INFLUENCE OF HUMIC ACID ON METAL SORPTION BY ESTUARINE SEDIMENTS

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PREFACE

The environment has become a global concern because of the growing apprehension about the potential effects of pollutants on various fauna and flora. The local, regional and global cycles of trace metals, the dominant and the most persistent contaminants, and their disturbances by human activities, and the fear on the role of these metals on the regulation of the environmental quality and human health has mushroomed the research on fundamental, applied and health aspects of trace metals. The major objectives of these research ponder over the two basic scientific questions: (1) to what extent the anthropogenic emissions have already altered the biogeochemical cycles of trace elements and (2) what is the scale of these perturbations?

Of the three basic compartments in the aquatic systems, water, sediment and biota, where the heavy metals can be accommodated, the sediment due to its high sorption capabilities controls the fate and effects of the metals in system. The organic matter associated with the sediments, its extensive complexing/chelating functional character, determines the sorption capacity and dynamicity. Humic substances, the major fraction of the organic matter, which are having complex structural as well as reactional characteristics, contain a strikingly high content of oxygen containing functional groups (carboxyl, phenolic and alcoholic hydroxyl groups, ketonic etc.) through which they can interact with the metals. Humic acids are the base-soluble and acid insoluble fractions of humic substances.

Present work illustrates some of the physico-chemical characteristics of humic acids, isolated from different environments as well as its role in the adsorption of copper, cadmium, manganese, zinc, mercury and lead by estuarine sediments.

The thesis consists of six chapters. Chapter I gives a general introduction about humic substances, and its importance in sedimentary trace metal speciation. Aim and scope of present study are also included in this chapter.

Chapter II describes the location of collection sites, sampling procedures of water and sediment. Analytical procedures for the determination of hydrographical parameters and sediment characteristics (dissolved oxygen, salinity, pH, sediment organic carbon, total phosphorus and humic acid), isolation and purification of functional group characteristics, spectral studies and adsorption studies are also given in this chapter. Results of hydrographical parameters and sediment characteristics are also included.

Chapter III deals with the acidic functional group characteristics, carbohydrate contents, phosphorus contents and spectral properties of isolated humic acids. The results are compared with that of the terrestrial humic acids.

Chapter IV illustrates the adsorption of copper, cadmium, manganese, zinc, mercury and lead by an estuarine sediment, sediment after alkali extraction, an estuarine humic acid and a terrestrial humic acid samples. Effect of salinity on the adsorption capacity is also included. Desorption studies for explaining the nature of adsorbed metal ions are also described in this chapter.

Chapter V gives a kinetic approach to the heterogeneity analysis of the sedimentary sorption of metals. The kinetic data of the sorption was applied in the model evolved.

The overall features of the present study are summarised in Chapter VI followed by a list of references.

CONTENTS

INTRODUCTION

The mounting scientific and public awareness of environmental issues have stimulated investigations on the complex and diverse natural systems. On the global scale, the hydrosphere has attracted a highly significant place as it includes several distinct ecosystem types- freshwater streams, lakes, ponds and rivers; estuaries and coastal and deep ocean waters within which exist many different biotic and abiotic components. The biotic or living components consist of plants, animals, and micro organisms that inhabit specific ecological niches in each ecosystem. The abiotic or non living components include the physical environment i.e., water, suspended material and sediments within the boundaries of ecosystem. Each aquatic system is thus a product of complex interaction of living and non living components. The physical and chemical properties of aquatic ecosystem can have a profound effect on the impact of chemicals and other xenobiotics on the biological activity.

Estuaries are unique water systems as they are the interphase between fresh river water and saline coastal water. They have a high biological productivity and are often subjected to high degree of pollution.Estuaries are characterized by pronounced spatial heterogeneities and temporal variabilities in the composition of the water body.

Sediment is a suitable substrata for biological and chemical reactions, it plays an important role in biochemical and geochemical processes, and also serves as a surface for adsorption process and bacterial activity.

A. Sediment-Metal Interactions

Heavy metals from first row of transition elements including chromium, manganese, iron, cobalt, nickel, copper and zinc are natural constituents of sediments. A large part of the anthropogenic discharge of heavy metals into the environment becomes part of the suspended matter in rivers, which acts as an efficient scavenger for these metals. The chemical forms in which heavy metals are present in the aqueous environment and sediments are

Dissolved suspended matter
Deposited sediment
Interstitial water

Adsorbed organic matter,Fe and Mn oxides lattice positions
Discrete minerals

Sediments are widely used as indicators of environmental contamination by trace metals in rivers, lakes, estuaries and the oceans. This has generated interest among ecologists to study the heavy metal concentration in the bottom sediment of aquatic biotope exposed to pollution and environmental degradation. Most of the informations relating to the distribution of heavy metals available from India pertains to water and living organisms and data on the concentration of heavy metals in polluted estuarine sediments are scanty.

The heavy metals in sediments are thus present in a wide variety of forms. Since competition arises between the various components of the sediments capable of binding the heavy metals, the elemental portion depends on the mineralogical and chemical composition of sediment and additionally on the chemical environment of the sediments during transport and after deposition.

B. Process Affecting the Heavy Metal Content of Estuarine Sediments

Process affecting the heavy metal content of estuarine sediments are two fold. First there are the chemical processes of mobilization and secondly the physical process of mixing of contaminated fluvitile sediments with sediments which have a lower heavy metal content. The latter may be marine sediments or reworked estuarine sediments.

Chemical process

When transported from river to estuary, the sediments are transferred from freshwater conditions, through brackish water to the marine environment. As with the transformation from oxidising environment prevailing during transport to reducing environments developing after deposition, the heavy metal compounds in the sediment will react to the change in salinity, tending towards chemical equilibrium with their new environment.

The desorption of cobalt and silver present in clay minerals and freshly prepared $Fe(OH)_2$, when these minerals are brought into contact with sea water, was studied by Kharkar *et al.*(1968). About 40-70% of the adsorbed cobalt and 20-30% of adsorbed silver was desorbed from clay minerals. Very small amounts of these metals were desorbed from the $Fe(OH)_2$.

The formation of stable chloro complexes in the presence of sea water may cause the release of mercury from sediments in an estuary, as was experimentally shown by Feick *et al.*(1972). It was also found by Lockwood and Chen (1973) that the adsorption of mercury on hydrous MnO_2 is prevented by the presence of Cl^- and their concentration in sea water. Reimers and Krenkel (1974) have reported that only illite at high Cl^- concentrations loses its adsorbed mercury while desorption of mercury was negligible from clays and organic compounds. According to Rickard (1971) source of the copper present in sediments is desorbed upon introduction into sea water.

In the sediments of Rhine-Mense and Ems estuaries, decrease in concentration of heavy metals in the seaward direction is paralleled by a decrease in organic content. Part of this decrease is caused by the decomposition of unstable land-derived organic matter. Therefore, there is some release of heavy metals,associated with the organic matter, from sediments.

Field evidence for desorption process has been given by Evans and Cutshall (1973) and Turekian (1977). Turekian (1977) reported high concentrations of nickel, cobalt and silver in the estuarine waters of Long Island Sound, which could not be explained by simple mixing of river and sea water, but were due to the release of metals from sediments introduced into the marine environment.

Physical process

When contaminated fluviatile sediments are mixed with uncontaminated sediments in an estuary, should also observe a decrease in heavy metal content of the estuarine sediment in the seaward direction. In the Rhine-Mense and Ems estuaries the mixing of marine and fluviatile sediments may partly explain decrease in the contents of heavy metals in the seaward direction (Salomons, 1973). At present it is not possible to determine which of the two processes, chemical (desorption) or physical (mixing) is more important cause of decrease in the heavy metal content of estuarine sediments in seaward direction. The results of the relevant investigations are conflicting in this respect.

In natural waters, trace metals are distributed between the dissolved and solid phase. In the dissolved phase, these trace metals exist as hydrated ions, labile complexes, predominantly with the respective anionic inorganic constituents of water and non labile complexes with dissolved organic matter. Among these complexing dissolved organic matter, humic and fulvic acids are considered to be of major significance in speciation of heavy metals in natural water system. In solid phase, trace metals are adsorbed on suspended particles and sediments. The concentration of metal ions in aquatic system is controlled by the ability of sediments to strongly adsorb them. Organic components of sediments can greatly influence heavy metal ion levels in aqueous phase through their ability to both form soluble complexes and participate in solute-solid interactions.

The distribution of heavy metals in natural water system and their concentrations in them are determined by two competing processes (Davis and Leckie, 1978; Vuceta and Morgan, 1978). One is precipitation of insoluble organic compounds of the heavy metals, which are adsorbed on suspended particles, leading to the removal of heavy metals from the solution; the other is the complexation of heavy metals by inorganic and organic ligands and the formation of colloids which help to increase the heavy metal concentration in the water. These processes can be affected by the concentration of the metal, pH, ionic strength (salinity) of water, concentration of metal binding sites associated with organic material and partitionate material. These processes proceed most rapidly in the zone where mixing of river and sea water take place, where there is a rapid change in conditions, expressed in a change in salinity of water. This results in active precipitation of high molecular weight organic substance, colloids and heavy metals bound to them. As a result, large proportion of dissolved form of heavy metals in river waters pass into estuarine sediments (Turekian, 1977). Thus trace metal interaction with organic matter plays an important role in biogeochemical cycling of metals in aquatic systems. The nature and extend of interaction between heavy metals and naturally occurring organic matter has been continuous to be investigated by ever expanding number of research groups.

Soil/sediment organic matter has been of particular interests in studies of heavy metal retention by sediment, because of its significant impact on adsorption capacity and more important, the tendency of trace metal cations to form stable complexes with organic ligands. In a study with 24 British soils, Mc Laren and Crawford (1973) reported that the majority of available copper exists as an organically bound form. Udo *et al.* (1970) noted a strong correlation between total solid zinc and organic matter content and concluded that it was an important soil component responsible for zinc retention in calcareous soil.

C. Sedimentary Organic Matter

Sediments have been the principal depositories of posthumous organic debris. Organic materials embedded in sediments are mainly classified into compounds that are survivors of diagenesis (non humic substances) and product of diagenesis (humic substances). Diagenesis is the post-depositional transformation process both physical and chemical that alter sedimentary matter under low temperature and low pressure conditions. The first group includes all those organic molecules which are chemically similar to living matter, whereas second group comprises of compounds that arise during diagenesis from organic debris but generally not a part of plants and animals. The organic pigments from plants and animals are resistant to wide range of diagenetic processes.

During diagenesis, most of the organic matter loses its biological identity. Polymers gradually break down into their monomeric building blocks and these in turn can be modified by elimination of functional groups, which destroy the sequence of arrangement of biochemical compounds. These breakdown products can reorganize and polymerise into chemically stable substances; typical examples are the humic acids, coal, kerogen etc.

Non humic substances include a large number of relatively simple compounds of known structures and belong to well known groups such as carbohydrates, proteins, peptides, aminoacids, fats, waxes, resins, pigments and other low molecular weight organic substances. In general, these compounds are relatively easily attached by micro organisms in the soil and have a relatively short survival rate.

Humic substances are organic materials that occur widely in aquatic and terrestrial environments. Humic substances are heterogeneous polyfunctional yellowish black organic macro molecules. Since humic substances comprise a major part of the organic matter in aquatic system, they are considered to have an important role in geochemical processes like carbon cycle of the earth, transportation of metals, and other inorganic and organic compounds.

Though humic substances have been studied by soil scientists for two centuries, clear picture of its structure is still in dark. Humic substances do not correspond to a unique chemical entity. So it can't be represented by a general structural formulae. Similarly, humic substances are not biologically predestined to carry out a specific biochemical action and thus can't be defined in functional terms. As a result, humic substances are only operationally defined. Humic substances represent a general category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in colour of high molecular weight and refractory (Aiken, *et al*., 1985). Based on their solubility in alkali and acids, humic substances are usually divided into three fractions,

1) humic acids, soluble in dilute alkaline solution but is precipitated by acidification,

2) fulvic acids, soluble in both in acid and alkali and

3) humin, which can't be extracted by dilute alkali and acid. The insolubility of the third fraction is ascribed to the firmness with which it combines with inorganic soil and water constants (Schnitzer and Khan, 1972).

Process of Humus Formation

The concept of the origin of humus can be roughly divided into purely chemical and biochemical theories. Chemical theory (Beckley, 1921) suggests that the action of mineral acids on carbohydrate forms hydroxy methyl furfural which on condensation may produce humus. Another theory put forward by Waksman (1938) stated that condensation of carbohydrate with amino acids or polypeptides leads to the formation of humus. Humic acids can be developed from lignin by opening the heterocyclic ring.

Phenolic substance formed by the decomposition of protein was shown to be responsible for many of the dark coloured materials in humus. In brief chemical transformations during humus formations are:

1) formation of hydroxy methyl furfural

2) condensation of amino acid or polypeptides with carbohydrates

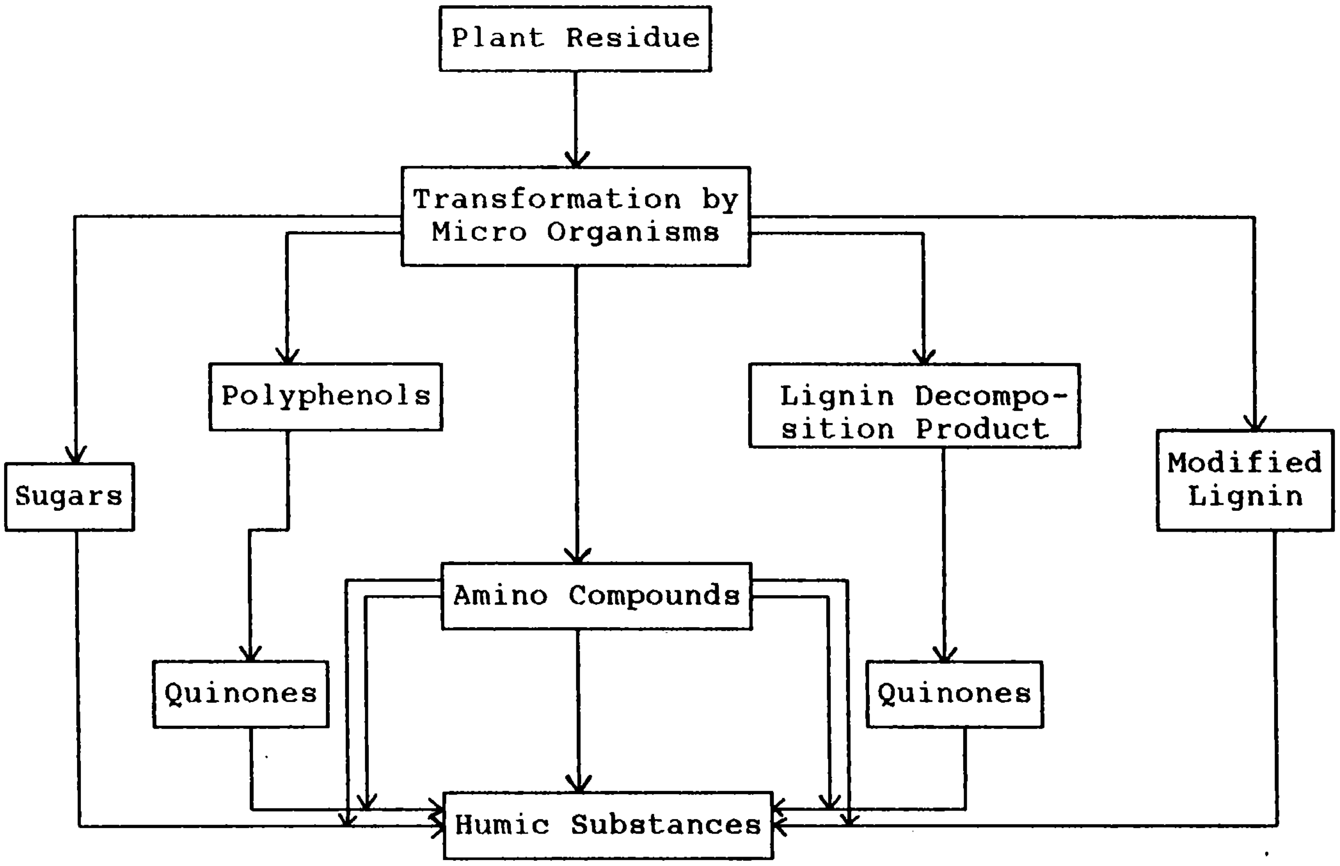
3) aldol condensation of methyl glyoxal with aminoacids

4) oxidation of aromatic compounds such as phenols, quinone and hydroxy quinone of natural occurrence .

Biochemical transformation of lignin to humus is another process for the formation of humus

Microbiological formation of humus is as follows : Carbohydrates serve as a source of energy for the micro organisms, the proteinaceous and hemicellulose cell substances are synthesised as a result of their activity. Lignin also undergoes modification; altogether these products form humus. Autolysis of the micro organisms itself produces considerable amounts of humus. Fungi are important in the production of forest humus and, therefore, probably play a major role in humus formation in the regions of brown water lakes and rivers.

Eventhough several reports have been published, the composition of humic material is not fully understood at present. A variety of synthetic routes have been suggested (Stevenson, 1982; Aiken *et al.*, 1985).



The physico-chemical properties of humic substances have been studied extensively for almost the last 200 years, the recent being reported by Aiken *et al.* (1985) and Ishiwatari (1992). In spite of this, much remains unknown about their nature and structure, particularly in their state of field. The three fractions of humic material (humic acid, fulvic acid and humin) are not individual chemical compounds, but can be regarded as group of macro molecules broadly similar in composition, properties and structure, but covering a wide range of molecular weight. One of the major analytical difficulties in characterization of humic substances is the lack of any fractionation procedure which will yield pure components which can be subsequently characterized by standard techniques. Data available suggest that structurally the three fractions are similar to each other, but that they differ in molecular weight and functional group content (Schnitzer and Khan, 1972).

Molecular weight ranging from a few hundred to several millions have been reported for humic substances (Rashid and King, 1969; Rashid, 1971). Humic substances contain carbon, hydrogen, nitrogen, sulphur and oxygen as major elements; carbon and oxygen being predominant ones (Schnitzer, 1977). The elemental analysis of soil humins is about the same order of magnitude as that for humic acids (Khan, 1971). Compared to soil humic substances, humic material in water generally contain considerably less carbon and nitrogen (Gjessing, 1976). But Stuermer and Payne (1976) have found that sea water fulvic acids possess relatively lower oxygen content, higher nitrogen content and higher H/C ratios as compared to terrestrial ones. The major oxygen containing functional groups in humic substances are carboxyl, hydroxyl and carbonyl groups. Total acidity is the sum of carboxyl and phenolic hydroxyl groups.

Most of the humic materials in soils as well as sediments, occurs in insoluble forms. Humic substances are found to include

1. insoluble macro molecular complexes

2. macro molecular complexes bound together by di- and trivalent cations, such as Ca^{++}, Fe^{+++}, and Al^{+++}

3. in combination with clay minerals through bridging by polyvalent cations (clay-metal-humus), hydrogen bonding and van der Waal's forces (Greenland, 1971; Theng, 1979).

Metal-Humic acid Interactions

Humic acids form one of the important sink/source system for metal ions in a number of environmental compartments including soil and sediments in both fresh and saline waters. The distribution of metals in aquatic system can be influenced by variations in the extent to which these elements are retained by humic acids. Since humic acids occur almost in every aquatic environment determining the fate of metals should be evaluated.

The ability of humic substance to form stable complexes has been well established (Stevenson, 1982). The great importance of humic and fulvic acids in modifying the chemical properties of trace metals in the sediment environment requires that some consideration be given to the mechanisms where by they combine with metal ions. Their ability to form complexes with metal ions can be attributed to their high content of oxygen containing functional groups, -COOH, phenolic-alcoholic -OH and >C=O structures of various types. Amino groups may also be involved. Stevenson (1982) suggested that Phthalate type complexes are formed with adjacent -COOH groups in humic acids.

Results of I.R spectroscopic studies have confirmed that -COOH groups or more precisely carboxylate ion $-COO^-$ play a predominant role in interaction of metal by humic acids. -OH, >C=O and > NH also involved in the interaction (Boyd *et al.*, 1981a;b). In addition to the above, interactions between metal and conjugated ketonic structures are reported.

Importance of Humic material in Estuaries

The importance of humic material lies in its probable ability to stimulate plant growth. Aspects of the direct physiological effects of humic material on aquatic plants reported by Prakash (1971) indicated the importance of organic material in the sea in chelating various trace metals and making them available to organisms, and subsequent studies by Nielsen and Anderson (1971) suggested that the natural levels of metals such as copper in sea water would exhibit toxic effect on algae if they were not complexed with organic material. Prakash and Rashid (1968) and Prakash *et al.* (1973) have shown that humic materials have stimulating effect on marine phytoplankton cultures and Rashid (1971) had discussed the role of marine humic material in complexing divalent and trivalent metal ions.

The studies by Nissenbaum and Kaplan (1972), Gardner and Menzel (1974) and Nissenbaum (1974) suggested that most of the humic material in the sea is autogenic, but that in estuaries the terrestrial contribution is dominant. Most of the terrestrially derived humic material probably precipitates out within the estuary or close inshore (Sieburth and Jensen,1966; Gardner and Menzel, 1974). Estuaries are regions which receive extensive inorganic and organic loads from terrestrial run off. Even though the natural content of humic material is important in regulating the concentrations of inorganic constituents, particularly metal, to levels which are beneficial to plant growth, the degradation of humic materials comes to the formation haloforms, which can cause serious health problems at rather low concentrations. Prakash (1971) suggested that the high fertility of coastal waters is attributable to the presence of humic material and other organics.

Aim and Scope of Present Work

Elevated trace element concentrations in natural water systems derived from industrial sources pose a severe threat to the environment. Many studies have been undertaken to ascertain the sources and in particular the chemical behaviour and speciation, speciation determining toxicity and the toxic effect on biota (Venugopal *et al.*, 1982; Shibu *et al.*, 1990; Babukutty, 1991; Nair *et al.*, 1991; Geetha, 1992; Nair, 1992; Shibu, 1992; Sujatha, 1992). Humic substances which constitute the major fraction of organic matter form one of the important source/sink for metal ions in a number of environmental compartments, including soil and sediments of both fresh and saline waters. Even though studies on distribution of humic acid was conducted by Nair (1992), no work has yet been reported on structural characteristics of humic acids and its significance on metal adsorption by sediment.

Humic substances are chemically very complex materials whose structure and reactions are not fully understood. They can't be represented by a general formula. These are amorphous, brown or black, hydrophilic, acidic, polydisperse substances of molecular weight ranging from several hundreds to tens of thousands. They contain a wide variety of functional groups, which may react with metals. One of the most striking characteristics of these materials is their relatively high content of oxygen containing functional groups through which they can interact with metals.

The physico-chemical properties of humic substances have been studied extensively for almost the last 200 years. In spite of this, much remains unknown about their nature, structure, and particularly their state in the field. The highly complex and diverse nature of the humic acid limited the studies on these substances to concentrate mainly on the distribution properties.

Present study mainly focus on assessing the role that the humic acid contribute to adsorption of heavy metals by estuarine sediment. Comparison of some physico-chemical properties of humic acids isolated from different stations and from a forest soil is also included in this study. The purpose of the investigation was to obtain more detailed information on the sorption of metals on humic acid with special emphasis on effect of salinity. In the present study, the adsorption selectivity sequence of copper, cadmium, manganese, zinc, mercury and lead was documented for estuarine sediment, sediment after alkali extraction, an estuarine humic acid sample and a terrestrial humic acid for comparison. The principal objective was to ascertain the metal retention sequence for sediment and explore its relationship to humic acid content.

CHAPTER II

MATERIALS AND METHODS

Description of the Study Area

A brief description of the study area and the various methods employed are described in this chapter.The hydrographical conditions and the general sedimentary characteristics of the study area are also included in this chapter.

The Cochin estuary, extending between $09^{\circ}40' - 10^{\circ}12'$N and $76^{\circ}10' - 76^{\circ}30'$E is a tropical positive estuary. Two main rivers discharge fresh water into the estuarine system; the Periyar river flows into the northern parts and the Muvattupuzha river into the southern part.

A large number of industrial units are situated on the banks of Periyar river and hence the northern part of the estuary receives large quantities of treated and untreated industrial effluents. This part also receives organic wastes from the sewage treatment plant of Cochin city, coconut retting yards and fish processing units. Whereas the southern part of the estuary receives water that drains through agricultural land and also effluents from Hindustan News Print factory.

The area of investigation and station locations are given in Fig.2.1. The stations were fixed as to obtain a fairly good coverage of the prevailing complex environmental conditions.

Station 1 is situated in the river Muvattupuzha which drains into Cochin estuary through the southern side . It is about 20 Km away from bar mouth and represents purely freshwater system.

Station 2 is 1 Km down to the discharge site of effluents from Hindustan News Print Factory. The characteristic of the station indicated that settling of most of the waste from the paper mill takes place in this region.

Station 3, River mouth where Muvattupuzha joins the estuary. During monsoon months, the station behaves as a freshwater zone.

Station 4 is located in the same arm of the estuary. This regions is always estuarine and the sediments are characterized by sand, silt, clay or silt clay. Numerous irrigation channels carrying discharges from various industrial and sewage out falls drain into the location.

Station 5 is located in the northern arm of the estuary where the river Periyar empties its flux into the sea. The station is always typically estuarine. Sewage and wastes from oil mills, food industries etc., are discharged into this area.

Station 6 also represents an estuarine station in the northern arm of the estuary. The retting of coconut husks is the major activity in this area and huge amount of plant pith get accumulated in this region. The sediment of this region contains large amount of pith and yarns of coconut husk.

Station 7 is located in the Periyar river near the industrial complex FACT and it is here that the effluent from industrial concerns are discharged. Sediments contain oil and grease and when dried become grey in colour.

A terrestrial soil sample from a typical ever green forests,Shudurunny at Kollam district and a coastal sample from 20 Km off Cochin bar mouth were also collected for isolating humic acid.

Sampling Procedure

Water and sediment samples were collected from different stations marked in the Fig.2.1.

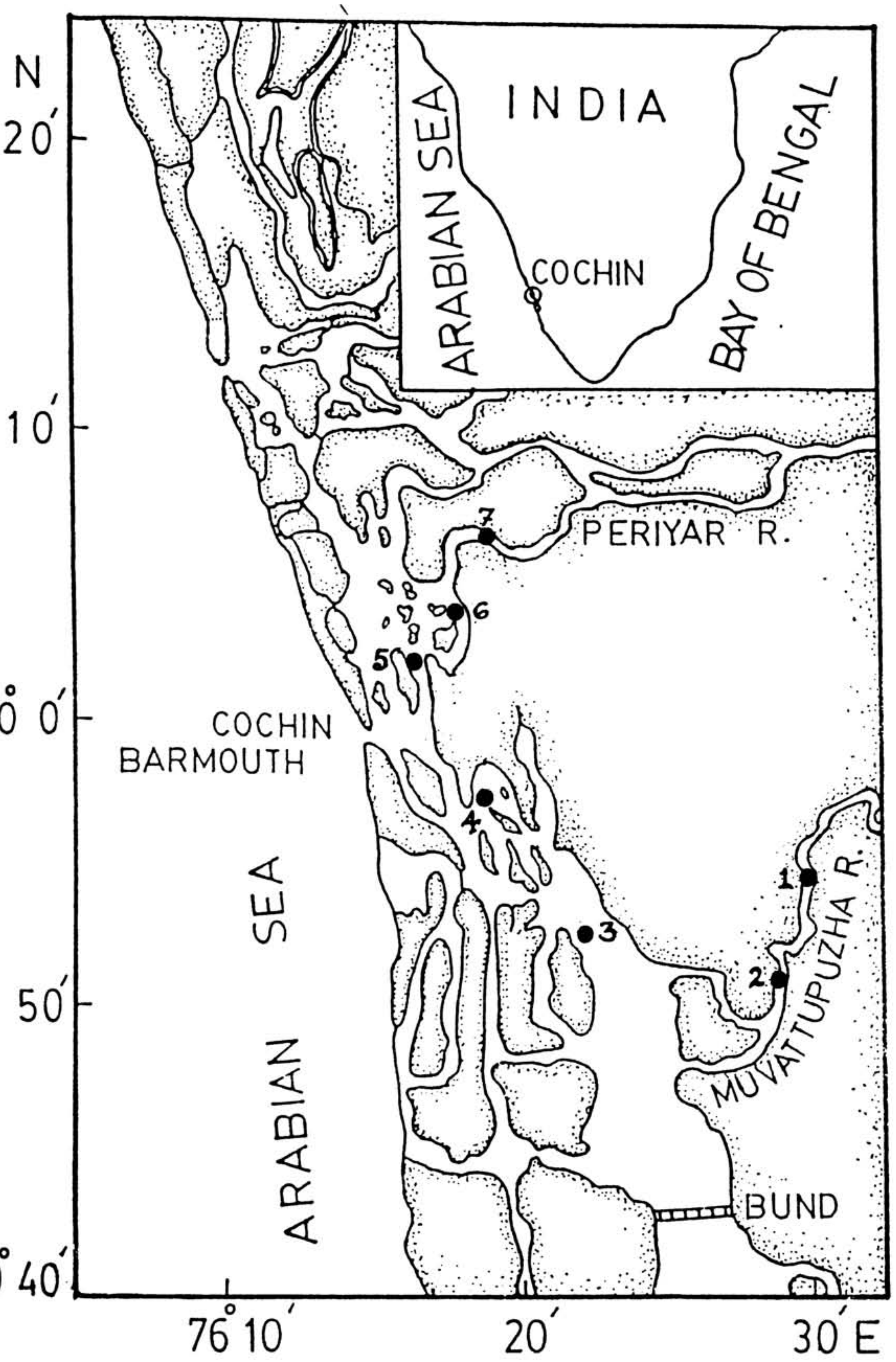


Fig. 2.1 Map of Cochin Estuary showing Location of Stations

Surface water samples were collected by using a clean bucket and bottom water samples by using a Hytech water sampler. Collected samples were stored in clean plastic bottles.

A stainless steel plastic lined van-Veen grab was used to collect the sediment samples. From each location three grab full of sediment had been collected. Samples were homogenized after removing the decayed leafy particles and were stored at -5^0C in polythene cover.

Laboratory Procedures

Glasswares and plastic bottles used for the experiments were soaked in nitric acid (1:1) and washed with double distilled water. All reagents were of analytical grade. Reagent solutions and standard solutions were prepared in deionised double distilled water.

A. Hydrographical parameters and Sediment Characteristics

Hydrographic parameters such as salinity and dissolved oxygen were determined by employing standard procedures (Grasshoff, 1983). pH was measured using a Philips portable pH meter. Sediment organic carbon was determined by dichromate method (El Wakeel and Riley, 1957). The texture analysis was conducted by subjecting the dried sample to mechanical sieving and pipette analysis as described by Krumbein and Pettijohn (1938). Total phosphorus in the sediments was estimated by the modified method of Murphy and Riley (1962). Humic acid in the sediment was analysed in spectrofluorimeter. A known quantity of the dried sediment was extracted with 1N NaOH for 24h, filtered and acidified. The precipitated humic acid was redissolved in NaOH and fluorescence intensity was measured (λex =362nm, λem =462nm) in a spectrofluorimeter (Hitachi F- 3010) (Nair, 1992).

B. Isolation, Purification and Analytical Characteristics of Humic acid

Isolation and Purification

Humic acid is the alkali soluble, acid insoluble fraction of organic matter in soils and sediments. It can be isolated from sediments and soils by alkali and precipitated by acidification.

The method used for the extraction of humic acid is same as those described by Chen *et al.*(1978). Sediment/soil samples were taken in 2 litre beakers and treated with 0.5N NaOH for 24 h with occasional stirring. The mixture was then filtered. On acidification of alkali extract with 6N HCl, humic acid got precipitated. The precipitated humic acid was purified by employing repeated base-dissolution, acid-precipitation cycles. Finally, humic acid was suspended in dilute HCl-HF mixture (5 ml HF + 5 ml HCl + 990 ml distilled water)to eliminate weak base insoluble materials. Humic acid was separated, washed with deionised double distilled water and dialysed against deionised double distilled water for five to seven days. The purified humic acid thus obtained was then dried in oven below 60^0C, kept in a desiccator.

Analytical Characteristics

Total acidity, carboxylate acidity carbohydrate content, total phosphorus and total metal associated with isolated humic acids were estimated as follows:

(a) Total Acidity (Baryta Absorption Method)

The phrase `total acidity ' conveys all acidic hydrogen in humic acid. Total acidity was estimated by barium hydroxide method, suggested by Schnitzer and Khan (1972). 20mg of humic acid was added to 20ml of 0.1 M $Ba(OH)_2$ in a glass stoppered Erlenmeyer flask. The air in the flask was displaced with nitrogen and kept for 24 h with occasional shaking. Simultaneously a blank containing 20 ml $Ba(OH)_2$ was equilibrated for 24 h. The suspensions were filtered and the residue was washed with CO_2 free distilled water. The filtrate and washings were titrated to pH 8.4 potentiometrically against standard HCl. The difference between the titre value for the blank and the sample, represented the total acidity.

$$Ba(OH)_2 + 2H^{+}Humate \longrightarrow Ba\ (Humate)_2 + 2H_2O$$

(b) Carboxyl Group (Calcium acetate Exchange Method)

A major portion of the total acidity of humic acid is believed to be attributable to carboxyl groups. The method was suggested by Bloom *et al.* (1957) and modified by Schnitzer and Khan (1972). 20mg humic acid was suspended in 50 ml of 0.1 M calcium acetate solution in an Erlenmeyer flask. The air in the flask was displaced with nitrogen and allowed to equilibrate for 24 h. A reagent blank (50 ml calcium acetate) was also maintained simultaneously. The suspension was filtered, and the residue thoroughly washed. The filtrate and the washings were potentiometrically titrated against standard NaOH. The difference between the titre values for sample and blank represented the carboxyl group.

$$2\ RCOOH + (CH_3COO)_2Ca \longrightarrow (RCOO)_2Ca + 2CH_3COOH$$

(c) Carbohydrate

Acid hydrolysis is often used to release carbohydrates from sediment and isolated humic acid. This method was first applied by Degens *et al.* (1964). Known weight of (10-20 mg) humic acid was hydrolysed with 20 ml of 1N H_2SO_4 at 100^0C 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).

(d) Phosphorus

The method adopted for the extraction of phosphorus from humic acid is that suggested by Nissenbaum (1979). In this method phosphorus analysis was carried out by exhaustive oxidation of humic acid with perchloric acid, followed by colorimetric estimation, (molybdenum-blue method). About 20-40mg of humic acid sample was accurately weighed and transferred to 250ml conical flask. 3 ml of concentrated HNO_3 was added to effect primary oxidation. Then 4 ml of 70% $HClO_4$ was added and digestion was carried out until the solution became colourless. After cooling, the solution was made upto 20 ml. Aliquots were taken and phosphorus was determined by using molybdenum blue method (Grasshoff, 1983).

