



## Saline domestic sewage treatment in constructed wetlands: study of plant selection and treatment characteristics

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### ABSTRACT

A series of investigations was conducted to treat saline domestic sewage using constructed wetlands. Twelve emergent plant species were planted in experimental units and fed with saline domestic sewage. All species were classified into three clusters using cluster analysis based on the average values of relative growth rate, nutrient uptake, root biomass and activity. The species of Cluster I, including *Canna indica*, *Phragmites australis* and *Scirpus validus*, had strong potential for the purification. The above plants were employed again to treat saline domestic sewage under different influent salinities concentrations. For the influent salinity of 0.5, 1.0 and 1.5‰, average treatment performances of planted units were found to be 61.5–70.5% for COD, 59.3–68.4% for  $\text{NH}_4^+\text{-N}$ , 61.9–70.4% for TN and 40.4–47.3% for TP. With increasing influent salinity to 2.0‰, the removal efficiencies were dropped significantly. It was similar to the change of the soil enzyme activity in the experiment units. Activities of urease and cellulase declined significantly when influent salinity increased to 2.0‰. The lower soil enzyme activity in the treatments receiving wastewater at 2.0‰ indicated that saline domestic sewage had an adverse effect on microbial activities.

**Keywords:** Constructed wetland; Saline sewage; Salt-tolerant plant; Pollutant removal; Enzyme activity

### 1. Introduction

Fresh water shortage is a global problem. In recent years, seawater has been used for toilet flushing to save fresh water in many coastal cities in China, such as Shenzhen, Tsingtao, Tianjin and so on. However, the domestic sewage in these cities transforms into saline

as a result of seawater use. Treating saline wastewater is regarded as difficult due to adverse effects of salt on organisms. To date most studies of saline wastewater treatment adopt activated sludge method, only a few studies have investigated the constructed wetland (CW) for saline wastewater treatment [1,2].

CWs, treating wastewater through an integration of microbial, physical and chemical reactions, are capable of removing suspended solids (SS), organic

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matter, nitrogen, phosphorus and heavy metals from many kinds of wastewater with relatively lower costs for construction and operation [3,4]. Currently many kinds of wastewater are treated by CWs, such as municipal sewage, industrial wastewater, aquaculture effluent, landfill leachate, mine drainage and so on [5–8]. Saline domestic sewage contains high level of contaminants and salt, constituting a new object for treatment in wetlands. Salt has negative influence on the wetland plant growth, and the running efficiency of CWs is reduced accordingly [1,2,9]. So, it is necessary to select the suitable salt-tolerant wetland plants for the saline sewage treatment.

The role of plants as an essential component of CWs is well established [10,11]. The plants accelerate the performance of CWs by giving more oxygen supply and a support medium for microbial degradation. The main active reaction zone of CWs is the rhizosphere. This is where physico-chemical and biological processes take place. In addition, nutrient assimilation

of plants promotes the removal efficiency of nitrogen and phosphorous in CWs [11]. The suitable plants for use in CWs should be able to grow well under hypertrophic waterlogged conditions and have a high pollutant removal capacity. Furthermore, they should have a well-developed root system to provide a large surface area for attached microbial growth and oxygen supply. In addition, they must possess strong resistance to adverse conditions [12–14].

In this study, 12 emergent plant species were used for the treatment of saline domestic sewage. The specific objectives of this study are twofold: first, selecting the suitable salt-tolerant plant according to its growth, nutrient uptake and root system, and second, investigating the effect of influent salinity on the performance of CWs.

## 2. Methods

### 2.1. Experimental plants and set-up

Through the investigation of local salt-tolerant wetland plants in Zhejiang province, China, 15 emergent plants were collected from the open country and transplanted to a horizontal subsurface CW. The growth of plants was investigated, 3 out of the 15 species which were *Rhizoma alismatis*, *Zizania aquatica* and *Scripus triquetter* appeared dwarfed, dry and yellowish, and 12 well-grown plants (Table 1) were selected for further research. For each objective plant, the similar size individuals were transplanted to experimental units, respectively, with a density of 15 individual plants  $m^{-2}$ . Each species-specific unit was replicated three times. The experimental units were constructed with dimensions of 0.5 m deep, 1.5 m long and 1.0 m wide (Fig. 1). Each unit was filled from bottom to top with 5 cm of pebbles (diameter: 50–100 mm), 20 cm of

Table 1  
List of tested plant species

Common name	Scientific name
Yellow iris	<i>Iris wilsonii</i> C.H.Wright
Umbrella plant	<i>Cyperus alternifolius</i> Linn.
Softstem bulrush	<i>Scirpus validus</i> Vahl.
Rice grass	<i>Spartina anglica</i> Hubb.
Common rush	<i>Juncus effuses</i> Linn.
Canna	<i>Canna indica</i> Linn.
Spiked loosestrife	<i>Lythrum salicaria</i> Linn.
Cattail	<i>Typha orientalis</i> Presl.
Cress	<i>Oenanthe javanica</i> (Blume) DC.
Water spinach	<i>Ipomoea aquatica</i> Forsk.
Reeds	<i>Phragmites australis</i> Trin. Ex Steud.
Calamus	<i>Acorus calamus</i> Linn.

