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Growth and physiological responses of *Kandelia candel* and *Bruguiera gymnorrhiza* to livestock wastewater

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Abstract

Growth and physiological responses of two mangrove species (*Kandelia candel* and *Bruguiera gymnorrhiza*) to livestock wastewater under two salinity conditions (seawater with salinity of 30þ and freshwater) were examined in greenhouse pot-cultivation systems for 144 days. Wastewater treatment significantly enhanced growth of *Kandelia candel* and *Bruguiera gymnorrhiza* in terms of stem height, stem basal diameter, leaf production, maximum unit leaf area and relative growth rate. Wastewater discharges and salinity levels did not significantly change biomass partitioning of *Kandelia candel*, however, more biomass of *Bruguiera gymnorrhiza* was allocated to leaf due to wastewater discharges. In *Bruguiera gymnorrhiza*, contents of chlorophyll *a* and chlorophyll *b* increased with wastewater discharges but such increase was not observed in *Kandelia candel*. On the other hand, livestock wastewater increased leaf electric conductance in *Kandelia candel* but not in *Bruguiera gymnorrhiza*. The peroxidase activity in stem and root of *Kandelia candel* under both salinity conditions increased due to wastewater discharges, while the activity in root of the treated *Bruguiera gymnorrhiza* seedlings decreased under freshwater condition but increased at seawater salinity. The superoxide dismutase activity in treated *Bruguiera gymnorrhiza* decreased but did not show any significant change in *Kandelia candel* receiving livestock wastewater.

Introduction

Natural and constructed wetlands including mangroves have been considered to be low-cost and effective wastewater treatment systems and are especially efficient in nutrient removal (Clough et al., 1983; Corbitt & Bowen, 1994; Breaux et al., 1995). Nutrients, especially nitrogen and phosphorus are often deficient in coastal inter-tidal areas such as mangrove wetlands (Boto & Wellington, 1983; Clough et al., 1983; Lin, 1999). Therefore, mangrove wetlands are particularly suitable for the treatment of wastewater rich in nutrients. In a mangrove ecosystem, mangrove plant is the most important component. If mangroves were to be used as the treatment system for nutrient-rich wastewater such as livestock sewage, suitable mangrove plant species that is most efficient in nutrient removal should be selected. The species should also be tolerant to wastewater stress. So growth and physiological responses of the wastewater treated plants should be an important consideration when selecting the appropriate species for wastewater treatment.

Mangroves are naturally distributed in areas with a wide range of water salinity, from nearly freshwater to seawater (Lin, 1999). Habitat salinity may influence the N uptake by mangrove plants, and affect their growth and physiological responses to wastewater addition. Naidoo (1990) suggested that growth enhancement of *Bruguiera gymnorrhiza* in response to N addition occurs only at a low salinity. The present investigation therefore aims to compare growth and physiological responses of two dominant mangrove plant species in Hong Kong SAR and South-east Asia, *Bruguiera gymnorrhiza* and *Kandelia candel*, to live-

Table 1. Characteristics of livestock wastewater, tap water and seawater used in this experiment

Parameter	Wastewater	Tap water	Seawater
pH	7.24 (7.00∼7.35)	7.64 (7.10∼8.03)	8.01 (7.32∼8.26)
Conductivity (ms cm^{-1})	1.39 (1.14∼1.77)	0.22 (0.18∼0.23)	36.60 (34.83∼38.49)
TOC (mg l^{-1})	65.9 (48.1∼88.3)	2.3 (0.5∼5.4)	5.2 (2.1∼7.0)
NH_4^+-N (mg l^{-1})	36.1 (21.2∼58.7)	0.3 (0.0∼1.0)	1.9(0.0∼5.9)
NO_x^--N (mg l^{-1})	<0.05	<0.05	<0.05
PO_4^{3-}-P (mg l^{-1})	53.7 (22.5∼94.6)	0.2 (0.0∼0.5)	0.2(0.0∼0.7)

Mean and range (in brackets) values of 9 samples are shown.

stock wastewater. The effect of salinity on these responses was also evaluated. This work is of significant importance to the selection of the most suitable plant species of mangrove wetlands to treat wastewater rich in nutrients.

