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Halophytes for the treatment of saline aquaculture effluent

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Abstract

We determined the feasibility of using salt-tolerant plants (halophytes) as biofilters to remove nutrients from saline aquaculture wastewater. *Suaeda esteroa*, *Salicornia bigelovii* and *Atriplex barclayana* (Chenopodiaceae), species with potential as forage and oil seed crops, were grown in sand in draining containers (lysimeters) in a greenhouse experiment. They were irrigated to meet evapotranspiration demand and to produce a 0.3 leaching fraction, using aquaculture effluent generated from an intensive tilapia culture system. The effluent salinity was increased with NaCl to make salinity treatments of 0.5, 10 and 35 ppt. The plant–soil system removed 98% and 94% of the applied total and inorganic nitrogen, respectively. It removed 99% and 97% of the applied total and soluble reactive phosphorus, respectively. High removal rates occurred despite the high leaching fraction. Salt inhibited ($P < 0.05$) the growth rate, nutrient removal, and volume of water that all three plant species could process. *Suaeda* and *Salicornia*, which are succulent salt marsh species, performed better than the desert saltbush, *Atriplex*, at the higher salinities. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Halophytes; Effluent; *Suaeda esteroa*; *Salicornia bigelovii*; *Atriplex barclayana*

1. Introduction

The large volumes of wastewater discharged from coastal aquaculture projects can become a serious source of pollution. Untreated effluent may damage coastal ecosystems

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and fisheries (Gowen and Bradbury, 1987; Bell et al., 1989; Iwama, 1991; Handy and Poxton, 1993; Hopkins et al., 1995; Wu, 1995; Costa-Pierce, 1996; Dierberg and Kaittisimkul, 1996). Several methods of biological treatment of effluent have been tested; however, no single treatment method always works. Seaweeds can be effective biofilters in intensive, closed systems (Neori et al., 1996; Shpigel and Neori, 1996). However, in open systems where effluent is discharged into ditches or canals, seaweeds can become light-limited and fouled with settled solids (Phillips, 1995). Constructed wetlands, utilizing higher plants, have also been used to treat aquaculture effluent (Schwartz and Boyd, 1995). These wetlands filter solids out of the wastewater stream, but they are less effective at removing inorganic N and P than they are at removing solids, and they do not provide an economic return to the grower. Integrating vegetable hydroponics with fish culture is another effective but expensive technique to remove nutrients from a fish culture system (Rakocy and Hargreaves, 1993).

Another option that has been used successfully for the disposal of freshwater aquaculture effluent is application to field crops (Olsen et al., 1993; D'Silva and Maughan, 1996). Crop plants can remove a significant fraction of the nutrients in the irrigation stream and provide an economic return to the grower. Unlike seaweed scrubbing systems, this method actually reduces effluent water volume through plant evapotranspiration. It could be extended to saline effluent streams if suitable, salt-tolerant crops were available. Such crops are being developed from wild, salt-tolerant plants (halophytes) with the potential to produce biomass, forage and oilseed crops using saltwater irrigation (Glenn et al., 1991, 1994, 1997a; Miyamoto et al., 1996; Swingle et al., 1996).

Under high-salinity irrigation, halophytes require a leaching percentage of 30–50% above consumptive use in order to flush excess salts below the root zone (Miyamoto et al., 1996). This flushing is necessary because the build-up of salts in the root zone inhibits plant growth (Miyamoto et al., 1996). However, this leaching fraction could result in significant discharge of N and P to the aquifer if these nutrients are not efficiently absorbed by the soil–plant system. We tested the capacity of three halophyte species grown at three salinities to remove N and P from a highly concentrated aquaculture effluent source when irrigated at a rate of 30% above evapotranspiration demand.

2. Methods and materials

2.1. Experimental design and procedures

The study was conducted in July–October 1995 in a controlled environment greenhouse in Tucson, AZ. Plants were started from seed (*Salicornia bigelovii* and *Suaeda esteroa*) or cuttings (*Atriplex barclayana*) from wild plants collected at Estero Morua, near Puerto Peñasco, Sonora, Mexico (Glenn and O'Leary, 1985; Glenn et al., 1991). Seedlings or rooted cuttings were transplanted into 20 l, white plastic pots (drainage lysimeters), containing 25 kg (dry weight) of washed river sand and equipped with bottom tubes leading to collection bottles. Water, salt and nutrients leaching past the root zone were quantified (Miyamoto et al., 1996).

