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**Quality Wash Water for Carrots
and Other Vegetables: Insurance
for Clean Food and Minimising
Environmental Impact**

Martin Mebalds and Andrew Hamilton
Agriculture Victoria

Project Number: VG99005

VG99005

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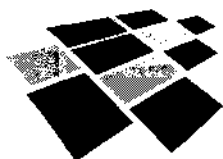
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Horticulture Australia

Final Report for HAL Project VG99005

**Quality wash water for carrots and other vegetables: insurance for
clean food and minimising environmental impact**



Horticulture Australia

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December, 2002

Horticulture Australia Project Number: VG 99005

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This publication reports the research and extension program which addressed the lack of information and treatment protocols for the safe re-use of vegetable wash water on farm.

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Media Summary

This project has found that if basic precautions are adopted, waste water from vegetable washing sheds can be re-used, saving many millions of litres of water annually. It is estimated that 4.4 million ML of water is used in Australia every year for washing vegetables, yet very few growers are willing to re-use the water for fear that it may contain plant pathogens, human pathogens such as *E. coli* or significant residues of agrochemicals. Growers are most concerned about the re-use of waste water used to remove soil from root crops as it is highly coloured and often produces foul odours.

Australia-wide surveys of vegetable farm waste-water derived from washing root crops showed that there were very few cases of excessive agrochemical residues but there was a slight increase in levels of plant pathogens, *E. coli*, nitrates and phosphorus. The water was shown to be unsuitable for discharge into rivers and streams but could be treated economically and effectively on farm for re-use. The most common agrochemical residues were residual pre-emergent herbicides. Consideration should be given to recent herbicide application history and if recent applications were made, then the water should be tested for herbicide residue concentrations. In some instances, excessive linuron levels in waste water had the potential to harm sensitive crops.

A set of guidelines were developed to assist growers in designing effective waste water treatment systems to remove excess organic matter, plant and human pathogens and nutrients. Safe re-use of waste water has the benefit of reducing farm costs and the requirement of water from rivers and bores.

A system of settling pits and ponds can adequately reduce excessive loads of organic matter provided that the capacity of the system can allow for a sufficient holding time to improve water quality. However, some larger packing houses have insufficient holding capacity in their settling ponds to cope with the volumes of water used by the washing system. The end result is thus little improvement of water quality after settlement pond treatment. Improvements in waste water treatment such as aeration and constructed wetland treatment may overcome the short-comings of existing water treatment methods for the removal of organic matter and nutrients.

If waste water is to be re-used to wash harvested crops, it should be disinfested considering it is highly likely to have elevated levels of human pathogens. The waste water is highly coloured and so is unsuitable for disinfestation by UV light, however, micro-organisms in the water may be best controlled using chlorine dioxide, which works more effectively than other forms of chlorine treatments in water with high levels of organic matter.

Technical Summary

Washing vegetables is an essential part of the postharvest treatment to remove soil adhering to root vegetables and to clean the product ready for sale. It has been estimated that Australia-wide, the process requires 4.4 million megalitres of water annually. Wash water re-use has the potential to significantly reduce the demand for water from our rivers and catchments and to alleviate water restrictions in our drier vegetable producing regions. Saving water has a direct financial benefit to growers in lower water costs and in having the ability to utilise waste water for washing soil off product or for irrigation. The re-use of waste water has not been widely practiced due to fears that the water could harm the crop by recirculating plant pathogens or because the water may be contaminated with agrochemicals or excessive salts.

This project examined the quality of water used for washing vegetables before and after the washing process and examined treatment methods aimed at improving water quality to a standard suitable for re-use on farm. Guidelines were produced for safe waste water re-use on farm.

A survey of waste vegetable wash water showed that field and postharvest pathogens were present in wash water. There was some risk that water reuse for washing or irrigation could redistribute the spores of pathogens either on product after harvest or on growing plants in the field. The survey also showed that coliform bacteria, including *E. coli* were sometimes found in source waters, including dams, bores and rivers such as the Murray River. The coliform bacteria concentrations in the wash waters were generally very high in waste water and may present a hazard to the consumer if not rinsed off in a final rinse. However, the presence of these bacteria in the waste water indicates that higher numbers of bacteria are being washed from soil encrusted root crops and that residual wash water on product would contain far fewer bacteria than the soil it replaced. The final rinse in chlorinated water immediately after the soil removal process generally removes most of the remaining bacteria. The concentrations of bacteria found in wash water ($0-2,800 E.coli.100 mL^{-1}$ and $0 - 6.8 \times 10^6$ coliforms. $100 mL^{-1}$) exceed WHO guidelines of $<10^3$ faecal coliforms. $100 mL^{-1}$) for the re-use of treated water on vegetable crops. Wash water should therefore undergo a form of treatment to reduce bacterial numbers before re-use either for irrigation or for use in the soil removal step in product washing. The waste water is highly coloured and so is unsuitable for disinfection by UV light, however, microbes in the water may be best controlled using chlorine dioxide which works better in water with high levels of organic matter than other chlorine treatments.

The most commonly found pesticides in wash waters surveyed were chlorpyrifos, prometryn, linuron and endosulphan. Of these, chlorpyrifos and linuron were the only chemicals found to be in a significantly higher concentration than source water. Pesticide levels were generally well below those which would be likely to cause the product to exceed maximum residue limits or to be of concern if the water were to be released into streams. However, in some cases, the concentration of linuron in the wash water was so high that carrot, lettuce and tomato crops would have been adversely affected. Adoption of low cost technologies such as horizontal flow constructed wetlands may be an efficient, cost effective method of reducing agrochemical, nutrient, and coliform bacteria concentrations in agricultural wastewater.

Treatment of water in settling pond systems did not significantly reduce the nutrient concentrations in water so additional treatments would be required if the water were to be discharged. Re-use of treated waste water for irrigation however, is practicable as the salt

concentrations were generally not likely to be harmful to crops such as carrots, although there were exceptions, and some crops are much more sensitive to salt than carrots. Generally salt levels in source and treated water did not vary greatly and were not affected by treatment in settling ponds. Where known salt concentrations in source water is close to the limit tolerated by crops, then waste water from washing should be carefully monitored for salinity before re-use.

There were very low concentrations of plant pathogens in the waste water, however, they were more numerous than in source waters. If waste water is being considered for crop irrigation then it should be monitored on a regular basis for the presence of plant pathogens. Where plant pathogens in waste water are of concern, water sanitation systems suited to high organic matter load should be considered.

Introduction

Vegetable growers face increasing pressures of decreasing water allocations, increasing charges for water and higher levels of accountability for environmental impact of their farming operations by councils, state EPAs and by the general community.

The importance of reducing the contamination of surface and ground waters from agrochemicals is recognised worldwide (Holt 2000, Yuones and Galal-Gorchev 2000) and in Australia (ARMCANZ and ANZECC 1995), but is of particular concern to rural communities who depend on these water sources for their drinking water. Furthermore, overseas buyers are increasingly concerned that the product they buy has been produced in a way that has minimal environmental impact. In Australia, reduced environmental flows of water and degraded water quality have major environmental impacts. In this context, the safe re-use of wash water would help to alleviate these concerns. It has been estimated from growers statements on water use per tonne of product, that 4.42 million megalitres of water is used per annum to wash vegetables. One of the larger growers uses 320,000 L water/week washing root vegetable crops. Recycling this quantity of water will have a significant impact on water use efficiency on farm, with less water required from Australia's river systems and underground water supplies.

The re-use of water used to wash soil off vegetables has been practiced by many in the industry where water is scarce for many years, however the build up of colour and development of unpleasant odours restricts this practice and discourages others from re-using this water. There is a lack of detailed knowledge of the changes in water quality parameters once it has been used to remove soil from vegetables. There is no information on the effectiveness of existing vegetable industry water treatment practices in removing organic matter and other undesirable components of waste wash water

Washing root crops such as carrots, parsnips and potatoes results in rapid deterioration of water quality. The waste water carries away not only soil but organic matter, spores and mycelium of fungi, bacteria, traces of nutrients, salts and chemical residues that are associated with the crop. The high load of organic matter begins to break down through microbial action, depleting oxygen in the water. In the absence of dissolved oxygen, anaerobic fermentation begins, causing the evolution of unpleasant smelling gases such as hydrogen sulphide (Wensloff 1998, Gross 1995). Growers have tried to overcome the problems associated with re-use of this water by installing relatively inexpensive systems of settlement pits, screens and settlement ponds where heavier material is allowed to settle out and lighter plant material is trapped by screens.

A further discouragement for water re-use is the fear that the water is not fit for the soil removal process or for irrigation onto crops because it may contain excessive agrochemicals, human or plant pathogens washed from diseased plants or contaminated soil. Similar concerns have recently been expressed about the use of recycled water in Queensland (Higgins *et al.* 2002). Blumenthal *et al.* (2000) however have recently reviewed, from a human health perspective, the WHO guidelines for the safe re-use of wastewater in agriculture. They present clear guidelines for the maximum allowable concentrations of coliforms and *E. coli* present in waste water for different crops. Studies had shown that consumers who ate raw vegetables irrigated with 10^4 faecal coliforms (FC)/100 mL did not develop infection with diarrhoeal disease or *E. coli* related diseases and this water was considered acceptable for use on root crops.

Water treatment options

The aim of water treatment is to improve water quality parameters (chemical and microbial) to a level where the water is fit for its intended use. The extent of water re-use on farm therefore will depend on the cost of treatments for the removal of organic matter, excessive residues of agrochemicals and nutrients, and the removal of bacteria and fungi which may compromise the safety of the crop in ground, of the harvested vegetables and of agricultural workers or consumers.

Removal of organic matter may be achieved in a variety of ways:

- by trapping particulates with screens and filters or break down and removal with the use of settlement in ponds and dams (Kolarik and Booker 1995, Gross 1995, Clear water solutions 1998);
- constructed wetlands (Headley *et al.* 2001);
- aerobic (Torrijos and Moletta 1997) and anaerobic microbial breakdown (Rintala and Lepistö 1997, Di Bernardino *et al.* 2000).

Headley *et al.* (2001) found that the removal efficiency for phosphorous and nitrogen was increased with increasing retention time. A hydraulic retention time (HRT) of 5 days, 86% of all nitrogen and phosphorous was removed from nutrient rich runoff from a nursery. Industrial style batch reactors may also be used to reduce organic matter in farm waste waters. Torrijos and Moletta (1997) developed a relatively inexpensive sequencing batch reactor which can reduce Biological Oxygen Demand (BOD₅) by 97.5% from highly polluted winery waste water. The system is suitable for small operators in terms of cost and management. The costs of the processes per megalitre of water vary greatly, depending on the cost of equipment, infrastructure and running costs.

Removal of 85–94% of enteric bacteria from waste water was achieved using surface flow constructed wetlands (Perkins and Hunter 2000) and efficiency of removal was inversely proportional to the flow rate or retention time in the system. Furthermore, Perkins and Hunter (2000) found that when flow rates became excessive, the wetlands became inefficient at removing bacteria. The findings of Headley *et al.* (2001) and Perkins and Hunter (2000) have immediate implications for growers who want to install water treatment ponds or wetlands. They must ensure that they have sufficient capacity in their settling pond/wetland systems to adequately retain the volume of waste water flowing from the washing facility for a minimum retention time (eg 3 days), otherwise the system will fail. Where high volumes of water are processed during the harvest season, aeration of wastewater in settling ponds will accelerate breakdown of organic matter (Wensloff 1998).

A part of this project was to examine the efficiency of existing water treatment systems for the removal of agrochemicals and harmful microbes, and to consider cost effective methods to improve the treatment of water on farm.

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SECTION 1

A survey of microbial, agrochemical and physical characteristics of wastewater from the carrot washing process, and implications for its re-use in washing and irrigation

Introduction

The availability of good quality water for agricultural use and domestic consumption is a key economic and environmental issue in Australia. It has been estimated that 4.4 million ML water is used in washing vegetables every year yet few growers are re-using this water, as there is little knowledge of the microbial and chemical quality of the water. Clearly, re-use of wash water by the vegetable industry will contribute to a significant saving of a precious resource and improving the vegetable grower's profitability by reducing water costs. The key questions facing growers with respect to water re-use are:

- 1) is wastewater suitable for re-use in irrigation, with respect to salinity, crop health and quality and food safety?
- 2) if the water is to be re-used for washing, are postharvest or human pathogens likely to contaminate the product and is this likely to affect postharvest quality and safety of food?

The carrot washing and water management systems used in Australia vary greatly between regions and among properties within regions. For example, some growers in southern Victoria use bore water for the soil removal process, and then discharge all the waste water to un-used land. Others divert the wastewater to a holding dam where it may later be used for irrigation. In drier areas, such as Northern Victoria, water is often obtained from a river. In the Sunraysia District, the requirement for water by agricultural industries for irrigation and vegetable washing is competing with demands for town water and for re-establishment of environmental flows.

The aim of this survey was to determine if wastewater from the carrot washing process needs to be treated before it is re-used for either washing or irrigation.

Materials and methods

Sampling protocol

Water was sampled from 19 farms throughout the major carrot growing districts of Australia—Mornington Peninsula, Victoria (2 properties) and Gippsland, Victoria (1), Mallee, along the Murray River in north-western Victoria (4), sandy plains around Perth, Western Australia (3), Tasmania (4), New South Wales (3), South Australia (4) and Queensland (1). Sampling was somewhat opportunistic, and three farms were sampled twice. Sampling was conducted only when washing was in progress and usually between mid-morning and mid-afternoon, when washing had been in progress for at least a few hours. For each operation, both 'source' and 'waste' water samples were collected.

- (i) Source samples were collected from a point immediately before the water was used to wash the soil off the carrots (e.g. from a sprinkler head over a hopper or a pipe entering a tumbler).
- (ii) Waste samples were intended to represent the quality of water potentially available for re-use.

Four litre, acid washed glass bottles were used for samples destined for chemical analysis. Sterilised 0.5 L Schott bottles were used for water samples collected for microbial analysis. These bottles were packed in ice in polystyrene boxes for shipment to the microbiology laboratory at the Institute for Horticultural Development, Knoxfield.

All operations had a drain for water leaving the processing shed, and this is where waste-water samples were taken. Most of the water passing through this drain was from the soil removal wash, although there were usually minor inputs from a final spray rinse. However, these inputs would be expected to have minimal impact on the survival of micro-organisms, because of the massive dilution factor and the high levels of organic matter, which would have reacted with all the available chlorine. In addition, at the end of the day the floor of the shed is typically washed, and this water is usually diverted to the same common drain as the washing waste-water. Thus, oil from fork-lifts and mechanical equipment, as well as detergents, can also enter the waste-water at the end of the day, but this was not investigated in this study. In addition to these water samples, a soil sample was collected from each property.

Analysis of water samples

Nutrient analyses

All nutrients were analysed using the Hach® DR/2000 spectrophotometric analysis system. Details of the tests can be found in the Hach reference manual (Hach 2000), and all tests are based on APHA *et al.* (1998) approved methods. In summary, the following methods were used:

- nitrite—diazotization method;
- nitrate—cadmium reduction method;
- ammonia—salicylate method;
- dissolved reactive phosphorus (DRP)—molybdovanadate method;
- total phosphorus—acid persulphate digestion followed by DRP test.

At this point, it should be noted that the concentrations of all chemicals (nutrients and toxicants) henceforth will be expressed taking into consideration the mass of the molecule minus the hydrogen atoms. This is the standard approach used in water chemistry and is the approved APHA *et al.* (1998) method. This approach is used because the number of hydrogen ions attached to the molecule will change depending on the equilibrium status of the chemical. Concentrations expressed in this manner are usually denoted as, for example, NH₃-N or NH₃ (as N). The former terminology will be used henceforth.

