Was monochloramine responsible for widespread lettuce crop failures at a major recycled water irrigation scheme?

S. F. Barker, R. Faggian, J. Blackbeard, G. Hepworth and A. J. Hamilton

ABSTRACT

In 2008, vegetable growers observed stunted lettuce plants showing signs of chlorosis and wilting. It was suspected that monochloramine in the recycled water used for irrigation, in combination with extreme environmental conditions (high irrigation water salinity and extreme heat), was responsible for these crop failures. A series of glasshouse studies was conducted to evaluate the impact of monochloramine concentration alone on iceberg lettuce seedlings, as well as in combination with high salinity and hot ambient temperatures. Monochloramine concentrations up to 9 and 15 mg L^{-1} Cl_2 for continuous and initial irrigation only, respectively, did not affect the weight of iceberg lettuce heads ($p > 0.05$), while the combination of monochloramine (4–5 mg L^{-1} Cl_2) and salinity (3,500 μS cm^{-1}) did not significantly affect harvest measurements ($p > 0.05$). We therefore conclude that it is unlikely that monochloramine was responsible for the observed crop failures.

Key words | Australia, disinfectant, *Lactuca sativa*, lettuce, recycled water, Werribee

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INTRODUCTION

Wastewater irrigation has been practised for centuries and in recent years has gained importance as a critical irrigation source in many countries (Hamilton *et al.* 2007). While in many countries irrigation with untreated or minimally treated wastewater is common, often out of necessity, treatment of wastewater for irrigation of food crops is a standard practice. In countries such as the USA, Israel and Australia, wastewater is highly treated to comply with strict regulations for the irrigation of food crops. In Australia, wastewater use in agriculture has increased steadily over the last decade, and is now commonplace in Victoria, particularly in Melbourne's peri-urban vegetable growing areas. The Werribee Irrigation District (WID), approximately 30 km west of Melbourne's city centre, is an important vegetable growing region that receives recycled water from the Western Treatment Plant (WTP) (Melbourne's first large commercial recycled water scheme). While the scheme has been crucial to the continuing production of vegetables in the WID, it has also featured prominently in local debate over the fit-for-purpose status of the wastewater in general, and has struggled with a range of difficulties including production and supply to meet growing demand, as well as water quality, particularly salinity levels (Barker-Reid *et al.* 2010a, b).

doi: 10.2166/wrd.2013.019

The Werribee incident

In January 2008, 10 vegetable growers in the WID observed stunting of their iceberg lettuce crops, covering ~15 hectares (Rodda & Kent 2008), coinciding with a period of extreme heat and the use of both recycled water and high salinity river water. The primary symptoms included chlorosis or yellowing of leaves and stunting or lack of growth, while those plants that were severely affected were wilted with older leaves that had died and shrivelled (Taylor 2008). Symptoms were observed within 1 or 2 weeks of transplanting seedlings (rosette stage; first 2 weeks of January

2008), although the worst affected plants were transplanted between 1 and 3 January 2008. In these extreme cases, the plants failed to grow, turned yellow and wilted. Distribution of affected plants was patchy, with symptomatic plants interspersed between healthy plants. This was the second reported lettuce crop failure (the first, in 2006, affected 15 growers over 50 hectares) and in both instances there was widespread stunting of iceberg lettuce crops and some degree of patchiness (other crops in this area, such as cauliflower, were affected to a lesser extent). The 2006 investigation was inconclusive, although results were hampered by the delay in commencing the investigation (DPI 2007).

At the time of the 2008 incident, there were several conditions that might have affected plant health, including the use of high salinity river water, high ambient temperatures (multiple days $>35\,^{\circ}$C), low relative humidity (13% daytime minimum; BOM 2009) and monochloraminated recycled water. Growers in the WID typically have access to surface water (Werribee River water from the Melton Reservoir), groundwater and recycled water from the WTP. River and recycled water are delivered to irrigators through the same network of channels on alternate days and stored (and therefore mixed) in dams for multiple days. Since July 2007, there has been a full ban on groundwater extraction (Rodda & Kent 2008) and river water allocations have been exceedingly low. Saline ephemeral tributaries flow into the Werribee River and, during periods of low river flow, contribute to a significant increase in salt load. In early January 2008, river water was released into the irrigation system and, due to the accumulation of salts, water provided to growers was highly saline, particularly in early January 2008 (up to 3,210 μS cm^{-1} on 3 January 2008; Werribee Weir, site code 231204, www.vicwaterdata.net). While the WTP typically operates in free chlorine mode, during this period ammonia levels exceeded treatment plant capacity for free chlorine mode and therefore the treated water was chloraminated. Growers that reported lettuce crop failures had access to both recycled and river water, while those that had access only to river water did not report any symptoms. An investigation of the incident concluded that, based on observed plant symptoms, plant and soil analyses and the extreme environmental conditions at the time, it was likely that a combination of factors, namely high salinity water, hot ambient temperatures and plant root damage caused by monochloramine in the recycled water, caused the observed plant symptoms (Taylor 2008).

