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Winery wastewater inhibits seed germination and vegetative growth of common crop species

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ABSTRACT

The ability to reuse winery wastewater would be of significant benefit to the wine industry, as it could potentially be a cost-effective method of wastewater management, whilst at the same time providing a valuable water resource. This study investigated the effects of different dilutions of a semi-synthetic winery wastewater on the growth and germination of four common crop species in a glasshouse study; barley (*Hordeum vulgare*), millet (*Pennisetum glaucum*), lucerne (*Medicago sativa*) and phalaris (*Phalaris aquatica*). The wastewater caused a significant delay in the germination of lucerne, millet and phalaris, although overall germination percentage of all species was not affected. Vegetative growth was significantly reduced in all species, with millet being the most severely affected. The germination index of barley correlated very highly ($r^2 = 0.99$) with barley biomass, indicating that barley seed germination bioassays are highly relevant to plant growth, and therefore may be of use as a bioassay for winery wastewater toxicity.

1. Introduction

Worldwide agricultural production is likely to increase in coming years to meet the needs of an expanding global population [1]. This increase in population will further add to the pressure on already over-utilised water resources, from urban, industrial and agricultural practices. This situation is likely to be further exacerbated in many regions of the world, with current climate projections predicting that many regions of the world will have lower and more sporadic rainfall, and increased severity of droughts [2]. Together, these factors are major driving forces behind efforts to improve water use efficiency in all sectors, including the agriculture sector.

Processing of raw agricultural products creates significant wastewater streams, and the effective management of these wastes is perceived as a major component of achieving agricultural sustainability [3]. One such wastewater stream is the processing of grapes to wine. It has been estimated that the annual worldwide production of wine was 2.6–3.0 billion L between 2003 and 2007 [4]. Whilst the amount of winery wastewater (WWW) produced varies greatly between different wineries, average values are ca. 1.6–2.0 L/L wine [5], indicating that winery wastewater is a major wastewater stream.

Winery wastewater has a high organic load, generally low pH, high salinity and low nutrient levels, all of which indicate that the wastewater poses an environmental risk. Winery wastewater arises mostly from cleaning operations within the winery, and therefore primarily contains wine, grape juice and solids (vintage season only) and cleaning agents. The carrier for these wastes is water sourced from the mains, groundwater or rainwater. The wastewater in most wineries is of high organic strength, containing, in approximate order of abundance sugars, organic acids (acetic, tartaric, propionic), esters and polyphenols [6]. Inorganic ions present are predominantly potassium and sodium, with low levels of calcium and magnesium [7].

The combination of high organics and inorganics, as well as the variability in wastewater composition, makes the treatment of winery wastewater challenging [8]. There are numerous different treatment options for winery wastewater, but all aim to achieve a significant reduction in the level of organic matter and solids, and some also to reduce inorganic load. Whilst there are several effective treatment options available, costs associated with the construction, maintenance and operation of such facilities are often seen as prohibitive, especially for smaller wineries [8].

Abbreviations: GI, germination index; MTG, mean time to germination; RDW, root dry weight; SDW, shoot dry weight; WWW, winery wastewater.

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Consequently, most winery wastewater, treated to greater or lesser degrees, is disposed of either by way of municipal sewerage in populated areas, or to land irrigation in rural areas [6].

Land disposal of winery wastewater onto field crops, tree lots, pastures and vineyards is a widely used means of wastewater management [9]. It is simple, low cost, and requires minimal technical expertise; however there is limited understanding of the consequences of such a practice, and variable success. Several components of winery wastewater, particularly sodium and polyphenols, have been shown to have phytotoxic effects on plants [10,11] and soil microorganisms [12]. Whilst WWW is anecdotally known to restrict plant growth, there are few rigorous studies that have been performed to date. Furthermore, whilst winery wastewater is currently a waste stream requiring management, it may be considered a potentially valuable resource if phytotoxic compounds and other adverse chemical characteristics can be readily removed and/or counteracted. Wastewater reuse in agriculture has the potential to both treat a waste product at the same time as harnessing a valuable water source. Whilst climate change forecasts are variable, they generally agree that rain and snowfall patterns will be altered, which is likely to affect the water allocation system in many winegrowing areas [13], meaning that the ability to reuse WWW is highly relevant to water limited wine regions such as south-eastern Australia, California and South Africa.

Here, we present the results of a diagnostic glasshouse study in which we investigated the effects of semi-synthetic winery wastewater application on the germination and growth of four commonly grown crops or pasture species: barley (*Hordeum vulgare*), millet (*Pennisetum glaucum*), lucerne (*Medicago sativa*) and phalaris (*Phalaris aquatica*). These crops assessed could potentially be used as cover crops within a vineyard, or could be grown in a separate area as a method of wastewater utilisation. The key aim of the study was to develop a rapid assessment methodology for assessing winery wastewater toxicity, by investigating the toxicity of the wastewater to both seed germination and vegetative plant growth, and determining whether any relationships between plant growth and seed germination exist. Other rapid assessment tools for winery wastewater have used onions and cress in bioassays [14]. These are not necessarily a good surrogate for field grown crops, however; as such a range of typical crops on which winery wastewater could be applied were tested here.

2. Experimental

2.1. Semi-synthetic winery wastewater preparation

A semi-synthetic winery wastewater was used to assess the impacts of WWW on plants. This approach was selected due to the high variability in industrially generated wastewater, and the need to use a WWW with consistent properties that could be used across experiments. A controlled wastewater, consistent with industrial output, was generated by combining 2.5% (v/v) wine (Cabernet Sauvignon, Morris, Australia), 2.5% (v/v) grape juice (P&N, Australlia), and a combination of sodium salts with appropriate counterions to achieve a total sodium concentration of 200 mg/L in deionised water. The counterions of these salts also provided anions of organic acids, which are found in significant quantities in WWW. The sodium salts added were 110 mg/L sodium hydroxide, NaOH (Sigma); 350 mg/L sodium tartrate dibasic dehydrate, $C_4H_4Na_2O_6{\cdot}2H_2O$ (Sigma); and 285 mg/L sodium citrate tribasic dehydrate, $HOC(COONa)(CH_2COONa)_2{\cdot}2H_2O$ (Sigma).

