SOME ASPECTS OF THE DECOMPOSITION PROCESS IN THE MANGROVE SWAMP AT GAZI BAY, KENYA.

BY

HAMADI IDDI BOGA

A thesis submitted in partial fulfillment of the requirements for the degree of **Masters of Science (MSc.)(Microbiology)** of Kenyatta University.

DEPARTMENT OF BOTANY.

FACULTY OF SCIENCE.

1993.

D E D I C A T I O N.

I dedicate this Thesis to my mother, **Sophia Mohammed Bokoko** and my father **Iddi Hamadi Boga**, two mortals who brought me into this world and nartured me into the person I have grown to be.

D E C L A R A T I O N.

1. I, **Hamadi Iddi Boga**, hereby declare that, this thesis is my original work and has not been presented for a degree in any other University.

Signature .....

Date 4th Dec. 1993.

2. This thesis has been submitted for examination with our approval as University supervisors.

1. DR. Wanjiru .E. Mwatha.

Signature .....

Date 16th Dec. 1993.

2. DR. Francis .M. Muthuri

Signature .....

Date 4th Dec., 1993

ACKNOWLEDGEMENT.

I wish to express my sincere heartfelt gratitude first, to my supervisors Dr Wanjiru E. Mwatha and Dr Francis M. Muthuri for their contributions, criticisms and suggestions which enabled this thesis to reach the stage it has. A lot of thanks also go to Rhodah Nyange a student of Mombasa Technical Training Institute attached to the Kenya Marine and Fisheries Research Institute (K.M.F.R.I) who served as a very able assistant. I would also wish to express my thanks to Mwangi, Monica, Ohowa, Gwada, Gonda, Kamau and Tunje all of K.M.F.R.I for their support and assistance during the course of my research. Special thanks are due to Anne-Francois Woitchik of European Economic Community, for her technical assistance and suggestions. I would also wish to thank my friends Suleyman Madzengo, Mwasiwa Boga, and Abdalla "Guy" who went out of their way to assist me and ensured that my stay in Mombasa was a happy and comfortable experience.

A lot of thanks are also due to Matano Ndaro for proof reading my thesis and to Margaret Mbato for assisting me with cartography. I also wish to express my sincere gratitude to K.M.F.R.I for allowing me to use their excellent laboratory facilities, East Africa Wildlife Society for their financial assistance and DAAD (Deutscher Akademischer Austauschdienst) for sponsoring my Msc. studies at Kenyatta University. Finally, I am grateful to the Department of Resource Surveys and Remote Sensing (D.R.S.R.S.) for allowing me to make use of their excellent computer facilities.

A B S T R A C T.

The number and activity of several physiological groups of heterotrophic bacteria in Gazi Bay, were estimated between September 1992 and February 1993. The study was done in three plots made of pure stands of Aviccenia marina, Ceriops tagal and Rhizophora mucronata trees. Average numbers of aerobic heterotrophic bacteria in sediments ranged between 7.44×10^7 and 4.70×10^8 cfu/g dwt of sediment. Average numbers of anaerobic heterotrophic bacteria in sediments ranged between 2.00×10^6 and 1.86×10^8 cfu/g dwt. There was no significant difference between the three plots with respect to the numbers of aerobic bacteria in the sediment. The average number of aerobic heterotrophic bacteria in water ranged between 8.6×10^4 and 8.90×10^6 cfu/ml of water. Water in the Rhizophora plot had a significantly higher population of aerobic heterotrophic bacteria than water in the other plots. Aerobic heterotrophic bacteria populations on leaves ranged from 2.00×10^2 - 9.75×10^5 cfu/cm^2 of leaf area. A. Marina leaves had the highest number of heterotrophic bacteria while C. tagal leaves had the least. Numbers of nitrate reducing bacteria in the sediment ranged from 2.37×10^2 - 9.21×10^3 bacteria cells/g dwt. The highest numbers of nitrate reducing bacteria were observed in the Rhizophora plot. The fastest cellulolytic activity was observed in the Aviccenia plot where 92% of filter paper dry weight was lost in 28 days.

The slowest cellulolytic activity was in the Ceriops plot where only 11% of filter paper dry weight was lost in 28 days. Rates of acetylene reduction ranged between 0 and 51.474 n moles of C_2H_4/h/g dwt of sediment. The Ceriops plot showed the lowest rates of nitrogen fixation while the Rhizophora plot had the highest.

Aviccenia marina leaves in their native plot decomposed faster than either Rhizophora mucronata or Ceriops tagal leaves in their native plots. Organic matter in the mangrove swamp sediments ranged from 2.25 - 37.17%, while water content ranged from 17.48 - 76.36%. The levels of combined NO_3^- and NO_2^- in sediment pore water ranged between 0.85 and 113.92 $\mu g/l$. Sediment in the Rhizophora plot had the highest levels of nutrients compared to the other plots. Characterization of bacteria revealed that, the majority of the heterotrophic bacteria strains in the mangrove swamp were Gram positive and spherical in shape.

NB: cfu = colony forming units.

g dwt = gram dry weight.

g wwt = gram wet weight.

Table of Content.

page.

Chapter Two.

Chapter Three.

List of Figures.

List of Tables.

List of Plates.

CHAPTER ONE.

1.0 INTRODUCTION AND LITERATURE REVIEW.

1.1 INTRODUCTION.

The term mangrove is used to describe an ecological group of halophytic species which belong to some twelve genera in eight different families (Lugo and Snedaker, 1974). These halophytic species form evergreen forests on tropical and subtropical coastlines. They are the tropical equivalent of salt marshes (Chapman, 1974). Despite their large taxonomic variability all mangroves exhibit marked similarities in physiological characteristics and morphological adaptations under similar environmental conditions (Lugo and Snedaker, 1974). The unique physiological and morphological adaptations which include possession of aerial roots, salt secreting glands and xerophytic features are well documented (Beekman et al., 1990; Chapman, 1974; Lugo and Snedaker, 1974).

Mangroves normally occupy sheltered tropical and subtropical coastlines (Chapman, 1974). It is common to see mangrove vegetation in bays, lagoons, estuaries and behind spits, offshore bars and islands where they are protected from strong wave action (Chapman, 1974; Forsberg, 1974).

Mangroves are the only marine macrophytes characterized by the storage of aerial biomass in the form of trees and shrubs which form a forest (Mann, 1982). Barnes and Hughes (1982) estimated primary production in mangrove swamps to be around 350-500 g C m^{-2} yr^{-1}. Much of the carbon produced is seldom consumed to any significant degree because grazing is of minor importance in mangrove swamps (Mann, 1982). The main primary consumers are a few species of birds, insects, monkeys and crabs (Barnes and Hughes, 1982).

Odum and Heald (1975) observed that most of the food chains in mangrove swamps and near shore coastal waters are detritus based. Detritus material formed from decomposition has a low C:N ratio and serves as a rich source of nutrients for organisms in the higher trophic levels in the food chains. These include various species of fish, crustaceans and molluscs (Boaden and Seed, 1985).

The nearshore coastal waters which are not very productive benefit from the export of an estimated 0.5-2.4 g C m^{-2} d^{-1} in the form of detritus (Barnes and Hughes, 1982). Indeed Martosubrato and Naamin (1977) observed a positive correlation between the extent of mangroves and the size of fishery yields from adjacent waters.

Decomposition of organic matter in estuarine environments is essentially viewed as a microbially mediated process, although crabs and other macrofauna may assist in physical breakdown. An estimated 80-90% of carbon mineralized in the estuarine environment may be oxidized by bacteria (Mann, 1982). Fungi and actinomycetes also play an important role in decomposition in mangrove areas (Fell and Master, 1984; Subramanian, 1988).

The discussion above shows that the decomposition process in mangrove swamps is of extreme importance to the coastal environment. However, indiscriminate exploitation of mangroves by man has led to a gradual decrease in the total acreage of mangrove forests in this century (Beekman et al., 1990; Ruwa, 1992).

Cutting of mangroves for building poles, clearing of mangrove areas for salt pans and fish ponds and destruction caused by oil spills pose a serious threat to the existence of this unique and important ecosystem (Ruwa, 1992).

Over the past decade public and scientific interest in the role of mangroves in nature and their value to mankind has resulted in an increase in the number of research studies done on mangroves (Snedaker, 1984). A lot of information exists on the mangroves of South East Asia, Australia, New Zealand and America (Agate, 1988; Albright, 1976; Alongi, 1988; Chandramohan, 1988). However, there is a scarcity of information on the East African mangroves.

Thus, this study seeks to deepen our understanding of the ecology of the East African mangroves with a specific reference to the decomposition process in the mangrove swamp at Gazi Bay, Kenya.

1.2 LITERATURE REVIEW.

1.2.1 The Geography of Mangroves.

Geographically, extensive mangrove formations are found in two regions. These are the Indo-Pacific region which includes the mangroves of South East Asia, Eastern Africa, Australia and New Zealand, and the New world West African region which includes the mangroves of the western coast of Africa and those of the Americas (Walter and Steiner, 1936).

South East Asia (Indo-Malayan peninsula) holds the most extensive areas of mangrove forests with 36 species of mangrove reported (Lugo and Snedaker, 1974). Floristically, the East African mangroves resemble an impoverished South East Asian System with only eight mangrove species (Lind and Morrison, 1974). In America and West Africa, only 10 mangrove species have been reported (Lugo and Snedaker, 1974). Thus, the South East Asian system is the most complex mangrove formation in the world.

1.2.2 The Kenyan Mangrove Swamps.

Mangrove species which have been recognised in Kenya include Rhizophora mucronata Lam., Ceriops tagal (Perr.) C.B. Robinson, Aviccenia marina (Forsk) Vierh., Sonneratia alba Sm., Lumintzera racemosa Wild.,Heritiera littoralis Dryand. in Ait., Xylocarpus granatum (Koen) and Bruguiera gymnorrhiza (L) Lam. (Lind and Morrison, 1974; Ruwa, 1992). Different mangrove species occupy specific zones in the forest depending on the species and ecological conditions like salinity, nature of substrate and tidal influence. The zones may be composed of single species or may be a mixture of several species (Beekman et al., 1990; Ruwa, 1992).

According to Graham (1929), R. mucronata is the most common tree in the Kenyan mangrove swamps forming about 90% of the stock. However, A. marina is the most widely distributed species in the East African region because of its ability to tolerate high ranges of salinity, varied flooding regimes and substrate types (Ruwa, 1992). The Kenyan mangrove forests cover a total area of 52,980 hectares along the coast (Doute et al., 1981). Extensive areas of mangrove forests occur along creeks and around islands.

In the south the mangroves occur at Vanga, Shirazi(Shimoni) and Gazi; in the central region in the Mombasa area especially along Tudor Creek and its branches, in Mtwapa, Kilifi and Mida Creeks.

The most extensive forests however occur in the north on the mainland and around islands of the Lamu archipelago (Fig 1) (Beekman et al., 1990). The bulk of these mangroves occupy intertidal areas where underground water discharge occur, as is evidenced by their location away from the two major rivers at the coast i.e. Tana and Sabaki (Ruwa and Polk, 1986 ; Ruwa, 1992).

1.2.3 Importance of Mangroves.

Mangrove swamps are of extreme importance to the tropical and subtropical coastlines ecologically and economically . They play a crucial role in fish production by producing detritus which enhances offshore productivity and by providing shelter to juvenile fish and crustaceans (Beekman et al., 1990). In addition, they filter land runoff and help in land formation. The mangroves absorb floodwater thereby reducing danger to people and agriculture.
In Bangladesh a flood prone area, the government has planted 25,000 hectares of mangroves to protect embarkments and new land (Anon, 1985).

Economically, mangroves are a source of food, fuel, wood, medicine, tannin and dyes. Fish ponds and salt pans are often created within mangrove swamps for fish culture and salt production. Along the coastal towns mangrove poles are very popular building materials (Beekman et al., 1990; Ruwa, 1992). The great economic value of mangrove products has resulted in the over-exploitation of mangroves currently being experienced all over the world.

1.2.4 Decomposition in Mangrove Swamps.

Decomposition is the process where dead organic matter gradually disintegrates into simpler substances like water, carbon dioxide and mineral salts (Mason, 1976).

In mangrove forests nearly 50% of the litterfall is carried away to the coastal waters during the high tide (Rao and Nair, 1984). The litter that is left behind then undergoes decomposition during which a succession of decay phases all of which influence depletion of the litter can be elucidated.

Cundell et al., (1979), observed four decay phases during microbial degradation of mangrove leaves in the Floridan waters of U.S.A. In the first fourteen days a rapid leaching of sugars and tannins from the leaves was observed, which was followed by depletion of sugars and continued leaching of tannins, together with microbial colonization of the leaf surfaces in the next fourteen days. The third phase lasted twenty one days and was characterized by depletion of tannins and complete colonization of the leaf surfaces by bacteria, fungi and protists.

Finally the leaves became rich in microbial communities of cellulolytic bacteria, fungi, meiofauna and protists. The four phases lasted a total of seventy days after which the leaves started fragmenting hence increasing the surface area for further microbial action. Similar accounts of mangrove litter decomposition were given by Fenchel (1977) and Valiela (1984).

During the leaching phase the leaves lose soluble organic compounds like sugars, proteins, amino acids and tannin which are depleted quickly and are released into the water to be attacked by bacteria for further mineralization (Valiela, 1984). Recalcitrant compounds like celluloses, waxes and phenolic compounds like lignins remain resistant to breakdown.

Through time the levels of these compounds in the detritus rises. Ultimately the remaining detritus contains large concentrations of fulvic and humic acids. These decay phases are however not unique to mangrove litter and can be observed in decomposition of organic matter from other sources (Valiela, 1984). The decomposability of detritus is closely linked to the composition of the source material, although the phases for all organic material undergoing decomposition are much less the same (Valiela, 1984).

As the decomposition process progresses through the various phases, there is mechanical fragmentation by wave action, and animal activities like feeding, burrowing and movement. This helps by reducing the sizes of detrital particles hence exposing new areas to bacterial attack (Fenchel, 1977).

Woitchik et al. (1993) observed that Rhizophora leaves decomposed faster than Ceriops leaves in their native plots. The decomposition rates of the leaves of both species were higher during the rainy season. Steinke and Ward (1987) working in St. Lucia estuary, South Africa found Aviccenia marina leaves to degrade faster than Bruguiera gymnorrhiza leaves in their native plots. Albright (1976) reported that mangrove leaves decompose faster in the sediment-water interface, than when buried in the mud.

Thus, in the mangrove swamps, litter decomposition varies according to season, species and according to location of the litter in the mangrove swamp.

1.2.5 Nutrient Enrichment by Decomposing Leaves.

For any organic materials undergoing decomposition changes in the C:N ratio are observed. At first carbohydrates and hence C is lost during the leaching process but colonization by the microbial saprophytes enriches the N-content. Thus, the C:N ratio decreases over time making aged detritus a suitable food for grazing animals (Odum and de la Cruz, 1967; Mann, 1982).

Fell and Master (1984) found the C:N ratio in senescent leaves of Rhizophora mangle was 120:1 in senescent leaves and 43:1 in partially decomposed leaves. Initially nitrogen was 0.2-0.4% of the dry weight of the leaves but increased during decomposition to 0.5% via microbial nitrogen immobilization. Woitchik et al. (1993) attributed 60% of the increase in nitrogen on decomposing leaves in Gazi Bay during the rainy season to nitrogen fixation by bacteria occurring on those leaves.

1.2.6 Decomposers.

In the soil, microorganisms are the primary agents for the mineralization of organic matter. It is logical therefore to conclude that the same role is played by marine microorganisms (Litchfield, 1976). The decomposition of organic matter in estuarine environments is essentially viewed as a microbially mediated process, although crabs and other macrofauna may assist in the physical breakdown. Approximately 80-90% of the carbon mineralized in the estuarine environment may be oxidized by bacteria (Mann, 1982).

A subject of controversy over the years has been the relative importance of bacteria and fungi in the decomposition of organic detritus (Padgett et al., 1985). Early research emphasized the role of bacteria as principle decomposers of salt marsh detritus (Buckholder and Bornside, 1957; Odum and de la Cruz, 1967). Recent findings by Montagna and Ruber (1980); Rublee et al. (1978) have reaffirmed this view.

Bacteria have unique properties which explain their dominant role in primary decomposition of organic detritus in mangrove forests. These include ability to utilize dissolved organic and inorganic nutrients, possession of enzyme systems which enable them to hydrolyse and decompose plant tissues and possession of efficient systems of anaerobic metabolism (Chandramohan, 1984). The role of fungi as decomposers in anaerobic environments is limited since they are either rare or inactive under such conditions of limited oxygen supply (Valiela, 1984).

Decomposition of leaves begins while they are still attached to the plant. Yeasts and bacteria are the initial colonizers, using up sugars exuded from leaf surfaces (Lee, 1980). As the leaves senesce the phylloplane (leaf surface) microflora attack with enzymes such as cutinases, pectinases and cellulases. Eventually the enzymes penetrate the cuticle, attack middle lamellae and disintegrate cell walls. Once, on the ground additional organisms attack the leaves. Initial colonization is by bacteria followed by fungi, algae, ciliates, flagellates and finally large grazers. Small crustaceans, nematodes, rotifers and tubellarians may also be seen colonizing detrital particles (Barnes and Hughes, 1982; Kennish, 1986). Primary colonizers include members of the genera Pseudomonas and Bacillus (Chandramohan, 1984).

These are later followed by filamentous fungi like members of the genera Cladosporium, Aureobasidium and Alternaria (Chandramohan, 1984).

In the mangrove swamp sediment, the surface layer (0-2 cm) is well aerated. It is dominated by aerobic chemoorganotrophic bacteria which make use of the organic input. These carry out aerobic mineralization using oxygen as the terminal electron acceptor (Vosjan and Olanczuk-neyman, 1977). Aerobic chemolithotrophic bacteria like Thiobacillus thiooxidans and Chromatium also occur in surface sediments and utilize inorganic substrates like H_2S and N_2 originating from anaerobic processes within the sediment, which they oxidize to SO_4^{2-} and NO_3^- respectively (Agate, 1988; Vosjan and Olanczuk-neyman, 1977).

The aerobic bacteria hydrolyse organic matter using extracellular enzymes like proteinases, esterases, β-glucosidases, alginases, agarases, dehydrogenases and amylases degrading particulate organic matter (POM) into dissolved organic matter (DOM) (Corpe and Winters, 1972; Kim and ZoBell, 1974; Meyer-Reil, 1984).

Thus, aerobic heterotroph bacteria are able to degrade a wide range of natural substrates like sugars, proteins, celluloses, alginic acid, chitin and lignin (Austin, 1988; Wood, 1965). These aerobic bacteria e.g. Pseudomonas, Micrococcus, Coyrnebacterium and Lactobacillus occur in the sediments, water column and on the surfaces of dead leaves and twigs (Mann, 1982).

Some centimetres below the sediment surface, conditions become anaerobic and anaerobic mineralization takes over. Obligate and facultative anaerobic bacteria replace obligate aerobes. Anaerobic bacteria e.g. Thiobacillus denitrificans, Desulfovibrio and Methanobacterium use NO_3^-, SO_4^{2-} and CO_2 as terminal electron acceptors. Organic molecules can be used as electron acceptors in the process of fermentation resulting in production of alcohols, lactate, fatty acids and H_2 gas.

The products of anaerobic metabolism are later used by aerobic bacteria and are broken down to CO_2, H_2O and mineral salts (Vosjan and Olanczuk-neyman, 1977). Thus, different niches are created in the environment which are inhabited by different microbial groups (Mann, 1982; Vosjan and Olanczuk-neyman, 1977).

Sulphate reduction and methanogenesis are the most common anaerobic processes associated with mangrove leaves which have sunk below the sediment surface (Mann, 1982).

1.2.7 Bacterial Abundance.

Fine sediments have been found to harbour large populations of bacteria when compared to coarse sediments because of there larger surface area. This is because most bacteria occur attached to the surface of soil particles (Dale, 1974).

The increase in bacterial numbers with decrease in grain size maybe related to the nutrient absorption on the particle surfaces or to the greater proportion of particulate organic matter in the fine sediments. Indeed, a significant negative correlation has been observed between bacterial abundance and both carbon and organic nitrogen content of sediment particles (Rublee, 1982).

Early attempts to determine bacterial numbers in the seawater relied on culturing water samples on agar plates and counting resultant colonies. However, the diversity of bacterial types in the sea is so great that any one culture technique will leave out a number of bacterial types (Morita, 1977).

Dale (1974), obtained 10^8 to 10^{10} cells g^{-1} dwt of sediment particles in an estuary in Nova Scotia. Rublee and Dornseif (1978), while working in a salt marsh obtained an average population of 8.39 X 10^9 cells cm^{-3} of sediment samples. Alongi (1988) while working on surface sediments of a mangrove swamp in Australia observed bacteria numbers averaging 1.1 X 10^{11} cells/g dwt of sediment. All these workers used the direct count method.

Bacteria populations in the water column are usually less than bacteria populations observed in sediments. Fenchel (1984) reported a population of between $2 \times 10^5 - 10^6$ cells per ml in offshore water samples, while Morita (1977) and Seiburth (1976) reported populations of between $10^4 - 10^5$ cells per ml of water.

Coastal waters which are more productive than offshore waters were reported to have around 3×10^6 cells per ml (Fenchel, 1984; Rublee, 1982). Palumbo and Ferguson (1978) found estuarine waters to have bacteria populations of between $10^6 - 10^7$ cells per ml, which were 1000 times less than the population of bacteria they found in sediments of the same estuary.

1.2.8 Heterotrophic Bacteria in the Mangrove Swamps.

The mangrove swamps have many bacteria which have the ability to hydrolyse and decompose carbohydrates, proteins and other organic compounds. These are the heterotrophic bacteria which are unable to manufacture their own organic carbon. The heterotrophic bacteria are thus equipped with different metabolic mechanisms for attacking the various substrates they encounter in their environment (Chandramohan, 1988).

There are two groups of heterotrophic bacteria associated with the utilization of organic carbon in the mangrove environment. These are the aerobic heterotrophic bacteria and the anaerobic heterotrophic bacteria. The former use oxygen as their terminal electron acceptor during the process of respiration, and are found in the sediments, water column and on the surfaces of dead organic matter (Mann, 1982).

Among the aerobic bacteria are the facultative aerobes which can do with or without oxygen as a terminal electron acceptor. These use oxygen when it is present in the environment and in its absence they use another electron acceptor mostly nitrate ions (Chandramohan, 1988).

The obligate anaerobic bacteria do not require oxygen as an electron acceptor and therefore carry out their metabolism in the absence of oxygen. Such bacteria include the sulphate reducers and the methanogenic bacteria. These use sulphate ions and CO_2 respectively as electron acceptors (Boto, 1988; Chandramohan, 1988). The sulfate reducing bacterium Desulfovibrio desulfuricans is an obligate anaerobe which converts lignin and cellulose in wood into a gray fibrous sediment (Subramanian, 1988)

1.2.8.1 Saccharolytic Bacteria.

Saccharolytic bacteria are heterotrophic bacteria which hydrolyse carbohydrates like sugars, cellulose, alginic acid and lignin and they are prevalent in the mangrove swamps. Cellulose is the most abundant carbohydrate in nature and its rate of degradation is very slow compared to other substrates. The mineralization of cellulose is dependant upon the rate at which it can be hydrolysed to glucose (Colwell et al., 1975). Cellulose decomposing bacteria which have been isolated from sea water and sediments include members of the genera Cytophaga, Sporocytophaga, Micrococus and Pseudomonas (Chandramohan, 1984).

Most of the cellulolytic bacteria are strict aerobes, have a gliding movement and grow at mesophilic temperatures (Schneider and Rheinheimer, 1988). Cellulose decomposers do not occur in isolation and can be found together with other bacteria which are able to hydrolyse other substrates like starch, sugars, agar, lignin, chitin and alginic acid (Wood, 1965). Some anaerobes like Desulfovibrio desulfuricans and some species of the genus Clostridium can hydrolyse cellulose and they have been reported in mangrove swamps (Subramanian, 1988).

1.2.8.2 Proteolytic Bacteria.

These are heterotrophic bacteria which have enzyme systems that enable them to hydrolyse proteins and protein derivatives. These bacteria hydrolyse substrates like gelatin, casein, peptides, polypeptides, amino acids, urea and thio compounds.

Proteolysis leads to the formation of ammonia and ammonium compounds (Wood,1965). Most of the ammonia is later converted into nitrates in the upper layers of the sediments (Chandramohan, 1988). Members of the genera Pseudomonas and Corynebacterium have been identified as proteolytic bacteria (Wood, 1965).

1.2.8.3 Nitrate Reducing Bacteria.

Nitrate reducing bacteria are heterotrophic facultative aerobes which use nitrate ions as terminal electron acceptors in the absence of oxygen. In so doing they reduce the nitrates to other nitrogen oxides or nitrogen (Chandramohan, 1988).

Nitrate reduction is energetically superior to other anaerobic processes like fermentation, sulfate reduction and carbon dioxide reduction (Chandramohan, 1988). The nitrate reducers have the enzyme nitrate reductase on their membranes for catalyzing the reduction. In the process a biological reductant is essential; a role usually played by organic compounds, since most of the nitrate reducers are heterotrophic. In this case, organic material from mangrove litter serve as the reductants during nitrate reduction, donating the electrons which are accepted by nitrate ions leading to their reduction (Chandramohan, 1988).

During nitrate reduction there is complete oxidation of the organic substrate to carbon dioxide and water, while nitrate is dissimilated to nitrite which is further reduced to nitrogen gas.

Other bacteria may in the process of dissimilating nitrate reduce it to ammonia hence denitrification fails to occur (Nedwell, 1973). Bacillus licheniformis, Aeromonas and Klebsiella are examples of heterotrophic nitrate reducing bacteria found in the mangrove environment (Chandramohan, 1988).

Other nitrate reducers which are not heterotrophic exist. These include the chemolithotrophic Thiobacillus denitrificans which is an autotroph using reduced sulfur compounds like hydrogen sulfide as electron donors instead of organic compounds. However, these do not participate in the hydrolysis of organic matter hence are not important in decomposition.

Very few studies into the extent of nitrate reduction in natural samples have been done around the world. Srensen (1978) amended sediments from Limfjorden in Northern Denmark with ^{15}N labelled nitrate and showed that reduction to ammonia occurred at a rate ranging from 0.12-0.75 μmoles N cm^{-3} d^{-1}. Similar results were obtained by Koike and Hattori (1978) for marine sediments.