Wastewater treatment and chloramination

To comply with microbiological guidelines for irrigation water quality, wastewater is often disinfected with chlorine. Chlorination is a complex chemical process that is affected by a wide range of environmental parameters such as pH, temperature and quality of the water being treated (Tchobanoglous *et al.* 2003). Chlorination is popular because of its relatively low cost and its long history of effectiveness. Of the chlorine that is added to water, a portion is consumed or reduced by oxidation or substitution reactions with inorganic and organic materials; these reactions represent the chlorine demand of the water. Any chlorine that remains is 'residual chlorine' and wastewater treatment plants often have a target chlorine residual that must be met during the treatment process. When ammonia is present in wastewater, it reacts with chlorine to form monochloramine. Monochloramine is widely used as an alternative to chlorine for the disinfection of drinking water as it is more persistent and provides a longer disinfection time than free chlorine. In wastewater treatment, ammonia is often present in high concentrations and the formation of monochloramine may occur unintentionally if ammonia levels exceed treatment capacity.

While there is some understanding of the toxicity of chlorine to terrestrial plants, there is little research that has specifically evaluated monochloramine. Datnoff *et al.* (1987) found that chlorine was phytotoxic to cabbages at 200 mg Cl L^{-1} (total chlorine) while Carillo *et al.* (1996) found that, using chlorine dioxide, a concentration of 26 mg L^{-1} free chlorine was toxic to radish and lettuce seedlings. In experiments where soil-less media was used, growth of capsicum and tomato declined at 8 mg L^{-1} free chlorine while lettuce declined at 18 mg L^{-1} (Frink & Bugbee 1987). This experiment also found that germination of vegetable seeds was not affected by chlorine treatments.

Research into the effects of monochloramine on plants is limited to a series of hydroponic studies published by a team in Japan (Date *et al.* 1995, 1999, 2002, 2005) and a significant report published by the Urban Water Research

Association of Australia (UWRAA 1990). Date *et al.* (1999) found that regardless of nutrient solution or environmental conditions, the addition of sodium hypochlorite (thus forming chloramine in hydroponic solution) decreased lettuce growth rates and caused root browning and wilting of plants. In 2002, they were able to verify that lettuce root browning was caused by solutions containing both hypochlorous acid and ammonium ions, concluding that it was the formation of chloramine (presumed to be predominantly monochloramine) that caused the root browning (Date *et al.* 2002). Further investigation refined these results, reporting that at chloramine concentrations >0.21 mg Cl L^{-1} lettuce seedlings exhibited intensive root browning, slight wilting of outer leaves and significant reduction in growth rate, while at chloramine concentrations >0.28 mg Cl L^{-1}, most mature leaves wilted completely; as the concentration of chloramine increased, the severity of wilting and degree of growth inhibition increased (Date *et al.* 2005).

Research conducted by the UWRAA (1990) demonstrated that the dry weight of wheat and peas was not significantly affected by foliar applications of monochloramine up to 71 mg L^{-1}. Soil applications of monochloramine had limited effect on plant dry weight and the authors reported that, for plants grown in fine sandy clay loam, only the highest monochloramine concentration (225 mg Cl_2 L^{-1}) decreased dry weight. For plants grown in a sandy soil, lower concentrations appeared to decrease plant dry weight. The authors reported that the dry weight of peas and wheat decreased at concentrations of 22 mg Cl_2 L^{-1} and greater, although they indicated that iron deficiency in peas may have contributed to the lower plant dry weights at concentrations down to 2.2 mg Cl_2 L^{-1}. Unfortunately, the UWRAA report did not provide any statistical analysis for the soil applications of monochloramine; therefore the above-mentioned decreases in plant dry weights may not be significant.

Victorian guidelines (Australia) for the use of recycled water stipulate a limit of <1 mg L^{-1} chlorine residual (free chlorine) at the point of application, but no value is provided for monochloramine (EPAV 2003). Australian national guidelines provide a target for irrigation water of 1–5 mg L^{-1} of chloramine or free chlorine, depending on the plants grown (NRMMC *et al.* 2006) while the USEPA guidelines for water reuse (USEPA 2004) indicate that <1 mg L^{-1} of free chlorine residual should not pose problems to plants (no reference to monochloramine is given). In recycled water suitable for vegetable irrigation, a target of 3–5 mg L^{-1} monochloramine has been set (EPAV 2003), resulting in a likely monochloramine concentration at the point of use of 1 mg L^{-1} (EPAV 2004).

Due to the paucity of research on the impacts of monochloramine on plant health, a series of glasshouse studies was conducted to determine whether the overhead irrigation of lettuce plants with monochloramine-containing water could result in similar symptoms to those observed in the WID.

MATERIALS AND METHODS

To evaluate the impact of monochloramine on iceberg lettuce (*Lactuca sativa* L.), two glasshouse experiments were conducted. The monochloramine dose-response experiment evaluated a range of monochloramine concentrations in an effort to determine the maximum concentration with no impact on plant health, and the monochloramine + salinity experiment evaluated the combined effects of monochloramine with high salinity irrigation water.

