Tolerance of Macrophytes and Grasses to Sodium and Chemical Oxygen Demand in Winery Wastewater

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Winery wastewater often contains elevated concentrations of sodium (Na), and has a high chemical oxygen demand (COD). In constructed wetlands, Na may be removed through phytoremediation by such macrophytic plants as *Typha latifolia*, *Juncus acutus* and *Scirpus maritimus*. The relative abilities of these plants to absorb Na, and to tolerate high COD wastewaters was determined in a glasshouse pot trial. Also tested were *Pennisetum clandestinum* (Kikuyu) and *Vetiveria zizanioides* (Vetiver) grass, which are used on wastewater disposal sites. Treatments consisted of factorial combinations of Na and COD. Toxicity symptoms were not apparent below 16.2 µM Na/L < 5 000 mg COD L, but were marked at ≥ 40 µM Na/L and ≥ 15 000 mg COD/L. Of the macrophytes, *J. acutus* was the least affected by high Na and COD levels. Averaged across the Na and COD treatments, total plant dry mass (DM) in the macrophytes peaked at 40 µM Na/L, as did ability to tolerate COD. The total Na in the top growth was greatest in *J. acutus* from which 61.4 mg Na/plant (767 mg/m^2) could potentially be harvested after six months. Equivalent figures for *T. latifolia* and *S. maritimus* were 38.8 and 25.0 g/plant, respectively. Of the grasses, *P. clandestinum* produced 35% more total DM than *V. zizanioides*. Top-growth Na contents were, respectively, 1427 and 29.1 mg/plant. To maximise Na uptake in harvestable plant components, *J. acutus* should be planted in wetlands, and *P. clandestinum* in pastures used for wastewater disposal.

Wastewaters from wineries contain elevated concentrations of mineral elements, notably sodium (Na) (Van Schoor & Rossouw, 2004). This is largely derived from cleaning and sterilising agents such as sodium hydroxide, sodium metasilicate and sodium carbonate (Papini, 2000). Winery wastewater also contains organic material (Shepherd, 1995), which confers high levels of chemical oxygen demand (COD) (Van Schoor, 2000). The disposal onto land or into streams of wastewater characterised by high Na concentrations and high COD values is regulated by the National Water Act (1998). Compliance invariably necessitates implementation of measures which reduce element concentrations and COD to acceptable levels before disposal. A method that has proved cost effective in South Africa (Mulidzi, 2008) and internationally (Bulc *et al*., 1997) is the treatment of wastewater in constructed wetlands. In such wetlands mineral elements are taken up by macrophytes (plants that grow in water but whose shoots project above the water surface) (Moshiri, 1993), a process known as phytoremediation (McCutcheon & Schnoor, 2003). Some of the mineral elements taken up from water, sediment or soil (Moshiri, 1993; Salt *et al*., 1998), usually by the roots, are translocated to the shoots and reproductive structures (Wainio *et al*., 2003), together with which they may be removed by periodic harvesting. In addition to the removal of nutrient elements, macrophytes contribute to water purification by providing substrates and habitats where organic compounds may be broken down (Gale *et al*., 1994), reducing COD. Such breakdown is mainly brought about by microbes associated with the macrophyte root systems (Brix & Carter, 1986).

Regardless of whether winery wastewaters undergo treatment or not, the water must be reused or disposed of. A common method is that of pasture irrigation (Mills *et al*., 2005). However, this practise may lead to increased soil salinity and sodicity, structural degradation, and the onset of anaerobic conditions in the subsoil (Van Schoor, 2000). A mitigating factor in such pastures is that plants may intercept and adsorb some of the minerals before they enter the soil (McCutcheon & Schnoor, 2003). In constructed wetlands and disposal pastures effective phytoremediation necessitates the use of plants that are able to function in environments that may be acidic, nutritionally unbalanced, brackish and contain organic materials that contribute to high COD levels (Salt *et al.*, 1998; Batty *et al.*, 2002). Some plants reportedly flourish under these conditions, finding the wastewater a rich source of nutrients (Karpiscak *et al*., 1999). Others may lack adequately developed tolerance mechanisms and not flourish in such conditions (Marschner, 1995). Identification of suitable plants is therefore a necessity. *Typha latifolia*, an emergent wetland macrophyte (Quick, 1987), has already been used in wastewater treatment wetland trials (He & Mankin, 1989; Moshiri, 1993; Zingelwa & Wooldridge, 2009). Other potentially usable macrophytes are *Scirpus maritimus* and *Juncus acutus*. *Typha species* are found in a variety of often saline habitats throughout the world, and may have evolved a genetic potential to

