Uptake and Accumulation of Mineral Elements from Winery and Distillery Effluents by *Typha latifolia* and *Phragmites australis*

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Mineral element uptake by the macrophytes *Typha latifolia* (bulrush) and *Phragmites australis* (common reed) from effluent (waste water) was investigated in a two-year sampling program carried out in constructed wetlands at a winery near Stellenbosch (33°55'S, 18°52'E), and at a distillery near Worcester (33°32'S, 19°13'E) in the Western Cape Province. Factors considered were: season of growth, site (distillery or winery), plant kind, wetland retention time and position in the wetland (inflow, outflow). Effluent nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) concentrations were lower at the outflow than at the inflow at the winery, but not the distillery. Dry mass increases in both macrophytes were greater at the distillery than the winery. The distillery effluent contained higher concentrations of N and K than that from the winery. Tissue N, P, K, Na, Ca, Mg, copper (Cu) and zinc (Zn) concentrations were higher in plants at the distillery than at the winery. Tissue N and K concentrations were, respectively, higher and lower in *P. australis* than in *T. latifolia*. Retention time, and position within the wetland, had either no, or inconsistent, effects on tissue element concentrations. The total element contents of the macrophytes were small in relation to the quantities of elements in the effluent. Where differences in effluent composition across the winery wetland were observed, these were probably due to biological activity in the effluent itself, on the limestone gravel surfaces, or on the plant roots.

The treatment of effluent (waste water) is a major concern for wineries and distilleries, and strict regulations govern the disposal of such waters (National Water Act, 1998; van Schoor, 2005). Worldwide, as in South Africa, most winery wastewaters contain excessive levels of suspended solids, organic carbon and such elements as sodium (Na), potassium (K), magnesium (Mg), nitrogen (N) and phosphorus (P) (Papini, 2000; Melamane *et al*., 2007). Discharge of such wastewaters into streams or onto land could result in environmental degradation. Methods that have been used by the wine industry to improve wastewater quality involve aerobic, anaerobic and other chemical and biological processes. Notable amongst these, is the use of constructed wetlands which mimic the water purifying effects of natural wetlands (Van Schoor, 2002). In most constructed wetland studies the levels of nitrate, nitrite and ortho-phosphate in effluents at the wetland inlet were found to be higher than at the outlet (Bulc & Vrhovsek,1997; Mitsch & Wise, 1998). Levels of turbidity, total dissolved solids, hardness, biological and chemical oxygen demands, sulphate, chloride, fluoride and Na in industrial effluent have also been found to decrease across a wetland treatment system (Barman, 2001), though most of these factors will not be discussed in this paper. The effectiveness of effluent treatment in constructed wetlands is affected by the nature of the substrate (gravel, soil, or sand) and by the kinds of plants (macrophytes) grown in the wetland (Moshiri, 1993).

Typha latifolia (bulrush) and *Phragmites australis (Common reed) are* emergent macrophytes (vascular plants living in water or wetlands, either free-floating or attached to a surface) that commonly occur in natural wetlands. Since growth and productivity of these macrophytes is thought to be stimulated by the high nutrient content of polluted waters (Brix & Carter, 1986), they would appear to be potentially suitable for wastewater amelioration studies. Macrophytes nevertheless differ in their effectiveness in improving effluents (Ye *et al*., 2001). *Typha latifolia* is known to be tolerant of heavy metals, and is able to colonise industrially degraded habitats (Ye et al., 1997). *Phragmites australis* may also be metal tolerant, either through total exclusion of elements, or storage of these elements in non-toxic forms in the tissues (Massaci et al*.,* 2001).