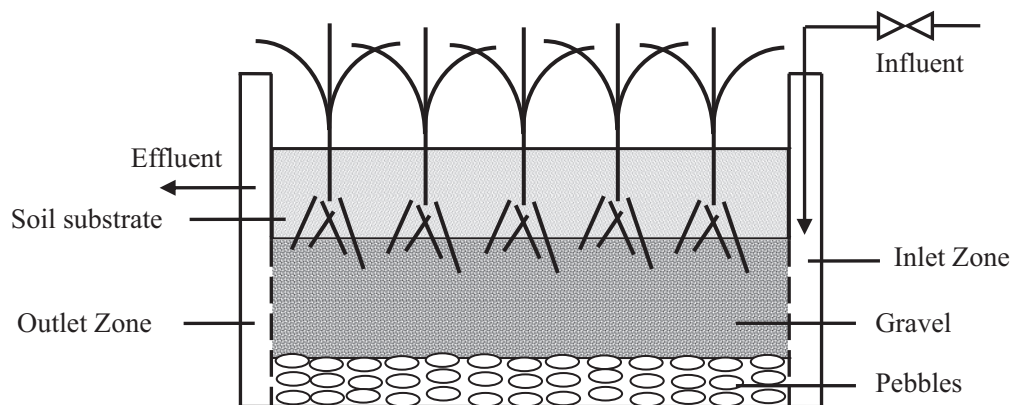


Fig. 1. Schematic diagram of the constructed experimental unit.

gravel (diameter: 10–30 mm) and 10 cm of soil substrate (mixture of local soil, sand and coal ash). And the effective depth of liquid in the bed was 30 cm. The units were operated in horizontal subsurface flow mode during the experiment.

The study site is located in Zhejiang Ocean University, Dinghai, China. Dinghai is located between 121°38′ and 122°15′ E, and 29°55′ and 30°15′ N, and belongs to the subtropical monsoon climate of the north. The annual average air temperature is 15.6–16.6°C. The mean temperature in the hottest month (August) is 25.8–28.0°C and in the coldest month (January) is 5.2–5.9°C.

## 2.2. Experimental procedure

The experiment was divided into two stages. The research aim of stage I was to select the suitable salt-tolerant plants for the disposal of saline sewage. In the beginning, the units were watered by tap water every day until the plants were adjustable. After plant acclimatization, all units operated in horizontal flow mode were fed with saline domestic sewage, which was prepared by spiking the students' dormitories sewage with sea salt to simulate the saline condition of 1.0%. Mean and standard deviation values of principal chemical compounds concentrations (mg l<sup>-1</sup>) of this saline domestic sewage were as follows: COD, 189.6 ± 26.7; BOD<sub>5</sub>, 82.0 ± 15.6; NH<sub>4</sub><sup>+</sup>-N, 21.3 ± 9.0; total nitrogen (TN), 26.2 ± 10.2; total phosphorus (TP), 2.4 ± 0.9 and SS, 161.0 ± 38.1. The sewage was discharged to each unit for two hours once a day at a hydraulic loading rate of 0.01 m<sup>3</sup> m<sup>-2</sup> h<sup>-1</sup> during April 10, 2009–June 10, 2009. All plants were harvested at the completion of stage I.

In stage II of the experiment, three well-grown species of stage I were selected for further research. Each species-specific unit was replicated three times. And three unplanted units were also set as a control. All the units were operated in horizontal subsurface flow mode. The aim of stage II was to study the effects of influent salinity on the treatment performance of the CWs. A continuous-flow mode was employed in this experiment stage. The hydraulic loading rate was 0.1 m<sup>3</sup> m<sup>-2</sup> d<sup>-1</sup> and the hydraulic retention time (HRT) was 3 d. Artificial sewage at different salinities (0.5, 1.0, 1.5 and 2.0%) was prepared by spiking the sewage with different amounts of sea salt. Pollutants concentrations of the sewage were similar to stage I. The experiments started from July 4, 2009 to October 25, 2009 including 30 d for system acclimatization under tested conditions and 83 d for system operation.