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tolerate such conditions (Von Oertzen & Finlayson, 1984). *Juncus acutus* and *S. maritimus*, however, are salt-marsh macrophytes. Both have been used in salt-tolerance studies (Coultas & Hsieh, 1997). *Scirpus maritimus* is a facultative halophyte. It lacks morphological adaptations to salt tolerance, but is nevertheless able to accumulate Na (Kantrud, 1996). *Scirpus maritimus* ranked highly in sodium tolerance studies, and shows positive growth responses to NaCl (Ungar, 1991).

Since wastewater salt concentrations do not decrease appreciably during passage through constructed wetlands (Zingelwa & Wooldridge, 2009), the plants used in disposal areas must, like those in artificial wetlands, be tolerant of poor water quality and able to take up minerals. *Vetiveria zizanioides* (vetiver grass) is widely used in hedges to alleviate erosion and improve soil structures in degraded land (Greenfield, 2002). *Vetiveria zizanioides* is tolerant of soil salinity, sodicity, and high heavy metal concentrations over a wide pH range (Greenfield, 2002), and tolerates up to 15% NaCl (Nanakorn *et al.,* 2005), whereas most plants have a threshold of 1% NaCl. When supplied with municipal effluent spiked with NaCl, *V. zizanioides* plants thrived, due to the nutrients provided by the wastewater (Klomjek & Nitisoravit, 2005). Another grass, *Pennisetum clandestinum* (kikuyu grass) is a common pasture crop that has been used in experiments to investigate tolerance and accumulation of salts (Mulidzi *et al.*, 2002). In a study by Mills *et al*. (2005), *P. clandestinum* pastures irrigated with saline wastewater showed exceptional tolerance to salinity. Information concerning the tolerance of *P. clandestinum* to high COD levels is nevertheless sparse.

The objective of this work was to determine the relative abilities of certain macrophytes and grasses to survive, generate dry mass (DM), and accumulate mineral elements in environments containing a range of Na and COD concentrations.

MATERIALS AND METHODS

Cuttings of the macrophytes *T. latifolia*, *J. acutus* and *S. maritimus*, and of the grasses *P. clandestinum* and *V. zizanioides* were established in coarse sand in 40 x 20 x 20 cm cement troughs. These were arranged in a glasshouse using a design that made provision for a fully randomised trial in which each plant x water quality treatment was represented in two blocks. Each treatment received two L of a simulated winery wastewater twice each week. Wastewater treatments consisted of five concentrations of Na (0, 16, 40, 162 and 400 µM/L), as in salinity tolerance trials by Marschner (1995), in factorial combination with five levels of COD (0, 5 000, 10 000, 15 000 and 20 000 mg/L). The Na was derived from NaCl, and the COD from distilled wine. The treated plants were allowed to grow for a six month period, by the end of which some of the shoots showed physiological responses to the treatments. Response severity was graded on a 4-point scale (0, dead; 1, weak shoots; 2, shoots showing toxicity symptoms, die-back and necrosis apparent; 3, all shoots green and apparently healthy). After grading, each plant was removed from the sand, quickly washed and divided into components (shoots, rhizomes [*T. latifolia* only] and roots). These were dried to constant mass (± 1%) in a fan oven at 70°C, weighed to determine DM and passed through a Wiley mill. A 0.40 g sample of each component was digested using a mixture of sulphuric acid and hydrogen peroxide (Allen, 1989). Concentrations of calcium (Ca), Na, magnesium (Mg) and potassium (K) in the volumetric solutions were then determined using a Pye Solaar Atomic Absorption Spectrophotometer. Phosphorus (P) was determined by the method of Murphy and Riley (Olsen & Sommers, 1982), using a Shimadzu spectrophotometer. Kjeldahl distillation and titration (Bremner & Mulvaney, 1982) were used to quantify the nitrogen (N) contents. The DM and analytical data were subjected to an analysis of variance using SAS (SAS, 2003). Least significant difference (LSD) values were calculated at the 5% probability level to facilitate comparison between treatment means. Treatments which differed at $P = 0.05$ were regarded as significantly different. Co-efficients of determination (r^2) (Rees, 2001) were calculated to enable tissue element concentrations and DM's to be related to the concentrations of Na and COD in the treatment solutions.