Decay of monochloramine in growth media

In an effort to replicate field conditions of the 2008 lettuce crop failure, an initial evaluation of growth media was conducted to determine an appropriate mixture that closely represented Werribee soils (Red Sodosol) but allowed sufficient drainage in a pot system. Four different types of growth media – Werribee soil, coarse sand, standard potting mix (a mixture of fine sand and composted pine bark) and 50:50 soil:sand mixture – were evaluated with a range of monochloramine solutions (24–25 mg L^{-1} Cl_2) using a modification of the standard soil/water extract method (method 3A1; Rayment & Higginson 1992). Approximately 10 mL of growth medium was added to a 50 mL Falcon tube and then the tube was filled to 15 mL with monochloramine solution. Each tube was shaken by hand ten times and then centrifuged at 14,000 rpm for 30 min at 25 °C. Supernatant was then removed to another Falcon tube and centrifuged for a further 30 min; the monochloramine concentration of the resulting supernatant was measured using an indophenol method. Samples of Werribee soil and the 50:50 mixture were analysed for total C, total N and organic matter (Table 1).

Very little residual monochloramine was detected in growth medium solution extracts, even with high starting concentrations of monochloramine (Table 2). Vikesland *et al.* (1998) found an increase in the loss of monochloramine with increasing concentrations of natural organic matter. Similar results were observed in this study; the lowest rate of decay (55%) occurred with sand, which had negligible organic matter content. Werribee soil had 3.45% organic matter and experienced 99% decay, while the 50:50 mixture had 1.45% organic matter and 98% decay. The potting mix results were very similar to those of Werribee soil. Based on these results, we selected the 50:50 mixture as the growth medium for all experiments as it approximated the rate of monochloramine decay while providing sufficient drainage to prevent waterlogging in pots.

Crop management

The studies were conducted in a controlled environment growth room, where light and temperature were programmed to simulate the environmental conditions of the WID in January 2008, with high temperatures immediately after transplanting and a 16-hour photoperiod. Temperature and humidity were recorded every 5 min with a Lascar High Accuracy Humidity and Temperature USB Logger (EL-USB-2 + ; MicroDAQ.com, Ltd, Contoocook, NH, USA). Lettuce seedlings, sourced from Boomaroo Nurseries (a major supplier of seedlings to the WID), were transplanted into individual 20-cm diameter pots with ~6 kg (dry weight) of a 50:50 mix (by volume) of coarse sand and Werribee soil (unsterilised surface soil collected from the site of a former recycled water irrigation trial; Engleitner *et al.* 2008). A controlled release complete fertiliser with trace elements (Osmocote® Plus Pots, Planters and Indoors; N:P:K of 15:4.4:10) was applied at the recommended rate (23 g per 20-cm diameter pot) immediately after the initial irrigation. Pots were irrigated with dechlorinated tap water to saturate the soil prior to transplanting seedlings. Saucers were placed under each pot to enable soil saturation and to ensure that root exposure to monochloramine treatments (if any) was prolonged. Irrigation rates were informed by standard district practice and evapotranspiration rates at the time of the 2008 incident (between 4.7 and 8.8 mm day^{-1}; BOM 2009). Irrigation treatments were applied using manual overhead irrigation to simulate overhead irrigation with sprinklers, ensuring that all water was captured by plant surfaces and the growth medium.

Table 1 | Analytical results for growth media

Growth medium	Total C (g 100 g^{-1})	Total N (g 100 g^{-1})	Organic matter (g 100 g^{-1})
Werribee soil	1.90	0.20	3.45
50:50 soil:sand mixture	0.78	0.08	1.45

Preparation and analysis of water treatments

Secondary-treated (waste stabilisation ponds and activated sludge treatment) recycled water was sourced from the WTP and manually chloraminated to the concentration levels specified for each trial. Monochloramine is more stable in water with pH ≥ 7, with the fastest reaction time (conversion of hypochlorous acid to monochloramine) at a pH of ~8.4 (Kirmeyer *et al.* 2004). All batches of recycled water exceeded pH 7; therefore, no pH adjustment was required. A Hach DR 2800 spectrophotometer (USA Hach Company, Loveland, Colorado, USA) was used to test each water sample using two different methods to measure monochloramine concentration. Test kits for total and free

Table 2 | Monochloramine decay in growth medium solution extracts (mean ± standard deviation) for $n = 6$. Decay (%) is defined as the difference between initial and final concentrations, divided by the initial concentration

	Werribee soil	50:50 soil:sand	Sand	Potting mix
Residual monochloramine (mg L^{-1} Cl_2)	0.2 ± 0.1	0.6 ± 0.3	11.0 ± 0.7	0.2 ± 0.1
Decay (%)	99.1 ± 0.3	97.6 ± 1.5	55.0 ± 2.9	99.2 ± 0.6

chlorine (Hach methods 8167 and 8021 respectively; www.hach.com), equivalent to Standard Method 4500-Cl G for drinking water and wastewater analyses (Eaton *et al.* 2005), were used and it was assumed that the difference between total and free chlorine represented predominantly monochloramine. We also used a monochloramine-specific test kit that uses an indophenol method (Hach chloramine (mono) indophenol method 10172; www.hach.com) and avoids measurement of organic monochloramines; this test was used primarily to confirm the results of the former test. Dilute solutions of ammonia (Merck GR analytical grade, Kilsyth, Victoria, Australia; ~0.1% NH_3) and sodium hypochlorite (Chem-Supply, Port Adelaide, South Australia, Australia; ~1,000 mg L^{-1} Cl_2) were prepared with MilliQ water and stored at 4 °C. In the dose-response experiment, dilute ammonia and sodium hypochlorite were added to vigorously stirred recycled water to form monochloramine of the required concentration. In the monochloramine + salinity experiment, stock monochloramine solutions were prepared by adding dilute ammonia and sodium hypochlorite to vigorously stirred recycled water and stored at 4 °C. Treatment solutions were prepared by adding stock solution to vigorously stirred recycled water to achieve the required monochloramine concentration. Samples were allowed to mix for a minimum of 1 hour prior to irrigation.

