.2 mg/L

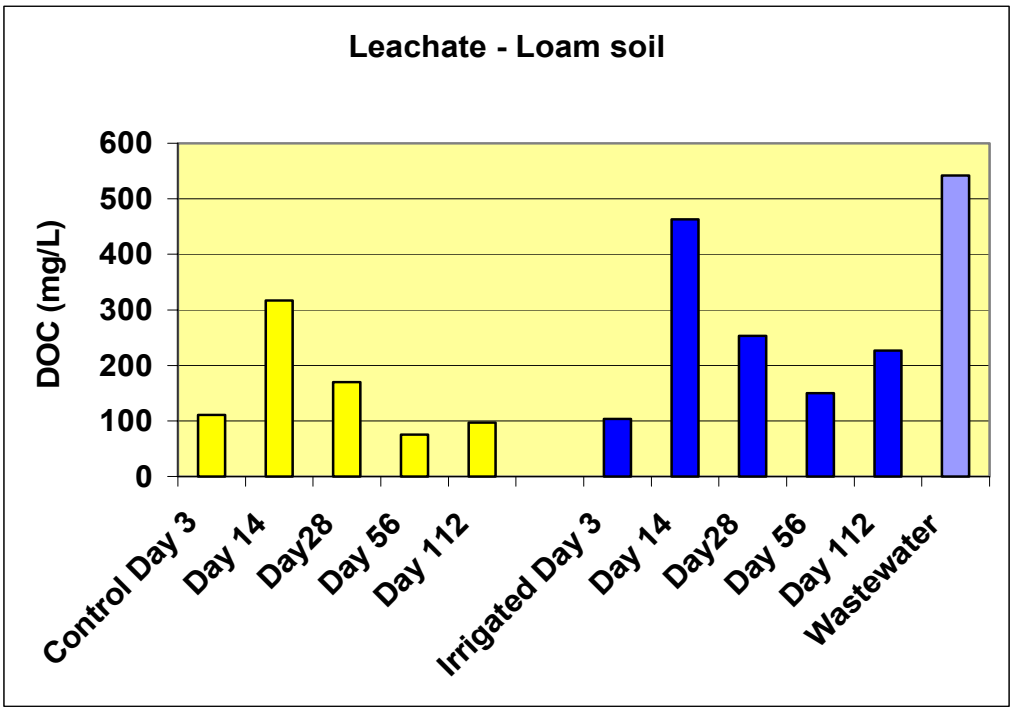


Figure 7.15 Dissolved organic carbon (DOC) concentrations in leachate from 20 cm columns of loam soil with increasing volume of wastewater irrigation (please refer to Table 7.2)