RESULTS AND DISCUSSION

Macrophytes

Physiological responses and survival

From Table 1, it is apparent that there is a broad diagonal relationship such that the macrophyte shoots remained healthy where both Na and COD were low (≤ 16 mM/L Na; ≤ 5 000 mg COD/L), whereas at high Na and COD concentrations (≥ 40 mM/L Na, ≥ 15 000 mg COD/L), most of the macrophyte plants died. In treatment combinations between these extremes, macrophyte shoot growth was generally weak or characterized by toxicity symptoms. This observation agrees with Xu *et al*. (2006), who found that high COD can cause shoot burning or even plant death, and with Marschner (1995) who commented that excess Na can stunt growth or even kill, either through its osmotic effect or through antagonism with essential mineral nutrients such as K and Ca. Dead *S. maritimus*, *T. latifolia* or *J. acutus* plants were observed in eight, four and three treatments, respectively. If the number of dead plants is regarded as an indication of ability to tolerate Na and COD in wetlands, then potential suitability for wetland use is likely to decrease in the sequence: *J. acutus* > *T. latifolia* > *S. maritimus*. At all levels of COD, *T. latifolia* and *J. acutus* tolerated higher concentrations of Na than *S. maritimus*.

TABLE 1

Effect of sodium (Na) concentration and chemical oxygen demand (COD) on growth and survival of *Typha latifolia*, *Scirpus maritimus* and *Juncus acutus*. Visually evaluated after six months using the scale: 0, dead; 1, weak shoots; 2, shoots showing toxicity symptoms; 3, healthy shoots.

COD mg/L (x 10 ^{3})	Species	Na (µM/L) 0	16	40	162	400
0	*S. maritimus*	3	3	2	2	1
	T. latifolia	3	3	3	3	3
	J. acutus	3	3	3	3	2
5 000	*S. maritimus*	3	3	2	1	1
	T. latifolia	3	3	2	2	2
	J.acutus	3	3	3	2	2
10 000	*S. maritimus*	2	2	2	1	0
	T. latifolia	3	2	2	1	1
	J. acutus	2	2	2	1	1
15 000	*S. maritimus*	1	1	0	0	0
	T. latifolia	3	2	1	0	0
	J. acutus	2	2	1	1	0
20 000	*S. maritimus*	1	0	0	0	0
	T. latifolia	1	1	1	0	0
	J. acutus	2	1	1	0	0

TABLE 2

Effect of species and plant component, and of sodium (Na) concentration and chemical oxygen demand (COD), on dry mass (DM), element concentration, and total element contents of *Typha latifolia (Tl)*, *Juncus acutus (Ja)* and *Scirpus maritimus (Sm)*.