Agrochemicals

The concentrations of fifteen agrochemicals were determined, using gas chromatography, for each source and waste-water sample. Concentrations were measured directly from the water for all agrochemicals, with the exception of dithiocarbamates, where the headspace was sampled. The State Chemistry Laboratories (DNRE) conducted all analyses. The chemicals tested for were: fenamiphos, chlorpyrifos, diazinon, dimethoate, malathion, phorate, trifluralin, chlorothalonil, dithiocarbamates, metalaxyl, prometryn, linuron, alpha-endosulphan, beta-endosulphan and endosulphan sulphate. These represent all the agrochemicals registered for use on carrot crops with the National Registration Authority.

Physico-chemical parameters

Various physico-chemical parameters were analysed for each sample. The parameters were: pH, electrical conductivity, total organic carbon, biochemical oxygen demand (total, i.e.

carbonaceous and nitrogenous), colour and total copper. All of these analyses were conducted by Australian Water Technologies.

Faecal indicator bacteria

The total number of coliform bacteria and *Escherichia coli* was determined for all source and waste-water samples using Petri Film® (1) and a membrane filtration method. The membrane filtration method was used for enumeration of low numbers of *E. coli*, beyond the limit of detection for Petri Film® (i.e. < 100/100ML). Eighty to 100 mL of sample was filtered through a 0.45µm filter. The filter was placed on a moist endonutrient pad (Sartorius) and incubated at 37°C. For both methods, *E.coli* counts were made at 48 hours respectively.

Fungi

The presence of fungi in source and waste-water was assessed by plating out two 0.5 mL aliquots of sample water from a dilution series from 0 to 10⁻³ onto potato dextrose agar, malt extract agar and water agar. All fungi growing on plates were identified at least to genus level. Fungi that were considered to be potentially pathogenic were identified to species level. Pathogenicity of selected isolates was assessed by placing a small portion of mycelium into a wound on a surface sterilised carrot (wiped with 70% ethanol, then air dried) made by piercing the carrot with flamed forceps. The inoculated carrots and uninoculated controls were then incubated at 20°C in a humid chamber for two weeks. Carrots were regularly assessed for symptoms of infection. A pear baiting test for *Pythium* and *Phytophthora* spp. was also undertaken for every water sample. In addition to isolating specific fungi, the total concentration of yeasts and moulds was determined for each sample using Petri Film®

Analysis of soil samples

The pH (as 1:5 water and 1:5 CaCl₂), electrical conductivity (as 1:5 water) and total soluble salts concentration were measured for all soil samples. Standard descriptive analyses were also conducted; these included: colour, texture, gravel content (estimated as volume) and visual carbonates. All soil parameters were analysed by the State Chemistry Laboratories.

Statistical analysis

The effect of water type, source or waste, on the various water quality parameters was analysed using the non-parametric Friedman test (Friedman 1937, 1940). For many of the variables, especially the agrochemicals, the data were highly heteroschedastic and non-normal even after transformation. Thus, parametric analysis of variance (ANOVA) was not appropriate. Where there were less than five samples in which any one chemical was detected, statistical analysis was not performed. For the other parameters, ANOVA on log₁₀ transformed data were performed in addition to the Friedman test. Tied ranks were accounted for in the Friedman tests. For properties that were sampled on more than one occasion, the mean value for each parameter was used.

Results

General trends across the industry

The soil removal process greatly changed the physical properties of the water. The pH of the waste-water was significantly lower than that of the source-water ($P < 0.01$) (Figure 1). However, the absolute difference was not great; the median pH for the source and waste samples were 7.3 and 7.1 respectively. Whilst the conductivity of the waste-water samples

was significantly higher than the source-water samples ($P < 0.01$), there was only a difference of $25.3 \mu\text{Scm}^{-1}$ between the medians of the two sample types. The colour of the waste-water was significantly greater than that of the source-water ($P < 0.01$). Exceptionally high colour, 1200 units, was observed in one of the waste samples. Turbidity levels of the waste-water were often very high, sometimes in excess of 1000 nephelometric turbidity units (NTU), and overall the waste-water was significantly more turbid than the source-water ($P < 0.001$). Both total organic carbon and BOD₅ (biochemical oxygen demand, an indirect measure of carbon oxidised over a five day period at 25°C) were significantly higher in the waste-water than the source water ($P < 0.05$ and < 0.01 respectively). One waste-water sample was found to have outstandingly high levels of organic carbon and BOD₅, with respective values of 190 and 320 mgL^{-1} .

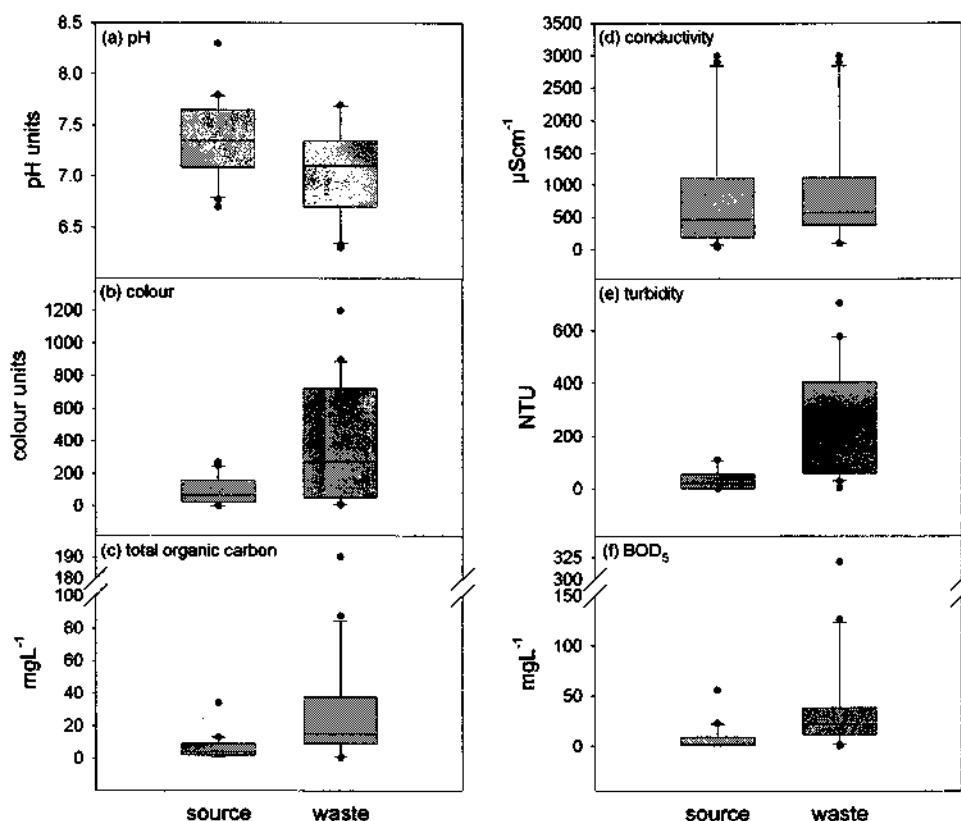


Figure 1. Box and whisker plots of physico-chemical parameters in source and waste waters. Median is marked as a line within each box. The bottom and top of the box represent the 25th and 75th percentiles respectively. The whiskers indicate the 10th and 90th percentiles, and data lying beyond these limits are marked as dots. NTU = nephelometric turbidity units.

The contamination indicator bacterium, *E. coli*, was found in significantly higher concentration in the waste-water than the source-water ($P < 0.01$) (Figure 2). Up to 2800 *E. coli* cfu mL^{-1} were observed in the waste-water.

Of the 15 agrochemicals analysed only five were detected in more than five samples (Table 1), and thus the statistical analysis was restricted to these five chemicals—alpha-endosulphan, chlorpyrifos, endosulphan sulphate, linuron and prometryn. There were no significant differences between the source and waste-water concentrations of alpha-endosulphan, prometryn or endosulphan sulphate ($P > 0.05$).

In contrast, the concentrations of linuron and chlorpyrifos were significantly higher in the waste-water than the source water ($P > 0.05$).

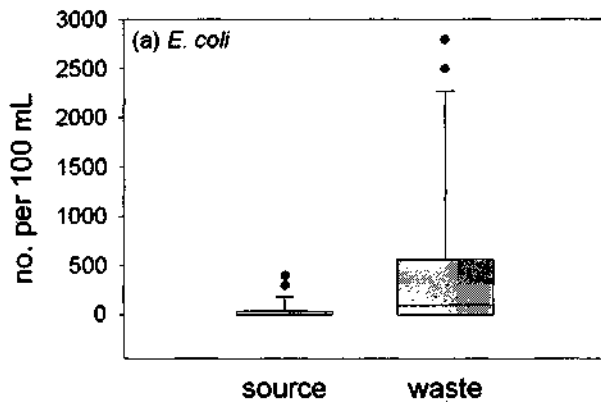


Figure 2. Box and whisker plots of *Escherichia coli* in source and waste waters. Median is marked as a line within each box. Presentation as per Figure 1

On one occasion a very high concentration of linuron, $34 \mu\text{gL}^{-1}$, was recorded in the waste-water, but on all other occasions waste-water concentrations were below $7 \mu\text{gL}^{-1}$ and source-water concentrations never exceeded $3.5 \mu\text{gL}^{-1}$. Prometryn concentrations in both the source and waste-waters were generally below $1 \mu\text{gL}^{-1}$, but substantially higher concentrations were detected in two of the waste-water samples (37 and $45 \mu\text{gL}^{-1}$) and one of the source-water samples ($38 \mu\text{gL}^{-1}$). Phorate and Chlorothalonil were not detected in any of the source or waste-water samples.

Table 1. Percentage of properties where agrochemicals were detected in the source and waste waters. Maximum concentrations (μgL^{-1}) are presented in parentheses. $n = 19$ for source and waste for every chemical.

	Source	Waste
Chlorothalonil	0.0	0.0
Chlorpyrifos	5.3 (0.58)	36.8 (2.60)
Diazinon	0.0	15.8 (0.53)
Dithiocarbamates	5.3 (16.00)	5.3 (21.00)
Dimethoate	5.3 (13.00)	5.3 (16.00)
α endosulphan	10.5 (0.01)	31.6 (0.10)
β endosulphan	10.5 (0.02)	15.8 (0.19)
Endosulphan sulphate	15.8 (0.39)	26.3 (1.80)
Fenamiphos	10.5 (0.36)	10.5 (0.54)
Linuron	21.1 (3.5)	47.4 (34.0)
Malathion	5.3 (0.22)	10.5 (1.90)
Metalaxyl	10.5 (3.60)	5.3 (3.70)
Phorate	0.0	0.0
Prometryn	26.3 (38.00)	31.6 (45.00)
Trifluralin	0.0	5.3 (0.54)

The concentrations of nitrate and oxides of N (nitrate plus nitrite) were significantly greater in the source-water than the waste-water ($P < 0.01$). Nitrite levels were generally very low,

and there was no significant difference in the concentrations between the source and waste samples ($P > 0.05$).

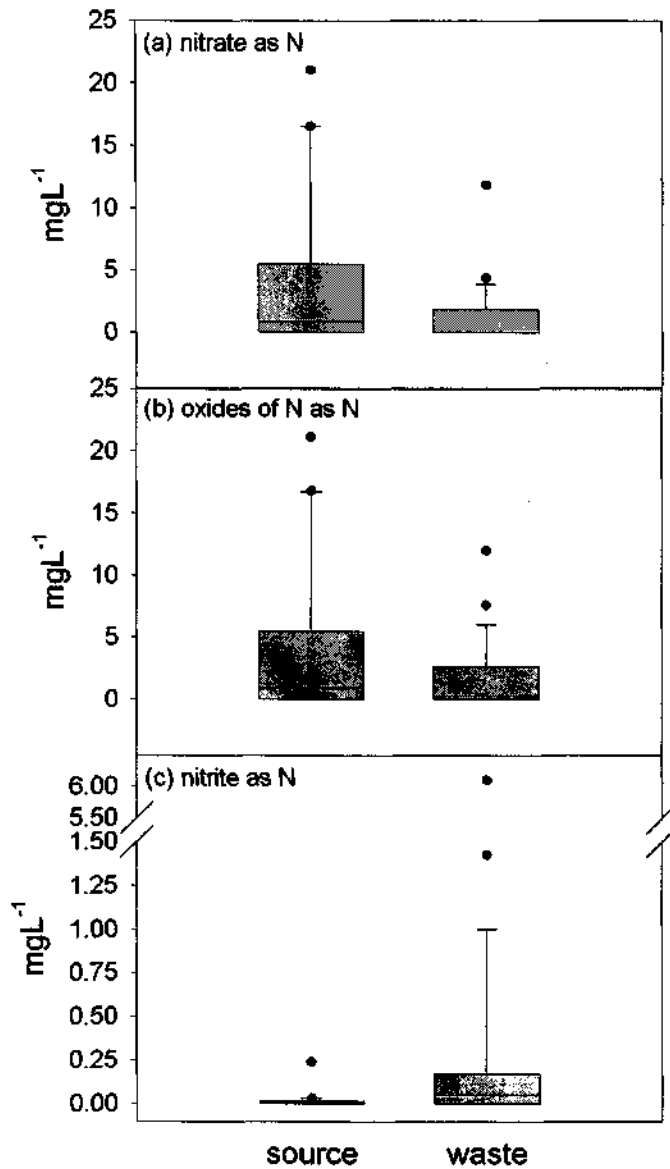


Figure 3. Box and whisker plots of the forms of nitrogen in source and waste waters. Median is marked as a line within each box. Presentation as per Figure 1.

We could only measure phosphorus concentrations in three carrot waste-water samples, due to technical reasons (too many interfering substances in the water). In these samples the average total phosphorus and dissolved reactive phosphorus concentrations were 5,833 and 1,967 μgL^{-1} respectively. In contrast, the source-water concentrations for these samples were 6,200 and 1,533 μgL^{-1} respectively.

Fungal isolations from source and waste waters

Pear baiting: Pear baiting was undertaken on all water samples however, *Pythium* and *Phytophthora* species were not isolated from any of the source or water samples.

Fungi isolated from source water and waste water

Forty nine fungal taxa were isolated from water found on vegetable farms (Table 2). This result confirms that growers are correct in the assumption that waste water has a higher load of fungal spores than the source water. Twelve of the taxa, including *Alternaria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum*, *F. sporotrichioides*, *Geotrichum candidum*, *Mucor* sp., *Phoma* sp., *Pythium* sp., *Rhizoctonia solani* and *Rhizopus* spp. are potential field or postharvest pathogens. Of these taxa, *A. alternata*, *F. oxysporum*, *Phoma* sp. and *Penicillium* spp. occurred frequently in waste water and in such numbers as to pose a risk to crops and product if exposed to recycling waste water. The incidences and concentrations of *Pythium* spp, *Rhizoctonia solani*, *Mucor* sp. and *F. sporotrichioides* were low in water samples and these species were not considered to be a significant threat to crops or harvested product. The source water generally contained fewer fungal colony forming units (cfus) than waste wash water or treated waste water however the incidences of specific taxa of fungi were so highly variable that a statistical analysis (ANOVA) would not be appropriate.

Pathogenicity of fungal isolates

A *Pythium* species isolated from waste water and sent to Dr Elaine Davison was not found to be pathogenic in her tests (pers. comm.) or in postharvest inoculation tests performed at the Institute for Horticultural Development.

