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Phytotoxicity testing of winery wastewater for constructed wetland treatment

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ABSTRACT

Rapid and inexpensive phytotoxicity bioassays for winery wastewater (WW) are important when designing winery wastewater treatment systems involving constructed wetlands. Three macrophyte wetland species (*Phragmites australis*, *Schoenoplectus validus* and *Juncus ingens*) were tested using a pot experiment simulating a wetland microcosm. The winery wastewater concentration was varied (0.5%, 5%, 10%, 25%, 50%, 75% and 100%) and pH was corrected for some concentrations using lime as an amendment. The tolerance of the three aquatic macrophytes species to winery wastewater was studied through biomass production, total chlorophyll and nitrogen, phosphorous and potassium tissue concentrations. The results showed that at greater than 25% wastewater concentration all the macrophytes died and that *Phragmites* was the least hardy species. At less than 25% wastewater concentration the wetland microcosms were effective in reducing chemical oxygen demand, phenols and total soluble solids.

We also evaluated the performance of two laboratory phytotoxicity assays; (1) Garden Cress (*Lepidium sativum*), and (2) Onion (*Allium coepa*). The results of these tests revealed that the effluent was highly toxic with effective concentration, EC_{50}, inhibition values, as low as 0.25%. Liming the WW increased the EC_{50} by 10 fold.

Comparing the cress and onion bioassays with the wetland microcosm results indicated that the thresholds for toxicity were of the same order of magnitude. As such we suggest that the onion and cress bioassays could be effectively used in the wine industry for rapid wastewater toxicity assessment.

1. Introduction

The Australian wine industry includes many hundreds of small producers set in rural environments [1]. Constructed wetlands with plants that can tolerate and detoxify wastewater can be a viable treatment option. Plants and bacteria can assimilate variable and large organic loadings with low maintenance and operational costs, mainly due to the activity of bacteria that live on root surfaces which purify the wastewater (WW) by breaking down the organics and removing colloidal solids [2]. Roots and gravel serve as a substrate supplying the bacteria with sugars and oxygen [2]. After the wastewater has been treated and filtered through a wetland, it may be reused for irrigation.

Establishing the phytotoxicity of WW is fundamental for the proper design and sustainability of a wetland system, but almost no documented information exists [2]. The chemistry of winery effluent is complex and variable, containing numerous inorganic as well as organic compounds whose individual and combined contribution to plant phytotoxicity is not known [3]. This complexity makes it difficult to carry out a phytotoxicity assessment of WW based on chemical analysis alone.

The organic content of winery wastewater consists of highly soluble sugars, alcohols, acids and recalcitrant high molecular weight compounds (e.g. polyphenols, tannins, and lignins). These are not easily removed by physical or chemical treatment alone [4] and tannins in particular can inhibit microbial digestion [5]. Detailed studies of the composition of winery wastewater have revealed that ethanol and, to a smaller extent and on a temporary basis, sugars (fructose and glucose) represent more than 90% of the organic load [6]. Organic acids, alcohol, and phenols have variable degradation rates. Biodegradable contaminants (e.g. sugars and alcohols) tend to degrade first, leaving behind wastewater containing less easily degraded compounds (e.g. phenols and tannins). These compounds have a high chemical oxygen demand (COD) of about 25,000 mg L^{-1} [7] and can also cause soil deterioration through pore blockage, resulting in anoxia [8]. Winery wastewater also contains significant amounts of sodium (Na) and potassium (K) with a K:Na ratio of 3:1 and K concentrations up to 1000 mg L^{-1}. Nitrogen and phosphorous content is usually low compared with other agricultural effluents,

Abbreviations: WW, wastewater; EC_{30} and EC_{50}, respectively, effective concentration causing 30 and 50% of root length reduction; COD, chemical oxygen demand; Chl a, chlorophyll a; Chl b, chlorophyll b; EC, electrical conductivity; TSS, total suspended solids; ANOVA, analysis of variance; LSD, least significant difference; L, limed; NL, non-limed.

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ranging from 8 to 35 mg L^{-1} and 2 to 20 mg L^{-1} respectively [6,7]. All these components may limit the microbial metabolism of winery wastewater.