The experiment was designed as a split plot with salinity as the main plot, and plant species as the subplot. Three salinity treatments (0.5, 10, 35 ppt) were used with three replicates per species per salinity treatment level. A set of control lysimeters (soil without plants) was included at each salinity (total lysimeters = 36). The lysimeters were placed in six rows (two rows per each of the three blocks) near the middle of the greenhouse in a randomized complete block design.

Wastewater for irrigation was generated in two 1 m^3 tanks that each held 10 kg hybrid tilapia in freshwater. Fish were fed pelleted food at 2% of body weight per day. Each tank contained a 100 l bead filter with 20 l of beads. Water was recycled within the tanks. Once a week the filters were backflushed, and solids and wastewater were withdrawn to irrigate the lysimeters. The irrigation water was thoroughly mixed then split into three batches to keep the nutrient content equal among treatments. NaCl was added to each batch to produce the treatment salinities. NaCl was used instead of synthetic sea salt in order to avoid using an undefined mixture of salts, some of which can precipitate at high salinities. The three salinities, 0.5, 10 and 35 ppt, simulated the salinity of non-saline irrigation water used in the western United States, brackish estuarine water and seawater, respectively.

The lysimeters were weighed weekly on a balance to estimate water use over the past week. Lysimeters were irrigated weekly with saline effluent to replace evapotranspiration (ET) losses and to provide for leaching fraction (LF) = 0.3, a typical value for irrigated crops in arid regions (Miyamoto et al., 1996). The irrigation volume (W_i) was calculated as: $W_i = (\mathrm{ET}/1 - \mathrm{LF})$. This design simulated best management practices for irrigating with saline water and kept the fraction of water discharged past the root zone as a constant among treatments. A sample of weekly irrigation water was retained for water quality analysis. Lysimeters were allowed to drain overnight and the leachate from all treatments was collected on the morning following irrigation. At the end of the 12-week experiment, above-ground biomass was harvested and dried at 60°C to determine dry matter yield per lysimeter.

2.2. Water, plant and soil analyses

Electrical conductivity, pH, total ammonia-nitrogen (TAN), nitrate-nitrogen, total nitrogen, soluble reactive phosphorus, and total phosphorus were measured in irrigation and leachate water using methods of the American Public Health Association (1995). Nitrite-nitrogen was measured initially, but was later dropped because concentrations were minor compared to the other nitrogen species. Total nitrogen was measured by first reducing nitrate to ammonia followed by the Kjeldahl nitrogen procedure (Bremner and Mulvaney, 1982). Soil samples were analyzed for electrical conductivity, pH, TAN, nitrate-nitrogen, total nitrogen, plant-available phosphate (sodium bicarbonate extraction), and total phosphorus following Page et al. (1982). Total nitrogen in samples of plant shoot tissue was measured with a CNS analyzer and total phosphorus was analyzed by acid digestion followed by colorimetry (Jones et al., 1991). Plant relative growth rate (RGR) was calculated as RGR = (ln(final weight (g)) − ln(initial weight (g)))/86 days (length of experiment). Plant water use efficiency (WUE) was calculated as WUE = plant dry weight accumulated (g)/water consumed (l).

The amount of nutrients removed by the plant–soil system was calculated by subtracting the total amount of nutrients that exited the lysimeter in the leachate water from the total amount added from the irrigation water. The percentage of the total amount of nutrients applied that were removed was calculated by $(1-(\text{total out}/\text{total in}))\times 100$.

2.3. Statistical analyses

Data were first tested for homogeneity of variances using Bartlett's test. Data with heterogeneous variances were transformed using either log, reciprocal or square root transformations. Percentages were arcsine or logit transformed to ensure normality (Sokal and Rohlf, 1981). Data were analyzed by parametric methods using a split plot analysis of variance with salinity as the main plot (F value: MS salinity/MS replications × salinity) and plant species as the subplot (F value: MS salinity/MS replica-

Table 1
Mean values (±SE, $n = 3$) for plant shoot final dry weight (DW), plant relative growth rate (RGR), water use over the experiment and plant water use efficiency (WUE) by three species of halophytes at three salinities