Materials and methods

Experimental design

In April 1997, mature propagules of *Bruguiera gymnorrhiza* were collected from Mai Po Mangrove Nature Reserve (114° 05′ E, 22° 32′ N) in Hong Kong SAR and planted into plastic pots (18 cm in diameter and 20 cm in height), with four individuals per pot, in a greenhouse. In November 1998, 1.5 years old *Kandelia candel* seedlings were transplanted from Wong Chuk Wan mangrove swamp of Hong Kong into greenhouse pots with three individuals per pot. Each pot contained 4 kg soils freshly collected from Sai Keng mangrove swamp, HKSAR. The soil is a loamy-sandy soil with 73.11%, 15.93% and 10.96% of sand, silt and clay particles, respectively. Each pot was daily irrigated with 300 ml tap water and excess water was able to drain freely from the six draining holes (0.6 cm in diameter) at the bottom by gravitational force.

On 3 March 1999, 12 pots of *B. gymnorrhiza* and 12 pots of *K. candel* were used for treating livestock wastewater. The livestock sewage was collected from Ta Kwu Ling Pig Farm of Hong Kong SAR after being primarily treated by sedimentation and secondarily treated by aeration. The wastewater had high P content and low NH_4^+-N/PO_4^{3-}–P ratio of 1:1.5 (Table 1). The stem basal diameter and stem height of *Bruguiera gymnorrhiza* at the start of the experiment were 0.82 ± 0.08 cm and 24.2 ± 3.2 cm, respectively (*n*=48), while the basal stem diameter of *Kandelia candel* was 0.64 ± 0.05 cm and the stem height was 25.2 ± 3.9 cm (*n*=36).

During the treatment period, each pot was daily watered with 300 ml treatment liquid. For each plant species, the following four treatments, each in triplicate and lasted for 144 days, were set up:

(1) F, each pot was irrigated with tap water every day,
(2) FW, each pot was irrigated with wastewater in every three days and tap water in every other day,
(3) S, each pot was irrigated with artificial seawater (salinity of 30þ, prepared by dissolving a commercial salt purchased from Instant Ocean, Aquarium Systems, Inc., Mentor, Ohio) every day,
(4) SW, each pot was irrigated with wastewater in every three days and seawater in every other day.

Growth and biomass partitioning analysis

At the beginning and at the end of the experiment, the stem basal diameter (D) at the first stem node and the stem height (H) (excluding hypocotyl) of each plant were measured. At the end of the experiment, biomass partitioning was determined by 105 °C dry weights of shoot (leaf and stem) and root of each individual plant. Hypocotyl was excluded in either shoot or root biomass measurement, mainly because the weight of hypocotyl did not change during the experiment. Data on total biomass per pot (B), average stem basal diameter per pot (D) and average stem height per pot (H) collected at the end of the experiment were used to fit the non-destructive allometric equations suggested by Snedaker & Snedaker (1984). The calculated equations for the two species were as follows:

$$B.\ gymnorrhiza:\ \log B = 0.9998 + 0.5416 \log (D^2H)(n = 12, p < 0.01)$$

$$K.\ candel: \log B = 0.6108 + 0.6450 \log (D^2H)(n = 12, p < 0.01)$$

The initial biomass per pot was then estimated according to the equations. The relative growth rate (RGR) was calculated as

$$\mathrm{RGR} = \frac{\ln B_2 - \ln B_1}{t_2 - t_1}.$$

B_1 and B_2 were total biomass at the beginning (t_1) and at the end (t_2) of the experimental period (Hunt, 1978).

Physiological analysis

About 0.1 g fresh tissues of the third pair of leaves from the top were ground in cold mortar with 10 ml 80% acetone. The homogenate was centrifuged at 10 000× ***g*** for 3 min. The contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and total carotenoid were determined according to Lichtenthaler & Wellburn's method (1983). The methods used to determine leaf electric conductance and root activity were the same as described by Zhang (1990).

The methods for extraction and determination of peroxidase (POX) and superoxide dismutase (SOD) activity were similar to those described by Liu & Zhang (1994) with minor modifications. Portions of fresh plant materials were ground and homogenized with five times of 62.5 mmol l^{-1} phosphate buffer (pH 7.8, including 0.4% polyvinyl pyrrolidone) in an ice bath. The homogenate was centrifuged at 1500 × ***g*** for 20 min. The supernatant, the extract of POX and SOD, was stored at 4 °C before the enzyme activity was determined.