(e) Total Metal

20 mg of powdered humic acid was digested with a 3:1 mixture of $HNO_3$and $HClO_4$. The digest was made up to 20 ml and trace metals were determined in the supernatant liquid by using AAS.

(f) Spectroscopic Studies

Eventhough spectroscopic methods, like all other investigational techniques have certain limitations when applied to humic acid, because of their complex nature, they are useful for determining the overall functionality in these materials. I.R spectroscopy is extensively used to identify the presence of functional groups.

Solid samples were used for I.R. spectral studies.

UV-VIS spectral measurements were performed on Hitachi 150-20 spectrophotometer. All measurements were made on solutions of humic acid dissolved in 0.5N NaOH. 0.5N NaOH was used as reference medium. Fluorescence spectra of humic acid samples were taken on a Hitachi spectrofluorimeter (F-3010). The excitation maxima was found out by scanning between 200-900 nm. In emission spectrum,the excitation maximum was fixed as excitation wave length. Spectra were taken at pH 5 to investigate the effect of pH on the spectra.

C. Sorption Studies

Adsorption studies were conducted to evaluate the significance of humic acid on metal adsorption by sediment. As both the substrates required for this evaluation, humic acid and the sediment, are in the solid phase and in the natural system the humic acid is an integral part of the sediment, mixing of the sediment with humic acid will never be identical with the natural systems. So in this study the role of humic acid was evaluated by a three step evaluation procedure. In the first step the substrate in the sediment itself. In the second step the alkali extractable organic matter is removed from the sediment using 0.5N NaOH and the sediment after the extraction was used as the adsorbent. In the third step purified humic acid was used as the substrate which was found to constitute about 50% of the alkali extract and about 90% of the ligand activity which is worked out from the percentages of adsorption and humic acid content of the sediments. Sediments used in the study are, (1) estuarine sediment collected from station number 4 (ES) and (2) sediment from same station left after alkali extraction (SAAE). SAAE was prepared as follows: sediment collected from station 4 was leached with 0.5N NaOH several times till alkali extract is almost colourless. The supernatant was removed and the residue was then washed thoroughly with distilled water. ES and SAAE were passed through a sieve of mesh size 250 μ to get uniform size. Two types of humic acid samples, estuarine humic acid (denoted as EHA) and terrestrial humic acid (designated as THA) were also used in the adsorption studies.

Influence of salinity on adsorption process was determined by conducting the experiments at different salinities. The method described in APHA (1985) was used for the preparation of synthetic sea water of salinity 35 x 10^{-3} (Table 2.1).

Table 2.1 The Composition and concentration of constituents in synthetic sea water of salinity 35X10^{-3}

Compound	Concentration (g/lit)
NaF	0.003
$SrCl_2.6H_2O$	0.020
H_3BO_3	0.030
KBr	0.100
KCl	0.700
$CaCl_2.2H_2O$	1.470
Na_2SO_4	4.000
$MgCl_2.6H_2O$	10.78
NaCl	23.50
$Na_2SiO_3.9H_2O$	0.020
$NaHCO_3$	0,001

Stock solutions (1000 ppm) of the metals, copper, cadmium, manganese, and zinc were prepared from 99.9 % pure metals (BDH Analar grade). The oxide coating on the surface of the metal was cleaned off using 0.1 N HNO_3 and the purified metal was dried using acetone. 1 g each of the metals was dissolved in minimum amount of 1:1 HNO_3. Any excess of acid present was carefully evaporated off, and diluted to 1 litre with double distilled deionised water. Stock solutions (1000 ppm) of mercury and lead were prepared from $HgCl_2$ and $PbNO_3$ respectively (BDH AnalaR). The stock solutions were then diluted to the concentrations required for the experimental studies.

(a) Adsorption studies

Adsorption Capacity

Using sediment and sediment left after alkali extraction

0.25 g each sediment was allowed to equilibrate with 20 ml of varying concentrations of each of the metals viz. copper, zinc, manganese, mercury, and lead (1,5,10,15 and 20 ppm) and of cadmium (0.5, 1,2,3,4, and 5 ppm) in acid washed 100 ml beakers with occasional shaking. Proper controls consisting of similar aliquots of the metal solutions, without the sediment samples and same amount of sediment without metal in the liquid phase, were also set to find whether there was any adsorption of metals by glasswares and any loss due to evaporation and any addition by desorption from sediment respectively. Duplicates were also run simultaneously.

After 24h, the suspensions were centrifuged and metal content in the centrifugate was determined in atomic absorption spectrophotometer (Perkin Elmer 2380). Mercury was estimated using AAS having an hydride generation system, MHS 10 as an accessory.

Salinity dependence

To evaluate the influence of salinity, experiments were repeated at salinities 0, 5, 10, 15 and 25 x 10^{-3}. Synthetic sea water was diluted with deionised double distilled water to get required salinity. 0.25 g of each sediment was allowed to equilibrate with solutions of copper, manganese, zinc, mercury and lead (1, 5, 10, 15 and 20 ppm) and cadmium (0.5, 1, 2, 3, and 4 ppm) in synthetic sea water of varying salinity. After 24 h , metal content in the supernatant was determined as in the above experiment.

(b) Using Humic Acids

10 mg of humic acid was suspended in 10 ml of metal

solutions of different concentrations (1, 2, 3, 4, 5 ppm) for 24 h with occasional shaking. After equilibration, the suspensions were centrifuged and the metal content in the liquid phase was estimated in AAS.

Salinity dependence

Salinity dependence was determined by repeating the experiments at salinities 0, 5, and 15 x 10^{-3}.

(b) Desorption Studies

Desorption studies were carried out to determine the nature of adsorption. Four different extractants, $CH_3COO\ NH_4$ (2N), HCl (0.5N), and EDTA (0.05M) and NaH_2PO_4 (0.01M) were used in desorption studies. 4 g of sediment and 200 mg of humic acid were equilibrated with 50 ml solution (50 ppm) and 20 ml (25 ppm) each metal solutions respectively for 24 h and then centrifuged. Residue was then washed with deionised double distilled water and dried. These sediment and humic acids were used for the desorption studies. 0.25 gm sediment was treated with 20 ml of each of the four different extractants in 100 ml beaker. Similarly 10 mg of humic acid was suspended in 10 ml of each of the four extractants. After 24 h the suspensions were centrifuged and the metal content in the extractant was estimated in AAS.

(c) Adsorption Kinetics

Kinetic studies were conducted at time intervals, 0, 10, 20, 40, 60, 120 minutes and 24 hour.

Using sediments

0.25g of each sediment was suspended in 20 ml of each of the six metal solutions of concentrations 5,and 15 ppm in distilled water (salinity 0 x 10^{-3}). These suspensions along with duplicates were kept aside for different time intervals 0, 10, 20, 40, 60, 120 minutes and 24 h. At the expiry of each designated time interval, the respective solutions were centrifuged and the amount of the metal in the centrifugate was analysed in AAS.

Experiments were repeated at salinity 15 x 10^{-3}.

Using Humic Acids.

Adsorption kinetic studies were carried out in distilled water using 10 mg of humic acid and metal solutions of concentration 1 and 5 ppm at the end of time intervals, 0, 10, 20, 40, 60, 120, minutes and 24 h. At the end of each time interval, the suspensions were centrifuged and the metal content in the centrifugate was determined by using AAS.

Experiments were repeated at salinity 5 x 10^{-3}.

Results of Hydrographic Parameters and Sediment Characteristics

The study of the general hydrographic parameters has great importance in characterizing the general features of an estuarine system. The hydrographical conditions in an estuary mainly depend on the intrusion of sea water associated with tides and influx of freshwater from rivers, precipitation and evaporation processes and also weather. In addition, the bottom topography and geographical condition in the estuary exert a profound influence on the hydrographical condition of the estuary. The sedimentary characteristics are more or less governed by the hydrography of the overlying water.

The hydrography of the Cochin estuary has been well documented (Qasim and Reddy, 1967; Qasim *et al.*, 1968;

Sankaranarayanan and Qasim, 1969; Josanto, 1971; Haridas *et al.*, 1973; Shynamma and Balakrishnan, 1973; Lakshmanan *et al.*, 1982; Sankaranarayanan *et al.*, 1986; Anirudhan, 1988; Babukutty, 1992; *Nair, 1992; Nair, 1992; Shibu, 1992). Although the information available gives seasonal variations of temperature, salinity, dissolved oxygen, pH, alkalinity and nutrients of the region as whole, the study on hydrography is essential for explaining present estuarine conditions.

Hydrographic parameters (dissolved oxygen, salinity and pH) of the collection sites are given in (Table 2.2). Salinity has been considered as an index of the estuarine mixing processes and the tidal effects. Stations 1, 2, and 7 exhibited freshwater characteristics. Highest values of salinity (26.9 x 10^{-3} and 28.18 x 10^{-3}) were recorded for surface and bottom waters at station 4.

Dissolved oxygen is the most valuable water quality parameter in assessing water pollution. It is also important since the existence of aquatic life is intimately linked with the availability of oxygen for their survival. The amount of dissolved oxygen in natural waters depend on various factors such as temperature, partial pressure and salinity. Dissolved oxygen may be helpful to explain the various physical, chemical and biological processes taking place in the estuarine system (Desousa and Sen Gupta, 1986).

During August dissolved oxygen values ranged from 3.96-5.85 ml/lit at surface and 4.61-5.96 ml/lit at the bottom. The values decreased as the season advanced i.e., on January. This may be attributed to high salinity and temperature and due to the utilization of oxygen for the degradation of organic matter.

Many of the processes in the aquatic system ~~are~~ depend on the pH of the surrounding medium. The pH of the medium varies with factors like photosynthetic activity, rainfall, nature of dissolved materials, discharge of effluents etc. Therefore

Table-2.2. Hydrographical parameters (Salinity, Dissolved oxygen and pH)

PARAMETERS	MONTHS		STATIONS						
			1	2	3	4	5	6	7
SALINITY (‰)	MAY	S	0	0	2.47	3.42	0.56	0.83	0
		B	0	0	3.75	8.85	0.83	0.17	0
	AUGUST	S	0.04	0.04	1.46	2.45	0.61	0.14	0.11
		B	0.04	0.04	1.73	4.07	0.51	0.08	0.08
	JANUARY	S	0.16	0.80	23.70	26.90	20.41	6.68	0
		B	0.41	1.18	24.34	28.18	26.90	15.62	0
DISSOLVED OXYGEN ml/l	MAY	S	4.43	4.93	4.09	3.65	2.54	4.09	4.81
		B	5.34	3.70	3.98	3.32	2.82	4.21	4.98
	AUGUST	S	5.85	4.83	4.95	4.27	4.72	3.94	5.85
		B	5.85	5.73	5.28	4.61	5.06	5.51	5.96
	JANUARY	S	5.13	5.14	3.32	3.59	4.16	3.19	4.62
		B	4.60	5.14	3.50	3.72	4.74	4.96	4.21
pH	MAY	S	7.04	6.97	7.00	7.02	7.01	6.94	7.05
		B	7.05	6.97	7.01	7.01	7.09	7.04	7.02
	AUGUST	S	7.00	7.10	8.00	7.50	6.80	6.70	7.80
		B	7.00	7.15	7.60	7.60	6.80	6.60	8.00
	JANUARY	S	7.68	7.56	7.53	7.56	7.37	7.36	6.45
		B	7.42	7.17	7.52	7.71	7.31	7.15	6.58

S-surface, B-Bottom

measuring of pH values is essential for monitoring water quality. During May, August, and January pH values in all stations remained around 7. Station 2 during May and station 5 and 6 during August and station 7 during January exhibited a lower pH values. This decrease may be due to acidic effluents from nearby industrial units.

Sediment acts as a source and sink for many chemical components in an aquatic system. Biological availability of sediment components plays a vital role in regulating the ecology of the aquatic ecosystem. Monitoring of sediment characteristics is essential for predicting the features of aquatic ecosystem.

Grain size is one of the basic characteristics of sediments and its determination is necessary to delineate the sedimentary environments. The concentration of carbon, nitrogen and phosphorus in the water and the sediment depends upon the texture of the sediment. The nature of sediment varied from station to station. Seasonal variation of grain size at each station was minimum. The riverine region composed of sandy sediment during the three months whereas stations 3 and 4comprised of sand-slit-clay. Sand represents particles of size greater than 63μ, silt indicates size between 63μ and 4μ clay includes particles less than 4μ. Texture analysis indicated that sediments of the estuarine region were composed of fine grained sediments whereas those of riverine region made up of sand.

The biogeochemical cycle of organic carbon and its distribution regulate the aquatic ecosystem. Higher carbon content was observed at all stations except station 1 and 2 (Table 2.3). The maximum value of SOC (71.44mg/g) observed in station 6 during August may be due to the texture of the sediment as well as to incorporation of retting coconut husks.

Phosphorus is a limiting nutrient in primary production and play a vital role in the eutrophication processes (Golterman, 1975). Biogeochemistry of phosphorus in an estuary is controlled by physico-chemical as well as biological process. The studies on distribution of sediment phosphate in the Cochin estuary (Ansari and Rajagopal, 1974; Remani *et al.*, 1981; Reddy and Sankaranarayan,1972; Anirudhan, 1988) showed a marked seasonal variation influenced by local precipitation and land runoff. Lower values of phosphorus was observed in southern region. Among the seven stations, station 7 showed a maximum value of total phosphorus i.e., 8.39, 7.31 and 6.39mg/g in May, August and January respectively. This may be due to the effluent discharging from FACT. Total phosphorus content in other stations during three months was within the range 0.17-2.81mg/g. No significant seasonal variations in total phosphorus content in stations 1, 2 and 4 was observed. In station 3 values are 0.69, 1.93 and 0.62mg/g, in station 5, 1.92, 2.81 and 0.77mg/g and in station 6, 1.5, 2.37 and 1.22mg/g during May, August and January respectively. Lower values of total phosphorus observed in the sediments of southern part of the estuary may be due to the leaching of the phosphorus from the mud.

Humic matter which consists of the complex, heterogeneous brown or yellow acidic polymers is a relatively stable by product of the microbial transformation of great variety of biochemical compounds derived from the dead cells and extra cellular products of various organisms- mainly land plants and aquatic algae.

The concentration of sedimentary humic acid at station 1-7 are givenin Table 2.3 (Nair, 1992). Sediments from estuarine region showed higher amount of humic acid than that from riverine region and also higher values of humic acid was noticed at all stations during May (3.24-17.00 mg/g). The concentration of humic acid is influenced by particle size of the sediments. The estuarine sediments are composed of sand -silt - clay with high percentage of clay. Bader *et al.* (1960) established that clay particles adsorb higher amounts of organic matter.

Table.2.3. Sediment Characterstics.

PARAMETERS	MONTHS	STATIONS						
		1	2	3	4	5	6	7
SEDIMENT ORGANIC CARBON (mg/g)	MAY	6.13	5.32	11.73	27.30	29.24	20.00	39.48
	AUGUST	0.63	4.72	29.28	23.40	31.93	71.44	46.64
	JANUARY	1.85	3.33	9.88	25.48	34.18	20.90	30.68
TOTAL PHOSPORUS (mg/g)	MAY	0.26	0.27	0.69	1.64	1.92	1.50	8.39
	AUGUST	0.17	0.24	1.93	1.57	2.81	2.37	7.31
	JANUARY	0.19	0.32	0.62	1.76	0.77	1.22	6.39
CARBO-HYDRATES (mg/g)	MAY	2.26	1.00	2.10	4.00	5.51	4.00	0.33
	AUGUST	0.53	2.00	8.18	4.00	10.64	18.00	13.74
	JANUARY	1.71	3.00	3.01	6.00	8.25	5.00	9.00
HUMIC ACID (mg/g)	MAY	3.64	3.24	11.4	17.00	16.08	7.97	11.50
	AUGUST	0.34	2.82	11.41	11.01	10.48	4.02	6.61
	JANUARY	1.09	5.27	4.51	8.03	6.95	6.33	6.05

CHAPTER III

PHYSICO-CHEMICAL CHARACTERISTICS OF HUMIC ACIDS

Humic substances are biogenic refractory, yellowish black organic substances that are ubiquitous, occurring in all terrestrial and aquatic environments. Their role in diverse environment is important because they serve as a major reservoir of organic carbon in soils, and sediments and water for a global water cycle (Nissenbaum and Kaplan, 1972). Humic substances are also involved in chelation of trace metals of biological importance (Siegel, 1971; Lewis and Cave, 1981) and also in growth regulation of terrestrial and aquatic organisms (Prakash and Rashid, 1968; Prakash *et al.*, 1973).

The first attempt to isolate humic substances from soils was made by Achard during 18th century (1786), who extracted peat with alkali and obtained a dark amorphous precipitate on acidification.

The structure of humic substances was of considerable interest to coal soil scientists as well as to water chemists for many years (Mantoura and Riely, 1975; Weber and Wilson, 1975; Schnitzer and Khan, 1978; Gillam and Wilson, 1983). At the early stages itself, it was established that these materials contain acidic functional groups, but until recently little detailed information was known concerning the amount of various forms of carbon in humic substances. The attempts in this direction includes functional group analysis, pyrolytic studies, and degradation studies (Perdue *et al.*, 1980; Abdul-Halim *et al.*, 1981; Mikita *et al.*, 1981; Reuter and Perdue, 1981; Saleh *et al.*, 1983; Wilson *et al.*, 1983; Yamaoka, 1983 a, b; Hayase and Tsubota, 1985; Poutanen and Morris, 1985; Sohn and Weese, 1986; Szpakowska *et al.*, 1986; Newman *et al.*, 1987; Shinozuka and Hayano 1987; Filip *et al.* , 1988; Gadel and Torri, 1988; Sardessai, 1989; Grasso *et al.*, 1990). However, studies on these materials have been hindered by the difficulty in isolating them in sufficient quantities for extensive chemical investigation. Though recently with the advent of new spectral techniques like ^{13}C NMR, ^{1}H-NMR etc., a few efforts on elucidation of the structure are reported (Ruggiero *et al*., 1979a, b; Mikita *et al* 1981; Wilson *et al*., 1983; Poutanen and Morris, 1985; Newman *et al*., 1987; Calender *et al*., 1988; Grasso *et al*., 1990). The structural details are still at infancy. The main objective that confronts researchers in this field today is to develop a valid concept of their chemical structures.

Spectroscopic investigations of lignin, humic substances, and peat carried out by Ziechmann (1964) established an inter-relationship, along with certain difficulties, on the basis of physical and chemical properties of the material.

Eventhough studies on the structure of humic acids were on retardation by the lack of suitable degradation procedures, Burges *et al*.(1964) carried out studies on phenolic constituents of humic acid and their relation to lignin. A relatively mild degradation under reducing conditions liberated upto 30% ether soluble material from soil humic acids and lignin. Phenolic and phenolic acid monomers in the ether soluble fraction were identified by thin layer chromatography. Vanillic, syringic, p - hydroxy benzoic, guaiacyl, and syringyl propionic acids were identified. The contribution of lignin from overlying vegetation to the formation of humic acid was demonstrated by the presence of relatively unchanged lignin residues.

Chemical study of humic acids in Western Pacific bottom sediments was carried out by Vasilevskaya *et al*.(1977). The oxidative degradation studies with alkaline nitrobenzene and potassium permanganate followed by chromatograph analysis of the products gave p-coumaric, guaiacilic, and syringilic structural units, typical of the lignin of terrestrial plants. They suggested that polysubstituted and polycondensed aromatic systems with a minor proportion of aliphatic structure are the basic structural units of humic acid of abyssal sediments. But

Stuermer and Harvey (1977) indicated that lipids are the important structural components of fulvic acid isolated from Sargaso sea and coastal waters, and that terrestrial sources are minor even in coastal samples. Also a series of aromatic structures was obtained.

Nissenbaum (1979) estimated phosphorus content of marine and non-marine humic substances. The phosphorus content of marine humic acid was in the range of 0.1-0.2 % whereas marine fulvic acid phosphorus content was 0.4-0.8 % . Soil humic acid resembled marine humic acid in phosphorus content and soil fulvic acids were three to five fold enriched in phosphorus relative to humic acid.

A simple analytical procedure was described by Lawrence (1979) for the simultaneous semi quantitative determination of fulvic acid, tannin, and lignin by UV spectrophotometry. The absorbance was measured at three different wavelengths and concentrations were calculated using an expanded form of Beer's law. The best results were obtained by measuring the absorbance at definite wavelengths between 280 and 400 nm and computing the concentrations for each combination of three wavelengths. This multiple wavelength approach has the advantage of detecting the presence of other interfering compounds.

Mikita *et al.*(1981) reported a method for characterization of hydroxyl functional group in humic substances using ^{13}C enriched-methylating reagents utilizing ^{13}C NMR spectroscopy. Carboxyl, aromatic- *o*-alkyl structures were recognised in humic acid isolated from fresh water of Lake Celyn by Wilson *et al.* (1981) using ^{13}C NMR spectroscopy. The results showed that 24 % Lake Celyn humic acid was carboxyl and 40 % was aromatic. The high proportion of aromatic carbon indicates the Lake Celyn humic acid was largely formed from terrestrial surrounding peaty water shed.

The molecular size distribution of humic substances was investigated in two sediment samples of different ages by Cronnin and Morris (1982) and showed that near surface younger samples contained predominantly high molecular weight humic acid and little fulvic acid, while, the deeper older samples contained relatively less humic acid and more fulvic acid. Riffaldi *et al.* (1982) investigated the distribution and composition of humic and fulvic acids in ten sewage sludges from various treatment plants. The results showed that only about 17% total organic carbon was represented by humic carbon, with a great variability in the carbon content of the humic fractions. The low content of carbon and functional groups and the low E_4/E_6 ratio revealed a relatively high degree of aromatic condensation.

Gillam and Wilson (1983) studied the structural group analysis of humic substances including determinations of aromaticities of humic substances by ^{13}C NMR spectroscopy. Harvey *et al.* (1983) isolated humic and fulvic acids from near surface water samples from five biologically diverse sites for detailed NMR and chemical studies. The PNMR spectra of all humic and fulvic acid studies were similar and differed mainly from with published spectra and the chemical data suggested a class structure and general mechanism of formation of marine humic substances. The proposed structures were a cross linked, auto-oxidized, polysaturated fatty acids.

The structure of humic acid from a New Zeland pasture top soil was studied by Wilson *et al.* (1983) using ^{1}H NMR spectroscopy. The result showed that the proton aromaticity of the humic acid (exchangeable protons) was 17% . Considerable amounts of protons in the humic acids were associated with carbohydrates (35%).

Analysis of humic substances by means of I.R spectroscopy, elemental analysis and CP MAS ^{13}C NMR spectroscopy, and pyrolytic GC MS experiment by Peschel and Wildt (1988) revealed that anthropogenous humic substance from the running of biological waste water treatment plants were very similar to autochthonous aquatic humic materials isolated from river waters, but were quite different from terregenous humic materials. The similarities were caused by the strong influence of carbohydrates and proteins which are the precursors of anthropogenous and autochthonous humic substances.

Sardessai (1989) conducted studies on humic and fulvic acids in sediments of Hooghly estuary and some coastal area in northern Bay of Bengal and reported that humic acid fraction was the major fraction in riverine system whereas fulvic acid constituted major fraction of humic substance in estuarine system.

Estuarine sediment is a complex biological, chemical, and physical system. However, the sediment serves as an important sink for many substances that enter an estuarine environment. The organic matter in the sediment consists of detritus derived from terrestrial (allochthonous) and aquatic biota (autochthonous) and it is considered that the detritus is modified by microbial activity both before and after deposition in the aquatic environment. The diversity of these sources and processes is undoubtedly responsible for the characteristics of humic acid in these sediments. So the characterization of the humic acid is very important since they are concerned with many geochemical processes. Distribution of biogeoorganic compounds in Cochin estuarine sediments has been reported (*Nair, 1992) but no work has yet been reported on the physico-chemical properties of humic acid.

Available data suggest that structurally, the three humic fractions (humic acid, fulvic acid and humin) are very similar to each other, but they differ in molecular weight, elemental analysis and functional group content (Schnitzer and Khan, 1972). Present chapter deals with the analysis of humic acid samples present in the sediments of the Cochin estuary with respect to the acidic functional groups, phosphorus and carbohydrates and the spectral characteristics (UV-VIS, fluorescence and I.R).

Materials and Methods

Details of the various methods employed in the present chapter are given in Chapter II.

Results and Discussion

Acidic Functional Groups

The results of the acidic functional groups analyses are given in Tables 3.1 and 3.2. In the present study, there is no considerable variations in total acidity values of humic acids between estuarine and freshwater samples (Table 3.1, Fig.3.1). The values ranged from 6.82 to 8.76 milli equivalents/g in May. During August and January except for a few observations, the total acidity values remained without any significant variations. During August, stations 2 and 4 recorded high values of 12.07 and 11.57 milli equivalents/g respectively. Similarly during January stations 5 and 6 recorded 12.11 and 12.09 milli equivalents/g. Terrestrial and coastal humic acid possess total acidity values 9.31 and 6.92 milli equivalents/g respectively.

In the case of carboxylate acidity, the northern arm of the estuary always recorded a high value when compared to southern arm (Table 3.2, Fig.3.2). In May the values ranged from 3.55.to 4.94 milli equivalents/g in northern arm and 2.33 to 4.46 milli equivalents/g in the southern arm. During August carboxylate acidity values ranged from 1.17 to 2.67 milli equivalents/g of humic acid and its value in the northern arm was higher than that of southern arm. But there was no significant variations in carboxyl group during January except in station 2, where the value ranged from 1.04 to 1.87 milli equivalents/g. The coastal sample showed high carboxyl content (5.13 milli equivalents/g), than estuarine and terrestrial samples. Carboxyl acidity value of terrestrial humic acid was 1.49 milli equivalents/g of humic acid.

Table.3.1. Total Acidity of Humic acid from different Stations in Cochin estuary (milliequivalents/g).

MONTHS	STATIONS								
	1	2	3	4	5	6	7	8	9
MAY	8.55	8.18	6.82	8.38	8.29	8.76	7.71	9.31	6.92
AUGUST	12.55	12.07	7.31	11.57	7.10	6.49	5.60		
JANUARY	9.83	8.15	7.05	8.97	12.11	12.09	9.92		

Table.3.2. Carboxylate Acidity of Humic acid from different Stations in Cochin estuary (milliequivalents/g)

MONTHS	STATIONS								
	1	2	3	4	5	6	7	8	9
MAY	4.46	3.70	2.59	2.34	3.96	4.94	3.55	1.49	5.13
AUGUST	2.11	1.42	1.26	1.27	2.31	2.17	2.67		
JANUARY	1.44	3.22	1.04	1.32	1.40	1.47	1.87		

Station 8-Terrestrial HA, Station 9-Coastal HA

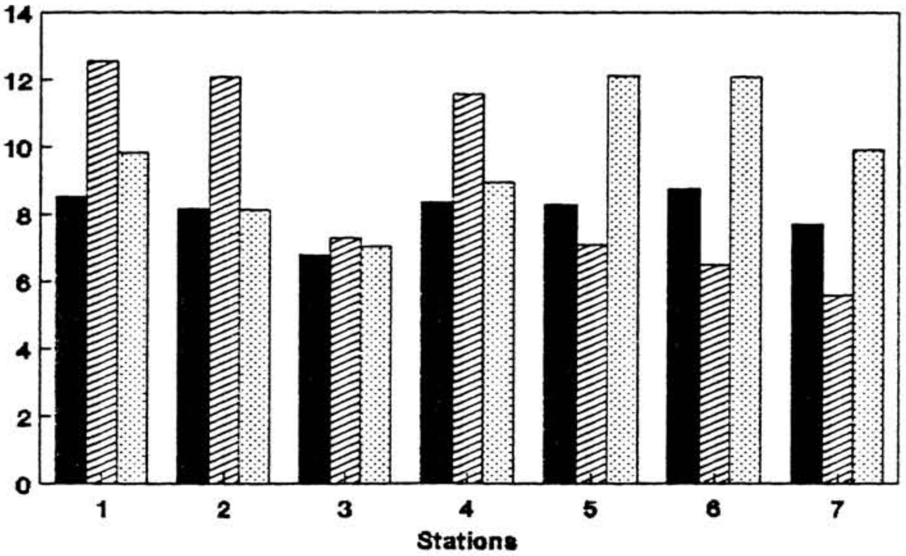


Fig.3.1 Stationwise variations of total acidity of humic acid (milli equivalents/g)

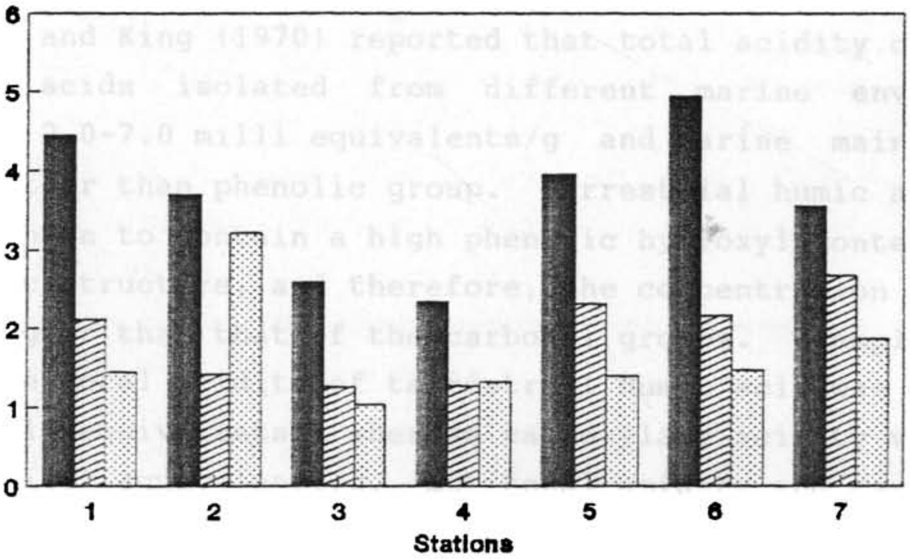


Fig.3.2 Stationwise variations of carboxylate acidity of humic acid (milli equlivalents/g)

■ May ▨ August ░ January

Humic materials originate by biochemical transformations followed by incorporation of plant and animal tissues into the sediment. Most of the materials on account of their fairly high content of phenolic and carboxyl groups are acidic in nature. Analysis of acidic functional groups increases our understanding of the chemical structure of humic acid and can be used to explain the behaviour of humic acid in various humification processes (Gjessing, 1976). Results of the present study coincides with the results of the earlier studies (Schnitzer and Gupta, 1964; Rashid and King, 1969; Volk and Schnitzer, 1973; Schnitzer, 1977). The humic acids from a podozol soil showed an acidity of 5.6-5.7 milli equivalents/g (Wright and Schnitzer, 1960). However, Schnitzer and Gupta (1965) have reported a total acidity of 8.7-8.9 milli equivalents/g for humic acids of grey wooded soils. These published results suggest that the humic acids of well developed soils possess considerably higher acidity than does marine humic acid.

Rashid and King (1970) reported that total acidity of humic and fulvic acids isolated from different marine environment ranged from 2.0-7.0 milli equivalents/g and arise mainly from carboxyl rather than phenolic group. Terrestrial humic acid has long been known to contain a high phenolic hydroxyl content with its aromatic structure, and therefore, the concentration of this group is higher than that of the carboxyl groups. Complimentary to this ,the total acidity of terrestrial humic acid was found to be 9.31 milli equivalents/g whereas carboxylate acidity value is only 1.49 milli equivalents/g. But total acidity and carboxylate acidity values of coastal sample was observed to be 6.91 and 5.13 milli equivalents/g respectively.

Total acidity of organic matter is a function of the activity of carboxyl and phenolic hydroxyl groups. Therefore phenolic hydroxyl groups were quantified by the difference between the total acidity and carboxylate acidity. Here terrestrial and estuarine humic acids possessed low carboxylate acidity and the acidity associated with these samples were mainly due to phenolic hydroxyl groups whereas, in coastal samples most of the acidity arise from carboxyl group. Similar results were reported by Rashid and King (1970). The relative proportions of carboxylic and phenolic functional groups are used as one of the indicators of the degree of humification or evolution of the humic matter.

Carbohydrates

Variations of humic carbohydrates in the estuarine stations during May, August and January, are given in Table.3.3 and Fig.3.3. Carbohydrate content recorded high values in January. In May, values ranged from 64.20 to 93.37 µg/g; in August 67.65 to 146.79 µg/g; and in January 83.77 to 151.95 µg/g. Humic acid bound carbohydrate in terrestrial and coastal humic acids were 92.22 and 93.69 µg/g respectively. Carbohydrates associated with terrestrial as well as coastal humic acids were comparable with those of estuarine humic acid.

Carbohydrates are polyhydroxy aldehydes or ketones or compounds that can be hydrolysed to these compounds. Carbohydrates which are the most abundant class of compounds produced in the biosphere form a fraction of humic acid. Data of humic carbohydrates provide information on the biological input to the sediment and the diagenesis of carbohydrates in terrestrial and aquatic environment.