1.2.8.4 Sulfate Reducing Bacteria.

Sulfate reducing bacteria are heterotrophic obligate anaerobes which use sulfate ions as electron the acceptors instead of oxygen. In so doing they reduce sulfate ions to sulfides using either lactate, malate or ethanol as electron donors which are themselves oxidized to acetate and carbon dioxide.

Sulfate reducing bacteria are obligate anaerobes and most of their activity is restricted to the bottom layers of the sediments where conditions are conducive to their proliferation (Chandramohan, 1988). The products of their mineralization diffuse to the surface layers where they are further mineralized by aerobic heterotrophic bacteria to carbon dioxide and water. Sulfate reducing bacteria isolated in mangrove swamps include members of the genera Desulfovibrio and Desulfotomaculum (Chandramohan, 1988). Sulfate reduction to sulfide requires a large supply of organic carbon as energy source, with up to 6 moles of organic carbon being utilized for each mole of sulfate reduced. Indeed, most workers regard this as the major pathway through which organic matter is utilized in anaerobic soils (Boto, 1988).

Vosjan and Olanczuk-neyman (1977), reported that more than 50% of mineralization in the sediments of salt marshes is carried out via sulfate reduction, which is partly due to the abundance of sulfur in seawater.

1.2.8.5 Nitrogen Fixing Bacteria.

Most of the nitrogen fixers in nature are heterotrophic bacteria which use atmospheric nitrogen as a nitrogen source and organic compounds as a carbon source. In the marine environment nitrogen fixation is also carried out by autotrophic bacteria and algae. Free living heterotrophic nitrogen fixing bacteria belong to the families Azotobacteriaceae, Enterobacteriaceae and Bacillaceae. Photosynthetic nitrogen fixers include members of the families Thiorhodaceae, Athiorhodaceae Chlorobacteriaceae and Cyanobacteria (Chandramohan, 1984). Since mangrove sediments are anaerobic, most of the earlier researchers isolated anaerobic diazotrophs giving the wrong impression that most of the nitrogen fixation in these swamps is done by the anaerobic bacteria. However, recent findings have shown that most of the nitrogen fixation is by aerobic or microaerophilic organisms e.g Azotobacter, photosynthetic bacteria and cyanobacteria (Chandramohan, 1988; Potts, 1984).

A few anaerobic bacteria such as the sulphate reducers Desulfovibrio and Clostridium fix atmospheric nitrogen in the mangrove swamps (Chandramohan, 1988; Potts, 1984).

1.2.9 Nitrogen Fixation in Mangrove Swamps.

Most of the research on mangrove microbiology has been done in South East Asia, Australia, New Zealand and America. Significant nitrogen fixation has been recorded in mangrove swamps in these areas.

Zuberer and Silver (1978), reported that more nitrogen fixation occurred closer to mangrove roots and in sediments associated with roots than in root free sediments. They attributed this to the rhizosphere effect of the roots which releases exudates into the sediments thus stimulating bacterial multiplication and activity. They also observed that nitrogen fixation is species specific and was influenced by light, suggesting the role of cyanobacteria and photosynthetic bacteria in the process. In their experiment, they observed nitrogen fixation in the range of 0.01-1.84 nmole C_2H_4/g wet weight/h, in plant free sediments.

Hicks and Silvester (1984) working in New Zealand, recorded a high correlation between nitrogen fixation and the dry weight of decomposing particulate organic matter in the surface sediments of a mangrove swamp. Acetylene reduction activity was higher under aerobic conditions than under anaerobic conditions. This served to prove further that aerobic diazotrophs play a more significant role in the nitrogen fixation in the mangrove swamps. Further experiments carried out on decomposing leaves collected from the surface of sediments during the study showed a mean acetylene reduction activity of 32.4 nmole C_2H_4 /g dwt litter/h.

Woitchik et al. (1993), recorded acetylene reduction rates of up to 300 nmole C_2H_4 /g dwt litter/h, on Ceriops leaves and up to 1200 nmole C_2H_4 /g dwt litter/h on Rhizophora leaves in Gazi Bay. The leaves had been left to decompose in their native plots for 1 month. The highest rates of nitrogen fixation on the leaves were observed during the rainy season.

1.2.10 Nutrient Levels In Mangrove Swamps.

The major nutrients which have a direct influence on the microbial population in a mangrove swamp include sulfates, nitrates, phosphates, organic matter and sediment water content.

Of these, nitrates and phosphates are the most limiting in the marine environment. Seawater contains large concentrations of sulfates, hence this nutrient is never limiting in any marine ecosystem (Subramanian, 1988).

I.2.10.1 Organic matter.

Mangrove swamp sediments are rich in organic material (Heald, 1971). Most of this organic matter results from fallen mangrove leaves and twigs while a small proportion of it is formed from dead animals, fungi and algae (Subramanian, 1988).

The bulk of organic matter is usually suspended in the water column and reaches the mangrove sediments by sedimentation. This is a very pronounced process in the shallow waters characteristic of mangrove swamps (Chapman, 1976). Allochthonous sources which contribute very little organic matter include terrestrial runoff and inflowing tidal currents from adjacent coastlines (Subramanian, 1988).

1.2.10.2 Nitrates.

Nitrogen enters the mangrove swamps via rainfall, terrestrial runoff, tidal currents from the sea, human activities like industrial and sewage release into coastal

areas, nitrogen fixation and through mineralization of organic matter. Mineralization and nitrogen fixation are the most important, while the other processes have little impact on the overall nitrogen content of the mangrove swamps (Chandramohan, 1988). Mangrove swamps sediments usually contain an average of 50 µg NO_3-N/l of pore water (Chandramohan, 1988).

Processes like, tidal export, leaching, volatilization, and denitrification remove nitrogen from the mangrove swamps. In sandy mangrove soils free exchange between the interstitial waters and tidal waters may occur resulting in loss of inorganic nitrogen, while in clay soils which are less permeable lack of nutrient exchange results in nutrient conservation (Chandramohan, 1988).

In anaerobic sediments, denitrification predominates leading to a lowering of the nitrate levels of the sediments. At the surface of the sediments where the substrate is more aerated ammonia and nitrites are usually oxidized to nitrates (Subramanian, 1988). Thus, nitrate levels in the swamp rely on the balance of the processes of nitrogen input and nitrogen output.

CHAPTER TWO.

2.0 OBJECTIVES AND THE STUDY AREA.

2.1. OBJECTIVES OF STUDY.

Considerable research work has been done on the Kenyan mangrove swamps (Graham, 1929; Kokwaro, 1986; Ruwa, 1990; Ruwa 1992). Recently, more research aimed at understanding the dynamics of the Kenyan mangrove ecosystem has been going on at Gazi Bay. These studies focused on vegetation composition, zonation, litterfall, macro- and meiofaunal composition, tidal cycles, nutrient cycles and regeneration prospects. Most of the research was being done by officers of Kenya Marine and Fisheries Research Institute (K.M.F.R.I), European Economic Community (E.E.C), Kenya Belgium Program (KBP), and the University of Nairobi. Some of the research findings are contained in the final report on the Dynamics and Assessment of the Kenyan mangrove Ecosystem (n^0 TS2-024-C (GDF)) published in April 1993 by the Netherlands-Belgium Kenya mangrove ecosystem project.

However, the area of microbiology has been ignored in studies done on the Kenyan mangrove ecosystem. The only study that looked at microbiological processes was done by Woitchik et al. (1993) at Gazi Bay and it focused on the decomposition process in the Ceriops and Rhizophora areas of the Gazi mangrove swamp. The study also focused on the influence of nitrogen fixation on nutrient build up on the decomposing leaves. In the experiment oven dried leaves were used and 60% of the build up of nitrogen on the decomposing leaves was attributed to nitrogen fixation.

This study attempts to enhance our knowledge of the decomposition processes at Gazi Bay. The mangrove species Aviccenia marina which has the widest vertical distribution in Gazi Bay has been included in the study alongside the other two species previously studied by Woitchik et al.,(1993). The rate of nitrogen fixation in mangrove sediments has been estimated and heterotrophic bacteria numbers in different localities in the mangrove swamp have been determined. The concentrations of some organic and inorganic nutrients in the mangrove swamp sediments have also been studied.

Thus, this study was designed to achieve the following objectives:-

(i) To estimate the population densities and distribution of the main groups of decomposing bacteria in the mangrove swamp.

(ii) To determine and compare the decomposition rates of leaves that belong to different mangrove species.

(iii) To determine the activity of nitrogen fixers in the mangrove swamp sediment.

(iv) To determine the levels of some organic and inorganic nutrients in the mangrove sediment.

(v) To isolate and characterize the dominant bacteria in the mangrove swamp.

2.2 STUDY AREA.

The study was carried out in the mangrove swamp at Gazi Bay in Kinondo Location of Kwale District.

2.2.1 Location.

Gazi Bay is located 50 km south of Mombasa on the Kenyan south coast (39^0 30'E; 4^0 24'S) (Survey of Kenya, 1967) (Fig 1). The Gazi Bay (Maftaha Bay) mangrove swamp covers an area of 15 km^2 of which 6.61 km^2 is covered by mangroves and seagrasses (Woitchik et al., 1993).

Fig 1. A map of the Kenyan coastline showing the location of mangroves in the shaded areas (after Isaac and Isaac, 1968).

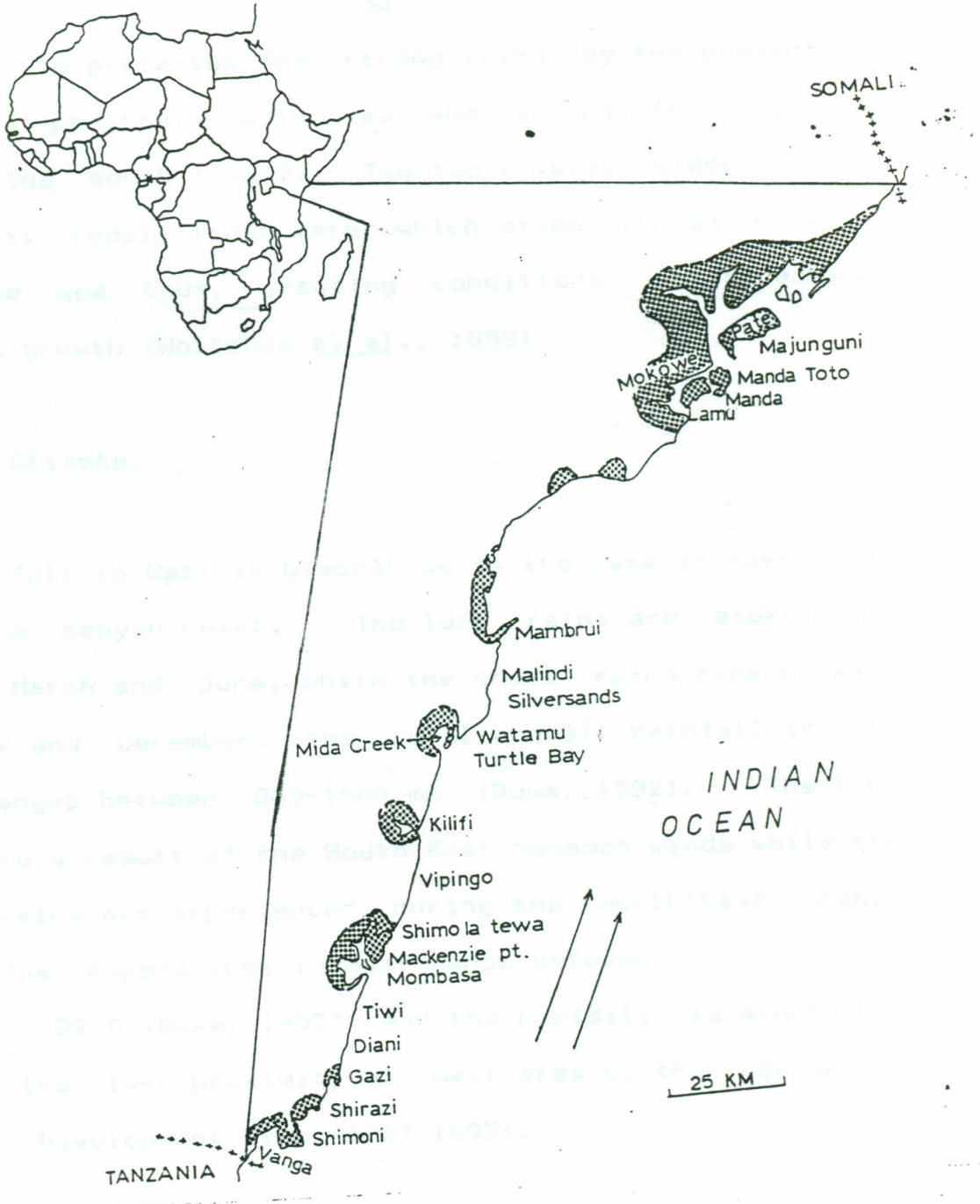

SOMALI
Majunguni
Manda Toto
Manda
Lamu
Mambrui
Malindi
Silversands
Watamu
Turtle Bay
Mida Creek
Kilifi
Vipingo
Shimo la tewa
Mackenzie pt.
Mombasa
Tiwi
Diani
Gazi
Shirazi
Shimoni
Vanga
TANZANIA
INDIAN
OCEAN
25 KM

Fig 1.

Gazi Bay is protected from strong waves by the presence of the Chale peninsula to the east and a coast fringing coral reef to the south (Fig 2). The two rivers, Kidogoweni and Mkurumudzi supply fresh water which mixes with saline water from the sea thus, creating conditions which enhance mangrove growth (Woitchik et al., 1993).

2.2.2 Climate.

The rainfall in Gazi is bimodal as is the case in most areas along the Kenyan coast. The long rains are experienced between March and June, while the short rains come between November and December. The total annual rainfall in the area ranges between 800-1500 mm (Ruwa, 1992). The long rains are a result of the South East Monsoon winds while the short rains are experienced during the North East Monsoon winds. The temperatures in Gazi range between 20^0 C and 32^0C (Ruwa, 1992) and the humidity is around 95% due to the close proximity of Gazi area to the sea (Kwale District Development Plan, 1987-1993).

Fig 2. A map of Gazi Bay and its surroundings.

Key.

Mangrove areas.

Mainland.

Inter-tidal seagrasses.

Sub-tidal seagrasses.

Intertidal flat

A *Aviccenia* plot

C *Ceriops* plot

R *Rhizophora* plot

LT Mean Low Tide (Neap).

EHT Extreme high tide

Source: Kenya Marine and Fisheries Research Institute (K.M.F.R.I).

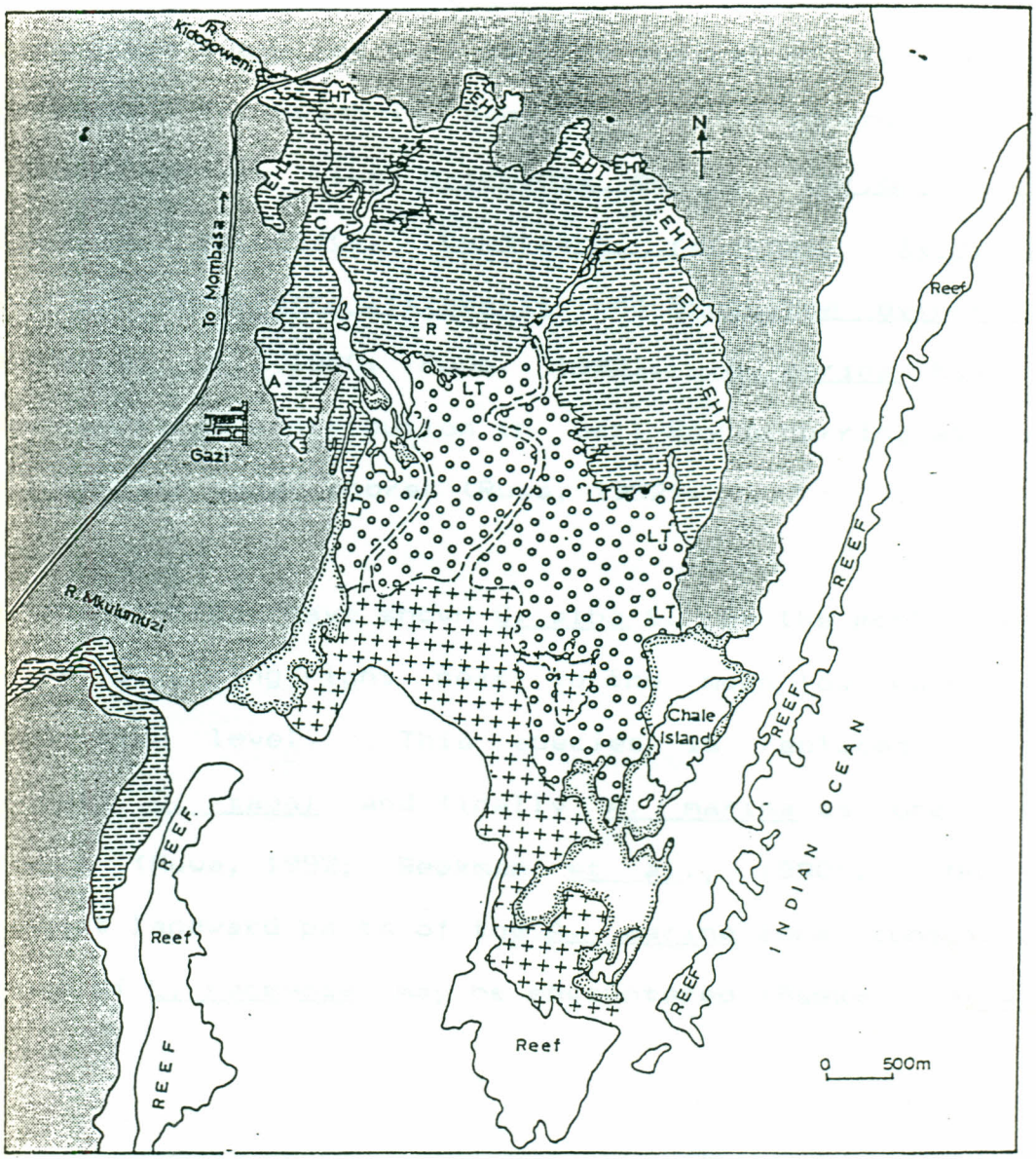

R. Kidogoweni
To Mombasa
Gazi
R. Mkuiumuzi
EHT
C
A
R
LT
N
Reef
REEF
Chale Island
INDIAN OCEAN
0 500m

Fig 2.

2.2.3 Vegetation.

The mangrove swamp at Gazi Bay is typical of East African mangroves. The swamp is poor in species composition and contains species like Aviccenia marina, Ceriops tagal, Rhizophora mucronata, Lumntzera racemosa, Xylocarpus granatum, Heritierra littoralis and Bruguiera gymnorrhiza (Beekman et al., 1990; Ruwa, 1992). A. marina has the widest vertical distribution in Gazi Bay occurring at both the lower and upper shores (Ruwa, 1992).

Zonation studies have shown S. alba to be the most seaward species occurring right next to the mean low water neap (MLWN) tide level. This species is replaced by R. mucronata, C. tagal and finally A. marina as one moves landward (Ruwa, 1992; Beekman et al., 1990). On the outermost landward parts of the A. marina zone conspicuous fringes of L. racemosa may be encountered (Beekman et al., 1990).

The zonation pattern of Gazi Bay mangroves is typical of East African Creek mangroves. Other mangrove species like B. gymnorrhiza, H. littoralis and X. granatum do not form pure stands and may be found mixed with other mangrove species in transition areas (Beekman et al., 1990).

Non-mangrove vegetation observed in Gazi Bay includes several halophytes like Sesuvium portulacastrum L., Sarliconia herbacea L., and Fimbristylis polytrichoides (Rezt) Vahl. These normally grow between sparsely populated Aviccenia vegetation (Beekman et al., 1990).

2.2.4 The Fauna of Gazi Bay.

The dominant animals on the Gazi mangrove forest floor are crabs mostly of the genera Uca (fiddler crabs), Sesarma, Dernadus (Hermit crab) and Thalamita (Ruwa, 1990; Slim et al., 1993). The gastropod Terebrelia palustris is also common on the forest floor.

The role and distribution of each of the animal species have been explained by Ruwa (1990) and Slim et al. (1993). Other macrofauna observed in Gazi Bay include the edible oyster (Crossostrea cucullata) and the barnacles which are found attached on the surfaces of the most seaward mangrove species S. alba and R. mucronata (Ruwa, 1990). Indeed the heavy felling of these two mangrove species for their excellent poles results in considerable loss of the edible oyster.

2.2.5 Anthropological Factors in Gazi Bay.

Gazi is a small village with two streets and about 100 homesteads. The lives of the people of Gazi are mostly sustained by the sea and the neighbouring mangrove swamp. Most of the people in Gazi and its surroundings are fishermen. Finfish, lobsters, shellfish, tigershells and sea cucumbers are caught throughout the year. The fish caught in Gazi are sold as far as Mombasa. The Kenya Marine and Fisheries Research Institute is currently constructing an artificial oyster farm along the Gazi creek (Plate 1).

Cutting of mangroves for building poles and fuelwood is also a common occupation for the people of Gazi. Cutting is done at low tide and the poles are transported to the harbour in canoes at high tide (Plate 2).

There is very little commercial agriculture going on at Gazi. Most of the area around Gazi Bay is a coconut plantation owned by Msambweni Development Farm, while the villagers grow a few food crops like cassava and peas. Recently a tourist hotel was established on Chale Island.

Plate 1. Part of the Oyster farm used by K.M.F.R.I for artificial cultivation of edible oysters.

Plate 2. Mangrove poles awaiting collection at the beach in Gazi Bay.

2.2.6 The Extent of Research in Gazi Bay.

Gazi Bay has attracted a lot of researchers because of its easy accessibility from Mombasa. Ruwa (1992); Beekman *et al.* (1990) did vegetation and zonation studies in the area. Woitchik *et al.* (1993) studied mangrove litter as a nutrient source, while Ruwa (1990) studied the distribution of macrofauna in the area. Litterfall studies for *Ceriops* and *Rhizophora* species were done by Slim and Gwada (1993). Currently a lot of research activity is going on in Gazi and more information on various topics will soon be out.

2.3 SAMPLING STATIONS.

Three sampling stations were selected in the Gazi mangrove swamp, depending on the dominant species of mangrove trees in the area namely, *Rhizophora mucronata*, *Ceriops tagal* and *Aviccenia marina*. These three make the bulk of the trees at the swamp covering about 80% of the area. They form pure stands from which the study sites were chosen. At each study site a plot was delineated, measuring 20 m X 20 m in area and a rope was used to mark the plot. The plots were then named the *Rhizophora* plot, the *Ceriops* plot and the *Aviccenia* plot. The position of the sampling stations in the swamp are shown in Figure 2.

2.3.1 The *Rhizophora* plot.

The plot lies next to the low water mark, just after *Sonneratia alba* which lies right next to the ocean. The *Rhizophora* trees are tall measuring about 20 metres in height. The plot is always submerged under water every high tide and its muddy black soils are always water-logged. A high prop root density is characteristic of the plot and the soils usually have very little decomposing litter evident on the surface (Plate 3).

2.3.2 The *Ceriops* plot.

The plot lies at about mid- tide level in the swamp and is also inundated every high tide. The plot has a sandy sediment in its border with the *Aviccenia*-*Ceriops* mixed stand, while on the lower side where the plot borders the *Rhizophora*-*Bruguiera* mixed stand the sediment is muddy. The trees measure about 1.5 - 2.5 metres (Plate 4).

2.3.3 The *Aviccenia* plot.

The plot is comprised of giant *Aviccenia marina* trees measuring about 5-10 metres. It occurs next to the extreme high water mark, where it borders tropical forests and coconut trees.

Plate 3. The Rhizophora plot during low tide.

Plate 4. The *Ceriops* plot during low tide.

The plot is usually inundated once every 15 days during the high spring tide. The sediment in this plot is light grey in appearance. Many A. marina seedlings and decomposing leaves could be seen on the surface of the sediment (Plate 5).

Plate 5. The Aviccenia plot during low tide revealing the large number of pneumatophores and Aviccenia marina seedlings.

CHAPTER THREE.

3.0 MATERIALS AND METHODS.

3.1 SAMPLING.

(i). Sediment samples for enumeration of bacteria and for estimation of nitrogen fixation were collected in triplicate using a plastic corer, diameter 1.5 cm , to a depth of 1 cm. The samples were collected from different parts of the plots and they were put into sterile bottles for onward transport to the laboratory. Sampling was always at low tide and the samples were not pooled.

(ii). Water samples from each plot were collected in triplicate by placing plastic containers tied to roots in different parts of the plot. At high tide water collected in these containers and during sampling (low tide), the water was transferred to sterile sample bottles.

(iii). Leaves were plucked off from mangrove plants using sterile forceps and were put into sterile petri-dishes. In every plot, senescent leaves which were judged to be near abscission were collected from plants situated in different parts of the plot. The petri-dishes were sealed with a cellotape to prevent contamination.

(iv). Sediment samples for analysis of organic matter, water content, and nitrate-nitrite concentration were collected in triplicate from a quadrat measuring 30 cm X 30 cm to a depth of 1 cm. Large quantities of sediments were required for this experiment. The sediment was usually scrapped off the surface using a scoop and was packed into clean (new) nylon bags which were tightly closed to prevent entry or loss of water.

3.2 ENUMERATION OF BACTERIA.

3.2.1 Aerobic Heterotrophic Bacteria in the Sediment.

A weighed sediment sample (1 g) was diluted serially in Sterile seawater to 10^{-6} with vigorous shaking at each dilution to ensure mixing. Then 0.05 ml samples were withdrawn from dilutions 10^{-4}, 10^{-5} and 10^{-6} using sterile pipettes and were transferred onto seawater nutrient agar (Labm) plates where they were spread using a sterilized bent glass rod. The plates were then incubated at 27^{0} C for three days, after which the colonies on the plates were counted using a colony counter (Gallenkamp). Plate counts between 20 and 300 colonies were used for calculations.

3.2.2 Aerobic Heterotrophic Bacteria in Water.

Bacteria suspensions in water samples were serially diluted in sterile seawater to 10^{-5}. Then, 0.05 ml samples from dilutions 10^{-3}, 10^{-4} and 10^{-5} were then transferred on to seawater nutrient agar plates. The sample was spread on the agar surface and incubated at 27^{0} C for 5 days.
The resultant colonies were enumerated using a colony counter (Gallenkamp). Plate counts between 20 and 300 colonies were used for calculations.