	Species			*Tl*			*Ja*		*Sm*		<	Na (µM/L)			>	<	COD mg/L x 10^3			>
	Tl	*Ja*	*Sm*	Shoot	Rhizome	Root	Shoot	Root	Shoot	Root	0	16.2	40	162	400	0	5	10	15	20
	Dry mass (g DM / plant or plant component)																			
DM	8.7b	10.9a	5.7c	6.7b	9.9a	9.4 a	12.4a	9.4b	1.8b	9.7a	9.6a	8.0b	8.3b	8.5ab	7.9b	9.5a	6.8b	8.9a	9.7a	7.3b
	Element concentration in tissue (%)																			
N	7.9 a	7.58a	7.4a	5.3c	8.2b	10.4a	5.1b	10.0a	5.2b	9.6a	7.5a	7.6 a	8.1a	7.8a	7.6a	7.6a	7.4a	7.9a	7.9 a	7.7a
P	0.64a	0.56a	0.58a	0.98a	0.58b	0.46b	0.85a	0.26b	0.86a	0.29b	0.55bc	0.60b	0.78a	0.56bc	0.49c	0.53b	0.56b	0.74a	0.58b	0.57b
K	8.4a	8.5a	7.2a	12.5a	8.2 b	4.6 c	12.5a	4.6b	9.0a	5.3b	8.3a	8.2a	7.0b	8.1a	8.9a	8.91a	8.4ab	8.9a	7.8b	6.5c
Ca	12.9a	11.2a	10.4a	12.8a	13.0a	12.9a	9.2b	13.2 a	9.3a	11.4a	16.7a	14.9a	7.5c	9.5bc	9.8b	11.6a	12.0a	11.6a	12.1a	11.2a
Mg	2.2a	2.2a	1.3b	1.9a	1.8 a	2.7a	2.8a	1.6b	1.5a	1.0b	1.0c	1.9b	1.5bc	3.3a	1.8b	1.7b	2.4a	1.9ab	2.0ab	1.5 b
Na	13.5a	14.7a	12.4a	20.1a	11.2b	9.31b	20.1a	9.3b	17.0a	7.9b	15.5a	12.6b	13.9ab	12.9b	12.9b	7.4c	11.3b	12.7b	13.3b	23.1a
	Total element in plant (mg/plant or mg/plant component)																			
N	71a	78a	52b	36b	83b	94a	64b	93a	10b	94a	74a	64a	70a	69a	61a	73ab	51c	74ab	80a	60c
P	4.6a	3.5a	3.6a	4.8a	4.0a	4.8a	4.3a	2.6b	4.5a	2.6b	3.8b	4.1b	5.2a	3.5b	3.3b	3.5b	3.6b	5.1a	4.1b	3.6b
K	5.6a	5.8 a	4.4a	11.0a	3.7b	2.1b	10.4a	1.2b	7.3a	1.5b	4.6b	5.6a	5.6	5.5a	5.3ab	5.3b	5.7ab	6.4a	5.1b	4.1 c
Ca	111a	87ab	70b	172a	104b	56c	109a	64b	79a	60a	133a	124a	51c	71b	82b	96a	98a	106a	94a	67b
Mg	31.4a	21.9ab	10.8b	28a	25a	41a	23a	21a	11a	11a	17bc	38a	14c	29ab	17bc	22ab	34a	21ab	23ab	15b
Na	26b	38a	17c	39a	19b	20b	61a	14b	25a	8b	16c	24b	24b	44a	25b	14b	26a	28a	31a	34a

Values in the same row, and in the same block of data, that are followed by the same letter, do not differ at P = 0.05.

Dry mass production

As was the case for potential suitability, total (whole plant) DM production decreased in the sequence: *J. acutus* > *T. latifolia* > *S. maritimus* (Table 2). Ideally, wetland macrophytes should retain most of their DM in the top growth, since this can be harvested without killing the plant (McCutcheon & Schnoor, 2003). Relationships between shoot and below ground DM's were not consistent. Shoot DM in *T. latifolia* was lower than in the rhizome and root, whilst shoot DM was considerably smaller than root DM in *S. maritimus*. Only in *J. acutus* did shoot DM exceed root DM. Shoot DM was greatest in *J. acutus* and least in *S. maritimus*. Production of abundant shoot, relative to below ground biomass may indicate tolerance of high concentrations of elements, in water or sediments (Ye *et al.,* 2001). If so, the fact that *J. acutus* was the only macrophyte in which shoot DM exceeded below ground DM may be further evidence in support of the suitability of this plant for use in wetlands used for the treatment of winery wastewater. The low total DM produced by *S. maritimus*, and its high mortality rate relative to *T. latifolia* and *J. acutus*, may indicate a higher level of physiological sensitivity to Na and COD. Such sensitivity would render *S. maritimus* relatively unsuitable for phytoremediation purposes.