	Final DW (g)[a]	RGR (per day)[a]	Water use (l)[a]	WUE (g/l)[a]
0.5 ppt				
Suaeda	66.77 (10.23)	0.049 (0.002)	35.79 (1.61)a	1.68 (0.22)
Salicornia	34.83 (5.51)	0.041 (0.002)	24.74 (1.89)b	1.30 (0.11)
Atriplex	58.17 (14.18)	0.047 (0.003)	26.39 (2.85)b	2.06 (0.29)
Soil control	–	–	17.22 (1.12)c	–
Species P-value	ns	ns	0.002	ns
0.5 ppt mean	53.26A	0.046A	26.03A	1.68A
10 ppt				
Suaeda	43.40 (3.11)	0.043 (0.001)	26.05 (1.01)a	1.47 (0.08)
Salicornia	40.97 (2.42)	0.043 (0.0007)	23.38 (1.22)ab	1.65 (0.02)
Atriplex	45.07 (5.77)	0.044 (0.002)	20.29 (0.85)b	2.02 (0.25)
Soil control	–	–	14.95 (0.48)c	–
Species P-value	ns	ns	0.0013	ns
10 ppt mean	43.15A	0.043A	21.17B	1.71A
35 ppt				
Suaeda	5.56 (0.25)a	0.028 (0.002)a	12.30 (0.29)a	0.89 (0.09)a
Salicornia	2.55 (0.14)a	0.030 (0.0008)a	11.45 (0.26)b	1.13 (0.09)a
Atriplex	0.81 (0.05)b	0.0079 (0.0006)b	10.23 (0.20)c	0.19 (0.008)b
Soil control	–	–	10.79 (0.30)c	–
Species P-value	0.0017	0.0005	0.0002	0.001
35 ppt mean	2.97B	0.022B	11.19C	0.74B

P-values are shown for species (or control) treatment within each salinity treatment. Salinity treatment means within each column followed by the same upper case letter are not significantly different ($P \geq 0.05$). Species (or control) means within each salinity treatment followed by the same or no lower case letter are not significantly different ($P \geq 0.05$).

[a] Salinity treatment had a significant effect on this variable ($P < 0.05$).

tions). The analyses were carried out using statistical software packages (JMP IN and Costat). Student–Neuman–Kuels tests were conducted to identify significant differences among means. The significance criterion was $P < 0.05$.

3. Results

3.1. Greenhouse environmental conditions

Mean weekly maximum and minimum air temperatures in the greenhouse over the course of the experiment were (mean ± SEM) 36.4 ± 1.02°C and 22.6 ± 0.75°C, respectively. Light transmission through the greenhouse was about 59% of ambient, and the

Table 2
Mean values (±SE, $n = 3$) for applied total nitrogen, the percent of the applied total nitrogen, the applied inorganic nitrogen (IN = total ammonia-N + nitrate-N), and the percent of the applied inorganic nitrogen removed by the lysimeters with three species of halophytes at three salinities

	TN removed (mg)[a]	% TN removed[a]	IN removed (mg)[a]	%IN removed[a]
0.5 ppt				
Suaeda	3992.9 (189.63)a	96.18 (1.23)	640.7 (18.34)a	93.44 (2.33)a
Salicornia	2691.1 (263.89)b	96.35 (1.13)	392.6 (50.26)b	83.48 (4.16)a
Atriplex	2972.7 (333.07)b	97.53 (0.24)	481.4 (42.89)b	95.73 (1.57)a
Soil control	1763.3 (107.98)c	95.26 (1.38)	138.5 (18.12)c	45.4 (8.82)b
Species *P*-value	0.0028	ns	0.0002	0.0029
0.5 ppt mean	2855.0A	96.33C	413.3A	79.51B
10 ppt				
Suaeda	2878.2 (122.76)a	98.02 (0.23)a	476.6 (19.43)a	97.00 (0.58)a
Salicornia	2632.0 (158.29)ab	98.44 (0.03)a	433.0 (23.57)ab	97.10 (0.49)a
Atriplex	2251.2 (92.91)b	98.58 (0.21)a	374.6 (13.38)b	97.94 (0.52)a
Soil control	1544.1 (28.68)c	94.40 (1.10)b	151.0 (5.37)c	55.07 (2.15)b
Species *P*-value	0.0009	0.004	< 0.0001	< 0.0001
10 ppt mean	2326.4B	97.36B	358.8A	86.78A
35 ppt				
Suaeda	1345.6 (40.98)a	99.09 (0.11)	214.5 (4.85)a	94.27 (0.57)a
Salicornia	1249.8 (35.38)b	99.06 (0.18)	202.8 (5.83)a	95.79 (0.96)a
Atriplex	1092.5 (33.11)c	98.73 (0.14)	169.5 (6.01)b	90.69 (0.09)a
Soil control	1154.7 (41.90)c	98.17 (0.37)	142.2 (11.09)c	72.6 (3.25)b
Species *P*-value	0.012	ns	0.0005	0.0003
35 ppt mean	1210.6C	98.76A	182.2B	88.34AB

P-values are shown for species (or control) treatment within each salinity treatment. Salinity treatment means within each column followed by the same upper case letter are not significantly different ($P \geq 0.05$). Species (or control) means within each salinity treatment followed by the same or no lower case letter are not significantly different ($P \geq 0.05$).
[a]Salinity treatment had a significant effect on this variable ($P < 0.05$).

mean daily quantum flux over the course of the experiment in the greenhouse was 28.6 E m^2/day (Brown and Russell, 1996). Relative humidity ranged from 60 to 80% (the greenhouse was evaporatively cooled throughout the experiment).

