STUDIES ON CHARACTERISATION AND COMPLEXATION OF HUMIC ACIDS FROM YAMUNA SEDIMENTS

Dissertation submitted to the Jawaharlal Nehru University
in partial fulfilment of the requirements
for the award of the degree of

MASTER OF PHILOSOPHY

PIUS KURIAN

SCHOOL OF ENVIRONMENTAL SCIENCES
Jawaharlal Nehru University
New Delhi-110 067 (India)
July, 1987

JAWAHARLAL NEHRU UNIVERSITY

Gram : JAYENU

Telephone : 652282
652114

New Mehrauli Road,
NEW DELHI-110067.

CERTIFICATE

This dissertation entitled "Studies on characterisation and complexation of Humic Acids from Yamuna Sediments" embodies the work carried out at the school of Environmental Sciences, Jawaharlal Nehru University, New Delhi. This work has not been submitted in part on full for any degree or diploma of any university.

Pius Kurian
(Candidate)

D.K. Banerjee
(Supervisor)

July, 1987
School of Environmental Sciences
Jawaharlal Nehru University
New Delhi - 110 067.

V. Asthana
(Dean)

ACKNOWLEDGEMENT

I wish to thank my supervisor Dr. D.K. Banerjee, Associate Professor, School of Environmental Sciences, for the instructions, encouragement and constructive criticism throughout this research study. My special thanks go to Prof. J.M. Dave for his help and encouragement. I am thankful to Prof. R.P. Gandhi, Department of Chemistry, Indian Institute of Technology, Delhi for providing me with the instrumentation facilities. I am also thankful to Dr. M.P. Singh, Indian Institute of Technology, Delhi for the helpful discussions.

I express my sincere thanks to my senior research students Dr. H. Shanmukhappa, Dr. P.A. Azeez, Dr. R.M. Sharma, Dr. S.P. Mahapatra, Mr. S. Pangrahi and fellow research students Mr. A.K. Tripathi, S. Sahu, Anwar Hussain, Mukesh Kumar, Vithianathan, Rana, Vinky and Sajid. I am also grateful to many others who helped me in different ways during the course of my research work.

I would like to thank the University Grants Comission for awarding me a Junior Research Fellowship, and Dean, School of Environmental Sciences for extending me all facilities.

I express my thanks to Mr. Deepak Chakravorty for typing the Dessertation.

PIUS KURIAN

CONTENTS

CHAPTER - I

INTRODUCTION

Humic substances are the most widely distributed groups of organic matter on the earth's surface and occur in soils, fresh waters and sea. They constitute about 70-80% of the organic matter in most inorganic soils and are formed by the chemical and biological degradation of plant and animal residues and also from the synthetic activities of micro-organisms (Shntizer and Khan 1972).

Based on their solubility in alkali and acid, humic substance are classified into the following three main fractions (i) Humic Acid (HA), (ii) Fulvic Acid (FA),and (iii) Humin (Schnitzer 1986). Out of these, the first fraction, i.e. HA was chosen for the present study.

Previous studies (Guy et al 1975) indicated that HA is a group of compounds of a complex nature, whose structures involve large portions of more or less condensed aromatic nuclei, with large number of - OH and - COOH groups fixed on them. They are among the most important complexing agents of metal ions in natural waters, soils and sediments for, they have a high affinity for metal ions

(Stevenson 1977). Because of this HA may markedly modify the chemical properties of metal ions and thus affect the natural processes such as rate of growth of plants or the accumulation of metals in sediments (Miele 1986).

Despite the fact that a great deal of work has been done to find the nature of HA, their structure is not known, In particular, only a few studies have been made to find out the nature of the molecules which are specifically compexing in HA (Buffle et al 1977). The initial studies showed that only some of the molecules present in the mixture, mainly low molecular weight compounds were strongly complexing (Prakash et al 1972).

Most of the studies done with the complexing properties of HA (mainly their stability constants and influence of pH) were initially developed for theoritical studies in complexation (Zunino et al 1972; Stevenson 1977; Takamatsu et al 1978). But since HA complexes with metal ions present in water and soil, it has direct impact on the vegetation present in that environment. Among the environmental manifestations, eutrophication is one of the most important aspect to be considered (Prakash et al 1972). High productivity of coastal waters was attributed to drainage from land of iron rich humic compounds. The dissolved organic compounds present in natural waters and sediments particularly HA plays an important role in the solubility of various physiologically important trace metals through the processs of chelation and complexing (Vaughan 1986).

The prsence of HA in fresh water system is of great ecological significance as it enhances the productivity of several fresh water algae (Prakash et al 1972).

HA efficiently promotes soil structure and there by improves its aeration water holding capacity, permeability, tilth and other vital characterstics of crop production. HA can have direct physiological effects on plants like increase in the permeability of plant memberanes and there by promoting the absorption of nutrients. Thus metal HA complexes can accelerate or decelerate the plant growth according to the type of metal chelated with HA. HA complexes of Cu, Mn and Zn reduce considerably the availability of these nutrients to the plants (Mantoura et al 1978).

Also HA tends at least in hydroponics, to reduce the activity of Cd and its absorption by plants where as growth simulating properties of these complexes is also reported. It can hold trace metals in such a form that can be asimilated plants (Cacco et al 1984).

Of the several metals release to the environment, Cu and Cd were taken for the present study on account of their toxicvity (Wood 1974), higher release to the environment (Nriagu, 1979) and their high affinity towards organic materials due to their high borderline characteristics (Rainbow 1985).

Several workers have tried to charectarize HA using different physical and chemical methods. Extensive use has been made of i.r. spectrophotometry in the fundamental chemisity of HA (Gessa et al 1983; Raspor et al 1984). Although UV-VIS spectra of HA shows a monotonous trend that is decreasing optical density with increasing wavelength, certain parmeters like E_4/E_6 ratio (OD at 465 nm/OD 665 nm) has been used to determine the degree of humification of HA from various sources (higher the value of E_4/E_6, less humified is the material,(Shanmukhappa et al 1987). More recent techniques used in the characterisation of HA are p.m.r. (proton magnetic resonance) spectroscopy and Carbon-13 n.m.r. (nuclear magnetic resonance) spectroscopy. p.m.r. has been used by Barton and Schnitzer (1963) and Gessa et al (1983) for the characterization of HA. C-13 n.m.r. now a days is being extensively used for HA characterisation since it has several advantage over p.m.r. in that it provides information on the carbon skelton of organic substances. In addition larger chemical shifts recorded by this technique allow signals to be resolved for individual carbon atoms with only slightly different magnetic environments (Wilson and Goh 1977; Preston and Schnitzer, 1984).

Considering the importance of humic acids in the environment, the present study was undertaken to pursue the following:

1. Isolation of HA from river Yamuna sediments from three different sites, viz. okhla (HAO), Old Yamuna Bridge (HAY), Wazirabad(HAW).

2. Determination of the degree of complexation of these three HA with two metals viz, Cu and Cd using ion selective electrodes.

3. Computation of stability constants from degree of complexation data.

4. Chemical characterisation of the HA using some standard analytical methods viz. UV-VIS spectrophotometry, IR, Proton NMR and C.H.N. analysis.

5. Correlation of some of the physico-chemical parameters of the river sediments with HA.

Since HA has an enormous influence on soil and aquatic productivity as also nutrient transport by chelation (Boyle et al 1977), the present study incorporated investigations of complexation properties and characterisation of HA isolated from the three sediment samples mentioned above. The information generated from the present study can be helpful in exploring the possibilites of using natural organic decomposition products in the abatement of metal pollution.

CHAPTER - II

LITERATURE REVIEW

INTRODUCTION:

Humic substances are probably the most widely distributed natural organic products on the earths surface, occuring in soils, lakes, rivers and sea. They constitute 70-80% of organic matter in most inorganic soils (Schnitzer and Khan, 1972). Prakash et.al. (1972) reported 1-20 mg/l of HA concentration in natural waters. A study conducted by Shanmukhappa (1980) showed widely varying concentrations of HA in three different types of biotopes namely estuary, mangrove and sea. In estuarine sediments the concentration reported by him varied from 1.5 to 4.5 percent.

Based on the solubility in alkali and acid, humic substances are classified into three fractions (1) Humic acids (HA), soluble in dilute alkali, but is coagulated by acidification of the alkaline extract (2) Fulvic Acids (FA), the humic fraction which remains in solution when the alkaline extract is acidified (soluble in both dilute acid and dilute alkali) and (3) Humin, the humic fraction which cannot be extracted

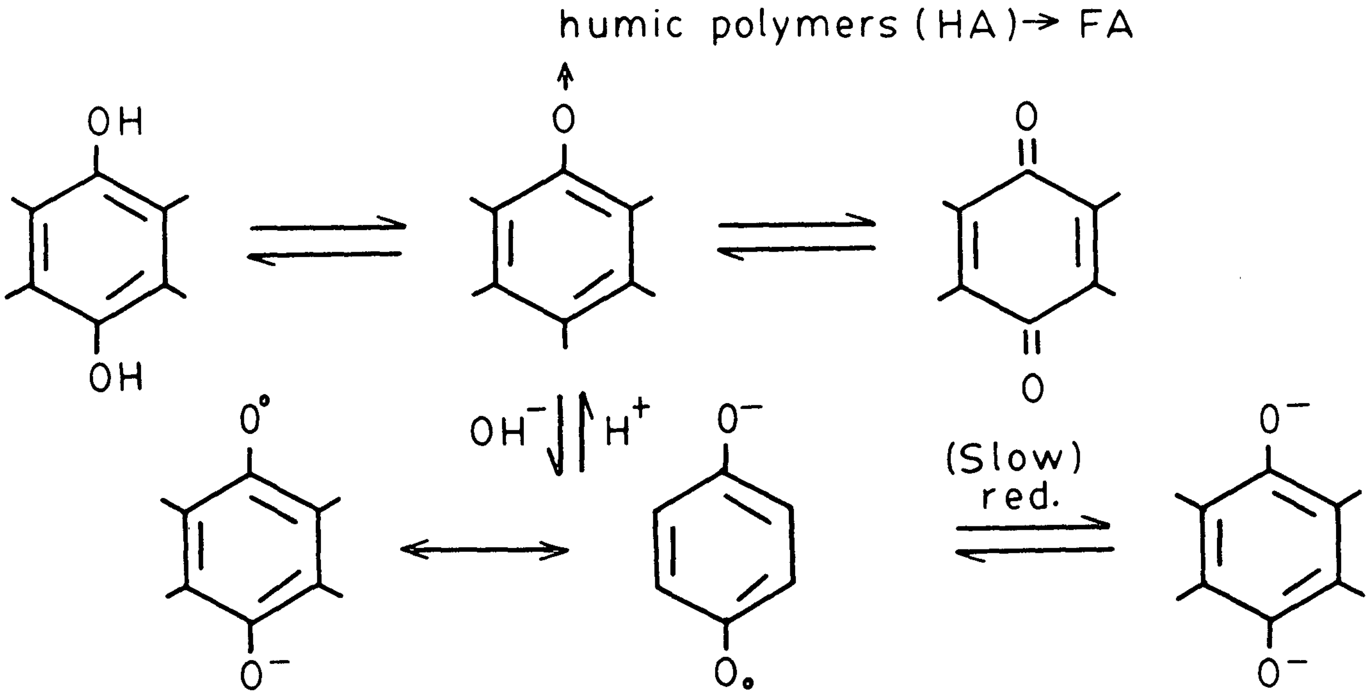

Fig. 1. Synthesis of humic substances

from soil either by dilute base or by dilute acid (Schnitzer and Khan, 1972).

FORMATION OF HUMIC SUBSTANCES

Humic substances arise from the chemical and biological degradation of plant and animal residues and from synthetic activities of microorganisms (Schnitzer and Khan, 1972; Martin and Haider, 1971). Martin and Haider, 1971 and Flaig, 1972 elaborated the activities of microbial population on compounds like lygnin resulting in formation of phenolic radicals and hydroxy benzoquinone the microbe mediated polymerization of which leads to the formations HA. The products of microbial decomposition of lignin like materials of plant origin tend to associate into a complex organic structure which are more stable than the source materials. There are indications (Mathur and Schnitzer, 1978) that the formation of a semiquinone free radical (fig. 1) is the rate determening step in the synthesis of HA from phenols and phenolic acids by oxidative polymerization. With one electron transfer reaction phenol is oxidized to the semiquinone. Flaig (1972) reports that the conversion of phenol to semiquinone is enhanced by the nitrogenous compounds derived from proteins of microbial origin. If another electron can be transferred, the semiquinone will be oxidized to quinone.

However normally these electron essential for semiquinone to quinone transformation is often not available and hence the quinone accumulation generally is not in detectable level.

The semiquinones which is normally unstable, will then couple with other semiquinones to form a stable humic polymer. As coupling of radicals require no activation of energy in contrast to electron transfer reaction, coupling of semiquinones rather than the formation of quinones is the preferred reaction path. The free radicals are formed by the chemical and biological dehydrogenation of phenolic-OH groups to yield semiquinones while the free radicals are formed more readily at higher pHs. They are also present in lower concentractions in aqueous solutions and solids of HA at pH levels normally occuring in soils (Sensi and Schnitzer, 1977). Schnitzer (1986) states that the free redicals can be used a very sensitive probes to explore the mechanism of humification. Eventually HA is degraded oxidatively into smaller but more reactive units which have the characterstics of FA.

CHEMICAL NATURE OF HUMIC ACIDS

The elucidation of the chemical structure of HA has posed a demanding question to the workers in the field for the last two decades. The high complexity and variability of the material depending upon varying environmental factors under-which the substances are formed have given ample thrust for the use of widely varying chemical and physical method for the study.

CHARACTERIZATION OF HA USING PHYSICAL METHODS

The main physical methods used in the characterization of HA are UV-VIS spectrophotometry, IR spectrophotometry and N.M.R. spectrophotometry.

a) UV-VIS SPECTROPHOTOMETRY

This has been used as a tool for analytical, structural and physico chemical investigation of humic substances. Light absorption of HA appear to increase with increase in (a) the degree of condensation of the aromatic ring they contain (b) total carbon content (c) the ratio of carbon in aromatic nuclei to carbon in aliphatic and alicyclic side chains (d) molecular weight (Schnitzer and Khan, 1972).

The absorption spectra of lignin fractions change with the increase in the period of humification. For example the maximum absorption in the region of 275-285 nm attributed to benzene ring substituted with oxygen and the maximum between 300-350 due to the presence of chromophoric group of the side chain-CO, olefinic double bond, in the case of rotted rise straw were found to become less prominent with the duration of humification (Flaig et.al., 1975).

It has frequently been observed that UV spectra of HA are featureless for, they achieve no prominent maxima or minima (Zeichmann, 1964). Pronounced differences in colour between HA extracts and fractions have often been noted. It is generally accepted that higher molecular weight of HA have dull grey brown colour, and with decreasing molecular weight the colour changes through brown to rich red brown. Based on this colour variations molecular weights are detectable in visible spectra (Kumada, 1965).

Ladd (1969) has reported that the optical extinction coefficients of the lower molecular weight humic acid fractions are greater than the higher molecular weight portions. However Dubashp and Mehta (1963) have reported an inverse relation to that observed by Ladd (1969). Swift et.al. (1970) also reported the optical extinction coefficients increasing with decreasing molecular weight similar to Ladd (1969). It is proposed that the degree of aromaticity increases as molecular weight decreases and that low molecular weight components formed are the end product of humification processes.

Summers et.al. (1987) describes that the differences in the UV-VIS spectra, as measured by the ratio of absorbances at 465 and 665 nm (E_4/E_6), can be correlated to molecular size fractions. However, the usefulness of the E_4/E_6 ratio is limited as a process-monitoring parameter, because of its strong concentration dependence in dilute concentration range (below 10 g/M^3). UV absorbance at 254 nM was found to be a good surrogate parameter for dissolved organic carbon.

b) INFRARED SPECTROPHOTOMETRY

Extensive use has been made of IR spectrophotometry in fundamental studies of nature, origin and structural chemistry of soil HA (Gessa et.al., 1983 and Raspor et.al., 1984). Both mull and pellet methods have been used. However pellet method are more popular than the other.

An undesirable feature of the pellet technique when KBr is used is that it causes interference due to hygroscopic moisture (Wagner and Stevensen 1965). The conventional technique of recording spectrum against a blank prepared in the same manner does not guarantee the absence of uncompensated moisture in the experimental sample (Theng et.al., 1966). In the technique adopted by Theng et.al. (1966) the absorbed moisture was removed by evacuating the dye at 75°C for 30 min before forming the pellet under pressure. Stevensen and Goh (1974) has also described that heating KBr pellets effetively eleminated interference due to hygroscopic moisture. The structural changes resulting from the heating process were limited only to the formation of cyclic anhydride from carbonyl groups present in HA. KBr catalysed the formation of cyclic anhydrides by mild thermal treatment in case of acid tnreated and methylated, saponified HA but not of untreated HA.

c) NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

i. PROTON NMR

Barter and Sehnitzer (1963) recorded NMR spectra of methylated HA dessolved in CCL and $CDCl_3$. The most remarkable fact about this spectra was the absence of aromatic and olefinic protons. Bands at 9.1 and 8.7 T were due to alphabatic CH (methyl and methyline) protons. The band at 6.3 T was due to OCH_3 and that at 6.1 T was due to CO_2CH_3. The total number of hydrogen at 6.3 and 6.1 T was in good agreement with values obtained from methoyl analysis.

Felback (1965) used NMR spectroscopy to determine the structure of a n - C_{25} or n-C_{26} hydrocarbon produced by high pressure hydrogenolysis of organic matter from a muck soil. From the study Felback was able to identify the organic matter in the muck soil of N -C_{25} length. He noted the absence of protons on N-atoms that were exchangable with duterium. About one third of N in the non hydrolysable fraction appeared to exist in the form of tertiary amine. Felback (1965) concluded from the study that the non-hydrolysable fraction of the organic matter in the muck soil on hydrogenolysis produced long unbranched carbon chains.

Gessa et.al. (1983) made use of PMr spectra to charactarize humic materials extracted by different methods. Their study shows that the use of different solvents for extraction caused difference in the spectra, some being characterstic of the prevalence of alphatic structures and others for aromatic structures.

It was seen by Gessa et.al. (1983) that the same solvent extracted structurally similar component from humic and fulric acids. Harvey et.al.(1984) who attempted the synthesis of marine humic substances from unsaturated lipid used PNR for comparing the synthesised from with the oceanic form.

ii CARBON -13 NMR

Carbon -13 nmr is a recent method used by different workers for structural characterization of HA (Mikita et.al., 1981; Hatcher et.al., 1981; Newman et.al., 1980). Preston and Schnitzer (1984) studied the effect of methylation on two HA as well as the effect of extraction by 0.5 M NaOH and 0.1 M HCl on HA. The spectra obtained for methylated HA indicate that almost all $-CO_2H$ groups are attached to aromatic structures and also that phenolic -OHs are the important functional groups. The spectra also exhibit fine structures in 127-131 ppm region possibly due to benzene carboxylic acid type structures, which have been proposed as the major building block of humic material. C-13 n.m.r spectra of hydrolysed HA indicate agreement with chemical analysis that 6.0 M HCL hydrolysis remove all amino acids and most cabohydrates. This treatment result in the increase of the aromatic residues.

The structure of some HA and lipid fractions of soil organic matter have been investigated by Wilson and Goh (1977) using C-13 n.m.r spectroscopy. In their study it was noted that in humic and fuluic acids, the signals from aromatic groupings were normally absent. There were differences in spectra from different sources C-13 n.m.r spectra of lipid fraction were generally similar to each other, however with significant differences which can be used to 'fingerprint' the lipids.

Newman et.al. (1980) also made use of C-13 n.m.r. to charactarize soil humic substances from a Newzealand pasture top soil. They concluded that the 26% of the total carbon present in the humic material is in the form of aromatic rings. Milcita et.al. (1981) noted that the ambiguity observed in case of many C-13 n.m.r signals of humic substances can be solved by premethylation of C-13 enriched reagents and they unambiguously identified four distinct bands with fine structural resolution of alphatic and aromatic carboxyl groups, phenolic groups, alphatic hydroxyl groups and amino nitrogen. They proposed that studies with coupled proton -C-13 n.m.r. can reveal hyperfine structures related with the immediate proton environment of OCH_3 group. The use of mild methylating agent for enhancement of specific functional group absorbants are also proposed by the above workers.

Sehnitzer and Preston (1983) studied the effect of acid hydrolysis on HA using C-13 n.m.r. spectra. It was seen that 6 M HCl hydrolysis was more efficient for removing protinaceous materials and cabohydrates than with 12M -0.5 M H_2SO_4 hydrolysis. The latter appeared to preserve better the structural integrity of humic material especially in the lower aliphatic region i.e, 0-50 ppm. Hydrolysis with the two acids led to increase in the aromaticity of the humic materials and it also lowered CO_2H content of two out of three humic preparations, probably because of acid decarboxylation. This study emphasises the potentials of C-13 n.m.r spectroscopy in the structural investigation of humic substances.

Steelink and Petson (1987) used C-13 NMR spectra of nature aspen and nature spruce lignini to interpret the spectra of lcraft lignins and selected humic substances. The nature and extent of side chain degradation, as well as changes in phenolic content, were clearly revealed in the kraft lignin spectra. Evidence for residual lignini fragments was also observed in the case of peat humic substances.

d) CHEMICAL NATURE OF HUMIC ACIDS

Most n.m.r spectra of humic substances indicate the presence of significant aliphatic structures that generally were not observed by chemical oxidation studies (Cranwell and Haworth, 1972). Chemical techniques have over estimated the degree of aromaticity of humic substances. Phenolic carbon have been considered as the major building block of HA. Hatcher et.al. (1981) reported that no signal for phenolic groups are observed in the n.m.r studies and their conclusion are contradictory to those of many workers (Kramwell and Haworth 1972) in that the phenolic carbon may be a minor component in HA.

According to Felback (1965) the HA is basically a polyhetro cyclic monomer consisting of $\alpha - \alpha^1$ condensation of α pyrone units into a linear chain. It consists of mainly phenolic and alcoholic hydroxyl, Carbonyl and Ketonic groups as revealed by i.r spectroscopic analysis (Schnitzer and Stiner 1965; Wagner and Stevens 1965; Theng et.al. 1965; Gessa et.al. 1983).

Elementary analysis performed by Haworth (1971) of these substances from various soils shows that HA contain 49-62% carbon, 3-6% hydrogen, .45 to 5% nitrogen. The humic acid studied by Haworth was found to have molecular weight varying from 1000 to 3000 dalton.

Mukherji and Lahiri (1958) concluded that HA are polyelectrolytes with flexible linear configurations and also showed that HA, colloidal in purely aqueous solutions became spheroids in presence of sufficient amount of natural electrolytes. Khan (1971) suggested that HA are mixtures of linear and sphero colloids, a proposal opposed by many workers. According to Ghosh and Schnitzer (1980) the assumption that the HA are mixtures of linear and sphero colloids seems unlikely and lacks sufficient supportive experimental data.

Chen and Schnitzer (1976) indicate that at very low pH humic substances behave like uncharged polymers where as at higher pH they exhibit polyelectrolytic character. Ghosh and Schnitzer (1980) observed that the macromolecular configuration of HA vary with changes in the medium. The controlling parameters were sample concentration, pH of the system and the ionic strength of the medium.

Molecular size distribution was investigated by Tsutsuki and Kuwatsuka (1984) using permeation chromatography on porous silica and porous glass. HA get fully expanded at low ionic strength at pH 7.5. With increasing ionic strength of the medium the HA underwent considerable shrinkage. This behaviour of HA molecules was analogous to that of poly electrolytic molecules.

Tsutsuki and Kuwatsuka (1984) noted increase relations between molecular size and Rf values of HA. They also observed an increase in carbonyl group with increase in humifications.

REACTION OF HUMIC ACIDS WITH METAL ION

The formation and properties of complexes of HA with metal ions are important for, it would provid us with useful information on the role of humic substances in soil forming processes, soil structure, nutrient availability, nutrient mobilization and transport in aquatic environment (Schnitzer and Khan, 1972).

It is well established that HA have the capacity to find substantial amount of metals and hence can exert considerable control over the supply and bio availability of nutrient elements to plants and animals in soil and water (Prakash et.al. 1972) Metals of the first transition series formed complexes with HA and the order of stability of different metal complexes followed that of Irwing Williams series ($P6^{2+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Cl^{2+} > Fe^{2+} > Mn^{2+} > Mg$) (Irwing Williams 1948, 1953). The most widely distributed functional groups in HA that have been shown to participate in metal complexation are -Co_2H, pheolic -OH and possibly CO and NH_2 (Schnitzer and Khan 1972).

Several methods were developed to measure the complex formation of metals with HA. The most important among them are (1) potentiometric method (2) ion selective electrode method (3) ion exchange equilibrium method and (4) anode stripping voltametry.

Potentiometric titrations have been shown to be a valuable tool for examining metal complexes of synthetic and natural polyelectrolytes. However in studies of complexation with naturally occuring polyelectrolytes only limited application has been made of this technique. Stevenson et. al. (1973) made use of this method to determine the formation constants of Cu^{2+} with HA. Stevenson (1977), Takamatsu and Yoshida (1978) Schnitzer and Steinner (1963) and Khanna and Stevenson (1962) are few among those who made use of potentiometric method for the study of complex formation.

Buffle et.al. (1977), Bresnahan et.al. (1978) Robert and James (1980) used ion selective electrodes for the study of complexation. Buffle et.al. (1977) compared the results obtained by ion selective electrode with that of vapour pressureos mometary in determining the complexation parameters of humic and fluric acids. They suggest that the ion selective electrods are very efficient to measure the complexing ability of organic matter and also to determine some perameters like molecular weight, stability constant,s degree of complexations etc. without modifying the medium and pretreatment.

Another recent technique used for the complexation studies is the ion exchange method (Vandenberg, 1984; Schnitzer and Skinner 1967; Schnitzer and Skinner, 1966; Randhawa and Broadbent, 1965). The ion exchange method which was developed by Schubert (1948) was found to be effecient to determine the stability constants of different types of humic and fulvic acids.

Nurnberg (1984) has stated that the Anode Stripping Voltametry (ASV) has contributed significantly to the study of metal interaction with organic substances. He also states that similar voltametric methods will remain as a promising method in future for the se studies. Varney et.al. (1984) observes that ASV is one of the most popular technique used in the field because of its high sensitivity and ease of use. However these workers note that the ASV does not simply responsd to metal ion concentration. It also gives signals to sufficiently labile complexes which can dissociate during the time scale of measurements. Simoesgonsalves et. al. (1983) Nelson and Mantoura (1984).

Studies conducted by Schnitzer and Skinner (1965) have shown that the important functional group involved in the complexation are $-CO_2H$ and phenolic -OH group. Blocking of either of these groups cause significant reduction in the metal retention. Both groups appeared to react simutaneously with the metal ions by a reaction similar to that between ferric iron and salysilic acid. Alcoholic hydroxyls did not participate in the organo-metallic reactions. In a later study they noted the stability constants of metal HA complexes varying with the metals (Schnitzer and Skinner, 1966). The association between humus and trace elements depends to a great extent on the concentration factor and pH. The association is probably very low for halogens and alkali ions. Benes et.al. (1976) states that alkaline earth and particularly the valent metals complexes with humus to a rather high degree. The stability constants and the chemical species of metal complexed vary with the pH of the medium, e.g. Randhawa and Broadbent (1965) observed that at pH 7, 70% of the zinc retained by HA was in divalent form and at pH 3.6, 75% monovalent. The total zinc retained increased with increase in pH. The stability constants determind by these workers varied from 4.42 at pH 3.36 to 6.18 at pH 5.6.

Tan et.al. (1971) noted that the amount of organic matter comlexed by a mole of metal and the stability of metal complexes increased with increase in pH in the case of Cu, Mg and Al complexes. However in case of Zinc no change was observed. These workers report that with respect to the divalent ion the amount of organic matter chelated and the stability of the divalent metal - complexes decreased in the order Cu > Zn > Mg.

Potentiometric studies by Khanna and Stevenson (1961) showed that the HA formed stable metal complexes with added transition metal and were bound primarily by the carbonyl group. Handerson - Hasselbach plot gave results similar to that of acids with weak complexing abilities. The complexes formed differed in its constitution with pH as explained by Schnitzer and Skinner (1962). Fe^{2+}, Al^{3+} and Cu^{2+} formed water soluble 1:1 molar complexes at pH - 3 ; 2:1 water soluble complexes were formed at pH - 5 between Ferric Iron, Cu, Ca and HA. These experiments also suggests the formation of molar complexes varying from 1:1 to 6:1 and becoming increasingly water insoluble as more metal is complexed.

It is widely established that free copper ion is the main toxic fraction to aquatic organisms. Complexation reactions may reduce abiotic and biotic sorption considerably. The effect of complexing agents on a biotic adsorption processes appears to vary from metal to metal and this aspect is mainly determined by the affinity of the metal to the complexants. Sylva (1976) observed the enhanced adsorption of Cu(II) by lake sediments due to the presence of nitrilotriacetic acid. However in case of iron and manganese the presence of nitrilotriacetic acid decreased adsorption.

Electron spin resonance spectral studies of a Cu - H complex and some adducts with electron doners revealed the mechanism of Cu^{2+} binding with with HA. Cu^{2+} formed two equatorial bonds with oxygen doner atoms originating from the functional groups of HA. The e.s.r. data also indicated that the Cu - HA oxygen bond occupied cis-position in the square plane of Cu^{2+}, a result consistant with the formation of Cu chelate in the original Cu-HA complex (Boyd et.al., 1981).

Stevenson (1976) conducted potentiometric studies and reported that the stability of complexes followed the order Cu > Pb > Cd. The constants obtained by potentiometric titration are empirical at the best. Hence many of the concepts developed for coordination chemistry in simple molecules do not apply directly to macro-molecules such as HA and result of such study must be integreated with caution. Cu^{2+} and Pb^{2+} were found to be considerable more

thermodynamic calculations it was concluded that the variation of the ratio from 10 to 70 % depend on the variation of stability constants of the copper with the organic matter.

The degree of interaction between HA and metals in the sea-water is related to the structure of particular HA. An index of hetero atom substitutions derived from p.m.r. spectra of these materials when combined with equivalent weight correllated with the nature and degree of metal interaction. The interactions of fulvic acid with copper seems to be related with structure while HA appear to interact with Cadmium regardless of the structure. cadmium interacts preferably with mature highly cross-linked HA (Piotrowicz, et.al. 1984).

Montgomery et.al. (1977) has described that the concentration of Zn and Cu in particulate fraction range from 30 % to 80 % of the total metal present in all fractions. The concentration in soluble inorganic legand fractions remained fairly constant at 10 to 20 % of the total metal present. The concentration in the soluble organic legand fraction range from 11 to 53 %. These later concentrations increased in the coastal zone as compared to river stations.

In natural waters trace metals are distributed between the disolved and solid phase. In the dissolved phase they exist as hydrated ions, labile complexes predominantly with the respective amonic inorganic constituents of water and the non labile complexes with dissolved organic matter. Humic acids are considered to be a major constituents of the dissolved organic matter in water.

Raspor et.al. (1984a) states that the dissolved HA will contribute significantly to the speciation of heavy metals where dissolved concentration is more than 1 mg dm^3. Raspor et.al. (1984b) emphasized that the trace metals interactions with dissolved organic matter play an important role in the biogeochemical cycle of aquatic eco-system. They observed that the abundance of trace metals in the HA decreases in the order of Al > Cu > Zn > Pb > Cd.

Tan et.al. (1971b) studied the HA's Zn complexing capacity by ion exchange equilibrium method and i.r. spectroscopy. The number of moles of organic metals complexed by one mole of Zn at constant pH was low in the case of high molecular weight fractions and was twice for low molecular weight fractions. The stability constants varied between 1.8 to 6.8 for the low molecular weight complexes.

Zumino et.al. (1972) introduced a novel concept of Maximum Complexing Ability (M.C.A.) to estimate the stability constant of water soluble complexes of unknown molecular weight. The M.C.A. represents the potential complexing ability of a solution of a complexioned in its active complexing sites. The higher the stability of a complex the lower is the metal concentration that has to be used to attain M.C.A. The limiting factor in this proposed method is the formation constants of the complex. If this constant is very low, very high concentration of metal have to be used to reach M.C.A.

The overall stability constants increase with increasing humification and the values correspond to the contents of very weakly acidic functional groups (Takamatsu and Yoshida, 1978). These workers state that the stability constant increases with increasing pH. Of the three metals they compared the stability constants varied in the following order $Cu^{2+} > Pb^{2+} > Cd^{2+}$. The overall stability of cadmium complexes ranged from 4.3×10^5 to 4.6×10^7 at pH 6. Kerven et.al. (1984) describe that the copper present in soil solution is present as organic complexes. Raising pH of aqueous exrtract to about 6 increase the degree of complexation of the soluble copper in the extract.

The average molecular weight of the fractionated aquatic humus are of the order of 10^3 and the acidic character of the humic polymers enable them to interact with cations of heavy metals forming complex linkages of various types by ion exchange, surface adsorption and chelation. The stabilities of metal humic complexes in natural waters are higher than those corresponding inorganic metals. The extent of complexation of heavy metals by aquatic humus is dependent on the concentration of humic substances and the competition for available complexing sites between trace metals and major cations (Reuter and Perdue, 1977).

Mantoura et.al. (1978) state that in sea water < 99% of humic metals is complexed by calcium and magnesium because of their relatively high concentration. Metal chelator is only appreciable for copper ($\simeq$ 10%). Humic substances from the various natural environments show a wide range of values of K_o in their interactions with any particular metal.

Boyle and Edmond (1977) has described the mechanism of iron removal in estuaries. They explained that the river borne dissolved iron consists of entirely mixed iron oxides organic matter colloids of diameter < .45 um stabilised by dissolved organic matter. Precipitation occurs on mixing because the sea water cations neutralise the negatively charged iron bearing colloids allowing flocculation. The effectiveness of precipitation by different sea salt cations increased in the sequence $NaCl << MgCl_2 << CaCl_2$ at equimolecular concentration.

Fitch and Stevenson (1984) has compared the various complexations models using a common set of experimental data. Their study revealed that the approaches based on assumption of linearity yield poor estimates of stability constants. Of the various models they examined the Hill plot is found to be the least reliable. The stability constants obtained by the study performed by Stevenson (1977) were in excellent agreement with those obtained by conventional titrations and followed the Irwing Williams order. The values of stability constant varied with pH but increased greatly with decreasing concentration of the supporting electrolytes and at least two major sites were inferred to be involved in complex formations.

ROLE OF HUMIC ACID IN ENVIRONMENT

Humic substances which are formed mainly from lignin like compounds by microbial activity under aerobic conditions play a critical role in the detrital cycle in soil (Odum 1971). These substances during the various steps of decomposition release nitrogen to the soil environment and according to Flaig et.al. (1975) these organic substances are the natural resorvoirs for the nitrogen supply of plants. As various steps of degradation of these compounds proceed the nitrogen contant also get reduced. This released nitrogen are also being used by microbial protein synthesis (Flaig et.al. 1975). The importance of humic materials on the microbial growth are elaborated by Burns et.al. (1986).

These organic materials in the soil exhibit both a direct and indirect impact on the growth of higher plants. Mostly the presence of sufficient amount of humic material lead to a stimulation of plant growth for, they modify many of the physical, chemical and biological properties of the soil system (Visser 1986). They modify the soil structure, water economy of the soil and its water retaining capacity, bufering and exchage cxapacities etc. Humic materials for their high complexation ability also help in mobilization and making available the bound nutrients (Swift and Haynes 1986). Another aspect by which the humic materials can stimulate the plant growth is by their ability to bind width many of the toxic trace elements (Saar and Weber 1980) and also pesticides which by the present day agricultural practices are added more and more in the soil system (Visser 1986).

Vaughan (1986) states that HA can function as mediator beween nutrient solution and roots. It is also reported that it can affect the metabolic use by its activity on some enzymes (Burns 1986). The probable metabolic action observed in the case of humic materials are assumed to be due to low molecular weight fraction of humic material which has got considerable hormone like activity (Burns 1986).

Tan and Binger (1986) conducted green house experiments on corn seedlings to study the effect of HA on aluminium toxicity. When Al treated plants developed marginal chlorosis and necrosis, they found that addition of HA alongwith same concentratrion of aluminium led to the development of healthy and green plants. They also observed that the phosphorus contents in the leaves in the presence of HA were normal while the plants treated with Al alone developed phosphate defficiency symptoms.

