

# Growth Response of Selected Mangrove Species to Domestic Sewage and Abiotic Stress

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**Abstract**—The sewage system of Dar es Salaam City, Tanzania, serves only 15% of the population, making sewage one of the leading sources of marine pollution. This study was initiated to assess the potential of peri-urban mangrove forests as filters and phyto-remediators of sewage and the growth of two mangrove species under sewage-inundated conditions. One-year old *Avicennia marina* and *Rhizophora mucronata* wildlings were planted in 10 l buckets and treated with 0, 25, 50, 75 and 100% domestic sewage in triplicates for 12 h, 24 h or 1 week under a split-split plot design. Growth and physico-chemical characteristics of the system were recorded monthly for four months. Treated mangroves grew better than untreated plants and *A.marina* outperformed *R. mucronata*. Inundation with 75-100% sewage was more effective than <50% sewage but no significant differences were observed due to the relatively brief experimental period. Irrespective of the species, pH and electric conductivity (EC) increased by the end of each inundation. P and N removal by plants was highest in 50 and 75% sewage. Additionally, three sulfide levels of 0, 0.75 x 10<sup>-3</sup>, 1.5 x 10<sup>-3</sup> M NH<sub>2</sub>S affected mangrove growth when applied in seawater concentrations of 0, 50 and 100%.

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## INTRODUCTION

The Tanzanian mainland has 115,500 ha of mangrove forest (Semesi, 1991) along its 800 km coastline. These forests are concentrated at Wami, Rufiji, Pangani, Kilwa and Mtwara. Eight mangrove species occur in Tanzania, including *Avicennia marina*, *Lumnitzera racemosa*, *Xylocarpus granatum*, *Rhizophora mucronata*, *Ceriops tagal*, *Sonneratia alba*, *Heritiera littoralis* and *Barringtonia racemosa*

Peri-urban coastal areas of the developing world receive extensive amounts of untreated sewage, which is typically discharged into creeks lined by mangrove forests. Mangroves filter this discharged wastewater, thereby limiting coastal sewage pollution. Several authors have thus proposed the use of mangrove swamps as natural sewage treatment plants (Corridor and Morell 1994;

Boonsong *et al.*, 2002) to provide low-cost sewage treatment for tropical coastal communities (Corridor and Morell, 1994). This is based on evidence that the mangrove environment is nutrient-deficient, especially of nitrogen and phosphorus (Holguin *et al.*, 2001), and may be tolerant of high pollutant concentrations over long periods (Clough *et al.*, 1983; Wong *et al.*, 1995; Boonsong *et al.*, 2002). These plants may also trap suspended solids and nutrients in untreated sewage (Corridor and Morell, 1994; Wong *et al.*, 1997, Tam *et al.*, 1999) and prevent their entry into the aquatic environment (Kivaisi, 2000; UNEP, 2003). Sewage disposal into mangrove forests is expected to prolong the inundation time of mangroves and impact their growth (Ye *et al.*, 2002), which in turn might affect their efficiency in pollutant removal.

Mangroves typically grow in an environment with a salinity between that of fresh water and the sea, which normally has ~35 g/l salt (including 483 mM Na<sup>+</sup> and 558 mM Cl<sup>-</sup>), equivalent to an osmotic potential of -2.5 MPa (Hogarth, 2007). Mangroves have adopted three main strategies to maintain their water balance in saline situations. Some have physiological mechanisms that exclude salt uptake; exclusion of up to 80-90% of the salt in seawater has been demonstrated in *Bruguiera*, *Rhizophora*, *Sonneratia*, *Ceriops* and *Acrosticum* (Ilka and Sitnik, 2002). Other species such as *Avicennia* may allow substantial amounts of salt to enter the plant through the roots which they then secrete. Finally, some merely tolerate the salt by other physiological mechanisms (Hogarth, 2007).