3.2.3 Anaerobic Heterotrophic Bacteria in Sediment.

Sediment samples weighing 1 g were serially diluted with sterile seawater to 10^{-6}. Then 0.05 ml of diluted sample from dilutions 10^{-4} and 10^{-5} were transferred and spread on seawater nutrient agar plates. The plates were incubated in an anaerobic jar at 27^{0} C. Anaerobic conditions were achieved by burning candles in the jar to consume the air after which the jar was purged with nitrogen gas. A methylene blue strip was earlier on placed in the jar as a visible check on anaerobic conditions. The jar was always closed tightly to keep the conditions anaerobic.

3.2.4 Aerobic Heterotrophic Bacteria on Senescent Leaves.

A leaf was spread out in a sterile petri-dish, and then a 1 cm^2 piece was cut from the leaf using sterile forceps, a sterile Rule, and a sterile blade. The piece of leaf was further chopped into smaller bits and was put into a test tube containing 9 ml of sterile sea water. These were shaken vigorously for about 3 minutes since bacteria do not come off easily from leaf surfaces, and were then serially diluted to 10^{-2}. Then, 0.05 ml samples from dilutions 10^{-1} and 10^{-2} were plated on nutrient agar plates and incubated for 5 days at 27^0 C. Resultant colonies were counted using a colony counter (Gallenkamp).

3.2.5 Nitrate Reducing Bacteria in the Sediment.

The population of nitrate reducing bacteria was estimated using the MPN method (Colwell et al., 1975). Sediment samples were serially diluted with sterile seawater to 10^{-3}. Then 0.1 ml samples from dilutions 10^{-1}, 10^{-2} and 10^{-3} were inoculated into test-tubes containing nitrate broth as in Colwell et al.(1975). The tubes were incubated at 35^0 C for 3 days and were tested for nitrate reduction using sulfalinic acid and α-naphthalmine solutions as described by Kerr (1985). The numbers of nitrate reducers were then interpreted using MPN tables (Refai, 1979).

3.2.6 Activity of Cellulolytic Microorganisms.

Filter papers (Whatman No 1) were weighed and then placed into carefully labelled litterbags of mesh size 1 mm. Eight sets of 5 litterbags were placed in each plot.

The filter papers in the litterbags were then left to decompose afterwhich the litterbags were retrieved from the plots at given intervals. They were taken to the laboratory where the filter papers were removed and carefully washed to remove sand and animals attached to them. The filter papers were then dried to constant weight in an oven at 105^0 C. Percentage weight loss from filter papers was taken as the measure of cellulose decomposition and it was calculated as follows.

$$\frac{FWB - FWA}{FWB.} \times 100.$$

Where: FWB is filter paper weight before decomposition.

FWA is filter paper weight after decomposition.

3.2.7 Nitrogenase Activity in the sediment.

The rate of nitrogen fixation in each plot was estimated by the acetylene reduction method (Drevon, 1983; Hardy et al., 1968; Hardy and Holsten, 1977; Knowles, 1980; Postgate, 1972).

One gram of a wet sediment sample was placed in a 10 ml serum bottle which was then tightly sealed using a rubber stopper and special aluminium cap. Then, 1 ml of acetylene was introduced into the bottles using special glass syringes which are fixed to a needle and the samples were incubated for 30 minutes at room temperature.

After incubation 100 µl of gas were retrieved from the bottle after careful mixing using a syringe. The gas samples were then analyzed on a Gas Chromatograph (GC) (Varian 3300) using a flame ionization detector (Drevon, 1983; Hardy and Holsten, 1977). A Pora Pak R column was used and its temperature was set at 50^0 C. The flow rate of nitrogen the carrier gas was 30 ml/minute. Three sediment samples from each plot were analysed for acetylene reduction activity. Control incubation bottles containing sediment samples from the three plots but, without acetylene were used for detecting endogenous ethylene.

The gas phase from from each bottle was sampled at regular intervals gas chromatographic analysis. The ethylene concentration in each sample bottle was calculated using a standard of known ethylene concentration. A graph of time against C_2H_4 concentration was then drawn for each sample and the gradient was taken as the rate of acetylene reduction (Knowles, 1980).

3.3 DECOMPOSITION OF MANGROVE LITTER.

Over 200 senescent leaves observed to be near abscission were picked from each of the three species. For each species, the leaves were split into two groups, a reference group and an experimental group (Fell and Master, 1984). The reference group was used to predict the dry weight of leaves in the experimental group. The reference group were split into eight sets, whose fresh weight were taken. These were then dried separately in an oven at 105^{0} C to a constant weight. The values were then used to calculate the relationship between dry weight and fresh weight of leaves for each species. Once known, the relationship was used to predict the initial dry weight of leaves in the experimental group.

Fresh senescent leaves of known weight were placed in litterbags. For every species there were 8 sets of 5 litterbags placed in their native plots. The bags were firmly tied to roots so as to float during high tide and to lie on the sediment surface at low tide. One set of litterbags was then retrieved from each plot every one or two weeks until all the litterbags had been completed. The retrieved leaves were taken to the laboratory where they were washed carefully and oven dried to a constant weight.

Decomposition was estimated as percentage dry weight loss. The percentage dry weight loss from leaves was calculated as follows.

$$\frac{PDWB - DWA}{PDWB} \times 100.$$

Where: PDWB is predicted dry weight before incubation.

DWA is dry weight after incubation.

3.4 NUTRIENT ANALYSIS OF MANGROVE SEDIMENT.

3.4.1 Concentrations of Combined NO_3^- and NO_2^- in the Sediment.

Sediment samples were squeezed using a vacuum filter pump and the pore water was collected in clean plastic bottles. The water was then analyzed for levels of combined nitrates and nitrites in the Autoanalyzer (Technicon, New York, U.S.A) which uses the Cadmium reduction method described in Golterman et al. (1978). Standards were prepared by dissolving KNO_3 in deionized water so as to have concentrations between 0-5 µg/l. Resultant peaks for the standards were used to draw a regression graph of concentration against peak heights.

From this graph the concentration of combined nitrate/nitrite in the sample could be obtained by reading from the peak height for that sample. Mangrove sediment samples have low nitrite concentration in their sediments.

The nitrites are very unstable and are quickly converted to nitrates or gaseous nitrogen by various biological processes (Chandramohan, 1988). Thus, in this study due to frequent delays experienced in nutrient analysis caused by the large amount of work that had to be accomplished, it was decided that the nitrates and nitrites be analyzed together.

3.4.2 Water Content in the Sediment.

Three sediment samples from each plot were weighed, oven dried at 105^{0} C and their percentage water content calculated as detailed in Allen et al. (1974).

3.4.3 Organic Matter Content in the Sediment.

Sediment samples (3 g) dried at 105^{0} C from each plot were, ignited in a muffle furnace at 450^{0} C as described in Allen et al. (1974). After ignition the sediments were allowed to cool in a desiccator and then weighed. The amount of organic matter present in each sample was calculated as a percentage of the dry weight (Allen et al., 1974).

3.5 CHARACTERIZATION OF BACTERIA ISOLATES.

3.5.1 Isolation of Pure Cultures.

The dominant colonies obtained from each plot during enumeration were picked and streaked across nutrient agar plate so as to get pure cultures using an inoculation loop. Streaking was repeated until it was evident that the colonies on the plates were pure cultures (Kerr, 1985). These isolates were then subjected to morphological examination, physical and biochemical tests with the aim of characterizing them.

3.5.2 Colony Characteristics.

Three day old colonies were observed for the following features; colour, elevation, margin type, and colony shape.

3.5.3 Salt Tolerance.

Isolates were tested for growth on nutrient agar plates prepared with 0%, 10%, 15%, 20% and 24% NaCl (Kerr, 1985). The various salt concentrations stated above were obtained by dissolving 0 g, 10 g, 15 g, 20 g and 24 g of NaCl in distilled water and diluting the solution to 100 ml. The salt solutions were then used to prepare the media with the respective salt concentrations.

Each isolate was streaked across the media with various salt concentrations and the cultures were incubated at 27^0 C for 48 h. Plates were then observed for growth of the various cultures.

3.5.4 pH Range of Growth.

Isolates were tested for growth on nutrient agar adjusted to pH 4.5, 6.3 and 9.0. The original pH for seawater nutrient agar was 6.3, while pH 9.0 was obtained by adding 10 ml of 1M Na_2CO_3 into 100 ml of the seawater nutrient agar. Media with pH 4.5 was obtained by adding 0.25 ml of 5% sulphuric acid to 100 ml of seawater nutrient agar.

3.5.5 Gram Stain.

Gram staining of 48h old cultures was done as described by Kerr (1985).

3.5.6 Cell Size.

The length for rods and diameter for cocci bacteria were measured from smears of 48h old cultures using stage and ocular micrometers (Olympus) under oil immersion (Kerr, 1985).

3.5.7 Spore Staining.

Staining for endospores was done on one week old colonies of all rod shaped bacteria as in Kerr (1985).

3.5.8 Motility.

Tests for motility were done by observing 48h old broth cultures using the hanging drop technique under the compound light microscope (Olympus) (Kerr, 1985).

3.5.9 Carbohydrate Fermentation.

Pure cultures of the isolates were tested for their ability to ferment xylose, maltose, glucose, lactose and mannitol to produce acid and/or gas. Isolates were inoculated into carbohydrate media containing the above sugars. In all tests, 0.002% of methyl red was added as an indicator. Cultures that were able to ferment any of the sugars to produce acid turned the indicator from red to yellow. Any gas produced was trapped in Durham's tubes (Kerr, 1985).

3.5.10 Starch Hydrolysis.

To test for their ability to degrade starch, isolates were grown on nutrient agar containing 1% starch for 3 days at 27^{0} C. The cultures were tested for starch hydrolysis using a dilute iodine solution. Where the isolates had hydrolysed starch clear zones were observed, while a dark blue colour was evident where starch had not been hydrolysed (Kerr, 1985).

3.5.11 Gelatin Hydrolysis.

Pure cultures were grown on gelatin nutrient agar plates prepared by adding 2% gelatin solution onto nutrient agar before autoclaving. The cultures were incubated for 3 days after which they were tested for ability to hydrolyse gelatin using a saturated ammonium sulfate solution (Kerr, 1985). Clear zones were observed where the isolates had hydrolysed gelatin, while a white precipitate was evident where the isolates had not hydrolysed gelatin.

3.5.12 Oxidase Test.

A filter paper was impregnated with freshly prepared Kovak's Oxidase reagent (n,n,n'n'-tetramethyl-p-phenylene diamine dihydrochloride) in a petri-dish.

With the help of a platinum loop each organism was smeared on a filter paper. Colonies which developed purple colour within 10 - 60 seconds were oxidase positive (Kerr, 1985).

3.5.13 Catalase Activity.

The activity of the enzyme catalase in each colony was tested by adding a portion of the pure isolate to 3% hydrogen peroxide solution on a slide and observing for effervescence (Kerr, 1985).

3.5.14 Nitrate Reductase.

The ability of each isolate to reduce nitrate was tested by growing the isolate on nitrate broth and testing for presence of nitrites using sulfalinic acid and α-naphthalamine solutions as described by Colwell et al. (1975); Kerr (1985). A violet or maroon colour is the positive test for reduction of nitrate to nitrite.

For test-tubes which gave a negative reaction zinc powder was added. If the colour failed to develop after the addition of zinc powder, then the isolates had reduced the nitrate to either ammonia or gaseous nitrogen. Where the maroon colour developed after addition of zinc powder the isolates had not reduced the nitrate.

3.5.15 Haemolysis.

All bacteria isolates were tested for ability to haemolyse red blood cells by growing them in blood agar (GIBCO) and observing for zones of clearing around the bacteria colonies (Kerr, 1985).

3.5.16 Cellulose Hydrolysis.

All the isolates were grown on the cellulose medium described by (Schneider and Rheinheimer, 1988), where pieces of cellulose filter paper was the only carbon source provided. As a source of nitrogen, KNO_3 and NH_4Cl were added to the medium. All colonies that were able to grow on this medium were judged to be cellulose hydrolysers.

3.5.17 Urea Hydrolysis.

Colonies were tested for urease activity using media incorporated with urea (Kerr, 1985). Phenol red was used as an indicator and turned from yellow to pink where urea was hydrolysed. Urea was filter-sterilized and was added to the rest of the media when the temperature had cooled to about 45^0 C

3.6 RAINFALL DATA.

Daily rainfall figures observed during the sampling period for the nearest weather station, Msambweni Agricultural Office (Ref No. 9439014) were obtained from the Meteorological Department. The weather station lies about 5 km south of Gazi Bay.

3.7 DATA ANALYSIS.

The data collected was analyzed using the SPSS computer package as two factor anovar with replication. Where there was a significant difference the LSD (Least Significant Difference) test was done to compare the means.
Bar and line graphs were used to present trends of various parameters during the sampling period and where necessary correlation between variables was computed.

CHAPTER FOUR.

4.0 RESULTS.

4.1 ENUMERATION OF BACTERIA.

4.1.1 Aerobic Heterotrophic Bacteria in the Sediment.

The average number of aerobic heterotroph bacteria in sediment collected in the Rhizophora plot ranged between 1.09×10^7 and 2.10×10^8 cfu/g dwt. The number of aerobic heterotrophic bacteria/g dwt of sediment in the Aviccenia plot ranged from $1.38 \times 10^7 - 2.18 \times 10^8$ cfu/g dwt, while in the sediment collected from the Ceriops plot bacteria numbers ranged from $7.44 \times 10^6 - 4.70 \times 10^8$ cfu/g dwt (Table 5).

The numbers of aerobic heterotrophic bacteria observed during the sampling period are presented in Fig 3. Higher numbers of bacteria were observed in October, November and early December in all the three plots. On 19th January all the plots recorded their lowest numbers of aerobic heterotrophic bacteria.

Fig 3. Average number of aerobic heterotrophic bacteria (cfu/g dwt) in sediment collected from Gazi Bay between October 1992 and February 1993.

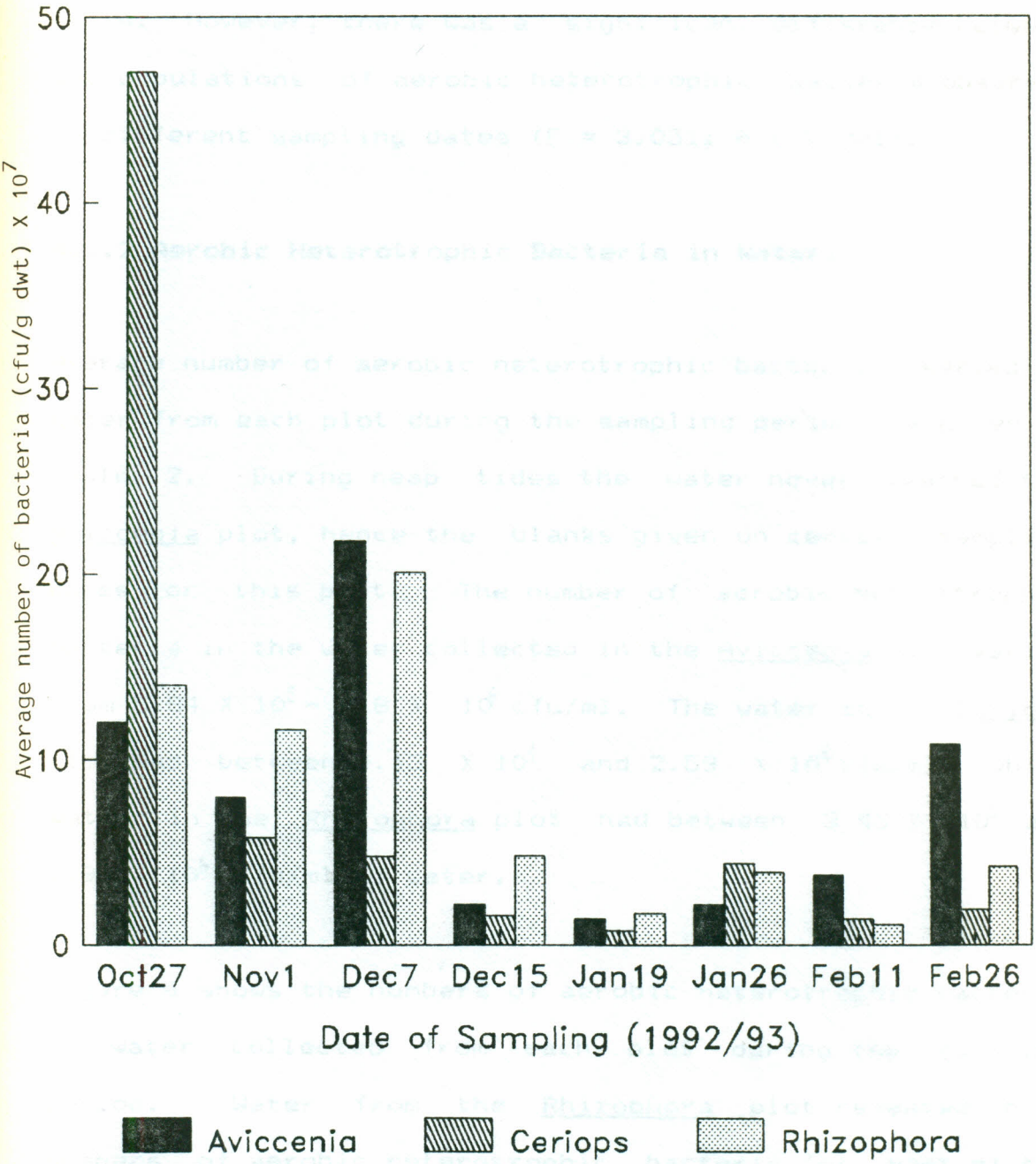

50
40
30
20
10
0
Average number of bacteria (cfu/g dwt) X 10^7
Oct27
Nov1
Dec7
Dec15
Jan19
Jan26
Feb11
Feb26
Date of Sampling (1992/93)
Aviccenia
Ceriops
Rhizophora

Fig 3

Analysis of variance showed no significant difference in the numbers of aerobic heterotrophic bacteria among the three plots. However, there was a significant difference between the populations of aerobic heterotrophic bacteria observed on different sampling dates ($F = 3.051$; $P < 0.001$).

4.1.2 Aerobic Heterotrophic Bacteria in Water.

Average number of aerobic heterotrophic bacteria observed in water from each plot during the sampling period are given in Table 2. During neap tides the water never reached the Aviccenia plot, hence the blanks given on certain sampling dates for this plot. The number of aerobic heterotrophic bacteria in the water collected in the Aviccenia plot varied from $5.84 \times 10^5 - 6.8 \times 10^6$ cfu/ml. The water in the Ceriops plot had between 4.13×10^4 and 2.59×10^6 cfu/ml, while water in the Rhizophora plot had between 3.43×10^5 and 8.90×10^6 cfu/ml of water.

Figure 4 shows the numbers of aerobic heterotrophic bacteria in water collected from each plot during the sampling period. Water from the Rhizophora plot revealed high numbers of aerobic heterotrophic bacteria for most of the sampling dates.

Fig 4. Average number of aerobic heterotrophic bacteria (cfu/ml) in water collected from Gazi Bay between October 1992 and February 1993.

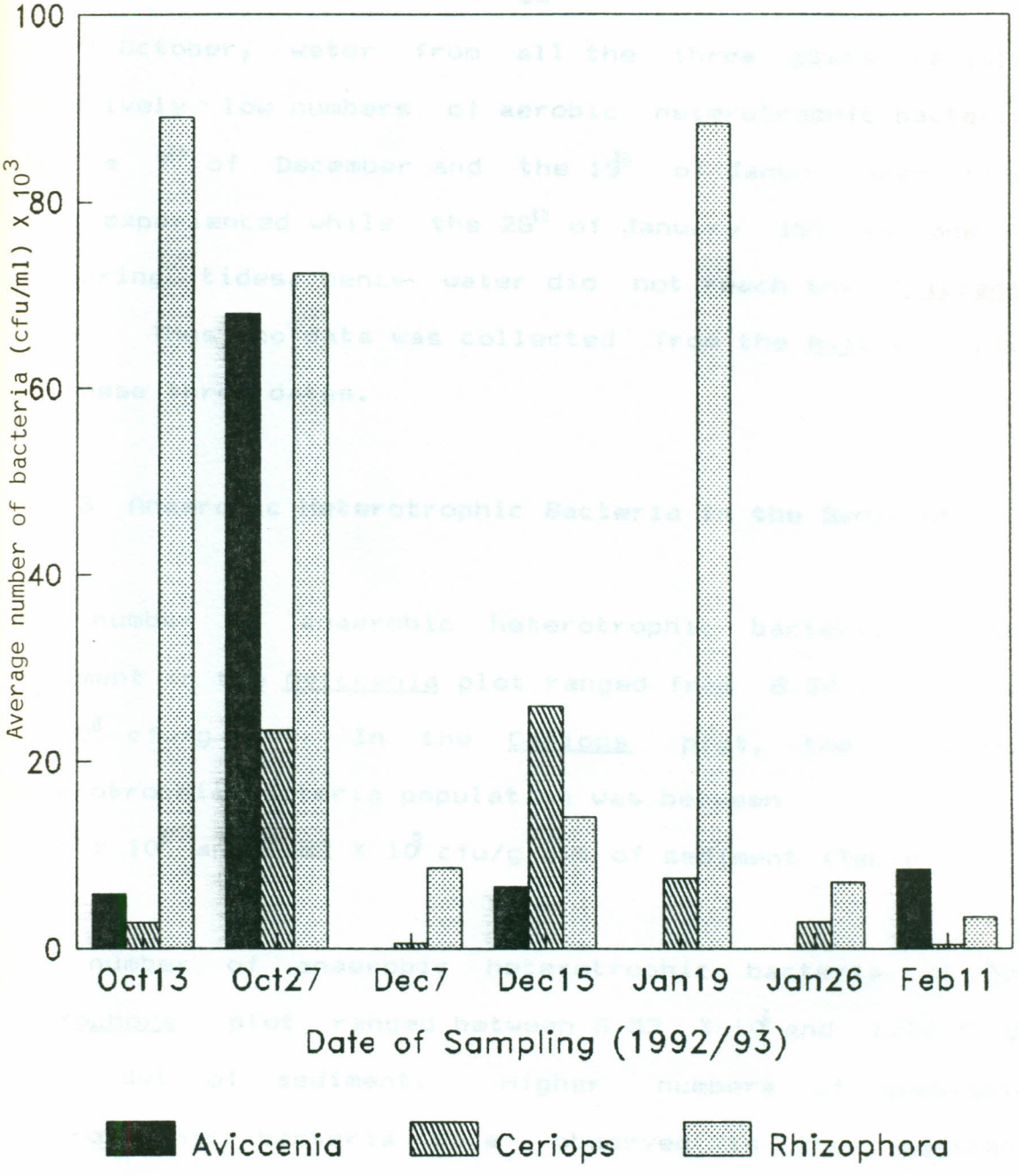

100
80
60
40
20
0
Average number of bacteria (cfu/ml) X 10^3
Oct13
Oct27
Dec7
Dec15
Jan19
Jan26
Feb11
Date of Sampling (1992/93)
Aviccenia
Ceriops
Rhizophora

Fig 4

After October, water from all the three plots revealed relatively low numbers of aerobic heterotrophic bacteria. On the 7th of December and the 19th of January neap tides were experienced while the 26th of January 1993 was one day to spring tides, hence water did not reach the Aviccenia plot. Thus, no data was collected from the Aviccenia plot on these three dates.

4.1.3 Anaerobic Heterotrophic Bacteria in the Sediment.

The number of anaerobic heterotrophic bacteria in the sediment in the Aviccenia plot ranged from $8.20 \times 10^6 - 1.44 \times 10^8$ cfu/g dwt. In the Ceriops plot, the anaerobic heterotrophic bacteria population was between 7.45×10^6 and 1.82×10^8 cfu/g dwt of sediment (Table 3).

The number of anaerobic heterotrophic bacteria in the Rhizophora plot ranged between 8.53×10^6 and 1.74×10^8 cfu/g dwt of sediment. Higher numbers of anaerobic heterotrophic bacteria were observed in the sediment collected from the Aviccenia plot, than in the other two plots. Sediment from all the three plots had their highest number of anaerobic heterotrophic bacteria between October and early December.

Fig 5. Average number of anaerobic heterotrophic bacteria (cfu/g dwt) observed in sediment in Gazi Bay on different sampling dates.

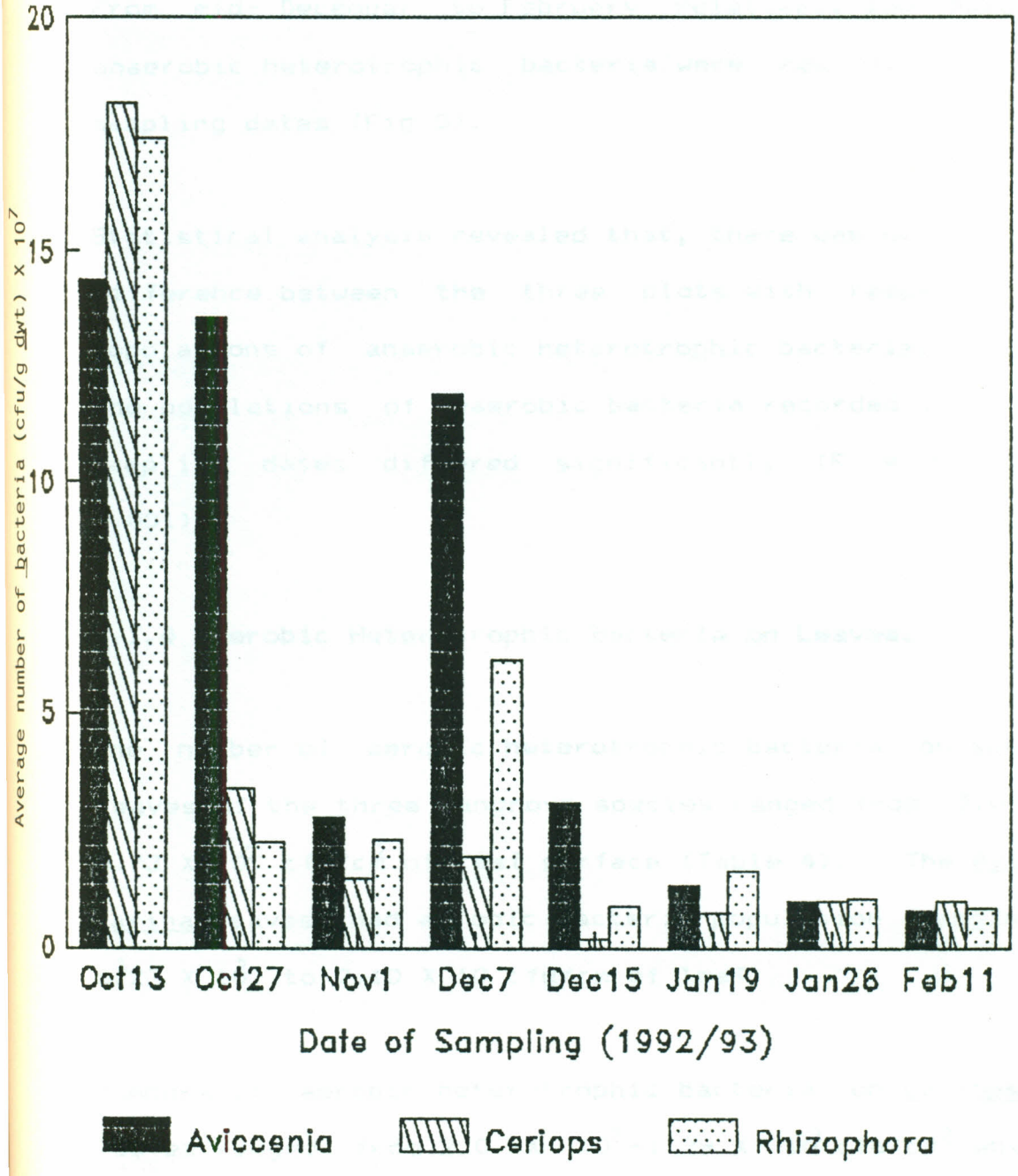

20
15
10
5
0
Average number of bacteria (cfu/g dwt) x 10^7
Oct13
Oct27
Nov1
Dec7
Dec15
Jan19
Jan26
Feb11
Date of Sampling (1992/93)
Aviccenia
Ceriops
Rhizophora

Fig 5

From mid- December to February relatively low numbers of anaerobic heterotrophic bacteria were recorded on all the sampling dates (Fig 5).

