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STUDIES ON THE METAL BINDING CHARACTERISTICS OF HUMIC ACIDS

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C E R T I F I C A T E

This dissertation entitled 'Studies on the Metal Bending characteristics of Humic Acids' embodies the work carried out at the school of Environmental sciences, Jawaharlal Nehru University, New Delhi. This work has not been submitted in part or full for any degree or diploma of any University.

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CHAPTER I

INTRODUCTION

Environmental concern for water resources has in recent years led to intensive research on the chemistry of natural waters. While water is the most abundant natural resources on earth, its distribution is not uniform. As world population increases, the demand for water for food production, industrial activities and domestic purposes grows and leads to heavier withdrawals of the limited renewable freshwater resources. Simultaneously, these very same human activities generate wastes which are discharged into the depleted water resources, thus polluting them. Thus the world at large is facing the dual problem of increasing demands on limited freshwater resources coupled with the growing degradation of those very same water resources which may seriously reduce their quality and utility. Thus today, the question of preserving and managing water resources is coupled intimately with the question of preserving and managing waterquality.

Both natural and man-made processes release the various elements in the earth's crust into the hydrosphere among which the metals constitute a sizeable section. The metals usually involve geochemical as well as biological recycling processes thereby forming the earth's biogeochemical cycles. Except the metals such as Ca, Mg, K, etc. which act as nutrient elements for both plant and animal life, others like Hg, Pb, Cu, Zn, Cd, etc. known as heavy metals are toxic enough to cause harmful effects for the life system. These toxic heavy metals generally find their way into the water system through industrial activities, agriculture and urbanisation. Thus the heavy metal pollution has become a grave danger for the substenance of life system on the earth's surface. The chemical, biological and ecological consequences of excessive toxic heavy metal discharges entering the ecosphere, particularly aquatic systems have become the aspects of intensive research in recent years.

Natural water is generally regarded as a complex heterogeneous electrolyte system containing inorganic and organic species distributed between aqueous and solid phases. Heavy metals entering natural water become part of this system and their distribution processes are controlled by a dynamic set of physico-chemical interactions and equilibria. The heavy metals distribute themselves mostly along with the organics which constitute an important part of the soil and water systems.

Although a classification of aquatic organic matter is difficult, an examination of recent literature reviews indicate that the major groups of organics are polysaccharides,proteins and peptides, pedogenic (soil derived), and aquogenic (formed in situ in the water body) organic matter. The last two groups form the so called "humic substances" which represent a large proportion (70 - 80 %) of the organic matter in natural waters (Buffle, et.al.,1984). Humic substances are probably the most widely distributed natural products on the earth's surface, occuring in soils, lakes, rivers, and the sea. These are amorphous, brown or black, hydrophilic, acidic, polydisperse substances of molecular weights ranging from several hundreds to tens of thousands (Schnitzer, et.al.,1972). Humic substances arise from the chemical and biological degradation of plant and animal residues and from synthetic activities of microorganisms. When an organic redidue is incorporated into water or soil and environmental conditions are favourable, the microorganisms immediately begin to utilize it as a source of carbon and energy. The plant and animal polymers, including poly-saccharides, lignins and proteins are fast degraded to smaller organic molecules. These are utilized by organisms for synthesis of cell substances, or other metabolic products which are further degraded into simple inorganic substances such as CO_2, H_2O and NH_3 or undergo enzymatic and chemical reactions to form new polymers. These new polymeric substances are relatively more stable than the starting material and are popularly known as "humic substances" (Martin, et.al. 1971).

Based on their solubility in alkali and acid, humic substances are usually partitioned into the following three main fractions: (a) Humic acid (HA), which is soluble in dilute alkali, but is coagulated by acidification of the alkaline extract; (b) Fulvic acid (FA), which is that humic fraction which remains in solution when the alkaline extract is acidified, that is, it is soluble in both dilute alkali and dilute acid and (c) Humin, which is that humic fraction which can not be extracted from the soil by dilute acid or (Schnitzer, 1972). Recent studies show that the humic and fulvic acids are a group of compounds of a complex nature whose structures involve a large proportion of more or less condensed aromatic nuclei, with a large number of functional groups such as carboxyls (-COOH), phenolic and alcoholic hydroxyls (-OH), and carbonyls (>C=0) fixed on them (Schnitzer, 1970).

The humic and fulvic acids dissolved in natural waters have attracted increasing interest in recent years not only because of their involvement in marine food chains and organic geochemical cycles, but also because of their ability to form complexes with metals (Mantoura, et.al., 1978). These acids participate in complex formation with metallic elements through their ionizable functional groups with a wide range of differential acidities (Reuter, et.al. 1977). What is important is that they have the capacity of binding substantial amounts of metals and can thus excert considerable control over the supply and availability of nutrient as well as toxic metals to plants and animals in soils and waters. The complexes so formed are either water-soluble or water-insoluble and they play a significant role in the migration or the accumulation of metal cations. The absorption of toxic heavy metals by microorganisms and higher plants and animals is very much influenced by the binding capacities of HA's and FA's.

Although the purely inorganic species present in fresh water have been extensively studied, the interactions of metals with HA's and FA's present in such waters have not been investigated. Recently, studies are now being concentrated upon the distribution of the metal and HA & FA species with respect to each other, the side reactions with other macro-components of water, the rate of complex formation, and in general, the mechanism of trace metal complex formation in water and soil media.

The application of several quantitative models for the prediction of the behaviour of heavy metals towards HA's and FA's has received special attention in recent days. The models include various types of plotting methods (Fitch, et.al. 1984) such as:

a) Scatchard Plot,
b) Single surface Langmuir plot,
c) Double surface Langmuir plot,
d) Double reciprocal plot,
e) Hill plot.

These models were previously used to examine the ligand binding characteristics of proteins and macromolecules and are now being used to determine the mechanism of complexation reactions between heavy metals and HA's and FA's. According to the recent studies (Fitch, et.al. 1984; Bresnahan, et.al,1978; Zunino, et.al. 1977; Buffle, et.al. 1977; Sposito, et.al.1979; Saar, et.al. 1979, 1980a, 1980b, 1980c; Gamble, et.al. 1970; Cornish-Bowden, et.al. 1975 and 1970; Klotz, 1982; Munson, et.al. 1983; Perdue, et.al. 1983; Sanders, 1980; Sposito, 1982, etc.) these mathematical models are found to explain satisfactorily the various physico-chemical aspects of complexation between heavy metals and humic substances. Proliferation of modelling techniques for calculating different theoretical parameters of metal complexes with humic bubtances has led to confusion in interpreting published data and in comparing stability constants of metal-humate and metal-fulvate complexes from various sources. However, these recent models can be shown to have a common basis in that they represent modifications of Adair's equation, which has been used extensively to examine the ligand-binding properties of proteins and other macromolecules of biological importance (Fitch, et.al.1984).

CHAPTER - II

REVIEW OF LITERATURE

The physiological, physico-chemical and biochemical properties of humic substances have received careful attention only in the recent past. The significance of humic substances such as humic and fulvic acids as regards to their origin in water and soil environments, molecular structure, physico-chemical properties, especially their interaction with heavy metal cations in water and soil have been the topics of interest by reseachers in the last decade.

The origin of humic and fulvic acids in soils and natural waters have been studied by many workers. There is increasing evidence that the primary source of aquatic humus are soil and marsh leachates, polyphenolic components of plant exudates and decomposition products of plankton and benthos (Prakash, et.al., 1972). Serra, et.al., in 1972 noted that the soil and aquatic humus is a complex heterogeneous mixture of biological residues in various stages of biological and chemical alteration. Cellulose, proteins, and fats are readily available carbon sources for the micro-organisms where as such compounds as lignin and other phenolic plant constituents are decomposed more slowly (Flaig, et.al., 1975). Studies with C-14 labelled plant material have shown that glucose and other low molecular weight organic compounds are much prone to decomposition under aerobic conditions whereas celluloses, hemicelluloses, and lignins are very slowly decomposed by microorganisms which can remain in the water and soil system of a longer period (Flaig, et.al., 1975). They also noticed some amount of lignin, or its degradation products, such as P-hydroxybenzaldehyde, Vanillin, etc. in the isolated humic acid fractions. After a series of investigations, Martin, et.al. (1971) concluded that lignins are phenolic polymers which form a major plant constituent and are relatively resistant to microbial decomposition. They are generally considered to be a major, it not the primary source of phenolic units for humic acid synthesis. Numerous organisms are able to slowly decompose lignin or its partial decomposition products. According to Martin, et.al. when an organic residue is incorporated into water or soil and environmetal conditions are favourable, the micro-organisms immediately begin to utilize it as a souce of carbon and energy. The plant and animal polymers, including polysaccharides, lignins and proteins are fast degraded to smaller organic molucules. These are utilized by organisms for synthesis of cell substances, or other metabolic products are further degraded into simple inorganic subnstances such as CO_2, H_2O and NH_3 or undergo enzymatic and chemical reactions to form new polymers. Some of the new substances are relatively resistant to decomposition or form resistant complexes with metal ions or clays and constitute an important fraction of humus. They also found that many of the simple phenolic compounds, derived from lignin or synthesized by soil organisms can be transformed into active phenolic radicals and quinones by the action of phenoloxidases produced by white rot fungi and other microorganisms. These enzymes remove a hydrogen atom from a phenolic hydroxyl. The radicals thus formed stabilize by linking together or by further oxidation to quinones. The latter compounds react with other phenolic compounds, by nucleophilic attack or with amino acids or peptides to form humic acids. Model polymers formed either upon oxidation of phenols with phenolases or mild oxidising reagents have properties similar to HA's, especially if amino acid compounds are present in the reaction mixture. Flaig, et.al. (1975) examined the elementary composition of lignin and humic acids and showed that the carbon content of the lignin of different plants was higher than that of humic acids. They also concluded that the decrease of the carbon content and the increase of the oxygen content are the results of oxidative processes which play an important role during the transformation of lignin into humic acids.

Roletto, et.al. (1985) have extracted humic acids from humified poplar bark, which has substantial amounts of cellulose, lignin, fats, waxes, resins, and tanins. They also extracted humic substances from composted lignocellulosic residues. Haider, (1987) pointed out that during biodegradation of plant materials and their constituents, lignins can acquire properties that are characteristics of soil humic compounds. Kogel, et.al. (1987) found that the lignin content decreases with increasing decomposition in forest humus layers and the caboxyl carbon atom of the HA's is due to the oxidation of lignin. Kyuma (1987) studied humification in Paddy soils and found that the humification started with the decomposition of rice roots, and stubbles, aquatie weeds and phytoplanktons and the resultant organic debris under anaerobic condition possesses the character of humic acids with more or less lignin like nature. Neue, H.U.(1987) also investigated the decompositon of rice straw in flooded soils in the above manner. Lax, et.al. (1987) studied extensively on the humification of organic wastes used as manures. Fillip, et.al. (1987) found that Spartina alterniflora (Loisel), the dominant plant species of the tidal salt marsh on the Atlantic coast of the USA is a potential source of salt marsh humic acids. Saiz-Jimenez (1987) studied the nature and fate of plant components involved in soil humus formation with special reference to the formation of humic acid-like polymers from Olive vegetation matter. Gadel, (1987) isolated humic acid from deposits of a blue green lake on the ice-cap (Greenland), where there is a profound growth of blue algae. Shanmukhappa, et.al. (1987) studied the origin and distribution of humic acids in coastal aquatic biotopes of Porto Novo, India which is surrounded by thick mangrove and other vegetations.

Firstly, in 1972, Schnitzer pointed that humic materials in soils are very much similar to those occuring in fresh water and in the ocean. Visser, (1983) conducted a comparative study of humic acids of aquatic origin and from soils and microbial substrates and found that aquatic humus had lower carbon and nitrogen and higher oxygen contents that humic compounds isolated from soils. Reuter, et.al. (1977) also found striking similarities between aquatic humics and soil humic substances and suggested a soil origin for at least a part of the aquatic humic matter. In freshwater ecosystems, the submerged plants which represent the basic structural constituents of many aquatic ecosystems contribute mostly to the total humus content (Ulehlova, 1978).

Many investigators have adopted different extraction procedures for isolating humic acids from soil, peat, humified organic matter and natural waters. Of the large number of extractants that have been tested, dilute aqueous sodium hydroxide solution remains the most commonly used and quantitatively the most effective reagent for extracting humic acids from soils or sediments (Schnitzer and Khan, 1972). According to them and several other workers, NaOH solution of strength 0.1 N is generally preferred for exraction. Schnitzer and Khan made a critical review of various other extraction procedures which include extractants like $Na_4P_2O_7$ solution, cation exchange resins

(Dowex A-1, Amberlite IRC-50, Dowex-50, etc), organic solvents (aqueous solutions of acetylacetone, hexamethylenetetramine, dodecylsulphate, urea, formic acid, and phenols). Tan, et.al. (1986), and Takamatsu, et.al. (1977) used 0.1 M NaOH solution as the extractant for the isolation of humic acids. Cheshire, et.al.(1977) prescribed 0.2 N NaOH solution for extracation. The second most important extractant $Na_4P_2O_7$ solution has been used effectively by many other workers such as Stevenson; et.al. (1973), Fitch, et.al. (1984) (0.15 M $Na_4P_2O_7$ solution) and Cacco et.al. (1984) (0.1 N $Na_4P_2O_7$ solution).

The chemical structure of HA's and FA's have been investigated by many researchers with the use of modern analytical techniques such as molecular spectroscopy (UV, IR, NMR, etc.). A review of Cranwell, et.al. (1972) shows that the IR slpectrum of humic acids shows peaks corresponding to -OH & -COOH groups and a band at 1615 cm^{-1} is attributable to $-COO^-$ or to the aromatic structure. They suggested a perylene quinone structure for humic acids as revealed by visible and UV spectra. The x-ray powder photographs show that the humic material is amorphous in nature and the NMR spectra is attributed to a low concentration of aromatic protons. Hatcher, et.al. (1981) used 13C NMR technique and found the aromaticity of humic acids from 35 to 92% which indicated the presence of significant aliphatic structures. Schnitzer, et.al. (1965) found the presence of ketonic C=O group and C=N bond as revealed by spectrophotometric studies. 13C NMR studies by Mikita et.al. (1981) show four bands which indicate aliphatic, hydroxyl, aromatic and carboxyl regions in humic acid molecules. 13C NMR spectra by Preston, et.al. (1984) of methylated HA's and FA's indicat that almost all -COOH groups are attached to aromatic structures and that phenolic -OH's are significant functional groups. The spectra also exhibit fine structures possibly due to benzene-carboxylic acid-type structures which have been proposed as major building blocks of HA's and FA's. Wagner, et.al. (1965) concluded that one third of the -COOH groups in HA's occured in positions close enough to form cyclic anhydrides and two-thirds of the -OH groups are phenolic as revealed by IR spectra.

The interaction of humic acids with heavy metals have been studied by many workers only in the recent past. All through these years, a number of experimental methods have been developed and used successfully to determine the nature of heavy metal-humic acid complexation. These include:

a) Potentiometric titrations,
b) Job's method of continuous variation,
c) Ion exchange equilibrium method,
d) Ion selective electrodes (ISE),
e) Gel permeation chromatography,
f) Differential pulse polarography, and
g) Differential pulse anodic stripping voltametry.

Stevenson (1977) used the method of potentiometric titration for determining stability constants which involved sequential addition of metal ions to solutions of humic acids at constant pH. Sposito, et.al. (1978) also used this method for studying the metal-FA interactions. Other researchers who have used effectively this method are Van Dizk (1971), Smith, et.al. (1973), Sposito (1977). Stevenson (1976, 1973), etc. The ion exchange equilibrium method has been adopted by unino, et.al. (1972), Ardakani, et.al. (1972), Schnitzer, et.al. (1966), Randhawa, et.al. (1965) and Gamble, et.al. (1970). Job's method of continuous variation was used by Schnitzer, et.al. (1970) and Crosser et.al. (1977). The method of ion-selective electrodes has been used most extensively to investigate metal -HA and -FA interactions by many researchers such as: Fitch, et.al. (1984), Takamatsu, et.al. (1978), Kerven, et.al. (1984), Cheam, et.al.(1974), Saar, et.al. (1980), Bresnahan, et.al. (1978), and Buffle, et.al. (1977). Mantoura, et.al. (1975) used successfully gel permeation chromatographic method to metal-organic interactions. Ernst, et.al. (1975) used the methods of Differential pulse polarography and differential pulse anodic stripping voltametry to determine stability constants of heavy metal-Humic acid systems.