Carbohydrates in humic and fulvic acids from Hiroshima Bay sediments have been analysed by gel filtration, and found that carbohydrates were more abundant in fulvic acid than in humic acid (Yamaoka, 1983a). The compositional differences in the content of carbohydrates in humic and fulvic acids were greater in top section of 0-20 cm than 50-70 cm of the sediment core sample. Yamaoka (1983b) also carried out similar studies on the vertical distribution of carbohydrates in humic and fulvic acids from sediments of Suo Sound, Japan by gas chromatography and Sephadex gel chromatography. Fulvic acid carbohydrates decreased

Table.3.3. Carbohydrate associated with Humic acid from different Stations in Cochin estuary (µg/g)

MONTHS	STATIONS								
	1	2	3	4	5	6	7	8	9
MAY	84.63	70.23	67.38	64.27	93.37	91.50	80.88	92.22	93.69
AUGUST	131.71	78.92	67.64	121.52	107.26	105.89	146.79		
JANUARY	111.42	83.77	96.00	125.36	137.91	131.71	151.95		

Table.3.4. Total Phosphorus associated with Humic acid from different Stations in Cochin estuary (µg atom/g)

MONTHS	STATIONS								
	1	2	3	4	5	6	7	8	9
MAY	50.17	56.78	40.29	35.66	35.15	33.99	37.31	39.91	10.23
AUGUST	44.33	35.25	44.78	41.37	50.49	44.69	29.91		
JANUARY	72.32	60.33	65.17	50.19	48.29	30.83	42.68		

Station 8- Terrestrial HA, Station 9- Coastal HA

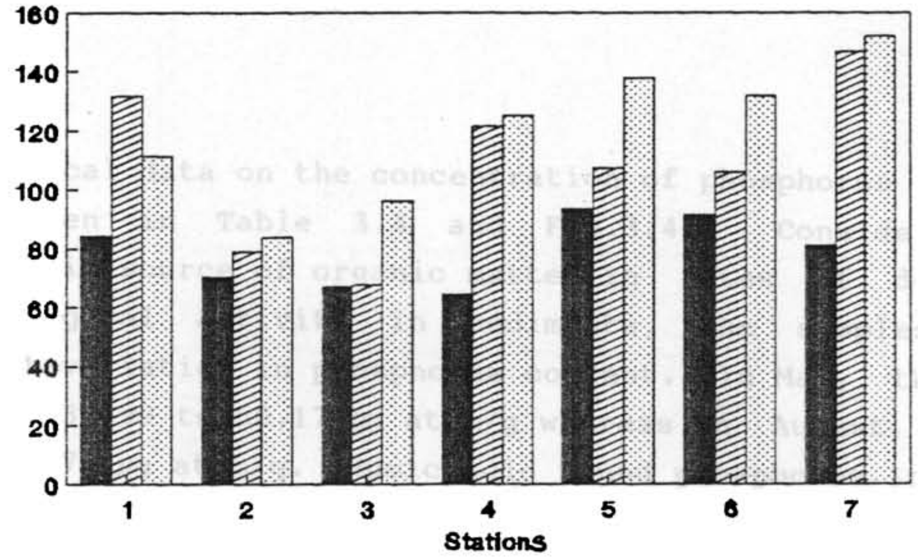


Fig.3.3 Stationwise variations of carbohydrates in humic acid (µg/g)

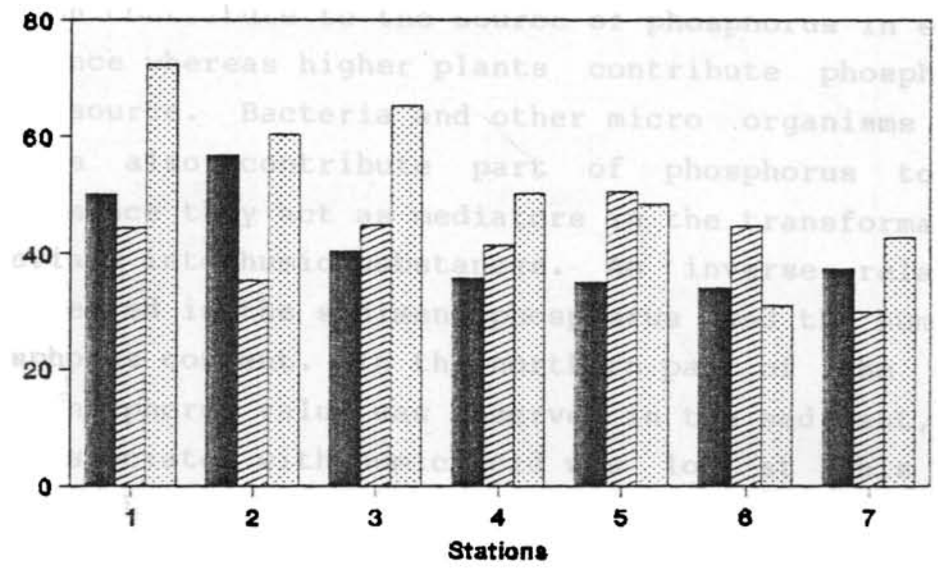


Fig.3.4 Stationwise variations of total phosphorus in humic acid (µg atom/g)

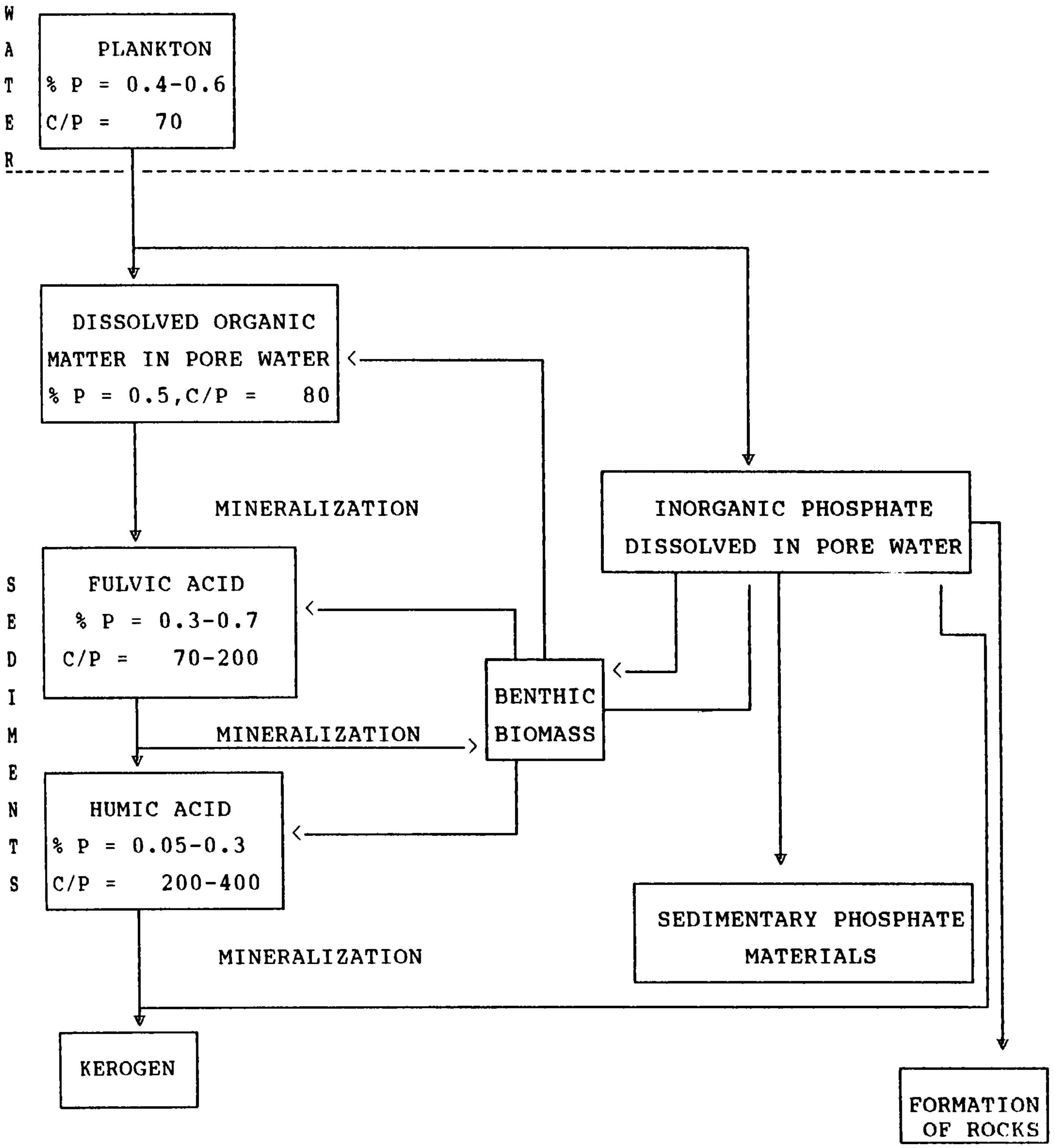
■ May ▨ August ░ January

with increasing depth. A decrease of humic carbohydrate was observed between the surface layers up to 20 cm depth, while below 20 cm there was no change in carbohydrate.

Phosphorus

Analytical data on the concentration of phosphorus in humic acids is given in Table 3.4 and Fig.3.4. Considering the differences in source of organic matter in rates of deposition and in biological activity in sediments, the samples showed significant variation in phosphorus content. In May, the value ranged from 33.99 to 50.17 μg atom/g whereas in August, it was 29.91 to 61.78 μg atom/g. Humic acid bound phosphorus in January was higher than that in August and May except in humic acid from stations 5 and 6. Phosphorus content of terrestrial humic acid was 39.90 μg atom / g which was within the range of estuarine humic acid. But coastal sample recorded a value of 10.26 μg atom /g lower than that of terrestrial and estuarine samples.

Plankton contribute to the source of phosphorus in estuarine humic substance whereas higher plants contribute phosphorus in terrestrial source. Bacteria and other micro organisms present in sediments also contribute part of phosphorus to humic substances, since they act as mediators in the transformation of plant materials into humic substances. An inverse relationship could be observed in the sediment phosphorus and the humic acid bound phosphorus content. In the northern part of the estuary, the higher phosphorus value was observed in the sediment, whereas phosphorus associated with humic acid was low at this region. Also sediment phosphorus was higher during August while humic acid phosphorus showed a maximum value during January. Phosphorus content of the marine and non-marine humic acids was estimated by Nissenbaum (1979)and found to be in the range of 0.1-0.2% whereas marine fulvic acid phosphorus content was 0.4-0.8%. Soil humic acid resembled marine humic acid in phosphorus content i.e., phosphorus associated with terrestrial and estuarine samples was comparable while coastal humic acids contained comparatively lesser amount of phosphorus.



A schematic representation of behaviour of phosphorus during the humification in marine sediments is given above. This generalised scheme on the formation and diagenesis of sub aquatic (marine and lacustrine) humate is based on chemical and isotopic evidence (Nissenbaum and Kaplan,1972; Kemp and Mudrochova,1973; Nissenbaum,1974; Krom and Sholkovitz, 1977).

The potential importance of the humic phosphorus is also enhanced by its geologically transient nature since it may constitute an intermediate reservoir which could generate digenetic transformation of geologically important deposit of phosphorites.

Total Metals

Table 3.5 and Fig.3.5 give the heavy metal levels in isolated humic acids. Very small amounts of copper, manganese and zinc were present in all samples, while cadmium and lead were found to be absent. Estuarine humic acid possessed higher metal content than coastal and terrestrial humic acids. During May and August, the amount of copper was higher than that of manganese and zinc in humic acids from the southern part of the estuary.

Spectral Studies

UV-VIS, Fluorescence and I.R spectral studies of terrestrial, estuarine and coastal samples were conducted.

UV-Visible

UV-VIS spectra of terrestrial, coastal and estuarine humic acids were featureless. Absorbance increased with decrease in wavelength. UV-VIS spectra of all samples showed similarity in spite of differences in elementary composition. Similar observation has been reported by earlier workers (Kumuda, 1955; Zeichmann, 1964; Butler and Ladd, 1969; Schnitzer, 1971, 1978; Schnitzer and Khan,1972; Stevenson, 1982).

Table 3.5. Total metal in Humic acid from different Stations in Cochin estuary (mg/g)

MONTH	METAL	STATIONS						
		1	2	3	4	5	6	7
MAY	Cu	0.061	0.042	0.051	0.142	0.223	0.101	0.030
	Mn	0.013	0.054	0.040	0.040	0.054	0.013	0.034
	Zn	0.019	0.028	0.034	0.047	0.348	0.107	0.058
AUGUST	Cu	0.081	0.081	0.162	0.101	0.162	0.081	0.020
	Mn	0.040	0.027	0.027	0.013	0.020	0.027	0.013
	Zn	0.047	0.013	0.011	0.036	0.226	0.023	0.030
JANUARY	Cu	0.081	0.122	0.041	0.122	0.081	0.142	0.061
	Mn	0.013	0.108	0.013	0.067	0.027	0.027	0.000
	Zn	0.107	0.146	0.026	0.159	0.168	0.271	0.064

	Cu	Mn	Zn
TERRESTRIAL	0.020	0.054	0.030
COASTAL	0.020	0.027	0.019

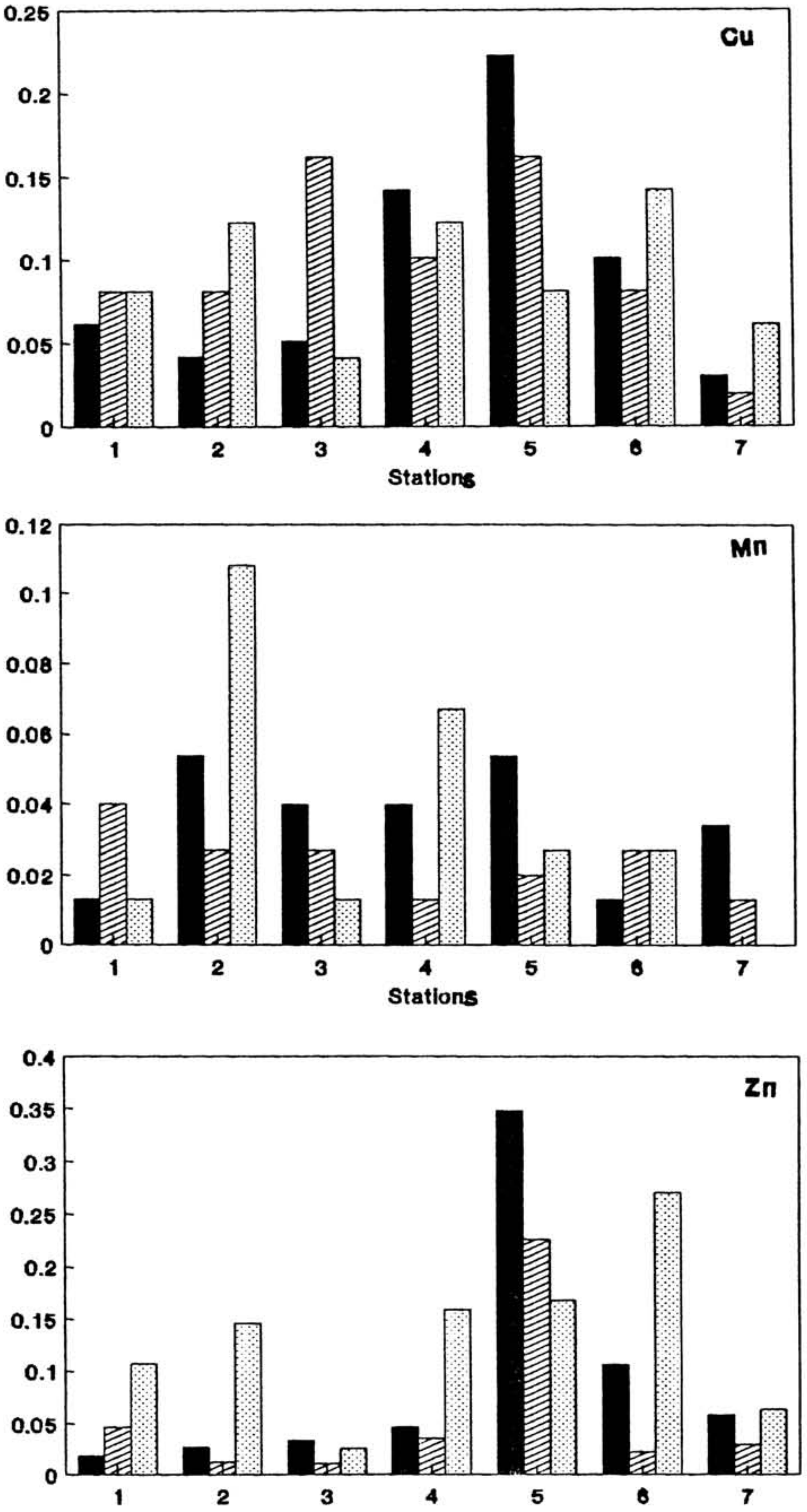


Fig.3.5 Stationwise variations of total metal in humic acid (µg/g)

Fluorescence

Fluorescence excitation spectra of different humic acid samples, at slight alkaline condition (pH 9) were more or less identical with an excitation maxima at 362, 364 and 363 nm for estuarine, coastal and terrestrial humic acid respectively. On scanning, the emission spectrum at respective excitation maxima, estuarine and coastal samples showed a narrow range peak at 417 nm and fluorescence emission peak at 462 nm and 461 nm whereas in terrestrial soil humic acid samples, emission maxima occurred at 456 nm. The detailed investigation with respect to the quantitative assessment of humic acid indicated that the narrow range peak observed can not be considered as characteristic of the humic acid (Table 3.6, Fig.3.6). Fluorescence maxima varied with pH, i.e., as pH decreases emission maxima also decreases. At lower pH emission, maxima shifted from 462 to 453.8, 461 to 449.6 and 456 to 453 nm in estuarine, coastal and terrestrial samples respectively.

Estuarine and coastal humic acid samples had fluorescence at higher wave length than soil humic compounds. No consistent effect of season on excitation and emission spectra could be observed.

For a molecule to fluorescence, it must possess chromophores which absorb the incident radiation. Compounds containing conjugated double bonds or aromatic rings give rise to fluorescence. Groups capable of donating electrons, such as - OH or $-NH_2$ enhance fluorescence. Electron accepting groups such as -COOH, tend to diminish fluorescence in aromatic compounds (Wehry and Rogers, 1966).

Humic substances exhibit fluorescence property (Seal *et al.*, 1964; Schnitzer, 1971; Flaig *et al.*, 1975; Hayes and Swift, 1978; Stevenson, 1982) which is a result of the poly functional characteristics of humic acid. This property can be made use of in the estimation of humic acid by scanning fluorescence

Table.3.6. Wavelength of Maximum Excitation and Emission in the Fluorescence Spectra of Different Types of Humic Acids

Type of Humic Compound	Wavelength Maximum Excitation Band (nm)	Wavelength Emission Maximum (nm)	References
Soil humic acids			
Various origins	470	500-540	Datta *et al.*, 1971
Black cotton soil		500-540	Seal *et al.*, 1964
Chernozem	465		Ghosh and Schnitzer, 1980
Podozol	335	440	Muller-Wegener, 1977a
Swamp	~360	460	Muller-Wegener, 1977b
Swamp		~460	Kleist and Mucke, 1966
Aquatic humic acids	270	460	Brun and Milburn, 1977
Present study			
Esturaine	362	462	
Coastal	364	461	
Terrestrial	363	456	

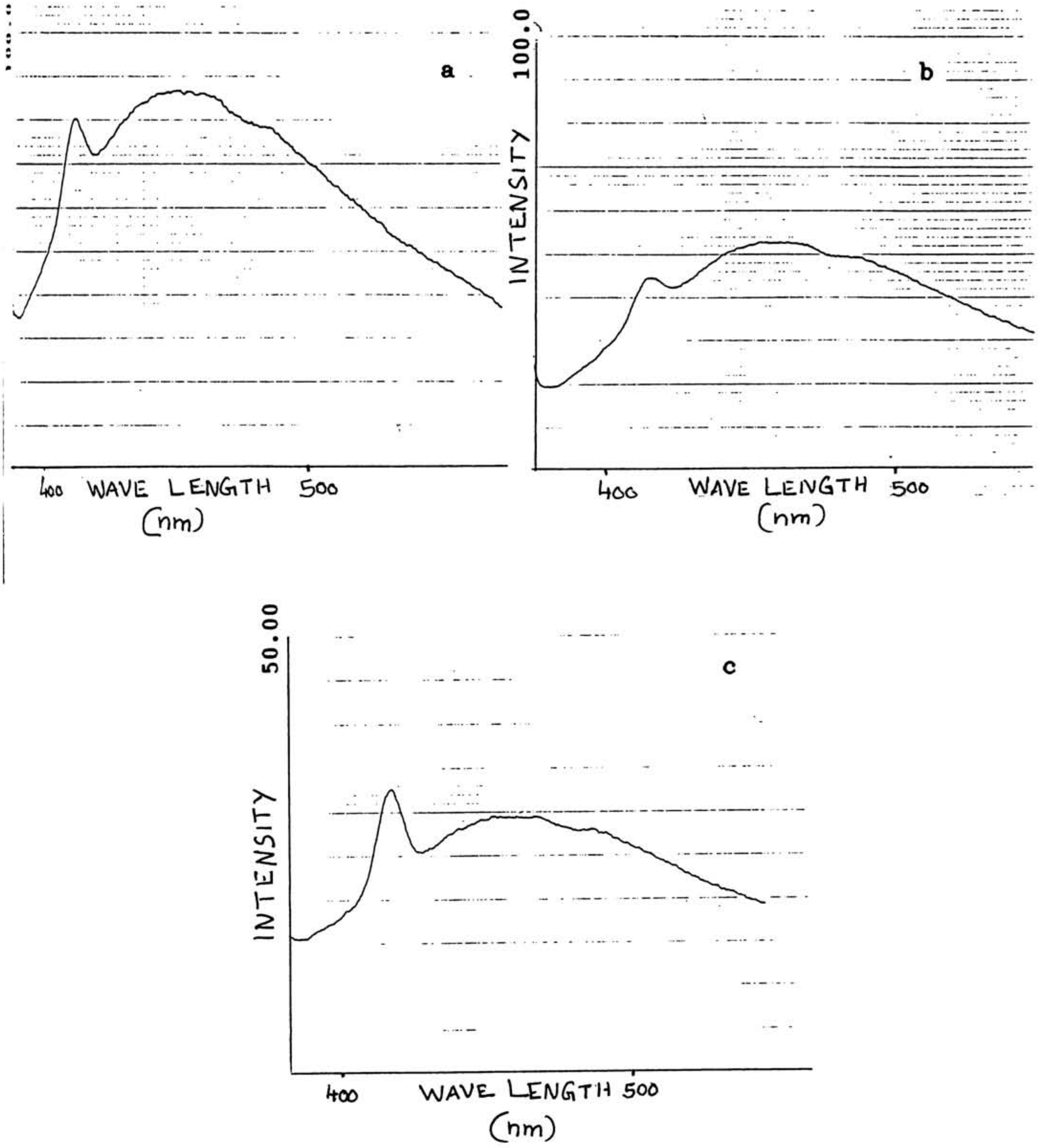


Fig.3.6 Fluorescence spectra of humic acids (a) Terrestrial (b) Estuarine (c) Coastal

intensity at emission maxima (Nair, 1992). As reported earlier (Schnitzer and Khan, 1972) it was found that pH affects the fluorescence intensity.

The maximum excitation and emission wave lengths were independent of molecular weight. It is suggested that fluorescence maxima can be utilized to differentiate between sedimentary humic acid and fulvic acid. Studies on fluorescence and absorption spectra of humic acid and fulvic acid with molecular weights ranging from < 10,000 to > 30,000 at 20° C and pH 8 showed that maximum excitation and emission wavelengths of humic acid was longer than that of fulvic acid (Hayase and Tsubota, 1985). Fluorophors in humic acid were of higher molecular weight aromatic groups than those in fulvic acid. Ghosh and Schnitzner (1980a) also unfolded the application of the fluorescence spectra to differentiate between soil humic acid and fulvic acid. Fluorescence phenomena of humic acids of aquatic origin and microbial cultures were studied by Visser (1983). Microbially formed humus fluorescence more strongly than the aquatic compounds. The excitation maximum of microbially formed humic acid occurred at lower wavelength than that reported in the literature for soil humic acid. The excitation and emission maxima can be used to differentiate humic acid and fulvic acid.

Infra red (I.R)

The absorption of infra red radiation by matter corresponds to the vibrational and rotational transitions within the material. In the case of solids and liquids one can generally observe only the vibrational bands, and these are the only bands of relevance in the study of humic substance.

The main absorption bands in the I.R spectra of different humic acids are given in Table 3.7 and Fig.3.7. I.R spectra of five different humic acids (three estuarine, one coastal and one terrestrial samples) showed a close resemblance. Among the five samples, terrestrial and coastal sample showed a strong band at

Table.3.7. Infrared Band Assignments

RIVERINE	KUMBALAM	THEVARA	COASTAL	TERRESTRIAL	Possible Assignments*
1010-1150		1030-1050	1000-1050	1020-1150	Stretching of polysccharide/polysaccharide like substances
1380-1410		1360-1410	1370-1450	1300-1420	Bonding vibrations of alcohols and carboxylic acid
1500-1580	1500-1580	1500-1550	1500-1570	1550-1600	Amide / Aromatic ring Stretching Vibrations
1600-1670	1600-1750	1620-1700	1600-1750	1600-1700	>C=O stretching vibrations of carbonyl group
2320-2400	2000-2300	2320-2400	2060-2260	2300-2400	Amine derivatives and aminoacids
2940-3000	2800-3000	2820-2980	2860-3000	2940	Stretching vibrations of aliphatic -CH, -CHO, CH_3 groups of side chain aromatic nuclei
3000-3500	3000-3500	3000-3500	3000-3600	3000-3600	Hydrogen bonded hydroxyl groups and -OH stretching vibrations

* (Farmer and Morrison, 1960; Schnitzer, 1965; Wagner and Stevenson, 1965; Stevenson and Goh , 1971)

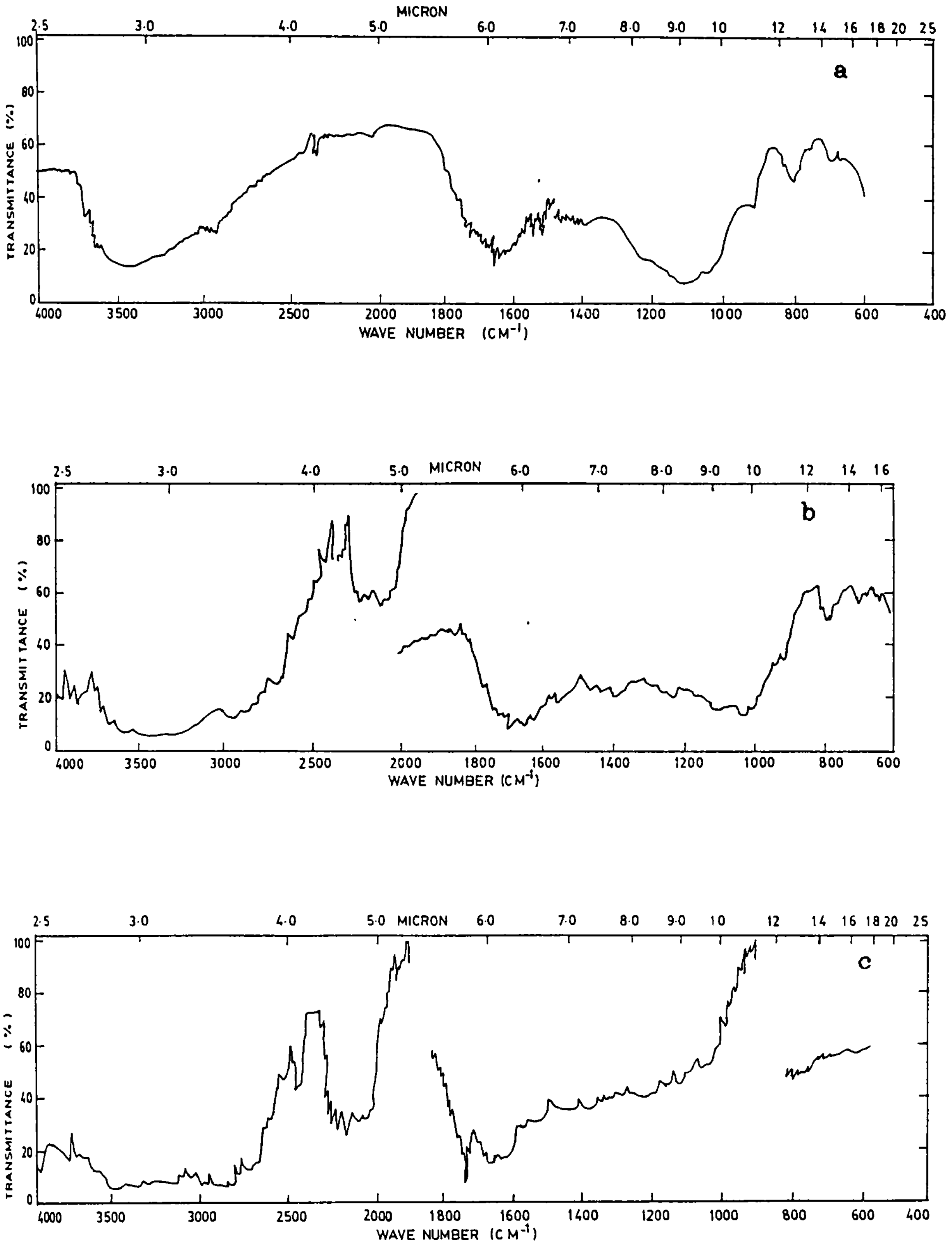


Fig.3.7 IR spectra of humic acids
(a) Terrestrial (b) Estuarine (c) Coastal

3450 cm^{-1} region.

The most striking feature of the infra red spectra of humic acid is the overall simplicity of the spectra. A broad absorption band that occurred in the range of 3000 - 3600 cm^{-1} indicated the hydrogen bonded -OH groups. Wagner and Stevenson (1965) reported that on methylation or acetylation there was a decrease in absorption intensity at this region. The -NH groups can also contribute to the absorption in this region.

Small shoulder near 2900 - 3000 cm^{-1} region is indicative of the aliphaticity of humic acids. Asymmetric and symmetric stretching vibrations of aliphatic -C-H bonds in methyl or methylene groups also contribute to the absorptions in this region (Theng *et al.*, 1966). Wagner and Stevenson (1965) observed that there was an increase in absorbance at this band on methylation of the sample, supports the above explanation. This band was prominent in the terrestrial sample. However, in all samples this band was relatively weak, indicating the absence of large amount of $> CH_2$ and $- CH_3$ groups.

The most characteristic feature of humic acid spectra was given by the strong but relatively broad absorption of the C=O stretching vibrations of several functional groups, which was present in all the five samples such as carboxylic acids, cyclic and acyclic aldehydes and ketones at 1700 $cm^{-1}$and of the C=C stretching vibrations of double bonds in aliphatic and aromatic compounds at 1615 cm, also pointing to the different types of benzene ring substitution. In the direction of higher wavelengths there were some minor inflection at 1550 cm^{-1} and 1504 cm^{-1} which may have developed from C=C as well as from C=N stretching vibrations of possible nitrogen containing heterocycles.

Humic acids that had been fractionated by solvent extraction from phragmites peat were investigated by Farmer and Morrison (1960). They studied the changes in the infrared absorption following methylation and reduction in order to get information on the acidic unsaturated structures. They found that about 40% of the humic acids were converted by alkaline hydrolysis to water soluble products, presumably sugars and aminoacids. This was shown by a marked drop in absorption near 1052 cm^{-1}, ascribed to the loss of carbohydrates, 1653 cm^{-1} and 1538 cm^{-1}, attributed to the loss of peptides. According to their results, the C=O absorption band at 1709 cm^{-1} must originate principally from carboxylic acids, since this band is absent in the spectrum of sodium salts of both humic acids and alkaline hydrolysed humic acids. By treatment with sodium borohydride, a reagent that reduces most aldehydes and ketones but not carboxyl groups, the intensity of this band was found to be reduced to only 10%. The authors assumed that the changes were due to the contribution of strongly hydrogen bonded α, β- unsaturated carboxyl groups.

For the determination of the number of arrangements of functional groups, Wagner and Stevenson (1965) subjected humic acids to methylation, saponification and acetylation. By treatment with diazomethane they found an increase in intensity of the C=O stretching vibrations at 1720 cm^{-1} and a sharpening of the C-O stretching vibration at 1250 cm^{-1} as a result of ester formation. The addition of the methyl groups, further more, caused the introduction of bands in the region C-H stretching vibrations at 2920 cm^{-1} and of hydrogen bonding vibration at 1440cm^{-1}. After the saponification and reacidification, a demethylated product was isolated whose characteristic features were similar to those of the initial material. Acetylation of this material gave four new bands in the region of the C=O absorption vibrations with approximate bands at 1840 cm^{-1}, 1815 cm^{-1}, 1775 cm^{-1} and 1740 cm^{-1}. The new bands were assigned to C=O vibrations of phenol acetates and anhydrides (Wood *et al.*, 1961). The acetylation of the unsaponified but diazomethane treated sample presented a strong band at 1365 cm^{-1} assigned to the C-CH_3 vibration of the added acetyl groups and several faint bands as shoulders in the region of the carbonyl vibration at about 1720 cm^{-1}.

I.R spectra of the present study were similar to those reported by Ishiwatari and Hanya (1965), Ishiwatari *et al.*(1966), Otsuki and Hanya (1967), Ishiwatari (1970), Stevenson and Goh (1971), Kemp and Mudrochova (1973, 1975) and Bourbonniere and Meyers (1978). The similarity of I.R spectra of humic acid from different lakes suggests a similarity of the aspects of chemical structure that are related to their I.R absorption. However I.R spectroscopy is not sensitive enough to uncover minor structural difference among humic acid. When I.R is applied to humic substance a number of functional groups can be determined.

Humic and fulvic acids from different sources were categorized into three spectral types depending on their infra red spectra by Stevenson and Goh (1971). One of them showed equally strong bonds at 1720 and 1600 cm^{-1}, with no discernible absorption being evident at 1640 cm^{-1}. Distinguishing characteristics of the second type was a very strong band at 1720 cm^{-1}, shoulder at 1650 cm^{-1} and the absence of 1600 cm^{-1} band. Third type pronounced spectral band indicative of protein and carbohydrates.

Szpakowska *et al.*(1986) compared the physico-chemical properties of humic substances isolated from different aquatic ecosystems on the basis of elemental analysis and I.R and UV-VIS absorption spectra. They reported that humic acid of land origin contained more aromatic rings and had more acidic functional groups compared with marine humic acid.

Shanmukhappa *et al.* (1987) conducted physical investigations on humic acids from different ecological environments. Humic acid samples from two mangrove habitat and an estuary were subjected to photoacoustic spectrometry to study possible components and humification process. From the UV-VIS and the I.R spectra they reported that both the mangrove habitats showed similar pattern in UV-VIS and I.R spectra, while estuarine humic acid showed a different pattern compared to the above samples. Filip *et al.* (1988) compared salt marsh humic acid with humic like substances from fresh and dead plant material (*Spartina alterniflora*). The results showed that plant humic substances consisted of a higher portion of aliphatic compounds than that from mud in which plants were growing. However, the overall similarities between the extracted material indicated that *S. alterniflora* had a direct contribution to the humic substances in the salt marsh ecosystem.

The overall observations of the general structural investigations offers the following conclusions:

(1) Though there was slight variations in the contributions by the carboxylic and phenolic acidities between the terrestrial, estuarine and coastal samples of humic acids, no significant variations in the composition as well as interactive structural components can be identified from the spectral as well as chemical studies.

(2) The fluorescence spectra was found to be a better quantitative tool than U.V- Visible spectra for the analysis of humic acids.

(3) Though simple, the I.R spectra was indicative of a highly complex structural pattern for the humic acid with a large number of functional groups making the assignment of the bands to specific groups very difficult.

(4) The comparison of the bands obtained in the I.R spectra with the earlier reports suggests that there is no significant variation in the general composition and the organic structural components of the humic acids of this study with the humic acid isolated from other environmental systems.