According to Reddy *et al.* (1982), total P removal efficiency in reservoirs containing macrophytes was 69-86%, and that of ammonium N was 86-89%, whereas in reservoirs with no plants, nitrogen (N) removal decreased to 54%. Healthy *P. australis* plants are reputed to be tolerant of overloading with N and P in aquatic environments, this loading leads to enhanced growth of the shoots and rhizomes. The removal ratio for P and N is around 1:10 (Massaci *et al.*, 2001). Oxidation reactions with root bacteria help to break down waterborne organic compounds, liberating elements for uptake (Lakatos *et al.*, 1999) by the roots and rhizomes of macrophytes. Translocation may result in high concentrations of nutrient elements and metals accumulating in the shoots and reproductive structures of plants (Whitton *et al.*, 1981).

The aim of this study was to determine the effects of two macrophyte species (*T. latifolia* and *P. australis*), wetland retention time and plant position in the wetland on element removal from distillery and winery wastewater in constructed wetlands.

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MATERIALS AND METHODS

Experiment design and layout

Three similar wetlands were constructed at a distillery in Rawsonville, near Worcester (33°32'S, 19°13'E), and at a winery near Stellenbosch (33°55'S, 18°52'E). The vinyl-lined wetlands were 6.0 m long, 2.4 m wide and 1.0 m deep, and were filled with coarse, angular limestone gravel (mean length 30 mm, 45% porosity), to a depth of 0.9 m in accordance with the free water flow surface wetland design of Shepard & Grismer (1997). Flow rates in the three wetlands at each locality were adjusted to give retention times (RT's) of 4.5, 9.0 and 18.0 days (42, 21 and 10.5 L/hour, respectively). In spring 2002 rhizome cuttings of *T. latifolia* and *P. australis* were obtained from neighbouring natural wetlands and planted in alternating transverse rows along the length of each constructed wetland. Borehole water was supplied during the period of establishment. The first effluent was supplied in February 2003. At each site a natural wetland containing *T. latifolia* and *P. australis* was used as a control.

Sampling and analysis

Plant sampling was carried out at 28-day intervals over a two year period, commencing in early March, approximately 28 days after effluent was introduced. At that time the plants were fully grown. On each occasion whole plants were taken from locations close to the inflow and outflow points of each constructed wetland, or from the centre of the natural wetland, in the case of the controls. Each plant was washed in tap water to remove sediment, oven-dried to constant mass at 70˚C, weighed to determine dry mass, milled and digested with a combination of sulphuric acid and hydrogen peroxide (Allen, 1989). The concentrations of calcium (Ca), Na, Mg and K were determined by atomic absorption spectrophotometry, as were copper (Cu), zinc (Zn), manganese (Mn), iron (Fe) and aluminium (Al). Phosphorus and N were respectively determined by the method of Murphy & Riley (as described in Olsen & Sommers, 1982) and by Kjeldahl distillation (Bremner & Mulvaney, 1982). Total plant element contents were calculated from the plant mass and concentration data. Effluent samples were taken from the inflow points at 7-day intervals over the same period, and analysed by the same methods as the plant samples.

Data analysis

The data were grouped for statistical analytical purposes on the basis of season, plant growth stage and appearance (phenology), and effluent flow (Table 1). Data for each interval thus defined were averaged over the 2-year trial period to take into account seasonal variations in growth, and the different activities in the winery and distillery over the course of the annual cycle. These data, and the averaged raw effluent data, were subjected to analysis of variance (ANOVA) using SAS 9.1.3 (SAS Institute Inc., 2003). Student's *t* LSD values were calculated at the 5% probability level to facititate comparison between treatment means. Means which differed at P=0.05 were considered to be significantly different.

RESULTS AND DISCUSSION

Effluent analysis

Averaged over the trial period, the wastewater that entered the constructed wetlands at the distillery contained appreciably more N, K and Na than at the winery (Table 2). Inflow K, Mg and Na concentrations at the winery were significantly greater than outflow concentrations, irrespective of RT, implying that a portion of each of these elements was removed from solution during its passage through the wetland. At the distillery, inflow and outflow of N, K and Na concentrations did not differ. These elements may have been too abundant in the distillery effluent for the wetland to bring about any significant reduction. At the winery wetland, but not the distillery, N concentrations decreased in the sequence: inflow (significantly) > 4.5 days RT > 9.0 and 18.0 days RT. Calcium concentrations were not consistently affected by RT or sample position at either site. At the distillery, outflow Ca and Mg concentrations after 18 days RT exceeded the Ca concentration at the inflow. These elements may have been leached from the limestone gravel by the more concentrated distillery effluent. Phosphorus was not measured in these wastewaters.