In recent years, many quantitative models have been developed to predict and characterize the behaviour of heavy metals towards HA's and FA's. These mathematical procedures help in determining certain important complexation parameters such as:

a) degree of complexation,

b) Maximum binding ability (MBA),

c) a quantity 0, which is defined as the number of sites bound per total no. of reactive sites.

d) Total number of binding sites, and

e) conditional stability constants.

Buffle, et.al. (1977) made the use of ion selective electrodes for the measurement of the complexation properties of HA's and FA's. The model proposed by them helps in determining the values for the mean molecular weight of the ligand (HA's or FA's), the stability constants of the complexes, number of ligands fixed per metal ion, and the dependency of the stability of the complexes on pH. Buffle, et.al. calculated 'α', the degree of complexation by using the following equation:

$$\alpha = \frac{[Me]_t}{[Me]} \qquad \text{...............} \quad (1)$$

Where $[Me]_t$ = total concentration of the metal ion and
$[Me]$ = concentration of metal ions present in the ionic form.

The equation (1) can also be expressed as:

$$\alpha = [Me]_t \times 10^{(Eo - E)/P} \qquad \text{................} \quad (2)$$

where $\frac{1}{[Me]} = 10^{(Eo-E)/P}$

Here Eo = Standard electrode potential of the electrode.

E = electrode potential measured during the titration, and

P = the slope of the electrode.

The same method was followed by Kerven, et.al. (1984) for the calculation of 'α', the degree of complexation.

Buffle, et.al. (1977) also derived the following mathematical expression for the determination of average molecular weight of humic and fulvic acids as well as the conditional stability constants of metal-humate and-fulvate complexes.

$$Y = \frac{[Lt]}{[Me]_t} \cdot \frac{\alpha}{\alpha - 1} = \frac{mw}{n} + \frac{mw}{n} \cdot \frac{[H]^x}{\beta_1{}^*} \cdot \frac{\alpha}{[Me]_t} \quad \ldots.(3)$$

Where [Lt] = the concentration of HA's or FA's in gm/L,

mw = the molecular weight of HA or FA,

n = number of binding sites,

x = number of displaced protans, and

β_1^* = the formation constant.

The factor $\frac{\beta_1^*}{[H]^x}$ in the above equation is a measure of the conditional stability constant.

Zunino, et. al. (1977) calculated maximum binding ability (MBA) by applying single surface Langmuir equation to metal-humic acid systems:

$$\frac{c}{x} = \frac{c}{k_1} + \frac{1}{k_1 k_2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \ (4)$$

Where x = solute adsorbed per unit weight of the colloid.

c = concentration of solute at equilibrium,

k_1 = a constant that represents a maximum adsorption,

k_2 = a constant indicating the affinity between the adsorbate and the adsorbant.

Plotting c/x against c yields a straight line whose slope is $1/k_1$ and the intercept is $1/k_1k_2$. From the slope k_1 (MBA) can be calculated.

Fitch, et.al. (1984) reviewed and applied some more theroetical methods for determining conditional stability constants of metal complexes with humic and fulvic acids. These methods were shown to represent graphical modifications of the basic equation, i.e.:

$$\theta = Ko\ (M)\ /\ 1 + Ko\ (M) \qquad \ldots\ldots\ldots\ldots\ldots\ldots \ (5)$$

where Ko = conditional stability constant,

M = concentration of the metal present in the ionic form,

θ = Sites bound/total number of reactive sites

= $(Mb)/n(Lt)$, where

Mb = concentration of the metal present in the bound form, and

$n(Lt)$ = The maximum binding ability (MBA).

The eqn. (5) is actually a modification of the Adair's eqn. which was previously used to explain the metal-binding properties of proteins and other macromolecules. The modified form of Adair's equation (5) can be arranged in a number of ways in order to get different equations which yield information about the number of classes of sites and the stability constant for each class. Plotting can be done as a Scatchard plot ($\theta/(M)$ vs. θ), a reciprocal plot

{(M)/(Mb) vs. (M)}, a double reciprocal plot {(Lt)/(Mb) vs. 1/(M)} and in some cases, as a Hill plot (Log $\theta/(1-\theta)$ vs. Log (M)}. The various equations and plotting variable are listed in the following table:

PLOT TITLE	FORM OF THE EQUATION	PLOT
		X Y
Scatchard	$\theta/(M) = Ko - \theta\ Ko$	θ/M vs. θ....(6)
Reciprocal	$(M)/(Mb) = \frac{M}{n(Lt)} + \frac{1}{n(Lt)Ko}$	$\frac{M}{(Mb)}$ vs.(M)...(7)
Double reciprocal	$\frac{(Lt)}{(Mb)} = \frac{1}{n} + \frac{1}{n\ Ko(M)}$	$\frac{1}{(Mb)}$ vs. $\frac{1}{(M)}$...(8)
Hill	$\mathrm{Log}\frac{\theta}{(1-\theta)} = \mathrm{Log}\ K^{*} + n\ \log(M)$	Log $\frac{\theta}{(1-\theta)}$ vs.Log(M) ...(9)

The double reciprocal plot was shown to be identical with the model proposed earlier by Buffle, et.al. (1977). Similarly, the reciprocal plot has the same form as the single surface Langmuir equation as proposed earlier by Zunino, et.al. (1977), thus showing that the quantity k_2 in equation (4) is equal to the conditional stability constant Ko. The Hill plot method was also derived in another way by Zunino and Martin (1977) to determine conditional stability constants for the reaction of Cu (II) with a soil humic acid:

$$\text{Log} \frac{(\text{Mb})}{\text{MBA} - (\text{Mb})} = \text{Log K} + \text{n Log (M)} \text{ ....(10)}$$

It should be noted here that the Ko, in Scatchard, Langmuir and Double reciprocal plots is micro-scopic stability constant whereas the K used in the above equation is macroscopic stability constant. The Hill plot was found to be useful in explaining cooperative binding of metal ions with HA's (Fitch, et.al. 1984 and Cornish-Bowden, et.al., 1975).

Sposito (1982) and Fitch, et.al. (1984) found that the application of single surface Langmuir equation produced non-linear curve with metal-humic acid systems. The linear treatment of Langmuir plots to obtain estimates for MBA, as done by Zunino, et.al. (1977) would appear to be of questionable value for most studies of the binding of metal ions to HA's. Therefore, they suggested a double-surface Langmuir equation which has the form:

$$(M) = [\, b_1 - (Mb)/K_1(M)\,] + [b_2 - (Mb)/K_2(M)] \text{.....(11)}$$

where b_1 and b_2 are site concentrations and K_1 and K_2 are constants related to the binding energy. b_2 corresponds to the binding at high metal ion saturation and is a correct measure of the maximum binding ability (MBA).

Fitch, et.al. (1984), after a series of investigations concluded that the Scatchard plot method for determining conditional stability constants is the most. Hill plot is least reliable but still helpful in explaining certain other properties of metal-humic acid interactions.

Sposito, et.al. (1979) studied complexes formed between Cu^{2+} and fulvic acids (K/H fulvate) by potentionmetric titration and calculated formation functions giving the number of moles of Cu^{2+} bound per mole of K/H fulvate. From the scatchard plot method, they concluded that (i) a relatively strong 1:1 complex (log K=3.9) is formed between Cu^{2+} and the most acidic functional groups in fulvic acid, and (ii) a weaker set of complexes (log K = 2.00) is formed between Cu^{2+} and the more weakly acidic functional groups acting as both unidentate and bidentate ligands. Saar, et.al. (1980) investigated lead (II) - fulvic acid (aquatic origin) complexes by ion selective electrode method and found that the conditional stability constants increased with increase in pH.

Bresnahan, et.al. (1978) discussed the coordination of Cu^{2+} to soil FA's studied by electron paramagnetic resonance (EPR) spectroscopy, differential pulse polarography (DPP) and a Cu^{2+} ion selective electrode. The Scatchard plot constructed for each experiment indicated the presence of two classes of binding sites. From pH 4.0 to 6.0, the total number of binding sites per molecule increased from 0.8 to 4.2 and 0.6 to 2.6 for all the soil and water FA's, respectively. They also concluded that Pb^{2+}, like Cu^{2+}, forms strong complexes with FA's where Cd^{2+} fulvate complexes are much weaker.

Stevenson, et.al. (1973) used a modification of the well-known Bjerrum's potentiometric titration method and calculated formation constants for Cu^{2+} complexes with HA's and FA's. He found that highly stable complexes were formed with formation constants of the order of those observed for synthetic polycarboxylic acids.

Saar, et.al. (1979) studied the stability constants of Cd(II) bound to fulvic acids derived from water and soil, and found that (i) the stability constants increased with increasing pH and (ii) the stability constants decreased with increasing FA concentration. From pH 4.0 to 8.0, the overall conditional stability constant increases from 1.4 to 12×10^3 for water derived FA and from 1.7 to 43×10^3 for soil derived FA.

Gamble, et.al. (1970) concluded that, Cu^{2+} reacts with FA to form a site bound chelate on the FA polymer molecules. The strength of Cu^{2+} -FA chelate is comparable to that of other bidentate Cu^{2+} chelates.

Schnitzer, et.al. (1967) found the FA/metal ratios to be increased with increase in pH and the log K values increased with increase in pH which can be ascribed to increased ionization of functional groups, especially carboxyl groups with increase in pH. They noted that the order of stabilities at pH 3.5 was:

$$Ni^{2+} > Pb^{2+} > CO^{2+} > Mn^{2+} > Mg^{2+}$$

Khanna, et.al (1961) showed that the pH drop on addition of metals of the first transition series follows the order:
$Mn < Co < Ni < Cu < Zn$

Sylva (1976) after a series of studies came to the conclusion that the inorganic and organic complexation and adsorption and precipitation processes in most aquatic systems are capable of reducing free copper levels to very low values even in the presence of high levels of total copper.

Stevenson (1976) determined apparent stability constants for the complexes between Cu^{2+}, Pb^{2+} and Cd^{2+} and three Humic acids from diverse sources. Complexes of Cu^{2+} and Pb^{2+} were found to be considerably more stable than those for Cd^{2+}. Log K values were 8.9 for Cu^{2+}, 8.7 for Pb^{2+} and 6.9 for Cd^{2+}.

Ardakani, et.al. (1972) used Schubert's ion exchange equilibrium method for the determination of stability constants of metal - soil organic matter complexes. Log K of Zn (II) - HA complexes ranged from 3.13 to 5.13 at pH 6.5.

Tan, et.al. (1971) investigated metal complexing capacity and the nature of the chelating ligands of water extract of poultry litter with respect to divalent ions. The amount of organic matter chelated and the stability of the divalent metal complexes were found to be decreased in the order Cu > Zn > Mg. The IR-spectra also revealed the fact that the formation of the stable metal-complexes involved carboxyl electrovalent linkages and probably hydroxyl and/or amino coordinate linkages.

Cheam, et.al. (1974) determined the conditional stability constants of metal-fulvate complexes at 25°C using specific ion electrode. The log K values were 4.86 and 5.08 at pH 3.0 and 4.0 for Hg complexes; 3.04 and 3.64 at pH 4.9 and 5.95 for Cd complexes; and 3.22, 3.72. and 4.35 at pH 3.0, 4.0 and 5.0 for copper complexes. The order of metal affinity toward FA was Hg > Cu > Cd.

Dijk (1971) used potentiometric titration method and judging from the magnitude of the pH drop on addition of inorganic salts concluded that there was at pH 5.0 no large difference in bond strength for the divalent ions Ba, Ca, Mg, Mn, Co, Ni, Fe, and Zn (in this order only slightly increasing). Pb

(II), Cu(II) and Fe(II) ions, however, were (in this order) more firmly bound.

Pitrowicz, et.al. (1984) studied the interactions in sea water of Cd, Cu and Zn with natural levels of marine humics (HA'S and FA'S) by DPASV method. Both the marine FA's and HA's were found to be appreciably interacted wirh Zn. In contrast, the extent of interactions of FA'S with Cu(II) appeared to be related to their structure while HA'S appeared to be interacted with Cu(II) regardless of structure. Cd(II) interacted only with matured, highly crosslinked HA's.

Takamatsu, et.al.(1978), with the help of potentiometric titration combined with ion selective electrode determined apparent stability constants (successive and overall) of divalent metal cations (Cu^{2+}, Pb^{2+} and Cd^{2+}) complexes with HA'S. The stability constants increased with an increase in pH, the order was $Cu^{2+} > Pb^{2+} >> Cd^{2+}$. The log Kn of soil HA'S were 8.65 for Cu^{2+}, 8.35 for Pb^{2+} and 6.25 for Cd^{2+} at pH 5.0 The log Kn of Cd^{2+} complexes ranged from 4.3×10^5 to 4.6×10^7 at pH 6.00. The overall stability constants increased with increasing humification in the higher humification range. The binding mechanism of the metal cation complexes with HA'S seemed to accompany the coordinate bonds of undissociated very weakly acidic fuctional groups, in addition to the ionic bonds through -COOH and/or -OH groups.

Kerven, et.al.(1984) using ISE method found that the degree of complexation increased in peats to more than 98% with increasing pH to about 6.0.

Mantoura, et.al. (1978) computed stability constants by using a gel filteration chromatographic technique which revealed that the order of increasing strength of binding of the metals followed the Irving -William's order.

Cheshire, et.al.(1977) studied the EPR spectra of metal-humate and fulvate complexes and came to the conclusion that in the HA fraction, copper was present partly as a copper-porphyrin-type complex but in the FA it was in some other complexed form.

Stevenson (1977) found that the order of stability constants for some divalent metal ions was, CU > Pb >> Cd > Zn.

CHAPTER - III

MATERIALS AND METHODOLOGY

PREPARATION OF SAMPLES

Samples of humic acid were isolated from three different sources such as garden soil (from J.N.U. Nursery), peat and humified water hyacinth (*Eichhornia crasipes*). The preparation of the sampling sources before the extraction of humic acids was carried out in the following manner.

First Source :

Four Kilograms of garden soil (upper 6 cm) were collected from J.N.U Nursery and kept in a rectangular glass container moistened enough with distilled water. The soil was kept for three months with intermittent mixing is order to maintain aerobic conditions. It was constantly being kept moistened with distilled water throughout this period.

Second Source :

Two kilograms of soil from the same source as above were collected and mixed throughly with two kilograms of crushed water hyacinth (1:1 ratio), kept in a glass container and enough distilled water was added just to moisten it. It was maintained throughout the period of three months. The contents were throughly mixed periodically in order to enable aerobic conditions.

Third Source :

This was prepared by decomposing water hyacinth under aerobic conditions in aqueous medium. About four kilograms of water hyacinth were collected, crushed thoroughly and kept in a glass container moistening it with enough quantity of distilled water. The contents were thoroughly mixed periodically and water was added as and when required. This was also kept for three months.

The three months of humification period as maintioned above covered the months of May, June and July 1985, when the average room temperature was about 30 to 35°C.

EXTRACTION OF HUMIC ACIDS :

The extraction of humic acids was carried out by following the method proposed by Takamatsu, et.al.(1978).

The source samples (soil, peat and humified water hyacinth) were air dried and sieved (2 mm). 250 gms. of each sample was extracted with 1 L of 0.1N NaOH for 1 hour at 95°C with stirring. Each extract was filtered through a G-4 glass filter and centrifuged to remove suspended flounlants. The pH of the solution wa adjusted to slightly lower than 2 with HCl to precipitate humic acid. The precipitate of HA was collected by centrifugation and washed three times with 0.1N HCl and twice with distilled water successively and then dissolved with 0.1N NaoH. After the purification procedure of dissolution and precipitation three times, the HA precipitate was washed repeatedly with water until dissolution was no longer observed. The prepared HA was then freeze-dried for use in the experiment.

POTENTIOMETRIC TITRATION :

The potentiometric titration method using ion selective electrodes (Buffle, et.al.1977, Kerven, et.al. 1984; and Fitch, et.al.1984) was adopted in the present study to determine some characteristic parameters of complexation between HA's and two toxic heavy metals, viz Cu(II) and Cd(II). The instrument used for this purpose was Orion Ionalyzer, 901 with Orion Cu- and Cd- ion selective electrodes.

