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Sulfide Concentration and Redox Potential Patterns in Mangrove Forests of Dar es Salaam: Effects on *Avicennia Marina* and *Rhizophora Mucronata* Seedling Establishment

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Abstract—The mangrove species *Avicennia marina* and *Rhizophora mucronata* occur in coastal areas with reducing sediment that contain high sulfide concentrations. However, in this study a glasshouse experiment demonstrated that the establishment of seedlings from these species did not occur in sediment with high sulfide concentration (0.5-6 mM) and low redox potential (-27 to -198 mV). *In situ* measurements, conducted at Mtoni and Mbweni, Dar es Salaam, showed similar ranges. However, sediments with mangrove roots had significantly lower ($P = 0.03$) sulfide concentrations than the adjacent areas without roots. Corresponding redox potential was higher in the sediment with roots than without roots. At Mtoni, the sulfide concentrations ranged between 0.0025 to 0.96 mM and 1.5 to 24.5 mM in sediment with roots and without roots, respectively. At Mbweni, sulfide concentrations between 0.01-0.97 mM and 1.09-16.59 mM were detected in sediment with roots and without roots, respectively. The conclusion of these results is that spatial and temporal variation in the soil redox potential and sulfide concentrations, which are results of microbial activities in the sediment, influence mangrove seedling establishment. These soil factors are modified by the root systems, whereby sediments in areas with plenty of mangrove roots are oxidized and thus conducive for mangrove regeneration.

INTRODUCTION

Mangrove forests occur along coastlines of tropical seas in sheltered mud flats, lagoons and river mouths. Distribution of the different mangrove species depends on factors like the amount of water in the mud, the salinity level (Lind & Morrison, 1974; Semesi, 1998), the amount of shade (Macnae, 1968; Lind & Morrison, 1974), evaporation rate, supply of nutrients, sulfide concentrations (Nickerson & Thibodeau, 1985), tides, substrate composition (Semesi, 1998), pH, water temperature, waves, rainfall, topography and soil redox potential (Boto & Wellington, 1984). Low redox potential (Eh) and high sulfide concentrations are characteristic of waterlogged sediment whereby decomposition processes become anaerobic due to low penetration of oxygen into the sediment. In marine sediments, more than 50% of the organic matter may be oxidised by sulfate reducing bacteria (SRB) (Jørgensen, 1982; Holguin *et al.*, 2001). SRB reduce sulfate to sulfide, which is usually in the form of hydrogen sulfide (H_2S) or, if iron is available, precipitated as black ferrous sulfide. Extensive production of H_2S results in a reduced (low Eh) and sulfidic sediment that is hostile to aerobic organisms, including mangrove seedlings. The concentration of H_2S known to cause a 50% inhibition of cytochrome oxidase in plant roots is

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13 µM (Allam & Hollis, 1972). Seawater provides an ample supply of sulfate for reduction, producing sulfide concentrations that may exceed 10 mM in sediment (McKee, 1993; Lyimo *et al.*, 2002a). However, most of the H_2S eventually escapes either as gas from the sediment, is precipitated with iron or oxidized back to sulfate. Biological sulfide oxidation is mainly by sulfide-oxidizing-bacteria which require O_2 as an oxidant. A few sulfide-oxidizing bacteria, such as *Thiobacillus denitrificans* are capable of using nitrate as an oxidant under anoxic conditions. Because oxygen penetration is low, major oxidation process takes place at the very surface but may also occur around the roots of the mangrove trees, which extend into the sediment over a vast area, and can transport oxygen (Lacerda *et al.*, 1993; Holguin *et al.*, 2001). Previous studies on mangrove forestry in the Western Indian Ocean have revealed extensive cutting of mangrove trees due to increased human population and economic pressures (Dahdouh-Guebas *et al.*, 2000; Kairo *et al.*, 2002). In many forests, vast cleared areas remain without trees, taking a long time to re-establish the forest. McCusker (1971) noted that regeneration in undisturbed communities is higher than in disturbed ones simply because the presence of trees prevent seedlings from being washed away with the tide. Various efforts have been made to conserve mangrove ecosystem but also to re-establish degraded forests (Toledo *et al.*, 2001; Wagner *et al.*, 2001; Kairo *et al.*, 2002). However, Wagner *et al.* (2001) reported that a number of mangrove areas along the Tanzanian coast were degraded to such an extent that they were unlikely to recover naturally. Lyimo *et al.* (2002a) reported that certain open areas, cleared by cutting of trees were devoid of any tree regeneration, did not host fauna such as crabs and had large amounts of undigested litter. Furthermore, these areas were highly sulfidic and reduced. The working hypothesis for the present study was that cutting of trees significantly reduces oxygen input in the mangrove sediment which results in high SRB activities leading to sulfidic conditions in the sediments. Thus, the reduced sediments are not conducive for mangrove seedling establishment. Our specific objectives were to assess the influence of Eh and sulfide concentration on the establishment of *Avicennia marina* (Forsk.) Vierh. and *Rhizophora mucronata* Lamk. seedlings and to measure *in situ* levels of sulfide and Eh during rainy and dry seasons along transects in sediment with and without roots.