Although peri-urban mangrove wetlands absorb excess nutrients and sequester other pollutants, preventing their entry to the sea and other water bodies (Boonsong *et al.*, 2002), the mechanisms of pollutant removal, inundation time and sewage loading for optimal growth are not well known. Studies in Puerto Rico and China have shown that mangrove ecosystems can tolerate a considerable sewage load without suffering any damage to their growth (Tam and Wong, 1993). However, no such studies have been undertaken in Western Indian Ocean countries

to assess the effects of sewage loading and inundation time on the removal efficiency of pollutants by the region's mangroves. The present study was thus initiated to provide information for application in constructed mangrove wetlands. The study formed part of a regional project on peri-urban mangrove forests as potential filters and phyto-remediators of sewage in East Africa, known as PUMPSEA. The study compared the effect of sewage loading and inundation time on the efficiency of pollutant removal between two predominant mangrove species in Tanzania, *Avicennia marina* and *Rhizophora mucronata*.

## MATERIALS and METHODS

### Experimental plants

One-year *Rhizophora mucronata* and *Avicennia marina* wildlings with five nodes were collected from Kunduchi mangrove forest and potted in 10 l buckets, 25 cm in diameter and 30 cm in height. Soil from Kunduchi mangrove forest was used as the potting medium. A 15 cm space was left above the potting medium in the buckets to accommodate 3 l of treatment mixture. Seawater used for dilutions was collected in a 1500 l tank from Kunduchi beach and domestic sewage from the University of Dar es Salaam (UDSM) waste stabilization treatment ponds. The seawater tank was placed in the shade of a tree to minimize evaporation. The experiment was conducted at the Botany Department Nursery of UDSM.

### Effects of sewage and inundation time

Different sewage concentration were allocated to 90 experimental pots, of which 18 were controls (seawater only), the balance being treated in a split-split plot design in three replicates. The two mangrove species *R. mucronata* and *A. marina* were allocated to the main plots, inundation regimes were allocated to the sub-plots and sewage concentration to the sub-sub plots. Seawater and sewage were mixed to sewage concentration levels of 0%, 25%, 50%, 75% and 100% by volume (Table 1).

Table 1. Experimental design on the effect of sewage loading and inundation time on *Avicennia marina* and *Rhizophora mucronata* seedlings.

Mangrove species	Inundation time	Sewage concentration			
		25%	50%	75%	100%
<i>Avicennia marina</i>	1 week (1st series)	A1 A2 A3	A4 A5 A6	A7 A8 A9	A10 A11 A12
	24 hours (2nd series)	A13 A14 A15	A16 A17 A18	A19A20 A21	A22 A23 A24
	12 hours (3rd series)	A25 A26 A27	A28 A29 A30	A31A32 A33	A34 A35 A36
<i>Rhizophora mucronata</i>	1 week (1st series)	R1 R2 R3	R4 R5 R6	R7 R8 R9	R10 R11 R12
	24 hours (2nd series)	R13 R14 R15	R16 R17 R18	R19R20 R21	R22 R23 R24
	12 hours (3rd series)	R25 R26 R27	R28 R29 R30	R31R32 R33	R34 R35 R36
Control	1 week (1st series)	C1	C2	C3	C4
	24 hours (2nd series)	C5	C6	C7	C8
	12 hours	C9	C10	C11	C12

The plants and controls were subjected to inundation periods of 12 h, 24 h or 1 week with a resting period of 48 h between successive inundations to allow for aeration. This was intended to mimic the natural inundation regime in the mangrove forest (the normal inundation regime around Kunduchi Bay comprises fortnightly neap and spring tides with semi-diurnal low and high tides every 6 h). The seedling height (cm), number of leaves, number of nodes and total biomass (g) were measured at the start of the experiment and each month thereafter. In situ measurements of pH, electric conductivity (EC) and salinity of the sewage samples were recorded on sewage loading and after each inundation period. A Fisherbrand Hydrus 200 pH-meter was used to measure pH and an YSI Model 85 handheld meter was used to measure conductivity and salinity. In both cases, measurements were taken by dipping the probe about 3-5 cm into the 15 cm sewage layer in the bucket and at the interface of the sewage and sediment.