For the POX activity assay, 20 μl of the enzyme extract was diluted to 1 ml with deionized water, mixed with 3 ml combined reagent (consists of 500 ml 0.1 mol l^{-1} phosphate buffer at pH 7.0, 280 μl guaiacol and 190 μl hydrogen peroxide). The absorbance at 470 nm was measured at every minute interval. An increase of 0.01 absorbance per minute was considered as one unit of POX activity. The SOD activity was determined by the degree of inhibition to photo-reduction of nitro blue tetrazolium (NBT). The following reagents in a sequence of 2.4 ml 62.5 mmol l^{-1} phosphate buffer, 0.2 ml 0.06 mmol l^{-1} riboflavin, 0.2 ml 30 mmol l^{-1} methionine, 0.1 ml 0.003 mmol l^{-1} Na_2EDTA, 20 μl enzyme extract, and 0.2 ml 1.125 mmol l^{-1} NBT were mixed. In a blank set up to determine the maximum photo-reduction of NBT, the enzyme extract was substituted by the buffer solution. The reaction was carried out under illumination (400 lux) for 1 h, and the absorbance at 560 nm was measured. An enzyme unit was calculated as 50% inhibition of the maximum photo-reduction of NBT.

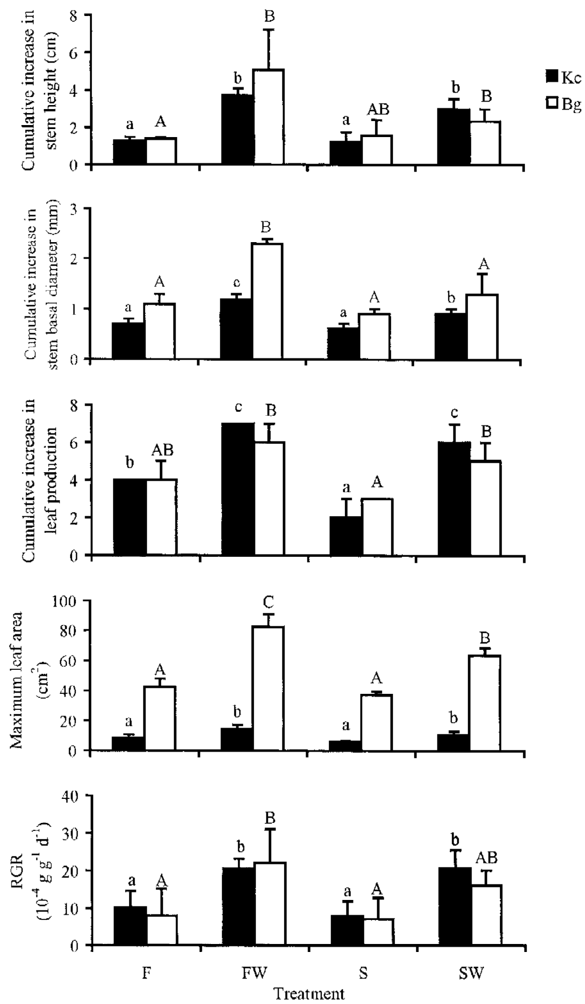

Figure 1. Growth of *Kandelia candel* (Kc) and *Bruguiera gymnorrhiza* (Bg) at the end of 144 days treatment (for each species, means with different letters are significantly different at $p<0.05$ level).

Data analysis

Mean and standard deviation values of triplicates were calculated. A parametric two-way analysis of variance (ANOVA) test was employed to examine any significant difference among wastewater treatments, between salinity conditions, and interactions between wastewater and salinity. The Student–Newman–Keuls multiple comparison method was used if significant difference was found among treatments.

Table 2. Results of two-way ANOVA tests showing the effects of livestock wastewater treatments and salinity conditions on the growth (cumulative increase in stem height: ΔH; cumulative increase in stem basal diameter: ΔD; cumulative increase in leaf number production: ΔL; maximum unit leaf area: MLA; relative growth rate: RGR) and biomass partitioning (root weight ratio: RWR; stem weight ratio: SWR; leaf weight ratio: LWR; biomass ratio of root – shoot: R/S) of *Kandelia candel* (Kc) and *Bruguiera gymnorrhiza* (Bg)

Source of variance	ΔH	ΔD	ΔL	MLA	RGR	RWR	SWR	LWR	R/S
Kc									
Salinity (S)	NS	*	***	NS	NS	NS	NS	NS	NS
Wastewater (W)	***	***	***	***	***	NS	NS	NS	NS
S×W	NS	NS	NS	NS	NS	NS	NS	NS	NS
Bg									
Salinity (S)	NS	***	*	**	NS	NS	NS	NS	NS
Wastewater (W)	*	***	***	***	*	***	NS	***	***
S×W	NS	*	NS	NS	NS	NS	NS	NS	NS

Note: NS (not significant), *($p<0.05$), **($0.05<p<0.01$), ***($p<0.005$).