3.2. Plant growth and water use

The mean experiment-wide leaching fraction (0.308 ± 0.021) was close to the desired 0.3 target and did not differ significantly ($P > 0.05$) among species or salinity treatments. Mean salinity values for the leachate water for the 0.5, 10 and 35 ppt treatments were 1.6 ± 0.039, 28.6 ± 0.81 and 84.7 ± 0.52 ppt, respectively. The ratio of irrigation salinity to leachate salinity can be used as a check of the volumetric measurement of LF. LFs calculated from leachate salinity were 0.31, 0.35 and 0.41, indicating that some salt storage occurred at the middle and high salinity treatments.

Water consumption was greatest in the low salt treatment and lowest in the high salt treatment ($P < 0.0001$). *Suaeda* consumed more water than the other species (Table 1). At 35 ppt salinity, *Atriplex* did not use significantly more water than the bare-soil, control lysimeters. Plants produced more dry matter at the lowest and middle salinity than plants at the highest salinity ($P = 0.0016$). *Atriplex* plants were significantly smaller than the other two species at 35 ppt ($P = 0.0017$). RGR followed the same trend

Table 3
Mean values ($\pm$SE, $n = 3$) for plant shoot nitrogen, percent of the applied total nitrogen sequestered by the plant shoot of three species of halophytes at three salinities

	Plant N (mg)[a]	% TN Removed by plant[a]
0.5 ppt		
Suaeda	877.94 (89.85)	21.49 (3.44)
Salicornia	659.20 (37.59)	23.83 (1.46)
Atriplex	683.67 (123.14)	22.11 (1.69)
Species *P*-value	ns	ns
0.5 ppt mean	740.27A	22.47A
10 ppt		
Suaeda	533.99 (26.52)	18.21 (0.82)
Salicornia	582.05 (18.11)	21.88 (1.04)
Atriplex	427.26 (49.49)	18.72 (2.18)
Species *P*-value	ns	ns
10 ppt mean	514.43B	19.60A
35 ppt		
Suaeda	182.36 (7.36)a	13.43 (0.40)a
Salicornia	192.55 (1.75)a	15.28 (0.31)a
Atriplex	35.61 (3.83)b	3.20 (0.24)b
Species *P*-value	< 0.0001	< 0.0001
35 ppt mean	136.84C	10.64B

P-values are shown for species treatment within each salinity treatment. Salinity treatment means within each column followed by the same upper case letter are not significantly different ($P \geq 0.05$). Species means within each salinity treatment followed by the same or no lower case letter are not significantly different ($P \geq 0.05$).
[a]Salinity treatment had a significant effect on this variable ($P < 0.05$).

as plant dry weight. WUE was reduced by salinity because soil evaporation dominated water loss at high salinity due to low plant water use.

3.3. Irrigation water quality

Nitrogen and phosphorus parameters in the irrigation source throughout the experiment were: total nitrogen 77.22 ± 0.47 mg/l; TAN 9.69 ± 0.91 mg/l; nitrate-N 3.33 ± 0.28 mg/l; total phosphorus 25.27 ± 0.27 mg/l; soluble reactive phosphorus-P 6.40 ± 0.94 mg/l. The pH was 7.31 ± 0.0009.

3.4. Nitrogen removal

Removal of both total N and inorganic N (TAN + nitrate-N) by the plant–soil system was significantly ($P < 0.0001$, and $P = 0.0012$, respectively) inhibited by increasing

Table 4
Mean values (± SE, $n = 3$) for concentrations of total ammonia-nitrogen (TAN), nitrate-N, and inorganic nitrogen (IN = TAN + nitrate-N), in the leach water averaged over the course of the experiment, and soil inorganic nitrogen concentration (mg nitrate-N + TAN/kg of soil) with three species of halophytes at three salinities