3.2 mg of each of the three HA's were accurately weighed and dissolved in 100 ml of 0.1N NaOH solution. These solutions were prepared freshly in each case before the titration. The HA solutions were adjusted to the required pH levels (3.0, 3.5, 4.0, 4.5 for Cu(II) and 3.0, 3.5, 4.0, 4.5, 5.8 for Cd(II) with the help of 0.1N NaoH and 0.1N HNO_3. 2 ml of 5 N $NaNO_3$ solution was added to each sample in order to make the total ionic strength of the solution 0.1 in each case.

STANDARDISATION :

The orion ionalyzer was standardised first by $Cu(NO_3)_2$ (for titration with Cd(II) and then by $Cd(NO_3)_2$ (for titration with Cd(II) standard solutions each of three different concentrations such as : 10^{-1}, 10^{-2} and 10^{-3}M at an ionic strength of 0.1 by using 5N $NaNO_3$ solution.

TITRATION PROCEDURE :

After the adjustment of ionic strength and pH to the required level, the titration of HA solutions was carried out at room temperature. The titrants used were 10^{-3}M $Cu(NO_3)_2$ for Cu(II) and 10^{-3}M Cd $(NO_3)_2$ solution for Cd(II). Stepwise additions of 0.2 ml of the corresponding metal ion solution was carried out with the help of microburetes. After each step, the pH of the solution was readjusted by 0.1N NaoH and 0.1N HNO_3 back to within ± 0.05 units of the original value in order to maintain a constancy throughout the titration. The solution was continuously stirred with the help of a magnetic stirrer in order to facilitate the attainment of equilibrium between metal ion and HA. When the reading became stble, the electrode potential (E) at each step of titration was directly recorded from the instrument. For each HA, titrations were performed at four different pH levels (3.0, 3.5, 4.0, 4.5) for Cu(II) and five pH levels (3.0, 3.5, 4.0, 4.5, 5.8) for Cd(II). The choice of the upper limit of pH levels were restricted to 4.5 for Cu(II) and 5.8 for Cd(II) in order to eliminate the possibility of metal hydrolysis at higher pH levels, which might give erratic results. Simultaneously duplicate titrations wee run in the absence of HA's.

The slope was calculated as the difference of potential values of two different concentrations of metal ion solution (a difference of 10-fold in the concentration). The concentrations chosen were 10^{-1}M and 10^{-2}M for each of Cu(II) and Cd(II) metals. The difference of electrode potential values of these two concentrations give the slopes of the electrodes. The standard electrode potentials (Eo) were determined from the calibration curves for blank titrations.

CALCULATION OF DEGREE OF COMPLEXATION (α) :

The degree of complexation (α) for each of the HA's with respect to cu(II) and Cd(II) respectively at various pH levels mentioned above were calculated by following the methods of Buffle, et.al. (1977) and Kerven, et.al.(1984) which is expressed as equation (1) and (2) (chapter II).

CALCULATION OF BOUND METAL [Mb] :

The concentration of bound metal was taken as the difference between the total and free metal ion concentrations (Fitch, etal.1984), i.e.

$$[Mb] = [Mt] - [M^{++}] \quad .... (12).$$

MODELS AND PLOTTING METHODS :

1. Single surface langmuir Plot :

The single surface Langmuir equation was used to determine maximum binding ability of HA's (Zunino, etal. 1977; Fitch, etal.1984). The modified form of this equation as applied to HA's suggested by Fitch, etal.1984, that is, equation (7) was used for plotting graphs between $[M^{++}]/[Mb]$ and $[M^{++}]$.

2. Double surface Langmuir Plot :

The double surface langmuir equation (11) (Chapter II) as given by Fitch, et.al.(1984) and Sposito (1982) was used which gives a plot of $[M^{++}]$ vs. $[Mb]/[M^{++}]$ and from the straight line segments, b_1 and b_2 are obtained by extrapolation. The value of b_2 at higher metal ion concentration is generally taken as the MBA.

Calculation of θ :- Fitch, et.al.(1984) defined 'θ' as the sites bound per total number of reactive sites which can be expresses as :

$$\theta = \frac{\text{Sites bound}}{\text{Total number of reactive sites}} = \frac{[Mb]}{n[Lt]}$$

or

$$\theta = \frac{[Mb]}{MBA} \quad \ldots\ldots \; (13)$$

Where n[Lt] is the maximum binding ability.

3. Scatchard Plot :

The Scatchard plot using equation (6) (Chapter II) (Fitch, et.al. 1984) was used to determine conditional stability constants (Kn).

A plot of $\theta/[M^{++}]$ vs. θ gives a non-linear curve which have linear segments in the initial as well as final stages. The linear segment in the final stage corresponding to higher metal ion concentration is extrapolated and Ko (conditional stability constant, Kn) was calculated from the slope and intercept.

4. **Double Reciprocal Plot** :

The double reciprocal plot is based on the equation (3) (Chapter II) derived independently by Buffle, etal.(1977) and Fitch, et.al.(1984).

A plot of [Lt]/[Mb] vs. $1/[M^{++}]$ gives non-linear lines from which Ko and mw are calculated from the slope and intercept of the tangent drawn towards high metal ion concentrations.

5. **Hill Plot** :

The Hill equation (9) (Chapter II) as applied to metal-HA interactions given by Fitch, etal. (1984) was used. to plot $\log [\theta/(1-\theta)]$ vs. $\log [M^{++}]$ from which n and kn (macroscopic binding constant) were calculated from slope and intercept.

CHAPTER IV

RESULTS

Table 1

Concentration of ionic copper [Cu^{2+}] and bound copper [Cub] in HA (soil) solution at 0.1 M ionic strength

pH ------>	3.0		3.5		4.0		4.5	
Steps	[Cu^{2+}] x 10^{-6}M	[Cub] x 10^{-6}M	[Cu^{2+}] x 10^{-6}M	[Cub] x 10^{-6}M	[Cu^{2+}] x 10^{-6}M	[Cub] x 10^{-6}M	[Cu^{2+}] x 10^{-6}M	[Cu^{2+}] x x10^{-6}M
1	0.61	0.70	0.59	0.72	0.78	0.53	0.62	0.69
2	1.32	1.29	1.30	1.31	1.67	0.94	1.56	1.05
3	2.15	1.76	2.26	1.67	2.70	1.21	2.64	1.27
4	3.09	2.11	3.24	1.96	3.77	1.43	3.87	1.33
5	3.98	2.51	4.31	2.18	4.91	1.58	5.07	1.42
6	4.94	2.84	5.42	2.36	6.05	1.73	6.33	1.45
7	6.08	2.99	6.62	2.45	7.23	1.84	7.58	1.49
8	7.10	3.20	7.79	2.51	8.38	1.92	8.78	1.52
9	8.29	3.31	8.96	2.64	9.66	1.94	10.07	1.53
10	9.35	3.55	10.21	2.69	10.90	2.00	11.34	1.56
11	10.58	3.62	11.47	2.73	12.13	2.07	12.62	1.58
12	11.71	3.69	12.63	2.77	13.28	2.12	13.79	1.61
13	12.97	3.73	13.90	2.80	14.51	2.19	15.07	1.62
14	14.21	3.79	15.12	2.88	15.73	2.27	16.35	1.65
15	15.28	3.92	16.28	2.92	16.87	2.33	17.53	1.67
16	16.56	3.94	17.55	2.95	18.12	2.38	18.81	1.69
17	17.75	3.95	18.71	2.99	19.29	2.41	19.98	1.72
18	18.97	4.03	19.98	3.02	20.56	2.44	21.25	1.75
19	20.11	4.09	21.16	3.04	21.73	2.47	22.42	1.76
20	21.36	4.14	22.43	3.07	23.08	2.48	23.71	1.79
21	22.52	4.18	23.61	3.09				
22	23.66	4.24	24.78	3.12				
23	24.93	4.27	26.06	3.14				
24	26.08	4.32	27.24	3.16				
25	27.22	4.38	28.42	3.18				
26	28.39	4.41	29.60	3.20				
27	29.66	4.44	30.88	3.22				
28	30.82	4.48	32.06	3.24				
29	31.99	4.51	33.25	3.25				
30	33.15	4.55	34.41	3.29				

Table 2

Concentration of ionic copper [Cu^{2+}] and bound copper [Cub] in HA (peat) solution at 0.1 M ionic strength

pH ------>	3.0		3.5		4.0		4.5	
Steps	[Cu^{2+}] x 10^{-6}M	[Cub] x 10^{-6}M	[Cu^{2+}] x 10^{-6}M	[Cub] x 10^{-6}M	[Cu^{2+}] x 10^{-6}M	[Cub] x 10^{-6}M	[Cu^{2+}] x 10^{-6}M	[Cu^{2+}] x10^{-6}M
1	0.24	1.07	0.31	1.00	0.62	0.69	0.47	0.84
2	0.68	1.93	0.84	1.77	1.61	1.00	1.26	1.35
3	1.35	2.56	1.54	2.37	2.69	1.22	2.20	1.71
4	2.21	2.99	2.43	2.77	3.79	1.41	2.23	1.97
5	3.06	3.43	3.38	3.11	4.94	1.55	2.43	2.06
6	4.07	3.71	4.32	3.46	6.12	1.66	5.60	2.18
7	5.08	3.99	5.53	3.54	7.35	1.72	6.78	2.29
8	6.03	4.27	6.54	3.76	8.53	1.77	7.93	2.37
9	7.14	4.46	7.61	3.99	9.75	1.85	9.187	2.42
10	8.33	4.47	8.84	4.06	11.02	1.88	10.43	2.47
11	9.41	4.79	10.07	4.13	12.30	1.90	11.69	2.51
12	10.34	5.06	11.14	4.26	13.48	1.92	12.87	2.53
13	11.59	5.11	12.37	4.33	14.74	1.96	14.14	2.56
14	12.08	5.20	13.59	4.41	16.01	1.99	15.39	2.61
15	13.93	5.27	14.75	4.45	17.18	2.02	16.56	2.64
16	15.15	5.35	16.01	4.49	18.46	2.04	17.82	2.68
17	16.29	5.41	17.15	4.55	19.62	2.08	18.97	2.73
18	17.55	5.45	18.40	4.60	20.87	2.13	20.24	2.76
19	18.72	5.48	19.54	4.66	22.03	2.17	21.39	2.81
20	19.96	5.54	20.81	4.69	23.30	2.20	22.64	2.86
21	21.08	5.62	21.97	4.73	24.48	2.22	23.79	2.91
22	22.19	5.71	23.14	4.76	25.66	2.24	24.95	2.95
23	23.44	5.76	24.39	4.81	26.94	2.26	26.20	3.00
24	24.57	5.83	25.56	4.84	28.11	2.29	27.36	3.04
25	25.72	5.88	26.71	4.89	29.30	2.30	28.53	3.07
26	26.85	5.95	27.85	4.95	30.48	2.32	29.69	3.11
27	28.10	6.00	29.09	5.01	31.75	2.35	30.96	3.14
28	29.22	6.08	30.24	5.06	32.92	2.38	32.12	3.18
29	30.35	6.15	31.38	5.12	34.10	2.40	33.27	3.23
30	31.50	6.20	32.54	5.16	35.28	2.42	34.44	3.26

Table 3

Concentration of ionic copper [Cu^{2+}] and bound copper [Cub] in HA (humified water hyacinth) at 0.1 M ionic strength

pH ------>	3.0		3.5		4.0		4.5	
Steps	[Cu^{2+}] x 10^{-6}M	[Cub] x 10^{-6}M	[Cu^{2+}] x 10^{-6}M	[Cub] x 10^{-6}M	[Cu^{2+}] x 10^{-6}M	[Cub] x 10^{-6}M	[Cu^{2+}] x 10^{-6}M	[Cu^{2+}] x10^{-6}M
1	0.86	0.45	0.75	0.56	0.45	0.86	0.33	0.98
2	1.91	0.70	1.45	1.16	0.75	1.86	0.66	1.95
3	2.97	0.94	2.47	1.44	1.62	2.29	1.56	2.23
4	3.99	1.21	3.50	1.70	2.56	2.64	2.57	2.63
5	5.10	1.39	4.61	1.88	3.65	2.84	3.70	2.79
6	6.09	1.69	5.73	2.05	4.72	3.06	4.93	2.85
7	7.24	1.83	6.95	2.12	5.79	3.28	6.12	2.95
8	8.24	2.06	2.40	6.85	3.45	7.28	3.02	2.37
9	9.38	2.22	9.13	2.47	8.08	3.52	8.53	3.07
10	10.51	2.39	10.30	2.60	9.242	3.66	9.79	3.11
11	11.73	2.47	11.54	2.66	10.48	3.72	11.05	3.15
12	12.83	2.57	12.65	2.75	11.63	3.77	12.23	3.17
13	13.92	2.78	12.81	2.85	13.85	3.51	13.19	2.56
14	15.12	2.88	15.08	2.92	14.07	3.93	14.78	3.22
15	16.22	2.98	16.19	3.01	15.20	4.00	15.96	3.24
16	17.47	3.03	17.43	3.07	16.45	4.05	17.24	3.26
17	18.56	3.14	18.58	3.12	17.61	4.09	18.43	3.27
18	19.72	3.28	19.84	3.16	18.84	4.16	19.72	3.28
19	20.79	3.41	21.01	3.19	19.90	4.30	20.91	3.29
20	21.95	3.55	22.27	3.23	21.11	4.39	22.20	3.30
21	23.10	3.60	23.43	3.27	22.21	4.49	23.39	3.31
22	24.23	3.67	24.57	3.33	23.29	4.61	24.57	3.33
23	25.50	3.70	25.85	3.35	24.50	4.70	25.85	3.35
24	26.66	3.74	27.00	3.40	25.62	4.78	27.03	3.37
25	27.84	3.76	28;16	3.44	26.69	4.91	28.20	3.40
26	29.00	3.80	29.32	3.48	27.80	5.00	29.38	3.42
27	30.26	3.84	30.58	3.52	29.00	5.10	30.66	3.44
28	31.43	3.87	31.75	3.55	30.13	5.17	31.83	3.47
29	32.60	3.90	32.90	3.60	31.25	5.25	33.01	3.49
30	33.77	3.93	34.04	3.66	32.38	5.32	34.20	3.50

Table 4

Concentration of ionic cadmium [Cd^{2+}] and bound Cadmium [Cdb] in HA (Soil) solutions and 0.1 M ionic strength

pH ------>	3.0		3.5		4.0		4.5	
Steps	[Cd^{2+}] x 10^{-6}M	[Cdb] x 10^{-6}M	[Cd^{2+}] x 10^{-6}M	[Cdb] x 10^{-6}M	[Cd^{2+}] x 10^{-6}M	[Cdb] x 10^{-6}M	[Cd^{2+}] x 10^{-6}M	[Cdb] x x10^{-6}M
1	0.74	0.64	0.67	0.61	0.70	0.73	0.58	0.74
2	1.68	1.49	1.12	1.44	1.17	1.36	1.25	1.31
3	2.72	1.19	2.47	1.44	2.40	1.51	2.25	1.66
4	3.82	1.38	3.54	1.66	3.42	1.78	3.23	1.97
5	4.92	1.57	4.64	1.85	4.48	2.01	4.24	2.25
6	6.08	1.70	5.58	1.93	5.60	2.18	5.37	2.41
7	7.26	1.81	7.09	1.98	6.82	2.25	6.48	2.59
8	8.44	1.86	8.31	1.99	7.92	2.88	7.69	2.61
9	9.67	1.93	9.59	2.01	9.21	2.39	8.92	2.68
10	10.80	2.08	10.81	2.09	10.40	2.51	10.20	2.71
11	12.12	2.08	11.54	11.89	12.26	1.61	12.59	1.48
12	13.31	2.12	12.99	2.39	12.78	2.62	12.62	2.81
13	14.50	2.19	14.26	2.42	14.22	2.56	13.91	2.84
14	15.69	2.27	15.52	2.51	15.53	2.46	15.08	2.91
15	16.84	2.41	16.63	2.59	16.71	2.50	16.90	3.02
16	18.00	2.50	17.81	2.68	18.00	2.53	17.54	3.03
17	19.21	2.54	19.90	2.81	19.22	2.54	18.58	3.10
18	20.38	2.59	20.29	2.72	20.39	2.58	19.81	3.19
19	21.63	2.64	21.41	2.80	21.59	2.61	20.90	3.28
20	22.82	2.71	22.90	2.83	22.69	2.10	23.40	3.30
21	23.76	2.88	23.73	3.02	23.81	2.88	23.41	3.33
22	24.89	3.02	24.79	3.11	25.11	2.79	24.52	3.36
23	26.10	3.13	26.16	3.04	26.30	2.90	25.79	3.39
24	27.09	3.29	27.30	3.13	27.62	2.81	27.01	3.42
25	28.16	3.40	28.44	3.18	28.70	2.92	28.09	3.48
26	29.32	3.49	29.51	3.27	29.76	3.01	29.33	3.51
27	30.44	6.68	23.33	3.08	23.12	3.50	23.60	3.44