The final wastewater had a pH of 6.0, electrical conductivity (EC) of 0.88 dS/m and chemical oxygen demand (COD) of 14,600 mg/L. These are consistent with typical values reported in the literature for WWW [15,16]. In the experiments described below, the wastewater was used undiluted (i.e. 100% WWW), and at concentrations of 75%, 50%, 25%, 10% and 0% (control). Dilutions were performed using reverse osmosis (RO) water.

2.2. Seed germination and plant growth studies

The effects on WWW on the germination and growth of four plant species were considered. The species assessed were: barley (*H. vulgare*; supplied by De Bortoli), millet (*P. glaucum*; supplied by Queensland Agricultural Seeds, Toowoomba, Queensland), lucerne (*M. sativa*, SARDI 10; supplied by Heritage Seeds, Mulgrave, Victoria) and phalaris (*P. aquatica*; supplied by Smyth Seeds, Benalla, Victoria)

WWW effects on seed germination were assessed in two separate experiments. In both experiments, seeds of each species were placed on a filter paper moistened with 2 mL of the appropriate WWW (0–100%, see above) concentration in a 90 mm Petri dish. Dishes were placed in a controlled temperature facility (15/25 °C min/max) in the dark. In the first experiment, germination, defined as the seed having a radicle of ≥5 mm [17], was assessed 12-hourly over a 13-day period. The total percentage germination and mean time to germination (MTG) of the seeds that germinated were calculated, as described previously [18]:

$$\mathrm{MTG} = \sum \frac{n \times d}{N}$$

where n is the number of seeds that germinated between scoring intervals, d is the incubation period in days at that time point and N is the total number of seeds germinated in the treatment.

In the second experiment, seeds were germinated (as above), and the percentage germination and radicle length were measured at 48 h for millet, lucerne and barley, and 72 h for phalaris. These data were used to calculate the germination index [19].

$$\text{Germination index(GI)} = 100 \times \frac{Gs}{Gc} \times \frac{Ls}{Lc}$$

where Gs and Gc are the number of seeds germinated in the sample and the control (distilled water, dH_2O), and Ls and Lc are the root lengths in the sample and the control, respectively; the germination index is measured as % of control.

The effects of WWW addition on the plant growth was assessed in a glasshouse study. Seeds of each of the four study species were sown into separate plastic, free-draining pots containing 660 g of a coarse textured seed raising mix constituted of sand and pine bark, with no additional nutrients (Debco, Australia). Seedlings were thinned to one per pot within 3 weeks of planting. A total of 24 pots of each species were established. Four replicate pots were watered with each WWW concentrations (described above) to field capacity as required, typically three times per week. Plants were grown in a glasshouse (Monash University, Clayton Campus), where the temperature range was maintained between 15 and 25 °C. Supplemental lighting was provided, giving an average light intensity of 460 μmol s^{-1} m^{-2} with a 16 h photoperiod. The pots were arranged in a randomised complete block design.

Plants were destructively harvested 11 weeks after sowing. At the time of harvesting, plants were removed from the pots, and the soil was gently washed away from the roots and rinsed with RO water. The roots and shoots were separated, and fresh weights determined. Tissue samples were dried at 65 °C for >48 h, and dry masses recorded. Dry samples were then ground to a fine powder, and stored in airtight vials prior to digestion using a microwave digester (Anton Parr Multiwave 3000). Briefly, samples (0.1 g) were digested using 5 mL HNO_3, 1 mL HCl and 2 mL H_2O_2, as described previously [20]. The temperature was ramped up to 175 °C over a

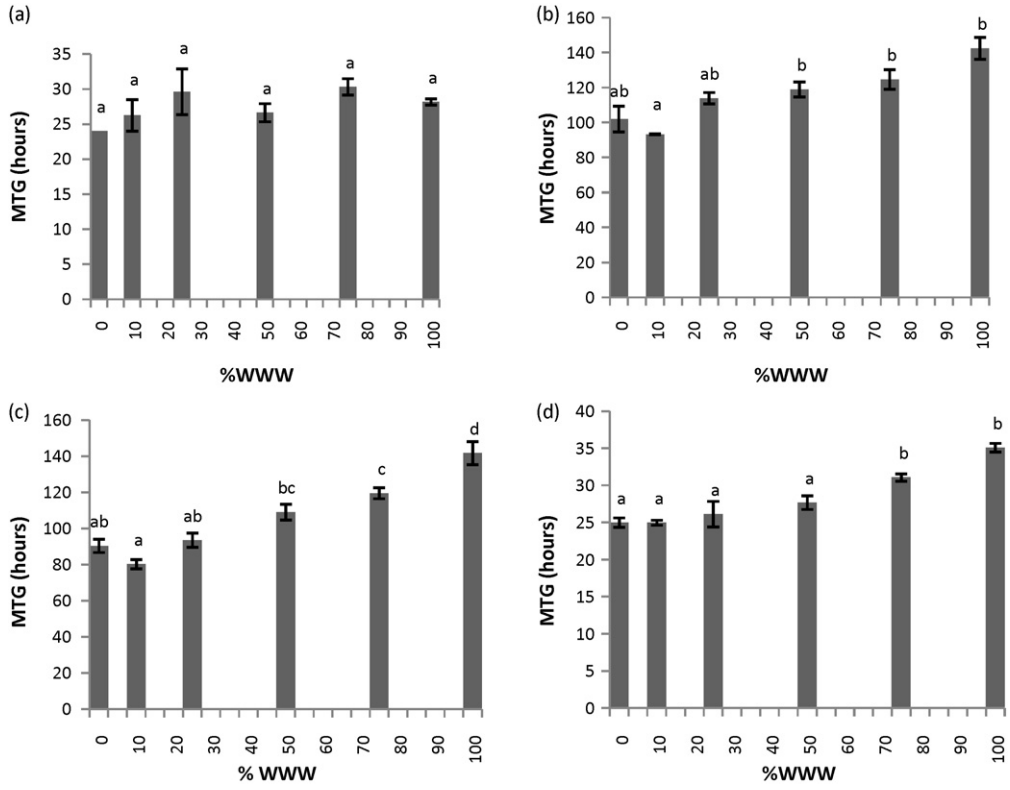

Fig. 1. Mean time to germination of (a) barley, (b) phalaris, (c) millet and lucerne (d) in the presence of increasing WWW concentrations. Means (±S.E.) followed by the same letter are not significantly different at the $P < 0.05$ level, as determined by Tukey's HSD test.