Dry mass production

Total plant dry masses in the constructed wetlands followed an annual cycle, decreasing from high values in summer (before effluent flow commenced), remaining high in autumn, then decreasing to significantly lower values through May with the onset of senescence, remaining low through the winter, then recovering in spring (Table 3). Such a seasonal pattern has previously been reported for *T. latifolia* by Garver *et al*. (1988). Average plant dry masses were greater at the distillery than at the winery, which tends to support the view of Brix & Carter (1986) that growth in macrophytes is promoted by the nutrient content of polluted waters. As averaged over the season, dry mass in *T. latifolia* exceeded that in *P. australis*, implying that *T. latifolia* may be better than *P. australis* for use in wetland treatment systems. Effluent RT, and location within the wetland had no effect on plant dry mass, and no treatment interactions were observed.

The dry mass production pattern in the controls differed from that in the constructed wetlands (data not shown), probably because the natural wetlands tended to dry out in summer after the spring growth flush, leading to early senescence, whereas the plants in the artificial wetlands remained in a permanently effluent-saturated environment and experienced an extended growing season. For this reason the control data will not be discussed further in this article.

TABLE 1

Intervals (defined in terms of months, effluent flow and phenology) used for statistical analysis adapted from Zingelwa (2003).

Months	Effluent flow	Phenology
November to January	Not flowing	Fully grown, healthy shoots
February to April	Flowing	Shoots becoming senescent
May	Flowing	Senescent
June to mid September	Flowing	Dead top growth
Mid September to end October	Not flowing	New growth

TABLE 2

Concentrations (mg/L) of elements in winery and distillery wastewaters flowing into wetlands, and leaving the wetlands after retention times of 4.5, 9.0 and 18 days.

Element	Inflow	Outflow		
		4.5 days	9.0 days	18 days
		Winery		
N	6.5a*	4.57b	3.1c	3.1c
K	453.0a	205.8b	191.5b	166.8b
Ca	168.0a	115.1c	161.5ba	130.4bc
Mg	55.1a	37.1b	39.9b	34.9b
Na	136.9a	127.3bc	98.6c	99.6c
		Distillery		
N	414a	442.7a	393.5a	389.5a
K	889.5a	972.a	988.4a	909.5a
Ca	72.1b	94.6a	82.5b	116.4a
Mg	46.3c	67.5b	61b	83.9a
Na	208.1a	203a	207.2a	198.4a

*Values in the same row, that are followed by a different letter, differ significantly at the 5% level of probability.

Mineral element concentrations in plant tissues

Tissue element concentrations varied with season, tending to be higher in spring and summer, when effluent was not flowing, and in some cases also in autumn, than in winter. In spring and summer Ca was the dominant element, followed by K, then N. In autumn and in May (a transitional month), N predominated over K and Ca. Whether this increase in N content was phenological or a reflection of the N content of the effluent is unclear. During the winter months, the tissue element concentrations tended to decrease in the sequence: Ca > N > K. Averaged over the year, concentrations of P, Na, Ca, Mg, Cu and Fe were significantly higher at the distillery than at the winery. Zinc (Zn) concentrations at the winery exceeded those at the distillery, and were higher at the inflow than the outflow. In contrast, Mn was higher at the outflow. Tissue P concentrations did not vary significantly with season. *Typha latifolia* contained more Ca and Mn than *P. australis*. With the exception of manganese (Mn), which was highest at the outflow, and Zn, which was highest at the inflow, the position of the plant in the wetland had no effect on mineral concentration. The highest and lowest observed K concentrations occurred, respectively, in *T. latifolia* at the distillery, and in *P. australis* at the winery. Sodium concentrations were significantly higher in *T. latifolia* at nine and 18 days RT, than in *P. australis*, over the same retention periods. Trace element concentrations were generally low.