Table 5

Concentration of ionic cadmium [Cd^{2+}] and bound Cadmium [Cdb] in HA (Peat) solutions and 0.1 M ionic strength

pH ------>	3.0		3.5		4.0		4.5	
Steps	[Cd^{2+}] x 10^{-6}M	[Cdb] x 10^{-6}M	[Cd^{2+}] x 10^{-6}M	[Cdb] x 10^{-6}M	[Cd^{2+}] x 10^{-6}M	[Cdb] x 10^{-6}M	[Cd^{2+}] x 10^{-6}M	[Cdb] x x10^{-6}M
1	0.35	0.92	0.10	0.40	0.91	0.50	0.81	0.57
2	0.89	1.72	0.78	1.84	1.13	1.49	1.28	1.33
3	1.61	2.30	1.42	2.49	2.03	1.88	1.22	1.69
4	2.46	2.74	2.24	2.96	3.02	2.18	3.29	1.91
5	3.40	3.09	3.14	3.55	4.11	2.88	4.33	2.16
6	4.42	3.36	4.14	3.64	5.19	5.56	2.22	5.72
7	5.60	3.47	5.27	3.80	6.43	2.64	6.82	2.25
8	6.82	3.48	6.36	3.94	7.55	2.75	7.98	2.32
9	7.95	3.65	7.58	4.02	8.79	2.81	9.21	2.39
10	9.21	3.69	8.841	4.06	10.00	2.90	10.50	2.41
11	10.51	3.71	10.10	4.09	11.20	3.03	11.80	2.45
12	11.59	1.19	14.17	2.29	13.10	2.90	12.49	3.00
13	12.92	3.81	12.41	4.28	13.51	3.21	14.11	2.57
14	14.11	3.88	13.72	4.33	14.82	3.23	15.22	2.79
15	15.20	4.01	14.79	4.40	15.89	3.33	16.50	2.68
16	16.42	4.07	16.01	4.48	17.08	3.40	17.69	2.78
17	17.59	4.12	17.09	4.61	18.20	3.48	18.82	2.88
18	18.80	4.20	14.71	9.31	18.69	9.88	23.01	0.01
19	19.92	4.27	19.41	4.77	20.42	3.81	21.01	3.28
20	21.33	4.22	20.68	1.62	23.92	2.22	23.30	3.30
21	22.30	4.38	21.90	4.82	22.88	3.84	23.30	3.41
22	23.51	4.41	22.99	4.86	23.90	3.99	24.29	3.58
23	24.68	4.46	4.976	5.11	24.08	5.73	23.54	5.58
24	25.64	4.58	25.42	5.02	26.19	4.17	26.71	3.42
25	27.06	4.60	26.53	5.08	27.33	4.29	28.00	3.61
26	28.09	7.67	25.13	8.40	24.40	9.11	23.62	8.89
27	29.30	8.88	25.19	9.52	24.61	0.34	23.75	0.08

Table -6

Concentrationsof Ionic Cadmium (Cd^{2t}) and Bound cadmium (cd b) in HA (hunified water hyacinth) solutions at 0.1 M Ionic Strength

Ph.	3.0		3.5		4.0		4-5		5.8	
Steps	(Cd^{2+}) x10^{-6}M	Cdb x10^{-6}M	Cd^{2+} x10^{-6}M	Cdb x10^{-6}M	Cdb x10^{-6}M	Cdb x10^{-6}M	Cd^{2+} x10^{-6}M	Cd b x10^{-6}M	Cd b x10^{-6}M	Cdb x10^{-6}M
1.	0.91	0.34	0.83	0.48	0.74	0.57	0.64	0.67	0.57	0.74
2.	2.05	0.55	1.84	0.77	1.63	0.98	1.47	1.14	1.32	1.29
3.	3.20	0.71	2.92	0.99	2.61	1.30	2.37	1.54	2.15	1.75
4.	4.33	0.87	4.09	1.11	3.66	1.54	3.35	1.85	3.10	2.10
5.	5.55	0.94	5.28	1.21	4.77	1.72	4.39	2.10	4.08	2.41
6.	6.77	1.01	6.48	1.30	5.89	1.89	5.48	2.30	5.12	2.66
7.	7.96	1.11	7.69	1.38	7.09	1.98	6.62	2.45	6.21	2.86
8.	9.12	1.18	8.80	1.50	8.24	2.06	7.74	2.56	7.30	3.00
9.	10.40	1.21	9.99	1.61	9.51	2.09	8.92	2.68	8.41	3.19
10.	11.60	1.29	11.21	1.69	10.82	2.19	10.19	2.70	9.56	3.34
11.	12.81	1.38	12.40	1.78	12.02	2.21	11.40	2.79	10.80	3.40
12.	13.78	1.55	13.40	1.99	13.07	2.28	12.52	2.89	11.91	3.51
13.	15.01	1.70	14.59	2.08	14.21	2.46	13.74	2.99	13.12.	3.59
14	16.32	1.74	15.82	2.19	15.50	2.50	14.91	3.10	14.33	3.69
15	17.39	1.81	16.81	2.36	16.61	2.59	16.10	3.14	15.39	3.82
16	18.59	1.92	18.20	2.30	17.79	2.68	17.28	3.19	16.60	3.88
17	19.62	2.09	19.29	2.39	18.84	2.88	18.43	3.30	17.69	3.99
18	20.81	2.17	20.52	2.49	20.21	2.85	19.61	3.38	18.91	4.08
19	21.91	2.31	21.63	2.60	21.31	2.92	20.79	3.42	20.11	4.14
20.	23.11	2.43	22.81	2.70	22.48	2.99	22.02	3.50	21.33	4.19
21.	24.20	2.54	23.90	2.79	23.62	3.09	23.11	3.61	22.40	4.30
22.	25.27	2.61	25.00	2.18	24.66	3.20	24.18	3.68	23.51	4.42
23.	26.52	2.70	6.21	3.02	25.90	3.31	25.54	3.74	24.88	4.35
24	27.73	2.72	27.43	3.04	27.10	3.34	26.60	3.82	25.93	4.48
25	28.71	2.89	28.52	3.11	28.21	3.40	27.68	3.90	27.01	4.60
26	30.02	2.80	29.62	3.19	29.30	3.49	28.81	3.99	28.11	4.68
27	31.20	2.91	30.79	3.28	30.52	3.61	30.11	4.03	29.42	4.72

Table - 7

Percentage of TotalCopper present as Cu^{2+} in HA Solutions at 0.1M Ionic Sgrength

	HA (Soil)				HA (Peat)				HA (humified water hyacinth			
PH.	3.0	3.5	4.0	4.5	3.0	3.5	4.0	4.5	3.0	3.5	4.0	4.5
1.	46.46	45.11	59.24	47.63	18.32	23.66	47.25	35.73	65.57	57.02	34,35	24.96
2.	50.57	49.81	64.02	59.77	26.05	32.18	61.69	48.28	73.18	55.55	28.74	25.29
3.	54.99	57.80	69.05	67.52	34.53	39.39	68.80	56.27	75.93	63.17	.41.43	39.90
4.	59.42	62.31	72.50	74.42	42.50	46.73	72.88	62.72	76.73	67.31	49.23	49.42
5.	61.32	66.41	75.65	78.12	47.15	52.08	76.12	68.26	78.58	71.03	56.24	57.01
6.	63.50	69.67	77.76	81.36	52.30	55.53	78.66	71-98	78.28	73.65	60.67	63.37
7.	67.03	72.99	79.71	83.57	56.01	60.97	81.04	74.75	79.82	76.63	63.84	67.48
8.	68.93	75.63	81.36	85.26	58.54	63.50	82.82	76.99	80.00	76.70	66.50	70.68
9.	71.47	77.24	83.28	86-81	61.55	65.60	84.05	79.14	80.86	78.71	69.66	73.53
10.	72.48	79.15	84.50	87.91	64.57	68.53	85.43	80.85	81.47	79.84	71.63	75.89
11.	74.51	80.77	85.42	88.87	66.27	70.92	86.62	82.32	82.61	81.27	73.80	77.82
12	76.04	82.01	86.23	89.55	67.14	72.34	87.53	83.57	83.31	82.14	75.52	79.42
13	77.66	83.23	86.89	90.24	69.40	74.07	88.26	84.67	88.35	83.17	76.95	80.90
14	78.94	84.00	87.39	90.83	67.11	75.50	88.94	85.50	84.00	83.78	78.17	82.11
15	79.58	84.79	87.86	91.30	72.55	76.82	89.48	86.25	84.48	84.32	79.17	83.13
16	80.78	85.61	88.39	91.76	73.90	78.10	90.05	86.93	85.22	85.02	80.24	84.10
17	81.80	86.22	88.89	92.07	75.07	79.03	90.42	87.42	85.53	85.62	81.15	84.93
18.	82.48	86.87	89.39	92.39	76.30	80.00	90.74	88.00	85.74	86.26	81.91	85.74
19.	83.10	87.44	89.79	9 .64	77.36	80.74	91.03	88.39	85.91	86.82	82.23	86.40
20.	83.76	87.96	90.51	92.98	78.27	81.61	91.37	88.78	86.08	87.33	82.78	87.06
21.	84.34	88.43			78.95	82.28	91.69	89.10	86.52	87.75	83.18	87.60
22.	84.80	88.92			79.53	82.94	91.97	89.43	86.85	88.06	83.48	880.6
23.	85.38	89.25			80.27	83.53	92.26	89.73	87.33	88.53	83.90	88.53
24.	85.79	89.61			80.82	84.08	92.47	90.00	87.70	88.82	84.28	88.91
25	86.14	89.94			81.39	84.53	92.72	90.28	88.10	89.11	84.46	89.24
26.	86.55	90.24			81.56	84.91	92.93	90.52	88.41	89.39	84.76	89.57
27	86.98	90.56			82.40	85.31	93.11	90.79	88.74	89.68	85.04	89.91
28	87.31	90.82			82.78	85.67	93.26	90.99	89.04	89.94	85.35	90.17
29	87.64	91.10			83.15	85.97	93.42	91.15	89.32	90.14	85.62	90.44
30.	87.93	91.27			83.55	86.31	93.58	91.35	89.58	90.29	85.89	90.72

TABLE-8

Percentage of Total Cadmium present as Cd^{2+} in HA solutions and O.I.M. Ionic Strength.

	HA (Soil)					HA (Peat)					HA (humified)				
pH.	3.0	3.5	4.0	4.5	5.8	3.0	3.5	4.0	4.5	5.8	3.0	3.5	4.0	4.5	5.8
1.	56.18	49.01	46.72	44.66	43.89	26.34	22.90	30.84	38.02	43.66	69.24	63.28	56.18	49.01	43.66
2.	64.37	57.09	55.17	52.11	40.19	34.25	29.50	43.30	49.04	53.26	78.54	70.50	62.45	56.32.	50.57
3.	69.57	63.17	61.38	57.54	54.73	41.18	36.32	51.92	56.78	58.82	81.84	74.68	66.75	60.61	55.25
4.	73.46	68.08	65.77	62.12	58.85	47.31	43.08	58.08	63.27	64.42	83.27	78.65	70.38	64.42	59.62
5.	75.81	71.49	69.03	65.33	63.33	52.39	48.38	63.33	66.72	69.03	85.52	81.36	74.50	67.64	62.87
6.	78.15	75.19	71.98	69.02	66.20	56.81	53.21	66.71	71.47	73.52	87.02	83.29	75.71	70.44	65.81
7.	80.04	78.17	75.19	71.44	69.02	61.74	68.10	70.89	75.19	76.96	87.76	84.79	78.17	72.99	68.47
8.	81.94	8068	76.89	74.66	71.94	66.21	61.75	73.30	77.48	79.32	88.54	85.44	80.00	75.15	70.87
9.	83.36	82.67	79.40	76.90	77.32	68.53	65.34	75.78	79.40	81.20	89.66	86.12	81.98	76.90	72.50
10.	83.72	83.72	80.62	79.07	76.36	70.40	68.53	77.52	81.40	82.17	89.92	86.82	83.72	79.07	74.11
11.	85.21	83.80	81.69	8099	78.17	73.94	71.13	78.87	83.10	83.80	90.14	87.32	84.51	80.28	76.06
12.	86.36	84.42	83.12	81.82	80.52	75.32	73.73	79.87	83.77	84.42	89.61	87.01	85.06	81.17	77.27
13.	87.83	85.63	85.03	83.23	82.04	77.25	74.25	80.84	84.43.	84.43	89.82	87.43	85.03	82.04	78.44
14.	87.52	86.11	86.11	83.89	83.33	78.33	76.11	82.22	84.34	85.00	91.56	87.78	86.11	82.78	79.44
15.	87.50	86.46	86.98	84.38	84.90	79.17	77.08	82.81	85.94	85.42	91.63	87.50	86.46	83.85	80.21
16.	87.80	86.83	87.80	85.37	85.37	80.00	78.05	83.41	86.34	86.34	91.73	88.78	86.83	84.39	81.98
17.	88.48	87.10	88.48	85.71	87.10	81.11	78.80	83.87	86.64	86.64	91.32	88.94	86.64	84.79	81.57
18.	88.70	88.26	88.70	86.09	87.83	81.74	79.57	83.91	86.54	86.96	92.43	89.13	87.83	85.22	82.17
19.	89.26	88.43	89.26	86.36	87.60	82.23	81.17	84.30	86.78	86.78	92.50	89.26	88.02	85.95	83.06
20.	89.41	88.63	89.41	86.67	88.63	83.53	81.18	84.71	87.06	86.67	91.51	89.41	88.24	86.27	83.53
21.	89.14	88.76	89.14	87.64	89.14	83.52	82.02	85.77	87.27	87.27	91.64	89.51	88.39	86.52	83.90
22.	89.25	88.89	89.96	87.81	89.25	84.23	82.44	85.66	87.10	87.46	91.68	89.61	88.53	86.74	84.23
23.	89.38	89.73	90.07	88.36	89.38	84.59	82.88	8596	88.01	87.67	90.75	89.73	88.70	87.33	85.27
24.	89.14	89.80	90.79	88.82	89.14	84.87	83.55	86.18	87.83	87.83	91.12	90.13	89.14	87.50	85.20
25.	89.24	89.87	90.82	88.92	90.19	85.34	83.86	86.39	88.61	87.97	91.82	90.19	89.24	87.66	85.44
26.	89.33	89.94	90.85	89.33	90.85	85.67	84.45	87.59	88.72	88.11	91.46	90.24	89.33	87.18	85.67
27.	89.15	91.32	90.91	89.44	90.91	85.92	84.75	86.51	88.86	88.27	91.50	90.32	89.44	88.27	86.22