Results and discussion

Growth responses to livestock wastewater

Discharges of livestock wastewater significantly enhanced all growth parameters, including stem height, stem basal diameter, leaf production, maximum unit leaf area and relative growth rate of *Kandelia candel* and *Bruguiera gymnorrhiza* seedlings (Fig. 1, Table 2). For the controls, the two salinity levels had no significant effects on the increases of stem height and relative growth rates of both species. For wastewater treatments, *Bruguiera gymnorrhiza* seedlings had significantly more increases in stem diameter and leaf area than *Kandelia candel* seedlings under both salinity conditions. McKee (1995) also indicated that higher nutrient levels resulted in greater investment in leaf area and leaf production of seedlings of three mangrove species, namely *Rhizophora mangle*, *Avicennia germinans* and *Laguncularia racemosa*.

In general, *Kandelia candel* seedlings had higher RWR (root weight – total biomass ratio), SWR (stem weight – total biomass ratio) and R/S ratio (root – shoot biomass ratio) but lower LWR (leaf weight to total biomass ratio) than *Bruguiera gymnorrhiza* seedlings (Fig. 2). Salinity levels and livestock wastewater treatments did not significantly change biomass partitioning of *Kandelia candel* (Table 2). On the contrary, *Bruguiera gymnorrhiza* seedlings receiving livestock wastewater had a sharp decrease in their RWR and R/S

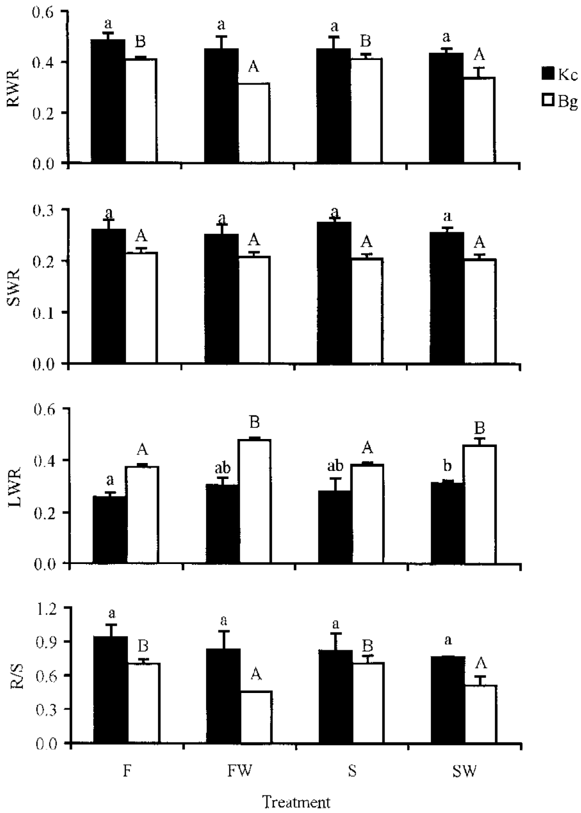

Figure 2. Biomass partitioning in terms of root weight ratio to whole biomass (RWR); stem weight ratio to whole biomass (SWR); leaf weight ratio to whole biomass (LWR); biomass ratio of root – shoot (R/S) of *Kandelia candel* (Kc) and *Bruguiera gymnorrhiza* (Bg) at the end of 144 days treatment (for each species, means with different letters are significantly different at $p<0.05$ level).

