Biogeochemistry **35:** 367–375, 1996.

Effects of mangrove soil ageing on the accumulation of hydrogen sulphide in roots of *Avicennia* spp

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Received 17 August 1995; accepted 23 May 1996

Key words: accumulation, hydrogen sulphide, mangroves, root, soil

Abstract. The possibility for accumulation of hydrogen sulphide gas in roots of mangroves (*Avicennia* spp) which had colonized reclaimed coastal areas in Singapore was investigated using a simple potentiometric technique preceded by an extraction step. The study showed that detectable amounts of hydrogen sulphide gas were present in the underground cable roots of the mangroves and that the concentrations increased with the age of the plant. Furthermore concentrations of hydrogen sulphide in the roots were 30–40 times higher than the concentrations of the gas which were simultaneously generated by anaerobic processes in the surrounding, ageing mangrove sediment. The reasons for these patterns and their possible impact on the succession of *Avicennia* spp by a different type of mangrove, *Rhizophora* spp, are discussed.

Introduction

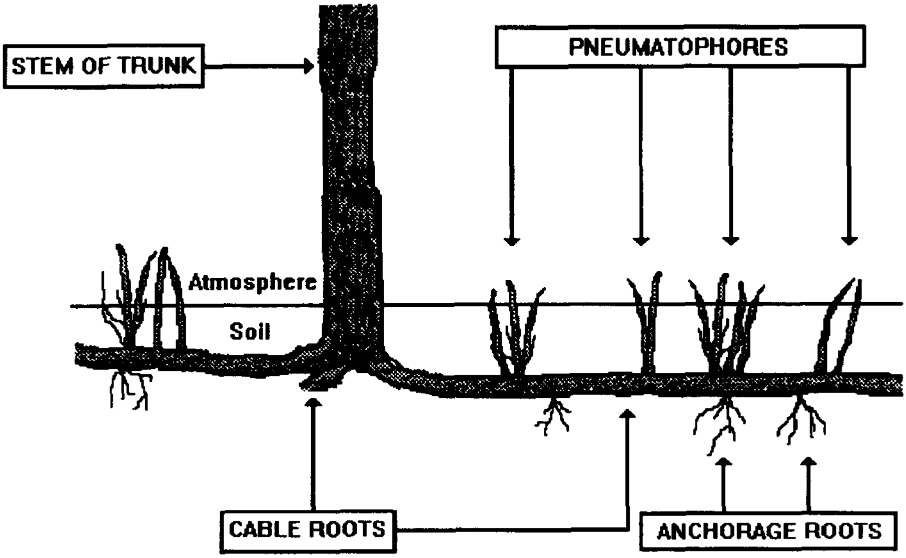
Mangroves and mangrove ecosystems are among the most important features of the coastal environment in many tropical and subtropical areas. Human populations in these areas frequently utilize the marine organisms of the mangrove ecosystems as sources of protein, and benefit from the natural coastal protection provided by the mangrove vegetation itself.

Because of population growth in many tropical areas, land reclamation is a steadily growing trend, which may affect the original balance of mangrove ecosystems. Since the 1960s, in order to create more land to meet development needs, Singapore has undertaken such programs, and many sections of the foreshores are now useful sites for studies of the natural regeneration of the coastal mangrove ecosystem, including chemical changes in the mangrove soil (Kryger & Lee 1995) and patterns of colonization of reclaimed land with new mangrove vegetation (Lee et al. 1996).

The present study is focused on the hydrogen sulphide gas which is gradually accumulated in the soils of reclaimed land (Kryger & Lee 1995) and, most notably, on the possibility for accumulation of hydrogen sulphide in the roots of mangroves. Furthermore the possible effects of hydrogen sulphide on the succession of mangrove species on reclaimed lands are assessed.