	TAN (mg/l)[a]	NO_3-N (mg/l)[a]	IN (mg/l)[a]	Soil IN (mg/kg)[a]
0.5 ppt				
Suaeda	0.32 (0.055)a	2.96 (1.05)c	3.29 (1.09)bc	5.34 (0.89)
Salicornia	0.19 (0.003)b	7.31 (1.94)b	7.49 (1.93)b	7.68 (0.77)
Atriplex	0.26 (0.015)ab	1.94 (0.77)c	2.20 (0.78)c	7.50 (1.61)
Soil control	0.19 (0.006)b	22.36 (3.54)a	22.55 (3.55)a	8.44 (1.79)
Species *P*-value	0.0145	0.0021	0.0022	ns
0.5 ppt mean	0.24B	8.64A	8.88A	7.24A
10 ppt				
Suaeda	0.21 (0.023)	1.34 (0.22)b	1.55 (0.22)b	0.90 (0.056)b
Salicornia	0.22 (0.024)	1.25 (0.18)b	1.48 (0.17)b	1.57 (0.78)b
Atriplex	0.20 (0.009)	0.88 (0.23)b	1.09 (0.24)b	1.26 (0.35)b
Soil control	0.21 (0.018)	17.31 (1.43)a	17.53 (1.41)a	4.83 (0.56)a
Species *P*-value	ns	< 0.0001	< 0.0001	0.008
10 ppt mean	0.21B	5.19B	5.41B	2.14B
35 ppt				
Suaeda	0.32 (0.100)	2.32 (0.21)bc	2.63 (0.295)b	1.54 (0.23)c
Salicornia	0.48 (0.216)	1.66 (0.37)c	2.14 (0.58)b	1.51 (0.081)c
Atriplex	0.31 (0.049)	3.76 (0.16)b	4.06 (0.19)b	2.48 (0.24)b
Soil control	0.47 (0.105)	11.20 (1.34)a	11.67 (1.33)a	3.39 (0.21)a
Species *P*-value	ns	0.0003	0.0006	0.0005
35 ppt mean	0.39A	4.73AB	5.12AB	2.23B

P-values are shown for species (or control) treatment within each salinity treatment. Salinity treatment means within each column followed by the same upper case letter are not significantly different ($P \geq 0.05$). Species (or control) means within each salinity treatment followed by the same or no lower case letter are not significantly different ($P \geq 0.05$).

[a]Salinity treatment had a significant effect on this variable ($P < 0.05$).

salinity (Table 2). The lysimeters at the lowest salinity treatment processed the most water and therefore removed the most total nitrogen. *Suaeda* removed more total and inorganic nitrogen than *Atriplex* or *Salicornia* at the lowest salinity. *Atriplex* did not remove significantly more total nitrogen than the soil control at the highest salinity. All planted lysimeters removed 96% or more of the applied total nitrogen. Removal efficiency declined slightly at the lower salinities (i.e., although lysimeters in the lower salinity treatments received and removed a greater amount of nitrogen, the percentage of nitrogen removed relative to the amount applied declined). Lysimeters with plants removed a significantly greater percentage of inorganic nitrogen than the control lysimeters; the overall mean percent inorganic nitrogen removed by lysimeters with plants was 94%, compared to 58% for soil control lysimeters.

Salinity had a significant inhibitory effect on total nitrogen content in the plant shoot ($P < 0.0001$). At the highest salinity, *Atriplex* contained significantly less nitrogen than the other two species (Table 3). The percentage of the applied total nitrogen incorpo-

Table 5
Mean values ($\pm$SE, $n = 3$) for applied total phosphorus (TP), the percent of the applied total phosphorus, the applied soluble reactive phosphorus-P (SRP), and the percent of the applied SRP removed by the lysimeters with three species of halophytes at three salinities

	TP removed (mg)[a]	% TP removed[b]	SRP removed (mg)[a]	% SRP removed[a]
0.5 ppt				
Suaeda	1396.9 (70.62)a	98.76 (0.39)bc	355.6 (17.11)a	94.00 (1.59)b
Salicornia	927.3 (93.54)b	99.62 (0.07)ab	236.3 (24.64)b	97.88 (0.40)ab
Atriplex	1020.2 (119.41)b	99.24 (0.23)bc	260.8 (30.67)b	96.11 (1.11)ab
Soil control	589.9 (44.30)c	99.74 (0.003)a	144.0 (11.09)c	98.45 (0.25)a
Species *P*-value	0.0022	0.0105	0.0018	0.0303
0.5 ppt mean	983.6A	99.34	249.2A	96.61B
10 ppt				
Suaeda	977.8 (42.68)a	98.95 (0.07)b	250.4 (11.12)a	95.98 (0.33)b
Salicornia	892.1 (55.19)ab	99.56 (0.10)a	229.1 (14.40)a	98.09 (0.42)a
Atriplex	751.3 (30.68)b	99.54 (0.08)a	190.1 (7.81)b	98.19 (0.37)a
Soil control	510.6 (15.01)c	99.66 (0.08)a	124.2 (4.24)c	98.49 (0.22)a
Species *P*-value	0.0010	0.0099	0.0007	0.0276
10 ppt mean	782.9B	99.43	198.4B	97.69B
35 ppt				
Suaeda	435.4 (15.66)a	99.71 (0.02)	107.6 (4.48)a	99.03 (0.06)
Salicornia	401.7 (13.73)b	99.51 (0.16)	98.2 (3.60)ab	98.28 (0.70)
Atriplex	350.1 (11.51)c	99.72 (0.02)	86.2 (3.11)c	99.13 (0.05)
Soil control	374.8 (16.26)bc	99.68 (0.02)	93.4 (4.24)bc	99.03 (0.11)
Species *P*-value	0.0034	ns	0.0082	ns
35 ppt mean	390.5C	99.65	96.3C	98.87A