## 2.3. Plant sampling and analysis

In the experimental stage I, for each species, six plants were sampled randomly from the three parallel species-specific units (two from each unit). Biomass of above-ground plant tissues were measured at the beginning and the end of the experimental stage. The sampled above-ground plant tissues were oven dried for 48 h at 80°C before the analysis of nutrient content and the calculations of biomass increment and relative growth rate (RGR):

$$\text{RGR}(\text{d}^{-1}) = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where  $W_1$  and  $W_2$  are dry weight of above-ground plant tissues at the beginning ( $t_1$ ) and at the end ( $t_2$ ) of experimental stage, respectively.

Biomass and activity of root were measured at the end of experimental stage. Roots of the sampled plants were separated, cleaned, dried for 48 h at 80°C and weighed. Root activity was measured using  $\alpha$ -naphthylamine oxidation method and expressed by  $\alpha$ -NA oxidizing power [15].

## 2.4. Pollutant removal measurement and analysis

The pollutant removal efficiency was evaluated by measuring organic and inorganic parameters during continuous-flow study. The influent and effluent water samples were analysed for ammonium nitrogen (NH<sub>4</sub><sup>+</sup>-N), TN, TP and COD according to the standard methods for the examination of wastewater [16]. Water samples were collected from the inlet and outlet of the units at three day intervals. The analyses were done immediately after sample collection. For the calculation of removal efficiency, the following equation was used.

$$r(\%) = \frac{C_i - C_e}{C_i} \times 100$$

where  $r(\%)$  is the removal efficiency in %,  $C_i$  is the concentration in the inflow to the unit and  $C_e$  is the concentration in the outflow from the unit.

## 2.5. Soil enzyme activity measurement

At the end of continuous-flow study, cellulase activity and urease activity in soil of each species-specific unit and unplanted unit were measured to analyse the effects of influent salinity on biological activity in soil. For each unplanted unit, six

10 cm × 10 cm plots were sampled from the CW. Soil samples were collected from the depth of 0–10 cm using a soil sampler. For each species-specific unit, soil samples were collected from the rhizosphere using the method of Wu et al. [2]. Six plants were pulled out and shaken gently to remove the bulk soil. The soil still adhering to the plant roots, around 1 mm thick, was defined as the rhizosphere soil. All the soil samples from each unit were lyophilized, sieved through 1.0 mm mesh to remove plant material and sand, mixed thoroughly and then stored at 4°C prior to use. Urease activity was measured using the method of [17] and expressed as  $\mu\text{g NH}_4^+\text{-N g}^{-1} 24 \text{ h}^{-1}$ . Cellulase activity was assayed according to [18] and expressed as  $\mu\text{g glucose g}^{-1} 72 \text{ h}^{-1}$ .

### 2.6. Statistical analysis

Statistical analysis was performed on the software of SPSS 16 (SPSS Inc., Chicago, IL, USA) program. Analyses of variance were performed to compare the treatment performance and soil enzyme activities of the species-specific CWs. Differences between means were tested using paired-sample *t*-tests.

## 3. Results and discussion

### 3.1. Plant growth and nutrient assimilation

#### 3.1.1. Plant growth

RGR can reflect the health of plants, so it is one of the most commonly used scientific measurements of CWs plants screening [1,12].

After the two month intermittence-flow study, the RGR values differed among the tested species, which were in the range 0.0097–0.0234  $\text{d}^{-1}$  as shown in Fig. 2, and *Phragmites australis* showed the highest value. Besides *P. australis*, other plants which were *Scirpus validus* and *Oenanthe javanica* relatively grew better than the rest of the tested species in the saline CWs, and the RGR values were higher than 0.0200  $\text{d}^{-1}$ . By contrast, plants of slow growth included *Acorus calamus*, *Ipomoea aquatica* and *Juncus effuses* with average RGR value less than 0.012  $\text{d}^{-1}$ .

#### 3.1.2. Nutrient assimilation

The uptake of nutrient into the plant biomass is one of the ways for nitrogen and phosphorous removal in CWs, and its removal quantity depends on the above-ground biomass increment and nutrient concentration of plant. Table 2 shows the nutrient content and nutrient uptake of tested plants. Nutrient concentration was in the range 8.75–23.30  $\text{mg g}^{-1}$  dry mass for nitrogen and 1.83–4.22  $\text{mg g}^{-1}$  dry mass for phosphorus. These nutrient content values were found to resemble the nutrient content of 41 wetland plant species, which were 2.5–21.4  $\text{mg g}^{-1}$  dry mass and 1.3–5.1  $\text{mg g}^{-1}$  dry mass for nitrogen and phosphorus, respectively, when they were cultured in the standard nutrition conditions (N:P:K = 7:11:27) [19]. This indicated that the effect of salinity on the nutrient content of plants was negligible.