Mangrove vegetation is adapted to the inter tidal zone of the coastal environment and is characteristic by its aerial roots, which constitute a pathway for gasses across the interface between the atmosphere and the sediment. Consequently, although mangrove sediment, because of frequent inundation by sea water, is typically anoxic in nature, the presence of mangrove vegetation may significantly affect redox processes in the soil. Figure 1 illustrates the differences in the root systems of *Avicennia* spp and *Rhizophora* spp which are frequently found along the shores of Singapore. As shown the root system of *Avicennia* spp (Figure 1a) is characterized by its underground cable roots and anchorage roots and by the upright pneumatophores (or breathing roots) which protrude from the cable roots into the atmosphere thus allowing atmospheric oxygen to diffuse into the cable roots. *Rhizophora* spp (Figure 1b), on the other hand, is characterized by its rhizophores (aerial prop roots), that also help in transport of oxygen. The interaction between sulphides in the mangrove environment and the mangrove vegetation itself has been studied by a number of workers. Nickerson and Thibodeau proposed that the distribution of *Avicennia* spp and *Rhizophora* spp may be related to the soil concentrations of hydrogen sulphide and to the different abilities of these two mangrove species to oxidize the anaerobic substrate (Nickerson & Thibodeau 1985). McKee and co-workers in their work with *Avicennia germinans* (black mangrove) and *Rhiozophora mangle* (red mangrove) on a mangrove island in the Belizian barrier reef system showed that the pore water sulphide concentration and the soil redox potentials were significantly correlated with the presence of mangrove roots (aerial roots and pneumatophores) (McKee et al. 1988). It was shown that sulphide concentrations were three to five times lower near the prop roots of *Rhiozophora mangle* and the pneumatophores of *Avicennia germinans* than in adjacent (<1 meter away) unvegetated sediment. It was also discovered that sulphide concentrations in areas occupied by *Avicennia germinans* was a decreasing function of the pneumatophore density. This observation was in line with results reported earlier by Thibodeau and Nickerson (Thibodeau & Nickerson 1986) who discovered that *Avicennia germinans* created oxidized rhizospheres substantially larger than those described for other plant species. However, if the pathway for atmospheric air through the pneumatophores was blocked (e.g. by silt), the rhizospheres became as reduced as nearby unvegetated soil.

In Singapore studies on reclaimed land of increasing ages suggested that the regeneration of mangrove vegetation and the succession pattern of mangrove species might be related to the patterns of hydrogen sulphide build up in the mangrove soil. Most notably colonies of *Avicennia* spp (*Avicennia*



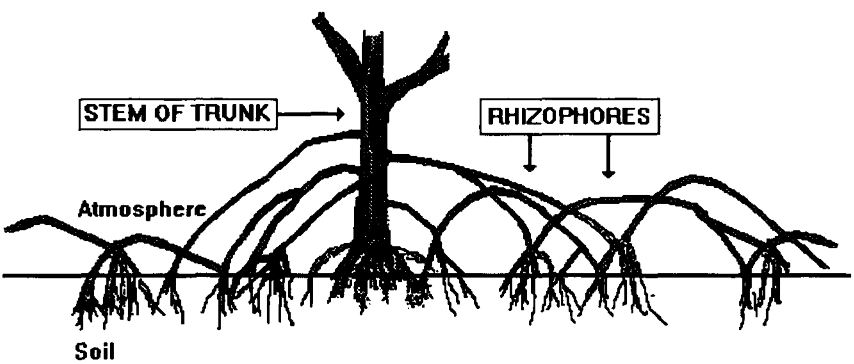


Figure 1. (A) Roots of *Avicennia* spp and (B) *Rhizophora* spp.

alba and *Avicennia lanata*) were found to establish well shortly after land reclamation. However, 12–14 years after land reclamation, these colonies began to degenerate with several individuals dying or in the moribund state (Lee et al. 1996). Furthermore, in line with the observations of Thibodeau and Nickerson (Thibodeau & Nickerson 1986) the pneumatophores of the degenerating *Avicennia* spp were found to be abnormally short due to deposition of silt. By contrast, on locations where *Avicennia* spp degenerated, saplings of a different type of mangrove *Rhizophora* spp were observed to establish well, and in the mature mangrove plots along the original coastline