TABLE - 9

Degree of Complaxation (α) of Cu^{2+} with Humic Acids and 0.1 M Ionic Strength

	HA (Soil)				HA (Peat)				HA (humified water hyacinth)			
H→	3.0	3.5	4.0	4.5	3.0	3.5	4.0	4.5	3.0	3.5	4.0	4.5
	2.15	2.22	1.69	2.10	5.47	4.23	2.12	2.80	1.52	1.75	2.91	4.01
	1.97	2.00	1.56	1.67	3.85	3.10	1.62	2.06	1.37	1.79	3.48	3.94
	1.82	1.73	1.45	1.48	2.90	2.54	1.57	1.77	1.32	1.58	2.40	2.50
	1.68	1.61	1.37	1.34	2.35	2.14	1.37	1.61	1.30	1.49	2.03	2.03
	1.63	1.54	1.32	1.28	2.12	1.92	1.31	1.46	1.27	1.40	1.77	1.75
	1.61	1.43	1.29	1.23	1.91	1.80	1.28	1.39	1.28	1.36	1.65	1.57
	1.49	1.37	1.26	1.20	1.78	1.64	1.24	1.34	1.26	1.31	1.56	1.48
	1.45	1.32	1.23	1.18	1.71	1.57	1.20	1.30	1.25	1.30	1.50	1.42
	1.40	1.30	1.20	1.15	1.63	1.53	1.19	1.27	1.24	1.27	1.43	1.36
0	1.38	1.27	1.18	1.13	1.55	1.46	1.17	1.24	1.23	1.26	1.40	1.32
1	1.34	1.24	1.17	1.12	1.51	1.41	1.16	1.21	1.21	1.23	1.35	1.28
2	1.32	1.22	1.16	1.12	1.49	1.38	1.14	1.20	1.20	1.22	1.33	1.26
3	1.29	1.20	1.15	1.10	1.44	1.35	1.13	1.19	1.20	1.20	1.30	1.23
4	1.27	1.19	1.15	1.10	1.48	1.32	1.12	1.17	1.19	1.19	1.28	1.22
5	1.25	1.18	1.14	1.10	1.38	1.30	1.11	1.16	1.19	1.19	1.26	1.20
6	1.24	1.16	1.13	1.09	1.35	1.28	1.11	1.15	1.17	1.17	1.25	1.19
7	1.22	1.16	1.12	1.09	1.33	1.26	1.11	1.14	1.17	1.17	1.23	1.18
8	1.21	1.15	1.12	1.09	1.31	1.25	1.10	1.14	1.16	1.15	1.22	1.16
9	1.20	1.14	1.12	1.08	1.29	1.24	1.10	1.13	1.16	1.15	1.22	1.16
0	1.20	1.13	1.11	1.07	1.28	1.22	1.10	1.12	1.16	1.14	1.21	1.15
1	1.19	1.13			1.26	1.21	1.09	1.12	1.16	1.14	1.21	1.14
2	1.17	1.13			1.26	1.20	1.09	1.12	1.15	1.14	1.20	1.14
3	1.17	1.12			1.25	1.19	1.09	1.11	1.15	1.13	1.19	1.13
4	1.17	1.11			1.23	1.19	1.08	1.11	1.14	1.12	1.19	1.12
5	1.16	1.11			1.23	1.19	1.08	1.11	1.13	1.12	1.18	1.12
6	1.16	1.10			1.22	1.18	1.08	1.10	1.13	1.12	1.18	1.11
7	1.15	1.10			1.21	1.18	1.07	1.11	1.12	1.11	1.18	1.11
8	1.14	1.10			1.21	1.16	1.08	1.10	1.13	1.11	1.17	1.11
9	1.14	1.10			1.20	1.17	1.07	1.10	1.12	1.10	1.17	1.10
0	1.14	1.10			1.20	1.16	1.07	1.10	1.11	1.11	1.17	1.11

TABLE - 10

Degree of Complexation (α) of Cd^{2+} with Humic Acids at 0.1 M Ionic strength

	HA(Soil)					HA(Peat)					HA(humified water hyacinth)				
PH	3.0	3.5	4.0	4.5	5.8	3.0	3.5	4.0	4.5	5.8	3.0	3.5	4.0	4.5	5.8
1	1.78	2.04	2.14	2.24	2.28	3.80	4.37	3.24	2.63	2.29	1.35	1.58	1.78	2.04	2.29
2	1.55	1.75	1.81	1.92	2.00	2.92	3.39	2.32	2.04	1.88	1.27	1.42	1.60	1.78	1.97
3	1.44	1.58	1.63	1.74	1.83	2.43	2.75	1.93	1.76	1.70	1.22	1.34	1.50	1.65	1.81
4	1.36	1.47	1.52	1.61	1.70	2.11	2.32	1.72	1.58	1.55	1.20	1.27	1.42	1.55	1.68
5	1.32	1.40	1.45	1.53	1.58	1.91	2.07	1.58	1.50	1.45	1.17	1.23	1.36	1.48	1.59
6	1.28	1.33	1.39	1.45	1.51	1.76	1.88	1.50	1.40	1.36	1.15	1.20	1.32	1.42	1.52
7	1.25	1.28	1.33	1.40	1.45	1.62	1.72	1.41	1.33	1.30	1.14	1.18	1.28	1.37	1.46
8	1.22	1.24	1.30	1.34	1.39	1.51	1.62	1.36	1.29	1.26	1.13	1.17	1.25	1.33	1.41
9	1.20	1.21	1.26	1.30	1.34	1.46	1.53	1.32	1.26	1.23	1.12	1.16	1.22	1.30	1.38
10	1.19	1.19	1.24	1.27	1.31	1.40	1.46	1.29	1.23	1.22	1.11	1.15	1.20	1.27	1.35
11	1.17	1.19	1.22	1.24	1.28	1.35	1.40	1.27	1.20	1.19	1.11	1.15	1.18	1.25	1.30
12	1.16	1.18	1.20	1.22	1.24	1.33	1.38	1.25	1.19	1.18	1.12	1.15	1.18	1.23	1.29
13	1.15	1.17	1.18	1.20	1.22	1.29	1.35	1.24	1.18	1.18	1.11	1.14	1.18	1.22	1.27
14	1.15	1.16	1.16	1.19	1.20	1.28	1.31	1.22	1.18	1.18	1.10	1.14	1.16	1.21	1.26
15	1.14	1.16	1.15	1.19	1.18	1.26	1.30	1.21	1.16	1.17	1.10	1.14	1.16	1.19	1.25
16	1.14	1.15	1.14	1.17	1.17	1.25	1.28	1.20	1.16	1.16	1.10	1.13	1.15	1.18	1.23
17	1.13	1.15	1.13	1.17	1.15	1.23	1.27	1.19	1.15	1.15	1.11	1.12	1.15	1.18	1.23
18	1.13	1.13	1.13	1.16	1.14	1.22	1.26	1.19	1.16	1.15	1.11	1.12	1.14	1.17	1.22
19	1.12	1.13	1.12	1.16	1.14	1.22	1.25	1.19	1.15	1.15	1.11	1.12	1.14	1.16	1.20
20	1.12	1.13	1.12	1.15	1.13	1.20	1.23	1.18	1.15	1.15	1.10	1.12	1.13	1.16	1.20
21	1.12	1.13	1.12	1.14	1.12	1.20	1.22	1.17	1.15	1.15	1.10	1.12	1.13	1.16	1.19
22	1.12	1.13	1.11	1.14	1.12	1.19	1.21	1.17	1.15	1.14	1.10	1.12	1.13	1.15	1.19
23	1.12	1.11	1.11	1.13	1.12	1.18	1.21	1.16	1.14	1.14	1.10	1.11	1.13	1.15	1.17
24	1.12	1.11	1.10	1.13	1.12	1.18	1.20	1.16	1.14	1.14	1.10	1.11	1.12	1.14	1.17
25	1.12	1.11	1.10	1.12	1.11	1.17	1.19	1.16	1.13	1.14	1.10	1.11	1.12	1.14	1.17
26	1.12	1.11	1.10	1.12	1.10	1.17	1.18	1.15	1.13	1.13	1.09	1.11	1.12	1.14	1.17
27	1.12	1.11	1.10	1.12	1.10	1.16	1.18	1.15	1.13	1.13	1.09	1.11	1.12	1.13	1.16

TABLE - 11

Maximum Binding Ability (MBA) of Humic Acids with respect to $Cu(II)$ at 0.1M Ionic, Strengrth (As Determined from Double surface langmuir PLots).

pH	HA (soil)	HA (peat)	HA(humified water hyacinth)
	x 10^{-6}M	x 10^{-6}M	x 10^{-6}M
3.0	5.67	7.45	6.40
3.5	3.70	5.60	4.27
4.0	3.48	2.94	7.55
4.5	2.28	4.28	3.60

TABLE - 12

Maximum Binding Ability (MBA) of Humic Acids with respect to Cd(II) at 0.1M Ionic, Strengrth (As Determined from Double surface langmuar PLots).

pH	HA (soil)	HA (peat)	HA(humified water hyacinth)
	x 10^{-6}M	x 10^{-6}M	x 10^{-6}M
3.0	4.47	5.67	2.57
3.5	4.13	4.47	2.95
4.0	3.90	4.47	2.95
4.5	3.63	4.37	3.65
5.8	3.78	3.67	4.35

TABLE - 13

"Q" (Sites bound/Total number of reactive sites) of Humic Acids with respect to Cu(II) at 0.1 M ionic strength.

Concentration of Cu^{2+} increasing

PA	HA (Soil)				HA (Peat)				HA (humified water hyacinth)			
	3.0	3.5	4.0	4.5	3.0	3.5	4.0	4.5	3.0	3.5	4.0	4.5
1.	0.12	0.19	0.15	0.30	0.14	0.18	0.24	0.20	0.07	0.13	0.11	0.27
2.	0.23	0.35	0.27	0.46	0.26	0.32	0.34	0.32	0.11	0.27	0.25	0.54
3.	0.31	0.45	0.35	0.56	0.34	0.42	0.41	0.40	0.15	0.34	0.30	0.65
4.	0.37	0.53	0.41	0.58	0.40	0.49	0.48	0.46	0.19	0.40	0.35	0.73
5.	0.44	0.59	0.45	0.62	0.46	0.56	0.53	0.48	0.22	0.44	0.38	0.78
6.	0.50	0.64	0.50	0.64	0.50	0.62	0.56	0.51	0.26	0.48	0.41	0.79
7.	0.53	0.66	0.53	0.65	0.54	0.63	0.59	0.54	0.29	0.50	0.43	0.82
8.	0.56	0.68	0.55	0.67	0.57	0.67	0.60	0.55	0.32	0.56	0.46	0.84
9.	0.58	0.71	0.56	0.67	0.60	0.71	0.63	0.57	0.35	0.58	0.47	0.85
10.	0.63	0.73	0.57	0.68	0.61	0.73	0.64	0.58	0.37	0.61	0.48	0.86
11.	0.64	0.74	0.59	0.69	0.64	0.74	0.65	0.59	0.39	0.62	0.49	0.88
12.	0.65	0.75	0.61	0.71	0.68	0.76	0.65	0.59	0.40	0.64	0.50	0.88
13.	0.66	0.76	0.63	0.71	0.69	0.77	0.67	0.60	0.43	0.66	0.51	0.89
14.	0.67	0.78	0.65	0.72	0.70	0.79	0.68	0.61	0.45	0.68	0.52	0.89
15.	0.69	0.79	0.67	0.73	0.71	0.79	0.69	0.62	0.47	0.70	0.52	0.90
16.	0.69	0.80	0.68	0.74	0.72	0.80	0.69	0.63	0.47	0.72	0.54	0.91
17.	0.70	0.81	0.69	0.75	0.73	0.81	0.71	0.64	0.49	0.73	0.54	0.91
18.	0.71	0.82	0.70	0.77	0.73	0.82	0.72	0.64	0.51	0.74	0.55	0.91
19.	0.72	0.82	0.71	0.78	0.74	0.83	0.74	0.66	0.53	0.75	0.57	0.91
20.	0.73	0.83	0.71	0.79	0.74	0.84	0.75	0.67	0.55	0.76	0.58	0.92
21.	0.74	0.84			0.75	0.84	0.76	0.68	0.56	0.77	0.59	0.92
22.	0.75	0.84			0.77	0.85	0.76	0.69	0.57	0.78	0.61	0.93
23.	0.75	0.85			0.77	0.86	0.77	0.70	0.58	0.78	0.62	0.93
24.	0.76	0.85			0.78	0.86	0.78	0.71	0.58	0.80	0.63	0.94
25.	0.77	0.86			0.79	0.87	0.78	0.72	0.59	0.81	0.65	0.94
26.	0.78	0.86			0.80	0.88	0.79	0.73	0.59	0.81	0.66	0.95
27.	0.78	0.87			0.81	0.89	0.80	0.73	0.60	0.82	0.68	0.96
28.	0.79	0.88			0.82	0.90	0.81	0.74	0.60	0,83	0.68	0.96
29.	0.80	0.88			0.83	0.91	0.82	0.75	0.61	0.84	0.70	0.97
30.	0.80	0.89			0.83	0.92	0.82	0.76	0.61	0.86	0.70	0.97

TABLE - 14

"θ" (Sites bound/Total number of reactive sites) of Humic Acids with respect to Cd(ii) at 0.IM Tonic Strength

	HA (Soil)					HA(Peat)					HA (Humuified water hyaeinth				
	3.0	3.5	4.0	4.5	5.8	3.0	3.5	4.0	4.5	5.8	3.0	3.5	4.0	4.5	5.8
1.	0.13	0.16	0.18	0.20	0.19	0.17	0.21	0.20	0.19	0.20	0.12	0.18	0.19	0.18	0.17
2.	0.21	0.27	0.30	0.34	0.34	0.30	0.38	0.33	0.30	0.33	0.20	0.29	0.33	0.31	0.30
3.	0.27	0.35	0.39	0.46	0.47	0.41	0.52	0.42	0.39	0.44	0.26	0.37	0.44	0.42	0.40
4.	0.31	0.40	0.46	0.54	0.57	0.48	0.61	0.49	0.44	0.50	0.32	0.42	0.52	0.50	0.48
5.	0.35	0.45	0.52	0.62	0.63	0.54	0.69	0.53	0.49	0.55	0.34	0.46	0.58	0.58	0.55
6.	0.38	0.47	0.56	0.66	0.70	0.59	0.75	0.58	0.51	0.56	0.37	0.49	0.64	0.63	0.61
7.	0.40	0.48	0.58	0.71	0.74	0.61	0.79	0.59	0.51	0.57	0.40	0.52	0.67	0.67	0.66
8.	0.42	0.48	0.61	0.72	0.76	0.61	0.82	0.62	0.53	0.58	0.43	0.57	0.70	0.70	0.69
9.	0.43	0.49	0.61	0.74	0.78	0.64	0.83	0.63	0.55	0.59	0.44	0.61	0.71	0.73	0.73
10.	0.47	0.51	0.64	0.74	0.81	0.65	0.84	0.65	0.55	0.63	0.47	0.64	0.75	0.74	0.77
11.	0.47	0.56	0.67	0.74	0.82	0.65	0.85	0.67	0.55	0.63	0.51	0.68	0.75	0.77	0.78
12.	0.47	0.58	0.67	0.77	0.79	0.67	0.87	0.69	0.57	0.65	0.58	0.75	0.78	0.79	0.80
13.	0.49	0.58	0.64	0.77	0.78	0.67	0.89	0.72	0.59	0.71	0.62	0.79	0.85	0.82	0.83
14.	0.51	0.61	0.64	0.80	0.79	0.69	0.89	0.72	0.64	0.74	0.62	0.83	0.85	0.85	0.85
15.	0.54	0.63	0.64	0.83	0.77	0.71	0.91	0.74	0.62	0.76	0.65	0.91	0.88	0.85	0.87
16.	0.56	0.65	0.64	0.83	0.79	0.72	0.93	0.76	0.64	0.76	0.69	0.87	0.92	0.88	0.90
17.	0.56	0.68	0.64	0.85	0.74	0.72	0.95	0.78	0.66	0.79	0.76	0.91	0.98	0.90	0.92
18.	0.58	0.65	0.67	0.88	0.74	0.74	0.97	0.83	0.71	0.81	0.80	0.94	0.95	0.93	0.94
19.	0.58	0.68	0.67	0.91	0.79	0.76	0.99	0.85	0.73	0.87	0.84	0.98	0.98	0.93	0.94
20.	0.60	0.70	0.69	0.94	0.77	0.74	0.99	0.87	0.76	0.93	0.87	1.02	1.02	0.96	0.97
21.	0.65	0.73	0.74	0.91	0.77	0.78	0.99	0.85	0.78	0.93	0.91	1.02	1.05	0.99	0.99
22.	0.67	0.75	0.72	0.94	0.79	0.78	1.01	0.89	0.89	0.82	0.95	0.95	1.09	1.01	1.01
23.	0.69	0.73	0.74	0.94	0.82	0.79	1.04	0.92	0.80	0.98	0.98	1.13	1.12	1.01	0.99
24.	0.74	0.75	0.72	0.94	0.87	0.81	1.04	0.94	0.85	1.01	0.98	1.13	1.12	1.04	1.03
25.	0.76	0.77	0.74	0.96	0.82	0.81	1.06	0.96	0.82	1.04	1.05	1.17	1.15	1.07	1.06
26.	0.78	0.80	0.77	0.96	0.79	0.83	1.06	0.98	0.85	1.06	1.02	1.21	1.19	1.10	1.08
27.	0.83	0.80	0.79	0.99	0.82	0.85	1.08	1.03	0.87	1.09	1.05	1.25	1.22	1.10	1.08

TABLE - 15

Conditional Stability Constants (kn) of Cu(II) humate complexes as Determined by Scatchard Plot Method at 0.1M Ionic Strength.