### Analysis of chemical parameters

Chemical parameters of the sewage were measured colorimetrically at the beginning and the end of each inundation period according to Stewart (1989) and APHA (1998) using a spectrophotometer. Sewage samples were filtered in the laboratory before chemical analysis. When immediate analysis was not possible, samples were preserved by acidifying with sulphuric acid to a pH of less than 2 according to Stewart (1989) and APHA (1998).

Phosphates were determined using the ascorbic acid method (Emteryd, 1989; APHA, 1998); this method was also used to analyse total phosphorous after digestion with persulphate ( $K_2 S_2O_8$ ) (Emteryd, 1989; APHA, 1998).

The ammonium-nitrogen content was determined using the indophenol blue method (Allen, 1989) in which ammonium-nitrogen is oxidized by sodium hypochlorite, then coupled with a phenolic compound (sodium salicylate) and catalyzed by sodium nitroprusside to develop the indophenol-blue colour (APHA, 1998).

### Effects of salinity and sulfide

Mangrove seedlings were exposed to different salt and sulfide concentrations for a week. Sets of six seedlings were exposed respectively to tap water (control), 50% seawater and 100% seawater. A further 18 seedlings were dosed in batches of six in low sulfide concentrations ( $0.75 \times 10^{-3}$  M  $\text{NH}_2\text{S}$ ), again in tap water, 50% seawater and 100% seawater. A third batch of 18 seedlings was similarly dosed with high sulfide concentrations of  $1.5 \times 10^{-3}$  M  $\text{NH}_2\text{S}$ .

The sulfide was dosed by spiking it slowly into the sediment in several places using a pipette and immediately sealing the pot in a plastic bag to prevent evaporation. Monthly growth of the seedlings was measured in terms of plant height (cm), number of nodes, number of leaves and total biomass. Total biomass was determined by destructive harvesting, careful washing the roots followed by drying the plant in an oven at  $85^\circ\text{C}$  until constant dry weight. Physico-chemical parameters (salinity, EC and pH) were measured monthly for the four month duration of the experiment.

