SORPTIONAL BEHAVIOUR OF METALS ON THE SEDIMENTS AND HUMIC ACID OF MANGROVE ECOSYSTEM

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CERTIFICATE

This is to certify that the thesis titled " Sorptional behaviour of metals on the sediments and humic acid of mangrove ecosystem" is an authentic record of the research work carried out by Sr. Janat Augustine K under my supervision and guidance in the Department of Chemical Oceanography, School of Marine Sciences, Cochin University of Science and Technology, in partial fulfillment of the requirements for PhD degree of Cochin University of Science and Technology and no part of this has been presented before for any degree in any University.

Kochi 16
September, 2002

Dr. N. Chandramohanakumar
(Supervising guide)

DECLARATION

I hereby declare that this thesis entitled " Sorptional behaviour of metals on the sediments and humic acid of mangrove ecosystem" is an authentic record of the research work carried out by me under the guidance and supervision of Dr. N. Chandramohanakumar, professor and Head, Department of Chemical oceanography, School of Marine sciences, Cochin University of Science and Technology, and no part of this has previously formed the basis of the award of any degree, diploma, associateship, fellowship or any other similar title or recognition.

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"The human creature feels very little before the wonder of the divine providence manifested in creation and history. At the same time she/he realises that she/he is the recipient of a message of love that calls her/him to responsibility. People are indeed appointed by God as administrators of the earth to cultivate and protect it. From here stem that which we might call the 'ecological vocation' which in our time has become more urgent need than ever".

- Pope John Paul II

At the world summit on Sustainable Development
at Johannesburg, South Africa.

Preface

There is a growing awareness among many on the mangrove ecosystem, its role in the intricate web of marine life, its value as a natural resource for local population the protection it affords to easily eroded coastlines and its importance as a buffer deriving its wealth from both the land and the sea. The scientists from different parts of the world have recognised and realized that this intertidal or tidal habitat of mangrove ecosystem is threatened and endangered. Alarming situations are ever increasing due to population pressure on these coastal and estuarine areas which has turned these mangals most vulnerable and fragile. In addition to it, pollutant discharges from industries, oil refineries, tanneries and urban areas have caused dramatic changes in these mangrove ecosystems through out the world. Conversion of agricultural lands, renovation of brackish water fisheries, prawn and shrimp farms, salt pans, development of ports, harbours, tourist spots, cities and discharges of pollutants from cities have several detrimental effects on the natural regeneration process of mangroves The scientific knowledge may solve the problems for developing an ideal ecosystem in the intertidal zone.

In view of the importance of mangroves it is good to anticipate some possible consequences of human intrusion. One predictable effect of more people and more human intervention is higher loads of metal into the ecosystem. This study was in part intended to throw some light on the efficiency of mangrove sediments and humic acid to bind metals. Humic acid is highly functionalised carbon rich biopolymer that stabilizes soils and sediments as soil or sediment organic matter. In addition they are effective acid–base buffers, they bind metals, molecules and other biopolymers. The study of the sorptional behaviour of sediment and humic acid from mangrove ecosystem in relation to the structure of Humic acid, has revealed to some extent the significance of humin and its function as a scavenger.

For the study mangrove samples were collected from three stations in Cochi area. Station i is Mangalavanam which is surrounded by brackish waters can be considered as a closed system with minimum human intervention. There is one outlet in the form of a canal opening to backwaters. Avicenia, Rhizphora and Acanthus are the three main mangrove species seen here. Station ii is Vypeen which is semi-enclosed system and lies adjacent to the brackish waters. The Vypeen island has a number of mangrove species. This is closer to the sea than the other two. Station iii is Nettor which can be considered as a vanishing mangrove ecosystem with a long strip of mangrove plants along the canal. This is an ecosystem with much anthropogenic input. The increasing prawn culture and the urbanization affect this area more than the other two. Bimonthly collections of both water and sediments were made from all the three different stations from Nov'99-Nov 2000

The thesis is divided into six Chapters. The first chapter gives a brief introduction on mangrove ecosystem, organic matter, characteristics of sediments and humic acid. The aim and scope of the present work are also included in this chapter.

The second chapter gives a description of the study area, sampling protocols for water and sediment samples and the various analytical methods employed in the estimation. Besides this the results of hydrographical parameters are given in this chapter.

The third chapter deals with the analytical characteristics of humic acid. The elemental composition of humic acid, seasonal variation of acidity, total phosphorus, carbohydrate, and metals in the humic acids are given in this chapter.

The fourth chapter summarises the results of spectral studies on humic acid. ^{13}C Nuclear magnetic resonance studies, Infra red spectroscopy, Ultraviolet spectroscopy and Scanning electron microscopy are used for the elucidation of the structure of humic acid.

The fifth chapter illustrates the sedimental parameters and the sorptional characteristics of sediment. Details of adsorption and desorption studies are included in this Chapter.

The sixth chapter is on sorptional behaviour of metals on humic acid as well as the sediment after the removal of humic acid. Batch wise adsorption results of metals, Cu, Fe and Mn at different pH and concentrations along with the results of desorption studies are given in this chapter. The references are included at the end of each chapter.

A brief summary of the work given at the end shows how the results have helped in the investigation of the structure of humic acid and its molecular interactions with metals. The sorptional study on the sediments and so called 'ambiguous substance' humic acid, separated from the mangrove sediments has thrown some light on this endangered ecosystem of Mangroves.

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Chapter 1

INTRODUCTION

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1.1 Mangrove Ecosystems

Mangrove ecosystems are dominant ecosystems along sheltered tropical coasts, comprising the "ecotone" between the coast and tropical rainforests. This ecosystem can be defined as the complex plant community below the high tide level and, typically found on flat coastal land in areas of high, year-round rainfall. They are one of the world's most productive ecosystems in terms of gross primary production and production of leaf litter. (Lugo and Snedaker, 1974) They are important transformative interfaces between land and sea which import inorganic matter from the terrestrial system and transport organic matter both dissolved and particulate to marine ecosystems (Sardessai, 1999). Odum *et al.*, (1972) has described the mangrove ecosystem as a "pass through" type of system because of large flow of organic matter transported through the mangrove coastal zones by physical activities of tides, runoff and rainfall, and the intense biological activities of decomposition, uptake, and bioturbation occurring in the mangroves that are important downstream in the marine ecosystem.

The sources of primary productivity include mangrove vegetation themselves, algal colonies associated with the mangrove root surfaces, moist forest floor and the phytoplankton communities in the associated bay and lagoons. The primary food source for aquatic organisms in mangrove dominated estuaries occurs in the form of particulate organic matter derived chiefly from the litter fall. Mangrove litter fall has been estimated to account for 30-60% of total primary production (Bunt *et al.*, 1979). The annual litteralfall normally ranges from 10,000 to 14,000 kg dry weight per hectare (Gopinathan and Selvaraj, 1996). The mangrove detritus and the subsequent mineralized nutrients are exported out of the mangrove ecosystem through tidal flushing. The leaf detritus not only provides the base of the major mangrove community food chain but also an important food source in adjacent deeper waters. These are found in the food base for marine micro organisms and these in turn support the valuable estuarine and near shore fishery (Naskar, 1989). In poorly mixed estuaries with sharp salinity gradients, flocs form the settling and coagulation of suspended organic matter in the water column. These organic materials are very valuable foods for crustaceans, mussels and benthic feeding fish like mullets. Dissolved organic compounds of mangrove origin provide an additional nutrition.

Mangrove ecosystems serve as the reservoir of a variety of species of plants and animals. The mangrove environment provides living space for a dependent biota of more than two thousand species of flora and fauna of resident, semi resident or migratory mode of life (Gopinathan and Selvaraj, 1996). These mangrove plants intake nutrients from the tidal sea water, river sources and these ecosystems provide natural food to the mangrove dwelling fauna (Odum *et al.*, 1972). The vegetation and its associated biota play a major role in contributing organic matter.

Mangrove swamps serve as vital nursery grounds for the economically important nearshore species. MacNae (1968) has reported that without the mangrove ecosystem several species of shrimps and prawns may not exist and strongly concluded that if there is no mangrove there is no prawn. Some prawn species may breed and complete their life cycle in the shallow mangrove dominated water. As many of such prawn species may require more saline water and depart offshore to spawn and their juveniles then migrate back to mangrove forest zone which serve as their grazing ground, till their maturation.

These ecosystems are valued not only for their resource value but for their ecosystem services in soil building, watershed stablilsation and coastal protection. Because of the high sedimentation rates in the mangroves, they build land. As such mangroves are an important "pioneer" species extending into the coastal zone and connecting the marine ecosystems to the edge of the rain forest. MacNae (1968) has reported that the undisturbed and natural mangrove ecosystems may act as the seaward barrier and check considerably the coastal erosion and minimize the tidal thrust or strong storm-hit arising from the sea. On the other hand the well developed mangrove habitats may also accelerate the siltation and accretion process by arresting the water transport silt and clay particles, which ultimately build up or extend the coastal zone. Mangrove coverage may act as a buffer agent and protect or minimize the barrier cyclone or surges of the bay considerably.

Mangroves are capable of trapping large quantities of heavy metals, without much significant effect on the vegetation. Environmental behaviour of trace metals is receiving increased attention with added emphasis on sources, sinks, and bioavailability. The various bio-geochemical processes that the trace metals undergo during their residence times in the ecosystem ultimately determine their distribution and fate in marine environment (Nair *et al.*, 1993). Since cycling of trace meal in an estuarine system is intensively coupled with the sedimentation processes of a particulate material, a knowledge of trace metal concentrations in sediment can play a key role in detecting source, distribution, mechanisms in coastal areas (Vollmer *et al.*, 1990).

Mangrove plants mostly grow within the sheltered intertidal flat deltaic lands, funnel shaped bays, broad estuarine mouths, shallow or frequently tidal-inundated coast lines (Thom, 1982) These mangroves prefer mostly the humid atmosphere (60%-90%) with moderate to high annual rainfall, between 1000mm - 3000mm). The factors like water level or tidal inundation, water and soil salinity, pH, sediment flask, oxygen potential, availability of anions and cations, hydrodynamics, stresses etc are important in determining the habit and habitat selection, individual species distribution, succession pattern and inter specific competition among these mangroves. Mangrove plants are very much distinct from other plants with respect to their morphological, anatomical, physiological, seeds and seedlings developmental, physiognomical adaptations and succession mechanisms. Mangroves are recognized by their characteristic presence of prop roots, pneumatophores and viviparous seeds. All these habits and habitat differences are governed by climatic, edaphic and salinity factors of both soil and water phases and the physicochemical properties of the soil itself. Each mangrove species has its own optimum salinity preference and its respiration rates have been found to be related to salinity. At salinities higher than optimal, respirational rates increase and net growth and leaf litter production decrease. As a result each mangrove species occupies a salinity zone which it is best adapted (Snedaker, 1978).

The total true mangrove species from the Indian mangals were estimated as 35 species, while another 35 mangrove associates or back mangroves were also identified by Naskar (1983). The Honourable supreme court of India has categorized mangrove habitats under the Coastal Zone Regulation –1(CRZ-1) area to ensure that they are sustained and conserved.

1.1.1 Mangroves of the Kerala Coast

The length of Kerala coast is about 560 km extending from north to south parallel to the 'Western Ghat.' The Kerala coast has beautiful beaches, minor estuaries and lagoons and the population density is very high as 2362/km^2. This higher population density in the Kerala coast has resulted in tremendous pressure on the natural ecosystem, particularly the mangroves. For urbanization, construction of harbours, ports, prawn farming, coconut plantation and ricefish culture, vast mangal lands were cleared and reclaimed (Gopalan, 2002). These reclaimed lands of the coastal areas are underdeveloped and rice culture or brackish water fish and prawn culture are commonly practised here. Ramachandran *et al.*, (1986) have reported several mangrove species from Kerala coast and have also reported that for fuel, wood harvest, the mangrove habitat in the Kerala state has declined. Kerala mangroves are distributed in eight coastal districts of which Kannur (755 Ha) has the largest area followed by Kozhikode (293 ha), Ernakulam (260ha), Alappuzha(90ha) and Kottayam (80ha) (Subramanian 2002). Sunilkumar (1993) has identified *Rhizophora mucronata, Rhizophora apiculata, Avicenia officinalis, Acanthus ilicifolius, Bruguiera cylindrica, Acrostichum aureum, Clerodendum inerme, Cerebra odollom, Derris trifoliata, Sonneralia apocctala* from the mangals of Cochin.

The rapid expansion of coastal cities throughout the world is exerting major impacts on all coastal ecosystems. There is a massive rural to urban migration occurring in the developing nations. Coastal aqua culture development has accelerated the loss of this extremely important habitat in many countries. There are also reports of extensive mangrove destruction caused by oil pollution, herbicides, and war.

1.1.2 The nature of Organic Matter in the Mangrove Ecosystem

In the tropical mangrove ecosystem, the vegetation and its associated biota play a major role in contributing organic matter. The average sediment organic matter in the mangrove environment is comparatively higher than the values reported elsewhere (Jagtap, 1987). The organic matter in the marine sediments is composed of a wide variety of more than 1000 different organic molecules (Degens and Mopper, 1976). The composition and origin of soil organic matter have been the subjects of controversy. The organic compounds found in water and sediment are mixture of biopolymers such as carbohydrates, lipids, hydrocarbons like alkanes, cycloalkanes, and iso-prenoids; fatty acids; fatty alcohols; Ketones and wax esters; steroids; triterpenoids; tetraterpenoids; pigments; amino acids and peptides; purines and pyramidines; aromatic hydrocarbons; natural polymers like chitin, cellulose, lignin and tannine; branched and cyclic hydrocarbons; proteins, phenols, as well as not well characterized macromolecules, generally referred to humic substances (Chester, 2000). It is the major part of Organic matter of most water sources. Humic substances are very important in aquatic systems as they induce phytoplankton multiplication, nutrient transportation, and sediment formation. It is known that humic acid form complexes with metal ions, hydrous oxides and interacts with clay minerals and organic chemicals that may be toxic pollutants. Most workers have attempted to make a distinction between the humified components of soil organic matter that have been called humus or humic substances and non humified components of soil organic matter. A major fraction of the organic matter in modern sediments cannot be readily described in terms of chemical composition. It is described by operational definitions based upon solubility properties. These are alkali insoluble humins, and alkali extractable humic compounds. Further more it is subdivided into humic acids, which insoluble under acid conditions especially where the pH is less than 2.0 and fulvic acids which are soluble at all pH values. (Rashid, 1975; Aiken *et al.*, 1985) Some 70-80% of the organic matter in surficial marine sediments may consist of humic and fulvic acids. (Nissenbaum and Kalpan, 1972). Humic and fulvic acids are heterogeneous and disorganized assemblages of aliphatic and aromatic compounds that together form complex high molecular weight macromolecules (Juhani, 1997).

1.2 Hypotheses of Humification

According to Kononova (1966), soil humic acid which is the main component of humic substances is a high molecular weight compound, which is formed as a result of the synthesis from simpler, chemically individual substances formed by the decomposition of tissue components of dead organism. In other words, soil humic acid is considered to be a heteropoly condensate(Christl *et al.*, 2001) of aromatic compounds with products of protein decomposition with the possible participation of substances of carbohydrates nature. The humification process is considered as involving condensation of various degradation products resulting from decomposition of plants, animals and bacterial biomass (Robert *et al.*, 1998).

Humic substances are the result of biological and chemical processes. Labile plant materials decompose rapidly on entering aerobic soil environments with adequate water supplies, but more resistant components transform slowly in the same environment. The highly transformed amorphous dark coloured material is known as humic substances. (Hayes, 1998). Aiken *et al.*, (1985) states that humic substances are a general category of naturally occurring heterogeneous organic substances that can generally be characterized as yellow to black in colour of high molecular weight and are refractory.

A variety of synthetic routes have been suggested, based both on attempts to synthesize humic materials and on their reactions. Detailed discussions of these are given by Konnova (1966). Current thinking is discussed by Stevenson (1994), Aiken *et al.*, (1985) and (Robert *et al.*, 1998). Presently the view is that humic substances are produced by condensation of quinones or phenolic compounds, which are themselves formed by the biological transformation of plant residues. The above hypotheses may be restated more concisely in the following forms.

a. The plant alteration hypotheses

Those fractions of the plant tissue that are resistant to microbial attack, especially the lignified tissues, are altered more or less superficially in the soil to produce humic substances. According to this view the nature of the original plant compound strongly influences the nature of the final humic substance. The higher molecular weight humic substances then represent the first stages of humification followed by subsequent microbial attack to degrade the humin to humic acid, then fulvic acid, and finally mineralisation to CO_2 and H_2O.

b. The chemical polymerisation hypothesis

Plant material is degraded microbially to small molecules which are absorbed by the microbes as sources of carbon and energy. The microbes synthesize various products, usually phenols and amino acids, which are excreted into the surrounding medium where chemical oxidation and polymerisation to humic substance take place.

c. The cell autolysis hypothesis

Humification is thought to be the product of the autolysis of plant and microbial cells after the death of a cell. The product is a heterogeneous substance formed by the random condensation and polymerisation of cellular debris such as sugars, amino acids, phenols and other aromatic compounds that are converted to free radicals by autolytic enzymes released on the death of the cell.

d. The microbial synthesis hypothesis

The early stages of microbial attack on plant tissues are seen to be the same as in the chemical polymerisation hypothesis in that a microbe utilizes the plant tissue only as a source of carbon and energy. However, the microbe synthesizes various high molecular humus like compounds intracellularly and these compounds are only released to the soil in which the microbe dies and its cells are lysed. According to this view the higher molecular weight compounds are present in the first stages of humification followed by extracellular microbial degradation to humic acid, fulvic acid and final mineralisation.

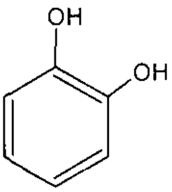
1.3 Humic Substances

Humic substances are important because this organic matter can affect soil fertility, mineral weathering, water acidity; and are involved in the transport, sequestration, and mitigation of contaminants and may even have an impact on atmospheric chemistry through the carbon cycle, in which carbon is constantly recycled between plants, animals, soil, air and water. Humic substances are divided into three fractions based on their solubility in aqueous acids and bases; humin, humic acid and fulvic acid. Humic substances from different environment such as from soil, interstitial waters, streams, ground waters, sea and ocean water differ widely from one another.

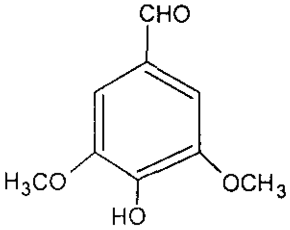
1.3.1 Structure of Humic Acid

A number of factors may be important in determining the structure of the organic matter in any environment. Nature of the parent material is important because the decomposing plant life contains a larger proportion of one class of chemical compounds than another and so the organic matter will contain different degradation products. Geographical and geochemcal factors are also important. For example humic substances from mangrove ecosystem differ from that of fresh water and so on. Another factor is the degree of microbiological activity, which influences the rate of transformation of plant and animal debris.

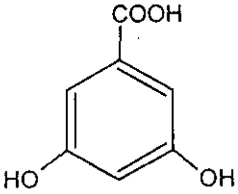
The researchers found a great deal of structural variety, including sheets and globular configurations, thread and net like shapes and small uniform aggregates in contrast to the previously held belief that the molecules form rings in acidic or strong electrolytes solutions and elongate in dilute alkaline solutions. Various reviews indicate that the microstructures of humic substances are different under different chemical conditions. The carbon skeleton of these macromolecules consists of a complex three-dimensional network of cross linked paraffinic structures covalently bonded and in association with various amounts of aromatic moieties. The basic structure consists largely of condensation products of compounds such as catechol, syringaldehyde and 3,5 dihydroxy benzoic acids shown in Fig 1.1 (Harrie *et al.*, 1991) Their structural core is surrounded by a variety of labile hydrophilic complexes including oxygen containing functional groups such as carboxyl, phenolic, and alcoholic hydroxyls, carbonyls and also acetyl, methoxyl, and aminogroups. (Sparks, 1997; Harrie *et al.*, 1991)



Catechol



Syringaldehyde



3,5-dihydroxybenzoic acid

In any given soil, the humic material is believed to consist of molecules ranging in molecular weight from a few hundred to several hundred thousand. Individual fractions such as humic acid merely represent a particular part of this molecular weight range. Since there is a wide variety of possible precursor molecules, molecules of humic material need not be chemically and structurally identical.

1.3.2 Humic acid - Metal Ion interaction

The ability of humic acid to form complexes with metal ions can be attributed to their high content of oxygen containing functional groups including carboxyl, phenolic, alcoholic, enolic, and carbonyl structures. In addition to these various functional groups, humic acids may contain nitrogen, phosporus and sulphur. The association of positively charged metal ions with negatively charged organic ions goes beyond the concept of cation exchange. Humic acids behave as negatively charged species due to the ionization of their acidic carboxyl and hydroxyl groups. That is why they can attach to positively charged metal ions and form complexes (Bettina, 2001). It is suggested that humic compounds of different origins differ in their metal binding behaviour because of different functional group contents. Structures commonly present in humic substance and have the potential for binding with metal ion are shown in fig 1.1.

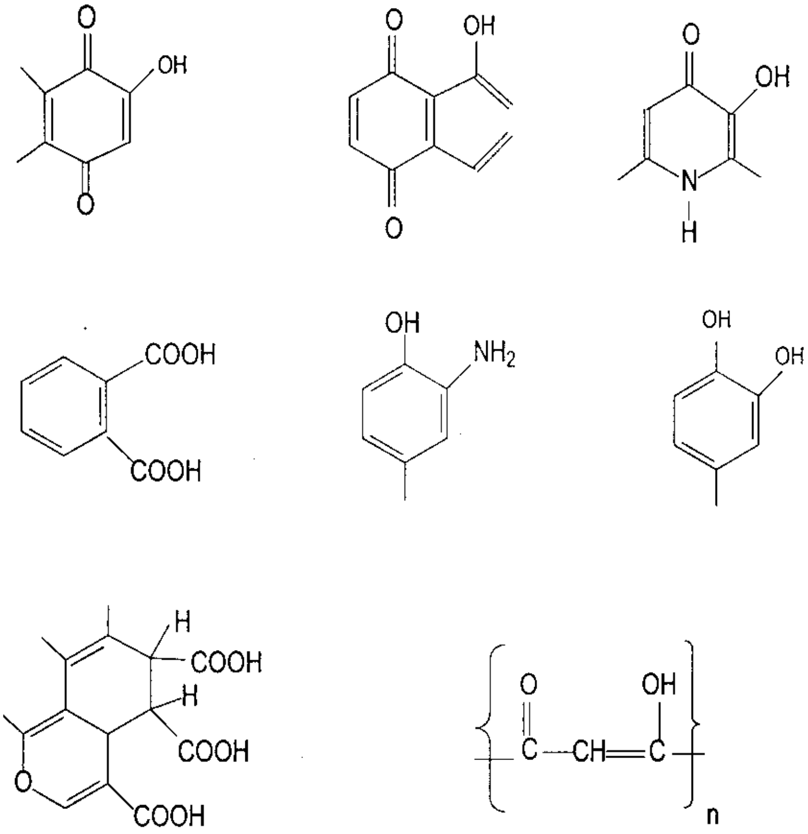


Fig 1.1

1.4 Roles of Humic Substances in Soil and Water Environment

The role of humic acid in soil fertility and terrestrial plant growth has been extensively reviewed. Attenuation of toxicity of heavy metals and other toxic compounds, simulation of the various physiological and biochemical processes associated with cellular metabolism, and increase in growth, root initiation, yield, nutrient uptake, chlorophyll synthesis, seed germination etc are worth mentioning. Even though the sediment organic matter composition of mineral soils is usually in the range of 1-5%, it would be impossible to achieve the agricultural productivity that is needed to sustain the world population in the absence of humic substance. Sediment organic matter is vital to the formation and stabilization of soil aggregates. Humic substances have direct role in the stabilization of the aggregates. Stevenson (1994) listed properties of Humic substances that are important for soil conservation and for crop growth. The role of humic substances in soil fertility and plant growth is well established. There are strong evidences given by Nardi, *et al.* (1996) that humic substances stimulate root cell elongation and proliferation. Increase in growth and yield of plants resulting from humic enrichment has been explained on the basis of direct or indirect effects. Direct effects involve the entry of the humic substances through plant roots and participation in the metabolic activities of the plant. Indirect effects on the other hand involve humic substances as suppliers and regulators of plant nutrients.

From the viewpoint of terrestrial productivity, the role of humic compounds in providing nutrients such as nitrogen, potassium and phosphorus is noteworthy. (Clapp *et al.*, 1998). Through complexation properties of humic compounds the solubility, mobility, migration, recycling and accumulation of trace metals play significant roles in the mineral nutrition of terrestrial plants.(Rashid, 1973). In the presence of humic substances the solubility of metals increases and the productivity of waters becomes higher.

Humic substances play an important role in the circulation of energy. Certain bacteria, fungi and other microorganisms are reported to be capable of utilising humic acid as a source of energy (Shapiro, 1957). Humic substances by virtue of their ability to precipitate alone or together with other organic molecules such as proteins and acidic polysaccharides may provide additional sources of energy to higher aquatic organisms.

Being strong organic ligands, they introduce into cycles large amounts of cations, forming with them chelates and organometallic complexes. They can also improve soil chemical properties by increasing cation exchange capacity or buffering soil pH to provide more suitable soil environments. The toxicity of heavy metals towards many organisms living in these waters vanishes or reduces and availability of trace elements necessary for normal functioning of aquatic organisms increases. Most of these beneficial effects can be explained on the basis of complexation reactions.

Analysis of Humic substances derived specifically from aquatic plants in waters and sediments provides a means for characterizing the autochthonous source of these humic compounds which can then be differentiated from allochthonous sources derived from soil leaching.

1.5 Aim and Scope of the Present Work

In short 'Mangrove ecosystem ' may be expressed as the 'coastal ecosystem in a holistic manner, including its common habitat and inhabitant fauna. Humic substances constitute a major fraction of the dissolved and particulate organic matter in natural ecosystem. The ubiquitous presence of humic substances together with their ability to provide multiple sites for chemical reaction makes them relevant to numerous biogeochemical processes. The functional group chemistry and microstructure which are influenced by the surrounding media determine the reactivity of humic substances. Humic acid is one of the most important source and sink for metal ions in a number of environments including soil and sediments in both fresh and saline waters and hence it deserves special attention.

Different factors may control the partitioning and also the bioavailability of the hydrophobic and heavy metals within the benthic ecosystem. These factors include various sediment characteristics, such as grain size distribution, mineral composition and organic content especially the humic acid content. For the above reasons it is important to have a clear picture about the structure and sorptional characteristics of humic acid in the sediment.

It has been practically impossible to define in detail the structural composition of humic acid because of its macromolecular and largely heteropolymeric nature. In the past structural studies were limited to determining the C.H,O and N percentages and functional group contents. With the discovery of sophisticated instrumentation for structural analysis an effort is made to unravel in detail the structure of humic acids from mangroves ecosystem. IR spectrometry has provided valuable information about the functional groups. With the new advances in the design of NMR techniques one can now observe the spectrum of both liquids and solids. Increased sensitivities and higher resolution have made it an attractive tool for structural studies of humic substances. Because of C-H dipolar broadening and chemical shift anisotropy, ^{13}C NMR of solids had provided very little information. Although dipolar broadening was eliminated with the advent of high power ^{1}H decoupling and cross polarization, chemical anisotropy still hindered the ^{13}C resolution. Cross polarization has recently been combined with magic angle spinning making it possible to achieve better ^{13}C resolution. For this reason CP/MAS ^{13}C NMR was used as a primary tool for the structural characterization of humic acids. Other chemical analyses such as, elemental analyses, carbohydrate analyses, determination of such as COOH and OH, SEM and IR studies were used to substantiate the results of ^{13}C NMR studies. Sorptional studies are done to have a clear view of the binding nature of humic acid with metals.

The organic matter exist in a number of forms which include; a. discrete organic particles e.g. vascular plant debris and planktonic tissues b. surface films on inorganic substrates, e.g. humic clay complexes; and c. integral components of inorganic matrices. However, almost 90% of the organic matter cannot be physically separated from its mineral matrix.

(Chester, 2000). Though indirectly, the present study on humic acid intends to give a bird's-eye-view on the sorptional characteristics of this part of the organic matter which is considered to be inert and inseparable.

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Chapter 2

MATERIALS AND METHODS

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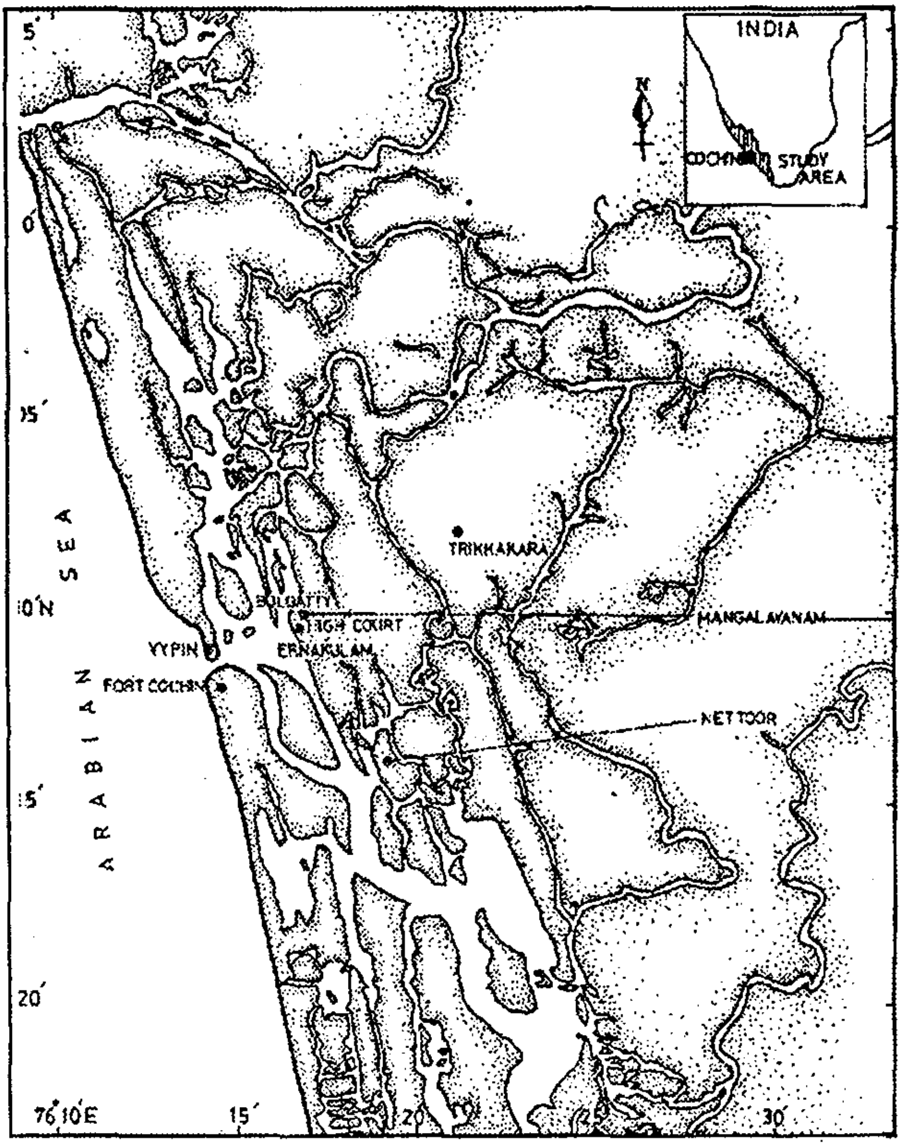
A brief description of the study area, sampling protocols for water and sediment samples, the various methods employed for analysis and the hydrographical parameters are given in this chapter.