Similarly, the isolates of *Geotrichum candidum*, *Phoma* sp. and *Verticillium* sp. were not active as postharvest pathogens on carrot.

Fusarium sporotrichioides known to be pathogenic to parsnips (Snowdon 1991), was found to be pathogenic to carrots in inoculation tests. Similarly, *Alternaria alternata* is known to be pathogenic to other root or tuber vegetables (e.g. potato Stevenson *et al.* 2001) and we found it to be pathogenic to carrots, causing small black lesions around the point of inoculation. An isolate of *Alternaria radicina* from carrot seed was found to be pathogenic as a postharvest pathogen but was not isolated from any of the waste water samples.

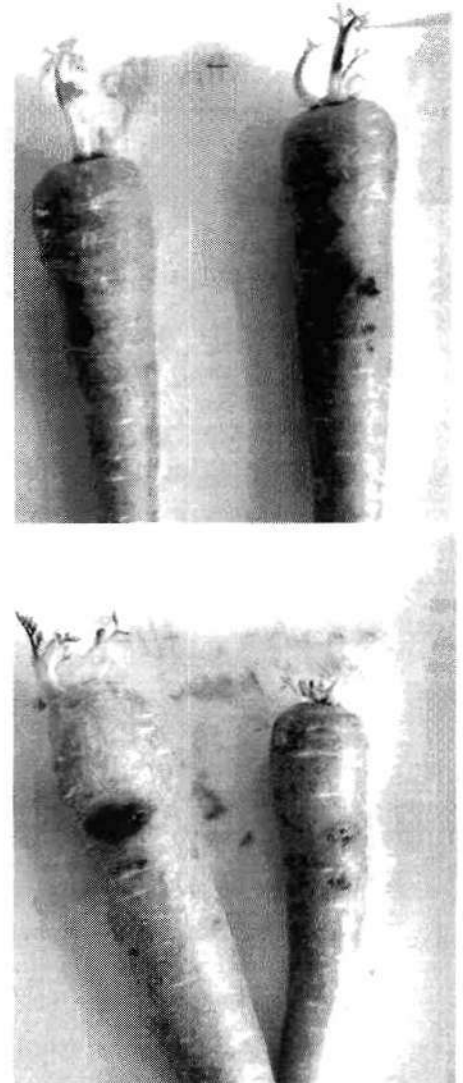


Plate 1 Pathogenicity tests for *Alternaria alternata* (top) and *A. radicina*

Table 2 Percentage Incidence and concentration of colony forming units (cfu) per mL of water sampled from source (n = 32) and waste (n = 30) and settlement pond treated (n = 6) waters from vegetable farms Australia wide. Highlighted rows represent recorded field or postharvest pathogens (Snowden 1991).

Fungi isolated	Source Water (n = 32)			Waste water (n=30)			Treated water (n = 6)		
	% incidence	Mean cfu.mL ⁻¹	Maximum cfu.mL ⁻¹	% incidence	Mean cfu.mL ⁻¹	Maximum cfu.mL ⁻¹	% incidence	Mean cfu.mL ⁻¹	Maximum cfu.mL ⁻¹
<i>Acremonium sp.</i>	34	1.2	5.4	44	2.9	17.1	13	1.0	1.5
<i>Acladium sp.</i>	3	0.3	0.3	0	0.0	0.0	0	0.0	0.0
<i>Alternaria alternata</i>	13	5.6	19.5	25	0.6	2.1	6	0.3	0.3
<i>Alternaria spp.</i>	13	2.4	5.7	28	3.1	12.6	0	0.0	0.0
<i>Aphanocladium sp.</i>	16	1.1	1.5	0	0.0	0.0	6	0.3	0.3
<i>Aspergillus niger</i>	25	1.0	58.0	28	2.0	8.7	3	0.3	0.3
<i>Aspergillus spp.</i>	19	1.2	2.4	25	1.0	2.1	3	0.3	0.3
<i>Aureobasidium pullulans</i>	13	1.4	4.2	22	0.6	1.8	6	0.3	0.3
<i>Botrytis cinerea</i>	0	0.0	0.0	0	0.0	0.0	3	0.3	0.3
<i>Calciopodium sp.</i>	3	1.2	1.2	6	2.4	3.9	3	1.5	1.5
<i>Candelabrella – like sp.</i>	1	0.3	0.0	0	0.0	0.0	0	0.0	0.0
<i>Chaetomium spp.</i>	0	0.0	0.0	3	0.3	0.3	0	0.0	0.0
<i>Cladosporium sp.</i>	28	2.9	39.0	28	2.2	7.8	0	0.0	0.0
<i>Cladosporium cladosporioides</i>	19	2.4	6.9	19	5.5	28.2	9	6.4	9.6
<i>Colletotrichum sp.</i>	3	0.6	0.6	0	0.0	0.0	0	0.0	0.0
<i>Coniella sp.</i>	0	0.0	0.0	3	0.3	0.3	0	0.0	0.0
<i>Cytospora sp.</i>	0	0.0	0.0	0	0.0	0.0	0	0.0	0.0
<i>Fusarium moniliforme</i>	6	0.6	0.6	13	1.3	3.6	0	0.0	0.0
<i>Fusarium oxysporum</i>	13	0.9	2.1	50	2.5	6.6	6	0.6	0.6
<i>Fusarium sambucinum</i>	3	0.9	0.9	3	1.2	1.2	0	0.0	0.0
<i>Fusarium solani</i>	9	2.3	3.9	31	1.5	3.3	3	0.3	0.3
<i>Fusarium sporotrichioides</i>	0	0.0	0.0	3	0.3	0.3	0	0.0	0.0
<i>Fusarium sp.</i>	19	1.1	2.4	31	1.5	8.7	3	0.6	0.6
<i>Geotrichum sp.</i>	3	0.3	0.3	3	0.3	0.3	0	0.0	0.0
<i>Geotrichum candidum</i>	13	1.7	5.4	9	5.5	15.6	9	0.6	1.2
<i>Gliocladium sp.</i>	3	1.8	1.8	6	0.5	0.6	0	0.0	0.0
<i>Gliocladium roseum</i>	0	0.0	0.0	9	4.7	9.0	0	0.0	0.0
<i>Gliomastix murorum</i>	6	0.5	0.6	16	0.7	1.2	3	0.6	0.6
<i>Humicola sp.</i>	0	0.0	0.0	3	0.6	0.6	0	0.0	0.0
<i>Mariannaea elegans</i>	3	0.6	0.6	0	0.0	0.0	0	0.0	0.0
<i>Microdochium sp.</i>	6	0.3	3.9	3	0.3	0.3	0	0.0	0.0
<i>Microspheariopsis sp.</i>	3	0.3	0.3	13	0.4	0.6	6	0.6	0.9
<i>Myrothecium sp.</i>	3	0.3	0.3	6	0.3	0.3	0	0.0	0.0
<i>Mucor sp.</i>	19	0.8	2.1	47	1.1	4.5	13	1.5	3.0
<i>Paecilomyces sp.</i>	13	1.7	3.0	22	2.7	9.3	6	3.0	5.1
<i>Penicillium sp.</i>	53	8.8	29.1	72	9.8	38.4	13	14.1	30.6
<i>Phialophora sp.</i>	0	0.0	0.0	6	0.6	0.9	0	0.0	0.0
<i>Phoma sp.</i>	22	3.2	10.2	56	3.3	31.5	9	0.9	1.5
<i>Pythium spp.</i>	3	0.3	0.3	9	0.3	0.3	3	0.6	0.6
<i>Rhizoctonia solani</i>	3	0.3	0.3	0	0.0	0.0	0	0.0	0.0
<i>Rhizopus sp.</i>	3	1.2	1.2	31	1.4	3.0	0	0.0	0.0
<i>Rhizopus stolonifer</i>	0	0.0	0.0	3	0.9	0.9	0	0.0	0.0
<i>Stemphylium sp.</i>	3	0.3	0.3	13	0.4	0.6	0	0.0	0.0
<i>Trichocladium asperum</i>	3	0.6	0.6	19	1.0	2.7	3	0.3	0.3
<i>Trichoderma sp.</i>	28	2.0	6.0	50	3.0	7.2	13	1.5	3.0
<i>Trichothecium roseum</i>	0	0.0	0.0	3	0.3	0.3	0	0.0	0.0

<i>Ulocladium sp.</i>	0	0.0	0.0	6	0.8	1.2	0	0.0	0.0
<i>Verticillium spp.</i>	13	1.0	1.8	31	0.8	2.7	9	1.1	2.4

Discussion

Human health considerations

E. coli, a contamination indicator bacteria, was found in significantly ($P < 0.01$) higher concentrations in the wastewater suggests that in the washing process there is greater potential for increased human pathogen loadings on product when recycled waste-water rather than 'fresh' water is used. However, it should also be acknowledged that in most systems the produce passes through a final chlorine rinse, and thus the pathogen load is likely to be reduced. The issue is complex and warrants further investigation.

It would probably be reasonable to assume that most of the *E. coli* in the waste-water originated from chicken manure, which was used on most farms as a fertiliser. Since the survey, however, new regulations have come into force banning the use of uncomposted chicken manure on vegetable crops, so now there may be less *E. coli* evident in waste waters (pers com David Element). However, there were a few properties where *E. coli* and coliform bacteria concentrations were very high in the source-water. These were the only properties where the source-water was obtained from a dam. Waterfowl were observed on these dams, and several studies (Hussong *et al.* 1979; Standridge *et al.* 1979) have shown that they may contribute to the *E. coli* load.

Agrochemicals

Most of the agrochemicals tested for were not detected frequently. The main exceptions were alpha-endosulphan, chlorpyrifos, endosulphan sulphate, linuron and prometryn. Of these, chlorpyrifos and linuron were the only chemicals where there was a significant difference in concentration between the source and waste-water samples. In both cases concentrations were higher in the waste-water. It is likely that the elevated concentrations in the waste-water are a result of the soil removal process. Linuron is one of the most commonly used herbicides in carrot fields, and chlorpyrifos is a widely used insecticide in horticulture.

Being a herbicide, linuron has the potential to damage carrot or other vegetable crops if the concentration in the irrigation water is too high. However, even though linuron was found in almost half of the waste-water samples analysed, the concentrations were generally well below those likely to cause crop damage if the water was to be re-used for irrigation. Carrot crops are not usually damaged if irrigated with water containing less than $12.4 \mu\text{gL}^{-1}$ linuron (Caux *et al.* 1998). With the exception of one sample that contained $34.0 \mu\text{gL}^{-1}$, the concentration of linuron was $7.0 \mu\text{gL}^{-1}$ or less for all the samples. Whilst the concentrations of linuron found in the waste-waters would not usually cause a problem when irrigating carrots, they could preclude the use of such water for irrigating other vegetable crops. For example, lettuces, turnips, parsnips and cucumbers should not be irrigated with water containing more than 4.9, 1.89, 8.9 or $3.3 \mu\text{gL}^{-1}$ of linuron respectively (Caux *et al.* 1998). Tomatoes are particularly sensitive to linuron, and should not be irrigated with water containing greater than $0.071 \mu\text{gL}^{-1}$. For all the waste-water samples where linuron was detected in the survey, the concentration was well above $0.071 \mu\text{gL}^{-1}$ (minimum = $0.44 \mu\text{gL}^{-1}$). Aside from being potentially toxic to crops, linuron can also cause damage the environment.

There are no Australian guidelines for acceptable concentrations of linuron in the environment. Furthermore, Caux *et al.* (1998) conducted a literature search of water quality guidelines throughout the world and found that there were no specific guidelines for linuron.

They developed interim Canadian Water Quality Guidelines for linuron. According to these guidelines, for the protection of aquatic life the concentration of linuron in fresh surface water should not exceed $7\mu\text{g/L}$. With the exception of one sample, the concentration of linuron in carrot waste-water in this survey was $7\mu\text{g/L}$ or less. Thus, based on this guideline, in most circumstances waste-water from the carrot washing process may not cause environmental problems. However, research on the toxicity of linuron to Australian plant and animal species needs to be conducted before any firm conclusions can be made.

Being an insecticide, chlorpyrifos is not likely to cause direct damage to horticultural crops if it is present in irrigation water. However, it does have the potential to disrupt natural food-webs. According to the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (henceforth referred to as 'Australian and NZ Water Guidelines'—ANZECC & ARMCANZ 2000) chlorpyrifos concentrations in freshwater should not exceed $0.00004\mu\text{gL}^{-1}$ or $0.01\mu\text{gL}^{-1}$ to ensure 99% and 95% ecosystem protection, respectively. 'Ecosystem protection' simply refers to percentage of species that are expected to be protected at these concentrations. Because the extent of dilution is unknown, it is difficult to determine if the concentrations of chlorpyrifos detected in the waste-water samples are likely to cause problems if the water is discharged to natural waterways. The maximum concentration of chlorpyrifos found in waste-water was $2.60\mu\text{gL}^{-1}$, and thus this water would need to be diluted by at least 1 in 65,000 in order to satisfy the 99% ecosystem criterion. Such a high dilution would not be expected if waste-water was to be discharged to a small stream, as is sometimes the case. Thus, considering that chlorpyrifos was detected in the waste-water of 37% of the properties, it's potential to cause environmental damage should not be disregarded. In most circumstances, the waste-water from carrot washing is discarded to land rather directly to a waterway. In some instances crops are irrigated with the water, and in others spare land is simply flooded. In either case, the potential to cause contamination of natural waterways would be expected to be much lower than direct disposal to waterways, as the soil acts as natural filter where agrochemicals would be expected to be broken down to some degree. Nevertheless, even these diffuse sources of agrochemical contamination have the potential to be detrimental to aquatic food-webs.

Linuron and chlorpyrifos were the only agrochemicals that were found in significantly higher concentrations in the waste-water than the source-water. For the other agrochemicals (∞ endosulphan, endosulphan sulphate and prometryn) the soil removal process did not appear to lead to elevated concentrations in the waste-water. Thus, in many cases the source of the contamination appeared to be from outside the property, as the source-water itself had the same concentration as the waste.

Regardless of the source of the contamination, if the waste-water contains agrochemicals it still has the potential to cause either crop or environmental damage. None of the other agrochemicals included in our survey were encountered as frequently as either linuron or chlorpyrifos. Aside from these two, the most common in the waste-water were ∞ endosulphan (31.6% of samples), endosulphan sulphate (26.3 %) and diazinon (15.8%). Both endosulphan and diazinon are general purpose insecticides/acaricides, and are therefore unlikely to cause direct crop damage if present in irrigation water. However, they do have the potential to cause damage to natural ecosystems.

According to the Australian and NZ Water Guidelines the concentrations of total endosulphan and diazinon should not exceed 0.03 and $0.00003\mu\text{gL}^{-1}$ respectively for 99% species protection, and 0.2 and $0.01\mu\text{gL}^{-1}$ for 95% protection (ANZECC & ARMCANZ 2000). Furthermore, bio-accumulation and secondary poisoning can occur with endosulphan. Individually, the various endosulphan analytes analysed in the survey were found in concentrations in the waste-water that exceed the 99% protection level for total endosulphan, and thus total endosulphan would clearly have often been above this concentration. However, it must also be acknowledged that the endosulphan analytes were only detected in a

small proportion of the samples (see Table 1). Also, unlike chlorpyrifos, only a moderate dilution would normally be required to bring the concentrations of these chemicals below the 99% protection criterion of $0.03 \mu\text{gL}^{-1}$. Diazinon was found in 16% of the waste-water samples, and at a maximum concentration of $0.53 \mu\text{gL}^{-1}$ (average concentration in samples that it was detected in = $0.35 \mu\text{gL}^{-1}$) which is well above the 99% and 95% species protection concentrations of 0.0003 and $0.01 \mu\text{gL}^{-1}$ respectively. Thus, it appears that like chlorpyrifos, in some circumstances substantial dilution of waste-water would be needed to prevent environmental damage.

