

## Halophilic (aerobic) bacterial growth rate of mangrove ecosystem

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**Abstract:** Mangroves are woody specialized trees of tropics and are valuable flora contributing to economical, ecological, scientific and cultural resources. They thrive in salty environments like coastal regions and are aid towards disaster management facing the onslaught of giant waves such as Tsunami. Analysis of mangrove soil on the banks of the Adyar river, behind the Theosophical society campus, Adyar, Chennai, India, gave a startling revelation of microorganisms that can tolerate different salinity ranges. Previous studies in Pichavaram delta, have reported bacterial isolates such as nitrogen fixing bacteria, halophiles and several others. However their efficiency in the growth of mangrove forest has been studied to a lesser extent. The present study has been designed and formulated to estimate halophilic(aerobic) bacterial load from mangroves soil sample based on depth and salinity of the soil and further the efficiency if any of these isolates in the growth of mangroves. Results have been correlated and a cohesive conclusion reached for further intensive research. This study throws light on the ecology of the bacterial population in the coastal marine environment inhabited by mangroves and its possible role in disaster mitigation.

**Key words:** Mangroves, Halophiles, Disaster mitigation  
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### Introduction

Mangroves are woody, specialized types of trees of the tropics that can live on the edge, where rain forests meet oceans. The term Mangrove may have been derived from a combination of the Malay word "Manggi Manggi" (Huang and Shedakar, 1984) for *Avicennia*. They are one of the most valuable biomes providing an economic, ecological, scientific and cultural resource. Mangroves have very specialized adaptations that enable them to live in salt waters. It exists under very hostile and inhospitable conditions (Khan and Ali, 2007a). The pneumatophores of certain species of mangroves such as *Avicennia marina* or black mangrove contain many small breathing pores called lenticels that allow them to survive in anaerobic sediment (Chandramohan, 1988). Microbes play an important role in governing the biogeochemical cycles of any ecosystem. Being very rich in organic matter the presence of microbes especially bacteria are active participants in the mangrove ecosystem. Different groups of bacteria that get nourished by detritus and in turn, help the mangrove ecosystem in different ways (Holguin *et al.*, 2001). Distribution of bacteria depends on changes in water temperature, salinity and other physico-chemical parameters (Alavandi, 1990). Due to high salinity, halophilic bacteria are believed to be predominant in this ecosystem. It serves as important source of food for a variety of marine organisms and maintains pristine nature of the environment. It also acts as a biological mediator through their involvement in the bio-geochemical process (Capone, 2002). Thus, Mangroves are highly productive areas contributing to the food chains of coastal oceanic areas and form a barrier against the waves and tides with their long spreading root systems. Today, mangroves

are among the most threatened habitats in the world disappearing at an acceleration rate, yet with little public notice. Biological diversity is a key issue of natural conservation (Ozcelik *et al.*, 2008) and with continuing degradation and destruction of mangroves, there is a critical needs to understand them better (Kathiresan, 2002) and now it is a need of the hour to concentrate and protect mangroves. In the present study bacterial population and growth rate have been investigated.

### Materials and Methods

**Sample collection and grouping:** Samples of soils from mangroves were collected from the southern bank of the Adyar river, behind the Theosophical Society Campus, Adyar, Chennai, Tamil Nadu, India. Soil samples were collected in and around Mangrove trees at nine random locations labeled as A to I in three different depths ranging from 0-5, 5-10 and 10 -15 cm as top, middle and bottom segments respectively and grouped (Table 1) using a soil corer. The samples were collected in sterilized polythene bags and transported to the laboratory without delay. The pH of the soil at the field was recorded to be between 7.5 and 8.