Statistical analysis revealed that, there was no significant difference between the three plots with respect to the populations of anaerobic heterotrophic bacteria. However, the populations of anaerobic bacteria recorded in different sampling dates differed significantly ($F = 8.126$; $P < 0.001$).

4.1.4 Aerobic Heterotrophic Bacteria on Leaves.

The number of aerobic heterotrophic bacteria on senescent leaves of the three mangrove species ranged from 2.0×10^2 - 9.73×10^5 cfu/cm^2 of leaf surface (Table 4). The Aviccenia marina leaves had aerobic bacteria population ranging from 6.523×10^2 to 2.10×10 cfu/cm of leaf.

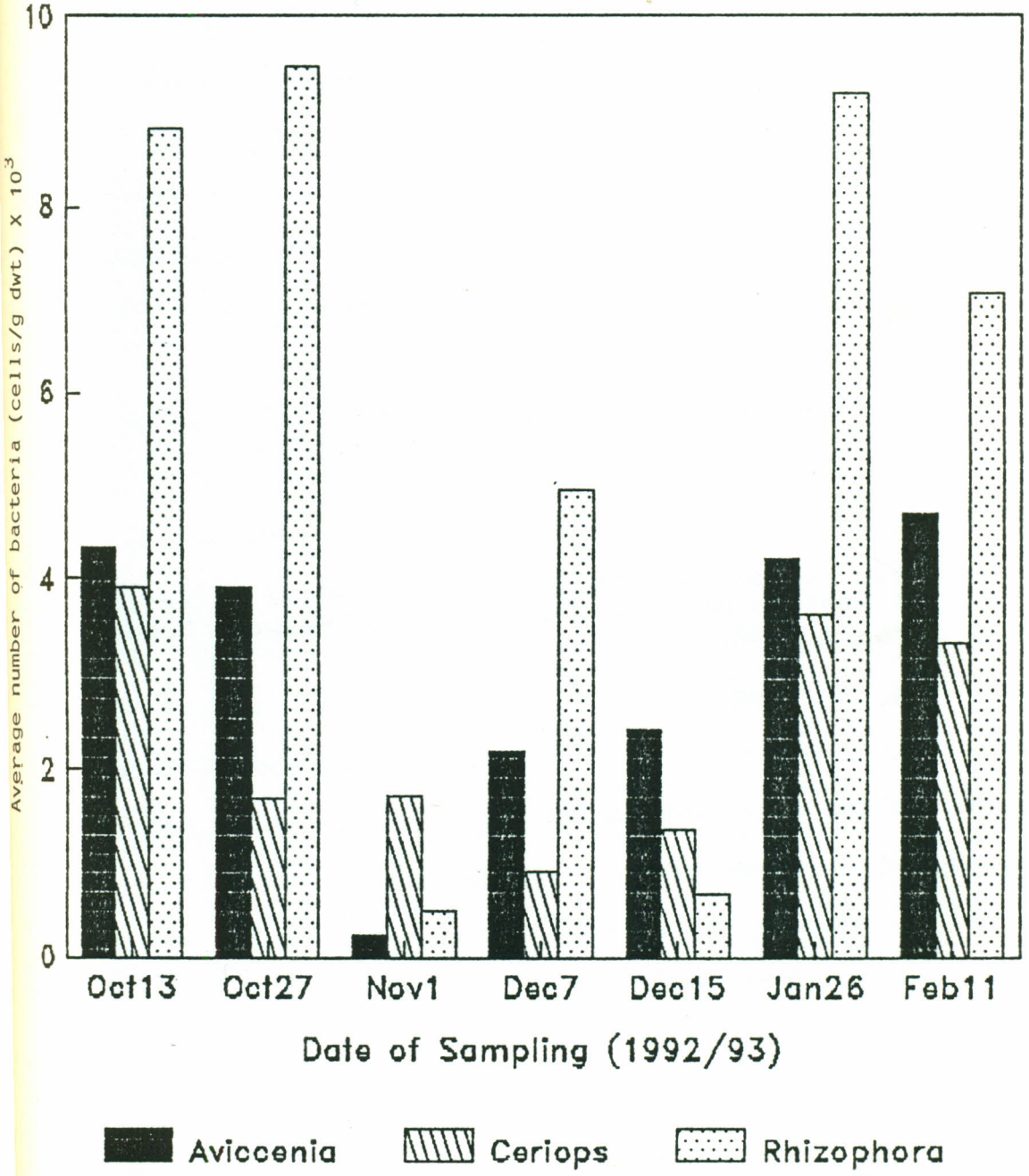
Numbers of aerobic heterotrophic bacteria on Ceriops tagal leaves ranged from 2.0×10^2 - 1.39×10^3 cfu/cm^2 while on Rhizophora mucronata leaves the numbers ranged from 4.0×10^2 - 1.42×10^5 cfu/cm^2 of leaf surface.

Ceriops tagal leaves had relatively low numbers of aerobic heterotrophic bacteria on their surfaces compared to the other mangrove species that were studied. There was a significant difference between the three mangrove speciess with respect to the populations of aerobic heterotrophic bacteria ($F = 61.922$; $P < 0.001$).

4.1.5 Nitrate Reducing Bacteria in the Sediment.

Average number of nitrate reducing bacteria in the sediments at Gazi Bay ranged between 2.37×10^2 and 9.21×10^3 bacteria/g dwt of sediment (Table 5).

The population of nitrate reducing bacteria in the Rhizophora plot ranged from 5.00×10^2 - 9.21×10^3 bacteria/g dwt, while the population in the Aviccenia plot ranged from 2.37×10^2 -4.33×10^3 bacteria/g dwt of sediment. Numbers of nitrate reducing bacteria in the Ceriops plot varied between 8.99×10^2 and 9.21×10^3 bacteria/g dwt of sediment. Figure 6 shows numbers of nitrate reducing bacteria recorded in each plot during the sampling period.


10
8
6
4
2
0
Average number of bacteria (cells/g dwt) X 10^3
Oct13
Oct27
Nov1
Dec7
Dec15
Jan26
Feb11
Date of Sampling (1992/93)
Aviccenia
Ceriops
Rhizophora

ig 6

Fig 6. Average number of nitrate reducing bacteria (cells/ g dwt) in sediment in Gazi Bay in the period between October 1992 and February 1993.

In the Rhizophora plot the numbers of nitrate reducing bacteria were high in October, they decreased from November to December and were high again from January to February. The same trend was observed for the populations of nitrate reducing bacteria in the Aviccenia and Ceriops plots. The population of nitrate reducing bacteria observed in the Rhizophora plot was higher than in the other plots for most of the sampling dates.

4.1.6 Activity of Cellulolytic Microoganisms.

Cellulose decomposition was faster in the Aviccenia plot where 90% of the filter paper dry weight was lost in 28 days. In the Rhizophora plot the filter papers lost about 72% of their dry weight in the same duration while not more than 20% dry weight was lost from filter papers in the Ceriops plot (Table 6). Thus, the cellulose decomposition curve for the Aviccenia plot rises faster than the curves for the other two plots. This is an indication of a high activity of cellulolytic microorganisms in the Aviccenia plot (Fig 7).

Fig 7. Cellulose decomposition in Gazi Bay expressed as percentage dry weight loss from filter papers.

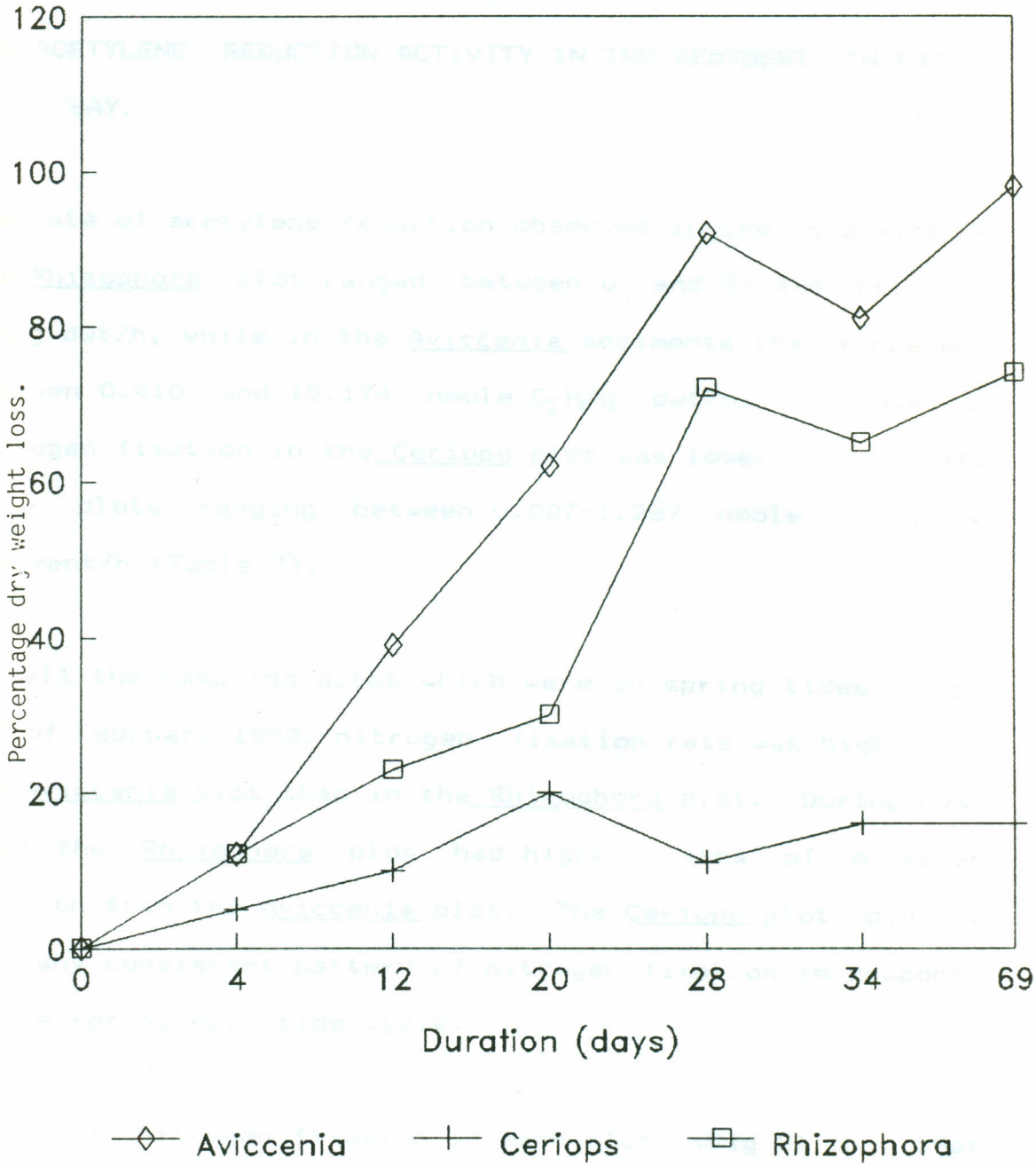

120
100
80
60
40
20
0
Percentage dry weight loss.
0
4
12
20
28
34
69
Duration (days)
Aviccenia
Ceriops
Rhizophora

Fig 7

4.2 ACETYLENE REDUCTION ACTIVITY IN THE SEDIMENT IN GAZI BAY.

The rate of acetylene reduction observed in the sediment in the Rhizophora plot ranged between 0 and 51.474 nmole of C_2H_4/g dwt/h, while in the Aviccenia sediments the range was between 0.410 and 15.174 nmole C_2H_4/g dwt/h. The rate of nitrogen fixation in the Ceriops plot was lower than in the other plots ranging between 0.007-1.287 nmole C_2H_4/g dw sediment/h (Table 7).

For all the sampling dates which were on spring tides except 11th of February 1993, nitrogen fixation rate was higher in the Aviccenia plot than in the Rhizophora plot. During neap tides the Rhizophora plot had higher rates of nitrogen fixation than the Aviccenia plot. The Ceriops plot did not show any consistent pattern of nitrogen fixation in response to the spring-neap tide cycle.

Trends of nitrogen fixation in each plot (Fig 8) show that all the plots experienced their highest rates of nitrogen fixation on 7th of December among the sampling dates. There was a significant difference between the plots with respect to the rate of nitrogen fixation ($F = 13.533$; $p < 0.001$).

Fig 8. Rates of acetylene reduction activity (nmole C_2H_4/g dwt/h) in sediment associated with Aviccenia, Ceriops and Rhizophora plots during the study period.

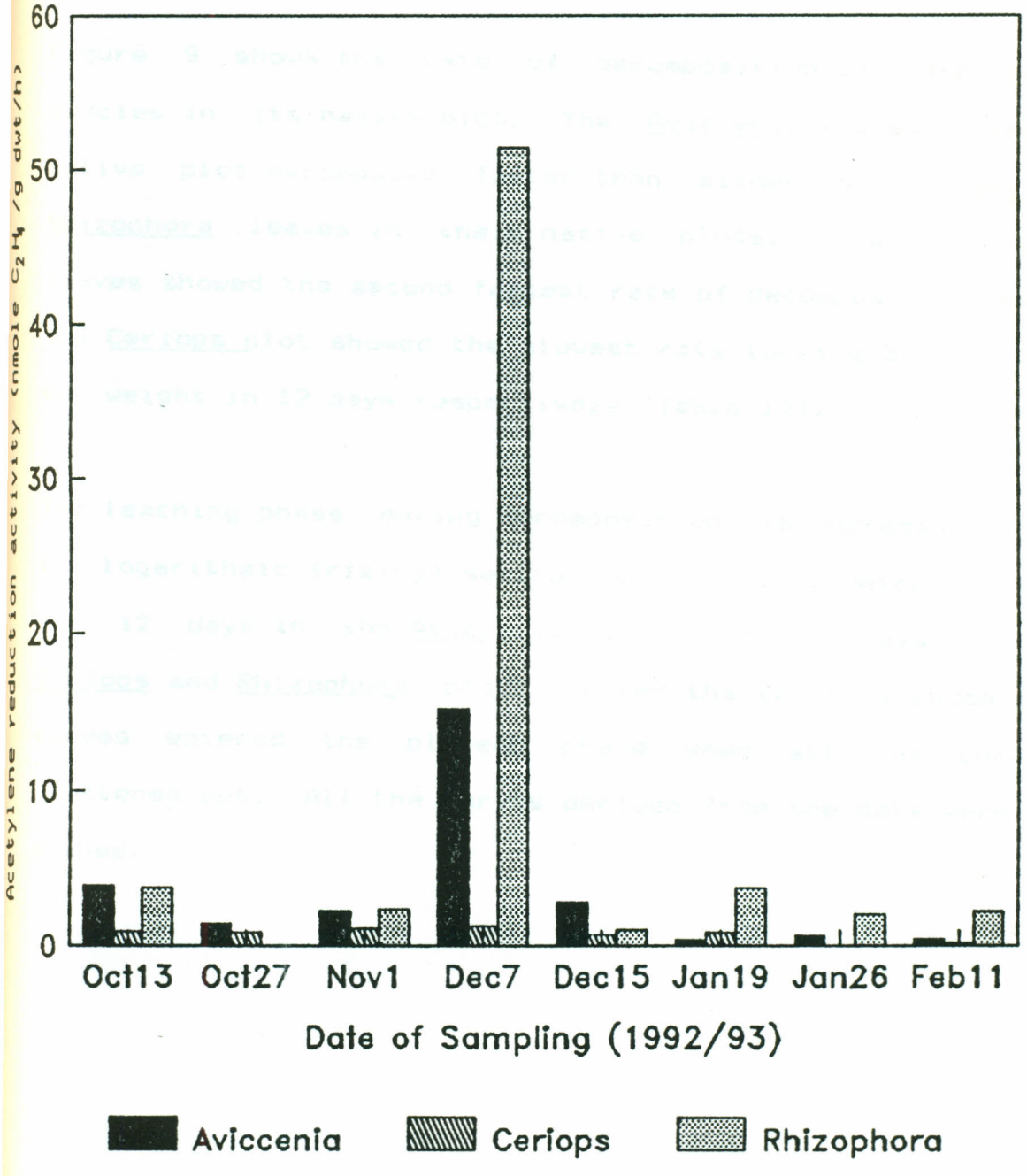

Acetylene reduction activity (nmole C_2H_4 /g dwt/h)
60
50
40
30
20
10
0
Oct13
Oct27
Nov1
Dec7
Dec15
Jan19
Jan26
Feb11
Date of Sampling (1992/93)
Aviccenia
Ceriops
Rhizophora

Fig 8

4.3 LEAF DECOMPOSITION.

Figure 9 shows the rate of decomposition of each leaf species in its native plot. The Aviccenia leaves in their native plot decomposed faster than either the Ceriops or Rhizophora leaves in their native plots. The Rhizophora leaves showed the second fastest rate of decomposition while the Ceriops plot showed the slowest rate loosing 32% and 22% dry weight in 12 days respectively (Table 12).

The leaching phase during decomposition is represented by the logarithmic (rising) section of the curve which lasted for 12 days in the Aviccenia plot, and 20 days in the Ceriops and Rhizophora plots. After the leaching phase the curves entered the plateau phase when all the curves flattened out. All the curves derived from the data were S-shaped.

Fig 9. Decomposition of leaves in Gazi Bay expressed as percentage dry weight loss in a given period of time.

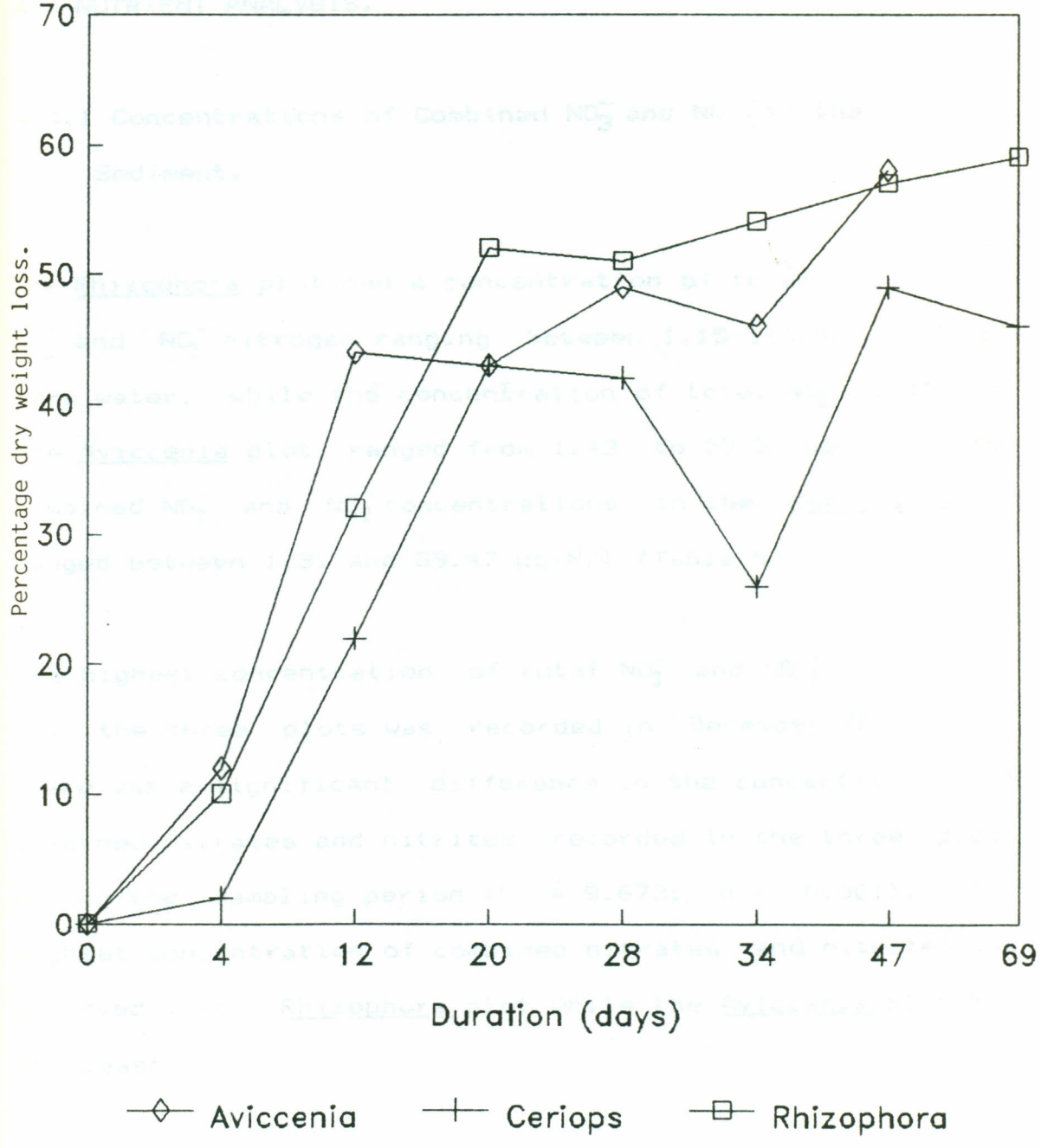

70
60
50
40
30
20
10
0
Percentage dry weight loss.
0
4
12
20
28
34
47
69
Duration (days)
Aviccenia
Ceriops
Rhizophora

Fig 9

4.4 NUTRIENT ANALYSIS.

4.4.1 Concentrations of Combined NO_3^- and NO_2^- in the Sediment.

The Rhizophora plot had a concentration of total NO_3^- and NO_2^- nitrogen ranging between 1.15-113.92 µg-N/l of pore water, while the concentration of total NO_3 and NO_2 in the Aviccenia plot ranged from 1.43 to 53.39 µg-N/l. The combined NO_3^- and NO_2^- concentrations in the Ceriops plot ranged between 1.31 and 69.47 µg-N/l (Table 9).

The highest concentration of total NO_3^- and NO_2^- nitrogen in all the three plots was recorded in December (Fig 11). There was a significant difference in the concentration of combined nitrates and nitrites recorded in the three plots during the sampling period ($F = 9.673$; $p < 0.001$). The highest concentration of combined nitrates and nitrites was observed in the Rhizophora plot while the Aviccenia plot had the least

Fig 10. Concentrations of combined nitrates and nitrites (µg/litre) in sediment collected in Gazi Bay in different sampling dates.

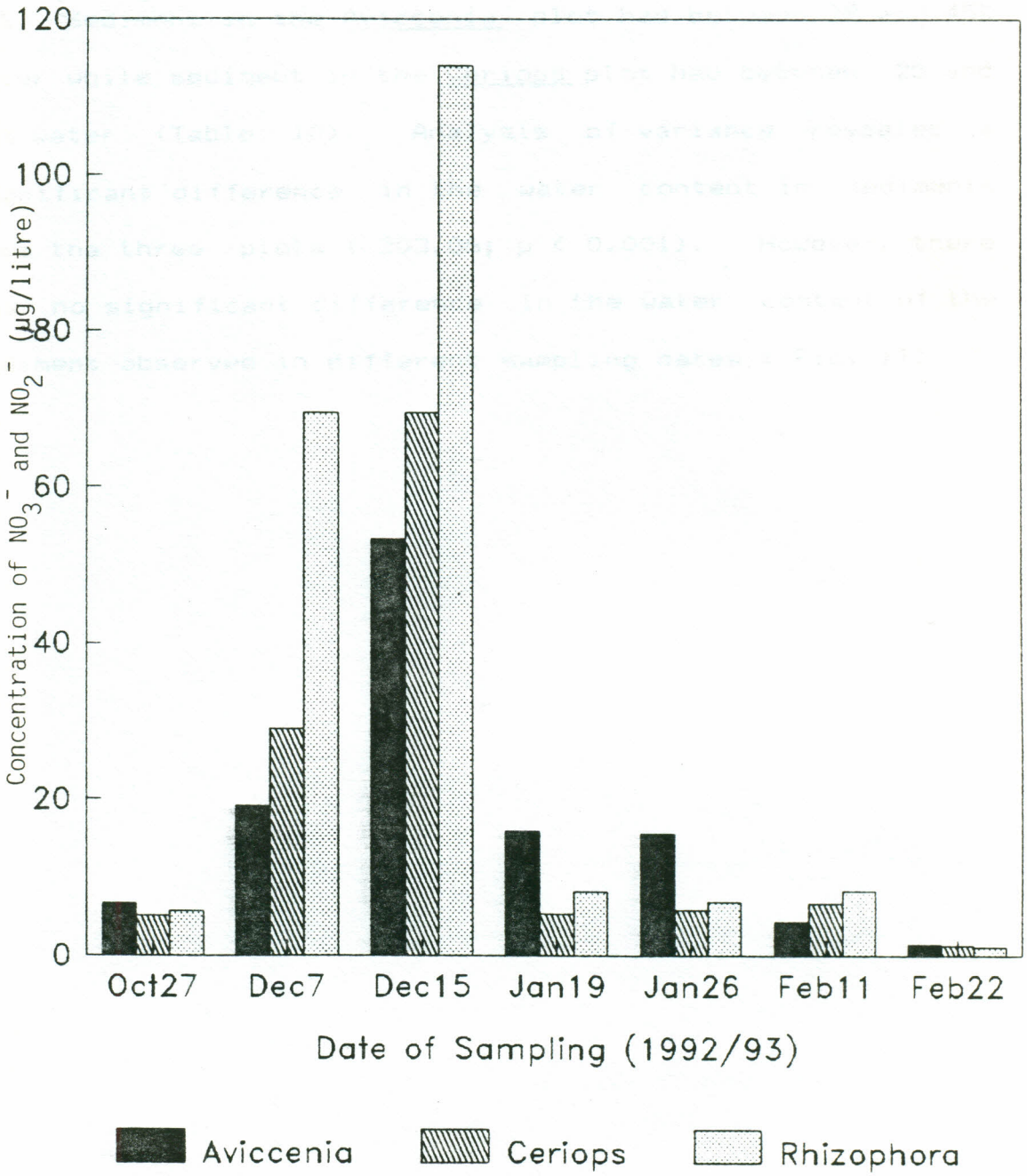

120
100
80
60
40
20
0
Concentration of NO_3^- and NO_2^- (µg/litre)
Oct27
Dec7
Dec15
Jan19
Jan26
Feb11
Feb22
Date of Sampling (1992/93)
Aviccenia
Ceriops
Rhizophora

Fig 10

4.4.2 Water Content in the Sediment at Gazi Bay.

The Rhizophora plot had high water content varying from 69-76%. Sediment in the Aviccenia plot had between 38 and 45% water while sediment in the Ceriops plot had between 25 and 32% water (Table 10). Analysis of variance revealed a significant difference in the water content in sediments from the three plots (203.06; $p < 0.001$). However, there was no significant difference in the water content of the sediment observed in different sampling dates (Fig. 11)

Fig 11. Percentage water content in sediment collected in Gazi Bay in the period between October 1992 and February 1993.