Other agrochemicals that were only occasionally detected in the waste-water (Table 1), and for which there are Australian and NZ freshwater species protection guidelines, are dimethoate, malathion and trifluralin. The 99% and 95% species protection concentrations for these chemicals are: dimethoate, 0.1 and $0.15 \mu\text{gL}^{-1}$; malathion, 0.002 and $0.05 \mu\text{gL}^{-1}$; trifluralin, 2.6 and $4.4 \mu\text{gL}^{-1}$. The highest concentrations of dimethoate found in waste water was $16 \mu\text{gL}^{-1}$ and for malathion, $1.9 \mu\text{gL}^{-1}$, indicating that these waste waters could present a hazard to fish and amphibians if released into surface waterways.

It is not known how the concentrations detected in the waste-water are related to levels in the vegetable (e.g. carrot), or whether these concentrations would be likely to lead to unsafe levels according to the Food Standards Code.

Nutrients

The waste-water from the carrot washing process generally contained high concentrations of nitrogen and phosphorus. In our survey of carrot waste-waters we found that nitrite levels were generally low in both the source and waste waters, although on two occasions very high concentrations were detected in the waste-water (Fig 3). It may be that much of this nitrite was derived from fertilisers that were washed off the carrot surface during the soil removal wash. In nature, nitrite is a transient form of nitrogen and is usually only present in very low concentrations. Many commercial fertilisers, and chicken manure, contain very high concentrations of nitrite. Nitrate however, was more concentrated in the source-water samples than the waste-water samples (Fig 3). Nitrate and nitrite concentrations are often reported as oxidised nitrogen, which is the sum of nitrate and nitrite (Fig 3). In south-eastern Australia total oxidised nitrogen concentrations in lowland rivers and freshwater lakes/reservoirs should not exceed 50 and $10 \mu\text{gL}^{-1}$ respectively (ANZECC & ARMCANZ 2000). Similarly, in south-western Australia, total oxidised nitrogen should not exceed 150 and $10 \mu\text{gL}^{-1}$ in lowland rivers and freshwater lakes/reservoirs respectively. The concentrations of oxidised nitrogen in the carrot waste-waters were substantially higher than these values, but the concentrations in the source water were even higher. This suggests that overall, the carrot washing process is not adding to the oxidised nitrogen loading of the water. Whether or not the nitrogen, specifically nitrite, in carrot waste-water is likely to cause eutrophication of waterways in the environment would depend on several factors. Firstly, in most situations the waste-water is not discharged directly to a waterway. By disposing of the water by land irrigation some of the nitrogen may be removed. Soil can act as a natural filter. This is a strong reason for encouraging waste-water disposal by land irrigation. Secondly, the degree of dilution of the waste-water when discharged into streams needs to be taken into account when assessing the risk of eutrophication.

In south-eastern Australia total phosphorus concentrations in lowland rivers and freshwater lakes/reservoirs should not exceed 50 and $10 \mu\text{gL}^{-1}$ respectively (ANZECC & ARMCANZ 2000). Similarly, in south-western Australia total phosphorus concentrations should not exceed 65 and $10 \mu\text{gL}^{-1}$ in lowland rivers and freshwater lakes/reservoirs respectively. We could only measure phosphorus concentrations in three carrot waste-water samples, due to technical reasons (too many interfering substances in the water). In these samples the

average total phosphorus and dissolved reactive phosphorus concentrations were 5,833 and 1,967 μgL^{-1} respectively. In contrast, the source-water concentrations for these samples were 6,200 and 1,533 μgL^{-1} respectively. Thus, unlike nitrogen, the concentration of total and of dissolved reactive phosphorus does not appear to change greatly as a result of the soil removal process, although further data would be needed to confirm this. As with nitrogen, the dilution of the waste-water with a natural water body would need to be taken into account when trying to determine the likelihood of eutrophication.

Fungal pathogens in waste water

The concentrations of all potential pathogens was always very low, the highest being 66 cfu.mL^{-1} *Pencillium* spp. and is generally considered too low to act as inoculum on healthy plants. Most pathologists generally rely on 10^5 to 10^6 cfu.mL^{-1} to achieve infection however recent work has shown that at least 10^4 to 10^5 *Botrytis cinerea* spores. mL^{-1} are required for field infection and that lower concentrations do not result in infections higher than background disease incidence (Warren *et al.* 1999). There was only one colony of *B. cinerea* isolated from all samples and this came from a settling pond, therefore *B. cinerea* from waste waters is not considered a threat to crops or harvested product. The concentrations of potential pathogen cfus in waste water therefore suggest that there is a minimal risk of developing field infections by irrigating waste water onto crops. An experiment was conducted to test this hypothesis and is presented in Section 3 of this report.

The results indicate that a major concern of carrot growers who consider that they may increase risk of crop disease by irrigating with waste water is valid. The pathogens of concern however, are not *Pythium* or *Geotrichum*. Isolates of these fungi were found to be non-pathogenic to carrots and they were not found to be present in high cfu.mL^{-1} concentrations. *Alternaria alternata*, the most common fungal species isolated, however, was found to cause postharvest lesions in carrots and was found to be present in a significant proportion of source and waste waters. *Alternaria* species produce spores that are relatively resistant to water sanitisers such as chlorine, chlorine dioxide chloro-bromine and ozone. The removal of *Alternaria* will require higher doses of sanitisers than the removal of many of the other fungal contaminants (Mebalds *et al.* 1996). A table of effective water disinfection treatments against *Pythium* spp., *Alternaria* and *Fusarium* species is included in Appendix 1.

Conclusions

Waste water from vegetable washing facilities is often loaded with high concentrations of nitrates, phosphates, human and plant pathogens. Of the agrochemicals registered for use on carrots, only four were consistently identified in waste water: the herbicides linuron and prometryn and the insecticides chlorpyrifos and endosulphan.

Levels of herbicide residue in waste water may pose a threat to growing crops if irrigated onto them. There are two kinds of threat:

- (i) Long term residual herbicides may affect a normally insensitive crop in high concentration, eg, high linuron residues in water irrigated onto carrots, or,
- (ii) Low levels of herbicide residue may affect sensitive crops in rotation, eg low linuron residues in water irrigated onto tomatoes

It is recommended that agrochemical residues in waste water should be determined before use on crops. Furthermore, waste water should not be used as a final rinse for root crops due to possible elevated levels of human and plant pathogens in the water. Soil removal from root crops however may not be adversely affected by the re-use of waste water if followed by a final rinse and if monitoring of microbial quality of the water does not show excessive levels of coliform bacteria or plant pathogens. Chlorinated source water should be used in the final rinse of root crops.

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Section 2 Settling pond studies

Introduction

In the first phase of this project we surveyed the quality of the wastewater from the carrot washing process. We found that the wastewater was typically highly turbid, which would be expected when washing carrots since much soil is removed. The high turbidity of the wastewater makes it difficult to disinfect. We also found several plant pathogens in the wastewater, and in some cases the faecal indicator bacterium *E. coli* was present. In the study described here, we investigated the effectiveness of settling pond systems at improving water quality. Aside from the settling of suspended solids, which reduces turbidity, settling ponds may also be a useful means of improving other water quality characteristics. Settling pond systems were studied because they are already used on many properties as a means of treating water, and they are a simple, low technology method of treatment that can be installed on any farm. The aim of this study was to determine if the types of settling pond systems currently in use in the industry could be used to treat wastewater effectively so that it can be re-used for either washing or irrigation.

Methods

Data from two different settling pond systems are presented in this study. Washing operations and settling pond systems differ substantially from one property to the next, and thus it is difficult to generalise across the industry. Furthermore, it was not possible to obtain detailed engineering data on these systems, such as hydraulic retention time or mixing patterns. For most systems, the volume of ponds was not even known. Here we present the results from two systems, one from a relatively small-scale operation and the other from a large-scale operation.

System 1

System 1 consisted of a series of three ponds on a small-scale production operation. Carrots were washed using an industry standard barrel tumbler, and they were also passed through a final chlorine rinse. Water for washing was mostly obtained from a bore. Rainwater collected from the washing shed roof was also sometimes used in addition to the bore water, but this would have contributed very little to the total volume of water used. Waste-water from the washing process was directed to a settling pond series. The first pond was next to the washing shed. This pond rapidly filled with sediment and was dredged regularly. Water exited this pond via an overflow pipe that was situated at the top of the pond, and then flowed to the next pond. Water moved from the second pond to a third in the same manner. The entire settling pond system was gravity driven. The second and third ponds were adjacent to each other, and they were located a few hundred meters downhill from the first. The settling ponds were all of approximately similar size. The exact volumes of the ponds are not known but an estimate is provided in Table 3. It must also be acknowledged that the depth of the Pond 1 would be expected to change as sediment accumulated. The depth measurement presented in Table 3 represents the depth at the start of the washing season, just after the pond had been dredged. Water leaving the last settling pond was not re-used for washing. Rather, it was directed to a larger storage dam that was used for irrigating the crops. This dam (referred to as "drainage and bore dam") was supplied with water from several sources, of which the waste wash-water was only a part. No wastewater was re-used for washing.

The settling pond system was studied on three dates, one just before the carrot harvest started, one just after (day 3 into the harvest) and about 2 weeks into the harvest. No washing had taken place for about three months before the first sampling date. By sampling before

washing had started we were able to gain an appreciation of how washing affected the functioning of the ponds.

Table 3. Dimensions of ponds at System 1. Depth was measured depth at the approximate centre of pond. Volume has been estimated assuming that the walls of the ponds were vertical, and thus it is likely to be a slight overestimate.

	length (m)	width (m)	surface area (m ²)	Depth (m)	volume (m ³)
Pond 1	25	10	250	1.1	275
Pond 2	27	7.5	202.5	0.93	188
Pond 3	8.8	18.2	160.16	0.7	112

On the first two dates, sampling was conducted throughout the day so that we could determine how the ponds changed over the day and if the operating efficiency of the system was maintained throughout the day. Samples were taken at 9am, 11am, 1pm, 3pm and 5pm. On the first date, there was no flow from one pond to the next because there was no washing in progress. Thus any changes in water quality parameters would be related to processes happening within each pond. On the second date, when washing was in progress, there was a continual flow of water from one pond to the next. However, because we sampled at the start of the harvest season, the ponds were not full, and water did not start flowing from the second to the third pond until after the 1 pm sample. This provided us with an opportunity to look at how the water quality of the third pond changed after receiving wastewater from the second. On the third date, only one sample, at 1 pm, was taken. This was taken in order to confirm the operating efficiency of the system after 2 weeks of washing. This was important, especially considering that on the second date the third pond did not receive wastewater until after 2pm.

The following parameters were measured for every sample: turbidity, dissolved oxygen, biochemical oxygen demand (and indicator of the level of organic matter), sulphide, *E. coli* and total yeasts and moulds.

System 2

This settling pond system was associated with a large-scale carrot washing facility. Unlike System 1, after water had passed through the settling ponds it was re-used for washing. Immediately after harvesting, carrots were dumped into a hopper at the washing shed where they were spray-rinsed with re-cycled water to remove some of the soil. After this they passed through a barrel tumbler to remove the remaining soil, and then they went through a final chlorine rinse. The waste-water from all these washing processes was collected in a common drain, and it was then sent to the settling pond system via a pipe. Unlike System 1, the settling ponds were not arranged in a simple series. After the Pond 1, the water was pumped into two other ponds (Ponds 2a and 2b). Approximately equal volumes are diverted to each of these ponds. These two ponds are connected at the far end (see Figure 4). Water flowed from Pond 2b into a final settling pond (Pond 3), which was about twice as large as the other ponds (Figure 4). Water was then pumped from this pond through a sand filter. This water is pumped back to the washing shed where it is re-used for washing. In order to top up the system, river water was used for washing instead of re-cycled water one day each week.

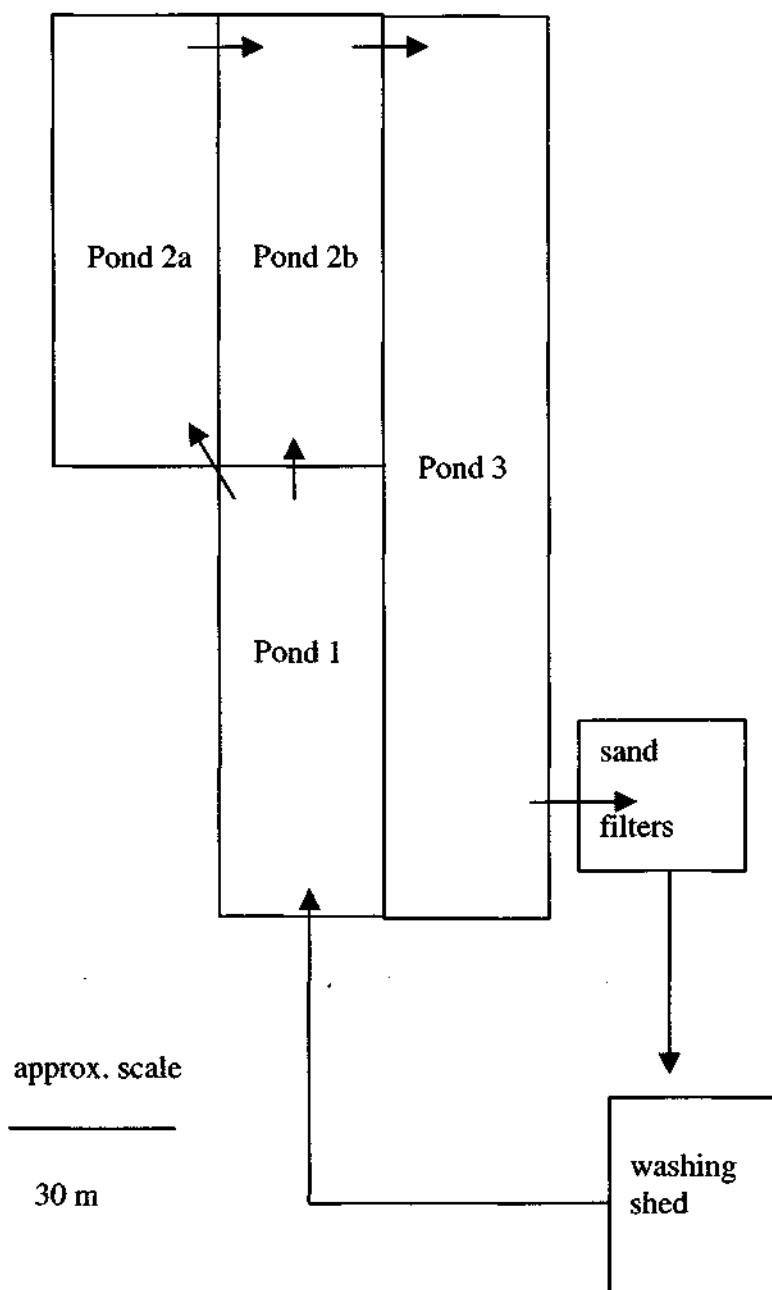


Figure 4 Schematic diagram of settling ponds in System Two.

In order to determine the effectiveness of the entire settling pond system, water entering and leaving the system was analysed. This involved sampling from the mouth of the pipe entering the first pond, and in the final pond near the mouth of the pipe that collected the water from this pond and pumped it to the sand filter. A sample was also taken of water that had been through the sand filter. This enabled the effectiveness of the sand filter to be assessed.