MATERIALS AND METHODS

Study sites

Sampling and field measurements were conducted at two sites: Mtoni and Mbweni mangrove forests both situated along the coastal region of Dar es Salaam, Tanzania (Fig. 1). At Mtoni, sampling was done at Mzinga creek, approximately 39° 41' E and, 6° 45' S, 10 km south of Dar es Salaam city center. The mangroves *Sonneratia alba*, *A. marina*, *R. mucronata* and *Ceriops tagal* are the common species at this site. Mbweni mangrove forest is located at 39° 25' E and 6° 34' S, about 30 km to the north of Dar es Salaam city center. The dominant species at Mbweni forest are *R. mucronata*, *C. tagal* and *A. marina*. Freshly cut mangrove tree stumps were seen, indicating that the trees were frequently cut, probably for fuel wood and building poles etc. (Wagner *et al.*, 2001; Semesi, 1998). Many parts of the forest had been clear-cut in previous years and the cleared areas have remained open for long time because of lack of natural regeneration. By visual observation, most sediment without aerial roots was characterized by the lack of fauna, the presence of tree stumps, decayed leaves and branches and blacker sediments.

Sampling and *in situ* measurements of physical-chemical parameters

Sampling, including *in situ* measurements of physical-chemical parameters, was conducted at both sites, along one transect running perpendicular to the shoreline towards the center of the forest. In each transect three sampling stations were set. The first station was positioned at the "edge" (close to the land), the second in between the land and forest center, referred here as "intermediate", and the third at the "center" of the forest. The edge and center stations were approximately 100 m apart.