CHAPTER IV

SORPTIONAL CHARACTERISTICS OF SEDIMENT AND HUMIC ACID

Metals are intrinsic component of earth's crust. With the rapid development, the evolution of metal based industries has led to the contamination of environment with heavy metals. The dual role played by the sediment in regulating the contaminants in the overlying water and biota by acting both as a sink and source is well established. Sediments have only recently been considered as a non point source of environmental contaminants. The various processes operating in the system cause contaminants to migrate to the sediments with relatively long residence times. Contaminants which enter as dissolved atoms or molecules are removed from the water column through geochemical processes such as precipitation and co precipitation, adsorption and uptake by biota. The resulting particles are mixed with those already in place to create a wider spectrum of different species in quality and sizes. These particles then undergo a series of geochemical processes like transport, settling, resuspension and deposition. All these processes are accompanied by a number of chemical reactions which modify both particle matrix and particle associated contaminants.

Adsorption/desorption process, degradation of ions, transformation of iron and manganese oxyhydrates to sulphides and vice versa and biotransformations of contaminants ingested by organisms produce a steady change in the absolute and relative concentrations and forms of contaminants. The concentrations in the sediments are generally much greater than in overlying water, thereby acting as a source of contaminants to the water column.

The fate of heavy metals in the aquatic environment is of extreme importance because of their impact on the ecosystem. Sediments are usually regarded as the ultimate sink for heavy metals discharged into the environment (Gibs, 1973). The investigation on the distribution of heavy metals in the bottom sediments of aquatic systems, thus warrants a knowledge on the mechanisms of the exchange processes of heavy metals between the sediments and the overlying water. The adsorption is considered as one of prime mechanisms of uptake. Sediment organic matter, particularly humic substances, plays a predominant role in their partitioning of metals between the solid and liquid phases.

Humic substances are widely distributed over the earth's surface, occurring in terrestrial as well as in aquatic environments. Humic substances are chemically very complex materials whose structure and reactions are not fully understood. They are believed to be macro molecules, spanning a wide range of molecular weights, which are formed from quinones and phenolic compounds. One of the most striking characteristics of these materials is their relatively high content of oxygen-containing functional groups (carboxyl, phenolic and alcoholic hydroxyl groups, ketonic and quinonoid C=O) through which they can interact with metals. The colloidal properties of humic materials also affect their interaction with metals. Reaction with humic substances profoundly affects the environmental behaviour of metals.

Cations adsorbed to solid surfaces are in equilibrium with their respective dissolved, free ion concentrations so that changes in the dissolved matrix will result in a predictable change of the adsorbed ion fractions. These equilibrium processes can be parameterized by adsorption isotherms (Stumm and Morgan, 1970). Adsorption isotherms are widely used in the study of heavy metal reaction with sediments, soils and humic substances. The extent of metal ion retention by sediment varies in accordance with adsorption isotherm parameters (i.e., solute concentration, nature and concentration of competing cations and bonding constant value) and other factors such as pH, texture and nature of organic matter present in the sample. Much remains to be learned about the adsorption of the metals by sediments, even though many investigations to determine the factors affecting the adsorption of heavy metals by soils have been conducted (Udo *et al.*, 1970; John, 1972; Soldantini *et al.*, 1976; Petruzzelli *et al.*, 1978). Nriagu and Cocker (1980) described that humic acid and fulvic acid acquired most of their trace metal burden in the overlying water. Perturbation of the natural cycle of heavy metals due to the discharge of waste water into Cochin estuarine system has been intensively studied during recent years (Venugopal *et al.*, 1982; Nair *et al.*, 1990; Shibu *et al.*, 1990; Nair *et al.*, 1991; Nair, 1992; Shibu, 1992).

Present chapter reveals the significance of humic acid on the adsorption of some selected heavy metals by estuarine sediments. This work was carried out by using an estuarine sediment (ES), sediment left after alkali extraction (SAAE), estuarine humic acid (EHA) and a terrestrial humic acid (THA). Metal solutions of copper, cadmium, manganese, zinc, mercury and lead were used in this study. The influence of salinity on these adsorption processes was also determined. Desorption studies for explaining the chemical forms of adsorbed metal ions were also conducted using extractants 2N CH_3COONH_4, 0.5N HCl, 0.05M EDTA and 0.01M NaH_2PO_4.

Materials and Methods

Details of the procedure employed in this studies are illustrated in Chapter II.

Results and Discussion

A. Adsorption Studies

The present study envisages the sorptional behaviour of heavy metals, copper, cadmium, manganese, zinc, mercury and lead on estuarine sediment in different saline conditions and the role of humic acid in these sorption processes. An estuarine sediment sample (ES), sediment left after alkali extraction (SAAE), estuarine humic acid (EHA) and terrestrial humic acid (THA) were exposed to five different concentrations of above mentioned six metals for 24h. During these periods, the pH of the medium was kept at 7, the average pH shown in this estuary.

The evaluation of the data was generally carried out with the four basic concepts; the partition, Langmuir and Freundlich adsorption isotherm and the equilibrium mass balance.

(1) It is well known that the partitioning of a substance between the two phases of solution and sediment is a reversible phenomenon. It can be explained in terms of partition coefficient Kp, where Kp is the ratio of the concentration of the substance in the sediment to its concentration in the liquid phase.

(2) Adsorption isotherms are widely used in the study of heavy metal interactions with soils and sediments. An adsorption isotherm expresses the relationship between the amount of the substance adsorbed per unit weight of the adsorbent and its equilibrium concentration in the solution at constant temperature. The Langmuir adsorption isotherm is of particular utility because both a capacity factor and an intensity factor can be calculated.

$$\frac{Xe}{X} = \frac{1\ X_e}{K_1} + \frac{1}{K_1K_2}$$

X is the amount of metal adsorbed per unit weight of adsorbent, Xe is the equilibrium concentration of the metal in the solution and K_1 and K_2 are constants. A plot of Xe/X against Xe gives a straight line if the data were fitted to the Langmuir equation. The constants K1 and K2 can be calculated from the slope and the Y intercept respectively.

The basic concept of Langmuir adsorption isotherm is mono layer adsorption. The area of each site is a constant, and depends only upon the geometry of the surface. The adsorption energy is same at all sides. More over, the adsorbed molecules can not migrate across the surface, or interact with neighbouring molecules.

(3) Freundlich adsorption isotherm can be described by the equation

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

X is the amount of metal adsorbed per gram of 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 model is consistent with a heterogeneous surface. It is assumed that each class of surface sites adsorb individually according to the Langmuir equation, the Freundlich isotherm can be shown (1) to correspond a unique distribution of relative adsorption site affinities which is essentially log normal; (2) that the empirical parameters K and n may be used to mathematically characterize the size distribution, thus providing information about surface heterogeneity of the adsorbent. Linear form of the isotherm is described for 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.

(4) Equilibrium constant, K of the adsorption process

$$M + X \longrightarrow MX$$

can be calculated using the equation

$$K = \frac{[MX]}{[M]\ [X]}$$

$$K = \frac{[MX]}{[Me]\ \{X_T - [MX]\}}$$

X is the number of available sites for adsorption at equilibrium. It is calculated by subtracting adsorbed metal concentration [MX] from total number of sites X_T. [MX] is the amount of adsorbed metal per unit weight of adsorbent and Me is the equilibrium metal concentration.

Above equation can be rearranged as,

$$\frac{[Me]}{[MX]} = \frac{1}{KX_T} + \frac{[Me]}{X_T}$$

which is same as Langmuir isotherm. Hence, K and X_T are related to the intercept and slope of the Langmuir equation. The discussion of the results of this study therefore considers only the Langmuir and Freundlich isotherms and the partition coefficient. During the evaluation of the data with these principles, the random odd values were not considered.

Salient characters of the adsorption process are defined by the constants, derived from Langmuir and Freundlich isotherms. In the Langmuir relation, K_1 represents adsorption maxima which inturn is a measure of the effective adsorption sites that will take part in the adsorption process and K_2, the equilibrium coefficient, is a function of the energy and by definition an increase in K_2 represents a decrease in the energy involved in the adsorption process.

By definition,

$$K = A\,e^{\frac{-E}{RT}}$$

Where A is a constant, E is the activation energy, R, the gas constant and T, temperature.

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 inturn is a measure of effective adsorption.

In general, a decrease in the partition coefficient (Kp) (Table.4.1) and adsorption maxima along with an increase in adsorption capacity can be observed with the removal of humic acid from the sediment. This can be due to the exposure of the inorganic clay minerals for the exchange process which have a greater adsorption capacity. The increased adsorption capacity was found not to contribute to an increase in adsorption maxima due to the restriction of higher energy requirements. K_2 values support the higher energy requirements for the adsorption of SAAE.

Copper

Copper is the first element of the sub group I B of periodic table with atomic number 29. Copper is one of the important, essential elements for plants and animals.

In fresh and saline waters of Cochin estuary, copper was extensively associated with organic colloidal matter (Shibu *et al*., 1990). Distribution of copper in the surface sediments exhibited wide spatial seasonal variability. Values of total copper varied from 1.3 to 50 mg/Kg and 8 to 49% of total copper

Table.4.1. K_p Values for the adsorption of metals on ES and SAAE.

SALINITY	ADSORBENT	METAL					
		Cu	Cd	Mn	Zn	Hg	Pb
0	ES	0.878±	0.145±	0.021±	0.330±	0.212±	0.370±
	SD	0.227	0.060	0.016	0.027	0.035	0.042
	SAAE	0.046±	0.148±	0.309±	0.224±	0.005±	0.132±
	SD	0.01	0.034	0.20	0.096	0.003	0.06
5	ES	0.594±	0.020±	0.003±	0.055±	5.047±	0.825±
	SD	0.078	0.006	0.0007	0.04	0.70	0.089
	SAAE	0.441±	0.084±	0.015±	0.369±	0.059±	0.366±
	SD	0.153	0.008	0.003	0.11	0.035	0.092
10	ES	0.398±	0.007±	0.002±	0.054±	6.274±	0.361±
	SD	0.059	0.001	0.0007	0.025	3.14	0.161
	SAAE	0.427±	0.035±	0.007±	0.240±	0.065±	0.336±
	SD	0.22	0.003	0.002	0.68	0.06	0.126
15	ES	0.312±	0.004±	0.001±	0.037±	15.697±	0.358±
	SD	0.08	0.0006	0.0004	0.027	1.02	0.144
	SAAE	0.421±	0.020±	0.005±	0.194±	0.031±	0.273±
	SD	0.10	0.001	0.001	0.062	0.005	0.100
25	ES	0.249±	0.002±	0.0009±	0.026±	6.384±	0.170±
	SD	0.039	0.0002	0.0003	0.010	3.02	0.064
	SAAE	0.336±	0.009±	0.001±	0.123±	0.017±	0.145±
	SD	0.11	0.0008	0.0005	0.04	0.012	0.050

Table.4.2. K_p Values for the adsorption of metals on EHA and THA.

SALINITY	ADSORBENT	METAL					
		Cu	Cd	Mn	Zn	Hg	Pb
0	EHA	4.402±	1.414±	0.058±	0.100±	15.428±	12.274±
	SD	1.3	0.49	0.002	0.061	2.76	2.4
	THA	1.894±	0.913±	0.120±	0.059±	87.707±	5.323±
	SD	1.01	0.34	0.09	0.038	15.42	2.27
5	EHA	0.800±	0.029±	0.061±	0.058±	6.588±	0.599±
	SD	0.15	0.005	0.02	0.043	1.99	0.23
	THA	0.720±	0.007±	0.020±	0.063±	16.534±	0.509±
	SD	0.14	0.003	0.008	0.024	1.29	0.16
15	EHA	1.068±	0.017±	0.021±		0.325±	0.336±
	SD	0.22	0.005	0.008		0.29	0.076
	THA	0.072±	0.493±	0.007±		18.546±	0.406±
	SD	0.03	1.7	0.004		1.33	0.07

and was found to be associated with organic phase (Nair *et al.*, 1990).

The results of the copper adsorption studies with ES, SAAE, EHA, and THA are given in Tables 4.3 and 4.4 and their respective graphs in Fig.4.1, 4.2, 4.3 and 4.4. The copper adsorption on the ES and SAAE did not completely follow the Langmuir and Freundlich isotherms. However, for a general comparison, the constants of these isotherms were calculated from the best fit data. SAAE showed a lower Kp value (partition coefficient) than that of ES, and also its value decreased in saline conditions (Table 4.1). From the K_p values it is obvious that humic acids play a significant role in copper adsorption. The slope of the Langmuir adsorption isotherm, which is a measure of the adsorption maxima, decreased with salinity. The Constant in Langmuir isotherm which deals with the energetics of the process decreased with salinity in ES, whereas it decreased when salinity changed from 0×10^{-3} to 5×10^{-3} and then remained almost constant in higher salinities in the case of SAAE.

The adsorption capacity factor, K in Freundlich isotherm decreased with salinity and K of SAAE is higher than that of ES at saline conditions. This is in agreement with K_p values. ES showed a higher adsorption intensity values.

Partition coefficient of EHA is greater than THA and also showed a lower value in saline waters (Table 4.2). The adsorption data of the EHA was observed to fit into the Langmuir and Freundlich. Values of Langmuir constant K_1, the adsorption maxima, increased and K_2 the energy factor decreased with salinity. Values of n, adsorption intensity and K, adsorption capacity in the Freundlich equation were decreased in EHA in saline conditions.

Partition coefficient indicated the major role of humic acid in the fate of copper in the aquatic system. In marine sediments, copper level in humic acid fraction tends to fall into

Table.4.3 Langmuir and Freundlich constants for adsorption of Cu on ES and SAAE

SALINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	ES[a]	0.064	14.289	1.795	0.140
	SAAE[b]	-0.011	-1.978^{**}	0.666	0.101^{***}
5	ES	0.173	3.913	1.106	0.437^{***}
	SAAE	-0.002	-30.255^{**}	0.339	532.350^{**}
10	ES	-1.345	-0.325	0.827	1.123
	SAAE	-0.003	-27.710^{**}	0.360	253.860^{***}
15	ES	-0.024	-8.275	0.658	1.743^{**}
	SAAE	-0.004	-19.737	0.371	108.140^{*}
25	ES	-0.054	-3.533	0.787	0.535^{***}
	SAAE	-0.001^{*}	-19.820	0.196	106684.1^{*}

[a] n=10 [b] n=5, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table.4.4 Langmuir and Freundlich constants for adsorption of Cu on Humic acids.

SALINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	EHA	0.172	58.076	1.728	0.875^{*}
	THA	0.140	46.819^{*}	2.501	0.290^{***}
5	EHA	0.194	7.717^{*}	1.561	0.350^{***}
	THA	0.156	7.222^{*}	1.576	0.265^{**}
15	EHA	0.234	6.810^{*}	1.390	0.486^{***}
	THA	0.165	11.956^{*}	1.675	0.339^{**}

n=5, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

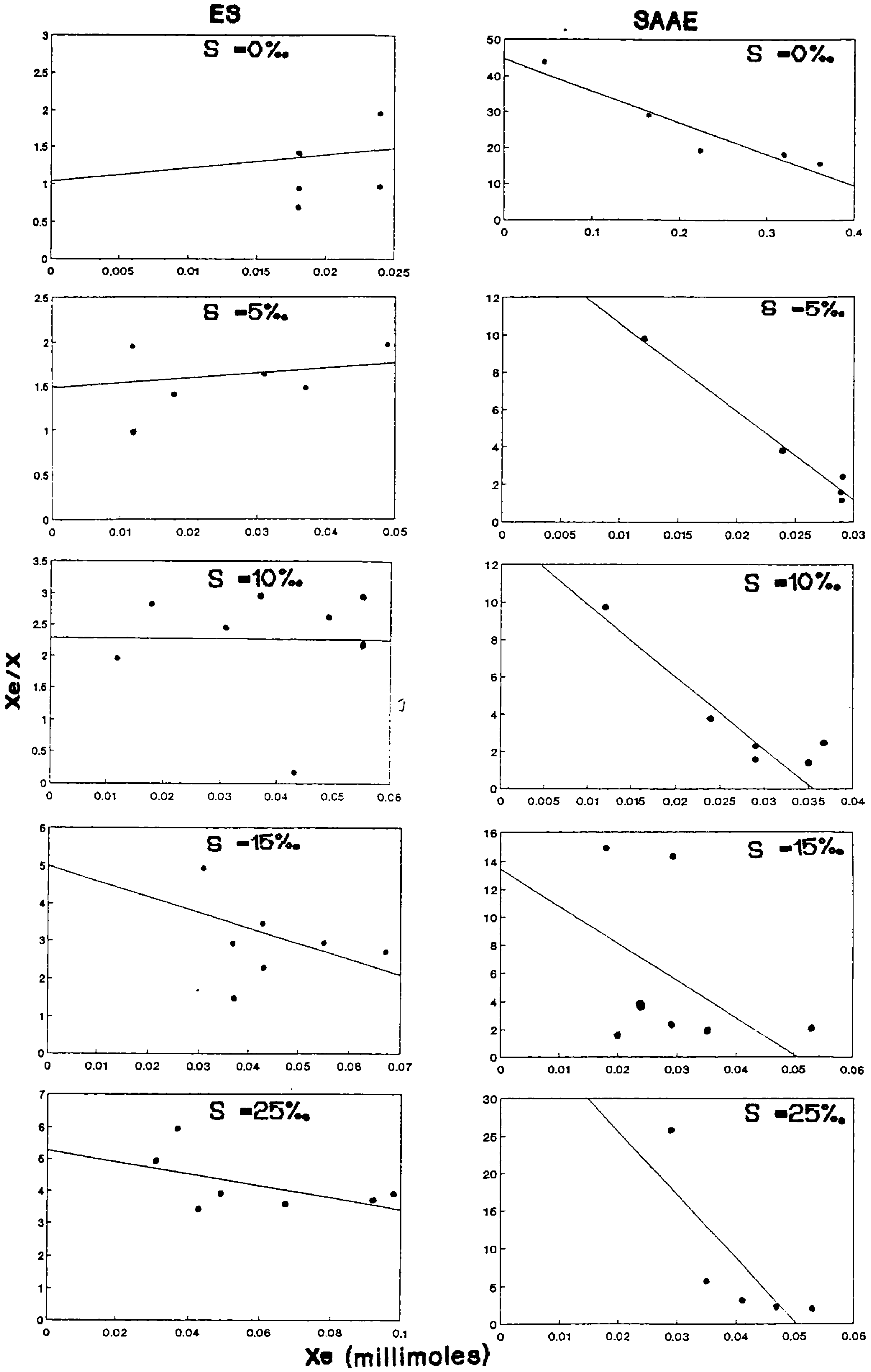


Fig.4.1 Langmuir isotherm for the adsorption of copper by ES and SAAE.

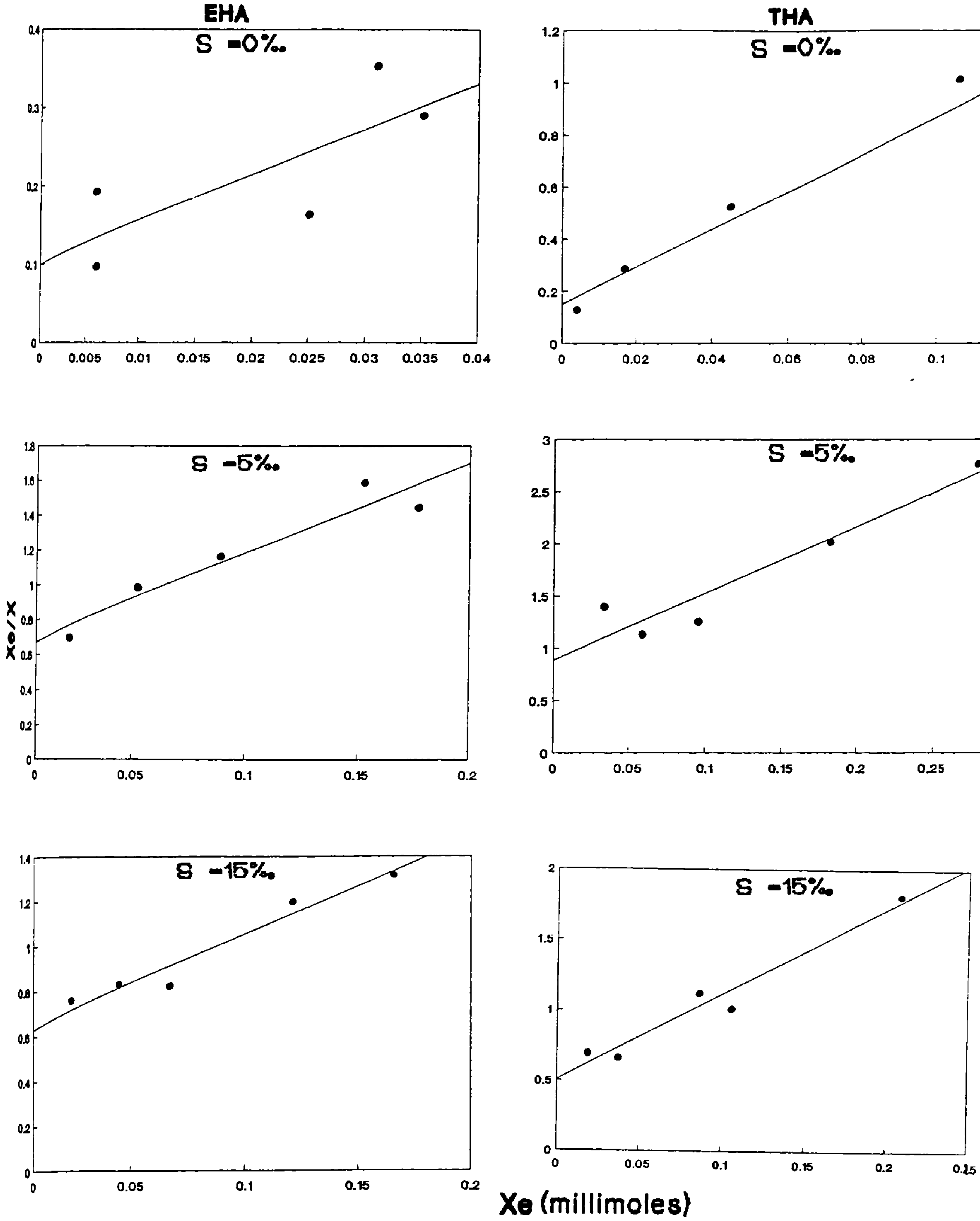


Fig.4.2 Langmuir isotherm for the adsorption of copper by EHA and THA

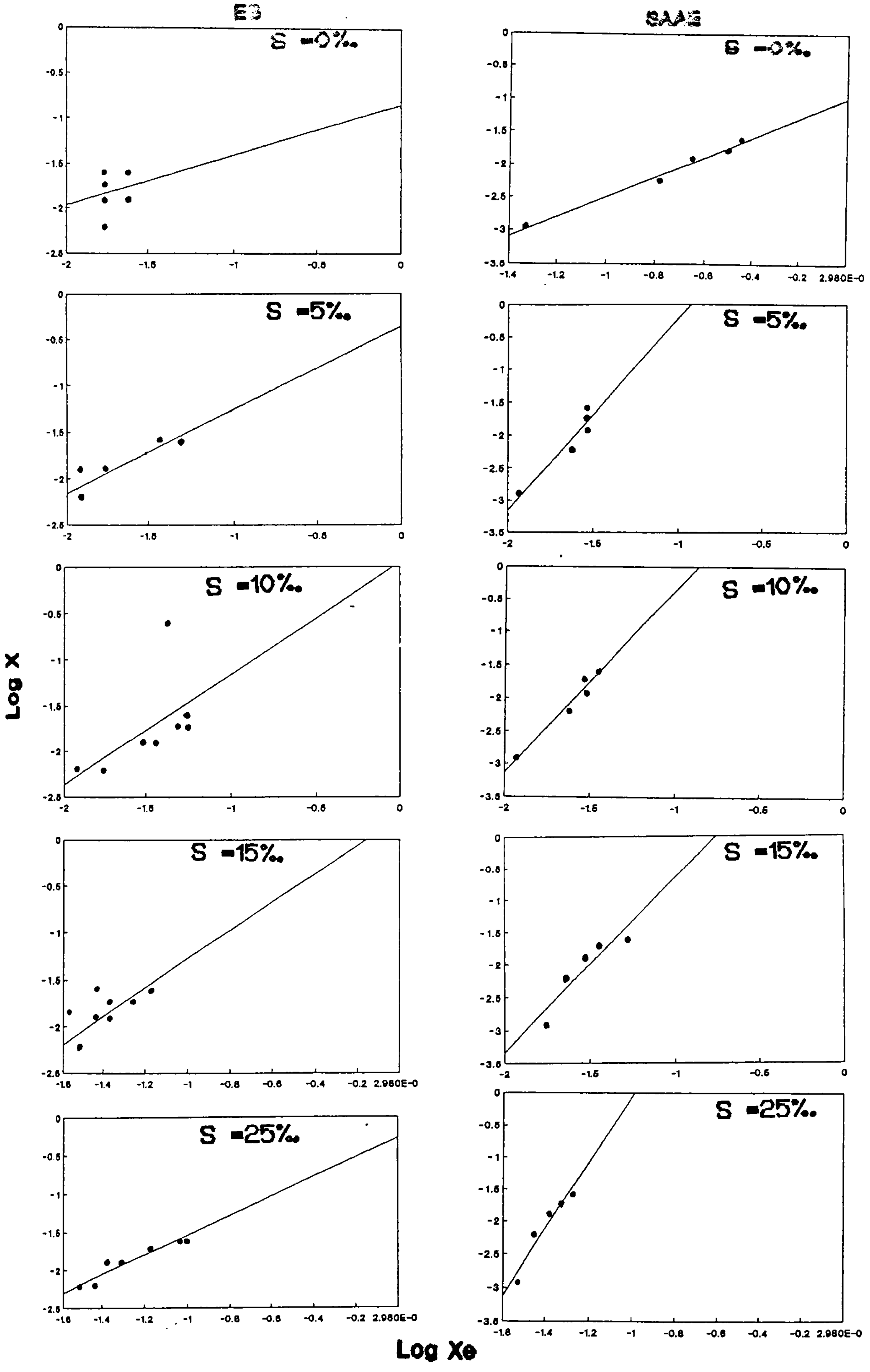


Fig.4.3 Freundlich isotherm for the adsorption of copper by ES and SAAE

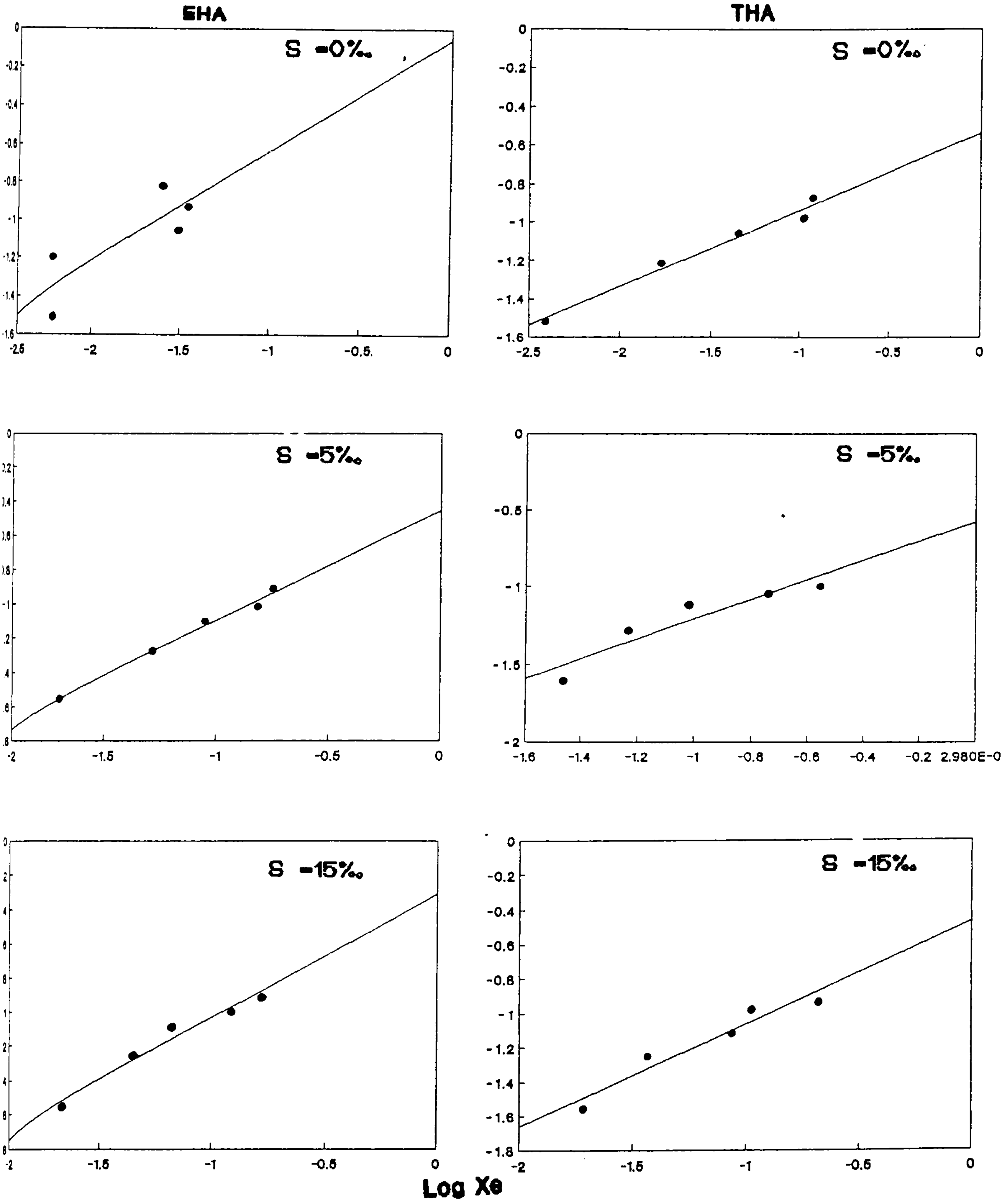


Fig.4.4 Freundlich isotherm for the adsorption of copper by EHA and THA

600 to 400 ppm range. Similarly the interstitial water complexes contain most of the copper and zinc in this phase (Jakson, 1975; Nissenbaum and Swaine, 1976).

It was found that copper adsorption by both humic acid samples obeyed Langmuir and Freundlich isotherms. This was in agreement with the results published by Guy and Chakrabarti (1976).

Bilinski *et al.*(1991) reported that copper was not practically adsorbable on kaolinite and bentonite in natural waters and UV irradiated waters of salinity 20 x 10^{-3} if the copper ion concentration was lower than complexation capacity value, and copper adsorption was of medium intensity at salinity 38 x10^{-3}. Copper adsorption on sedimentary materials decrease in the following order HA > montmorillonite > illite > Kaolinite (Reimer and Toth, 1970). Humic acid in particular is an excellent scavenger for copper.

Cadmium

Among the environmental pollutants, cadmium a heavy metal, merits a special reference as a potentially toxic element of high mobility in the environment, and of long biological half life in man (WHO, 1982). Outbreak of cadmium pollution, affecting a large population in Japan in 1972 and other parts of the world, has aroused much concern about the toxicity of this heavy metal. Cadmium, a crystalline silver white malleable metal belongs to the group II B of the periodic table, and is relatively a rare metal. It has no essential biological function and is highly toxic to plants and animals. The major hazard to human health from cadmium is its chronic accumulation in the kidneys where it can cause dysfunction. Cadmium pollution of the environment has been rapidly increasing in recent decades as a result of rise in consumption of cadmium by industries. Unlike lead, copper and mercury which had been utilized for centuries, cadmium has only been used in this century. Sources of environmental contamination by cadmium are the mining and smelting of cadmium and zinc, atmospheric pollution from metallurgic industries, the disposal of wastes containing cadmium such as the incineration of plastic containers and batteries, sewage, sludge etc. The average concentration in the earth crust is estimated to be in the range of 0.1 mg/kg. Cadmium is closely associated with zinc its geochemistry; both elements have similar ionic structures and electronegativity.

Evaluation of available data of Cochin estuary indicated that the estuary receives anthropogenic inputs of cadmium (Paul and Pillai, 1983a; Nair *et al*., 1990; Shibu *et al*., 1990; Nair *et al*., 1991). Bioavilability of cadmium in the native flora and fauna has been reported (Paul and Pillai, 1983b; Babukutty, 1991). Analysis of surficial sediments (Nair *et al* ., 1990) revealed accumulation arising out of anthropogenic inputs especially in the northern parts. Shibu *et al*. (1990) observed gradual decrease of dissolved cadmium as the river water enters in the estuarine region. About 15-25% dissolved cadmium in the water was associated with organic compounds. Cadmium was found to the least abundant of the trace metals determined in the estuarine sediment during the study of Nair (1992). A unique feature in the partitioning of cadmium fraction was the preponderance of organic bound cadmium over other fractions.

K_p of cadmium decreased as salinity increased in the case of ES SAAE, EHA and THA (Tables 4.1 and 4.2). Langmuir constant, K_2, which indicate adsorption energy and slope, K_1, which represents adsorption maxima decreased as salinity increased in ES, EHA and THA, but from r values it was clear that data do not fit for Langmuir equation. Freundlich model is fitted in the case of cadmium sorption by four samples. As salinity increased adsorption capacity increased in all four samples (Table 4.5 and 4.6). Respective graphs are given in Fig. 4.5, 4.6, 4.7 and 4.8.

Bilinski *et al*.,(1991) observed that cadmium adsorption on kaolinite and bentonite in natural and UV irradiated waters of ble.4.5 Langmuir and Freundlich constants for adsorption of Cd on ES and SAAE

SALINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	ES[a]	0.008	17.754^{***}	1.928	0.013^{***}
	SAAE[b]	-0.015	-8.304	0.951	0.181^{*}
5	ES	0.004	5.626^{*}	1.238	0.010^{***}
	SAAE	-0.028	-2.730	0.918	0.118^{**}
10	ES	0.004	2.110^{*}	1.150	0.005^{***}
	SAAE	0.009	4.624^{**}	1.193	0.021^{***}
15	ES	0.003	1.594^{***}	1.189	0.003^{***}
	SAAE	0.027	0.760	1.033	0.018^{**}
25	ES	-0.017	-0.114	0.974	0.002^{***}
	SAAE	0.012	0.858	1.087	0.008^{**}

n=10 [b] n=5, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table.4.6 Langmuir and Freundlich constants for adsorption of Cd on Humic acids.

SALINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	EHA	0.051	66.225^{*}	1.436	0.465^{*}
	THA	0.037	75.346^{**}	2.546	0.095
5	EHA	0.010	3.915	1.345	0.150^{**}
	THA	0.002	7.616	3.623	0.0017
15	EHA	0.001	84.994^{*}	3.316	0.0026
	THA	0.013	-2194.470^{**}	6.431	0.038

n=5 , * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

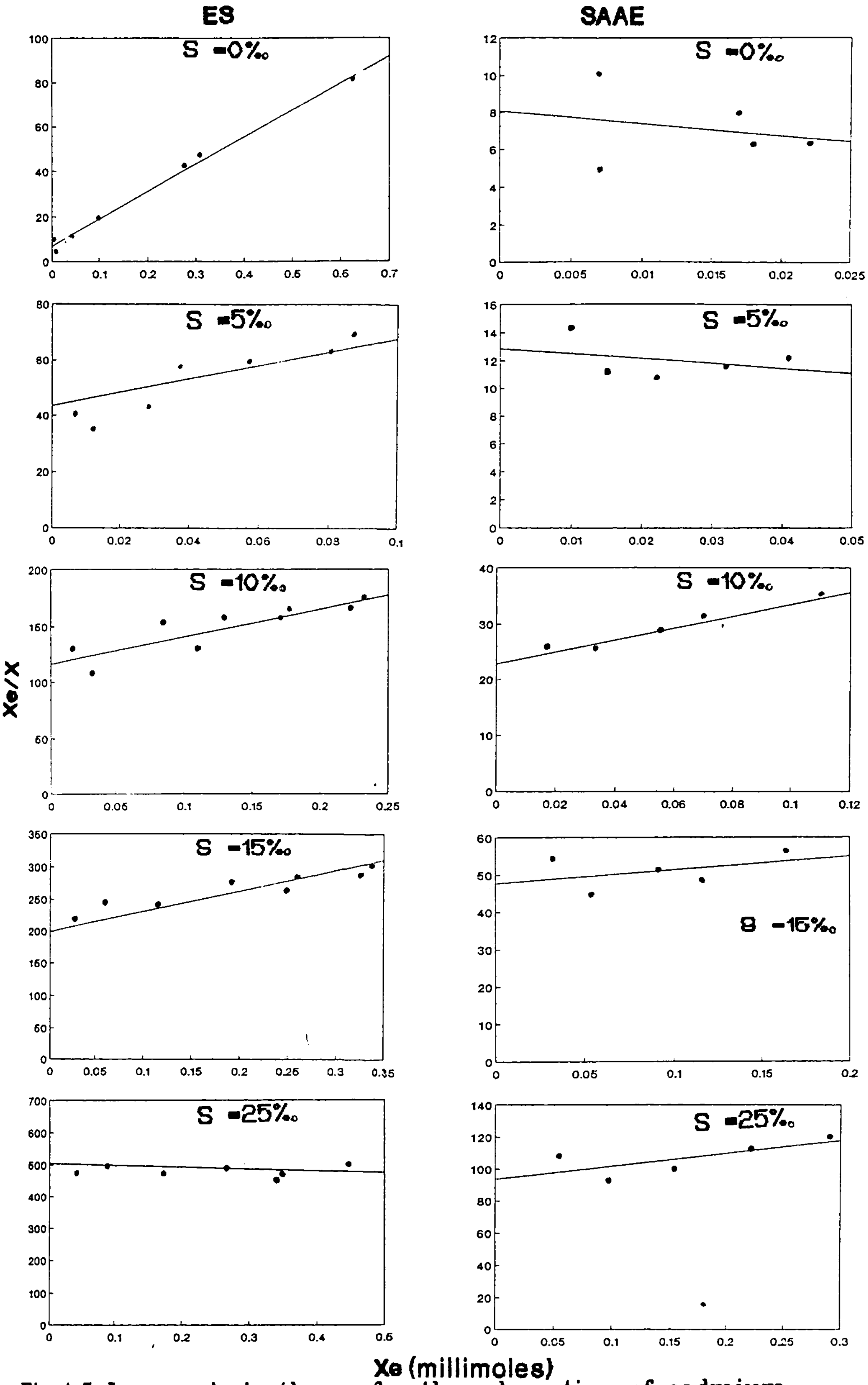


Fig.4.5 Langmuir isotherm for the adsorption of cadmium by ES and SAAE.

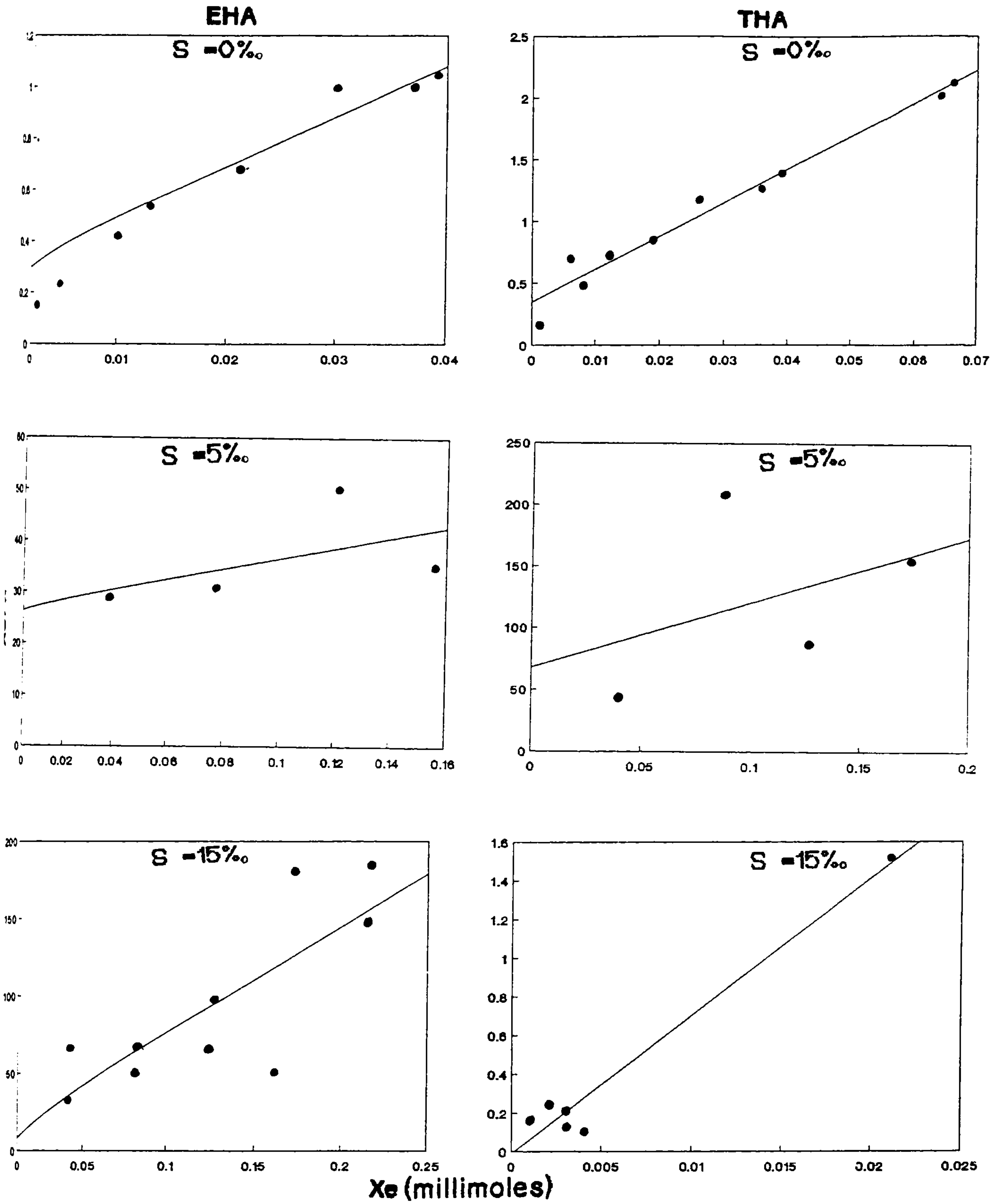


Fig.4.6 Langmuir isotherm for the adsorption of cadmium by EHA and THA

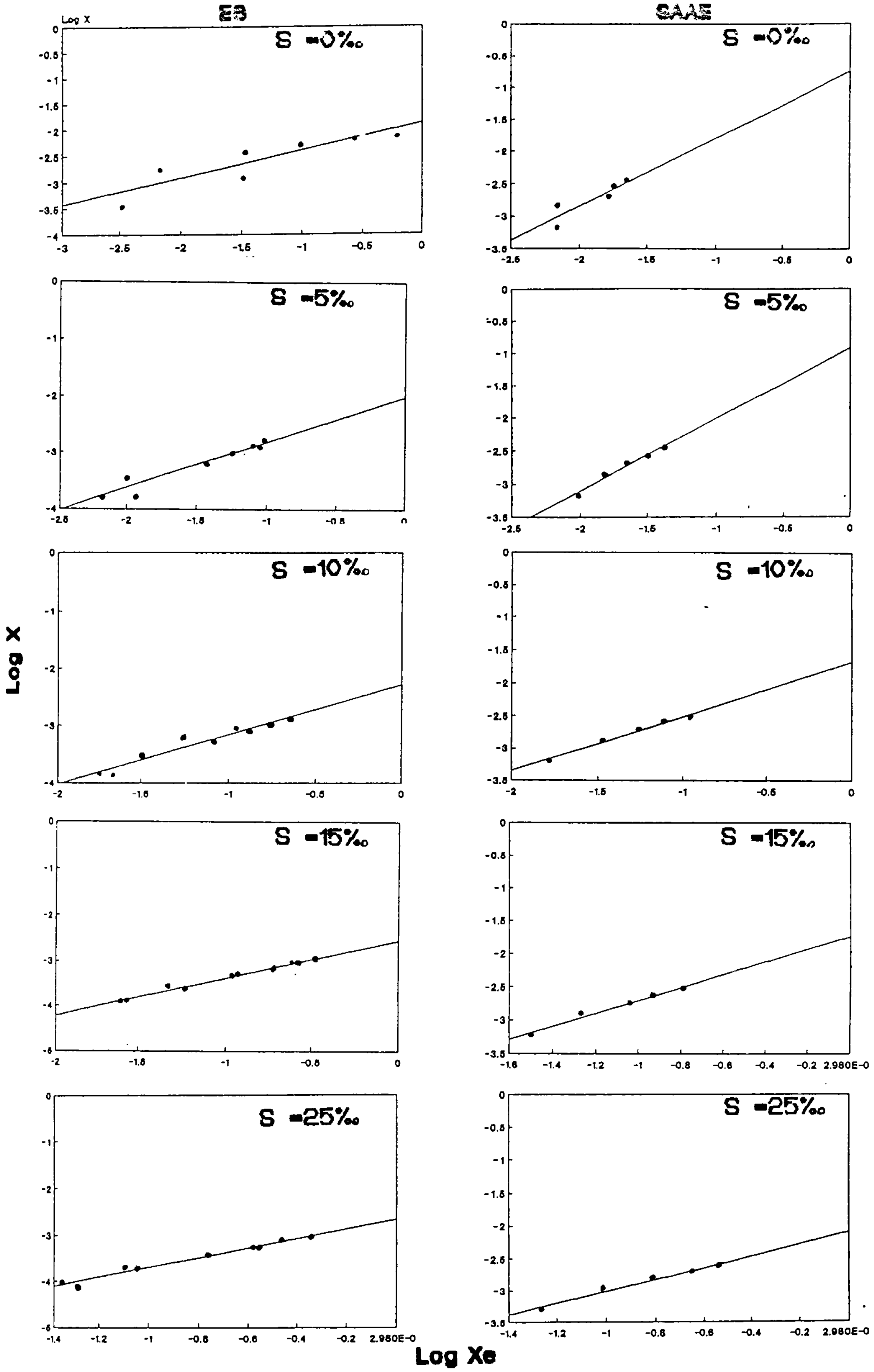


Fig.4.7 Freundlich isotherm for the adsorption of cadmium by ES and SAAE

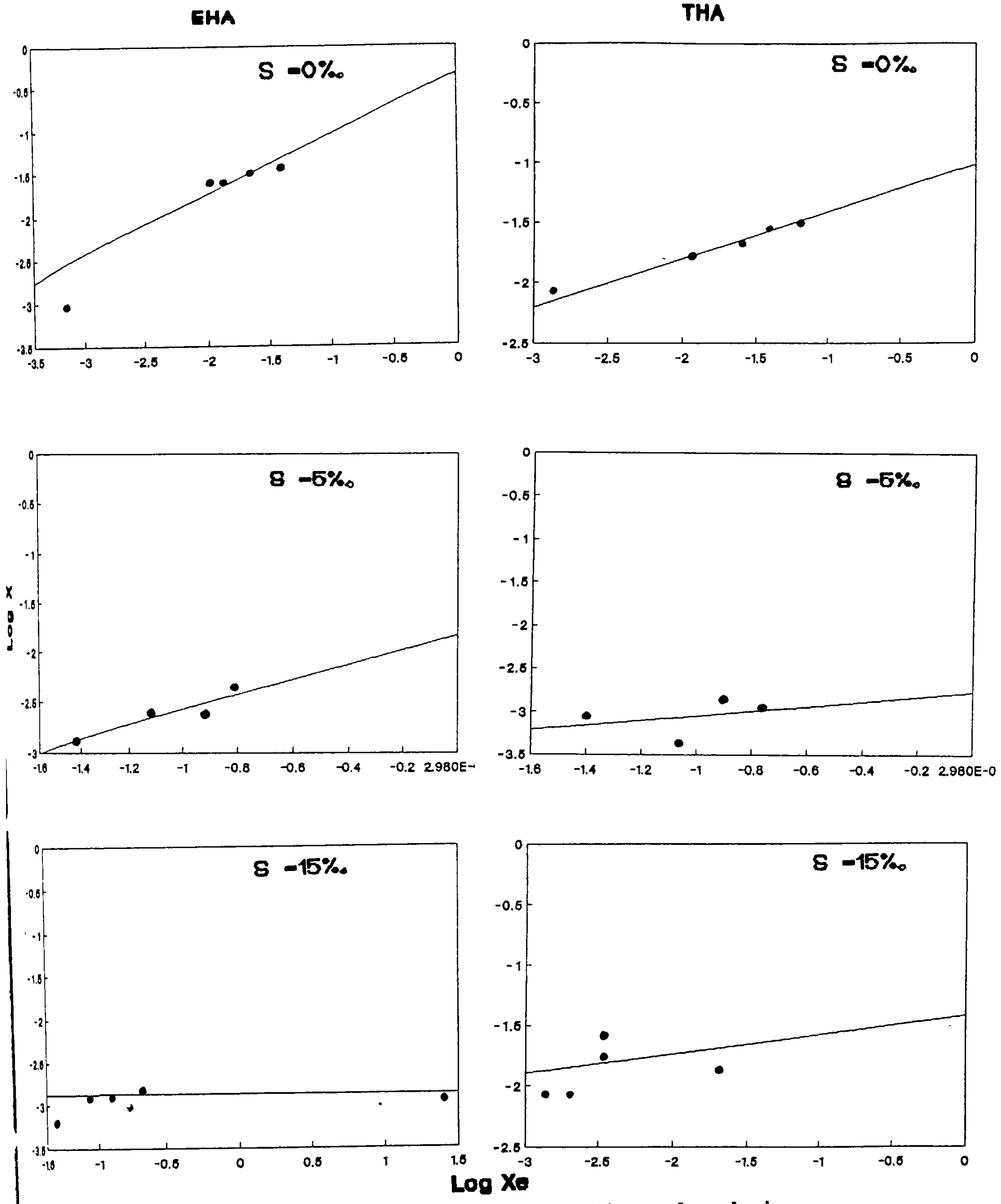


Fig.4.8 Freundlich isotherm for the adsorption of cadmium by EHA and THA

salinity 20 x 10^{-3} was of medium intensity.

Manganese

Manganese is important to our industrial civilization and also to the maintenance of life within it. Manganese is mined in large quantities, and finds important applications in engineering and products such as steel and electrical storage batteries. Environmental pollution problems are relatively insignificant when compared with those associated with some other heavy metals. The most common biological toxic effects are found in plants exposed to excess natural manganese in the soil under some conditions, particularly flooding.

In Cochin estuary, the abundance of manganese was only next to zinc. Total manganese varied from 7-170 mg/Kg with a mean value of 66 mg/Kg. An appreciable quantity (10-38%) of total manganese was found to be associated with organic phase (Nair, 1992).

Partition coefficient decreased as salinity increased as in the case of cadmium. Adsorption maxima, K_1, increased in ES and decreased in SAAE, EHA, and THA and K_2, energy factor, decreased as salinity increased in ES and SAAE. Constant related to adsorption capacity, K, in Freundlich showed a decreasing trend but results showed a significant r value (Tables 4.7 and 4.8; Fig.4.9, 4.10, 4.11 and 4.12). The addition or removal of organic matter or iron oxides in soils greatly influenced exchangeable manganese (Misra and Mishra, 1968). MnO_2 additions had little effect on copper and zinc (Mckenzie, 1980). Adsorption capacity values for SAAE in manganese sorption were found to be higher than ES as in the case of cadmium. K values were 0.014 and 0.083 (at salinity 0 x 10^{-3}), 0.003 and 0.11 (at salinity 5 x 10^{-3}), 0.002 and 0.007 (at salinity 10 x 10^{-3}), 0.002 and 0.005 (at salinity 15 x 10^{-3}) and 0.0005 and 0.002 (at salinity 25 x 10^{-3}) for ES and SAAE respectively. This may be due to the creation of new sorption sites. Sequi and Aringhieri ble.4.7 Langmuir and Freundlich constants for adsorption of Mn on ES and SAAE

SALINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	ES^a	0.209	3.222***	2.241	0.014***
	SAAE^b	0.034	15.157**	1.543	0.083*
5	ES	0.018	0.256***	1.381	0.003***
	SAAE	0.024	1.033**	1.565	0.011***
10	ES	0.022	0.125*	1.128	0.002***
	SAAE	0.026	0.433**	1.347	0.007***
15	ES	0.079	0.022	1.137	0.002***
	SAAE	0.024	0.287**	1.236	0.005***
25	ES	-0.004	-0.144***	0.575	0.0005***
	SAAE	0.009	0.187*	1.755	0.002*

=10 ^{b}n=5 , * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table.4.8 Langmuir and Freundlich constants for adsorption of Mn on Humic acids.

SALINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	EHA	0.021	-15.436^*	33.557	0.026
	THA	0.023	-24.923^*	4.435	0.072
5	EHA	-0.077	-0.510	0.814	0.068^*
	THA	-0.037	-0.185	4.207	0.0074
15	EHA	-0.003	-1.099	0.384	0.033^*
	THA	-0.0006	-1.209	0.285	0.020^{**}

n=5 , * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

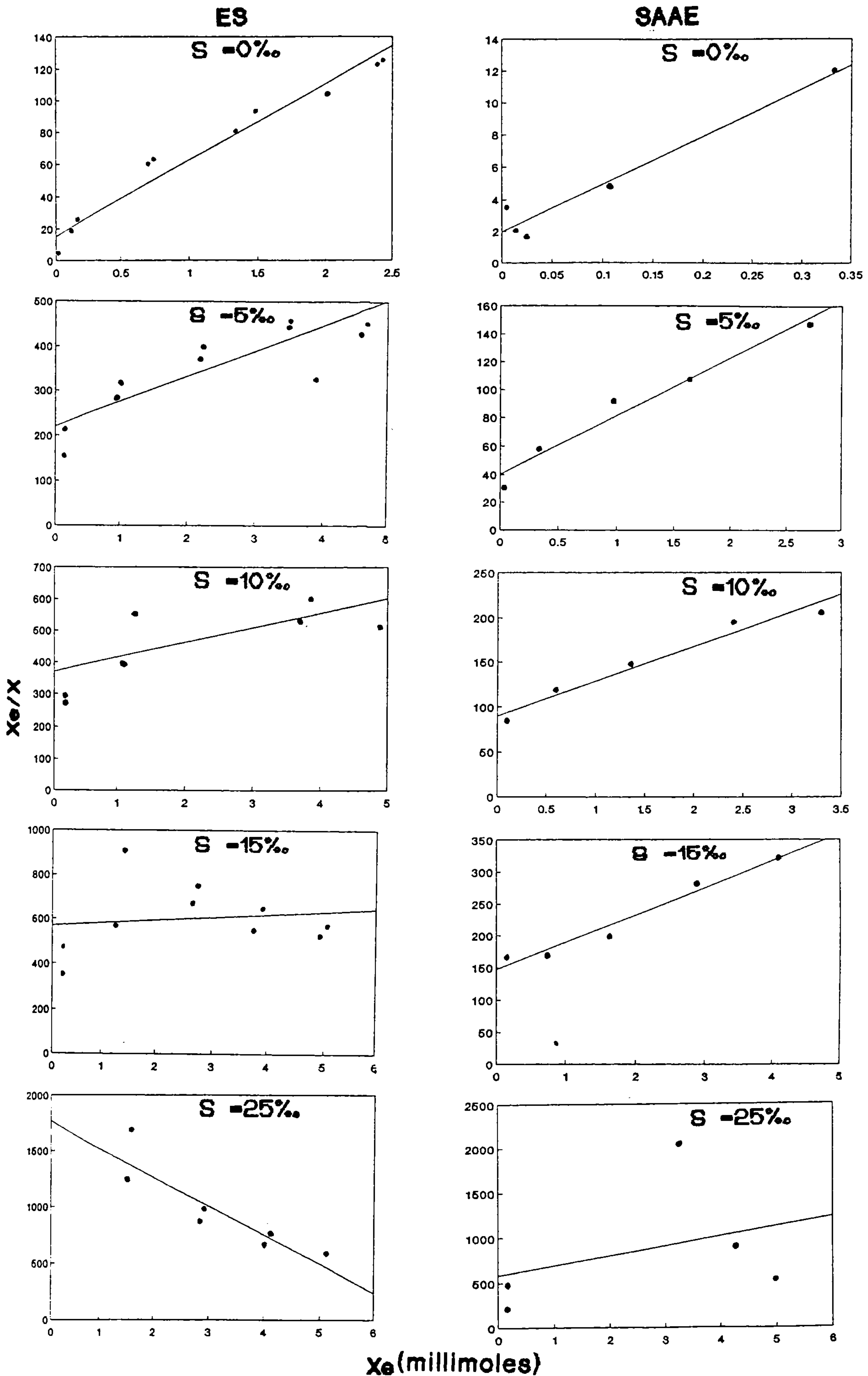


Fig.4.9 Langmuir isotherm for the adsorption of manganese by ES and SAAE.

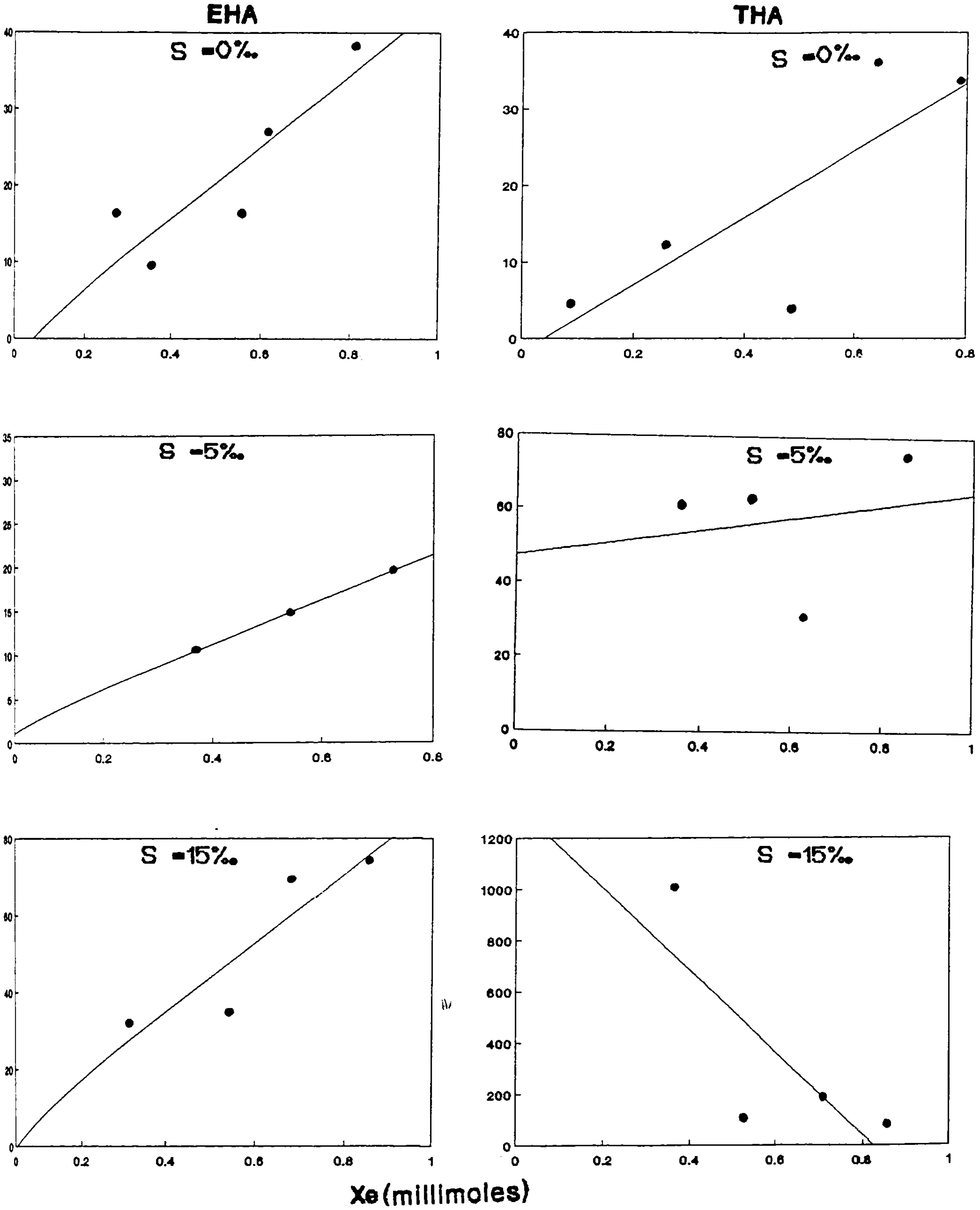


Fig.4.10 Langmuir isotherm for the adsorption of manganese by EHA and THA

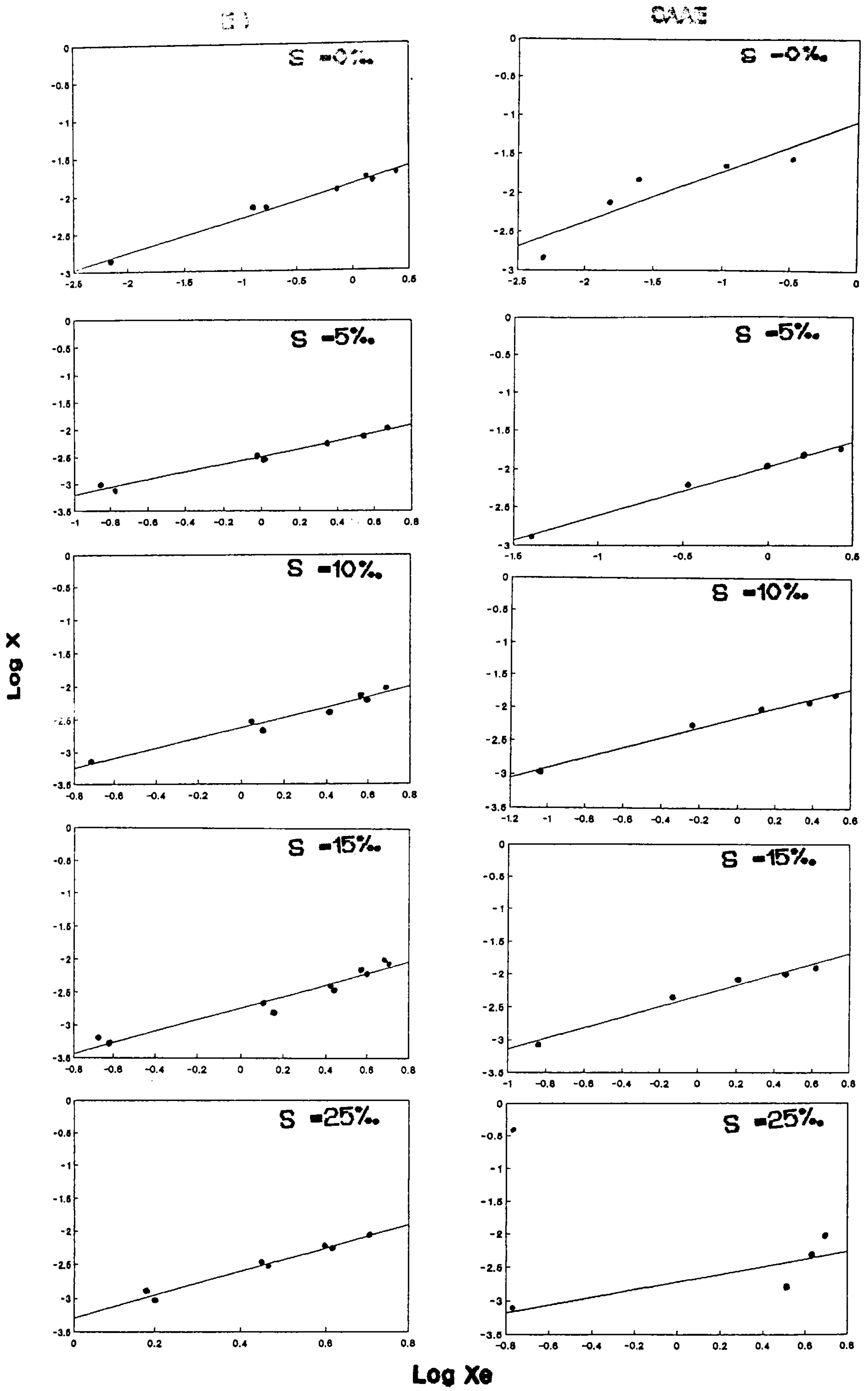


Fig.4.11 Freundlich isotherm for the adsorption of manganese by ES and SAAE

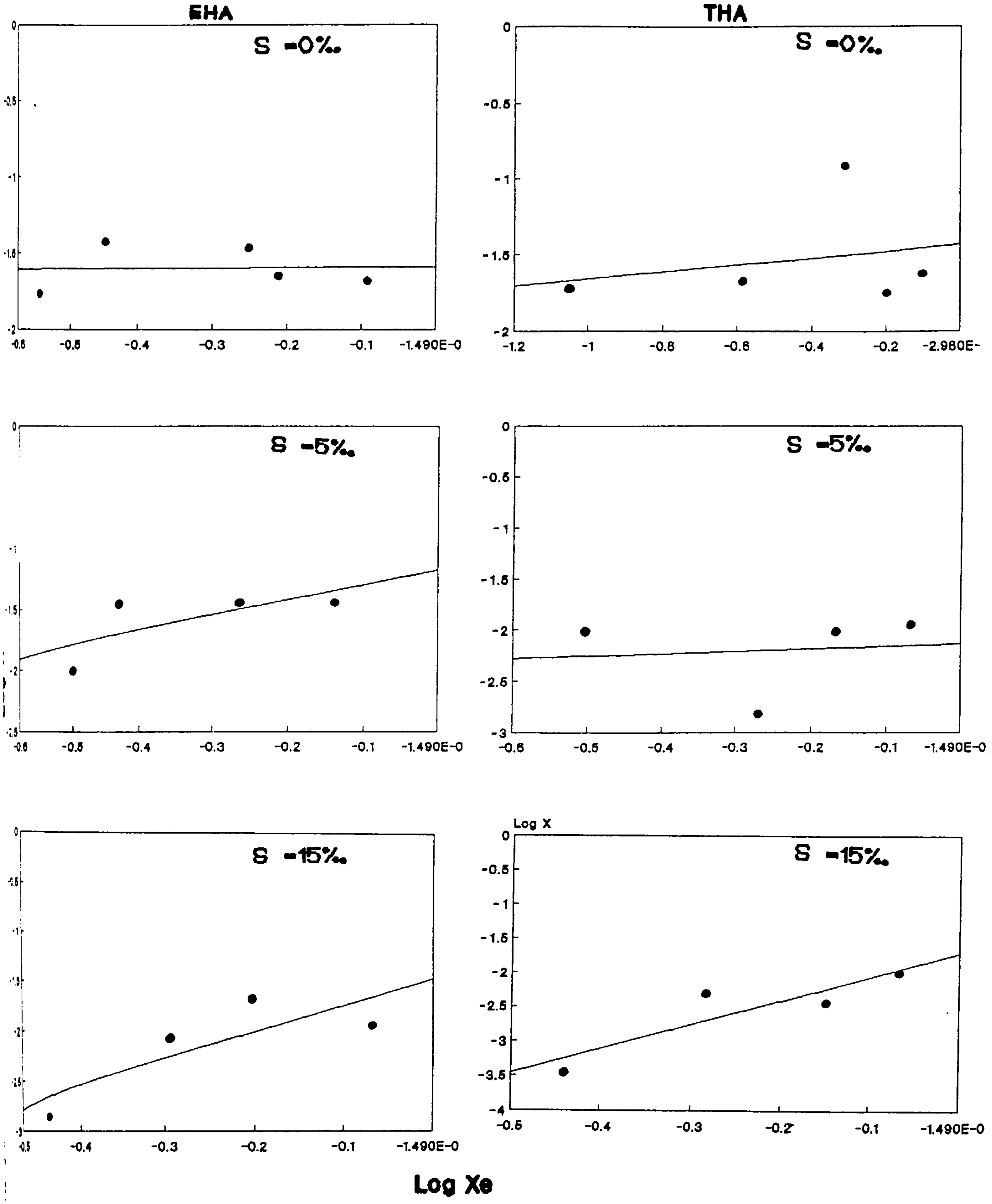


Fig.4.12 Freundlich isotherm for the adsorption of manganese by EHA and THA

(1977) found that organic matter destruction by H_2O_2 released new sorption sites. They reported that metals may also precipitate during organic matter destruction.

Recently, Gamble *et al.* (1976) have shown by NMR measurements that Mn(II) forms an outer sphere complex with fulvic acid, while Fe(III) forms an inner sphere complex. Gamble *et al.* (1977) and Mc Bride (1978) concluded from ESR measurements that Mn(II), rather than forming chelates with humic materials, was bound in fully hydrated form [as $Mn(H_2O)_6^{2+}$] by electrostatic binding only. An outer sphere complex is one in which the ion is bound electrostatically to the poly anion (such as HA) without displacement of water of coordination from the ion by a negatively charged functional group. In an inner sphere complex, ligand functional groups may enter into coordination positions and displace strongly coordinated water molecule. Mc Bride (1978) concluded from ESR investigations that HA does not form highly covalent metal organic bonds with copper or manganese. He suggested that a single electrostatic bond formed between the humic acid and metals is consistent with the ESR results, which appears to put some doubts on chelation as a predominant mechanism.