5 min period, held for 25 min, and cooled at a maximum cooling rate. The samples were diluted to 100 mL using Milli-Q H_2O prior to analysis by inductively couple plasma optical emission spectroscopy (ICP-OES) (Varian ICP-OES Vista-Pro, CCD simultaneous, with axial torch orientation).

2.3. Data analysis

Data were analysed using one-way analysis of variance (ANOVA) to determine whether there were any differences between the means of the different treatments. Where significant differences were found, treatment means were compared using Tukey's HSD test. ANOVA and Tukey's analyses were performed using SPSS 16.0 (SPSS, Chicago, IL). The concentration required to effect a 50% reduction in plant biomass, the EC50 value, was determined using SigmaPlot 11 (Sysstat, Chicago, IL).

3. Results

3.1. Seed germination

In the first germination experiment, the total proportion of seeds that germinated was not affected by WWW concentration (data not shown); however, the mean time to germination (MTG, Fig. 1) increased with increasing WWW concentration for all species except barley.

Phalaris seeds showed a significant increase in MTG between 10% WWW and concentration of 50%, or greater. Furthermore, there was no significant difference between the dH_2O control and any of the WWW treatments. Lucerne and millet seeds showed a significant increase in the MTG (compared with the dH_2O control) when treated with WWW at concentration ≥75%.

In the second germination experiment, the germination index (GI, Fig. 2) decreased with increasing WWW concentration for all species. For the species considered here, the GI was significantly lower where seeds were treated with high WWW concentrations in comparison with the dH_2O control, with decreases of 2-, 11- and 25-fold recorded for barley, millet and lucerne, respectively; the fold decrease for phalaris was unable to be calculated due to the GI value of zero in the 100% WWW treatment.

3.2. Plant biomass

WWW had a phytotoxic effect on all species studied here (Fig. 3), with all species showing a similar trend of decreasing biomass (roots and shoots) with increasing WWW concentrations. The dry weights of barley shoots and roots steadily decreased over the range of WWW concentrations tested here. For shoot dry weights, there was a 4-fold decrease between the 0% and 100% WW treatments, and the EC50 value was 54%. Similarly, for root dry weights there was an 11-fold decrease between 0% and 100% WWW, and an EC50 value of 14%.

The shoot and root biomass of millet and phalaris plants decreased with increasing WWW concentrations. In response to WWW addition millet exhibited EC50 values of 13% and 15% for root and shoot dry weights, respectively, and a biomass decrease of 10-fold for both root and shoot biomass. Phalaris showed an 8- and 7-fold decrease in shoots and roots, respectively; the EC50 values calculated were unreliable due to the variability in the samples (data not shown).

Lucerne was very sensitive to WWW application with a sharp decrease in shoot and root biomass between the 0% and 10% WWW treatments, but there were no significant differences in biomass at higher WWW concentrations. EC50 values were not significantly different for shoots, however, the EC50 value for lucerne roots was 11% WWW; decreases in biomass were 8- and 7-fold, respectively for shoots and roots.

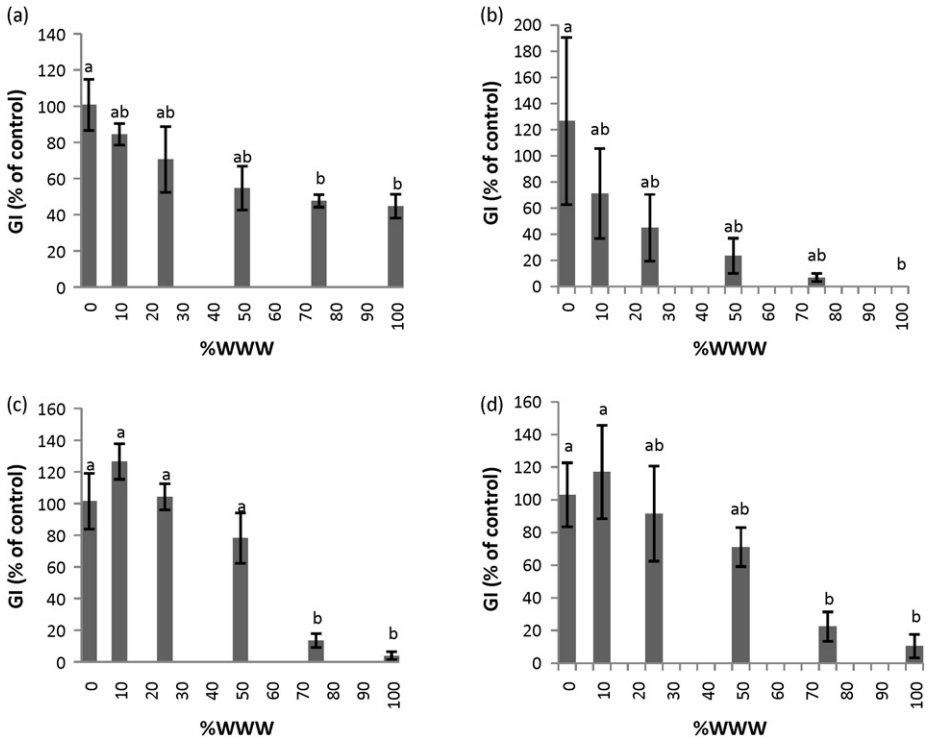

Fig. 2. Germination index of (a) barley, (b) phalaris, (c) millet and lucerne (d) in the presence of increasing WWW concentrations. Means (±S.E.) followed by the same letter are not significantly different at the $P < 0.05$ level, as determined by Tukey's HSD test.