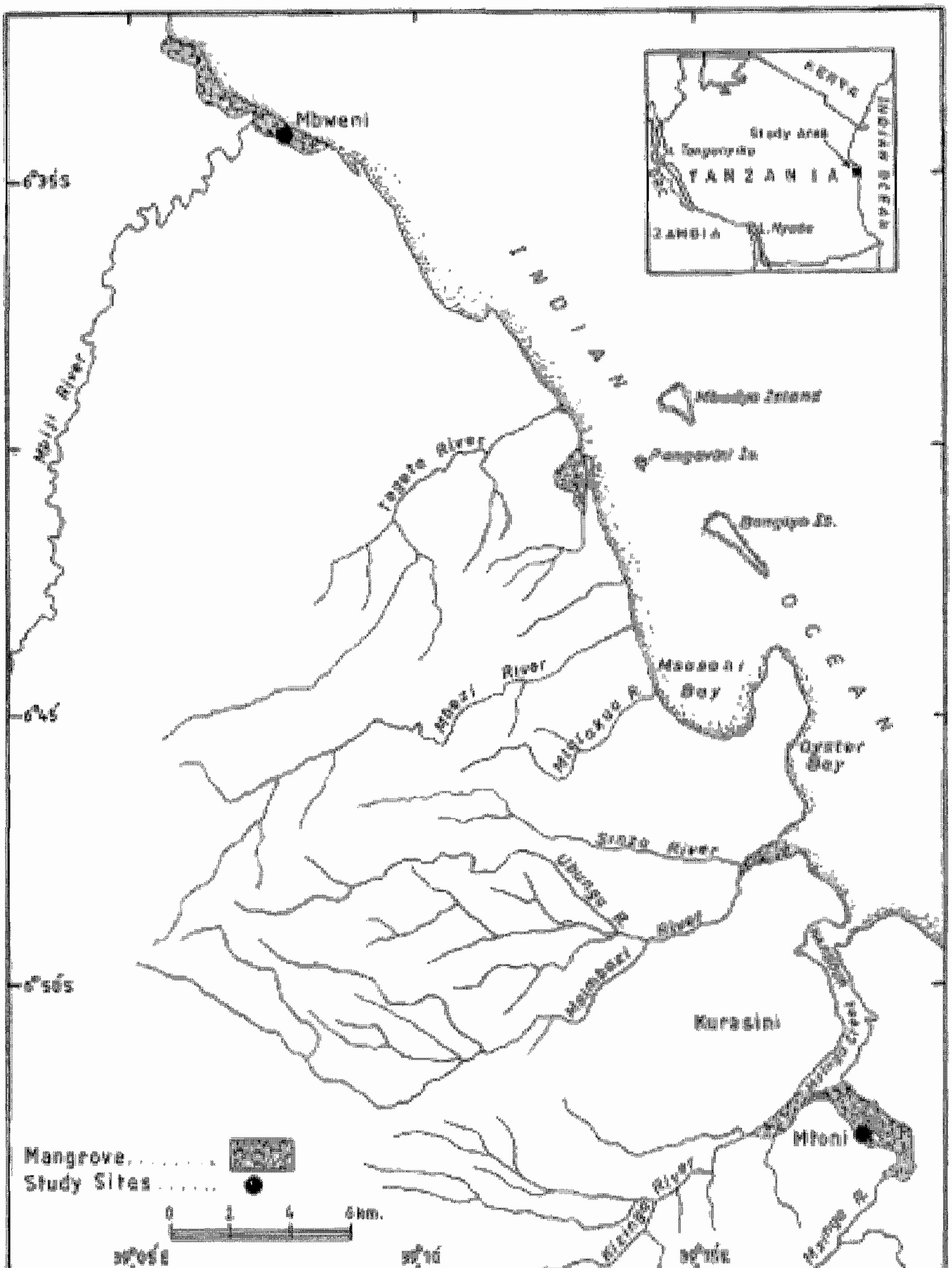

Fig. 1. Map of Dar es Salaam coastal area showing study sites

At each station (with mangrove trees and roots) there were open spaces (without trees and roots). In both areas with and without roots, triplicate samples and measurements were taken twice during low tide in rainy (April 2003) and dry (August 2003) seasons, resulting in four data sets for each station.

The percentage of pore water and organic matter contents were determined from fresh weights (fw) of sediment samples that were placed in porcelain crucibles and dried at 80°C to a constant weight. The percentage loss in weight was used to determine the percentage of pore water. Subsequently, each sample was baked at 550°C for 4 hours and thereafter cooled in desiccators and weighed. The loss in dry weight was used to calculate the percentage of organic matter per dry weight of sediment.

Temperature, pH and salinity were measured from tidal pool water using a methanol thermometer, portable pH meter (Sentron model 1001, The Netherlands) and an ACADO hand refractometer (Salt Refractometer 300011 SPER SCIENTIFIC, made in China), respectively.

Sediment samples for grain size determination were taken in duplicate to a depth of 10 cm using a corer (plastic cylinder, 5.5 cm inside diameter and 45 cm length). The samples were then transported to the laboratory and oven dried at 60°C for 72 hours and sieved to obtain the following fractions: >2, >1, >0.5 >0.25, >0.125, >0.063 and <0.063 mm. Each fraction was weighed separately and calculated as percentage of total sample weight. The proportion silt/clay (<0.063 mm), sand (0.063-2 mm) and coarse sand (>2 mm) was calculated for each stations (see Eklöf *et al.*, 2005 and references therein).

Numbers of culturable sulfate reducing bacteria

The number of culturable sulfate reducing bacteria (SRB) from 0-5 cm depths sediment samples was determined using the Most Probable Number (MPN) technique. The substrate and incubation conditions were carried out as shown by Lyimo *et al.* (2002b). Analysis of H_2S, used as a surrogate measure of SRB growth, was done using Gas Chromatograph (Agilent 6890) equipped with flame photometric detector and a silica plot wide-bore capillary column (CP 8569) packed with Carbopack BHT100. The nitrogen, hydrogen and air flow rates were 55, 50 and 60 ml/min, respectively. The oven, injection port and detection port temperatures were set at 70°C, 150°C and 225°C, respectively.

Measurement of sulfide concentrations and redox potential

Dissolved sulfide ($H_2S + HS^- + S^{2-}$) concentration and redox potential (Eh) profiles were determined using micro-electrodes encased in stainless steel needles of 1 mm tip, 60 cm length (Microscale measurements, the Netherlands). Calibrations of the electrodes were done according to the manufacturers protocol. Field measurements were obtained by gently lowering the needle electrode into the sediment (as described by Vischer *et al.*, 1991) to a depth of 20 cm at the three stations along the mangrove transects, in sediment with and without roots.

Glasshouse experiment

In January 2003, mature seedlings (20 each) of *A. marina* and *R. mucronata* were collected from Mtoni mangrove forest and transported to the glasshouse (Botany Department, University of Dar es Salaam) where they were grown in 20 l pots. During a preliminary experiment, sandy sediment in 2 l pots was treated by addition of different concentrations of sulphide, however, following rapid disappearance of the sulfide due to oxidation and evaporation, silt/clay and sandy sediments from Mtoni site were used instead. From this site, material with high and low sulfide concentrations was collected. Ten pots were prepared with sandy (low concentration of sulphide ranging from below detection limit to 1.5 mM) and ten pots with silt/clay sediments (high concentration of sulfide ranging from 0.5 - 6 mM) in which to grow the seedlings. Thus, five pots from silt/clay and five pots from sandy sediment were used for *A. marina* and the same for *R. mucronata*. The sediment in the pots was kept wet with diluted seawater (1:4 seawater: tap water) with added inorganic nutrients contained (mg/l): $Ca(NO_3)_2$ (354), KNO_3 (101),

$MgSO_4$ (185), KCl (146), NH_4NO_3 (80), Fe-EDTA (35), H_3BO_3 (2.9), $MnCl_2$ (1.8), $CuSO_4$ (0.2), $ZnSO_4$ (0.2), $(NH4)_6Mo_7O_{24}$ (0.04) and NaH_2PO_4 (50) (see Bi *et al.*, 2003). Sulfide concentrations and redox potentials were measured to a depth of 15 cm at two-week intervals as described above. In addition, the height of each seedling was measured each week for growth rate determination.