Plant species had the significant effects on nutrient assimilation, nitrogen and phosphorus uptake of above-ground tissues which were in the range

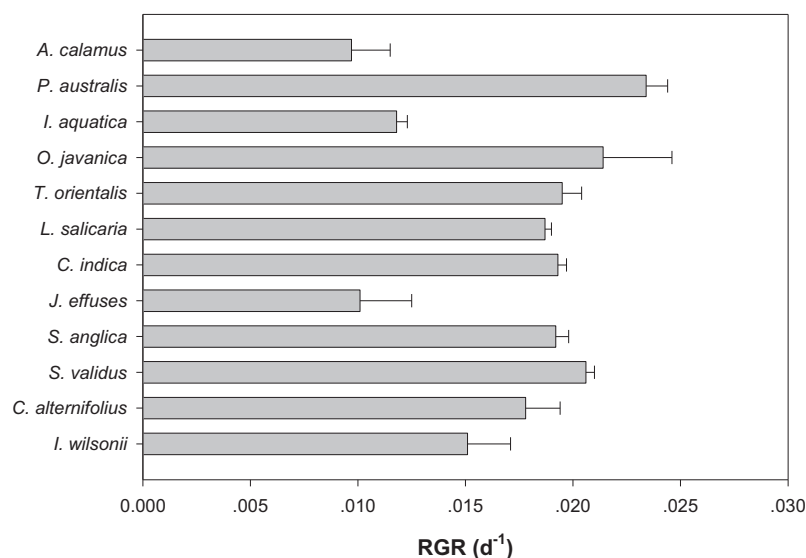


Fig. 2. RGR of tested plant species (mean ± SD, *n* = 6).

Table 2  
Nutrient assimilation of tested plant species

Plant species	Biomass increment (g per plant)	Nutrient concentration (mg g <sup>-1</sup> DW)		Nutrient uptake (mg per plant)	
		N	P	N	P
<i>I. wilsonii</i>	2.09 ± 0.58	8.75 ± 0.43	2.61 ± 0.07	18.42 ± 5.96	5.46 ± 1.64
<i>C. alternifolius</i>	3.01 ± 0.66	20.77 ± 0.79	3.12 ± 0.11	62.89 ± 16.11	9.45 ± 2.37
<i>S. validus</i>	10.91 ± 0.62	17.78 ± 0.38	3.61 ± 0.07	193.79 ± 7.93	39.36 ± 2.15
<i>S. anglica</i>	4.52 ± 0.35	23.28 ± 1.22	3.17 ± 0.07	105.45 ± 13.97	14.32 ± 1.20
<i>J. effuses</i>	0.94 ± 0.36	12.78 ± 0.36	2.77 ± 0.09	12.32 ± 4.33	2.67 ± 0.93
<i>C. indica</i>	9.19 ± 0.50	17.42 ± 0.86	3.05 ± 0.68	160.07 ± 11.32	28.03 ± 6.53
<i>L. salicaria</i>	5.09 ± 0.30	23.30 ± 0.41	3.13 ± 0.08	118.24 ± 8.72	15.20 ± 0.37
<i>T. orientalis</i>	4.69 ± 0.55	20.50 ± 0.84	4.22 ± 0.12	96.28 ± 13.49	19.79 ± 2.19
<i>O. javanica</i>	5.72 ± 1.14	14.35 ± 1.64	3.62 ± 0.40	82.21 ± 10.62	20.76 ± 1.17
<i>I. aquatica</i>	1.20 ± 0.28	15.77 ± 1.09	2.21 ± 0.17	18.71 ± 5.04	2.61 ± 0.82
<i>P. australis</i>	15.70 ± 1.96	11.56 ± 0.89	1.83 ± 0.12	180.85 ± 19.85	28.61 ± 2.53
<i>A. calamus</i>	1.18 ± 0.49	10.85 ± 0.62	3.52 ± 0.28	12.94 ± 4.51	4.16 ± 0.44

Means ± SD of the six replicates are shown for plant species variable.