While monochloramine is more stable than free chlorine, it is inherently unstable (Vikesland *et al.* 1998) and can be affected by a wide range of chemical and environmental parameters including pH, temperature, light and natural organic matter (UWRAA 1990; Kirmeyer *et al.* 2004). Preliminary laboratory experiments (conducted at room temperature and under normal daylight conditions) demonstrated a decay rate of >2 mg L^{-1} Cl_2 in the first 24 hours for a 5 mg L^{-1} Cl_2 starting solution while decay rates for 15 mg L^{-1} Cl_2 solutions were >5 mg L^{-1} Cl_2. Therefore, irrigation treatments were applied in such a way as to minimise any effect of decay over the duration of the irrigation event. In the dose-response experiment, all water treatments were prepared and tested immediately prior to irrigation to minimise decay. This meant that water treatments were not applied one block at a time but rather one treatment at a time. While this situation was not ideal, it was unavoidable due to the large number of treatments and the long time periods needed to prepare the treated water. In the monochloramine + salinity experiment, the small number of treatments meant that all water samples could be prepared at one time and therefore irrigation treatments were applied by block.

Salinity treatments were prepared by adding sodium chloride (Chem-Supply Analytical Reagent) to recycled water to attain the specified concentration. Salinity (as electrical conductivity), pH and temperature were recorded for all water samples using a hand-held meter (TPS WP-81 pH-cond-salinity; Microdaq.com, Ltd, Contoocook, NH, USA).

Plant measurements

At harvest, lettuce heads were cut at the base and weighed. Roots were gently teased from the growth medium ('intact roots') and washed with tap water while roots remaining in the growth medium were manually removed and washed ('root pieces'). Both intact and root pieces were weighed. Plant material (lettuce heads and roots) was placed into separate paper bags, dried at 70 °C for 48 hours and then reweighed.

Monochloramine dose-response experiment

This trial investigated a range of monochloramine concentrations to determine the critical concentration for observable impacts on lettuce seedlings. Treatments included nine monochloramine concentrations and two different dosing methods: (1) application of chloraminated recycled water for all irrigation events ('continuous dosing') and (2) a single application of chloraminated recycled water at transplanting ('spike'), followed by irrigation with MilliQ (deionised) water. The treatment combinations (and monochloramine concentrations in mg L^{-1} Cl_2) were as follows: continuous dosing (0.5, 1, 2, 3, 5, 9), spike (1, 2, 3, 5, 7, 9, 12, 15) and controls (recycled water – no monochloramine added, MilliQ – deionised water).

Monochloramine concentrations were selected based on concentration guidelines provided by Southern Rural Water (maximum monochloramine concentration of 5 mg L^{-1} Cl_2; EPAV 2004) and the results of previous trials on lettuce (hydroponics) that found very low level impacts

(Date *et al.* 2002, 2005). The highest concentrations were used to reflect the extreme worst-case scenario in the spike concentrations. There were 16 treatment combinations and four replicates of each treatment, resulting in 64 lettuce plants. The treatments were arranged in a lattice design which included blocking (to account for any impact from the opening and closing of the growth room door) and randomised location of treatments, thus ensuring no two treatments were co-located more than once. Lettuce seedlings (cv. 'Seagull') were transplanted on 24 March 2009 and grown for 15 days. Harvest measurements were carried out over 4 days between 8 and 11 April 2009 (15–18 days post-transplant). All harvest measurements were conducted one block per day and no further irrigations were applied (plants showed no visible signs of water stress).

Growth room conditions (temperature and humidity) are presented in Figure 1, showing sporadic elevated temperatures, designed to reflect environmental conditions in Werribee in January 2008. A computing failure resulted in a loss of logged temperature and humidity data from 27 March 15:00 to 2 April 12:30. Temperatures were manually checked at least daily; therefore, temperature and humidity profiles have been infilled from other dates based on the temperature program during that period. All environmental control equipment functioned normally during the experiment.

Irrigation treatments were prepared immediately prior to application, and monochloramine concentration, pH, salinity and temperature were recorded (Figure 2). Water quality parameters varied from day to day but monochloramine treatments remained discrete. Solution pH remained between 8 and 9, ideal for the formation of monochloramine. Salinity was very similar between treatments on any given day and temperatures remained between 15 and 25 °C.