Understanding the phytotoxicity of winery wastewater is fundamental to the design and implementation of constructed wetlands. The ideal plant should tolerate high organic loads and be capable of removing significant amounts of contaminants and purifying the effluent in a relatively short time period. Amongst wetland plants, *Phragmites spp* and *Schenoplectus spp* are the most commonly used and have different purification potentials [9,10]. *Schoenoplectus validus* has been reported to grow over a wide range of nutrient and organic matter strengths in winery, piggery and dairy farm wastewater [10].

In order to determine the phytotoxicity of winery wastewater, two rapid laboratory phytotoxicity bioassays were compared with a simulated wetland microcosm pot trial with three aquatic plant species.

2. Materials and methods

2.1. Wastewater

The winery wastewater (WW) was taken during the peak of the vintage season in March 2008 from a winery located near Griffith, NSW, Australia. The chemical and physical properties of the WW were: pH 4.5; electrical conductivity (EC) 5.1 dS m^{-1}; chemical oxygen demand 17,000 mg L^{-1}; total suspended solids (TSS) 1000 mg L^{-1} and total phenol content of 10.6 mg L^{-1}. For the bioassay toxicity test assessment the following concentrations of WW were used: 100%, 75%, 50%, 25%, 10%, 5%, 0.5% and 0%. Dilution being undertaken with deionized water. Half the treatments were amended with lime (L-WW) to pH 6.5, the others remaining at their original pH of about 4.5 (NL-WW). Lime (CaOH) was chosen as it is widely used in the wine industry as a neutralizing material. For the wetland plant species test, only the treatments at 100% and 25% wastewater concentration were lime amended.

2.2. Phytotoxicity analyses

2.2.1. Garden cress test

The toxicity of winery wastewater was assessed using the bioassay described by Saadi et al. [11]. The WW used in this test was passed through a 0.4 μm filter and four millilitres of the test solution were placed on glass microfibre filters (GF/A; Whatman) in 90 mm glass Petri dishes. Ten garden cress seeds were placed in each dish, using three dishes per sample. Germination was conducted over 5 days under darkness, at 25 °C. The dishes of each treatment were wrapped together with a polyethylene bag to prevent desiccation and passage of volatiles between treatments. As parameters of toxicity both root length (cm) and seed germination rate (%) were measured. EC_{50} and EC_{30} are expressed as concentrations of WW causing 50 and 30% root length reduction, and calculated by plotting the percentage of root growth reduction of treated *vs.* control plants against the $\log_e$ of concentrations of WW. The growth inhibition values, EC_{50} and EC_{30} were interpolated from a plot of root lengths as a percentage of the control, against the log of the concentrations [12].

2.2.2. Onion test

In this study we used the onion test [13] as a potential simple, rapid and low cost test for ecotoxicological evaluation of WW.

Commercial onion bulbs of *Allium coepa*, (15–22 mm in diameter) not treated with plant growth regulators were obtained from a local farm. A set of six onions was used for each concentration of WW and placed in test tubes filled with WW. The WW was recharged every day. At day 5 the experiment was terminated and the length of the root bundles measured. Growth inhibition EC_{50} and EC_{30} were determined as for the garden cress test.

2.2.3. Wetland plant species test

A simulated wetland microcosm pot experiment was carried out in a greenhouse illuminated with natural light at 20 °C. Nine plants of three autochthonous macrophyte species, common reed (*Phragmites australis*), bull rush (*Schoenoplectus validus*), and giant rush (*Juncus ingens*) at the same stage of growth (approximately 12 months) were used in a randomised factorial design with three replicates. Polyvinyl chloride boxes, 30 cm long, 20 cm wide, 25 cm deep, with no drainage holes and containing ~10 kg of 10 mm dry washed river gravel were used. The boxes were shaken during filling with gravel to obtain packing-bulk densities similar to that found in a constructed wetland. This resulted in porosities of 44%, a pore volumes of 4 L for each box. The boxes were filled with WW at concentrations of 100%, 75%, 50%, 25%, 10%, 5%, 2.5% and 0% and periodically refilled with WW to compensate for evapotranspiration. The effect of pH on water quality was determined by amending the 25% and 100% WW replicates with lime to a pH of 6.5. Water and plant samples from each box were taken 20, 40 and 60 days after transplantation to further analyse biomass and water quality (see paragraph 2.3). Shoots and roots from two plants in each pot were dried at 70 °C, ground in a stainless steel mill (screen diameter 0.85 mm) and weighed. Dry matter was analysed for N content by high temperature combustion in an atmosphere of oxygen using a Leco CNS-2000 analyser [14], and P and K contents by digestion with nitric acid, at 140 °C for 8 h and analysed by inductively coupled plasma optical emission spectrometry (ICPOES) [15].