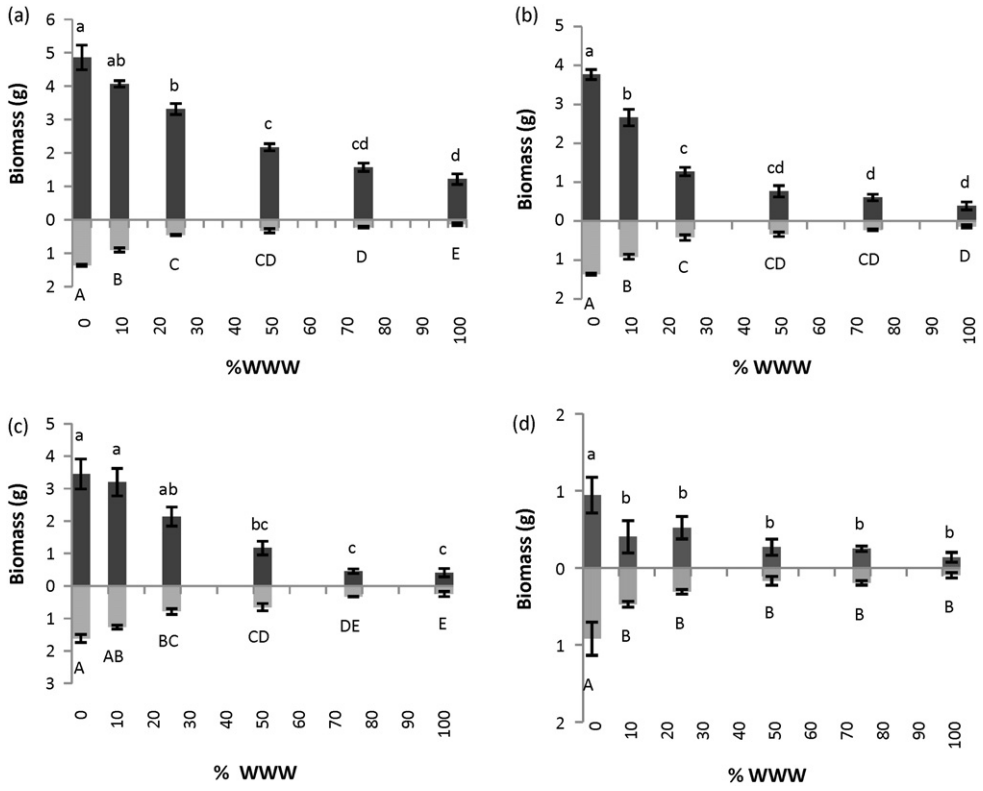

Fig. 3. The effect of synthetic wastewater application on shoot (above *x*-axis) and root (below *x*-axis) biomass (g dry weight) of (a) barley, (b) millet, (c) phalaris and (d) lucerne plants. Means (±S.E.) followed by the same letter are not significantly different at the $P < 0.05$ level, as determined by Tukey's HSD test ($P < 0.05$).

3.3. Plant tissue inorganic analysis

The concentration of sodium in the shoots (Table 1) of barley and phalaris increased significantly with increasing WWW concentrations. Whilst a similar trend was evident for millet and lucerne, the differences were not significant. There was a significant increase in lucerne root sodium concentrations, but not for any other species. The phosphorus concentration in the shoots of millet and phalaris, and the roots of barley and lucerne, increased with increasing WWW concentration. Calcium concentrations increased in barley roots, but decreased in phalaris roots. Magnesium concentrations in barley and millet roots decreased significantly with increasing wastewater concentrations, and concentration decreases were also seen for chromium in phalaris and lucerne roots, potassium in millet roots and iron in phalaris roots.

4. Discussion

These studies showed that winery wastewater has inhibitory effects on both seed germination and vegetative growth of millet, barley, phalaris and lucerne. This demonstrated toxicity therefore means that the release of WWW into the environment needs to be carefully considered and monitored in order to prevent environmental degradation. The data presented here provides some insight into the basis of this toxicity, and the opportunity to develop a rapid assessment tool that can be used in monitoring WWW prior to land application.

4.1. Effects of winery wastewater application on seed germination

Winery wastewater significantly reduced the mean time to germination but not the overall rates of germination. Such delays in germination have been reported in studies of olive mill wastewater [10] and diesel oil [21] but not, to our knowledge, WWW. The delay in germination differed between species and is likely due to variation in seed size, seed coat permeability, differential uptake of nutrients and toxins and metabolism [22]. The mechanisms of the phytotoxicity are difficult to ascertain, due the inherent complexities of a wastewater sample. Nevertheless, both phenols and salts have both been shown to be responsible for delaying seed germination [23,24], and are likely to be involved here. In addition to effects on MTG, the germination index (an indicator of early plant growth) was significantly reduced with increasing WWW concentration. Taken together, these data indicate that winery wastewater is negatively affecting the early growth and development of the species tested.

Seed germination and emergence were shown to be affected by winery wastewater, indicating that germination assays are a suitable method for determining toxicity. Germination studies have been commonly used as a basis for other toxicity assays [17,25,26]. Based on the results of the tests performed, overall seed germination does not distinguish between different wastewater treatments and therefore would not be suitable for ascertaining toxicity; the germination index does, however, have sufficient sensitivity to determine phytotoxicity [19]. In an applied sense, it is important to know the effects of wastewater application at different growth stages, to determine whether there are certain growth stages where WWW should be avoided. If seeds were to be irrigated using WWW, the time to emergence is likely to be affected, but the overall emergence is unlikely to be impacted upon.

4.2. Effects of winery wastewater application on plant growth and inorganic composition

The application of increasing winery wastewater to plants resulted in a significant decline in plant biomass production across all species, with 50% reduction in plant growth (i.e. EC50 values) noted at dilutions ranging from 54% WWW (barley shoots) to as low as 11% (lucerne, shoots and roots). Such a marked decrease in plant growth at relatively low WWW concentrations indicates that dilution of winery wastewater is not likely to be adequate to mitigate the phytotoxic effects observed here. Dilution has been shown to be effective in some cases, including reducing ammonia volatilisation following land application of animal manures [27]. However, since dilution is not a viable option in the case of WWW, efforts need to focus on the identification of the toxic components of WWW and treatment methods to eliminate or mitigate their toxic effects. Identifying the causal components of this diminished plant growth is essential if the wastewater is to be managed in a way that facilitates sustainable reuse.