Data analysis

Obtained data was compared using either unpaired t-test or Mann-Whitney U-test and various correlations by Spearman rank correlations as described in Zar (1999) using Graph Pad Instant tm 1990-1993 software. Probabilities (P) less than 0.05 ($P<0.05$) were considered significant.

RESULTS

Physical-chemical characteristics of the sediment

The different physical-chemical parameters, mangrove species, distribution and height and most probable number (MPN) of sulfate reducing bacteria (SRB) in the sediment at different sites and stations are shown in Table 1. The data presented in Table 1 were mean of the values from area with and without roots as there were insignificant differences. Sediment temperatures and salinity were in ranges of 28.2 - 31.8°C, and 32 - 38‰, respectively, with insignificant differences between sites and seasons. However, lower salinities were recorded during the rain season (April 2003) compared to the dry season (August 2003) and may be attributed to freshwater intrusion. Higher salinity and temperatures were observed at the edge of forest compared to the center.

Mtoni sediments had high organic matter content ranging from 14.7 - 29.3% with a mean of 26.2 ± 3.9 as compared to Mbweni sediments, which contained 15.2 - 25.3% organic matter with an average of 21.3 ± 3.6% (n = 24). Generally, higher organic matter content was found at the edge of the forest and it decreased towards the center of the forest. Although insignificant, the organic matter content was higher during the rainy season compared to the dry season. This was expected

Table 1. Various physical-chemical characteristics (determined at 0 to 5 cm depth of sediment) at the sampling stations (n = 24 except for MPN of SRB where n = 6; mean ± S.D.). The values are average in sediments with or without roots

Parameter	Mtoni			Mbweni		
	Edge	Intermediate	Center	Edge	Intermediate	Center
Mangrove species	*Avicennia, Rhizophora and Ceriops*	*Sonneratia and Rhizophora*	*Sonneratia*	*Avicennia Rhizophora and Ceriops*	*Rhizophora, Ceriops and Avicennia*	*Rhizophora and Ceriops*
Canopy height (m)	1-3	5-8	10-15	8-7	5-7	4-7
Soil type:						
Silt/clay	13.21 ± 0.58	23.07 ± 1.1	29.8 ± 1.24	3.91 ± 1.59	0.39 ± 0.13	0.41 ± 0.19
Sand	86.44 ± 12.9	71.54 ± 1.9	69.7 ± 11.87	95.87 ± 5.0	99.37 ± 11.01	99.25 ± 13.7
Course sand	0.35 ± 0.44	5.43 ± 0.5	0.32 ± 0.17	0.22 ± 0.25	0.24 ± 0.56	0.35 ± 0.042
Temperature (°C)	32.6 ± 0.2	30.4 ± 0.1	29.5 ± 0.3	33.2 ± 0.5	29.8 ± 0.3	29.1 ± 0.4
Salinity (‰)	38 ± 1.5	34 ± 1.0	31 ± 1.0	36 ± 1.0	33 ± 2.0	33 ± 0.6
Surface pH	7.0 ± 0.01	7.23 ± 0.2	7.64 ± 0.3	6.92 ± 0.2	7.09 ± 0.02	7.49 ± 0.6
Organic matter (%)	28.6 ± 0.6	25.4 ± 2.5	15.9 ± 0.9	24.2 ± 1.5	22.5 ± 0.7	17.1 ± 2.6
Pore water (%)	18.4 ± 1.2	30.0 ± 1.1	35.5 ± 2.4	17.8 ± 0.5	20.0 ± 0.3	31.9 ± 0.1
MPN of SRB (cells/g fw)	3.5×10^6	1.5×10^7	9.3×10^5	2.3×10^7	2.4×10^5	2.3×10^4

since organic matter from terrestrial environments is trapped by standing vegetation at the forest edge, and does not easily reach the forest center. However, the majority of the organic matter would probably be from the mangroves themselves. The percentage of pore water and the pH ranged from 16 - 36.9% and from 6.8 - 7.9%, respectively. Higher pore water content was recorded at the center compared to the edge of the forest. Typically, the sediments at Mtoni contained more pore water (29 ± 7.7%) than sediments at Mbweni (23.1 ± 7.3%; n = 24).

All stations were dominated by sand (0.06 - 2 mm), with varying proportions of coarse sand and silt/clay (Table 1). The highest percentages of sand were found in Mbweni site (ranging from 95.9 - 99.4%). The highest silt/clay soil occurred at the center station in Mtoni (30.0%) while the lowest (0.04%) was recorded at the center station at Mbweni.