Zinc

Zinc is a bluish white, relatively soft metal with a density slightly less than that of iron. Zinc is a moderately abundant element. Many organic compounds, including humic and fulvic acids and a wide range of biochemical compounds, form stable compounds with zinc.

Considerable anthropogenic inputs of zinc into the Cochin estuary was reported by earlier workers (Sankaranarayanan *et al.*, 1978; Paul and Pillai,1983a; Ouseph, 1987; Nair *et al.*, 1990; Shibu *et al.*, 1990). Speciation studies on zinc in estuarine river Periyar revealed that considerable quantities of labile zinc with high organic matter association (Shibu,1992). Nair *et* *al.* (1990) observed significant seasonal and spatial variation in the distribution of zinc in surficial sediments. Total zinc varied from 14-259 mg/Kg. Organic bound fraction of zinc contributing 6.7-43.4% of total zinc explaining the influence of organic matter both detrital and autochthonous (Nair, 1992).

K_p values decreased as salinity increased. Adsorption capacity in Freundlich equation showed a decreasing trend in saline condition in all four samples (Tables 4.9 and 4.10). Cavallaro and Mc Bride (1984) found that destruction of the organic matter in two soil clays had little effect on zinc and copper adsorption. This is supported by the observation, the lower value of adsorption capacity of SAAE compared to ES. Langmuir and Freundlich isotherms for zinc adsorption are given in Fig. 4.13, 4.14, 4.15 and 4.16. Salinity is one of the main factors influencing adsorption. Increase in adsorption capacity at lower salinities, may be due to the retention of the more highly charged cation, thus the Zn^{2+} would be favoured over the Na^+ at the lower ionic strengths. At higher ionic strengths the Na^+ offers more competition for adsorption sites due to mass action (Bohn *et al.*, 1979). Petruzzelli *et al.* (1978) also reported that less cadmium and copper were adsorbed with increasing ionic strength due to competition for the existing soil sites.

Zinc adsorption by whole soil usually yields two or more linear portions to the Langmuir isotherm, which can be interpreted as being caused by different types of adsorption sites (Shuman, 1975). Hydrous iron oxides are important in zinc adsorption, but removing them from soil gave a mixed results, in that for some soils it increased zinc adsorption and for others it decreased zinc adsorption (Shuman, 1976). Cavalaro and Mc Bride (1984) found that removal of the iron oxide fraction from two soil clays reduced zinc adsorption. Soil organic matter and manganese oxides also influence metal adsorption. Extraction of soil organic matter with NaOH increased zinc adsorption in soils (Mangaroo *et al.*, 1965). Here also a lower Kp value was observed

Table.4.9 Langmuir and Freundlich constants for adsorption of Zn on ES and SAAE

SALINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	ES^a	0.024	40.662^{***}	2.154	0.053^{**}
	SAAE^b	-0.009	-9.770^{*}	0.654	1.101^{**}
5	ES	0.021	6.931^{***}	1.923	0.021^{***}
	SAAE	-1.013	-0.319	0.907	0.510^{**}
10	ES	0.024	44.601^{***}	2.230	0.049^{**}
	SAAE	0.158	1.525	0.999	0.231^{**}
15	ES	0.020	6.351^{***}	2.393	0.017^{***}
	SAAE	0.157	1.174	0.956	0.211^{**}
25	ES	0.021	5.969^{***}	1.843	0.016^{***}
	SAAE	-0.104	-0.912	0.861	0.176^{**}

^{a}n=10 ^{b}n=5, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table.4.10 Langmuir and Freundlich constants for adsorption of Zn on Humic acids.

SALINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	EHA	0.021	-18.506**	-43.478	0.026
	THA	0.021	34.668*	23.294	0.020
5	EHA	0.019	15.157	-9.200	0.016
	THA	0.006	-6.994*	-8.650	0.011

n=5 , * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

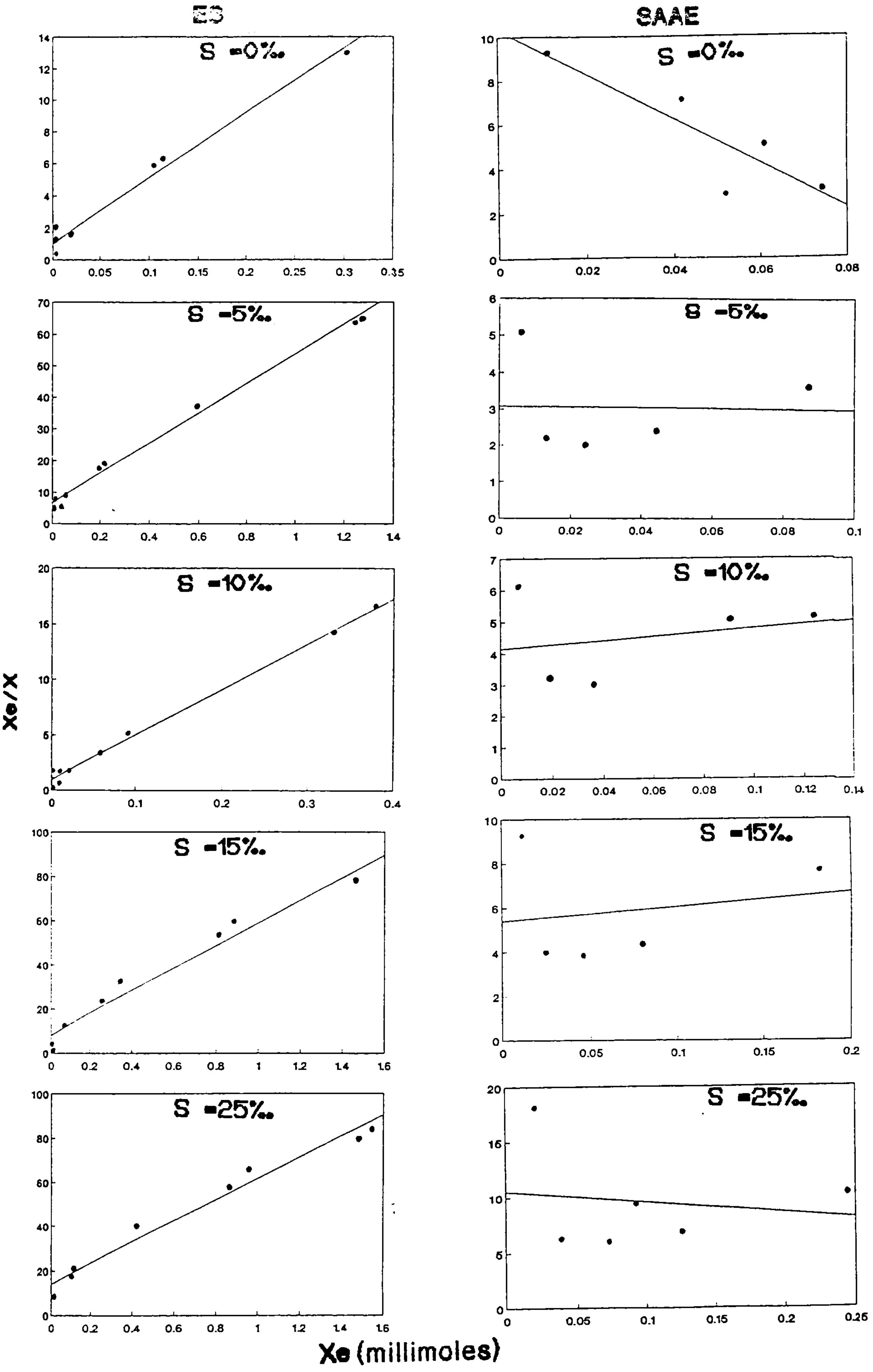


Fig.4.13 Langmuir isotherm for the adsorption of zinc by ES and SAAE.

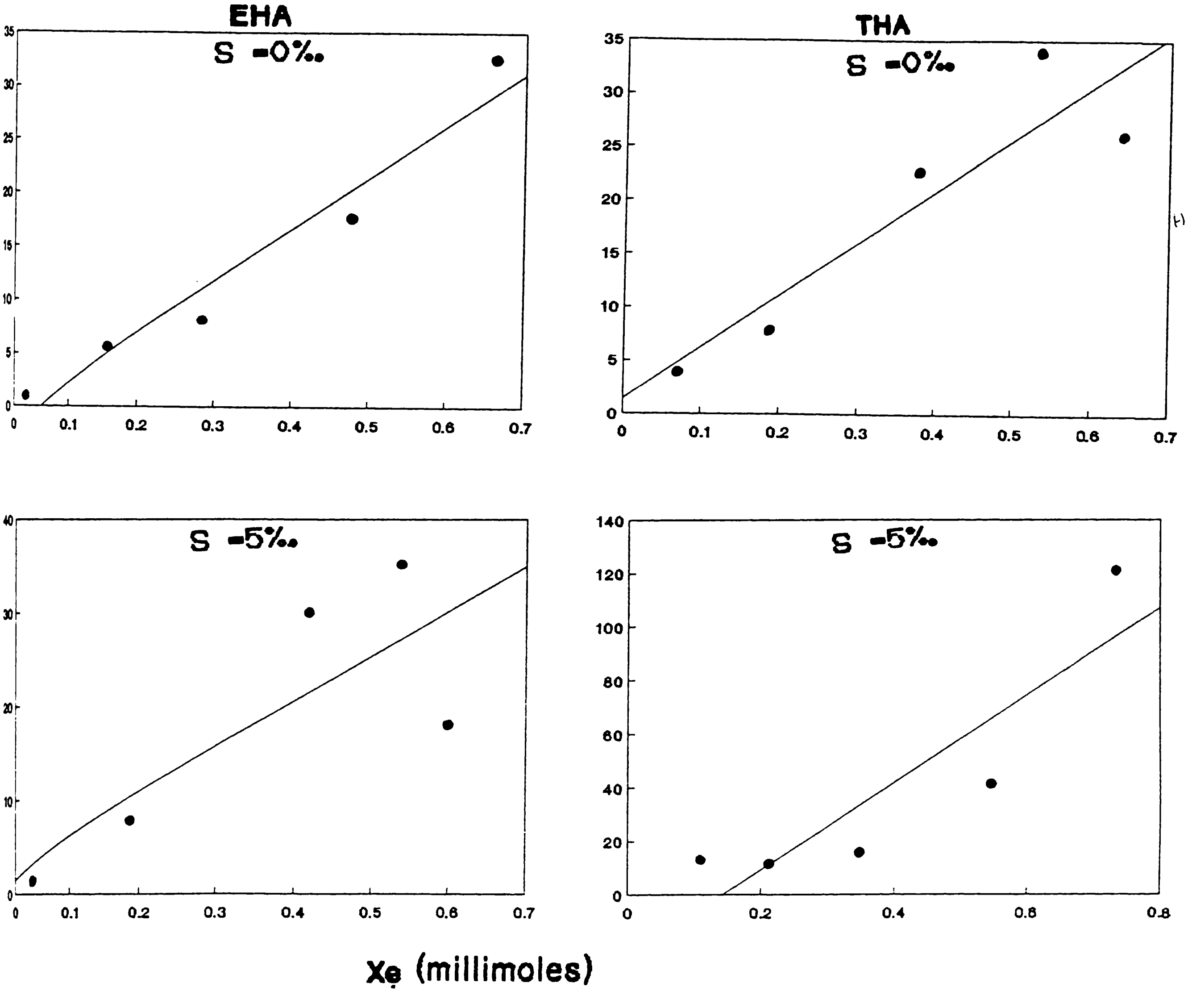


Fig.4.14 Langmuir isotherm for the adsorption of zinc by EHA and THA

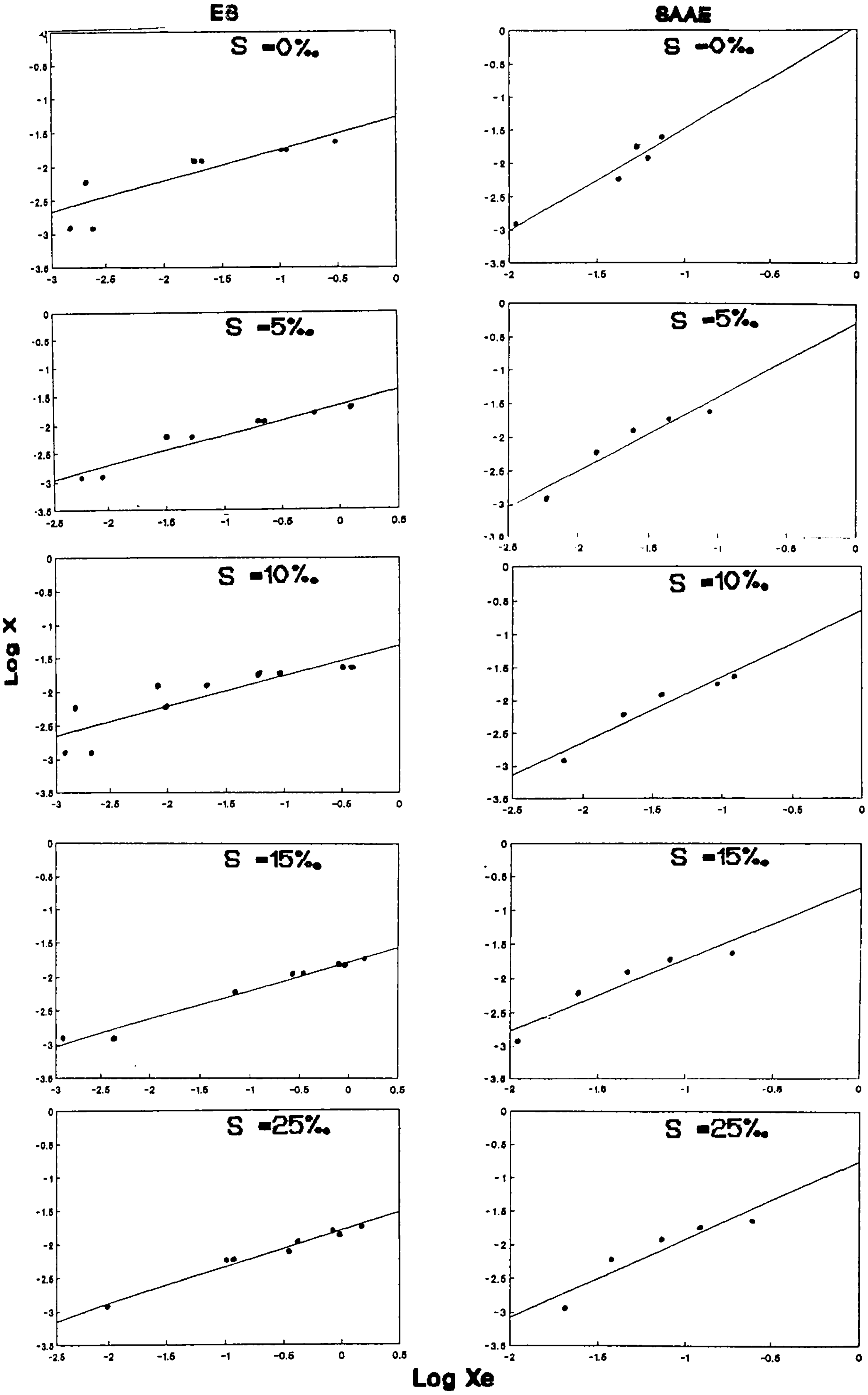


Fig.4.15 Freundlich isotherm for the adsorption of zinc by ES and SAAE

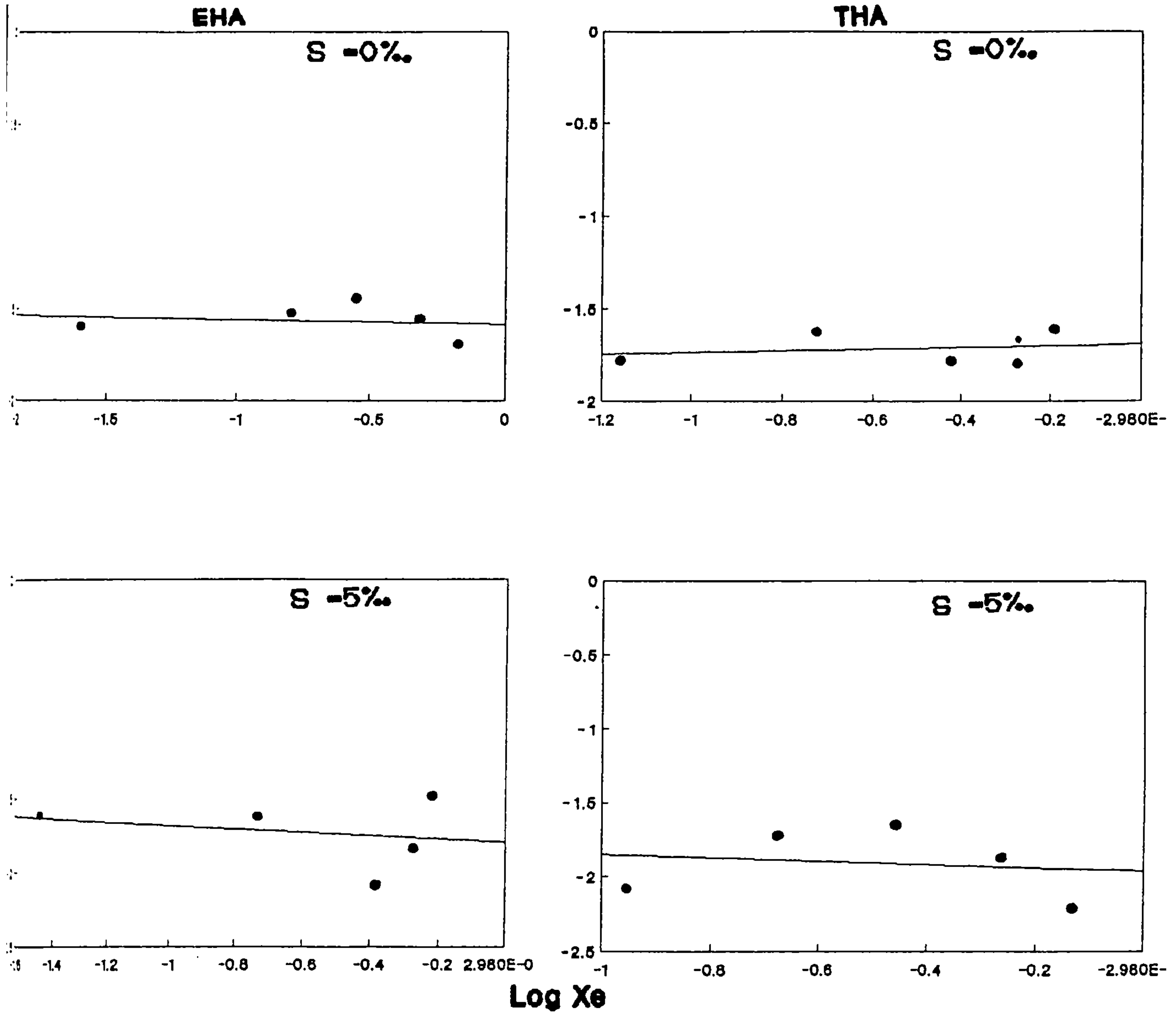


Fig.4.16 Freundlich isotherm for the adsorption of zinc by EHA and THA

in SAAE compared to ES.

Zinc-Humic Acid complexes can influence geochemical distribution. Randhawa and Broadbent(1965) and Schnitzer and Skinner (1963) and have suggested that humic and fulvic fractions of organic matter are very important in Zinc adsorption. Zinc can be fixed through solid phase adsorption (including complexation) or formation of insoluble precipitate. The hydrolysis and solubility constants for zinc hydroxides indicated that these insoluble forms of zinc can't be controlling the levels of soluble zinc. Most of the zinc immobilization was attributed to surface adsorption and complexation by organic matter. But Bilinski *et al.*(1991) observed a powerful adsorption of zinc on kaolinite and bentonite in natural and UV irradiated water.

Mercury

At present the most significant anthropogenic activities giving rise to emission of mercury to land, water and air appears to be the following :

1) Mining and smelting of ores, in particular copper and zinc smelting
2) Burning of fossil fuels, mainly coal
3) Industrial production process, in particular the mercury cell chloralkali process for production of chlorine and caustic soda.

Around 45% of the anthropogenic release of mercury was emitted to air, 7% to water and 48% to land. Mercury is among the most toxic elements to man and mercury salts show acute toxicity with a variety of symptoms and damages. Society became aware of environmental mercury poisoning during late 1950s afte Minamata Bay disaster, i.e., severe neurological disorder o inhabitants of Minamata arose by the consumption of fis containing high levels of methyl mercury.

able.4.11 Langmuir and Freundlich constants for adsorption of Hg on ES and SAAE

LINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	ES[a]	0.021	13.080^{*}	1.111	0.147^{***}
	SAAE[b]	0.008	0.645	1.804	0.004
5	ES	-0.010	-360.936	0.988	6.439^{*}
	SAAE	0.0004	-15.696^{*}	5.464	0.003
10	ES	-0.005	-248.528	0.758	3.668
	SAAE	0.0008	-39.325^{*}	-145.98	0.002
15	ES	0.008	2238.579^{***}	2.363	0.091^{**}
	SAAE	0.0005	-21.903^{*}	-6.262	0.001
25	ES	0.007	271.974^{***}	3.327	0.014^{**}
	SAAE	0.001	-16.954^{*}	2.237	0.005

n=10 [b] n=5 * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table.4.12 Langmuir and Freundlich constants for adsorption of Hg on Humic acids.

SALINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	EHA	0.029	2260.006	-1.639	0.0004
	THA	0.091	1205.792	1.248	15.624^{**}
5	EHA	0.017	-12235.7^{**}	30.637	0.019
	THA	0.016	-1.205	-1.701	0.0002^{*}
15	EHA	0.007	-80.641^{*}	-29.762	0.013
	THA	0.015	-969.757^{**}	94.967	0.021

n=5 , * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

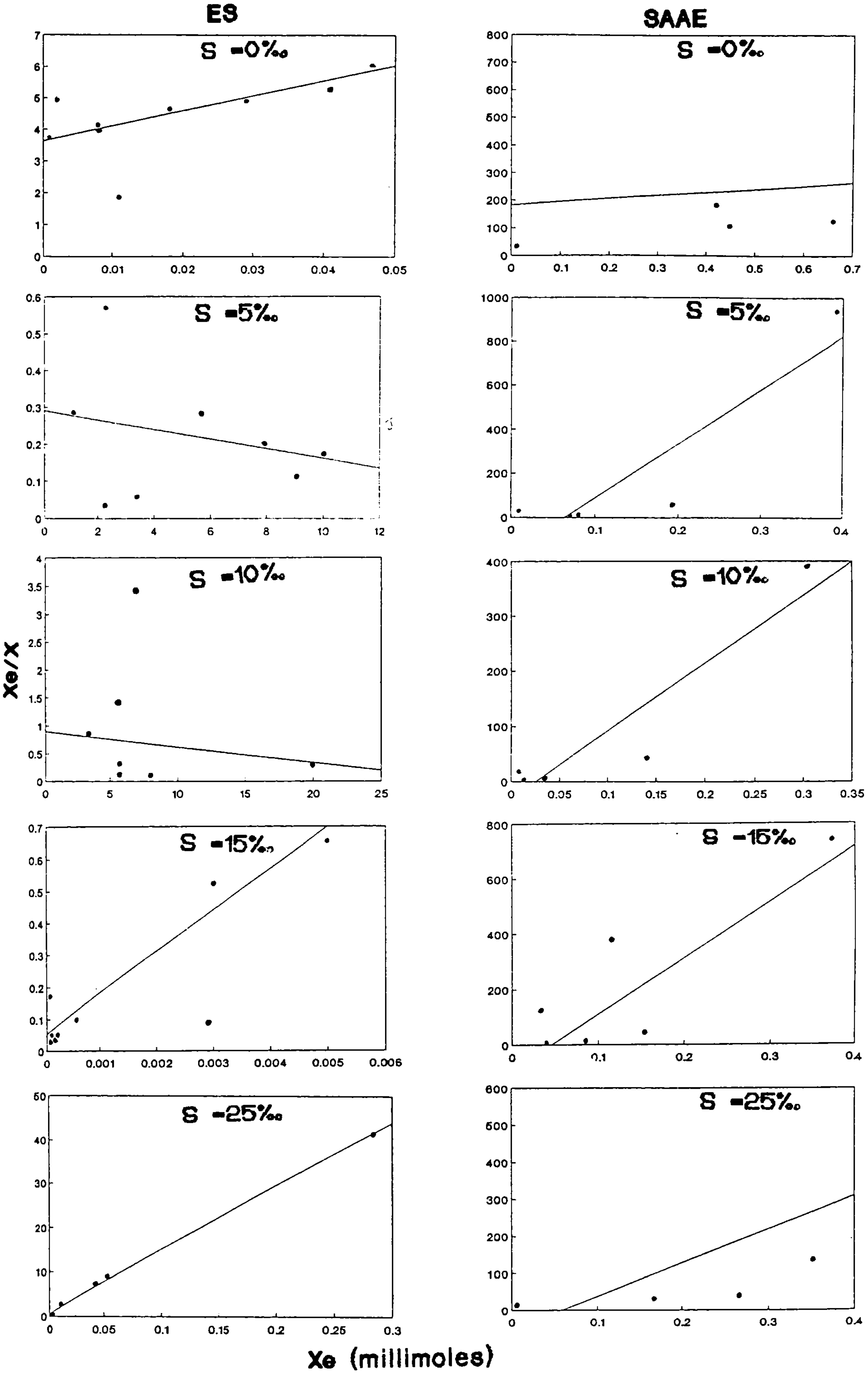


Fig.4.17 Langmuir isotherm for the adsorption of mercury by ES and SAAE.

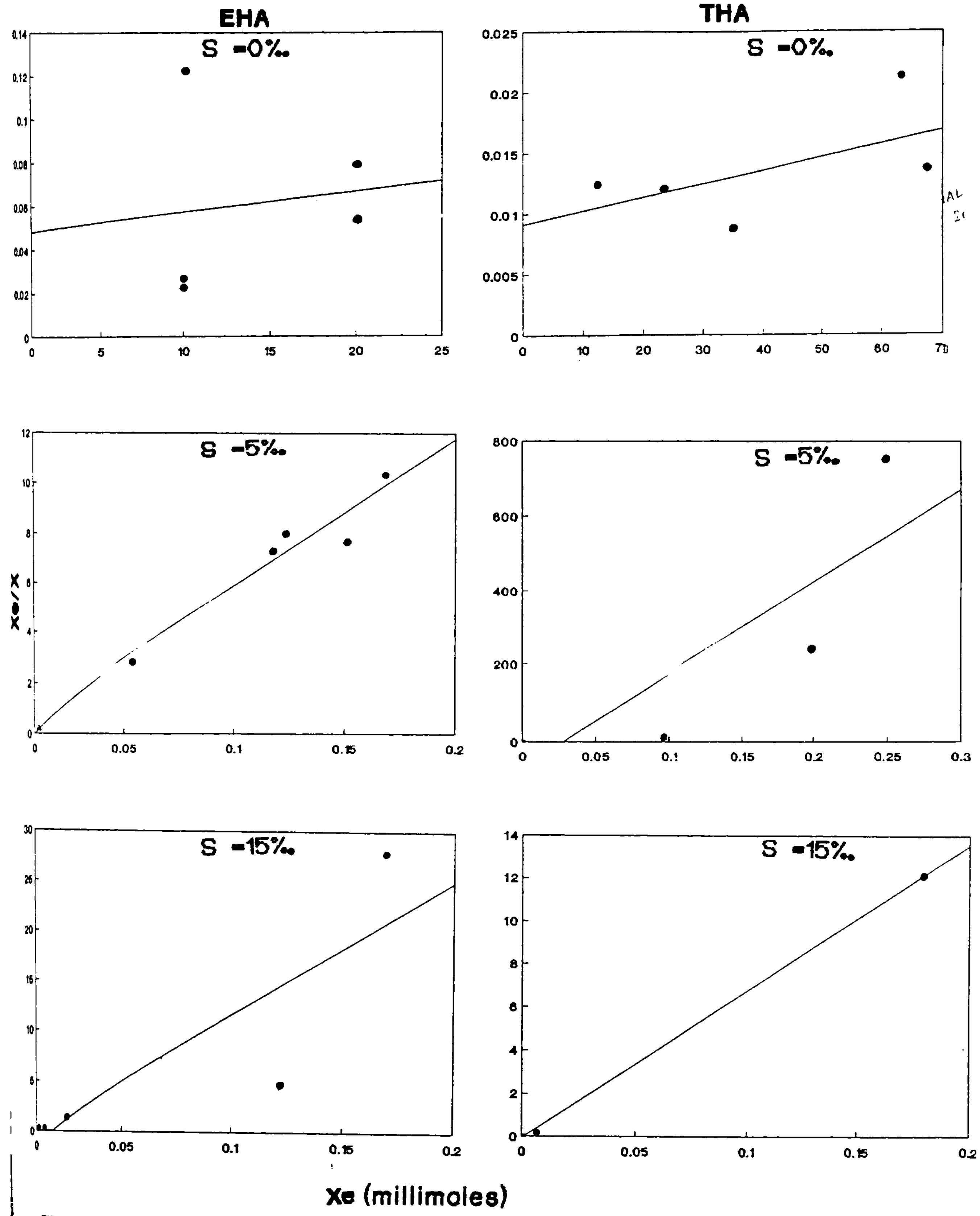


Fig.4.18 Langmuir isotherm for the adsorption of mercury by EHA and THA

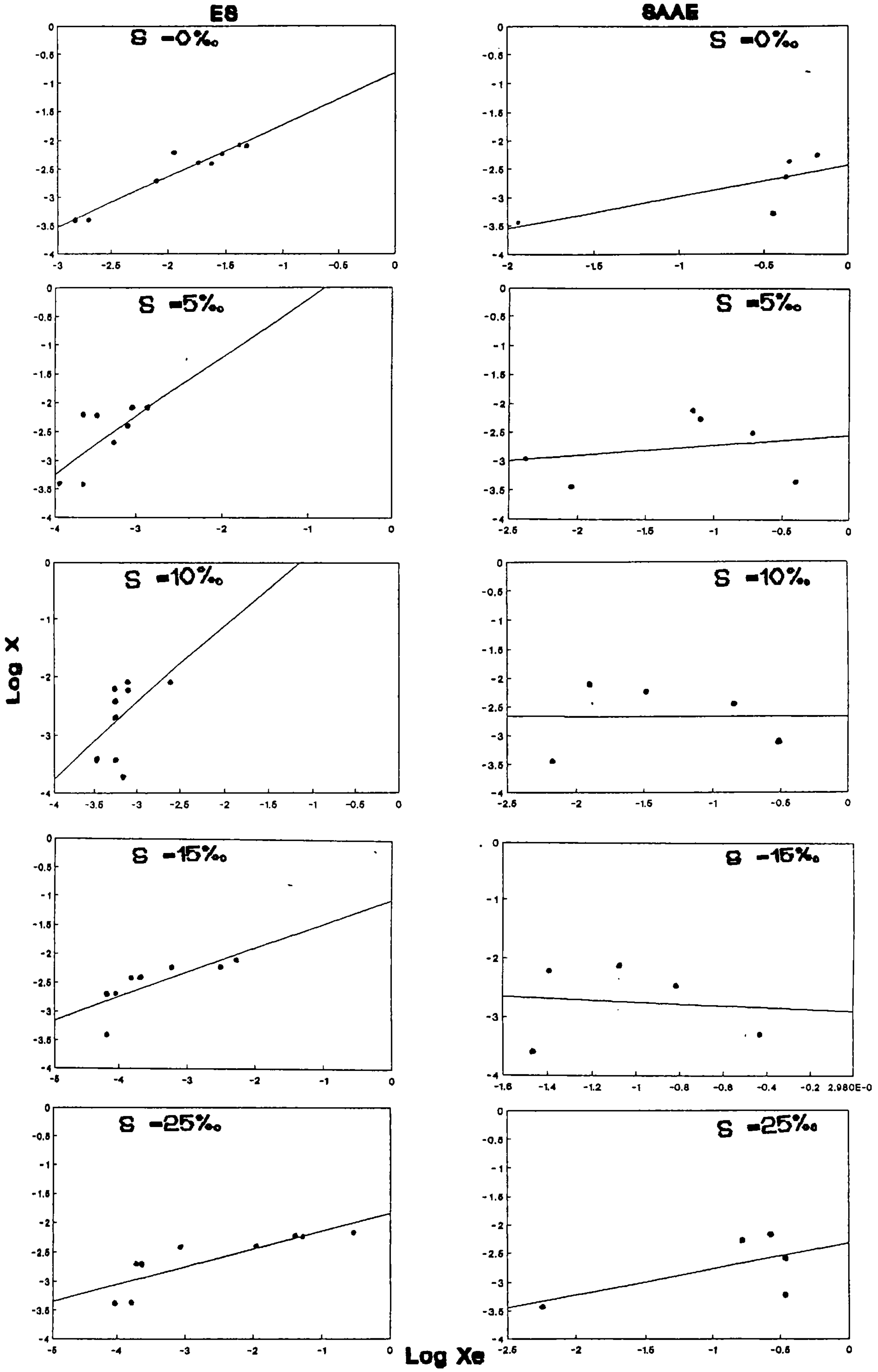


Fig.4.19 Freundlich isotherm for the adsorption of mercury by ES and SAAE

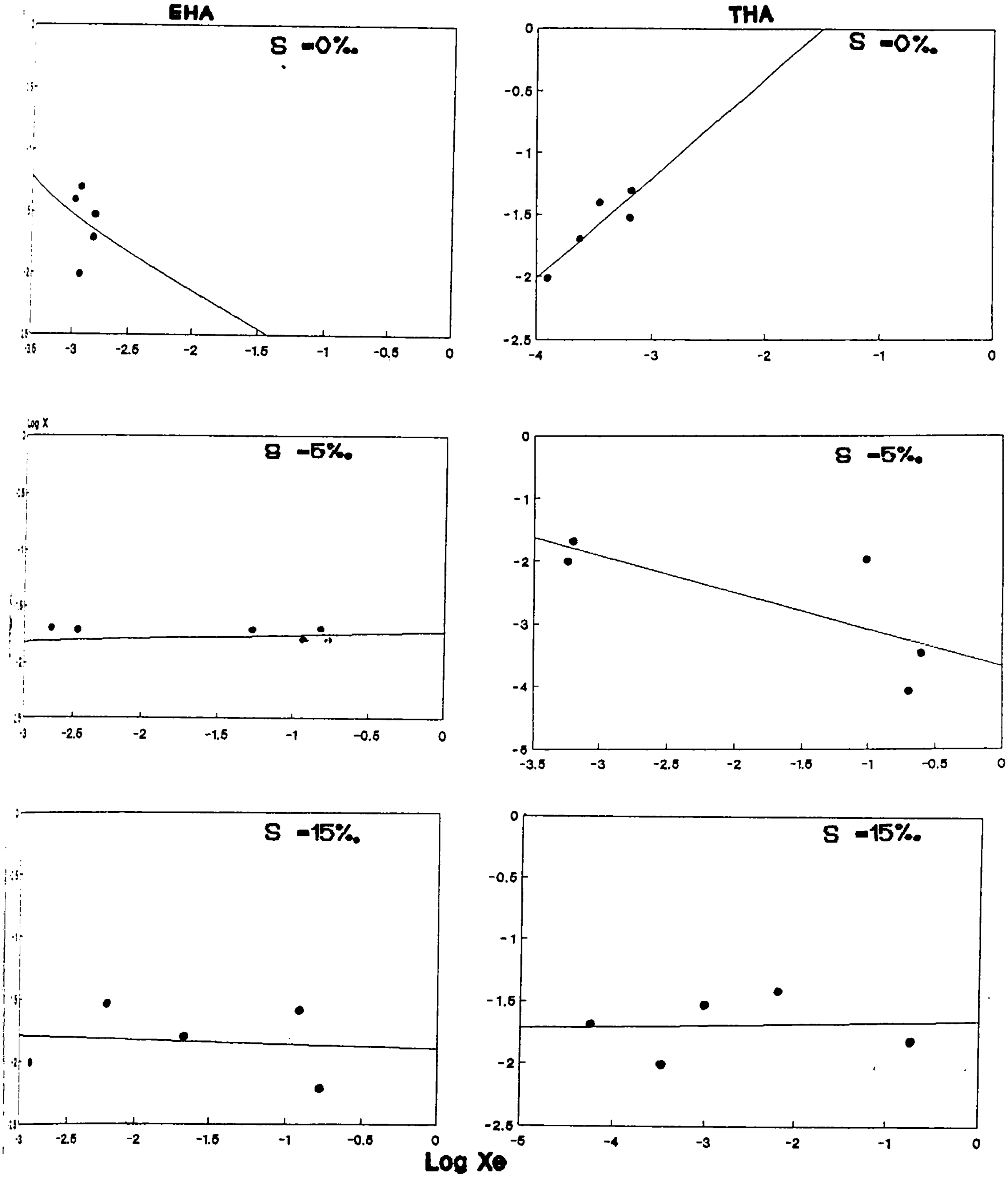


Fig.4.20 Freundlich isotherm for the adsorption of mercury by EHA and THA

Partition coefficient of SAAE and THA was less than that of ES and EHA respectively. Value of K_1 increased as salinity increased in the case of ES, EHA, and THA whereas in SAAE its value increased when salinity changed from 0-5 x 10^{-3} and then decreased. Freundlich adsorption capacity values exhibited a decreasing trend in saline conditions for SAAE, EHA and THA, while an opposite trend was observed in ES (Tables 4.11 and 4.12; Fig. 4.17, 4.18, 4.19, and 4.20).