Table 1
Phytotoxicity of non-limed (NL-WW), and limed wastewater (L-WW) to garden cress (*Lepidium sativum L.*) with 0.5–100% winery wastewater (WW), expressed as root length (cm), root length as % of control, and germination rate (%). Values are mean ± SE (n = 30 seeds). EC_{50} and EC_{30} are expressed as concentrations of WW causing 50 and 30% of root length reduction.

Concentration of WW	Non-Limed WW			Limed WW		
	Root length		Germination rate	Root length		Germination rate
	cm	% of Control	%	cm	% of Control	%
100%	0.0 ± 0.0	0.0	0.0	0.07 ± 0.01	1.5	60
75%	0.0 ± 0.0	0.0	0.0	0.90 ± 0.10	9.0	100
50%	0.0 ± 0.0	0.0	0.0	0.50 ± 0.10	20.0	100
25%	0.07 ± 0.01	1.03	56.6	1.43 ± 0.20	24.8	100
10%	1.23 ± 0.12	18.0	80	4.03 ± 0.85	70.6	100
5%	2.38 ± 0.21	33.9	96	4.00 ± 0.75	70.4	100
0.5%	4.23 ± 0.80	61.4	96	3.83 ± 0.25	67.9	100
Control 0%	6.92 ± 1.10	100	100	5.78 ± 0.95	100	100
EC_{50}		*2.5%*			*15%*	
EC_{30}		*0.25%*			*0.5–10%*	

Expressed as root length (cm) and percentage of control.

Prior to each harvest, plant chlorophyll was also measured by taking two discs (0.8 cm diameter) at the mid-length of the leaf, from both mid-rib sides. Chlorophyll a (Chl a) and chlorophyll b (Chl b) were extracted with acetone and the concentration determined in a UV–Visible spectrophotometer (Perkin Elmer Lambda 3B) at the absorbance of 470, 647, and 664.5 nm [16]. At each sampling time the WW was analysed for pH, electrical conductivity (EC), COD, total suspended solids and phenols.

2.3. Wastewater quality analysis

WW was analysed for pH, EC ($dS\,m^{-1}$), COD, ($mg\,L^{-1}$), TSS ($mg\,L^{-1}$) and phenols ($mg\,L^{-1}$). All assays were carried out in duplicate. Standard WW analysis methods [17] were used. COD was determined using a commercially available high range kit (Chemetrics; mercury containing; 0–15,000 $mg\,L^{-1}$ supplied by Water Test systems) and a single set wavelength photometer (640 nm). Total phenols were analysed according to the method outlined by Box [18]. Absorbance was measured at 725 nm in a UV–Visible spectrophotometer (Cary 50 Bio, Varian).

2.4. Statistical analysis

The phytotoxicity data were subjected to analysis of variance (ANOVA) to determine the significance of the treatment effects with the software Statgraph 5.1. Separation of means was performed using Separation of means was performed using LSD test at $P < 0.05$ level of significance.

3. Results and discussion

3.1. Phytotoxicity test responses

3.1.1. Garden cress test

The phytotoxicity of WW to garden cress (Table 1) is expressed as Effective Concentration (EC) values. The WW was found to be very toxic with EC_{30} and EC_{50} at 0.25 and 2.5% concentration of WW respectively. No seeds germinated when the WW concentration was >50%.

Addition of lime was beneficial as EC_{50} values were at least two fold higher than for the non-limed treatments, 0.5–10% and 5–15% respectively. When WW was further diluted the germination increased to 100% but root elongation was still negatively affected, even by WW concentrations as low as 0.5%. Toxicity in limed treatments was significantly lower than in the non-limed treatments. Even with undiluted WW liming resulted in 60% germination and a root elongation of 1.5%. At the lower concentrations (<10%) 100% germination was achieved and root elongation increased (25–70%) with liming.