2.1 Location of Study

The coastal wetlands in Kerala cover an area of 3313 Km^2 with a reticulate system of backwaters and canals. The estuarine system is comprised of a series of coastal brackish wetlands including lakes, lagoons, rivermouths, mud flats, tidal marshes and mangrove swamps. Cochin estuary is a tropical positive estuary that extends between 09°40'-10°12'N and 76°10'- 76°30'E. The major sources of fresh water to this estuary are Periyar in the north and Moovatupuzha river in the south. This estuary is connected to the Arabian sea through the barmouth which is of about 450m in width. Coastal area where the backwaters are situated is the cradle of civilization with an ever increasing density of human population. Their increasing activities like agriculture, fisheries, navigation, industries, tourism and disposal of wastes have marred the beauty of this natural ecosystem.

There are several mangrove patches of diverse species lying along the estuary. Mangrove area in Kerala is estimated to be 50Km^2 (Mohanan, 1997), which is only the remnants of about 700Km2 of mangroves which existed in Kerala earlier (Ramachandran *et al.*, 1986). The development of timber industry mainly plywood industry is the major reason for the degradation of mangroves of Kerala. Mangroves of Cochin area are slowly being degraded and they grow in isolated patches, confined to the inner reaches of inter-tidal margin of the back waters, and criss-cross along the coastal region The major species seen here are Avicenia, Rhizphora and Acanthus. Three different mangrove ecosystems in Cochin estuary Mangalavanam, Vypeen and Nettoor are chosen for the study. Fig 2.1 depicts the location of the sites.


INDIA
COCHIN
STUDY AREA
N
ARABIAN SEA
TRIKKAKARA
BOLGATTY
HIGH COURT
ERNAKULAM
VYPIN
FORT COCHIN
MANGALAVANAM
NETTOOR
5'
0'
05'
10'N
15'
20'
76°10'E
15'
20'
25'
30'

Mangalavanam

This is a preserved mangrove forest of 8.44 hectares in the middle of Ernakulam city at 9°59'N latitude and 76°11'E longitude. It is comprised of a shallow tidal pond in the center with its edges covered with thick mangrove vegetation. It is almost an enclosed system surrounded by brackish waters with one outlet in the form of a canal to the cochin backwaters. Mangalavanam which is considered as the 'green lung' of Ernakulam city has gained importance not only because of the mangrove vegetation but also due to the congregation of breeding birds. Wide stilt roots formations of Rhizphora, profuse spreading of the dwarf mangrove species Acanthus and bushy areas of Avicenia add life and beauty to this ecosystem. The thick forest and the water adjacent to it attract a number of migratory birds of different species which periodically find shelter during their breeding season and enrich the system with lot of organic input. Forty one species of birds were recorded from Mangalavanam representing 12 Orders and 24 families (Jayson, 2002). The most common species found here are Little cormorant and Night heron. Though this area is preserved and with less human intervention there are a few factors which are detrimental to this ecosystem (Jayson 2002).

a. Proposal to lease a part of the land to a Trust
b. Parking of oil tankers on the Salim Ali road
c. Dumping of waste in the lake
d. Air pollution due to the unloading of cement
e. Uncontrolled growth of weeds in the lake
f. Encroachment of Puramboke land on the eastern side

Vypeen

The largest single stretch of mangrove of Kerala comprising of about 101 hectares is present in Vypeen Island. This mangrove ecosystem can be considered as a semi enclosed system lying close to the brackish waters and is closer to the sea than the other two systems. Part of the Vypeen Island is reclaimed land and are underdeveloped. Rice culture commonly known as pokali, brackish water fish and prawn culture are commonly practiced here. Since the profitability of fish farming is naturally higher than rice culture there is a growing tendency for fish culture that might lead the conversion of rice lands into perennial fish farms. Though large areas of mangroves are destroyed for prawn, fish culture and for other human activities, still thick bushy mangrove zones are seen in the different parts of this island like Puthyvypu, Murikuumpadam etc. Mangrove formations are discontinuous in this region yet superior to the other two with regard to the diversity of mangroves and its associates. Rhizophora sp, Acanthus sp, Avicenia sp and Bruguiera sp are widely distributed in the different parts of this island.

Nettoor

It can be considered as a vanishing mangrove ecosystem with more anthropogenic input than the other two. This is a typical fringing type of mangrove ecosystem affected much by the various urbanization programmes. Mainly the coastal railway line to Alapuzha and the newly constructed Thevara -Kundannoor bridge have resulted in the destruction of a variety of species in the Nettoor region. Thuruths (islets) with cluster of mangrove plants are the peculiarities of this place but majority of them are being destroyed for the increasing prawn culture.

2.1.2 Collection and Analysis of Samples

Samples of both water and sediment were collected from all the three different stations from Nov '99-Nov 2000 on a bimonthly basis. Surface water samples were collected by using a clean bucket and were stored in acid washed plastic bottles. The surface sediment samples were collected using Teflon coated stainless steel grab. Samples were homogenized after removing the leaf and twig remains and were stored at a temperature of – 5°C in polythene covers.

Laboratory procedures

Glassware and plastic bottles used for the experiments.were soaked in 1:1 nitric acid and washed with milli Q water. All reagents were of analytical grade and standard solutions were prepared in milli Q water.

2.2 Hydrographical Parameters

Hydrographic parameters such as temperature, alkalinity, salinity, dissolved oxygen, pH and Eh were noted. Salinity was determined by Mohr-Knudsen method and dissolved oxygen was determined using the modified Winkler method. (Grasshoff, 1999). PH was measured using a Philips portable pH meter and the thermometer was noted using a sensitive thermometer. Measurement of Eh was carried out on a stable column of sediment *in situ*. A combination glass and platinum electrodes were used for this measurements following Kemp *et al.*,(1971). Alkalinity of the water samples were estimated using the method of Koroleff, (Grasshoff, 1999)

2.3 Sediment Characteristics

2.3.1 Water Content

The Water content is determined according to Boyce, (1976). The sediment is placed in pre-weighed weighing bottle with a tight fitting lid. The wet sediment and container are weighed. The container is placed in the oven with lid off and dried at 105°c to a constant weight. The sample is removed from the oven and the lid is replaced. These are then transferred to desiccator to cool for about 2hrs. The sample and the container are reweighed, and water content is calculated according to the equation

$$\Theta_w = M_{ws} - M_{ds} / M_{ws} \times 100$$

Θ_w is wet the water content (%), M_{ws} Mass of wet sediment(g), and M_{ds} Mass of the dry sediment(g)

Similarly the dry water content

$$\Theta d = M_{ws} - M_{ds} / M_{ds} \times 100$$

2.3.2 Bulk density

Bulk density of the sample is determined by Syringe technique described by Boyce (1973, 1976) The end of a 1cm^3 syringe is cut off, squared and sharpened so that the leading edge is flush with the inside diameter of the syringe. The cylinder and end of the plunger are placed with the surface of the sediment; the plunger is held stationary while the cylinder is slowly pushed into the sediment. The volume of the sediment is determined before the sediment is weighed. The sediment is then extruded and weighed wet. The wet bulk density is then calculated by the equation

$$\rho wb = Mws/Vws$$

Where ρwb is the wet bulk density (g/cm^3), Mws is the mass of the wet sample Vws is the volume of the wet sample.

2.3.3 Porosity

Boyce (1976) showed that Porosity can be determined from other physical parameters such wet bulk-density and wet water content.

$\phi = \rho wb \times \Theta_w$ ϕ is the porosity (%), ρ_{wb} bulk density and Θ_w is wet the water content.

2.3.4 Particle size distribution

Quantitative analysis of the size distribution of the particles were done. The oven dried samples were subjected to textural study by mechanical sieving and pipette method following Krumbein and Pettijohn (1938) and Carvar (1971).

2.3.5 Sediment Organic Carbon

The sediment samples were air dried and ground to a fine powder, stored in airtight polythene bottles in desiccators until analysis. They were analysed for total organic carbon with dichromate (Gaudette *et al.*, 1971).

2.3.6 Determination of Trace Elements in the Sediments

Samples were dried to constant weight at 40°-50° C powdered and the composite samples were digested with a 1:3 mixture of concentrated nitric acid and perchloric acid. Parallel replicate measurements were made on all the samples and the mean values have been tabulated. Determination of heavy metals was performed with an atomic absorption spectrophotometer (Perkin Elmer 3110). Stock standard solutions 1000 ppms of different metals were used in preparation of secondary standards.

Total phosphorus in the sediments was estimated by the modified method of Strickland and Parson (1977). A known quantity of the sediment dried was extracted with 1N NaOH to separate humic acid.

2.3.7 Extraction and Purification of Humic Acid

The method used for the extraction of Humic acid is the same as the one used for the extraction of IHSS soil humic acids (Swift, 1996). Sediment samples were hand sorted to remove macroscopic vegetative matter, placed in 4l. beakers and extracted for 24 hrs with occasional stirring with 10:1(v/v). 5M NaOH. The mixture was then filtered through No.2 whatman filter paper and the humic acid in the filtrate was precipitated by acidification to pH 1 with 0.6M HCl. The Humic acid was filtered and washed with 0.1M HCl, and then redissolved on 0.5M NaOH, reprecipitated, filtered and washed again as described above. The Humic acid thus obtained was suspended in a weak solution of HF and HCl (0.5ml con HCl +0.5ml con HF diluted to 100ml with distilled water) as suggested by Khan (1971)and stirred continuously for 5 to 8 hr. Khan reported that this treatment reduced the ash content of Humic acid to a few percent. Humic acid was then washed with deionised water and dialysed against milli Q water for five to seven days. The purified humic acid thus obtained was then dried in oven below 60° C and kept in a desiccator.

2.4 Functional Group Analysis of Humic Acid

2.4.1 Total Acidity

Accurately weigh 5-10mg of humic material into a 25 ml ground glass stoppered conical flask. Add 10ml of.01M $Ba(OH)_2$ solution and blanket it with nitrogen. Stopper the flask and shake it for 24hours. Filter the suspension and rinse the flask and filter with three 10ml aliquots of CO_2 free water. Combine the washings and filterate and titrate potentiometrically with standard 0.02M HCl. Calculate the total acidity in meq g^{-1} using the method of Schnitzer and Khan (1972).

2.4.2 Carboxyl Group

The Determination of the carboxyl group was done by following the method of Bloom *et al.*, (1957) modified by Schnitzer and khan (1972). Weigh accurately 5-10mg of humic material into a ground glass stoppered conical flask. Add 10 ml of. 01M Calcium acetate solution and blanket it with nitrogen. Stopper the flask and shake for 24 hours at ambient temperature filter and wash precipitate. Combine the filterate and washings and titrate potentiometrically with a standard NaOH.solution.

2.4.3 Phenolic Group

Phenolic groups are often assumed to be the difference between total acidity and carboxyl content (Perdue, 1985). The functional groups obtained by this method actually include all the weakly acidic groups as recommended by Stevenson (1982), probably phenols, Weaker carboxyl groups, alcoholic groups, alcoholic groups in carbohydrate entities, and enols.

2.4.4 Carbohydrate

About 10-20 mg of humic acid was hydrolysed with 20ml of 1N H_2SO_4 at 100°C for one hour. Polysaccharides within the humic acid were hydrolysed to monosaccharides which were then estimated by using phenol sulphuric acid method. (Dubois *et al.*, 1956)

2.4.5 Total Phosphorus

Phosphorus was extracted from humic acid as suggested by Nissenbaum (1979). Analysis of phosphorus was carried out by exhaustive oxidation of humic acid with perchloric acid followed by colorimetric estimation, (molybdenum blue method). Weigh about 20-40 mg of humic acid and this was treated with 3 ml of con HNO_3 to effect primary oxidation and then 4ml of 70% $HClO_4$ the sample was digested until the solution was colourless. After cooling the solution was made up to 20 ml and phosphorus was determined using molybdenum blue method (Strickland and Parson, 1977)

2.4.6 Total Metal

About 20 mg of powdered humic acid samples were digested with a 3:1 mixture of HNO_3 and $HClO_3$. This was made up to 20 ml and the trace metals were determined by using atomic absorption spectrophotometer (Perkin Elmer 3110).

2.4.7 CHN Analysis

The Elemental composition of the humic acid samples were determined using CHN Heraeus CHN – O – rapid analyser

2.5. Spectroscopic Analysis of Humic Acid

2.5.1 Infrared Spectrum

Infrared spectra of humic acid from the stations were obtained over the range 4000 to 400cm^{-1} with the spectrometer IR – Bruker Equinox 55. Samples were oven dried at 60°C and were finely powdered prior to analysis. KBr discs were prepared by mixing 98 mg of KBr with 2 mg of the respective samples.

2.5.2 UV- Visible studies

UV- Visible studies were done on UV spectrophotometer. All measurements were made on solutions of humic acid dissolved in 0.5N NaOH. The E4/E6 ratio was directly determined in the spectrophotometer.

2.5.3 Scanning electron microscope

The polymeric nature of humic acid are studied using a Scanning Electron microscope, Cambridge Stereoscan S 360, Scanning Electron microscope at a magnification of about 30-40 thousand.

2.5.4 ^{13} C NMR

Solid state^{13} C NMR with CPMAS (cross polarization magic angle spinning) was obtained with a NMR – BRUKER DSX 300, spectrometer operated at a proton frequency 100 MHZ and a 13 C frequency of 5 KHz. Other parameters were contact time 1 ms, recycle delay 0.8s, sweep width 562.5 ppm and line broadening 30Hz. Dried solid samples were placed in a rotor and spun at a frequency of 5 KHz at the magic angle 54.7° to the magnetic field.

2.6. Sorption Studies

Sorption studies were done on sediment, humic acid and sediment left after the alkali extraction, in view of understanding the nature of binding of metals on humic acid and sediment and thus to have a better understanding of the structure of humic acid and its interaction with the sediments in the natural systems. The experiments were done in three steps, first adsorption of metals (a) on the sediment (S), (b) on the humic acid(HA) and (c)on the sediment left after alkali extraction (SAAE) following Jayasree (1993). The SAAE was obtained by extracting the sediment repeatedly with 0.5.N NaOH till the alkali extract became colourless. It took more than month for each extraction so that the sediment sample was completely free of humic acid. The supernatant liquid was removed, the residue was washed well with milli Q till it was neutral. The sediment and SAAE were filtered through a sieve of mesh size of 250μ to get particles of uniform size. Humic acid was powdered well before adsorption.

2.6.1 Adsorption Capacity

About 0.25g of the sediment, 10mg of humic acid and 10mg of SAHA were allowed to equilibrate with 10ml of varying concentrations of the metals Cu, Fe and Mn for 24 hours with occasional shaking. Analytical grade Merck samples of Copper sulphate, Ferrous Ammonium sulphate and Manganese Nitrate were used for the preparation of standard metal solutions for adsorption. The experiments were performed in acid washed beakers. Controls consisting of similar aliquots of the metal without the samples were kept along to find out if there were any adsorption by glassware. Duplicates were also done simultaneously. After 24 hours 1 ml of solution from the supernatant liquid was withdrawn and the metal content in it was determined using atomic absorption spectrometer. (Perkin Elmer 3110). Stock standard solutions 1000 ppms were used in preparation of secondary standards for the analysis of metals by AAS.

2.6.2 pH Dependence

To study the influence of pH on adsorption the experiments were done with metal solutions different pH viz. 4,5,6,7,8. 0.05N HNO_3 and 1:1 NH_3 were used to bring about change in the pH of the medium. Once the metal solution of desired pH was prepared, 10ml of Ammonium citrate was added to buffer it.

2.6. 3 Effect of Concentration

The effect of metal concentration on adsorption was studied using metal solutions with varied concentrations such as 10 ppm,20ppm,50ppm and 100ppm for humic acid and 500ppm, 1000ppm, 1500ppm,2000ppm for the sediment and SAAE.

2.6.4 Desorption Studies

For a better understanding of the nature of the binding of metals on the sediment and humic acid desorption studies were done. Ammoniunm Acetate (2N) HCl (0.5 N) EDTA (.05N) and NaH_2PO_4 (.01N) were used for desorption studies. For desorption studies, the metal solution left after adsorption was filtered, the adsorbed samples were dried and a known amount of it was kept in contact with the extractant desired for desorption. After about 3 hrs. 1ml of the supernatant liquid was withdrawn and its metal content was determined using AAS.

2.7 Results of Hydrographical Parameters

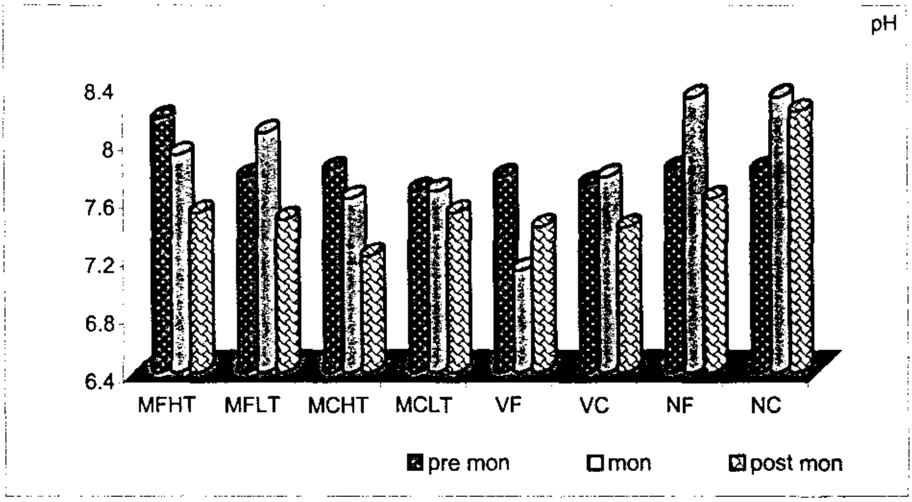
2.7.1 PH

pH is considered as one of the master variables in an estuarine system (Chester 2000). The various process taking place in the system, like flocculation, adsorption, precipitation and biological uptake of metals etc depend very much on the pH of the system. Complexation and chelation reactions with inorganic and organic ligands is influenced by this master variable.

The seasonal variation of pH in the three stations during the three seasons are shown in table 2.2. All the stations are alkaline and in all the three systems there is considerable varation in pH with the seasonal change. The values ranged from 7.65-8.15 during pre monsoon, 7.1-8.3 during monsoon and 7.2-8.2 during post monsoon. In Manglavanam forest the highest value is seen during the pre monsoon where as the canal shows lowest value during the monsoon. Same is the trend with Vypeen forest and canal. The highest value reported by Mangalavanam agrees with the earlier report made by Imelda and Chandrika (2000). Nettoor forest and canal shows the highest value of 8.3 during monsoon.

Table 2.1 Seasonal variation of pH of water

Stations	pre mon	mon	post mon
MFHT	8.15	7.9	7.5
MFLT	7.75	8.05	7.45
MCHT	7.8	7.6	7.2
MCLT	7.65	7.65	7.5
VF	7.75	7.1	7.4
VC	7.7	7.75	7.4
NF	7.8	8.3	7.6
NC	7.8	8.3	8.2



Seasonal variation of pH of water

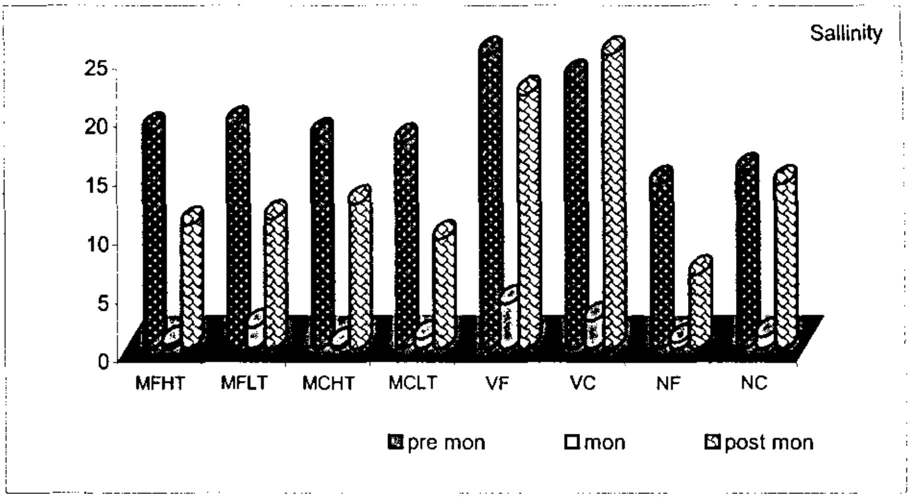
The highest value could be due to high photosynthetic activity in the mangrove region which result in an undersaturation with respect to CO_2. (Geetha, 2002). Humic acid tends to aggregate or disperse in aqueous solution depending specially on pH. The humic materials changed from fibers and bundles at low pH to a finely woven network at intermediate pH (Senesi *et al.*, 1972).

2.7.2 Salinity

Salinity is a measure of the degree to which the water is salty and it is a function of the total weight of the solids dissolved in a quantity of water. It is an important parameter which determine the character of Mangrove ecosystem. Salinity has been suggested as a possible agent of vegetation zonation within mangrove wetlands (Bunt *et al* 1982). The mangrove ecosystem system act as buffer between transitional near shore and estuarine environments with respect to the influence of fresh water discharge. The salinity values of the three systems during the three seasons are shown in Table 2.2. In Mangalavanam forest, the salinity varied from .33 to 18.5 ppt during high tide and 1.78-19 ppt during low tide. In all the stations the maximum value is observed during premonsoon and the minimum during the monsoon. In the adjacent canal of Mangalavanam salinity ranged from 0.2-18 during high tide and 0.78 –17.5 during low tide. Here too the maximum and minimum values are observed during the same season. Same is the case with Nettoor with the highest salinity values during pre monsoon and the lowest during monsoon.

Table 2.2 Seasonal variation of salinity (ppt)

Stations	pre mon	mon	post mon
MFHT	18.5	0.33	10.5
MFLT	19	1.78	11
MCHT	18	0.2	12.3
MCLT	17.5	0.78	9.3
VF	25	3.85	21.6
VC	23	2.35	25
NF	14	0.53	6.3
NC	15	0.99	14



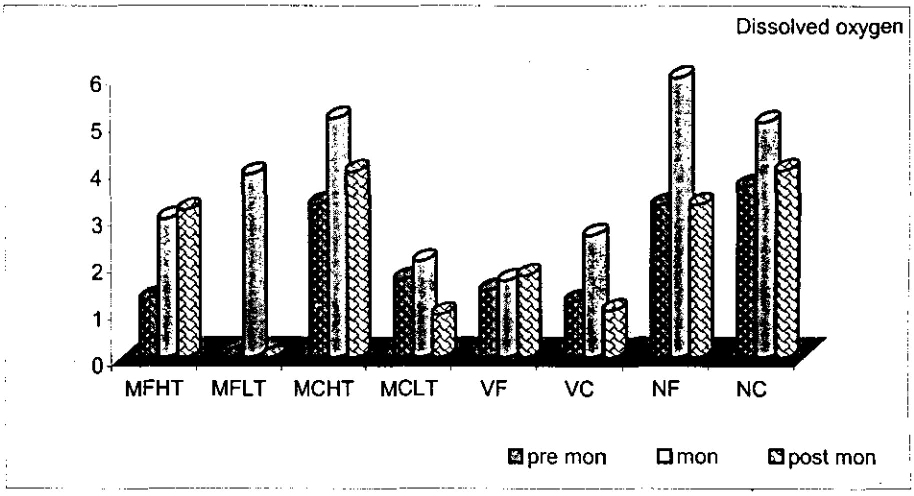
There is sharp salinity difference during the monsoon, post monsoon and pre monsoon agreeing with the observations made earlier. (Imelda and Chandrika, 2000. Untawale *et al.*, 1973)

2.7.3 Dissolved Oxygen

Dissolved oxygen is an important factor in aquatic life as they govern many physical and chemical changes. Many of the chemical changes that take place during early diagenesis are redox mediated (Chester 2000). Dissolved oxygen is a function of salinity and temperature. The seasonal variations of Dissolved oxygen are given in Table 2.3. Compared to the other two stations Nettoor shows highest dissolved oxygen and it is during the monsoon. The lowest value is zero and it is shown by Mangalavanam forest during both the pre monsoon and post monsoon low tide. Monsoon values are higher than the pre monsoon and post monsoon values except in the case of Vypeen where post monsoon values are higher than the other two seasons. When the rate at which oxygen is consumed by oxidation of organic matter present in the water exceeds the rate at which it is supplied by currents, reducing conditions are set up. The high organic matter in the mangrove sediments and the decomposition of mangrove litter can lead to anoxic conditions (Jagtap 1987). This might be the case of Mangalavanam. The low level of oxygen in the water might also be an important factor for the great percentage of organic matter in the mangrove sediment. The anoxic water column prevents oxidative alteration of the organic biomass before sedimentation and as a consequence, the highest degree of preservation of organic matter is observed in the more anoxic basins (Riley and Chester, 1971). Anaerobic organisms can use dissolved oxygen from the overlying or interstitial waters to burn organic matter.

Table 2.3 Seasonal variation of Dissolved Oxygen (ml l^{-1})

Seasons	pre mon	mon	post mon
MFHT	1.25	2.96	3.14
MFLT	0	3.91	0
MCHT	3.27	5.1	3.97
MCLT	1.66	2.06	0.92
VF	1.43	1.64	1.75
VC	1.2	2.62	1.01
NF	3.25	5.96	3.27
NC	3.66	5.02	4

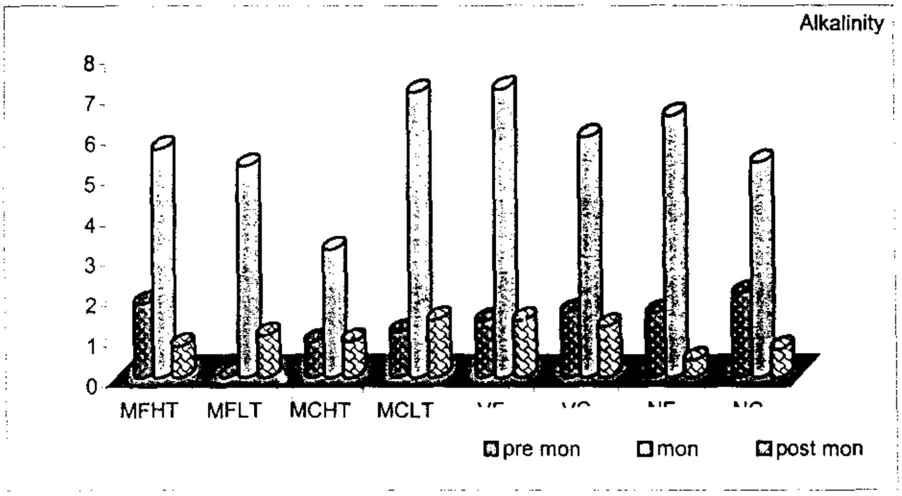


2.7.4 Alkalinity

Alkalinity values are shown in table 2.4. The low alkalinity value is a proof for the high productivity of mangrove sediments. The Low alkalinities are found in high productivity waters in which calcium utlising organisms proliferate or chemical precipitation of $CaCO_3$ occurs (Riley and Chester 1971). Lowest value is found in Mangalavanam forest during the low tide and the highest is seen in Vypeen forest during the monsoon season.

Table 2.4 Seasonal variation of alkalinity (m moles $^{-1}$)

Seasons	pre mon	mon	post mon
MFHT	1.82	5.66	0.77
MFLT	0.48.	5.23	1.04
MCHT	0.87	3.17	0.89
MCLT	1.11	7.06	1.42
VF	1.39	7.14	1.4
VC	1.62	5.95	1.25
NF	1.58	6.48	0.42
NC	2.07	5.33	0.69



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Chapter 3

ANALYTICAL CHARACTERISTICS OF HUMIC ACID

3.1 Abundance of Humic acid in the sediment

3.2 Elemental composition

3.3 Acidic functional groups

3.4 Carbohydrate

3.5 Phosphorus

3.6 Metal content in Humic acids

Mangrove areas are probable zones for humification since they are areas of high productivity. Analytical and experimental works have shown that phenolic compounds are particularly conducive to humification reaction (Flaig *et al.*, 1975). Mangrove areas have relatively high phenolic input as they contain more productive local sources such as vascular plants that use lignin as a structural components especially mangroves and microalgae such as *phaeophyta,* that make a variety of phenolic compounds. The abundance of polycarboxyl-substituted phenols shows a positive correlation with terrestrial inputs, indicating that lignins and probably also tannins are important in humic formation in terrestrial environments (Stephen *et al.*, 1993). These components are important in resistance towards biodegradation and so their dominance in aerobic environments is unexpected. The bulk of aromatic units in soil humic material are refractory lignified plant tissues, altered to varying degrees of microbial degradation. The by-products of the decomposer organisms and their remains are also likely to contribute to humic substance formation and may account for the major part of the aliphatic components supplementing the aliphatic contribution from higher plants (Stephen *et al.*, 1993).

3.1 Abundance of Humic Acid in the Sediments

The amount of humic substance and their composition also depend on the sedimentation in the environment and evolutionary stage of the organic matter in addition to the relative productivity of the environment (Aiken *et al.*, 1985). These factors influence both the nature of the organic input and the extent of pre-sedimentary alteration. The chemical composition of the organic input depends on the climate, the proximity of the shore, and the relative contribution of the marine and continental biomass to the sediment. The extent of alteration is related to the distance between the site of introduction of the biomass produced and the site of sedimentation and whether it occurs along with depth and oxygen content of the water.

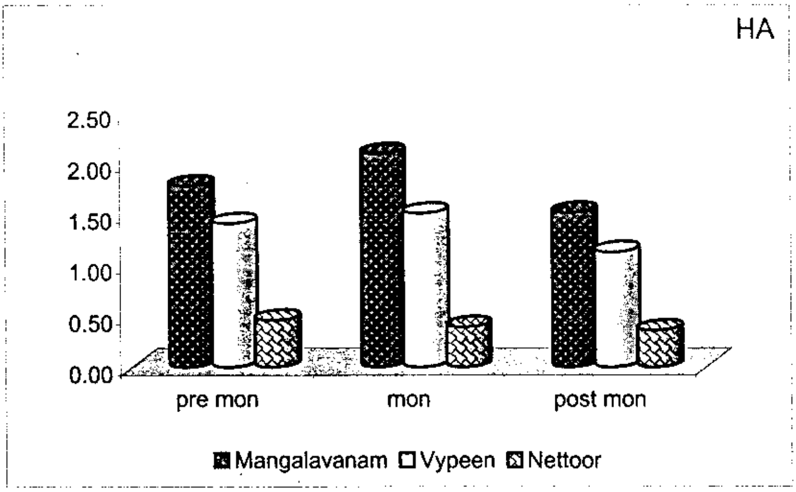
Environmental factors like primary productivity, sediment texture, microbial activity etc. play an important role in humification (Sugantha, 1995).

The percentage of Humic acid in the sediments and its seasonal variation are shown in table 3.1 The sediments of Mangalavanam have the highest percentage of Humic acid in all the months. Nettoor sediment has a very low Humic acid content compared with the other two. Humic acid in the sediments of Mangalavanam and Vypeen is higher in the monsoon and lower in the pre monsoon periods agreeing with the earlier observations made by Shanmukhappa *et al.* (1989). Whereas in the case of Nettoor sediment there is very little seasonal change in the amount of Humic acid. The low percentage of Humic acid in Nettoor compared to the other two stations can also be due to the high sand content and low water content of the sediment of this station.

The amount of humic substance per unit weight of the sediment is also comparable with previous observations made by Raspor *et al.* (1984) and Jayasree (1993). There is a positive correlation between the percentage of Humic acid content and the organic carbon in the sediment.