Similar to their role in their terrestial system, humic substances also play similar beneficial role in aquatic systems also. A number of studies have noted the relation between concentration of humic substances of water body and their productivity, For example Shanmukhappa (1984) has observed concentration of humic substances in the range of .1 to .45 mg/g in an estuary and .15 to .69 mg/g in mangroves which are considered to be highly productive aquatic eco-system. Odum (1971), Prakash et.al. (1972) also discussed about the relation between humic substances and aquatic productivity. They conclude that the main role of humus in eutrophication is probably as a chelator of nutrients preventing precipitation of phosphates.

HA are found to bind with ionic and non ionic herbicides like bromacil diuron, simazine, glyphosate and diquat, thus reducing the toxicity of these compounds in the soil system (Madhun et.al. 1986). Jensen-Korte et.al. (1987) observed that the photodegradation of some pesticides that are non digreadable or nearly non-degradable in standard laboratory tests were found to be photodegrading at an increased rate in presence of HA.

In natural waters in the transport of trace and macro elements like Ca, Mg, Zn, Mn, Co, Ni, Cu, Cd, Hg and Fe the humic materials are very important because a significant concentration of dissolved elements in natural waters are present in bound form either with dissolved or with colloidal humic materials Boyle et.al. (1977) (Mantoura et.al. 1978 ; Boyle et.al. 1977) notes that in estuaries the dissolved iron are in the form of iron oxide organic matter colloids of less than 0.45 um stabilised by the dissolved organic matter. The removal of iron in the estuary from the dissolved phase is actually effected by the flocculation due to the neutralisation of the negatively charged iron oxide bearing colloids by sea water cations. Campbell and Evans, (1987) found that Cd should be more bioavailable than Pb for, Cd binding to HA is much lower than that of Pb. An examination by them of concentration factors in 10 lakes in Canada indicates the significance of humic materials in controlling bioavailability.

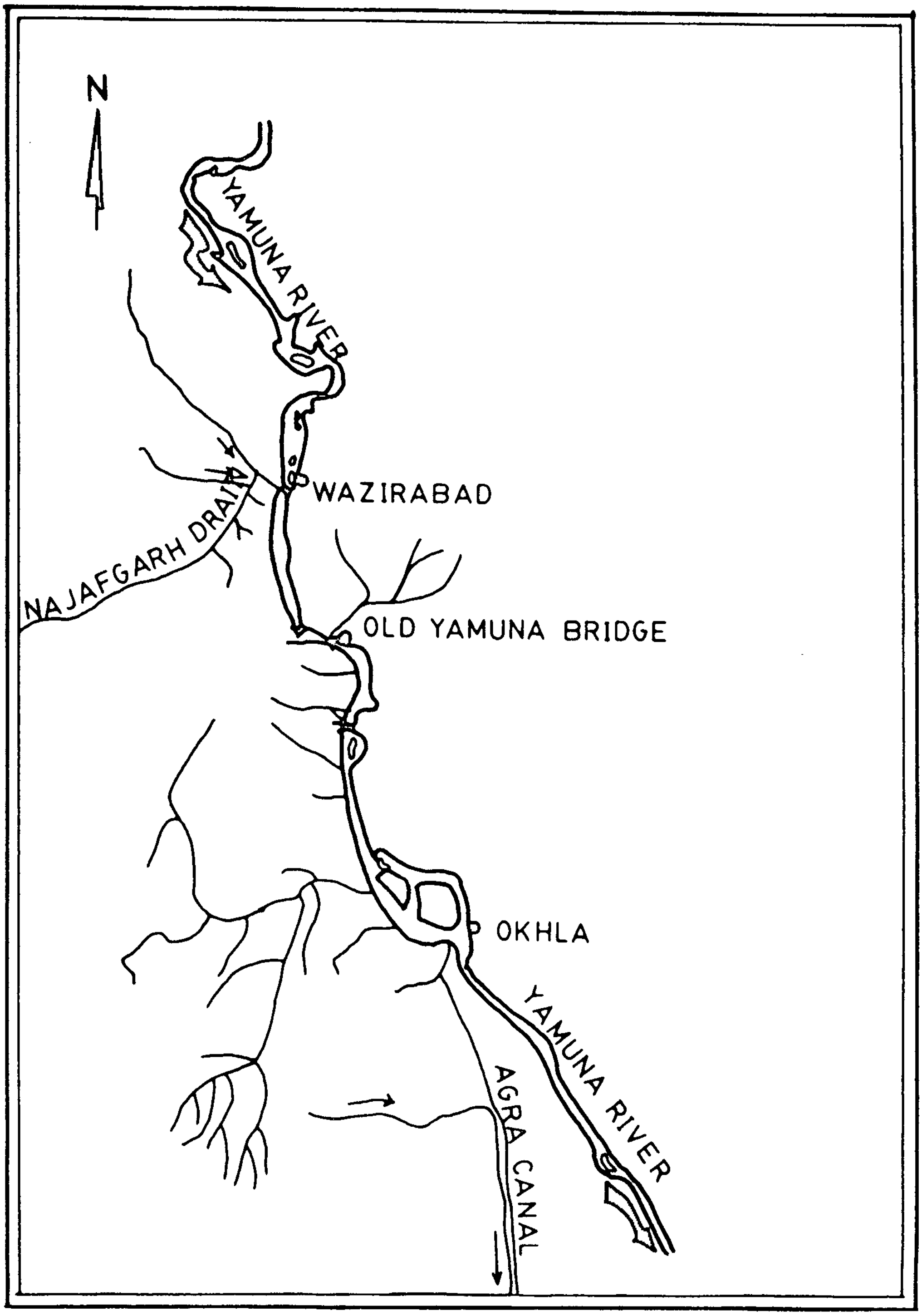

Fig. 2 River Yamuna around Delhi Showing the sampling stations.

CHAPTER III

MATERIALS AND METHODS

Sediment Grain size Analysis

The granulometric analysis of sediments were carried out the the laboratory using Attenbergs Cylinders described by Forstner and Wittman (1981).

The sediment sample was dried in over at 100°C and then it was gently crushed to break all the clumps, and shells were seperated. Then the sample was treated with hydrogen peroxide solution to remove the organic matter adhering to the sediment particles. A known quantity of sample was transfered to an Atterbergs cylinder and the speration was effected.

The HA for the present study was extracted from the river Y amuna sediments collected from three sites viz. Wazirabad, Old Yamuna Bridge and Okhla shown in Fig. 2. Wazirabad is the place where Yamnuna enters Delhi and Okhla where it leaves Delhi. Old Yamuna Bridge was chosen as it was almost at the middle of the course of the river through Delhi.

Another criteria for the selection of the sites was the sources of HA. At Wazirabad the major source of HA was the decay of aquatic vegetations and the phytoplanktons where as at Old Yamuna Bridge it was the decay of the ritualistic wastes like flowers, rice etc. At Okhla it was the decay of Eichornia dominated vegetation and the organic matter coming from the swagee, which act as main source of HA.

The different fractions collected were dried and weighed. The weight percentage of each fraction was calculated.

Organic matter in Sediments

The sample was first taken in known quantity, after drying the sediments in over at 100^{o} C for 24 hours, to remove moisture. Thus a known quantity of sediment was treated with Hydrogen perioxide to remove all organic matter in the form of CO_2. The treatment was repeated till all bubbing stopped. Sample was then dried at 110^{o}C in the oven till all the moisture evaporated. Then it was weighed and from the loss in weight, the percentage of organic matter was calculated.

Estimation of Organic Carbon in Sediments

The total organic carbon from the sediments was estimated by the method described by Walkley and Black (1934). The sample was first carefully washed with distilled water to remove the salts on a buchner funnel fitted with a fine textured, hardened filter paper. After drying at 105°C, the samples were ground to fineness and then used for the estimation of total organic carbon.

Estimation of Total Nitrogen in Sediments

The Total Nitrogen in the sediment was estimated by the method of Kjeldahl's micro-degistion technique, which has been described by Jackson(1973).

Estimation of Total Phosphorous in Sediments

The total phosphorous in sediments was estimated by the method described by John (1970). The phosphorous combined with other resistant minerals is not determined by this method.

Estimation of Cu and Cd in sediments by Atomic absorption Spectrophotometer

The sediment sample taken for the estimation of metals was first dried in over, shells and deries were removed. Then finally ground in an agate mortar passed through a 2mm sieve and again powedered to fineness.

Teflon bombs were used for the digestion of the sample. 0.5 gm of the sediment was accurately weighed and transfered to the Teflon bomb. For digestion, two acid mixtures were used viz (a) $HClO_4$ (b) HNO_3 in the ratio 1:3 (Enk and Mathias, 1977). All samples were digested at 100 - 110°C for 1 to 1 and half hours till a clear solution was obtained. It was colled to room temp and made up to 50 ml. with distilled water and analysed using 1L 457 Atomic Absorptions Spectrophotometer.

Estimation of Humic Acid in sediments

The HA was extracted from the sediment sample using 0.1 N NaOH (Takamatsu and Yoshida 1978). Then the precipitated HA was filtered through a pre weighed Whatman filter paper (pore size .45 u). The paper along with the HA was dried in the oven at 60°C, and the weight of HA was calculated by taking difference as mg/g of sediment sample.

Extraction of HA

Soil samples was collected from setes from river Yamuna viz. Okhla, Old Yamuna Bridge and Wazirabad. These soil samples were air dried and seieved (2mm). Two hundred and fifty grams of each soil sample was extracted with 1 l of O.I N. NaOH for 1 hour at 95°C with strring. The extract was centrifuged (at 27000g) (Sposito et.al. 1978) to remove suspended clay particles. The pH of the solution was adjusted to slightly lower than 2 with HCl to precipitate HA. The precipitate of HA was collected by centrifigation and washed three times with 0.1 N Hcl and twice with water successively and then dissolved in 0.1 N NaOH. HA was again precipitated and washed with water. (Takamatsu and Yoshida 1978) HA thus obtained was dried at 35°C in air oven.

Complexation studies of Cu^{2+} and Cd^{2+} with HA

The purified HA us dried in oven at 35°C for 2 hours and 0.02 gm of it was weighed accuratly. This was dissolved in 50 ml of NaOH. Out of this solution 12 ml was pippetted out, 3 ml of ionic strength adjuster (ISA) (5M $NaNO_3$) was added and the solution was made up to 150 ml. which gave 0.032

mg/l concentration of HA.

Stock solutions of Cu^{2+} and Cd^{2+} at a concentration of $10^{-3}M$ were prepared from orion metal standard. The stock solutions were acidfied with $HClO_4$ in order to prevent hydrolysis of these ions.

The complexation was carried out with the above metal solution with HA solution. The degree of complexation was measured with respect to ions present in the solution using an Orion ion analyser (model No 901) and Cu and Cd ion selective electrodes.

Determination of stability constants using Ion Selective Electrodes

Measurement of the Degree of complexation. The response of ion selective electrode (ISE) is given by

$$E = Eo' - E_R + E_1 + RT/zF \times \text{In } r\,Me + RT/zF \times \text{In } [Me]$$

$$Eo' - E_R + E_1 + RT/zF \times \text{In } r\,Me = Eo$$

$$E = Eo + p.\log([Me]) \qquad \text{--------} \ (1)$$

Where [Me] is the concentration of the hydrated ion Me^{z+} in the solution. Eo' is the standard electrode potential. z is the number of charges of ion Me^{z+}, E_R is the reference electrode potential and E_l is the liquid junction potential between the test solution and refence electrode solution. r. Me, R,T, and F have their usual meanings. At constant temperature and ionic strength, E_j,RT/F, and r Me will be constant and the potential can be expressed in the form given in equation 1, where the values of Eo and p (2.3 RT/zF) can be computed from calibration curve.

The degree of complexation of ion Me^{2+}, α is defined as

$$\alpha = \frac{[Me]_t}{[Me]} \qquad \text{-------} \quad (2)$$

Where $[Me]_t$ is the total concentration of Me^{z+}, irrespective of the form in which Me^{z+} is present. From equation 1, x may be computed by means of $\alpha = [Me]_t \times 10^{(Eo-E)/p}$ ------- (3) Hence by titrating a solution of HA with the Me^{z+} and measuring the coresponding values of E, a curve α = f (log $[Me]_t$) may be obtained.

Determination of M_w and B_n^*

The concentration $[Me]_t$ corresponding to the ligand saturation, as well as the values of α for $[Me]_t \to 0$, can be used to characterize the complexing ability of a given medium. However, if the medium contains only one type of

$$B_1^* = \frac{[Me\ L]\ .\ [H]^x}{[Me]\ .\ [L\ H_x]} \quad \text{---- (5)}$$

Here charges of the particles are omitted and [X] denotes the concentration of species X in Mol/l :

Mass balance equation is given as :

$$[Me]_t = [Me] + [Me\ L] \quad \text{---- (6)}$$

$$\frac{[L]_t}{M_w} = \frac{[LH_x]}{v} + \frac{[Me\ L]}{v} \quad \text{---- (7)}$$

Where $[L]_t$ is the total concentration of organic matter (in g/l), and V is 1 if 1:1 complexes are formed.

Combining ernation 5 to 7 are obtains :

$$Y = \frac{[L]_t}{[Me]_t} . \frac{\alpha}{\alpha-1} = \frac{M_w}{v} + \frac{M_w}{v} . \frac{[H]^x}{B_1^*} . \frac{\alpha}{[Me]_t} \quad \text{---(8)}$$

Hence, if titration is carried out at constant pH, a plot of the left hand term of equations 8 vs. $\alpha/[Me]_t$ should give a straight line and form the slope and intercept on the ordinate Mw/V and $B_1^*/[H]^x$ can be evaluated.

In the case of 1:2 complexes the curve may not be liniar for the above plot. Under these conditions, and since $[L] \simeq [L\ H_x]$ one has complexing agent, then by modifying the method initially proposed by scatchard can be used to obtain quantitative information as to the nature of the ligand.

Since the molecular weight of HA and FA, in natural waters may vary from a few hundreds to hundreds of thousands three types of reaction may be assumed: the formation of solible complexes with metal - ligand ratio l/n (where n>/1), by one of the v sites of a colloidal particle of high molecular weight (M_w); and the absorption of hydrolysed metal on colloidal organic matter or formation of mixed ligand complexes with OH and HA.

If 1:1 complexes are formed, or if the sites in the colloidal particles are far apart so that the interactions between the two site in particle is negligible, then the only reaction which could occur is:

$$Me^{2+} + LH_x \rightleftharpoons Me\ L + x\ H^+ \qquad \text{------------} \ (4)$$

where L H_x is either a ligand molecule or a complexing site of the molecule. Equilibrium constant for this reaction is given

$$\alpha = \frac{[Me]_t}{[Me]} = 1 + \frac{B_1^*}{[H]^x} [L]_t + \frac{B_2^*}{[H]^{2x}} \cdot [L]^2_t$$

Where B_2^* is defined as

$$B_2^* = \frac{[Me\ L_2]\quad [H]^{2x}}{[Me]\quad [L\ H_x]^2} \qquad \text{------ (9)}$$

Hence

$$M_w \cdot \frac{\alpha - 1}{[L]_t} = \frac{B_1^*}{[H]^x} + \frac{B_2^*}{[H]^{2x}} \cdot \frac{[L]_t}{M_w} \qquad \text{------ (10)}$$

Since v may be assumed to be 1.

The equation was checked by ploting M_w (α-1) / $[L]_t$ Vs $[L]_t$ / M_w for various samples. Good straight lines were obtained where excess of organic matter was used. Log K where K = B_1^* /$[H]^x$ calculated from equation (8) is used for the calculation of stability constant. The method discussed above was given by Buffle et.al. 1977.

Elemental analysis

C,H and N were analysed for all the three samples using Perkin Elmer 240c Elemental Analyser.

UV - VIS Spectrophotometry

10 ppm solution was prepared for all the three samples of HA in .1N NaOH and the spectra was recorded from 200 to 700 mm using Hitachi 330 UV-VIS Spectrophotometr.

Determination of the ratio E_4/E_6.

Optical density, E, at 465 and 665 nm was measured using a cecil double beam UV-VIS spectrophotometer of 0.1g HA l^{-1} in 0.01M NaOH.

IR Spectrophotometry

Sample preparation

The pellets were preheated to 65°C for 2 hours to eleminate the moisture interference 1mg of the pre dried sample at 45°C for about 18 hr., mixed with 300 mg. of similarly predried KB_r and ground for 15 sec. A control pellet is also formed in the same manner, following which both the pellets are placed in vaccum oven at 100°C for 2 hours. The pellet containing HA is seanned in double beam mode against the KB_r blank (Stevenson and Goh, 1974). The spectra was recorded using Nicolt 5AX - FT IR spectrophotometer.

Proton NMR Spectroscopy

10 mg of HA was dissolved in 2 ml. of Dimelthyl sulfoxide $-d_6$ (DMSO-d_6) and the spectra was recorded at 99.55 MH_z using Tetramethylsilane (TMS) as the internal standard. The spectra was recorded using Joel-JNM FX-100 NMR spectrometer.

All the statistical computations and curve fitting were performed using Hewlett - Packard 9000/236 computer.

CHAPTER - IV

RESULTS AND DISCUSSIONS

River Yamuna which is the main source of water for the metropolitan city of Delhi is also the main reciever of urban and industrial wastes. A number of studies regarding the physico-chemical characterstics of Yamuna water (Sharma,1985,Srinivasan,1986) and sediments have been reported(Sitasawad,1984;Jha,1986). However, a study with emphasis on the environmentally important organic fraction, namely HA, has not been done. The present study was undertaken with the intension of exploring the chemical nature and complexation properties of HA in Yamuna sediments. The study involved physico-chemical characterization of sediments which included the study of grain size,organic matter,organicarbon, Total nitrogen,phosphorus and their correlation with HA content.

Sediment characterstics

1. Grain size

The pattern of sediment grain size distribution is given (in tables 1,2 and 3) for each station (Okhla,old Yamuna Bridge and Wazirabad) for samples collected at fortnightly intervals. Three fraction were analysed, viz, sands >63U silt

TABLE - 1

PERCENTAGE OF SAND SILT CLAY AND ORGANIC MATTER

OKHLA

DATE	SAND	SILT	CLAY	O.M.
01-12-86	60.14	32.01	2.89	5.61
15-12-86	62.52	32.17	1.90	4.82
01-01-87	63.70	32.21	2.09	4.38
15-01-87	63.64	30.87	2.49	4.32
01-02-87	62.50	31.07	2.44	4.97
15-02-87	62.75	32.21	2.61	5.01
MEAN	62.54±1.18	31.75±0.56	2.40±0.32	4.85±.43

TABLE - 2

PERCENTAGE OF SAND SILT CLAY AND ORGANIC MATTER

OLD YAMUNA BRIDGE

DATE	SAND	SILT	CLAY	O.M.
01-12-86	90.31	8.29	0.50	1.66
15-12-86	91.86	7.06	1.08	1.31
01-01-87	90.90	7.82	1.28	1.62
15-01-87	91.65	7.74	1.49	1.97
01-02-87	79.69	17.32	1.68	2.31
15-02-87	69.75	26.64	1.64	2.76
MEAN	85.52±8.17	12.47±7.24	1.28±.4	1.93±.48

TABLE - 3

PERCENTAGE OF SAND SILT CLAY AND ORGANIC MATTER

WAZIRABD

DATE	SAND	SILT	CLAY	O.M.
01-12-86	86.15	10.61	1.92	2.17
15-12-86	81.37	17.01	1.91	2.36
01-01-87	82.57	11.52	2.82	3.76
15-01-87	79.80	17.82	1.10	2.57
01-02-87	92.72	4.66	1.61	1.01
15-02-87	84.25	11.88	1.08	3.05
MEAN	84.47±4.2	12.25±4.37	1.74±.59	2.48±.84

>2U and clay <2U. It was seen that Wazirabad and old Yamuna Bridge stations contained considerable amount of sand all through the period of study. The average percentage of sand fraction was 85%. At okhla station, the percentage of sand was always lesser than the other two stations and the mean sand percentage came to 62.54%. Okhla samples always contained higher percentage of silt and clay than the other two station. The approximate average percentage of silt at okhla was 31% and that of clay 2.4%. Old Yamuna Bridge samples contain the least amount of clay and silt. This is because of the strong current at this site. At okhla the maximum amount of lower size fraction were seen. This may be due to the blocking of water by okhla barrage and the settling down of these fractions. The sediment grain size distribution is important in acertaining the aboundance and variation of the biologically important materials like trace metals humic substances etc. with respect to water movements, biological activities and other physical activities (Shanmukhappa,1984). Fostner and wittman (1981) showed that the fine grain sediments possess higher capacity to sorb and retain higher concentration of trace metals and also organic matter.

2. Organic matter in sediments

Wide variations in organic matter content in sediments were noticed from the three different stations. The okhhla samples contained the highest amount of organic matter and Old Yamuna Bridge and Wazirabad, it was 1.94 and 2.49 mg/g respectively. The reason for the high value of organic matter at okhla station is due to the dense Eichornia dominated aquatic vegetation at this site. The sewage disposal from the okhla sewage treatment plant and the settling of suspended organic matter due to okhla barrage also contribute significantaly to the observed high value of organic carbon at this station. At Old Yamuna Bridge where the organic content was the least, the apparent aquatic vegetation density was also the least. This area also abounds with human activities which may cause physical mobilization of sediments. Wazirabad showed a value in between that of okhla and old Yamuna Bridge. This can be attributed to the relatively high amount of vegetation around the sampling station. Sing and Subramanian (1984) discussed on the sohibilization of certain trace metals due to the break down of organic contents which forms an important link in the aquatic detritus food web (wetzel,1983). The solubilization of trace metals result in the formation of soluble organo-metallic complexes which are released into the water. Pomeroy etal,(1976) notes that the sediments with high organic content will also be having a good population of benthos.

3. Carbon, Nitrogen and Phosphorous

Synthesis of carbon compounds in the presence of sunlight forms the basis for sustained productivity in any ecosystem. The cycles of carbon, nitrogen and phosphorous in the aquatic system is complex. Bulk of the organic matter which contains these elements, viz, C N and P is trapped in the sediments. It is released into the water by connection and biological activities. The total organic carbon estimated at each station varied considerably. At okhla it varied from 8.62 to 15.97 mg/g, at old Yamuna Bridge 3.30 to 10.14 mg/g and at Wazirabad 1.5 to 12.42 mg/g (Table 4). Generally a direct correlation of organic carbon with aquatic productivity is observed (Shanmukhappa 1984). Sediments sampled at okhla contained high concentration of organic matter. The sources of organic carbon in waterbodies can be of terrestrial orgin as also surface run off, biological productivity of the stream and human activities. Continuous exchage occurs between the organic carbon in the sediments and the overlying waterbody.

TABLE - 4

ORGANIC CARBON

DATE	OKHLA mg/g	OLD YAMUNA BRIDGE mg/g	WAZIRABAD mg/g
01-12-86	9.12	5.83	1.52
15-12-86	10.64	3.30	8.36
01-01-87	15.97	3.30	12.42
15-01-87	11.40	10.14	11.66
01-02-87	10.14	6.08	3.04
15-02-87	8.62	10.15	4.56
MEAN	10.98±2.64	6.47±3.08	6.69±3.72

TABLE - 5

TOTAL NITROGEN

DATE	OKHLA mg/g	OLD YAMUNA BRIDGE mg/g	WAZIRABAD mg/g
01-12-86	0.0896	0.0392	0.0280
15-12-86	0.0840	0.0336	0.0616
01-01-87	0.0952	0.0280	0.0728
15-01-87	0.0896	0.0448	0.0336
01-02-87	0.0784	0.0224	0.0224
15-02-87	0.0840	0.0504	0.0504
MEAN	0.0840 ± 0.009	0.0364 ± 0.009	0.0448 ± 0.02

TABLE - 6

PHOSPHOROUS

DATE	OKHLA mg/g	OLD YAMUNA BRIDGE mg/g	WAZIRABAD mg/g
01-12-86	0.025	0.032	0.013
15-12-86	0.026	0.031	0.067
01-01-87	0.075	0.019	0.093
15-01-87	0.073	0.019	0.083
01-02-87	0.063	0.045	0.016
15-02-87	0.031	0.052	0.050
MEAN	0.049 ± 0.024	0.033 ± 0.013	0.054 ± 0.03

TABLE - 7

FORTNIGHTLY VARIATION OF TRACE METALS IN SEDIMENTS FROM OKHLA.

DATE	Cu ppm	Cd ppm
01-12-86	34.1	0.98
15-12-86	32.3	1.32
01-01-87	36.3	1.21
15-01-87	22.8	1.1
01-02-87	36.1	1.4
15-02-87	21.7	1.37
MEAN	30.55 ± 6.3	1.23 ± .15

TABLE - 8

FORTNIGHTLY VARIATION OF TRACE METALS IN SEDIMENTS FROM OLD YAMUNA BRIDGE.

DATE	Cu ppm	Cd ppm
01-12-86	24.8	0.9
15-12-86	18.4	1.2
01-01-87	27.8	1.3
15-01-87	22.3	1.1
01-02-87	24.7	0.93
15-02-87	23.2	1.1
MEAN	23.53 ± 2.86	1.08 ± .14

TABLE - 9

FORTNIGHTLY VARIATION OF TRACE METALS IN SEDIMENTS FROM WAZIRABAD.

DATE	Cu ppm	Cd ppm
01-12-86	19.2	0.81
15-12-86	20.3	0.92
01-01-87	21	1.1
15-01-87	24.3	1.2
01-02-87	20.1	0.8
15-02-87	25.2	0.72
MEAN	21.68 ± 2.24	0.92 ± .17

In case of total nitrogen exactly similar trends as total organic carbon was observed. The maximum value obtained was in the case of Okhla sample. At Okhla the mean concentration was 0.084 mg/g at Wazirabad and Old Yamuna Bridge the values were 0.044 mg/g and 0.036 mg/g respectively (Table 5). Recently the domestic wastes and waste from agricultural practises have also become a significant contribution.

Like organic carbon and nitrogen, phosphorous in the sediment also varied considerably. High values were obtained at Wazirabad with a mean concentration 0.054 mg/g. At Okhla phosphorous was 0.0 49 mg/g and at Old Yamuna Bridge 0.033 mg/g. The range in phosphorous content at Okhla was 0.05 to 0.075 mg/g, Old Yamuna Bridge 0.019 to 0.052 Mg/g and at Wazirabad 0.013 to 0.093 mg/g respectively (Table 6) in aquatic systems. Phosphorous is one of the main limiting factors in phytoplankton productivity. Low concentration of phosphate in natural waters cause low productivity (Rhyther and Dustan 1971; Correl et al, 1975 Aston and Hewitt, 1977;). The trapped phosphorous in the sediments is assimilated by plants as phosphates which adds to the productivity.

Shanmukhappa,(1981) has demonstrated that low concentration of phosphate will reduce the phytoplankton population even in presence of sufficient other nutrients. As in the case of nitrogen, sewage forms one of the major sources of phosphate to aquatic systems at present, thus making the systems eutrophic and sometimes hyper and trophic.

4. Copper and Cadmium in Sediments

Release of excess heavy metals to the enviornment, by both natural and anthropurgic sources, is one of the most serious contemporary problems. In the present study the concentrations of two heavy metals Cu and Cd in the sediments were determined for all the three stations at fortnightly intervals (Tables 7,8 and 9). Of these two, Cu in low concentrtion is a nutrient while for Cd no important biological roles have been reported (Huntsman and Sunda, 1980). These elements which show borderline charecteristics (Nieboer and Richardson, 1980; Ranbow, 1985) are included by Wood (1974) in very toxic and relatively accessible elements. In the present study okhla samples have been found to contain these two metals in the highest concentration among the three sampling sites. 30.56 ppm and 1.23 ppm and of Cu and Cd were observed in

TABLE - 10

HUMIC ACIDS

DATE	OKHLA mg/g	OLD YAMUNA BRIDGE mg/gm	WAZIRABAD m/g
01-12-86	4.35	2.51	0.73
15-12-86	3.36	2.02	2.58
01-01-87	1.96	1.88	3.43
15-01-87	3.01	1.73	3.37
01-02-87	7.89	1.74	0.78
15-02-87	5.03	1.01	2.11
MEAN	4.27 ± 2.07	1.82 ± 0.45	2.17 ± 1.2

the Okhla sediment samples. Lowest concentration of all these metals were observed at Wazirabad. Sitasawad (1984) reports an average value of 21 ppm of Cu at Wazirabad and 34 ppm of Cu at Okhla, which is close to the present observation. Since Wazirabad is least disturbed by man made addition, it is having the least value for all these metals. Thus the bulk composition of the river sediment do not reflect entirely the natural geochemical cycle, but significant contribution of trace metals from various drains connected to river Yamuna along with some other secondary sources or changing water-sediment interaction at different location downtream (Sitasawad 1984).

Humic Acids in sediments

Okhla sediment has the highest concentration of HA, the main source of which may be the decaying aquatic vegetation aboundantly present in the area and also that carried by river Yamuna. The values of HA at these stations range from 1.96 to 7.89 mg/g. Old Yamuna Bridge samples were found to have the least amount of HA when compared to the other two stations. Although Delhi's biggest drain Najafgarh Nallah opens into the river upstream of the sampeling station the HA concentration was found to be low. The average HA content observed at this

TABLE - 11

CORRELATION COEFFICIENTS BETWEEN HA AND ORGANIC CARBON, TOTAL NITROGEN, PHOSPHOROUS, Cu, Cd, AND ORGANIC MATTER FROM ALL THE THREE STATIONS.

PARAMETERS	r	t	p	df
HA AND ORGANIC CARBON	.388	1.683	$p < 0.2$	16
HA AND TOTAL NITROGEN	.589	2.73	$p < .02$	16
HA AND PHOSPHOROUS	.299	1.25	N.S*	16
HA AND Cu	.513	2.39	$p < .05$	16
HA AND Cd	.58	2.85	$p < .02$	16
HA AND ORGANIC MATTER	.701	4.01	$p < .005$	16

* N.S. = Not Significant.

station is 1.82 mg/g. At Wazirabad, the concentration HA was 2.17 mg/g. The periodical variation of HA at the three sampling satations are given in Table 10 and the mean value calculated is also given there in. At Wazirabad the main source of HA is the decay of aquatic vegetation.

The correlation constants and their significance of HA with different parameters like, organic matter, organic carbon, total nitrogen, phosphorous and Metals are given in table 11. HA as expected showed a positive correlation with organic matter ($P <.005$). Organic carbon and HA correlations are found to be quite insignificant while with nitrogen the correlation was significant at $P < 0.02$ level. Both the metals examined viz Cu, Cd showed very strong correlation with HA content (Cu-$p < .05$,Cd-$P<.02$) This correlation is indicative of the high complexing capacity of HA, which is being discussed later.

The important source of HA in the river water autotrophic and heterotrophic metalbolism of which the former includes phytoplankton and other aquatic vegetation while the latter is from aquatic organisms. Another source of HA in the waterbody is of terrestrial origin and the input is mainly by the terrestrial run off. A third source of HA to the river water is that which is carried downstream by the river.

Complexation Properties Humic Acids

Stability constant is an important characteristic of a soluble metal organic complex, the value of which provides a quantitative measure of the affinity of the metal for the ligand (Ardakani and Stevenson, 1972). In the present study the stability constant of two metals, viz, Cu and Cd at pH 3 and 5 were expermentely determined using Ion Selective Electrod (ISE) technique. Response of the ISE to added Cu(II) and Cd(II) in presence and absence of HA is illustrated in Fig.3 to 8 (Appendix tables 1 - 12). An initial lag in the ISE response was followed by constent increase in potential as more of Cu(II) was added. A similar trend was observed in the case of Cd too. Values at the flattened portion of the curves correspond to the free Cu(II) values near the practical lower limit of detection of Cu(II) ISE (near pCu 8) (Fitch and Stevenson,1984). A similar explanation seems to be valid in the case of Cd also which shows a similar trend.

The saturation values of the ligand, viz, HAO, HAY AND HAW with the metals at pH/3 and 5 shows the following trends in general. The saturation values were found to be around-

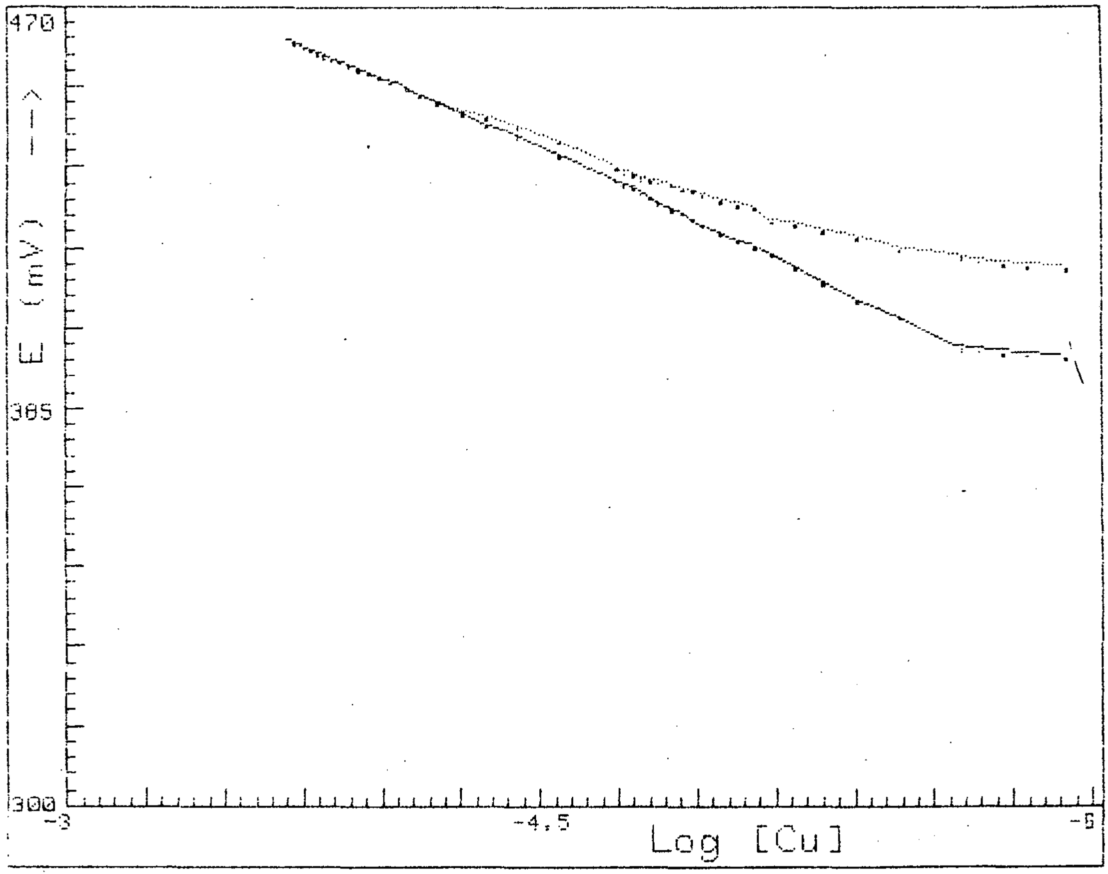

Fig. 3 (a)

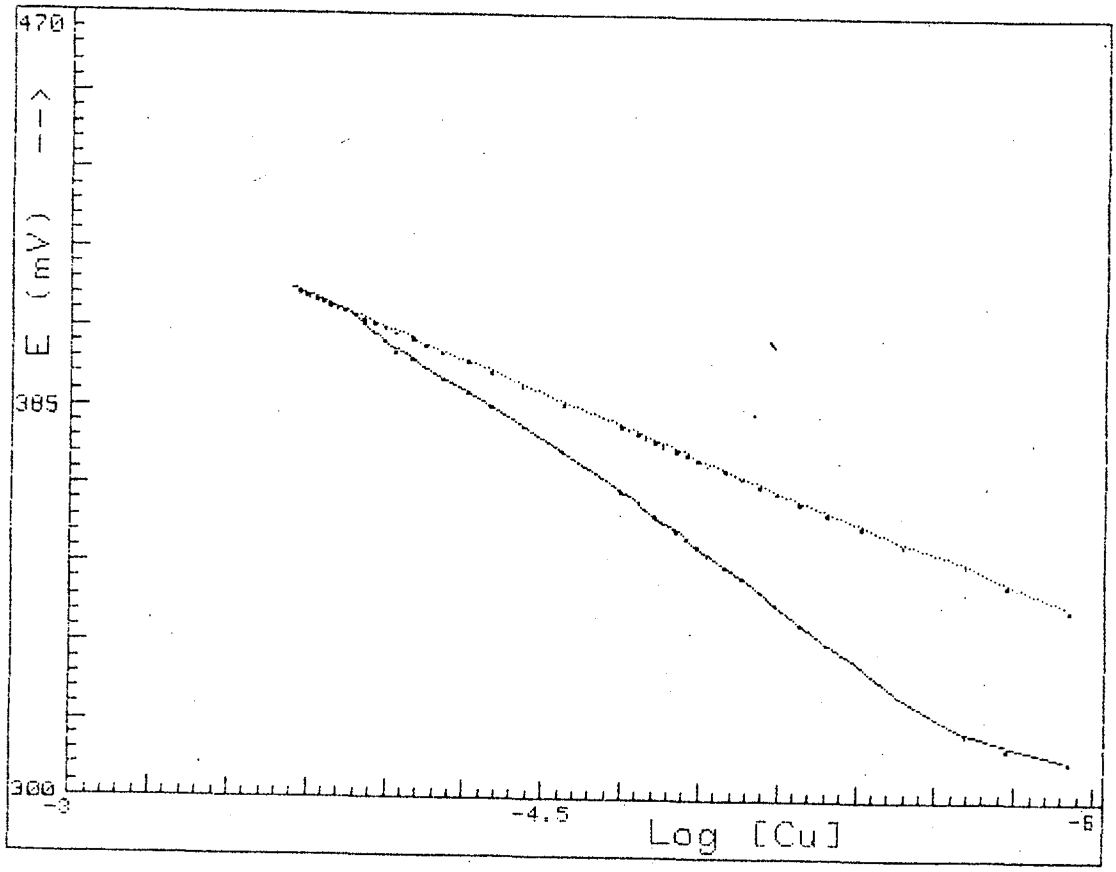

Fig. 3 (b)

Fig. 3 ISE response curves for Cu (II) - HA interactions at 25^{o}C for HAO (Ionic strength 0.1 M; p = 29.55). (a) pH = 3 (b) pH = 5.