In situ sulfide concentrations, redox potentials and number of sulfate reducing bacteria

The concentration of sulfide (Fig. 2) and the redox potential (Eh) (Fig. 3) in mangrove sediment varied between sites and with seasons. Comparatively, Mtoni had significantly higher (*P* = 0.03, *U*-test) concentrations of sulfide (maximum value of 24.5 mM during wet season) and a more reduced sediment (minimum Eh of -184 mV) compared to Mbweni where the sediment contained a maximum concentration of sulfide (16.6 mM) and lower Eh (-178 mV). These results reflect the values obtained for MPN of SRB (see above), which were slightly higher at Mtoni (7.8 ± 1.3 x 10^6 cells/g fw) compared to Mbweni (6.5 ± 3.4 x 10^6 cells/g fw). Sulfide concentrations at the edge of the forest were significantly higher (P = 0.03, *U*-test) than at the

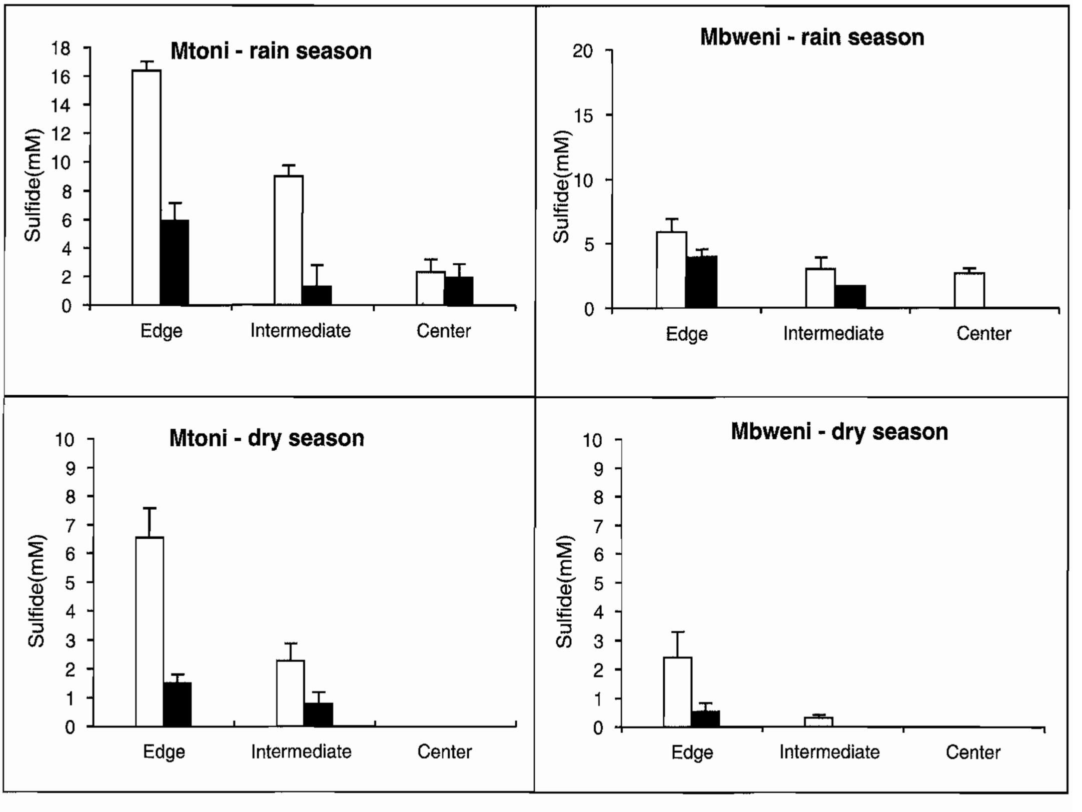

Fig. 2. Sulfide concentrations in sediment with roots (black column) and without roots (clear column) measured during rainy and dry season. Values are mean (n = 21) and error bars are standard deviation