Table 3. Results of two-way ANOVA showing the effects of livestock wastewater treatments and salinity conditions on leaf pigments, including chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl), ratio of chlorophyll *a* to chlorophyll *b* (*a*/*b*), total carotenoid (Car), leaf electric conductance (LC), leaf water content (LWC), and root activity (RA) of *Kandelia candel* (Kc) and *Bruguiera gymnorrhiza* (Bg)

Source of variance	Chl *a*	Chl	*b*	Chl*a*/*b*	Car	LC	LC	RA
Kc								
Salinity (S)	NS	NS	NS	***	NS	***	NS	*
Wastewater (W)	NS	NS	NS	***	***	**	NS	NS
S×W	NS	NS	NS	***	*	NS	NS	NS
Bg								
Salinity (S)	NS	NS	NS	*	NS	**	***	*
Wastewater (W)	***	***	***	NS	***	NS	***	NS
S×W	*	*	*	NS	NS	NS	NS	NS

Note: NS (not significant), *($p<0.05$), **($0.05<p<0.01$), ***($p<0.005$).

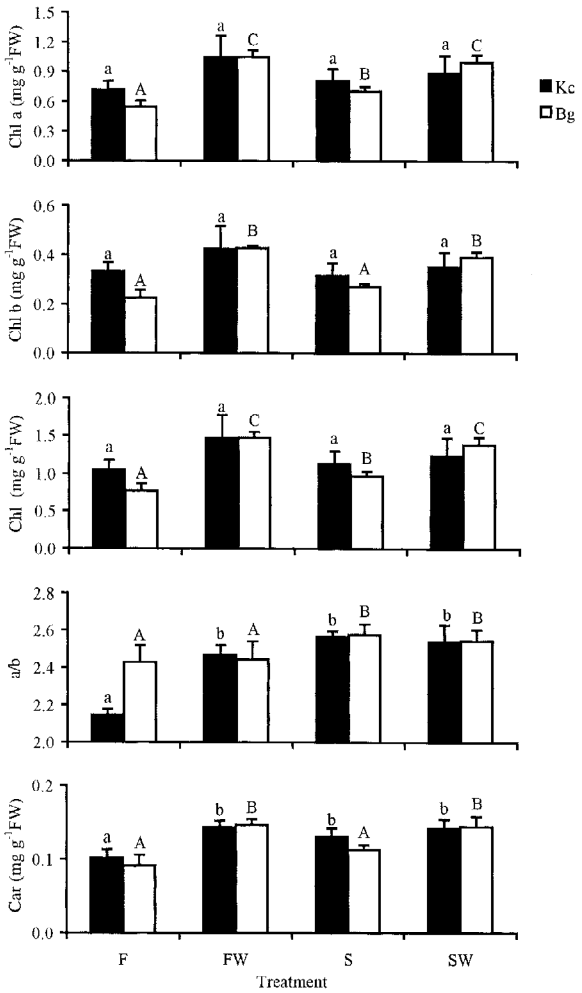

Figure 3. Leaf pigments, including chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl), ratio of chlorophyll *a* to chlorophyll *b* (*a*/*b*), and total carotenoid (Car) of *Kandelia candel* (Kc) and *Bruguiera gymnorrhiza* (Bg) at the end of 144 days treatment (for each species, means with different letters are significantly different at $p<0.05$ level).

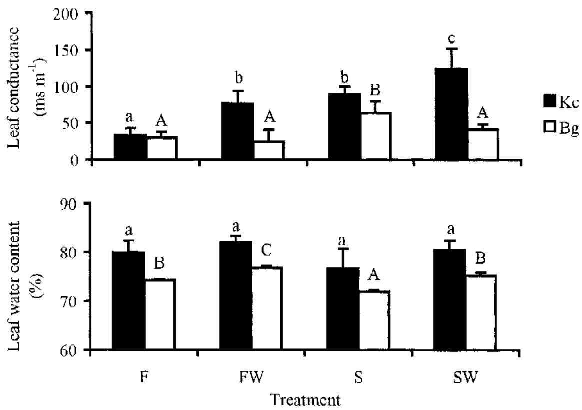

Figure 4. Leaf electric conductance and leaf water content of *Kandelia candel* (Kc) and *Bruguiera gymnorrhiza* (Bg) at the end of 144 days treatment (for each species, means with different letters are significantly different at $p<0.05$ level).

ratio but a significant increase in LWR, suggesting that more biomass was allocated to leaf.