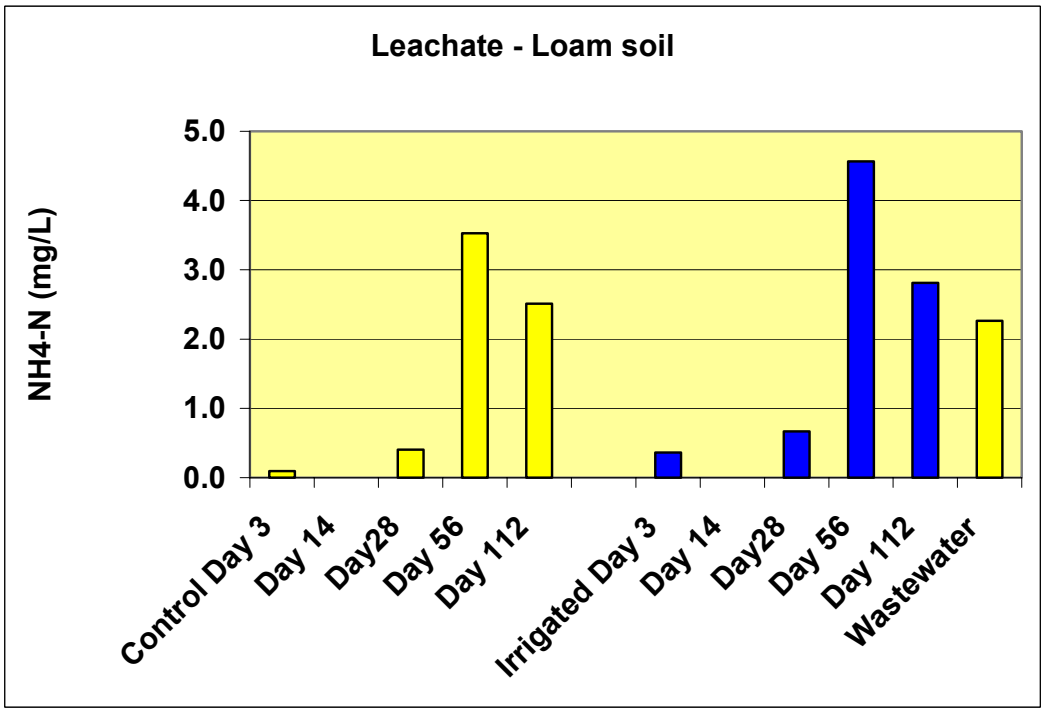


Figure 7.16 Ammonium concentrations in leachate from 20 cm columns of loam soil with increasing volume of wastewater irrigation (please refer to table 7.2*)*

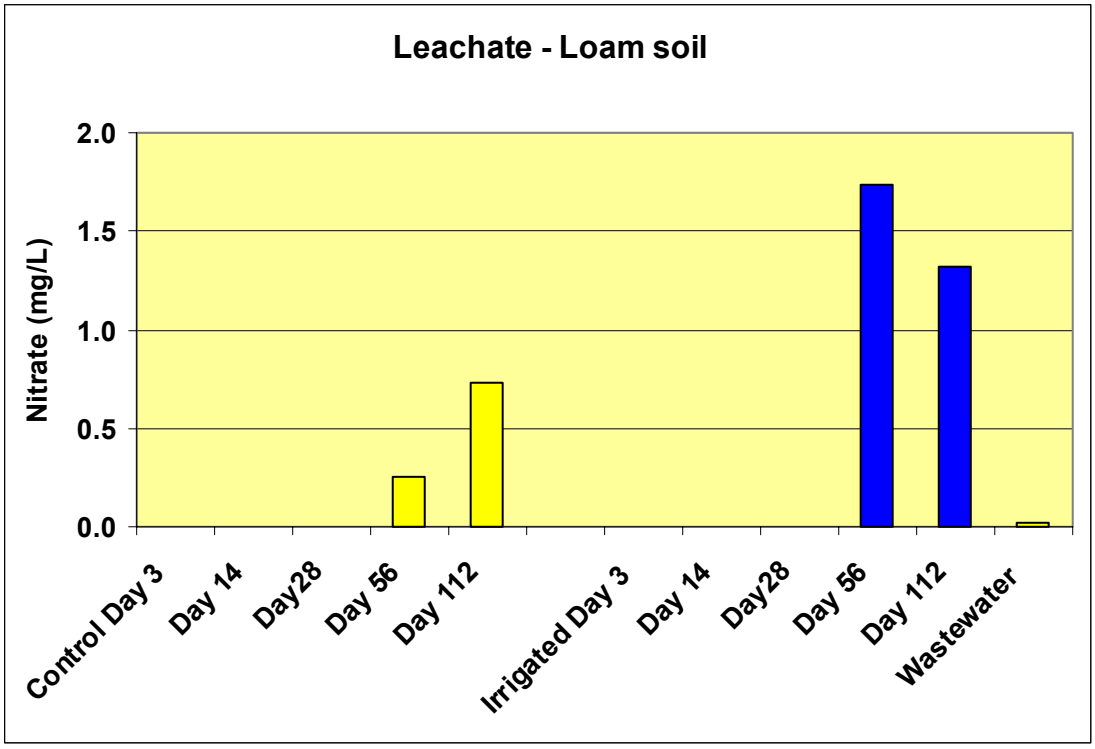


Figure 7.17 Nitrate concentrations in leachate from 20 cm columns of loam soil with increasing volume of wastewater irrigation (please refer to table 7.2)