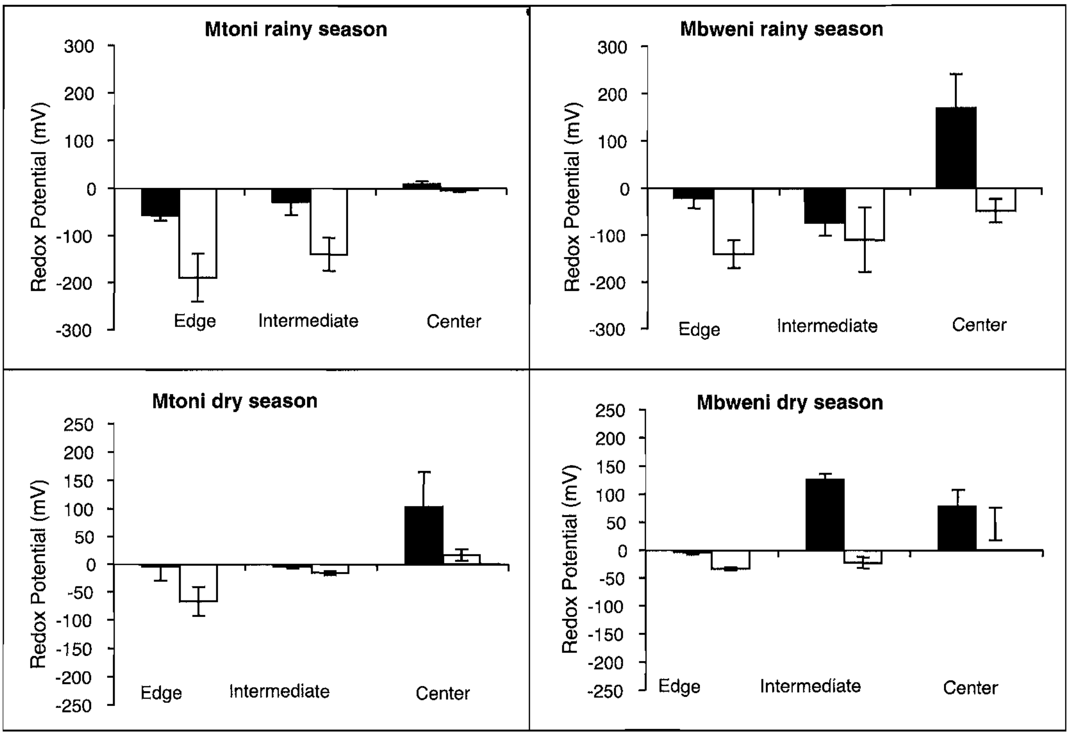

Fig. 3. Redox potentials in sediment with roots (black column) and without roots (clear column) measured during rainy and dry season. Values are mean (n = 21) and the error bars are standard deviation

center of the forest and were highly reduced. In addition, the sulfide concentrations during the rainy season were significantly higher (*P* = 0.04, t-test) than during the dry season. On average, the concentrations were twice as high during the rainy season (Fig. 2). This may be due to introduced dissolved organic matter as a result of runoff. When comparing sediment with roots and sediment without roots, the concentrations of sulfide were lower in sediment with roots than sediment without roots at all stations. Corresponding Eh was higher in the sediment with roots than without roots. The sulfide concentrations at Mtoni ranged from 2.5 - 960.0 μM in sediment with roots while in the sediment without roots the concentrations ranged from 1.5 - 24.5 mM. At Mbweni, sulfide concentrations ranged from 0.01 - 0.97 *mM in* sediment with roots while in the sediment without roots the concentrations ranged from 1.1 - 16.6 mM. It was clear that the sediment without roots (mainly due to mangrove cutting) had significantly higher sulfide concentrations (*P* = 0.03, t-test) compared to the sediment in rooted areas.

There was a positive correlation between sulfide concentrations and organic matter content of the sediment (r = 0.78, *P* = 0.04). Likewise, the most probable number of sulfate reducing bacteria that ranged from 2.4 x 10^4 to 2.3 x 10^7 cells/g.fw of sediment was positively correlated to organic matter content (r = 0.8, P = 0.02).

Influence of redox potential and sulfide concentration on mangrove seedling establishment

Glasshouse experiments showed that *A. marina* and *R. mucronata* seedlings in pots grew under conditions of low sulfide concentrations (below 1.5 μM), high Eh (-23 to 143 mV) and pH 7.8 ± 0.2 at the sediment surface (1 to 5 cm depth). Under these conditions, *A. marina* seedlings had significantly (*P* = 0.009, t-test) higher growth rate (1.1 ± 0.1 cm/day) compared to *R. mucronata* (0.4 ± 0.2 cm/day) (Fig. 4). No growth of seedlings was observed in pots with high sulfide concentrations (0.5-6 mM), low Eh (-27 to -198 mV) and average pH of