Physiological responses

Contents of Chlorophyll *a*, chlorophyll *b*, and total chlorophyll in leaves of *Kandelia candel* treated with livestock wastewater were not significantly different from those found in the controls (Fig. 3 and Table 3). This is similar to the results of *Aegiceras corniculatum* and *Kandelia candel* receiving synthetic sewage under simulated tidal conditions (Chen et al., 1995; Wong et al., 1997). However, the content of total carotenoids (pigments protect chlorophyll from oxidation) and the ratio of chlorophyll *a* to chlorophyll *b* in leaf of *Kandelia candel* seedlings treated with livestock wastewater increased dramatically under freshwater condition but such increase was not found under seawater condition. Unlike *Kandelia candel*, *Bruguiera gymnorrhiza* treated with wastewater had significantly higher contents of leaf chlorophyll *a*, chlorophyll *b*, total chlorophyll and total carotenoids than the controls under both freshwater and seawater conditions. Significant interactions between salinity levels and wastewater treatments were found in chlorophyll contents of *Bruguiera gymnorrhiza* but not in *Kandelia candel*.

The two species responded differently to livestock wastewater treatments in leaf electric conductance (Fig. 4 and Table 3). The electric conductance of *Kandelia candel* seedlings treated with wastewater was significantly higher than their respective controls. Similarly, Chen et al. (1995) and Wong et al. (1997) also reported that *Kandelia candel* and *Aegiceras corniculatum* seedlings treated with synthetic wastewater had higher electric conductance values. However, there was no significant increase in electric conductance in wastewater treated *Bruguiera gymnorrhiza* under freshwater condition and the value even dropped under seawater condition. These results indicated that *Bruguiera gymnorrhiza* was more tolerant to livestock wastewater than *Kandelia candel*.

Similar to that reported by Chen et al. (1995) on effects of artificial wastewater on water contents of mangrove seedlings, the present study also showed that livestock wastewater did not significantly change leaf

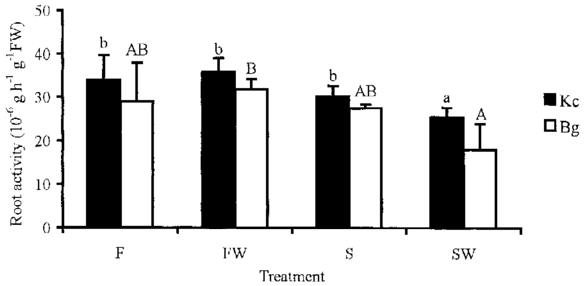

Figure 5. Root activity (μg α-naphthylamine per gram of fresh weight per hour) of *Kandelia candel* (Kc) and *Bruguiera gymnorrhiza* (Bg) at the end of 144 days treatment (for each species, means with different letters are significantly different at $p<0.05$ level).

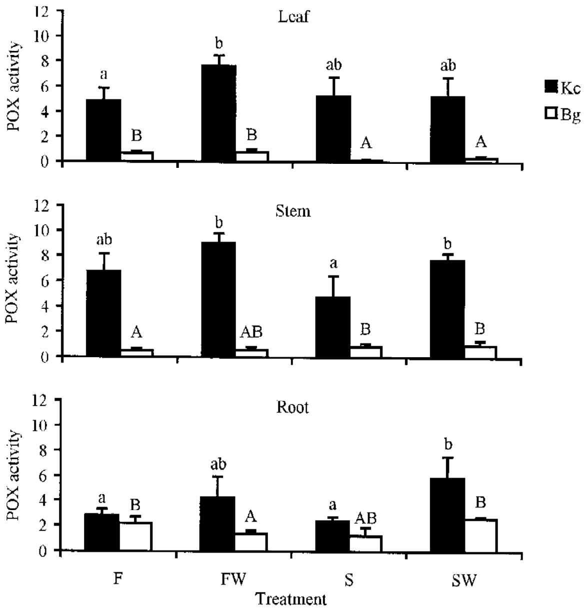

Figure 6. Peroxidase (POX) activity ($\times 10^3$ unit per gram of fresh weight) in leaf, stem and root of *Kandelia candel* (Kc) and *Bruguiera gymnorrhiza* (Bg) at the end of 144 days treatment (for each species, means with different letters are significantly different at $p<0.05$ level).

water contents of *Kandelia candel* (Fig. 4 and Table 3). However, water contents in leaf of *Bruguiera gymnorrhiza* seedlings treated with wastewater increased significantly under both freshwater and seawater conditions. It has been proposed that leaf water contents could reflect a plant's activity, and a higher value indicated the plant possessed a high metabolic rate and a rapid growth (Chen et al., 1995). Therefore, in terms of water content, both species were tolerant to livestock wastewater although *Bruguiera gymnorrhiza* appeared to be more tolerant than *Kandelia candel*.