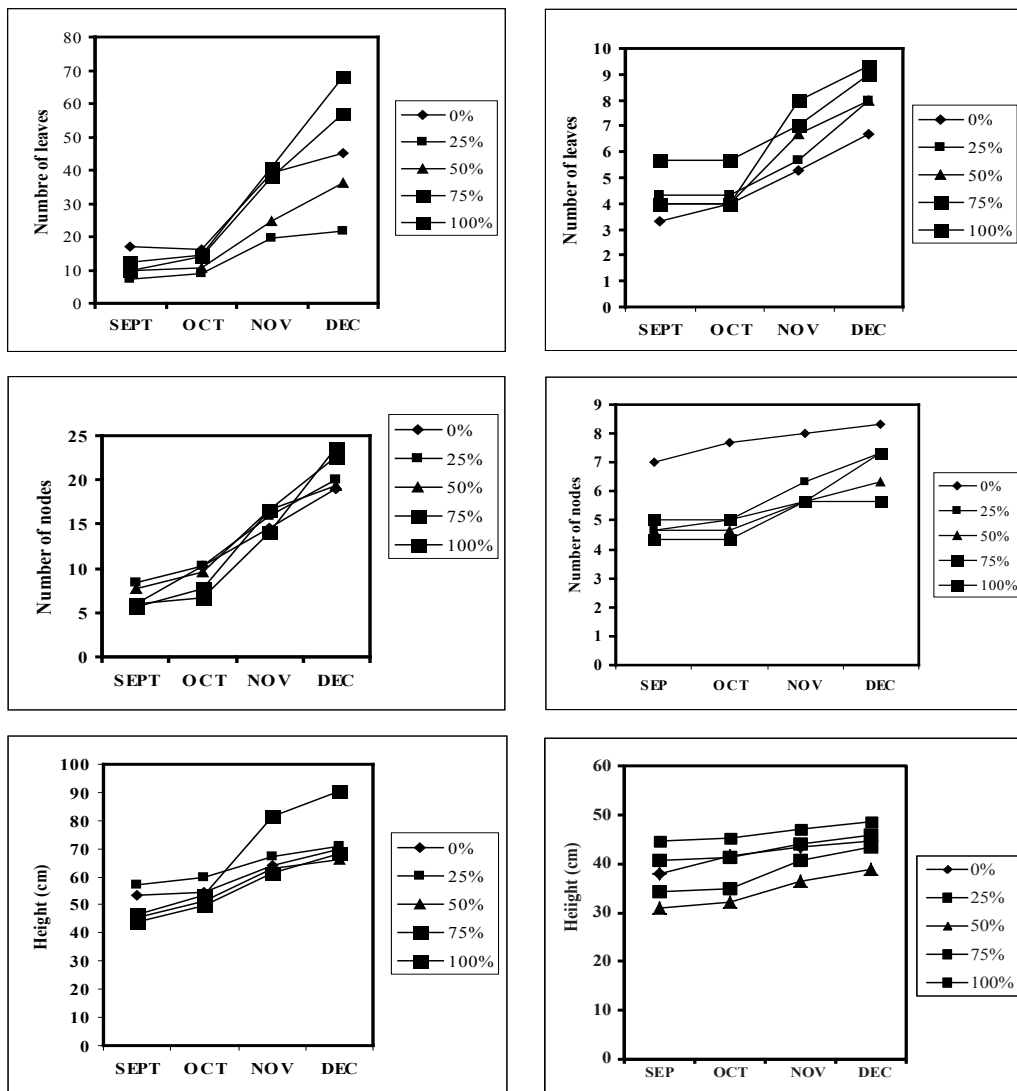


Figure 1. Growth parameters for *Avicennia marina* (left hand side) and *Rhizophora mucronata* (right hand side) seedlings inundated in sewage for 1 week.

## Data analysis

Data were analyzed using GENSTAT (Discovery version 2; 2007), the Tukey-Kramer Multiple Comparison test and Student-Newman-Keuls-Multiple-Comparison test to reveal differences between the treatments. In the interaction tables, A denotes inundation time, B species and C sewage concentration.

## RESULTS and DISCUSSION

### Effect of sewage on *A. marina* and *R. mucronata* seedling growth

Characteristics of the sewage used to inundate the two mangrove species are presented in Table 2. The *A. marina* and *R. mucronata* seedlings responded positively to sewage loading and inundation time (Table 3, Figure 1). Growth increased with increasing sewage concentration, indicating that it was tolerated by the two mangrove species and it enhanced their growth. Plants treated with sewage grew better in terms of plant height, number of nodes and number of leaves. Plants grown in 100% and 75% sewage manifested better performance than those treated with 50% and 25% sewage (Table 3 and Figure 1). The response of *A. marina* was higher than

Table 2. Characteristics of sewage used in mangrove inundation experiments.

Parameter	Value
Conductivity(ms/cm)	1.73
Salinity	1.35
pH	7.81
Ammonia-N (mg/l)	8.02
Total Phosphates (mg/l)	14.90
PO <sub>4</sub> (mg/l)	4.41

that of *R. mucronata*, a difference which was statistically significant, this being evident particularly in terms of plant height ( $p=0.05$ ; Table 3).

The interactions between mangrove species and inundation time (A\*B), inundation time and sewage concentration (A\*C), mangrove species and sewage concentration (B\*C) and mangrove species, sewage concentration and inundation time (A\*B\*C) on growth were not statistically significant in terms of the number of leaves, number of nodes. These findings are, in part, corroborated by those of Wong *et al.* (1995), who found no adverse effect on mangrove growth after a year of sewage discharge onto a mangrove stand.

Table 3. ANOVA of *Avicennia marina* and *Rhizophora mucronata* seedling height inundated for different times at different sewage concentrations.