Tissue element concentrations

With the exception of Mg, which was low in *S. maritimus*, relative to *T. latifolia* and *J. acutus*, tissue element concentrations did not differ between macrophyte species (Table 2). In *J. acutus, T. latifolia* and *S. maritimus*, top growth N concentrations were low, and P, K and Na were high, relative to the below ground structures.

Total elements in plant

Total element contents (DM x concentration) varied inconsistently with species, plant component and treatment with Na and COD (Table 2). Sodium decreased in the sequence *J. acutus* > *T. latifolia* > *S. maritimus*, whereas the Ca and Mg contents were greater in *T. latifolia* than in *S. maritimus*. Nitrogen was more abundant in *T. latifolia* and *J. acutus* than in *S. maritimus*, whereas P and K contents did not differ between species. Total shoot K, Ca and Na exceeded that in the below ground plant components in all three species. Total plant Na peaked at 162 µM Na/L, and was lowest at zero mg COD/L, tending to increase over the range from 5 000 to 20 000 mg COD/L.

Effects of Na and COD

The effects of Na and COD on DM in the individual macrophytes were inconsistent. Where the macrophyte data were pooled (Table 3), there was a tendency for tolerance to COD, as indicated by DM production, to increase with Na to a maximum at 40 µM/L, then decrease. Whole plant DM production at 40 µM Na/L peaked at 15 000 mg COD/L. These values (40 µm Na/L and 15 000 mg COD/L) may be close to the Na and COD concentrations in wetlands where such macrophytes are likely to perform best as agents of phytoremediation. The effects of Na on tissue element concentrations were either not significant or inconsistent, as were the effects of COD. The exception was tissue Na, which was highest in the zero µM Na/L treatment, and which tended to increase with increasing COD. Coefficients of determination exceeded 0.5 (50%) in the cases of Na in *J. acutus* and *T. latifolia* ($r^2 = 0.54$ in both), and total Na in *S. maritimus* ($r^2 = 0.50$) (data not shown). Magnesium also correlated with the Na and COD treatments in *S. maritimus* ($r^2 = 0.69$). No other element was significantly related to the Na and COD treatments, implying the absence of a direct suppressive effect of the treatments on these elements, contradicting Marschner (1995).

Juncus acutus, with its combination of high shoot DM, reasonably high tolerance of Na and COD, and high shoot N, P, K and Na concentrations, relative to the roots; plus its high total shoot Na (61.4 mg/L) as compared with 25.0 mg/plant in *S. maritimus* and 38.8 mg/plant in *T. latifolia*) (data not shown) was the best suited of the three macrophytes for use as a phytoremediant in wetlands. At a density of 12.5 plants/m^2, full harvesting of the top growth would result in the removal of 0.77, 0.31 and 0.49 g Na/m^2 of wetland per harvest by *J. acutus, S. maritimus* and *T. latifolia*, respectively. These masses are small relative to the amount of Na that could enter a wetland, which explains why wetlands are not usually effective in removing Na (Zingelwa & Wooldridge, 2009). A further drawback to harvesting top growth in wetlands is that any dead plant material that remains behind will decompose, promoting eutrophication (Quick, 1987).

TABLE 3

Effect of sodium (Na) concentration and chemical oxygen demand (COD) on dry mass production (g DM/plant) by *Typha latifolia, Juncus acutus* and *Scirpus maritimus* (data for the three species pooled).

CODmg/ L x 10^3	Na (µM/L) 0	16	40	162	400
0	13.63a	6.08c	6.53b	9.08ab	12.7a
5	6.97c	7.18bc	6.75b	7.79bc	5.48c
10	8.96bc	10.84a	6.86b	12.07a	6.16bc
15	8.33bc	9.54ab	15.50a	8.38bc	7.04b
20	10.34b	6.53bc	5.71b	5.53c	8.69

Values in the same column, which are followed by the same letter, do not differ at P = 0.05

LSD (P= 0.05) = 3.060 (rows).

Grasses

Physiological responses and survival

Neither of the grass species showed visible signs of physiological stress nor did the plants die (data not shown). Both *P. clandestinum* and *V. zizanioides* were therefore able to function across the range of Na concentrations, from zero to 400 µM Na/L, and from zero to 20 000 mg COD/L (data not shown).