Table 4. Results of two-way ANOVA showing the effects of livestock wastewater treatments and salinity conditions on activities of oxidation-resistant enzymes, including peroxidase (POX) and superoxide dismutase (SOD) of *Kandelia candel* (Kc) and *Bruguiera gymnorrhiza* (Bg)

Source of variance	POX			SOD		
	Leaf	Stem	Root	Leaf	Stem	Root
Kc						
Salinity (S)						
Wastewater (W)	NS	*	NS	*	*	*
S×W	NS	**	**	NS	*	NS
Bg	NS	NS	NS	NS	NS	NS
Salinity (S)						
Wastewater (W)	***	*	NS	***	NS	*
S×W	NS	NS	NS	***	*	***
	NS	NS	***	NS	NS	*

Note: NS (not significant), *($p<0.05$), **($0.05<p<0.01$), ***($p<0.005$).

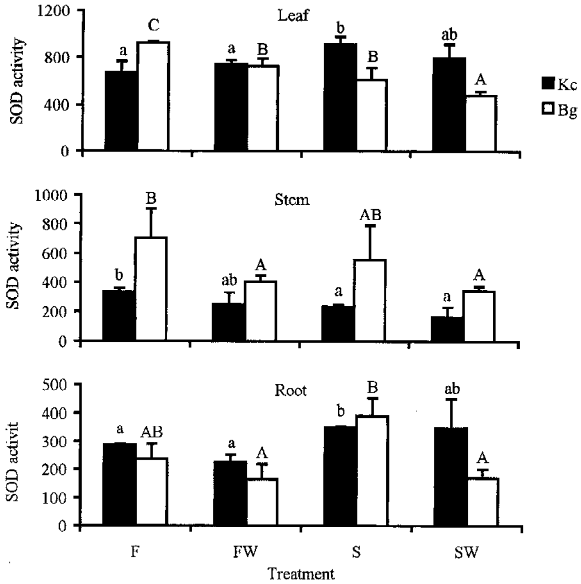

Figure 7. Superoxide dismutase (SOD) activity (unit per gram of fresh weight) in leaf, stem and root of *Kandelia candel* (Kc) and *Bruguiera gymnorrhiza* (Bg) at the end of 144 days treatment (for each species, means with different letters are significantly different at $p<0.05$ level).

The discharge of livestock wastewater did not significantly change the root activity of both *Kandelia candel* and *Bruguiera gymnorrhiza* (Fig. 5 and Table 3), similar to the result obtained by Chen et al. (1995) who examined the responses of *Kandelia candel* to artificial wastewater. This also indicates that livestock wastewater did not damage root systems of *Kandelia* *candel* and *Bruguiera gymnorrhiza*, and both species might be somewhat tolerant to sewage discharge.

Due to wastewater addition, the POX activity in stem and root of *Kandelia candel* increased under seawater condition but the changes in treated leaf were insignificant (Fig. 6 and Table 4). Chen et al. (1995) also observed that leaf POX activity did not significantly change due to artificial wastewater treatments. On the other hand, the leaf and stem POX activity of wastewater treated *Bruguiera gymnorrhiza* did not show any significant change from their respective controls, while the value in root of *Bruguiera gymnorrhiza* significantly decreased under freshwater condition but increased under seawater condition. For almost all organs of *Bruguiera gymnorrhiza*, wastewater discharge caused a significant decrease in their SOD activity but wastewater addition did not alter the SOD activity in *Kandelia candel* (Fig. 7 and Table 4). Both POX and SOD are enzymes responsible for the removal of oxygen radicals harmful to plants. After treated by livestock wastewater, *Bruguiera gymnorrhiza* had a more stable POX activity and a more decrease in SOD activity but better growth than *Kandelia candel*, indicating that *Bruguiera gymnorrhiza* accumulated less oxygen radicals from wastewater. This further suggests that *Bruguiera gymnorrhiza* was more tolerant to livestock wastewater than *Kandelia candel*.

In conclusion, *Bruguiera gymnorrhiza* would be a more suitable mangrove species for sewage treatment than *Kandelia candel* as reflected in its growth and physiological responses to livestock wastewater. Ye et al. (2001) also found that the mangrove system planted with *Bruguiera gymnorrhiza* had a higher N and P removal and was more efficient in treating wastewater than *Kandelia candel* systems.

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