12.32–193.79 mg N per plant and 2.61–39.36 mg P per plant, respectively (Table 2), and *S. validus* showed the highest nutrient assimilation. The nutrient uptake values of tested species were obviously lower than those were reported for CWs receiving sanitary wastewater, which were 65.16–6978.32 mg N per plant [20]. This is mainly due to the slow growth of tested species under saline condition. The above-ground biomass increments of tested plants were in the range 0.94–15.7 g per plant (Table 2). Compared to the study [20], the biomass increment of *P. australis* was 154.33 g per plant. This clearly indicated that the growth of plants was strongly inhibited under the combination effect of high salt concentration, flooded condition and anaerobic environment; they caused a great reduction in the biomass increment of nearly 90%. Thus, the nutrient assimilation of tested plant was influenced.

For tested plant species, the nutrient storage ratios of above-ground and total plant tissues were in the range 81.8–95.0% for nitrogen and 78.1–93.8% for phosphorous (data not shown). Nutrient was mainly stored in above-ground tissues, which can be removed through harvesting the above-ground biomass. Among the tested plant species, *S. validus*, *C. indica* and *P. australis* had high absorptive capacity of nutrient and the above-ground nutrient uptake of these species were higher than 160 mg per plant and 28 mg per plant for nitrogen and phosphorus, respectively.

### 3.2. Root biomass and activity

Plant root zone is the most active reaction zone of CWs. This is where the root provides the surface areas

for bacterial growth and adds oxygen to the water and to the substrate, and then the most intensive physico-chemical and biological processes take place. So, the root biomass and activity are important indexes for the CW plants screening [21]. The average root biomass and root activity of the tested species at the end of the intermittence-flow study are showed in Fig. 3. Six out of the tested species, which were *P. australis*, *C. indica*, *S. validus*, *Lythrum salicaria*, *Typha orientalis* and *O. javanica* showed well-developed root systems under saline condition, and the root biomass of each species was higher than 1.5 g per plant. By contrast, *Acorus calamus*, *I. aquatica*, *J. effuses*, *Cyperus alternifolius* and *Iris wilsonii* had relatively less roots, and the root biomass of each species was less than 1.0 g per plant.

With respect to different species, *P. australis*, *C. indica*, *L. salicaria*, *S. anglica* and *I. wilsonii* exhibited high average root activity under saline condition, and the  $\alpha$ -NA oxidation capacity higher than 80  $\mu\text{g g}^{-1} \text{h}^{-1}$ . Low-root activity plant included *C. alternifolius*, *J. effuses* and *T. orientalis* with average  $\alpha$ -NA oxidation capacity of root below 40  $\mu\text{g g}^{-1} \text{h}^{-1}$ .

Some studies reported that root growth affected the hydraulic quality of soils [22,23], so the root biomass does not suit being used as an index for the CW plants screening. Nevertheless, most of research results indicate that plant root plays a key role in the purification of CW [10,11,20,24]. In addition, root growth and the microbial degradation of dead roots cause the formation of new secondary soil pores [11].