Dry mass production

Pennisetum clandestinum produced 35% more total plant DM (roots, rhizomes and shoots) than *V. zizanioides* (Table 4). Root DM in both grasses exceeded shoot DM. However, *P. clandestinum* exhibited a higher ratio of shoot DM to root DM (1:1.739) than *V. zizanioides* (1:1.391), which accords with the observation by Greenfield (2002) that *V. zizanioides* has a relatively well developed root system. In the grasses, total DM's tended to decrease with increasing solution Na concentration, in accordance with Marschner (1995). According to Chapman (1974), excess Na may affect the Ca regime in the plant, thereby inhibiting cell wall formation and membrane integrity, resulting in reduced growth. Total plant DM increased with both COD and Na over the respective ranges zero to 20 000 mg COD/L and zero to 40 mg Na/L. At higher Na concentrations DM was suppressed in the higher COD treatments (Table 5). Coefficients of determination (r^2) between total plant DM and the combined effects of Na and COD were not significant for either grass species (data not shown).

Tissue element concentrations

Concentrations of N, P, Ca, Mg and Na, but not K, were significantly greater in *P. clandestinum* than *V. zizanioides* (Table 4). In *P. clandestinum* the concentrations of N, P, K, Mg and Na in the shoots exceeded those in the roots whereas, in *V. zizanioides* the root N, Ca and Na concentrations exceeded those in the shoots (Table 4). As with COD, the tissue N, P, K, Ca and Mg concentrations were lower at 162 and 400 µM Na/L than in the zero (mg/L) Na, which agrees with Marschner (1995) and Chapman (1974). In contrast, tissue Na concentrations increased with increasing solution Na concentration. Chemical oxygen demand

TABLE 5

Parameter estimates and standard errors for regression relationships between dry mass (DM), tissue sodium (Na) concentration and total plant Na, in the shoots of *Pennisetum clandestinum* as functions of solution Na concentration (mM/L) and solution chemical oxygen demand (COD) (mg/L). Model described by the relationship: DM, Na conc., Total Na = a + b(Na conc.) + c(Na conc.^{2}) + d(COD) + e(COD^2) + f (Na conc. x COD).

Parameter		a (Intercept)	b	c	d	e	f	P	r^2
DM (g/plant)	Par. est.	21.138	0.031	-1.183x10^{-4}	-1.796x 10^{-4}	2.343x10^{-8}	6.646x10^{-7}	0.010	28.2%
	Std error	2.371	0.027	6.145x10^{-5}	4.521x10^{-4}	2.111x10^{-8}	8.354x10^{-7}		
Na concentration (mg/kg^{-1})	Par. est.	26.461	0.557	6.132x10^{-4}	8.274x10^{-4}	5.543x10^{-8}	-6.010x10^{-6}	<0.01	80.6%
	Std error	7.772	0.088	2.014x10^{-4}	1.480x10^{-3}	6.918x10^{-8}	2.740x10^{-6}		
Total Na in plant (mg/plant)	Par. est.	415.663	15.840	-0.028	-0.018	1.930x10^{-6}	1.360x10-6	<0.01	94.5%
	Std error	262.234	2.966	6.800x10^{-3}	0.050	2.330x10^{-6}	9.239x10^{-5}		

TABLE 4

Effect of species and plant component, and of sodium (Na) and chemical oxygen demand (COD), on dry mass (DM), element concentration, total element content of plants and coefficients of determination (r^2) of *Pennisetum clandestinum* (Kikuyu, K) and *Vetiveria zizanioides* (Vetiver, V).