.... blank

---- in presence of HA

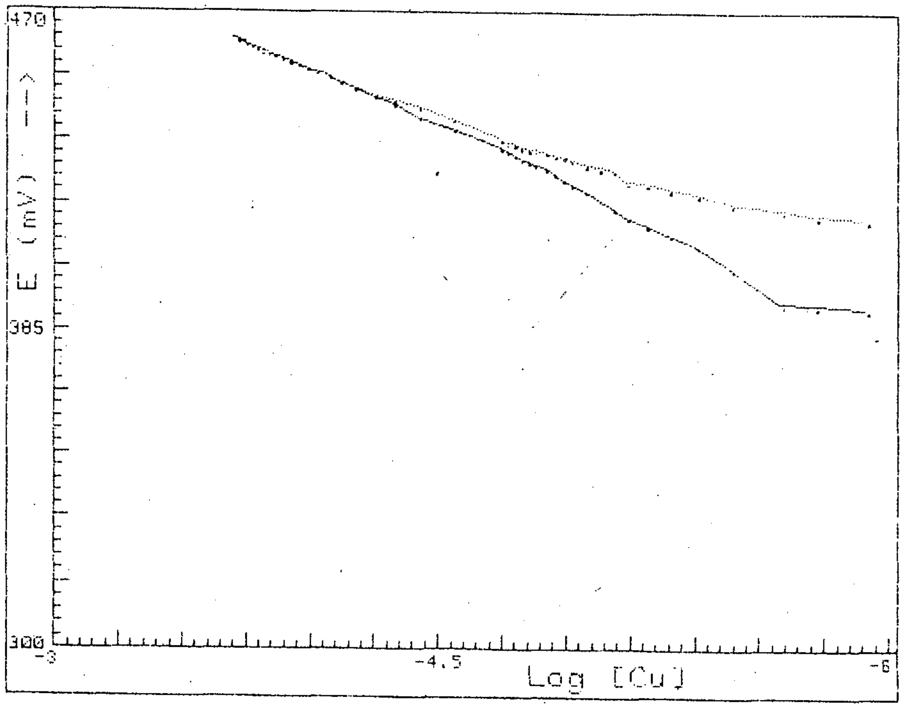

Fig. 4 (a)

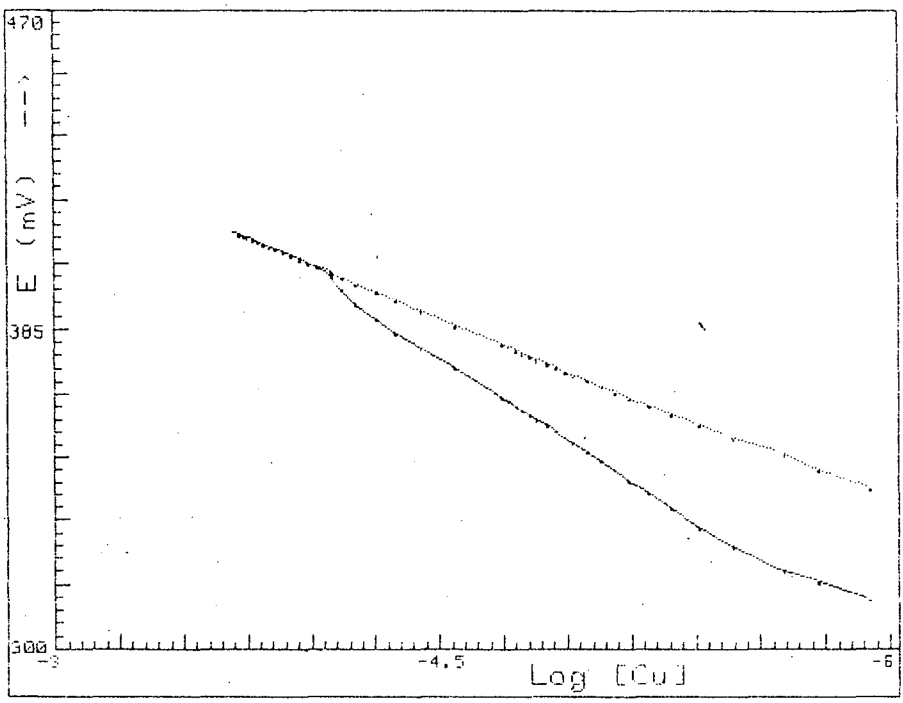

Fig. 4 (b)

Fig. 4 ISE response curves for Cu (II) - HA interactions at 25^{o}C for HAY (Ionic strength 0.1 M; p = 29.55).
(a) pH = 3 (b) pH = 5.
..... blank
----- in presence of HA

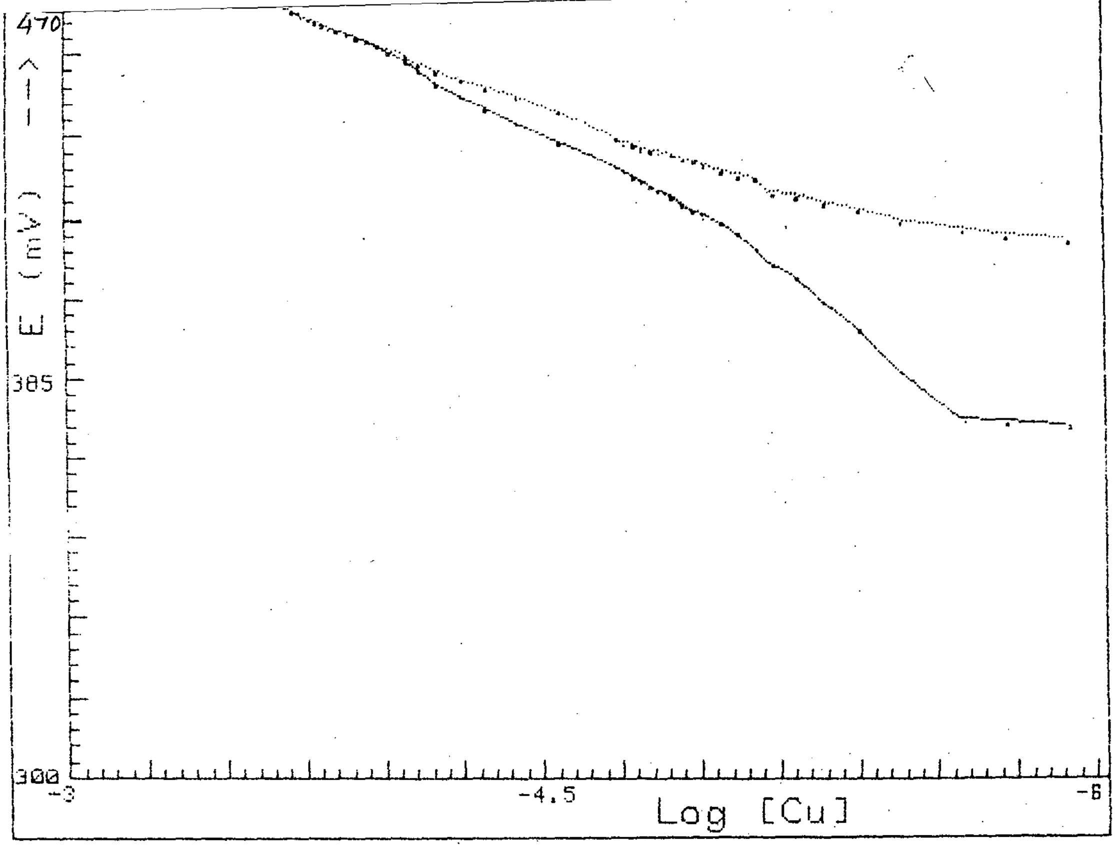

Fig. 5 (a)

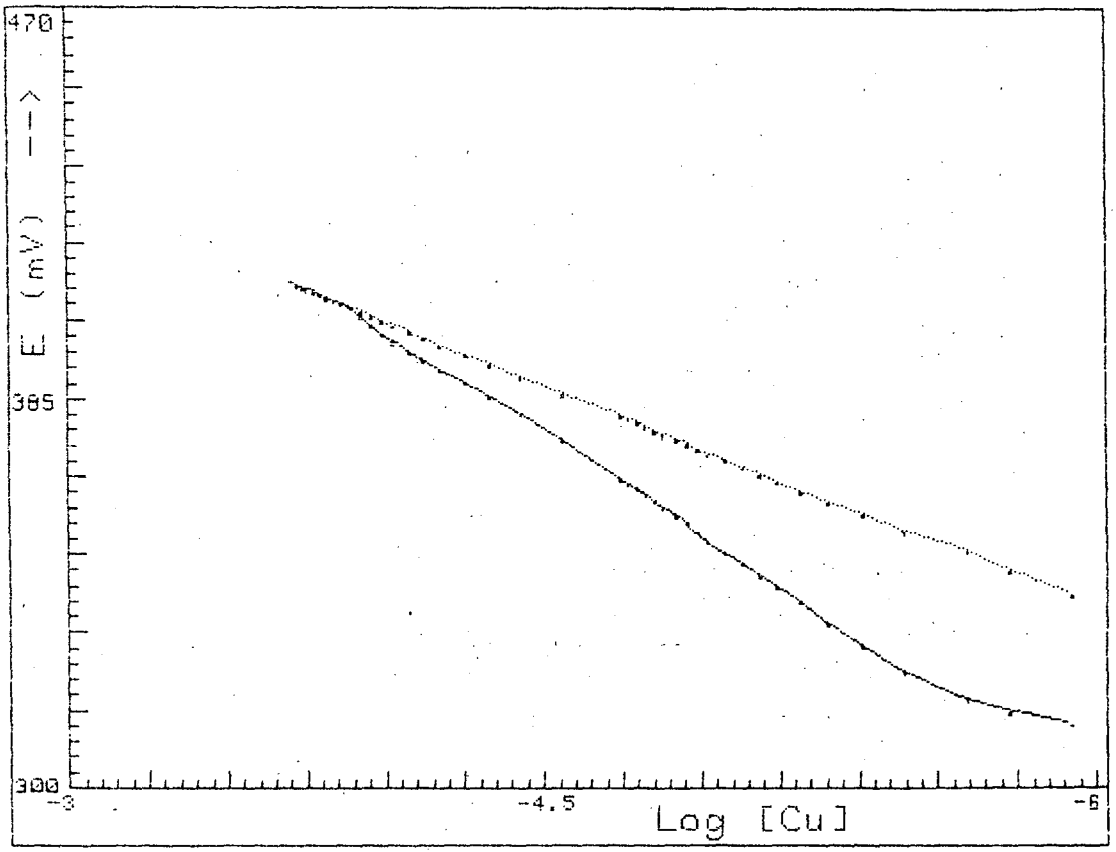

Fig. 5 (b)

Fig. 5 ISE response curves for Cu (II) - HA interactions at 25^{o}C for HAW (Ionic strength 0.1 M; p = 29.55). (a) pH = 3 (b) pH = 5.

.... blank

---- In presence of HA

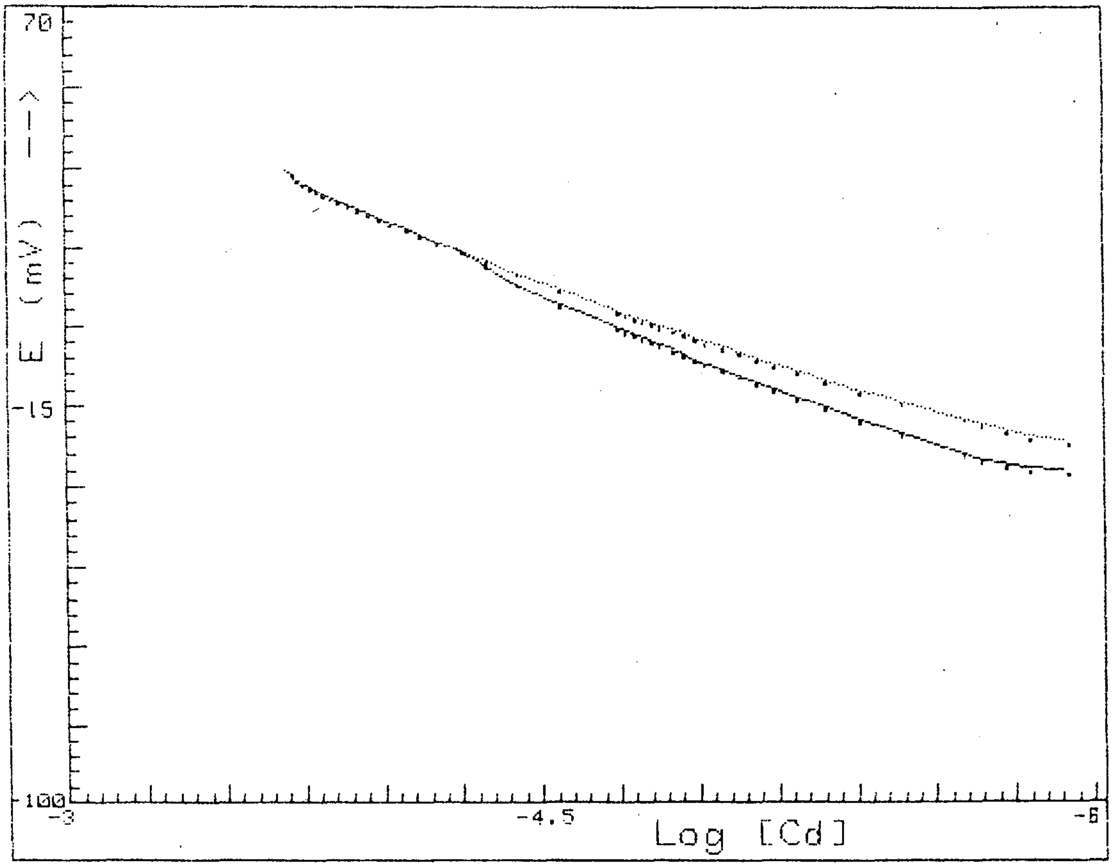

Fig. 6 (a)

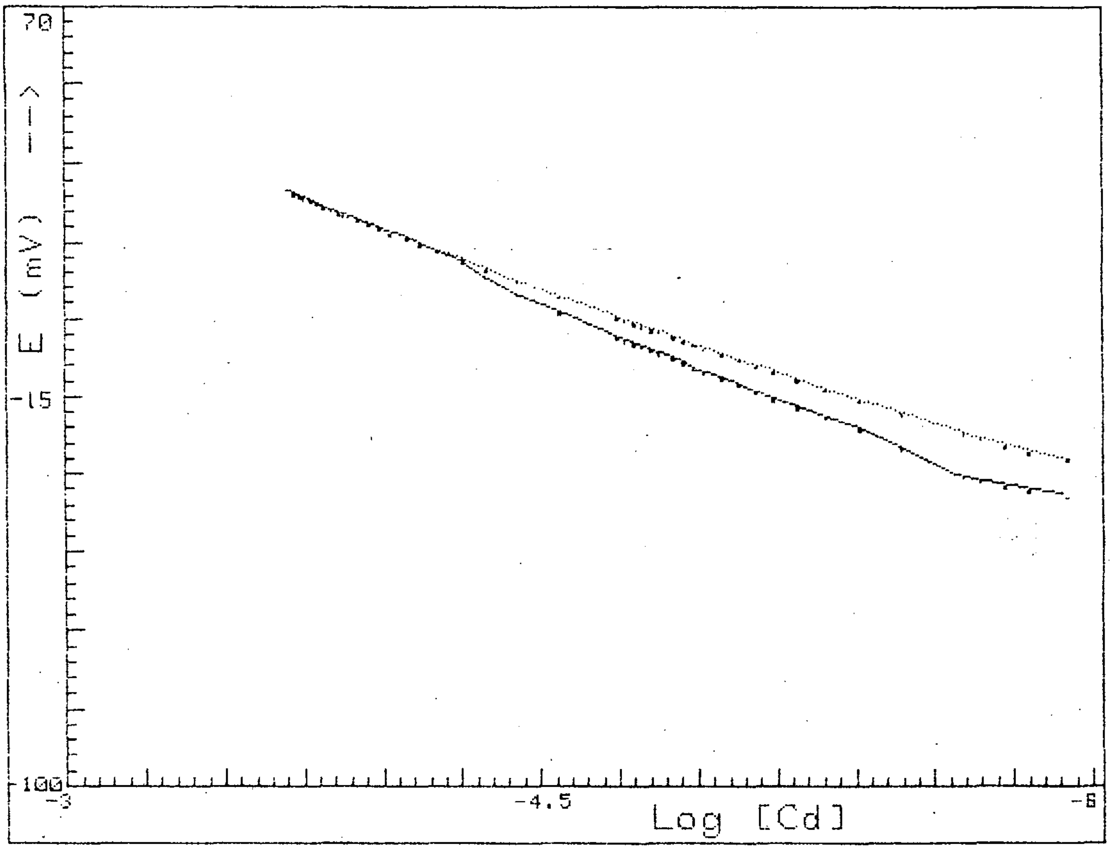

Fig. 6 (b)

Fig. 6 ISE response curves for Cd (II) - HA interactions at 25^{o}C for HAO (Ionic strength 0.1 M; p = 29.20). (a) pH = 3 (b) pH = 5.

.... blank
---- in presence of HA

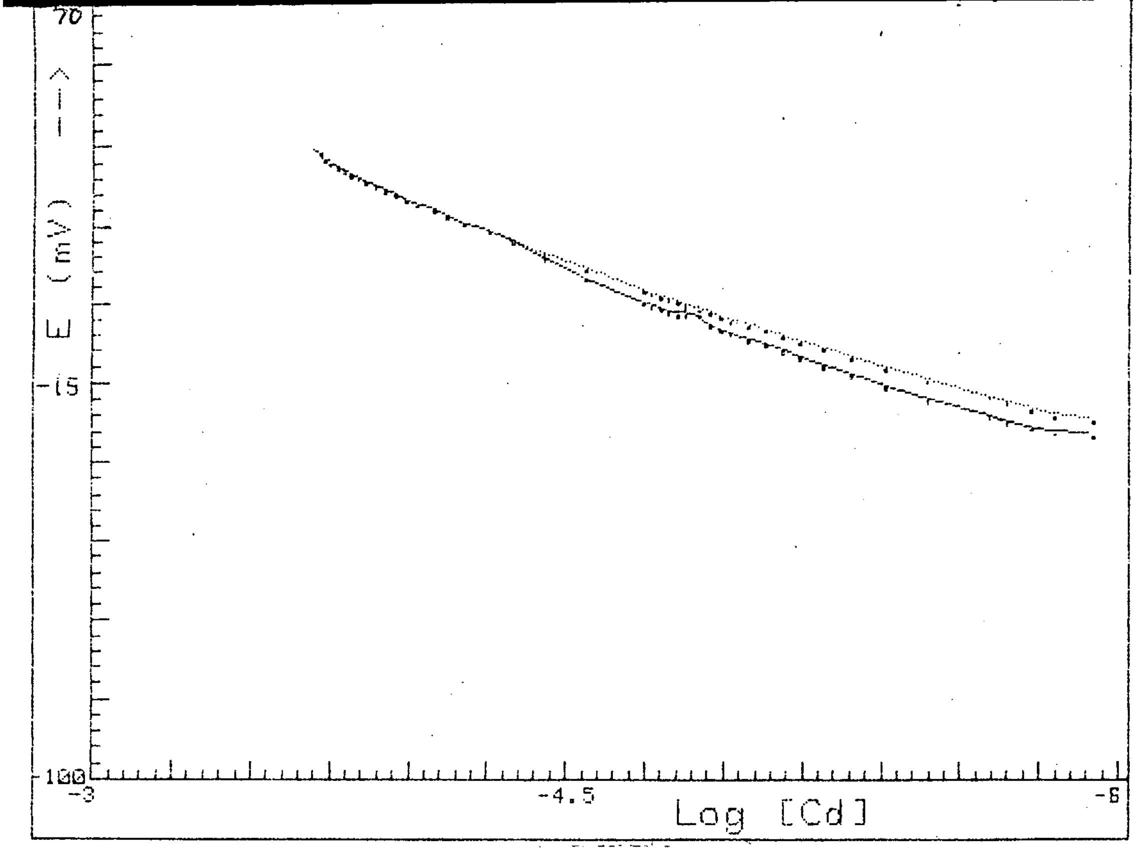

Fig. 7(a)

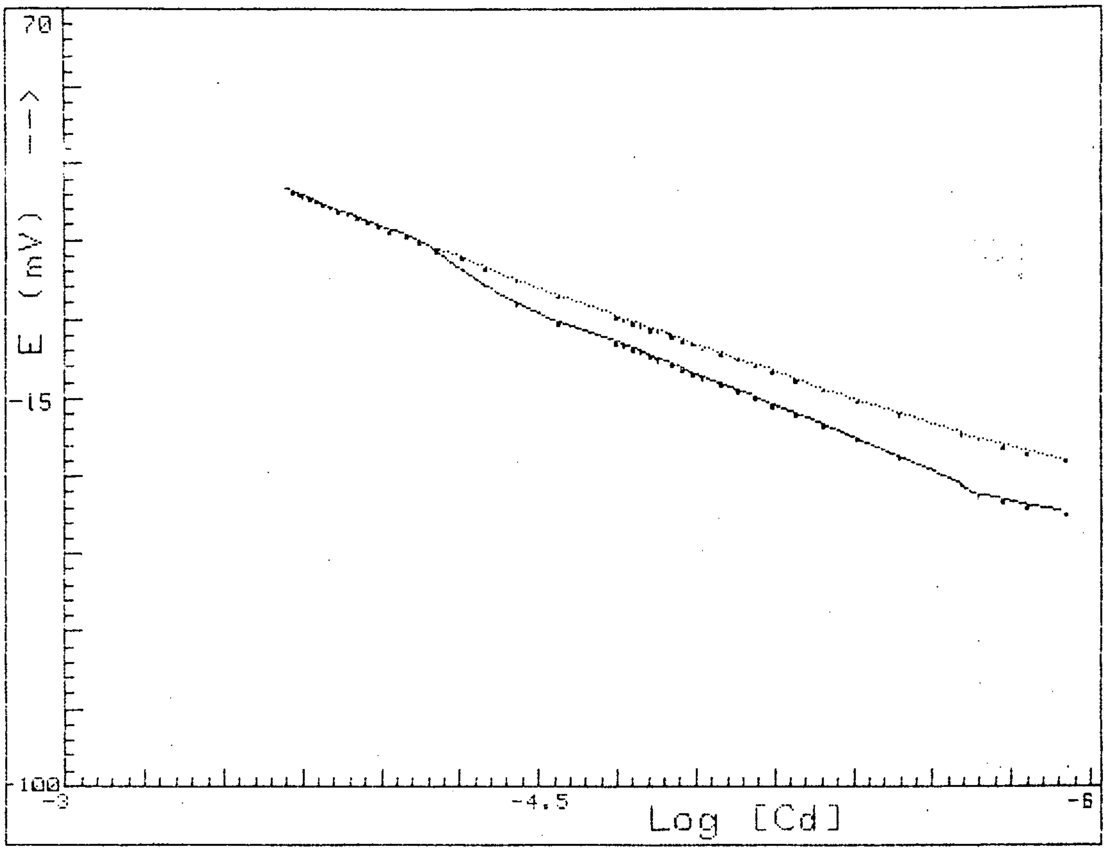

Fig. 7 (b)

Fig. 7 ISE response curves for Cd (II) - HA interactions at 25^{O}C for HAY (Inoic strength 0.1 M; p = 29.20). (a) pH = 3 (b) pH = 5.

.... blank

---- in presence of HA

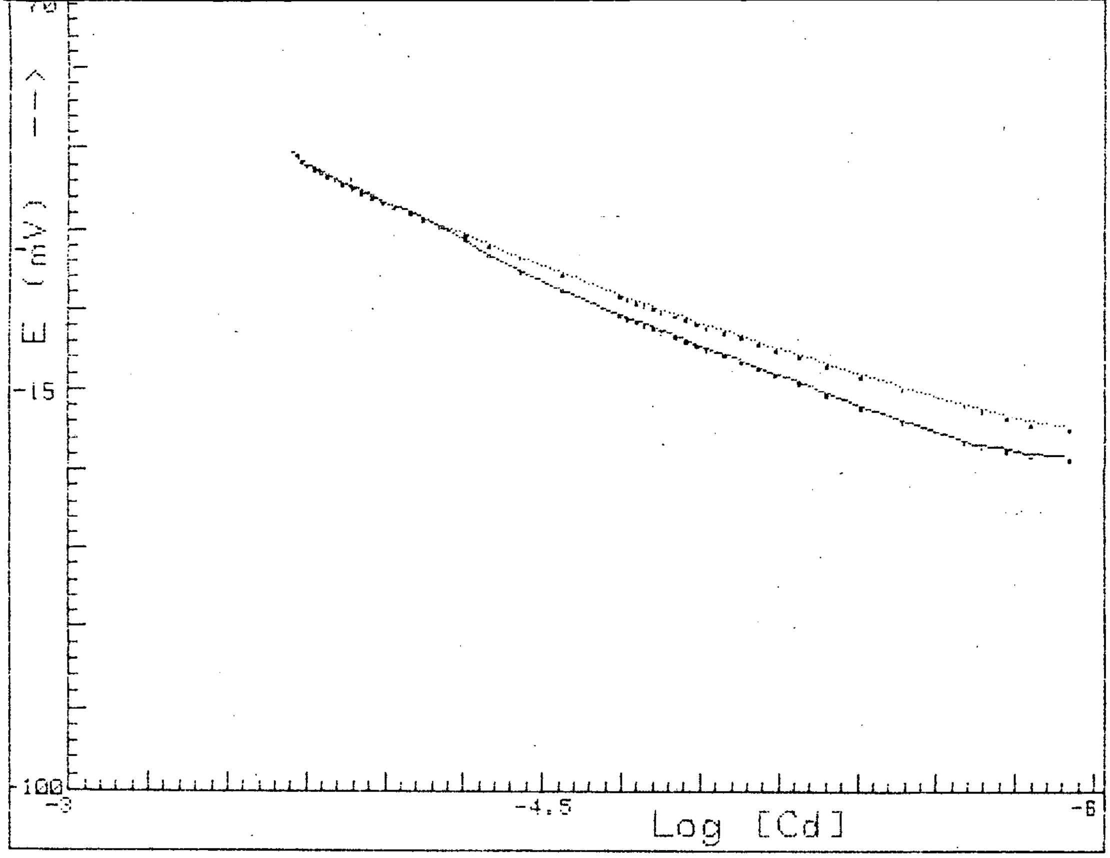

Fig. 8 (a)

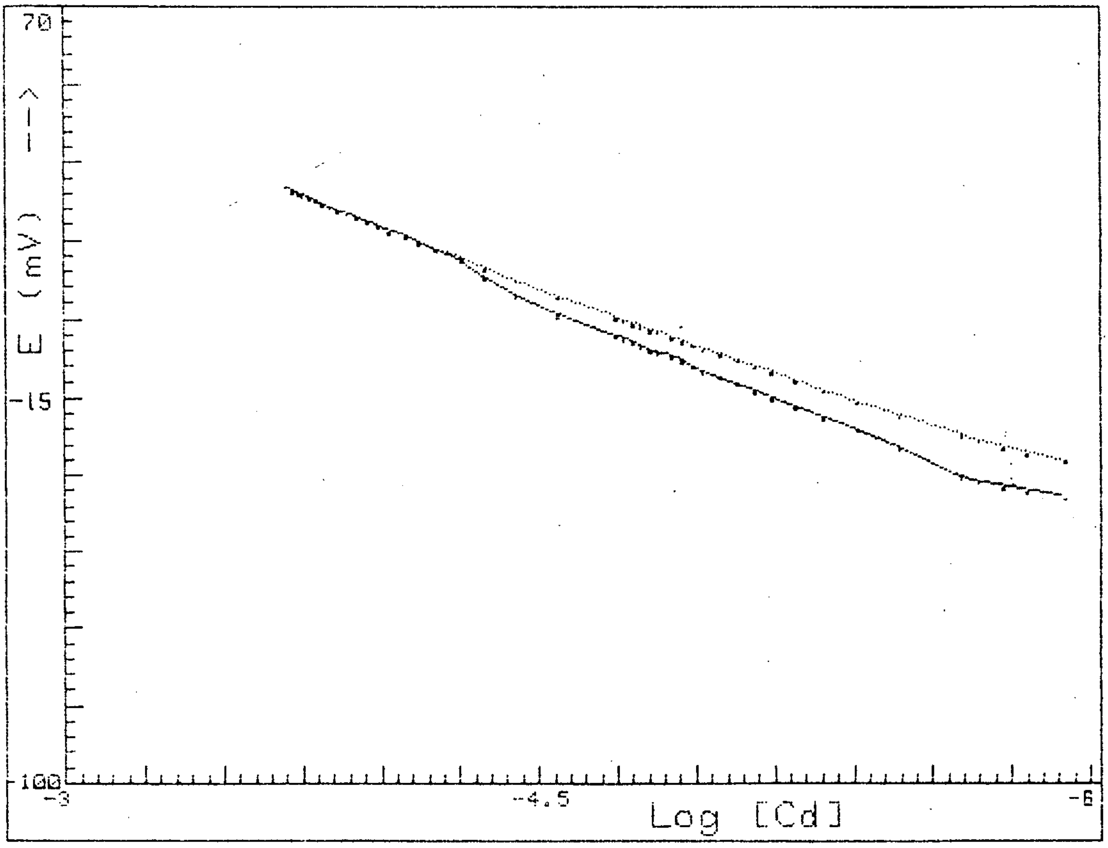

Fig. 8 (b)

Fig. 8 ISE response curves for Cd (II) - HA interactions at 25°C for HAW (Inoic strength 0.1 M; p = 29.20).
(a) pH = 3 (b) pH = 5.