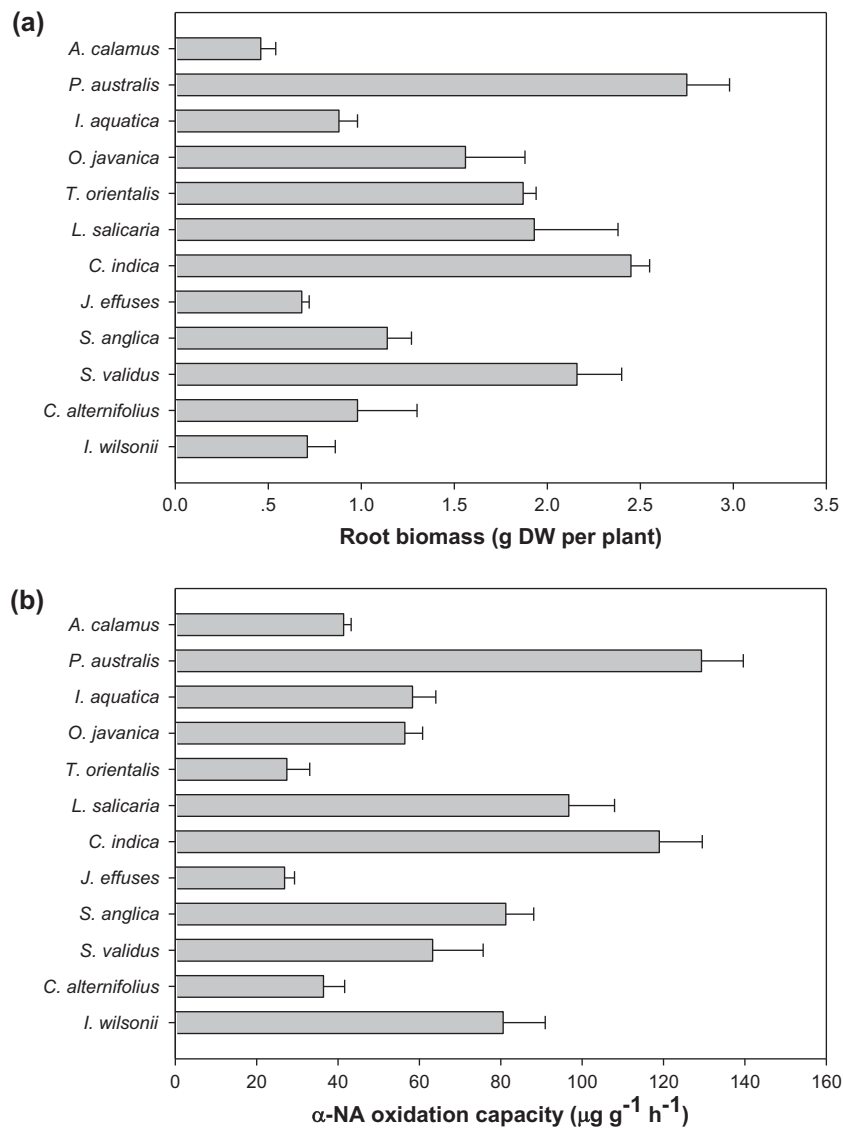


Fig. 3. Root biomass (a) and root activity (b) of tested plant species measured at the end of stage I experiment (mean  $\pm$  SD,  $n = 6$ ).

### 3.3. Cluster analysis of the species

Above results showed that the plant rankings had something in common but also differ for different screening index. So, the single index could not fully reflect the performance of the CW plants under saline condition. In this study, the 12 tested species were classified using cluster analysis based on the average values of RGR, nitrogen uptake, phosphorous uptake, root biomass and root activity. The tested species could be classified into three clusters using a criteria value of rescaled distance between 10 and 15 (Fig. 4). The species of Cluster I included *C. indica*, *P. australis* and *S. validus* which had some

common characteristics, such as growing and uptaking nutrient fast, a better developed root system and so on. So, they could be regarded as the most effective species for the purification of saline domestic sewage among the tested species. Cluster II contained five plant species that were *S. anglica*, *L. salicaria*, *C. alternifolius*, *O. javanica* and *T. orientalis*, which had relatively slower growth and fewer roots than the plants of Cluster I. The rest species belonged to Cluster III, which grew strong restrainedly under the condition of saline domestic sewage. So, these plant species could not be considered for the purification of saline wastewater.

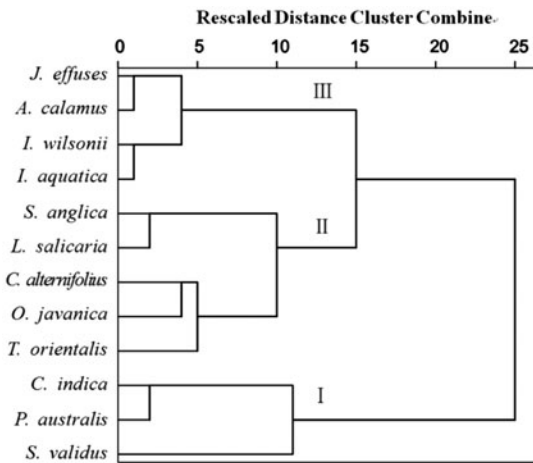


Fig. 4. Cluster analysis of the tested plant species.

3.4. Treatment performance of the species-specific units at different salinities

As reported above, *P. australis*, *S. validus* and *C. indica* showed the highest durability and effectiveness under saline condition in CWs, and they were employed again to treat saline domestic sewage under different influent salinity. The removal efficiencies of the species-specific units under the condition of the influent salinity of 0.5, 1.0, 1.5 and 2.0% are shown in Fig. 5.

The effects of salinity on the contaminant removal were insignificant when the influent salinities of the units were less than or equal to 1.5%. In terms of organic matter removal, the average COD removal efficiency of species-specific units for an influent salinity of 0.5, 1.0 and 1.5% varied between 68.2 and 70.5% for *P. australis*, between 61.5 and 63.2% for *S. validus*, and between 62.1 and 65.2% for *C. indica*. But when the influent salinity increased to 2.0%, COD removal efficiency of species-specific units dropped significantly ( $p < 0.05$ ). And average COD removal efficiency of planted units dropped to 53.7% for *P. australis*, 51.2% for *S. validus* and 49.3% for *C. indica*.