Rhizophora spp was the predominant species. This pattern of regeneration was suspected to be related partly to differences in the root systems of the two mangrove species, partly to the patterns of hydrogen sulphide build up in the mangrove soil. As regards the hydrogen sulphide build up in the above mentioned plots of reclaimed land obvious ageing patterns of generation and accumulation of gaseous hydrogen sulphide were also observed (Kryger & Lee 1995). This work showed that soil ageing was accompanied by anaerobic processes resulting in a gradual build-up and trapping of hydrogen sulphide gas in the mangrove soil with a mean concentration of 5 μg H_2S/g soil in 6 months old soils, 27 μg H_2S/g soil in 4 and 14 years old soils, and 258 μg H_2S/g soil in mature mangrove soil (i.e. soil from the original coastline). Since the degeneration of *Avicennia* spp took place where hydrogen sulphide concentrations in the soil were high, it was suspected that gaseous hydrogen sulphide from the soil might contribute to a condition of hypoxia thus causing the plant to die. Furthermore, because of the aerial roots, *Rhizophora* spp might be more fit for survival on soils with significant concentrations of toxic gasses, and hence be a natural successor of *Avicennia* spp. In order to test this idea and further understand the succession of species, it was decided to study whether the occurrence of hydrogen sulphide in roots of *Avicennia* spp could be detected.

The investigation showed that roots of *Avicennia* spp may indeed contain measurable concentrations of hydrogen sulphide, and that concentrations increase with the age of the soil and hence of the root material. Furthermore the contribution of hydrogen sulphide towards the phasing out of *Avicennia* spp in the process of species succession within the mangrove ecosystem is discussed.

For determination of hydrogen sulphide in the mangrove roots an electrochemical technique for sulphide was chosen. In conjunction with ion-selective elctrodes, thin film mercury electrodes, and chemically modified electrodes, electrochemical methods (potentiometric and voltammetric) have now reached a state, where they constitute a reliable, low-cost, and frequently portable alternative to spectroscopic and chromatographic techniques for determination of a variety of chemical substances in samples of environmental interest, e.g. sulphides in sediments (Revsbech & Jørgensen 1986; Kryger & Lee 1995) and traces of heavy metals in sea water (Jagner & Kryger 1975), decomposed industrial waste (Christensen et al. 1982), coal and coal liquids (Baldwin & Santos 1986), and a number of biological materials (Baldwin et al. 1986); (Kryger 1991). For the present purpose the quantification of sulphide was carried out using an ion-selective electrode approach preceded by extractive transfer of hydrogen sulphide from the mangrove roots and simultaneous conversion into sulphide ions in an antioxidant medium.

Materials and methods

Sampling of mangrove roots

The study was conducted during late 1993 and early 1994. Samples of roots from *Avicennia* spp were collected from three different mangrove communities. These communities had developed on plots of land, which were reclaimed in 1978 ("16 years old"), in 1988 ("6 years old"), and in 1992 ("1 year old"). Samples of pneumatophores (typical diameter 0.5 cm) were cut using secateurs. Underground roots, i.e. cable roots (typical diameter 2 cm) and anchorage roots (typical diameter 0.1 cm), were rapidly excavated with a spade and given a quick rinse in the water, which had previously surrounded the root. While still in contact with the water, each root sample was cut into sections, about 1 cm long. Immediately after cutting, all samples were transferred to a 90 ml plastic container (one for each root sample) which contained 50 ml of a sulphide antioxidant buffer (SAOB) (prepared from 20 g of sodium hydroxide, 80 g of sodium salicylate, and 18 g of ascorbic acid diluted with distilled water up to one (1) litre). The plastic containers were immediately sealed and vigorously shaken. This procedure ensured, that hydrogen sulphide gas, released from the interior parts of the roots was trapped in the buffer and converted to sulphide ions (S^{2-}).

Determination of hydrogen sulphide released from mangrove roots

The increasing concentration of sulphide ion, resulting from the gradual release of hydrogen sulphide from the mangrove roots to the SAOB medium was subsequently monitored using a silver/sulphide ion-selective electrode (Corning, catalog no. 476129), a double junction reference electrode (Ag/AgCl, KNO_3) (Corning, catalog no. 476370) and a mV/pH meter (Corning pH meter 240). In alkaline media, such as the SAOB, the ion-selective electrode responds only to sulphide ions. Prior to the measurements on the mangrove roots, calibrations had been carried out using a standard addition approach, where known micro amounts of sodium sulphide were added to 50 ml of the SAOB using microliter pipettes, and the resulting reading E (mV vs. Ag/AgCl) of the electrode potential had been recorded. A total of 14 measurements revealed that the reading correlated well with the logarithm of the added amount of sulphide. More specifically, in the range from 10 to 20,000, the concentration G (expressed as μg H_2S per 50 ml SAOB) could be derived from E using the linear relation (correlation coefficient 0.9994):