.... blank
---- in presence of HA

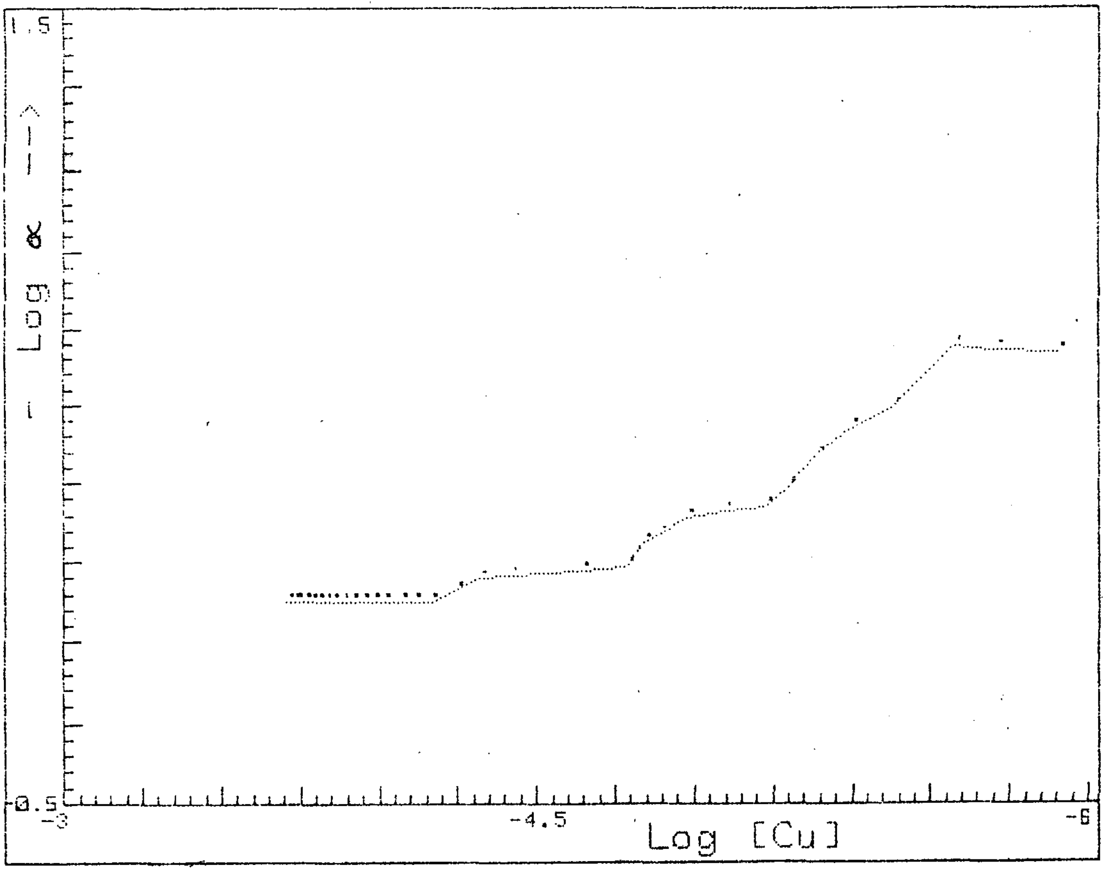

Fig. 9 (a)

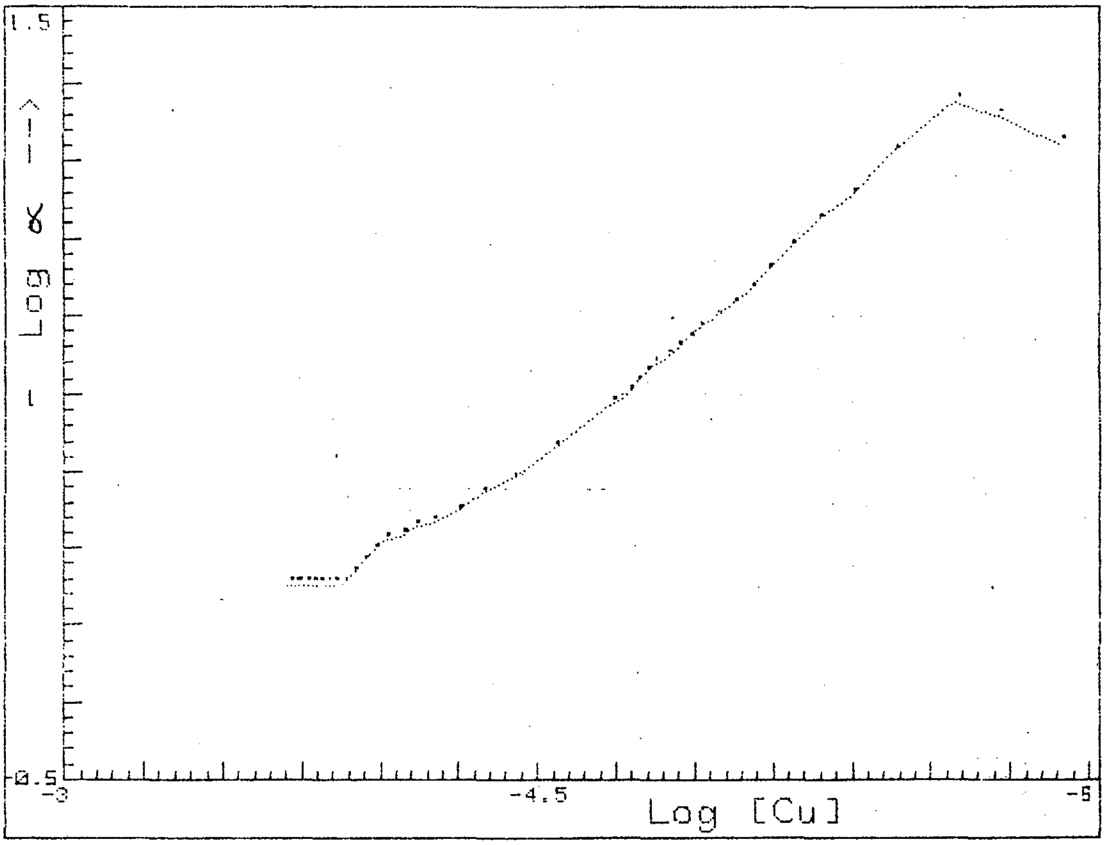

Fig. 9 (b)

Fig. 9 Log α Vs. Log $[Cu]_t$ for HAO. (a) pH = 3 (b) pH = 5

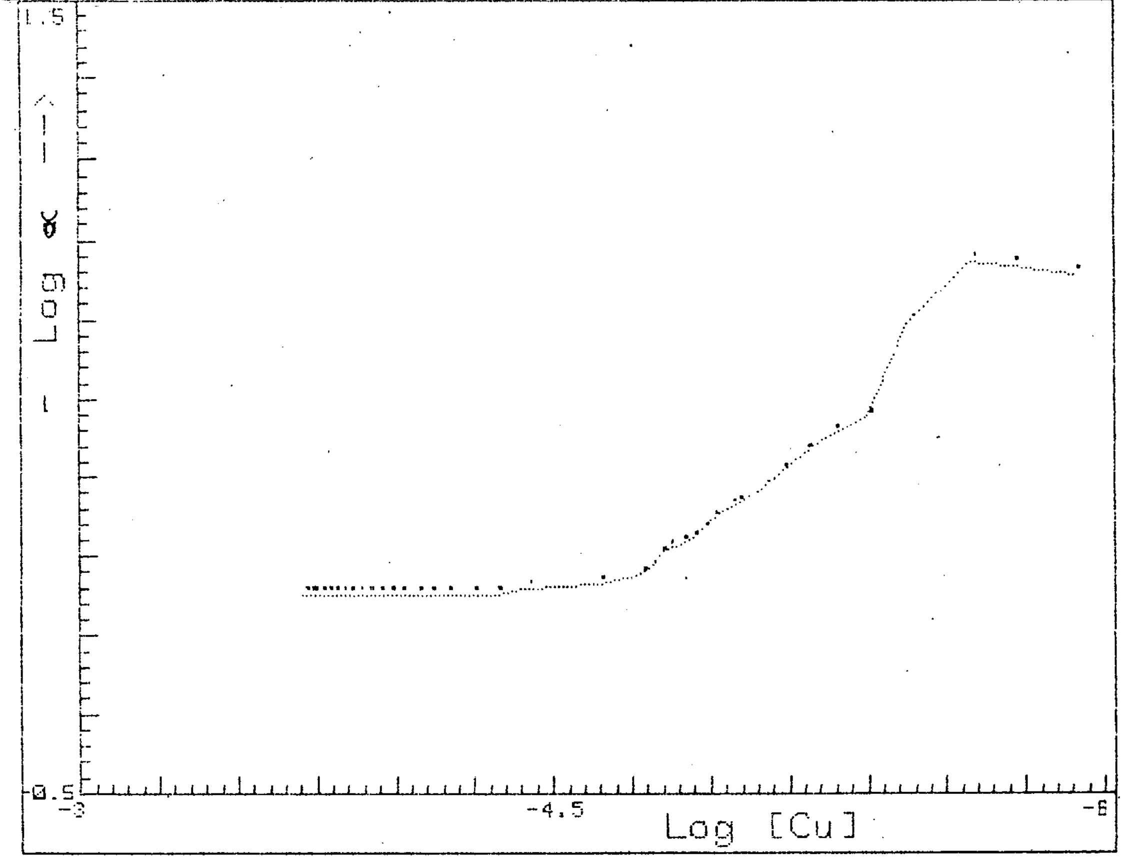

Fig. 10 (a)

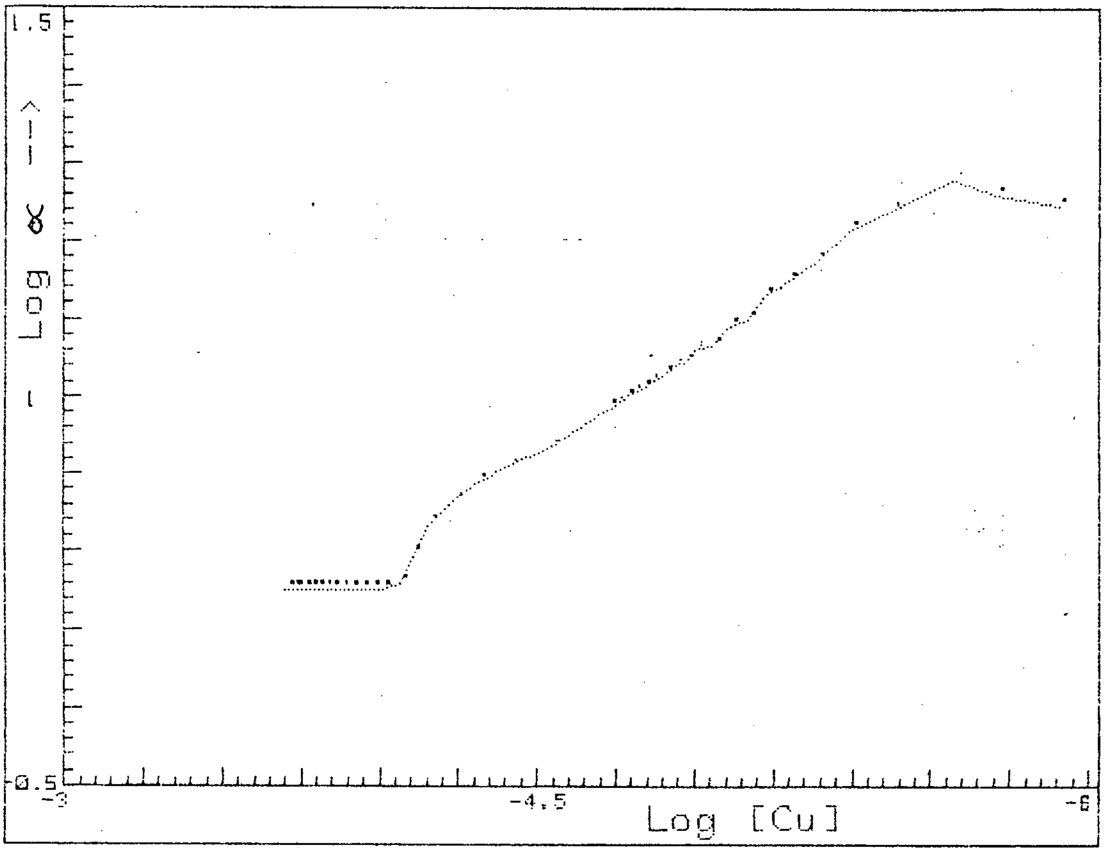

Fig. 10 (b)

Fig. 10 Log α Vs. Log $[Cu]_t$ for HAY.
(a) pH = 3 (b) pH = 5.

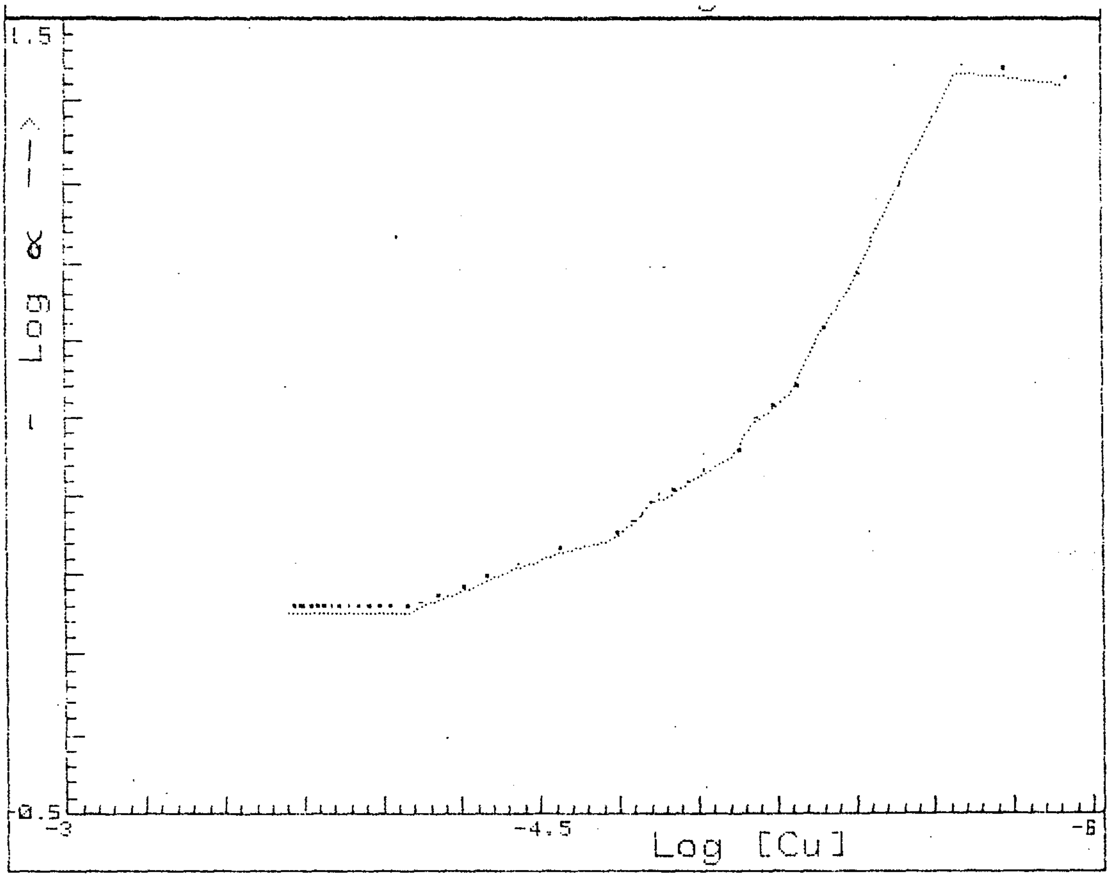

Fig. 11 (a)

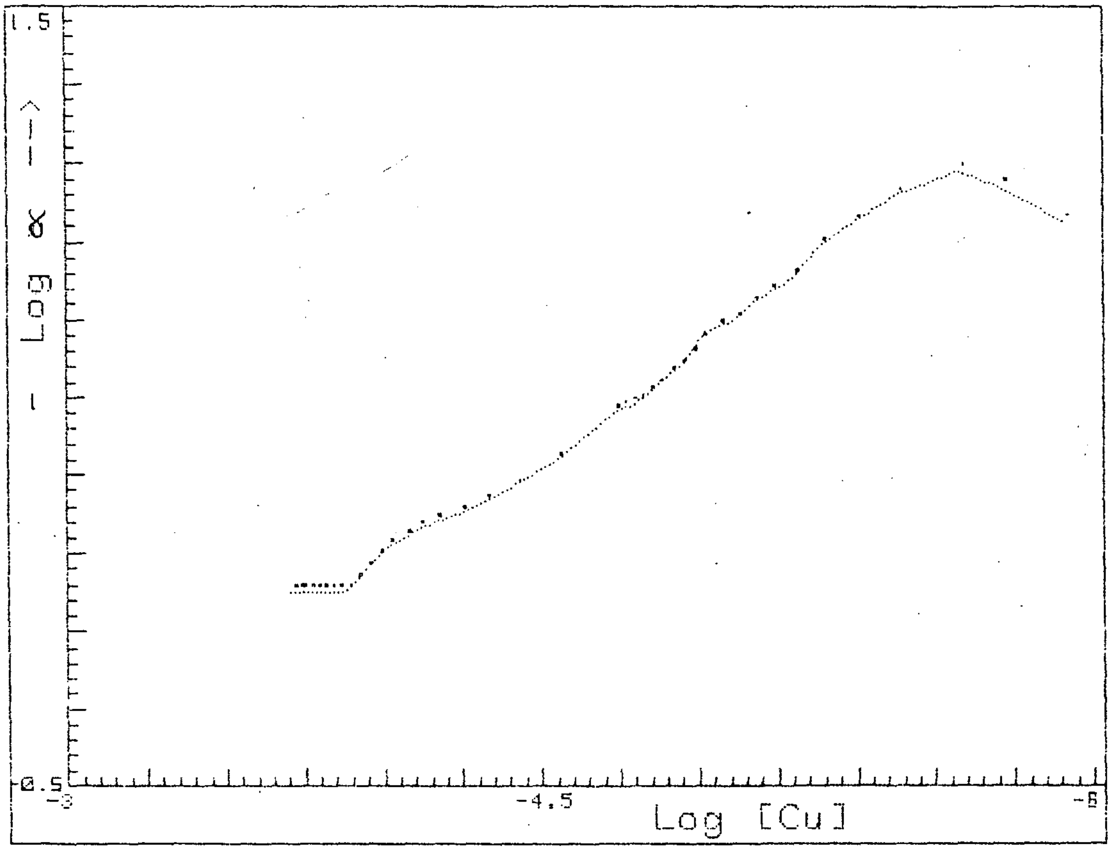

Fig. 11 (b)

Fig. 11 Log α Vs. Log $[Cu]_t$ for HAW.
(a) pH = 3 (b) pH = 5

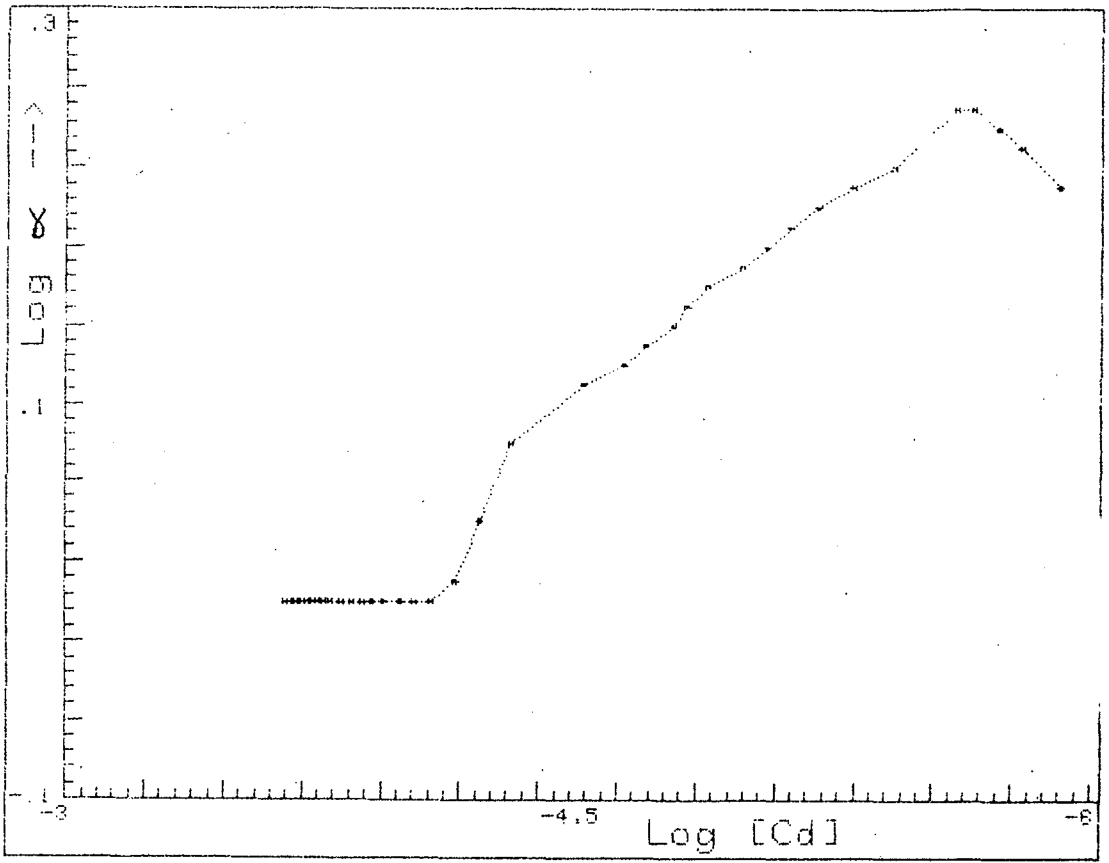

Fig. 12 (a)

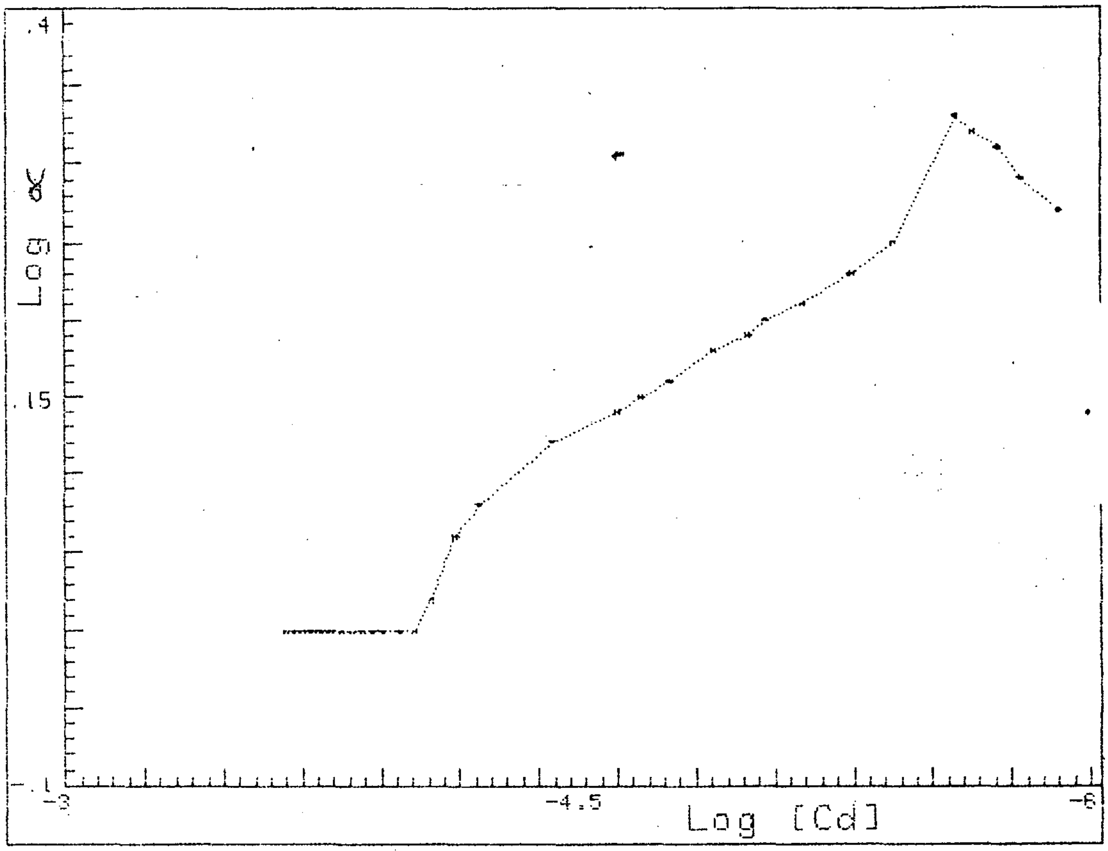

Fig. 12 (b)

Fig. 12 Log α Vs. Log $[Cd]_t$ for HAO.
(a) pH = 3 (b) pH = 5.

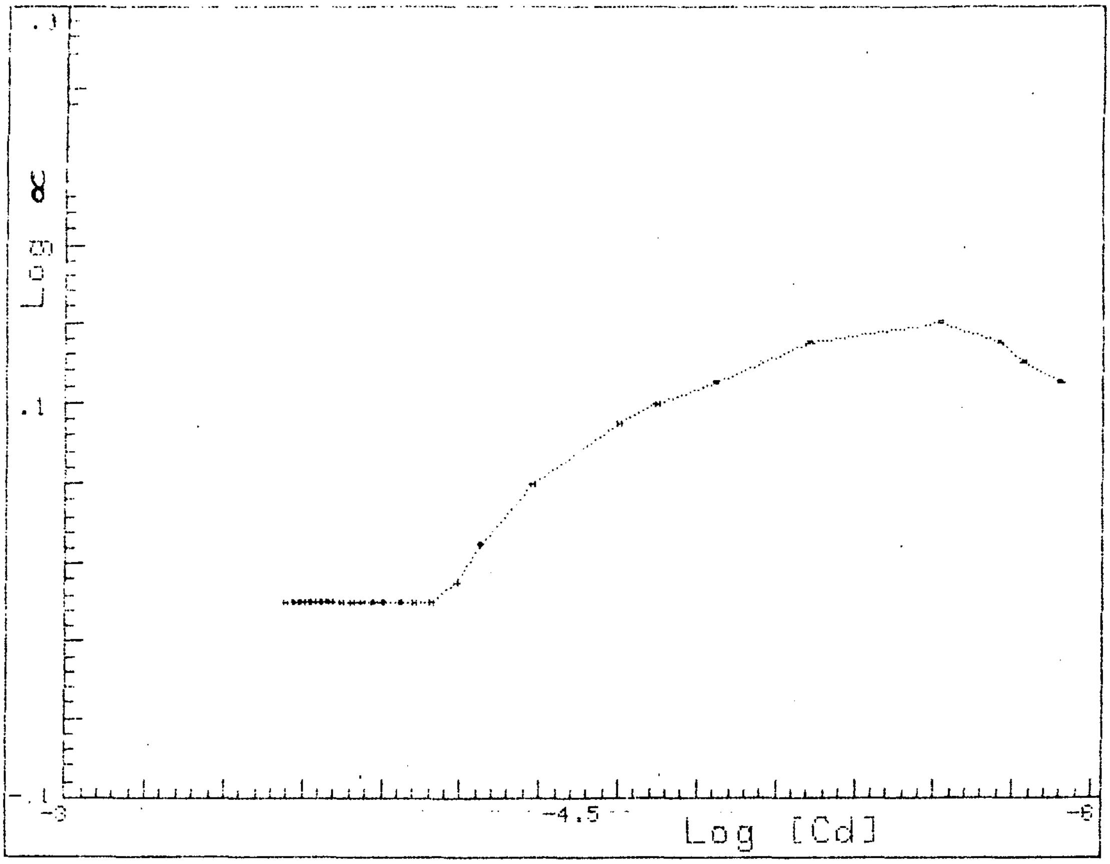

Fig. 13 (a)

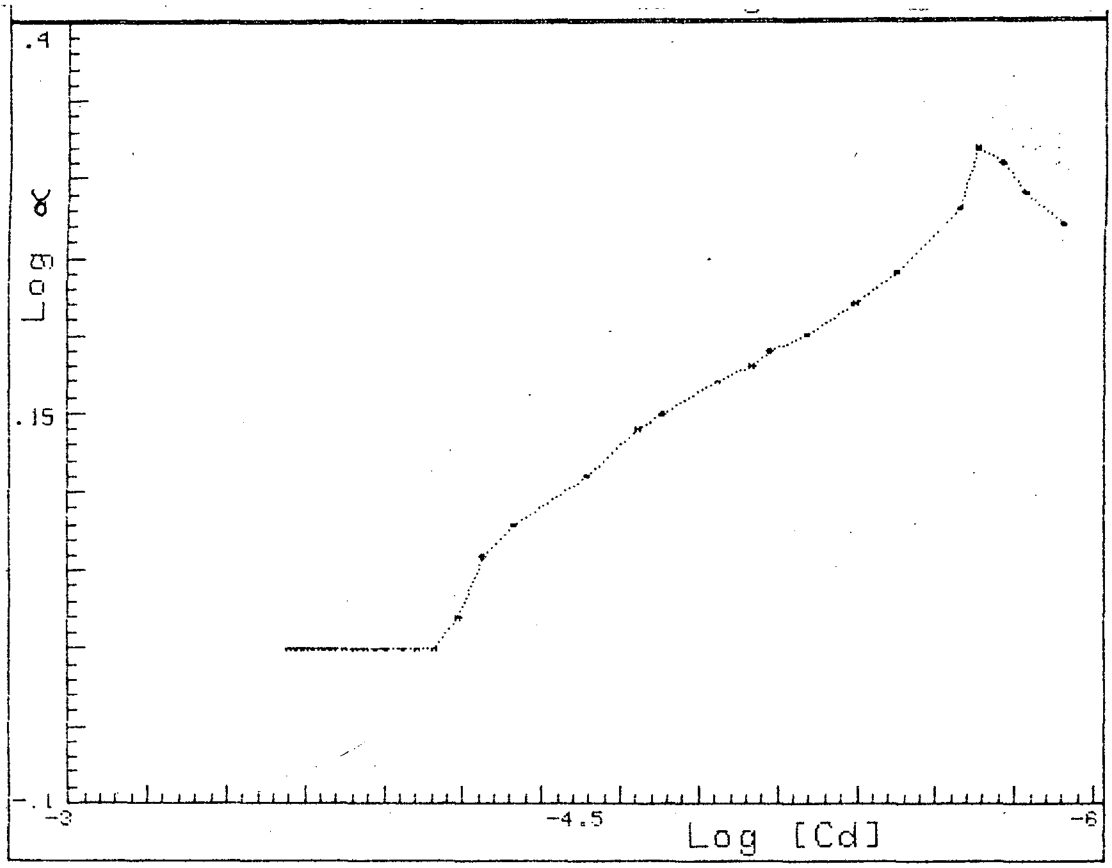

Fig. 13 (b)

Fig. 13 Log α Vs. Log $[Cd]_t$ for HAY.
(a) pH = 3 (b) pH = 5.

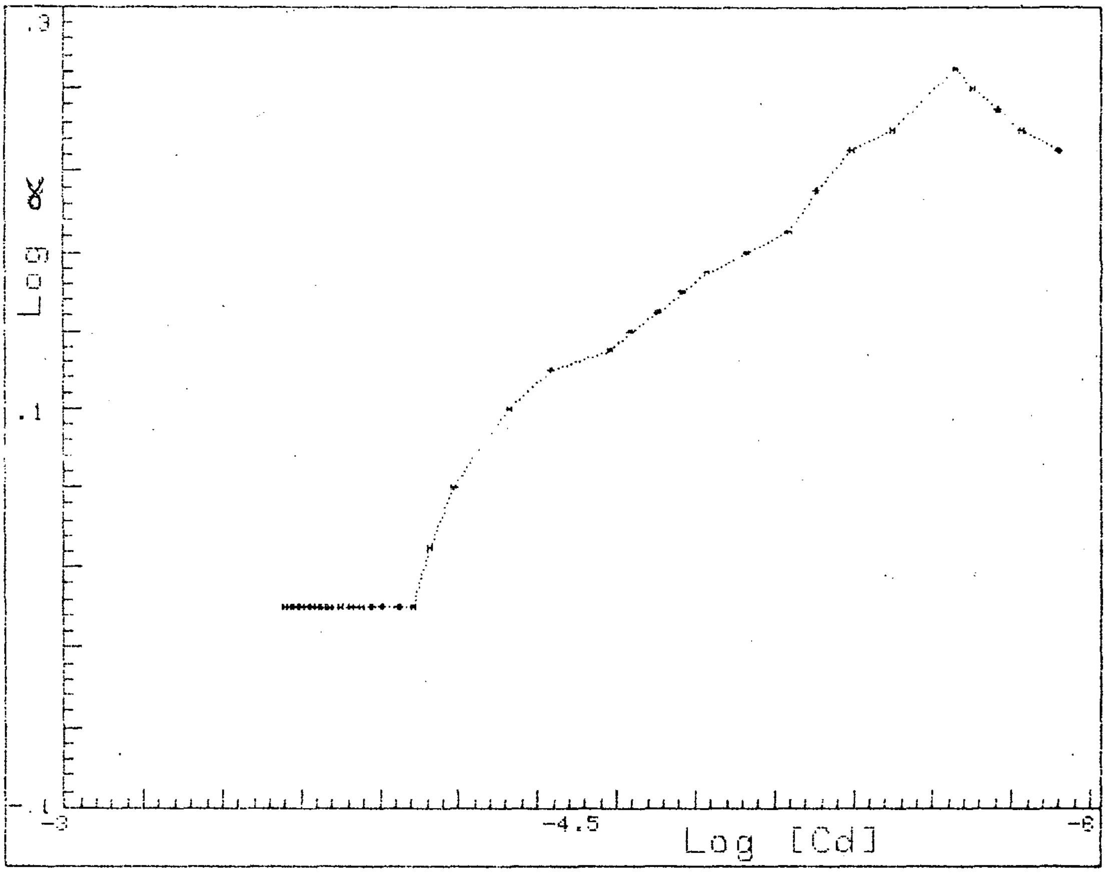

Fig. 14 (a)

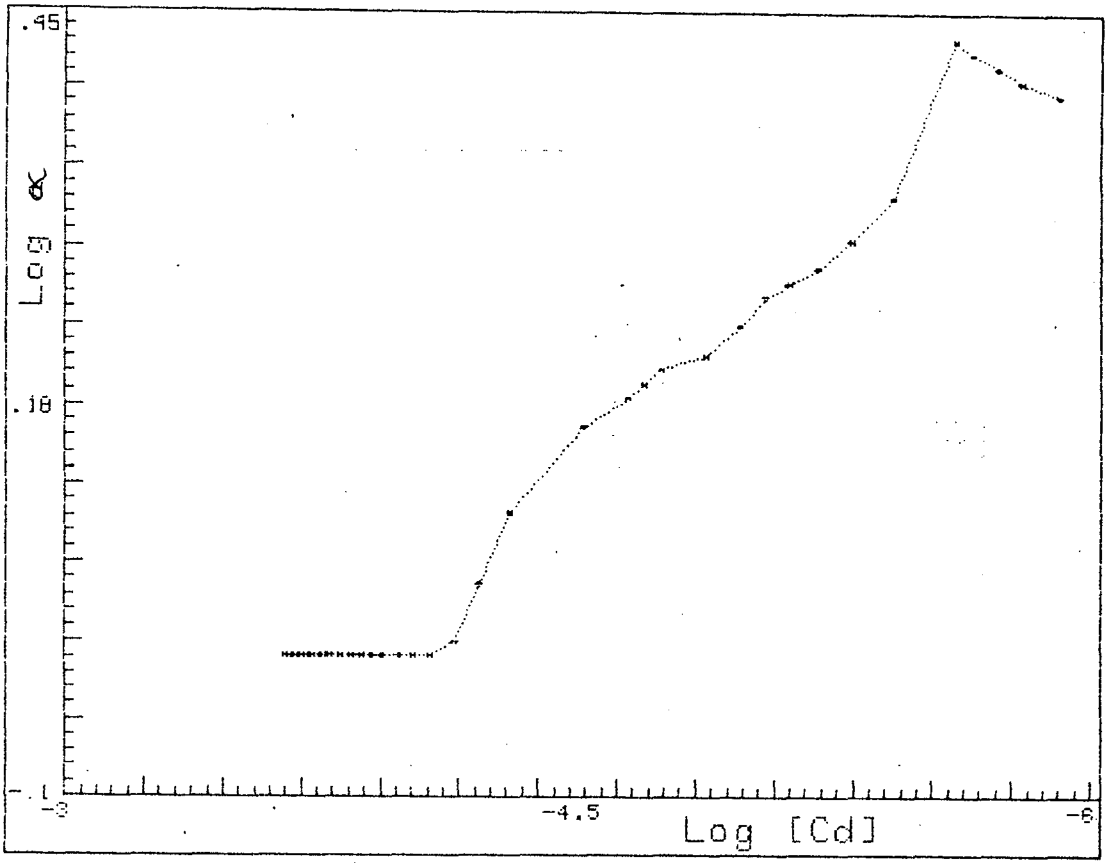

Fig. 14 (b)

Fig. 14 Log α Vs. Log $[Cd]_t$ for HAW
(a) pH = 3 (b) pH = 5.