**Estimation of salinity:** 5 ml of the sample was taken in a conical flask and 3 to 4 drops of potassium chromate indicator solution were added. This was titrated against silver nitrate with vigorous shaking of the conical flask. The end point is the color change from yellow to brick red. It was repeated for concordant values (Huber and Klimant, 2000).

$$\text{Salinity} = 0.03 + (1.805 \times \text{Chlorinity}) \text{ g kg}^{-1}$$

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**Table -1:** Grouping of soil samples

Top segment (0 - 5 cm)			Middle segment (5 - 10 cm)			Bottom segment (10 - 15 cm)		
Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III
A1 } B1 } C1 }	D1 } E1 } F1 }	G1 } H1 } I1 }	A2 } B2 } C2 }	D2 } E2 } F2 }	G2 } H2 } I2 }	A3 } B3 } C3 }	D3 } E3 } F3 }	G3 } H3 } I3 }
1			2			3		

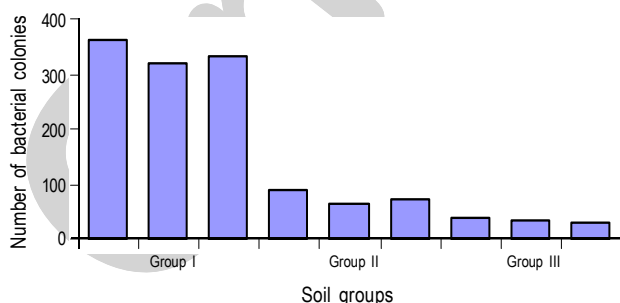
**Enumeration of bacterial density:** 1 g of sample was taken and added in 100ml of sterile saline to make up to  $10^{-2}$  dilution and serially diluted to  $10^{-12}$  dilutions. 1 ml of the diluted sample was plated by spread plate method in sterile nutrient agar medium maintaining the pH to be 8 and incubated at  $37^{\circ}\text{C}$  for 24 hr in an inverted position and the results were looked concordantly for standardization, in triplicates. After incubation the microorganisms (bacteria) were enumerated (Holt, 2001).

The number of organism = Average number of colony forming units X Dilution factor

**Growth rate of mixed halophilic (aerobic) bacteria at different concentrations:** 100 ml of Nutrient broth was modified with addition of three preferred concentrations of NaCl (0.5, 1.0 and 1.5M) in separate conical flasks for each of the samples from the different depths. The flasks were plugged, sterilized and cooled. 1ml each of the sample from  $10^{-2}$  dilution was added to the respective conical flask and incubated at room temperature in a shaker for 18 hr. After 18hrs the growth of halophiles was measured using a Spectrophotometer for every 2 hr until 28 hr. The values were noted and recorded (Valera et al., 1981).

### Results and Discussion

Soil in the mangrove region has salinity of  $6.3 \text{ g l}^{-1}$  and it is very rich in microbial (Halophilic aerobic bacterial) load as it gives too numerous to count (TNTC) colonies even at  $10^{-8}$  dilution (Kathiresan, 2001). Among the three segments studied, the top segment shows higher aerobic bacterial load compared to the middle and the bottom, which showed a decreasing bacterial load gradually. The top segments of Group I, II and III gave similar results; the same was found to be true in the middle and the bottom segments as well (Fig. 1). This shows that as the depth of the soil increases the



**Fig. 1:** Enumeration of Halophilic (aerobic) bacterial densities at different zones

bacterial load decreases. This could be due to the increasing anaerobic condition with respect to the depth of the soil.

The organisms from the three different segments were allowed to grow on 0.5, 1 and 1.5 M modified broths. When soil depths were taken in to an account top segment shows higher bacterial growth at 0.5 M and when compared 1 and 1.5 M concentrations (Fig. 2a). In the middle segment high bacterial density is observed in 1M concentrations. At 0.5 and 1.5 M it shows lower densities when compared to 1M concentration (Fig. 2b). In bottom soil there is a gradual decrease in bacterial densities from 0.5 to 1.5 M concentrations (Fig. 2c). When salinities were taken in an account the observations showed that at 0.5 M salt concentrations the bottom soil showed higher bacterial load (Fig. 3a). At 1 M and 1.5 M salt concentration the middle and topsoil showed higher bacterial load respectively (Fig. 3 b,c). From the above results it is evident that the topsoil shows Euryhaline (Odum, 1971) nature and higher fluctuation of bacterial growth due to the exchange of seawater and fresh water i.e. the circa-tidal rhythm, which shows wide fluctuation of salinity. The middle soil shows optimal salinity due to the deposition of salts and that the bacteria respond more to 1 M salt concentrations. Subsequently the bottom segment shows that the increase in salinity is inversely proportional to the bacterial density.