The following parameters were measured for each sample: biochemical oxygen demand, turbidity, pH, conductivity, sulphide, *E. coli*, coliform bacteria, and total yeasts and moulds. Nitrate and nitrite were only measured at the two sampling points at the end of the system, i.e. just before and after the sand-filter. Extremely high turbidity levels at the start of the system precluded analysis. UV transmittance was only measured in the samples taken after the sand filter. Washing was in progress when the samples were taken.

Results and Discussion

System 1

Before washing took place, turbidity was substantially higher in Pond 1 than the other two ponds, and it was roughly similar between Ponds 2 and 3 (Table 4). The fact that the first pond was more turbid than the others may be because it had been dredged and refilled about a week before the sample was taken. Much of the lighter suspended matter may have not yet settled. Once washing started the turbidity of the first and second ponds increased substantially (Table 5). It is interesting to note that the turbidity in Pond 3 was substantially higher on the second date, even before wastewater had started to flow into this pond (i.e. before 3pm sample). This high turbidity most likely represents an algal bloom that was observed in this pond, rather than a high concentration of suspended solids. A similar trend was seen on the third sampling date, although there was not an algal bloom in the third pond on this occasion and the turbidity was lowest in this final pond (Table 19).

Before washing commenced, the conductivity of the first pond was generally higher than Ponds 2 and 3, and there was no clear difference between these last two ponds (Table 6). It is possible that the slightly higher conductivity of the first pond was related to the fact that it had been dredged about a week before sampling. This would most likely have resulted in an increase in the amount of sediment, and ultimately salts, in the water column. The conductivity of the first pond did not show any clear change once washing commenced, but the conductivity of last two ponds tended to be higher on days of use (compare Tables 6 and 7).

Table 4. Turbidity (NTU) along a series of settling ponds over a day. The washing system was not operating and had not been used for at least two months.

	Pond 1	Pond 2	Pond 3
9 am	299	34.5	49.9
11 am	341	32.4	41.3
1 pm	403	28.5	37.4
3 pm	382	26.8	32.4
5 pm	358	27.8	29.7

Table 5 Turbidity (NTU) along a series of settling ponds over a day of washing. *Water started flowing into Pond 3 at around 2 pm.

	Pond 1	Pond 2	Pond 3
9 am	669	82	524
11 am	779	93	451
1 pm	779	164	388
3 pm*	>1000	297	392
5 pm	>1000	471	348

Table 6. Conductivity (μScm^{-1}) along a series of settling ponds over a day. The washing system was not operating and had not been used for at least two months.

	Pond 1	Pond 2	Pond 3
9 am	470	301	374
11 am	447	291	268
1 pm	429	285	260
3 pm*	417	280	259
5 pm	429	287	268

Table 7. Conductivity (μScm^{-1}) along a series of settling ponds over a day of washing. *Water started flowing into Pond 3 at around 2 pm. 'nr' = not recorded (meter malfunctioned).

	Pond 1	Pond 2	Pond 3
9 am	nr	nr	nr
11 am	417	397	350
1 pm	441	416	379
3 pm*	427	411	377
5 pm	398	390	362

When washing was not in progress, the concentration of dissolved oxygen tended to increase throughout the day in all the ponds, and this trend was particularly noticeable in the first pond (Table 8). The lower levels at the start of the day most likely resulted from the use of oxygen by bacteria and other heterotrophic microorganisms, such as fungi, overnight. This process would also be occurring during the day, but algae re-oxygenate the ponds in the presence of light. This is most likely why the oxygen concentration in all the ponds gradually increased throughout the day.

When the washing system was operating, the dissolved oxygen concentration in the first two ponds was substantially lower than when there was no washing. This was probably the result of higher levels of organic matter being present in the ponds. Much of this organic matter is most likely from the soil that was washed off the carrots. As mentioned above, microorganisms use this organic matter and oxygen for growth. Thus, higher levels of organic matter result in more oxygen being consumed by this microbial community, and the amount of oxygen that is consumed is often used as an indirect measure of the amount of organic matter present. This is referred to as biochemical oxygen demand (BOD_5), and it represents the amount of oxygen consumed by a sample over five days. It can be seen in Tables 10 and 11 that the BOD_5 of all the ponds was substantially higher when washing was in progress. It is also important to note that when washing was in progress, the dissolved oxygen levels in the first two ponds did not increase throughout the day like they did in the absence of washing. This is probably because oxygen was being consumed at a greater rate

(i.e. higher BOD₅) when washing was in progress. Also, less oxygen may also have been produced as a result of higher water turbidity, which inhibits photosynthesis. The dissolved oxygen concentration of the third pond increased until it started to receive the wastewater from the second pond, after which it started to decrease. Furthermore, it should be noted that after washing had been in progress for 2 weeks, the dissolved oxygen concentration in this pond was very low (Table 19).

Table 8. Dissolved oxygen concentrations (mgL⁻¹) along a series of settling ponds over a day. The washing system was not operating and had not been used for at least two months.

	Pond 1	Pond 2	Pond 3
9 am	4.74	5.12	3.75
11 am	12.78	4.15	5.18
1 pm	16.01	5.75	5.2
3 pm	18.13	6.26	6.16
5 pm	19.28	6.68	6.69

Table 9. Dissolved oxygen concentrations (mgL⁻¹) along a series of settling ponds over a day of washing. *Water started flowing into Pond 3 at around 2 pm.

	Pond 1	Pond 2	Pond 3
9 am	0.3	2.51	3.0
11 am	0.09	2.15	6.78
1 pm	0.4	3.19	13.55
*3 pm	0.4	2.95	11.0
5 pm	0.72	3.56	10.18

Table 10. Biochemical oxygen demand (mgL⁻¹) along a series of settling ponds at 5 pm. The washing system was not operating and had not been used for at least two months

Pond 1	Pond 2	Pond 3
5.09	1.85	2.24

Table 11. Biochemical oxygen demand (mgL⁻¹) along a series of settling ponds at the end of a day of washing (5 pm).

Pond 1	Pond 2	Pond 3
24.49	16.74	22.63

As seen in Table 9, the dissolved oxygen concentrations in ponds that receive the raw wastewater can drop to very low levels. In such cases, objectionable odours can develop. This is often the situation on properties where wastewater from washing goes into a concrete settling pit just outside the shed. Oxygen can become depleted to the extent that parts of pond, usually the lower layers of the water, become anaerobic (anoxic), that this, there is no dissolved oxygen. Under such conditions, certain anaerobic bacteria can produce hydrogen sulphide gas, which is most likely a major component of the foul smells emitted from these ponds and pits. This gas readily volatilises out of solution, especially when the water is mixed. In this study, the concentration of hydrogen sulphide gas in the air was not measured. However, dissolved concentrations in the water were measured. The levels of sulphide found in all the ponds during this study were generally very low (Tables 12 and 13). In comparison,

sulphide concentrations in raw sewage are often around 2 to 8 mgL⁻¹ (A. Hamilton, unpublished data).

Table 12. Sulphide concentrations (mgL⁻¹) along a series of settling ponds over a day. The washing system was not operating and had not been used for at least two months.

	Pond 1	Pond 2	Pond 3
9 am	0.218	0.044	0.144
11 am	0.124	0.048	0.13
1 pm	0.164	0.05	0.136
3 pm	0.166	0.106	0.084
5 pm	0.178	0.116	0.126

Table 13 Sulphide concentrations (mgL⁻¹) along a series of settling ponds over a day of washing. *Water started flowing into Pond 3 at around 2 pm.

	Pond 1	Pond 2	Pond 3
9 am	0.115	0.021	0.025
11 am	0.014	0.031	0.026
1 pm	0.160	0.023	0.021
*3 pm	0.160	0.021	0.020
5 pm	0.170	0.024	0.019

The concentration of total yeasts and moulds can be used as a rough indicator of the quality of the water with respect to its potential to cause postharvest or field disease problems. In general, the concentration of yeasts and moulds in the first pond was substantially higher when washing was in progress (Tables 14 and 15). It would be reasonable to expect that the soil that is washed off the carrots would contain high numbers of fungi, and considering the large quantities of soil that are washed off the carrots, it is most likely that this is why the concentrations were much higher during washing. This theory is also supported by the fact that the concentration of yeasts and moulds in Ponds 2 and 3 increased markedly in the 3pm and 5pm samples (on day of washing - Table 15); prior to these sampling periods these ponds did not receive washing waste-water.

Table 14. Concentrations of total yeasts and moulds (number per 100 mL) along a series of settling ponds over a day. The washing system was not operating and had not been used for at least two months.

	Pond 1	Pond 2	Pond 3
9 am	7000	5000	7500
11 am	4100	27600	3000
1 pm	5000	50800	7000
3 pm	11000	17000	7700
5 pm	5000	62700	13600

Table 15. Concentrations of total yeasts and moulds (number per 100 mL) along a series of settling ponds over a day of washing. *Water started flowing into Pond 3 at around 2 pm.

	Pond 1	Pond 2	Pond 3
9 am	40000	12000	3300
11 am	50000	14000	2000
1 pm	32000	6000	3900
*3 pm	40000	40000	37700
5 pm	40000	60000	30000

Table 16 Incidence of fungi in a series of waste water ponds prior to washing carrots

	Pond 2	Pond 4 (upstream)	Pond 4 (downstream)	Drainage and bore dam
<i>Acromonium</i> sp.		+		
<i>Aspergillus niger</i>	+	+	+	+
<i>Chaetomium</i> sp.		+		
<i>Cladosporium</i> sp.		+	+	+
<i>Fusarium</i> spp.	+	+		+
<i>Gliocladium</i> sp.			+	
<i>Microdochium</i> sp.	+			+
<i>Penicillium</i> sp.	+	+		+
<i>Phoma</i> sp.		+	+	+
<i>Rhizopus stolonifer</i>	+			
<i>Trichoderma</i> sp.	+	+	+	

The incidence of the fungi listed in Table 16 in a series of settling ponds for waste water indicated that there is little impact of the settling pond system on populations of fungi which may be pathogenic to the crop in the ground or to harvest carrots. The results also demonstrate that the fungi which cause post harvest diseases, *Phoma* sp., *A. niger* and *Fusarium* sp. are not easily removed from water during the settlement process. *Rhizopus stolonifer*, a postharvest pathogen, however was only found in Pond 2 and not further downstream and may indicate that it may have been eliminated from the waste water. The presence of *Gliocladium* spp. and *Trichoderma* spp. in the pond system may be of benefit in reduction of plant pathogenic fungi in waste water, as species of these fungal genera (*G. virens* and *T. harzianum*) are often used as bio-control agents for plant pathogenic fungi in soil (Samuels 1996). There is no evidence however that these fungi will have a beneficial effect in an aqueous environment.

Escherichia coli can be used as an indicator of faecal contamination, and the general 'health', of water. In the present study, samples from the pre-washing date (described above) were not analysed for the presence of *E. coli*. On the first washing date (Table 17) *E. coli* was substantially more numerous in the ponds that received the wastewater; numbers only reached high levels in Pond 3 once flow started to enter this pond after 2pm. Once washing had been in progress for 10 days, the ponds were sampled again and *E. coli* was detected in all three ponds. Thus, overall it appears that the settling pond system was not efficient at removing *E. coli*, and hence, it may also be ineffective at treating other potential human pathogens.

Table 17. Changes in concentration of *Escherichia coli* (number per 100 mL) over a day of washing along a series of settling ponds. *Water started flowing into Pond 3 at around 2 pm.

	Pond 1	Pond 2	Pond 3
9 am	5100	1300	100
11 am	2500	4000	0
1 pm	3700	3600	0
*3 pm	2900	2200	100
5 pm	1500	2300	2500

Table 18 Levels of various parameters along the settling pond series at midday, 2 weeks after the start of harvest. Washing was in progress throughout the day, and on each day for 10 days prior to this date.

	Pond 1	Pond 2	Pond 3
dissolved oxygen (mgL ⁻¹)	0.14	0.13	0.66
BOD ₅ (mgL ⁻¹)	18.39	26.43	19.77
sulphide (mgL ⁻¹)	0.532	0.202	0.108
<i>E. coli</i> (no. 100mL ⁻¹)	600	200	200
yeasts and moulds (no. 100mL ⁻¹)	340,000	180,000	300,00

System 2

In the second system, the turbidity of the water in the last settling pond was substantially lower than that of the raw waste-water (Table 19). However, the turbidity of the water after the filter was typically similar to that at the end of the pond system. It is highly probable that the greatest contributor to the turbidity of the water at the end pond was algal biomass rather than other suspended solids. Thus, the ponds may still have fulfilled their 'settling' function effectively, even though the turbidity of the water at the end of the system was relatively high. The concentration of chlorophyll *a*, an indicator of total algal biomass, was high in the last pond, as was the concentration of algal cells (Table 20).

The sand filter system did not appear to be efficient at removing algae, there was no clear difference in the algal cell count or chlorophyll *a* concentration before and after the filter. However, more samples would need to be taken to confirm this. In practice, the presence of algal blooms needs to be taken into account when measuring the turbidity of the water. It would not usually be practicable to analyse water samples for the presence of algae, but a distinct greenish colouration to the water, easily visible to the naked eye, indicates the presence of a bloom. Microscopic examination of the algae revealed that they were not 'blue-green algae' (cyanobacteria), which can have the potential to cause toxic blooms.

Table 19. Variations in turbidity (NTU) of waste water before and after settling pond treatment and after sand filtration over four sampling periods.

	raw waste-water	end pond system	after filter
13/2/00	> 1000	85.4	71.2
14/3/00	127	61.5	58.7
21/3/00	>1000	97.5	103.5
30/3/00	475.5	130.0	115.5

Table 20. Effect of sand filtration on chlorophyll *a* (mg.m³) and green algal cells (no. mL⁻¹).

	end pond system	after filter
chlorophyll <i>a</i>	946.1	856.4
algal cells	7815	10270

There was little variation in pH across sampling points and across dates (Table 21). The pH of the water has significant implications for its re-use if it is to be disinfected. A common disinfectant used in the industry is chlorine in the form of hypochlorous acid. The proportion of hypochlorous acid, the active form, in solution decreases with increasing pH. Thus, these chlorine disinfectants are less effective at high pH levels. Only 50% of the chlorine is present as hypochlorous acid at pH 7.5, and this rapidly decreases with pH values greater than this. It is therefore recommended that pH should at least be below 7.5 if hypochlorous acid based disinfectants are to be used. At pH 7 about 75 % of the chlorine is in the active form. Where water pH is above 7.5, but below 8.5, then a hypochlorous/hypobromous acid mix may be more effective as a disinfectant but still has the same limitation in the presence of high levels of organic matter. Another alternative is the use of chlorine dioxide, which is less affected by high levels of organic matter and is still effective at a pH of 9.5–10. Disinfection using chlorine dioxide is recommended in particular for waste/dam water if the pH is higher than 7.5.

Table 21. Variations in pH of waste water before and after settling pond treatment and after sand filtration over four sampling periods.

	raw waste-water	end pond system	after filter
13/2/00	7.91	7.85	7.88
14/3/00	6.73	6.88	6.87
21/3/00	7.17	7.27	7.22
30/3/00	7.37	7.84	7.67

Overall, the conductivity of waste-water remained at a reasonably consistent level across both sampling points and dates (Table 22). If waste-water is to be re-used for irrigation then it is important to ensure that it is not so saline so as to be injurious to crops. Salinity can affect plants through either indirect means, namely the reduction in availability of water to the plant, and direct routes, such as toxicity of specific ions to the plant.