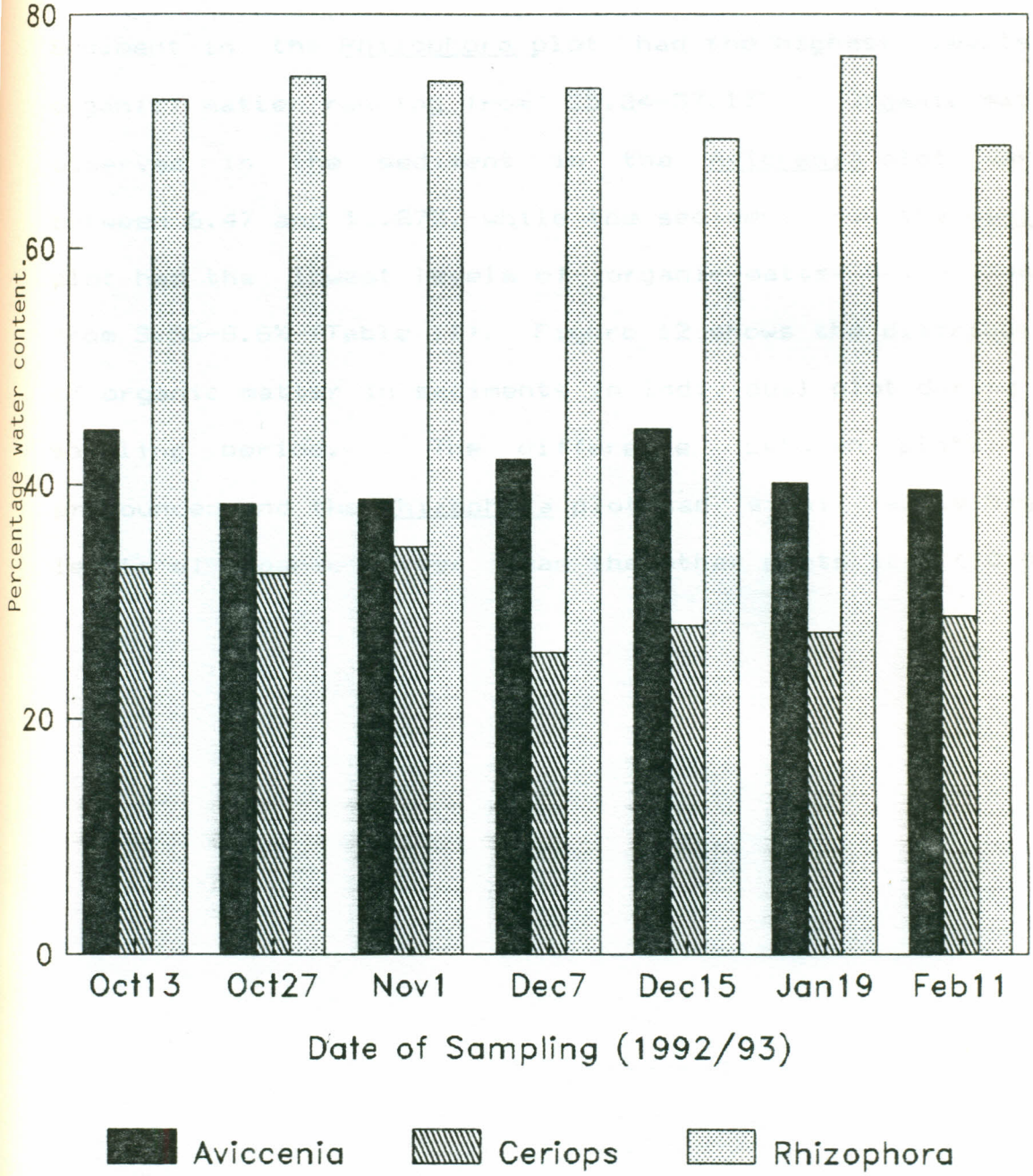

80
60
40
20
0
Percentage water content
Oct13
Oct27
Nov1
Dec7
Dec15
Jan19
Feb11
Date of Sampling (1992/93)
Aviccenia
Ceriops
Rhizophora

Fig 11

4.4.3 Organic Matter in the Sediment.

Sediment in the Rhizophora plot had the highest levels of organic matter ranging from 25.34-37.17%. Organic matter observed in the sediment in the Aviccenia plot varied between 6.47 and 11.87%, while the sediment from the Ceriops plot had the lowest levels of organic matter which ranged from 3.53-8.6% (Table 11). Figure 12 shows the distribution of organic matter in sediments in individual plot during the sampling period. The difference between plots was pronounced and the Rhizophora plot had significantly higher levels of organic matter than the other plots at $P < 0.001$.

Fig 12. Percentage organic matter in sediment collected in Gazi Bay in different sampling dates.

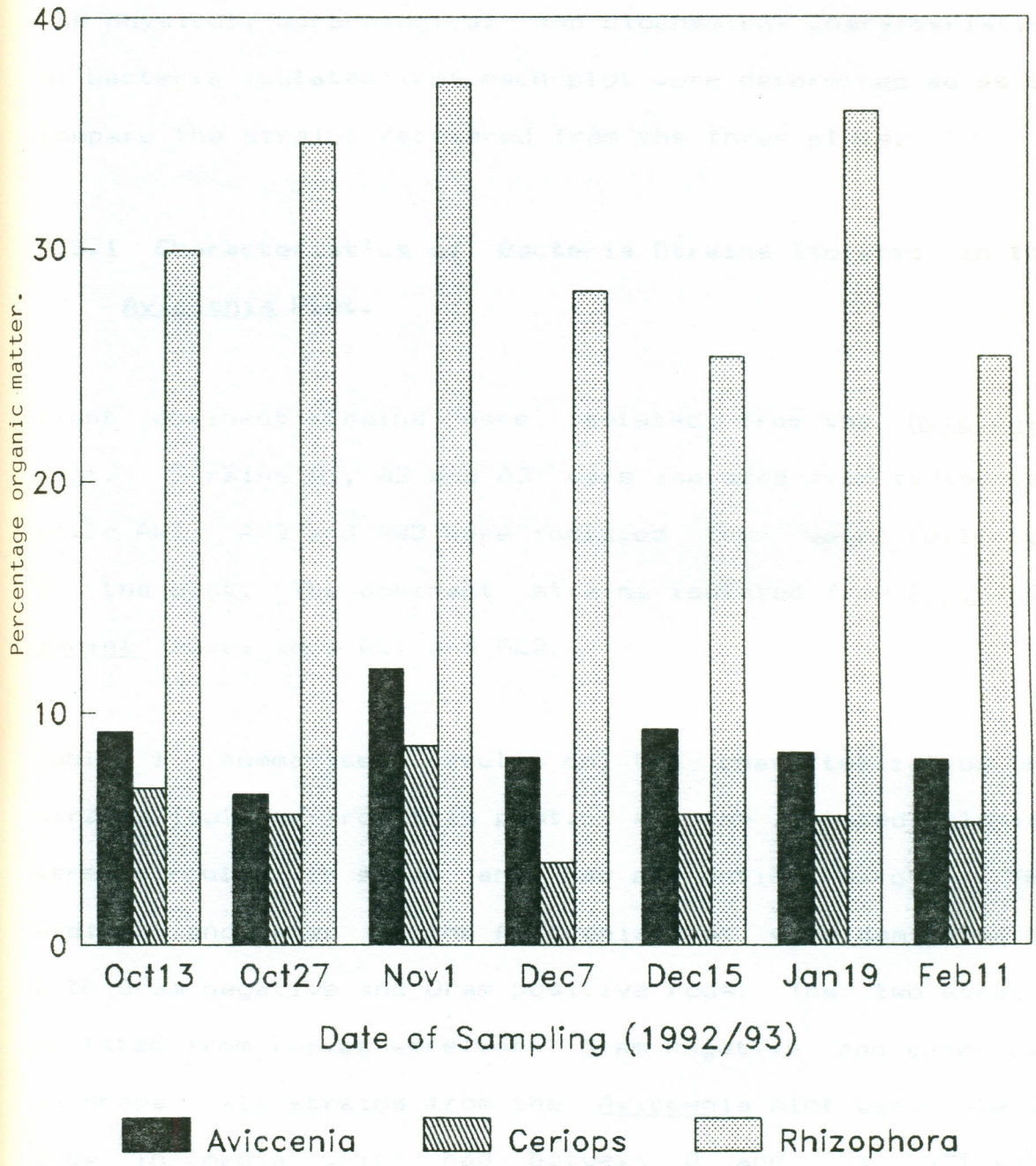

40
30
20
10
0
Percentage organic matter.
Oct13
Oct27
Nov1
Dec7
Dec15
Jan19
Feb11
Date of Sampling (1992/93)
Aviccenia
Ceriops
Rhizophora

Fig 12

4.5 CHARACTERIZATION OF BACTERIA ISOLATES.

The physical, morphological and biochemical characteristics of bacteria isolated from each plot were determined so as to compare the strains recovered from the three plots.

4.5.1 Characteristics of Bacteria Strains Isolated in the *Aviccenia* Plot.

Eight dominant strains were isolated from the *Aviccenia* plot. Strains A1, A2 and A3 were isolated from sediments, while AW1, AW2 and AW3 were isolated from water collected in the plot. The dominant strains isolated from *Aviccenia marina* leaves were AL1 and AL2.

Table 1 summarises results on the characterization of strains isolated from this plot. All the isolated colonies were circular in shape and had an entire margin. The sediment and water in the *Aviccenia* plot were dominated by both Gram negative and Gram positive rods. The two strains isolated from leaves were both Gram negative and spherical in shape. All strains from the *Aviccenia* plot were able to grow in media which had between 0 and 24% NaCl salt concentration.

The eight strains also grew very well in media of pH ranging from 6.3-9.0, but only A1 and AW3 grew at pH 4.5.

Many of the strains were oxidase and catalase positive (Table 1), while only A1 could hydrolyse starch. None of the strains from the plot could reduce nitrate, while strains AW1, AW2, AL1 and AL2 could all hydrolyse gelatin. None of the bacteria isolated from this plot were able to ferment Xylose while only strain AW2 could ferment glucose.

Table 1 shows that strains AL1 and AL2 resemble each other in all characteristics except that the former ferments lactose yielding acid and gas while the latter yields only acid.

Table 1 .

Characteristics of bacteria isolated from the Aviccenia plot.

	Colony characteristics				Growth conditions		Cell properties				Biochemical properties						Carbohydrate fermentation										Cellulose utilization
Strain	Colour	Elev.	Marg.	Shape	pH range	Salt range	Gram stain	Shape	Size (µm)	Motility	Urease	Oxidase	NO_3 Reductase	Catalase	Amylase	Gelatinase	Xylo.		Gluco.		Malt.		Mann.		Lact.		
																	G	A	G	A	G	A	G	A	G	A	
A1	Cream	Conv.	Undu.	Circ.	4.5-9	0-15%	-ve	Rods	0.286	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-	-	-	-	+	+	-	-	-	-	-ve
A2	Brown	Flat	Ent.	Circ.	6.3-9	0.24%	+ve	Cocci	0.286	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-	-	-	-	-	-	-	-	-	-	-ve
A3	Brown	Flat	Ent.	Circ.	6.3-9	0-24%	+ve	Rods	0.286	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-	-	-	-	-	-	-	-	-	-	-ve
AW1	Brown	Rais.	Ent.	Circ.	6.3-9	0-24%	-ve	Rods	0.858	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-	-	-	-	-	-	-	-	-	+	+ve
AW2	Brown	Flat	Ent.	Circ.	6.3-9	0-24%	+ve	Rods	0.572	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-	-	-	+	-	-	+	+	-	+	-ve
AW3	Cream	Rais.	Ent.	Circ.	6.3-9	0-24%	+ve	Cocci	0.286	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-	-	-	-	-	+	-	-	-	-	-ve
AL1	Cream	Flat	Ent.	Circ.	6.3-9	0-24%	+ve	Cocci	0.286	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-	-	-	-	-	-	-	-	+	+	+ve
AL2	Cream	Flat	Ent.	Circ.	6.3-9	0-24%	+ve	Cocci	0.286	-ve	-ve	-ve	-ve	+ve	-ve	+ve	-	-	-	-	-	-	-	+	-	+	+ve

Where: +ve = positive reaction; -ve = negative reaction; + = presence of a parameter; - = absence of a parameter; G = gas; A = acid; Circ. = circular; Rais. = raised; Conv. = convex; Marg. = margin; Elev. = elevation; Undu. = undulate; Ent. = entire; Xylo. = xylose; Gluco. = glucose; Malt. = maltose; Mann. = mannitol and Lact. = lactose.

4.5.2 Characteristics of Bacteria Strains Isolated in the *Ceriops* Plot.

Seven dominant strains of bacteria were isolated in the *Ceriops* plot. Strains C1, C2 and C3 were predominant in sediments, CW1 and CW2 were predominant in water and CL1 and CL2 were predominant on leaves.

Table 2 shows the characteristics of bacteria strains isolated from the plot. All the colonies were circular with variable margin types. Gram positive and Gram negative rods were the most common types of bacteria. Most of the colonies isolated in this plot could not grow on media with NaCl salt concentration above 20%. Biochemical tests show that only C2 and CW2 were oxidase positive, while strain CL1 was the only catalase negative isolate. Most of the strains in the *Ceriops* plot were able to ferment glucose and hydrolyse starch.

Table 2.

Characteristics of bacteria isolated from the Ceriops plot.

	Colony characteristics				Growth conditions		Cell properties			Biochemical properties						Carbohydrate fermentation										Cellulose utilization
Strain	Colour	Elev.	Margin	Shape	pH range	Salt range	Gram stain	Shape	Size (µm)	Motility	Urease	Oxidase	NO_3 Reductase	Catalase	Amylase	Gelatinase	Xylo.		Gluco.		Malt.		Mann.		Lact.		
																	G	A	G	A	G	A	G	A	G	A	
C1	Cream	Flat	Erose	Circ.	6.3-9	0-10%	-ve	Rods	0.572	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-	-	-	+	-	+	-	+	-	-	-ve
C2	Brown	Flat	Entire	Circ.	6.3-9	0-24%	+ve	Rods	0.286	-ve	-ve	+ve	-ve	+ve	+ve	+ve	-	-	-	+	-	-	-	-	-	-	-ve
C3	White	Rais.	Entire	Circ.	6.3-9	0-20%	-ve	Rods	0.286	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-	+	-	+	-	+	-	+	-	-	+ve
CW1	Cream	Flat	Erose	Circ.	4.5-6.3	0-20%	+ve	Cocci	0.286	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-	-	-	+	-	+	-	-	-	-	-ve
CW2	Brown	Flat	Curled	Circ.	6.3-9	10-20%	+ve	Rods	0.429	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-	-	-	-	-	-	-	-	-	-	-ve
CL1	Yellow	Flat	Undu.	Circ.	6.3-9	0-10%	+ve	Rods	0.143	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-	+	-	+	-	+	-	+	-	+	-ve
CL2	Pink	Rais.	Entire	Circ.	4.5-9	0-24%	+ve	Cocci	0.286	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-	-	-	+	-	+	-	-	-	-	+ve

Where: +ve = positive reaction; -ve = negative reaction; + = presence of a parameter; - = absence of a parameter; G = gas; A = acid; Elev. = elevation; Circ. = circular; Undu = undulate; Xylo = xylose; Gluco. = glucose; Malt. = maltose; Mann. = mannitol; Lact. = lactose; Rais. = raised.

4.5.3 Characteristics of Bacteria Strains Isolated in the *Rhizophora* Plot.

Eight dominant strains were isolated from the *Rhizophora* plot. Strains R1 and R2 were dominant in sediment, RW1 and RW2 dominated in water, while RL1, RL2, RL3 and RL4 dominated on *Rhizophora mucronata* leaves.

Table 3 shows the characteristics of bacteria strains isolated from the *Rhizophora* plot. Most of the strains were Gram positive cocci. All the isolates grew well in media of pH ranging from 6.3-9.0. Only a few could grow at pH 4.5. Most of the colonies grew poorly on media with more than 15% salt concentration. Strains R2, RW2, RL2, RL3 and RL4 were oxidase positive, while strains R2 and RL1 were the only catalase negative isolates. All the colonies could hydrolyse gelatin, while only strains R, RW, RL and RL could hydrolyse starch.

Fermentation tests showed that strain R1 could ferment all the sugars producing acid and gas except for mannitol which it fermented to yield acid only. Strain RW1 also fermented all the sugars producing acid but not gas. Strains RL1 and RL3 could not ferment any of the sugars.

Table 3.

Characteristicts of bacteria isolated in the Rhizophora plot.

	Colony Characteristics				Growth conditions		Cell properties				Biochemical Properties						Carbohydrate fermentation										Cellulose utilization
Strain	Colour	Elev.	Marg.	Shape	pH range	Salt range	Gram stain	Shape	Size (µm)	Motility	Urease	Oxidase	NO_3 Reductase	Catalase	Amylase	Gelatinase	Xylo.		Gluco.		Malt.		Mann.		Lact.		
																	G	A	G	A	G	A	G	A	G	A	
R1	Brown	Rais.	Ent.	Irre.	6.3-9	0-15%	+ve	Cocci	0.286	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+	+	+	+	+	+	-	+	+	+	-ve
R2	Cream	Conv.	Ent.	Circ.	6.3-9	0-10%	+ve	Cocci	0.143	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-	-	-	+	-	-	-	-	-	-	-ve
RW1	Brown	Flat	Ent.	Irre.	6.3-9	0-20%	+ve	Cocci	0.572	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-	+	-	+	-	+	-	+	-	+	-ve
RW2	Brown	Rais.	Undu.	Irre.	6.3-9	0-20%	+ve	Rods	0.429	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-	+	-	-	-	+	-	-	-	-	+ve
RL1	Cream	Flat	Ent.	Circ.	4.5-9	0-15%	+ve	Rods	0.572	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-	-	-	-	-	-	-	-	-	-	+ve
RL2	Yellow	Conv.	Ent.	Circ.	4.5-9	0-24%	+ve	Cocci	0.572	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-	+	-	-	-	-	-	-	-	-	-ve
RL3	Brown	Rais.	Undu.	Circ.	6.3-9	0-24%	+ve	Cocci	0.286	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-	-	-	-	-	-	-	-	-	-	-ve
RL4	Brown	Rais.	Ent.	Circ.	4.5-9	0-24%	+ve	Rods	0.429	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-	-	-	-	-	+	-	-	-	-	-ve

Where: +ve = positive reaction; -ve = negative reaction; + = presence of a parameter; - = absence of a parameter; G = gas; A = acid; Circ. = circular; Rais. = raised; Irre. = irregular; Undu. = undulate; Elev. = elevation; Conv. = convex; Xylo. = xylose; Gluco. = glucose; Mann. = mannitol; Malt. = maltose; Lact. = lactose and Ent. = entire

4.6 RAINFALL EXPERIENCED AROUND GAZI BAY.

Table 4. Daily rainfall (mm) data for the period between September 1992 and February 1993 from Msambweni District Agricultural Office (Ref. No. 9439014) situated about 5 km south of Gazi Bay. (Source: Kenya Meteorological Department).

Day	Sept	Oct	Nov	Dec	Jan	Feb
1	0.0	0.0	0.0	0.0	0.0	0.0
2	0.0	0.0	19.5	10.0	0.0	0.0
3	5.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	8.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0
6	2.0	0.0	0.0	12.0	2.0	0.0
7	8.0	0.0	0.0	0.0	0.0	0.0
8	6.0	0.0	27.0	0.0	0.0	0.0
9	19.0	0.0	0.0	10.0	0.0	0.0
10	5.0	0.0	0.0	0.0	0.0	0.0
11	0.0	0.0	27.0	0.0	0.0	0.0
12	2.5	0.0	0.0	0.0	27.0	0.0
13	20.0	0.0	0.0	8.0	0.0	0.0
14	0.0	0.0	0.0	0.0	27.0	0.0
15	0.0	0.0	13.0	2.0	0.0	0.0
16	20.0	0.0	0.0	0.0	25.0	0.0
17	0.0	0.0	0.0	6.0	5.0	0.0
18	10.0	0.0	12.0	0.0	0.0	0.0
19	0.0	0.0	0.0	0.0	0.0	0.0
20	26.0	0.0	0.0	0.0	2.0	0.0
21	0.0	0.0	0.0	0.0	0.0	0.0
22	5.0	2.0	13.0	0.0	0.0	0.0
23	0.0	0.0	0.0	13.0	0.0	0.0
24	5.0	4.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0
26	0.0	5.0	0.0	0.0	0.0	0.0
27	0.0	11.0	0.0	0.0	0.0	0.0
28	0.0	14.0	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0	0.0
30	0.0	0.0	0.0	0.0	0.0	
31		0.0		0.0	0.0	
Total	113.5	36.0	111.5	69.0	88.0	0.0

The month of September experienced the highest rainfall amount totaling 133.5 ml and had the highest number of rainy days, while the month of February experienced no rainfall at all. In the five month within which the study was conducted a total of 438 mm of rainfall was recorded.

CHAPTER FIVE:

5.0 DISCUSSION.

5.1 BACTERIA POPULATIONS IN GAZI BAY.

5.1.1 Aerobic Heterotrophic Bacteria in the Sediment.

The number of aerobic heterotrophic bacteria in the mangrove sediment at Gazi Bay ranged between 7.44×10^7 and 4.70×10^8 cfu/g dwt (Table 5). There was no significant difference between the three plots with respect to the number of aerobic heterotrophic bacteria in the sediment. This implies that the Rhizophora, Ceriops and Aviccenia zones in Gazi Bay had almost the same numbers of aerobic heterotrophic bacteria.

Alongi (1988) observed that the average number of bacteria in a mangrove swamp in Australia was around 1.1×10^{11} cells/g dwt of sediment, while Boto et al. (1989) observed an average of $7.3\text{-}49.0 \times 10^{10}$ bacteria cells/g dwt of sediment.

Rublee and Dornseif (1978) counted bacteria in sediment collected from a salt marsh and observed an average of 8.39 X 10^9 bacteria cells/cm^3 of sediment. All these studies were conducted using the direct count method.

The direct count method usually gives values 100-1000 times more than the plate count method (Mwatha, 1991). This is because the plate count method enumerates only the viable aerobic heterotrophic bacteria, while the direct count method includes other groups of bacteria such as the anaerobes and photosynthetic bacteria. The direct count method also includes dead bacteria cells. Another reason why the plate count method usually gives lower values of bacteria numbers compared to the direct count method is that there is a great diversity of bacteria in the sea. Therefore, any culture technique will leave out a number of bacterial types (Morita, 1977). Thus, the number of aerobic heterotrophic bacteria observed in Gazi Bay during this study are comparable with those by Alongi (1988) and Boto et al. (1989) if the differences caused by the methods are accounted for.

Although several studies on bacteria numbers and bacterial activity in mangrove swamps have been conducted, the only study that attempted to look at the zonation of bacteria populations in the swamps was done by Alongi (1988). However, the study focused only on the zonation of the total number of bacteria and did not consider zonation of specific physiological groups.

The fact that there was no significant difference between the Aviccenia, Ceriops and Rhizophora plots in Gazi Bay indicates that there is no zonation in the mangrove swamp with respect to the numbers of aerobic heterotrophic bacteria. Most studies done in temperate areas revealed no consistent zonation patterns with respect to bacteria numbers in salt marsh areas (Alongi, 1988). However, studies done in Australian mangrove swamps revealed that the number of bacteria differed significantly between the different intertidal zones.

Alongi (1988) studied zonation of bacteria numbers in several estuaries and observed a decrease in the number of bacteria with elevation. However, there were differences in the zonation patterns of bacteria numbers in the estuaries studied.

In the Morgan/McIvor estuary bacteria numbers were not significantly different between the mid-intertidal and the low-intertidal zones; and between the mid-intertidal and high-intertidal zones. However, there was a significant difference between the high-intertidal and low-intertidal zones (Alongi, 1988).

This situation was observed both in summer and in winter. In the Lockhard estuary a different zonation pattern was observed. In winter the zonation pattern for bacteria numbers in the sediment was (Low = mid > high), while in summer it changed to (Low < Mid = High) (Alongi, 1988).

The above studies show different zonation patterns for total bacteria counts in the two estuaries. In the Lockhard estuary seasonal changes influence the zonation pattern while in the Morgan/McIvor estuary the zonation pattern was the same for both winter and summer. In the study at Gazi Bay there was no zonation pattern observed with respect to the number of aerobic heterotrophic bacteria. When the data for each sampling date was analyzed separately as a single factor anovar, still there was no significant difference observed between the plots on any of the sampling dates.