Table 3.1 Monthly variation of Humic acid content in the sediment (g dry wt /100g sediment)

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	1.78	1.41	0.46
mon	2.09	1.51	0.39
post mon	1.52	1.13	0.36



Monthly variation of Humic acid content in the sediment
(g dry wt /100g sediment)

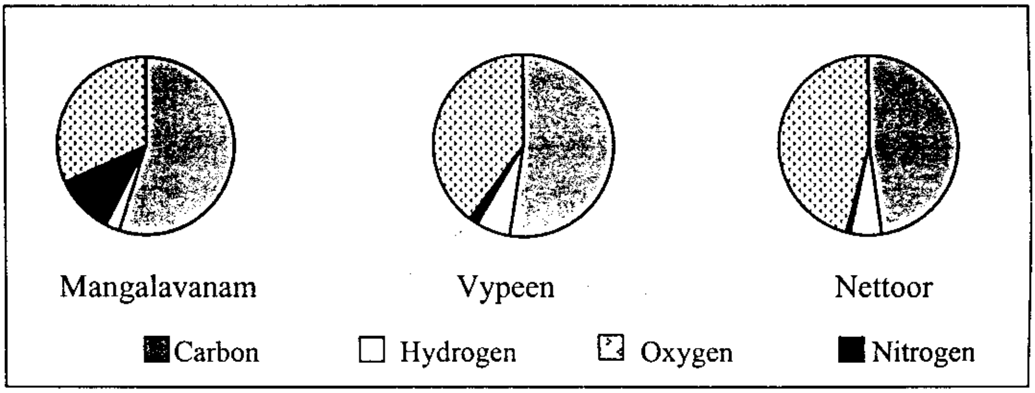
3.2 Elemental Composition of Humic Substances

Elemental composition is the most essential characteristic of the humic substance. The first information on the elemental composition of Humic acids dates back to 175 years. According to the analysis of Berzilius and Mul'der the carbon content of Humic acid fluctuated in the range 56-62% that of hydrogen 2.5-5% and of nitrogen 2-8%(up to 14) (Orlov, 1985). These historically important data are difficult to compare with the modern values as they used different values even for equivalent and atomic weights in their calculations. Samples are frequently compared according to their H/C, O/C and N/C ratios. H/C ratios in marine sediment Humic acid may vary between 0.9-1.7 O/C ratios between 0.2-1 and N/C ratios between 0.02-0.1. Elemental composition changes as a function of geological evolution. Elemental composition of Humic acids from the three stations is given in table 3.2.

Table 3.2 Elemental composition of Humic acids

Percentage	Mangalavanam	Vypeen	Nettoor
Carbon	56.9	52.46	47.74
Hydrogen	2.8	6.11	5.57
Nitrogen	11.2	1.63	0.85
Oxygen	32.9	39.8	45.84

It can be noticed that the most abundant elements in humic substances are carbon and oxygen while the contents of hydrogen and nitrogen are much less. This also agrees with the results of Raspor *et al* (1984).



Percentage of elements in Humic acids from the three stations

The highest percentage of carbon (56.9%) is seen in the Humic acid from Mangalavanam. The relatively high carbon content is typical of Humic acids isolated from plant tissues (Orlov, 1985). There is significant positive correlation between the sediment organic carbon and carbon content in Humic acid. The low value of the percentage of carbon in the Humic acid from Nettoor sediment may be due to the poor condition of the development of microflora due to increasing human intervention in this area. This might have led to the decrease in the rate of mineralization of nonspecific organic substances and to their relative accumulation. This situation is responsible for a higher proportion of aliphatic side chains in the composition of Humic acids and relative reduction in the carbon content. (Orlov, 1985). The variation of carbon content in the three stations can be due to variability of territorial distribution of plant residues, moisture etc.

The oxygen content varies between (29.1-45.8%). The Humic acid from Nettoor shows the highest percentage of Oxygen. The calculation of the O:C or C:O does not yield any substantial information since these two elements together constitute 90%of the total mass of the Humic acids. For this reason a clear negative correlation is observed between the amounts of these elements, which is in fact purely an arithmetic effect.

The hydrogen content varies between (2.7-6.1%) and that from Vypeen has a hydrogen percentage little higher than the other two. Humic substances act as suppliers and store houses of nitrogen for plant roots and microorganisms. Nitogen components in Humic acids have been identified as proteins, peptides, amino acids, amino sugars, purins, pyramidines, and nitrogen compounds that have become integral constituents of humic substances (Schnitzer, 1985). The nitrogen content varies much between the three ecosystems (0.85-11.16%). The Vypeen and Nettor Humic acid shows comparatively much lower nitrogen percentage and C/N ratio is much higher than Mangalavanam Humic acid.

The highest percentage nitrogen content (11.16%) from Humic acid of Mangalavanam may be explained as contributions from the migratory birds, algal and bacterial materials. The composition of humic substances appears to be influenced by the availability of various potential precursor substances in each environment (Stephen *et al.*, 1993).

Humic like substances from the plants have much lower C:N ratios than the corresponding whole plant tissues. According to Dell'Agnola and Ferrari (1979) and Nissenbaum and Kalpan (1972) the C:N ratio is an index of the humification process and the value of this ratio decreases with time when compared with that of the parent material

3.3 Acidic functional groups

In general the maximum amount of any given metal ion that can be bound is approximately equal to the content of acidic functional groups, primarily COOH. Humic substances have a considerable ion exchange capacity typically 4-14meq/gC resulting primarily from carboxyl and phenolic hydroxyl groups (Sardessai, 1989). The results of acidic functional groups are shown in tables 3.3 a, b & c

Table 3.3a. Total acidity (meq g^{-1})

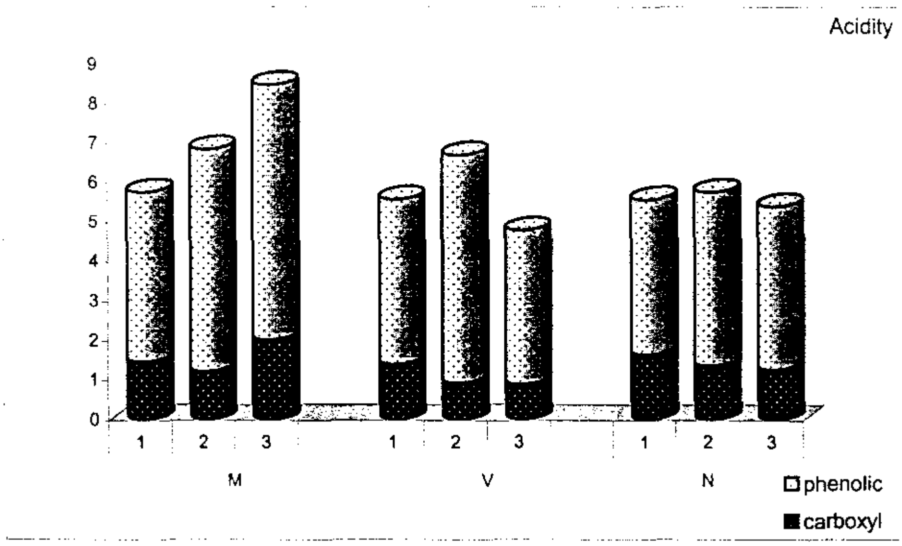
Seasons	Mangalavanam	Vypeen	Nettoor
Pre mon	5.75	5.56	5.56
mon	6.83	6.69	5.75
Post mon	8.47	4.77	5.39

Table 3.3b. Carboxylate acidity (meq g^{-1})

Seasons	Mangalavanam	Vypeen	Nettoor
Pre mon	1.45	1.39	1.64
mon	1.21	0.92	1.36
Post mon	2.01	0.87	1.26

Table 3.3c. Phenolic acidty (meq g^{-1})

Seasons	Mangalavanam	Vypeen	Nettoor
Pre mon	4.30	4.17	3.92
mon	5.62	5.77	4.39
Post mon	6.46	3.90	4.13



Variation of Acidity of Humic acids

1. Pre monsoon 2. Monsoon 3. Post Monsoon
2. M- Mangalavanam V- Vypeen N-Nettoor

Chemical analysis reveals higher acidity in the Humic acids of Mangalavanam than the other two in all the seasons. During the pre-monsoon the values do not differ much between the three stations. However Mangalavanam shows the highest (5.75 meq g^{-1}) during this season. The highest value is observed in the Mangalvanam Humic acid during the post monsoon (8.47meq g^{-1}). In the Humic acid of Mangalavanam acidity is lowest in the premonsoon and highest in the post monsoon period, where as in the case of Vypeen and Nettoor highest acidity is seen during monsoon. In the case of carboxylate acidity, Humic acid of Mangalavanam during post monsoon period shows the highest value(2.01meq g^{-1}) and in the post monsoon the values of Mangalavanam ranged from (1.26-2.01meq g^{-1}). During the monsoon highest carboxylate acidity is seen the Nettoor Humic acid and the value ranges from (0.92-1.36meq g^{-1}). There is no significant variation in the carboxyl acidity in Humic acid of Nettoor during the three seasons. The values of total acidity and carboxyl acidity agree with the acidity values of estuarine Humic acid by Jayasree (1993). There is significant positive correlation between the total acidity and the phenolic acidity. The elements Carbon and Nitrogen show a significant positive correlation with the total acidity and the Hydrogen and Oxygen show a significant negative correlation. The same trend follows in the case of phenolic acidity. Whereas in the case of carboxylate acidity only Nitrogen shows a significant positive correlation, the other elements do not show any significant correlation. Total acidity and phenolic acidity are correlated positively with sediment organic carbon.

Titration data indicate that the acid groups in Humic substances provide a continuum of dissociable protons. The strongest acids are carboxylic and some are activated by appropriate adjacent groups. Phenolic hydroxyls also contribute to total acidity and they are most abundant in the Humic acid fraction. Their contribution to the total acidity is greatest in the newly formed humic substances and especially in those with origins in lignified components of plants. As oxidation takes place, the phenols are oxidized and eventually carboxylic acids are formed. Some of the phenolic substituents can have enhanced acidity because of the influences of their substituents on the aromatic structures. Enols and other weakly dissociable groups also contribute to the charge characteristic under alkaline conditions representing a range of acid strengths, from the strong to the very weak.

3.4 Carbohydrates

Carbohydrates and proteins are precursors in the formation of humus (Nissenbaum and Kalpan, 1972). The monosaccharides in Humic and fulvic acids are Rhamnose, Ribose, Arabinose, Xylose, Mannose, Ggalactose, and Glucose (Yukiho, 1983). Data of humic carbohydrate give an idea on the biological input to the sediments and the diagenesis of carbohydrate in terrestrial and aquatic environment. Carbohydrates were found to account for 1-4% of organic carbon. (Aiken *et al.*, 1985). Humic substances can play an important role in aquatic ecology as they control the bioavailability of dissolved proteinaceous material and carbohydrates. Stabel (1977) and Steinberg (1977) showed that carbohydrates associated with humic substances had a greater microbial availability than proteinaceous matter associated humic substances and postulated that this was due to different kinds of binding to the humic substances. Steinberg (1977) expressed that there could only be weak labile bonds between carbohydrates and humic substances. The results of carbohydrate analysis are shown in table 3.4. Carbohydrate content of Vypeen Humic acid is much higher than the other two Humic acids. The highest value is shown by the Humic acid of Vypeen during the monsoon (246.83 mg/g) and this value decreases towards the post monsoon. The carbohydrate content of other two Humic acid shows the same variation with the highest value in June decreasing towards November. The above value for Mangalavanam and Nettoor during the pre monsoon is very low (2.44 and 2.27 mg/g). Carbohydrate values reported are comparable with those reported by Jayasree (1993).

Seasonal variation of Carbohydrate in Humic acid (mg/g)

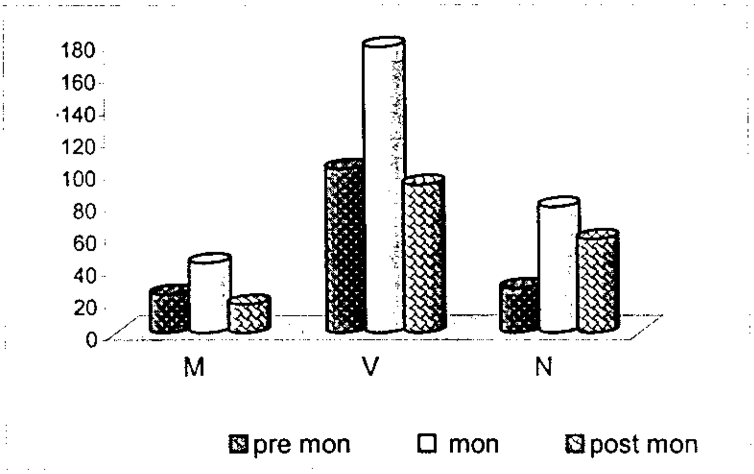


Table 3.4 Seasonal variation of Carbohydrate in Humic acid (mg/g)

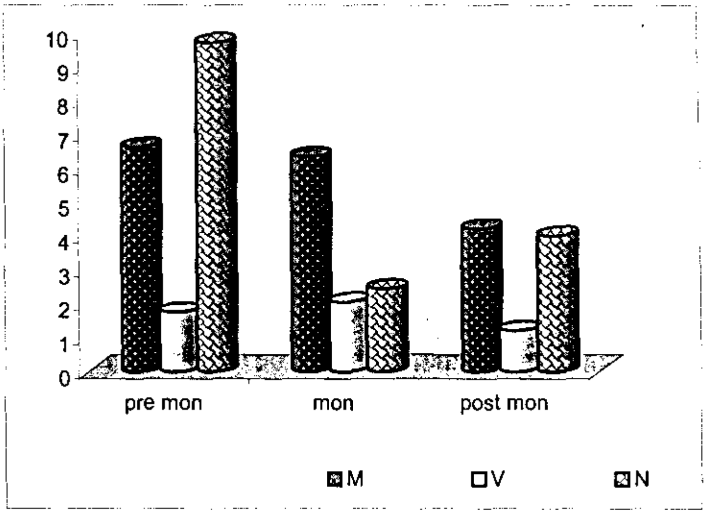
Seasons	Mangalavanam	Vypeen	Nettoor
Pre mon	23.84	101.77	27.46
mon	43.32	177.62	78.28
Post mon	17.59	91.96	58.13

3.5 Phosphorus

Mangrove plants contribute to the source of phosphorus along with bacteria and other microorganism which act as mediators in the transformation of plant material into humic substances. Nissenbaum (1972) has reported that marine and non marine Humic acid contain phosphorus in the range of 0.1%-0.2%

Table 3.5 Seasonal variation of Phosphorus in Humic acid

Seasons	Mangalavanam	Vypeen	Nettoor
Pre mon	6.63	1.76	9.71
mon	6.35	2.06	2.45
Post mon	4.22	1.21	4.01



3.6 Metal Content in Humic Acid

Total metal contents are excellent criteria to judge the extent of metal accumulation. The ability of humic substances to form stable complexes with polyvalent cations has been well established (Stevenson, 1982).The formation of these complexes facilitates the mobilization, transport, segregation and deposition of trace metals in soils, sediments, and biogenic deposits of various types. Organic complexing agents play a key role in the chemical weathering of rocks and minerals, and they function as carriers of metal cations in natural waters (Minhan, 1995). In general, the maximum amount of any given metal ion that can be bound is approximately equal to the content of acidic functional groups, primarily COOH (Aiken *et al.*, 1985). Other factors influencing the quantity of metal ions bound by humic substances are pH, ionic strength, molecular weight, and the different functional groups. For any given pH and ionic strength, trivalent cations are bound in greater amounts than divalent cations. Those forming strong coordination complexes will be bound to a greater extent than the weakly coordinated ones.

The formation of ionic salts is not the only way in which these metals can react. Co-ordination compounds can also occur in which ligands provide an electron pair to bond or to a metal atom. Many of the functional groups identified as components of humic molecules contain suitable atoms like nitrogen and oxygen which are capable of acting as ligands. Given the variety of functional groups in humic molecules and the various ways in which they can interact with metals infinite number of metal humic complexes are possible in principal. Humic acid ligands are highly selective for a particular ion, even in the presence of other, chemically similar ions. Such species play an important role in some biological process. Plant uptake of trace metals can be greatly affected by the organic matter in the soil (Shibu *et al.*, 1990).

The concentrations of Cu, Zn, Mn, Fe, Co, Ni and Cr were determined in the samples of all the Humic acids. Comparing trace metal contents of

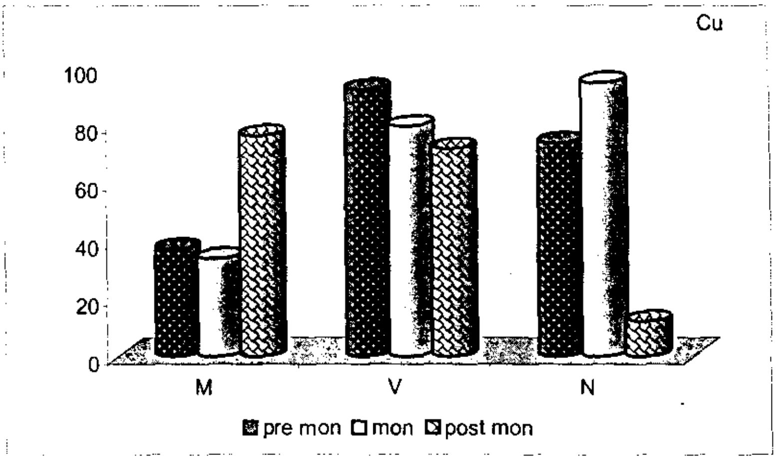
Humic acids isolated from the various sediments, it is seen that all the Humic acids have the highest concentration of Fe. The next frequently abundant metal is Zn followed by Cr, Co, Ni, Mn and Cu.

Copper

The Humic acid from Nettoor during the monsoon shows highest amount (94.71µg/g) of Cu and the pre monsoon sample of Vypeen (92.34µg/g) comes next. The lowest amount (33.90µg/g) is found in the post monsoon sample of Mangalavanam. In Mangalavanam the highest amount is found during post monsoon (76.32 µg/g). The high Cu content might be due to the industrial effluents. Antifouling paints used on the ship are another important source of Copper in the Cochin estuaries. (Jayasree *et al.*, 1995). There is significant positive correlation between the Cu present in the sediment and Humic acid.

Table 3.6a Seasonal variation of Cu in Humic acids (µg/g)

Seasons	Mangalavanam	Vypeen	Nettoor
Pre mon	37.12	92.34	73.91
mon	33.90	79.63	94.71
post mon	76.32	71.85	12.16

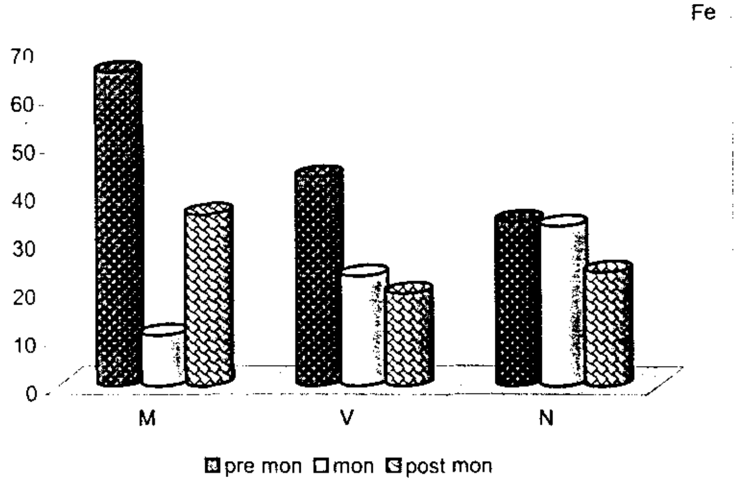


Iron

The iron content in the Humic acids in all the seasons and in all the stations are very high compared to the other metals. Pre monsoon Humic acid from Mangalavanam has comparatively very high iron content and during pre monsoon it is low. Vypeen and Nettoor follow the same trend of having the highest during premonsoon, then monsoon and the lowest during post monsoon. The very high concentration of Fe must be due to the precipitation of Fe as sulphide which is common in mangrove environment (George *et al.*, 1997). The anoxic conditions in the system favour such precipitation.

Table 3.6b Seasonal variation of Fe in Humic acids (mg/g)

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	65.09	43.32	34.07
mon	10.56	22.76	33.14
post mon	35.53	19.15	23.72

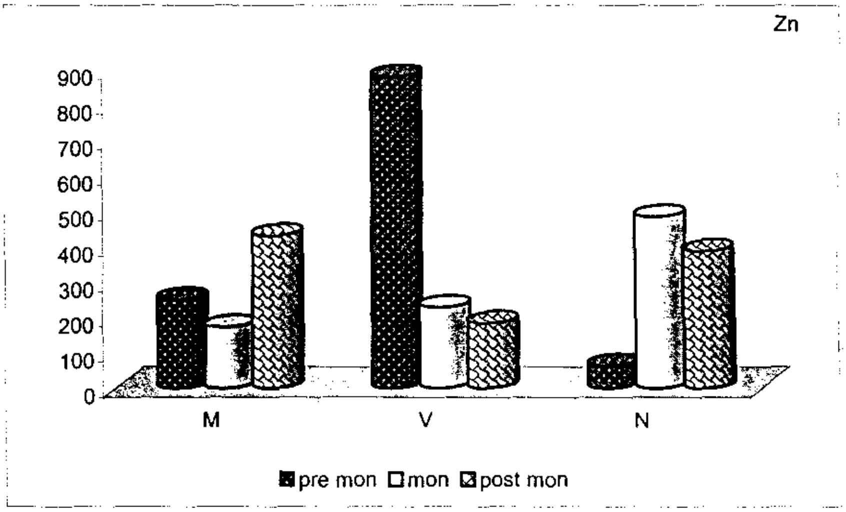


Zinc

The presence of Zinc is comparatively much higher in the pre monsoon Humic acid of Vypeen (880.09μg/g). Mangalavanam shows the highest (430.69 μg/g) during post monsoon and Nettoor shows the highest (484.86 μg/g) during the monsoon season. Lowest amount of Zn (63.37μg/g) is found in the post monsoon Humic acid of Nettoor.

Table 3.6c Seasonal variation of Zn in Humic acids (μg/g)

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	251.02	880.09	63.67
mon	172.59	227.98	484.86
post mon	430.69	183.30	387.76

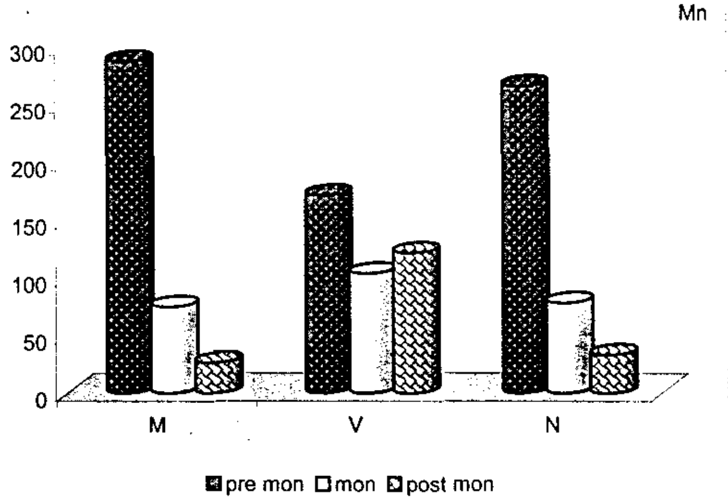


Manganese

In the Humic acid separated from Mangalavanam the highest amount of Mn is found in Pre monsoon period (286.73μg/g) and the lowest amount is found in post monsoon. In Humic acid of Vypeen too the highest value is found during the premonsoon (171.26µg/g) and the lowest (103.44µg/g) during the monsoon. But in the Nettoor Humic acid the highest amount of Zn is found in pre monsoon (265.58µg/g) and the lowest in the Humic acid of post monsoon (30.08µg/g). When all the seasons and all the stations are compared Pre monsoon Humic acid of Mangalavanam and Nettoor rank high.

Table 3.6d Seasonal variation of Mn in Humic acids (µg/g)

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	286.73	171.26	265.58
mon	74.67	103.44	78.06
post mon	26.04	121.41	33.08



Seasonal variation of Mn in Humic acids (µg/g)

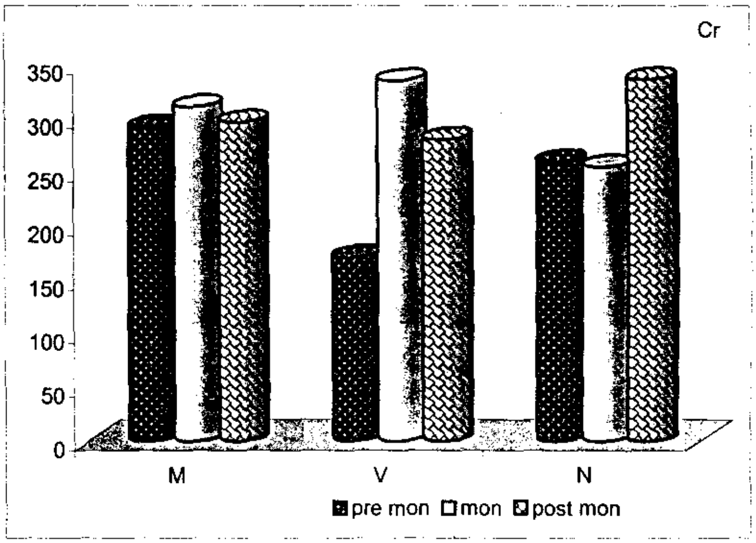
Chromium

The highest amount of Chromium (336.92 mg/g) is seen in the Humic acid of Nettoor during the post monsoon period whereas the lowest (171.16

μg/g) is seen in the Monsoon Humic acid of Vypeen. Chromium content of all the stations are comparable to one another.

Table 3.6e Seasonal variation of Cr in Humic acids (μg/g)

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	292.20	171.61	260.31
mon	311.06	335.37	255.38
post mon	296.91	280.94	336.92

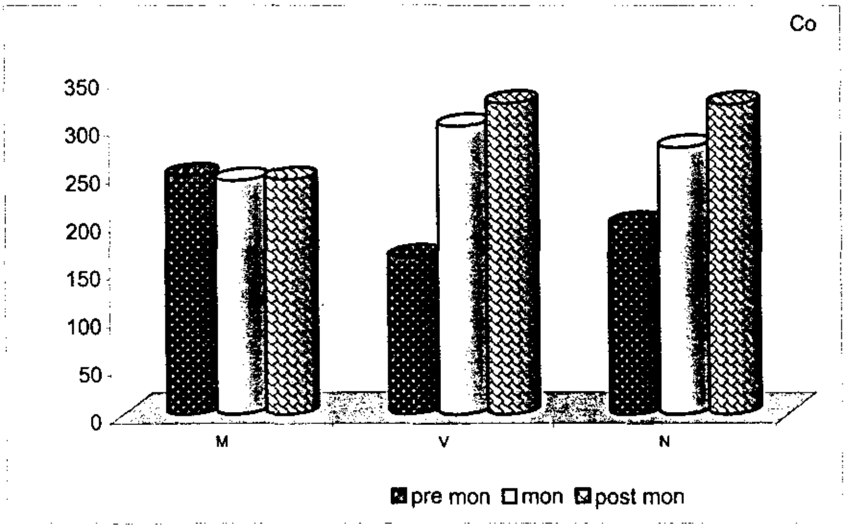


Cobalt

Like Chromium, Cobalt too do not show much variation from station to station or from season to season. The monsoon Humic acid of Vypeen (325.03 μg/g) and the post monsoon Humic acid of Nettor (323.42μg/g) stand high. In Vypeen and Nettoor the lowest cobalt content is found during the pre monsoon period (163.96μg/g, 198.41μg/g), whereas in Mangalavanam the lowest amount of cobalt is found during the monsoon period (243.96μg/g).

Table 3.6f Seasonal variation of Co in Humic acids (μg/g)

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	248.86	163.96	198.41
mon	243.96	300.60	278.48
post mon	245.30	325.03	323.42

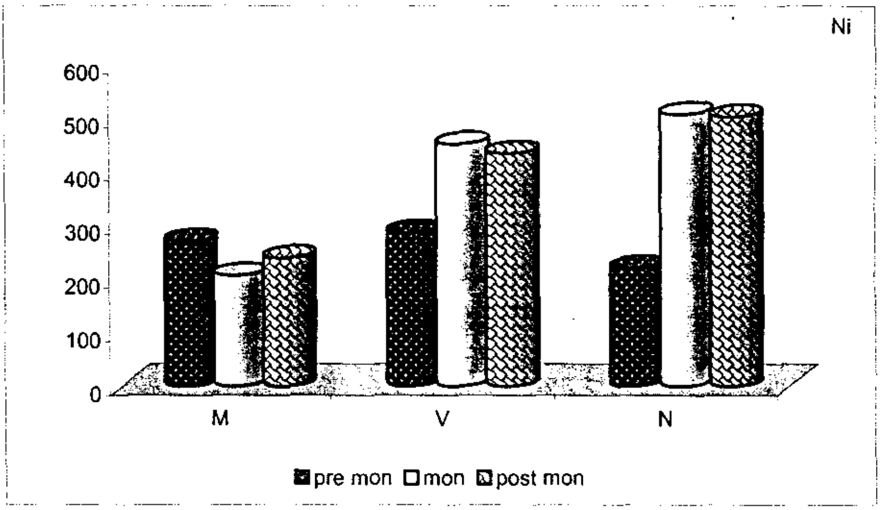


Nickel

There is a wide difference between the lowest (219.46μg/g) and the highest (502.76μg/g) value of Nickel in the Humic acids of Nettoor. Among the Mangalavanam samples the highest value is found in the Humic acid from the pre monsoon sediment (269.5μg/g) and the lowest (205.46μg/g) is found during the monsoon. Vypeen shows the highest during the Monsoon (451.7 μg/g) and the lowest during the pre monsoon season (285.86μg/g). In Nettoor the monsoon and post monsoon values are higher and they do not differ much. The lowest value (219.46 μg/g) of Nettoor is seen in the pre monsoon sample.

Table 3.6g Monthly variation of Ni in Humic acids (μg/g)

Seasons	M	V	N
pre mon	269.50	285.86	219.46
mon	205.46	451.70	507.69
post mon	238.04	434.13	502.76



Seasonal variation of Ni in Humic acids (μg/g)

Very few have attempted to link the metal concentration in the sediments of mangrove ecosystem to that of Humic acid from the same sediments. Of the metals studied, Fe is the most abundant metal and Cu and Mn are the least abundant. The amount of metal in the sediments is comparable with the same in Humic acids the values of which are given chapter V. There is significant positive correlation between the amount of Cu, Ni and Fe in the sediment and Humic acid. Whereas there is significant negative correlation between the amount of Cr in the sediment and Humic acid. Mangrove sediments are considered as a biochemical sink for metals due to their high concentration of organic matter and sulphides under permanently reducing conditions. The distribution of these metals in the sediments are controlled by hyrographic parameters, pH, salinity and grain size of the sediment (Jayasree *et al.*, 1995; Carmen *et al.*, 1997) which in turn might have influenced their distribution in Humic acids. (James, 1995). It seems that in sediments, Humic acid is the store house of metals.

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Chapter 4

SPECTROSCOPIC ANALYSIS OF HUMIC ACID

4.1 ^{13}C NMR

4.2 Infra Red Spectroscopy

4.3 Scanning Electron Microscopic Studies

4.4 UV-Visible

A detailed knowledge of Humic acid and its structure is the key to understand a wide variety of significant environmental phenomena, since they are responsible for some of the major processes in soils and in waters (Aiken *et al.*, 1985). Humic acids are remarkably diverse biopolymers with multiple functionalities. The diversity of Humic acid is illustrated by the many studies of their metal binding capacities, metal exchange rates and affinities (Mark *et al.*, 1998). Humic acid is regarded as being made up of a series of molecules of different sizes, few having the same structural configuration or array of reactive functional groups. According to current concepts, humic acid consists of micelles of a polymeric nature, the basic structure of which is an aromatic ring of the di- or tri hydroxy-phenol type bridged by -O-, $-CH_2-$, -NH-, -N=, -S- and other groups containing both free OH groups and the double linkages of quinone(Aiken *et al.*, 1985).