	Species		Kikuyu		Vetiver		.<		Na (µM/L)		>	<		COD (mg/L x 10^3)		>	r^2	
	K	**V**	**Shoot**	**Root**	**Shoot**	**Root**	**0**	**16.2**	**40**	**162**	**400**	**0**	**5**	**10**	**15**	**20**	**K**	**V**
									Dry mass (g/plant or g/component)									
DM	31.6a	23.4a	23.1b	40.1a	19.5b	27.1a	30.2a	25.9bc	29.2ab	27.9bc	24.4c	27.7ab	24.3b	26.4ab	29.2a	30.2a	0.282	0.096
									Element concentration (mg/kg $^{-1}$)									
N	12.9a	6.9b	14.5a	11.2b	5.9b	7.8a	10.7a	10.8a	10.5ab	9.6b	7.9c	10.4a	10.2ab	10.7a	9.3bc	8.9c	0.64	0.191
P	2.6a	0.66b	3.3a	1.8b	0.81a	0.52b	1.9a	1.9a	1.7b	1.5c	1.2d	1.5c	1.6bc	1.8a	1.7ab	1.6bc	0.603	0.030
K	9.5a	7.3a	13.4a	5.7b	11.3a	3.4b	9.7ab	10.5a	8.9bc	7.7c	5.5d	8.3a	8.4a	8.3a	8.5a	8.8a	0.411	0.057
Ca	26.7a	6.4b	25.4a	28.0a	4.9b	7.9a	20.2a	16.4b	16.4b	16.6b	14.0b	11.5b	16.6a	19.0a	18.2a	18.5 a	0.289	0.177
Mg	2.98 a	1.0b	4.2a	1.8b	1.2 a	0.81b	2.3a	2.14ab	2.2ab	1.9b	1.5c	2.1a	1.9 a	2.1a	2.1a	1.89 a	0.280	0.141
Na	40.9 a	2.9b	64.9a	16.9 b	2.22 b	3.72a	6.78 d	12.2cd	16.9c	31.1b	43.9a	23.5a	23.8a	19.9a	21.9a	22.0 a	0.806	0.481
									Total element (mg/plant or mg/component)									
N	392 a	162a	337 b	447 a	115 b	209a	327 a	275 b	317 ab	277 b	200 c	301a	250 b	290 ab	279 ab	276 ab	0.434	0.055
P	75.7 a	15.1b	77.7 a	73.8 a	16.0 a	14.2a	56.1 a	50.7ab	50.1ab	43.1 b	29.5c	43.3ab	37.7 b	48.4 a	51.0 a	49.1 a	0.443	0.043
K	273a	165a	316a	231b	236a	95b	279 a	245 ab	248 ab	204 b	123.5c	221 ab	188 b	205 ab	236 ab	248a	0.431	0.086
Ca	835 a	158b	589b	1081a	92.2 b	223a	605 a	447 bc	558 ab	529 ab	368c	336c	440bc	555 ab	559 ab	626a	0.250	0.210
Mg	85.4a	23.0b	97.2 a	73.6 b	24.1 a	21.9a	66.6 a	52.7 b	63.5ab	54.6ab	35.6c	60.5	44.1b	53.2ab	57.7a	58 a	0.264	0.069
Na	1027a	69.0b	1427a	628b	39.1b	98.5a	176c	304bc	445 b	906a	947a	513 a	520 a	513 a	606a	625a	0.606	0.510

Values in the same row, and in the same block of data, that are followed by the same letter, do not differ at P = 0.05.

did not affect the concentrations of K, Mg and Na in the grasses. Contrary to Chapman (1974), the Ca concentration was low at zero mg COD/L relative to the higher COD treatments. Tissue P peaked in the 10 000 mg COD/L treatment, in which it was higher than in the zero and 20 000 mg COD/L treatments. Nitrogen concentrations did not differ over the range from zero to 10 000 mg COD/L, but declined at higher COD's. In *P. clandestinum*, significant correlations (r^2) were observed between tissue N, P and Na concentrations and the combined effects of solution Na and COD (data not shown). *Vetiver zizanioides* showed no such significant relationships, implying that it is more likely to be effective in bringing about phytoremediation in disposal site pastures than *P. clandestinum*.