Source of variation	df	SS	MS	F value
<i>Main plot analysis</i>				
Time (A)	2	1392.26	696.13 ns	0.182
Error (a)	4	1037.32	259.33	
<i>Sub-plot analysis</i>				
Species (B)	1	14317.52	4317.52**	<0.001
Time x Species (A*B)	2	114.19	57.1ns	0.743
Error (b)	6	1096.51	182.75	
<i>Sub-sub plot analysis</i>				
Sewage Concentration (C)	4	3833.02	958.26ns	0.007
Time x Concentration (A*C)	8	3947.21	493.4ns	0.059
Species x Concentration (B*C)	4	305.87	107.98ns	0.864
Time x Species x Concentration	8	863.86	107.98ns	0.885
Error (c)	48	11526.39	240.13	

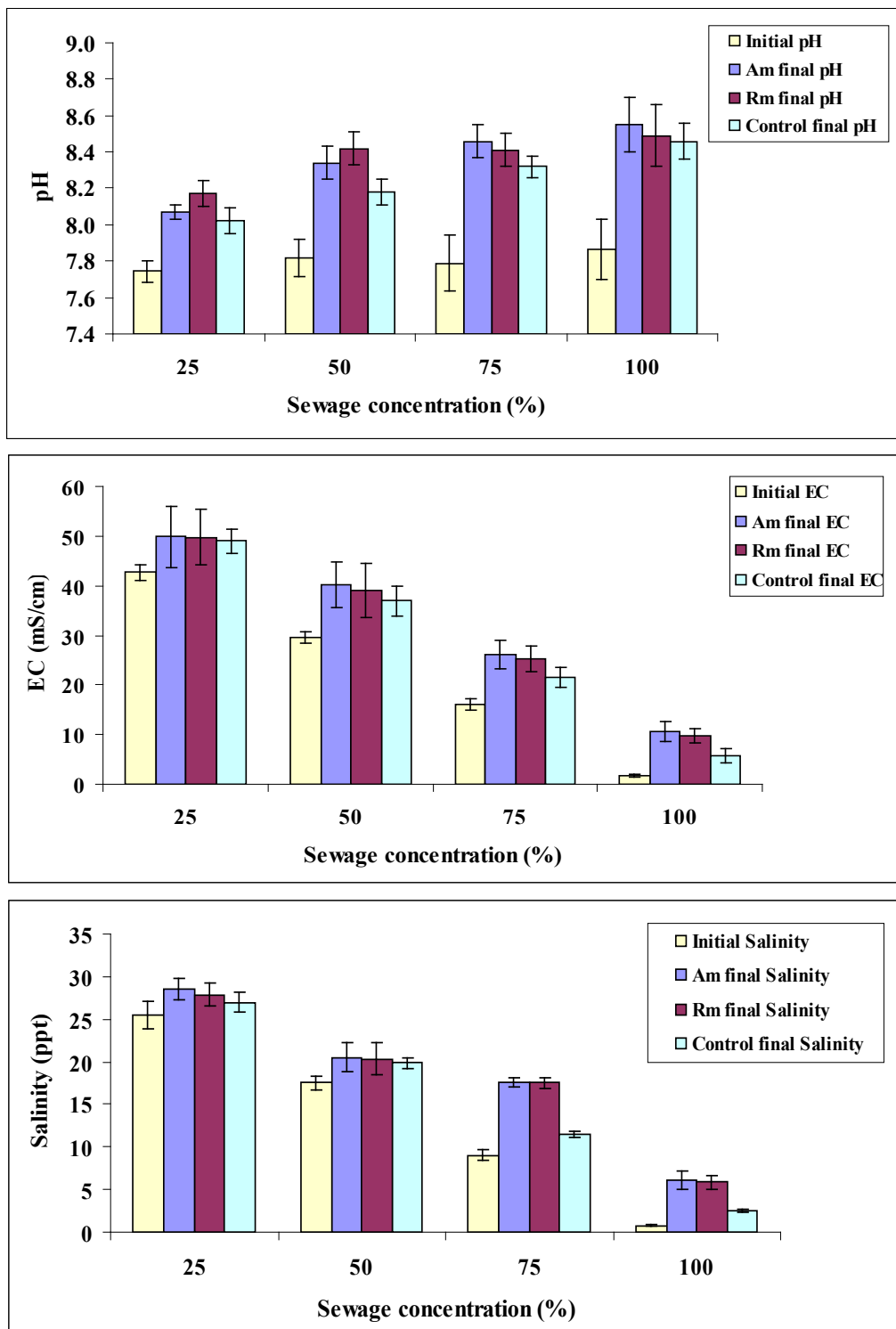


Figure 2. Variation of a) pH, b) EC and c) salinity in *Avicennia marina* and *Rhizophora mucronata* rooting medium inundated with various sewage concentrations for 1 week.

The greater growth of mangrove seedlings in sewage may be attributed to the presence of nutrients (Lester, 1996; Frederk, 2005). Chia (2000) reported that about two-thirds of the solids that are present in domestic sewage are organic, consisting mainly of nitrogenous compounds (proteins and urea) and carbohydrates (sugars, starches). Chingege (2005) found that the two most important nutrients for aquatic plant growth are phosphorus and nitrogen, while Hessen *et al.* (1997a) noted that nitrogen is a key element in marine primary production and the worldwide production of agricultural crops and forests.

### Physico-chemical responses of the system to sewage loading

The pH at the sediment surface increased after each inundation as a result of sewage loaded onto the potted seedlings and bare sediment controls, although this increase was not statistically significant ( $df=2$ ;  $p>0.05$ ; Figure 3a). The increase in pH might have been caused by increased alkalinity due to addition of compounds such as  $\text{CaCO}_3$  and  $\text{MgCO}_3$  in the admixture of sewage and sea water. The addition of these salts would have ensured continuous formation of ionized bicarbonate ( $\text{HCO}_3^-$ ) and carbonate ions ( $\text{CO}_3^{2-}$ ), thereby increasing the pH (Brady & Weil, 1999).