The cause of the very high toxicity of this winery wastewater is not known definitively, but is probably a combination of high COD (between 80 and 16,800 $mg\,L^{-1}$), high total phenols concentrations (between 0.52 and 12.1 $mg\,L^{-1}$), low pH, and high salinity (Table 2). However, at low concentrations (0.5–5% WW) salinity is low and suggesting this not the primary cause of phytotoxicity. Lime addition resulted in COD values up to 50% lower than in non-limed treatments. However, TSS levels increased up to 10 fold due to the dissolution of solids at high pH. The lower phytotoxicity with liming could be due to the adsorption of the dissociated strong organic acids, mainly tartaric and malic, to the positively charged surface sites of calcium carbonate, thereby increasing the stability of the organic acids. Ethanol which accounts for about 70% of the COD of winery wastewater [3] would not have been affected by adsorption processes due to its very low dissociation constant (pK_a 16.0). Kumar and Kookana [19] reported a similar decrease in toxicity when they increased the pH of WW from 3.0 to 9.0 by adding

Table 2
Mean chemical properties (±SE) of non-limed (NL-WW) and limed wastewater (L-WW) diluted from 0 to 100%, used for the garden cress, onion and wetland plant species tests. BDL represents below detection limits.

WW %	Non-Limed WW				Phenols ($mg\,L^{-1}$)	Limed WW				Phenols ($mg\,L^{-1}$)
	pH	EC ($dS\,m^{-1}$)	TSS ($mg\,L^{-1}$)	COD ($mg\,L^{-1}$)		pH	EC ($dS\,m^{-1}$)	TSS ($mg\,L^{-1}$)	COD ($mg\,L^{-1}$)	
0	6.0 ± 0.2	0.09 ± 0.02	BDL	BDL	BDL	5.7 ± 1.1	0.04 ± 0.005	35 ± 12	BDL	BDL
0.5	6.1 ± 0.3	0.13 ± 0.02	50 ± 5.8	300 ± 10	1.21 ± 0.2	7.1 ± 1.2	0.34 ± 0.1	1080 ± 100	61 ± 20	0.41 ± 0.1
5	5.5 ± 0.5	0.65 ± 0.1	400 ± 24	400 ± 15	1.86 ± 0.5	7.2 ± 0.7	0.55 ± 0.1	3360 ± 110	300 ± 18	0.25 ± 0.08
10	5.3 ± 0.8	0.58 ± 0.1	200 ± 14	460 ± 12	3.09 ± 1.1	7.3 ± 0.8	0.95 ± 0.2	2900 ± 110	460 ± 10	0.81 ± 0.2
25	5.6 ± 0.7	1.42 ± 0.5	275 ± 18	6080 ± 65	4.9 ± 1.1	7.6 ± 1.5	1.56 ± 0.2	2200 ± 120	3000 ± 75	2.3 ± 0.5
50	4.7 ± 0.6	2.51 ± 0.5	555 ± 24	7550 ± 75	5.74 ± 1.2	7.0 ± 1.4	3.73 ± 1.1	4200 ± 135	5420 ± 120	5.2 ± 1.1
75	4.6 ± 0.4	3.93 ± 0.7	575 ± 25	13,050 ± 60	9.54 ± 1.8	7.6 ± 1.2	5.66 ± 1.1	3750 ± 160	9900 ± 130	8.5 ± 1.1
100	4.5 ± 0.5	5.15 ± 0.8	1000 ± 45	16,800 ± 58	10.6 ± 2.5	7.1 ± 0.4	8.54 ± 1.2	10,850 ± 140	15,400 ± 145	12.1 ± 1.2
LSD ($P \leq 0.05$)	1.5	0.45	150	100	1.3	1.3	0.52	1050	240	1.8

lime. Adjusting the pH results in reduction of COD values by up to 50%, and hence this might explain the decrease in phytotoxicity. Our analysis did not reveal any significant correlation between phytotoxicity, pH, salinity, and total phenol concentration. This suggests that the phytotoxicity may be related to different classes of compounds.

COD and phenolic compounds are usually considered responsible for reduction in plant growth [20]. However, Kumar and Kookana [19] also reported that heavy metals and organic contaminants could be major toxic agents in WW. In addition to organic loading (i.e. COD), other components of WW that represent a concern to regulatory agencies include TDS, sulphur, tannin and lignin [8]. There is a lack of information on the contribution of organic acids and tannins toward toxicity. Ethanol phytotoxicity has been reported by some studies [21,22]. Murin [21] indicated a low value of LC_{50} of ethanol for *Vicia sativa*, 3 mL L^{-1}. Stutte et al. [22] tested ethanol toxicity to radish seedlings and suggested that damage could be due to membrane disruption associated with phospholipid extraction from the cell. Our results and previous literature indicates that WW is a complex toxic mixture. However, the literature does not report any comprehensive study on the characterization of the principal components of toxicity in WW.