P-values are shown for species (or control) treatment within each salinity treatment. Salinity treatment means within each column followed by the same or no upper case letter are not significantly different ($P \geq 0.05$). Species (or control) means within each salinity treatment followed by the same or no lower case letter are not significantly different ($P \geq 0.05$).
[a] Salinity treatment had a significant effect on this variable ($P < 0.05$)
[b] Salinity treatment did not have a significant effect on this variable ($P \geq 0.05$).

rated into the plant shoot was significantly greater at the 0.5 and 10 ppt salinity treatments than at the 35 ppt salinity treatment.

Higher concentrations of ammonia were found in leachates from the 35 ppt lysimeters than the other salinities (Table 4). However, 96% of leachate inorganic nitrogen was in the form of nitrate. The mean concentrations of both the inorganic nitrogen and the nitrate in the leach water were significantly greater (six-fold higher) in the control lysimeters without plants, than in the lysimeters with plants. Soil inorganic nitrogen content was significantly greater ($P = 0.0006$) in the 0.5 ppt treatment than in 10 and 35 ppt treatments. In the 10 and 35 ppt treatments, soil inorganic nitrogen content was significantly higher in the control lysimeters containing no plants than in planted lysimeters ($P = 0.008$, $P = 0.0005$, respectively) (Table 4).

3.5. Phosphorus removal

The lysimeters in the lowest salinity treatment removed significantly more total and soluble reactive phosphorus than the lysimeters in the 10 ppt treatment, which in turn removed significantly more phosphorus than the lysimeters in the 35 ppt treatment ($P < 0.0001$) (Table 5). *Suaeda* removed a significantly greater amount of total and soluble reactive phosphorus at the lowest salinity treatment ($P = 0.0022$, $P = 0.0018$,

Table 6
Mean values ($\pm$SE, $n = 3$) for plant shoot phosphorus, and the percent of the applied total phosphorus sequestered by the plant shoot of three species of halophytes at three salinities

	Plant P (mg)[a]	% TP removed by plant[a]
0.5 ppt		
Suaeda	208.91 (21.41)	14.93 (2.03)
Salicornia	136.52 (26.84)	14.44 (1.43)
Atriplex	170.93 (14.09)	16.79 (0.93)
Species *P*-value	ns	ns
0.5 ppt mean	172.12A	15.39A
10 ppt		
Suaeda	105.95 (17.11)a	10.68 (1.57)b
Salicornia	168.59 (6.76)a	18.90 (0.92)a
Atriplex	105.34 (11.11)a	13.94 (1.32)ab
Species *P*-value	0.0468	0.0490
10 ppt mean	126.63B	14.51A
35 ppt		
Suaeda	23.07 (2.38)a	5.27 (0.42)a
Salicornia	21.33 (2.25)a	5.31 (0.62)a
Atriplex	3.49 (0.33)b	0.99 (0.10)b
Species *P*-value	0.006	0.0056
35 ppt mean	15.96C	3.86B

P-values are shown for species treatment within each salinity treatment. Salinity treatment means within each column followed by the same upper case letter are not significantly different ($P \geq 0.05$). Species means within each salinity treatment followed by the same or no lower case letter are not significantly different ($P \geq 0.05$).
[a]Salinity treatment had a significant effect on this variable ($P < 0.05$).

respectively). *Atriplex* did not remove significantly more total and soluble reactive phosphorus than the soil control at 35 ppt. Overall, the lysimeters removed 99.4% and 97.4% (mean of all non-control lysimeters over all treatments) of the applied total and soluble reactive phosphorus, respectively. Unlike nitrogen removal, the control lysimeters without plants had similar removal efficiency as lysimeters with plants. Mean removal efficiency for soluble reactive phosphorus was significantly greater at the 35 ppt treatment compared to the two lower salinity treatments ($P = 0.0074$).