Monochloramine + salinity experiment

Based on results from the dose-response experiment, the monochloramine + salinity experiment was designed to determine if the combination of high temperature, salinity and monochloramine concentration affected plant health. The trial used a Latin square design, with four treatment combinations in a 2 × 2 factorial arrangement and 16 replicates of each treatment, for a total of 64 lettuce plants. The treatments were as follows:

1. Recycled water (~2,500 μS cm^{-1} as received), no monochloramine
2. Recycled water (~2,500 μS cm^{-1} as received), 4–5 mg L^{-1} monochloramine as Cl_2
3. Recycled water (3,500 μS cm^{-1}), no monochloramine

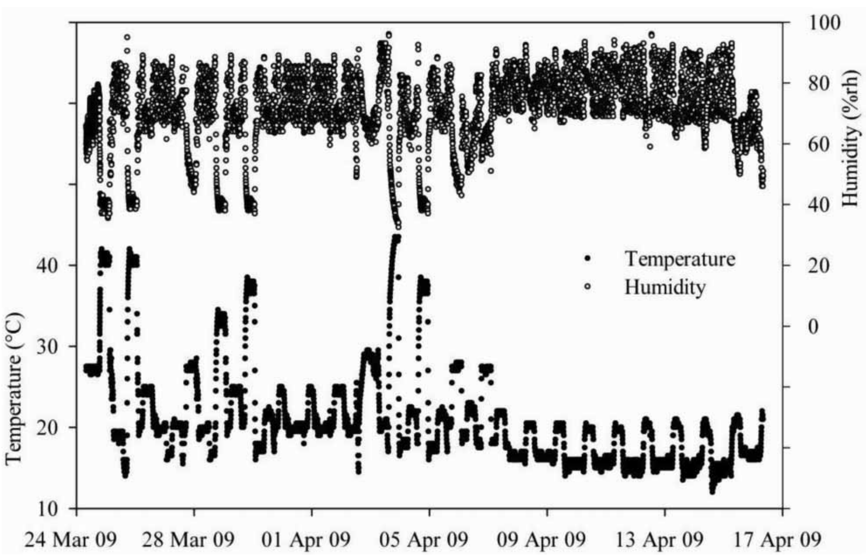

Figure 1 | Temperature and humidity profile from the monochloramine dose-response experiment. Data between 27 March 15:00 and 2 April 12:30 were infilled from other profiles, based on the temperature program.

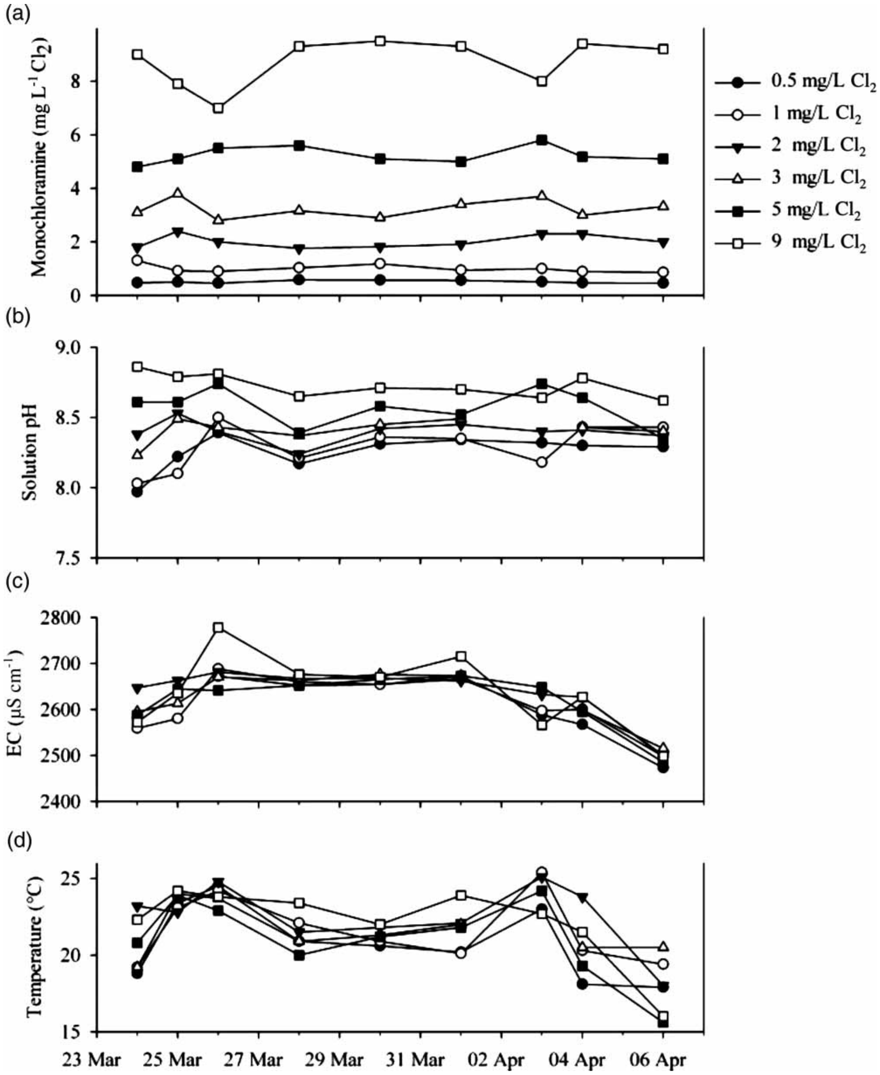

Figure 2 | Irrigation water quality for the monochloramine dose-response experiment: (a) concentration of monochloramine (mg L^{-1} Cl_2), (b) solution pH, (c) salinity (µS cm^{-1}) and (d) temperature (°C) for the duration of the trial.