7.4 Conclusions

- The results did not indicate any direct adverse effect due to winery wastewater irrigation on a range of soil microbiological indicators used in this study. However, indirect effects on microorganisms due to excessive water application leading to structural decline and prolonged waterlogging conditions resulting in depletion of oxygen were not tested in this study.
- Salt build up in the soil profile, especially sodium and potassium, with continuous usage of winery wastewater, is the key concern in terms of soil health. This can lead to structural decline, which in turn can cause severe soil physical and biological detriments as well as poor soil productivity and biodiversity.
- To avoid excessive salt built up, leaching of salts from the soil profile is necessary. This would need to be achieved without adverse impact on groundwater.
- Threshold values for tolerance of sodium and potassium in soils need to be established with respect to soil chemical, physical and biological standpoints.

8. OVERALL CONCLUSIONS

The study provided some definitive data on the winery wastewater characteristics, especially in relation to their ecotoxicological impacts on both aquatic and terrestrial ecosystems. For aquatic ecosystems, it became evident that during vintage the wastewater produced had a higher toxicity and the toxicity was greatest in the case of smaller wineries not employing any designed treatment process. It was also noted, however, that existing wastewater treatment processes helped to reduce the toxicity of wastewater for aquatic organisms but led to greater salt load in the wastewater which is of greater consequence to the terrestrial ecosystem.

The study identified that the bulk of the toxicity was associated with the organic fraction of the wastewater which when removed rendered the wastewater suitable for sensitive aquatic organisms. Copper and zinc ions in winery wastewater could be of some concern. The use of toxic polymers were noted to have a major effect on wetland ecosystem. Interventions such as pH adjustment were found to be very helpful in removing toxicity associated with polymers. Preliminary investigation on polymer toxicity to the selected aquatic organisms revealed that current cationic polymers used in the wineries can be classified as being highly to moderately toxic. Greater care is needed in choosing the right polymer for use in treatment process. In general, the species diversity and water quality were poorer at the treatment wetland system in comparison to any natural wetland system. However, the tested wetland system was able to improve the winery wastewater quality in the dam making it more suitable for irrigation purposes.

For terrestrial ecosystems, the data set showed that there was no adverse measurable impact on soil microbiological activity or selected functions that were tested. On the other hand soil physical and chemical properties were significantly impacted by the use of winery wastewater. Indeed, the greatest impact of winery wastewater irrigation on land is likely to be through the build up of salts, especially sodium and potassium. The build up of monovalent ions in the soil profile can result in deterioration of soil structure and consequently can adversely impact soil productivity. This aspect needs a thorough investigation to clearly establish the threshold of different soil types in tolerating winery wastewater.

9. RECOMMENDATIONS

The following recommendations can be made based on this study.

1. Based on the extensive characterisation of winery wastewater in this study pH, EC, SAR and TOC can be recommended as four important key indicators of winery wastewater quality.
2. An integrated approach is needed to identify issues at source, treatment and reuse steps for better management of winery wastewater. Opportunities need to be exploited for minimizing the wastewater volume and the separation of product/waste streams (e.g. spills of juice, wine). These are being explored in a new GWRDC/CSIRO project on management of winery wastewater.
3. Simple treatment steps such as filtration, aeration and pH adjustment are desirable in improving winery wastewater quality. However, pH adjustment may lead to greater salt loading.
4. The pH of winery wastewater should be carefully adjusted when cationic polymers are being used as flocculants. Because little ecotoxicological information is available on the polymer toxicity to terrestrial organisms. Similarly there is a variety of chemicals such as cleaning agents and flocculants used across the wine industry, with little information on the toxicity of these chemicals. Further studies should focus on the toxicity and environmental rating of all cleaning agents and flocculants used.
5. Removal of solids from winery wastewater entering the wetland ponds, pH adjustment and aeration of the first the pond should be used as important management strategies for wetland systems receiving winery wastewater.
6. The build-up of monovalent ions such as sodium and potassium in the soil profile can result in deterioration of soil structure and consequently can adversely impact the soil productivity. This aspect needs a thorough investigation to clearly establish the threshold of different soil types in tolerating the winery wastewater with respect to soil chemical, physical and biological perspectives.
7. The treatment processes have historically been driven by an aquatic ecosystem as a receiving environment. The changed reuse pattern to an irrigation resource for vineyards or other land-use need to be considered. In particular the criteria such as BOD are of lesser importance than salt loading, when the wastewater is to be used for irrigation purposes. There is a need to tailor the treatment steps to match the intended reuse.