Recently a number of workers have proposed that humic substances result from the partial degradation of plant polymers (Wershaw *et al.*, 1998). The major organic constituents of terrestrial and aquatic plants are carbohydrates, proteins and lipids. In addition to the above, terrestrial plants contain lignin. When plant residues are incorporated into sedimentary deposits, the organic components are altered and as a result of humification, complex macromolecular humic substance results. Studies show that structures of humic substances derived from terrestrial plants differ from those derived from aquatic plants. (Hatcher et. al, 1981, Schnitzer *et al.*, 1981; Santa *et al.*, 1998; Mao *et al.*,1998) Terrestrial humic substances are largely aromatic and contain methoxyl, hydroxyl, carboxyl, and ether functional groups. (Schnitzer *et al.*, 1972 Schnitzer *et al.*, 1980). These groups are derived from lignin residues which are predominantly aromatic in structure and occur in vascular plants. Humic substances derived from aquatic flora such as algae and microorganisms, are very different and the main distinguishing feature is their aliphatic structures. Because of this terrestrial and aquatic humic substances can be chemically distinguished from one another. Malcom *et al.*, (1989) found that humic substances are a mixture of plant degradation products including lignin, terpenoids, flavonoids and hydrolysable tannins.

Different structures have been suggested for humic acid though none can entirely satisfy all the expectations. Dragunov's structure of humic acid has been recorded by Kononova (1966) which contains aromatic rings of the di- and trihydoxybenzene type part of which has the double linkage of a quinone group (fig 4.1). Nitrogen is present as part of a ring structure or in the peripheral chains. Carbohydrate residues are part of the structure.

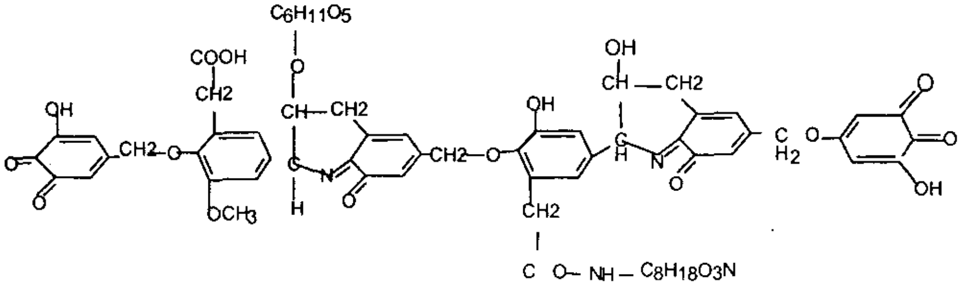


Fig 4.1

Flaig (1960b) suggested a structure which also is highly aromatic with a number of substituted benzene rings (fig 4.2). These two structures contain insufficient number of COOH groups relative to phenolic group

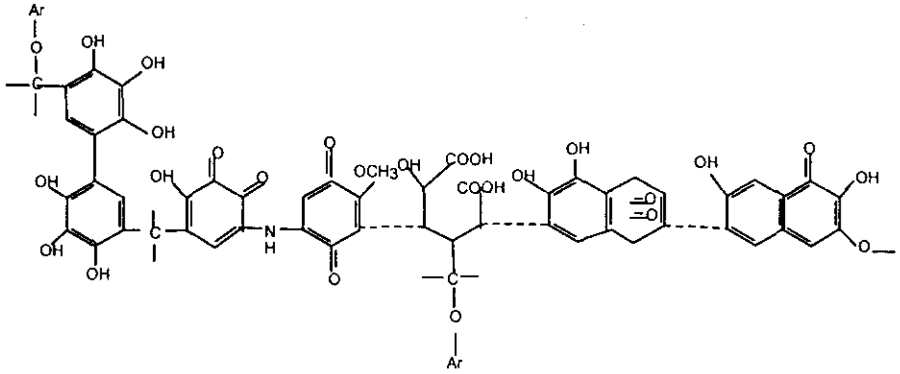


Fig 4.2

The hypothetical structure of humic acid suggested by Stevenson (1982) has number of phenolic groups both free and bound oxygen as bridge units with a number of aromatic carboxyl groups (fig 4.3). Carbohydrate and protein residues are part of this structure as it does in Dragunov's model. All three structures show nitrogen as a structural component.

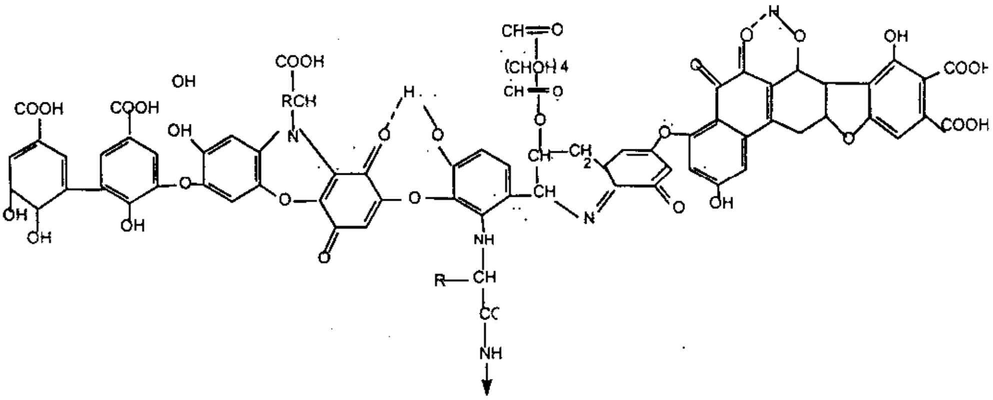


Fig. 4.3

Lewis and Davin (1994) suggested structures which include oxidized coal structures, structures reminiscent of common biosynthetic transformation, and lignin like frame works. The latter two structures are similar as they form some common amino acid building blocks and similar enzymatic processes. However humic acid like materials are found in plants and algae that lack the necessary enzymes for lignin biosynthesis and even from plants containing no lignins at all. Santa K *et al.*, (1998) suggested some sample structures for plant derived and coal derived humic acids. They are characterized by fused rings that have undergone a fairly significant number of mild oxidation processes. The structure of plant derived humic acid is shown in fig 4.4. This model for the humic acid aponomer is not distant from lignin models.

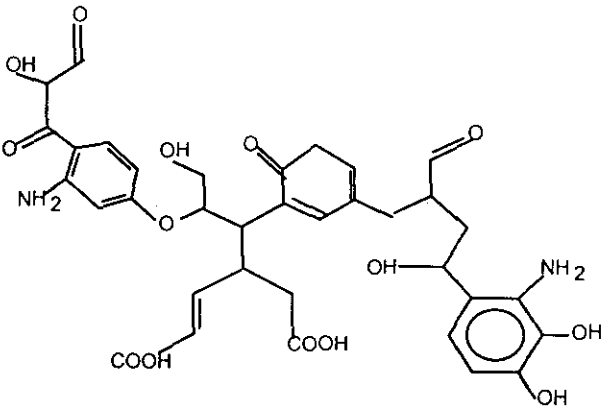


Fig 4.4

The purpose of this chapter is to characterize and identify structurally the humic acid separated from the mangrove ecosystems using three spectroscopic techniques such as Infra red, Fluorescence, Ultra violet and ^{13}C NMR and the results of other analytical procedures discussed in the previous chapter. With the sophisticated methods an attempt is made to provide a rough insight into the structure of Humic acid from mangrove ecosystem, which is mainly the humification product of mangrove plants. The quantitative structural data available in ^{13}C NMR can provide information from which we can begin to draw molecular frame work models of humic acids (Hatcher *et al.* 1983). Structures are constructed based on the quantitative signals offered by ^{13}C NMR for aromatic and paraffinic structures, carboxylic, carbonyl and ether functional groups and carbohydrates. The structure thus formed is verified with the other experimental data.

The number average molecular weights of humic acids reported in literature vary from 3000 to 1000,000 and that of fulvic acid range from 500-5000 (Stevenson, 1982) Many different techniques have been applied to measure molecular weights each based on a different technique. In addition all the techniques have their own limitations. For example, colligative properties yield a number average molecular weight with no indication of polydispersity, where as low angle X ray scattering measures the molecular radius of gyration but only gives partial information of the molecular size range. (Ronald, 1987). Gel permeation chromatography (Swift *et al.*, 1971) and ultrafilteration (Buffle *et al.*, 1978) can give molecular weight distributions, but both suffer serious problems due to charge repulsion effects and solute adsorption. (Ronald, 1987). One reason for the very large molecular weights reported is the possibility that humic substances can aggregate even to the extent of forming micelle like structure (Buffle *et al.*, 1978). Juhani *et al.*, (1997) by high performance size exclusion chromatography (HPSEC) obtained molecular weight ranges. So the experiments to find the molecular weights done by the earlier studies gave only an average molecular weight either number, weight or viscosity average or a range. Such an average may give erroneous result while deriving a model for its structure.

Following Mark *et al* (1998) an attempt is made to suggest structures for humic acid apomonomer from the three different sites based on empirical formula derived from elemental determinations and other spectral and analytical data. Thus an average building block for humic acid from each site, that is consistent with all available chemical analyses and valency considerations, is suggested.

Using the percentage of elements in different humic acid samples their empirical formula and the degree of unsaturation are calculated and are shown in table 4.1. The degree of unsaturation can vary to a small extent based on the nature of the nitrogen which can be in an aromatic or aliphatic ring or chain as a 1°, 2°, 3° nitrogen

Table 4.1 Empirical formula

Sample	Empirical formula	Degree of unsaturation
Mangalavanam	$C_{24}H_{14}N_4O_9$	18
Vypeen	$C_{36}H_{51}O_{21}N$	12
Nettoor	$C_{35}H_{50}O_{25}$	11

4.1 ^{13}C NMR

Solid ^{13}CNMR is a powerful method for the determination and quantification of functional groups in humic substances. In the NMR spectra of solids usually the lines are broader than liquid samples. This situation arises due to anisotropic interactions mainly dipole – dipole coupling. To a large extent this can be eliminated by high power proton decoupling. Chemical shift anisotropy is eliminated by magic angle spinning and more sensitivity is attained by cross polarization. The above techniques are combined in cross polarization magic angle spinning (CP/MAS) method. (Andrew, 1972). The CPMAS technique enabled better functional group analysis and allowed quantification of the groups.(Malcom 1989). Hatcher *et al.* (1983) have attempted to measure the functional group concentration humic acids, fulvic acids, and humins of a number of fresh water samples, marine sediments, soil and plants and they noted that high aromaticity indicated vascular plant origin. Aliphatic structures are major components of most humic substances and predominate in humic substances from submerged sediments such as peat, poorly drained soils, algal remains and marine sediments. They concluded that the aliphatic structures indicate contributions from alga and other microorganisms. Preston and Ripmeester (1982) have found that the solid state spectra of acid hydrolysis residues of organic soils are much more aromatic than humic substances isolated from the soils. They pointed out that aromatic peaks in both liquid and solid state spectra may be reduced in intensity by line broadening caused by coordination of paramagnetic ions to aromatic or phenolic structures.

The Solid ^{13}CNMR of the humic acid samples are shown in figures 4.5 to 4.7 The^{13}CNMR spectra were assigned to five regions by the previous authors (Benny *et al.*, 1998, Hatcher *et al.*, 1983).

Aliphatic hydrocarbon bands (0-50 ppm)

This is an intense region in the spectra of all the three samples. Peaks are mainly of aliphatic carbon or carbons bound only to other carbons; All the three spectra have peaks centered around 30 ppm which is the chemical shift of methylene carbons in long chains or cycloparaffins. The strong peak at 30 ppm is observed by several other workers (Hatcher *et al.*, 1980; Poutanen *et al.*, 1985; Benny, et al., 1998; Robert *et al.*, 1998). The peaks at 20-25 ppm represents methylene C and methyl C. The bands in the aliphatic region 17 ppm, correspond to terminal methyl groups and 39-40 methylene groups near points of carbon branching. The band at 42 ppm was assigned to methylenes β to ester linkages (Robert, *et al.*, 1998; Benny, et al., 1998).

Aliphatic alcohol and ether bands (50-112 ppm)

The resonance between 60 and 110 ppm is characteristic of carbon atoms singly bonded to oxygen or nitrogen in alcoholic, etheric or amine carbons (Poutanen and Morris, 1985). The peaks in the 60-90 ppm region are mainly due to carbohydrates. The bands for C6 atoms are generally between 63-65 ppm for all carbohydrates (Gamble *et al.*, 1996). The chemical shifts of anomeric carbon atoms of carbohydrates occur in the region between 90-105 ppm (Robert, *et al.*, 1998). Aliphatic carbon atoms attached to protonated carbon atoms of some phenolic functional groups also have bands between 90 and 105 ppm. The peak at 56 ppm can be due to Methoxyl C or N-substituted aliphatic carbon.

Aromatic and phenolic carbons (112-163 ppm)

These are mainly aromatic and olefinic carbon bands. The Peak at about 130 ppm is that of ring carbons in which the ring is substituted by a strong electron donor such as oxygen or nitrogen. The chemical shift region of aromatic carbons substituted by functional groups containing O and N should be about 150 ppm (Poutanen and Morris, 1985). The spectra of Vypeen (sampe 2) and Nettoor (sample3) humic acids have peaks in this region suggesting that there are aromatic carbons substituted by functional groups such as $-OCH_3$, -OH, -OR, $-NH_2$. Terrestrial humic acid is found to contain a large proportion of phenolic, polyphenolic and methoxylated aromatic compounds (Schnitzer and Khan, 1972). Carbons 1,2 and 6 of parahydroxy phenyl lignin monomers and C1 carbons of 3,4-dihydroxy - or trihydroxy phenols and phenolic ethers have chemical shifts between 130 and 135 ppm (Robert, *et al.*, 1998)

Carbonyl bands

There is a considerable overlap of the chemical shifts of the carbonyl containing structures of the chemical components. The chemical shifts of the carbonyls of the carboxylic acids or carboxylate salts esters and peptides can all fall within the range 160-190 ppm. (Robert *et al.*, 1998) The carbonyl carbon of carboxylic acid bands are seen in the range at 175-184 ppm (Leyden and cox 1977). The bands between 163-170 ppm are most likely due to ester linkages. Aliphatic esters generally have band at 171 ppm and aromatic esters are expected to be between 163-169 ppm (Wooten, 1995). Aldehydes Ketones and quinones generally have chemical shifts between 180-220ppm. The chemical shifts of o- quinines are near 180 ppm. (Robert *et al.*, 1998) The various regions along with the respective groups are shown in table 4.2.

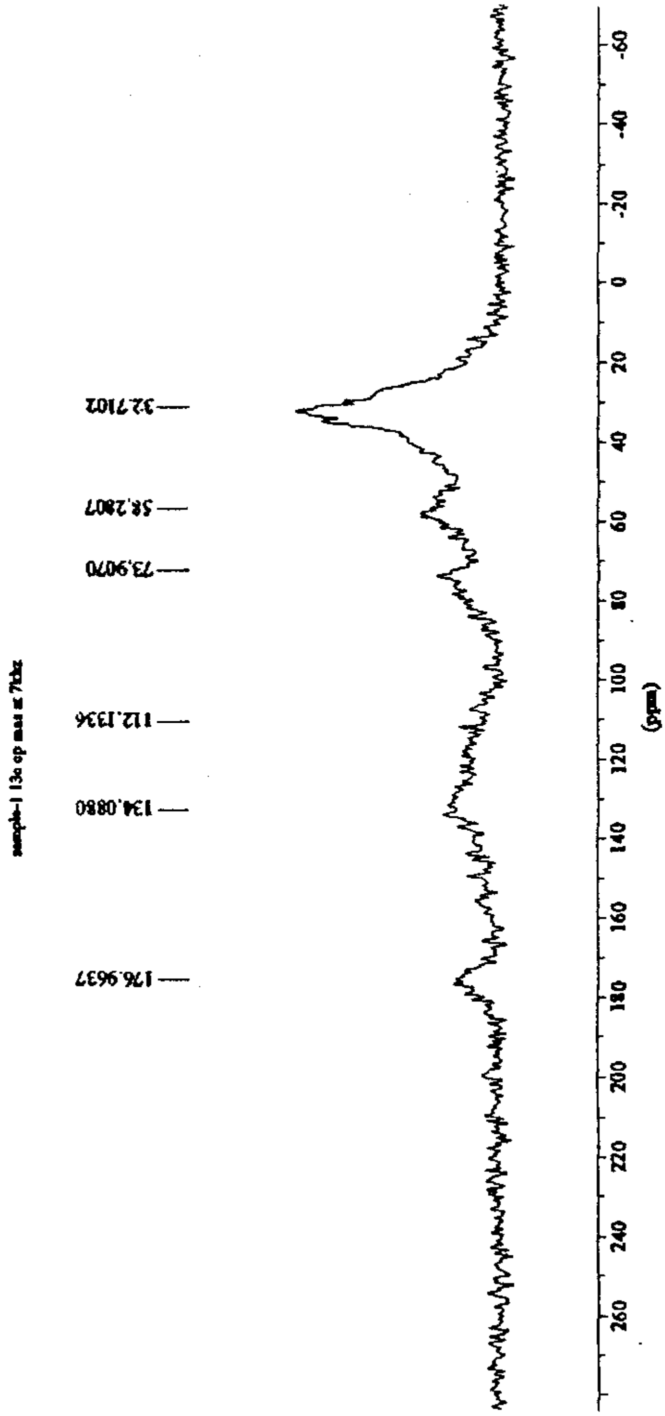
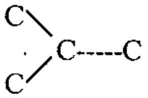


Fig. 4.5 Mangalavanam

Table 4.2 ^{13}C NMR Chemical shifts regions for various types of carbons typically present in humic acid

Region I	Region II	Region III	Region IV	Region V
0-50 ppm	50-110 ppm	110-160ppm	160-190ppm	190-220ppm
Paraffinic C: C-C*-C	Alcohols C–OH	Olefinic-C	Carboxyl C-OH	Aldehyde
	Amines (C-NH$_2$, C-NHR, C- NR$_2$)	Aromatic -C	Ester	Ketone
	Carbohydrates		Amide	
	Ethers (C-O-C)			
	Methoxyl (OCH$_3$)			
	Acetals			

The areas under the major peaks have been determined and based on this the number of carbon atoms possible in each area and thus in each group is calculated as shown in table 4.3 The region 0- 112ppm is calculated as aliphatic carbon, and 112-163ppm ppm as aromatic carbon. Total aromaticity is calculated by expressing aromatic carbon 112-163ppm as a percentage of aliphatic carbon 0-112ppm plus aromatic C. The area under the region 0-90 ppm represents the number of sp^3 and that under 90-120 ppm the number of sp^2 hybridised carbon atoms. Distribution of carbon in different groups are shown in table 4.4

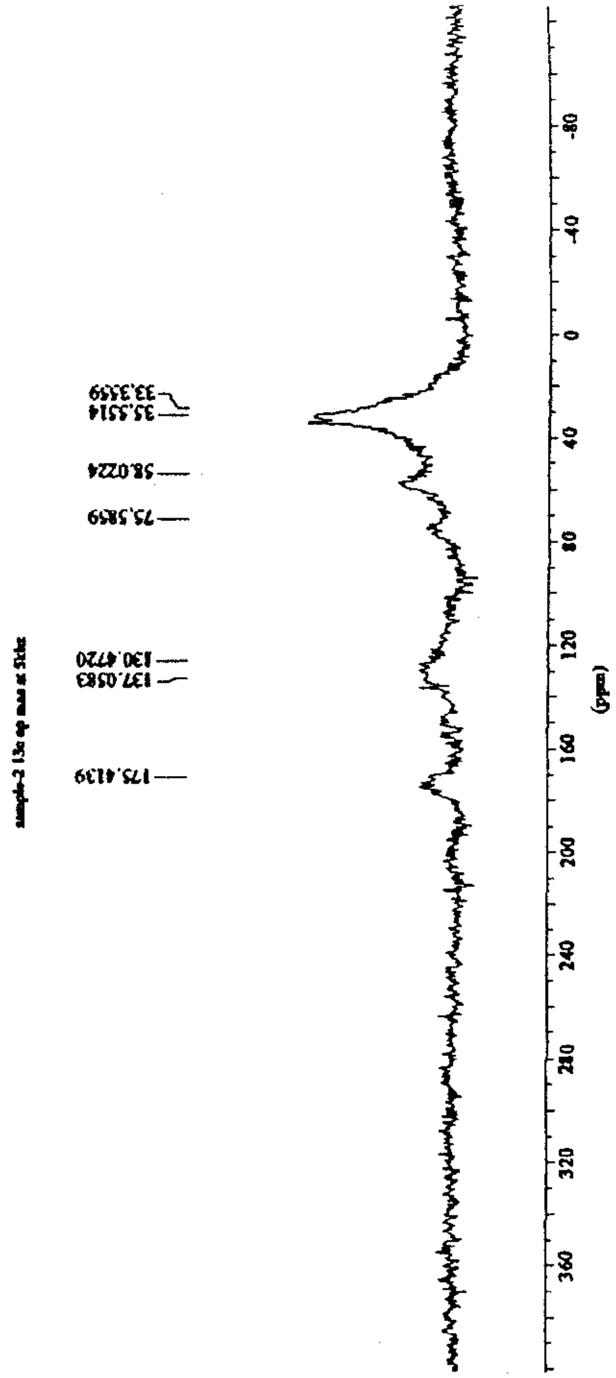


Fig. 4.6 Vypeen

Table 4.3 Area under the various regions

	Area under the peaks				Number of C atoms		
Regions	ppm	M	V	N	M	V	N
		cm^2			%		
I	0-50	4.19	2.07	1.82	45.05	34.96	45
II	50-112	2.15	1.2	1.34	23.12	20.27	33
III	112-163	2.1	1.7	0.59	22.58	28.71	15
IV	163-190	0.54	0.43	0.2	5.81	7.26	5
V	190-220	0.32	0.52	0.06	3.44	8.78	1
SP^3	0-90	5.97	3.24	3.04	64.19	54	76
SP^2	90-220	3.33	2.77	0.97	35.81	46	24

Table 4.4 Distribution of carbon in different groups

Regions	Characteristic groups	M	V	N
		$C_{24}H_{14}O_9N_4$	$C_{36}H_{51}O_{21}N$	$C_{35}H_{50}O_{25}$
I	Paraffinic	11	13	16
II	Carbohydrate, Esters, Ehers, Methoxy, Acetals Amines, Alcohols	6	7	11
III	Ring, phenolic	5	11	5
IV	carboxyl, ester, amide carbon	1	2	2
V	Carbonyl carbons	1	3	1
	sp3	15	24	27
	sp2	9	12	8

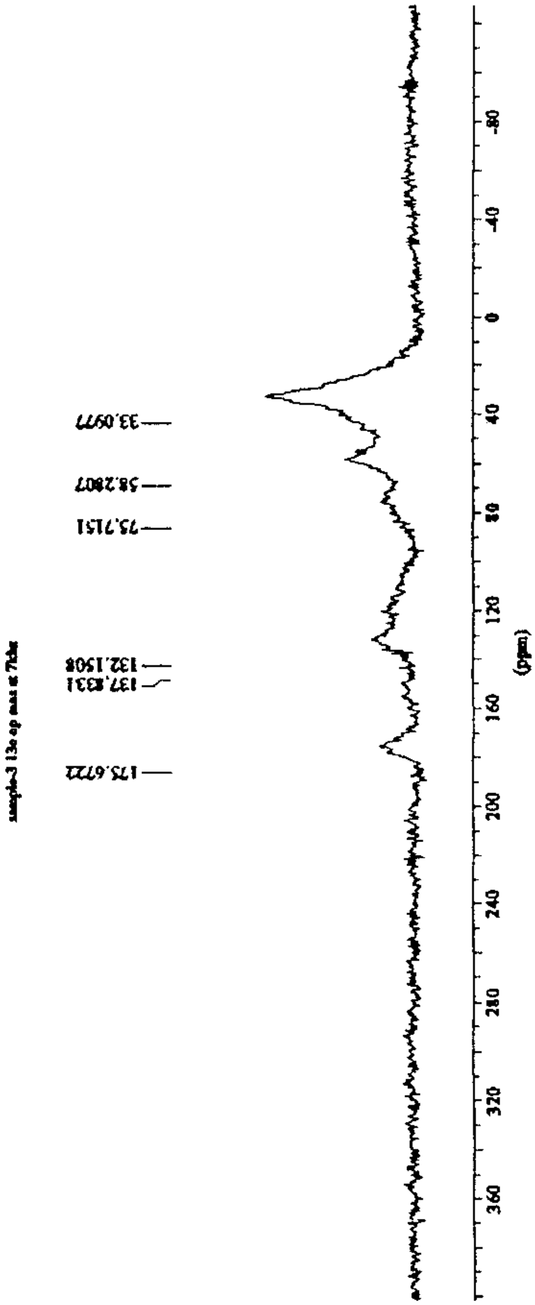


Fig. 4.7 Nettoor

Based on the above functional groups the possible structure for each humic acid is found out by various permutations and combinations.

Mangalavanam $C_{24}H_{14}N_4O_9$

The elucidated structures are shown in fig 4.8a and 4.8b The high nitrogen content and very low amount of hydrogen in this humic acid makes it unique in its structure. The migratory birds seen in this site for 4-6 months make it exceptionally rich in nitrogen. The comparatively higher nitrogen content might be due to significant contribution from uric acid from the excreta of the migratory birds. This humic acid aponomer is similar to the model of Santa *et al.,* 1998. The parraffinic contribution might be the breakdown products of terpenoids.

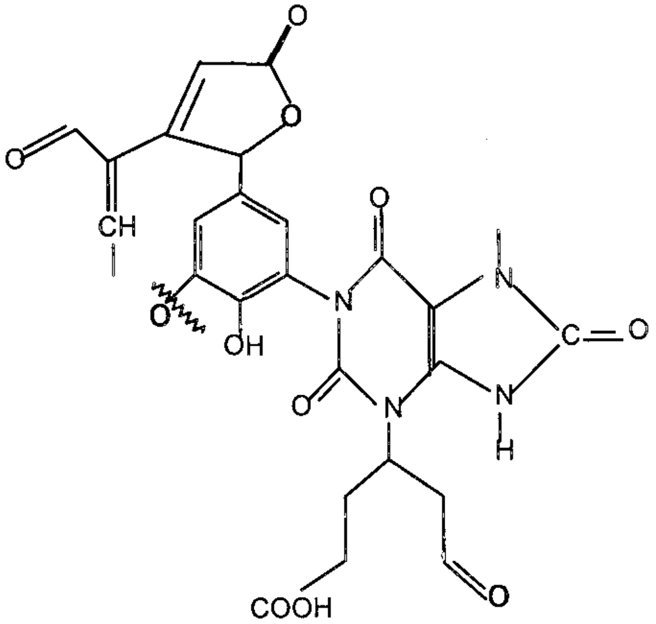


Fig 4.8a Mangalavanam $C_{24}H_{14}N_4O_9$

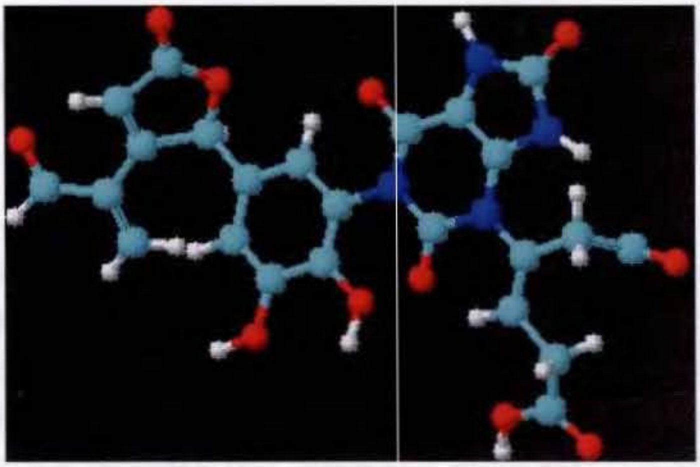


Fig. 8b : Three dimensional structure of Humic acid from Mangalavanam

Vypeen $C_{36}H_{51}O_{21}N$

The possible structure for the humic acid of Vypeen is given in fig 4.9a and 4.9 b. This structure has carbohydrate and lignin as part of its backbone. Hatcher et al. (1983) have noted that if lignin is assumed to contribute to humin of peats, carboxyl carbon will not be expected unless lignin were to oxidize. It is possible that the carboxyl group is associated with the paraffinic structures. Phenolic groups present in the aromatic ring answer the required phenolic acidity. Carboxylic acidity might be due to the hydrolysis of the lactone present in the carbohydrate residue.