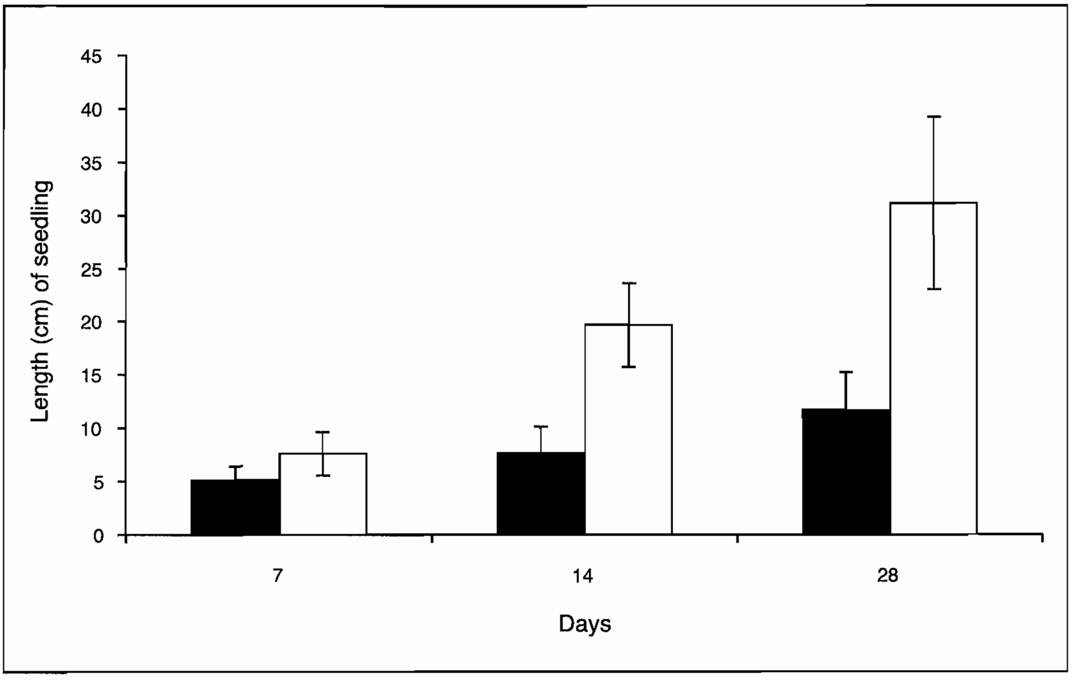

Fig. 4. Growth measurements for seedlings grown in low sulfide pots. (Black column for *Rhizophora mucronata* and clear column for *Avicennia marina*). Values are means of 10 (n = 10) independent seedlings. Error bars show the standard deviation

7.4 ± 0.4 at the surface (1 to 5 cm depth). Figure 5 shows the average sulfide concentration and the Eh at different depths measured for 30 days of experiment. The surface sediment (1 to 5 cm depth) of each pot had a significantly lower sulfide concentration ($P = 0.001$) than the deeper sediment (5 to 15 cm). Sulfide concentration gradually decreased with time (>50% after 30 days) probably due to reoxidation or release of H_2S to the atmosphere. At the start of the experiment the sulfide concentrations and Eh levels were comparable with the levels measured *in situ* (see also Fig. 2 and Fig. 3).

DISCUSSION

Results of the physical-chemical parameters were within the expected ranges and corresponded with findings of other studies (e.g. Matthijs *et al.*, 1999; Shunula & Whittick, 2001; Kairo *et al.*, 2002; Bosire *et al.*, 2003; Marchand *et al.*, 2004). Slight differences between sites may be due to differences in local geographic factors, natural and anthropogenic impacts.

The general trend of decreasing sulfide concentrations towards the center of forests in both sediments with aerial roots and without roots may reflect the interaction between several abiotic and biotic factors. In the centre of the forest more trees were present, which may have influenced the oxidation of the sediment through rhizosphore aeration as discussed by Boto & Wellington, (1984), Thibodeau & Nickerson (1986), Marchand *et al.* (2003 and 2004). In addition, higher organic matter contents were observed at the forest edges, probably due to increased input of dissolved organic material from terrestrial environment. This may have resulted in increased dissolved organic matter and in turn higher sulfate reduction and sulfide concentrations.

The observed low sulfide concentration and high redox potential in sediment with aerial roots corresponded with previous results (Lyimo *et al.*, 2002a) and is an indication that mangrove roots serve as conduits for oxygen to the sediment. In addition, the sediment with roots contained more fauna as indicated by the presence of several (personal observation) crab holes most probably