$$\log_{10}G = (-E - 668.5)/31.3 \quad (1)$$

The measurements of hydrogen sulphide trapped in the root samples were carried out by monitoring the gradual release of sulphide to the SAOB. Each day the plastic containers with the SAOB medium and the root samples were vigorously shaken, and left for a few minutes for the solutions to settle. The actual concentration of sulphide released to the SAOB in any particular container was measured in the following fashion: The lid of the container was removed, and the electrodes submerged into the SAOB medium. Once a stable reading (E mV vs. Ag/AgCl) had been observed and recorded (typically in less than 60 s), the electrodes were removed from the SAOB. The container was then re-sealed, and left until next day, where the above procedure was repeated. The monitoring was continued in this fashion until the E value had not decreased for three consecutive days, thus indicating, that hydrogen sulphide was no longer released from the root sample in this container. Once this situation had been encountered (after 4 days for some containers and no longer than 16 days for any container), the roots were removed from the SAOB medium and washed with water. The washing was continued until the (alkaline) SAOB was no longer extracted from the roots. The root samples were subsequently dried under vacuum at 130 °C and the dry weight of the root material determined.

Results and discussion

The E-readings eventually attained in the above measurements ranged from −702 to −781 mV vs. Ag/AgCl. Since the E values obtained from several measurements on the SAOB medium itself were always more positive than −500 mV, blank concentrations of sulphide in the medium could be neglected. As regards the root samples, it was noted that while cable roots, sampled from the 1 year old plot were generally spongy in nature, those obtained from the older (6 and 16 years old plots) showed obvious signs of lignification. The dry weights of the root samples varied between 0.26 g and 6.08 g. Table 1 shows the results of the analysis. Since the pneumatophore samples were all cut above the ground level, these samples had been exposed to atmospheric air for several hours per day. Consequently, as could be expected, their content of hydrogen sulphide was generally low and showed no significant variation with age. By contrast, the underground cable roots and anchorage roots contained significant concentrations of hydrogen sulphide. The mean concentrations of hydrogen sulphide found in the anchorage roots were invariably larger than those found in the cable roots. However, the uncertainty of the results for anchorage roots were very large, a fact which was attributed to the small diameter of the roots and to inevitable random losses of hydrogen sulphide during the cutting and washing of the roots. Consequently no correlation between the age of the anchorage roots and the content of hydrogen sulphide could be detected. The average content of hydrogen sulphide in the thicker

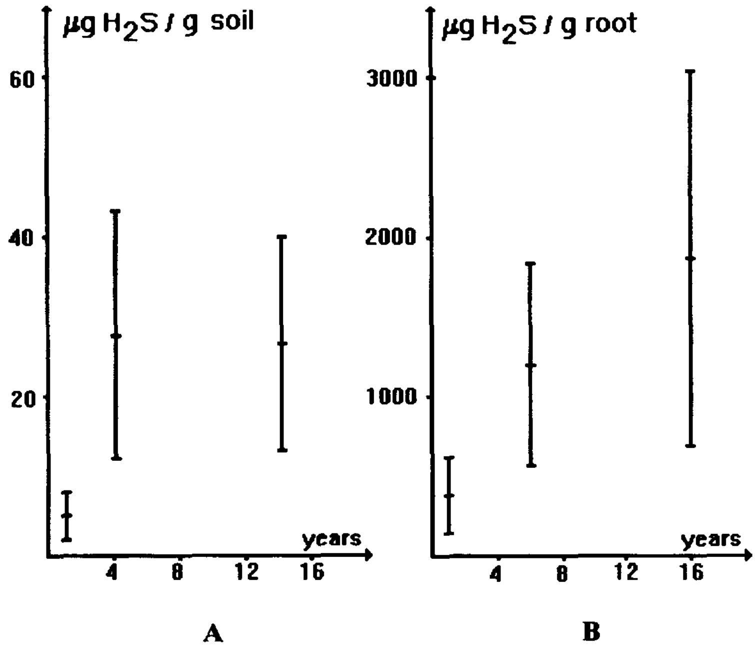


Figure 2. Development in time of the mean concentrations of hydrogen sulphide in (A) mangrove soil and (B) cable roots. The vertical bars indicate 95% confidence limits.