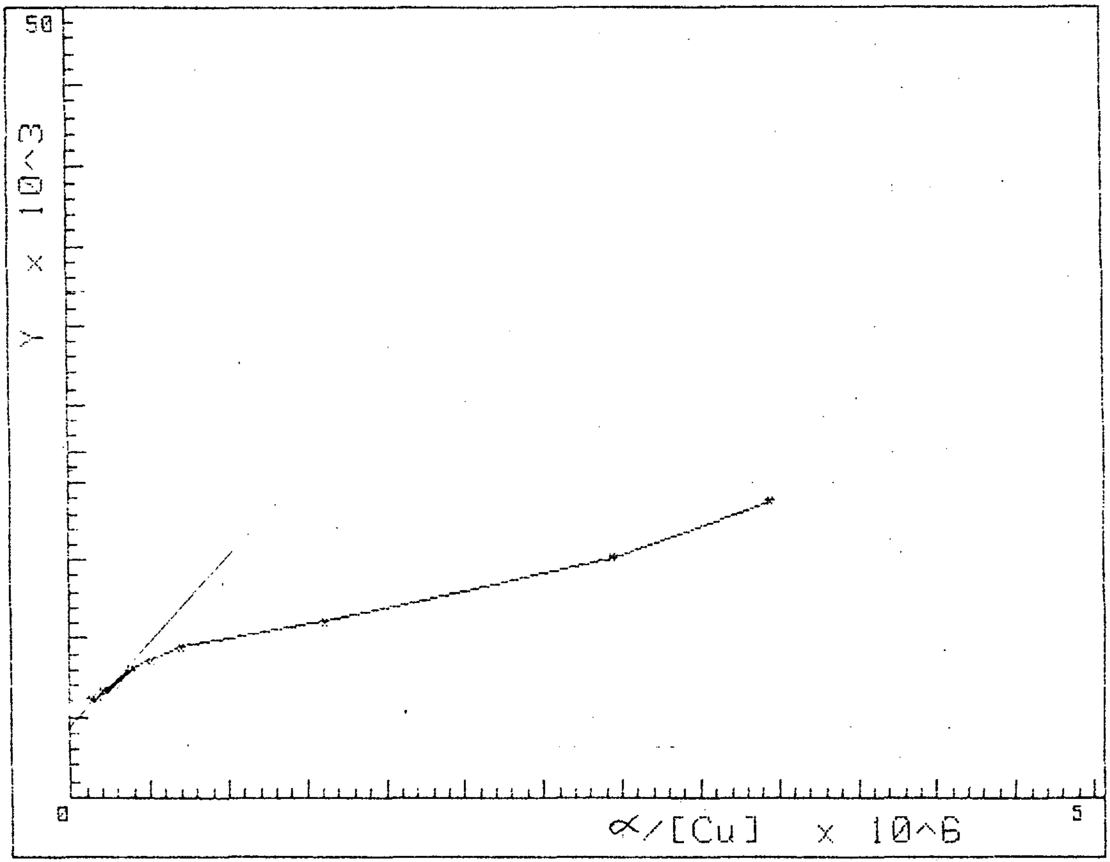

Fig. 15 (a)

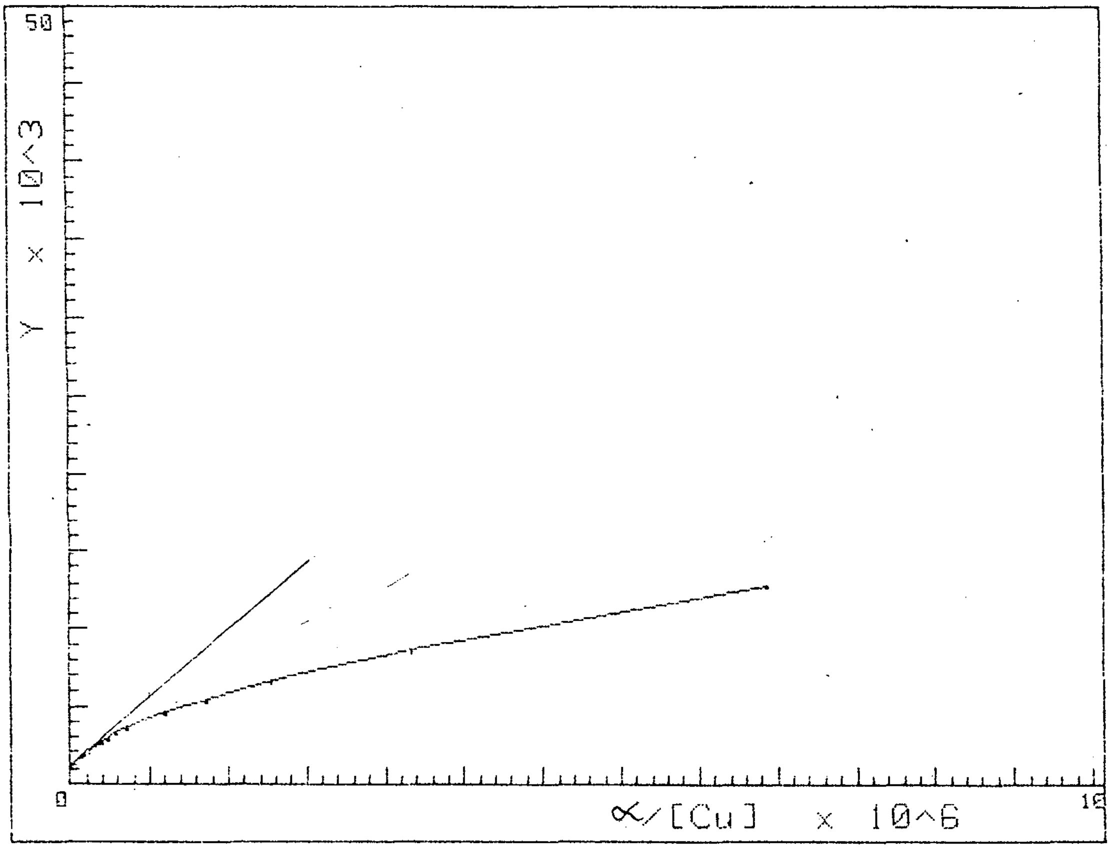

Fig 15 (b)

Fig. 15 Y Vs. $\alpha/[Cu]_t$ for HAO

(a) pH = 3 (b) pH = 5.

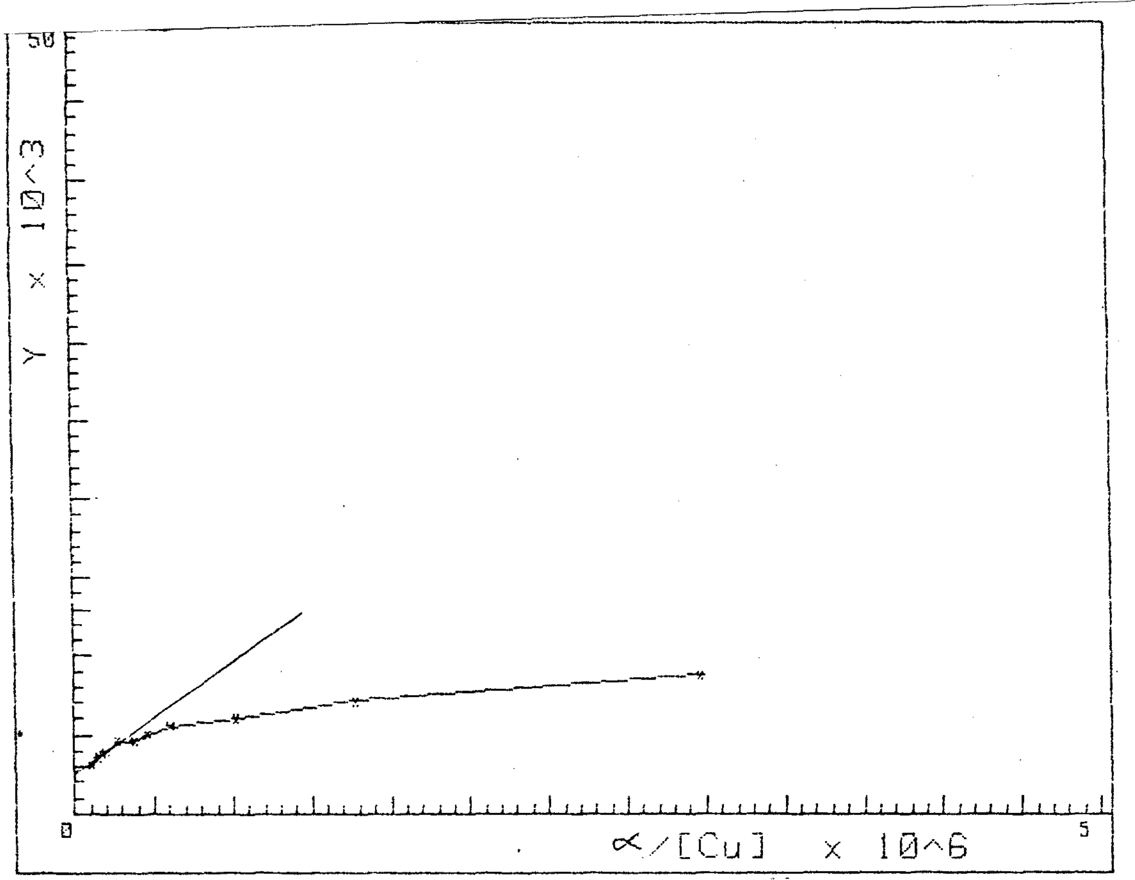

Fig. 16 (a)

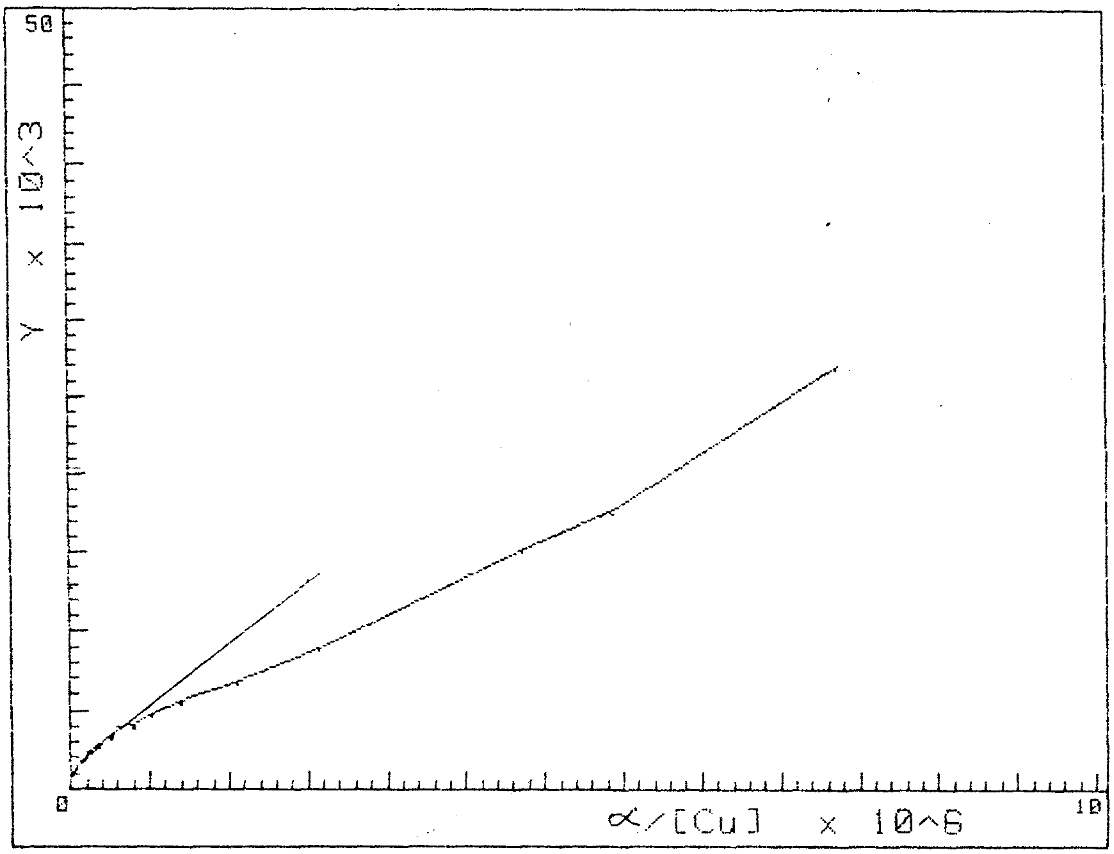

Fig. 16 (b)

Fig. 16 Y Vs. $\alpha/[Cu]_t$ for HAY
(a) pH = 3 (b) pH = 5.

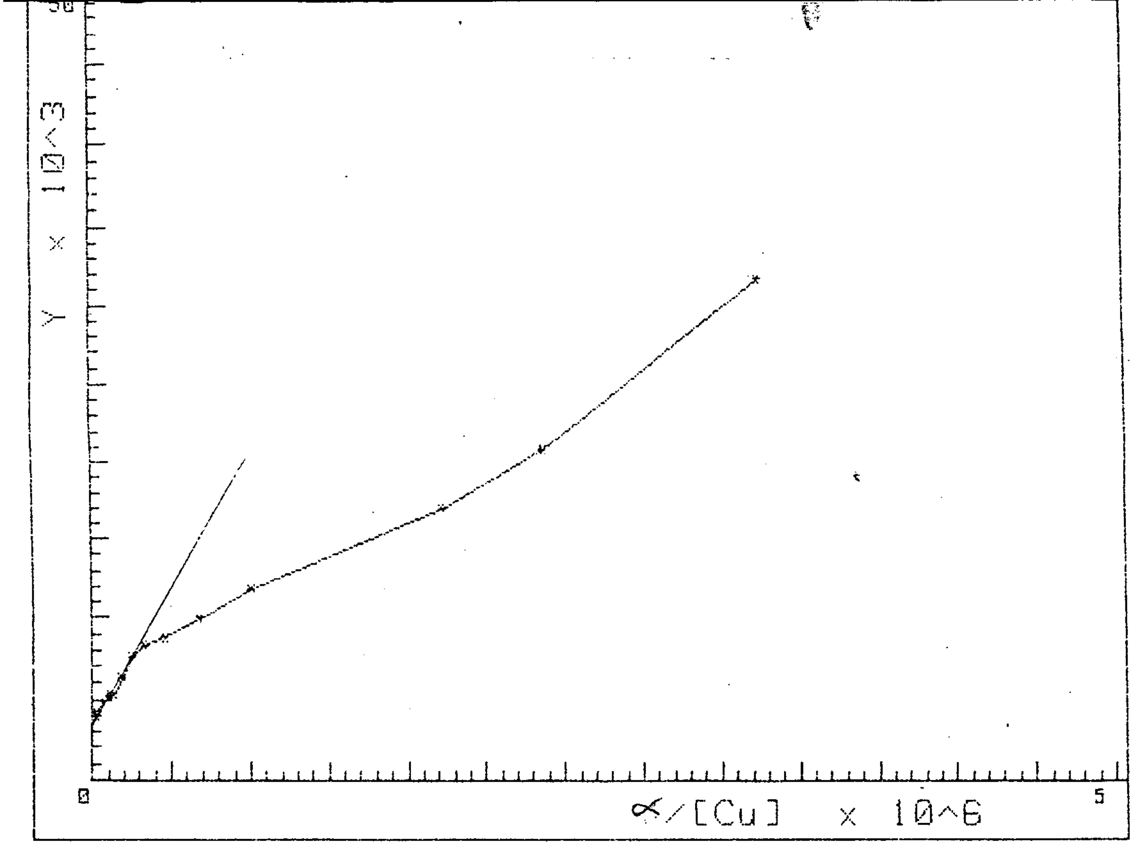

Fig. 17 (a)

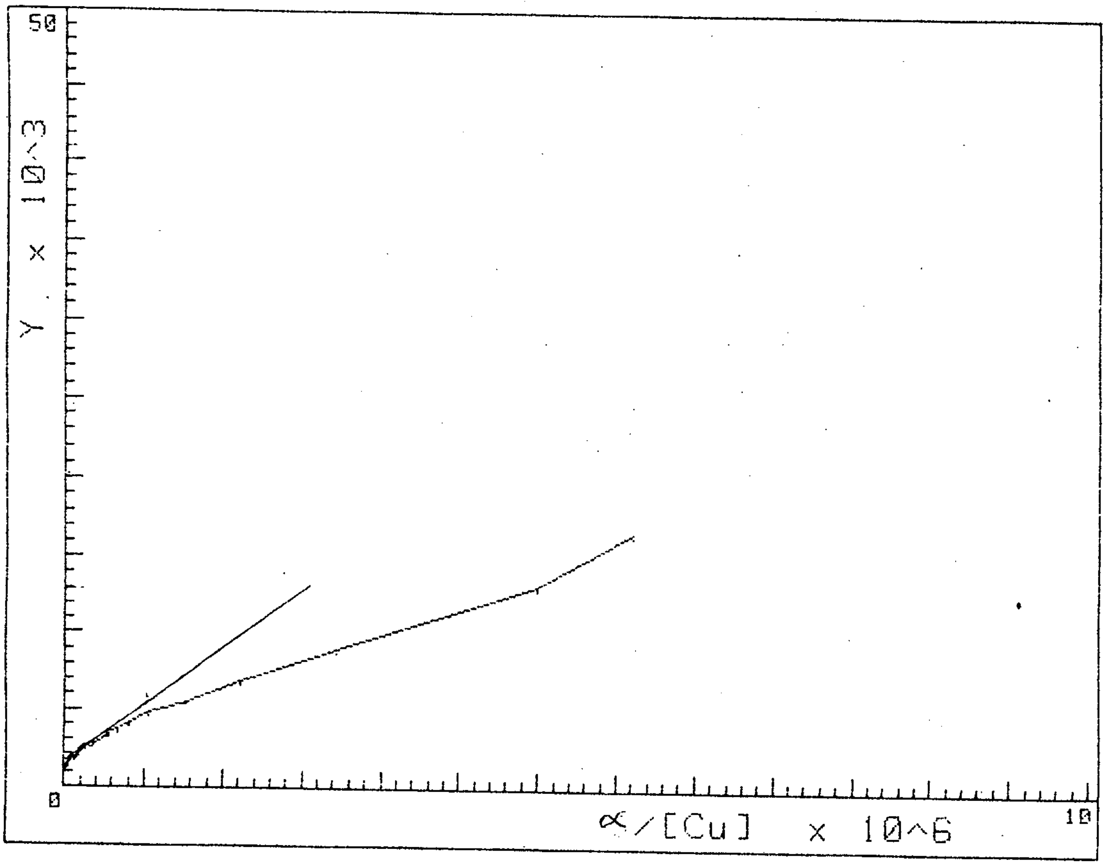

Fig. 17 (b)

Fig. 17 Y Vs. $\alpha/[Cu]_t$ for HAO
(a) pH = 3 (b) pH = 5.

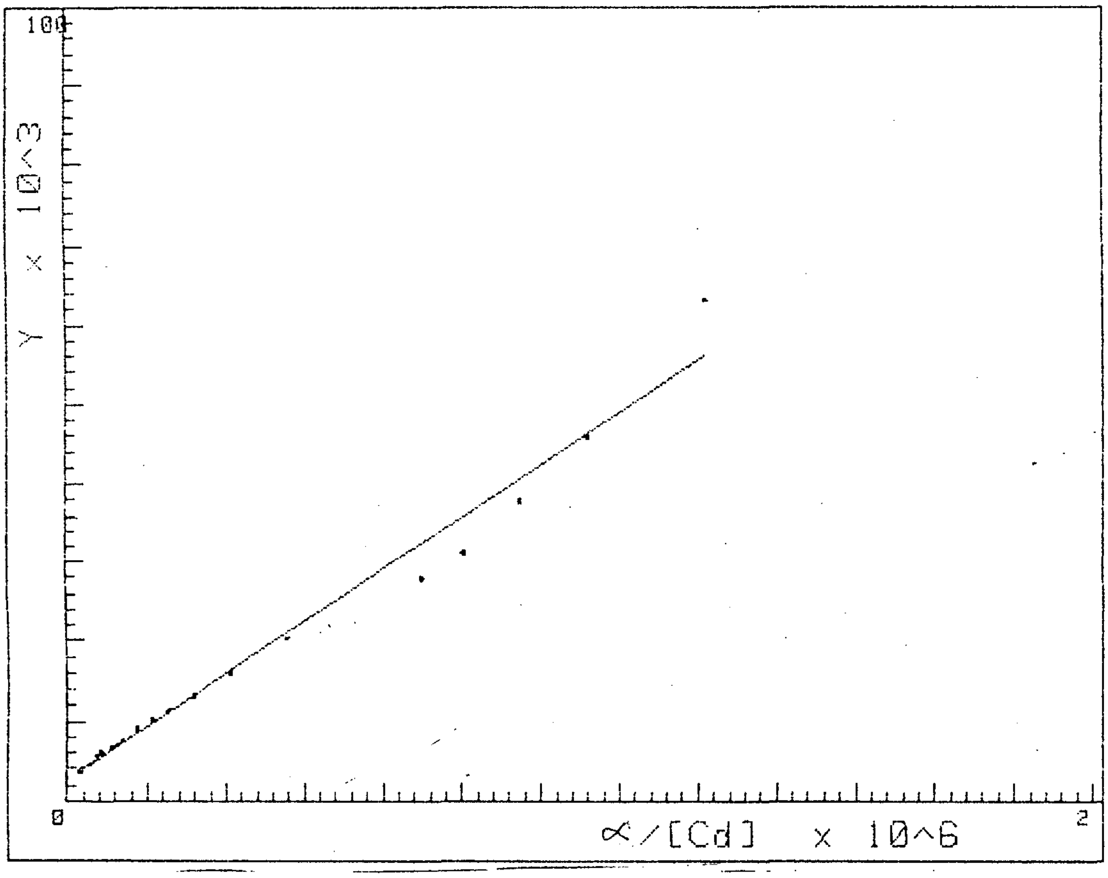

Fig. 18 (a)

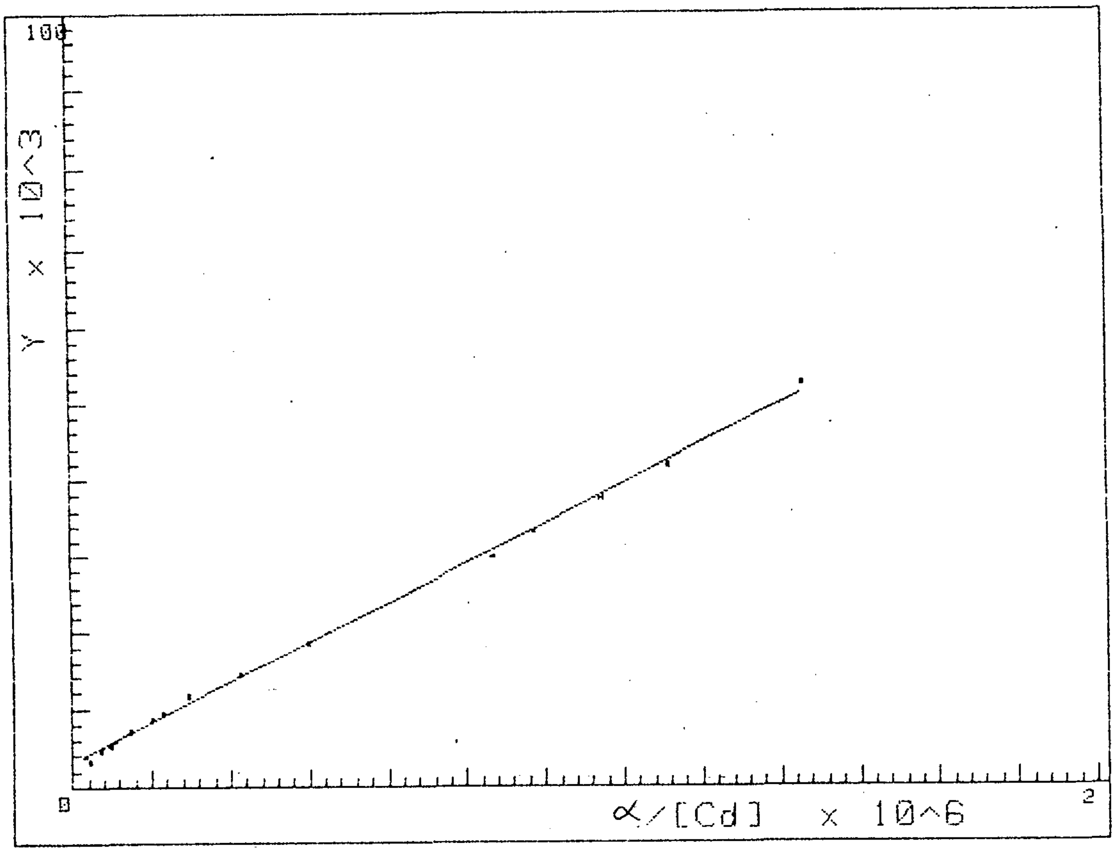

Fig. 18 (b)

Fig. 18 Y Vs. $\alpha/[Cd]_t$ for HAO

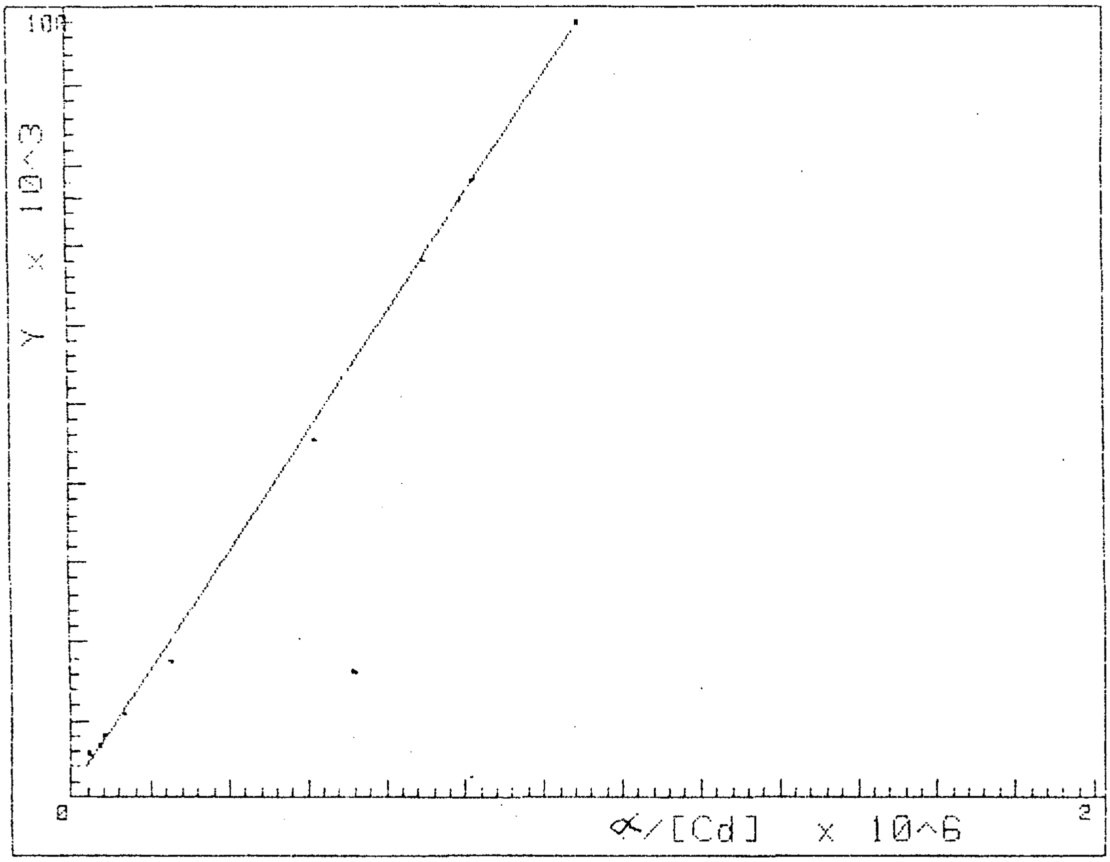

Fig. 19 (a)

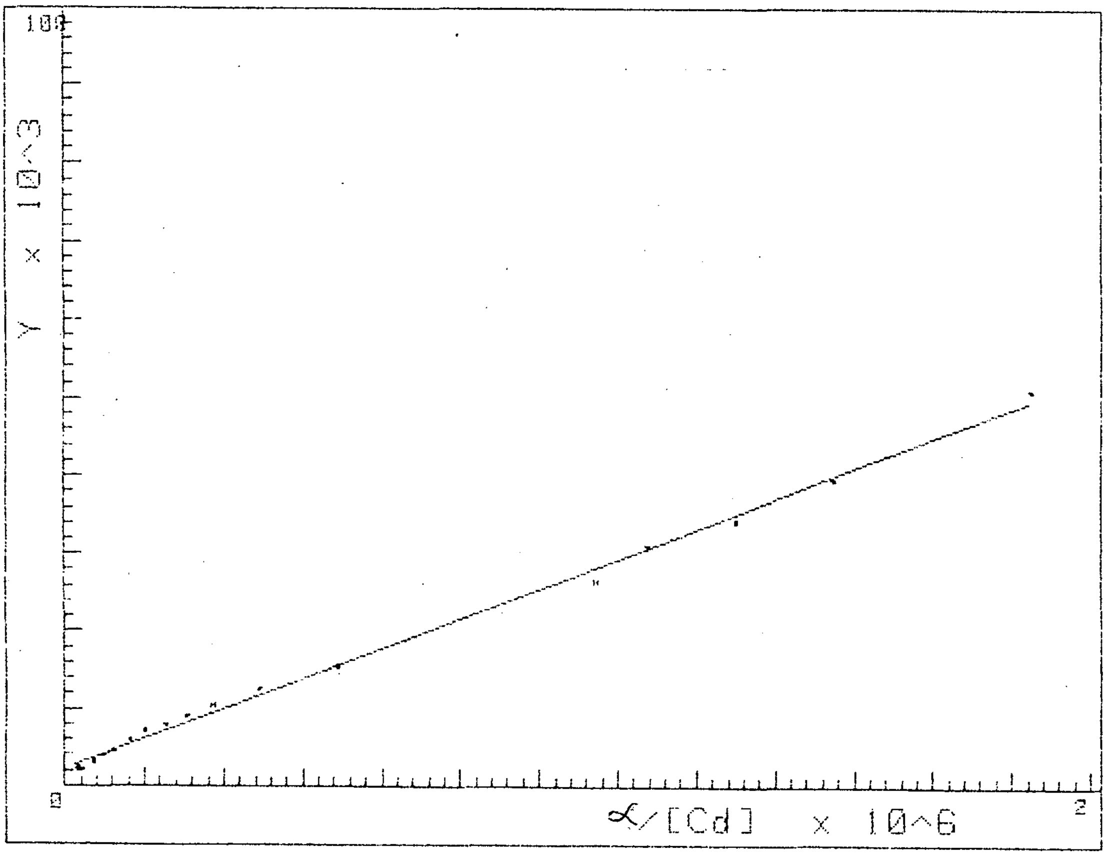

Fig. 19 (b)

Fig. 19 Y. Vs. $\alpha/[Cd]_t$ for HAY

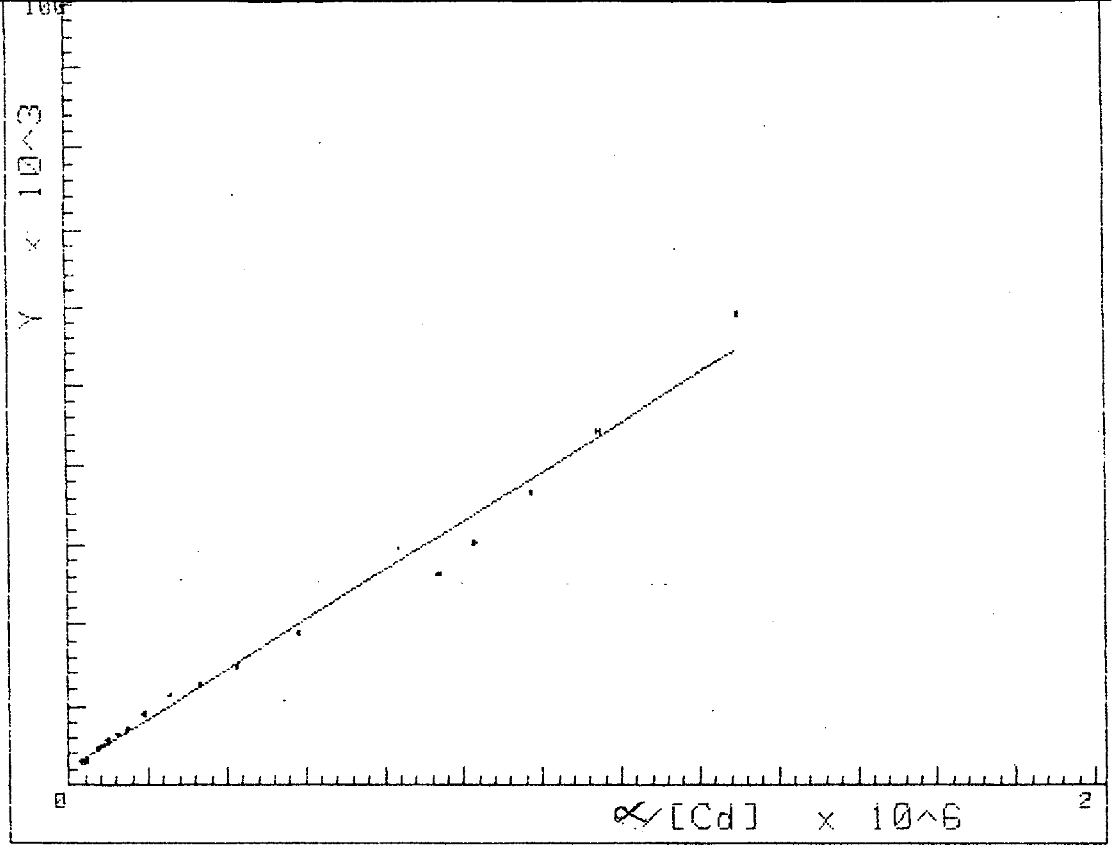

Fig. 20 (a)

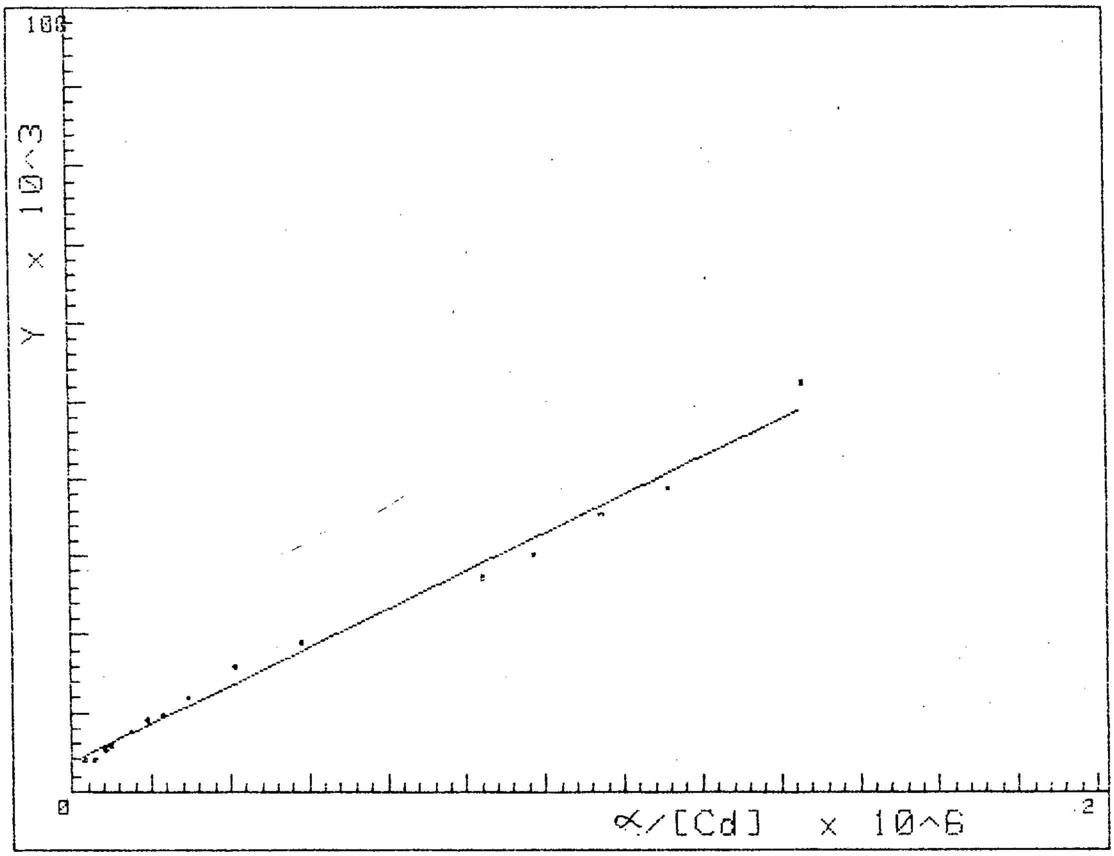

Fig. 20 (b)

Fig. 20 Y. Vs. $\alpha/[Cd]_t$ for HAW

4 (log concentration). The minimum saturation value for Cu was for HAY at pH3 and maximum for HAO at pH5, the log concentrations being -4.06 and -3.77 respectively. In the case of Cd the minimum value was for HAY at pH3 and maximum HAY at pH5, the values of log concentration being -4.20 and -4.01 respectively.

By using the values of p (slope of electrode) the corresponding degree of complexation can be computed using the equation.

$$\alpha = [Me]t \times 10^{(Eo - E)p}$$

Where L is the degree of complexation [Me]t is the molar concentration of metal added Eo is the standard electrode potential and E is the electrode response of the metal with HA. The plot of log α against log concentration showed a definite trend ie. first an increase and then a steady fall to a constant value O, which is shown in fig 9 - 14 (appendix tables 13 - 24). Fig 15 - 20 (appendix table 24 - 36) demonstrates the variation of Y with the reciprocal of concentration of the uncomplexed metal ion.

$$Y = \frac{[L]_t}{[Me]_t} \frac{\alpha}{\alpha - 1} = \frac{Mw}{V} + \frac{Mw}{V} \frac{[H]^x}{B^*} \frac{\alpha}{[Me]_t}$$

where $[L]_t$ is the total concentration of the organic matter (in g/L) and V=1 for 1:1 complex, Mw is the molecular weight and $B^*/[H]^x$ is the conditional stability constant. From the slop ($Mw/V \cdot [H]^x/B^*$) and intercept (Mw/V) the value for $B^*/[H]^x$ was found out which is the conditional stability constant(k). These values for Cu and Cd at pH 3 and 5 are given in table 13.

In the case of Cu the plot of Y with the reciprocal of concentration of the uncomplexed metal ions did not follow a straight line instead a bend was observed when it neared the y-axis. Hence the slope of the tangent was taken for the calculation of $B_i^*/[H]^x$. This bend was observed probably due to the formation of 1:2 complexes in the case of Cu (Buffle etal 1977).