According to Epstein (1972), adequate levels of N, P, K, Ca and Mg in plants are around 1.5%, 0.2%, 1.0%, 0.5% and 0.2%, respectively. Average values ([*P. australis* + *T. latifolia*]/2) for these elements in the wetlands were, respectively, 1.25%, 0.03%, 1.27%, 2.98% and 0.32%. Thus, relative to Epstein (1972), N and P were low in the wetland plants, whereas K, Ca and Mg were adequately, but probably not over abundantly supplied.

Total mineral content of plants

At the distillery the individual plants contained more N, P, K, Ca and aluminium than at the winery (Table 3), reflecting both the generally higher element concentrations in the distillery effluent, and the greater dry mass of the plants at the distillery, relative to the winery. Each *T. latifolia* plant contained more of these elements than the *P. australis* plants, which supports the view of Ye *et al*. (2001) that macrophytes are not equally effective in taking up elements from wastewaters. *Typha latifolia* may therefore be more effective for removing elements in wetlands than *P. australis*.

Total N, P, K, Ca, Mg and Al varied with season and, as was the case for concentration; tending to be higher in spring and summer than in the colder months. Retention time had no significant effect on plant element contents, but total plant Mn was higher at the outflow than at the inflow. The reason for this increase in Mn across the wetlands is not known.

Interactions

Plant N concentrations tended to increase with RT at the distillery but not at the winery (Table 4). Potassium concentrations, and total Na and Mg, were greater in *T. latifolia* than *P. australis* but, as for N concentration, these differences were only observed at the distillery where concentrations of K and Na (but not Mg) were relatively high (Table 5). At the winery total Na was lower in *P. australis* than in *T. latifolia*. Sodium concentrations in *T. latifolia*, but not in *P australis*, tended to increase with RT (Table 6).

Factors affecting element removal

That the N, K, Ca, Mg and Na concentrations in the effluent outflow were significantly lower than at the inflow at the winery implies removal of these elements from the flowing effluent during passage through the wetland. According to Moshiri (1993), macrophytes are storage sites for carbon and nutrients. The extent

TABLE 3

Dry mass (g/plant), element concentration (mg/g dry mass, whole plant) and total element mass (mg/plant).