	HA (soil)		HA(peat)		HA(humified) water hyacinth)	
pH	$K_n x10^5$	Log k_n	$k_n x10^5$	log kn	$knx10^5$	log kn
3.0	1.188	5.07	1.083	5.03	0.570	4.76
3.5	1.380	5.14	1.350	5.14	0.963	4.98
4.0	1.438	5.16	1.550	5.19	1.020	5.01
4.5	1.767	5.25	1.600	5.20	1.350	5.13

TABLE - 16

Conditional Stability Constants (kn) of Cd(II) humate complexes as Determined by Scatchard Plot Method at 0.1M Ionic Strength.

	HA (soil)		HA(peat)		HA(humified) water hyacinth)	
pH	$K_n x10^5$	Log k_n	$k_n x10^5$	log kn	$knx10^5$	log kn
3.0	0.550	4.47	0.467	4.67	0.430	4.63
3.5	0.583	4.77	0.550	4.74	0.850	4.93
4.0	0.917	4.96	0.567	4.75	1.040	5.02
4.5	1.200	5.08	0.650	4.81	1.080	5.03

TABLE - 17

Conditional Stability Constants (kn) of Cu(II) humate complexes as Determined by ~~Scatchard~~ Double Reciprocal Plot Method at 0.1M Ionic Strength.

	HA (soil)		HA(peat)		HA(humified) water hyacinth)	
pH	$K_n x10^5$	Log k_n	$k_n x10^5$	log kn	$knx10^5$	log kn
3.0	2.512	5.40	3.388	5.53	0.603	4.78
3.5	2.630	5.42	3.802	5.58	2.138	5.33
4.0	2.884	5.46	3.981	5.60	5.012	5.70
4.5	4.571	5.66	4.266	5.63	10.000	6.00

TAPLE - 18

Conditional Stability Constants (kn) of Cd(II) humate complexes as Determined by ~~Scatchard~~ Double Reciprocal Plot Method at 0.1M Ionic Strength.

	HA (soil)		HA(peat)		HA(humified) water hyacinth)	
pH	K_nx10^5	Log k_n	k_nx10^5	log kn	knx10^5	log kn
3.0	0.871	4.94	0.759	4.88	0.162	4.21
3.5	0.955	4.98	1.023	5.01	0.309	4.49
4.0	2.884	5.46	1.318	5.12	0.355	4.55
4.5	3.020	5.48	1.479	5.17	0.741	4.87
5.8	3.981	5.60	1.622	5.21	1.288	5.11

TABLE - 19

Molecular Weight per Total Number of Binding sites (mw/n) of humic minds as Determined by Double Reciprocal Plot Method [Cu(II)]-HA.

	HA (soil)	HA(peat)	HA(humified) water hyacinth)
3.0	6.33×10^3	5.00×10^3	5.50×10^3
3.5	9.00×10^3	6.00×10^3	8.50×10^3
4.0	11.50×10^3	13.67×10^3	7.00×10^3
4.5	17.00×10^3	10.50×10^3	9.25×10^3
Ave.	10.96×10^3	8.79×10^3	7.56×10^3

TABLE - 20

Molecular Weight per Total Number of Binding sites (mw/n) of humic minds as Determined by Double Reciprocal Plot Method [Cd(II)]-HA.

	HA (soil)	HA(peat)	HA(humified) water hyacinth)
3.0	8.00×10^3	3.75×10^3	3.76×10^3
3.5	7.67×10^3	4.50×10^3	4.87×10^3
4.0	9.67×10^3	6.33×10^3	8.83×10^3
4.5	8.83×10^3	8.25×10^3	5.67×10^3
Ave.	8.57×10^3	6.52×10^3	4.93×10^3

TABLE - 21

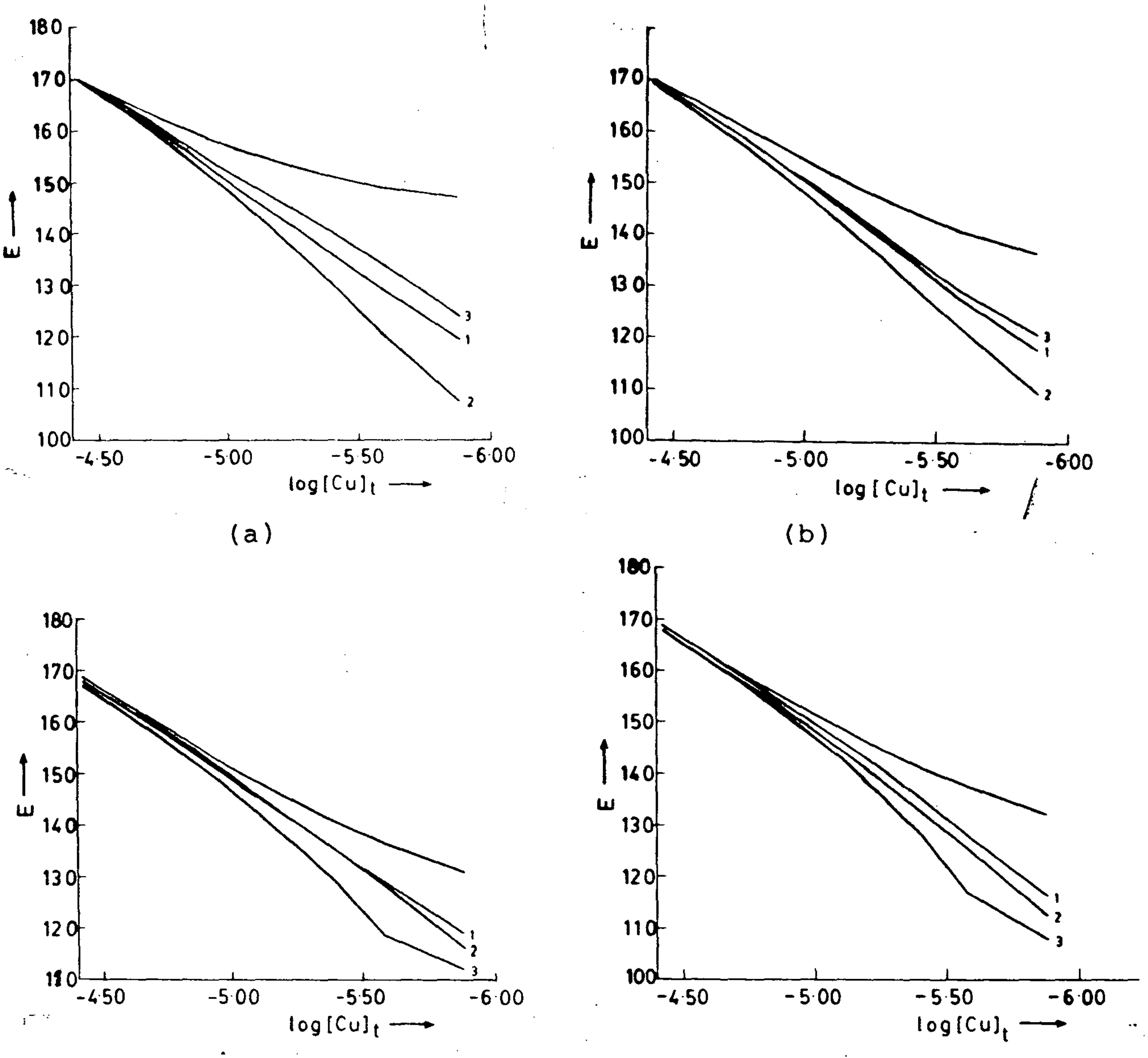
Total number of Binding sites (n) and Macroscopic Binding Constant (kn) of Humic Acids and Cu(II) - humate complexes respectively as Determined by Hill Plot Method.

	HA (soil)		HA(peat)		HA(humified) water hyacinth)	
pH	n	Log kn	n	log kn	n	log kn
3.0	1.11	4.99	1.43	5.04	1.67	4.81
3.5	0.91	5.03	1.67	5.06	1.25	4.87
4.0	1.00	5.08	0.77	5.08	0.50	4.93
4.5	0.77	5.10	0.40	5.14	0.56	5.29

TABLE - 22

Total number of Binding sites (n) and Macroscopic Binding Constant (kn) of Humic Acids and Cd(II) - humate complexes respectively as Determined by Hill Plot Method.

	HA (soil)		HA(peat)		HA(humified) water hyacinth)	
pH	n	Log k_n	n	log kn	n	log kn
3.0	1.11	4.82	0.71	4.94	1.67	4.74
3.5	1.00	4.92	1.00	5.04	0.56	4.80
4.0	0.83	4.96	0.63	5.04	0.83	4.85
4.5	1.00	5.04	0.36	5.07	1.25	4.96
5.8	1.00	5.08	0.69	5.08	1.00	5.06



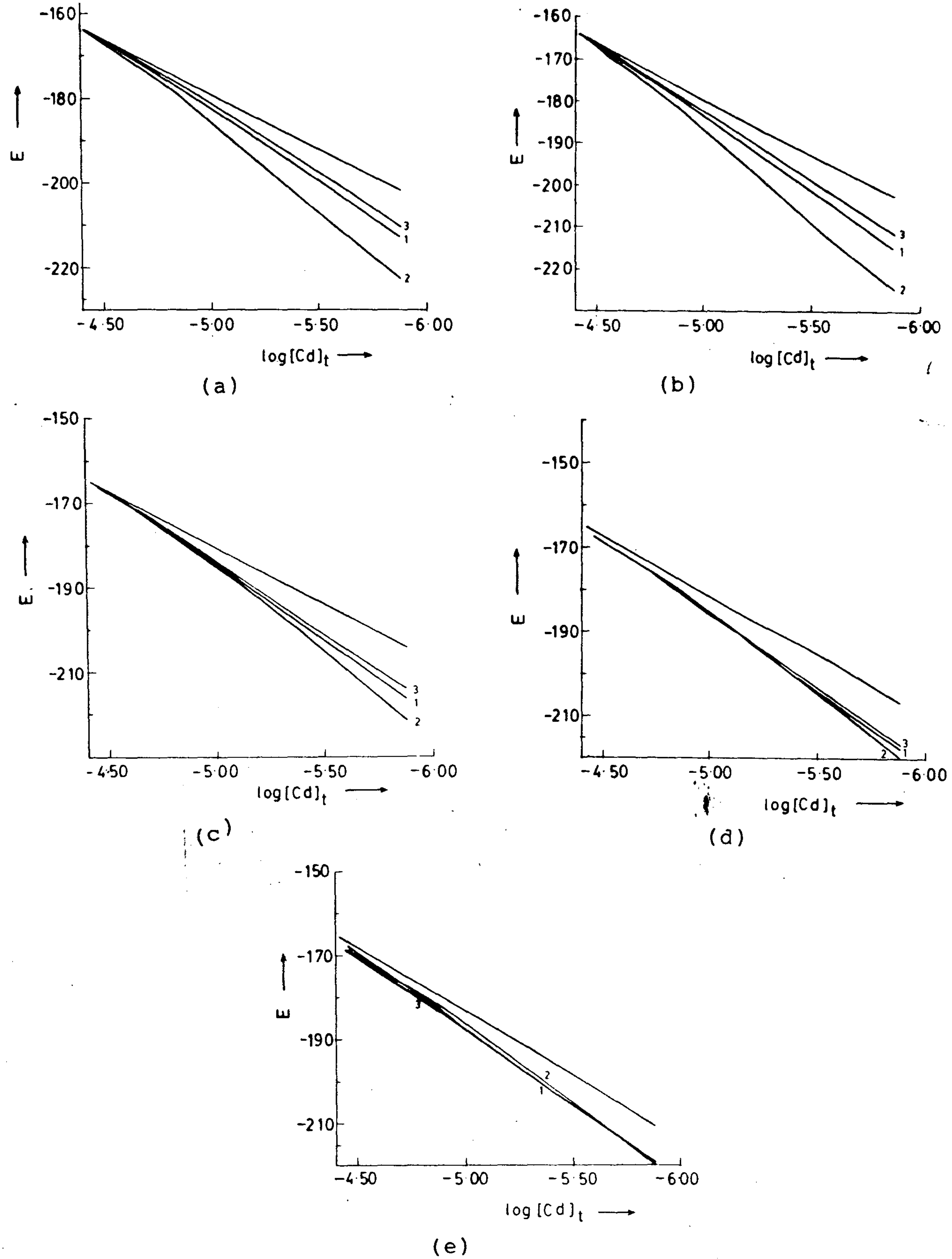
(a)

(b)

(c)

(d)

Fig. 1. ISE Response Curves for Cu(II) - HA interactions (Ionic Strength = 0.1M : P = 29.55). pH : 3.0 (a), 3.5(b), 4.0(c), 4.5(d). 1 = HA (soil); 2 = HA (peat); 3 = HA (humified water hyacinth) and Blank (HA absent).

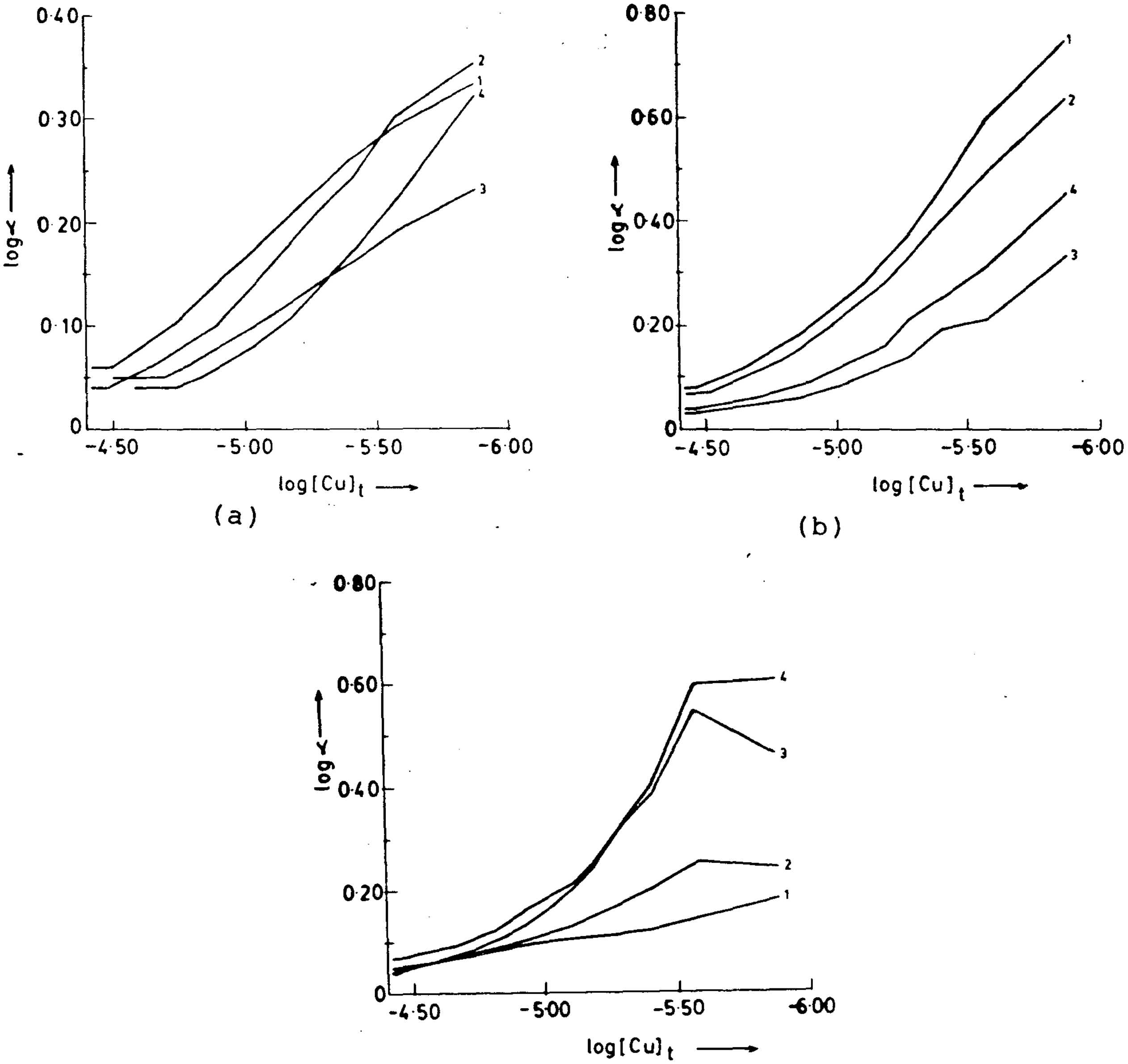


(a) (b)

(c) (d)

(e)

Fig. 2. ISE Response Curves for Cd(II) - HA interactions (Ionics strength = 0.1M; P = 29.20) pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d). 5.8(e) and blank (HA absent). 1 = HA (soil); 2 = HA (peat); 3 = HA (humified water hyacinth) and Blank (HA absent).