It is not yet known which component(s) of the wastewater are likely to be responsible for the chronic phytotoxicity observed, however there is some evidence suggesting that sodium [28], ethanol [29] and polyphenols [10] are all potentially phytotoxic constituents. The detailed analysis of inorganic components in the root and shoot tissue, presented here, provide some insights. Phosphorus and sodium concentrations showed a fairly consistent increase with WWW concentration, in both roots and shoots. The increase in phosphorus concentration is unlikely to be associated with toxicity [30], and therefore is more likely a reflection of reduced plant biomass due to other factors, leading to a tissue concentration effect. Co-transport of phosphorus with sodium may also be important [31], as phosphorus is only elevated in the tissues where sodium is also elevated. Concentrations of some other inorganic elements showed decreases with increasing WWW concentrations, however effects across species and tissue types were not consistent, and none are likely to be associated with toxicity [30].

High sodicity levels in soils are known to result in inhibited plant growth [28], and this is likely to be consistent with the increased sodium levels observed in most species tested here. However, the highest sodium concentrations in phalaris shoots was determined to be approximately 6 mg/g (100%WWW, corresponding to 88% reduction in growth). In similar studies investigating the effects of salinity alone, using approximately 15-fold higher sodium concentrations, shoot Na concentrations ranged from 13 to 25 mg/L, and corresponded with between 0% and 63% shoot biomass [11]. Thus, the large reduction in growth of phalaris cannot be attributed to Na toxicity alone. Sodicity impacts on plant growth are complex, however, and elevated soil sodium levels can limit plant growth by affecting soil structure and plant water and oxygen uptake [32]. Although the concentrations of inorganic elements observed are unlikely to be causes of immediate plant toxicity, the long term effects of WWW application on sodium accumulation in soils, and the resultant impacts on soil health and potential groundwater contamination are important areas to consider. Longer term studies are required to identify and quantify any such changes.

Whilst polyphenols are commonly present in WWW, this synthetic sample had relatively low polyphenol concentrations (<0.04 mg/L), thereby suggesting that polyphenols are also not solely responsible for the observed phytotoxicity. As such, it is highly likely that the phytotoxicity arises from the complex combination of organic matter in the wastewater, which has also been demonstrated in the case of olive mill wastewater [33].

4.3. Seed germination as a predictor of plant growth

Given the phytotoxic nature of WWW reported here, it is essential that land discharge onto crops be carefully considered. Due to the highly variable nature of WWW throughout the year, there may be times when the WWW is not phytotoxic. To this end, the ability to rapidly assess the toxicity of WWW would be very valuable to

Table 1
Inorganic element concentrations (mg/g) of roots and shoots at different concentrations of WWW. Means (±S.E.) followed by the same letter are not significantly different at the $P < 0.05$ level, as determined by Tukey's HSD test (no letter indicates that there is no significance at any level).