Similar phenomenon was also found in the removal of nitrogen ( $\text{NH}_4^+\text{-N}$  and TN); and when the influent salinity increased from 1.5 to 2.0%, the removal efficiency of all the species-specific units dropped significantly ( $p < 0.05$ ). For the influent salinity of 0.5, 1.0 and 1.5%, the average removal efficiency varied from 59.3 to 68.4% for  $\text{NH}_4^+\text{-N}$  and 61.9 to 70.4% for TN. For the influent salinity of 2.0%, the average removal efficiency varied from 43.2 to 48.6% for  $\text{NH}_4^+\text{-N}$  and from 45.2 to 50.5% for TN.

However, phosphate removal was less sensitive to influent salinity in all the species-specific units. The TP removal efficiency varied between 40.4 and 47.3% for an influent salinity of 0.5, 1.0 and 1.5%, and between 34.2 and 38.9% for an influent salinity of

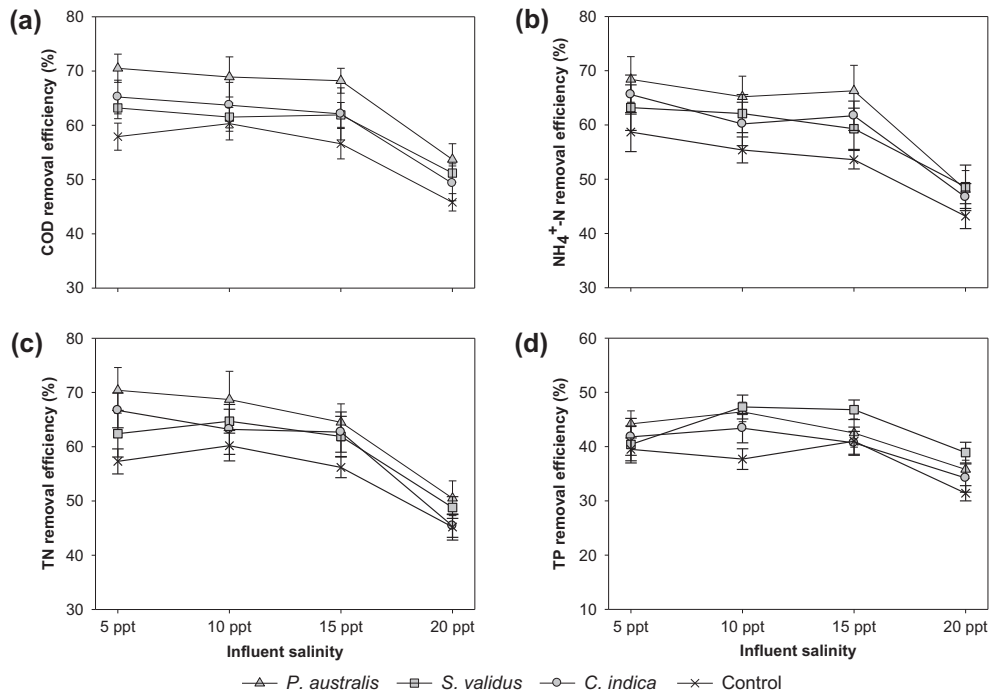


Fig. 5. COD (a),  $\text{NH}_4^+\text{-N}$  (b), TN (c) and TP (d) removal efficiencies of the CWs during 83 days of the continuous-flow study.

2.0%. This decrease in removal of TP obviously was less than that of COD,  $\text{NH}_4^+\text{-N}$  and TN. It is generally known that phosphorus is removed by the filler, microorganism and plant in CWs. While the major loss of phosphate from wastewater is through the adsorption and precipitation of the filler, uptake by emergent plants and microbes accounts for a small proportion [25]. This was why the phosphate removal in the units was less affected by influent salinity.

As Fig. 4 shows, in comparison with the control units, the planted units outperformed the control units in terms of organic matter removal efficiency by about 10% when the influent salinity was less than or equal to 1.5%. Similar phenomenon was also found in

the removal of  $\text{NH}_4^+\text{-N}$  and TN. So, this just suggests that plant plays an important role in the removal of organic and nutrients contaminant. As it has been reported that the macrophytes accelerate the performance of CWs by giving more oxygen supply and a support medium for microbial degradation to occur [26,27].