4. Recycled water (3,500 µS cm^{-1}), 4–5 mg L^{-1} monochloramine as Cl_2

Growth room conditions (temperature and humidity) were similar to those presented in Figure 1 showing high-temperature events at the start of the trial and then temperate conditions (~28 °C daily maximum, ~20 °C daily minimum). Irrigation treatments were prepared immediately prior to application, and monochloramine concentration, pH, salinity and temperature were recorded (Table 3). Elevated salinity and monochloramine levels were selected to match concentrations reported during the January 2008 incident. Iceberg lettuce seedlings (cv. Marksman) were transplanted on 22 April 2009 and irrigated immediately after transplanting (one block at a time). Marksman is not a typical warm weather variety, but it was the best option available at that time of year. Irrigation treatments were applied for 1 week, after which all plants were irrigated with recycled water (as received). Lettuce heads were harvested on 26 May 2009. All pots with intact roots were kept at 10 °C prior to root harvest and measurement to minimise any

Table 3 | Mean solution characteristics (±standard deviation) during first week of the monochloramine + salinity experiment ($n = 5$)

Treatment	pH	Salinity (μS cm^{-1})	Temperature (°C)	Monochloramine (mg L^{-1} Cl_2)
Recycled	7.98 ± 0.32	2,297 ± 60	19.9 ± 5.7	na
Recycled + Cl_2	8.51 ± 0.28	2,211 ± 33	15.2 ± 1.7	4.76 ± 0.18
Recycled + salt	7.98 ± 0.09	3,300 ± 7	13.6 ± 2.0	na
Recycled + salt + Cl_2	8.45 ± 0.21	3,308 ± 8	16.7 ± 1.8	4.78 ± 0.22

na = not applicable.

degradation of roots over the 3 days of harvesting. Root harvesting was conducted from 27 to 29 May 2009 (35–37 days post-transplant).

Data analysis

All data were analysed with GenStat software (11th edition; VSN International, Hemel Hempstead, UK) using analysis of variance, and post hoc comparisons between means were made using Fisher's least significant difference. For each experiment, all data were analysed together such that in the dose-response trial both continuous and spike treatments were analysed together. Transformation of data (log_{10}) was required to stabilise the variance for the monochloramine + salinity experiment measurements of head and root weights, but all analyses of the dose-response experiment were performed on the raw data.

RESULTS

Monochloramine dose-response experiment

All plants grew rapidly, regardless of treatment. In January 2008, symptoms of the lettuce crop failure (stunting, chlorosis and wilting) were observed very quickly after transplanting. Therefore, when similar symptoms were not observed within 2 weeks of transplanting, there was little to be gained from continuing the experiment through to commercial harvest size. Significant differences were detected in fresh weights of intact roots ($p = 0.043$) and all roots ($p = 0.019$), but there was no clear trend in post-hoc differences between means (Figure 3). There were no significant differences ($p > 0.05$) between any other harvest measurements.

Monochloramine + salinity experiment

Plant growth was much less vigorous than in the dose-response experiment. No obvious treatment differences were observed throughout the duration of the experiment. In late May it became apparent that lettuce plants were suffering some kind of infection (most likely soil-borne); therefore, lettuce heads were harvested immediately on 1 day. Root harvesting revealed very limited root development and unusual nodule-like growths on many roots although the cause could not be determined. There was no significant treatment effect ($p > 0.05$) on any harvest measurements (Figure 4).

DISCUSSION

Monochloramine concentrations up to 9 mg L^{-1} Cl_2 (continuous irrigation) and 15 mg L^{-1} Cl_2 (initial irrigation only) did not affect the weight of iceberg lettuce heads grown in a sand:soil mixture and did not induce stunting similar to that observed in field-grown crops in the WID. We had hypothesised that lettuce seedlings might be more sensitive to monochloramine than 1-month-old pea or wheat plants (UWRAA 1990), but our results are similar to those reported by the UWRAA, where concentrations <22 mg L^{-1} Cl_2 had no effect on plant health. While previous experiments investigating the impact of chlorine on lettuce found higher thresholds (26 (Carillo *et al.* 1996) and 18 (Frink & Bugbee 1987) mg L^{-1} free chlorine), the maximum monochloramine concentrations investigated in this experiment were based on an estimated worst-case scenario for the recycled water, making higher concentrations irrelevant. A threshold monochloramine concentration for iceberg lettuce was not determined.