			% WWW					
			0	10	25	50	75	100
Barley	Shoots	Ca	3.41 ± 0.18 a	2.91 ± 0.38 a	3.58 ± 0.16 a	4.07 ± 0.20 ab	4.32 ± 0.09 ab	5.79 ± 0.033 b
		Cr	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.01
		Fe	0.23 ± 0.09	0.015 ± 0.04	0.13 ± 0.03	0.14 ± 0.01	0.18 ± 0.02	0.28 ± 0.04
		K	24.3 ± 2.06	20.6 ± 1.87	21.3 ± 1.16	16.9 ± 2.34	16.9 ± 1.82	19.4 ± 1.62
		Mg	3.11 ± 0.10	3.26 ± 0.33	3.28 ± 0.13	3.41 ± 0.20	3.35 ± 0.13	3.59 ± 0.15
		Mn	0.23 ± 0.03	0.24 ± 0.03	0.23 ± 0.01	0.20 ± 0.03	0.17 ± 0.02	0.20 ± 0.01
		Na	0.98 ± 0.34 a	0.70 ± 0.09 a	1.11 ± 0.05 a	1.96 ± 0.45 a	1.94 ± 0.23 a	4.64 ± 0.59 b
		P	0.18 ± 0.04	0.33 ± 0.17	0.17 ± 0.03	0.24 ± 0.03	0.25 ± 0.01	0.28 ± 0.03
		Zn	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.05 ± 0.01
	Roots	Ca	4.91 ± 0. 18	3.39 ± 0.16	3.13 ± 0.49	3.05 ± 0.58	3.28 ± 0.65	2.95 ± 0.21
		Cr	0.05 ± 0.01	0.03 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	0.08 ± 0.01
		Fe	1.47 ± 0.11	0.93 ± 0.12	0.95 ± 0.15	0.75 ± 0.15	0.56 ± 0.07	0.72 ± 0.12
		K	2.79 ± 0.78	1.28 ± 0.19	1.31 ± 0.31	4.74 ± 1.07	4.46 ± 0.55	2.20 ± 0.74
		Mg	1.00 ± 0.07 a	0.83 ± 0.02 a	0.61 ± 0.07 a	0.50 ± 0.04 a	0.57 ± 0.11 a	0.54 ± 0.03 b
		Mn	0.20 ± 0.02	0.18 ± 0.01	0.14 ± 0.02	0.15 ± 0.19	0.14 ± 0.01	0.17 ± 0.02
		Na	0.85 ± 0.15 ab	0.58 ± 0.06 a	0.70 ± 0.12 a	1.45 ± 0.14 ab	1.91 ± 0.20 b	1.61 ± 0.51 a
		P	0.34 ± 0.01 a	0.33 ± 0.01 ab	0.34 ± 0.01 a	0.48 ± 0.03 b	0.51 ± 0.03 bc	0.51 ± 0.03 bc
		Zn	0.09 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.05 ± 0.01	0.06 ± 0.01
Millet	Shoots	Ca	1.39 ± 0.17	1.24 ± 0.16	1.68 ± 0.26	2.40 ± 0.12	1.75 ± 0.25	1.89 ± 0.16
		Cr	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.04 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
		Fe	0.16 ± 0.02	0.09 ± 0.01	0.18 ± 0.06	0.22 ± 0.02	0.04 ± 0.01	0.04 ± 0.00
		K	12.8 ± 0.24	12.82 ± 0.29	12.60 ± 0.31	12.73 ± 0.44	13.06 ± 1.00	12.70 ± 0.42
		Mg	2.29 ± 0.19	1.98 ± 0.13	2.04 ± 0.18	2.46 ± 0.12	1.95 ± 0.21	2.21 ± 0.11
		Mn	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.04 ± 0.01	0.10 ± 0.01
		Na	0.00 ± 0.02	0.03 ± 0.01	0.11 ± 0.02	0.40 ± 0.09	0.36 ± 0.05	0.89 ± 0.23
		P	0.70 ± 0.04 a	0.79 ± 0.05 a	0.93 ± 0.11 a	1.37 ± 0.09 a	1.78 ± 0.30 a	1.84 ± 0.15 b
		Zn	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.00	0.03 ± 0.00	0.04 ± 0.01
	Roots	Ca	1.90 ± 0.24	1.91 ± 0.17	2.46 ± 0.51	2.23 ± 0.41	1.20 ± 0.14	1.44 ± 0.39
		Cr	0.08 ± 0.05	0.03 ± 0.01	0.18 ± 0.05	0.44 ± 0.22	0.00 ± 0.00	0.00 ± 0.00
		Fe	0.50 ± 0.29	0.23 ± 0.03	0.86 ± 0.09	1.31 ± 0.32	0.03 ± 0.01	0.07 ± 0.02
		K	15.7 ± 0.68 a	13.9 ± 0.62 ab	14.8 ± 0.36 ab	13.0 ± 1.39 ab	10.5 ± 0.85 ab	8.38 ± 0.90 b
		Mg	3.80 ± 0.16 ab	4.19 ± 0.26 ab	5.12 ± 0.26 ab	4.15 ± 0.62 ab	2.12 ± 0.28 b	2.52 ± 0.07 b
		Mn	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.07 ± 0.00	0.03 ± 0.01	0.03 ± 0.00
		Na	2.01 ± 0.20	2.40 ± 0.25	1.79 ± 0.11	2.09 ± 0.44	0.78 ± 0.26	1.52 ± 0.24
		P	0.42 ± 0.02	0.42 ± 0.01	0.48 ± 0.02	0.50 ± 0.08	0.57 ± 0.15	0.36 ± 0.24
		Zn	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
Phalaris	Shoots	Ca	1.68 ± 0.11	1.42 ± 0.19	1.83 ± 0.21	2.33 ± 0.16	3.67 ± 0.71	3.95 ± 0.46
		Cr	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.11 ± 0.01	0.01 ± 0.01
		Fe	0.09 ± 0.01 a	0.10 ± 0.02 a	0.12 ± 0.01 a	0.19 ± 0.06	0.57 ± 0.04 b	0.09 ± 0.04
		K	19.1 ± 1.63	15.3 ± 1.00	14.0 ± 0.52	14.5 ± 1.46	16.2 ± 0.42	21.0 ± 2.47
		Mg	1.88 ± 0.18	1.73 ± 0.15	1.88 ± 0.09	2.69 ± 0.12	2.99 ± 0.78	3.10 ± 0.30
		Mn	0.11 ± 0.01	0.11 ± 0.02	0.13 ± 0.01	0.19 ± 0.02	0.28 ± 0.05	0.29 ± 0.04
		Na	0.71 ± 0.09 a	0.88 ± 0.10 a	1.47 ± 0.20 ab	2.99 ± 0.50 bc	4.03 ± 0.65 c	6.27 ± 0.58 d
		P	0.68 ± 0.05 a	0.64 ± 0.04 a	0.82 ± 0.04 a	1.36 ± 0.13 b	2.09 ± 0.19 c	2.17 ± 0.14 c
		Zn	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.13 ± 0.03	0.09 ± 0.02
	Roots	Ca	3.89 ± 0.31 ab	3.29 ± 0.30 ab	3.49 ± 0.34 ab	4.09 ± 0.63 a	1.78 ± 0.22 ab	1.21 ± 0.46 b
		Cr	0.08 ± 0.03 ab	0.07 ± 0.02 ab	0.11 ± 0.01 ab	0.19 ± 0.02 a	0.04 ± 0.01 b	0.00 ± 0.00 b
		Fe	1.23 ± 0.23 a	0.73 ± 0.13 ab	0.91 ± 0.10 ab	1.23 ± 0.17 a	0.35 ± 0.02 ab	0.11 ± 0.04 b
		K	8.06 ± 1.08	9.27 ± 1.37	13.19 ± 1.36	16.47 ± 1.05	14.6 ± 2.17	11.0 ± 3.74
		Mg	1.23 ± 0.05	1.28 ± 0.16	1.31 ± 0.19	1.29 ± 0.03	1.19 ± 0.35	0.58 ± 0.19
		Mn	0.19 ± 0.01	0.18 ± 0.02	0.15 ± 0.03	0.19 ± 0.02	0.15 ± 0.03	0.13 ± 0.05
		Na	0.97 ± 0.17 a	1.42 ± 0.28	2.60 ± 0.71	2.29 ± 0.32	1.66 ± 0.35	0.96 ± 0.33
		P	0.95 ± 0.06 a	0.93 ± 0.11 a	0.90 ± 0.07 a	1.39 ± 0.10 ab	1.43 ± 0.09 b	0.96 ± 0.31 b
		Zn	0.08 ± 0.00	0.06 ± 0.01	0.06 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.05 ± 0.02
Lucerne	Shoots	Ca	17.9 ± 2.79	19.0 ± 2.89	14.9 ± 2.76	11.3 ± 2.16	12.1 ± 1.19	
		Cr	0.01 ± 0.00	0.04 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
		Fe	0.21 ± 0.05	0.35 ± 0.11	0.05 ± 0.01	0.04 ± 0.04	0.08 ± 0.03	
		K	21.1 ± 1.03	22.5 ± 3.18	22.2 ± 1.34	21.8 ± 5.14	25.1 ± 2.26	
		Mg	5.61 ± 1.10	7.37 ± 0.42	6.46 ± 1.08	5.44 ± 0.88	5.70 ± 0.77	
		Mn	0.11 ± 0.01	0.11 ± 0.01	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	
		Na	0.32 ± 0.11 a	0.46 ± 0.02 a	1.15 ± 0.22 ab	2.37 ± 0.72 b	2.17 ± 0.26 b	
		P	0.80 ± 0.09	0.95 ± 0.07	0.86 ± 0.06	2.31 ± 0.75	1.92 ± 0.35	
		Zn	0.09 ± 0.01	0.09 ± 0.01	0.06 ± 0.00	0.09 ± 0.02	0.07 ± 0.01	
	Roots	Ca	1.72 ± 0.13	2.05 ± 0.21	2.17 ± 0.08	2.35 ± 0.22	2.05 ± 0.20	
		Cr	0.02 ± 0.00 a	0.02 ± 0.00 a	0.05 ± 0.01 b	0.00 ± 0.00 a	0.00 ± 0.00 a	
		Fe	0.32 ± 0.04	0.44 ± 0.04	0.57 ± 0.08	0.36 ± 0.05	0.31 ± 0.07	
		K	10.7 ± 0.42	12.6 ± 0.76	9.84 ± 1.31	11.1 ± 1.30	12.1 ± 1.00	
		Mg	11.8 ± 1.83	12.9 ± 0.67	11.3 ± 0.77	11.8 ± 2.81	12.3 ± 1.76	
		Mn	0.25 ± 0.05	0.33 ± 0.06	0.33 ± 0.07	0.36 ± 0.10	0.50 ± 0.19	
		Na	0.29 ± 0.08 a	0.35 ± 0.09 a	1.12 ± 0.26 b	0.97 ± 0.14 ab	1.23 ± 0.06 b	
		P	0.87 ± 0.02 a	0.96 ± 0.05 a	0.98 ± 0.10 a	1.25 ± 0.19 a	1.88 ± 0.13 b	
		Zn	0.25 ± 0.03	0.30 ± 0.03	0.20 ± 0.02	0.34 ± 0.08	0.39 ± 0.09	