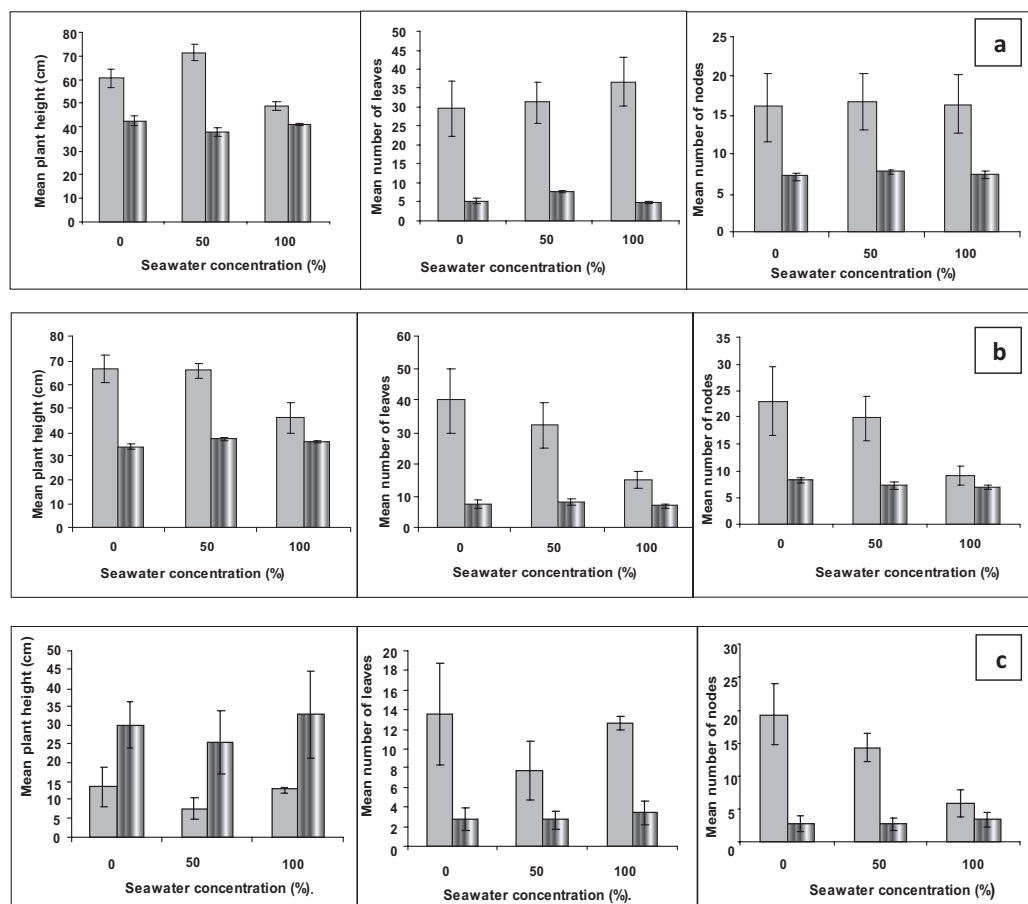


Figure 3. Growth characteristics of *Aecinia marina* (light grey) and *Rhizophora mucronata* (dark grey) seedlings at different a) seawater concentrations b) with sulphide added at  $0.75 \times 10^{-3}\text{M NH}_2\text{S}$  and c)  $1.5 \times 10^{-3}\text{M NH}_2\text{S}$ .

Overall, the EC increased in both planted and bare sediment. However, the level of increase was reduced at increased sewage concentrations and ANOVA using the Tukey-Kramer Multiple Comparison test showed these differences to be significant ( $df = 4$ ;  $p < 0.0001$ ; Figure 3b). This is probably attributable to the high concentration of soluble salts in the sea water used to dilute the sewage.

Salinity varied with EC, i.e. the higher the salinity the higher the EC. Accordingly, it followed the same trend of decreasing at increased sewage concentrations (Figure 3c). Again the Tukey-Kramer Multiple Comparison Test revealed these results to be significant ( $p < 0.0001$ ).

### Effect of reducing conditions on *A. marina* and *R. mucronata* seedling growth

The effects of sulfide on the growth of the two mangrove species are shown in Figures 4a-c, revealing that the application of sulfide was unfavourable. At a concentration of  $0.75 \times 10^{-3} \text{M}$   $\text{NH}_2\text{S}$ , *A. marina* did not show any significant adverse effects ( $p = 0.05$ ), unlike *R. mucronata* (Figure 4b). At  $1.5 \times 10^{-3} \text{M}$   $\text{NH}_2\text{S}$ , about 94.5% of the *A. marina* seedlings died, while all the *R. mucronata* seedlings died during the fourth month after commencing treatment (Figure 4c). Lyimo and Mushi (2005) found that sulphide adversely affected *Avicennia marina* and *Rhizophora mucronata* seedlings at concentrations as low as 0.5-6 mM in greenhouse experiments. They encountered

a range of sulphide concentrations of 0.0025-0.96 mM in seeded and 1.5-24.5 mM in bare sediments at a sewage impacted site at Mtoni (South of Kunduchi); the sulphide levels were 0.01-0.97 mM and 1.09-16.59 mM respectively in seeded and bare sediments in a pristine mangrove forest at Mbweni (North of Kunduchi). They related their findings to high microbial activities in the seeded sediment.

This trend of decreased plant growth at high sulphide levels may be due to severe physiological dysfunctions, including possible disruption of the glyoxalase pathway that results in cessation of the detoxification of reactive oxygen species. Hogarth (2007) attributed reduced growth to difficulties in carbon fixation and metabolic maintenance under such extreme conditions of survival. Detailed studies are needed to evaluate mangrove seedling establishment and growth under reduced conditions in Tanzania.