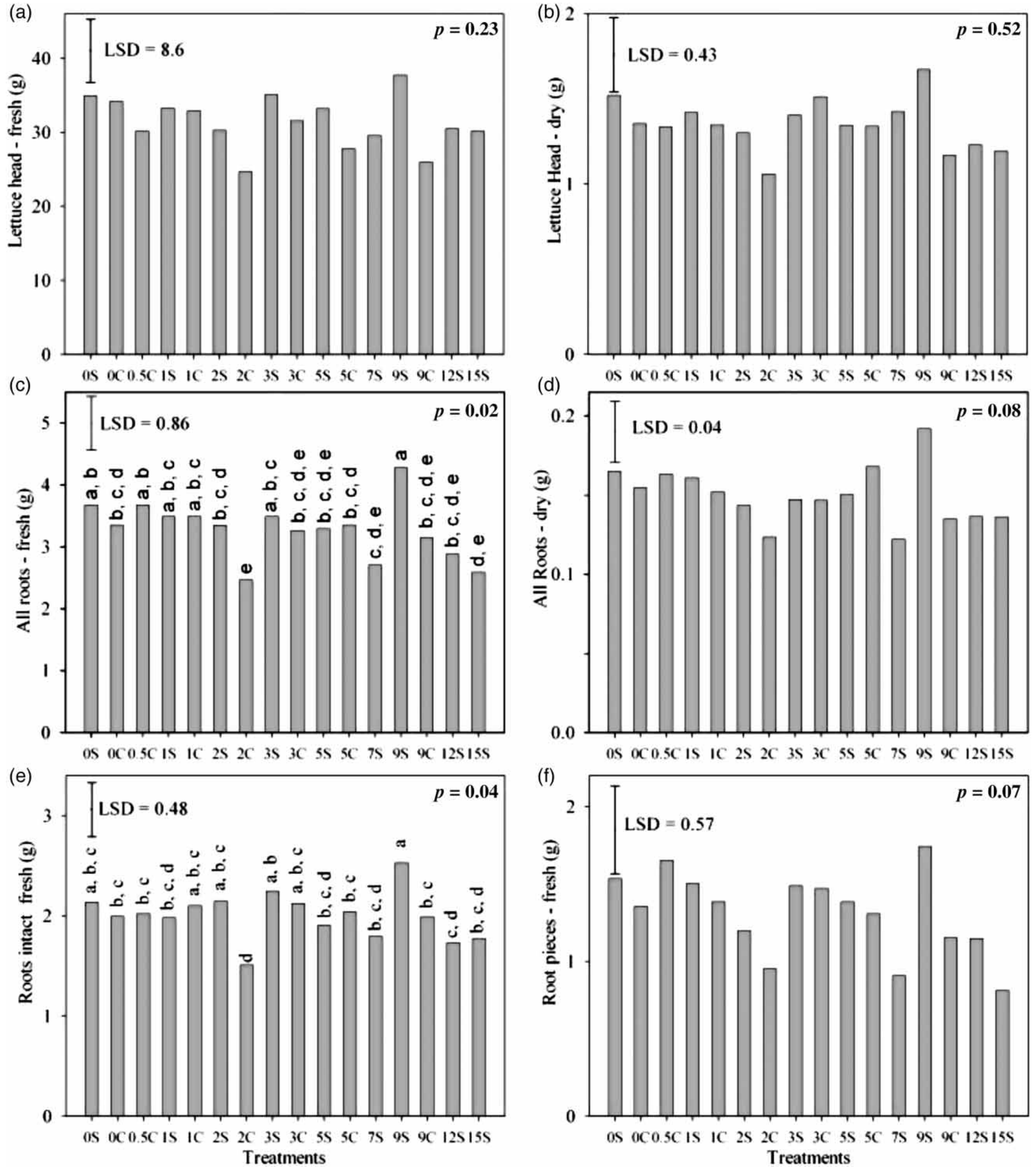

Figure 3 | Harvest weights (g) for the monochloramine dose-response experiment: (a) lettuce head fresh and (b) dry weight, (c) all roots fresh and (d) dry weight, (e) intact roots and (f) root pieces. Note for the treatments, *S* refers to spiked treatments, *C* refers to the continuous treatment and the number is the monochloramine concentration mg L^{-1} Cl_2. *p* refers to the *p*-value for testing the hypothesis of no difference between the treatment means, and for $p \leq 0.05$, significant differences between treatments occur where bars have different letters.

The monochloramine + salinity experiment attempted to mimic the conditions of the 2008 lettuce crop failure by applying highly saline, chloraminated water under high temperature conditions. We had anticipated that, regardless of chloramine treatment, a salinity effect would be observed. Grattan (2002) reported an irrigation