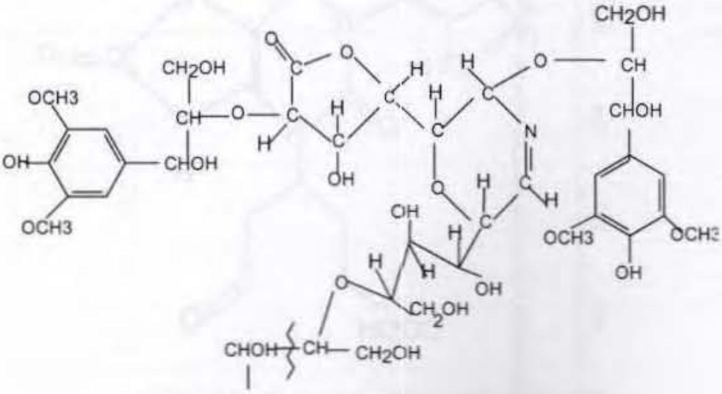


Fig 4.9a Humic acid Vypeen $C_{36}H_{51}O_{21}N$

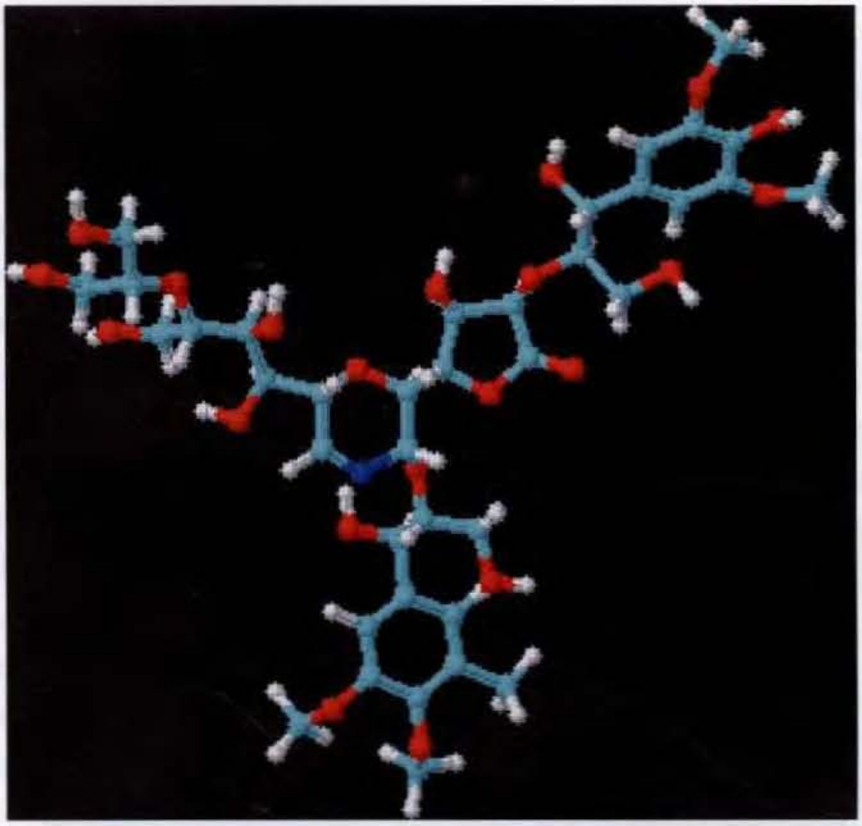


Fig. 4.9 b : Three dimensional structure of Humic acid from Vypeen

Nettoor $C_{35}H_{50}O_{25}$

The presence of intense signals for oxygen substituted aromatic carbons and methoxyl carbons is a strong indication that unaltered lignin residues are present in large amounts. It appears that the anaerobic environment has influenced the aromatic structures of humic acid to resemble their parent material namely lignin (Hatcher *et al.*, 1983). The structures are shown in fig 4.10a and 4.10b

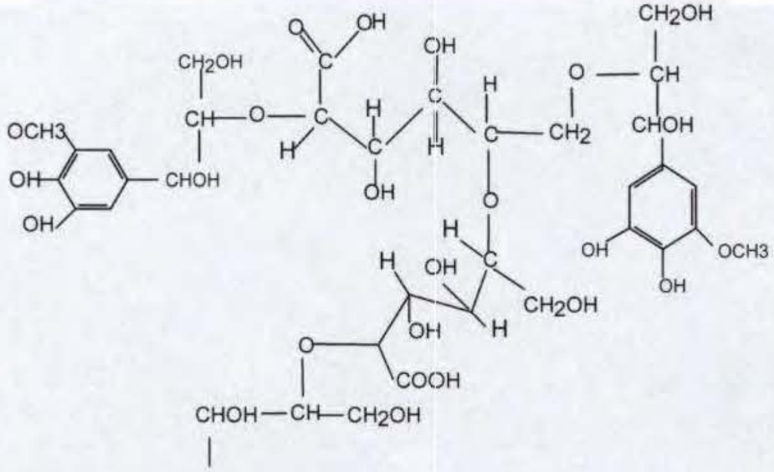


Fig 4.10a Nettoor $C_{35}H_{50}O_{25}$

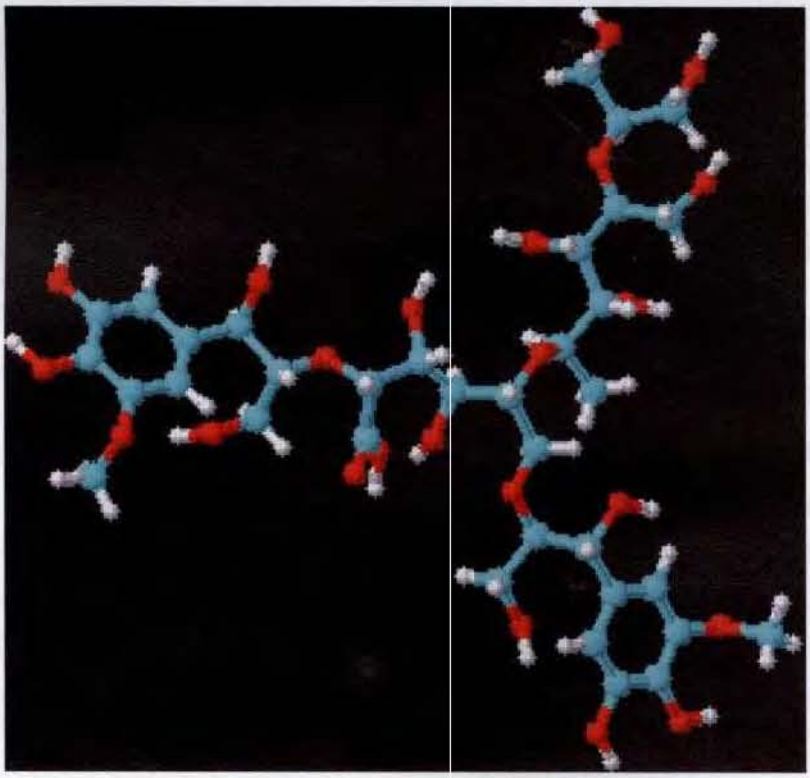


Fig. 4.10b : Three dimensional structure of Humic acid from Nettoor

4.2 Infra Red Spectroscopy

No error proof methods are available for the analysis of various functional groups. Infrared spectra provide valuable information on the nature and arrangement of functional groups in humic acids. It is most effective in analyzing functional groups containing oxygen and nitrogen. I R of the three samples are given in fig 4.11-4.13. The most striking feature of the infrared spectra of pure humic compounds is the overall simplicity of the spectra. This simplicity is more apparent than real because the broadness of the bands result from the fact that one is dealing with a complex mixture in the case of humic substances. A particular type of functional group in humic substance can exist in a wide variety of chemical environments each characterized by slightly different force constants for its bonds (Aiken *et al.*, 1985). On the basis of the data available in the literature and the experimental data a table is compiled (Table 4.5). For wide bands and shift of the absorption maxima the range of wave numbers are given. Spectra fig 4.7-4.9

The 3400 cm^{-1} Region

The broad band seen in this region may be due to OH stretching vibrations alcohols (Wagner and Stevenson, 1965). The broadening may be due to hydrogen bonding or the complicated structure of humic acids and the wide variety of chemical environments which might have contributed much for the broadening of OH group. (Aiken *et al.*, 1985). Wagner and Stevenson (1965) observed a decrease in the intensity of absorption by methylation or acetylation and increase to its original value while saponification. All the three IR spectra have this broad band and that might be due to the hydrogen bonded OH groups.

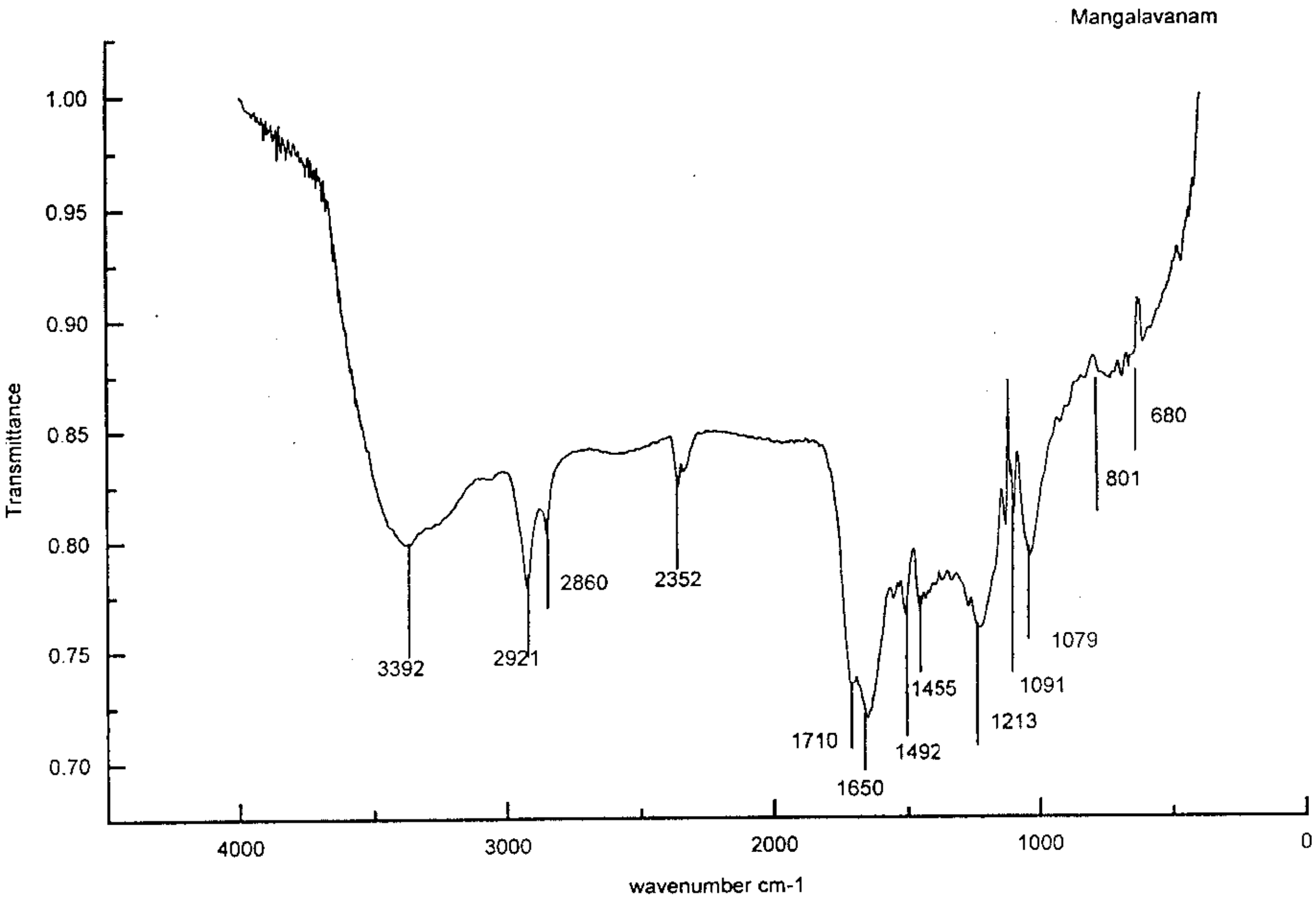


Fig. 4.11 Managalavanam

The absorption in this region also can be due to NH groups. Secondary amines show single weak band in 3350-3310cm^{-1} region due to N-H stretching. (Silverstein *et al.*, 1981). These bands are shifted to longer wavelength due to hydrogen bonding.

The 3100 cm^{-1}- 3000cm^{-1} Region

The shoulder at 3100 could be of aromatic C-H stretching bands occurring between 3100 and 3000cm^{-1} (Aiken *et al.*, 1985). Sharp absorption at 3000cm-1 serves as a diagnostic criterion for the =C-H groups of aromaic compounds. With a large number of substituents the intensity of the 3030 cm-1 band becomes very low. The aromatic and aliphatic groups have a significant role in the formation of the spectra of humic acids (Filips *et al.*, 1988 Aiken *et al.*, 1985). The 3090 peak in the Mangalavanam and 3100 peak in the Vypeen sample point out the presence of Aromatic ring in the humic acids from these sites

The 3000and 2840 cm^{-1} bands

Absorption arising from C-H stretching in the alkanes occurs in general in the region of 3000-2840 cm^{-1}. Saturated hydrocarbons containing methyl groups show two bands occurring at 2962 cm^{-1}and at 2872 cm^{-1}, the first of these results from the asymmetrical stretching mode in which the two C-H bonds of the methyl group are extending while the third one is contracting (Silverstein *et al.*, 1981). The second one arises from symmetrical stretching in which all the three C-H bonds contract in phase. The C-H bands seen at 2924 and 2842 confirms the presence of paraffinic carbon in humic acid. (Filips *et al.*, 1988, Aiken *et al.*, 1985). The bands 2921 cm^{-1} and 2924 cm^{-1} of Mangalavanam, 2924 cm^{-1}and 2842 cm^{-1} of Vypeen and 2986 cm^{-1} and 2674 cm^{-1} of Nettoor assure the presence of aliphatic C-H bonds.

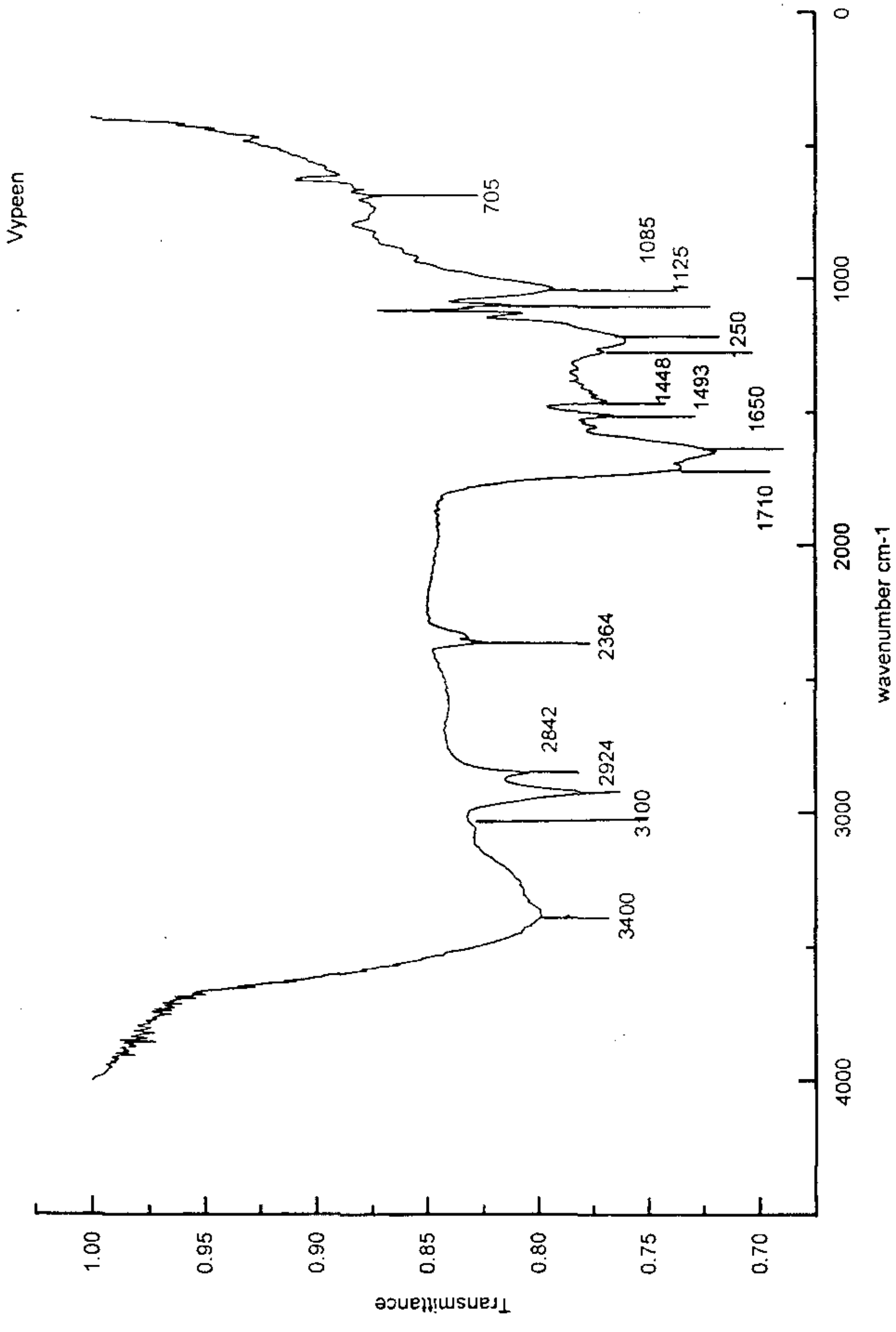


Fig. 4.12 Vypeen

The 1870-1540 cm^{-1} band

Ketones, aldehydes, carboxylic acids, carboxylic esters and amides show a strong C=O band in the 1870-1540 cm^{-1} region. Its relatively constant position, high intensity, and relative freedom from interfering bands make this one of the easiest bands to recognise in infrared spectra. The position of the C=O stretching band is determined by the following factors like the physical state, electronic mass effects of neighbouring substituents, conjugation, hydrogen bonding and ring strain. Consideration of these factors leads to a considerable amount of information about the environment of the C=O group. Conjugation of C=O with C=C bond results in delocalisation of the π electrons of both unsaturated groups. Delocalisation of the π electrons of the C=O group reduces the double bond character of the C to O bond causing absorption at lower wave numbers. Conjugation with phenyl group also could have reduced the absorption. But steric effect which has reduced the co planarity of the conjugated system reduce the effect of conjugation (Silverstein *et al* 1981). The C=O stretching bands of carboxylic acids are considerably more intense than ketonic C=O stretching bands (Silverstein *et al.*, 1981, Aiken *et al.*, 1985). In IR of the samples the spectra of the peaks at 1717 cm^{-1} (Mangalavanam), 1710 cm^{-1} (Vypeen) 1802 cm^{-1} (Nettoor) could be resulting from a C=O group present in these humic acids.

The 1650-1540 cm^{-1} band

Though this band is not very clear in the spectra of many humic acids under favourable conditions, the amide 1 band appears near 1650 cm^{-1} (Orlov, 1985). Many samples of humic acids particularly when the nitrogen content is high, there are separate bands at 1650-1640$cm^{-1}$and 1550-1540cm^{-1} (Orlov, 1985). These are typical of amide groups. The amide I band may be attributed to the carbonyl group of the amide. The amide II band is

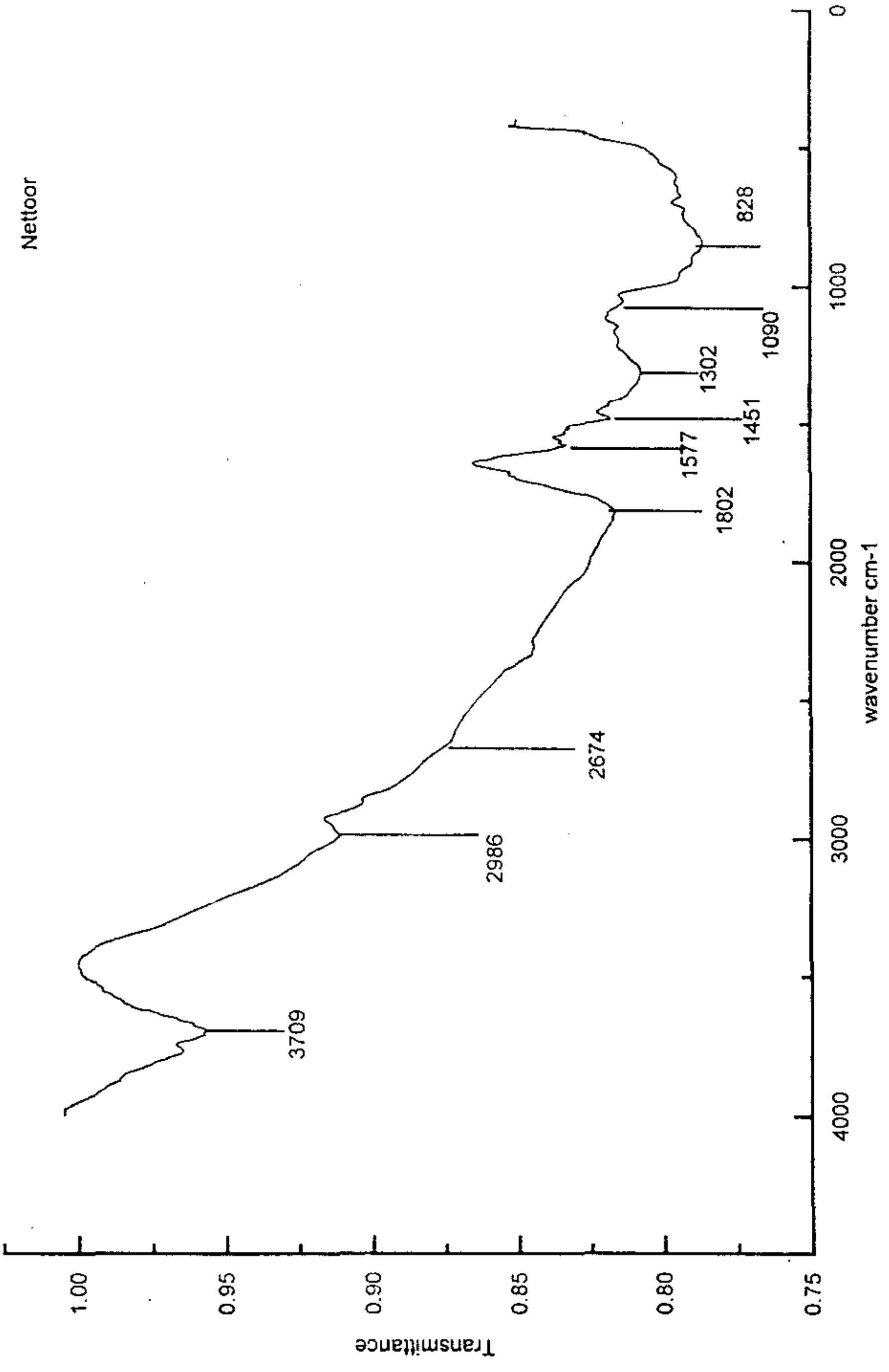


Fig. 4.13 Nettoor

due to deformational oscillations of NH or shifting oscillations of OCN and NH. The appearance of the amide band corresponds to secondary amides, which allows the entire set of amide bands to polypeptides. In many cases the carbonyl band passes directly into an absorption band at 1620-1610 cm^{-1}. So this might be the amide band or the carboxylic band. In the humic acid of Mangalavanam a clear absorption band is seen in the region1650 cm^{-}1and that of Vypeen shows absorption at 1640 cm^{-1}

Among the groups absorbed near 1625 cm^{-1} there are aromatic C=C, carbonyl C=O bond by hydrogen bonds and quinines also bound to OH groups by hydrogen bonds. The various literature on this subject emphasize the presence of C=C and C=O groups or the influence of polar substituents. The band intensity may decrease during methylation or during the formation of complex esters.

In humates the ionized carboxyl group shows absorption near 1590 and 1400cm^{-1} but it does not appear always as separate bands. The 1590 band often fuses with the 1600cm-1 band and 1400cm-1 band is superimposed on the absorption region of deformational oscillations of the CH_2 and CH_3 groups.

1620-1590 cm^{-1}

Weak combination and overtone bands appear in the 1620-1590 cm^{-1} region characteristic of the substitution pattern of the ring. Weak bands in the this region confirm highly substituted aromatic ring. The weak bands seen may be of C-C stretching of carbon in the aromatic ring (Silverstein *et al.* 1981).

Amines

The N-H bending band is seldom detectable in the spectra of aliphatic secondary amines whereas secondary aromatic amines absorb near 1515cm^{-1} (Silverstein *et al.*, 1981).

Table 4.5

Mangalavanam cm^{-1}	Vypeen cm^{-1}	Nettoor cm^{-1}		Possible assignment
3385	3400		br	O-H stretch, N-H stretching
3090	3100		sh	Aromatic C-H stretching
2900	2924			Aliphatic asymmetrical C-H stretching
2820	2842			Aliphatic symmetrical C-H stretching,
2340	2345			
2330	2334		sh	
1725	1717		sh	C=O stretching of COOH, ketone and ester C=O,
1600	1640			Amide linkages of protein
1588	1588			C-C stretching (ring)
1450	1493			substitution in the ring
	1448			O-H bending
1368	1380			CH3, CH2 stretch of aliphatic compounds
1358	1360			CH3 stretch, O-H stretch in alcohols
1210	1250			C-O stretching
				C-N stretching of amines
1085	1085			Asymmetrical C-O-C stretching in ethers
				C-O stretch in aromatic compounds
1120-1045	1125-1045			C-O stretch of alcohols, carbohydrates
900-670	900-670			C-H bending in aromatic compounds

sh –shoulder br- broad

1493 cm^{-1} Skeletal vibrations involving carbon-carbon stretching within the ring absorb 1500-1400cm^{-1}.

1448 cm^{-1} The band in this range may be due to deformational oscillations of C-H, deformational oscillation of O-H groups.

1500 cm^{-1}-1200 cm^{-1}

Aromatic ring In-plane bending bands ring C-H bands appear in the 1300-1000 cm^{-1} regions.

Carboxylic acid Two bands arising from C-O stretching and O-H bending appear in the spectra of carboxylic acids near 1320-1210cm^{-1} and 1440-1395cm^{-1}. C-H bending vibrations are seen in the region 1450cm^{-1}-1375cm^{-1} (Silverstein *et al.*, 1981)

Amines C-N stretching of amines occur in the region 1250cm^{-1} (Filips *et al.*, 1988)

1150 cm^{-1}-1000 cm^{-1}

Ethers : The most characteristic of ethers is a strong band in the 1150-1085 cm^{-1} because of asymmetrical stretching. Symmetrical stretching band is usually weak. The C-O-C group in a 6 membered ring absorbs at the same frequency as in an acyclic ether (Silverstein *et al* 1981) This must be due to the OCH_3 group.

Alcohols and Carbohydrates : C-O stretching of alcohols and carbohydrates are in the region 1125-1045 cm^{-1} (Ertel and Hedges, 1983). Attempts have been made to explain absorption in the region 100cm-1 as oscillations of alcohols (Orlov, 1985). The weak bands at 955 $cm^{-1}$and 752 cm^{-1} are probably due to carbohydrates.(Robert and Kay, 1998).

Below 1000 cm^{-1}

Humic acid do not show any strong absorption in this region but comparatively weak diffused bands are observed in this region. This could be due to the C-H stretching band of aromatic compounds which are seen in the region 900-670cm^{-1} (Silverstein *et al.*, 1981)

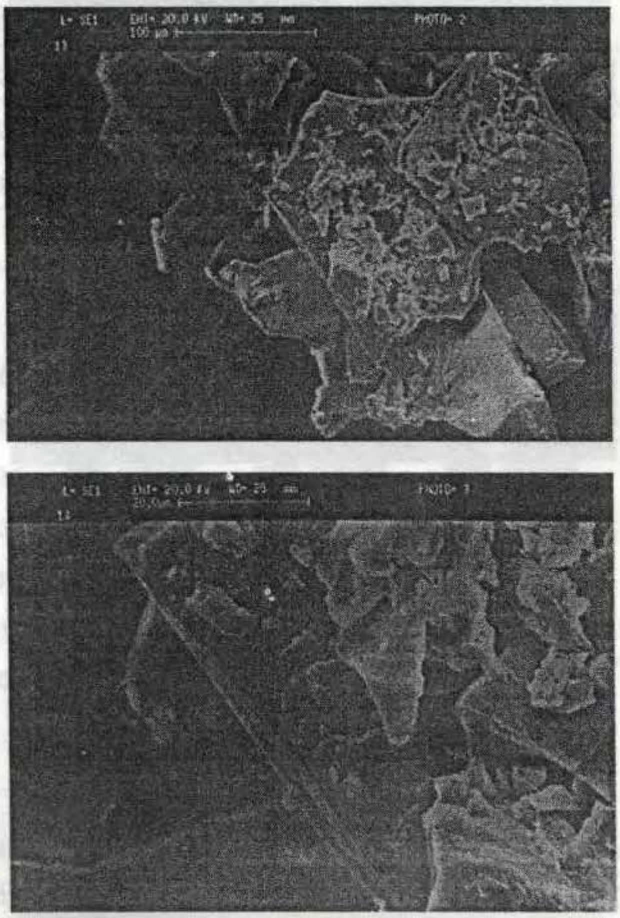
In short a close examination of their IR spectrum shows the presence of aliphatic chain agreeing with observation made in the NMR study. The aromaic ring with many substitution (3or 4) is also evidenced by the IR spectrum. The presence of twin carboxyl band at 1717 $cm^{-1}$and 1672 cm^{-1} may be due to simultaneous presence of many C-O links. The high ketone content observed in the ^{13}C NMR study is assured again. The IR spectra also pinpoint the presence of carbohydrate group and thus supports the earlier study made by spectrophotometric analysis and ^{13}CNMR. The presence of OCH_3 groups in the structure is supported by the ether band in the IR spectra. IR spectra gives valid support for the presence of alcoholic group in the structure. From the above it is clear that the results are in tune with the proposed structures. Though these may not be 'the real' structures the ones that are elucidated are suitable to explain the various behaviours of humic acid especially its sorptional characteristics with metals.

4.3 Scanning Electron Microscopic Studies

The additional evidence for the polymeric structure of Humic acid is seen from the SEM of the samples (Fig 4.10-4.14). The plate-like repeating units could be considered as different polymeric units of humic acids. The different dimensions of these platelets reflect the nature of Humic acid as heterogeneous polymeric mixtures. The surface area of these platelets is compared with the surface area of humic acid samples, obtained from the three dimensional structures. The area of the plate line unit in the humic acid of Mangalavanam is 136×10^{8} $Å^2$ and the are of the monomer unit as revealed by the three dimensional structure is 79 $Å^2$. The surface area of the platelets in the SEM is

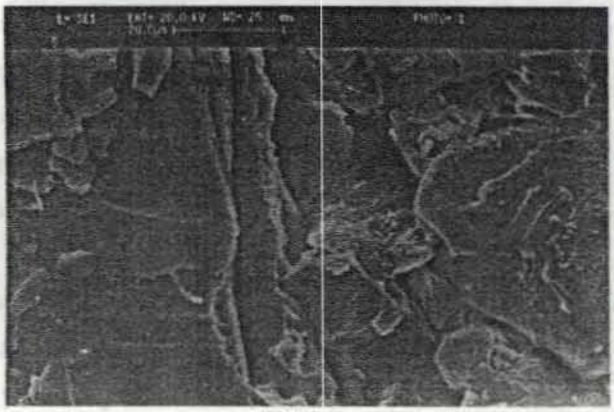
10^8-10^{10} times more than a single monomeric unit. So each platelet like unit could be considered as polymer containing 10^8 to 10^{10} times monomer units. Though there are differences, there is a lot of similarity between the SEM of the three samples. Noticeable change in the structure is observed after adsorption of metals. The polymeric structure seems to be disrupted after adsorption. The plate-like structure could also be due to Humic acid crystals formed during its dissolution in Sodium Hydroxide. Further surface structural studies are needed to have a clear picture in this regard.

SEM of Metal Adsorbed Humic acid

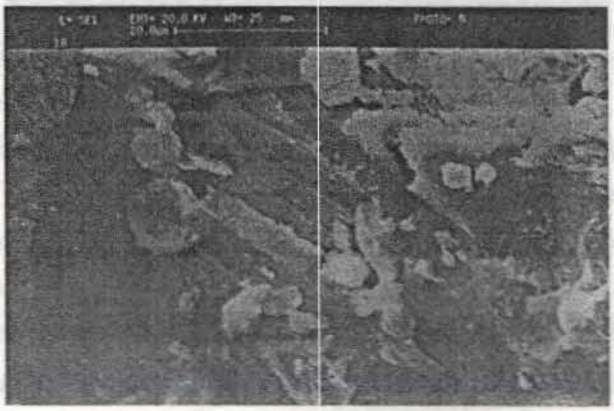


SEM of Humic Acid

Mangalavanam



Vypeen



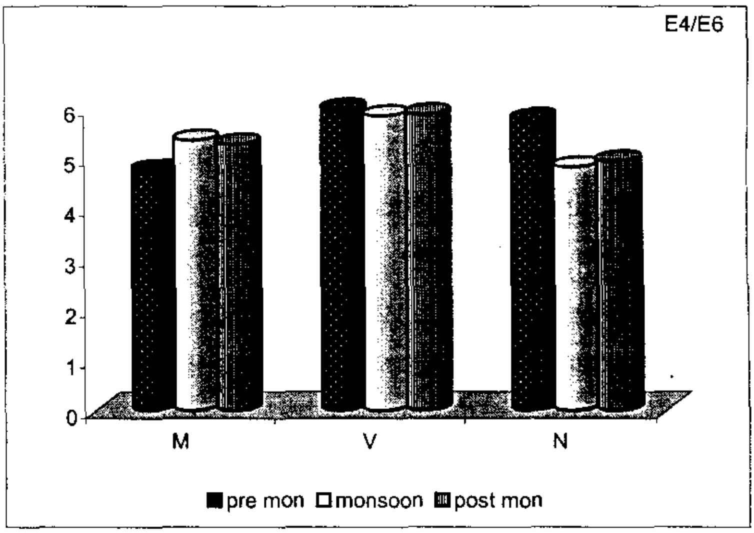
Netoor

4.4 UV-Visible

UV- Visible spectra of Humic acids are featureless. Absorbance increased with the decrease in wavelength but no minima or maxima were observed. The ratio between the optical densities are 465 and 665nm. It has been used as indicator of the degree of condensation of humic substances. The lower the E4/E6ratio the higher the degree of condensation. E4/E6 ratios are listed in the table 4.6.