10. Acknowledgements

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12. KEY PUBLICATIONS 2004-05

1. Kumar A, Camilleri C, Sisson N and Kookana R. 2004. Waste Not, Want Not! - Workshop at the 12th Australian Wine Industry Technical Conference, July 2004, Melbourne

2. Kumar A, Saison C, Doan H, Grocke S, Correll C and Kookana R. 2005. Environmental impact of winery wastewater. In the Proceedings of the Environmental Conference, Adelaide, 2-4 Feb 2005.

3. Kumar A,, Doan H, Grocke S and Kookana R. 2003. Toxicity of winery wastewater to waterflea, *Ceriodaphnia dubia*. In the Proceedings of the SETAC/ASE Conference, Christchurch, New Zealand, 27 Sep - 1 Oct 03.

Details in the attached Appendices (I and II)

APPENDIX I

WORKSHOP RECOMMENDATIONS - WASTE NOT, WANT NOT!

As a part of this project, a workshop was organised by the CSIRO team of project scientists at the 12th Australian Wine Industry Technical Conference, Melbourne. The key speakers at the workshop were experts from the industry, State EPAs, CSIRO and universities.

Thirty-five participants attended the workshop and also took part in group discussions for addressing the following R&D issues relevant to winery wastewater:

1. Cleaner production and treatment technology
2. Environmental impacts
3. Soil management

The key recommendations on R&D needs from the workshop are listed below

Cleaner production and treatment technology

1. Consider options for the management of solid waste.
2. Identify sources of different constituents of winery wastewater (www) within winery processes for efficient waste management.
3. Establish the best treatment technology available that works across the industry.
4. Develop decision support tool for assessment of various options in the treatment processes.
5. Report R&D findings to wineries and regulators for more cohesive and uniform regulations across Australia.
6. Network within the industry to exchange/share information. Upfront help for start-up wineries and helping small to medium wineries that often struggle to find relevant information.
7. Use effective tools for communication and training regarding environmental issues, especially for small and medium wineries. Develop consistency and accessibility of guidelines for planning, designing, operating waste management system such as EMS (Environment Management System).

Environmental impacts, including soil management

1. Assess polymer use for the winery wastewater treatment as polymers are toxic to aquatic organisms and can also have effects on soils.
2. Employ targeted treatments based on the disposal mechanism. BOD treatment has aquatic focus, for soils there may be totally different approaches.
3. Address perception vs. reality.

4. Develop decision support tool for the assessment of various options available for the disposal of winery wastewater.
5. Use regional approach for regional issues.
6. Determine impact of CCA treated posts on soil ecosystem.
7. Investigate BOD load and its impact on soil properties.
8. Investigate effect of salinity of on soil structure (long-term effect, Na-K balance).
9. Determine impact of potassium on wine quality and soil properties.
10. Assess role of biodiversity in the soil health management.
11. Provide information on the beneficial use of grape marc.
12. Validate existing flow and nutrient models and develop improved models.
13. Compile a database on winery wastewater characteristics and soil properties across industry.
14. Extend this workshop to regional and state industry forums

APPENDIX II