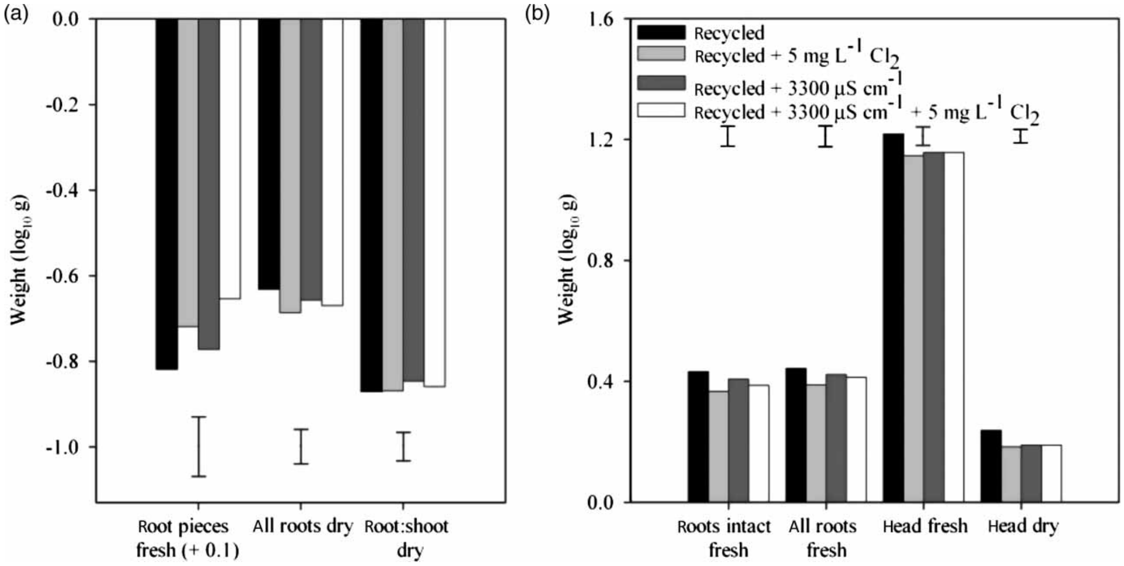

Figure 4 | Mean harvest measurements from the monochloramine + salinity experiment. Weight (log_{10} g) of (a) root pieces fresh (+0.1), roots dry, and root:shoot ratio dry; and (b) roots intact fresh, all roots fresh, head fresh and head dry. Error bars represent least significant difference.

water salinity threshold for lettuce of 900 μS cm^{-1}, where irrigation water was used continuously to achieve a leaching fraction of 15–20%, resulting in the maximum soil salinity level that the crop can tolerate without a decline in yield (1,300 μS cm^{-1}). All treatments exceeded the irrigation salinity threshold, but no differences related to salinity level were observed. It is likely that the trial was not of sufficient duration to significantly elevate soil salinity levels (the primary driver of reduced yields). Plant growth was much less vigorous across all treatments, likely due to the fact that the lettuce planted was a cool weather variety (Marksman), less suited to warm temperatures. Nonetheless, symptoms of chlorosis and wilting of leaves were not widely observed. While roots displayed unusual symptoms (such as short laterals and strange nodules), these were not related to the applied treatments and were likely caused by an unidentified soil-borne plant pathogen.

The fresh weight of roots (all and intact) from the dose-response experiment showed a significant treatment effect that did not correspond to increasing concentrations of monochloramine (Figure 3). On reflection, it is possible that this apparently random effect is an artefact of the water treatment method used in this experiment. Individual aliquots of ammonia and chlorine were added to recycled water to prepare solutions for each treatment. It is possible that mixtures had differing concentrations of unreacted ammonia that may have provided a varying fertilisation effect, although fertiliser was applied to all treatments so this effect is likely to have been minimal. The ammonia concentration was not measured as it was not expected to vary; therefore this theory cannot be confirmed.

While every attempt was made to mimic conditions experienced by growers in early 2008, we were unable to fully replicate hot, drying winds; therefore, it is likely that we inadequately represented the extreme transpiration conditions experienced by lettuce plants. It is possible that such conditions might alter the lettuce plant reaction to monochloramine. Further research should investigate the impact of transpiration rate on the impact of monochloramine on lettuce plants. As well, the interaction of soil organic matter and monochloramine decay should be further evaluated, particularly in light of vegetable production on sandy soils.

The results of these trials indicate that monochloramine, at concentrations commonly used for wastewater treatment, does not affect the health of iceberg lettuce seedlings, alone or in combination with high salinity.

CONCLUSIONS

This trial was conducted to determine whether monochloramine in recycled water could have been responsible for lettuce crop failures observed on commercial farms in the WID. Lettuce head weight was not affected by monochloramine concentrations up to 15 mg L^{-1} Cl_2, and the combined effects of monochloramine and high salinity irrigation water did not significantly affect harvest measurements. Results obtained from this study demonstrated that monochloramine should not affect the growth of iceberg lettuce seedlings, grown in soil, at typical water treatment concentrations (up to 5 mg L^{-1} Cl_2). Overall, the results of this study suggest that it is unlikely that monochloramine was a contributory agent, let alone the primary cause, of the lettuce crop failures experienced by Werribee vegetable growers.

ACKNOWLEDGEMENTS

This research was partially supported by Melbourne Water, The University of Melbourne's Warren Clark Scholarship and the Victorian Department of Primary Industries Post Graduate Program. Heritage Victoria is thanked for supplying all the funds for the purchase of the Hach DR 2800 spectrophotometer. The authors would also like to thank Yvonne Rogers for her assistance in conducting harvest measurements, Boomaroo Nurseries for providing the lettuce seedlings, Clinton Rodda for his advice and input, and Ben Pratt for his technical assistance.

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First received 15 January 2012; accepted in revised form 1 August 2012. Available online 27 February 2013