This bacterial growth profile study reveals that a perfect stratification exists between the depths of soil in the mangrove ecosystem and salt tolerance nature of the bacteria. This stratification may be responsible for a perfect nutritive management of the mangrove forests. Thus they provide unique ecological niche to variety of microorganisms (Khan, 2007b). Being very rich in organic matter microorganisms especially bacteria are active participants in the mangrove ecosystems, these bacteria not only maintain pristine nature of the environment but also acts as a biological mediators through their involvement in the biogeochemical cycle. This specialized adaptation enables them to live in salty environments like coastal regions, estuaries and river deltas. Thus, the conservation and management program of mangroves should also concentrate on mangrove soil management taking into consideration the microbial density and diversity, which influence the mangrove rhizosphere and its possible role in disaster mitigation.

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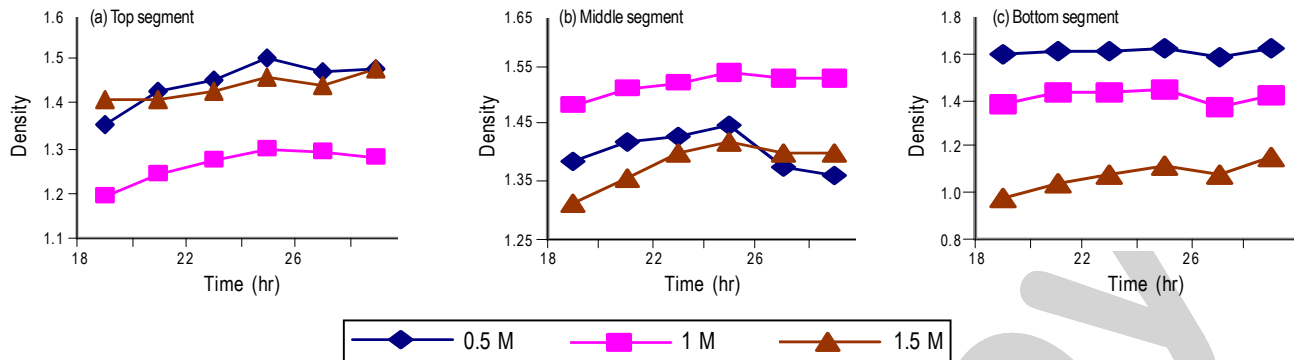


Fig. 2: Halophilic (aerobic) bacterial growth rate at various soil segments

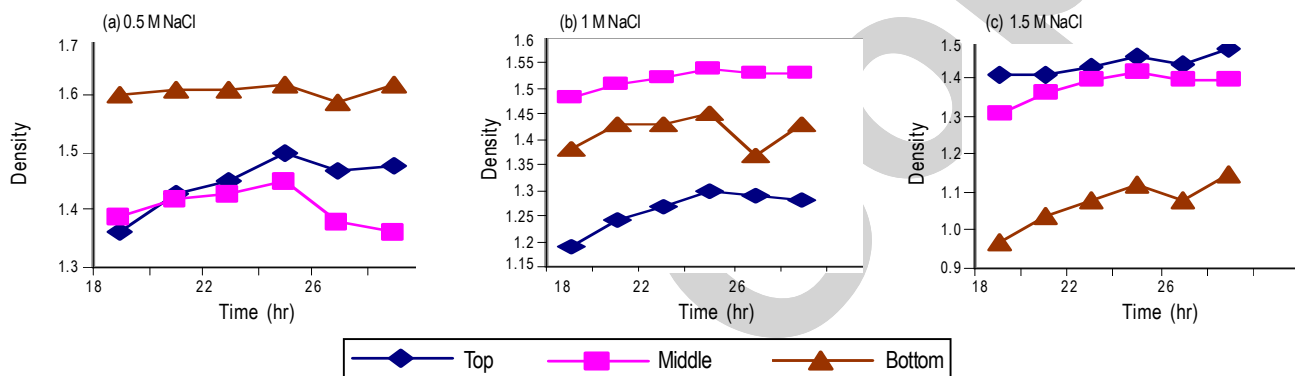


Fig. 3: Halophilic (aerobic) bacterial growth rate at various salinities

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