Table 4.6. E4/E6 ratios

Seasons	M	V	N
pre mon	4.76	5.98	5.79
monsoon	5.35	5.85	4.82
post mon	5.28	5.88	4.95



The values agree with those observed by Raspor *et al.*, 1984. The values show high degree of condensation. (Schnitzer *et al.*, 1972)

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Chapter 5

SORPTIONAL BEHAVIOUR OF METALS ON THE SEDIMENT

5.1 Sediment Organic carbon
    5.2 Porosity
    5.3 5.3 Bulk density
    5.4 Textural characteristics
    5.5 Total metal in the sediment
        5.5.1 Copper
        5.5.2 Iron
        5.5.3 Manganese
        5.5.4 Zinc
        5.5.5 Chromium
        5.5.6 Nickel
        5.5.7 Cobalt
5.6 Adsorption studies
        5.6.1 Partition coefficient
        5.6.2 Langmuir adsorption isotherm
        5.6.3 Freundlich adsorption isotherm
5.7 Desorption studies

Mangrove sediments are chemically and physically dynamic regions. Many metals enter mangrove ecosystems and become subject to a variety of complex biogeochemical process. These are important sources or sinks for trace metals (Harbinson, 1986). Many mangrove species are reported to contain very high concentrations of certain heavy metals such as Fe and Mn.(Untawale *et al.*, 1980). The toxic effects of these metals are reduced due to the action of chelating substances present in these plants (Untawale *et al.*, 1980).

Metal partitioning in sediments results from many factors like physico chemical properties of the metal considered, contamination modalities, relative importance of sediment sorbents, abiotic parameters etc. These are able to induce metal transfers between the upper layers of sediments and water columns either through mechanical stirring or by bioturbation. The fate and bioavailability of heavy metals in contaminated soil is largely controlled by sorption reactions to soil mineral surfaces and humic substances (Christl *et al.*, 2001). Heavy metals are known to form stable complexes or chelates with a variety of ligands, the average stability of which decreases with increase in the electronegativity in the order Pb>Cu>Ni>Co>Zn>Cd>Mn>.

It is worthwhile to mention the recent works on the mode of occurrence of trace metals on sediments. The use of theoretical Eh-pH diagrams to examine the stability fields of trace compounds is a satisfying approach (Hem, 1972) but the role of organic matter is not included among the other factors (Fengler et al., 1994). The chemical extraction techniques in which the sediment is sequentially leached with progressively more aggressive extractants in order to have a number of trace element bearing phases, exchangeable, carbonate, Fe and Mn oxide, organic and residual is another development. All such extraction techniques are largely empirical, and limitations of the approach must always be considered in data interpretation (Whalley and Grant, 1994, Rae,1997). The recent techniques for investigating the binding sites for trace metals in sediments involve the use of computer modeling. The model GEOCHEM (Mattigod and Sposito, 1979) goes some way in establishing the speciation in waters sediments and soils but it does have shortcomings because it ignores the macroionic nature of humic and fulvic acids (Rae,1997). More recently the model for fulvic acid MINTEQ code (Allison and Perdue, 1994) the available data base for metal interaction is limited. Another model WHAM, Windermere humic aqueous Model (Tipping, 1994) is particularly useful since it can be applied to equilibrium speciation problems involving waters, sediments and soils with humic substances present in dissolved and particulate form (Rae, 1997).

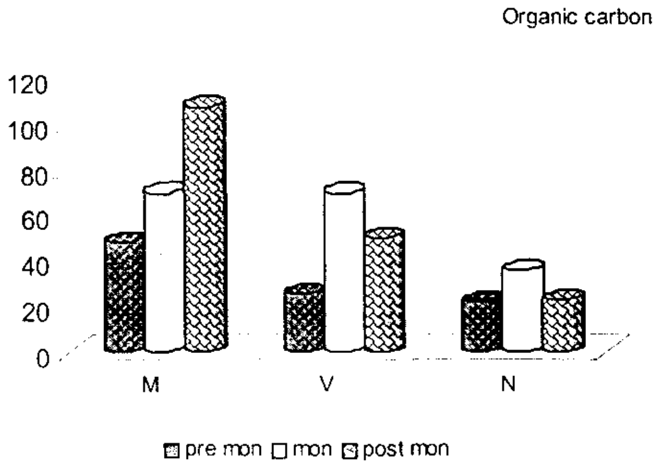
The objective of this chapter is to have a clear picture of the nature of the various types of binding involved between the mangrove sediment and metals. For this the natural physical characteristics of the sediments like porosity, bulk density and the chemical parameters like sediment organic carbon, the metals already present in the sediment samples etc were determined. The sorptional characteristics of the sediment samples were assessed. The samples were kept in contact with metal solutions of Cu, Fe and Mn of different concentrations and pH. The influence of these parameters on sorptional behaviour of metals is determined. To understand the competing nature of the metal ions sorptional studies were also done with a mixture of metal solution. To supplement the above results desorption studies were done with four different extractants, Ammonium acetate, EDTA, HCl and Sodium dihydrogen phosphate.

5.1 Sediment Organic Carbon

Different factors may control the partitioning and also the bioavailability of the hydrophobic organic compound and heavy metal within the benthic ecosystem. The factors include various sediment characteristics, such as grain size distribution, mineral composition and organic content (Forstner, et al., 1979). A number of studies have demonstrated that the sorption of hydrophopbic organic pollutants and heavy metals is well correlated with the organic mater in content and the particle size(Al Ghadban *et al.*, 1994). The organic matter in the sediment can be quantified by the total organic carbon and it plays an important role in the accumulation and release of different metals in the sediments (Sardessai, 1994). The mangrove swamps are rich in dense evergreen vegetation and along with their associated biota, they play an important role in the contribution of organic matter. The seasonal variation of organic carbon in the sediments of the three stations are given in Table 5.1.

Table 5.1. Seasonal variation of organic carbon in the sediment

Seasons	Mangalavanam	Vypeen	Nettoor
Pre mon	48.22	25.24	21.65
mon	69.62	69.62	35.74
post mon	107.20	50.03	22.52



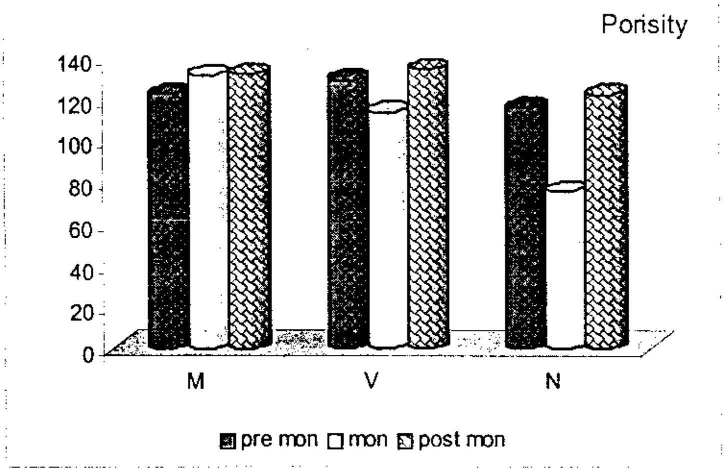
In Mangalavanam the organic carbon varies from 48.22 mg/g to 107.2mg/g, having the lowest during premonsoon and the highest during post monsoon period. In Vypeen the values range from 25.24mg/g to 69.62mg/g and in Nettoor they vary from 21.65mg/g to 35.74mg/g. The sediments of Vypeen and Nettoor show the highest value during the monsoon season, 69.92mg/g and 35.74mg/g respectively. In all the seasons Mangalavanam sediments have higher organic carbon than the sediment of the other two stations. The lowest value (21.65mg/g) is observed in the Nettoor sediment during the pre monsoon season. The high sediment organic carbon in the Mangalavanam can be due to the thick mangrove vegetation of this area.

5.2 Porosity

Porosity is an important dynamic feature and very much dependant on the sediment structure. It also changes with water content, and its stability is related to the nature and content of organic material and biological activity. (D' aqui *et al.*, 1998). The pores are the spaces in which biological and chemical activities and exchange of air and water take place. The porosity variations of the sediments of the three stations are given in table 5.2. The porosity values are comparable and they vary from 115.46 %-133.88 % excluding the sediment of Nettoor during the monsoon season which has a very low porosity value of 75.52%.

Table 5.2 Seasonal variation of porosity of the sediments

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	122.25	128.84	115.46
mon	131.26	113.43	75.52
post mon	131.94	133.88	122.15

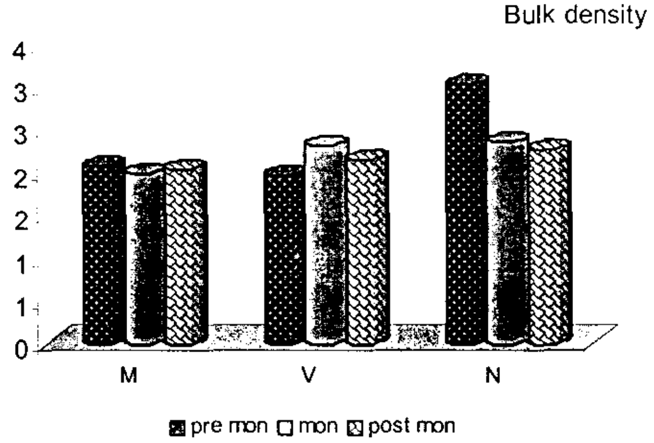


5.3 Bulk density

Like porosity this is a fundamental physical property of the sediment. Bulk density is the mass of the material divided by its volume, including the volume of its pores. Here the volume of the pore space is considered unlike specific gravity, which excludes this volume. Dadey *et al.*, (1992) stated that the determination of bulk density of a sedimentary deposit is a necessary component to estimate sediment accumulation rates. Jones and Bowser (1978) stated the importance of bulk density for the determination of accumulation rates of nutrients and heavy metals in sediments. The bulk densities of the sediments during the three seasons are given in table 5.3

Table 5.3 seasonal variation of bulk density of the sediments

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	2.06	1.95	3.02
mon	1.97	2.31	2.34
post mon	2.03	2.13	2.25



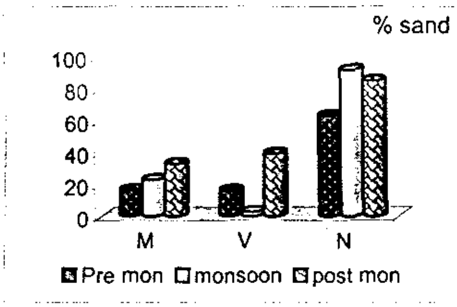
The highest bulk density is shown by the sediment of Nettoor during the pre monsoon season 3.02 gm/cm^3 and the lowest is shown by the sediment from Mangalavanam during the monsoon season. In the case of the Mangalavanam sediments the values vary very little from season to season (1.97 gm/cm^3 –2.06 gm/cm^3).

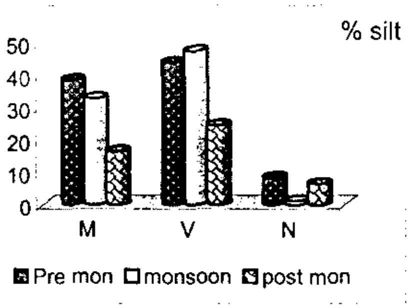
5.4 Textural characteristics

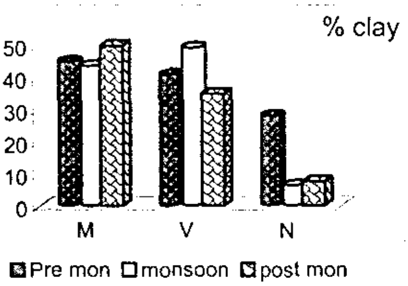
The key sediment characteristic is surface area or particle size, since many of the trace metal binding components like organic matter, Fe and Mn oxides and hydroxides are very well correlated with these characteristics (Rae, 1997). Trace metals are held in the sediments in different 'sites'. In addition to the metals in the mineral lattices, they are associated particle surfaces. Hence there is significant correlation between the trace metal content and grain size or surface area. The textural characteristics of the sediments are given in table 5.4

Table 5.4 Textural characteristics of the sediments

Seasons	Mangalavanam			Vypeen			Nettoor		
	sand	silt	clay	sand	silt	clay	sand	silt	clay
pre mon	16.47	38.62	44.77	16.19	44.12	40.81	63.41	8.53	27.92
mon	23.03	32.98	43.56	2.84	47.56	49.13	91.87	1.32	6.31
post mon	33.06	16.56	49.83	39.32	24.65	34.81	85.13	6.72	7.57

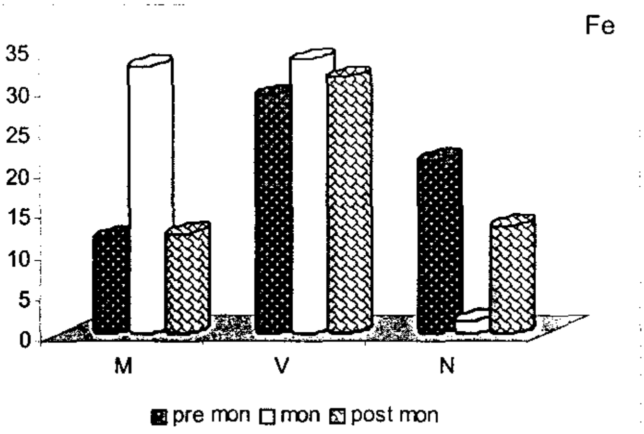






5.5 Total Metal in the sediments

Metal fluxes to sediments are strongly dependent on anthropogenic inputs. Metals exist in sediments in different forms which include water soluble species, exchangeable species, metal bound in organic compounds, silicates, Fe-, Al-and Mn oxides and hydroxides; and metals bound to phosphates. The soluble, the exchangeable and partly the organic bound



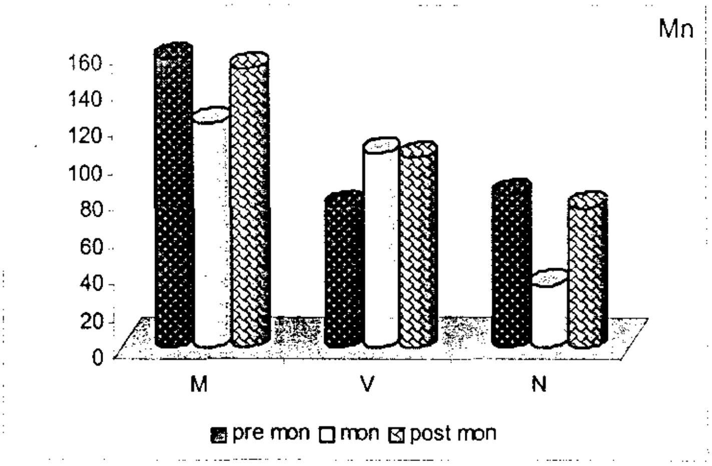
Seasonal variation of Fe in the sediments (mg/g)

5.5.3 Manganese

Like Copper, Manganese content too is highest in the Mangalavanam sediment, Vypeen ranks second and Nettoor has the least amount. The highest value (155.56μg/g) is during the pre monsoon season and the lowest value (32.78 μg/g) is that of Nettoor during the monsoon season. All the sediments show lower manganese content during the monsoon season.

Table 5.7 Seasonal variation of Mn in the sediments (μg/g)

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	155.66	75.59	81.89
mon	121.23	104.82	32.78
post mon	150.35	102.48	75.17



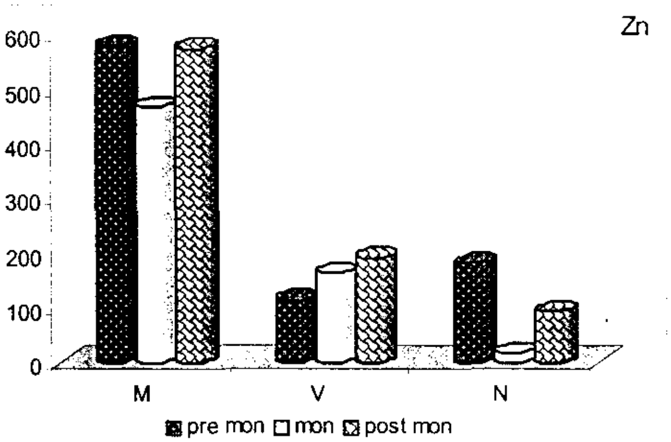
Seasonal variation of Mn in the sediments (µg/g)

5.5.4 Zinc

Zinc is classified as border line element according to HSAB (Hard-Soft Acid Base) classification in binding ligands. The border line character of zinc is reflected in its ability to form bonds with oxygen as well as nitrogen and sulphur donor atoms. The higher amount of zinc in the sediment and humic acid can be attributed to the above-mentioned complexing nature. There is no regular trend regarding the presence of this metal either in the sediment or in humic acid. The highest value is for the sediment of Mangalavanam during the premonsoon season (579.15µg/g) and the lowest for the Nettoor sediment during the Monsoon season. Zinc content is rather high in humic acid than the sediment.

Table 5.8 Seasonal variation of Zn in the sediments (µg/g)

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	579.15	113.64	180.51
mon	467.47	161.84	17.67
post mon	574.41	190.27	93.26



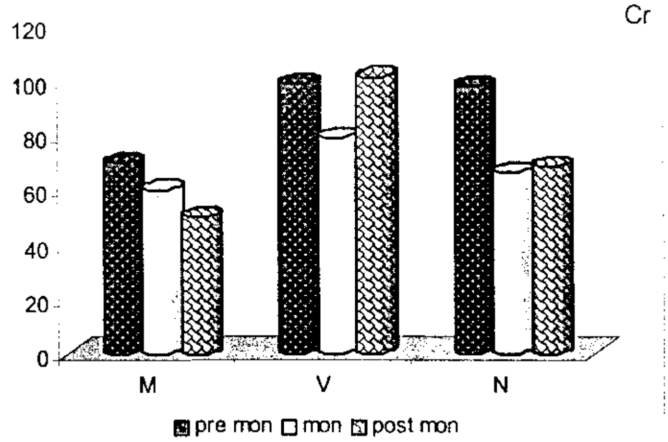
Seasonal variation of Zn in the sediments (μg/g)

5.9 Chromium

The seasonal variations of Chromium in the sediments are given in table 5.9. Chromium content in humic acid is comparatively much higher than that in the sediment. Unlike the other metals, Vypeen or Nettoor sediments have a higher percentage than the Nettoor sediment. The highest value (100.91μg/g) is found for the sediment from Vypeen during the post monsoon season and lowest value (49.82μg/g) for the sediment from Mangalavanam during the same season.

Table 5.9 Seasonal variation of Cr in the sediments (μg/g)

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	69.86	98.40	97.87
mon	59.99	78.63	66.29
post mon	49.82	100.91	68.69



Seasonal variation of Cr in the sediments (μg/g)

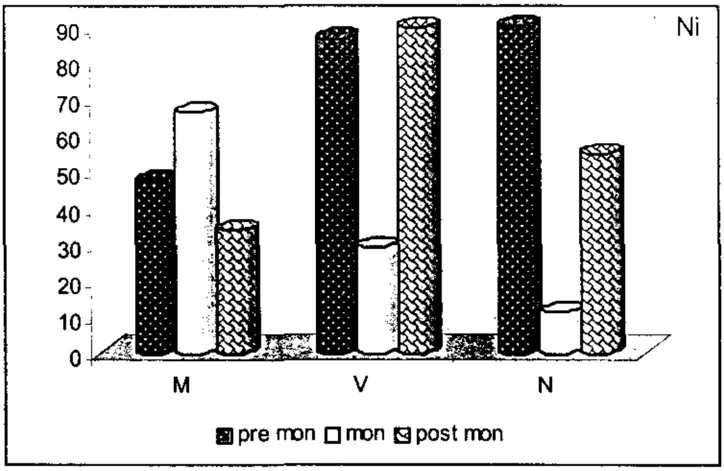
5.5.6 Nickel

Nickel (+2) forms stable complexes with inorganic and organic ligands. Organic ligands with oxygen, nitrogen, and especially sulphur donor atoms form strong complexes with nickel (James *et al.*, 1984). Humic acid shows much higher percentage than the sediment, once again reflecting its greater ability for complexation.

The highest chromium content (89.89 μg/g) is found in the sediment of Nettoor in the pre monsoon season and the value for the sediment from Vypeen (86.16μg/g) during the same season is not far from this. The lowest Nickel content (11.06μg/g) is seen in the Monsoon sediment of Mangalavanam.

Table 5.10 Seasonal variation of Cr in the sediments (μg/g)

Seasons	Mangalavanam	Vypeen	Nettoor
pre mon	47.47	86.16	89.89
mon	66.09	28.99	11.06
post mon	33.70	89.37	54.79



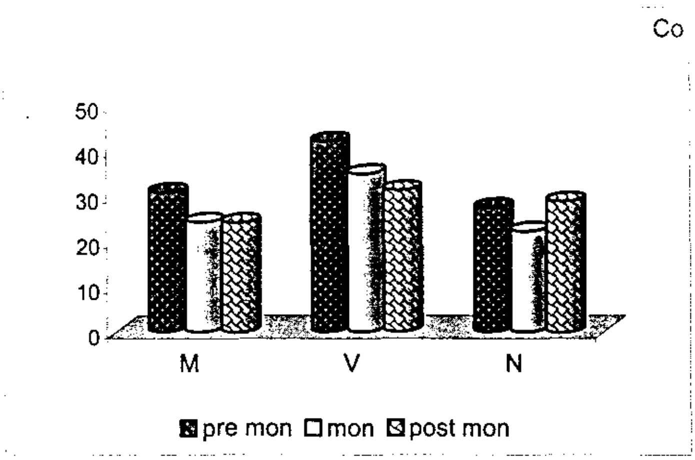
Seasonal variation of Cr in the sediments (µg/g)

5.5.7 Cobalt

The cobalt content in the sediment is much less than the other metals and the values do not vary much. But humic acid shows a much higher cobalt content which is almost 8-10 times more than that in the sediment. In all the seasons Vypeen values are higher and the highest (42.13µg/g) is found during the pre monsoon season. Like the other metals the lowest value is seen in the sediment from Nettoor.

Table 5.11 Seasonal variation of Co in the sediments (µg/g)

Seasons	M	V	N
pre mon	30.83	42.13	27.48
mon	24.25	34.93	22.24
post mon	24.23	31.30	29.01



Seasonal variation of Cr in the sediments (μg/g)

5.6 Adsorption studies

Trace elements and heavy metals in the sediments and natural waters participate in many processes which change their physico-chemical form or distribution in space. Some of the processes involved are adsoption/ desorption, precipitation/dissolution and aggregation/disaggregation (Forstner et al., 1979). The most important retention mechanisms are adsorption and precipitation. For metals such as copper, zinc, lead and cadmium adsorption is usually the most important mechanism governing the migration rate whereas precipitation is less important (Gustafsson, 2001). Metals may be adsorbed to soil particles through complexation or through exchange whereby metal cations are bound electrostatically to negative charges at the particle surface.

The evaluation of the data is carried out with three basic concepts- the partition, Langmuir and Freundlich adsorption isotherm.

5.7 The partition coefficient Kp

Kp is an empirical expression of equilibrium adsorption which is frequently used. It is the ratio of the concentration of the adsorbate bound to solids (C1) to the total dissolved concentration (C2) in equilibrium. Kp = C1/C2 Kp is an equilibrium constant that is too empirical (conditional) for some purpose (Schnoor, 1996). Chemical equilibrium models such as MINTEQA2/PRODEFA2 have improvised the concept by defining an activity Kd adsorption model such that $K_d$act = A1/A2 where A1 represents the activity of the adsorbed species and A2 represents the activity of the dissolved species. The extent of metal ion retention by sediment varies in accordance with adsorption isotherm parameters such as concentration, nature and concentration of competing cations and bonding constant values and other factors such as pH, texture and nature of organic matter present in the sample.

Kp values for the three metals at different pH and concentrations are given in the table 5.12-5.15 and are clearly depicted in fig 5.1-5.3. A gradual increase in Kp values with pH. is observed in most cases. At the acidic pH 4 and pH 5 there is a greater tendency for desorption of metals from the sediment and C1, the concentration of the metal in the sediment decreases and C2 concentration in the aqueous medium increases. Whereas at pH 6 the partition takes the upper hand and at a higher pH complexation predominates. Thus Partition coefficient at pH 6 can be equated with physical adsorption and its values are comparable with the constants of the Langmuir adsorption isotherm.

Table 5.12 Kp values for the adsorption of Cu on sediment

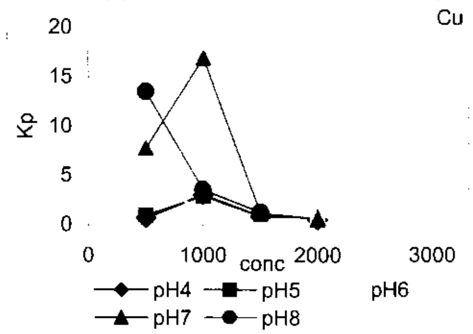
Concentration added in ppm	pH4	pH5	pH6	pH7	pH8
500	0.712	1.000	5.485	7.787	13.522
1000	3.049	2.906	1.004	16.912	3.566
1500	0.877	0.866	0.587	1.165	1.301
2000	0.548	0.553	0.672	0.639	

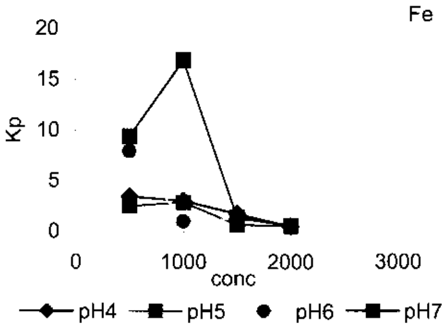
Table 5.13 Kp values for the adsorption of Fe on sediment

Concentration added in ppm	pH4	pH5	pH6	pH7
500	3.464	2.521	7.929	9.417
1000	3.049	2.906	1.004	16.912
1500	1.757	0.682	1.329	1.415
2000	0.548	0.553	0.672	0.639

Table 5.14 Kp values for the adsorption of Mn on sediment

Concentration added in ppm	pH4	pH5	pH6	pH7	pH8
500	0.222	0.272	0.433	0.706	0.634
1000	0.109	0.129	0.227	0.290	0.316
1500	0.076	0.109	0.514	0.558	0.316
2000	0.034	0.125	0.428	0.290	





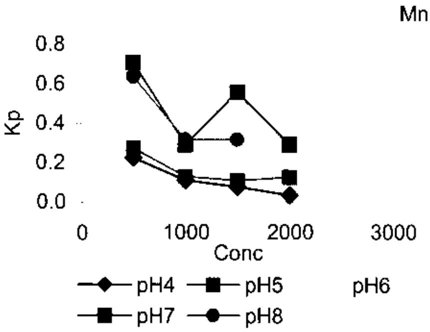


Table 5.15 Kp values for the adsorption of Cu, Fe Mn from a mixture of metals

Kp Cu	Kp Fe	Kp Mn
0.656	0.225	0.156
0.905	0.324	0.303
0.739	0.282	0.146

5.8 Langmuir adsorption isotherm

The Langmuir adsorption model is one of the most useful options in chemical equilibrium models, and the assumption of monolayer limited coverage is the representative of a wide range of equilibrium sorption isotherm data. This isotherm expresses the relationship between the equilibrium concentration of the metal ion and the amount of metal adsorbed by unit mass of the adsorbent. Here in this isotherm both capacity factor and the intensity factor are calculated and hence it is applicable to a large extent.

The Langmuir adsorption isotherm is expressed by the equation

$$Xe/X = Xe\ 1/k1 + 1/k1k2$$

X is the amount of metal adsorbed per gram of the adsorbent: Xe is the equilibrium metal concentration in the solution k1 and k2 are constants. A plot of Xe/X against Xe gives a straight line if the data were fitted in the equation. The constants k1 and k2 are calculated from the slope and the Y intercept respectively. In the Langmuir relation k1 represents the adsorption maxima which in turn is measure of the effective adsorption sites that will take part in the adsorption process and k2 the equilibrium coefficient is a function of the energy and by Arrhenius equation $k=Ae^{-E/RT}$, an increase in k2 represents a decrease in the energy involved in the adsorption process. (Jayasree, 1993).