3.1.2. Onion test

Table 3 shows the EC_{50} and EC_{30} concentrations causing growth inhibition using the same set of WW concentrations as that for the garden cress experiment, Table 1. The onion roots appeared to be much more sensitive than those of garden cress under the same experimental conditions, with an EC_{50} of 0.25 and EC_{30} of 0.1%. These are 2.5–10 fold lower than the inhibition values obtained with the garden cress test. Again the limed treatments (EC_{50} 5%) were found to be less toxic than the non-limed treatments (EC_{50} 0.5%). Compared to the cress test there is a clearer and more sensitive dose response.

Table 3
Toxicological effects on onion roots of *Allium coepa* after 5 days in non-limed (NL-WW) and limed wastewater (L-WW) expressed as root length (cm) and root length as % of control. Values are mean ± SE (n = 6 onion bulbs). The EC_{50} and EC_{30} values were determined by measuring the growth inhibition of the roots in relation to the control.

	Root length			
	Non-Limed		Limed to pH 6.5	
	cm	% Control	cm	% Control
100%	0.16 ± 0.08	8	2.66	6.3
75%	0.10 ± 0.02	5	0.16	6.3
50%	0.40 ± 0.12	20	0.50	18.8
25%	0.40 ± 0.13	21.7	0.66	22
10%	0.50 ± 0.12	25	0.66	25
5%	0.60 ± 0.10	33	1.33	50
0.5%	0.70 ± 0.15	35	2.33	87.5
0%	2.00 ± 0.21	100	2.66	100
EC_{50}		*0.25%*		*5%*
EC_{30}		*0.1%*		*2.5%*

3.1.3. Wetland plant species test

One week after transplanting, the treatments with wastewater concentrations of 50, 75 and 100% were found to be extremely toxic for all the wetland plant species. These plants showed marked symptoms of chlorosis, necrosis and death and were not sampled further. For the treatments with WW concentrations of 10 and 25% the plants showed significant reductions in biomass ($p < 0.05$), with an 80% reduction for the 25% WW concentration (Fig. 1). The treatments with WW concentrations of 10% and less displayed minimal phytotoxicity, thus indicating that a 10% concentration of WW is a threshold for plant health. This is close to the EC_{50} range observed for the garden cress and onion test. Limed treatments displayed higher biomass production relative to the corresponding non-limed sample. Only *Schoenoplectus* and *Juncus* showed biomass