It would be reasonable to expect that large amounts of soil that are removed from the carrots during washing, and which end up in the waste-water, would increase the concentration of salts in this water. Furthermore, if the water is recycled through a settling pond system and re-used repeatedly for washing, then salts may become concentrated in the water. However, in the present system the salinity of the raw waste-water, and the water at the end of the settling pond series was probably not prohibitively high so as to preclude its use for irrigation of most vegetable crops (ANZECC & ARMCANZ 2000).

Table 22. Variations in conductivity (μScm^{-1}) of waste water before and after settling pond treatment and after sand filtration over four sampling periods.

	raw waste-water	end pond system	after filter
13/2/00	444	612	618
14/3/00	594	535	554
21/3/00	679	656	654
30/3/00	649	628	638

In general, the concentration of yeasts and moulds tended to be lower at the end of the system than at the start. Species composition was not determined for these samples, so it is not possible to tell if potential pathogens were in fact found in lower concentrations at the end of the system. Nevertheless, the difference in concentration between the start and the end of the system/after the filter was substantial on all dates (Table 23), and thus the settling pond system, like those described above, does appear to help reduce fungal loads.

Table 23. Variations in yeasts and moulds (number per 100 mL) in waste water before and after settling pond treatment and after sand filtration over four sampling periods

	raw waste-water	end pond system	after filter
13/2/00	2000	290	300
14/3/00	1450	420	385
21/3/00	6700	675	1200
30/3/00	400	85	17

The highest concentrations of *E. coli* were observed at the start of the system, but *E. coli* was also detected at the end of the settling pond system and after the sand filter. Thus, the risk of contamination, whilst probably reduced, still exists at the end of the system. There was considerable variation in the concentration of *E. coli* across dates (Table 24).

Table 24. Variations in *E. coli* (number per 100 mL) in waste water before and after settling pond treatment and after sand filtration over four sampling periods

	raw waste-water	end pond system	after filter
13/2/00	56	1	0
14/3/00	1	0.5	0
21/3/00	4.5	5.5	7.5
30/3/00	1100	10	1

With the exception of the first date, sulphide concentration was lower at the end of the system than the start (Table 25). This could suggest that anaerobic processes are more dominant in the raw waste-water. However, there were no clear associated trends in BOD₅. The reason for this is not clear (Table 26).

Table 25. Sulphide concentrations (mgL⁻¹) variations in waste water before and after settling pond treatment and after sand filtration over four sampling periods

	raw waste-water	end pond system	after filter
13/2/00	0.029	0.040	0.086
14/3/00	0.189	0.109	0.097
21/3/00	0.513	0.135	0.131
30/3/00	0.270	0.130	0.199

Table 26. Biochemical oxygen demand (mgL^{-1}) variations in waste water before and after settling pond treatment and after sand filtration over four sampling periods

	raw waste-water	end pond system	after filter
13/2/00	11.84	15.08	24.20
14/3/00	54.48	37.12	42.16
21/3/00	100.64	100.8	71.36
30/3/00	60.64	57.12	47.84

Nitrate and nitrite (Tables 27 and 28) could not be measured in the raw waste-water because of turbidity interference. Nitrite would usually be expected to be found in very low concentrations, as it is a transient form of nitrogen. However, it is interesting to note that very high nitrite concentrations were recorded at the end of the pond system and after the sand filter on the last two dates. After consultation with the grower, we believe that this may be the result of run-off into the ponds after heavy rains. The surrounding plots had been fertilised with NPK and some of the excess run-off is directed to the settling pond system.

As nitrate and nitrite are soluble forms of nitrogen, it is not surprising that the sand filter did not appear to remove them. Neither the settling pond system nor the sand filters were effective at removing phosphorus (Table 29).

Table 27. Variations in nitrate (mgL^{-1}) in waste water before and after settling pond treatment and after sand filtration over four sampling periods

	end pond system	after filter
13/2/00	1.0	1.2
14/3/00	0.5	0.0
21/3/00	3.6	1.5
30/3/00	0.7	0.0

Table 28. Variations in nitrite (mgL^{-1}) in waste water before and after settling pond treatment and after sand filtration over four sampling periods

	end pond system	after filter
13/2/00	0.008	0.022
14/3/00	0.001	0.000
21/3/00	0.515	0.457
30/3/00	1.100	1.054

Table 29. Variations in total reactive P (mgL^{-1}) in waste water before and after settling pond treatment and after sand filtration over four sampling periods

	Raw waste-water	end pond system	after filter
13/2/00	5.5	5.5	5.3
14/3/00	7.1	4.2	4.8
21/3/00	7.9	5.4	5.5
30/3/00	4.2	4.9	5.2

The UV transmittance measurements of waste water at the end of the pond system and after the filter indicate that the filter did not significantly improve the transmittance of UV through the water (Table 30). The results indicate that very little UV can penetrate a one centimetre layer of water. Effective disinfection of the water using UV light can be achieved with highly turbid waters, however the UV system would require a significantly higher output of than required for relatively clean water, necessitating a significant capital outlay.

A two step coagulation/flocculation treatment combined with settlement may be required to further clarify water if a UV water disinfection system were to be adopted for the control of water-borne plant and human pathogens.

Table 30. Percentage UV transmittance

	End pond system	After filter
13/2/00	17.4	7.7
14/3/00	22.9	25.4
21/3/00	9.1	8.5
30/3/00	19.6	19.9

Conclusions

The settling pond systems have the potential to improve the quality of waste water if the hydraulic retention time is long enough for the breakdown of organic matter and to trap nutrients and agrochemicals. The two systems studied here did have a beneficial effect in improving water quality initially, but were soon receiving more water than they were capable of effectively treating and did not cope when vegetable washing was fully under way.

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Section 3 Effect of irrigating carrots with water contaminated with pathogenic fungi: a glasshouse experiment

Introduction

The use of waste water for the irrigation of crops may be carried out with little risk if the water is first treated to remove plant pathogens. The methods for water disinfection are well known (Mebalds *et al.* 1996, 1997) however the water quality of waste water from wash systems is of such quality that the cost of disinfection may be prohibitive due to the need for increased chemical or UV output from water disinfection systems. Given that the concentration of fungal pathogens in waste water was generally very low (<60 cfus.mL⁻¹) there is the possibility that disease development from such water may be insignificant. Most pathologists apply pathogens to plants in concentrations of 10^5 - 10^6 cfus.mL⁻¹ so there is little information to predict the outcome of irrigating waste water, loaded with low concentrations of plant pathogens onto crops. If there is little risk of disease development by applying untreated waste water onto crops, then growers could save significant quantities of water and money by reusing untreated waste water. If the disease risk is high, then growers may still use waste water but would need to install disinfection systems to treat their water.

The following experiment was designed to assess the impact of repeated applications of low concentrations of carrot pathogens on carrot plants. The experiment was undertaken in a glasshouse environment as known quantities of pathogen could be applied without confounding effects from pre-existing fungal populations in the soil or in crop debris close-by.

Materials and methods

Culture of carrot plants

The cultivar Tempo was used as it was known to be highly susceptible to cavity spot (McKay and Davidson 2000). Approximately ten seedlings were established in 10 cm pots filled with a standard pine bark/sand medium with a 6 month Osmocote fertiliser incorporated in the mix. The plants were maintained in a glasshouse and irrigated twice daily with microsprays below the canopy.

Pathogen inoculum:

All pathogens originated from carrots showing symptoms of disease. The *Pythium sulcatum* group F isolate (WAR 9689 ex QLD) was obtained from Dr Elaine Davison, Agriculture Western Australia. The following isolates were from the Victorian Plant Disease collection, *Alternaria radicina*, VPRI 341, *Fusarium oxysporum* VPRI 41, while the *Botrytis cinerea* and *Alternaria alternata* isolates were obtained from waste wash water samples.

All cultures were grown on Coons Agar except for *Pythium sulcatum* which was grown on V8 juice agar. *P. sulcatum* cultures did not produce oospores so cultures were placed in a Waring blender with deionised water and homogenised just enough to break up the agar and separate out mycelial fragments. The homogenate was examined on a haemocytometer slide for appressorial structures. The homogenate was diluted so that there were approximately 40 appressoria.mL⁻¹. The spores of *F. oxysporum*, *B. cinerea*, *A. alternata* and *A. radicina* from 7-14 day old cultures were suspended in sterile distilled water and spore concentration adjusted to 40 cfus.mL⁻¹ after determining the spore concentration with a haemocytometer.

Inoculation

Ten mL of sterile water or spore suspension was applied to leaves as a fine mist spray using a modified syringe at weekly intervals for thirteen weeks to each pot of ten carrots. Inoculation of *P. sulcatum* was similar except that a 10 mL suspension of 40 appressoria.mL⁻¹ was applied on all occasions except on the 7th inoculation where a suspension of 400 appressoria.mL⁻¹ was used. Spray drift was controlled using an upturned, bottomless pot that was resting on the treatment pot. Each fungal treatment had its own separate shield so that residual spray adhering to the shield would not cross contaminate neighbouring pots. Each treatment was replicated ten times.

Harvest and Assessment

Fourteen weeks after the beginning of the inoculation series, the plants were harvested, and any adhering potting medium removed. Fresh weight of all surviving carrots in each pot was determined. Sections of carrot and leaf were surface disinfested by dipping in 70% ethanol for one minute then allowed to dry in a laminar flow cabinet. Sections of carrot inoculated with *Pythium sulcatum* were plated onto V8 Juice Agar (V8JA) while leaves of all other treated and control plants were plated onto Coons Agar. All plates were then incubated at 20° C to seven days and cultures identified. One gram samples of potting media were taken from each pot and serially diluted in sterile deionised water to 1:100 dilution. Aliquots of 0.5mL of 1:10 and 1:100 dilutions then were spread onto Coons Agar and incubated at 20°C for seven days.

Fungal colonies growing on the plates were then identified and counted.

The experiment was layed out to a randomised block design and the results were analysed using Genstat to estimate the distribution of fresh weights, and average number of plants infected/pot and to compare treatments using ANOVA.

Results and Discussion

The average fresh weight of the carrot plants in pots inoculated with *Botrytis cinerea* (24g) *Alternaria alternata* (41g) or *Fusarium oxysporum* (41g) did not significantly ($P < 0.05$) differ from uninoculated plants (40 g) (Figure 5). The average fresh weight of *A. radicina* and *Pythium sulcatum* inoculated plants were the lowest fresh weights measured (21.8 and 21.2 g/pot respectively) however the variation in fresh weight between pots was too large to indicate a significant difference with uninoculated plants.

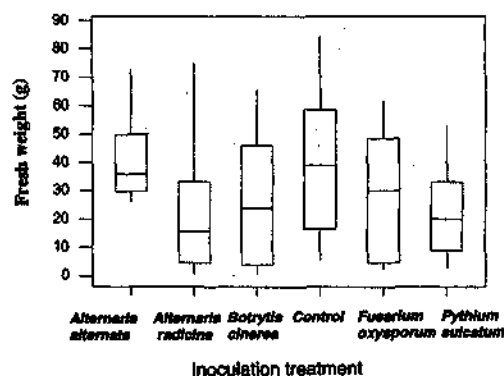


Figure 5 Box and whisker plots of fresh weight (g) of ten carrot plants inoculated with fungal pathogens. Median is marked as a line within each box. The bottom and top of the box represent the 25th and 75th percentiles respectively. The whiskers indicate the 10th and 90th percentiles, and data lying beyond these limits are marked as dots.

Pythium sulcatum was not reisolated from either carrot roots or from the potting mix. This indicates that the inoculum of mycelium and 40 appressoria.mL⁻¹, and on one occasion 400 appressoria.mL⁻¹, was not sufficient to cause colonisation of the soil or carrot roots. *P. sulcatum* rarely forms oospores, even in culture so the likelihood of spread by water is minimal.

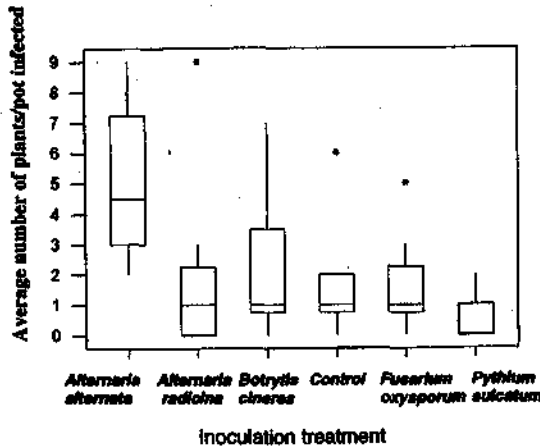


Figure 6 Average number plants /pot infected with *Alternaria alternata* in all inoculation treatments.

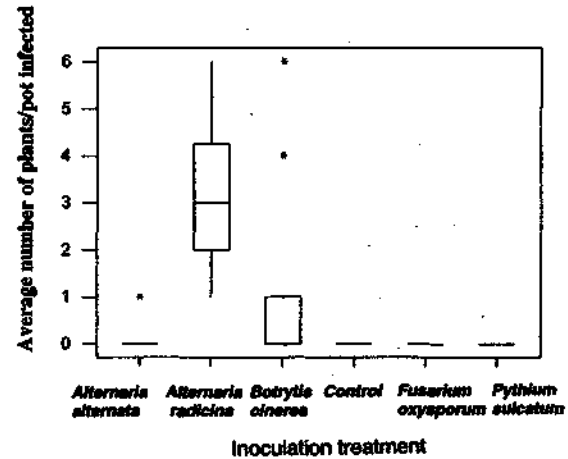


Figure 7 Average number plants /pot infected with *Alternaria radicina* in all inoculation treatments.

The weekly irrigation of water contaminated with 40 cfus.mL⁻¹ of *A. alternata* resulted in a significantly ($P < 0.05$) higher number of *A. alternata* infected plants than in other treatments (Figure 6). This indicates that on farm irrigation with waste water may result in higher levels of infection from *A. alternata*. Similarly, there were significantly ($P < 0.05$) higher numbers of plants infected with *A. radicina* where the fungus was inoculated than in any other treatments. The result indicates that, where there is a severe outbreak of *A. radicina* affected carrots, then irrigating crops with waste water contaminated with these conidia would lead to increased disease in the field (Figure 7).

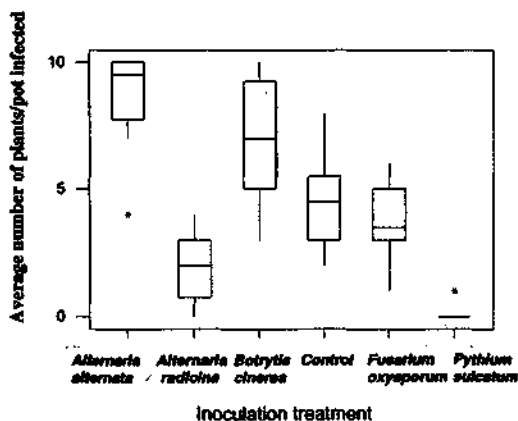


Figure 8 Average number plants /pot infected with *Botrytis cinerea* from all inoculation treatments.

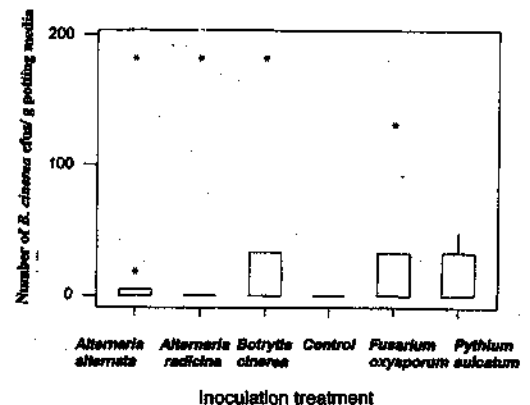


Figure 9 Number of colony forming units of *Botrytis cinerea* isolated from soil sampled all inoculation treatments.