Sorption of mercury by a number of sedimentary materials and organic substances with various functional groups was studied by Reimers and Krenkel (1974). They found the uptake and the capacity for adsorbing inorganic $HgCl_2$ to be in the following order R-SH > illite > montmorillonite > $R-NH_2$ > kaolinite > R-COOH > fine sand > medium sand > coarse sand.

Lead

Lead is a member of the IV B group of the periodic table. Two oxidation states Pb^{2+} and Pb^{4+} are stable, but the environmental chemistry of the element is dominated by the lead ion,Pb^{2+}. The Pb^{2+} in soils and water bodies comes from the break down of parent rock and from artificial sources such as industrial emissions, leaded gasoline and lead bearing insecticides applied to farm lands. When lead is released into the environment, it has long residence time compared with most other pollutants. As a result, lead and its compounds tend to accumulate in soils and sediments where due to their less solubility and relative freedom from microbial degradation, they will remain accessible to the food chain. Whatever the source, Pb^{2+} can be toxic to plants and animals. Therefore, it is important to understand how lead moves in the environment so that we may prevent dangerously high concentrations of it from entering the food chain.

Considerable seasonal and spatial variations in the distribution of lead in sediments of Cochin estuary was reported ble.4.13 Langmuir and Freundlich constants for adsorption of Pb on ES and SAAE

SALINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	ES [a]	0.034	8.865	1.376	0.101
	SAAE [b]	-0.011	-52.237	0.814	0.511
5	ES	0.001	-1092.55	-1.523	0.0001
	SAAE	-0.026	-3.414	1.922	0.024
10	ES	-0.011	-11.957	1.321	0.079
	SAAE	-0.002	-53.161	0.676	3.164
15	ES	0.0004	-167.133	-1.742	0.0001
	SAAE	-0.001	-31.038	0.461	17.709
25	ES	-0.002	-18.775	1.034	0.107
	SAAE	-0.001	-26.959	0.339	143.847

=10 [b] n=5 * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table.4.14 Langmuir and Freundlich constants for adsorption of Pb on Humic acids.

SALINITY	ADSORBENT	LANGMUIR		FREUNDLICH	
		K_1	K_2	n	K
0	EHA	-0.007	-193.406	0.503	0.292^{*}
	THA	0.092	68.891	3.758	0.105
5	EHA	-0.064	-6.922	0.765	1.705^{***}
	THA	0.031	66.806	3.007	0.070
15	EHA	-0.024	-7.906	0.627	2.004^{***}
	THA	-0.104	-3.184	0.840	0.729^{**}

n=5 , * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

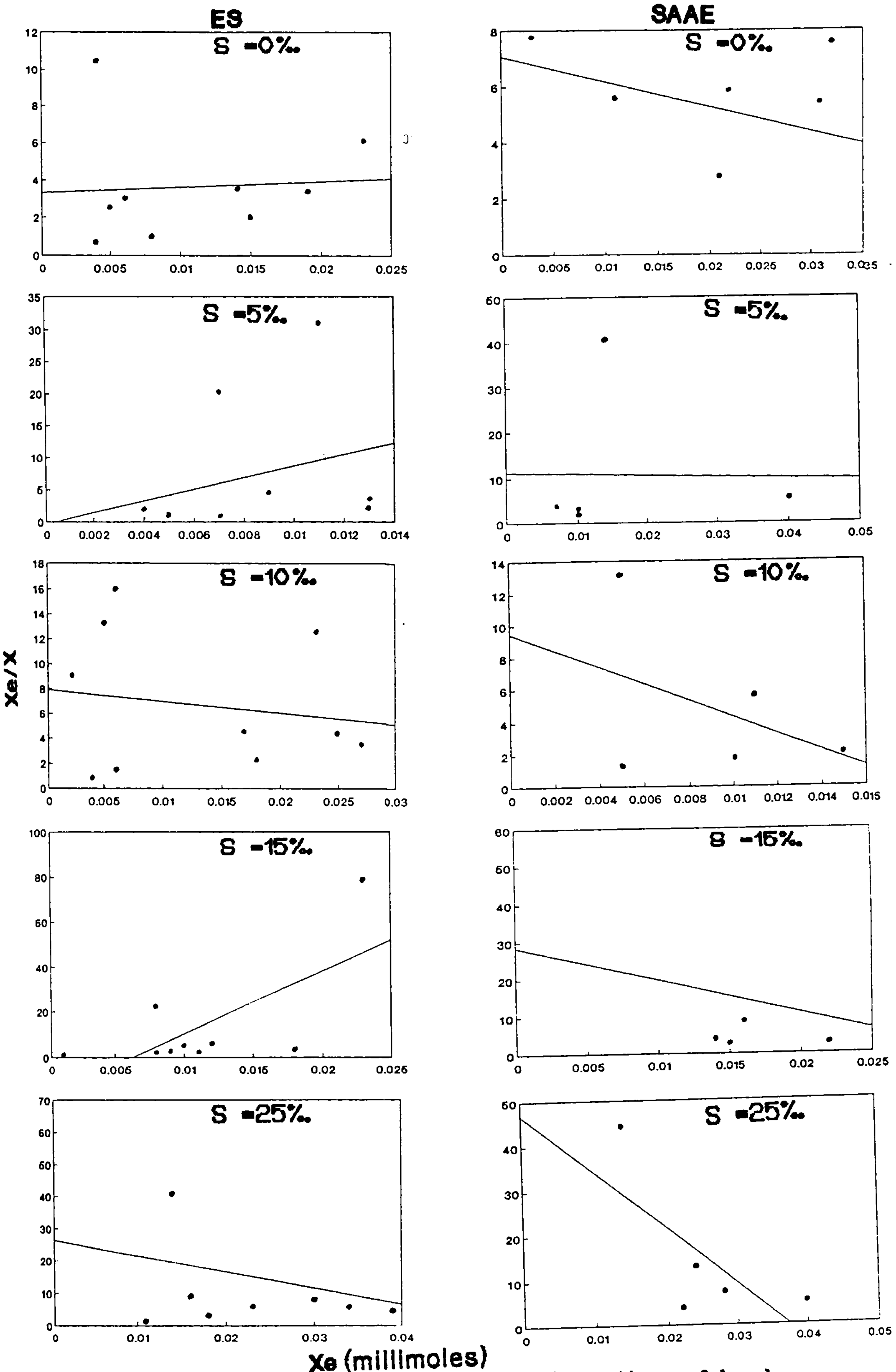


Fig.4.21 Langmuir isotherm for the adsorption of lead by ES and SAAE.

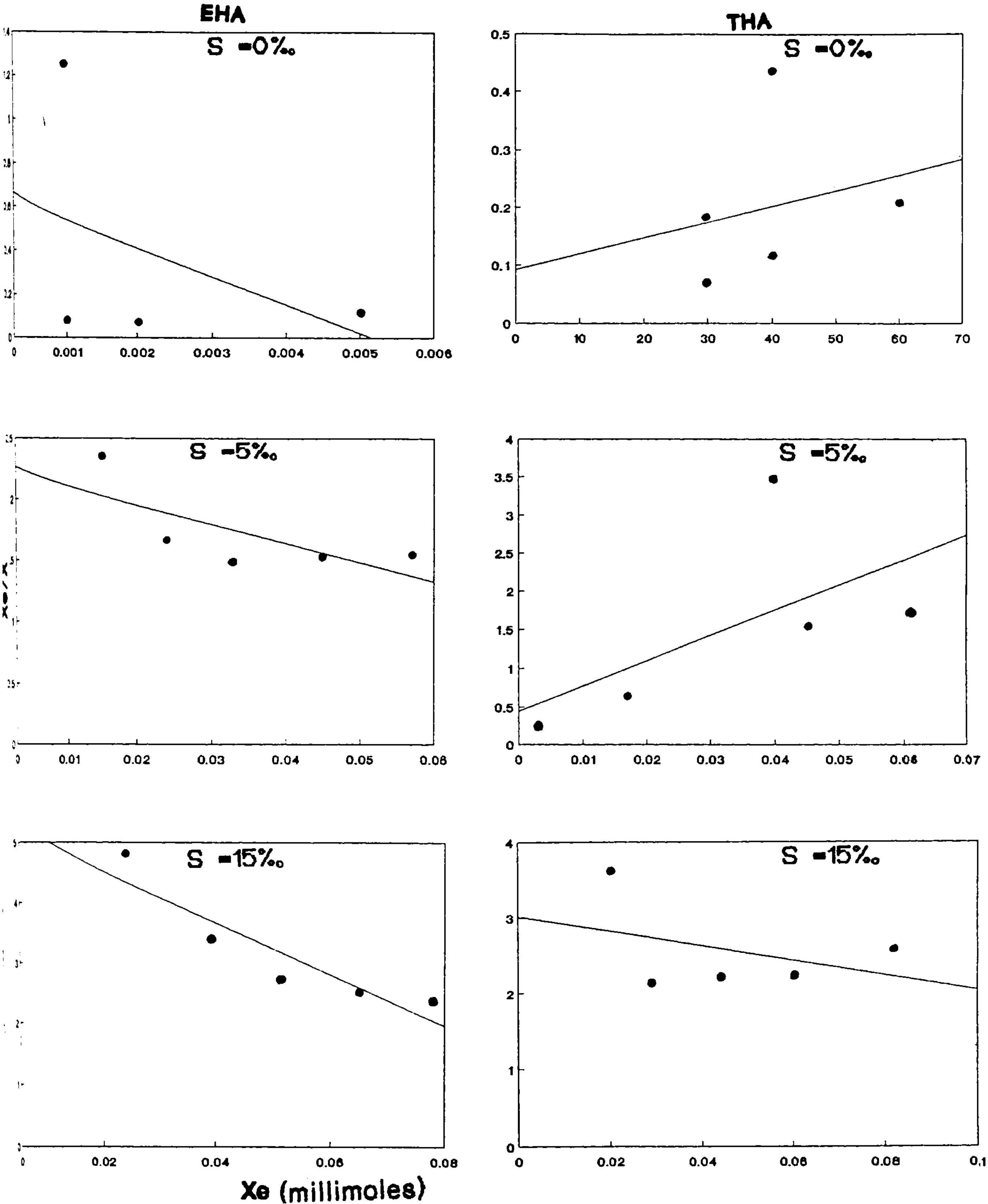


Fig.4.22 Langmuir isotherm for the adsorption of lead by EHA and THA

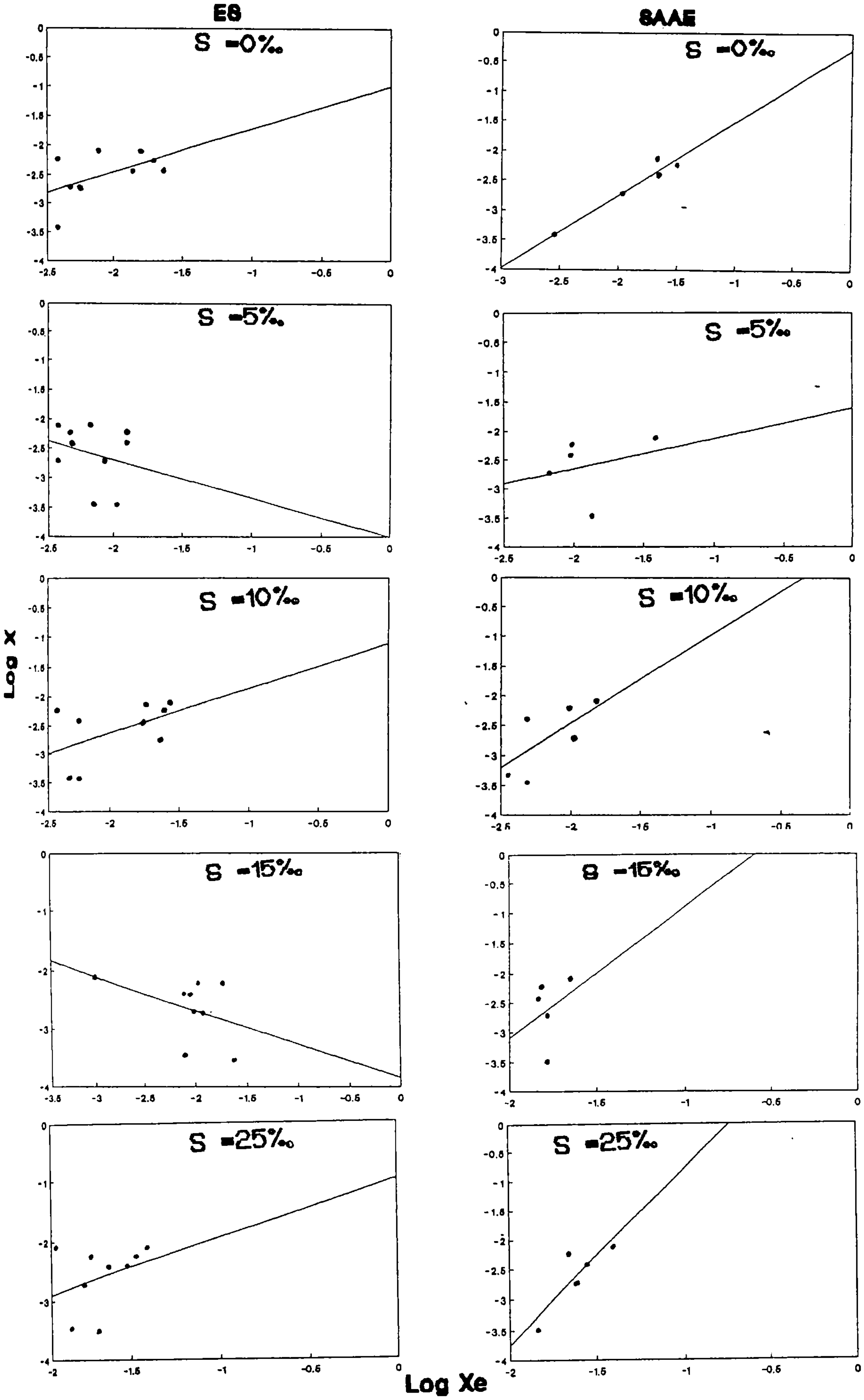


Fig.4.23 Freundlich isotherm for the adsorption of lead by ES and SAAE

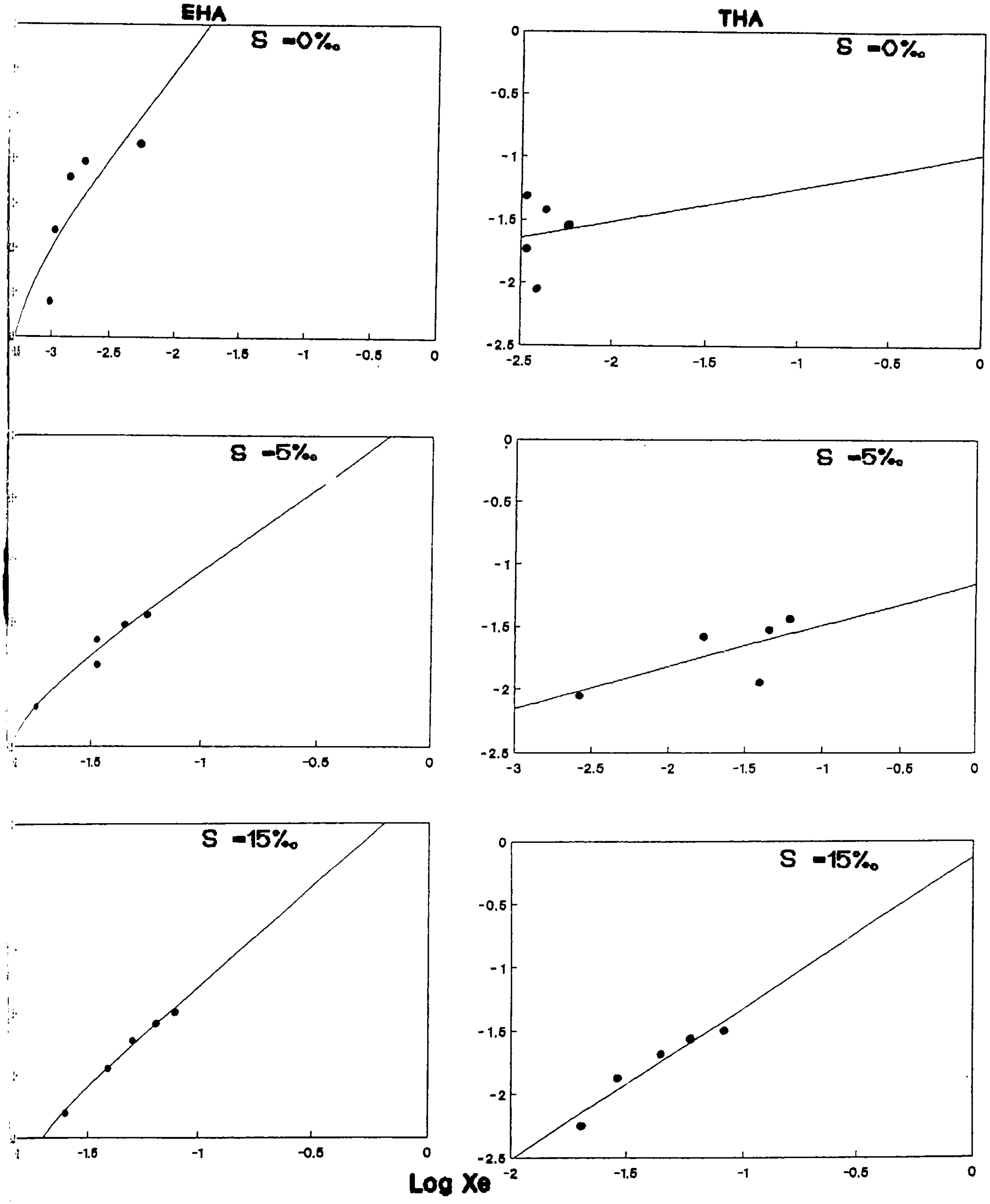


Fig.4.24 Freundlich isotherm for the adsorption of lead by EHA and THA

by Nair *et al.* (1990). Sewage discharges were considered to be an important anthropogenic source. Nair *et al.* (1991) reported significant spatial variation in the distribution of different forms of lead determined chemically in the sediments of the estuary. Total lead was quite low in comparison with values reported from other estuaries of India and amount of lead in the organic phase of the sediment contributed 1.4-28.6% of total lead.

Results of lead sorption are given in Tables 4.13 and 4.14 and Fig. 4.21, 4.22, 4.23 and 4.24 . Partition coefficient of SAAE was less than ES, while that of EHA and THA were almost constant. Equilibrium constant and K_1 (Langmuir slope) decreased with salinity in SAAE and ES. Freundlich constant exhibited a decreasing trend as ionic concentration of the medium increased. Petruzzelli *et al.* (1981) showed that lead adsorption capacity as measured by Langmuir isotherm was reduced by destroying the soil organic matter with H_2O_2. Soong (1974) who studied the competitive sorption of heavy metal onto the clay minerals established that lead was more competitive than copper and zinc. Forstner (1979) quoted the following affinity series for cation exchange on organic and inorganic substrates, Pb > Cu > Ni > Co > Zn. Elliot et al. (1986) also reported the high affinity of lead for adsorbing sites in soils. Recently Bilinski et al. (1991) reported high affinity of lead to estuarine particles. All these studies clearly demonstrated that lead had a high affinity for clay minerals and organic substances. Its high affinity to clay minerals would be attributed to its ionic radii (Soong, 1974) and to organic substances might be due to the high stability of lead-organic complexes (Irving and William, 1948).

B. Desorption Studies

A number of sediment forming materials with large surface area such as clay minerals, amorphous iron and manganese oxides as well as organic substances are capable of adsorbing trace metals either by cation exchange or as a result of inter molecular attraction (Forstner, 1979). In general, the bond forms of the adsorbed metal in mud are separated into the ion exchangeable form with minerals such as clay or sand, and the chemical form with organic matter such as humic acid (Riemer and Toth, 1970; Suzuki *et al.*, 1979). Therefore, ES, SAAE and humic acid were selected to determine the chemical form of the adsorbed metal in the sediment.

Table 4.15 and Fig 4.25 give the result of desorption experiment. Major fraction of the adsorbed metals, except mercury could be extracted with HCl and EDTA. mercury adsorbed in the four samples was not easily extracted with four extractants. In all cases NaH_2PO_4 released comparatively low amounts of metals.

Ammonium acetate (NH_4Ac) was the most suitable extractant to liberate the exchangeable metals from sediments and soils (Tessier *et al.*, 1979). NH_4Ac is strongly buffered around the natural point, and is thus suited for the determination of all exchangeable cations, but not efficient on silicates, sulphides or organic matter and therefore the metal extracted could be regarded as the best estimate of exchangeable trace metals present in the sediments. It was observed that copper, cadmium, manganese, zinc and lead which were experimentally adsorbed by ES were completely extracted (100%) and 11.2% of mercury with HCl, while NH_4Ac desorbed only 21.63% copper, 52.51% cadmium, 50.77% manganese, 25.41% zinc, 0.082% mercury and 9.81% lead. In the case of SAAE only 12.69-87.2% of adsorbed fraction was desorbed with HCl and 0.066-36.48% with NH_4Ac. Over 50% cadmium and manganese were removed from ES with NH_4Ac. These agree with the results of Petruzzeli *et al.* (1978), which states that cadmium in soil has a slight tendency to form stable complexes. Tada and Suzuki (1982) found that metals adsorbed on kaoline as ion exchangeable form were desorbed 87- 95% with NH_4Ac, but that those on humic acid were difficultly desorbed with one, where as these two forms could desorbed 81-100% with dilute HCl except for copper adsorbed on humic acid. Consequently it is found that the

Table.4.15. Percentage of metal release by extractants.

METAL	ADSORBENT	EXTRACTANT			
		NH_4Ac	E.D.T.A	HCl	NaH_2PO_4
COPPER	ES	21.63	99.38	100.00	0.82
	SAAE	11.90	68.19	71.4	0.00
	EHA	13.41	78.80	100.00	0.00
	THA	7.78	92.56	100.00	1.73
CADMIUM	ES	52.81	100.00	100.00	3.66
	SAAE	34.34	83.03	83.66	2.85
	EHA	46.61	94.75	90.19	4.95
	THA	51.34	94.05	88.71	6.16
MANGANESE	ES	50.77	100.00	100.00	17.02
	SAAE	36.48	86.04	87.21	18.79
	EHA	55.61	85.38	88.27	22.06
	THA	41.51	68.83	64.45	19.66
ZINC	ES	25.41	99.90	100.00	1.73
	SAAE	10.73	76.73	81.28	0.79
	EHA	31.45	100.00	92.60	7.05
	THA	41.86	96.42	100.00	10.39
MERCUARY	ES	0.08	3.09	11.20	0.43
	SAAE	0.07	5.69	12.69	0.24
	EHA	0.10	0.21	8.91	0.18
	THA	0.70	2.75	11.22	1.23
LEAD	ES	9.81	100.00	100.00	0.00
	SAAE	5.74	76.60	74.75	0.00
	EHA	29.04	56.88	89.55	0.00
	THA	31.75	74.89	94.83	0.00

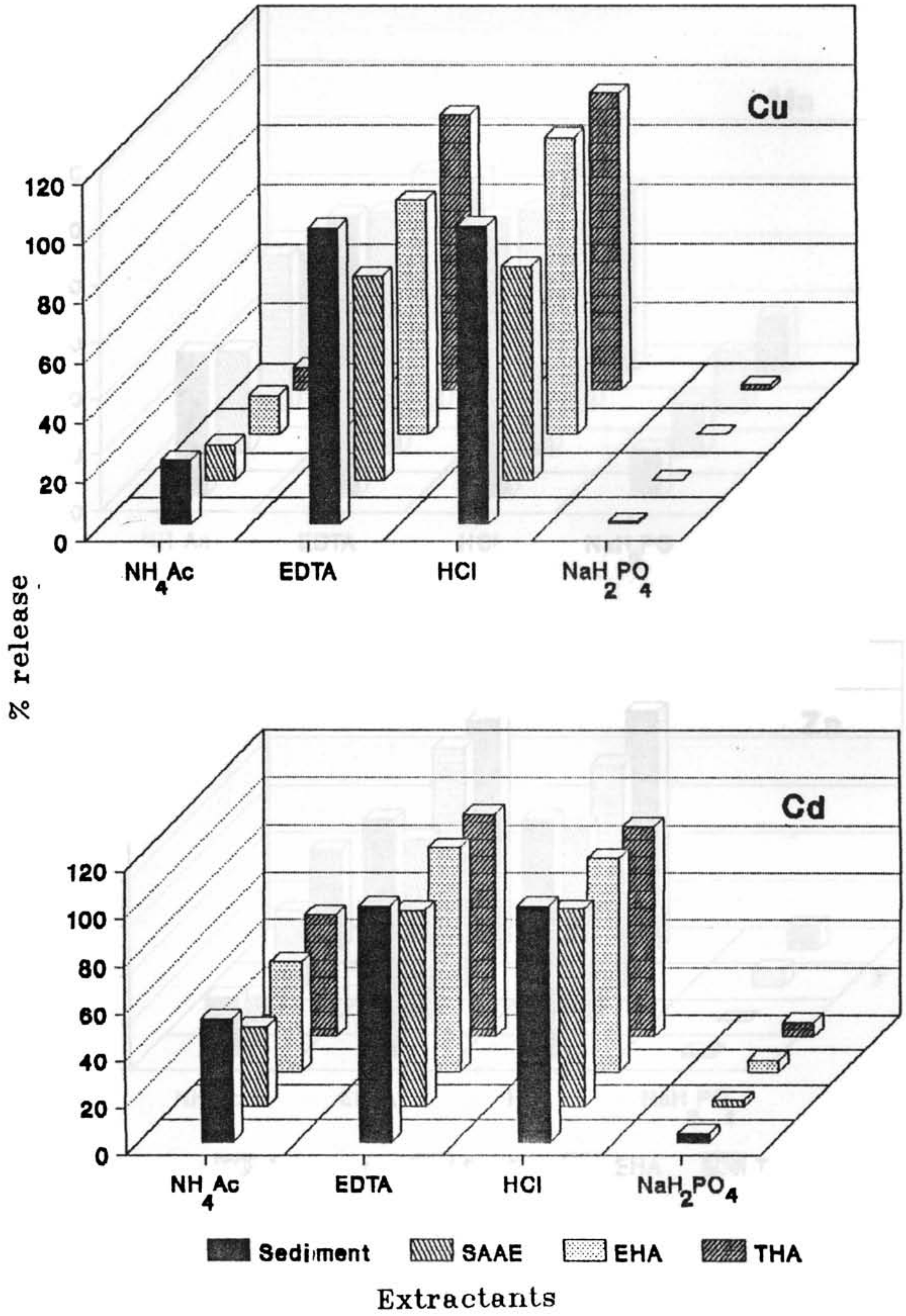


Fig.4.25 Percentage of metal release by the extractants

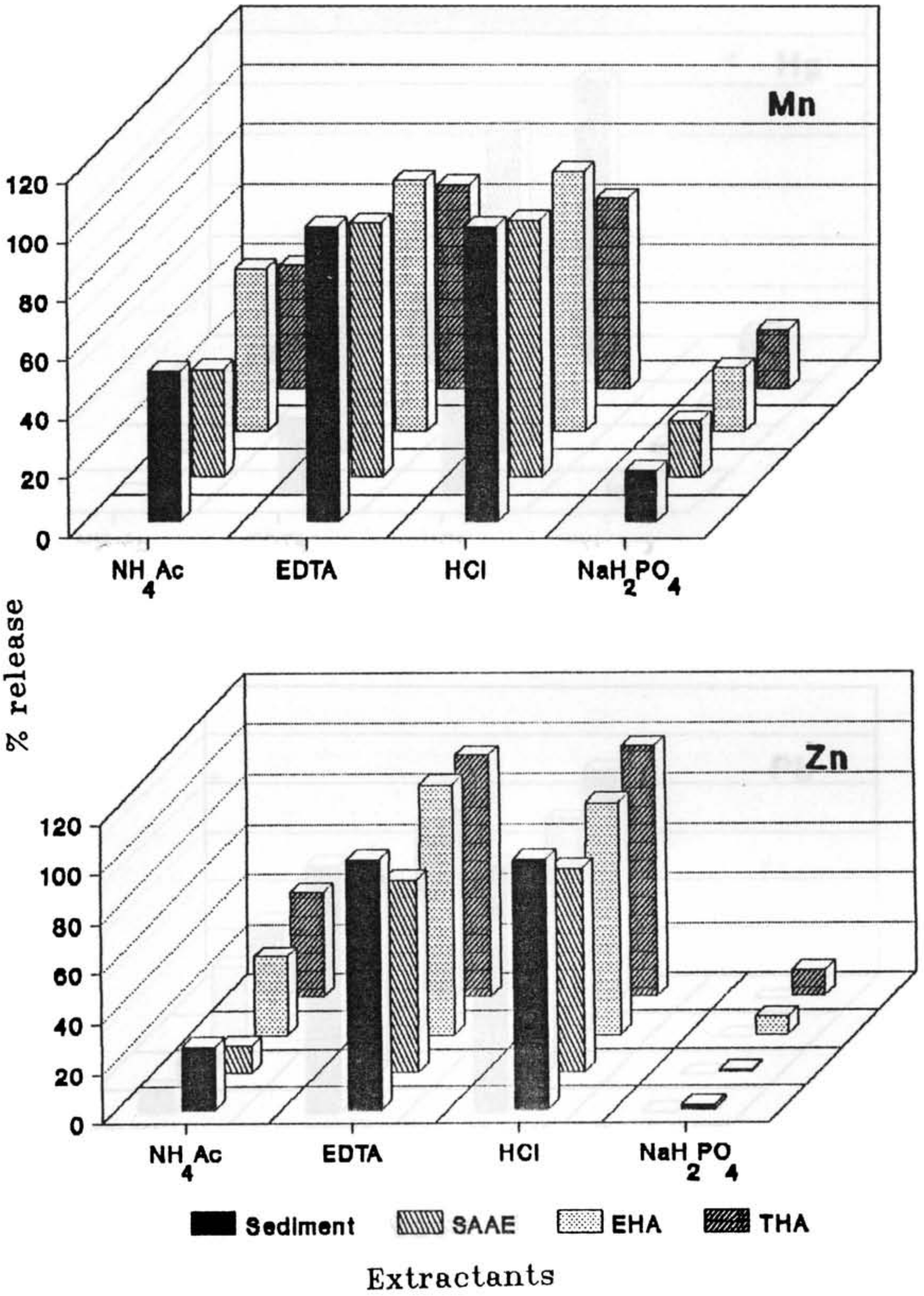


Fig.4.25 Percentage of metal release by the extractants

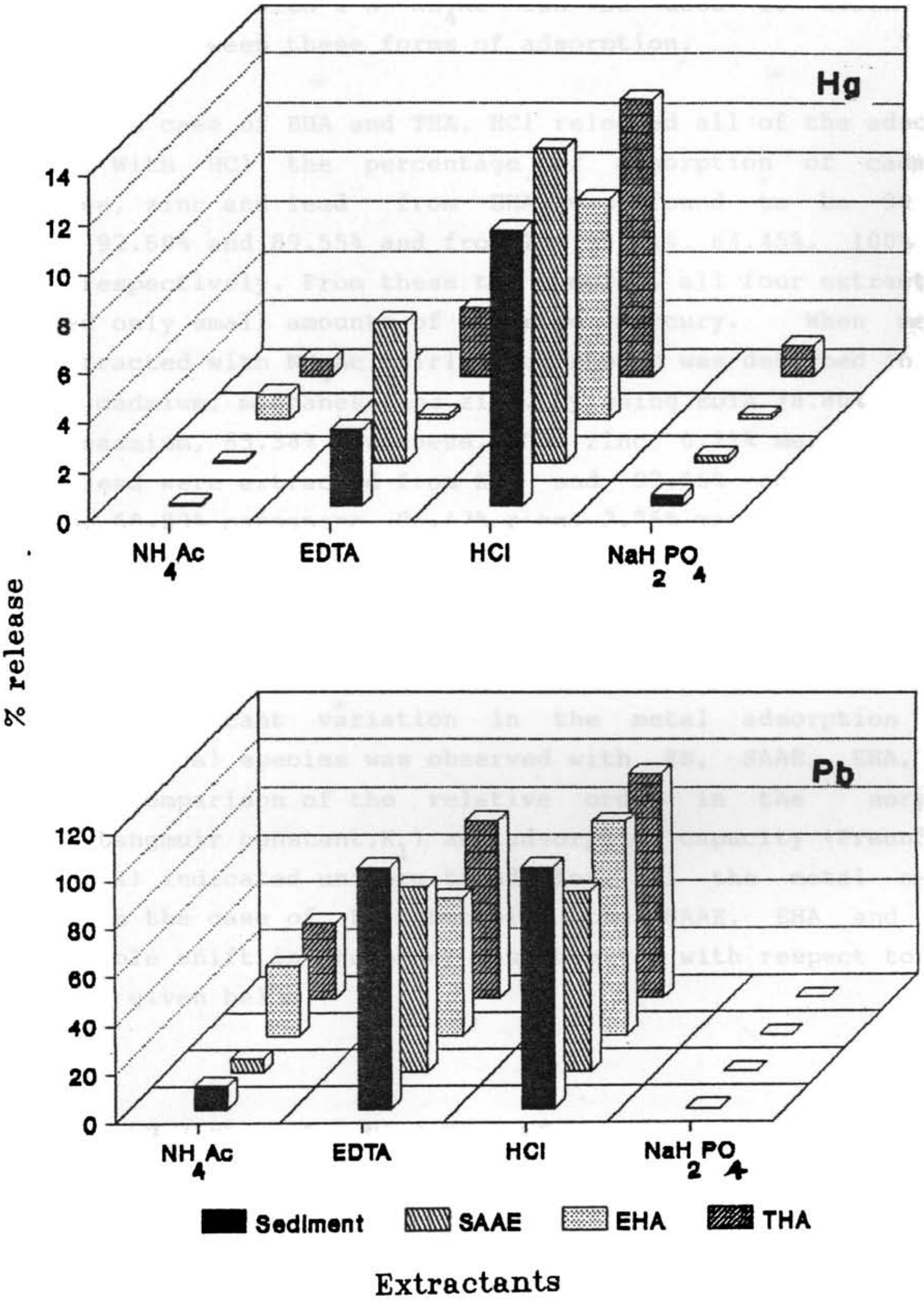


Fig.4.25 Percentage of metal release by the extractants

extraction method with 2 N NH_4Ac can be used to define the distinction between these forms of adsorption.