cable roots, on the other hand, clearly increased significantly with the age of the plants (379 µg/g, 1201 µg/g, and 1868 µg/g for roots from the 1 year old, the 6 years old, and the 16 years old soils, respectively). Most notably, as shown in Figure 2 these concentrations were 30 to 40 times higher than the average concentrations of hydrogen sulphide trapped in the soil, which surrounded the cable roots (Kryger & Lee 1995). It was therefore inferred that the transport of hydrogen sulphide from soil to cable roots could involve a mechanism, which was different from simple diffusion, and that the spongy texture of the younger roots and the more lignified ("woody") texture of the older roots could have contributed to chemisorption of hydrogen sulphide.

Combined with the findings of Thibodeau & Nickerson (1986) and McKee et al. (1988) these results suggest a reason for the survival of *Avicennia* spp in the young mangrove soil and its degeneration and death in older soils: In the younger plots (1 year and 6 years old) where the density and length of the pneumatophores were high, air was able to diffuse through the pneumatophores to the cable roots, thus creating oxidized rhizospheres and hence reducing the content of H_2S. In other words, young and healthy plants of *Avicennia* spp can maintain a lower H_2S in the root system through the oxidation by atmospheric air. However in the older (16 years) plot where the

Table 1. Mean concentrations of hydrogen sulphide in the roots of mangroves.

Age of plot (years)	Type of root[1]	Number of samples	Mean values and 95% confidence limits of H_2S concentration ($\mu g\ H_2S/g$ dry root)
1	P	7	144 ± 92
	C	8	379 ± 237
	A	6	2406 ± 3855
6	P	6	91 ± 69
	C	8	1201 ± 635
	A	7	5861 ± 4799
16	P	6	75 ± 65
	C	8	1868 ± 1177
	A	6	2061 ± 1796

[1] A: Anchorage roots, C: Cable roots, P: Pneumatophores.

pneumatophores of *Avicennia* spp were abnormally short as a result of accretion of silt, transport of air into the root system was inhibited, thus resulting in the rhizosphere becoming reduced and the build up of H_2S. The high concentration of H_2S in the soil in turn caused the accumulation in the cable roots. This effect may have blocked respiration in the root system of *Avicennia* spp for extended periods of time. Thus in summary the accumulation of H_2S in the cable roots may have caused the death of the plants at a faster rate.

The ability of *Rhizophora* spp to establish in older mangrove soil is possibly due to the ability of the prop roots to create oxidized rhizospheres (McKee et al. 1988). This observation supports the proposal by Nickerson and Thibodeau (Nickerson & Thibodeau 1985) that the distribution of *Avicennia* spp and *Rhizophora* spp may be related to the pattern of soil H_2S concentrations, the different abilities of the two mangrove species to oxidize the anaerobic substrate, and in particular the density and vigour of the pneumatophores.

Conclusion

The study confirmed that hydrogen sulphide could be detected in cable roots of *Avicennia* spp which had colonized reclaimed land and that a simple electrochemical approach was adequate to demonstrate that concentrations of hydrogen sulphide increased with the age of the plants and hence of the sediment. The pattern of hydrogen sulphide accumulation was closely correlated with the density and vigour of the pneumatophores and with the hydrogen sulphide gas which was simultaneously accumulating in the ageing mangrove sediment. It was therefore concluded that H_2S accumulation in the cable roots could be a contributing factor to the accelerating death of the trees in the older mangrove plots where the pneumatophores were partially covered by silt, and hence not sufficiently functional to maintain oxidized rhizospheres. Although it cannot be concluded at this stage that the increased content of hydrogen sulphide in the cable roots is the only factor which has caused the accelerated death of *Avicennia* spp and hence to the succession by *Rhizophora* spp the highly elevated concentrations found in the older Avicennia plants suggests that hydrogen sulphide may be a critical factor that causes the death of the plant. Studies are currently in progress to identify additional factors and to understand how *Avicennia* spp can tolerate (or possibly detoxify) significant concentrations of hydrogen sulphide before degenerating.

Acknowledgment

The authors are grateful to Professor Leo Tan for his encouraging interest in this study.

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