Total elements in plants and plant components

Total P, Ca, Mg and Na, but not N and K, were greater in *P. clandestinum* than *V. zizanioides*. The general tendency for *V. zizanioides* to contain lower masses of mineral elements than *P. clandestinum* therefore followed the same trend as mineral concentrations. Total N, Mg and Na in the roots exceeded that in the shoots in *P. clandestinum*, whereas total root N, Ca and Na exceeded that in the shoot of *V. zizanioides.* Total P in the shoots and roots did not differ in either species. Where both species were considered jointly, total N, P, K, Ca and Mg tended to drift irregularly downward with increasing solution Na concentration. The observed tendency for total Ca and Ca concentration to be low at high solution Na concentrations supports Chapman's (1974) contention that Na suppresses Ca uptake and DM production. In addition to Ca, total N, P, K and Mg were significantly lower in the 400 than in the zero µM Na treatment. As in the case of tissue concentration, total Na tended to increase with solution Na. The effects of COD were less consistent. Total Na was unaffected by COD, whilst Ca tended to increase irregularly with COD. Total N, P, K, and Mg followed no specific sequence with increasing COD.

Of the grasses tested, *P. clandestinum* appears to be better suited than *V. zizanioides* for use in disposal areas receiving winery wastewaters, mainly because *P. clandestinum* produces more shoot DM (grazeable or harvestable top growth) than *V. zizanioides* (2.92 and 2.44 t/ha, respectively, by extrapolation). Whereas the removal of *P. clandestinum* shoots and top growth from one ha removes 178.4 g of Na (at 12.5 plants/m^2), the removal of one ha of *V. zizanioides* top growth removes only 78.5 g of Na. *Pennisetum clandestinum* and *V. zizanioides* grasses removed from pastures used for the disposal of wastewaters over the range of compositions used in this trial can probably be used as fodder for cattle. Cattle consume salt liberally if given the choice, but are likely to stop if the concentration is too high

(C. Muller, personal communication, 2009). Grazing cattle on the pasture itself would result in Na being returned to the pasture in excreted material.

Effects of Na and COD

In *P. clandestinum*, shoot DM, shoot Na concentration and total shoot Na content were significantly affected by the Na concentration and COD treatments (Table 5). The relationships between shoot Na concentration and total shoot Na were characterised by higher r^2 values than was shoot DM. The Na and COD treatments had no significant effects on root DM, root mineral concentrations or total root Na in *P. clandestinum,* or on shoot or root DM and mineral contents in *V. zizaniodes* (data not shown). Potential Na removal in harvested top growth can therefore be calculated from a bivariate second order polynomial function for *P. clandestinum* (presented in Table 5), but not for *V. zizanioides*, where the wastewater Na concentration and level of COD are known.

CONCLUSIONS

This trial was carried out in a glasshouse using simulated winery wastewater. The results must therefore be verified under field conditions. It is nevertheless apparent from the trial results that the macrophyte *J. acutus* is, potentially, a better alternative to *T. latifolia* for use in wetlands, and even more suitable than *S. maritimus*. However, none of the macrophytes tested removed appreciable amounts of Na, or other elements, from the simulated winery wastewater. Post wetland disposal sites will therefore continue to receive mineral elements, notably Na, though in slightly lower concentrations than would be the case if passage through a wetland were omitted. Since total plant DM production by wetland macrophytes varies with both Na concentration and level of COD in the environment, the efficiency of a wetland containing these macrophytes will vary with wastewater quality, and probably also with temperature and season. Collectively, these factors increase the need for pasture plants in disposal areas as second lines of defence against Na entry into the soil. *Pennisetum clandestinum* accumulates considerably more Na in the top growth, is more tolerant of high Na concentrations and high COD treatment levels than *V. zizanioides,* and may be a better alternative for use in areas receiving winery wastewater than *V. zizanioides,* as well as providing feed for cattle. It must nevertheless be emphasised that the combination of macrophytes in wetlands and grasses in disposal pastures is unlikely to take up more than a limited percentage of the Na in the wastewater. Further, after harvest, the Na-containing plant tissues must be disposed of in such a way that the Na will not contribute to increased sodicity elsewhere. It is better not to allow Na to enter the system in the first place. For this reason every effort should be made to substitute Na-free products for those that contain Na. Lowering COD levels, as by reducing the amounts of solids that enter the wastewater treatment system, may also minimise future problems with the treatment process, either in constructed wetlands or in post constructed wetland systems.

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