### 3.5. Soil enzyme activity

It is generally known that the major loss of organic matter and nitrogen from water column in CWs is through the biodegradation of microbes. The negative effect of influent salinity on contaminants removal as

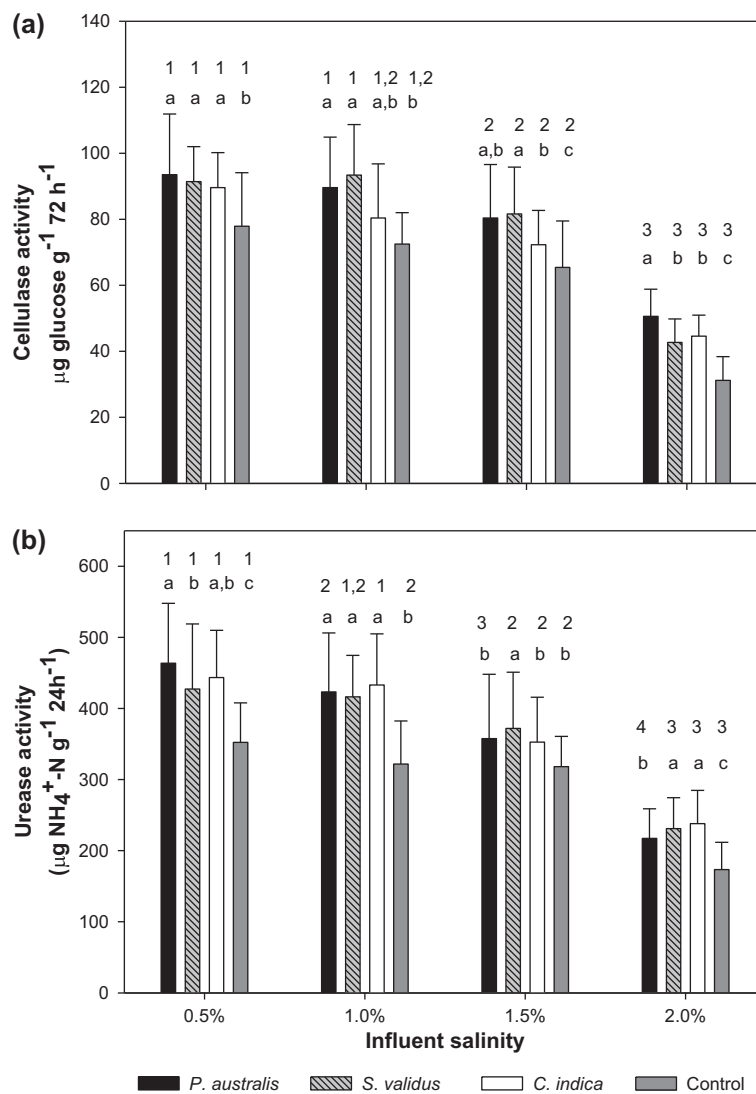


Fig. 6. Soil enzyme activity in the CWs at the end of the continuous-flow study. (a): cellulase; (b): urease. Different letters denote significant differences among CWs under the same saline condition at  $p < 0.05$  (i.e. *P. australis* vs. *S. validus* vs. *C. indica* vs. control under salinity of 0.5%, etc.). Different numbers denote significant differences of the same CWs under different salinity at  $p < 0.05$  (i.e. *P. australis* under salinity of 0.5, 1.0, 1.5 and 2.0%, etc.) (mean  $\pm$  SD,  $n = 3$ ).



presented in Fig. 5 may be due to its inhibition on the growth and activity of soil microbes, as reflected by decreases in soil enzyme activities at high influent salinity (Fig. 6). Soil enzymes are produced by living organisms and can enhance the biodegradation process in CWs [28,29]. Activities of urease and cellulase generally not declined significantly with influent salinity increased from 0.5 to 1.5%, and dropped significantly ( $p < 0.05$ ) when influent salinity increased to 2.0% (Fig. 6). These changing patterns were similar to that of contaminants removal (Fig. 5). Therefore, the low removal efficiency of organic matter and nitrogen in the CWs receiving saline domestic sewage at 2.0% might be explained by the adverse effect of influent salinity on microbial activities.

Under most of the tested salinity conditions, cellulase and urease activities were significantly higher ( $p < 0.05$ ) in the planted wetlands than in the control wetlands, and were generally not significantly different between the three planted wetlands (Fig. 6). This result indicates that the plant rhizosphere is crucial for CWs operating in a saline condition.

#### 4. Conclusions

This study demonstrated that the saline domestic wastewater had an adverse effect on growth of the tested plant species. The plants *C. indica*, *P. australis* and *S. validus* were the plant species better adapted to be used to treat saline domestic wastewater in terms of growth, nutrient uptake, root biomass and activity.

The removal of organic matter and nitrogen by the CWs was significantly inhibited when the influent salinity increased to 2.0%. The lower soil enzyme activity in the treatments receiving wastewater at 2.0% indicated that saline domestic sewage had an adverse effect on microbial activities.

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