Thus, the Rhizophora plot (low-intertidal), the Ceriops plot (mid-intertidal) and the Aviccenia plot (high-intertidal) were not significantly different from each other with respect to the number of aerobic heterotrophic bacteria. The fact that no zonation of aerobic heterotrophic bacteria was observed in Gazi Bay could mean that other physiological groups of bacteria might have caused the zonation patterns observed by Alongi (1988).

Several workers in mangrove areas have correlated bacteria numbers to organic carbon and grain size of the sediment. Alongi (1988) observed a positive correlation between bacteria numbers and the level of organic carbon ($r = 0.91$; $P = 0.001$). Rublee (1982) also observed a significant correlation between bacteria numbers and the level of organic carbon present in the sediment. Rublee (1982) observed a negative correlation between bacteria numbers and the grain size of the sediment but, Alongi (1988) observed a positive correlation for the same variables in a mangrove sediment ($r = 0.76$; $P = 0.01$).

In the study at Gazi Bay aerobic bacteria numbers did not correlate with the organic matter content of the sediment. The sediment in the Rhizophora plot had very high levels organic matter (25-38%) compared to either the Ceriops or Aviccenia plots (3-11.5%). Thus, the relatively low number of aerobic heterotrophic bacteria observed in the plot is surprising considering the correlation that most workers have observed between bacteria numbers and organic matter content in sediments.

As stated before, there were significant differences in the number of aerobic heterotrophic bacteria observed in different sampling dates. No single factor could be advanced to explain these differences. However, it is presumed that the interaction between the amount of rainfall, hydrographic factors (spring-neap tide cycle) and physico-chemical changes in the sediment were responsible. It is futile to attribute the differences on any single factor. Thus, it is my contention that seasonal variation of bacteria numbers in the mangrove swamps should be studied separately.

5.1.2 Anaerobic Heterotrophic Bacteria in the Sediment.

In Gazi Bay, the number of anaerobic heterotrophic bacteria ranged from $2.00 \times 10^6 - 1.82 \times 10^8$ cfu/g dwt of sediment. There was no significant difference between the three plots with respect to the number of anaerobic heterotrophic bacteria when the data collected on different sampling dates was analyzed together. However, when data for each sampling date was analyzed separately significant differences were evident on the 27th of October and 15th of December. The F-value for the former was 10.25, which was significant at $P < 0.05$ while, the F-value for the latter date was 29.320 at $P < 0.001$.

Thus, on these two sampling dates there was a zonation trend evident with respect to the number of anaerobic heterotrophic bacteria in the sediment at Gazi Bay. When the means were compared it was evident that on both dates Aviccenia had higher numbers of anaerobes compared to both the Ceriops and Rhizophora plots i.e Aviccenia > Rhizophora = Ceriops.

From the above observations it is evident that there is zonation of anaerobic bacteria in Gazi Bay on certain sampling dates and not in others. Most of the times the number of anaerobic bacteria in sediment samples from the three zones were almost the same. At certain times (i.e. 27th October and 15th December) there was a zonation pattern where higher numbers of anaerobes were observed in the Aviccenia zone compared to the Ceriops and Rhizophora zones.

On the two dates when zonation was observed there were spring tides which were preceded by some rainfall (Table 4). Thus, the plot was flushed with tidal water and at the same time, it was washed by rain water. This might have decreased oxygen concentration through a raise in the amount of pore water in the sediment. This could have led to the high number of anaerobes observed on these dates in the Aviccenia plot.

Little research has been done on anaerobic bacteria in other parts of the world. Most studies have concentrated on aerobic bacteria and total bacteria counts which unlike anaerobes do not require special growth conditions. Zuberer and Silver (1978), observed 8.6 X 10^6 cfu of anaerobic bacteria/g dwt of sediment collected from the Rhizophora zone of a mangrove swamp in Florida.

ZoBell and Morita (1977) reported an average of 10^5 anaerobic bacteria cfu/ml in deep sea sediments, while Campbell *et al*. (1988), reported around 1.95 X 10^3 anaerobic bacteria cfu/g of surface agricultural soil. Anaerobic bacteria numbers observed in Gazi Bay compare well with those observed by Zuberer and Silver (1978), but are 10^3 - 10^5 times higher than those observed in agricultural soils. Boto *et al*. (1989) describes mangrove sediment to be chemically reducing (Eh = +163 to +322 mV), because they have less oxygen tension compared to agricultural soils which have redox potentials of above +322 mV. This may be responsible for the higher number of anaerobic bacteria observed in the mangrove sediment in Gazi Bay compared to the population of anaerobic bacteria observed in agricultural soils by Campbell *et al*. (1988).

One observation which cannot be ignored is the fact that the number of aerobic heterotrophic bacteria and that of the anaerobes in each of the three plots were almost the same. On some dates the number of anaerobes observed in some plots were higher than the aerobes, although this was not common. For instance, on 27th October 1992 the population of anaerobic heterotrophic bacteria observed in the *Aviccenia* plot was 1.36 X 10^8 cfu/g dwt, while that of aerobic heterotrophic bacteria was 1.20 X 10^8 cfu/g dwt of sediment (Tables 5 & 7).

Zuberer and Silver (1978) made a similar observation. They found that, the number of aerobic heterotrophic and anaerobic heterotrophic bacteria in sediment collected in a Rhizophora zone was 2.6×10^7 and 8.6×10^6 cfu/g dwt of sediment respectively. They concluded that since mangrove sediments are chemically reducing most of the bacteria are facultative anaerobes. The same reason can be advanced to explain the observation made in Gazi where the number of aerobic and anaerobic heterotrophic bacteria were almost the same on most sampling dates.

Indeed, the range of reduction potential (+163-+322 mV) observed by Alongi (1988) on surface sediment in mangrove swamps is the most suitable for facultative anaerobic bacteria which use Fe^{3+}, Mn^{4+}, NO_3^- and O_2 as terminal electron acceptors (Boto, 1984).

There was a significant difference between the sampling dates with respect to the number of anaerobic heterotrophic bacteria in the sediment. Low numbers were evident in November and from mid-December to February. It is not clear what caused the low numbers of anaerobes on the said dates but, most probably the rainfall amount received might have interacted with the tidal cycle to bring about changes in oxygen levels in the sediment. This could have affected the population of anaerobes.

However, from the data available it is not easy to explain how all this could have happened. It is therefore suggested that a further investigation into these factors and their influence on the population of anaerobic bacteria should be done.

5.1.3 Aerobic Heterotrophic Bacteria in water.

The number of aerobic heterotrophic bacteria in water collected from the three plots ranged between 8.6×10^4 and 8.9×10^6 cfu/ml of water (Table 6). There was a significant difference between the three plots with respect to the number of aerobic heterotrophic bacteria observed in water ($F = 12.108$; $P < 0.001$). There was also a significant difference between the number of aerobic bacteria observed in water on the different sampling dates.

It is important to note that water entered the Aviccenia plot only during high spring tides. This happened once every 15 days, while the Ceriops and Rhizophora were inundated every high tide i.e. twice a day. Thus in the period between successive spring tides bacteria in the water column did not participate in any microbial processes in the Aviccenia plot since water did not reach the plot.

The number of aerobic heterotrophic bacteria observed in water were 10-100 times less than those observed in the sediment. This maybe a result of a higher concentration of nutrients in the sediment compared to the water column. During the study the concentration of combined nitrates and nitrites in the sediment ranged from 1.15-113.92 µg N/l.

On the other hand, Kazungu et al. (1993) observed nitrate concentrations ranging between 0.05 and 0.3 µg N/l, while nitrites ranged from 0.05-0.2 µg N/l in water samples collected in Gazi Bay. Thus, there is a relatively low concentration of the two nutrients in the water column compared to the sediment surface. Other nutrients like phosphates and organic carbon might also show a similar distribution. This is because in mangrove swamps there is always a net sedimentation of particulate organic matter from the water column onto the sediment surface (Chapman, 1976). Thus, the higher number of aerobic heterotrophic bacteria in the sediment when compared to those observed in water samples maybe explained by the high concentration of nutrients in the former.

Considerable research has been done on bacterial population in the water column in the marine environment. Fenchel (1984) observed 3 X 10^6 bacteria cells/ml in coastal waters using the direct count method.

Rublee (1982); Palumbo and Ferguson (1978) observed $10^4 - 10^7$ bacteria cells/ml of seawater. Considering the fact that the direct count method was used by these workers, the values obtained in their studies are relatively lower than the number of bacteria observed during this study in Gazi.

This could possibly result from the fact that Fenchel (1984); Rublee (1982); Palumbo and Ferguson (1978) were mainly counting bacteria on the surface of the water. The study in Gazi Bay dealt with the bacteria population in the water near the sediment surface. The depth of the water varried from ankle deep in the Aviccenia plot to about 2 m in the Rhizophora plot.

Thus, tidal waters might have resuspended fine sediment particles and bacteria from the sediment surface hence raising the number of bacteria in the water that lies immediately above the sediment surface. This was reflected in the high particle density observed in the water samples that were collected. Wiebe (1979) observed that resuspending of sediment and bacterial particles was a common occurrence in coastal waters.

The study in Gazi Bay shows that there were more aerobic heterotrophic bacteria in water collected in the Rhizophora plot compared to either the Ceriops or Aviccenia plots.

The high number of aerobic heterotrophic bacteria in water collected in the Rhizophora plot which lies close to the sea suggests that suspenion is felt more in this plot. This is probably a result of the fact that tidal water enters the Rhizophora plot before it reaches either the Ceriops and Aviccenia plots. Thus, the tidal water entered the Rhizophora plot with more force, causing more turbulence and suspenion of bacteria and sediment particles. When the tidal water finally reached the Ceriops and Aviccenia plots it may have lost most of its initial force thus, it was not able to upset the sediment very much.

5.1.4 Nitrate Reducing Bacteria in the Sediment.

The number of nitrate reducing bacteria observed in Gazi Bay ranged between 2.37×10^2 and 9.21×10^3 bacteria cells/g dwt of sediment (Table 8). There was a significant difference between the plots with respect to the population of nitrate reducing bacteria ($F = 20.445$; $P < 0.001$).

A comparison of the three study sites revealed that the number of nitrate reducing bacteria in the Rhizophora plot was significantly different from those in the Ceriops and Aviccenia plot. However, the number of nitrate reducing bacteria in the Aviccenia and Ceriops plots were not significantly different i.e (Rhizophora > Ceriops = Aviccenia).

Relatively few studies have been done on the number of nitrate reducing bacteria. Most researchers have preferred to measure their activity by estimating the rate of nitrate reduction using ^{15}N labelled nitrates. However, ZoBell and Morita (1977) counted nitrate reducing bacteria in deep sea sediments in the Philippine trench and observed $10^4 - 10^5$ cells/ml using the MPN method. Thus, the number of nitrate reducing bacteria observed during this study in Gazi Bay were almost 1000 times less than the observations made by ZoBell and Morita (1977).

The lower number of nitrate reducers observed in Gazi Bay could have resulted from the fact that being in the intertidal areas mangrove sediments are subjected to regular exposure to air. Thus, conditions are likely to be less reducing than they are at the bottom of the ocean. Due to the great depth involved, diffusion of oxygen to the bottom sediments in the ocean is most certainly limited. This makes the conditions at the bottom of the ocean more favourable for proliferation of anaerobic bacteria e.g the nitrate reducers, than in mangrove swamps.

A significant correlation was observed between the number of nitrate reducing bacteria and the concentrations of combined NO_3^- and NO_2^- in the Rhizophora and Ceriops plots at $p < 0.05$ ($r = -0.681$ and -0.516 respectively). Correlation coefficient for the Aviccenia plot was not significant.

The fact that correlation between the number of nitrate reducing bacteria and combined nitrates and nitrites in the Aviccenia plot was not significant could be explained by the duration of exposure to air experienced by this plot. Since the plot experiences the longest duration of exposure, the nitrate reducers on the sediment surface may receive enough oxygen from the atmosphere and thus rarely use nitrates as electron acceptors.

However, in the Ceriops and Rhizophora plots which are inundated every high tide, it is possible that there is more use of the nitrates as terminal electron acceptors. This is because the two plots which are flooded by tidal waters more often are likely to have less oxygen. However, there are no other studies which support or contradict this observation.

The zonation of nitrate reducing bacteria in the sediment in Gazi Bay indicate that the Aviccenia zone had the highest numbers of nitrate reducing bacteria, compared to the Ceriops and Rhizophora plots. There are no previous studies done on zonation of nitrate reducing bacteria in any mangrove ecosystem. However, the nitrate reducing bacteria which are anaerobes, are controlled by the redox potential of the sediment and the presence of nitrate ions (Chandramohan, 1988).

These two factors might be responsible for the zonation of nitrate reducing bacteria observed in Gazi Bay. The mean levels of combined nitrate and nitrites were significantly higher in the Rhizophora sediment (30.53 µg NO_3^- and NO_2^- N/l) than in the Ceriops (17.63 µg NO_3^- and NO_2^- N/l) and Aviccenia plots (16.59 µg NO_3^- and NO_2^- N/l). Thus, the relatively higher concentration of combined nitrates and nitrites in the Rhizophora plot could have been responsible for the higher numbers of nitrate reducing bacteria in the plot.

Data on reduction potential in the mangrove sediment in Gazi is not available. However, the average particle size in the Rhizophora sediment was observed to be around 56 µm, while in the Ceriops plot it was 256 µm (Slim and Gwada 1993). No figures are available for grain size in the Aviccenia plot. However in this study, a relatively high percentage water content was observed in the Rhizophora sediment (more than 70%) while the water content in sediment collected from the Ceriops and Aviccenia plot did not exceed 45%. Thus, there was very little pore space left for air in the Rhizophora plot hence, the conditions were likely to be more reducing than in the other plots.

This is because aeration and moisture are inversely related (Alexander, 1977). This might explain further the high number of nitrate reducing bacteria which were observed in this plot compared to the Aviccenia and Ceriops plots.

5.1.5 Aerobic Heterotrophic Bacteria on Leaves.

The number of aerobic heterotrophic bacteria on leaf surfaces ranged from $2.00 \times 10^2 - 9.73 \times 10^5$ cfu/cm^2 of leaf (Table 9). Analysis of variance revealed a significant difference between the three types of leaves with respect to the number of aerobic heterotrophic bacteria on their surfaces ($F = 61.922$; $P < 0.001$). A comparison of the means revealed that the population of aerobic heterotrophic bacteria on Aviccenia leaves was significantly higher than the population on Rhizophora and Ceriops leaves. However, Rhizophora and Ceriops leaves were not significantly different with respect to the number of aerobic heterotrophic bacteria i.e Aviccenia > Ceriops = Rhizophora.

Most studies on bacteria populations in the coastal environment have concentrated on bacteria in the sediment and water column. Thus, there was not a single study on bacteria numbers on leaf surfaces. Colonization of leaves by bacteria and fungi starts while they are still attached to the mother plant.

Lee (1980) reported that bacteria and fungi attack leaf surfaces and utilize sugars exuded from the leaves. The high number of aerobic heterotrophic bacteria observed in Aviccenia marina leaves is most probably a function of the nutritive value of the leaf.

Woitchik et al. (1993) observed A. marina leaves to have a C:N ratio of 88 ± 6 while Rhizophora mucronata and Ceriops tagal leaves had C:N ratios of 193 ± 45 and 218 ± 26 respectively. Thus, A. marina leaves were the most nutritious while R. mucronata and C. tagal leaves were of comparable nutritive value. Thus, it is possible that the high number of aerobic heterotrophic bacteria observed on A. marina leaves was a result of the low C:N ratio which makes the leaves more appealing to the bacteria. On the other hand the high C:N ratios observed in Rhizophora and Ceriops leaves were reflected in the lower number of bacteria observed on their surfaces.

5.2 DECOMPOSITION IN GAZI BAY.

5.2.1 Cellulose Decomposition.

The Aviccenia plot had the highest rate of cellulose decomposition compared to the Rhizophora and Ceriops plots (Fig 7). Filter papers incubated in the Aviccenia plot lost 92% of their dry weight within 28 days. In the Rhizophora plot filter papers lost 80% while in the Ceriops plot they lost less than 20% of their dry weight in 69 days.

Brinson (1977) used filter paper as an index for cellulose decomposition in an alluvial swamp forest. The fastest cellulose decomposition was observed in the river where 90% of filter paper dry weight was lost in 16 weeks.
Filter papers which were placed on the forest floor lost about 25% of dry weight in 16 weeks, while those in the Levee lost less than 20% dry weight in 24 weeks.
This led to the conclusion that, the high rate of cellulose decomposition in the river was the result of the high moisture content at that site. Cellulose decomposition was slow on the Levee which was relatively dry for most of the experiment period.

Brinson (1977) also correlated monthly cellulose decomposition rates to monthly minimum and maximum temperatures and obtained significant correlation coefficient ($r = 0.85$ and 0.84 respectively; $P < 0.05$). These observations led to the conclusion that the differences in rate of cellulose decomposition were mostly a result of differences in microbial activity between the three sites which are influenced by moisture and temperature (Brinson, 1977).

In the study at Gazi Bay, the differences in cellulose decomposition observed were also probably a result of differences in microbial activity. The fastest cellulose decomposition was in the Aviccenia plot which was exposed to air for the longest duration. The Rhizophora and Ceriops were inundated twice every day and hence, filter papers in those plots were moistened more often. However, the two plots had lower rates of cellulose decomposition than the Aviccenia plot (Fig 7). Thus, in Gazi Bay, exposure to air seems to accelerate cellulose decomposition.

Schneider and Rheinheimer (1988) observed that most of the cellulose decomposers known are strict aerobes. Thus, a high oxygen tension is essential for their activity and proliferation. Therefore, in the Aviccenia plot which was exposed more often oxygen tensions were probably high enough to facilitate the activity of cellulose decomposers hence the high rate of cellulose decomposition observed in the plot.

As a result of a longer duration of exposure, the temperatures in the Aviccenia plot were probably higher than in the other two plots due to heating from the sun, thus further favouring bacterial activity. Thus, temperature and exposure could have combined to bring the cellulose decomposition rates observed in Gazi Bay.

Another factor that could have led to the differences in the rates of cellulose decomposition are the strains of bacteria present in each plot. Strains AW1 from the water column and AL2 and AL3 from leaves in the Aviccenia plot were able to grow on cellulose media (Table 12). On the other hand, strains C2 and CL2; RW1 and RW2 from the Ceriops and Rhizophora plots respectively were also able to grow on cellulose media (Table 13 and 14).

Thus, the Aviccenia plot had more bacteria able to grow on cellulose media compared to the other plots. However, this should be treated with caution since only the dominant strains were recovered from each plot while the colonies which were present in low numbers were left out. It is possible that some of these colonies which were not characterized could hydrolyse cellulose.

Since the mangrove sediment is chemically reducing it is also possible that some of the cellulose decomposers are obligate anaerobes like Clostridium and Desulfovibrio desulfuricans (Agate, 1988). The anaerobic bacteria observed in Gazi Bay were also not characterized since special growth conditions are necessary for their continued cultivation. Thus, differences in cellulose decomposition in the three plots could also have resulted from differences in strains of anaerobic heterotrophic bacteria.

Fungi are present in mangrove swamps and have been reported to participate in decomposition (Fell and Master, 1984). It is possible that, fungi could also have caused the high rate of decomposition in the Aviccenia plot whose surface sediment are exposed more often.

This is because the sediment surface in the Aviccenia plot which was exposed for a longer duration may have had enough oxygen to support fungal activity. This is one area that needs to be looked into in order to get a clearer picture of events in the mangrove swamps.

Environmental factors like temperature and oxygen availability are likely to influence the speed of cellulose decomposition by each of the strains in the field. However, characterization of bacteria strains isolated from each plot reveal that the strains differed in their physical and biochemical properties. Thus, the strains in the Aviccenia plot could probably be the most active cellulose decomposers followed by those in the Rhizophora plot and finally the strains in the Ceriops plot.

Nitrogen levels in the sediment do not seem to influence the decomposition of cellulose in three plots. The highest NO_3^-/NO_3^- levels were observed in the Rhizophora plot (Table 9) but decomposition rates were faster in the Aviccenia plot. Activity of nitrogen fixers does not influence cellulose decomposition either.

5.2.2 Leaf Decomposition.

Aviccenia marina leaves decomposed faster than either Rhizophora mucronata or Ceriops tagal leaves in their native plots. The leaching phase of decomposition lasted for only 12 days in the case of Aviccenia leaves. In that duration nearly 44% of leaf dry weight was lost. The leaching phase in the case of Rhizophora and Ceriops leaves lasted for 20 days. The dry weight loss observed from Rhizophora and Ceriops leaves was 52% and 43% respectively (Fig. 9). There were more substances leached from the Rhizophora leaves than from either Aviccenia and Ceriops leaves. This indicates that there were more soluble substances in Rhizophora leaves compared to the other two species. This could be due to the presence of high levels of tannins present in Rhizophora tissue (Boto et al., 1989; Walter and Steiner, 1936)

Steinke and Ward (1987) studied mangrove leaf decomposition in St. Lucia estuary, South Africa. They observed that A. marina leaves decomposed faster than Bruguiera gymnorrhiza leaves both in the warm and cool seasons. In the warm season, A. marina leaves lost 55% of their dry weight in 2 months, while B. gymnorrhiza leaves lost around 20% of their dry weight in the upper shore.

In the lower shore, the decomposition rates of both species increased. A. marina leaves lost nearly 80% of their dry weight while B. gymnorrhiza lost only 45% in the same duration. Steinke and Ward (1987) attributed the faster decomposition rate observed in the lower shore to the fact that the litter was always moistened hence enhancing colonization and decomposition by bacteria.

Woitchik et al. (1993) studied the decomposition of Rhizophora mucronata and Ceriops tagal leaves in Gazi Bay. In their experiment oven dried leaves were used. The rate of leaf decomposition was faster in the Rhizophora plot than in the Ceriops plot. These are the same plots that were used in this study. Rhizophora mucronata leaves lost nearly 80% of their dry weight in 20 days, while Ceriops tagal leaves lost 65% in the same duration (Woitchik et al., 1993). The rates of leaf decomposition observed by Woitchik et al. (1993) was almost twice as high as the rate observed during this study in the two plots. The difference may have resulted due to the use of oven dried leaves by Woitchik et al. (1993) in their experiment. The drying of the leaves could have altered the leaf texture thus, making them easier for microbial degradation. The senescent leaves used in this study were placed in the field without any interference totheir texture.

It is therefore possible that, they proved to be harder for the microorganisms to attack and decompose. On the other hand, these represent the actual conditions in the field where leaves fall to the ground without being oven dried.

Steinke and Ward (1987) observed differences in decomposition rates of leaves according to species, location on the shore and season. The study in Gazi Bay did not look at the seasonal effect but the site and species were seen to influence decomposition of the leaves. Steinke and Ward (1987), observed that leaves on the lower shore decomposed faster than in the upper shore. The reverse was observed in Gazi. The decomposition of filter paper which was made of uniform material indicates that decomposition is faster in the Aviccenia (upper shore) plot.

Aviccenia marina leaves in their native plot also decomposed relatively faster than Rhizophora mucronata in their native plot. Both plots are exposed at low tide but, the Aviccenia plot experiences the longest duration of exposure to air since water reaches the plot only during high spring tide. Thus, in Gazi Bay exposure to air appears to accelerate decomposition. Hesse (1961) observed that decomposition rates were accelerated when drying of soils was followed by rewetting.

This situation is true of the Aviccenia plot in Gazi Bay where long periods of drying are followed by rewetting during spring tides. Lugo and Snedaker (1974) reported that exposure to air increased oxygen supply to the surface sediment in mangrove swamp hence enhancing decomposition.

However, there are other factors that might have influenced the decomposition of leaves in Gazi Bay. These are, the tree species, the bacteria population on leaf surfaces, activity of cellulose decomposers and the strains of heterotrophic bacteria in the plot.

From the study in Gazi Bay, it was observed that the number of aerobic heterotrophic bacteria was significantly higher on A. marina leaves than on R. mucronata and C. tagal leaves. Thus, since decomposition of leaves start while the leaves are still attached to the plant, the chance is that the A. marina leaves fell into the sediment while at a more advanced stage in decomposition than the leaves for the other two species. This is supported by the observation that senescent Aviccenia leaves had many darkened spots and holes on their surface while senescent Rhizophora and Ceriops leaves were uniformly brown and intact. The leaves for the latter started turning dark after staying for several weeks in the field.

The leaf chemical composition of A. marina leaves (C:N = 88 ± 6) makes them more nutritious than Rhizophora and Ceriops (Woitchik et al., 1993). This might have enhanced colonization of Aviccenia leaves by microorganism when the leaves were placed in the field. The Rhizophora and Ceriops leaves with higher C:N ratios (193 ± 45 and 218 ± 26 respectively) were thus less favourable to microorganisms as nutrient sources. This is another factor that could have contributed to the faster decomposition of Aviccenia leaves.

The higher activity of cellulose decomposers observed in the Aviccenia plot can also explain the high rate of leaf decomposition in the plot. The bulk of plant material is composed of cellulose and cellulose derivatives. Thus, leaves in the Aviccenia plot may have been attacked by cellulose decomposers destroying cell walls thus, making it easier for the soluble substances within the cells to be leached. The activity of cellulose decomposers in the Rhizophora plot was higher than in the Ceriops plot (Fig 7). This is reflected in the higher rate of decomposition of Rhizophora leaves compared to Ceriops leaves. Microbial cellulases are of utmost importance in plant tissue decomposition (Lugo and Snedaker, 1974).

The strains of heterotrophic bacteria that occur in each plot might also contribute to the differences in rates of decomposition observed in the plots. This is reflected in the fact that the dominant strains in each plot had different physical and biochemical characteristics (Tables 1, 2 and 3). The cellulose utilization experiment shows that bacteria strains AL1, AL2 and AW1 in the Aviccenia plot, were able to use cellulose as a carbon source. AL1 and AL2 were both isolated from leaves while AW1 was isolated from water. None of the strains that were dominant in the sediment could utilize cellulose as a substrate. However, it is possible that the bacteria strains from leaves and water could have been transferred to the sediment. Cellulose hydrolysing bacteria could also have been present in the sediment in low numbers. Thus, this data alone cannot explain the high activity of cellulose decomposers observed in the Aviccenia plot.

Strains AL1 and AL2 both of which were cellulolytic could have contributed to the higher rate of Aviccenia leaf decomposition. The two strains were dominant on Aviccenia marina leaves thus, much of the cellulose on the leaves may have been hydrolysed while the leaf was still on the mother plant. These strains are carried to the sediment when the leaves fall and may assist in further cellulose decomposition.