Salinity had a significant inhibitory effect on total phosphorus in the plant shoot ($P < 0.0001$). At the highest salinity *Atriplex* had significantly less shoot phosphorus than the other two species ($P = 0.006$) (Table 6). The percentage of the applied total phosphorus removed by the plant shoot was significantly greater ($P < 0.0001$) at the lowest and the middle salinity than at the highest salinity treatment.

Mean concentration of soluble reactive phosphorus in the leach water was significantly lower ($P = 0.0018$) in the highest salinity treatment than the concentration in the

Table 7
Mean values ($\pm$SE, $n = 3$) for concentrations of soluble reactive phosphorus-P (SRP) and total phosphorus (TP) in the leach water averaged over the course of the experiment, and soil plant-available phosphorus-P/kg soil with three species of halophytes at three salinities

	SRP (mg/1)[a]	TP (mg/1)[b]	Soil phosphate (mg/kg)[b]
0.5 ppt			
Suaeda	1.46 (0.399)a	1.16 (0.370)a	6.32 (0.014)
Salicornia	0.48 (0.105)ab	0.33 (0.069)b	6.21 (1.50)
Atriplex	0.93 (0.311)a	0.70 (0.240)a	5.04 (0.84)
Soil control	0.29 (0.045)b	0.20 (0.0033)bc	3.52 (0.52)
Species *P*-value	0.0281	0.0026	ns
0.5 ppt mean	0.79A	0.60	5.27
10 ppt			
Suaeda	0.95 (0.12)a	0.95 (0.088)a	6.73 (5.34)
Salicornia	0.44 (0.107)b	0.39 (0.098)b	4.79 (0.54)
Atriplex	0.39 (0.082)b	0.39 (0.070)b	4.54 (0.88)
Soil control	0.26 (0.02)b	0.23 (0.039)b	4.17 (0.44)
Species *P*-value	0.0143	0.0038	ns
10 ppt mean	0.51A	0.49	5.06
35 ppt			
Suaeda	0.19 (0.007)	0.23 (0.012)	7.38 (0.74)
Salicornia	0.31 (0.123)	0.37 (0.112)	5.72 (0.64)
Atriplex	0.16 (0.003)	0.21 (0.003)	5.57 (0.63)
Soil control	0.19 (0.018)	0.24 (0.012)	5.38 (0.71)
Species *P*-value	ns	ns	ns
35 ppt mean	0.21B	0.26	6.01

P-values are shown for species (or control) treatment within each salinity treatment. Salinity treatment means within each column followed by the same or no upper case letter are not significantly different ($P \geq 0.05$). Species (or control) means within each salinity treatment followed by the same or no lower case letter are not significantly different ($P \geq 0.05$).
[a]Salinity treatment had a significant effect on this variable ($P < 0.05$).
[b]Salinity treatment did not have a significant effect on this variable ($P \geq 0.05$).

two lower salinity treatments (Table 7). Concentrations of both soluble reactive and total phosphorus in the leachates from the control lysimeters were as low or lower than the concentrations in the leachates from planted lysimeters. The majority of the phosphorus leaching from the lysimeters was in the dissolved form, and the measured soluble reactive phosphorus was greater than the mean concentration of total phosphorus in six of the 12 treatments. This unexpected result was attributed to the different analytical techniques that were used to measure what was essentially the same quantity. Salinity did not affect the amount of plant-available phosphate in the soil, and there were no significant differences among the species within each salinity treatment with respect to plant-available phosphate in the soil (Table 7).

4. Discussion

Overall, the plant–soil system was effective in sequestering nitrogen and phosphorus from the applied effluent across treatments and species. The lysimeters with plants removed over 98% and 94% of the applied total and inorganic nitrogen, respectively; and 99% and 97% of the applied total and soluble reactive phosphorus, respectively. The high removal occurred in spite of the fact that 31% of the applied water leached from the lysimeters. In addition, the aquaculture effluent used for irrigation in this experiment was substantially higher in nitrogen and phosphorus concentration than typical mariculture effluent. In fact, the nutrient concentration of the irrigation water in this experiment was close to an order of magnitude greater than the nutrient concentrations found in typical shrimp pond effluent (Phillips, 1995). Removal efficiencies tended to increase with the higher salinity treatments probably because at lower salinities a much greater volume of water was processed than at higher salinities.

Removal efficiencies exceeded those reported by Schwartz and Boyd (1995) for a constructed wetland that received catfish pond-effluent. In addition to greater removal efficiency, the halophytes grown here might provide an economic return for the grower if they are utilized in animal feeds. Biomass of the three species used in our study has been used to replace grass hay in sheep fattening rations (Swingle et al., 1996).