Note: The biomass of lucerne in the 100% WWW treatment was insufficient for the determination of elemental concentrations.

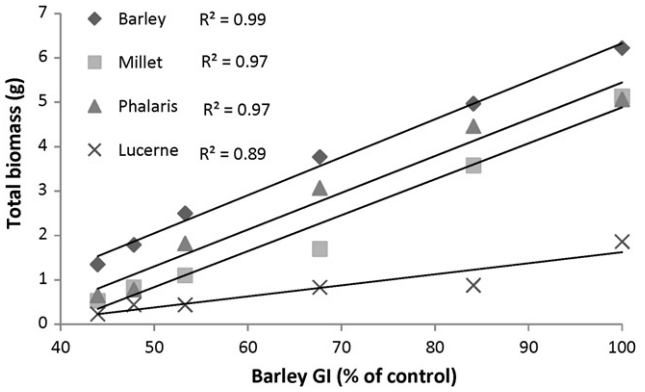

Fig. 4. Correlation of barley germination index with total biomass of plants harvested at 11 weeks.

industry. Plant growth studies, such as those undertaken here, are time-consuming, and are clearly not a viable option for rapid WWW phytotoxicity assessment. Conversely, the seed germination assays, specifically the GI, were rapid and showed a similar response to the plant growth experiments.

In an attempt to develop a rapid assessment tool, we sought to determine whether there were any correlations between seed germination and biomass parameters. Whilst germination and vegetative growth involve different physiological processes, the growth experiment incorporated the germination stage, and therefore may be considered to be linked to the germination experiments. There was no overall correlation between germination and biomass if the datasets were not first separated on the basis of species.

If the species were considered separately, the most meaningful correlations between germination and vegetative growth data (linear) were found to exist in barley and phalaris, relating the germination index to respective total plant biomass ($r^2 = 0.99$ and 0.91, respectively). There were no good correlations using the germination indices determined for millet or lucerne (0.51 and 0.49, respectively), suggesting that these species would not be suitable for using in a bioassay type situation. However, the relationship between the GI of barley and the total biomass of *all* species tested, is good (Fig. 4; $r^2 = 0.89$, 0.97, 0.97 and 0.99 for lucerne, millet, phalaris and barley, respectively). As such, it seems that barley germination could be used as a rapid WWW bioassay for all of these crops rather than having to undertake individual germinations, thus simplifying the test. On the basis of these results, barley germination could be tested for a greater range of crops to assess its predictive power for a greater range of crops.

5. Conclusions

This study has demonstrated that winery wastewater delays the germination and inhibits vegetative growth of barley, lucerne, phalaris and millet. Whilst overall germination was not affected, WWW was shown to increase the time to germination, and restrict early growth. Furthermore, WWW was shown to be toxic to plant growth, and with EC50 values ranging from 10% to 50%, high levels of dilution with fresh water would be required if WWW were to be used to irrigate such crops. The use of WWW with dilution is therefore practically limited based on freshwater availability and cost, in conjunction with overall volumes of wastewater production. A high correlation of barley seed germination index with total biomass following 11 weeks of growth was found, suggesting that barley seed germination tests could be a potential useful tool for use in industry, to determine whether the wastewater is suitable to use for irrigation at any given time.