Parameter	Place		Plant		Sampling period					Retention time (Days)			Location	
	Distillery	**Winery**	***Phragmites australis***	***Typha latifolia***	**November to January**	**February to April**	**May**	**June to mid September**	**Mid September to end October**	**4.5**	**9.0**	**18**	**Inflow**	**Outflow**
DM	8.22a*	6.85b	6.87b	8.87a	9.22a	8.81a	6.78bc	5.96c	7.91ab	7.92	7.64	7.59	7.74	7.70
Average concentration of element in plant tissue (mg/g dry mass)														
N	13.70a	10.68b	13.12a	11.86b	10.68b	15.60a	14.38a	11.77b	10.62b	11.97	12.91	12.88	12.61	12.56
P	0.36a	0.19b	0.29	0.31	0.36	0.30	0.27	0.19	0.43	0.35	0.30	0.26	0.27	0.33
K	15.88a	6.40b	10.54b	14.91a	14.71a	10.27b	10.67b	10.61b	16.87a	11.76	11.67	13.72	12.33	12.45
Na	2.17a	1.04b	1.28	2.39	1.73bc	1.14c	1.80b	1.85b	2.46a	1.67	1.66	1.91	1.79	1.71
Ca	32.05a	24.70b	26.50b	33.18a	44.79b	5.01d	5.81d	25.64c	69.42a	30.24	31.21	26.54	27.02	31.74
Mg	4.04a	1.65b	2.91	3.49	4.28a	2.11b	2.47b	2.25b	5.34a	3.41	3.27	2.79	3.41	2.89
Al	1.45	1.20	1.30	1.44	1.39b	0.76c	0.53c	1.41b	2.74a	1.27	1.40	1.40	1.46	1.25
Mn	0.14b	0.18a	0.13b	0.19a	0.25a	0.19b	0.09d	0.10cd	0.14bc	0.16	0.16	0.15	0.14b	0.18a
Cu	0.11a	0.04b	0.07	0.10	0.18a	0.02c	0.03c	0.06c	0.13b	0.09	0.08	0.08	0.11	0.06
Fe	0.45a	0.15b	0.31	0.38	0.20bc	0.54a	0.48a	0.33b	0.15c	0.33	0.36	0.34	0.34	0.34
Zn	0.22b	0.28a	0.23	0.26	0.27b	0.27b	0.06d	0.17c	0.44a	0.25	0.23	0.25	0.26a	0.22b
Element content in average plant (mg/plant)														
N	126.93a	79.79b	107.46	112.40	113.28b	156.79a	113.55b	74.11c	93.19bc	105.80	106.87	115.92	110.36	108.70
P	2.43a	1.40b	1.64b	2.61a	3.25a	1.42b	1.33b	1.13b	3.58a	2.15	2.16	1.85	2.00	2.10
K	180.07a	56.55b	101.27b	179.96a	187.74a	120.17bc	121.05bc	95.33c	165.29ab	128.03	120.28	155.11	137.00	131.92
Na	20.78	7.32	8.76	25.46	18.51	11.20	15.90	15.93	18.91	14.45	14.61	18.37	16.65	14.94
Ca	251.48a	166.88b	173.59b	284.11a	394.24b	26.59d	30.59d	160.48c	529.10a	240.07	220.75	200.26	203.18	238.15
Mg	20.33a	7.40b	11.58b	21.01a	20.08ab	12.63c	14.43bc	9.07c	26.23a	16.02	16.25	14.44	16.35a	14.75b
Al	10.22a	6.48b	7.63b	10.50a	10.17b	6.45c	5.10c	7.45bc	15.88a	8.96	8.61	8.96	9.45	8.21
Mn	1.01b	1.32a	0.78b	1.60a	1.89a	1.30b	0.58c	0.63c	1.15b	1.18	1.13	1.07	0.95b	1.31a

*Values in the same row and date set that are followed by different letters differ significantly at the 5% level of probability.

TABLE 4

Effect of site and retention time on nitrogen concentrations in macrophytes (mg/g).

Element	Distillery			Winery		
	4.5	9	18	4.5	9	18
N	12.91bc*	13.55ab	14.64a	10.38de	11.77cd	9.92e

*Values in the same row, that are followed by a different letter, differ significantly at the 5% probability level.

TABLE 5

Effect of site and macrophyte type on potassium concentration (mg/g), and on total sodium and total magnesium in macrophytes (mg/plant).

Element	Distillery		Winery	
	Phragmites australis	*Typha latifolia*	*Phragmites australis*	*Typha latifolia*
K	13.30b*	19.48a	5.69c	7.32c
Total Na	11.80b	33.31a	3.43c	12.46b
Total Mg	14.77b	28.08a	5.95c	9.31bc

*Values in the same row, that are followed by a different letter, differ significantly at the 5% probability level.

TABLE 6

Effect of plant type and retention time (days) on sodium concentration (mg/g) in macrophytes.