In the Ceriops and Rhizophora plots the strains C3 and CL2; RW2 and RL1 respectively were able to grow on cellulose media. Thus, each species had one strain on the leaves which could utilize cellulose. This, might explain the slower rate of decomposition of leaves belonging to the two species compared to A. marina leaves.

Thus, the rate of decomposition in the mangrove swamp could have been influenced by the bacteria population on leaf surfaces, leaf chemistry, duration of exposure (oxygen tension), activity of microorganisms and the strains of bacteria involved in the process.

5.3 ACETYLENE REDUCTION RATES.

The rates of acetylene reduction detected in the mangrove sediment in Gazi Bay ranged from 0.00-51.47 nmole C_2H_4 /g dw/ht (Table 11). There was a significant difference between the three study sites with respect to the rate of acetylene reduction ($F = 13.533$; $P < 0.001$).

A comparison of mean rates of acetylene reduction in the three plots revealed that, the Rhizophora plot was significantly different from the Ceriops plot.

However, the Ceriops and Aviccenia plot; and Aviccenia and Rhizophora plots were not significantly different from each other with respect to rate of acetylene reduction. The correlation coefficient between acetylene reduction and the levels of combined nitrates and nitrites was not significant.

Correlation between organic matter content of the sediment and acetylene reduction in each plot was also not significant. However, other workers in the mangrove environment observed a significant positive correlation between nitrogenase activity and the amount of organic carbon present in the sediment (Hicks and Silvester, 1984; Potts, 1984; Zuberer and Silver, 1978).

Various studies have been done focusing on nitrogen fixation in mangrove swamps. Zuberer and Silver (1978) observed fixation rates ranging between 0.01 and 1.84 nmole C_2H_4 /g wet weight/h in root free mangrove sediment in Florida, USA. On a wet weight basis, the rates of acetylene reduction in Gazi Bay ranged between 0.00 and 12.59 nmole C_2H_4 /g sediment/h. Thus, the values obtained in this study are higher than those reported by Zuberer and Silver (1978).

The difference may have been due to differences in environmental conditions in the field since Zuberer and Silver (1978) carried out their study in Florida, USA where the weather and even mangrove species may be different from the weather and mangrove species in Gazi.

There are no studies done, describing zonation of nitrogen fixation in the mangrove swamp. The only study that came close to describing zonation of nitrogen fixation in the mangrove environment was done by Woitchik et al. (1993). While studying leaf decomposition in Gazi Bay, they observed that, the rate of nitrogen fixation on decomposing Rhizophora mucronata leaves was as high as 1200 nmole C_2H_4/g dwt of litter/h. On the other hand, the rate of nitrogen fixation on decomposing Ceriops leaves in their native plot was as high as 300 nmole C_2H_4/g dwt/h. The Aviccenia plot was not included in the study. The values obtained by Woitchik et al. (1993) are many times higher than the rates of nitrogen fixation obtained in this study.

The rate of nitrogen fixation on decomposing leaves is always higher than that of the sediment (Potts, 1984). This might be due to the presence of readily available organic matter on the decomposing leaves which serve as a source of energy for the nitrogen fixers (Hicks and Silvester, 1984). However, the difference in the rates of acetylene reduction between the Rhizophora and Ceriops zones observed by Woitchik et al. (1993), reflect the observations made in this study. The mean rate of acetylene reduction in the Rhizophora plot was almost ten times higher than in the Ceriops plot.

Two factors seem to influence the rate of nitrogen fixation in the mangrove swamp at Gazi Bay. These are, the organic matter content of the sediment and the spring-neap tide cycle. The highest nitrogenase activity was observed in the Rhizophora plot, where the organic matter content was also high (25-38%). The Ceriops plot on the other hand showed relatively low rates of nitrogenase activity and relatively low levels of organic matter (5.84%). Thus, the difference in the rate of nitrogenase activity observed between the Rhizophora and Ceriops plot is most likely the result of the big difference in the organic matter present in their sediment.

The organic matter content in the sediment in the Aviccenia (8.73%) and Ceriops (5.84%) plots was not significantly different. This may be the reason why the two plots were not significantly different with respect to the rate of acetylene.

The data on acetylene reduction seems to indicate that the spring-neap tide cycle also exerted some influence on the nitrogen fixation in the Aviccenia and Rhizophora plot. Higher activity of nitrogenase was observed in the Aviccenia plot during spring tides compared to Rhizophora plot. During neap tides the reverse seems to occur.

Except for 11th February 1993, all the rates of acetylene reduction observed during spring tides were higher in the Aviccenia plot than in the Rhizophora plot (Table 11). Thus, the spring-neap tide cycle might have been responsible for the lack of a significant difference between the two plots with respect to the rate of acetylene reduction. This was despite the fact that the Rhizophora plot had higher levels of organic matter.

During spring tides the Aviccenia plot is inundated for the first time in 15 days. It is possible that the rewetting of the sediment in the plot may enhance the activity of nitrogen fixers by altering physical and/or chemical conditions of the substrate.

Nutrients like ammonia, nitrates and nitrite levels may accumulate in the Aviccenia plot in the period between successive spring tide due to accumulation of decomposing litter. It has previously been observed that during low tides nutrient levels rise in the sediment due to evaporation (Kazungu pers. comm). On the other hand, Kariuki (1992) reported that the level of nitrogen in water samples in Lake Naivasha was low (2.20 mg N/l) in the dry season when the lake level was low. However, in the rainy season the lake level rose and the concentration of nitrogen also rose to 6.30 mg N/l. This was attributed to the drawdown phenomenon whereby, nitrogen which had accumulated on the lake shores during the period when the lake level was low, was transferred to the water when the lake level rose. A similar situation may be existing in the Aviccenia plot in Gazi. In the period between successive spring tides, water does not enter the plot. Thus, decomposing leaves are left on the sediment surface where their decomposition ensures release of ammonium compounds into the sediment.

The ammonium compounds might raise the concentration of fixed nitrogen in the sediment which might subsequently suppress the activity of the enzyme nitrogenase.

During high spring tides, water enters the plot and "draws down" the nutrients with it as it recedes. This might cause the high rates of nitrogen fixation observed in the plots during spring tides. As the water recedes some of the fixed nitrogen collected in the Aviccenia and Ceriops plot may be deposited in the Rhizophora plot.

Thus, the the level of fixed nitrogen in the plot might raise, thereby inhibiting nitrogenase activity as observed in the Rhizophora plot during most spring tides. However, these conclusions about how the spring-neap tide cycle may influence nitrogen fixation in mangrove swamps should be taken with caution. Environmental factors like pH, salinity, temperature, amount of rainfall, oxygen tension and the level of organic matter may vary in different sampling dates. Some of these factors like pH, salinity and oxygen tension in the sediment are affected by the tides (Agate, 1988). These might affect the rate of nitrogen fixation in the mangrove swamp by either affecting the number of nitrogen fixers or affecting the activity of nitrogenase enzyme.

Thus, for a clear understanding of the reason why the acetylene reduction activity in the plots fluctuated as observed, a thorough investigation needs to be done on all the factors at the same time.

Apart from the heterotrophic nitrogen fixing bacteria, other groups of nitrogen fixers have been reported in mangrove sediment. These include, the Cyanobacteria like Oscillatoria, Microcoleus and Phormidium; the phototrophic bacteria like Chromatium and the anaerobic nitrogen fixers (Potts, 1984). The latter includes members of the genera Desulfovibrio and Desulfotomaculum (Chandramohan, 1988).

In the mangrove sediment, the phototrophic and anaerobic nitrogen fixers actually play a very limited role in the overall process of nitrogen fixation (Potts, 1984). Hicks and Silvester (1984), observed that nitrogen fixation in mangrove sediment was not significantly affected by the availability of light. In their experiment, they observed that sediment incubated in presence of light fixed 6.1 µmole $C_2H_4/m^2/h$, while those incubated in the dark fixed 6.2 µmole $C_2H_4/m/h$. This indicated that the contribution by light-dependent Cyanobacteria or phototrophic bacteria was not significant.

Hicks and Silvester (1984) also observed that nitrogenase activity in the mangrove sediment was actually depressed from 10.9 µmole $C_2H_4/m^2/h$ to 1.8 µmole $C_2H_4/m^2/h$ under anaerobic conditions. From this, they concluded that the most of nitrogen fixation in mangrove swamps is done by aerobic heterotrophic bacteria. It is possible that the same situation prevails in Gazi Bay.

5.4 NUTRIENT LEVELS IN GAZI BAY.

5.4.1 Concentration of Combined NO_3^- and NO_2^- in the sediment.

There was a significant difference between the three plots with respect to the levels of combined nitrates and nitrites ($F = 9.673$; $P < 0.001$). Correlation analysis between nitrogen fixation and the concentrations of combined nitrates and nitrites revealed no significant correlation in all the three plots.

Various researchers have studied nutrient levels in wetland ecosystems. Studies on total nitrogen, NO_3^-, NO_2^- and NH_3 levels have been done in several estuaries all over the world (Cerco, 1981; Kazungu *et al.*, 1986; Kazungu *et al.*, 1993; Nedwell, 1973).

Cerco (1981), observed that, concentrations of combined nitrates and nitrites ranged from 0.35-0.4 µg N/l of water, in St James river in Virginia, USA. Kazungu et al. (1986) observed that the concentrations of nitrates in Tudor creek, Kenya, ranged between 0.2 and 22.6 µg N/l in water samples.

In Gazi Bay, Kazungu et al. (1993), observed nitrate concentrations ranging from 0.05-0.30 µg NO_3^- N/l in water samples. The concentrations of nitrites in the same period were 0.05-0.2 µg NO_2^- N/l. It is evident that, most of the researchers concentrated their work in the water column, thus very little information on nutrient levels in the sediments is available. However, mangrove sediments are reported to have an average of 50 µg NO_3^- N/l (Chandramohan, 1988). The mean values of combined nitrates and nitrites obtained in this study are almost 2-2.5 times lower than the mean value given by Chandramohan (1988). However, sampling was done for only half the year and we therefore cannot conclude that this is always the case in Gazi Bay.

The peak levels of combined nitrates and nitrites occurred a week later than the peak for nitrogen fixation in all the three plots (Fig 8 & 10). Non-symbiotic nitrogen fixation is generally low and the nitrogen fixed usually remains locked up in bacterial cells until the cells die and become mineralized. The resultant ammonium compounds can the be nitrified into nitrates and nitrites. Thus, the one week lag period seems to be the time required for the death and mineralization of non-symbiotic nitrogen fixers and subsequent nitrification of ammonium compounds to nitrites and finally nitrates by nitrifying bacteria.

Rheinheimer (1977); Cerco, (1981) reported that nitrates in estuarine sediments including those in mangrove swamp are formed through the process of nitrification. Cerco (1981) observed that, the number of ammonia and nitrite oxidizers in bottom sediment in St. James river ranged between 10^2 and 10^5 MPN/ml. Thus, nitrification is common in estuarine surface sediment (Chandramohan, 1988). This confirms our own observations in Gazi Bay.

The Rhizophora plot had a significantly higher rate of nitrogen fixation than the Ceriops plot. This might explain the high levels of combined nitrates and nitrites observed in the former.

The rates of nitrogen fixation between the Ceriops and Aviccenia plot were not significantly different. This was reflected in the fact that there was no significant difference between the two plots with respect to the levels of combined nitrates and nitrites. Thus, the levels of nitrates and nitrites in each plot seem to be influenced by the processes of nitrogen fixation followed by nitrification.

However, the levels of nitrates and nitrites in any system are affected by other processes like denitrification, assimilation by plants and microorganisms and the rate of nitrification (Chandramohan, 1988). Thus, it is difficult to conclude how each of the above factors influenced the levels of combined nitrates and nitrites in the sediment in Gazi Bay.

5.4.2 Percentage Levels of Organic Matter and Water in the sediment.

The forces that influence the dynamics of bacteria populations in soil and the effects of these populations on their environment are governed by the physical and chemical properties of the soil (Alexander, 1977).

The clay fraction of the soil is the most influential in terms of microbial effects followed by the silt and sand fractions in that order.

The organic matter content in the sediment in the mangrove swamp at Gazi Bay ranged from 3.53-37.17% (Table 15). The amount of organic matter in the Rhizophora zone was significantly higher than the level of organic matter in the Ceriops and Aviccenia zones. However, the Ceriops and Aviccenia zones were not significantly different from each other with respect to the levels of organic matter i.e Rhizophora > Ceriops = Aviccenia. The average water content in the Rhizophora, Ceriops and Aviccenia plots was 72.84%, 29.95% and 41.19% respectively. Analysis of variance showed that the three plots were significantly different ($F = 203.06$; $P < 0.001$) with respect to the levels of water in the sediments.

Slim and Gwada (1993) studied the levels of organic carbon and nitrogen in the same plots. The level of organic carbon was 15.61% and 1.64% for the Rhizophora and Ceriops plots respectively. On the other hand, the levels of organic nitrogen were 0.832% and 0.088% for the Rhizophora and Ceriops plots respectively. The Aviccenia plot was not studied.

The levels of organic matter in the Rhizophora and Ceriops plots observed in the current study agree with the observations made by Slim and Gwada (1993). This is despite the fact that in their experiment only the organic carbon and organic nitrogen fraction of organic matter were measured.

In this study, the organic matter content of sediment in the Rhizophora plot was almost seven times higher than in the Ceriops plot and five times higher than in the Aviccenia plot. There was no significant difference between the level of organic matter in Ceriops and Aviccenia plot.

The distribution of organic matter in Gazi Bay seems to be influenced by the grain size of the sediment. Average grain size in the Rhizophora plot was 56 µm and in the Ceriops plot it was 256 µm (Slim and Gwada, 1993). A negative correlation exists between organic carbon and the grain size of the sediment (Alexander, 1977; Alongi, 1988). Thus, the higher organic matter content observed in the Rhizophora plot compared to Ceriops plot is a result of the smaller grain size of sediment particles in the former.

There is no data on the size of sediment grains in the Aviccenia plot but, field observations showed that the lower reaches of the Aviccenia plot were sandy while the upper reaches were muddy. Thus, the Aviccenia zone in Gazi Bay can be divided into a sandy Aviccenia and a muddy Aviccenia areas. The reverse situation is observed in the Ceriops plot where the lower reaches of the plot are muddy and contain as much organic matter as is present in the muddy Aviccenia area.

On the other hand the upper reaches of the Ceriops plot were sandy. Thus, the Ceriops zone in Gazi Bay can also be distinguished into a sandy Ceriops zone and a muddy Ceriops zone. This situation was probably the reason why there was no significant difference between the Aviccenia and Ceriops plots with respect to the amount of organic matter in the sediment.

Another factor which cannot be ignored is that of sedimentation of particulate organic matter (POM) from overlaying water in the swamp. Chapman (1976) noted that the high density of plants and pneumatophores in mangrove swamps enhances the sedimentation of organic matter from overlaying water.

In Gazi Bay, it is likely that the highest rate of sedimentation was in the Rhizophora plot compared to either Aviccenia or Ceriops plot. This is because seawater carrying particulate organic matter passes the Rhizophora plot before reaching the other two plots. Thus, the high prop root density in the Rhizophora plot (Plate 3) is likely to enhance the sedimentation of most of the load being carried by the seawater. This might have contributed to the high levels of organic matter in the plot. This is supported by the fact that most of the litterbags in the plot were seen to be buried under mud after two to three weeks in the field.

There was a significant correlation between the levels of organic matter and water content of the sediment in each plot ($r = 0.837$, 0.887 and 0.615 for the Rhizophora, Ceriops and Aviccenia plots respectively; $P < 0.05$). Thus, in the Rhizophora plot, the percentage organic matter content was high and subsequently the water content of sediment was also high. On the other hand, the Ceriops plot, with a lower organic matter content also had a lower percentage water content.

5.5 CHARACTERISTICS OF BACTERIA ISOLATED FROM GAZI BAY.

Characteristics of dominant heterotrophic bacteria isolated from Gazi Bay using nutrient agar are given in Tables 1 ,2 and 3. The possible identities of the isolates were determined using various identification keys and tables (Austin, 1988; Kerr, 1985; Starr et al., 1981).

5.5.1 Gram Positive Cocci (A2, AW3, AL1, AL2, R1, R2, RW1, RL2, RL3, CW1 and CL2).

Majority of the heterotrophic bacteria isolated in Gazi Bay fell into this group. All the gram positive spherical shaped bacteria except strains RL2 and RW1 were small in size (diametre $\leq$ 0.286 μm).

About half of the isolates were oxidase negative except, strains A2, AW3, RL2, R2 and RL3 which were oxidase positive (Tables 1 , 2 and 3). On the other hand, strains R1, R2, RW1, CL2 and CW1 could ferment glucose. Strain R1 could ferment glucose producing acid and gas while the rest yielded only acid. The ability to ferment glucose is a key factor in distinguishing between strains of gram positive cocci (Schleifer et al., 1981).

Thus, the eleven strains were split into those strains which could ferment glucose (R1, R2, RW1, CL2 and CW1) and those which could not (A2, AW3, AL1, AL2, RL2 and RL3). The genera Micrococcus, Staphylococcus and Streptococcus are the main gram positive cocci present in the environment (Mitruka, 1976). The three genera are usually distinguished on the basis of ability to ferment glucose. Staphylococcus and Streptococcus strains usually ferment glucose but Micrococcus species are unable to ferment glucose(Schleifer et al., 1981). Thus, those strains which were unable to ferment glucose closely resembled members of the genus Micrococcus.

All the eleven strains except R2 were catalase positive. Hill (1981) and Mitruka (1976) reported that members of the genus Streptococcus are the only Gram positive coccoid bacteria that lack catalase. However, some catalase negative Micrococcus have been reported which may be confused with Streptococcus (Hill, 1981). Therefore, the ability to ferment glucose by strain R2 distinguishes this strain from any Micrococcus spp that may be catalase positive. Thus, strain R2 closely resembled members of the genus Streptococcus. The other strains (R1, RW1, CL2 and CW1) which could ferment glucose and were catalase positive closely resemble staphylococcus (Kerr, 1985).

However, further studies are necessary before the true identity of these strains can be established. The genera Staphylococcus and Micrococcus have been reported in the marine environment (Chandramohan, 1988; Bottler, 1977) while the genus Streptococcus has not been reported in the marine environment.

The eleven strains could have played various roles in the decomposition of mangrove litter. All the strains except A2 and CL2 could have assisted in protein hydrolysis as they were able to hydrolyse gelatin (Tables 1 , 2 and 3). Strain A2, CW1, CL2, RW1, R1 and R2 which could hydrolyse starch probably participate in decomposition of carbohydrates present in the litter.

Strains AL1, AL2 and CL2 could all grow on cellulose media and probably participate in cellulose decomposition in the swamp. Cellulose forms the bulk of plant material hence these three strains may have an important role to play in the process of decomposition. Indeed, strains AL1 and AL2 which were dominant in Aviccenia marina leaves could probably have been responsible for the high rate of decomposition of A. marina leaves observed in Gazi Bay.

5.5.2 Gram Positive Rods (A3, AW2, CL1, RL4, RL1, RW2 and CW2).

The Gram positive rod shaped bacteria were second in abundance in Gazi Bay. All the strains lacked spores. All the strains except RL1 and CW2 were catalase positive. Strains RW2 and AW2 could ferment xylose and lactose respectively. Strain RL1 and CW2 which are catalase negative closely resemble Lactobacillus (Barksdale, 1981), but both strains were motile and failed to ferment glucose.

All known species of Lactobacillus can ferment glucose and lack motility (Barksdale, 1981). There are some catalase negative mutants in the Genus Corynebacterium (Barksdale, 1981). However, identification of the Gram positive rod shaped bacteria could not be established. More advanced techniques of identification are necessary before these strains can be assigned to any of the known genera.
Subsequently, the Gram positive non-spore forming rod shaped bacteria isolated in Gazi Bay could either belong to the genus Corynebacterium, Propionibcterium, Brevibacterium, Cellulomonas, Norcadia, Mycobacterium or Microbacterium.
All these genera are Gram positive non-spore forming rod shaped bacteria which are sometimes referred to as the coryneform bacteria (Barksdale, 1981; Goodfellow and Minnikin, 1981).

Several roles could probably be played by the seven strains of Gram positive non-spore forming rod shaped bacteria isolated in Gazi Bay. Strains C2, CW2, CL1 and RW2 could hydrolyse starch and probably assited in the decomposition of carbohydrates. Strains AW2, C2, RL1, CW2, RL4 and RW2 could hydrolyse gelatin and probably participate in protein hydrolysis. Strains RL1 and RW2 could grow on cellulose media and may be important in decomposition of cellulose which makes up the bulk of plant material.

5.5.3 Gram Negative Rods (AW1, A1, C1 and C3).

Only four strains of Gram negative rod shaped bacteria were isolated in Gazi Bay. Strains C1 and C3 were both oxidase negative, while AW1 and A1 were oxidase positive. Strain AW1 could hydrolyse gelatin and closely resembles the genus Pseudomonas (Kerr, 1985) although it was non-motile and could not reduce nitrates.

Strain A1 failed to hydrolyse gelatin and resembles members of the genus Alcaligenes (Kerr, 1985), but did not show an alkaline reaction with Simmon's Citrate Agar.

Strains C1 and C3 were both oxidase positive, motile and could ferment glucose and closely resemble members of the genus Proteus (Kerr, 1985), but could not reduce nitrates. At this stage it is futile to allocate the Gram negative rod shaped strains to any specific genus as additional tests need to be done.

The four strains of Gram negative rod shaped bacteria could play several roles in decomposition in the mangrove swamp. Strains C1 and C3 which hydrolysed starch probably takes part in carbohydrate decomposition. Strains C3 and AW1 which could hydrolyse gelatin and C1 which could hydrolyse urea probably take part in protein hydrolysis. Strains C3 and AW1 showed cellulolytic activity and were probably important in decomposition of cellulose present in mangrove litter.

5.5.4 Effect of Bacterial Characteristics on Decomposition Rates.

The majority of heterotrophic bacteria in the mangrove swamp at Gazi Bay were Gram positive spherical shaped bacteria. These were followed in predominance by the Gram positive rod shaped bacteria. Thus, the decomposition process in the mangrove swamp may largely be due to these two groups of bacteria isolates.

Most of the strains isolated from Gazi Bay were catalase positive (Table 1, 2 and 3) suggesting that they were either aerobic or facultative anaerobes. Thus, they could use oxygen as their terminal electron acceptor without suffering the adverse effects of hydrogen peroxide.

Important characteristics studied which relate to decomposition in the swamp include, cellulose utilization, starch hydrolysis and gelatin hydrolysis. Cellulose and starch form a large part of the plant cell constituents. Thus, the presence of bacteria strains which can hydrolyse these substrates may point at the relative utilization of those substrates in the three plots in the mangrove swamp.

The strains in the Ceriops plot except C1 could all hydrolyse starch. However, starch utilization does not seem to be important in influencing decomposition. This is because, the Ceriops plot which had more starch hydrolysing bacteria (Tables 1, 2 and 3), had the lowest rates of cellulose and leaf decomposition compared to the Aviccenia and Rhizophora plots. The ability by some strains to utilize cellulose may have caused the differences in decomposition rates observed in Gazi Bay. The Aviccenia plot which had 3 strains (AL1, AL2 and AL3) that were able to grow on cellulose media also had the higher rates of leaf and cellulose decomposition.

The Rhizophora and Ceriops plots each had two strains that could grow on cellulose media and these may partly be responsible for the lower rates of leaf and cellulose decomposition observed in the two plots.

5.6 SUMMARY.

The study in Gazi Bay, revealed that the numbers of aerobic heterotrophic bacteria in the Aviccenia, Ceriops and Rhizophora plots were not significantly different during the experiment period. The numbers of anaerobic heterotrophic bacteria were significantly different in certain sampling dates but not in others. The numbers of aerobic heterotrophic bacteria observed in water collected from the Rhizophora plot, were significantly higher than those observed in the Ceriops and Aviccenia plots. Numbers of nitrate reducing bacteria in the sediment were also significantly higher in the Rhizophora plot compared to the other two plots. The Rhizophora plot had the highest rate of nitrogen fixation, while the Ceriops plot had the lowest.

Cellulose and leaf decomposition was faster in the Aviccenia plot, followed by the Rhizophora and Ceriops plots in that order.

The differences in decomposition rates observed in the three plots are not due to size of the bacteria population, but may be due to an interaction of several factors. The most important factors seem to be, the activity of the heterotrophic bacteria, environmental factors especially aeration (exposure) and the species diversity of the bacteria involved.

Majority of the bacteria involved in decomposition in Gazi Bay were Gram positive cocci. The leaf texture and bacteria population on the leaf surface may also have contributed to the differences in leaf decomposition rates.

5.7 RECOMMENDATIONS.

From the current study, it is recommended that:

1. Bacteria strains in the mangrove swamps should be isolated and identified accurately using modern identification techniques like numerical taxonomy and chemotaxonomy. This is important but could not be achieved in this study as it was beyond the scope of this work.
2. The role of fungi and possibly actinomycetes in decomposition of mangrove litter should also be investigated since a number of fungal colonies were observed in the media during this study.

3. The process of nitrogen fixation in the East African mangroves needs thorough investigation. The strains of bacteria involved need to be isolated and identified and the effect of the spring-neap tide cycle on the rate of nitrogen fixation be thoroughly investigated.

4. The effect of seasonal variation on the following variables should be studied.

(i) Decomposition of leaves of different mangrove species.

(ii) Bacteria numbers.

(iii) Nutrient levels.

(iv) Rate of nitrogen fixation.

5. Decomposition experiments need to be done on other mangrove species like Bruguiera gymnorrhiza and Sonneratia alba which form a sizable proportion of the mangroves in Gazi and on the Kenyan coast.

REFERENCES.

Agate A.D. (1988). The sulfur and iron cycles. In; Agate A.D., C.V. Subramanian and M. Vannucci (eds). Mangrove microbiology. Role of microorganisms in nutrient cycling of mangrove soils and waters. UNDP/UNESCO Regional project on Research and its application to management of mangroves of Asia and the pacific. RAS/86/1988, New Delhi. pp 101-106.

Alexander M. (1977). Introduction to Soil Microbiology. John Wiley & Sons, New York. pp 3-203.

Albright L.J. (1976). In situ degradation of mangrove tissues. New Zealand J. Mar. Freshwater Res.10: 385-389.

Allen S.E., H.M. Grimshaw, J.A. Parkinson and C. Quarmby (1974). Chemical Analysis of Ecological Materials. Blackwell Scientific Publication, Oxford.pp 23-225.

Alongi D.M.(1988). Bacterial Productivity and Microbial Biomass in Tropical Mangrove sediments. Microbial Ecology 15: Springer-Verlag, New York. pp 59-79.