The incidence of *B. cinerea* on carrot plants was evident in all but the *P. sulcatum* treatments suggesting that the fungus has moved around in the air to cause generally high levels of infection (Figure 8). Similarly, *B. cinerea* was present in the potting mix in most treatments suggesting that the movement of *B. cinerea* by air may be more important in the field than by spread by water (Figure 9). None of the other test fungi were present in the potting media to such a consistent level that a statistical analysis was appropriate.

The experiment only considered the inoculation of a single species of pathogen in any one treatment yet in waste water, there was often more than one pathogen at any one time. This project did not investigate. Furthermore, limited time constraints precluded long term studies to examine the possibility of the build up of plant pathogen populations over time, for example over successive years of irrigation with waste water.

Conclusions

The repeated irrigation of carrot plants with a range of fungal pathogens at the low spore concentrations found in waste water did not result in a decline in fresh weight of the plant, although some pathogens were re-isolated from the leaf tissue. Irrigation of water containing *Pythium sulcatum* did not result in a discernible disease incidence, nor was the fungus re-isolated from the potting media or from surface sterilised root tissue. The results suggest that there is some movement of pathogens through water, which when irrigated onto crops may cause some infection. However, the level of infection remained low enough not to cause significant loss in yield in a glasshouse environment. Further work in the field may contribute further information, however, the confounding factors such as pre-existing soil and debris populations of pathogens and their variability over any given area may contribute to such high variability in field infections and crop yield that such an experiment may not yield any definitive answers.

References

- McKay, A. G. and Davidson, E. M. (2000). Carrot variety tolerance to Cavity Spot. *Proceedings of Carrot Conference Australia, Perth, Western Australia.*
- Mebalds, M.I., van der Linden, A., Bankier, M and Beardsell, D. (1996) Using Ultraviolet light and chlorine dioxide to control fungal plant pathogens in water. The Nursery Papers 5 1-2. *In Australian Nursery Magazine, March, 1996*
- Mebalds, M.I., Bankier, M., A., Beardsell, D., van der Linden, A., Tregear, W., Tawfik, F. and Roberts, G. (1997) Monitoring and treatment of recycled water for nursery and floriculture applications. HRDC Final Report Project NY515.

Technology Transfer

During the course of the project the information gathered on water quality, treatment and re-use was delivered to a wide range of audiences and in a range of media.

Conferences:

The results of our work were reported at 'Carrot Conference Australia' in Perth
Presentation: Hamilton, A. and Mebalds, M.I. (2000) The potential for recycling carrot wash water- water quality considerations

Fresh Conference: The Future in Food Safety and Processing Technologies for Value-added Horticultural Products Melbourne, 2001

Presentation Mebalds, M.I. (2001) Methods for the disinfestation of irrigation water.

Posters:

Hamilton, A. (2000) Coliform bacteria detected in carrot wash water.

Mebalds, M.I. (2000) The plant pathogenic fungi in carrot wash water and settling pond discharge

Articles in Industry Publications:

Two articles have been written for
Good Fruit and Vegetables Vol. 11, No. 2, p. 50 and
Good Fruit and Vegetables January 2001 issue.

Newsletters, *In the Wash*, This newsletter covers research findings and directions for both our project and the clean and safe handling systems for vegetables and tomatoes project (VX99004)

Grower workshops

The first vegetable grower workshop was held near Clyde, Victoria on 26 April 2002. The workshops were designed to deliver a 15 minute talk on Clean and Safe Handling Systems (VX99004) and a further 15 minute talk on recycling wash water (VG99005). A question time of approximately 30 minutes was then used to answer questions and concerns regarding the material presented. After a short break, demonstrations on water testing for turbidity and electrical conductivity using growers' water was used to develop further discussion on the implications of the test results on the most appropriate methods of water quality improvement and disinfestation.

A further demonstration on chlorination and the draw-down effect of various vegetables was held to illustrate the variability of chlorine demand and the importance of monitoring of disinfectant concentrations.

A Field Day notebook incorporating presentations and water treatment guidelines (Appendix 1) was produced and distributed to all participants. Further copies of the field day notebook were given to state vegetable IDOs for distribution.

Patrick Ulloa, the Victorian Vegetable Industry Development Officer, arranged the workshop with growers. It was attended by approximately 15 grower families representing the major vegetable growers on the Mornington Peninsula.

Further, Gatton Field Days at Gatton, Qld. were attended on 7, 8 and 9th May 2002 where a display booth was established with information on the project including a Power point presentation of results, Field day notes were distributed to interested vegetable growers and the project outcomes discussed.

A trip along the Murray River region from Swan Hill to Mildura by Sally-Ann Henderson, Martin Mebalds and Andrew Hamilton included visits to all the major carrot growers of the region where they explained the results of the project to growers.

Grower workshops were also held in Virginia, South Australia on 26 June, Yanco on 20th June and Cowra, New South Wales on 30th July Bundaberg Queensland on 18th June 2002. A further workshop for carrot growers was held in Perth on 4th October 2002.

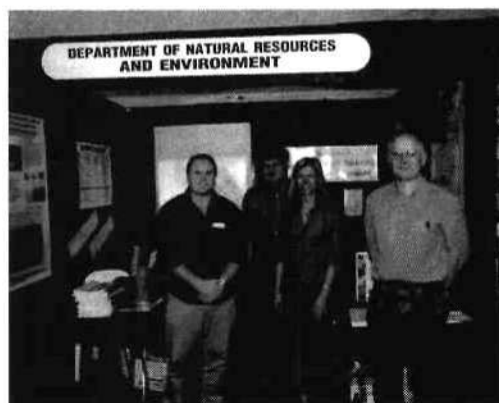


Plate 2 Explaining water recycling to grower **Plate 3** Gatton Field day booth with quality vegetable wash water project display



Plate 4 Explaining chlorine demand to the growers in Virginia SA



Plate 5 Grower workshop from the Mornington Peninsula, Victoria

Recommendations

Scientific

The project examined a range of existing water treatment strategies, as it was considered that an evaluation of the existing facilities has not been previously assessed. Once treatment strategies have been examined and analysed, recommendations could be made to improve water treatment processes within the vegetable industry. This approach has the best chance of adoption, as modifications of existing technologies are less expensive than installing new facilities. The pond systems were shown to reduce organic matter content if they were not overloaded with large volumes of wash water, reducing hydraulic retention time. The systems have a capability to reduce agrochemical concentrations in water however, in practice, there was little beneficial effect in existing systems when overloaded.

Further work needs to be undertaken in studying alternative and complimentary water treatment systems that may overcome current system inadequacies. In particular, extra aeration of water in settling ponds, and the addition of subsurface or surface horizontal constructed wetlands similar to those developed by Headley *et al.* (2001), have the potential to further reduce nitrates, phosphates, agrochemical concentrations and coliform bacteria levels with minimal additional cost. Further work on irrigation of crops with waste water over a period of years would help resolve the issue of cumulative effect of introduction of a range of pathogens at low concentration and plant disease development.

Industry

A draft of the Guidelines for the re-use of waste water from vegetable washing was distributed to all vegetable IDOs in 2001 for comment and feed back. Once comments were received, the guidelines were then incorporated into the field day notes and are presented in Appendix 1.

Acknowledgments

We acknowledge funding for this work from Horticulture Australia Limited, Department of Primary Industries Victoria (formerly the Department of Natural Resources and Environment) and AusVeg.

Many growers supported the project but in particular we are indebted to Mr Rocky Lamatina who provided advice and support for the project in the development stage.

We acknowledge the support of Dr Elaine Davison for the provision of *Pythium* cultures and the identification of isolates from waste water. Dr Davison and Dr Alan McKay also helped collecting water samples, arranged a grower's seminar in Perth and showed us carrot farms in the region.

We thank Fawzia Tawfik and Maresa Connell of the State Chemistry Laboratories, Werribee for her work on agrochemical detection in all water samples. I would like to acknowledge the work done by our collaborators in this national project. Their work was a key to the projects success.

The Industry Development Officers in each state contributed to the project, in particular Patrick Ulloa (Victoria) who helped with grower meetings and our technology transfer plan.

NSW Vegetable IDO Dr Alison Anderson assisted in planning the grower workshop in Cowra and provided transport from Sydney. Craig Feutrill SA Vegetable IDO organised the Virginia grower workshop. Judy Skilton, Executive Officer, Bundaberg Fruit & Vegetable Growers helped with the Bundaberg workshop. SallyAnn Henderson provided transport and drove us to carrot growers along the Murray River from Swan Hill to Mildura.

Dr Alison Anderson, NSW vegetable IDO and Joe Napoli of the Lachlan Valley Horticultural Network helped to organise the Cowra workshop and Samantha Hertiage and Julia Telford vegetable IDOs for Queensland help organise the Bundaburg workshop. The grower workshops were held in conjunction with a Mr Paul Harrup and Dr Robert Holmes who presented finding of their work on Clean and Safe Handling Systems for Vegetables and consequently, we shared the work load associated with the technology transfer package.

This project was largely dependant on the good will of the participating vegetable growers for access to farms and samples of their source and waste waters and to those that allowed detailed analysis of the performance of their waste water treatment systems. We thank all participating growers. Confidentiality agreements prevent me from naming them however, their participation was vital for the advancement of the industry through research and especially for the development of methods for the conservation of water within the vegetable industry.

If I have inadvertently left anyone out who should be acknowledged, I apologise. This project was a large team effort where all who participated showed enthusiasm and generosity of time and effort. Thank you.

Appendix I

Guidelines for the re-use of waste-water from vegetable washing

Why re-use vegetable waste-water?

In Australia each year about 4.42 million ML of water are used to wash carrots. In some instances this water is re-used for washing, and often it is disposed of by using it to irrigate crops, although sometimes it is simply flooded onto uncropped land.

Re-use of waste-water from vegetable washing is particularly important in drier areas where water is a highly valued resource, both environmentally and economically. For example, in the Sunraysia district of Northern Victoria, growers obtain their water from the Murray River. This river and its catchment have been under considerable pressure, and balancing the competing demands of agriculture and the environment is a topic of considerable debate. Growers pay to use this water; thus there are both economic and environmental incentives for its efficient use.

In our study of the quality of waste-water from the vegetable washing process, we used the carrot industry as a model. This industry uses particularly large volumes of water because soil has to be washed off the produce. The resulting 'dirty' waste-water can contain pathogens and is sometimes difficult to disinfect if highly loaded with organic matter. Thus, the carrot washing process can be seen as a worse case scenario when it comes to the quality of vegetable washing waste-water.

In these guidelines the following issues will be addressed:

- 1) potential spread of post-harvest diseases through the re-use of waste-water;
- 2) crop health issues related to the disposal of waste-water by means of crop irrigation;
- 3) potential food safety issues arising from re-use of vegetable waste-water;
- 4) methods of treating vegetable waste-water for re-use;
- 5) environmentally safe disposal of vegetable waste-water.

Contamination of food

Agrochemical residues

All of the agrochemicals in Table 1 were more frequently detected in waste-waters than source waters, suggesting that they entered the waste-water as a result of the soil removal process.

In Australia, there are prescribed levels for acceptable concentrations of agrochemicals in food—'The Food Standards Code' (National Food Authority 2001). The maximum residue limits (MRLs, maximum level of a chemical which is permitted to be present in food) of the most frequently detected agrochemicals in our survey are presented below.

Table 1. Maximum Residue Limits for some of the most commonly encountered agrochemicals in carrot waste-water. nsg = no specific guideline, therefore there must be no detectable residue in the product; * = all vegetables except asparagus, brassica vegetables, cassava, potato and tomato; ** = all vegetables except carrot, common bean (dry), lupin (dry), mung bean (dry), onion (bulb), potato, soya bean (dry), sweet corn (corn-on-the cob) and sweet potato.

conc. = $\mu\text{g/g}$	linuron	chlorpyrifos	prometryn	total endosulphan
carrot	nsg	nsg	nsg	200
brassica vegetables	nsg	500	nsg	nsg
asparagus	nsg	50	nsg	nsg
vegetables	50	10*	100	2000**

In our survey, most source and waste-waters contained few agrochemicals, and those that were present were typically found in very low concentrations. However, it is not possible from this information to determine if the low levels detected in the waste-water would lead to levels in the vegetable (e.g. carrot) in excess of the Food Standards Code levels, although specific studies would be need to confirm this. The four most commonly encountered agrochemicals were linuron, chlorpyrifos, prometryn and endosulphan sulphate.

Potential for spread of human pathogens

The presence of human pathogens in vegetable waste-waters was not measured directly in our study. Rather, we used an indicator bacterium, *Eschericia coli*. *E. coli*

belongs to a group of bacteria known as thermotolerant coliforms. Thermotolerant coliforms, including *E. coli*, are common in the gut of warm-blooded animals. Hence, their presence suggests that there is the potential for other pathogens to be present. According to the 'Guidelines for on-farm food safety for fresh produce' (Agriculture, Fisheries and Forestry – Australia 2001) the concentration of thermotolerant coliforms in farm irrigation water should not exceed 1000 cfu (colony forming units) / 100 mL, and the concentration of *E. coli* in produce should not exceed 20 cfu / g of produce. In our survey, we frequently found that in the waste-water the concentration of *E. coli* alone exceeded this recommended guideline for total thermotolerant coliforms.

Crop protection

Plant Pathogens

One of the primary concerns related to re-using vegetable washing wastewater for irrigation is that the practice may spread plant pathogens throughout a farm. If soil is washed off carrots from a diseased patch of ground, and this waste-water is used to irrigate various parts of the farm, then these pathogens can spread to previously uninfected areas.

The Australian and New Zealand Guidelines for Fresh and Marine Water Quality—henceforth referred to as 'Australian and New Zealand Water Guidelines'—(ANZECC & ARMCANZ 2000) have identified an urgent need for future research into the development of guidelines for acceptable levels of plant pathogens in irrigation water, particularly in intensive agricultural and horticultural industries where wastewaters are re-used. In our study we have taken the first step by identifying the most common plant pathogens found in vegetable wastewater.

In our survey of operations throughout Australia, wastewater was found to contain about ten times more fungi than source water. However, only some of these were potential plant pathogens. A list of the potential pathogens isolated, and the frequency at which they were found in source and waste-waters, is presented in Table 2. *Pythium*, the causative agent of cavity spot, was rarely isolated, although it was only found in waste-water.

Table 2. Potentially pathogenic fungi to carrots found in source and waste-waters. PH = potential postharvest pathogen; P = laboratory tests demonstrated these isolates to be pathogenic to carrots.

Fungus	disease	% source	% waste
<i>Alternaria alternata</i> ^P	potential PH pathogen	19	46
<i>Aspergillus niger</i>	black mould rot	31	46
<i>Fusarium sporotrichioides</i> ^P	PH pathogen	0	4
<i>Pythium</i> spp ^P .	cavity spot	4	15
<i>Rhizoctonia solani</i>	field root rot	8	0
<i>Rhizopus stolonifer</i>	rhizopus rot, minor PH rot	4	8

Because of the frequent presence of pathogens in waste-water, if this water is to be re-used then in most instances some form of treatment and disinfection will need to be applied. This treatment will not necessarily need to remove all plant pathogens, especially if the product is passed through a final disinfection rinse (e.g. chlorine). A reasonable objective would be to get the recycled water to a quality, with respect to plant pathogens, similar to that of water sourced from rivers and bores.