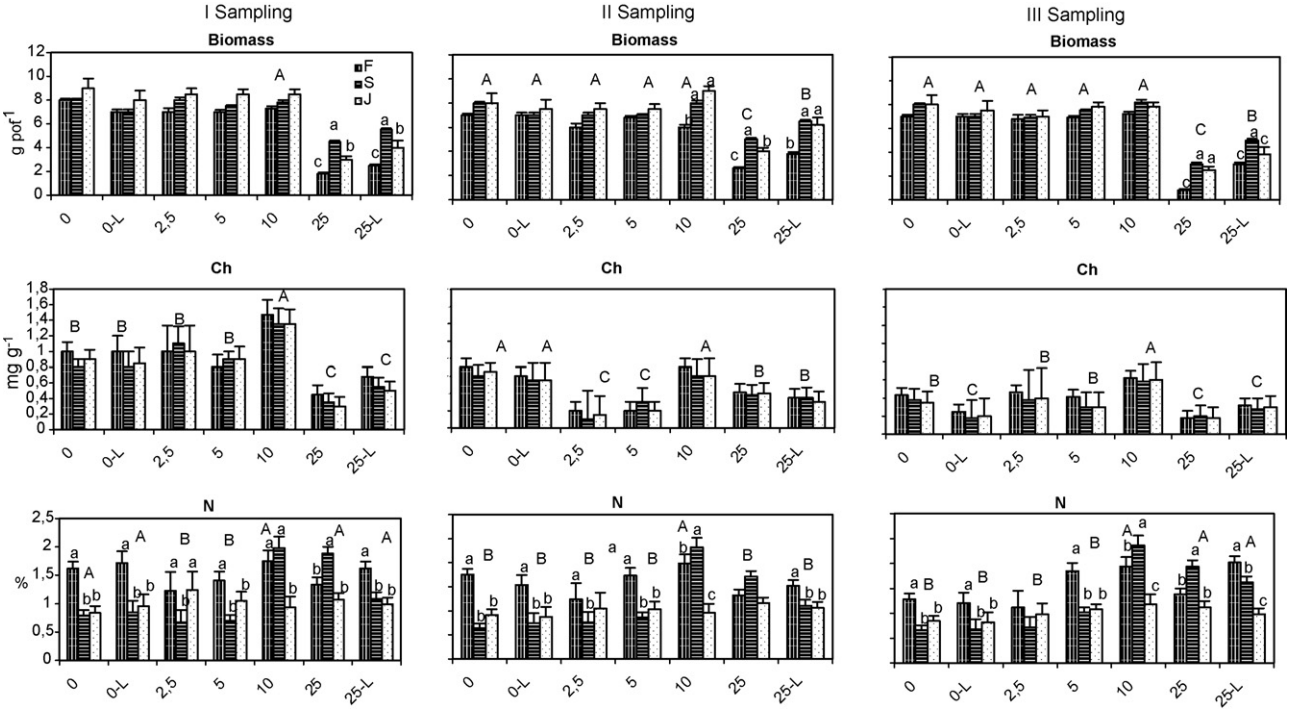

Fig. 1. Biomass (g pot^{-1}) production, total chlorophyll (mg g^{-1}) and nitrogen (% dry matter) concentrations of *Phragmites australis*, *Schoenoplectus validus* and *Juncus ingens* growing for 20, 40 and 60 days in winery WW diluted by factors of up to 200 with deionized water (0.5–100% WW). 0-L and 25-L represent limed water. Capital letters above the error bars represent significant differences among treatments and lower case letters represent significant differences between plant species (LSD test; $\alpha = 0.05$). Letters are not shown in cases where no differences were obtained at this level of significance.