Element	*Phragmites australis*			*Typha latifolia*		
	4.5	9	18	4.5	9	18
Na	1.47cd*	1.25d	1.11d	1.96bc	2.21b	2.95a

*Values in the same row, that are followed by a different letter, differ significantly at the 5% probability level.

to which macrophytes take up and store nutrient mineral elements is nevertheless unclear. The observation that passage through the wetland did not significantly or consistently affect mineral concentrations in the distillery effluent, in which inflow N, K and Na concentrations were higher than at the winery, suggests that wetlands are an effective means of remediation only where the effluent concentration ranges are lower than those which occurred at the distillery. The percentage of the total element throughflow that was intercepted by the plants, and therefore the efficiency of plant uptake, could not be calculated from the available data. However, based on a plant density of 25 plants/m^2 (20 cm x 20 cm) and the average element contents per plant cited for November to January in Table 3, the macrophytes in one m^2 of wetland will, in summer, contain approximately 2.8 g, 4.7 g, 9.9 g, 0.5 g and 0.5 g of N, K, Ca, Mg and Na, respectively. These quantities are broadly equivalent to the amounts of the respective elements in 43.1, 10.4, 58.9 9.1 and 3.7 L of winery effluent as calculated from the inflow values listed in Table 2. Uptake by macrophytes is therefore unlikely to contribute greatly to the removal of mineral elements from winery and distillery effluents unless the wetland is very large and the macrophytes are planted densely, grow actively and are regularly replaced, or the shoots are harvested, assuming that elements accumulate in the topgrowth, as implied by the work of Zingelwa (2003).

Element removal during passage through the winery wetlands (Table 1) was probably due, perhaps in large part, to interaction with the substrate, with organic and mineral materials associated with the roots or substrate, or with free living bacteria and algae in the water. Mitsch & Wise (1998) found that ion retention followed no consistent pattern in vegetated or in non-vegetated plots. This supports the contention that the role played by macrophytes in effluent remediation in wetlands may be small or non-existent. Alternatively, the main function of macrophytes in wetlands may be to supply organic residues and exudates needed by the bacteria and algae. A further factor requiring consideration is that the roots of macrophytes may block the flow of effluent over reactive surfaces. Trials involving the periodic removal of roots should be carried out to determine the effects of root density on wetland performance (Zingelwa, 2003).

Since most elements reach their peak abundance in the plant during summer and autumn, removal of the plants during these seasons would appear to be most effective from the viewpoint of removing mineral elements from the system. However, since late summer and autumn are periods of high effluent flow, removal at that time of year is likely to compromise the effectiveness of the wetland at the time when peak efficiency is most required. An alternative may be to replace the plants, or harvest the topgrowth after effluent flow has ceased.

Long wastewater RT's are presumed to lead to more effective removal of particulate matter (Brix, 1993) and nutrient elements than short RT's, the rationale being that the longer the period of contact between wastewater, plants and substrate, the more effective

the removal process (Mitsch & Wise, 1998). That retention time had no consistent, significant effects on element concentration or total element content in this trial may mean that extending the RT in constructed wetlands of the type, size and planting density used in this trial beyond 4.5 days does not confer further benefits.

CONCLUSIONS

This two-year sampling program showed that effluent from a distillery contained higher concentrations of N and K than effluent from a winery. That the element concentrations and total element accumulations in macrophytes growing in these effluents tended to reflect the element concentrations in the effluents suggests that macrophytes take up elements from solution and may therefore play a role in phytoremediation. However, mineral uptake by macrophytes appears to contribute little to phytoremediation relative to the purifying effects of organic residues, sediment and biological activity within the effluent itself, or associated with surfaces of the gravel fill material. *Typha latifolia* attained a greater dry mass than *P. australis* and may therefore be better suited to use in constructed wetlands. Effectiveness of removal of elements by *T. latifolia* and *P.* australis may increase with planting density. Conceivably, other wetland plants could remove mineral elements from effluents more effectively, and over a wider concentration range, than *T. latifolia* and *P.* australis. It is also possible that plants could be genetically engineered for phytoremediation perposes. Whereas wetlands of the type used in this survey appear to make a useful contribution to the purification of effluents from wineries, their value at distilleries where the concentrations of some mineral elements (notably N and K) in the effluent are much higher, is likely to be minimal.

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