Table 5.16 Langmuir constants for the adsorption of Cu, Fe and Mn on the sediment

pH	Cu		Fe		Mn	
	K1	K2	K1	K2	K1	K2
4	51.020	687.347	36.101	175.137	2.56	-300.59
5	44.444	437.444	28.818	120.865	12.58	1010.06
6	34.483	199.310	31.847	7.111	-40.65	-3312.36
7	34.247	21.709	36.364	18.404	0.37	-4.10
8	38.314	69.962	34.247	20.000		

Langmuir adsorption isotherm

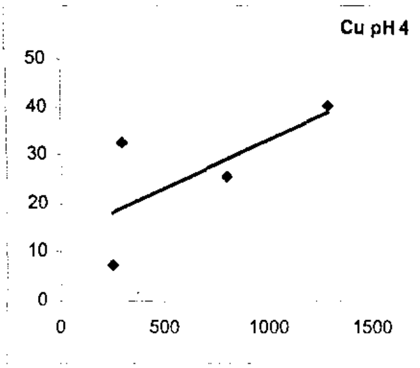


Fig. 5.4

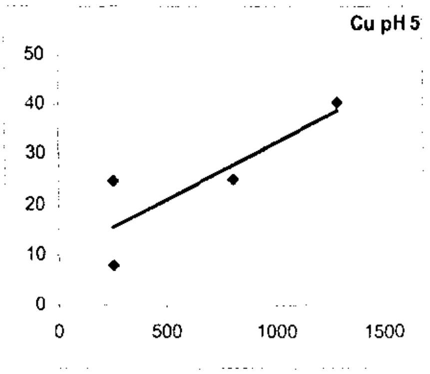


Fig. 5.5

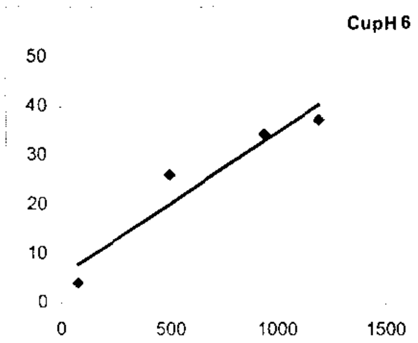


Fig. 5.6

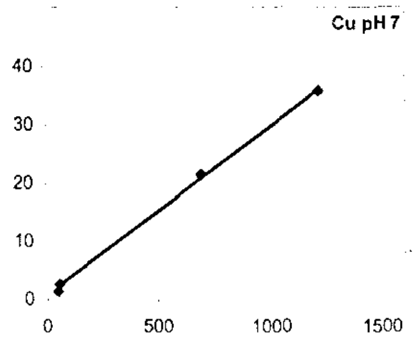


Fig. 5.7

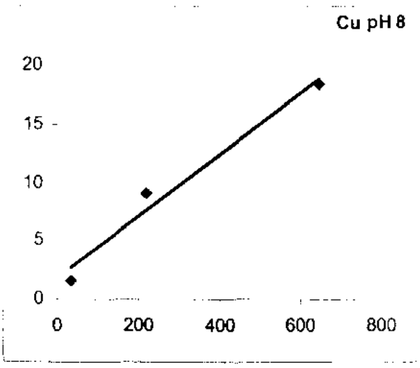


Fig. 5.8

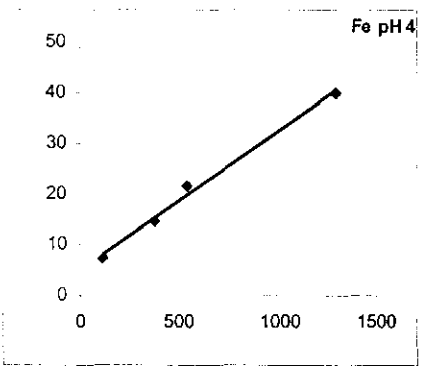


Fig. 5.9

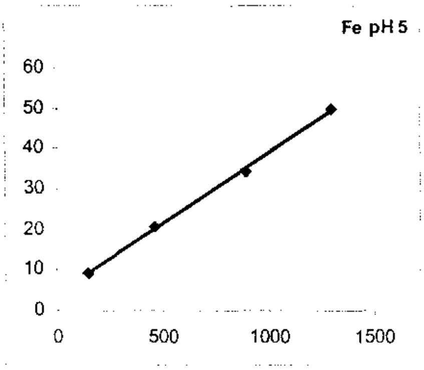


Fig. 5.10

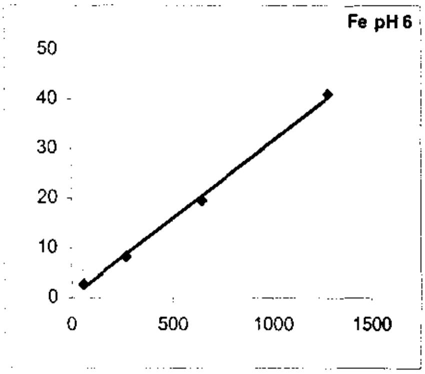


Fig. 5.11

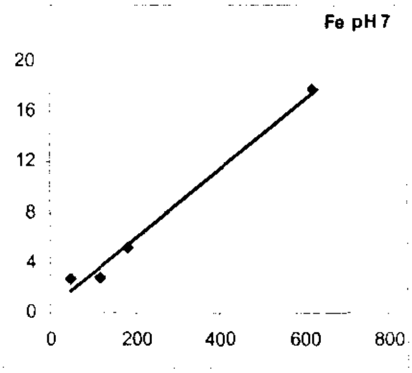


Fig. 5.12

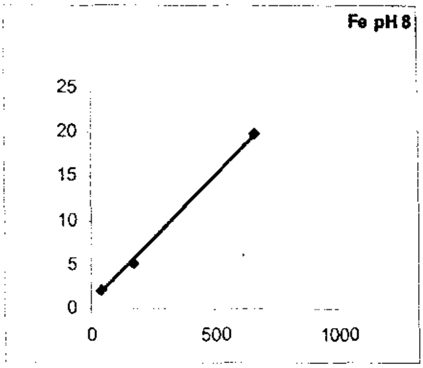


Fig. 5.13

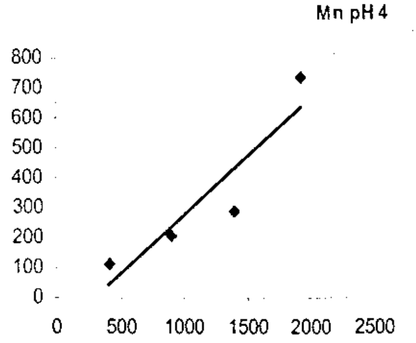


Fig. 5.14

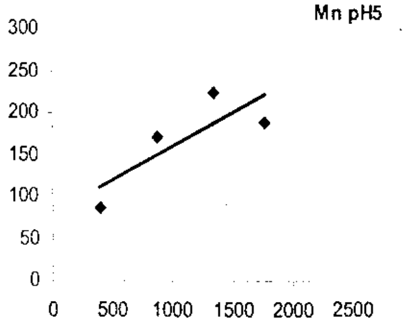


Fig. 5.15

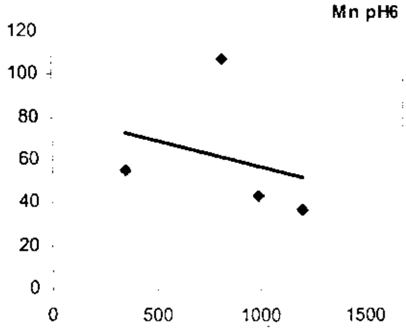


Fig. 5.16

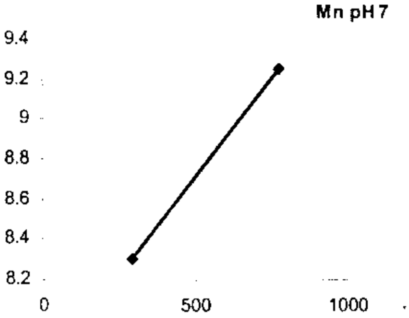


Fig. 5.17

Freundlich adsorption isotherm

The Freundlich model is used when more than monolayer coverage of the surface is expected and the sites are heterogeneous. An unlimited supply of unreacted sites is assumed to be available and they have different binding energies.

Freundlich isotherm is expressed by the equation

$$X = K\, Xe^{1/n}$$

X is the amount of metal adsorbed per gram of the adsorbent : Xe is the equilibrium metal concentration in the solution; K and n are empirical parameters representing relative indicators of sorption capacity and sorption intensity respectively. The linear form of the isotherm is given by the following equation

$$\log X = \log K + 1/n \log Xe$$

A plot of log X against log Xe gives a straight line with 1/n as slope and log K as the intercept. The empirical parameters K and n are used mathematically to characterise the size distribution and hence provide information about surface heterogeneity of the adsorbent. In the Freundlich adsorption isotherm, the constant K represents the adsorption capacity which is measure of all possible sites for adsorption and n is the sorption intensity which in turn is measure of effective adsorption (Jayasree, 1993). The Freundlich constants of the three metals are given in table 5.17 and the iostherm in fig 5.18 –5.29

Table 5.17 The Freundlich constants of the three metals

pH	Cu		Fe		Mn	
	n	K	n	K	n	K
4	2.774	2.472	3.401	4.038	4.66	0.86
5	2.589	2.182	4.065	4.699	2.37	0.33
6	5.935	8.452	6.289	1.274	0.81	0.00
7	15.699	2.137	4.202	9.078	0.81	0.67

For Cu the adsorption capacity is found to be highest at pH 6 (6.289), for Fe the highest value is at pH 9 (9.078) and for Mn adsorption capacity is very low at all pH.

For Cu the most effective adsorption is found to be at pH 7 and for Fe at pH6. Mn seems to have effective adsorption at the acidic pH 4.

Freundlich isotherms

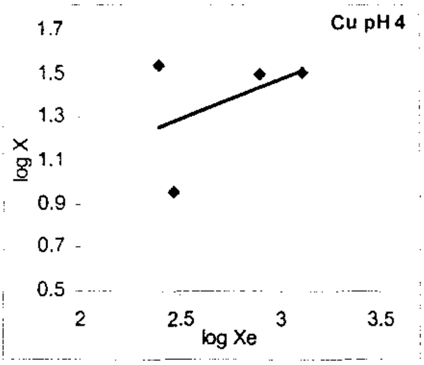


Fig. 5.18

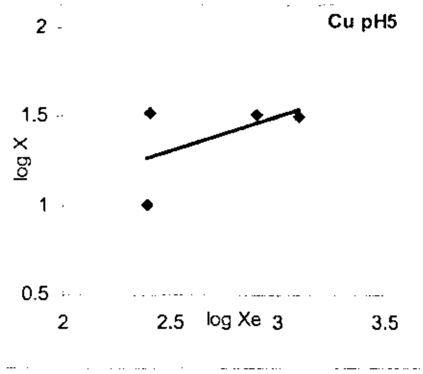


Fig. 5.19

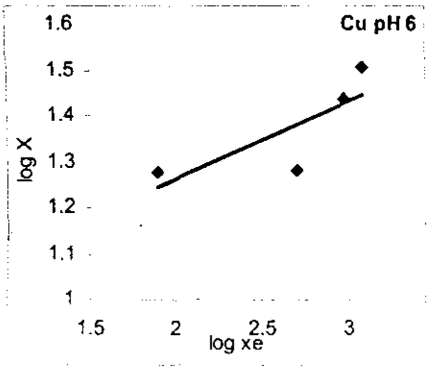


Fig. 5.20

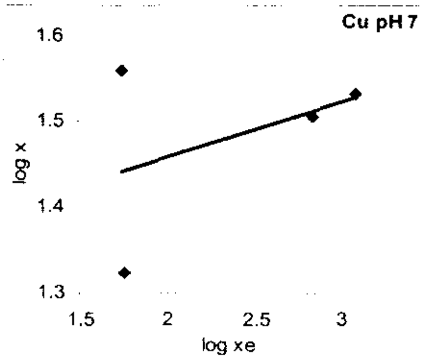


Fig. 5.21

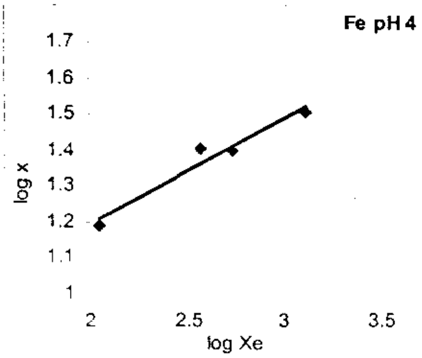


Fig. 5.22

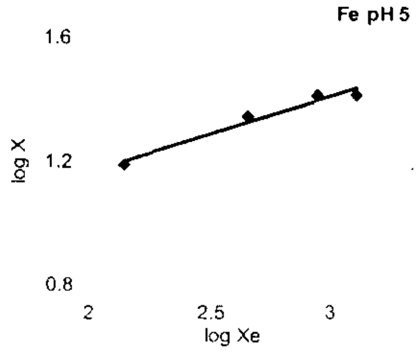


Fig. 5.23

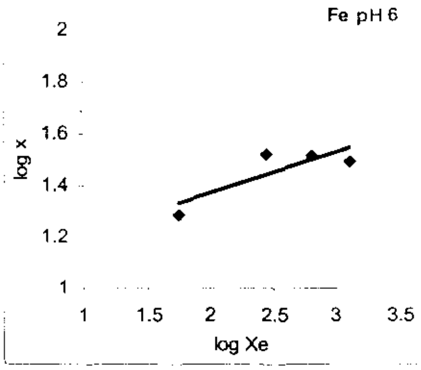


Fig. 5.24

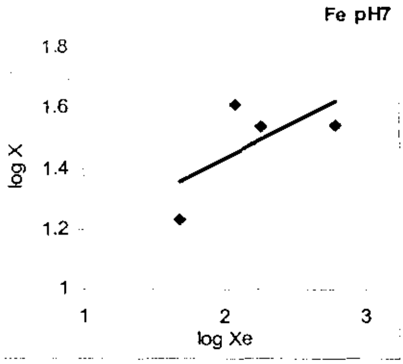


Fig. 5.25

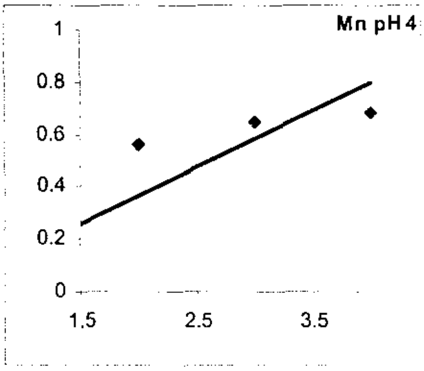


Fig. 5.26

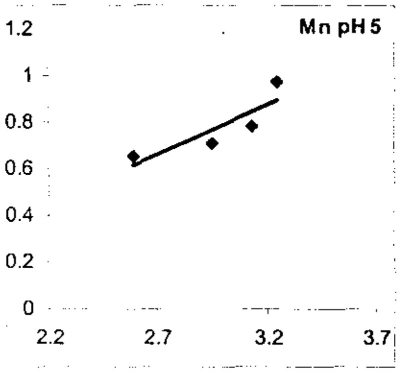


Fig. 5.27

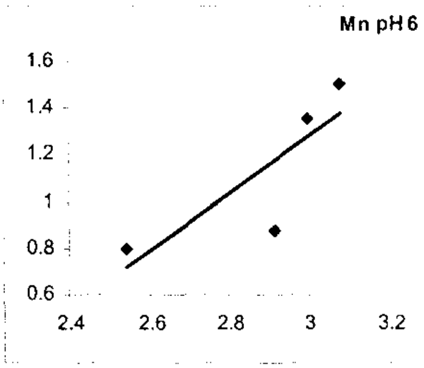


Fig. 5.28

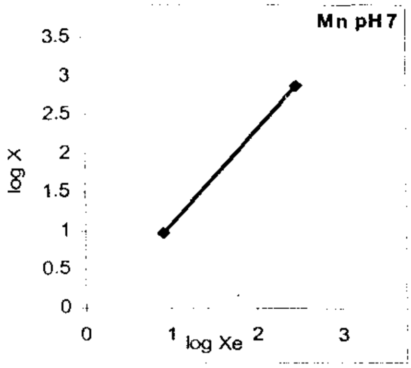


Fig. 5.29

5.9 Desorption

Four extractants 2N Ammonium acetate, 0.5 N EDTA, 0.5NHCl and Na_2HPO_4 are used for desorption. Desorption from these four extractants gives a clear insight into the various types of binding involved between the metal to the sediment and humic acid. Ammonium acetate which is being buffered at a neutral pH releases all labile fractions from the adsorbed species. EDTA is a very good complexing agent and it releases all the organically bound species through complexation. HCl can release all the metal present in the substrate with H^+ exchange with the corresponding metal ions. NaH_2PO_4 is buffered at an alkaline medium and can release only the inert fractions. The percentage of metal release by the various extractants are shown in table 5.18-5.21.

Ammonium acetate is a suitable extractant to liberate the exchangeable metals (Tessier *et al.*, 1979). The percentage release of Cu from the sediment with ammonium acetate varies between 21.92% to 24.05%. There is a gradual decrease in the percentage with increase of pH showing the stability of complexes with the increase of pH. Whereas this percentage release is much less in Fe (8.36-12.40%) and rather high in Mn (48.2-59.9%). The same trend is followed by Cu and Mn during desorption from a sediment, which is equilibrated with a mixture of metal solutions. Whereas Fe is released much less after it is being adsorbed from the metals solutions containing all the three metal species.

Table 5.18 Percentage of Cu release from the sediment

pH	NH_4Ac	E.D.T.A.	HCl	NaH_2PO4
4	24.05	99.97	100	1.58
5	25.26	100	100	2.56
6	22.24	100	100	2.61
7	21.91	99.19	100	1.52
8	21.92	98.55	100	1.91

EDTA releases the organically bound metals from the substrate. The release is almost 100% from the sediment showing that the metal is present in the sediment as organically bound species. The higher stability of the EDTA-metal complex than the organic-metal complex might have facilitated the desorption. The release of metals from the sediment by Ammonium acetate is much higher than that released from humic acid. This could be due to the following reasons. Humic acid present in the natural sediment may vary much from a humic acid extracted with NaOH, the structure of which has already been predicted in chapter IV. A structural change in humic acid is very much possible with NaOH extraction during which breaking of a glycosdic or amide like linkage or a stereochemical change etc is possible. The organic cover in the sediment also could prevent humic acid in the sediment for efficient complexation with metals. Thus in the sediment major complexation is done by organic species other than humic acid. The low percentage of humic acid in the sediment which is only 10-12% of the organic matter supports this argument.

Table 5.19 Percentage of Fe release from the sediment

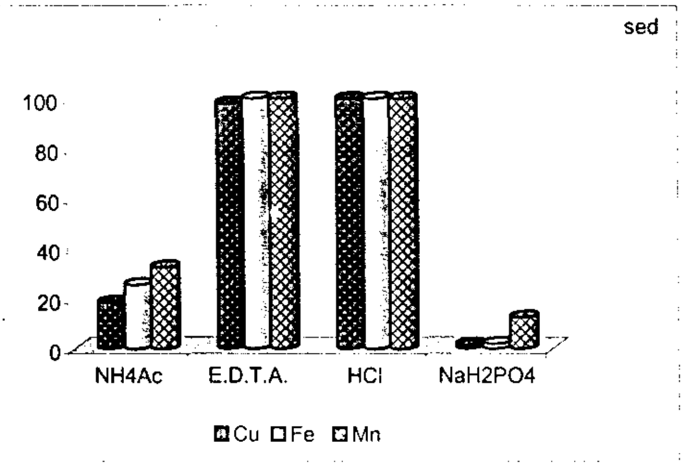
pH	NH_4Ac	E.D.T.A.	HCl	NaH_2PO4
4	8.36	100	100	0.18
5	12.02	100	100	0.22
6	10.21	98.75	100	0.10
7	9.76	98.75	100	0.09
8	12.40	99.28	100	0.09

Table 5.20 Percentage of Mn release from the sediment

pH	NH_4Ac	E.D.T.A.	HCl	NaH_2PO4
4	48.26	98.38	100	22.04
5	45.22	99.54	100	23.97
6	53.07	99.33	100	21.34
7	59.74	98.49	100	21.52
8	54.99	100.00	100	18.48

Table 5.21 Percentage release of metals from the sediment

Extractants	Cu	Fe	Mn
NH_4Ac	18.55	25.54	32.6
E.D.T.A.	98	100	100
HCl	100	100	100
NaH_2PO4	1.5	1.9	12.54



Percentage release of metals from the sediment

HCl releases all the metal from the sediment pointing to the absence of inert metal fractions. The same trend is followed by Cu and Mn during desorption and after adsorption from a mixture of metal solutions. Whereas Fe is released much less compared to its release after adsorption from a pure Fe solution than after it is being adsorbed from the solution containing all the three metal species. The percentage of release of metals are shown in fig 5.21.

In sediment the absorption is more of a physical adsorption than chemical adsorption or complexation. There is an indication of the absence of a stable complex formation in the sediment since there is 100% elution by E.D.T.A. Either the organic matter is less exposed for complex formation or it may not be giving a stable complex formation due to steric factors. While being desorbed after adsorption from a mixture of metals the percentage release shifted from 22.24 to 18.55 for Cu, 10.21 to 25.54 for Fe and 53.06 to 32.6 for Mn. If we assume uniform character to the adsorption site when there is adsorption from the mixture of metals, the significant trend is almost the same for Cu and Mn, but Fe behaves differently. While competing with the other metals Fe seems to be lagging behind, or in other words there is more release and less complex formation. Whereas Cu and Mn are adsorbed more from a mixture and for Mn the percentage release is much reduced. During adsorption from a mixture the affinity for complex formation in the sediment is in the order Cu>Fe>Mn.

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Chapter 6

CHARACTERISTICS OF METAL SORPTION ON HUMIC ACID

6.1 Adsorption studies

- 6.1.1 partition coefficient
  - a. Humic acid
  - b. Sediment left after alkali extraction
- 6.1.2. Langmuir adsorption isotherm
  - a. Humic acid
  - b. Sediment left after alkali extraction
- 6.1.3 Freundlich isotherm
  - a. Humic acid
  - b. Sediment left after alkali extraction

6.2 Desorption studies

Humic substances comprise of the most naturally occurring organic substances in soils, sediments, surface waters and ground waters. They are the decomposition products of plant tissue by microbes. The ubiquitous presence of humic substances combined with their ability to provide multiple sites for chemical reaction makes them relevant to numerous biogeochemical processes such as nutrient bioavailability and metal transport. Humic acids form one of the most important source/sink systems for metal ions in a number of environmental sediments including soils and bottom sediments (Sohn *et al.*, 1990). The role of humic material as a complicating factor in the uptake and utilization of metal ion has been recognised for years (Schnitzer *et al.*, 1972: Christman *et al.*, 1983; Aiken *et al.*, 1985). The metal ion interaction is of great ecological significance and it is one of the means for elucidating reactive functional groups. The fate and bioavailability of heavy metals in contaminated soil are largely controlled by sorption reactions to soil mineral surfaces and humic substances (Christl *et al.*, 2001).

There are many models for metal ion interaction in humic substances. Most of them depart from the idea that there are equivalent, noninteracting sites for bonding and suggest that strength of binding varies with the degree of metal loading on humic materials. (Mark et al., 1983). These authors consider numerical values of their binding constants distributed over several orders of magnitude, and their work clearly establishes the need for definition of this distribution. Other models developed by Marinsky and Ephraim, (1986); Ephraim *et al.*, (1986); Linder and Murray, (1985); de Wit *et al.*, (1990); Tipping, (1993,1994); Marshall *et al.*, (1995) and Tipping *et al.*, (1995); could explain to some extent the observed multi binding characteristics of humic acid. Information on chemical structures and functionalities, especially those of strong binding potential is a much valuable contribution in this regard. The density of reactive surface site is one of the key parameters in all complexation models. Another recent trend in metal ion organic interaction research has been the efforts made to obtain measures of "availability" by measuring the dissociation rate of metals bound to humic materials. Research in this area is heavily influenced by the developments in our fundamental understandings of humic acid.

The surface activity of humic substances arises from the simultaneous presence of both hydrophilic and hydrophobic surfaces (Chen and Schnitzer, 1978). The presence of oxygen containing functional groups (– COOH, – OH, $>$C =O) makes it hydrophilic and aromatic rings, fatty acids esters and aliphatic hydrocarbon etc provide hydrophobic sites in humic acids (Schnitzer, 1985). The main functional groups present in a sample of humic acid are alcohols, phenols, carbonyls, carboxyls and amides. All these groups can interact with metal species in solution (Senesi, 1992) The aliphatic and aromatic ring with hetero atoms like nitrogen and oxygen can also make it capable of acting as a very good ligand. Considering the variety of functional groups present in humic acid and the various ways by which they can interact with metals, a near infinite number of metal humic complexes are possible.

Present chapter reveals the significance of the sorptional behaviour of metals on humic acid. Based on the structure of humic acid developed in chapter iii and the results of batch-wise adsorption and desorption done on humic acid samples, a detailed study of the sorptional behaviour of Humic acid is done considering its various functional groups. Sorptional studies were also done on the sediment after the removal of humic acid (SAHA) in order to have a clear understanding of the sorptional behaviour of humic acid. With regard to its complexation and sorption, this model of humic acid mimic the macromolecule in the real system. The extent of metal ion retention by humic acid varies in accordance with the adsorption isotherm parameters such as concentration, nature and concentration of competing cations, bonding constant values and other factors such as pH and salinity. To have a basic understanding on these factors sorptional studies have been made at different pH, metal concentrations and salinities.

6.1 Adsorption studies

Sorptional behaviour of Cu, Fe and Mn on humic acid and sediment left after alkali extraction was carried out at different concentration, and pH. The evaluation of the data is done on the basis of the basic concepts like the Partition, Langmuir and Freundlich adsorption isotherms as in the previous chapter. The main features of the adsorption process are explained by the constants derived from Langmuir and Freundlich adsorption isotherms. The constants of these isotherms were calculated from the best fit curve.

6.1 Partition Coefficient

a. Humic acid

The Partition Coefficient parameterises the ratio of the concentration of the adsorbed metal ion in the sediment to the concentration of metal ion in the solution. Copper adsorption on sedimentary materials decreases in the following order Humic acid > montmorillonite > illite > Kaolinite (Reimer et al., 1976). So humic acid is considered as an excellent scavenger for Cu.

Table 6.1 Partition coefficient of Cu in Humic acid

Con	pH	
ppm	7	6
10	1.11	1.882
20	0.823	1.146
50	1.378	0.319
100	0.117	0.086

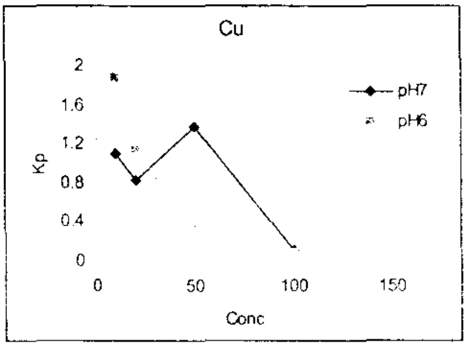


Table 6.2 Partition coefficient of Fe in Humic acid

Con	pH	
ppm	7	6
10	1.841	2.968
20	0.642	0.667
50	0.41	0.482
100	0.22	0.216

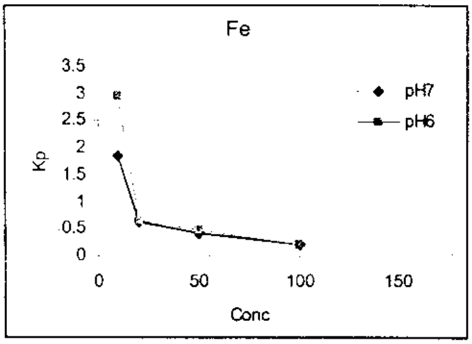
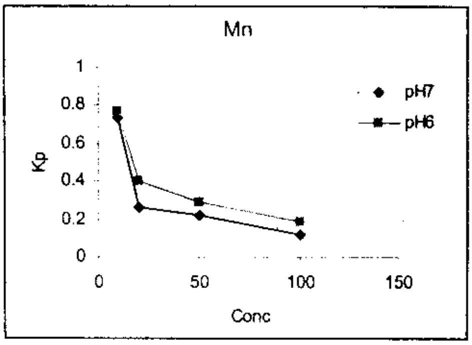


Table 6.3 Partition coefficient of Mn in Humic acid

Con	pH	
ppm	7	6
10	0.736	0.767
20	0.267	0.401
50	0.226	0.294
100	0.12	0.184



Using macroscopic solution factors such as viscosity, vapour pressure and osmometry researchers have predicted that humic substances will have a coil like structure at low pH and high ionic strengths and linear at high pH and low ionic strength. Recent studies on soil and fluvial humic substances using Xray microscopy do indeed show that pH ionic strength of the solutions change the macro - molecular structure of the humic substances (Sparks *et al.*, 1997). There is significant variation of metal adsorption with respect to metal species. The change in Kp with concentration and pH are shown in Tables 6.1- 6.3. No regular trend is observed for the changes in Kp of Cu with the changes in pH. Whereas Kp values of Fe and Mn at pH 6 is less than that at pH 7. For all the metals there is a decrease in Kp with the increase in concentration indicating that there is a decrease in concentration of the adsorbed metal ion on humic acid with the increasing concentration of the added metal solution. This is in agreement with the observations made earlier by Stevenson and Chen (1991); Milne *et al.*, (1995) and Spark *et al.*, (1997); that the interaction of humic acid with metal cations in solution increases with pH and decreases with metal concentration.

b. Sediment left after alkali extraction

With the sediment left after alkali extraction there is a reduction of Kp (Tables 6.4-6.6) of all the three metals with the increase in pH or concentration. Kp is an index of physical adsorption especially at lower pH. There is clearly a greater increase in Kp in the sediment left after alkali extraction than that of humic acid. Or in other words here there is more metal adsorption than in humic acid (Jayasree, 1993).

Table 6.4 Partition Coefficient of Cu in sediment left after alkali extraction

Con	pH	
ppm	7	6
500	2.268	4.155
1000	0.698	0.637
1500	0.506	0.381
2000	0.386	0.267

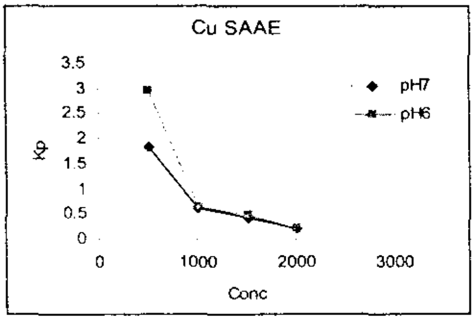


Table 6.5 Partition Coefficient of Fe in sediment left after alkali extraction

Con	pH	
ppm	7	6
500	1.841	2.968
1000	0.642	0.667
1500	0.41	0.482
2000	0.22	0.216

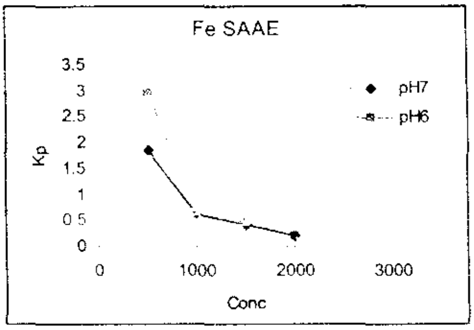
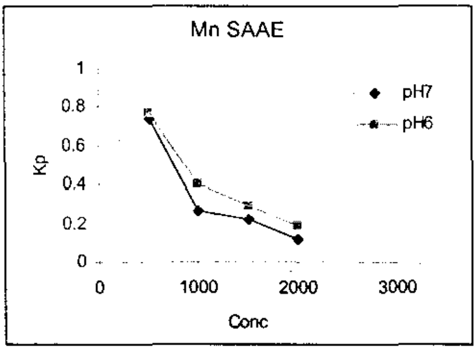


Table 6.6 Partition Coefficient of Mn in sediment left after alkali extraction

Con	pH	
ppm	7	6
500	0.736	0.767
1000	0.267	0.401
1500	0.226	0.294
2000	0.12	0.184



The sediment left after alkali extraction seems to be a better adsorbent than humic acid.

6.1.2 Langmuir adsorption isotherm

As already mentioned in the previous chapter in the Langmuir constant K1 represents the adsorption maxima which is a measure of the effective adsorption sites that will take part in the adsorption process and K2, the equilibrium coefficient, a function of the energy and by definition an increase in K2 represents a decrease in the energy of adsorption. A detailed analysis of K1and K2 reveals to some extent the sorptional pattern of humic acid and the sediment left after alkali extraction.

a. Humic acid

A mere glance at the Langmuir adsorption isotherms (fig6.7-6.10) reveals that the adsorption pattern of humic acid fits very well to this adsorption isotherm.

Langmuir isotherms for the adsorption of Cu, Fe, Mn, on Humic acid

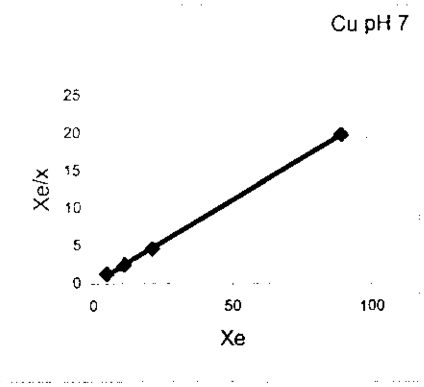


Fig 6.7

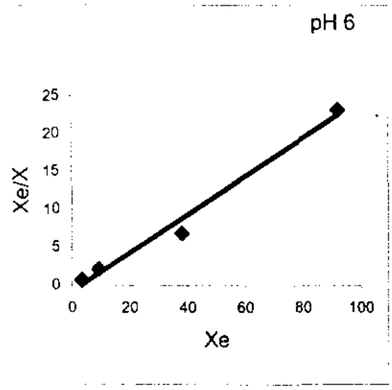


Fig 6.8

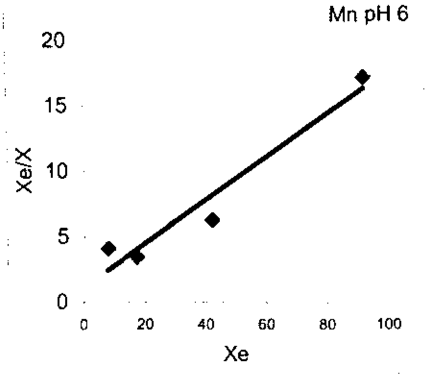


Fig 6.9

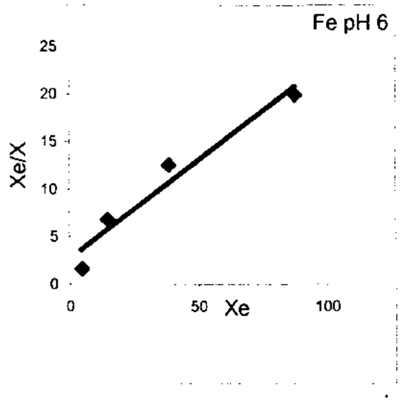


Fig 6.10

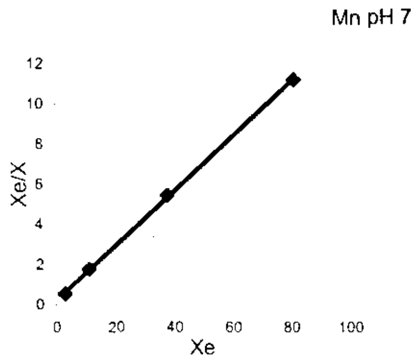


Fig 6.11

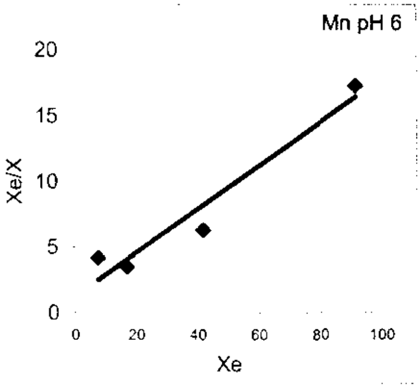


Fig 6.12

Table 6.7 Langmuir constants of the metals in Humic acid

Constants	Cu		Fe		Mn	
	pH 7	pH 6	pH 7	pH 6	pH 7	pH 6
K1	4.535	3.960	2.553	4.819	7.315	6.083
K2	2.616	-0.312	0.074	0.078	0.712	0.131

The Langmuir constants K1 and K2 are shown in table 6.7. For Cu and Mn the adsorption maxima tends to be higher at the neutral pH and so too the intensity of adsorption. For Fe, K1 is higher at pH 6 and K2, is higher at pH 7. A negative value of K2 for Cu adsorption on humic acid signifies the high amount of energy involved in the adsorption.

b. Sediment left after alkali extraction

The adsorption values of this fraction fit well in the Langmuir isotherm (Fig 6.13-6.18). All the metals have a higher K1 than that in humic acid. Evidently there are more adsorption sites in the sediment left after alkali extraction. This observation is very much in tune with the observation made earlier by Jayasree (1993). On considering the energy factor K2 for Cu at pH 7 there is decrease in K2 with the sediment left after alkali extraction. The same is observed for Mn at pH 6, pH 7 and Fe at pH 6. Thus the energy factor favours more adsorption on humic acid.