In the 0.5 and 10 ppt treatments, the plant shoot sequestered approximately 21% and 15% of the applied total nitrogen and phosphorus, respectively. Three percent of the applied total nitrogen and 1% of applied total phosphorus was lost from the system via leaching in these two treatments. The remainder of the nitrogen (76%) in the 0.5 and 10 ppt treatments was either incorporated into plant root tissues, retained in the soil as organic matter or inorganic nutrients, or lost to the system via ammonia volatilization or denitrification. Similarly, the remainder of the phosphorus and phosphorus (84%) was either in the plant root, or in the soil as organic matter or as inorganic phosphate. Thus, most of the total nutrient removal was not due to uptake into harvestable plant tissues, as might be expected, because plants can only take up inorganic nutrients. However, the ultimate fate of the nitrogen and phosphorus remaining in the soil, whether in organic forms such as uneaten feed, algae and fish feces, plant root tissue or in plant-available inorganic forms, can only be determined through multi-year experiments.

In the sand soil in this experiment, nitrate was the predominant form of inorganic nitrogen. Nitrate is typically very mobile in the soil water, and therefore a very high proportion of soil nitrate is available to the plant (Tisdale and Nelson, 1985). Therefore, the lysimeters with plants removed a much greater percentage of the applied inorganic nitrogen than did the control lysimeters without plants. By contrast, the soil itself was responsible for the majority of phosphorus removal from the wastewater. Phosphate is readily absorbed to soil particles and, therefore, typically only a very small percentage of soil phosphorus is available to the plant (Tisdale and Nelson, 1985). The control lysimeters (without plants) removed as much or more of the applied phosphorus on a percentage basis as the lysimeters with plants. However, the lysimeters with plants removed a greater amount of both nitrogen and phosphorus because they received more water to meet the evapotranspiration demands of the plant.

One of the main drawbacks of land disposal systems is the potential for nutrients to leach past the plant root zone and contaminate the aquifer. However, despite the uncharacteristically concentrated irrigation water used, the mean concentrations of ammonia and nitrate in the leach water were always lower than the mean concentration limits recommended by the U.S. Environmental Protection Agency for effluents (Schwartz and Boyd, 1994). The mean Environmental Protection Agency standards are 1.77 mg/l for TAN and 16.9 mg/l for nitrate-N. The mean leach water concentration for all plant-containing lysimeters was 0.28 mg/l for TAN and 2.6 mg/l for nitrate-N. On the other hand, the mean EPA standard for total phosphorus is 0.17 mg/l, a level that was exceeded in all the treatments (mean for plant-containing lysimeters = 0.53 mg/l). However, this concentration would have certainly been lower if the lysimeters had been irrigated with a more typical effluent or if the soil had been other than sand. A deeper soil profile or higher clay content would provide more binding sites for phosphorus than were present in the sand lysimeters.

Suaeda and *Atriplex* generally performed better than *Salicornia* at low salinity, whereas *Atriplex* performed very poorly relative to the other two species on high salinity water. *Atriplex* clearly is not as salt tolerant as the other two species. Plant dry matter production, RGR, ET and the amount of nitrogen and phosphorus removed decreased with increasing salinity. At seawater salinity, the plant–soil system was not capable of treating a large volume of water because the plants grew very little and therefore had low evapotranspiration rates. Slow growth occurred because root zone salinity reached levels high enough to cause serious inhibition. Had we designed the experiment to provide the same volume of wastewater to all treatments, the plants receiving 35 ppt water would have received more water and nutrients. These plants would have probably grown better because root zone salinity would decrease as the additional volume of water would flush salts from the soil. However, the volume of water leached from the high salinity treatments would have certainly been much greater than the volume leached from the low salinity treatments, and therefore would not represent a best management irrigation practice.

The weekly irrigation schedule was probably insufficient to keep soil salinity at levels that would support adequate plant growth. Irrigation strategies employing greater irrigation frequency will be necessary to achieve efficient biofiltration of nutrients if halophytes are to be used to treat aquaculture effluent approaching seawater salinity. The findings from the present experiment were used to conduct further experiments utilizing larger soil volumes and more frequent irrigations. At seawater salinity, much greater yields were produced than in this study (Brown and Glenn, Aquacultural Engineering, in press).

Extensive field testing and site considerations would be needed before saline aquaculture effluent could be used to grow crops at a given location, due to the potential hazards presented by the salt as well as the nutrient load. However, where coastal soils overlay saline aquifers with hydraulic connection to the sea, this strategy offers a potential low-cost and effective method of removing nutrients by using a soil–plant–water system for biotreatment of effluent.

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