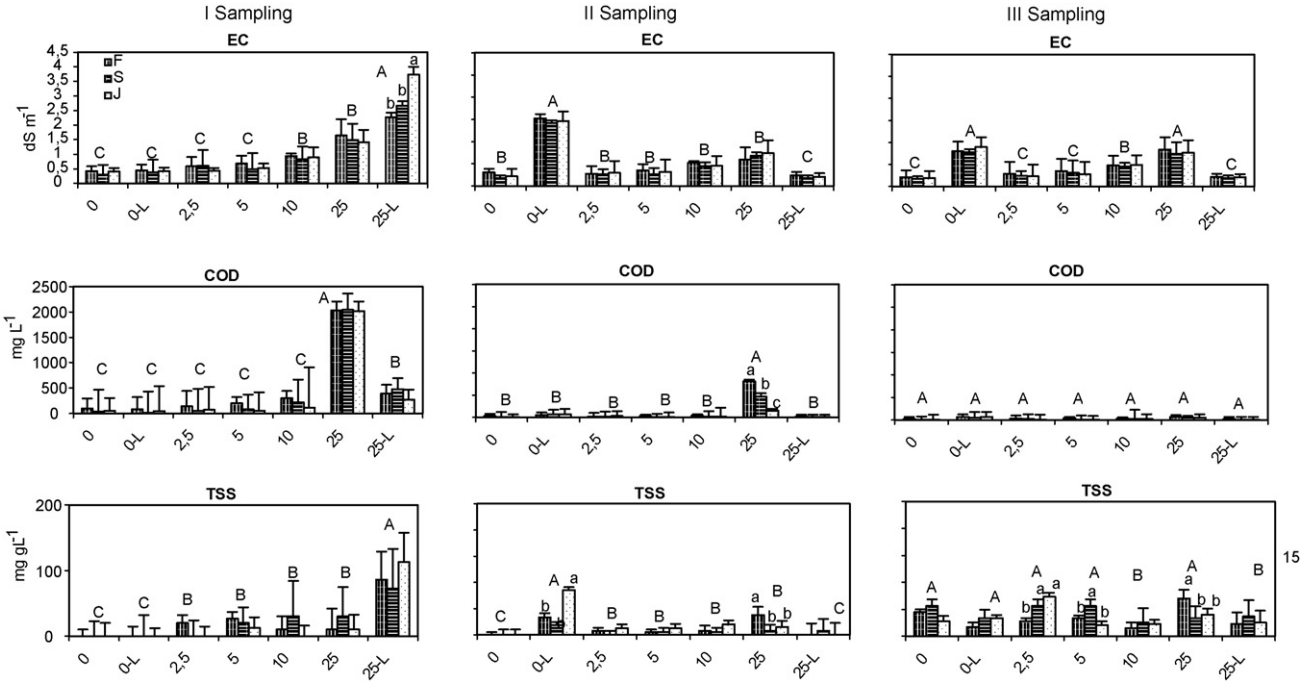

Fig. 2. EC (dS m^{-1}), COD (mg L^{-1}) and TSS (mg L^{-1}) concentrations of winery WW diluted by factors of up to 200 with deionized water (0.5–100% WW) at 20, 40 and 60 days of growing *Phragmites australis*, *Schoenoplectus validus and Juncus ingens*. 0-L and 25-L represent limed water. Capital letters above the error bars represent significant differences among treatments and lower case letters represent significant differences between plant species (LSD test; $\alpha = 0.05$). Letters are not shown in cases where no differences were obtained at this level of significance.

production of the same order of magnitude as the control. Although *Phragmites* has been reported to be a hardy plant species [23], in our experimental conditions it was found to be the most sensitive. The chlorophyll concentrations of *Phragmites* decreased for WW concentrations above 10% (Fig. 1). The chlorophyll content in all plant species was highest at 10% WW concentration. For *Phragmites* the chlorophyll was 1.5 *vs.* the control at 1.0 mg g^{-1} at 20 days ($p \leq 0.05$). No significant differences were observed in the chlorophyll concentrations between the limed and non-limed samples. Data on nitrogen (N), phosphorous (P) and potassium (K) concentrations in the plants, Figs. 1 and 2, indicated high levels of the elements in all three species. The total N concentration of the WW was ~35.0 mg L^{-1}. Nitrogen in this wetland microcosm, depending on the levels of oxygen and specific activity of plant root biomass, can be present in the form of ammonia and nitrate and adsorbed inorganic and organic nitrogen [9]. The mean level of N at the three sampling times in *Phragmites* and *Schoenoplectus* was ~2%, with the highest levels at 10% WW concentration. The maximum level of N found in *Juncus* did not exceed 1.25% and there were no significant differences between the limed and non-limed effluent. The levels of P in the plant tissue were low, 0.1–0.4%, over the three sampling periods, with the highest P concentration being 0.4% for *Schoenoplectus* at 10–25% WW concentration (Fig. 3). Concentrations of K in the plant tissue over the three sampling events were highest at 10–25% WW (Fig. 3). Potassium concentrations were up to 4.7% for *Schoenoplectus* and about two fold higher than the control (~2%). These levels of K seem to be comparable to those of up to 5% dry weight, reported by the literature for legumes, grasses and herbages grown with wastewater [24]. Since Potassium concentrations in WW have been reported to be an environmental issue, due to its potential negative effect on soil structure [25], this high uptake is a useful feature of these plants.

It is probably the organic constituents in the WW that accounted for the increased levels of N, P, and K compared to the control. The WW had strong yellow-brown colouration caused by the presence of humic substances (the absorption coefficient at 440 nm was greater than 25 mg m^{-1}), which have been shown to modify nutrient availability and toxicity to plants via complexation, chelation and ion exchange and to affect plant physiological processes via growth regulation [10].

3.2. Wastewater quality

Figs. 1 and 2 show the WW quality at 20, 40 and 60 days for WW concentrations of 0–25%. The salinity at 20 days tended to increase with WW concentration and were highest for the lime amended treatments, ~3.0 dS m^{-1}. At 40–60 days the salinity fell below 1 dS m^{-1}, with the lowest value (0.2 dS^{-1}) observed for the 25% WW treatment. This was 10 fold lower than the value of the control. This could be due to a more intense ion removal processes from the effluent by chelation, complexation and plant uptake.

Overall the results showed a rapid improvement in effluent quality. After 20 days the organic load of the WW reduced significantly, with COD mean values for the 25% WW treatment dropping to ~2000 mg L^{-1}, about 10 fold lower than the initial value, Table 2.