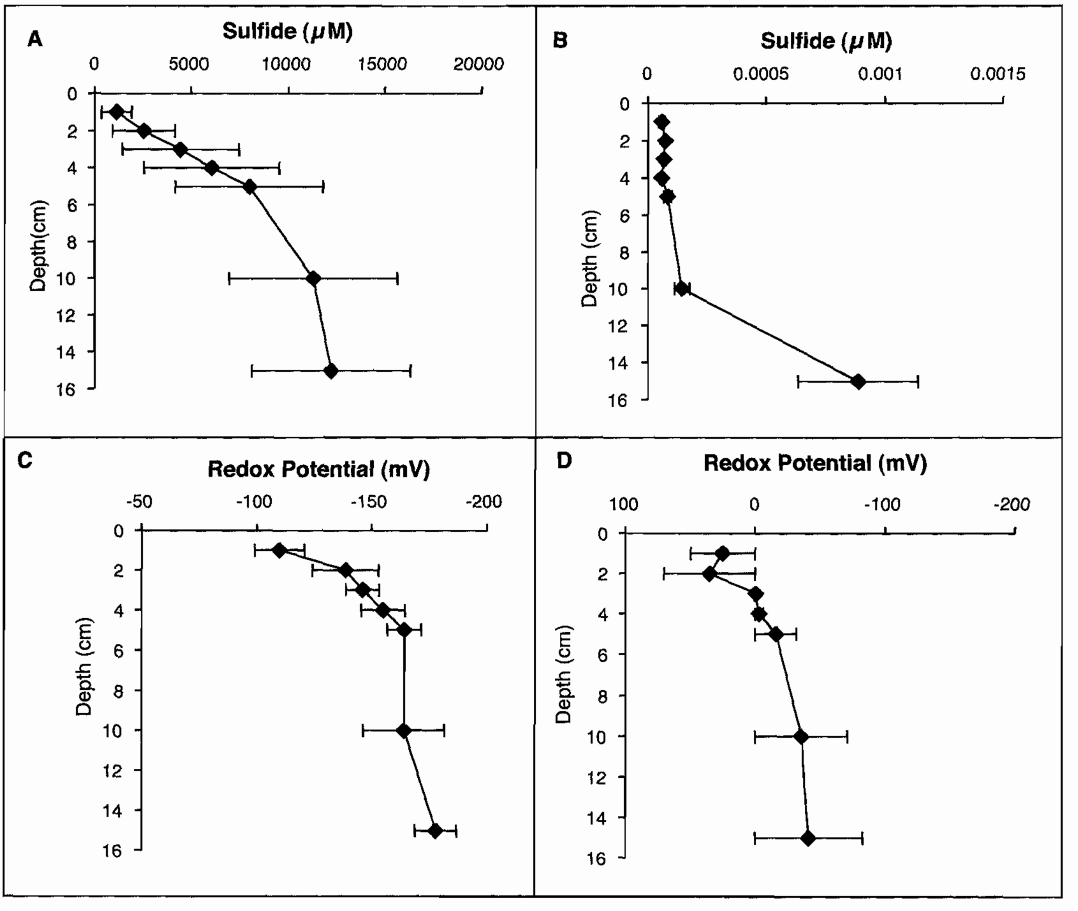

Fig. 5. Sulfide concentration and redox potential in pots containing high (A and C) and low (B and D) concentration of sulfide. Values are the mean of 30 (n = 30) independent profiles. Error bars show the standard deviation

due to favourable conditions created by roots. The presence of these crabs contributes further to the aeration of the sediment (see Holguin *et al.*, 2001). A number of seedlings were growing in between the roots of adult trees whereas in the adjacent sediment without roots there were no seedlings. This observation could be explained by the presence of sulfide and low Eh, which inhibit seedlings propagating in deforested areas. Except for sulfide concentration and Eh, other factors such as the retention of seeds by roots (McCusker, 1971; Clarke, 2004) and the shade provided by trees could be important for the establishment of the seedlings. For example, at a large clear-cut area at Mbweni, several attempts that have been made to replant the mangroves proved to be unsuccessful. According to the villagers it was due to the lack of shade. The observed higher salinities and temperatures at the edge of the forest compared to the center may be explained by heavy mangrove cutting at the easily accessible edge resulting in less shaded sediments with increased evaporation.

Although *A. marina* and *R. mucronata* occur in reduced sediments with high sulfide concentrations (Thibodeau & Nickerson, 1986; Matthijs *et al.*, 1999; Lyimo *et al.*, 2002a) seedlings from these species did not grow in laboratory experiments using sediments with high sulfide concentration and low redox potential. Possibly the seedlings were damaged by accumulated sulfides, a feature previously described by Allam and Hollis (1972). In addition, unsuccessful growth of these seedlings may be due to lack of oxygen, an important factor for seedling growth. These results showed that highly reduced and sulfidic sediments were not favourable for the establishment of mangrove seedlings. On the other hand, the *ex situ* experiment may explain the observed lack of seedling development in areas without mangrove roots in sediment that was highly reduced with high sulfide concentration. In addition, some studies have shown that several mangrove rhizosphore bacteria can significantly enhance plant growth and it may be possible to use these (as plant-growth-promoting bacteria) to speed up the development of mangrove seedlings for restoration of damaged areas (see Holguin *et al.*, 2001 and reference therein).

CONCLUSIONS

The effective management of mangroves requires thorough knowledge of how they function and interact with the surrounding environment. At present, the few strategies that exist are limited by our knowledge on this ecosystem. Insight into the associated microbial ecology will contribute to the understanding of the functioning of mangroves ecosystems. *In situ* and *ex situ* results obtained in this study clearly show that mangrove sediments that contain high concentrations of sulfide and chemically reduced, are not conducive for the establishment of seedlings. The presence of mangrove trees allows aeration of the sediment via the roots and thus reduces sulfide concentrations while increasing the redox potential through the input of oxygen, thus making conditions more favourable for the regeneration of these forests. Other factors, such as the removal of shade, evaporation and increased salinity may also have an impact on mangrove regeneration. The study provides yet more evidence that clear-cutting of mangrove forests not only endangers the forests but prolongs the time needed to re-establish naturally or be re-vegetated artificially.

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