Anonymous (1985). Even wastelands have their uses. Earthscan. The Standard (Kenya) April 5, 1986.

Austin B. (1988). Methods in Aquatic Bacteriology. John Wiley and Sons, Chichester. pp 72-81.

Barksdale L. (1981). The Genus Corynebacterium. In; Starr P.M, H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel (eds). The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria Vol II. Springer-Verlag, Berlin. pp 1827-1835.

Barnes R.S.K and R. Hughes (1982). An Introduction to Marine Ecology. Blackwell Scientific Publications, Oxford. pp 108-114.

Beekman H., E. Gallin and E. Coppejans (1990). Indirect Gradient Analysis of the Mangal Formation of Gazi Bay, Kenya. In; Silva Gandavensis 54: pp 57-72.

Boaden P.J.S. and R. Seed (1985). An Introduction to Coastal Ecology. Blackie, New York. pp 115-129.

Boto K.G. (1984). Waterlogged Saline Soils. In; Snedaker S.C. and J. G Snedaker (eds). The Mangrove Ecosystem: Research Methods. Monographs on Oceanographic Methodology 8: UNESCO. pp 114-130.

Boto K.G. (1988). The carbon cycle. In; Agate A.D., C.V. Subramanian and M. Vannucci (eds). Mangrove Microbiology. The Role of Microorganisms in Nutrient Cycling of Mangrove soils and waters. UNDP/UNESCO Regional Project on Research and its Application to Management of Mangrove of Asia and the Pacific. RAS/86/1988, New Delhi. pp 61-67.

Boto K.G., D.M. Alongi and L.J.N Alan (1989). Dissolved organic carbon-bacteria interaction at the sediment water interface in tropical mangrove system. In; Mar. Ecol. Prog. Ser.. Springer-Verlag, New York. pp 243-251.

Bottler M. (1977). Numerical Taxonomy and Characteristics of Saprophytic Bacteria isolated from Kiel Fjord and Kiel Bight. In; Rheinheimer G. (ed). Microbial Ecology of a Brackish Water Environment. Ecology of Estuaries 25: Springer-Verlag, New York. pp 168-171.

Brinson M.M.(1977). Decomposition and nutrient exchange of litter in an alluvial swamp forest. Ecology Vol 58 No 3: pp 601-609.

Burkholder P.R and G.H. Bornside (1957). Decomposition of marsh grass by aerobic marine bacteria. Bull. Torrey Bot. Club 84. pp 366.

Campbell R., R.W.C. Berkeley, A.H. Linton and M.F. Madelin (1988). Microbiology of the soil, air and water. In; Hawker L.E. and A.H. Linton (eds). Microorganisms: Function, Form and Environment. Edward Arnold, London. pp 243-269.

Cerco F.C. (1981). Nitrification in the Upper Tidal St. James River. In; Nelson J.B. and L.E. Cronin (eds). Estuaries and Nutrients. Humana press, Clifton, New Jersey. pp 413-423.

Chandramohan D. (1984). Microbiology of Mangrove Swamps. In; The UNDP/UNESCO Report on the Second Introductory Training Course on Mangrove Ecosystem. Goa, India. pp 63-69.

Chandramohan D. (1988). The nitrogen cycle. In; Agate A.D., C.V. Subramanian and M. Vannucci (eds). Mangrove Microbiology. The Role of Microorganisms in Nutrient Cycling in Mangrove Soils and Waters. UNDP/UNESCO Regional Project on Research and its Application to the Management of the Mangrove of Asia and the Pacific. RAS/86/1988, New Delhi. pp 61-67.

Chapman V.J. (1974). Mangrove Biogeography. In; Walsh G.; S. Snedaker and H. Teas (eds). Proceedings of an International Symposium on Biology and Management of Mangrove Vol I. Honolulu, Hawaii. pp 3-24.

Chapman V.J. (1976). The Coastal Vegetation. J. Cramer, Lenterhausen. pp 217-234.

Colwell R.R., R.K. Sizemore, J.F. Carney, J.D. Nelson jr, J.H. Pickar, J.D. Walker, R.Y. Morita, S.D.Valkenberg and R.T. Wright (1975). Marine and Estuarine Microbiology Laboratory Manual. University Press, Baltimore.

Corpe W.A. and H. Winters (1972). Hydrolytic enzymes of some periphytic bacteria. In; Can. J. Microbiol.18:1483.

Cundell, A.M., M.S. Brown, R. Stanford and R. Mitchell (1979). Microbial degradation of Rhizophora mangle leaves immersed in the sea. Est. Coastal Shelf Sci. 9:281-286.

Dale N. (1974). Bacteria in intertidal sediments: factors related to their distribution. In; Limnol.Oceanogr.19:105.

Doute R.N., N. Ochanda and H. Epp (1981). A Forest Inventory of Kenya Using Remote Sensing Techniques. KREMU Technical Report, Series No 30, Nairobi. Kenya Rangeland Ecological Monitoring Unit. pp 72.

Drevon J.J. (1983). To Evaluate the nitrogenase activity of legume nodules using acetylene reducing activity. In; FAO/GRET I Biol 5: pp 1-6.

Fell J.W. and I.M. Master (1984). Litter decomposition and nutrient enrichment. In; Snedaker S.C. and J.G. Snedaker (eds). The mangrove Ecosystem: Research Methods. Monographs on Oceanographic Methodology 8: UNESCO. pp 239-251.

Fenchel T. (1977). Aspects of Decomposition of Seagrasses. In; McRoy C.P. and C. Helfferich (eds). Seagrass Ecosystems. Marcel Dekker, New York. pp 123.

Fenchel T. (1984). Suspended marine bacteria as a food source. In; Fasham M.J.R. (ed). Flows of energy and materials in marine ecosystems: Theory and practice. Plenum press, New York. pp 301.

Forsberg F.R. (1974). Phytogeography of micronesian mangrove. In; Walsh G., S. Snedaker and H. Teas (eds). Proceedings of an International Symposium on Biology and Management of Mangrove Vol I. Honolulu, Hawaii. pp 25-42.

Golterman H.L., R.S. Clymo and M.A.M Ohnstad (1978). Methods for Physical and Chemical Analysis of Freshwater. IPB Handbook No. 8. Blackwell Scientific Publications, Oxford. pp 103-104.

Goodfellow M. and D.E Minnikin (1981). Introduction to the Coryneform Bacteria. In; Starr P.M., H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel (eds). The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria Vol II. Springer-Verlag, Berlin. pp 1800-1826.

Graham R.M. (1929). Notes on the mangrove swamps of Kenya. J.E. Afr.Nat. Hist. Soc. 36: 157-164.

Hardy R.W.F. and R.D. Holsten (1977). Measurement of N_2-fixation Alternative Substrate Methods. In; Hardy R.W.F. and A.H. Gibson (eds). A Treatise on Dinitrogen Fixation. John Wiley & Sons, New York. pp 468-471.

Hardy R.W.F., R.D. Holsten, E.K. Jackson and R.C. Burns (1968). The Acetylene Reduction Assay for N_2 Fixation: Laboratory and Field Evaluation. In; Plant Physiology 43: pp 1185-1207.

Heald E.J. (1971). The production of organic detritus in a South Florida estuary. Sea Grant Technical Bulletin No 6. University of Miami Sea Grant Program (Living resources), Miami, Florida. pp 110.

Hesse P. R. (1961). Some differences between the soils of Rhizophora and Aviccenia mangrove swamp muds. In; Nature 193; pp 295-346.

Hicks B.J. and W.B. Silvester (1984). Nitrogen Fixation Associated with the New Zealand Mangrove (Aviccenia marina (Forsk. f) Bakh). Appl. Environ. Microbiol. American Society for Microbiology. pp 955-959.

Hill B.G. (1981). The Family Streptococcaceae. In; Starr P.M, H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel (eds). The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria Vol II. Springer-Verlag, Berlin. pp 1631-1633.

Isaac W.E. and F.M. Isaac (1968). Marine Botany of the Kenya Coast 3. General Accounts of Environment, Flora and Vegetation. J.E. Afr. Nat. Hist.Soc. 27 (1): 2-28.

Kariuki F.W. (1992). Growth and dynamics of Eichhornia crassipes (Mart.) Solms in Lake Naivasha, Kenya. Msc. thesis, Kenyatta University. pp 75-95.

Kazungu J.M., F. Dehairs and L. Goeyens (1986). Nutrient Distribution Patterns in Tudor Estuary (Mombasa, Kenya), During the Rainy Season. To be published in; Kenya J. Sci. Series B Vol. 10.

Kazungu J.M., F. Dehairs and L. Goeyens (1993). Distribution of Nutrients and Particulate Organic Material in Gazi Bay. In; Woitchik A.F. (ed). Report on the Dynamics and Assessment of Kenyan Mangrove Ecosystems n° TS2-0240-182 (GDF). Netherlands-Belgium Kenya mangrove ecosystem project. pp 175-182.

Kerr T.J. (1985). Applications in General Microbiology. A Laboratory Manual. Hunter Textbooks inc., North Carolina. pp 13-149.

Kennish M.J. (1986). Ecology of Estuaries Vol I. CRC Press, Boca Raton, Florida.

Knowles R. (1980). Nitrogen Fixation in Natural Plant Communities and Soils. In; Bergersen F.J. (ed). Methods for Evaluating Nitrogen Fixation. John Wiley & Sons. pp 557-582.

Koike I. and A. Hattori (1978). Simultaneous determination of nitrification and nitrate reduction in coastal sediments by a ^{15}N dilution technique. Appl. Environ.Microbiol. 35: 853-857.

Kokwaro J.O. (1986). The distribution and economic importance of mangrove forests of Kenya. J. E. Afr. Nat. Hist. Soc. 75: 1-10.

Kim J. and C.E.Zobell (1974). Occurrence and activity of cell free enzymes in oceanic environments. In; Colwell R.R. and R.Y. Morita (eds). Effects of the ocean environment on microbial activities. University Park Press, Baltimore. pp 368.

Kwale District Development Plan (1987 - 1993). Min. of Plann. and Nat. Dev. Republic of Kenya. pp 1-20.

Lee J.J. (1980). A Conceptual Model of Marine Detrital Decomposition and the organisms associated with the process. In; Droop M.R. and H.W. Jannasch (eds). Adv.Aquat. Microbio. Vol 2: 257-281.

Lind E.M. and M.E.S. Morrison (1974). East African Vegetation. Longman, London. pp 128-136.

Litchfield C.D. (ed) (1976). Marine microbiology. Benchmark papers in microbiology/II. Dowden Hutchinson & Ross Inc. Strousberg, Pennsylvania. pp 280-285.

Lugo A.E. and C. Snedaker (1974). The Ecology of Mangroves. Ann. Rev. Ecol. Syst. Vol 5: 39-63

Mann K.H. (1982). Ecology of Coastal Waters. A Systems Approach. University of California Press, Berkeley. pp 1-46.

Martosubrato P. and N. Naamin (1977). Relationships between tidal forest (mangroves) and commercial shrimp production in Indonesia. Mar. res. Indonesia 18. pp 81-88.

Mason C.F. (1976). Decomposition: Studies in Biology No 64. Edward Arnold Publications, London. pp 1-46.

Meyer-Reil L.A. (1984). Bacterial biomass and heterotrophic activity in sediments and overlying waters. In; Hobbie J.E. and D.J. Le B. Williams (eds). Heterotrophic activity of the sea. Plenum Press, New York. pp 523.

Mitruka B.M. (1976). Methods of detection and identification of bacteria. CRC Press, Cleveland, Ohio. pp 25-95.

Morita R.Y. (1977). The role of microorganisms in the environment. In; Anderson N.R. and B. Zahuranec (eds). Oceanic Sound Scattering Production. Plenum Press, New York. pp 445.

Montagna P.A. and E. Ruber (1980). Decomposition of Spartina alterniflora in different seasons and habitats of a northern Massachusetts salt marsh, and a comparison with other Atlantic regions. Estuaries 3: pp 61.

Mwatha E.W. (1991). Microbial Ecology of Kenyan Soda Lakes. PhD. Thesis. University of Leicester. pp 243.

Nedwell D.B. (1973). Inorganic nitrogen metabolism in a eutrophicated tropical mangrove estuary. In; Water Res. Vol 9. 221- 231.

Odum E.P. and A.A. de la Cruz (1967). Particulate organic detritus in a Georgia salt marsh estuarine ecosystem. In; Lauff G.H. (ed). Estuaries. American association for advancement of science, Washington D.C. pp 383.

Odum E.P. and E.J. Heald (1975). The Detritus based food web of an estuarine mangrove community. In; Crown L.E. (ed). Estuarine research. Academic Press, New York. pp 265-286.

Padgett D.E., C.T. Hackney and R.K. Sizemore (1985). A technique for distinguishing between bacterial and non-bacterial respiration in decomposing Spartina alterniflora. Hydrobiologia 122: 113.

Palumbo A.V. and R.L. Ferguson (1978). Distribution of suspended bacteria in NewPort river estuary, North Carolina. Est. Coastal Mar. Sci. 7: 521.

Postgate J.R. (1972). The acetylene reduction test of N fixation. In; Methods in Microbiology (FAO) Vol 6B. Academic Press, London. pp 343-355.

Potts M. (1984). Nitrogen Fixation in Mangrove forests. In; Por F.D. and I. Dor (eds). Hydrobiology of the Mangal. Dr. W. Junk Publishers, The Hague. pp 155-161.

Rao T.S.S and S.S.R. Nair (1984). Trophic relationships in mangrove ecosystems. In; The UNDP/UNESCO second Introductory Training Course on Mangrove Ecosystem. Goa, India. pp 87-91.

Refai M.K. (1979). Microbiological Analysis. FAO Food and Nutrition Paper 14/4. Manuals of Food Quality Control. Rome. PP D3-D5.

Rheinheimer G. (1977). Regional and Seasonal Distribution of Saprophytic and Coliform Bacteria. In ; Rheinheimer G. (ed). Microbial Ecology of a Brackishwater Environment. Ecology of Estuaries 25: Springer-Verlag, New York. pp 121-137.

Rublee P.A. (1982). Bacteria and microbial distribution in estuarine sediments. In; Kennedy V.S. (ed). Estuarine Comparisons. Academic Press, New York. pp 159-182.

Rublee P.A. and B.E. Dornseif (1978). Direct counts of bacteria in the sediments of a North Carolina salt marsh. In; Estuaries 1: pp 188.

Rublee P.A., L. Cammen and J. Hobbie (1978). Bacteria in a North Carolina salt marsh: standing crop and importance in decomposition of Spartina alterniflora. University of North Carolina Sea Grant Publication. UNCSG-78-11 Raleigh.

Ruwa R.K. (1990). The effects of habitat complexities created by mangroves on macrofaunal composition in brackish water intertidal zones at the Kenya coast. Discovery and Innovation Vol 2 No 1: pp 59-55.

Ruwa R.K. (1992). Zonation and distribution of creek and fringe mangroves in semi arid Kenyan coast. In; Leith H. and A. Al-Masoom (eds). Towards a rational use of high salinity tolerant plants Vol 1. Khumer Academic Publishers,

Ruwa R.K. and P. Polk (1986). Additional information on mangrove distribution in Kenya; some observations and remarks. In; Kenya J. Sci. (Nairobi) B7 (2): 41-45.

Schleifer K.H., W.L. Kloos and M. Kocur (1981). The Genus Micrococcus. In; Starr P.M., H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel (eds). The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria Vol II. Springer-Verlag, Berlin. pp 1539-1547.

Schneider and G. Rheinheimer (1988). Isolation methods. In; Austin B. (ed). Methods in Aquatic Bacteriology. John Wiley & Sons, Chichester. pp 72-81.

Seiburth J.M. (1976). Bacterial substrates and productivity in marine ecosystems. Ann. Rev. Ecolog. Syst. 7: 259.

Slim E.J and P. Gwada (1993). The Mangrove Vegetation. In; Woitchik A.F. (ed). Report on the Dynamics and Assessment of the Kenyan Mangrove ecosystems n^0 TS2-0240-C (GDF). Netherlands-Belgium Kenya Mangrove Ecosystem Project. pp 194-211.

Slim E.J., B. Schonte and P. Gwada (1993) Removal of Fallen Litter by the Epibenthic Community. In; Woitchik A.F. (ed). Report on the Dynamics and Assessment of the Kenyan Mangrove ecosystems n^0 TS2-0240-C (GDF). Netherlands-Belgium Kenya Mangrove Ecosystem Project. pp 213-214.

Snedaker S.C. (1984). Introduction. In; Snedaker S.C. and J.G. Snedaker (eds). The Mangrove Ecosystem: Research Methods. Monographs on Oceanographic Methodology 8: UNESCO. pp xii-xv.

Srensen J. (1978). Capacity for denitrification and reduction of nitrate to ammonia in a coastal marine sediment. Appl. Environ. Microbiol. 35: 301-305.

Steinke T.D. and C.J. Ward (1987). Degradation of mangrove leaf litter in the St. Lucia Estuary as influenced by season and exposure. S. Afr. J. Bot. 53(5): 323-328.

Starr P.M., H. Stolp, H.G. Truper, A. Balows and H.G. Schlegel (eds)(1981). The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria Vol I & II. Springer- Verlag, Berlin. pp 500-1850.

Subramanian C.V. (1988). Organisms and processes involved in nutrient production, cycling and Transport in mangrove ecosystem. In; Agate A.D., C.V. Subramanian and M. Vannucci (eds). Mangrove microbiology. Role of microorganisms in nutrient cycling in mangrove soils and waters. UNDP/UNESCO regional project on research and its application to the management of the mangrove of Asia and the Pacific. RAS/86/1988, New Delhi. pp 3-6.

Survey of Kenya (1967). Map of Mombasa Series Y 503; sheet SB 37-3 edition 2-SK.

Valiela I. (1984). Marine Ecological Processes. Springer-Verlag, New York.

Vosjan J.H. and K.M. Olanczuk-neyman (1977). Vertical distribution of mineralization process in a tidal sediment. Netherlands J. Sea Res. 11(1): 14-23.

Walter H. and M. Steiner (1936). The Ecology of the East African Mangrove. In; Zeitschrift fur Botanik ed. 30. (Translated by Frau M.G. Jaenacke). pp 1-83.

Wiebe W.J. (1979). Anaerobic Benthic Microbial Processes: Change from Estuary to Continental Shelf. In; Livingstone R. (ed). Ecological Processes in Coastal and Marine System. Plenum press, New York. pp 469-481.

Woitchik A.F., B. Ohowa, R. Rao and F. Dehairs (1993). Mangrove litter as nutritive source in the mangrove ecosystem. In; Woitchik A. F. (ed). Report on the Dynamics and Assessment of Kenyan Mangrove Ecosystems n^0 TS2-0240-C (GDF). Netherlands-Belgium Kenya mangrove ecosystem project. pp 194-211.

Wood E.J.F (1965). Marine Microbial Ecology. Chapman & Hall, London. pp 116-119.

ZoBell C.E and R.Y Morita (1977). Deep Sea Bacteria. In; Thayer D.W. (ed). Microbial Interaction With the Physical Environment. Benchmark Papers in Microbiology. Dowden Hutchinson & Ross Inc., Pennsylvania. pp 267-282.

Zuberer D.A. and W.S. Silver (1978). Biological dinitrogen fixation (acetylene reduction) associated with Florida mangrove. Appl. Environ. Microbiol. : 567-575.

APPENDIX I.

Table 5. Average number of aerobic heterotrophic bacteria (cfu/g dwt sediment) in the Aviccenia, Ceriops and Rhizophora plot in Gazi Bay.

	P L O T S		
Date	*Aviccenia*	*Ceriops*	*Rhizophora*
27-10-92	1.20×10^8	4.70×10^8	1.40×10^8
01-11-92	7.98×10^7	5.79×10^7	1.16×10^8
07-12-92	2.18×10^8	4.78×10^7	2.01×10^8
15-12-92	2.14×10^7	1.59×10^7	4.77×10^7
19-01-93	1.38×10^7	7.44×10^6	1.69×10^7
26-01-93	2.16×10^7	4.37×10^7	3.91×10^7
11-02-93	3.73×10^7	1.38×10^7	1.09×10^7
26-02-93	1.08×10^8	1.92×10^7	4.22×10^7

Table 6. Average number of aerobic heterotrophic bacteria (cfu/ml) in water collected from the Aviccenia, Ceriops and Rhizophora plots in Gazi Bay.

	P L O T S		
Date	*Aviccenia*	*Ceriops*	*Rhizophora*
13-10-92	5.84 X 10^5	2.78 X 10^5	8.90 X 10^6
27-10-92	6.80 X 10^6	2.33 X 10^6	7.23 X 10^6
07-12-92	-	6.10 X 10^4	8.67 X 10^5
15-12-92	6.67 X 10^5	2.59 X 10^6	1.40 X 10^6
19-01-93	-	7.60 X 10^5	8.83 X 10^6
26-01-93	-	2.90 X 10^5	7.15 X 10^5
11-02-93	8.47 X 10^5	4.13 X 10^4	3.43 X 10^5

Table 7. Average number of anaerobic heterotrophic bacteria (cfu/g dwt) in sediment in the Aviccenia, Ceriops and Rhizophora plots in Gazi Bay.

	P L O T S		
Date	*Aviccenia*	*Ceriops*	*Rhizophora*
13-10-92	1.44×10^8	1.82×10^8	1.74×10^8
27-10-92	1.36×10^8	3.43×10^7	2.28×10^7
01-11-92	2.77×10^7	1.50×10^7	2.32×10^7
07-12-92	1.19×10^8	1.97×10^7	6.11×10^7
15-12-92	3.08×10^7	2.00×10^6	8.80×10^6
19-01-93	1.38×10^7	7.45×10^6	1.69×10^7
26-01-93	1.00×10^7	1.02×10^7	1.05×10^7
11-02-93	8.20×10^6	1.01×10^7	8.53×10^6

Table 8. Average number of aerobic heterotrophic (cfu/cm^2) on leaves belonging to Aviccenia marina, Ceriops tagal and Rhizophora mucronata in Gazi Bay .

	LEAVES		
Date	Aviccenia	Ceriops	Rhizophora
13-10-92	9.29×10^3	2.00×10^2	2.96×10^4
27-10-92	6.89×10^3	1.39×10^3	1.73×10^3
01-11-92	2.10×10^5	2.00×10^2	4.00×10^2
07-12-92	1.03×10^4	2.00×10^2	1.21×10^4
15-12-92	1.09×10^4	5.73×10^2	3.32×10^4
19-01-93	9.73×10^5	5.33×10^2	1.42×10^5
11-02-93	6.23×10^3	2.67×10^2	7.53×10^3

Table 9. Average number of nitrate reducing bacteria (cells/g dwt) in sediment in Gazi Bay.

	P L O T S		
Date	Aviccenia	Ceriops	Rhizophora
13-10-92	4.33×10^3	3.89×10^3	8.83×10^3
27-10-92	3.90×10^3	1.70×10^3	9.50×10^3
01-11-92	2.37×10^2	1.74×10^3	5.00×10^2
07-12-92	2.18×10^3	8.99×10^2	4.94×10^3
15-12-92	2.42×10^3	1.38×10^3	6.93×10^2
26-01-93	4.20×10^3	3.61×10^3	9.21×10^3
11-02-93	3.70×10^3	3.32×10^3	7.06×10^3

Table 10. Decomposition of cellulose in the Aviccenia, Ceriops and Rhizophora plots in Gazi Bay expressed as % dry weight loss from filter papers.

	P L O T S		
Duration (days)	Aviccenia	Ceriops	Rhizophora
0	0	0	0
4	12	5	12
12	39	10	23
20	62	20	30
28	92	11	72
34	81	16	65
69	98	16	74

Table 11. Average rates of acetylene reduction activity (nmole C_2H_4/g dwt/h) in sediment collected in the Aviccenia, Ceriops and Rhizophora plots in Gazi Bay.

	P L O T S		
Date	*Aviccenia*	*Ceriops*	*Rhizophora*
13-10-92[s]	3.808	0.925	3.687
27-10-92[s]	1.434	0.861	0.000
01-11-92[bn]	2.176	1.103	2.292
07-12-92[n]	15.174	1.287	51.472
15-12-92[s]	2.731	0.701	1.016
19-01-93[n]	0.410	0.836	3.653
26-01-93[bs]	0.671	0.007	1.961
11-02-93[s]	0.450	0.220	2.198

where:

[s] = spring tide; [n] = neap tide; [bs] = one day to spring tide and [bn] = two days to neap tide.

Table 12. Decomposition of Aviccenia, Ceriops and Rhizophora leaves in their native plots expressed as percentage dry weight loss in a given period of time.

	P L O T S		
Duration (days)	Aviccenia	Ceriops	Rhizophora
0	0	0	0
4	12	2	10
12	44	22	32
20	43	43	52
28	49	42	51
34	46	26	54
47	58	49	57
69	-	46	59

Table 13. Concentrations of combined NO_3^- and NO_2^- (μg-N/l) in sediment collected from the Aviccenia, Ceriops and Rhizophora plots.

	PLOTS		
Date	Aviccenia	Ceriops	Rhizophora
13-10-92	6.58	5.10	5.76
07-12-92	19.09	29.02	69.54
15-12-92	53.39	69.48	113.92
19-01-93	15.86	5.35	8.18
26-01-93	15.49	5.81	6.86
11-02-93	4.30	6.64	8.30
22-02-93	1.43	1.31	1.15

Table 14. Percentage water content recorded in the sediment collected from the *Aviccenia*, *Ceriops* and *Rhizophora* plots in Gazi Bay.

	P L O T S		
Date	*Aviccenia*	*Ceriops*	*Rhizophora*
13-10-92	44.60%	32.95%	72.69%
27-10-92	38.68%	32.40%	74.70%
01-11-92	38.76%	34.67%	74.27%
07-12-92	42.10%	25.67%	73.69%
15-12-92	44.67%	27.98%	69.37%
19-01-93	40.10%	27.40%	76.36%
11-02-93	39.56%	28.78%	68.85%

Table 15. Percentage organic matter in sediment in the Aviccenia Ceriops and Rhizophora plots at Gazi Bay.

	P L O T S		
Date.	Aviccenia	Ceriops	Rhizophora
13-10-92	9.18%	6.75%	29.95%
27-10-92	6.47%	5.60%	34.60%
01-11-92	11.87%	8.57%	37.17%
07-12-92	8.06%	3.53%	28.14%
15-12-92	9.29%	5.60%	25.34%
19-01-93	8.27%	5.53%	35.92%
11-02-93	7.97%	5.23%	25.37%