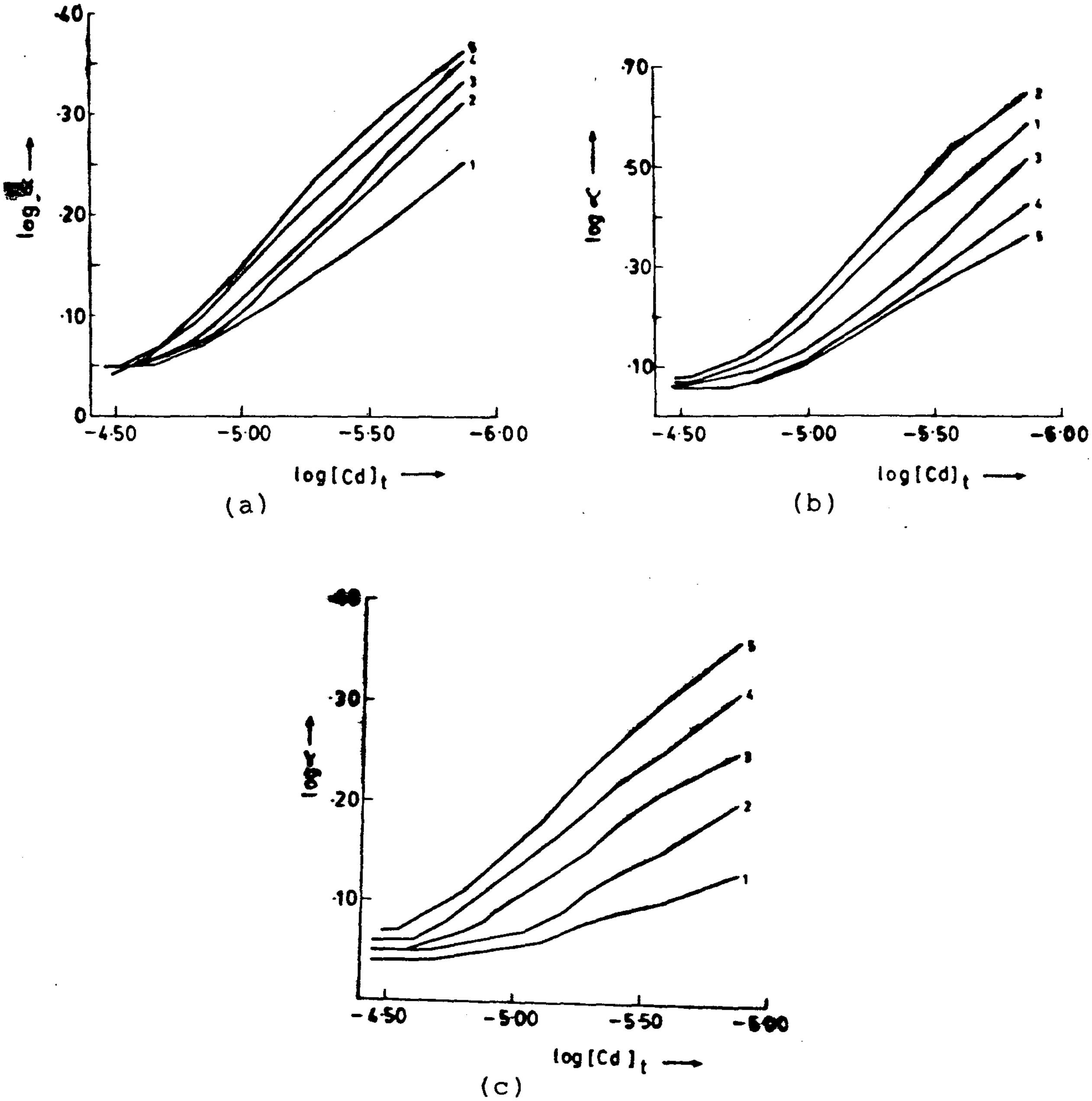


(a)

(b)

(c)

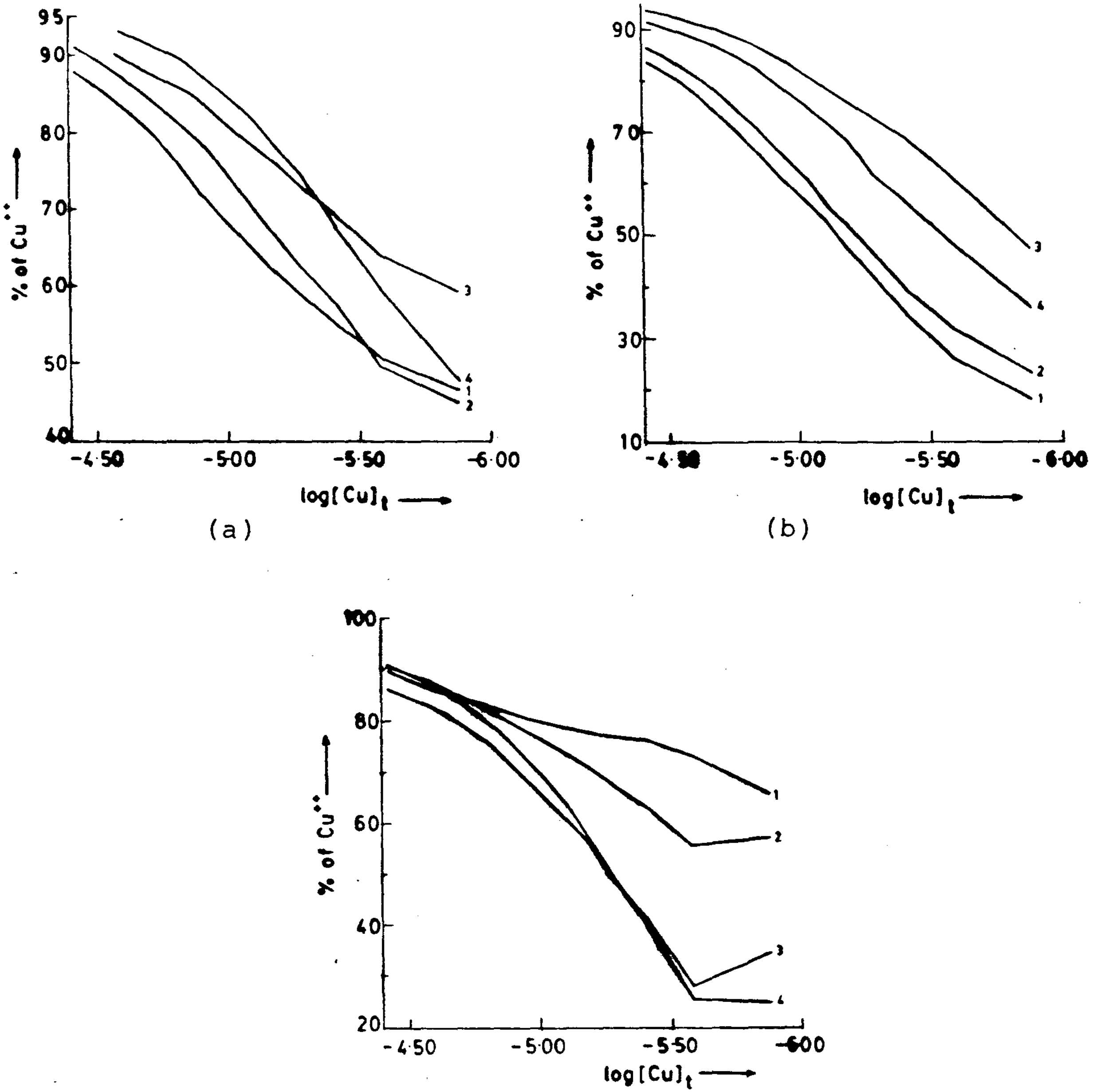
Fig. 3. Log x vs. Log $[Cu]_t$: (a) HA (soil), (b) HA (peat); (c) HA (humified water hyacinth) at pH : 3.0(1); 3.5(2); 4.0(3); 4.5(4).



(a) (b)

(c)

Fig. 4. Log x vs. Log $[Cd]_t$: (a) HA (soil); (b) HA (peat); (c) HA (humified water hyacinth) at pH : 3.0(1); 3.5(2); 4.0(3); 4.5(4); 5.8(5).



(a)

(b)

(c)

Fig. 5. % $[Cu^{++}]$ vs. Log $[Cu]_t$: (a) HA (soil); (b) HA (peat); (c) HA (humified water hyacinth) at pH : 3.0(1); 3.5(2); 4.0(3); 4.5(4).

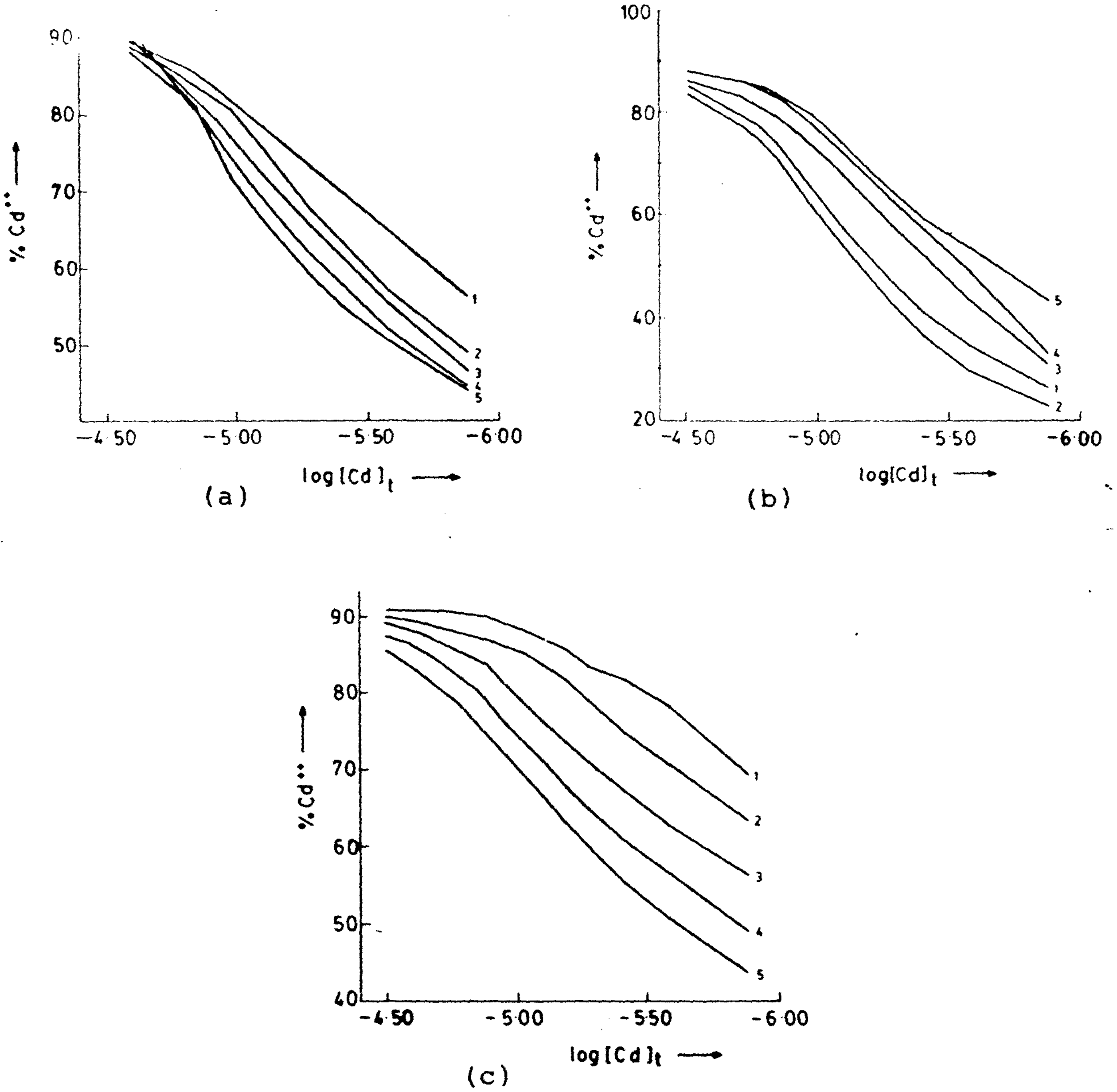


Fig. 6. % [Cd^{++}] vs. Log [Cd)$_t$: (a) HA (soil), (b) HA (peat); (c) HA (humified water hyacinth) at pH : 3.0(1); 3.5(2); 4.0(3); 4.5(4); 5.8(5).

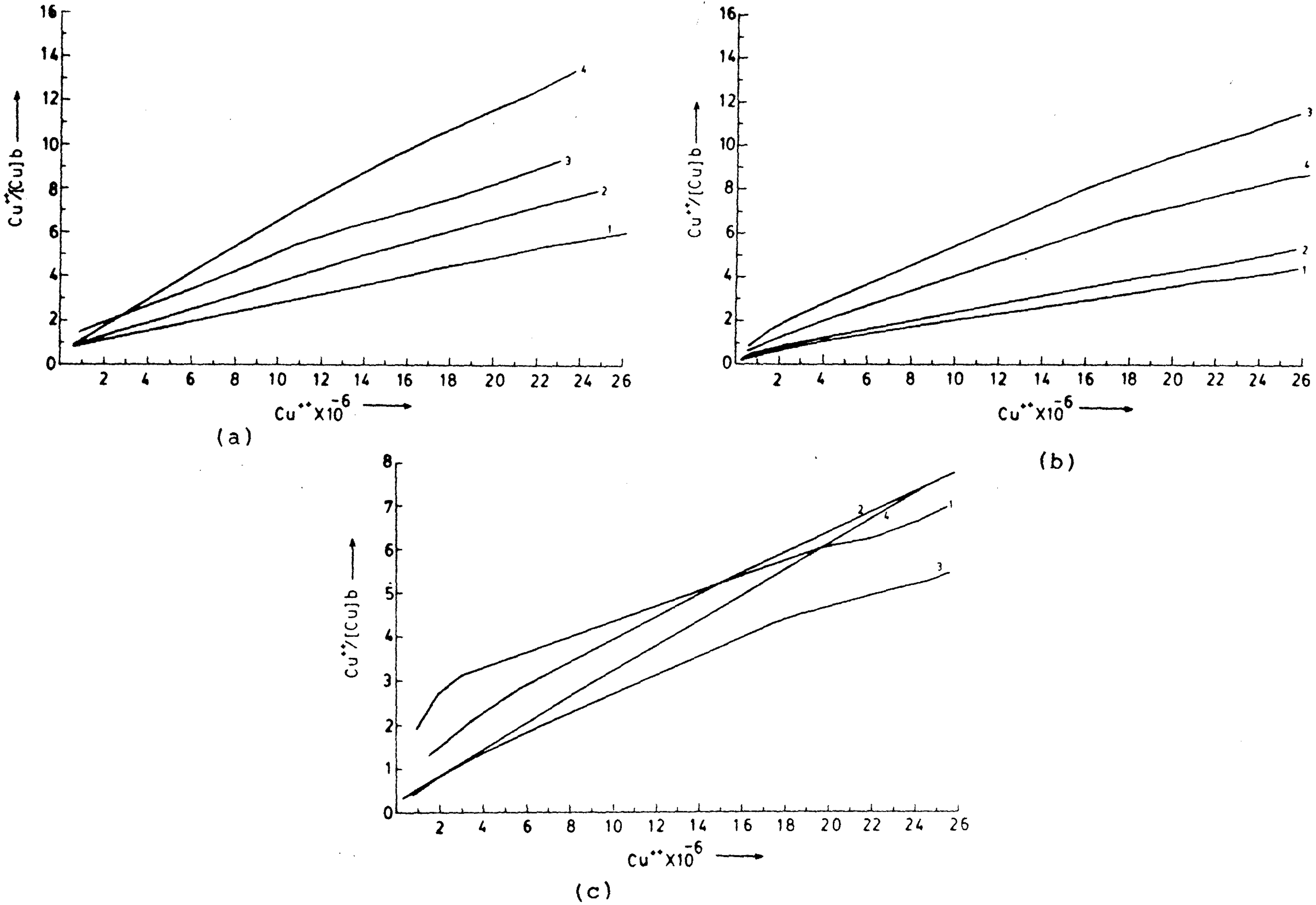


Fig. 7. Reciprocal (Single surface Longmuir) plots of Cu(II) binding by Humic Acids : (a) HA (soil); (b) HA (peat); (c) HA (humified water hyacinth) at pH : 3.0(1), 3.5(2). 4.0(3), 4.5(4).