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References

[1] D. Tilman, J. Fargione, B. Wolff, C. D'Antonio, A. Dobson, R. Howarth, D. Schindler, W.H. Schlesinger, D. Simberloff, D. Swackhamer, Forecasting agriculturally driven global environmental change, Science 292 (2001) 281–284.
[2] IPCC, Climate Change 2001: Impacts, Adaptation, and Vulnerability, International Panel on Climate Change, Cambridge, 2001.
[3] A.N. Angelakis, L. Bontoux, Wastewater reclamation and reuse in Eureau countries, Water Policy 3 (2001) 47–59.
[4] OIV, State of the Vitiviniculture World Market—2008, Organisation Internationale de la Vigne et du Vin, Paris, 2008.
[5] B. Fernandez, I. Seijo, G. Ruiz-Filippi, E. Roca, L. Tarenzi, J.M. Lema, Characterization, management and treatment of wastewater from white wine production, Water Sci. Technol. 56 (2007) 121–128.
[6] L. Malandra, G. Wolfaardt, A. Zietsman, M. Viljoen-Bloom, Microbiology of a biological contactor for winery wastewater treatment, Water Res. 37 (2003) 4125–4134.
[7] J.A. Chapman, R.L. Correll, J.N. Ladd, Removal of soluble organic carbon from winery and distillery wastewaters by application to soil, Aust. J. Grape Wine Res. 1 (1995) 39–47.
[8] W.C. Quayle, E.W. Christen, N.S. Jayawardane, J. Blackwell, R. Correll, A. Fattore, Innovation in management of wastewater in the Australian wine industry. 34th Annual Technical report, Australia and New Zealand Grapegrower and Winemaker (2006) 41–49.
[9] R. Crites, S. Reed, R. Bastian, Land Treatment Systems for Municipal and Industrial Wastes, McGraw Professional, 2000.
[10] A. Mekki, A. Dhouib, S. Sayadi, Polyphenols dynamics and phytotoxicity in a soil amended by olive mill wastewaters, J. Environ. Manage. 84 (2007) 134–140.
[11] R. Oram, J. Edlington, P. Gardner, Selection for resistance to salinity and waterlogging in *Phalaris aquatica* L., Aust. J. Agric. Res. 53 (2002) 391–399.
[12] R. Capasso, A. Evidente, L. Schivo, G. Orru, M.A. Marcialis, G. Cristinzio, Antibacterial polyphenols from olive oil mill waste waters, J. Appl. Microbiol. 79 (1995) 393–398.
[13] K. Hayhoe, D. Cayan, C.B. Field, P.C. Frumhoff, E.P. Maurer, N.L. Miller, S.C. Moser, S.H. Schneider, K.N. Cahill, E.E. Cleland, L. Dale, R. Drapek, R.M. Hanemann, L.S. Kalkstein, J. Lenihan, C.K. Lunch, R.P. Neilson, S.C. Sheridan, J.H. Verville, Emissions pathways, climate change, and impacts on California, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 12422–12427.
[14] M. Arienzo, E.W. Christen, W.C. Quayle, Phytotoxicity testing of winery wastewater for constructed wetland treatment, J. Hazard. Mater. 169 (2009) 94–99.
[15] A. Kumar, R. Kookana, Impact of Winery Wastewater on Ecosystem Health—An Introductory Assessment, CSIRO, 2006.
[16] T. Colin, A. Bories, Y. Sire, R. Perrin, Treatment and valorisation of winery wastewater by a new biophysical process (ECCF), Water Sci. Technol. 51 (2005) 99–106.
[17] USEPA, Seed germination/root elongation toxicity test, US Environmental Protection Authority, Washington DC, 1992.
[18] J.L. Brenchley, R.J. Probert, Seed germination responses to some environmental factors in the seagrass Zostera capricorni from eastern Australia, Aquat. Bot. 62 (1998) 177–188.
[19] F. Zucconi, M. De Bertoldi, Evaluating toxicity of immature compost, BioCycle 22 (1981) 54–57.
[20] R.N. Sah, R.O. Miller, Spontaneous reaction for acid dissolution of biological tissues in closed vessels, Anal. Chem. 64 (1992) 230–233.
[21] G. Adam, H. Duncan, Influence of diesel fuel on seed germination, Environ. Pollut. 120 (2002) 363–370.
[22] R.D. Williams, R.E. Hoagland, The effects of naturally occurring phenolic compounds on seed germination, Weed Sci. 30 (1982) 206–212.
[23] A. Jafarzadeh, N. Aliasgharzad, Salinity and salt composition effects on seed germination and root length of four sugar beet cultivars, Biologia 62 (2007) 562–564.
[24] A.T. Kuiters, Effects of phenolic acids on germination and early growth of herbaceous woodland plants, J. Chem. Ecol. 15 (1989) 467–479.
[25] N.F.Y. Tam, S. Tiquia, Assessing toxicity of spent pig litter using a seed germination technique, Resour. Conserv. Recycling 11 (1994).
[26] W. Guiga, P. Boivin, N. Ouarnier, F. Fournier, M. Fick, Quantification of the inhibitory effect of steep effluents on barley germination, Process Biochem. 43 (2008) 311–319.
[27] J.F. Moal, J. Martinez, F. Guiziou, C.M. Coste, Ammonia volatilization following surface-applied pig and cattle slurry in France, J. Agric. Sci. 125 (1995) 245–252.
[28] L. Bernstein, Effects of salinity and sodicity on plant growth, Annu. Rev. Phytopathol. 13 (1975) 295–312.
[29] G.W. Stutte, I. Eraso, S. Anderson, R.D. Hickey, Bioactivity of volatile alcohols on the germination and growth of radish seedlings, Hortscience 41 (2006) 108–112.

[30] D. Reuter, J. Robinson (Eds.), Plant Analysis: An Interpretation Manual, CSIRO Publishing, 1997.
[31] D.P. Schachtman, R.J. Reid, S.M. Ayling, Phosphorus uptake by plants: from soil to cell, Plant Physiol. 116 (1998) 447–453.
[32] D. Sparks, Environmental Soil Chemistry, Academic Press, 2003.
[33] R. Capasso, G. Cristinzio, A. Evidente, F. Scognamiglio, Isolation, spectroscopy and selective phytotoxic effects of polyphenols from vegetable waste waters, Phytochemistry 31 (1992) 4125–4128.