The addition of lime was extremely effective in reducing organic load, with a mean value of ~200 mg L^{-1} at 20–40 days. At 60 days COD values dropped further to below 100 mg L^{-1} with no significant differences between plant species. Also pH appeared to be effectively neutralized by the addition of lime.

The wetland microcosm system was also effective in reducing TSS concentration by up to 20 times from the initial value after 20 days (Table 2). Similar to salinity, TSS values at 20 days were significantly higher for the limed WW, being up to 100 mg L^{-1}. Phenol concentrations were also reduced by approximately 50 fold after 20 days and subsequently, phenol levels dropped below the detection value, 0.05 mg L^{-1} (data not shown). There were no significant differences between plant species.

Overall, the data for the limed WW at 25% concentration measured at 60 days showed good WW treatment with >95 removal of COD, TSS and phenols.

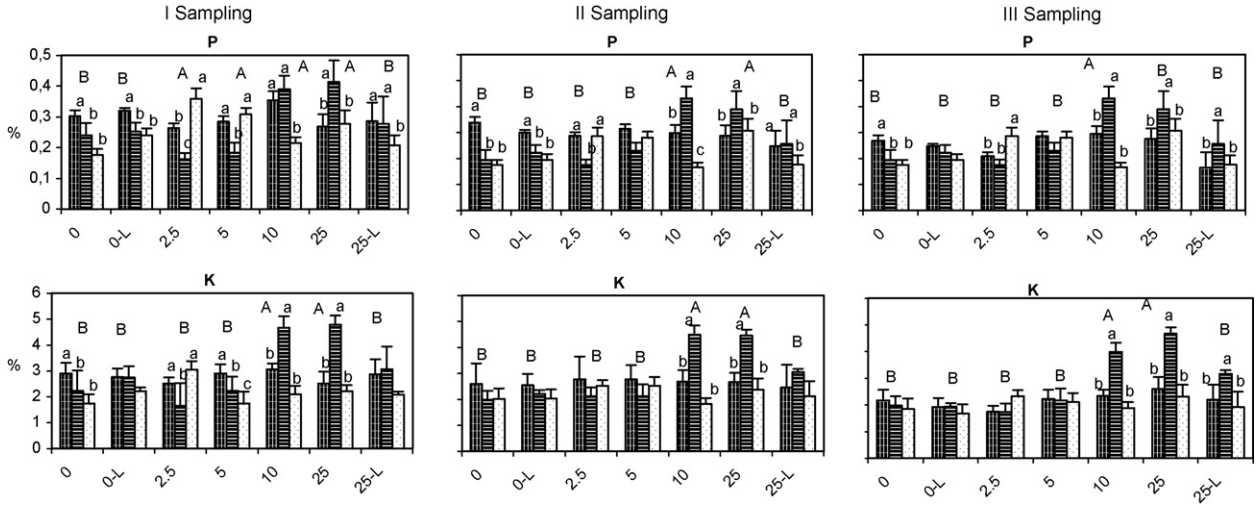

Fig. 3. Phosphorus and potassium (% of dry matter) concentrations of *Phragmites australis*, *Schoenoplectus validus* and *Juncus ingens* growing for 20, 40 and 60 days in winery WW diluted by factors of up to 200 with deionized water (0.5–100% WW). 0-L and 25-L represent limed water. Capital letters above the error bars represent significant differences among treatments and lower case letters represent significant differences between plant species (LSD test; $\alpha = 0.05$). Letters are not shown in cases where no differences were obtained at this level of significance.

4. Conclusions

The garden cress and onion bioassays provide a rapid phytotoxicity screening for WW. These tests provided comparable results to that of the wetland pot trial. The toxicity threshold for the wetland plants at 10% WW concentration was of the same order of magnitude as the EC_{50} observed for the garden cress test (2.5–15%) and the onion test (0.25–5%). As such the onion and garden cress tests could be used in the wine industry for rapid assessment of the toxicity of wastewater at different stages of treatment.

Adjustment of pH with lime and high dilution rates were required to reduce the phytotoxicity of the WW. However, dilution of WW is generally impractical, hence effective preliminary aerobic/anaerobic treatment is required to reduce the overall toxicity of WW before application to a wetland. *Schenoplectus* and *Juncus* appeared to be more tolerant to WW and had higher K uptake than *Phragmites*. All the microcosm wetland treatments for 25% wastewater concentration or less provided large improvements in water quality.

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