In the case of Cd a near straight line was observed and using a computer programme a best fit was made and the slope and intercept were calculated in order to compute the value of K. The formation of a straight line indicates that Cd-HA complexes more less follow the pattern of 1:1 complex formation (Buffle et al 1977).

As the information on the molecular charecteristics of HA which varies with different factors are scare, conditional

TABLE - 12

STABILITY CONSTANTS (K) OF Cu AND Cd WITH HA AT pH 3 AND 5.

SAMPLE	pH	K	
		Cu	Cd
HAO	3	5.26	4.77
	5	5.44	4.96
HAY	3	5.45	4.11
	5	5.48	4.96
HAW	3	5.44	4.73
	5	5.61	5.07

stability constants are used frequently in order to explain different complexation characters of the substance. ISE method is used for the present study although several other methods have been developed (Zunino et al 1975; Buffle et al,1977 and Stevenson et al,1977).

The stability constant (K) values for Cu-HA complexes at pH 3 were found to be 5.26, 5.45 and 5.44 for HAO HAY and HAZ respectively. At pH 5 the respective values were 5.44, 5.48 and 5.61. The K values for Cd - HA complexes at pH 3 were 4.77, 4.11 and 4.73 and at pH 5 4.95, 4.96 and 5.07 respectively for HAO, HAY and HAW. In all these cases it can be seen that the stability constant of Cu HA complexes were considerably higher than that of Cd-HA complexes. This agrees with the Irving Williams order (Irving and Williams 1948) which gives a higher K value for copper. Rainbow (1985) also reports that Cu shows a higher borderline characters than Cd which is indicative of its higher affinity with organic compounds. A different order to that of Irving - Withains series was observed by Schnitzer and Skiner (1966,1967), Takamatsu and Yoshida (1978).

The K values for different samples determined at different pH levels, given in Table 12, indicates an increase at higher pH in the case of the same sample. Similar observations were reported by many workers (Schnitzer and Skinner, 1967; Saar and Weber, 1979; Randhawa and Broadbent, 1965; Tan et al 1971). In the case of FA, Schnitzer and Skinner (1967) explains this phenomenon as due to the increased ionization of functional groups especially of the carboxyls with increase in pH.

Stevenson (1976) determined the stability constants of Cu^{2+} and Cd^{2+} at pH 4 and the apparent stability constant obtained for HA were 8.9 for Cu and 6.9 for Cd. The considerably lower value observed in the present study may be ascribed to the difference in the origin of HA studied.

The K of metal - organic complexes do not provide any information on the number of bonds formed between the metal and ligand. The magniude of the stability constants depend on the strength of the bond (Schnitzer and Skinner, 1966). These workers state that in metal organic complex formation two types of reactions are involved, the major one in which both carboxyl and phenolic hydroxyls participated and the minor one involving acidic carboxyls only. With the same metal ion, more than one functional group can involve in the process of binding. The same workers visualize a probable inverse relation between the

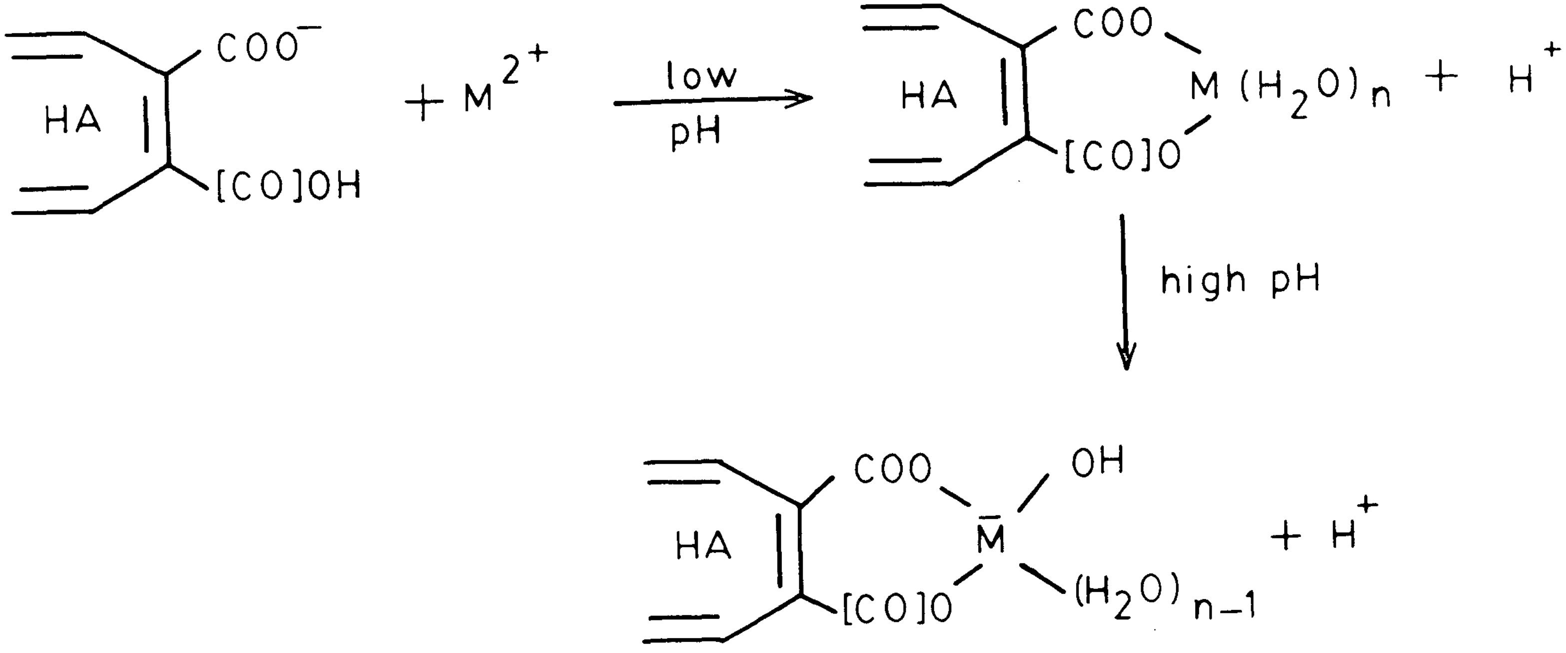

Fig. 21 General reaction leading to release of protons. Bracket means side groups may be phenolic OH or undissociated COOH.

number of functional groups and the stability constant in their study on the binding charecterstic of FA with Cu, pb and Fe. The bond formed between less number of group and a metal ion is supposed to show higher stability constant. The results of the present study also is suggested of a similar inference. Cu was always found to have higher stability constants.

The cupric ion is complexed strongly by HA and has been considered to involve chelation by o - hydroxy carboxylic acid and o - dicarboxylic acid moieties, the former being predominant (Schnitzer and Skinner, 1965; Gamble et al 1970; Van Dijk 1971 and Stevenson and Ardakni 1972). Alternatively the metal ions may complex with the same type of oxygen containing functional group without forming chelate ring (Boyd et al 1980). The general reaction during the process of chelation leading to a release of proton is given in Fig. 21. Stevenson (1976) observes that there is a relationship between titrable H^+ and apparent total binding capacity for Pb^{2+} which may be extended in the case of Cu also.

TABLE 13

Elementary composition of investigated humic material and ratios C/H and C/N.

Humic substance	Elementary Composition (%)				C/H	C/N
	C	H	N	O		
Okhla	50.46	6.53	2.54	40.72	7.72	19.86
Old Yamuna Bridge	52.89	5.89	2.61	38.54	8.99	20.29
Wazirabad	51.75	6.09	1.92	40.24	8.49	26.95

Elemental analysis of humic Acids

The results of the experimental analysis of **HAO, HAY and HAW** are presented in Table 13. The contents of carbon, hydrogen and nitrogen were determined experimentally, and the oxygen content was calculated as the difference from 100%. The C/H and C/N rates are also given in Table 13. As reported in an earlier study (Nissenbaum and Kaplan, 1972) the origin of a humic substance has an influence on its C/N ratio. It can also be noted from the table that the most abundant elements in HA are carbon and oxygen, while contents of hydrogen and nitrogen are relatively less. The carbon content is above 50% which agrees with the findings of Raspor et.al. (1984). According to Visser (1983) the average carbon content of HA of aquatic origin is lower than that of their terrigenous counterparts. The C/N ratio obtained in the present study varied from 19.86 to 26.95 which can be compared with the results obtained by Visser (1983) who reports C/N ratio to vary from 18.7 to 33.8.

UV - VIS Spectrophotometry

UV and VIS spectra of HA is found to be featureless, with optical density decreasing as the wavelength increases (Raspor 1984). Similar trend is observed from the samples

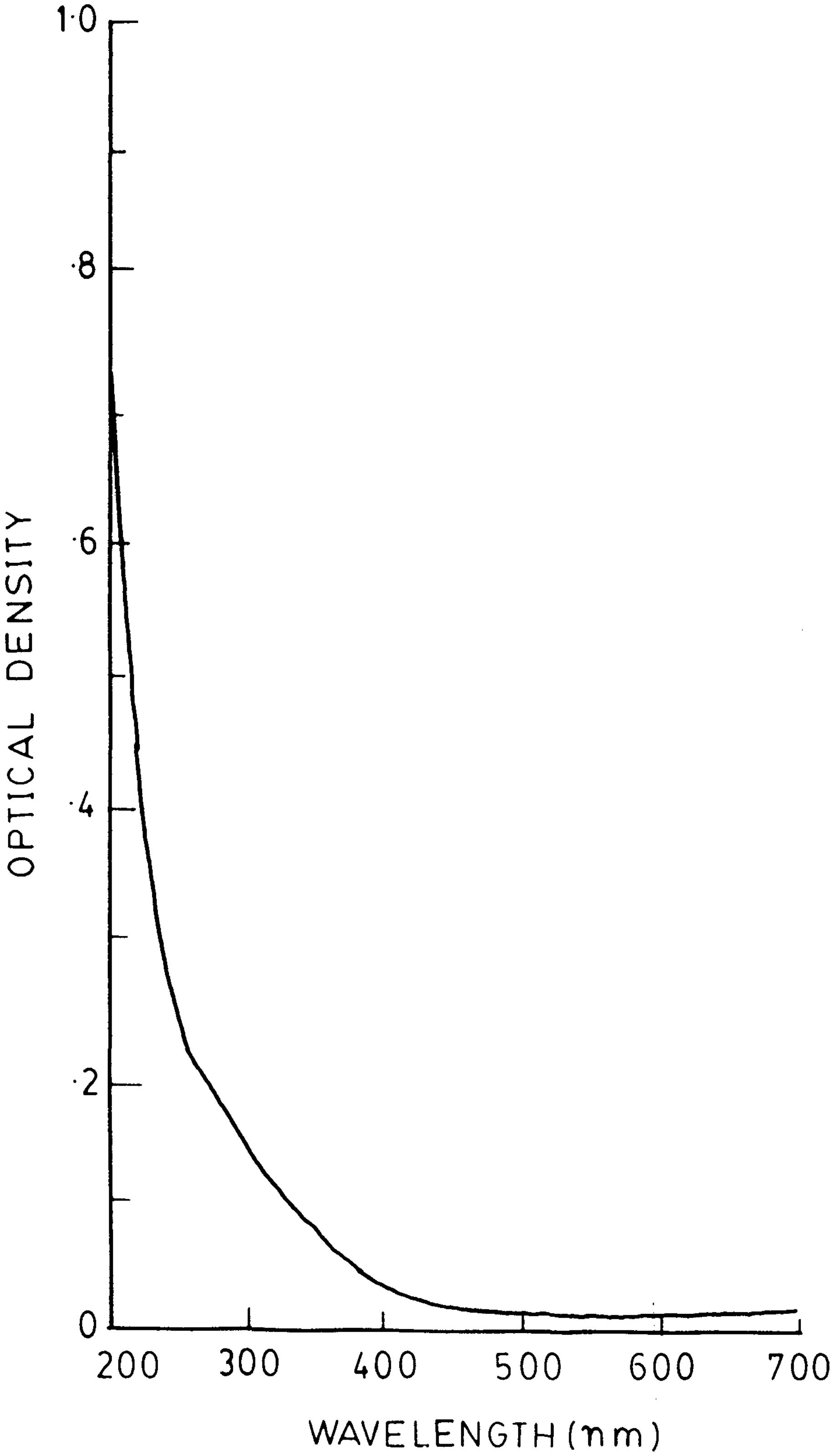

Fig. 22. UV - VIS spectra of HAO

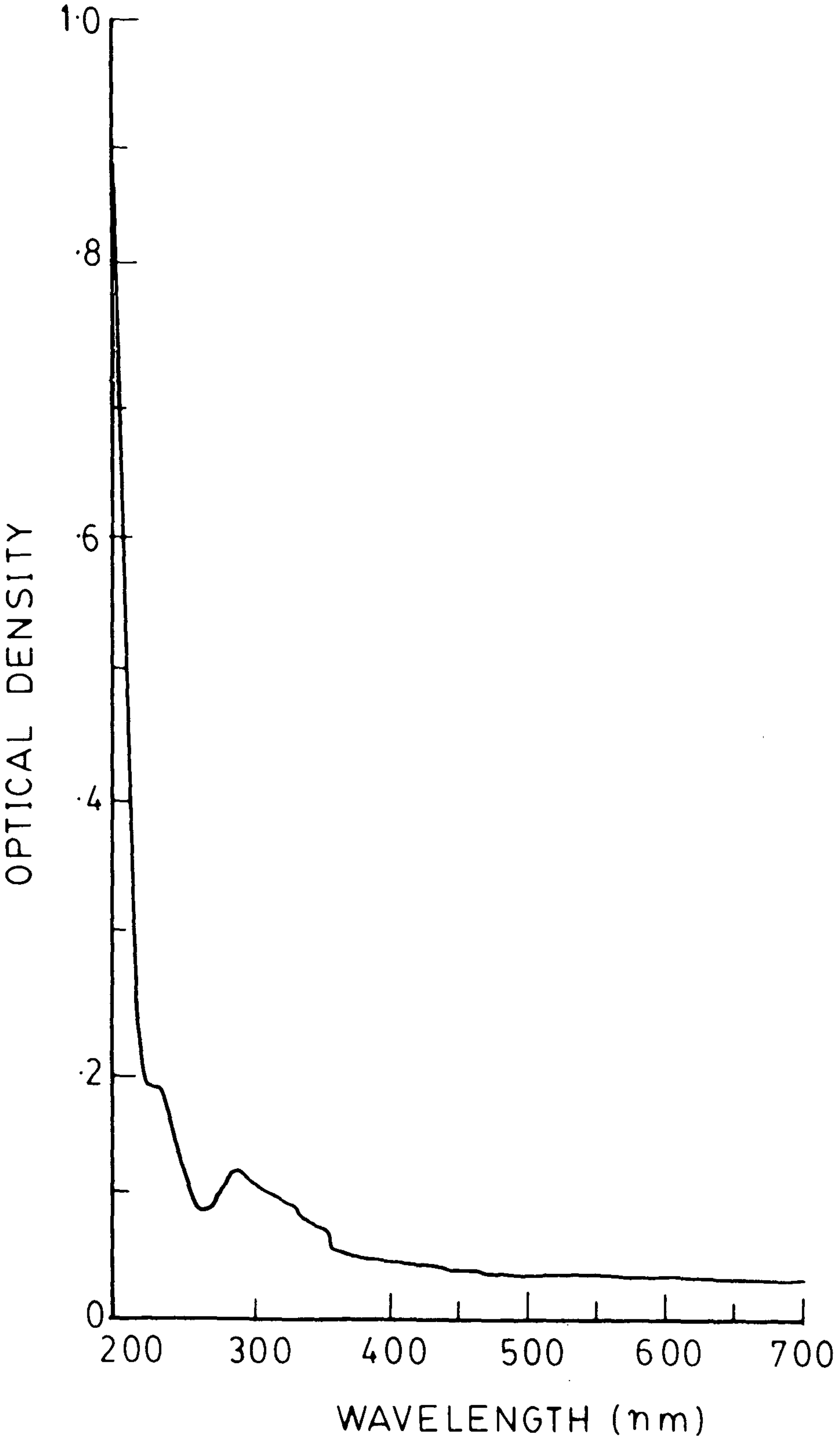

Fig. 23 UV - VIS spectra of HAY

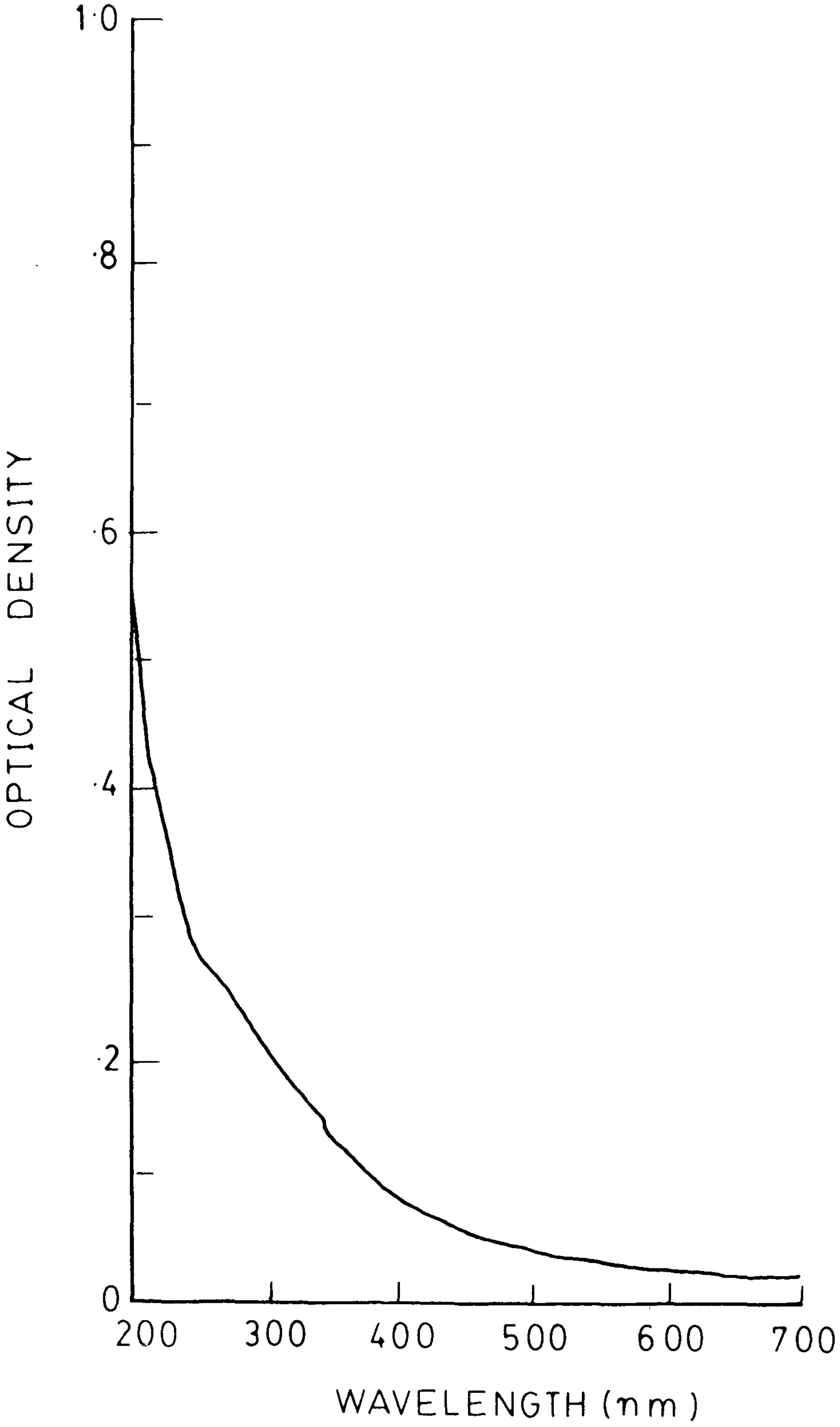

Fig. 24 UV - VIS spectra of HAW

TABLE 14

Optical densities, E, at 465 and 665 nm of 0.1g HA l^{-1} in 01M NaOH and E_4/E_6 ratios.

Humic Acid	E_{465}	E_{665}	E_4/E_6
Okhla	0.285	0.075	3.80
Old Yamuna Bridge	0.293	0.091	3.22
Wazirabad	0.327	0.112	2.92

obtained from Okhla and Wazirabad. The optical density decreases without any maxima. However, in the case of HA obtained from Old Yamuna Bridge there is an indication of a maxima at around 280 mm. Such maxima have been observed earlier also in certain isolated cases (Schnitzer and Khan 1972).

Typical chromophores known to occur in humic substances are C = C, and C = O; auxchromes that are likely to be present arè C - OH, C - N H_2 and others. If more than one chromophore is present, the interpretation of the spectra becomes difficult. Thus in the case of complex molecules such as HA, the chromophores must be well known before spectrocopy can be effectively applied to solve structral problems. Unfortunately, at the present time such information is not available for HA.

Although absorption spectra of HA in the visible region of the spectra do not proivide much detailed information on their chemical structure, the ratio of the optical densities at 465 mm and 665 mm is often used for charecterization of HA. This ratio referred as E4/E6, is independent of the concentration of the HA but varies for HA obtained from different origins. The E4/E6 values obtained are given in table 14. The value was 2.92 for

Wazirabad sample, 3.22 at old Yamuna Bridge sample and 3.80 for Okhla sample. This suggest that HA in the sediments of Wazirabad are more humified and condensed than those of Old Yamuna Bridge and Okhla (Shannukhappa et.al. 1987).

Infrared Spectrophotometry

Infrared spectrophotometry has been useful for the gross charecterization of HA of diverse origins, the evaluation of effects of different chemical extractants (Wagner and Stevenson, 1965), chemical modification such as methylation, acetylation, esterification, saponification and for the formation of derivatives (Schnitzer and Skinner 1965, Schntizer 1974).

The general charecter of the i.r. spectra obtained for all the three HA were found to be similar, which indicates a similar chemical strutures but differing mainly in the content of functional groups (Schnitzer and Skinner, 1965; Theng etal, 1966; Stevenson and Goh, 1977). The absorption bands which occur predominantly in the HA of present study are given in Table 15, 16 and 17.

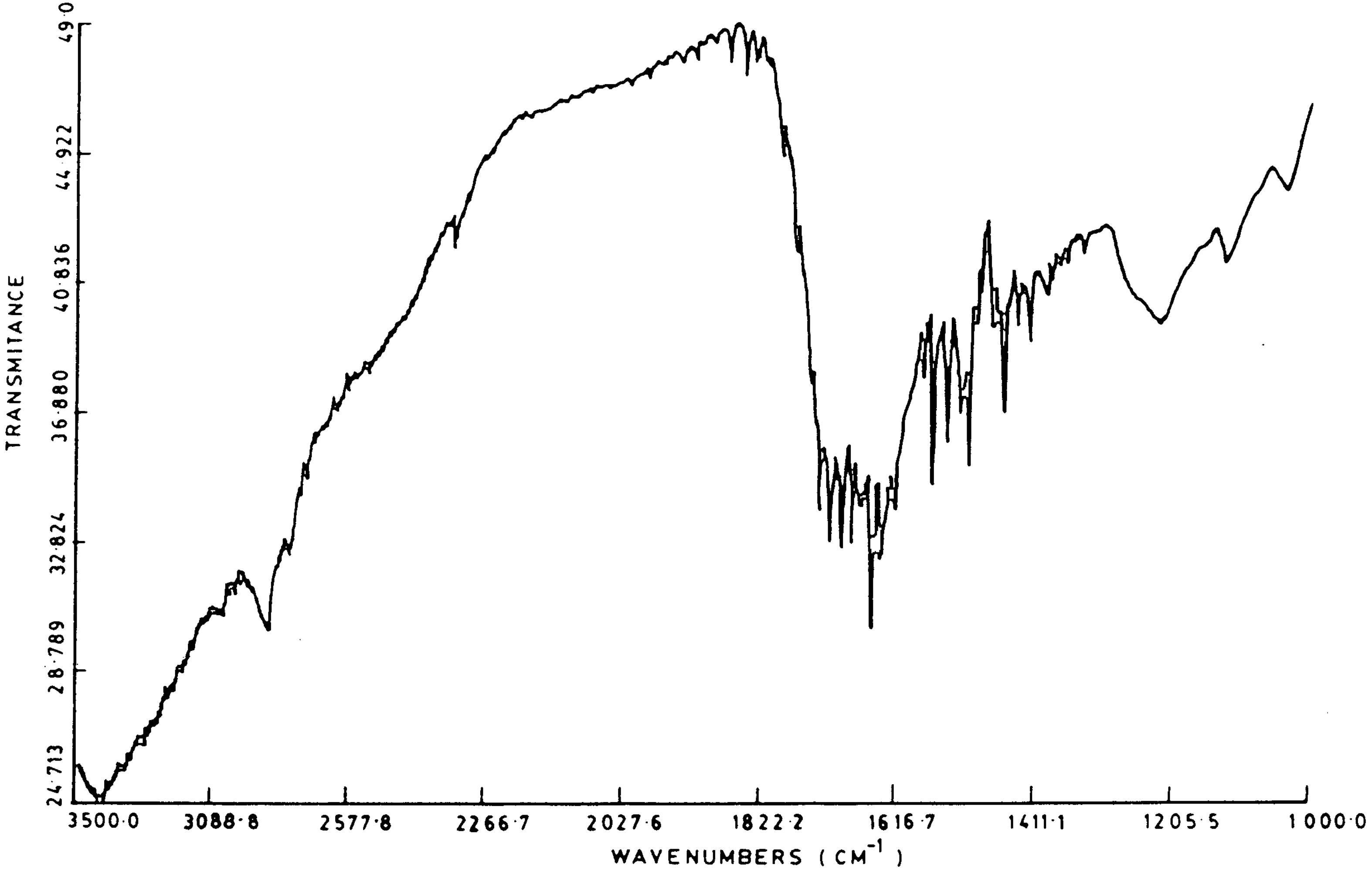

Fig. 25 IR spectra of HAO

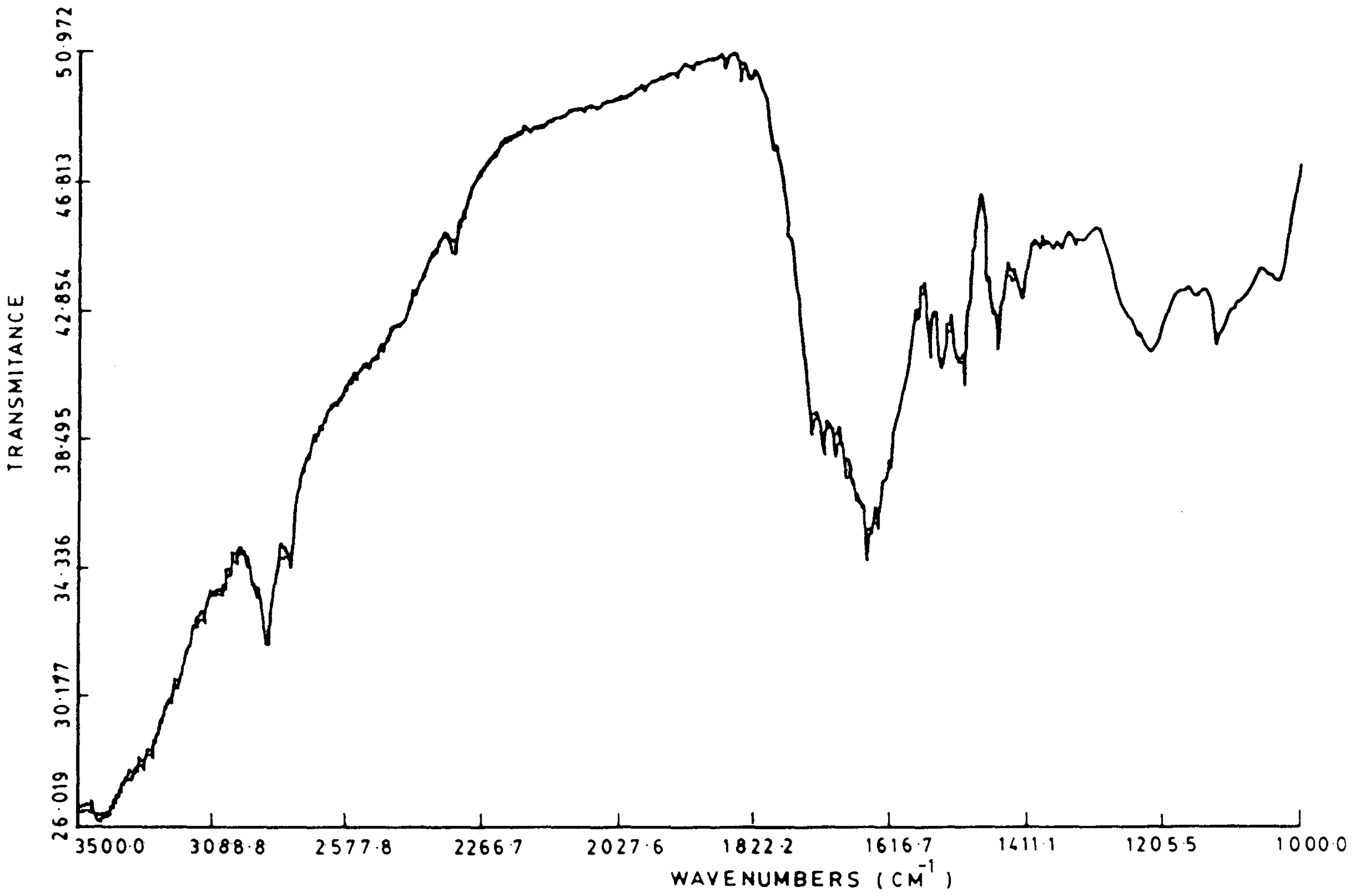

Fig. 26 IR spectra of HAY

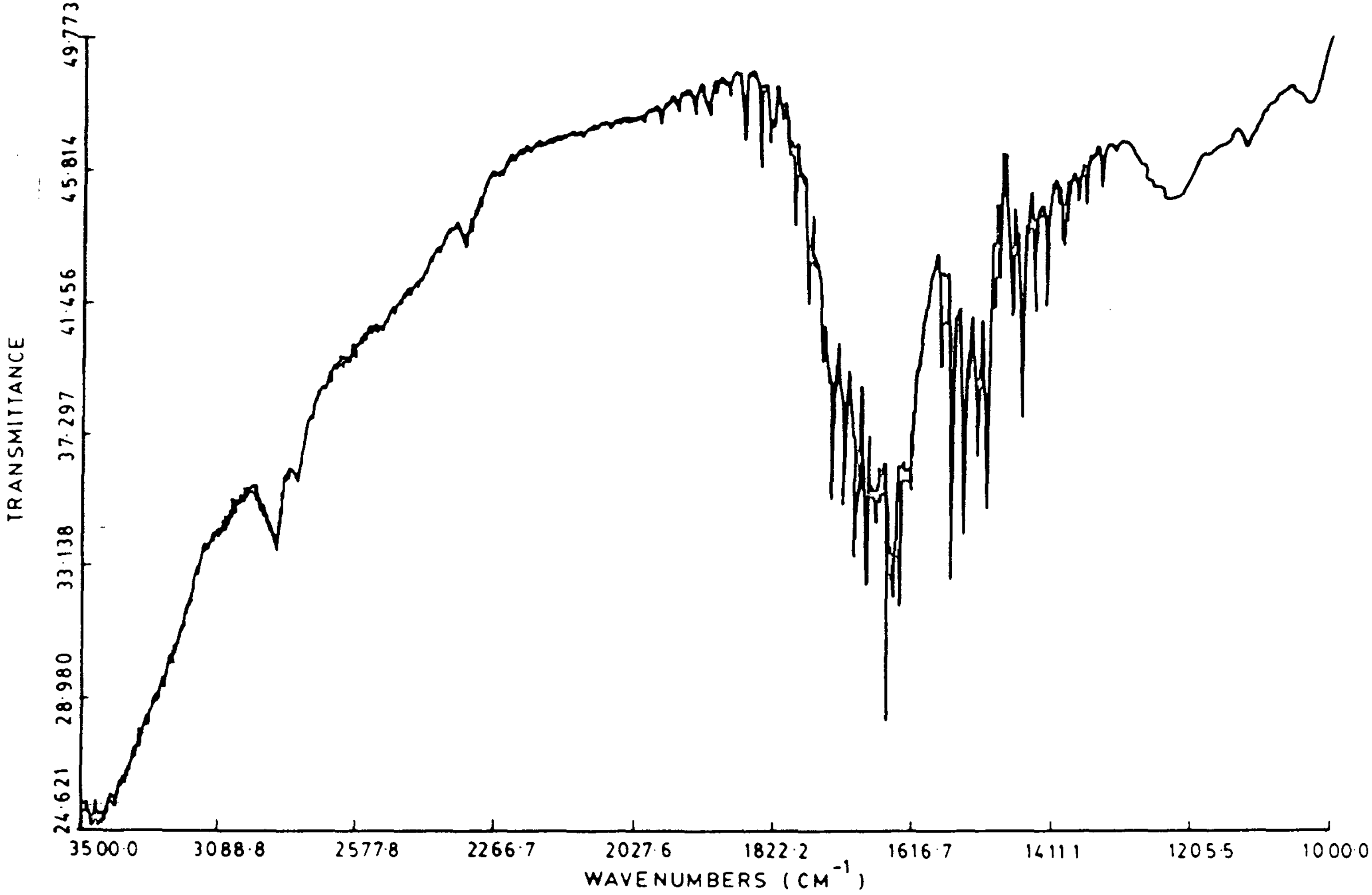

Fig. 27 IR spectra of HAW

TABLE 15

Main i.r. absorption bands of HAO.

Frequency cm^{-1}		Assignment
3420	(s,b)	Hydrogen bonded OH including those of - COOH and in some cases N - H groups.
2865	(m.s)	Aliphatic C - H
1725	(s)	C = o of CO$_2$H, C = O Ketonic Carbonyl
1655	(s)	Quinone Carbonyl groups, NH bending vibration of primary amine.
1640	(m)	aromatic C = C, double bond conjugated with carbonyl.
1630	(s,m)	N - H bending vibration of primary amine.
1468	(m)	Aliphetic C - H
1403	(s)	COO, aliphatic C - H, O - H bending C - o stretching vibration of phenols
1206	(m,b)	C = O stretch or OH deformation of Co$_2$H.
1055	(m,b)	Si-O of silicate impurities

s = strong, m = medium , b = broad

TABLE 16

Main i.r. absorption bands of HAY

Frequency cm^{-1}		Assignment
3430	(s,b)	Hydrogen bonded OH including those of - COOH groups and in same cases N - H groups
2853	(m,s)	Aliphatic stretching
1720	(s)	C = O of COOH, C = O stretch of atomic carbonyl
1658	(s)	Quinone Carbonyl group
1633	(s,m)	N - H bending vibrations of primary amine.
1460	(m)	Aliphatic C - H
1403	(s)	COO aliphatic C - H, O - H, C - O stretching of phenoles
1213	(m,b)	C = O or O - H deformation of Co$_2$H
1041	(m,b)	Si - O of silicate impurity

s = strong, m = medium, b = broad

TABLET 17

Main i.r. absorption bands of HAW.

Frequency cm^{-1}		Assignment
3432	(s,b)	Hydrogen bonded OH including those of - COOH groups and in some cases N - H groups
2880	(m,s)	Aliphatic C - H stretch.
1720	(s)	C = O of Co_2H, C = O of ketonic carbonyl
1666	(s)	Quinone Carbonyl groups
1638	(m)	Aromatic C = C, double bond Conjugated with carbonyl
1616	(s,m)	N - H bending vibration of primary amine.
1509	(v)	C = C of aromatic rings
1468	(m)	Aliphatic C - H
1394	(s)	COO^-, Aliphatic C - H, C - H bending, C - O stretching of phenol
1205	(m,b)	C - O stretch or OH deformation of CO_2H
1030	(m,b)	Si - O of silicate impurity

s = strong, m = medium, b = broad, v = variable

All the three samples show a strong absorption band around 3400 cm^{-1} which originates from the H bonded OH groups including those of COOH In some cases N - H groups may contribute to absorption in this region (Raspor et.al. 1984. The bands around 2900 cm^{-1} reflect the alphatic C -H stretching and this band is quite prominent in all three samples which indicates that the alphatic C-H group is quite prominent in all the three samples. The band around 1720 cm^{-1} is due to the carbonyl group and is of high intensity in the HAO and HAZ and relatively weak in the case of HAY. Band around 1660 cm^{-1} is quite prominent in all the three samples which denotes the presence of quinone carbonyl group and other x, B unsaturated carbonyl groups. Band around 1630 is attributable to C=C in aromatic ring and is observed in all the three samples. The C=O or OH deformation stretch band at around 1200 cm^{-1} is also found prominent in all the three samples. Si-O of silicate impurity was observed for all samples around 1050 cm^{-1}.