Table 6.8 Langmuir isotherms for the adsorption of Cu, Fe, and Mn on Sediment left after alkali extraction

	Cu		Fe		Mn	
Constants	pH 7	pH 6	pH 7	pH 6	pH 7	pH 6
K1	47.619	43.478	35.088	34.247	21.739	22.422
K2	0.090	-0.023	0.105	0.028	0.013	0.041

Langmuir isotherms for the adsorption of Cu, Fe, and Mn on Sediment left after alkali extraction

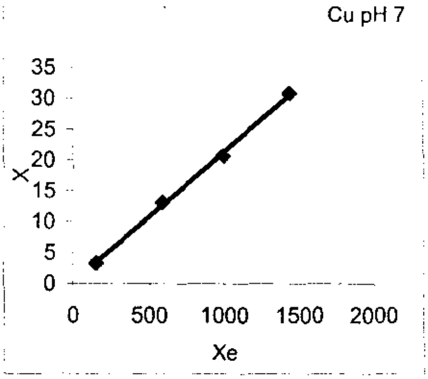


Fig 6.13

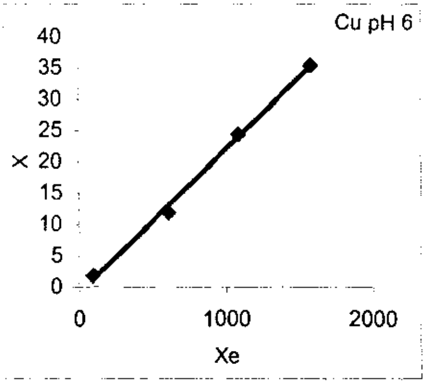


Fig 6.14

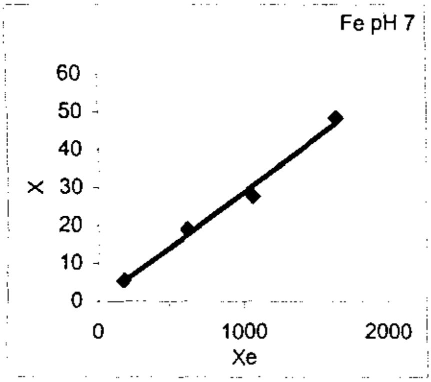


Fig 6.15

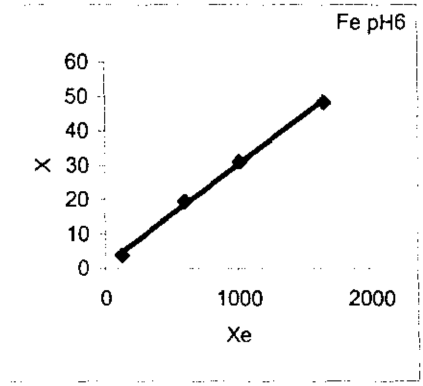


Fig 6.16

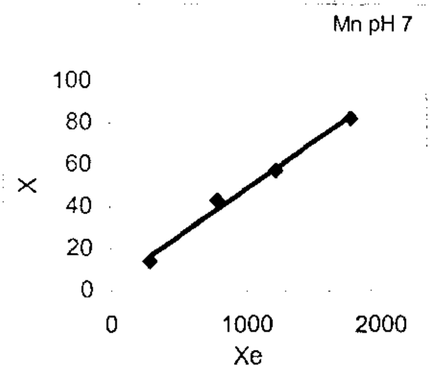


Fig 6.17

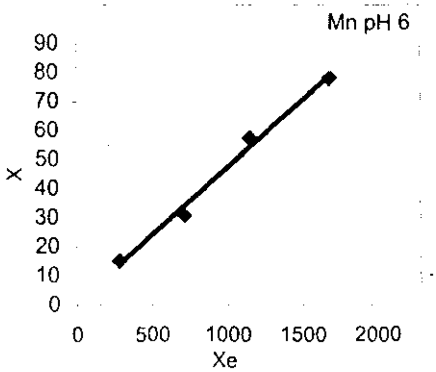


Fig 6.18

6.1.3 Freundlich Isotherm

In the Freundlich isotherm, the constant K represents the adsorption capacity which is a measure of all possible adsorption sites for the adsorption and ‘n’ is the sorption intensity which is a measure of effective adsorption.

a. Humic acid

The sorptional intensity of Cu seems to be very little in the acidic pH but increases at PH 7. Same is the case with Mn which increase in sorptional intensity towards the neutral pH. But for Fe the sorptional intensity is higher at pH 6. At pH 7, Cu has the highest sorptional intensity and Fe the lowest. The adsorption capacity increases with the increase in pH for Mn and reverse is the case for Cu and Fe. Freundlich isotherms of Humic acid is shown in figs 6.19-6.24.

Table 6.9 Freundlich constants of the metals in Humic acid

Freundlich constants	Cu		Fe		Mn	
	pH 7	pH 6	pH 7	pH 6	pH 7	pH 6
n	17.422	-30.395	2.991	6.305	14.837	2.356
K	3.648	5.161	0.520	1.851	5.399	1.051

The sorptional studies were done on humic acid at different pH and metal concentration agree with the recent trends which depart from the idea that there are equivalent non-interacting sites for bonding and suggest that the strength of binding on Humic acid 0 varies with the degree of metal loading and pH (Perude *et al.*, 1983; Chrisl *et al.*, 1999; Chrisl *et al.*, 2001a; Chrisl *et al.*, 2001b.)

Freundlich isotherms for the adsorption of Cu, Fe and Mn on Humic acid

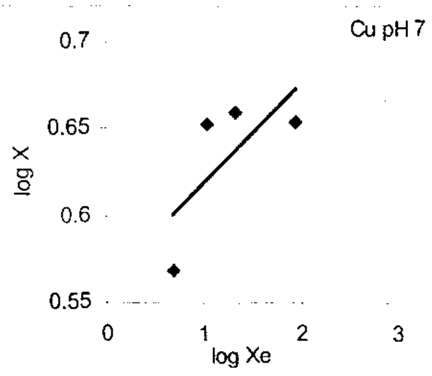


Fig 6.19

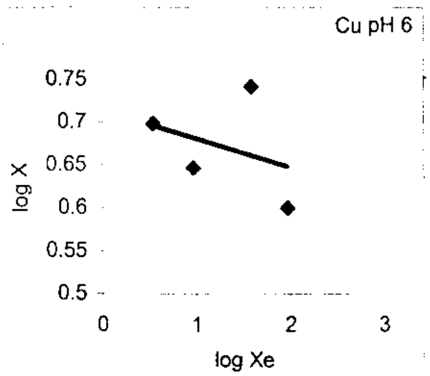


Fig 6.20

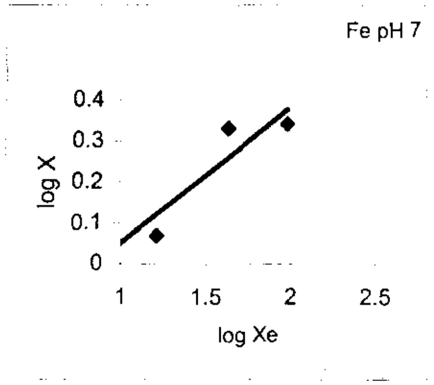


Fig 6.21

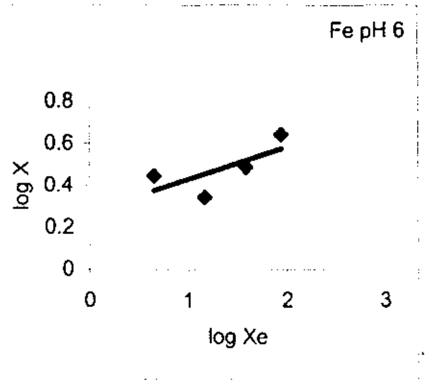


Fig 6.22

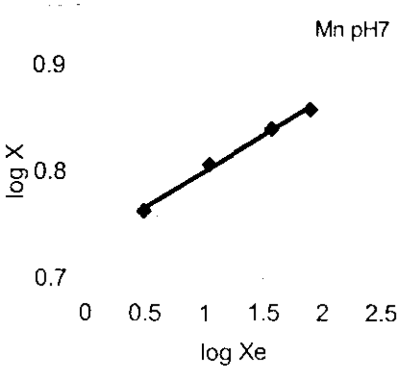


Fig 6.23

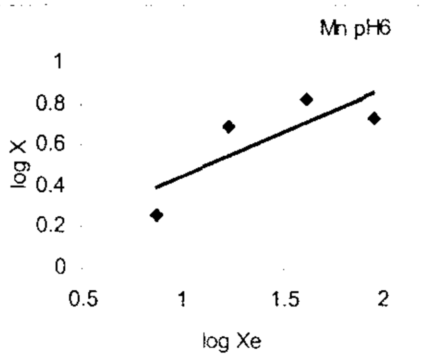


Fig 6.24

b. Sediment left after alkali extraction

Freundlich constants of the sediments left after alkali extraction is given in table 6.10 and Figures fig 6.25-6.30 depict the isotherms. The adsorption capacity and sorption intensity of Cu is higher at pH 7, but for Fe both values are higher at pH 7. But for Mn adsorption capacity is higher at pH 6 but the sorption intensity is higher at pH 7. On comparing the values with that of humic acid, the sediment left after alkali extraction has a higher adsorption capacity and sorption intensity as observed by Jayasree, (1993). This could be due to the exposure of inorganic clay for the exchange process.

Table 6.10 Freundlich constants for metals in sediment left after alkali extraction

Freundlich Constants	Cu		Fe		Mn	
	pH 7	pH 6	pH 7	pH 6	pH 7	pH 6
N	19.96008	123.4568	42.37288	22.42152	67.56757	17.69912
K	24.575	30.917	17.195	13.206	42.257	68.706

Freundlich isotherm for the adsorption of Cu, Fe and Mn on sediment left after alkali extraction

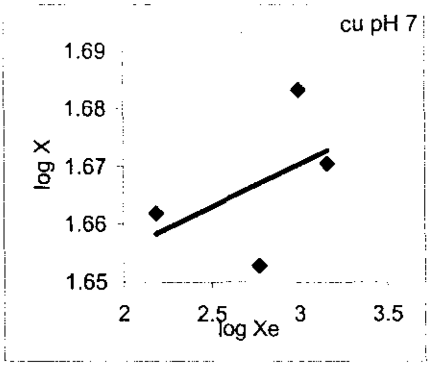


Fig 6.25

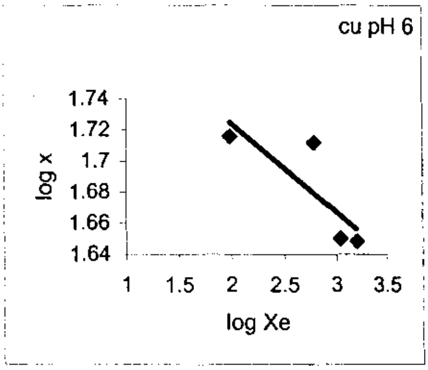


Fig 6.26

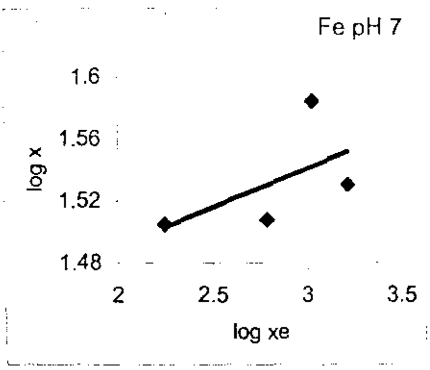


Fig 6.27

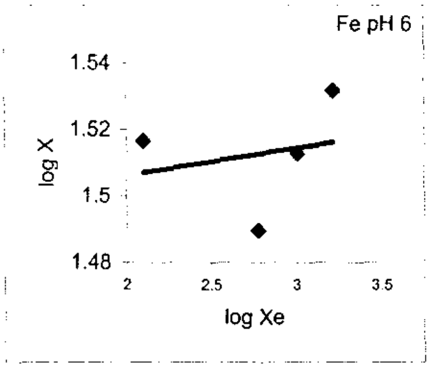


Fig 6.28

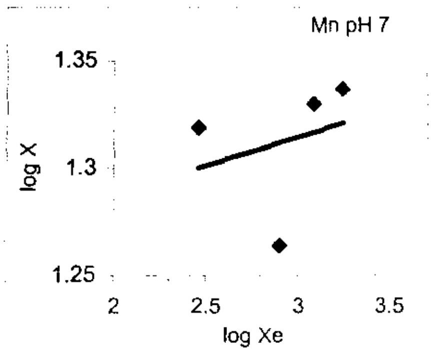


Fig 6.29

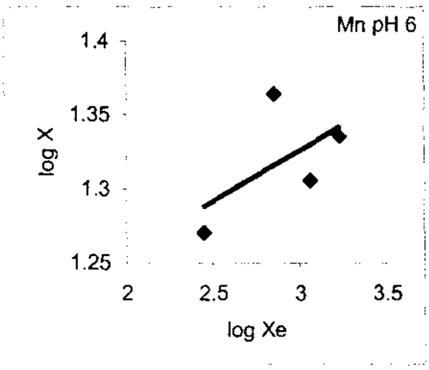


Fig 6.30

Desorption

Increasing pH of the system promotes dissociation of the weak acid functional groups present in the humic acid. If sorption process were chemical interaction involving proton displacement from functional groups uptake would increase with pH until solid dissolution. Complexation of humic acid with heavy metals in solution is an equilibrium process that is considerably dependent on the conditions under which it occurs. The principle effect on complex formation is exerted by the concentration of the interacting substances and pH of the solution and concentration of metal cations. The nature of the binding is revealed to some extent by the desorption studies.

a. Humic acid

For the desorption studies in Humic acid and sediment left after alkali extraction the same four extractants, which are used for sediments are employed. The desorption follows almost the same trend reported by Jayasree, (1993). The percentage release of metals from humic acid by the various extractants are shown in table 6.11 and figures 6.31 and 6.32. Only 8-10% of Cu is released by Ammonium acetate, which is much less than that released from the sediment. Similarly iron release is much less compared with that released from the sediment. Only 3-5% Fe is released from humic acid by Ammonium acetate. For Mn more metal is leached out from humic acid and sediment left after alkali extraction. The percentage released from the sediment and humic acid is comparable. The lower value indicates less labile fractions in humic acid, which point towards a more stable complex in humic acid.

If the sorption of the metal ions were just surface adsorption then there could have been a hundred percentage release with EDTA. Almost 73 % of Cu and 75 % Fe are released by EDTA, and the these values are less than the release from the sediment (100 and 99.3% respectively). This points to the presence of complexation while adsorption on humic acid. The additional stability of HA-metal complex than the metal EDTA-metal complex, the steric hindrance offered by the macromolecule Humic acid to EDTA, or the less favourable spatial orientation offered by humic acid fraction for the effective approach for EDTA etc might have contributed for the less percentage of release from humic acid. Whereas Mn shows a 100% release with EDTA indicating rather weak Humic acid –Manganese complex. HCl releases all the metals from humic acid by the high competence of H^-ions. Very little release of all the metals by Sodium disodium hydrogen phosphate either from humic acid or sediment left after alkali extraction indicates the presence of a negligible percentage of inert fractions.

Table 6.11 Percentage release of Cu, Fe and Mn from Humic acid

Extractants	Cu		Fe		Mn	
	pH		pH		pH	
	6	7	6	7	6	7
NH_4Ac	8.62	9.76	4.70	3.96	40.63	38.02
E.D.T.A.	72.43	75.13	76.65	73.58	100	100
HCl	100	100	100	99.50	100	100
NaH_2PO4	0.49	0.40	0.00	0.00	13.40	15.78

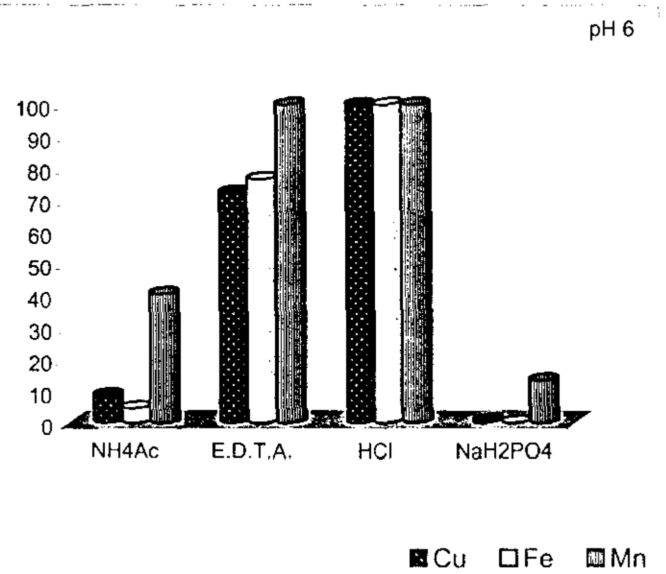


Fig. 6.31

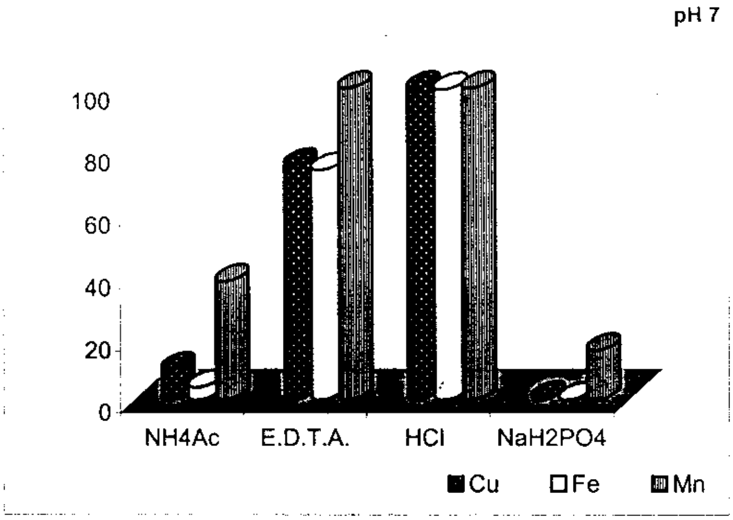


Fig 6.32

Table 6.12 Percentage release of Cu, Fe and Mn from Humic acid after adsorption from a mixture

	Cu	Fe	Mn
NH_4Ac	5.5	12.5	45.2
E.D.T.A.	78	100	100
HCl	100	100	100
NaH_2PO4	1.2	0	12.3

On desorption after adsorption it is being allowed to adsorb from a solution containing all the three metal ions (Table 6.12). Only 5.5% of Cu is released by ammonium acetate and 78% by EDTA revealing a stronger HA-Metal bond in humic acid. The release of Fe by ammonium acetate is 12.5% which is more than the release after it was being adsorbed from a pure iron solution. This indicates a weaker HA-Fe bond. For Mn the value has increased from 40.02% to 45.62% showing a weaker bond when adsorbed from a mixture of metals. So when there is a competition between these three metals, the strength of their bond with humic acid is in the following order Cu>Fe>Mn and when there is no competition the order is Fe>Cu>Mn.

b. Sediment after Alkali Extraction

The release Cu, Fe and Mn (Table 6.13 and Figs 6.33-6.34) by Ammonium acetate from this fraction is almost the same as that from the sediment but less than that from humic acid. This reflects the stability of metal humic complex than the organic–metal complex of the sediment or the clay-metal complex in the sediment left after alkali extraction. This sediment is clayey and metal adsorbed could occupy the interstitial sites or could get in with an exchange with other metals like Al present in the clay. Organic cover and steric hindrances were the factors which inhibited the formation of a less stable complex in the sediment than in humic acid.

Table 6.13 Percentage release of Cu, Fe and Mn from Sediment left after alkali extraction

Extractants	Cu		Fe		Mn	
	pH		pH		pH	
	6	7	6	7	6	7
NH_4Ac	27.85	27.15	9.58	10.66	32.46	33.91
E.D.T.A.	79.5	76.7	68.08	78.90	85.15	84.96
HCl	100	100	100	100	100	100
NaH_2PO4	0	1.55	1.05	1.33	20.27	21.22

The percentage of metals released by EDTA is much less than that released from the sediment showing the presence of stable organic complex with the sediment left after alkali extraction. The organic carbon content in this fraction too confirms the above conclusion.

The organic carbon content of the sediment left after alkali extraction is 142.5mg/g which is much higher than the organic carbon content of the respective sediment 107.2mg/g. The very high organic carbon content in this fraction favour the formation of stable organic metal complex with humin, the sediment left after alkali extraction.

Table 6.14 Percentage release of Cu, Fe and Mn from sediment after its adsorption from a mixture

Extractants	Cu	Fe	Mn
NH_4Ac	10.55	15.56	35.5
E.D.T.A.	70.1	82	100
HCl	100	100	100
NaH_2PO4	0.74	2.5	19.6

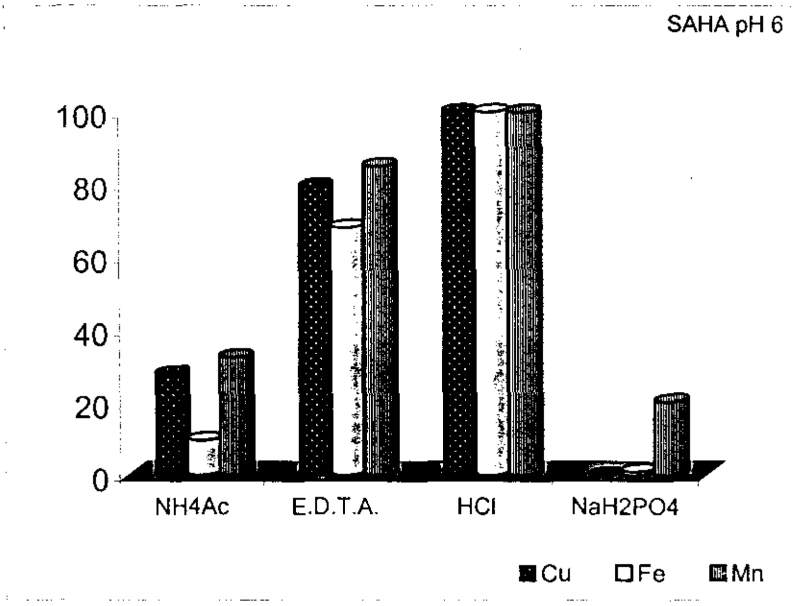


Fig 6.33

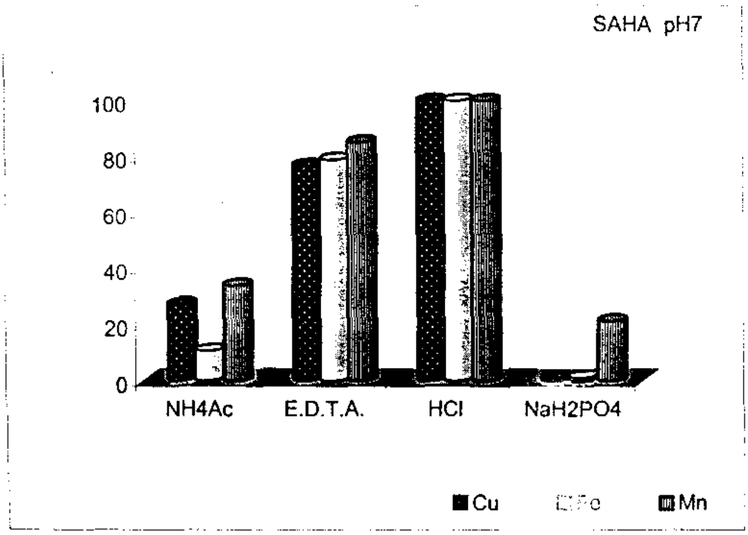


Fig 6.34

While considering desorption after adsorption from a mixture of metals, the percentage release of Cu has decreased whereas that of Fe and Mn have increased than the metal release after adsorption from a pure metal solution. This shows the possibility of stable Cu complex with the sediment left after alkali extraction. The strength of their bond is in the Cu>Fe>Mn. As observed earlier for the sediment and humic acid HCl could release almost all the metal from this fraction too. Very little Cu and Fe could be released by NaH_2PO_4. Following conclusions are derived from the above studies.

From the desorption studies it can be concluded that

a. The major portion of the organic matter is not extractable with sodium hydroxide, and humic acid is only a small portion of the total organic matter.

b. The organic matter left after alkali extraction exhibits significant complexation/adsorption and the desorption pattern indicates the presence of stable species.

c. Though we cannot have a definite picture of the structure and character of Krenkel, P.A. humin, the sediment left alkali extraction, the overall behaviour indicates the presence of an active component which acts like an ion exchanger. Adsorption or complexation takes place but the solid substrate is not extractable.

d. Considering its nonextractable nature this sediment left after alkali extraction is a better scavenger. The scavenger is one which can remove metal species from the system. Humic acid while forming soluble metal complexes brings back the metal to the system. The sediment left after alkali extraction is a better scavenger as it can form insoluble metal complexes.

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Summary

Mangrove ecosystems are among the most productive natural ecosystems on earth. The vegetation and its associated biota play a major role in contributing organic matter. The organic matter in the marine sediment can be divided into two general categories- Hydrolysable components which can be converted by hydrolysis into water soluble substances and non hydrolysable substance which make the bulk of organic matter and is composed of humic like components. As solids they retain water, they are effective acid base buffers, they bind metals, ions, molecule and other bio polymers. The most important products formed during the composition of organic substances are the humic acids. Humic acids are highly functionalised carbon rich polymers that stabilize soils as soil organic matter. They are anchored by metal binding and by attachment to clays and minerals which decrease their solubility at a given pH.

Humic substances are complex polymeric structures. No other polymers with such a wide range of properties are so widely distributed in nature. But still their molecular structures are unknown. Humic acid structures are closely related to their origin. A structural knowledge is essential in determining their reactivity with metals. In the present work structural elucidation of humic acids from three different mangrove ecosystems of Cochin area is done with the available data from functional group analysis and various spectroscopic methods. ^{13}C NMR spectra of the solid samples with CPMAS, IR and SEM are very promising in revealing the complex structures of these polymeric substances. Solid ^{13}CNMR serves as powerful tool in the determination and quantification of functional groups in humic substances. The IR spectroscopy is useful in confirming the presence of the functional groups predicted by ^{13}C NMR. The three dimensional structure reveals the spatial orientation of the atoms and the molecular volume of the monomer of each unit. SEM gives a clue regarding the polymeric nature of the humic acid.

The humic acid aponomer of Mangalavanam is similar to the model developed by Santa et al., (1998). The very high nitrogen content and its purine like structure can be attributed to the numerous species of migratory birds that were recorded from this mangrove vegetation. The parraffinic contribution might be from the breakdown products of terpenoids. The ability of this humic acid to form complexes with metal ions can be due to their high content of oxygen containing functional groups including COOH, phenolic, and C=O structures and the nitrogen containing imino and amino groups.

The structural core of the humic acids from Vypeen and Nettoor is surrounded by a variety of labile hydrophilic complexes including oxygen containing functional groups such as carboxyl, phenolic, alcoholic, carbonyls, methoxyl, and amino groups. The ability of humic acid to form complexes with metal ions can be attributed to their high content of oxygen containing functional groups. The presence of intense signals for oxygen substituted aromatic carbons and methoxyl carbons is a strong indication that unaltered lignin residues are present in large amounts. The basic difference between these two humic acid is in their nitrogen content. Very small percentage of nitrogen in the humic acid from Nettoor sediment rules out the possibility of having it as a structural component of the monomer unit. The very high carbohydrate content is explained as the very backbone of these humic acids.

The analysis of sorptional capacity with Langmuir and Freundlich adsorption isotherms shows that the humic acid and the sediment left after alkali extraction shows significant adsorption capacity. The sorptional studies done with humic acid at different pH and metal concentration agrees with the recent trends which depart from the idea that there are equivalent non- interacting sites for bonding and suggest that the strength of binding on Humic acid varies with the degree of metal loading and pH. For all the metals there is a decrease in Kp with the increase in concentration indicating that there is a decrease in concentration of the adsorbed metal ion on humic acid with the increasing concentration of the added metal solution. Langmuir adsorption isotherms reveal that the adsorption pattern of humic acid fits very well to this adsorption isotherm. For Cu and Mn the adsorption maxima tends to be higher at the neutral pH and so too the intensity of adsorption. For Fe, K1 is higher at pH 6 and K2, the intensity of adsorption is higher at pH 7. A negative value of K2 for Cu adsorption on humic acid signifies the high amount of energy involved in the adsorption On considering the energy factor K2 of Cu at pH 7 there is decrease in K2 with the sediment left after alkali extraction. The same is observed for Mn at pH 6, pH 7 and Fe at pH 6. Thus the energy factor favours more adsorption on humic acid. The sorptional intensity of Cu seems to be very little in the acidic pH but increases at PH 7. Same is the case with Mn for which too there is an increase in sorptional intensity towards the neutral pH. But for Fe the sorptional intensity is higher at pH 6. At pH 7. Cu has the highest sorptional intensity and Fe the lowest.

The observations of this study can be concluded as

a. The major portion of the organic matter is not extractable with Sodium hydroxide and humic acid only a small portion of the total organic matter.

b. The organic matter left after alkali extraction exhibits significant complexation/adsorption and the desorption pattern indicates the presence of stable species.

c. Though we cannot have any definite picture of the structure and character of humin, contained in the sediment left alkali extraction, the overall behaviour indicates the presence of an active component which acts like an ion exchanger. Adsorption or complexation take is possible but the solid substrate is not extractable.

d. Humic acid is a good complexing agent. Due to the nonextractable nature of the organic matter present with the sediment left after alkali extraction it is a better scavenger. The scavenger is one which can remove metal species from the system and the humic acid metal complex through its dissolution may bring back the metal to the system.

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List of Abbreviations

Pre mon - Pre monsoon

Mon - Monsoon

Post - mon Post monsoon

MF - Mangalavanam Forest

MC - Mangalavanam Canal

VF - Vypeen Forest

VC - Vypeen Canal

NF - Nettoor Forest

NC - Nettoor canal