Agrochemicals in irrigation water

If waste-water is to be re-used for irrigation then we need to know if it contains any herbicides in concentrations that may be toxic to crop plants. Linuron is a herbicide that is commonly used for the control of weeds in carrots and other vegetable crops, and we frequently detected it in carrot waste-waters.

There are no Australian guidelines for the concentration of linuron, or any of the other agrochemicals we surveyed, in irrigation waters. However, in the Australian and New Zealand Water Guidelines (ANZECC & ARMCANZ 2000) interim guidelines are provided for the levels of several other herbicides for various crops.

Despite the lack of official Australian guidelines, it is still important to gain some appreciation as to what acceptable levels of linuron, the most common herbicide in carrot waste-water, could be. Therefore, we look at guidelines from other countries. In a worldwide survey of regulatory agencies (Caux *et al.* 1998) found that there were no irrigation water quality guidelines for the protection of non-target (i.e. crop) plants from linuron. They developed maximum acceptable toxicant concentrations for linuron based on previously published research on the toxicity of linuron to various crops, and these can be used for vegetable production in Australia. These maximum acceptable concentrations for various vegetables are presented below (Table 3). It is important to note that even though we

found linuron in almost half of the carrot waste-water samples, the concentrations were generally well below those likely to cause damage to, or reduce productivity of, carrots. The average concentration of linuron in the samples where it was detected was only 5.3 µg/L, and there was only one sample where the maximum acceptable concentration for carrots was exceeded. Nevertheless, the concentration of linuron in this sample was very high (34 µg/L), and thus it can be seen that there is the potential for toxic levels of linuron to be present in waste-water being re-used for irrigation. It should also be noted that other vegetables are much less tolerant to linuron than carrots (Table 3.). In particular, tomatoes are highly sensitive. Therefore, in some instances carrot waste-water may be able to be used to irrigate carrots but not other crops.

Table 3. Maximum acceptable concentrations of linuron in irrigation water for the protection of various crop species. From Caux *et al.* (1998). These values are based on high irrigation rates.

	maximum acceptable linuron concentration (µg/L)
carrot	12.4
lettuce	4.9
turnip	1.89
parsnip	8.9
cucumber	3.3
tomato	0.071

Salinity of recycled water used for irrigation

Salinity is a measure of the concentration of salts in either water or soil. Salinity can affect plants through either indirect means, namely the reduction in availability of water to the plant, and direct routes, such as toxicity of specific ions to the plant. The overall effect of salinity on crops is dependent on many factors including soil type, soil moisture, soil salinity and climate. For this reason, there are no specific Australian guideline levels for irrigation water salinity for crops. However, the Australian and New Zealand Water Guidelines provide methods for estimating the potential effect of saline irrigation water for a specific site. In our survey, salinity in carrot waste-water ranged from 87 to 3,000 µS/cm (average = 730 µS/cm). Soil salinities of the farms ranged from 70 to 660 µS/cm (average = 206 µS/cm). Using a formula from the Australian and New Zealand Water Guidelines for the

properties investigated in our survey revealed that in nearly all circumstances the waste-water could be used for irrigation without causing salinity stress to carrots and other vegetables.

A computer program called SALT PREDICT can also be used to calculate permissible site specific irrigation salinity levels. This program takes into account many factors such as soil type, water-table depth, rainfall and irrigation rates, so a site specific threshold level for irrigation water salinity can be calculated. This software is available with the Australian and New Zealand Water Quality Guidelines and through the Queensland Department of Natural Resources. For a simple estimate of threshold salinities for various crops for three basic soil types, Table 4.2.5 in the Australian and New Zealand Water Quality Guidelines can be consulted. According to this table, irrigation water salinity for carrots should not exceed 2,200, 1,200 or 700 $\mu\text{S}/\text{cm}$ for sand, loam and clay soils respectively. However, it must be recognised that these values are just estimates and do not take into account other site specific factors such as rainfall or salinity from a rising water table.

Treatment of waste-water

Chemical disinfection

The raw waste-water from nearly all operations was too turbid (i.e. unclear) to enable effective disinfection using standard chemical disinfectants such as chlorine (e.g. sodium hypochlorite or calcium hypochlorite). High turbidity is typically an indicator of high suspended solids organic matter in the water. This matter can bind to chlorine and thus reduce the amount available to act upon pathogenic micro-organisms. We found that passing water through a series of settling ponds (see below) reduced the turbidity substantially.

The active agent in chlorine disinfectants is hypochlorous acid. The proportion of hypochlorous acid in solution decreases with increasing pH. Thus, chlorine disinfectants are less effective at high pH levels. Only 50% of the chlorine is in the active form (hypochlorous acid) at pH 7.5, and this rapidly decreases with pH values greater than this. It therefore recommended that pH should at least be below 7.5 if hypochlorous acid based disinfectants are to be used. In our survey we found that the average pH of waste-water was 7.0 (range 6.1–7.7), with 87% of the samples being below 7.5. At pH 7 about 75 % of the chlorine is in the active form. Thus, in most situations carrot waste-water is suitable for chlorine disinfection, provided that the turbidity has been reduced substantially. Where water pH is above 7.5, but below 8.5, then a hypochlorous/hypobromous acid mix may be more effective as a disinfectant but still has the same limitation in the presence of high levels of organic matter. Another alternative is the use of chlorine dioxide which is less affected by high levels of organic matter and is still effective at a pH of 9.5–10. Disinfection using chlorine dioxide is recommended in particular for waste/dam water if the pH is higher than 7.5.

Settling Ponds

A series of at least 3 settling ponds may provide an effective means of reducing the turbidity carrot waste-water, and also for removing pathogens. The size and number of settling ponds will have a critical effect on the effectiveness of the system, although it will not be feasible in most situations to conduct engineering studies to calculate optimal pond size and number. This will depend on many factors, including flow rates, pond mixing hydraulics and soil type. Nevertheless, some generalisations can be made with respect to using settling ponds to treat waste-water.

A screen trap to remove coarse material such as leafy material and broken pieces of root crops is a useful addition to any system, as this will greatly reduce the amount of organic matter entering the pond system.

The first pond in the series may become anaerobic, that is, it may lack oxygen (or have very low levels). If the organic load and throughput of the water is not matched with the size of the system, then the following ponds in a series may also become anaerobic, and this is generally undesirable for the removal of human and plant pathogens. Anaerobic ponds have a distinctive smell, similar to rotten eggs, which results from the emission of hydrogen sulphide gas. Hydrogen sulphide is produced under anaerobic conditions by bacteria in the water. A simple method for preventing a pond from becoming anaerobic is to aerate it. This may be done by pumping water from the pond, then spraying it through a fine nozzle, through the air, before returning it to the pond. Similarly, aeration of ponds can be encouraged by passing water over a rock-fall or some type of waterfall as it enters each pond.

We have studied three different sized settling ponds systems, and of these, two were highly inefficient at removing the faecal indicator bacterium *E. coli* whereas the other was reasonably efficient, although not all *E. coli* were removed. Based on these findings, it cannot be assumed that settling ponds are efficient at removing potential human pathogens, and thus disinfection should also be considered. In addition, these systems were generally only partially effective at reducing total fungal loads, and thus disinfection may also reduce the likelihood spreading post-harvest or field pathogens.

Artificial wetlands

A potential alternative to settling ponds is to pass water through artificial or constructed wetlands. This involves passing water through a series of wetlands that contain reed beds. Such systems have been demonstrated to be particularly efficient at removing nutrients from the water. Recent work in Australia has demonstrated constructed wetlands to be useful for cleaning nursery run-off water so that it can be re-used, or disposed of to the environment with greatly reduced nutrient concentrations (Headley *et al.* 2001).

Sand filters

Sand filtration may provide an additional benefit, although the degree of this benefit will depend on the quality of the final pond water. We believe that most carrot wastewaters would be too high in suspended solids to enable sand filtration without some form of initial sedimentation. The standard sand filtration system we analysed was not particularly effective at removing plant pathogenic fungi from the water. However, the effectiveness of any system will depend on flow rates and on how long it has been since the sand has been changed. Slow sand filters have previously been demonstrated as being effective at reducing loads of pathogenic fungi (Barth 1997).

Disposal of wastewater from vegetable washing: environmental considerations

Agrochemicals

According to the Victorian State Environment Protection Policy (Environment Protection Act 1970, Waters of Victoria, Schedule D, D1) 'All farm effluents from intensive animal industries, milking sheds and vegetable washing and processing shall be disposed of by land irrigation in such a manner as to preclude any polluting run-off to surface waters or pollution of groundwater.' Whilst the legislative requirements may vary slightly from one state EPA to the next, we believe that it is good practice always ultimately dispose of vegetable waste by land irrigation, particularly if it has not undergone any treatment. Direct release of wastewater to waterways, particularly without treatment, has the potential to cause environmental impacts. For example, wastewater can contain high levels of nutrients, such as nitrogen and phosphorous from fertilisers, and the addition of these nutrients to surface waters can lead to 'eutrophication'—the prolific growth of algae and aquatic plants. Eutrophication can bring about drastic changes in the structure of the natural biological community, and often results in greatly reduced biodiversity. In this project we found that in some circumstances vegetable waste-water contained levels of nutrients that were theoretically capable of causing eutrophication. The movement of nutrients into groundwaters is more likely on farms with sandy soils and with water tables close to the surface. Waste-water should be irrigated in such a way that it does not leach or pass the root zone of the crops.

Agrochemicals in waste-water also have the potential to cause environmental damage, through direct toxicity to aquatic organisms. For example, linuron, a commonly used herbicide for control of weeds in carrot production (e.g. Afolan, Dualin, Lorox, Linuron technical, Linuron 50W, Clean crop linuron, Checkmate EC herbicide), is known to be toxic to many aquatic plants and animals. This chemical was detected in about half of the wastewater samples surveyed in this project. There are no Australian guidelines for acceptable levels of linuron. Furthermore, Caux *et al.* (1998) conducted a literature search of water quality guidelines throughout the world and found that there were no specific guidelines for linuron. They developed interim Canadian Water Quality Guidelines for linuron that we can also use as a guide. According to these guidelines, for the protection of aquatic life the concentration of linuron in fresh surface waters should not exceed 7 µg/L. With the exception of one sample, the concentration of linuron in carrot waste-water in this survey was 7 µg/L or less. In one circumstance a concentration of 34 µg/L was detected. However, this wastewater was not being discharged directly to surface waters. Furthermore, even in

situations where this is the case, the dilution of the waste-water in the receiving water body needs to be considered.

Other agrochemicals detected in wastewater samples were fenamiphos, chlorpyrifos, diazinon, dimethoate, malathion, trifluralin, dimethoate, metalaxyl, prometryn, linuro, alpha-endosulphan, beta-endosulphan and endosulphan sulphate. Of these, the most commonly detected were chloryriphos and prometryn which were found in 38% and 27% of the waste-water samples respectively. For many of these chemicals, there are Australian and New Zealand Water Guidelines for the protection of freshwater life. These guidelines work on the concept of 'trigger values'. A trigger value represents the concentration of a chemical below which there is minimal risk that ecological damage will occur. "They indicate a risk of impact if exceeded and should 'trigger' some action..." The guidelines also define different 'levels of protection', and in this circumstance it signifies the percentage of species expected to be protected (ANZECC & ARMCANZ 2000). The trigger values for different levels of protection for the chemicals detected in our survey are presented below in Table 4.

Table 4. Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC & ARMCANZ 2000): trigger values for agrochemicals detected in carrot waste-water.

	trigger values for freshwater (μgL^{-1})			
	level of protection (% species)			
	99%	95%	90%	80%
Chlorpyrifos ^B	0.00004	0.01	0.11	1.2
Diazinon	0.00003	0.01	0.2	2
Dimethoate	0.1	0.15	0.2	0.3
Malathion	0.002	0.05	0.2	1.1
Trifluralin ^B	2.6	4.4	6	9 ^A
Endosulphan ^B	0.03	0.2 ^A	0.6 ^A	1.8 ^A

A = Figure may not protect key test species from acute toxicity (and chronic); trigger value > acute toxicity value—see Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Section 8.3.7.

B = Chemicals for which possible bio-accumulation and secondary poisoning effects should be considered.

Of the 15 agrochemicals analysed for, chlorothalonil and phorate were the only ones that were not detected in any of the 26 waste-water samples.

Physical characteristics of waste-water

Other problems associated with the disposal of vegetable wastewater are high turbidity or lack of clarity of the water. In south-eastern and south-western Australia the recommended turbidity ranges for lowland rivers are 6–50 NTU and 10–20 NTU respectively. The levels of turbidity of the waste-water in this study were clearly well in excess of these levels. Thus, if vegetable waste-water was to be discharged directly into a waterway, then this could potentially be environmentally damaging, particularly in smaller waterways where the dilution effect would be less marked.

The addition of salts to waterways can also cause environmental problems. However, in our survey we found that on average the soil removal process did not greatly increase the salinity of the water (624 $\mu\text{S/cm}$ in source-water compared to 730 $\mu\text{S/cm}$ in waste-water). Nevertheless, care needs to be taken when disposing of saline waste-water, regardless of the origin of the salts.

Nutrients

The waste-water from the carrot washing process generally contains high concentrations of nitrogen and phosphorus. In nature, nitrite is a transient form of nitrogen and is usually only present in very low concentrations, if at all. Many commercial fertilisers, and chicken manure, contain very high concentrations of nitrite. Nitrate was about 2.6 times more concentrated in the source-water samples than the waste-water samples. Nitrate and nitrite concentrations are often reported as oxidised nitrogen, which is basically the sum of nitrate and nitrite. In south-eastern Australia total oxidised nitrogen concentrations in lowland rivers and freshwater lakes/reservoirs should not exceed 50 and 10 µg/L respectively (ANZECC & ARMCANZ 2000). Similarly, in south-western Australia total oxidised nitrogen should not exceed 150 and 10 µg/L in lowland rivers and freshwater lakes/reservoirs respectively. The concentrations of oxidised nitrogen in the carrot waste-waters was substantially higher than these values, but the concentrations in the source water were even higher. This suggests that overall, the carrot washing process is not adding to the oxidised nitrogen loading of the water. Whether or not the nitrogen, specifically nitrite, in carrot waste-water is likely to cause eutrophication of waterways in the environment would depend on several factors. Firstly, in most situations the waste-water is not discharged directly to a waterway. By disposing of the water by land irrigation some of the nitrogen may be removed. Soil can act as a natural filter. This is another reason for encouraging waste-water disposal by land irrigation. Secondly, the degree of dilution of the waste-water needs to be taken into account.

In south-eastern Australia total phosphorus concentrations in lowland rivers and freshwater lakes/reservoirs should not exceed 50 and 10 µg/L respectively (ANZECC & ARMCANZ 2000). Similarly, in south-western Australia total phosphorus concentrations should not exceed 65 and 10 µg/L in lowland rivers and freshwater lakes/reservoirs respectively. We could only measure phosphorus concentrations in three carrot waste-water samples, due to technical reasons (too many interfering substances in the water). In these samples the average total phosphorus and dissolved reactive phosphorus concentrations were 5,833 and 1,967 µg/L respectively. In contrast, the source-water concentrations for these samples were 6,200 and 1,533 µg/L respectively. Thus, unlike nitrogen, the concentration of total and of dissolved reactive phosphorus does not appear to change greatly as a result of the soil removal process, although further data would be needed to confirm this.

References

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