### Nutrient extraction by mangrove seedlings

Results on nutrient ( $\text{PO}_4^{3-}$ , TP and  $\text{NH}_4^+\text{-N}$ ) removal efficiency by *A. marina* and *R. mucronata* relative to bare sediment controls are presented in Table 4. *A. marina* manifested slightly higher nutrient removal than *R. mucronata*. One of the adaptations of *A. marina* as other halophytes is its ability to reduce its osmotic potential relative to the surroundings, thereby creating a gradient that enables it to draw moisture and nutrients from an environment of higher salinity (Soarez *et al.*, 1998; Irfan and Khan, 2000).

Table 4.  $\text{PO}_4^-$ , TP and  $\text{NH}_4^+\text{-N}$  removal by *Avicennia marina* and *Rhizophora mucronata* at various sewage concentrations and inundation times.

Inundation time	Source (mg/d)	<i>Avicennia marina</i>				<i>Rhizophora mucronata</i>				Control: Bare sediment			
		25%	50%	75%	100%	25%	50%	75%	100%	25%	50%	75%	100%
1 week	$\text{PO}_4^-$	3.0	4.8	6.5	8.9	2.3	4.1	4.8	8.7	1.6	3.3	4.0	4.8
24 hours		4.2	6.8	7.9	12.6	3.6	6.1	7.0	12.1	2.4	4.0	4.1	9.2
12 hours		5.2	8.8	10.7	12.8	4.8	8.2	10.4	12.8	3.3	5.4	6.6	10.8
12 hours	TP	8.5	14.4	20.9	33.7	6.2	11.0	13.8	32.7	5.5	10.2	15.5	20.8
24 hours		10.7	18.0	22.0	35.0	9.8	16.9	20.7	33.2	6.4	11.7	14.6	21.7
1 week	$\text{NH}_4^+\text{-N}$	15.2	22.8	30.2	42.2	13.9	21.3	29.6	42.0	9.5	15.9	19.8	35.1
12 hours		4.7	9.4	11.5	17.8	4.4	8.8	11.1	17.8	2.7	5.0	6.5	11.0
24 hours		7.6	11.8	12.5	20.0	7.2	11.1	11.1	18.8	4.0	6.4	6.9	11.7
1 week		7.8	13.3	17.3	21.0	7.2	12.8	16.7	20.8	3.7	7.1	9.4	17.3



Significant increases in nutrient removal ( $p < 0.0001$ ) were encountered under increased sewage loads of 25% to 100%. The reduced nutrient removal at lower sewage loads may be attributable to the high salinity caused by sewage dilution with seawater. Normally, media with high salinity retard plant growth, which in turn reduces a plant's nutrient uptake causing a low water potential, ion toxicity in the media and a nutrient imbalance in the plants (Khan *et al.*, 2000). In our experiment, removal of phosphates, total phosphorous and ammonium-nitrogen was greatest at the 100% sewage concentration, probably due to the high nutrient availability and low salinity.

As observed by Aksornkoae (1993), greatest growth occurred where plants were exposed to seawater diluted with fresh water, a condition also found in estuaries and deltas. This might account for the higher removal of phosphates, total phosphorous and ammonium-nitrogen in 50% sewage concentrations compared to that in 75% sewage (Table 4). Khan and Aziz (2001) obtained similar results in a study on the salinity tolerance of some mangrove species in Pakistan, in which *A. marina*, *C. tagal* and *R. mucronata* yielded optimal growth in 50% seawater fortified with nitrogen. This might have been caused by nitrogen retention in the sediments, followed by uptake by the plants and microbes for their growth (Reddy & D'Angelo, 1997).

Sewage loading thus did not negatively affect the growth of *Avicennia marina* and *Rhizophora mucronata*. They both grew well, with *Avicennia marina* yielding slightly better growth. Mangrove species inundated for one week were found to remove more nutrients than those inundated for 12 and 24 h. Longer inundation times may provide more opportunity for interactions to occur in the system, an observation also recorded by Stephenson *et al.* (1980) and Crites (1994). Therefore, longer inundation of constructed mangrove wetlands by domestic sewage is expected to improve the removal of nutrients, corroborating their usefulness for wastewater bio-filtration.

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