Proton NMR Spectrometry

Recently n.m.r spectroscopy has become a powerful tool in elucidating the structure of organic compounds. The p.m.r. spectra obtained in the present study shows several

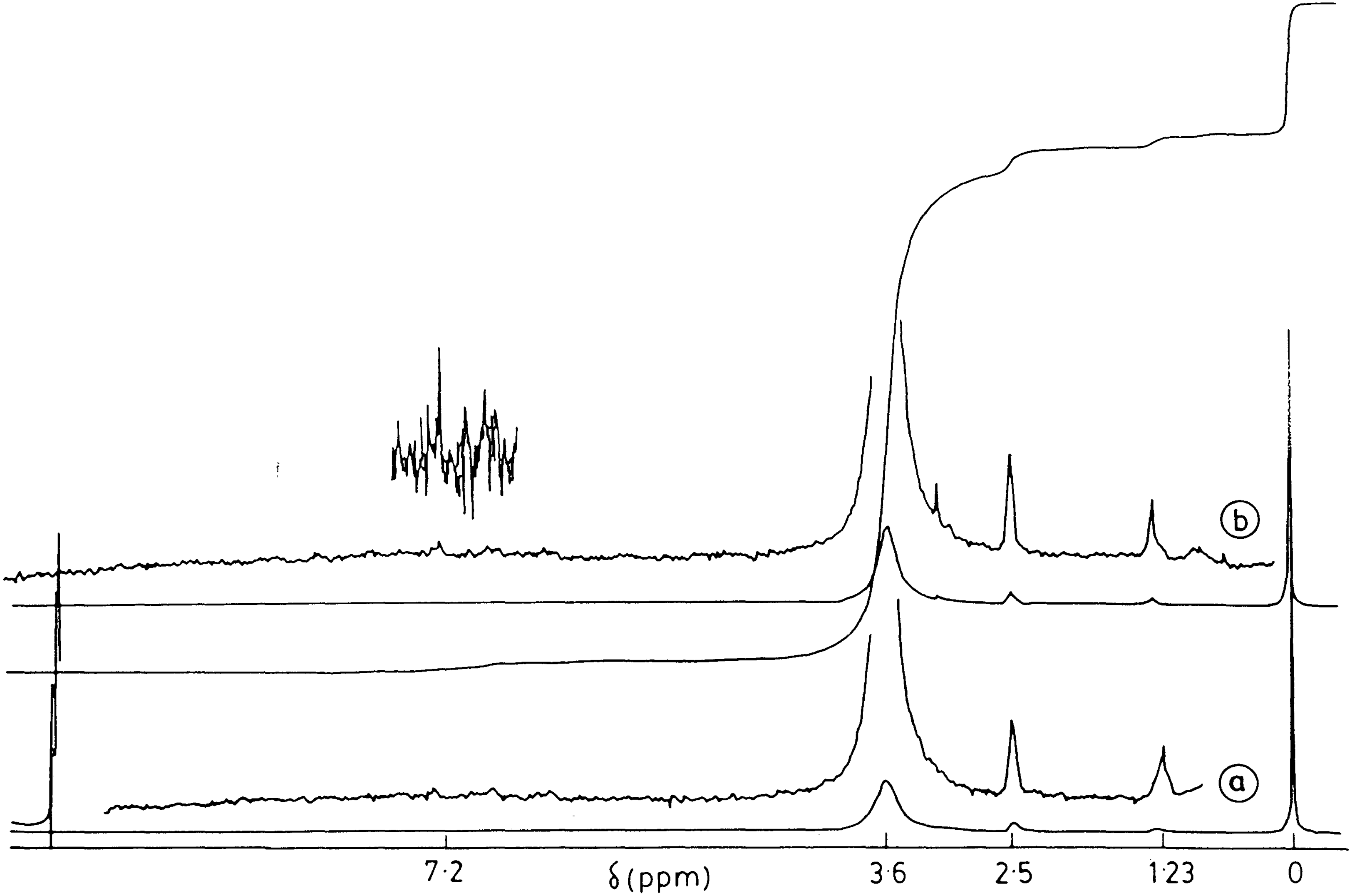

Fig. 28 NMR spectra of (a) HAO (b) HAY

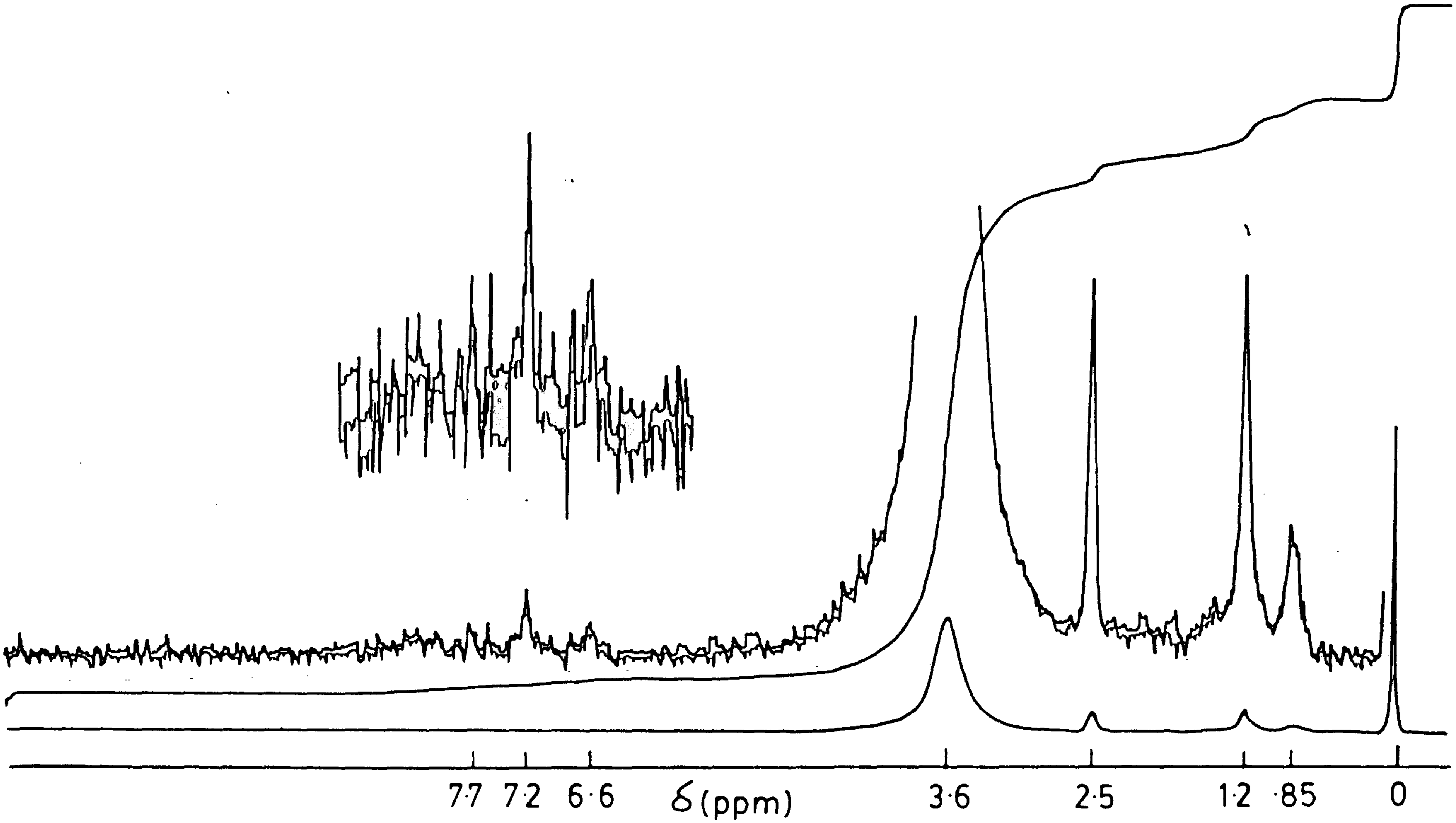

Fig. 29 Proton NMR spectra of HAW

TABLE 18

Tentative assignments of signals in p.m.r. spectra for HAO (All values in δ scale).

observed p.m.r. shift (ppm)	Assignment
0.8	- CH_3 groups
1.23	- CH_2 groups
2.5	$ArCH_3$ groups
3.6	Solvent peak (DMSO-d_6)
7.2	Aromatic (phenols) protons in polymeric association
7.69	De-shilelded aromatic proton.

TABLE 19

Tentative assignments of signals in p.m.r. spectra for HAY (All values in δ scale).

Observed p.m.r. shift (ppm)	Assignment
0.59 0.8 0.89	- CH_3 groups
1.23	- CH_2 groups
2.5	$ArCH_3$ groups
3.16	H - C = C - Conjugated
3.6	Solvent peak (DMSO-d_6)
7.2	Aromatic (phenols) proton in polymeric association
7.69	De- shielded aromatic proton

TABLE 20

Tentative assignments of signals in p.m.r. spectra for HAW (All values in δ scale).

Observed p.m.r. shift (ppm)	Assignment
0.858	- CH_3 groups
1.23	- CH_2 groups
1.79	- $CH_3 - C = C$ groups
2.5	- $ArCH_3$
3.6	Solvent peak (DMSO-d_6)
4.1	$ArNH_2$, OCH_3
6.66	- CH = CH - protons
7.1 7.5	Aromatic (phenols) protons in polymiric association
7.69	De-shielded aromatic proton.

similarities in structure, which can be predicted from the similar signals observed in the case of all the three samples. The signals observed and its assignments are given in Table 18, 19 and 20.

p.m.r spectra of all the three samples exhibited main signal at 1.23 ppm charecteristic of CH_2 groups in a long and poorely branched aliphatic chains (Gessa et.al. 1983). The signal at 0.8 ppm is also observed in all the three samples although very weak in the Okhla sample. This can ascribed to methyl groups on the chain. A strong main signal at 2.5 ppm is observed in all the three samples charecteristic of Ar CH_3 group which indicates that this group is quite prominent in all the three samples. The signal at 3.6 is the solvent peak. Similarly the peaks at 7.2 ppm and 7.69 ppm are also shown by the three samples which is due to the aromatic (phenol) protons in polymeric association and the de-shielded aromatic proton respectively.

Certain signals observed in one sample, were not found in other samples. For example, the main signal at 1.79 ppm is observed only in HAW which is ascribed to $CH_3 - C = C -$ group. This means that this group is present in more quantity in HAW and is not observed in HAO and HAY.

Another signal at 4.1 ppm observed at HAW is also not observed in other samples. This signal is due to $ArNH_2$ group or $-OCH_3$ group (Barton and Schnitzer, 1963). This is another indication that HAW contains more of Ar NH_2 or OCH_3 groups when compared to the other two samples.

Another signal which is observed only in HAW is at 7.5 ppm which is also assigned to ArO-polymeric association (Jackman and Sternhell, 1969). Although this signal was not observed in other samples similar peaks indicating a similar group were found in these samples also. HAY showed a signal which was not found in other samples at 3.16 ppm. This is due to the presence of conjugated H-C=C- group (Dyer 1984).

The intensities of signals in all the samples varied mainly because of the difference in solubilities of HA in DMSO-d_6. The maximum sohibility was for HAW which showed very intense peaks and the of very low least for HAO where peaks were of very low intensity. This difference in solubility can be ascribed to the length and crosslinking in the polymeric chain.

CHAPTER - V

SUMMARY AND CONCLUSIONS

(1) The present study covers measurement of various physico-chemical parameters in sediments of river Yamuna from three stations viz Okhla, Old Yamuna Bridge and Wazirabad, during the period, December 1986 to February 1987.

(2) The parameters analysed in sediments were HA, sediment grain size, organic matter, organic carbon, Total nitrogen, Phosphorous, trace metals (Cu and Cd); in from all the three sites.

(3) Complexation characteristics of HA were studied in all the three samples using ion selective Electrode technique with Cu and Cd and the stability constants were computed.

(4) Characterisation of HA was done using standard analytical techniques like UV-VIS spectrophotometry, IR spectrophotometry proton, NMR spectroscopy and elemental analysis for C,H and N.

(5) The Grain size analysis shows that samples from wazirabad contains maximum percentage of sand (85%). Clay and silt was found to be maximum at Okhla sation and the least at Old Yamuna Bridge.

(6) Organic matter in the sediments at Okhla was found to be the maximum due to the presence of large variety of plants and the release of seawage which contribute to the organic matter in sediments. The lowest value of organic matter was noticed at Old Yamuna Bridge. At Wazirabad the values obtained were in between that of Okhla and Old Yamuna Bridge samples.

(7) Organic Carbon and Total Nitrogen was found to be the highest in Okhla samples and the least in Old Yamuna Bridge samples. In the case of phosphorous a maximum was obtained for Wazirbad Sample and the minimum again for the Old Yamuna Bridge sample.

(8) Cu and Cd also was high in Okhla sample. The metal concentration decreased upstream and was the it least at Wazirabad. Old Yamuna Bridge showed an intermediate value.

(9) The stability constants determined for Cu and Cd, showed a higher value for copper than Cadmium.

(10) The stability constants were found to increase with the increase in pH in the case of all samples.

(11) C,H and N were analysed and O was calculated as difference from 100 for the three samples viz HAO,

HAY and HAW. The highest value for Carbon and Nitrogen was observed in the case of HAY where as H and O was found to more in the case of HAO.

(12) UV-VIS spectra was recorded and it optical density decreased with increasing wavelength. In case of HAO and HAY should maximum around 280 nm.

(13) E_4/E_6 was found to be the maximum for HAO and the minimum for HAW. This indicates that HAW is more condensed and humufied.

(14) i.r spectra showed most of the characteristic peaks expected in HA. All the samples showed characteristic peaks for OH, CO, quinone, aliphatic CH etc. and the HA isolated were found to be having more or less similar functional groups.

(15) The p.m.r. spectra also showed several characteristic peaks of HA and in the case of HAW the intensity was found to be more many which may be ascribed to solubility factor due to chain length and cross linking in the polymer.

BIBLIOGAPHY

Aston, S.R. and C.N. Hewitt (1977) Phosphorous and carbon distribution in a polluted coastal environment. **Est. Coastal Mar. Sci. 5 : 243 - 254**

Ardakani, M.S. and F.J. Stevenson (1972) A modified Ion-exchange technique for determination of stability constants of Metal Soil Organic matter complexes. **Soil Sci. Soc. Amer. Proc. 36 : 884 - 890.**

Barton D.H.R. and M.Schnitzer (1963) A new experimental approach to humic acid problem. **Nature 198 : 217 - 218**

Boyd A.S Lee E.S. Darrel W.N and Douglas X.W. (1981) The mechanism of copper (II) binding by humic acid : An electron spin responce study of coppa(II) - Humic Acid complex and some adducts with Nitrogen donors. **Soil Sci. Soc. Am. J. 45 : 745 - 749.**

Boyle E.A., Edmond J.M. and Sholkovitz E.R. (1977) The mechanism of iron removal in estuaries. **Geochim. Cosmochim. Acta 41 : 1313 - 1324**

Bresnahan, W.R., C.L. Grant, and J.H. Weber (1978). Stability constants for the complexation of copper(II) ions with water and soil derived fulvic acids measured by ion selective electrode. **Anal. Chem. 50 : 1675 - 1679.**

Buffle Jacques, France - Line Greter, and Werner Herdi (1977). Measurement of Complexation properties of Humic and Fulvic Acids in natural waters with lead and copper Ion selective Electrodes. **Analytical Chemistry, 49 : 216 - 222.**

Burnes, R.G. (1986) Humic substances : interaction with microorganisms and enzyme. In Humic substances, efforts on soil and plants. **Reda Ramo Editional degli agricoltori pp 35 - 39**

Cacco, G. and G. Dell' Agnola (1984) Plant Growth Regulation Activity of Soluble Humic Complexes. **Can. J. Soil Sci. 64 : 225 - 228**

Campbell, J.H. and R.D. Erans (1987) Inorganic and organic ligand binding of lead and Cadmium and resultant implications for bioavailability. **Sci. of total Environ. 62 : 219 - 227.**

Chen, Y and M.Schitzer (1976) Viscocity measurements on soil humic substances." **Soil Sci. Soc. Am. J. 40 : 866 - 872**

Correl, P.L., M.A. Faust, and D.J. Severn (1975) Phosphorous fuse cycling in estuaries. In estnarini research (corin ed) Vol.1. **Academic Press New York. pp 108 - 136**

Cramwell, P.A. and R.D. Haworth (1972). The chemical nature of Humic acids. **Proc. int. Meet. Humic substances Nieuwersluis, Pudoc Wageningen pp 13 - 42.**

Dubashp and Mehta N.C. (1963) The chemistry of soil humic substances **Soil Fertility 27 : 293 - 300.**

Dyer, J.R. (1974) Application of absorption spectroscopy of organic compounds. **Prentice Hall India Ltd. New Delhi.**

Enk, M.D. and Mathias, B.J. (1977) **Hydrobiologia 52 : 153 - 158.**

Felbeck Jr, G.T (1965) Studies on high pressure hydrogenolysis of the organic matter from a muck soil. **Soil Sci. Soc. Bm, Proc. 290 48 - 55.**

FELBECK Jr, G.T (1971) Structural hypothesis of Humic Acids. **Soil Sci 111 : 42 - 48.**

Fitch A. and F.J. stevenson (1984) Comparison of models for determining stability constants of metal complexes with Humic substances. **Soil Sci. Soc. Am J. 48 : 1044 - 1049.**

Flaig, W. (1972) An introductory review on humic substances; aspects of research on their genesis, their physical and chemical properties and there effect on organisms. **Proc. int. meet. Humic substances, Nieuwershis 1972 Pudoc, Wageningen.**

Flaig W. H. Butelspacher and E Rietz (1975) Soil Compoinents: Volume 1 Organic components [ed] John E. Gieseking **Springer - Verlag New York.**

Forstner, V. and Wittman, G.T.W. (1981) Metal pollution in the aquatic environment **Springer - Verlag p. 486**

Gadel, F. G.Torri and Bruchet (1987) Humic substances from Deposits of a Natural laboratory. A blue lake on the Ice-cap (Greenland). **The Science of the Total Environment 62 : 07 - 109.**

Gamble, D.S., Schnitzer, M. and Hoffmann I. (1970) Cu^{2+} fulvic acid chelation equibrium in 1 M KCl at 25°C. **Can. J. Chem. 48 : 3197 - 3204.**

Gessa, C., M.A Cabras G. Micera, M.Polemio and C. Testini (1983) Spectroscopic Characterization of extracts from Humic and Fulvic fractions : IR and 'H NMR spectra. **Plant and soil 75, 169 - 177.**

Ghosh, K and schnitzer (1980) Macromolecular structures of Humic substances. **Soil Sci. 129 : 266 - 276.**

Guy, R.D. C.L. Chabrabarti and L.L. Schramm (1975) **Can. J. Chem. 53 : 661 - 667.**

Harvey, G.R., D.A. Boran, S.R. Piotrowicz and C.P. Wiesel (1984) Synthesis of marine humic substanced from ansaturated lipids. **Nature 309 : 244 - 246.**

Hatcher, P.G. M. Schnitzer, L.W. Dennis and G.E. Maciel (1981) Armaticity of Humic Substances in soil. **Soil Sci. Soc. Am. J. 45 : 1089 - 1094.**

Haworth, R.D. (1971) Chemical nature of Humic Acid. **Soil Sci. 111 : 71 - 79.**

Huntsman, S.A. and Sunda, W.G. (1980) In Morris, I, (ed). **The physicological ecology of Phytoplankton. Black well Sci. Pub. pp 285 - 328.**

Irving, H. and Williams, R.J.P. (1948) Order of Stability of metal complexes **Nature** **<u>162</u> : 746 - 747.**

Jackson M.L. (1973) **Soil Chemical analysis Practice Hall, New Delhi.**

Jackman, L.M. and H. Sternwell (1969) Applications of nuclear magnetic Resonance spectroscopy in Organic Chemistry. **Pergamon press New York.**

Jensen - Korte, U., C. Anderson and M.Spiteller (1987) Photodegradation of pesticides in presence of humic substances. **Sci. of the total Envirm. <u>62</u> : 335 - 340.**

Jha, P.K (1986) Nature of Chemical and sediment load in the Yamuna River Basin. **Ph.D Thesis submitted to Jawaharlal Nehru University New Delhi.**

John, K.M. (1970) Colorismetric determination of phosphorous in soil and plant materials with ascorbic acid. **Soil Sci. <u>109</u> : 214 - 220.**

Kerven G.H, D.G. Edwards and C.J. Asher (1984) The determination of native ionic copper concentrations and copper complexations in peat soil extracts. **Soil Sci. <u>137</u> : 91 - 99.**

Khan, S.U. (1971) Distribution and characteristics of organic matter intracted from black solonetzic and black chernozemic soils of Alberta : The humic Acid fraction. **Soil Sci. <u>112</u> : 401 - 409.**

Kramer, C.J.M., J.C. Duniker (1984). **Complexation of Trace Metals in natural waters. Martins Nijhoff/Dr W.Jank Publishers.**

Kumada, K. (1955) Absorption spectra of Humic Acids. **Soil plant food <u>1</u> : 29 - 30.**

Kumada, K. and Kawamura, Y. (1968) On the fractionation of humic Acids by a gractional precipitation technique **Soil Sci. plant nutr. 14 : 198 - 200.**

Ladd J.N. (1969) The extinction coefficients of soil Humic acids fractionated by sephade gel filtration. **Soil Sci. 107 : 303 - 306.**

Madhum Y.A., V.H. Freed and J.L. Young (1986) Binding of ionic and neutral herbicides by soil humic acid. **Soil Sci. Soc. Am J. 50 : 319 - 322.**

Mantoura R.F.C., Andrew Dickson and J.P. Riley (1978). The complexation of metals with Humic materials in natural waters. **Est. Coastal marine Sci. 6 : 387 - 408.**

Martin, J.P. and K.Haider (1971) Microbial activity in relation to soil hummus formation. **Soil Sci. 111 : 54 - 63.**

Mathur, S.P. and Schnitzer, M. (1978) Effects of Chemical and spectroscopic characterization of some synthetic analouges of humic acid. **Soil Sci. Soc. Am. J. 42 : 591 - 596.**

Miele, S. (1986) The role of organic matter in agronomy practice and proposals for improving the humic balance of the soil. In Humic substances, effects on soil and plants. **Reda Ramo Editionale degli agrecoltori pp 136 - 170.**

Mikita, M.A. Cornelius Steelink and Robert, L - Dershaw (1981) Carbon - 13 enriched Nuclear magnetic Resonance Method for the Determination of Hydroxyl Functionality in Humic substances. **Anal. Chem. 53 : 1715 - 1717.**

Montgomery, J.R. and Santiago, R.J. (1978) Zinc and copper in 'particulate' forms and 'soluble' complexes with inorganic or organic legands in Guanajibo River and coastalzone, Puerto Rieo. **Esturaine and Coastal Marine science 6 : 111 - 116.**

Mukherji, P.N. and A. Lahiri (1958) Polyelectrolytic behaviours of humic Acids. **Fuel 37 : 220 - 226.**

Negishi, M. and K. Matsunga (1983) Organically - bound copper in lake and river waters in Japan **Water Res. 17 : 91 - 95.**

Nelson, L.A. and R.F.C Mantoura (1984) Anodic stripping voltammetry of copper in estuarnie media. **In complexation of trace metals in natural waters. (eds) C.J.M Kramer/J.C. duniker Martinus Nijhoff/ DR., W. Junk publishers The Hague.**

Newman, R.H., K.R. Tate, P.F. Barron and M.A. Wilson (1980). Towards a direct non destructive method of characterising soil humic substances using C - 13 NMR. **J. Soil Sci 31 : 623 - 631.**

Nikita, M.A., Steelink, C. and Wershaw R.L. (1981) Carbon - 13 enriched nuclear magnetic resonance method for the determination of hydroxyl functionality in humic substances. **Anal. Chem. 53 : 1715 - 1717.**

Nriagu, J.O. (1979) Global inventory of Natural and anthropogenic emmissions of trace metals to the atmosphere. **Nature 279 : 409 - 411.**

Nieboer, E and Richardson, D.H.S. (1980) **Environ. Poll. (Ser. B) 1 : 3 - 26.**

Nurnberg, H.W. (1984) Potentialities of voltmetry in the study of physico-chemical aspets of heavy metal complexation in natural waters. **In Complexation of Trace metals in natural waters (eds) C.J.M Kcramer and J.C Duniker Martinus Nijhoff/DR. W Junk publishers. The Hague.**

Odum, E.P. (1971) **Fundamentals of Ecology Saunders/Toppan Pub.**

Poitrowicz R.S, George R.H. Deborah A.B. Chifford P.W. and M.S. Young (1984) Cadmium, Copper and zinc interactions with marine ;humus as a function of ligand structure **Marine Chemistry 14 : 333 - 346.**

Pomeroy, L.R., E.E. Smith and C.M. Grant (1965) Exchange of phosphate between estmarine sediments and water. **Limnology and Occeanography 13 : 598 - 606.**

Prakash, A., Arne Jensen and M.A. Rashid (1972). Humic substances and aquatic productivity **Proc. int. Meet. Humic substances Nieuwersluis, Pudoc, Wageningen pp 259 - 267.**

Preston, C.M. and M.Schnitzer (1984) Effects of chemical modifications and extractants on Carbon - 13 NMR spectra of Humic Materials. **Soil Sci Soc. Am J. 48 : 305 - 311.**

Rainbow P.S. (1985) Biology of metals in the sea **inter. J. Environ. Stud. 25 : 195 - 211.**

Randhawa, N.S. and F.E. Broadbent (1965) Soil organic matter -metal complexes 6. Stability constants of Zinc-humic acid complexes at different pH values. **Soil Sci. 99 : 362 - 366.**

Raspor, B, H.W. Nurnberg, P. Valenta and Branica (1984 a). Significance of dissolved humic substance for heavy metal speciation in natural waters. **Kramer, C.J.M and Duninker J.C. (eds), Complexation of trace metals in natural waters. Martiness Nijhoff/Dr. W. Junk publishers The Haugue.**

Raspor, B, H.W. Nurnberg, P. Valenta and M.Branica (1984b) Studies in seawater and lake water on interactions of trace metals with humic substances isolat form marine and estuarine sediments. **Marine Chemistry 15 : 217 - 280.**

Ryther, J.H. and W.M. Dunstan (1971) Nitrogen phospherous and eutrophication in the coastal marine environment **Science 171 : 1008 - 1013.**

Saar, R.A. weber, J.H. (1979) Complexation of Cd(11) with water and soil derived-fulvic acids effect of pH and fulvic acid concentration. **Con. J. Chem. 57 : 1263 - 1269.**

Saar, R.A., and J.H. Weber (1980 a) Comparisons of spectrofluorometry and ion selective eleitrode potentiometry for determination of complexes between fulvic acid and heavy metal ions. **Anal. Chem. 52 : 2095 - 2100.**

Saar, R.A. and J.H. Weber 1980 b. lead (11) fulvic acid complexes conditional stability constants, solubility and implication for fb(11) mobility **Environ. Sci. Technol. 14 : 877 - 880.**

Schnitzer, M. (1965) The application of infrared spectroscopy to investigations on soil humic compounds. **Can.J Spectroscopy 10 : 121 - 127.**

Schnitzer, M. (1986) Synthesis, chemical structure, reactions and functions of humic substances, effects on soil and plants. **Reda Remo Editionale degli agricoltori pp. 11 - 29.**

Schnitzer, M. and S.I.M. Skinner (1963) Organo metallic interactions in soils : 2 **Soil Sci. 96 : 181 - 186.**

Schnitzer M. and S.I.M. Skinner (1965) Organo metallic interactions in soils : 4 Carboxyl and hydroxyl groups in organic matter and metal retention. **Soil Sci. 99 : 278 - 284.**

Schnitzer, M. and S.I.M. skinner (1966) Organo metallic interactions in soils : 5 stability constants of Cu^{2+}, Fc^{2+} and Zn^{2+} fulvic acid complexes. **Soil Sci 102 : 361 - 365.**

Schnitzer, Mand S.I.M SKINNER (1967) Organo metallic int4eractions in soil : 7 stability constants of $P6^{2+}$, Ne^{2+}, Mm^{2+}-, Co^{2+}, Ca^{2+}, and Mg^{2+} fulvic acid complexes. **Soil Sci. 103 : 247 - 252.**

Schnitzer, M. and C.M. Preston (1983) Effects of acid hydrolysis on 13C NMR spectra of Humic substances. **Plant and soil 75 : 201 - 211.**

Schnitzer M and AIM skinner (1965) The carboxyl group in soil organic matter preparation. **Soil Sci. Soc. Am. Proc. 29 : 400 - 405.**

Schintzer M and S.U. Khan (1972) Humic substances in the environment. **Marcel Dakker Inc. New York pp. 1 - 4.**

Sensi N. Chen, Y, and Schnitzer, M. (1977) The role of free redicals in the oxidation and reduction of fulvic acid. **Soil Biol. Biochem. 9 : 397 - 403.**

Shammukhappa, H.(1981) Primary productivity by phytoplanletion under controlled laboratory conditions. **M.Phil Dissertation Jawaharlal Nehru University, New Delhi p.78.**

Shammunkhappa, H. (1984) The humic acids and some elements of biological importance in the sea, Estuary and mangroves of Posto Noro, South India. **Ph.D Thesis Submitted to Jawaharlal Nehru University, New Delhi.**

Shammukhappa, H., D.K. Banerjee and K. Krishnamurthy (1987) Humic acids in coastal aqutic biotopes of Porto Novo, India. **Sci. to the total Environ. 62 : 469 - 475.**

Sharma, R.M. (1986) Effects of thermal pollution causes by the effluents of thermal power plant on selected phytoplantations. **Ph.D Thesis Submitted to Jiwaji University 1986.**

Schubert, J. (1948) Use of ion exchangers for the determination of physico - chemical properties of substances, perticularly radio tracers in solution : **Theoretical. J. Phys. Coll. Chem - 52 : 340 - 350.**

Simoesgonsalves M.L.S., P valenta and H.W Nurnberg (1983) Voltametric and potentiometric investigation on the complexation of Cd^{2+} by glycine in sea water. **J. Elecrtro anal. Chem. 149 : 249 - 262.**

Singh S.K. and V Subramanian (1984) Hydrous Fe and Mn oxides - Scavengers of heavy metals in the aquatic environment CRC **critical Reviews in Environmental control 14 : 33 - 90.**

Sitaswad, R.L. (1984) Distribution of Fe, Mn, Cu, Pb and Zn in Jamuna river sediments, Delhi. **Ph.D Thesis submitted to Jawaharlal Nehru University, New Delhi.**

Sposito G, K.M. Holtzclaw and C.S. levesque - Madore (1978) Calcium ion complexation by fulvic acid extracted from sewage sludge - soil mintures. **Soil Sci. Soc. Am. J. 42 : 600 - 606.**

Stevenson, F.J. (1976) Stability constants of Cu^{2+}, Pb^{2+}, and Cd^{2+} complexes with Humic Acids. **Soil Sci. Soc. Am. J. 40 : 665 - 672.**

Stevenson F.J. (1977) Nature of divalent transition metal complexes of humic acids as rivealed by a modified potentiometric titration method **Soil Sci. 123 : 10 - 17.**

STEVENSON, F.J. and K.M. GOH (1974) IR spectra of Humic acids eliminations of interference due to hygroscopic moisture and strectural changes acompanying heating with KBr. **Soil Sci. 117 : 34 - 41.**

Stevenson, F.J., S.A. Krastanov and M.S. Ardakani (1973) Formation constants of Cu^{2+} complexces with Humic and fulvic acids. **Geoderama 9 : 129 - 141.**

Sylva, R.N. (1976) The Environmental Chemistry of copper(II) is aquatic systems. **Water Research** **10** **: 789 - 792.**

Takamatsu, T. and T. Yoshida (1978) Determination of stability constants of metal humic acid complexes by potentiometric titrations and ion selective electrodes. **Soil Sci. 125 : 377 - 386.**

Tan, K.H. and A.Binger (1986) Effect of humic acid on alukminium toxicity in corn plants. **Soil Sci. 141 : 20 - 25.**

Tan, K.H., L.D. King and H.D. Morris (1971 a) complexe reactions of zinc with organic matter intereacted from sewage sludge. **Soil Sci Soc. Am. proceedings 35 : 748 - 751.**

Tan, K.H., R.A. Leonard, A.R. Bertrand, and S.R. Wilkinson, (1971 b) The metal complexing Capacity and the nature of the Electating lignands of water extracts of poultry litter. **Soil Sci. Soc. Am. Proc. 35 : 265 - 269.**

Theng, B.K.G, J.R.H Wake and A.M. Posner (1966) Infrared spectrum of humic acid. **Soil Sci. 102 : 70 - 72.**

Tsutsuki, K. and S. Kuwastscika (1984) Molecular size distribution of humic acids as affected by ionic strength and the ;degree of humification **Soil Sci. Plant Nutr. 30 : 151 - 162.**

Vanderberg, C.M.G. (1984) Determination of complexing capacities and conditional stability constants using ion exchange and by completion techniques. **In Kramer C.J.M. and J.C. Danker complexation of trace metal in Natural Daters martinus Nujhoff - Dr. W. Junk Publishers Hague pp. 17 - 32.**

Van Dijk. H. (1971) Cation binding of Humic Acids. **Geoderma 5 : 53 - 67.**

Varney, M.S., D.R. Turner, M. Whitefield and R.F.C. Mantoura (1984) The use of electrochemical techniques to monitor complexation capacity titrations in natural waters. **In complexation of trace metals in natural waters (edges) C.J.M Kramer/J.C. Diniker. MIartius Nijhoff/DR W junk publisher The Hague.**

Vaughan, D. (1986) Effect of humic substances on metabolic processes in plants. In humic substances effects on soil and plants. **Reda Ramo Editionale Degli agriecoltori pp 54 - 77.**

Visser, S.A. (1983) Comparative study on the elementary composition of fulvic and HUmic Acids of aquatic rigin and from soils and metrobial substrates. **Water Res. 17 : 1393 - 1396.**

Visser, S.A. (1986) Effect on humic substances on plant growth. In Humic substances, effects on soil and plants **Rada Ramo Editionale Degli agreoltori pp 89 - 136.**

Wagner, G.H. and F.J. Stevenson (1965) Structural arrangements of functional groups in soil humic acids as releaved by infrared analysis. **Soil Sci. Soc. Am. Proc. 29 : 43 - 48.**

Walkley, A. and Black, J.A. (1934) An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. **Soil Sci. 37 : 29 - 34.**

Wetzel R.G. (1983) Liminology second edition **Saunders College Publishing, New York.**

Wilson, M.A. and K.M. goh (1977) Proton decoupled pulse fourier transform C - 13 manetic resenance of soil matter. **J. Soil Sci. 28 : 645 - 652.**

Wood, J.M. (1974). Biological Cycles for toxic elements in the environment. **Science 15 : 1049 - 1052.**

Wood J.M. (1975) Metabolic cycles for toxic elements in the environments. A study of kinetics and mechanisms. IN Krenlect P.A. (ed) An international Conference. **Pergamon Press p.105**

Ziechmann, W. (1964). Spectroscopic investigation of lignin, humic substances and Peat. **Geochim. Cosmochim. Bcta <u>28</u> : 1555 - 1566.**

Zunino, H.P. Peirano, M. Aquilera and I. Esobar (1972) Determination of maximum complexing ability of water soluble complexants. **Soil Sci. <u>114</u> : 414 - 416.**

Zunino, H., P. Periano, M. Aguilera, and E.B. Schalescha (1975) Measurement of metal complexing ability of poly functional maeromoleules. A discussion of the relationship between the metal comlasing properties of extracted soil organic matter and soil genesis and plant nitrition. **Soil Sci. <u>119</u> : 210 - 216.**