In the case of EHA and THA, HCl released all of the adsorbed copper ~~metal~~. With HCl the percentage of desorption of cadmium, manganese, zinc and lead from EHA was found to be 90.19%, 88.27%, 92.60% and 89.55% and from THA 88.71%, 64.45%, 100% and 94.83% respectively. From these two samples, all four extractants released only small amounts of adsorbed mercury. When metals were extracted with NH_4Ac fairly good amount was desorbed in the case of cadmium, manganese and zinc. By using EDTA 78.80% copper, 94.75% cadmium, 85.38% manganese, 100% zinc, 0.21% mercury and 56.88% lead were extracted from EHA and 92.56% copper, 94.05% cadmium, 68.83% manganese, 96.42% zinc, 2.75% mercury and 74.89% lead from THA.

Comparative Analysis

The significant variation in the metal adsorption with respect to metal species was observed with ES, SAAE, EHA, and THA. The comparison of the relative order in the sorption maxima (Langmuir constant,K_1) and adsorption capacity (Freundlich Constant K) indicated uniform trend for all the metal except mercury in the case of ES. But with the SAAE, EHA and THA, considerable shift in the order was observed with respect to K_1, and K are given below.

ES

K_1 - Cu > Pb > Zn > Hg > Mn > Cd

K - Hg > Cu > Pb > Zn > Mn > Cd

SAAE

K_1 - Mn > Hg > Zn > Pb = Cu > Cd

K - Zn > Pb > Cd > Cu > Mn > Hg

EHA

K_1 - Cu > Cd > Hg > Mn = Zn > Pb

K - Cu > Cd > Pb > Mn = Zn > Hg

THA

K_1 - Cu > Pb > Hg > Cd > Mn > Zn

K - Hg > Cu > Pb > Cd > Mn > Zn

In the case of equilibrium coefficient (Langmuir constant, K_2) the observed pattern was

ES

Zn > Cd > Cu > Hg > Pb > Mn

SAAE

Mn > Hg > Cu > Cd > Zn > Pb

EHA

Hg > Cd > Cu > Mn > Zn > Pb

THA

Hg > Cd > Pb > Cu > Zn > Mn

The significant variation between the ES, SAAE and EHA clearly indicates the selectivity of the metal towards the adsorbing site. The sediment and SAAE represent two systems of heterogeneous adsorption sites of which the sediment is complemented by the presence of organic ligands. So the selectivity of the sediment towards the independent metal can only be considered as a result of the adsorption by the organic and inorganic exchanges.

The selectivity of the adsorbance was more clearly shown in the partitioning and retention of the metals. The order of partition in terms of partition coefficient for ES, SAAE, EHA, THA are found to be

ES

Cu > Pb > Zn > Hg > Cd > Mn

SAAE

Mn > Zn > Pb > Cd > Cu > Hg

EHA

Hg > Pb > Cu > Cd > Zn > Mn

THA

Hg > Pb > Cu > Cd > Mn > Zn

and the order of retention after extraction with NH_4Ac

ES

Hg > Pb > Cu > Zn > Mn > Cd

SAAE

Hg > Pb > Zn > Cu > Cd > Mn

EHA

Hg > Cu > Pb > Zn > Cd > Mn

THA

Hg > Cu > Pb > Mn > Zn > Cd

Mercury was found to be an exception in all the extraction procedures. Earlier studies on mercury sorption by humic acid indicated chemisorption and extraction procedures with acid and neutral extractants released only less than 50 percent of the adsorbed mercury. At the same time, EDTA was observed to be an effective extractant with extraction above 80 percent (Thanabalsingam and Pickering, 1985). In the study, the neutral and acidic extractants and EDTA failed to release significant amount of adsorbed mercury. The retention with neutral NH_4Ac indicated a heterogeneous character for the adsorption sites and a significant specificity of these sites towards individual metals. The NH_4Ac desorption is also indicative of the formation of ionic complexes during adsorption. The relative order of retention with EDTA (0.05 M) and HCl (0.5 N) is as follows:

EDTA

ES

0% except mercury

SAAE

Hg > Cu > Pb > Zn > Cd > Mn

EHA

Hg > Pb > Cu > Mn > Cd > Zn

THA

Hg > Mn > Pb > Cu > Cd > Zn

HCl

ES

0% except mercury

SAAE

Hg > Cu > Pb > Zn > Cd > Mn

EHA

Hg > Mn > Pb > Cd > Zn > Cu (0%)

THA

Hg > Mn > Cd > Pb > Zn = Cu (0%)

The effective quantitative recovery of the metals with EDTA and HCl (except mercury) is suggestive of a non specific chemisorption for any of these metals. And for the estuarine sediment, the metals can be considered as reversibly bound. The partial retention of the metals after EDTA and HCl extraction in the case of SAAE can be due to partial chemisorption.

The EHA and THA, though did not show any significant variation in the order of partitioning of the metals, the retention was found to exhibit some specificity towards individual metals.

The adsorption sequence was found to be the indicative of the highest adsorption for copper and lead. It is reported from the adsorption studies with soils and sediments that the sequence is Pb > Cu > Zn > Cd or Pb > Cu > Cd > Zn (Bidappa *et al.*, 1981; Eilliot *et al.*, 1986). Though there is an inter change between copper and lead the sequence is in tune with this earlier observation. The high desorption with NH_4Ac suggests the greater bioavailbility of this metal (Baker, 1990). The strong retention of lead and copper has been reported as a common character in the case of whole soils (Biddappa *et al.*, 1981; Scokart *et al.*, 1983) and sediments (Giblin *et al.*, 1980, Biddappa *et al.*, 1981). The rare phytotoxic effect shown by lead is considered to be due to this strong retention (Chaney, 1980). The significant role played by organic matter in this adsorption process is clearly indicated by the change in the positions of zinc, manganese and copper in the sequence observed with SAAE.With the copper, the removal of organic matter was shown to be shifting to the lowest adsorption (Elliot *et al.*, 1986). Stevenson (1977), from the potentiometric titrations of complexes of humic acids have reported that the order of stability is Cu > Pb > Cd > Zn and Bergseth and Stuanes (1976), from the complexation studies have shown that cadmium and zinc are bound equally with humic acid. It was shown that no definite pattern in the role of organic matter in zinc sorption can be observed. Zinc sorption decreased with addition of fulvic acid (Bartal *et al.*,1988) and removal of organic matter (Shuman, 1988). Chowdhury and Bose (1970) and Rashid (1974) suggested that preferential sorption of lead and copper on humic acid through the formation of stable complexes/chelates. In this study though lead was observed to be have a greater adsorption than the copper with EHA and THA, the order of retention was observed to be greater with copper. In the whole experiments, manganese was found to be positioned along with cadmium and zinc, except in the case of SAAE.

CHAPTER V

HETEROGENEITY ANALYSIS - A KINETIC APPROACH

Conceptually, the sediment is constituted of heterogeneous mixture of dissimilar particles interacting with the dissolved phase of the aquatic system. These solid phases in natural waters consist of a variety of components including clay minerals, carbonates, quartz, feldspar and organic solids. The matrix vehicle or the residual fraction (Jenne, 1977) is associated with more labile and thermodynamically unstable components such as carbonates, amorphous alumino silicates and organic matter (Martin *et al.*, 1987). These fractions are usually coated with iron and manganese oxides and living and non living organic materials. The primary medium responsible for the sedimentary sorption of inorganic components are metastable iron and manganese oxides and oxyhydrates, which have a high degree of isomorphic substitution (Jenne, 1977).

The transport of metals to sediments may be either gravitational, i.e.,settling of particulate materials with its associated metals (Sigg, 1985, Livett, 1988) or diffusive or sorptional (Cariganan and Tessier, 1985; Tessier *et al.*, 1989). In both cases, the overall result in both cases is a significant enhancement of metal concentrations in the upper strata of the sediments. Of the various approaches, for the determination of the metal partitioning in the sediments, the most accepted are the partial extraction and the equilibrium partition modeling. In the partial extraction method, mild selective reagents are used to extract specific metal forms from the sediment. The sensitivity to procedural variables, post extraction reabsorption, selectivity and sample preservation are the four major potential difficulties that one encounters in this procedure.

The basic assumption in the equilibrium partition modeling is that metals attain sorption equilibrium between the sediment interstitial water and the various solid surfaces available for sorption. A complete and comprehensive modeling requires (Luoma and Davis, 1983) the input data on the binding intensities and capacities of important sedimentary components, relative abundance of these components, the particle coatings and the multi component aggregation of binding capacity of each substrate, major competitors and kinetics of metal uptake and redistribution among sedimentary components. The effectiveness of modeling is still restricted, as the adsorption characteristics are related not only to the system conditions but also to the changes in the net system surface properties resulting from particle-particle interactions such as coagulations, due to the influence of organic ligands in the aqueous phase, due to the competition between various sorption sites and as the reaction kinetics of the individual constituents cannot be evaluated in a mixture of sedimentary components (Honeyman and Santschi, 1988). With the assessment of binding intensities and capacities for individual sediment components, though attempts have been made for modeling the complex multi component natural systems, the applications are limited by the virtual absence of field derived data and by the difficulties in experimentally verifying the model predictions.

The analysis of binding heterogeneity has given a new means for the evaluation of the complexities and difficulties in the mathematical modeling. Here also, the treatment so far available is limited to the analysis of the equilibrium adsorption was based on the Langmuir and Freundlich isotherms. In this study, an attempt is made for an evaluation of the complexities of the adsorption kinetics using the concept of heterogeneity analysis.

Basic Concepts of Heterogeneity Analysis

The sorption process plays an important role in assessing the behaviour of heavy metals in soils and sediments. Available reports on adsorption equilibria are based on the Langmuir and Freundlich equilibrium models. Even though several works are reported on adsorption equilibrium studies, only a few reports are available about adsorption kinetics. The lack of published reports may be due to the following complexities of the system.

(1) The sediment and SAAE represent heterogeneous systems, containing more than one chemically and physically distinguishable species of adsorbing entities like minerals (iron and manganese oxides, illites, geothites etc) and organic matter. The organic matter in general, and humic acid in particular also contain a mixture of reactive sites.

(2) Internal diffusion, or transport of the adsorbed metal ion within the solid phase can modify the adsorption process, the extent of which depends upon the conditions such as ionic radii and speciation of the adsorbate. Metals can exist as simple ionic form of different oxidation states, or as hydrated form or as ion pair form. The electronic configuration of the metal determines the stability of these species and their interactions with the adsorbing sites, and the internal transport.

(3) The mode and the rate of adsorption depend upon the nature of the site, the metal and the energy associated with the process. Thus, a selectivity or specificity of the metal is observed as a general trend which may get altered with a change in the general condition of the process.

(4) The general order of the reaction is the sum of the orders of the individual processes, as it takes place simultaneously, while the observed order will be the one which evolved from the sum of a weighed reaction process as in the case of complex chain reactions.

(5) The overall rate of the reaction will be a statistical sum of the individual reaction rates.

The poorly defined charge characteristics and processes leads to the evolvement of the "conditional" or "apparent" parameters. The heterogenic analysis can be considered as a valuable tool to get an insight into the binding behaviour, by providing realistic models for the description of the binding. The earlier analyses on these lines were based on the equilibrium or precisely on the Langmuir and Freundlich isotherms which start with the definition of the binding function of the reaction (Nederlof et al., 1991).

$$X + M \longrightarrow MX$$

The equilibrium coefficient of the reaction is given as

$$K = \frac{[MX]}{[X]\ [M]} \longrightarrow (1)$$

and the binding function is defined as

$$\text{Binding function}\quad \theta_i = \frac{MX}{X + MX} \longrightarrow (2)$$

By substituting the equilibrium coefficient, K

$$\theta_i = \frac{KM}{1 + KM} \longrightarrow (3)$$

This equation is known as local binding function or local isotherm which is equivalent to Langmuir isotherm and can be applied to a system of homogeneous polyfunctional ligands, each of which contains a number of equal sites. For a heterogeneous ligands system the overall binding function can be obtained from summation of the local binding functions weighed by the site fractions.

$$\theta_t = \Sigma\ f_i \theta_i \longrightarrow (4)$$

Substituting the definition of θ_i and when the difference between

K_i is infinitesimal above equation takes the form

$$\theta_t = \theta \text{ f } (\log K) \text{ d } \log K \longrightarrow (5)$$

where f(log K) is the distribution function and d denotes log K values present. The solution of this equation can be done in three semi analytical techniques; local isotherm approximation methods, the affinity spectrum methods and the differential equilibrium methods. The comparison of the measured binding curve with an ideal synthetic data set leads to the modification of the binding function to

$$\theta_t = \frac{(KM)^m}{1+(KM)^m} \longrightarrow (6)$$

where m is a measure for the heterogeneity. Nederlof et *al.* (1991) applied this heterogeneity analysis to a few of the systems and able to derive the distribution functions for the copper binding to the humic ligands. They also observed that the heterogeneity analysis can help to choose an appropriate binding model for the ligand system under consideration.

Evolution of the Kinetic Model

The heterogeneity analysis given above was evolved from the equilibrium concept, and so does not project the influence of heterogenic character on the kinetic part of the process. In fact, the effect of heterogeneity should have been more pronounced on the rate of the reaction. The general observations given in the earlier part of this discussion and the development of the heterogeneity analysis has combined for a mathematical modeling of the adsorption kinetics in the present study.

In this model the total adsorption process is split into individual adsorption reactions which can be given as

$X_1 + aM_1 \text{------>} M1_a\ X1$

$X_2 + bM_2 \text{------>} M2_b\ X2$ or

$Xi + n\ Mi \text{----->} Mi_n\ Xi$

where X1, X2 etc represents independent identical sites and M1, M2 etc represents fraction of the available metal concentrations for the respective sites.

The rate of the individual reactions with respect to the metal ion will be

$$\frac{dM1}{dt} = K_1\ M1^a\ X1 \longrightarrow (7)$$

$$\frac{dM2}{dt} = K_2\ M2^b\ X2 \longrightarrow (8) \quad \text{OR}$$

$$\frac{dMi}{dt} = K_i\ Mi^n\ Xi \longrightarrow (9)$$

The total reaction will be the sum of the individual reactions and is given as

$$\frac{dM}{dt} = \Sigma\ \frac{dMi}{dt} = \Sigma\ K_i Mi^n\ Xi \longrightarrow (10)$$

As the value of the available metal concentration Mi is limited to M, the total metal concentration, and assuming homogeneous distribution of metal ions in the solution and equal chance for a metal to interact with any of the sites, the Mi can be considered as a function of M at a specified time and is replaced by $f_i(M)$ in equation (10) and the equation takes the form

$$\frac{dM}{dt} = f_i\ M^{\alpha}\ \Sigma\ K_i\ Xi \longrightarrow (11)$$

As the K_i for a particular adsorption site is a constant and the distribution of Xi in the elemental limiting to the total adsorption sites (X_T) the summation term can be replaced by the integral form

$$\frac{dM}{dt} = f_i\ M^{\alpha} \int_{X_T} K_i dx_i \longrightarrow (12)$$

$$= fi\ M^{\alpha}\ Km \longrightarrow (13)$$

where Km represents the weighed sum of the product of the adsorption site variation and the reaction coefficient and is defined as the weighed activity of the adsorbent, $f_i M$ is the weighed means of the available metal concentration and α will be the statistical function of the overall order of the reaction which can be considered as a measure of the heterogeneity in this heterogeneous system. The integration and rearrangement of the equations (13) gives

$$\log t = \frac{1}{1-\alpha} \log (\alpha-1)\ Km + \frac{1}{(1-\alpha)} \log f\ M \longrightarrow (14)$$

By definition f M is a function of the total residual metal concentration at the time and for all practical purposes can be treated as equal to M. This relation can be used to get the overall effective rate coefficient and the heterogeneity of the adsorption process by plotting log M vs log t.

Application of the Model

The kinetic data obtained in the study for the adsorption of metal copper, cadmium, manganese, zinc and mercury by the substrate ES, SAAE, EHA and THA were applied to the model and the results obtained (Km and α) are given in Tables 5.1 and 5.2. A representative plot of log t Vs log M for the five metals at zero salinity are given in Fig. 5.1.

Km values for copper in SAAE were higher than that of ES and for the higher applied concentration its value decreased in ES from 2.806 and 0.433 and increased in SAAE from 211.59 and 91133.04 respectively. Saline condition were found to be lowering the Km values with both ES and SAAE, the value decreasing at applied higher concentration (0.039 and 0.0042 for ES and 48.057 and 0.608 for SAAE). EHA and THA were recorded considerably high Km values compared to ES and SAAE, Km value showed a sharp decrease with increase in applied concentrations and also at the saline conditions. α n showed a significant increase in its values with higher concentrations for ES, SAAE and with the saline condition for ES.

Km for cadmium adsorption decreased as the applied concentration increased in all the four adsorbents. Values of Km showed a sharp rise in saline conditions in ES and a sharp fall in SAAE (7474.29, 92.23, 2277403.1 and 1456.15 for ES and 3.19 x 10^{14}, 7.19 x 10^{8}, 107.42 and 6.65 for SAAE respectively). In EHA and THA, lower Km values were observed in saline conditions.

In manganese, the observed trend was similar to that of cadmium except in magnitude.

In ES and EHA Km increased in fresh water conditions, its value remains as constant in SAAE and decreased in EHA with applied mercury concentration. At saline conditions Km values decreased in SAAE, EHA and THA.

Value of α for copper showed a significant variation with applied metal concentration and ranged from 2.65-3.53 and 19.27-15.14 at 0 and 15 x 10^{-3}. In the case of cadmium, though there is an increase in α value in saline, α remains as a

Table.5.1. Kinetic parameters (α and K_m)for the metal adsorption on ES and SAAE.

METAL	SALINITY	ES		SAAE	
		α	K_m	α	K_m
Copper	0	2.65	2.81^{*}	4.14	211.59^{***}
		19.27	0.43^{*}	6.36	91133.04
	15	3.53	0.04^{**}	3.04	48.06^{***}
		15.14	0.004^{***}	2.47	0.61^{**}
Cadmium	0	3.99	7474.29^{***}	10.96	$3.19\ X10^{14}$
		3.23	92.23^{**}	8.88	$7.20\ X10^{8*}$
	15	12.02	$2.27\ X10^{6}$	4.41	107.42^{*}
		12.53	1456.15^{**}	4.19	6.65
Manganese	0	3.74	5.83^{***}	4.13	3823.20^{**}
		3.61	0.29^{*}	3.63	96.10^{***}
	15	5.88	0.015^{**}	10.28	2.65^{*}
		9.24	$2.02\ X10^{-5*}$	15.76	$8.22\ X10^{-4}$
Zinc	0	2.49	63.22^{**}	3.64	271.14^{***}
		3.83	604.57^{*}	4.00	483.65^{*}
	15	3.80	27.86^{***}	4.76	6470.66^{*}
		3.99	1.29^{**}		
Mercury	0	3.83	384.63^{*}	4.43	60.75
		5.59	793.96^{**}	5.39	60.25^{*}
	15	3.26	28.78^{*}	4.57	37.60^{*}
		6.19	51.27^{**}	5.66	16.30^{*}

n=6 , * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table.5.2. Kinetic parameters (α and K_m) for the metal adsorption on EHA and THA.

METAL	SALINITY	EHA		THA	
		α	K_m	α	K_m
Copper	0	29.71	8.28 X10^{30**}	33.75	1.61 X10^{24**}
		29.51	15.88	20.34	0.36***
	5	7.82	1.99 X10^{4}	25.45	1.58 X10^{16}
		50.58	232.52*	24.62	0.05
Cadmium	0	4.65	16432.88*	7.63	1.09 X10^{8}
		6.14	298.56*	4.85	32.05**
	5	6.49	10475.50***	8.93	9.53 X10^{6*}
		5.33	85.15**	7.09	42.97
Manganese	0	13.70	1.57 X10^{10}	22.04	5.02 X10^{13*}
		26.89	0.02	90.28	27.05***
	5	3.95	0.22*	10.47	74.34***
		12.38	7.44 X10^{-5*}	20.04	2.89 X 10^{-6*}
Zinc	0	5.08	2813.89	3.33	9.17
		3.91	0.36	29.13	2069.76*
	5	7.32	770.96*	15.11	1.86 X10^{10**}
		18.42	1.16*	32.07	1529.03*
Mercury	0	117.14	E	2.95	121.24*
		8.66	23208.08	5.43	782.39*
	5	25.55	1.98 X10^{28*}	3.11	176.48*
		27.14	6.74 X10^{11**}	5.49	78.72

n=6 , * P < 0.05, ** P < 0.01, *** P < 0.001

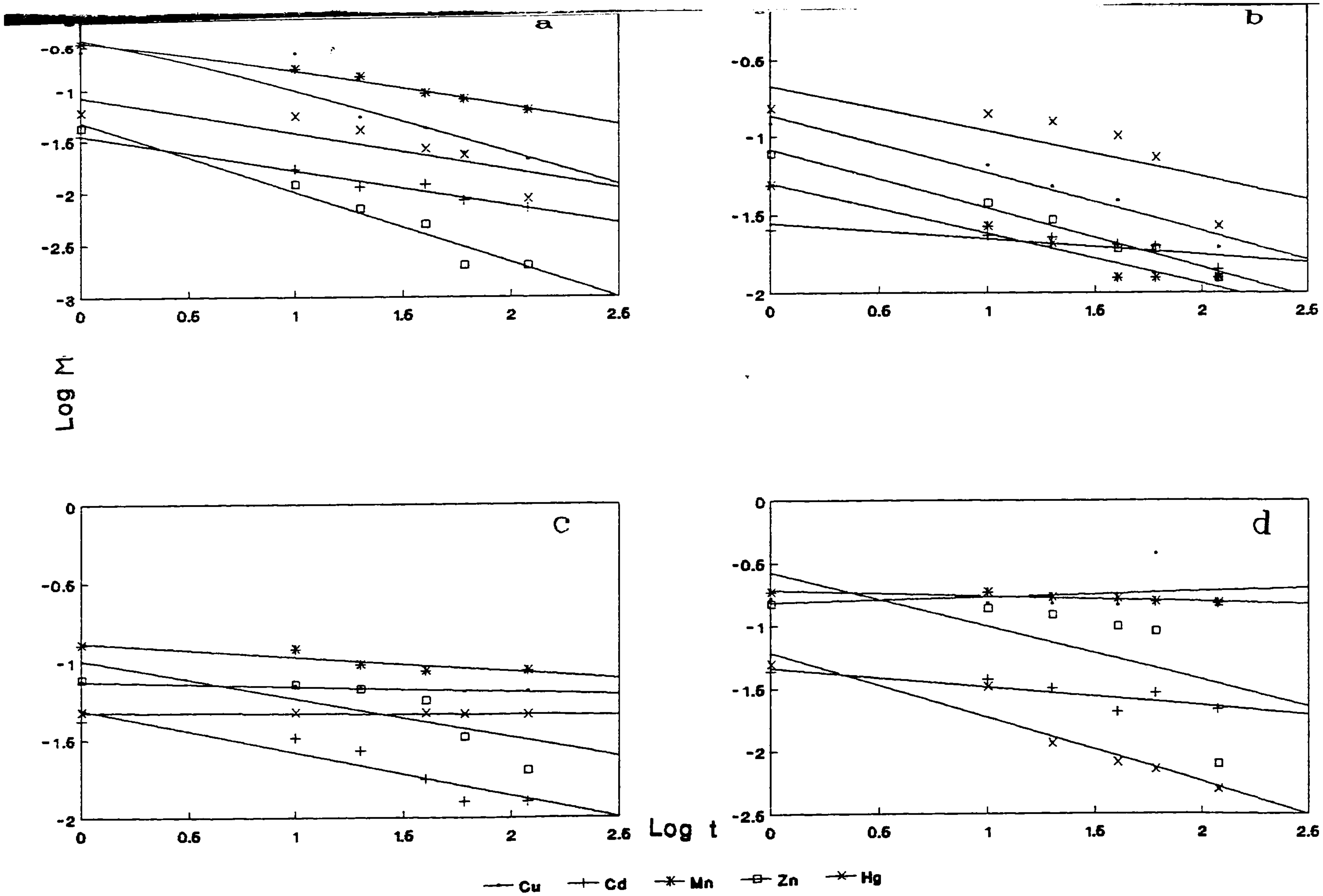


Fig.5.1 Plot of Log M *Vs* Log t for the metal adsorption
by (a) ES (b) SAAE (c) EHA (d) THA

constant at each salinity. α value for cadmium was found to be higher than that of copper both in the ES and SAAE at all the conditions, it was lower to the value of copper in the case of EHA and THA. For manganese, α is a constant at 0 x 10^{-3} (3.74 and 3.61) and increased in saline water as the applied concentration. All four α values are almost constant in the case of zinc and in mercury also there was not much variations. Values were 3.83 and 5.59 in 0 x 10^{-3} and 6.26 and 6.19 in 15 x 10^{-3}.

In SAAE also four values of α are almost constant in the case of zinc and mercury, α decreased with salinity in copper and cadmium and increased with salinity in manganese. However there was no significant variation in the values of α.

EHA and THA showed higher α values compared to ES and SAAE. Among the five metals, cadmium showed a lower α values for two humic acid samples and also α for mercury adsorption by THA is low.

Following conclusions can be derived from the values of α obtained.

(1) The close similarity that is given between the metals in the case of ES and SAAE is indicative of a non-specific and non selective adsorption process among the metals except for the adsorptions of copper by ES, and cadmium by SAAE.

(2) The α values that is given by copper in higher concentrations are indicative of a metal induced reactivity for the sediment.

(3) In the case of cadmium, significantly higher variation between ES and SAAE can be due to the preferential adsorption by inorganic constituents in the sediment system. Such a preferential adsorption inorganic components have been reported as earlier.

(4) Salinity was found to increase α values and in most the cases which can be due to the added heterogeneity by the competition of other inorganic cations.

(5) The decrease in the α value obtained for copper and cadmium for increase in salinity in the case of adsorption by SAAE may be due to the modification of the ionic species of the metal thereby limiting the effective adsorption.

(6) The humic acids exhibited considerably high heterogeneity compared to the sedimentary system. This can be attributed to the availability of all possible adsorption sites which in the sedimentary system is not available for the adsorption process. The greater affinity of the metal towards the ligandary groups of humic acids can also contribute for a higher heterogeneity. The considerable decrease in α values that is given with the salinity by the humic acids supports the above observation.

CHAPTER VI

SUMMARY

The understanding of the process governing the migration of trace metals in the aquatic system is essential for the prediction of the environmental impact on the spreading of these contaminants. The adverse effects of heavy metals are inseparably related to the sediment's ability to absorb and retain such elements. The management of the aquatic production practices is hindered by the lack of information on the processes regulating the partitioning of trace elements between the solution and solid phase. In the aquatic system, the sediment acts as a regulatory machine, acting both as a sink and a source. The organic matter, though constitutes only about 2-3% of the sediment, profoundly influences this regulatory mechanisms by virtue of its complexing/chelating capabilities.

Humic substances, the major fraction of organic matter, are large organic molecules which are formed by the degradation of biopolymers or polymerisation of smaller organic molecules in the environment (Hedges, 1988). Humic acids, the alkali soluble and acid insoluble fraction of humic substances form one of the most important source/sink systems for metal ions in a number of environmental compartments including soils and bottom sediments in both fresh and saline waters.

The ubiquity of humic substances in the terrestrial and aquatic environment is indeed impressive. However, despite their extensive distribution in soils, sediments, lakes, rivers and sea, and not withstanding a long history of diligent and systematic research, large gaps remain in our understanding of origin, nature, fate, chemical structure and function of humic substances. It has become increasingly clear that humic substances cannot be looked upon as immutable chemical entities, but as a dynamic group of heterogeneous compounds which are complex in their structural make up and prone to change with time and location.

The present work deals primarily with the comparison of some physico-chemical properties of sedimentary humic acids from different stations in the Cochin estuary and from a terrestrial soil and their interaction with some trace elements, selected as representative pollutant metals in the Cochin estuary.

In the case of total acidity, there is no considerable variation among estuarine, terrestrial and coastal samples. Terrestrial and estuarine humic acids possessed low carboxylate acidity and the acidity associated with these samples was mainly due to the phenolic hydroxyl groups, whereas in coastal samples most of the acidity arise from carboxyl group. Phosphorus content of coastal sample is lower than that of terrestrial and estuarine samples. Carbohydrates associated with terrestrial as well as coastal humic acids was comparable with those of estuarine humic acid. Copper, manganese and zinc were present in all samples while cadmium and lead were absent.

UV-VIS spectra were featureless as in earlier studies. Fluorescence spectra were more or less identical. IR spectra of all three samples, terrestrial, estuarine and coastal also showed a close resemblance, and were indicative of the presence of a number of functional groups containing oxygen. Specific assignments of the structural characteristics were not carried out due to the lack of supporting data. The main intention of the present work also did not warrant such an analysis of the structural pattern.

The analysis of the sorption data with the help of Langmuir and Freundlich isotherms indicated significant variations in the sorption capacity and the equilibration between metals and ES, SAAE, EHA and THA. EHA and THA showed significantly high sorptional capabilities and ES was found to be always presenting a higher sorption than the SAAE. The adsorption sequence was found to be indicative of the highest adsorption for copper and lead. The order of sorption ~~capacity~~ maxima for the metals was

ES

K_1 - Cu > Pb > Zn > Hg > Mn > Cd

SAAE

K_1 - Mn > Hg > Zn > Pb = Cu > Cd

EHA

K_1 - Cu > Cd > Hg > Mn = Zn > Pb

THA

K_1 - Cu > Pb > Hg > Cd > Mn > Zn

The sorption was found to be decreasing for all the metals and for all the substrates except mercury and lead with salinity. The competitive interaction of the major cations present in the saline water with the adsorbing sites may have resulted in the lowering of the metal sorption. In the case of mercury and lead, the saline conditions were favourable for the preferential precipitation of the respective chlorides/sulphates of these metals leading to a significant decrease of the concentrations of these metals in the media.

The significant variation between the ES, SAAE and EHA clearly indicated the selectivity of the metal towards the adsorbing site. The sediment and SAAE represented two systems of heterogeneous adsorption sites of which the sediment was complemented by the presence of organic ligands. So the selectivity of the sediment towards the independent metal can only be considered as a result of the adsorption by the organic and inorganic exchangers.

The adsorption and retention with neutral NH_4Ac indicated a heterogeneous character for the adsorption sites and a significant specificity of the these sites towards individual metals. The NH_4Ac desorption was also indicative of the formation of ionic complexes during adsorption.

The heterogeneity analysis, which was successfully applied by earlier workers to equilibrium sorption studies for the evaluation of the binding function and intensities, has been tried to extend the kinetics of sorption so as to get an insight into the sorptional process. The α obtained from the kinetic model by definition can be considered as a measure of the heterogeneity of the process. The values of α indicated a non-specific and non-selective adsorption process among the metals except for copper and cadmium. Copper showed a metal induced reactivity for ES and cadmium a preferential sorption by the inorganic constituents. Salinity increased the heterogeneity of the process by the involvement of the major cations in a competitive adsorption process. The humic acids, due to its structural specialities, presented significantly high heterogeneity.

In general, the present study indicated that the sorptional character of the sediment in this estuary is well regulated by the organic matter, especially from the contribution from humic acids. Of the metals studied, copper was found to be the most readily removed from the medium which is followed by lead, zinc, mercury, cadmium and manganese.

This study is the first comprehensive attempt on the sorption of metals in estuarine sedimentary systems. It has served to emphasis on the role of sediment as well as sedimentary organic matter, with special reference to humic acids, on the regulation of the metals in aquatic systems. More over, the importance of a heterogenic approach was fostered here to explain the sorptional processes. The total evaluation of the structural patterns of humic acid as well as the assessment of the exact contribution of humic acids to the sorption were not carried out in this work mainly because, both warrants extensive and rigorous investigations.

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