Zunino, H., and J.P. Martin (1977) Metal binding organic macromolecules in soil 2 Characterization of the maximum binding ability of the macromolecules. **Soil Sec. <u>123</u>. 188 - 202.**

APPENDIX

TABLE 1

pH 2

S. NO	Log[Cu]	Blank	Okhla
1	-5.88	415	396.2
2	-5.7	416	397.1
3	-5.58	417.2	398
4	-5.4	419.2	404.8
5	-5.28	421.4	408.4
6	-5.18	423	412
7	-5.1	424.2	415.5
8	-5.03	425	418
9	-4.98	428	419.7
10	-4.93	428.5	420.8
11	-4.88	429.5	422.5
12	-4.83	430.8	424.1
13	-4.8	432.7	425.6
14	-4.77	432.1	426.9
15	-4.74	433	427.8
16	-4.7	433.5	429
17	-4.68	433.8	430.3
18	-4.65	434.2	431.5
19	-4.63	435	432.3
20	-4.6	435.4	432.8
21	-4.58	436.5	434
22	-4.41	442	438.9
23	-4.29	445	443
24	-4.2	447	445.3
25	-4.13	448.6	447.8
26	-4.06	450.2	450.2
27	-4.01	451.8	451.8
28	-3.97	453.4	453.4
29	-3.92	454.6	454.6
30	-3.89	455.6	455.6
31	-3.86	456.6	456.6
32	-3.83	457.3	457.3
33	-3.8	458.3	458.3
34	-3.77	458.9	458.9
35	-3.75	459.6	459.6
36	-3.73	460.1	460.1
37	-3.71	460.6	460.6
38	-3.69	461.2	461.2
39	-3.67	462	462
40	-3.66	462.7	462.7
41	-3.64	463	463

TABLE 2

pH 3

S. NO	Log[Cu]	Blank	Old Yamuna Bridge
1	-5.88	415	391
2	-5.7	416	391.9
3	-5.58	417.2	392.5
4	-5.4	419.2	402.3
5	-5.28	421.4	408.1
6	-5.18	423	410.9
7	-5.1	424.2	413.4
8	-5.03	425	415.7
9	-4.98	428	418.4
10	-4.93	428.5	420.7
11	-4.88	429.5	422.7
12	-4.83	430.8	424.4
13	-4.8	431.7	425.9
14	-4.77	432.1	427.2
15	-4.74	433	428.7
16	-4.7	433.5	429.6
17	-4.68	433.8	430.3
18	-4.65	434.2	431.2
19	-4.63	435	432.5
20	-4.6	435.4	433.4
21	-4.58	436.5	434.1
22	-4.41	442	439.2
23	-4.29	445	442.5
24	-4.2	447	446
25	-4.13	448.6	448.1
26	-4.06	450.2	450.2
27	-4.01	451.8	451.8
28	-3.97	453.4	453.4
29	-3.92	454.6	454.6
30	-3.89	455.6	455.6
31	-3.86	456.6	456.6
32	-3.83	457.3	457.3
33	-3.8	458.3	458.3
34	-3.77	458.9	458.9
35	-3.75	459.6	459.6
36	-3.73	460.1	460.1
37	-3.71	460.6	460.6
38	-3.69	461.2	461.2
39	-3.67	462	462
40	-3.66	462.7	462.7
41	-3.64	463	463

TABLE 3

pH 3

S. NO	Log[Cu]	Blank	Wazirabad
1	-5.88	415	375.3
2	-5.7	416	376.1
3	-5.58	417.2	376.6
4	-5.4	419.2	387.4
5	-5.28	421.4	396.2
6	-5.18	423	402
7	-5.1	424.2	407.5
8	-5.03	425	410
9	-4.98	428	413.8
10	-4.93	428.5	416.8
11	-4.88	429.5	419.2
12	-4.83	430.8	420.6
13	-4.8	431.7	421.5
14	-4.77	432.1	423.1
15	-4.74	433	424.4
16	-4.7	433.5	425.5
17	-4.68	433.8	426.6
18	-4.65	434.2	427.7
19	-4.63	435	428.5
20	-4.6	435.4	429.7
21	-4.58	436.5	430.7
22	-4.41	442	435.5
23	-4.29	445	439.6
24	-4.2	447	442.6
25	-4.13	448.6	445.2
26	-4.06	450.2	447.9
27	-4.01	451.8	450.8
28	-3.97	453.4	452.4
29	-3.92	454.6	454.2
30	-3.89	455.6	455.6
31	-3.86	456.6	456.6
32	-3.83	457.3	457.3
33	-3.8	458.3	458.3
34	-3.77	458.9	458.9
35	-3.75	459.6	459.6
36	-3.73	460.1	460.1
37	-3.71	460.6	460.6
38	-3.69	461.2	461.2
39	-3.67	462	462
40	-3.66	462.7	462.7
41	-3.64	463	463

TABLE 4

pH 5

S. NO	Log[Cu]	Blank	Okhla
1	-5.88	343.1	309.5
2	-5.7	348.2	312.5
3	-5.58	352.5	315.5
4	-5.4	356.7	323.5
5	-5.28	360.3	330.6
6	-5.18	363.2	335.3
7	-5.1	365.5	339.9
8	-5.03	367.7	343.8
9	-4.98	369.4	347
10	-4.93	371	349.8
11	-4.88	372.5	352.1
12	-4.83	373.8	354.2
13	-4.8	374.9	356.2
14	-4.77	376	358.1
15	-4.74	377	359.6
16	-4.7	378	361.1
17	-4.68	378.8	362.6
18	-4.65	379.6	364.2
19	-4.63	380.6	365.8
20	-4.6	381.3	367.2
21	-4.58	382	368.1
22	-4.41	386.8	376.2
23	-4.29	390.4	382
24	-4.2	393.2	386.1
25	-4.13	395.5	389.2
26	-4.06	397.3	391.7
27	-4.01	398.8	394.8
28	-3.97	400.3	396
29	-3.92	401.5	397.7
30	-3.89	402.6	400
31	-3.86	403.6	401.6
32	-3.83	404.6	403.6
33	-3.8	405.5	405.4
34	-3.77	406.2	406.2
35	-3.75	407	407
36	-3.73	407.6	407.6
37	-3.71	408.3	408.3
38	-3.69	409	409
39	-3.67	409.3	409.3
40	-3.66	409.8	409.8
41	-3.64	410.3	410.3

TABLE 5

pH 5

S. NO	Log[Cu]	Blank	Old Yamuna Bridge
1	-5.88	343.1	313.7
2	-5.7	348.2	318
3	-5.58	352.5	321.1
4	-5.4	356.7	327.7
5	-5.28	360.3	332.8
6	-5.18	363.2	338
7	-5.1	365.5	342
8	-5.03	367.7	345.3
9	-4.98	369.4	348.5
10	-4.93	371	351
11	-4.88	372.5	353.4
12	-4.83	373.8	355.6
13	-4.8	374.9	357.4
14	-4.77	376	359
15	-4.74	377	360.5
16	-4.7	378	362
17	-4.68	378.8	362.3
18	-4.65	379.6	364.5
19	-4.63	380.6	365.8
20	-4.6	381.3	367
21	-4.58	382	368
22	-4.41	386.8	375.8
23	-4.29	390.4	381
24	-4.2	393.2	384.9
25	-4.13	395.5	388.7
26	-4.06	397.3	392.4
27	-4.01	398.8	396.2
28	-3.97	400.3	399.6
29	-3.92	401.5	401.5
30	-3.89	402.6	402.6
31	-3.86	403.6	403.6
32	-3.83	404.6	404.6
33	-3.8	405.5	405.5
34	-3.77	406.2	406.2
35	-3.75	407	407
36	-3.73	407.6	407.6
37	-3.71	408.3	408.3
38	-3.69	409	409
39	-3.67	409.3	409.3
40	-3.66	409.8	409.8
41	-3.64	410.3	410.3

TABLE 6

pH 5

S. NO	Log[Cu]	Blank	Wazirabad
1	-5.88	343.1	315.1
2	-5.7	348.2	317.6
3	-5.58	352.5	320.5
4	-5.4	356.7	326.4
5	-5.28	360.3	332.2
6	-5.18	363.2	336.9
7	-5.1	365.5	341.5
8	-5.03	367.7	344.8
9	-4.98	369.4	347.4
10	-4.93	371	350.2
11	-4.88	372.5	352.2
12	-4.83	373.8	354.7
13	-4.8	374.9	356.9
14	-4.77	376	358.9
15	-4.74	377	360.5
16	-4.7	378	362.2
17	-4.68	378.8	362.6
18	-4.65	379.6	365
19	-4.63	380.6	366.3
20	-4.6	381.3	367.4
21	-4.58	382	368.4
22	-4.41	386.8	376.9
23	-4.29	390.4	382.4
24	-4.2	393.2	386.4
25	-4.13	395.5	389.5
26	-4.06	397.3	392.1
27	-4.01	398.8	394.2
28	-3.97	400.3	396.2
29	-3.92	401.5	398.1
30	-3.89	402.6	400
31	-3.86	403.6	401.9
32	-3.83	404.6	403.8
33	-3.8	405.5	405.5
34	-3.77	406.2	406.2
35	-3.75	407	407
36	-3.73	407.6	407.6
37	-3.71	408.3	408.3
38	-3.69	409	409
39	-3.67	409.3	409.3
40	-3.66	409.8	409.8
41	-3.64	410.3	410.3

TABLE - 7

pH 3

S. NO	Log[Cd]	Blank	Okhla
1	-5.88	-22.3	-28.4
2	-5.77	-21.2	-27.9
3	-5.7	-19.8	-26.9
4	-5.63	-18.5	-25.8
5	-5.58	-17.2	-24.5
6	-5.4	-13.8	-20.2
7	-5.28	-11.2	-17.3
8	-5.18	- 9.	-14.8
9	-5.1	- 7.2	-12.8
10	-5.03	- 5.7	-11
11	-4.98	- 4.3	- 9.4
12	-4.93	- 3	- 7.9
13	-4.88	- 1.9	- 6.6
14	-4.83	- .9	- 5.5
15	-4.8	0	- 4.3
16	-4.77	.9	- 3.3
17	-4.74	1.6	- 2.4
18	-4.7	2.4	- 1.5
19	-4.68	3.1	- .7
20	-4.65	3.7	0
21	-4.63	4.3	.7
22	-4.6	4.9	1.4
23	-4.58	5.4	2.1
24	-4.41	10.3	7
25	-4.29	13.8	11.4
26	-4.2	16.5	15.4
27	-4.13	18.6	18.6
28	-4.06	20.4	20.4
29	-4.01	22	22
30	-3.97	23.4	23.4
31	-3.92	24.6	24.6
32	-3.89	25.7	25.7
33	-3.86	26.7	26.7
34	-3.83	27.6	27.6
35	-3.8	28.6	28.6
36	-3.77	29.4	29.4
37	-3.75	30.3	30.3
38	-3.73	31.1	31.1
39	-3.71	31.8	31.8
40	-3.69	32.4	32.4
41	-3.67	33	33
42	-3.66	34.4	34.4
43	-3.64	35.6	35.6

TABLE - 8

pH 3

S. NO	Log[Cd]	Blank	Old Yamuna Bridge
1	-5.88	-22.3	-25.5
2	-5.77	-21.2	-24.8
3	-5.7	-19.8	-23.8
4	-5.63	-18.5	-22.5
5	-5.58	-17.2	-21.3
6	-5.4	-13.8	-17.8
7	-5.28	-11.2	-15
8	-5.18	- 9.	-12.8
9	-5.1	- 7.2	-10.9
10	-5.03	- 5.7	- 9.1
11	-4.98	- 4.3	- 7.7
12	-4.93	- 3	- 6.3
13	-4.88	- 1.9	- 5.1
14	-4.83	- .9	- 4
15	-4.8	0	- 3
16	-4.77	.9	- 2
17	-4.74	1.6	.2
18	-4.7	2.4	.3
19	-4.68	3.1	.4
20	-4.65	3.7	1
21	-4.63	4.3	1.7
22	-4.6	4.9	2.3
23	-4.58	5.4	3
24	-4.41	10.3	8.1
25	-4.29	13.8	12.6
26	-4.2	16.5	16.5
27	-4.13	18.6	18.6
28	-4.06	20.4	18.6
29	-4.01	22	22
30	-3.97	23.4	23.4
31	-3.92	24.6	24.6
32	-3.89	25.7	25.7
33	-3.86	26.7	26.7
34	-3.83	27.6	27.6
35	-3.8	28.6	28.6
36	-3.77	29.4	29.4
37	-3.75	30.3	30.3
38	-3.73	31.1	31.1
39	-3.71	31.8	31.8
40	-3.69	32.4	32.4
41	-3.67	33	33
42	-3.66	34.4	34.4
43	-3.64	35.6	35.6

TABLE - 9

pH 3

S. NO	Log[Cd]	Blank	Wazirabad
1	-5.88	-22.3	-28.9
2	-5.77	-21.2	-28.1
3	-5.7	-19.8	-27
4	-5.63	-18.5	-26.2
5	-5.58	-17.2	-25.1
6	-5.4	-13.8	-20.8
7	-5.28	-11.2	-17.8
8	-5.18	- 9.	-15.1
9	-5.1	- 7.2	-12.8
10	-5.03	- 5.7	-11.1
11	-4.98	- 4.3	- 9.7
12	-4.93	- 3	- 8.2
13	-4.88	- 1.9	- 6.9
14	-4.83	- .9	- 5.8
15	-4.8	0	- 4.8
16	-4.77	.9	- 3.8
17	-4.74	1.6	- 2.9
18	-4.7	2.4	- 2
19	-4.68	3.1	- 1.1
20	-4.65	3.7	- .4
21	-4.63	4.3	.2
22	-4.6	4.9	.8
23	-4.58	5.4	1.5
24	-4.41	10.3	6.7
25	-4.29	13.8	10.7
26	-4.2	16.5	14.2
27	-4.13	18.6	17.7
28	-4.06	20.4	20.4
29	-4.01	22	22
30	-3.97	23.4	23.4
31	-3.92	24.6	24.6
32	-3.89	25.7	25.7
33	-3.86	26.7	26.7
34	-3.83	27.6	27.6
35	-3.8	28.6	28.6
36	-3.77	29.4	29.4
37	-3.75	30.3	30.3
38	-3.73	31.1	31.1
39	-3.71	31.8	31.8
40	-3.69	32.4	32.4
41	-3.67	33	33
42	-3.66	34.4	34.4
43	-3.64	35.6	35.6

TABLE - 10

pH 5

S. NO	Log[Cd]	Blank	Okhla
1	-5.88	-28	-36
2	-5.77	-26.3	-34.8
3	-5.7	-24.9	-34.9
4	-5.63	-23.4	-25.8
5	-5.58	-17.2	-24.5
6	-5.4	-13.8	-20.2
7	-5.28	-11.2	-17.3
8	-5.18	- 9.	-14.8
9	-5.1	- 7.2	-12.8
10	-5.03	- 5.7	-11
11	-4.98	- 4.3	- 9.4
12	-4.93	- 3	- 7.9
13	-4.88	- 1.9	- 6.6
14	-4.83	- .9	- 5.5
15	-4.8	0	- 4.3
16	-4.77	.9	- 3.3
17	-4.74	1.6	- 2.4
18	-4.7	2.4	- 1.5
19	-4.68	3.1	- .7
20	-4.65	3.7	0
21	-4.63	4.3	.7
22	-4.6	4.9	1.4
23	-4.58	5.4	2.1
24	-4.41	10.3	7
25	-4.29	13.8	11.4
26	-4.2	16.5	15.4
27	-4.13	18.6	18.6
28	-4.06	20.4	20.4
29	-4.01	22	22
30	-3.97	23.4	23.4
31	-3.92	24.6	24.6
32	-3.89	25.7	25.7
33	-3.86	26.7	26.7
34	-3.83	27.6	27.6
35	-3.8	28.6	28.6
36	-3.77	29.4	29.4
37	-3.75	30.3	30.3
38	-3.73	31.1	31.1
39	-3.71	31.8	31.8
40	-3.69	32.4	32.4
41	-3.67	33	33
42	-3.66	34.4	34.4
43	-3.64	35.6	35.6

TABLE - 11

pH 5

S. NO	Log[Cd]	Blank	Okhla
1	-5.88	-28	-39.5
2	-5.77	-26.3	-38
3	-5.7	-24.9	-36.9
4	-5.63	-23.4	-35.8
5	-5.58	-22.2	-32.9
6	-5.4	-18.1	-27.5
7	-5.28	-14.9	-23.4
8	-5.18	-12.5	-20.4
9	-5.1	-10.4	-18
10	-5.03	- 8.7	-15.9
11	-4.98	- 7.4	-14.2
12	-4.93	- 6	-12.6
13	-4.88	- 4.8	-11.3
14	-4.83	- 3.6	-10
15	-4.8	- 2.7	- 8.9
16	-4.77	- 1.8	- 8
17	-4.74	- 1	- 7.1
18	-4.7	.1	- 6.2
19	-4.68	.5	- 5.3
20	-4.65	1.2	- 4.4
21	-4.63	1.8	- 3.7
22	-4.6	2.5	- 3
23	-4.58	3.1	- 2.3
24	-4.41	7.7	1.8
25	-4.29	11	6
26	-4.2	13.6	10
27	-4.13	15.8	13.8
28	-4.06	17.6	17.3
29	-4.01	19.1	19.1
30	-3.97	20.5	20.5
31	-3.92	21.7	21.7
32	-3.89	22.8	22.8
33	-3.86	23.7	23.7
34	-3.83	24.7	24.7
35	-3.8	25.5	25.5
36	-3.77	26.3	26.3
37	-3.75	27	27
38	-3.73	27.6	27.6
39	-3.71	28.3	28.3
40	-3.69	28.9	28.9
41	-3.67	29.5	29.5
42	-3.66	30	30
43	-3.64	30.5	30.5

TABLE - 12

pH 5

S. NO	Log[Cd]	Blank	Wazirabad
1	-5.88	-28	-36
2	-5.77	-26.3	-34.8
3	-5.7	-24.9	-34
4	-5.63	-23.4	-32.8
5	-5.58	-22.2	-31.5
6	-5.4	-18.1	-25
7	-5.28	-14.9	-21
8	-5.18	-12.5	-18.3
9	-5.1	-10.4	-16.1
10	-5.03	- 8.7	-14.2
11	-4.98	- 7.4	-12.7
12	-4.93	- 6	-11.1
13	-4.88	- 4.8	- 9.7
14	-4.83	- 3.6	- 8.5
15	-4.8	- 2.7	- 7.2
16	-4.77	- 1.8	- 6.3
17	-4.74	- 1	- 5.4
18	-4.7	.1	- 4.5
19	-4.68	.5	- 3.7
20	-4.65	1.2	- 2.9
21	-4.63	1.8	- 2.1
22	-4.6	2.5	- 1.5
23	-4.58	3.1	- .8
24	-4.41	7.7	3.8
25	-4.29	11	8
26	-4.2	13.6	11.8
27	-4.13	15.8	15.3
28	-4.06	17.6	17.6
29	-4.01	19.1	19.1
30	-3.97	20.5	20.5
31	-3.92	21.7	21.7
32	-3.89	22.8	22.8
33	-3.86	23.7	23.7
34	-3.83	24.7	24.7
35	-3.8	25.5	25.5
36	-3.77	26.3	26.3
37	-3.75	27	27
38	-3.73	27.6	27.6
39	-3.71	28.3	28.3
40	-3.69	28.9	28.9
41	-3.67	29.5	29.5
42	-3.66	30	30
43	-3.64	30.5	30.5

TABLE - 13

pH 3

Okhla

S. No	Log[Cu]	Log a
1	-5.58	.63
2	-5.7	.64
3	-5.58	.65
4	-5.4	.49
5	-5.28	.44
6	-5.18	.37
7	-5.1	.29
8	-5.03	.24
9	-4.91	.23
10	-4.8	.21
11	-4.72	.17
12	-4.68	.15
13	-4.65	.12
14	-4.63	.09
15	-4.5	.08
16	-4.29	.07
17	-4.2	.06
18	-4.13	.03
19	-4.06	0
20	-4.01	0
21	-3.97	0
22	-3.92	0
23	-3.89	0
24	-3.86	0
25	-3.83	0
26	-3.8	0
27	-3.77	0
28	-3.75	0
29	-3.73	0
30	-3.71	0
31	-3.69	0
32	-3.67	0
33	-3.66	0
34	-3.64	0

TABLE 14

pH 3

Old Yamuna Bridge

S. No	Log[Cu]	Log a
1	-5.88	.81
2	-5.7	.83
3	-5.58	.84
4	-5.54	.69
5	-5.28	.45
6	-5.18	.41
7	-5.1	.36
8	-5.03	.31
9	-4.98	.27
10	-4.9	.23
11	-4.88	.22
12	-4.83	.19
13	-4.8	.16
14	-4.77	.14
15	-4.74	.13
16	-4.7	.12
17	-4.68	.1
18	-4.65	.07
19	-4.62	.05
20	-4.5	.03
21	-4.29	.02
22	-4.2	0
23	-4.13	0
24	-4.06	0
25	-4.01	0
26	-3.97	0
27	-3.92	0
28	-3.89	0
29	-3.86	0
30	-3.83	0
31	-3.8	0
32	-3.77	0
33	-3.75	0
34	-3.73	0
35	-3.71	0
36	-3.69	0
37	-3.67	0
38	-3.66	0
39	-3.64	0

TABLE 15

pH 3

Wazirabad

S. No	Log[Cu]	Log a
1	-5.88	1.34
2	-5.7	1.36
3	-5.58	1.37
4	-5.4	1.08
5	-5.28	.85
6	-5.18	.71
7	-5.1	.56
8	-5.03	.51
9	-4.98	.48
10	-4.93	.4
11	-4.83	.35
12	-4.78	.32
13	-4.74	.3
14	-4.7	.29
15	-4.68	.27
16	-4.65	.24
17	-4.62	.22
18	-4.58	.19
19	-4.41	.15
20	-4.29	.11
21	-4.2	.08
22	-4.13	.05
23	-4.06	.03
24	-4.01	.01
25	-3.97	0
26	-3.92	0
27	-3.89	0
28	-3.86	0
39	-3.83	0
30	-3.8	0
31	-3.77	0
32	-3.75	0
33	-3.73	0
34	-3.71	0
35	-3.69	0
36	-3.67	0
37	-3.66	0
38	-3.64	0

TABLE 16

pH 5

Okhla

S. No	Log[Cu]	Log a
1	-5.88	1.14
2	-5.7	1.21
3	-5.58	1.25
4	-5.4	1.12
5	-5.28	1.01
6	-5.18	.94
7	-5.1	.87
8	-5.03	.81
9	-4.98	.76
10	-4.93	.72
11	-4.88	.69
12	-4.83	.66
13	-4.8	.63
14	-4.77	.61
15	-4.74	.59
16	-4.7	.57
17	-4.68	.55
18	-4.63	.52
19	-4.63	.5
20	-4.6	.48
21	-4.58	.47
22	-4.41	.36
23	-4.29	.28
24	-4.2	.24
25	-4.13	.19
26	-4.06	.16
27	-4.01	.15
28	-3.97	.13
39	-3.92	.12
30	-3.89	.09
31	-3.86	.06
32	-3.83	.03
33	-3.8	0
34	-3.77	0
35	-3.75	0
36	-3.73	0
37	-3.71	0
38	-3.69	0
39	-3.67	0
40	-3.66	0
41	-3.64	0

TABLE 17

pH 5

Old Yamuna Bridge

S. No	Log[Cu]	Log a
1	-5.88	.99
2	-5.7	1.02
3	-5.58	1.06
4	-5.4	.98
5	-5.28	.93
6	-5.18	.85
7	-5.1	.8
8	-5.03	.76
9	-4.98	.7
10	-4.93	.68
11	-4.88	.63
12	-4.83	.62
13	-4.8	.59
14	-4.77	.58
15	-4.74	.56
16	-4.7	.54
17	-4.68	.52
18	-4.63	.51
19	-4.63	.5
20	-4.6	.48
21	-4.58	.47
22	-4.41	.37
23	-4.29	.32
24	-4.2	.28
25	-4.13	.23
26	-4.06	.17
27	-4.01	.09
28	-3.97	.02
29	-3.92	0
30	-3.89	0
31	-3.86	0
32	-3.83	0
33	-3.8	0
34	-3.77	0
35	-3.75	0
36	-3.73	0
37	-3.71	0
38	-3.69	0
39	-3.67	0
40	-3.66	0
41	-3.64	0

TABLE 18

pH 5

Wazirabad

S. No	Log[Cu]	Log a
1	-5.88	.95
2	-5.7	1.04
3	-5.58	1.08
4	-5.4	1.02
5	-5.28	.95
6	-5.18	.89
7	-5.1	.81
8	-5.03	.77
9	-4.98	.74
10	-4.93	.7
11	-4.88	.68
12	-4.83	.65
13	-4.8	.61
14	-4.77	.58
15	-4.74	.56
16	-4.7	.53
17	-4.68	.51
18	-4.63	.49
19	-4.63	.48
20	-4.6	.47
21	-4.58	.46
22	-4.41	.34
23	-4.29	.27
24	-4.2	.23
25	-4.13	.2
26	-4.06	.18
27	-4.01	.16
28	-3.97	.14
29	-3.92	.12
30	-3.89	.09
31	-3.86	.06
32	-3.83	.03
33	-3.8	0
34	-3.77	0
35	-3.75	0
36	-3.73	0
37	-3.71	0
38	-3.69	0
39	-3.67	0
40	-3.66	0
41	-3.64	0

TABLE - 19

pH 3

Okhla

S. NO	**Log[Cd]**	**Log a**
1	-5.88	.21
2	-5.77	.23
3	-5.7	.24
4	-5.63	.25
5	-5.58	.25
6	-5.4	.22
7	-5.28	.21
8	-5.18	. 2
9	-5.1	.19
10	-5.03	.18
11	-4.96	.17
12	-4.86	.16
13	-4.8	.15
14	-4.76	.14
15	-4.68	.13
16	-4.62	.12
17	-4.5	.11
18	-4.29	.08
19	-4.2	.04
20	-4.13	.01
21	-4.06	0
22	-4.01	0
23	-3.97	0
24	-3.92	0
25	-3.89	0
26	-3.86	0
27	-3.83	0
28	-3.8	0
29	-3.77	0
30	-3.75	0
31	-3.73	0
32	-3.71	0
33	-3.69	0
34	-3.67	0
35	-3.66	0
36	-3.64	0

TABLE - 20

pH 3

Old Yamuna Bridge

S. NO	**Log[Cd]**	**Log a**
1	-5.88	.11
2	-5.77	.12
3	-5.7	.13
4	-5.53	.14
5	-5.15	.13
6	-4.88	.11
7	-4.71	.1
8	-4.6	.09
9	-4.35	.06
10	-4.2	.03
11	-4.13	.01
12	-4.06	0
13	-4.01	0
14	-3.97	0
15	-3.92	0
16	-3.89	0
17	-3.86	0
18	-3.83	0
19	-3.8	0
20	-3.77	0
21	-3.75	0
22	-3.73	0
23	-3.71	0
24	-3.69	0
25	-3.67	0
26	-3.66	0
27	-3.64	0

TABLE - 21

pH 3

Wazirabad

S. NO	Log[Cd]	Log a
1	-5.88	.23
2	-5.77	.24
3	-5.7	.25
4	-5.53	.26
5	-5.58	.27
6	-5.4	.24
7	-5.28	.23
8	-5.18	.21
9	-5.1	.19
10	-4.98	.18
11	-4.86	.17
12	-4.79	.16
13	-4.71	.15
14	-4.64	.14
15	-4.58	.13
16	-4.41	.12
17	-4.29	.1
18	-4.13	.06
19	-4.06	.03
20	-4.01	0
21	-3.97	0
22	-3.92	0
23	-3.89	0
24	-3.86	0
25	-3.83	0
26	-3.8	0
27	-3.77	0
28	-3.75	0
29	-3.73	0
30	-3.71	0
31	-3.69	0
32	-3.67	0
33	-3.66	0
34	-3.64	0

TABLE - 22

pH 5

Okhla

S. NO	Log[Cd]	Log a
1	-5.88	.27
2	-5.77	.29
3	-5.7	.31
4	-5.63	.32
5	-5.58	.33
6	-5.4	.25
7	-5.28	.23
8	-5.14	.21
9	-5.03	.2
10	-4.98	.19
11	-4.88	.18
12	-4.75	.16
13	-4.67	.15
14	-4.6	.14
15	-4.41	.12
16	-4.2	.08
17	-4.13	.06
18	-4.06	.02
19	-4.01	0
20	-3.97	0
21	-3.92	0
22	-3.89	0
23	-3.86	0
24	-3.83	0
25	-3.8	0
26	-3.77	0
27	-3.75	0
28	-3.73	0
29	-3.71	0
30	-3.69	0
31	-3.67	0
32	-3.66	0
33	-3.64	0

TABLE - 23

pH 5

Old Yamuna Bridge

S. NO	Log[Cd]	Log a
1	-5.88	.39
2	-5.77	.4
3	-5.7	.41
4	-5.63	.42
5	-5.58	.43
6	-5.4	.32
7	-5.28	.29
8	-5.18	.27
9	-5.1	.26
10	-5.03	.25
11	-4.96	.23
12	-4.86	.21
13	-4.73	.2
14	-4.68	.19
15	-4.63	.18
16	-4.5	.16
17	-4.29	.1
18	-4.2	.05
19	-4.13	.01
20	-4.06	0
21	-4.01	0
22	-3.97	0
23	-3.92	0
24	-3.89	0
25	-3.86	0
26	-3.83	0
27	-3.8	0
28	-3.77	0
29	-3.75	0
30	-3.73	0
31	-3.71	0
32	-3.69	0
33	-3.67	0
34	-3.66	0
35	-3.64	0

TABLE - 24

pH 5

Wazirabad

S. NO	Log[Cd]	Log a
1	-5.88	.27
2	-5.77	.29
3	-5.7	.31
4	-5.63	.32
5	-5.58	.28
6	-5.4	.24
7	-5.28	.21
8	-5.14	.2
9	-5.03	.19
10	-4.98	.18
11	-4.88	.17
12	-4.72	.15
13	-4.65	.14
14	-4.5	.11
15	-4.29	.08
16	-4.2	.06
17	-4.13	.02
18	-4.06	0
19	-4.01	0
20	-3.97	0
21	-3.92	0
22	-3.89	0
23	-3.86	0
24	-3.83	0
25	-3.8	0
26	-3.77	0
27	-3.75	0
28	-3.73	0
29	-3.71	0
30	-3.69	0
31	-3.67	0
32	-3.66	0
33	-3.64	0

TABLE 25

Cu pH - 3

Okhla

S No	a/[Cu]	Y
1	3.24	31.71
2	2.19	20.8
3	1.7	16.98
4	.77	11.88
5	.52	9.97
6	.35	8.75
7	.25	8.27
8	.19	7.6
9	.14	6.33
10	.1	5.26
11	.08	5.19
12	.023	4.19
13	.018	3.93

TABLE 26

Cu pH - 3

Old Yamuna Bridge

S No	a/[Cu]	Y
1	3.39	18.82
2	2.63	15.22
3	1.23	11.1
4	.54	9.45
5	.39	8.63
6	.29	7.95
7	.22	6.99
8	.16	6.68
9	.13	6.18
10	.1	6.11

TABLE 27

Cu pH - 3

Wazirabad

S No	a/[Cu]	Y
1	3.02	8.77
2	1.35	7.1
3	.78	6.02
4	.46	5.56
5	.35	4.96
6	.29	4.57
7	.21	4.53
8	.15	3.91
9	.13	3.7
10	.11	3.53
11	.07	3.31
12	.02	3.0

TABLE 28

Cu pH - 5

Okhla

S No	a/[Cu]	Y
1	6.76	12.89
2	3.31	8.7
3	1.95	6.76
4	1.32	5.47
5	.93	4.66
6	.69	4.06
7	.55	3.7
8	.45	3.36
9	.37	3.05
10	.31	2.77
11	.27	2.64
12	.24	2.5
13	.21	2.37
14	.19	2.19
15	.15	2.05
16	.13	2
17	.12	1.9
18	.11	1.84
19	.06	1.46
20	.03	1.31
21	.02	1.19
22	.01	1.22

TABLE 29

Cu pH - 5

Old Yamuna Bridge

S No	a/[Cu]	Y
1	7.41	27.04
2	5.25	17.73
3	4.37	15.33
4	2.4	8.98
5	1.62	6.91
6	1.07	5.64
7	.79	4.79
8	.62	4.15
9	.4	4.02
10	.41	3.44
11	.32	3.17
12	.28	2.85
13	.25	2.72
14	.22	2.56
15	.2	2.43
16	.17	2.25
17	.16	2.19
18	.14	2.07
19	.13	2
20	.12	1.9
21	.11	1.84
22	.06	1.43
23	.04	1.2
24	.03	1.07
25	.02	1.059

TABLE 30

Cu pH - 5

Wazirabad

S No	a/[Cu]	Y
1	5.5	16.65
2	4.57	13.27
3	2.63	8.89
4	1.7	6.87
5	1.17	5.56
6	.81	4.77
7	.63	4.13
8	.52	3.74
9	.43	3.4
10	.36	3.07
11	.3	2.79
12	.26	2.68
13	.2	2.56
14	.17	2.43
15	.15	2.28
16	.14	2.22
17	.13	2.11
18	.12	2.04
19	.11	1.93
20	.06	1.86
21	.04	1.52
22	.03	1.35
23	.02	1.23
24	.02	1.17
25	.01	1.08

T A B L E - 31

Cd Okhla

pH - 3

S.No.	a/[Cd]	Y
1	1.23	63.31
2	1	45.83
3	.87	37.78
4	.76	31.19
5	.68	27.8
6	.42	20.22
7	.31	15.9
8	.24	13.12
9	.19	11.37
10	.16	10.11
11	.13	9.01
12	.1	7.52
13	.09	6.91
14	.08	6.68
15	.06	5.92
16	.05	5.53
17	.04	4.52
18	.02	3.71

T A B L E - 32

Cd Old Yamina Bridge

pH - 3

S.No.	a/[Cd]	Y
1	.98	98
2	.78	78.05
3	.68	68
4	.47	45.35
5	.19	17.47
6	.1	10.85
7	.06	7.98
8	.05	6.81
9	.03	5.76

TABLE - 33

Cd Wazirabad

pH - 3

S.No.	a/[Cd]	Y
1	1.29	59.04
2	1.02	44.38
3	.89	36.64
4	.78	30.3
5	.71	26.28
6	.44	18.93
7	.32	14.83
8	.25	12.63
9	.19	11.37
10	.14	9.01
11	.11	7.16
12	.09	6.4
13	.07	5.75
14	.06	5.07
15	.05	4.7
16	.03	3.41
17	.03	2.79
18	.02	3.01

TABLE - 34

Cd Okhla

pH - 5

S.No.	a/[Cd]	Y
1	1.41	52.43
2	1.15	41.68
3	1.02	37.43
4	.89	33.18
5	.81	29.86
6	.45	18.37
7	.32	14.34
8	.22	11.52
9	.17	9.29
10	.15	8.62
11	.11	7.15
12	.08	5.84
13	.07	5.12
14	.05	4.62
15	.03	3.8
16	.02	3.9

TABLE - 35

Cd Old Yamuna Bridge

pH - 5

S.No.	a/[Cd]	Y
1	1.86	50.96
2	1.48	39.31
3	1.29	34.25
4	1.12	31.02
5	1.02	26.36
6	.52	15.42
7	.37	12.52
8	.28	10.46
9	.23	8.94
10	.19	7.83
11	.15	7.1
12	.12	5.83
13	.09	4.48
14	.08	4.15
15	.07	3.85
16	.05	2.98
17	.03	1.93
18	.02	2.1

T A B L E - 36

Cd Wazirabad

pH - 5

S.No.	a/[Cd]	Y
1	1.41	38.43
2	1.15	38.69
3	1.02	35.43
4	.89	30.18
5	.79	27.33
6	.44	18.93
7	.31	15.9
8	.22	11.97
9	.17	9.63
10	.14	9.01
11	.11	7.49
12	.07	5.75
13	.06	5.19
14	.04	3.91
15	.02	3.9