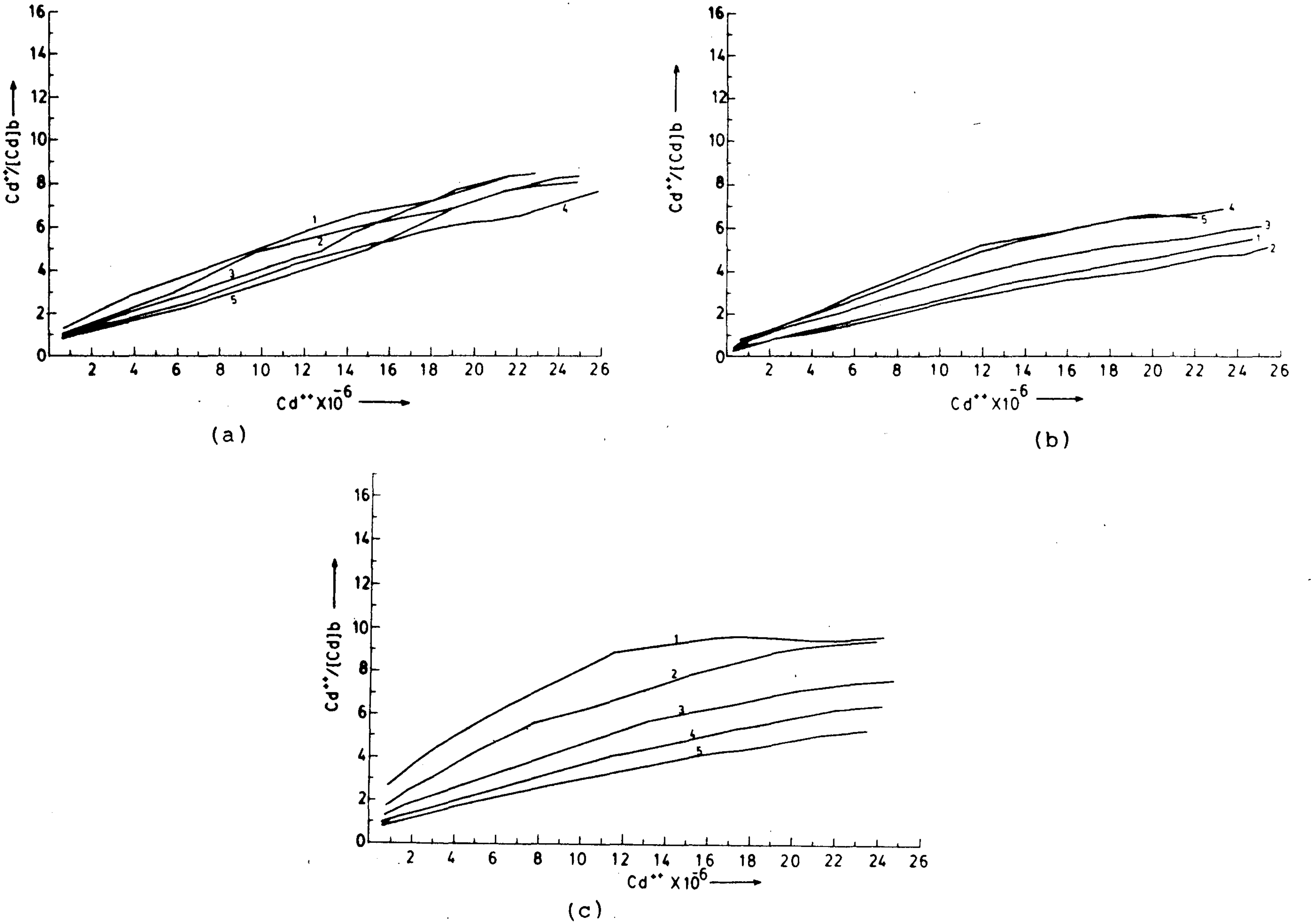


Fig. 8. Reciprocal (Single surface langmuir) plots of Cd(II) binding by Humic Acids : (a) HA (soil); (b) HA (peat); (c) HA (huminified water hyacinth) at pH : 3.0(1), 3.5(2), 4.0(3), 4.5(4), 5.8(5).

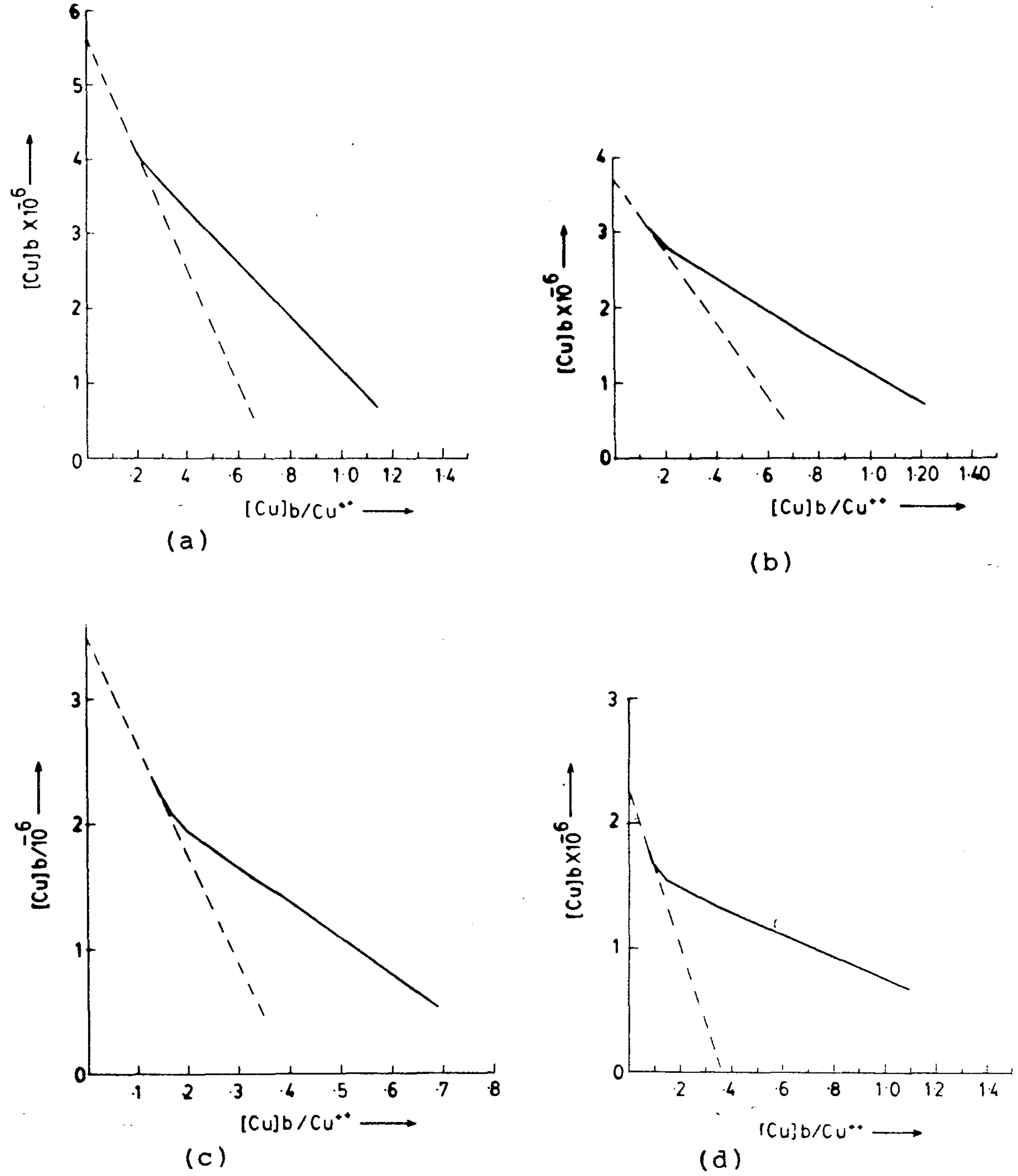
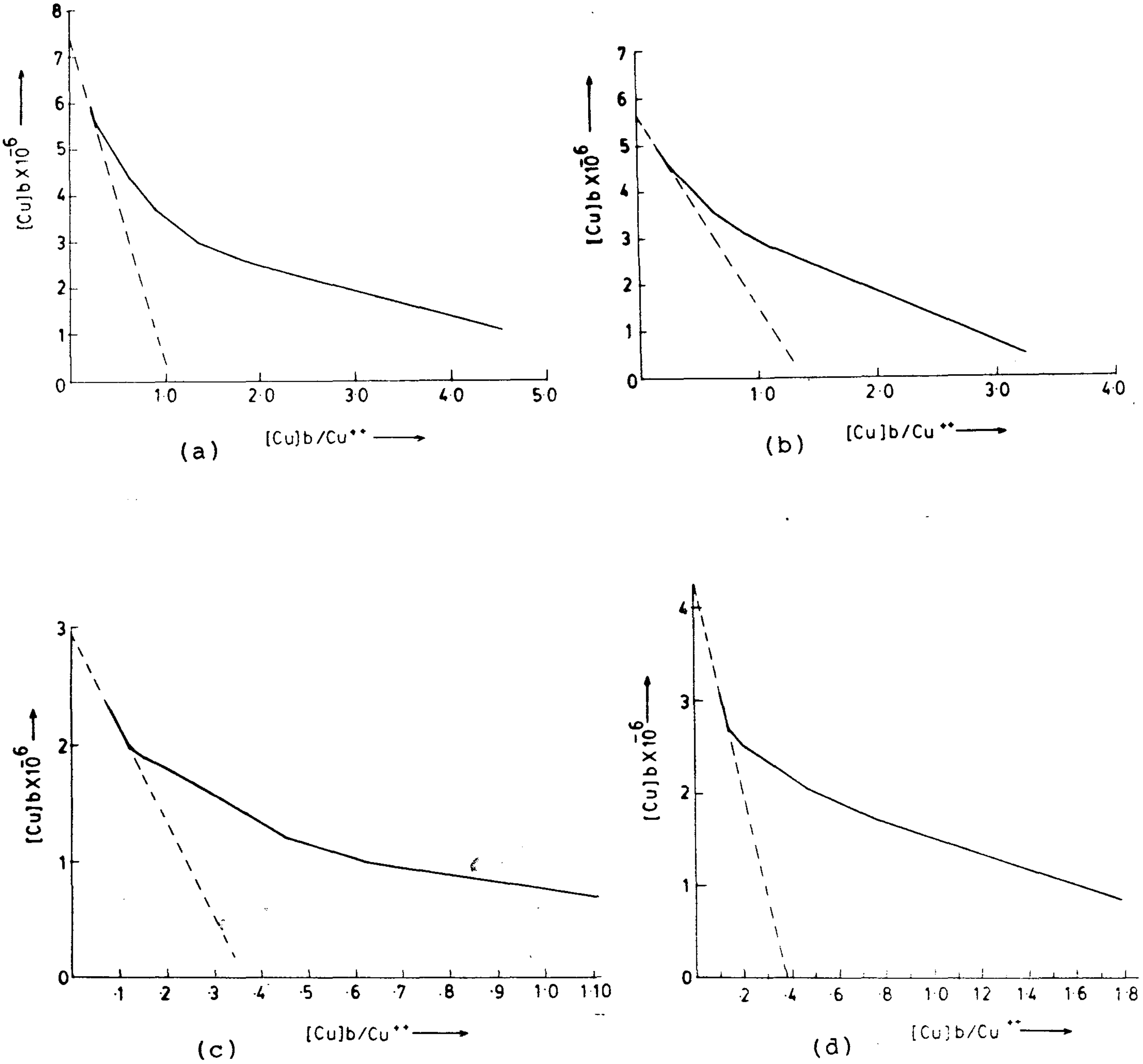


Fig. 9. Double surface Langmuir plots of Cu(II) binding by HA (soil) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d).



(a) (b)

(c) (d)

Fig.10. Double surface Langmuir Plots of Cu(II) binding by HA (peat) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d).

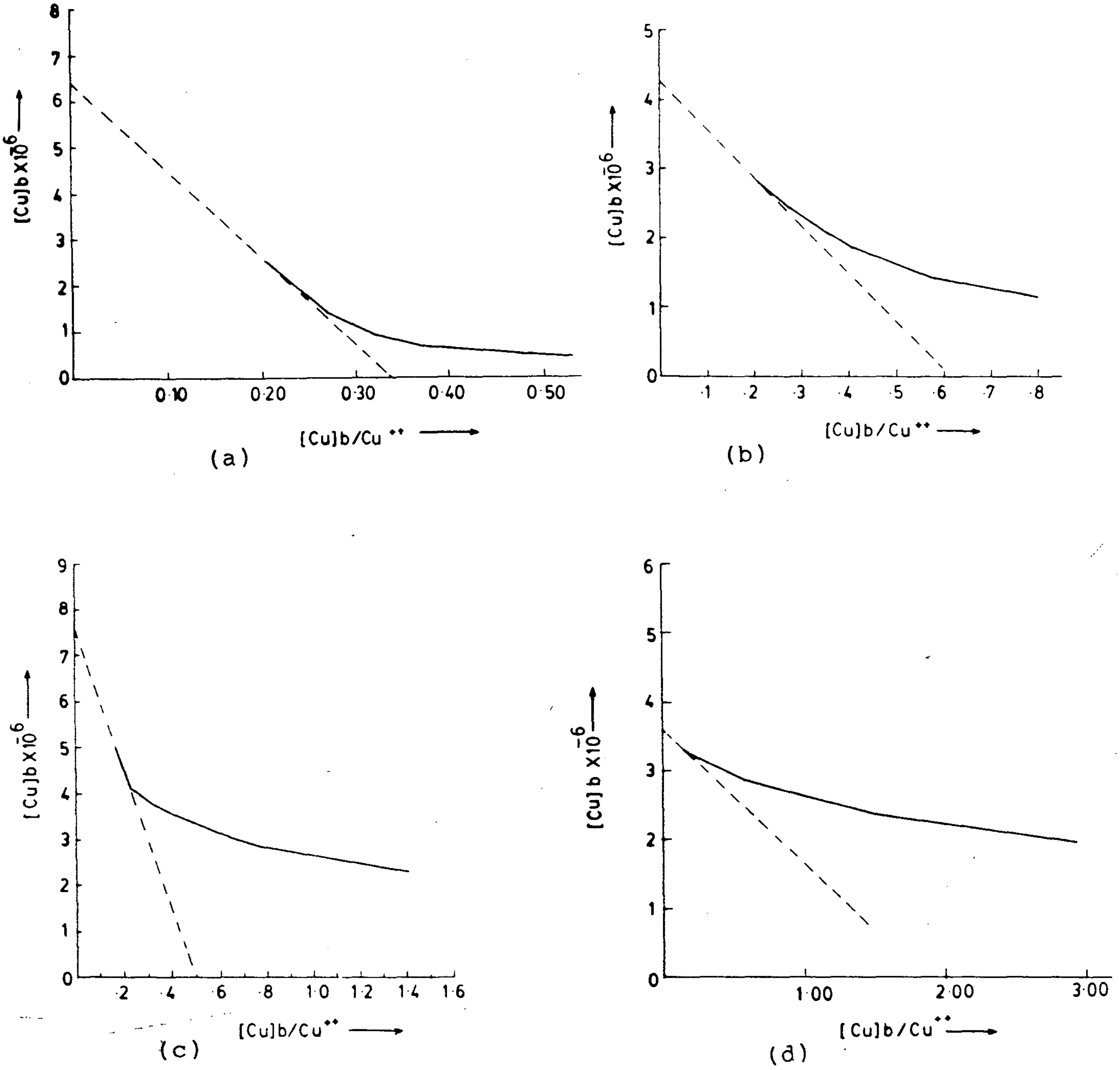


Fig.11. Double Surface Langmuir Plots of Cu(II) binding by HA (humified water hyacinth) at pH : 3.0 (a), 3.5(b), 4.0(c), 4.5(d).

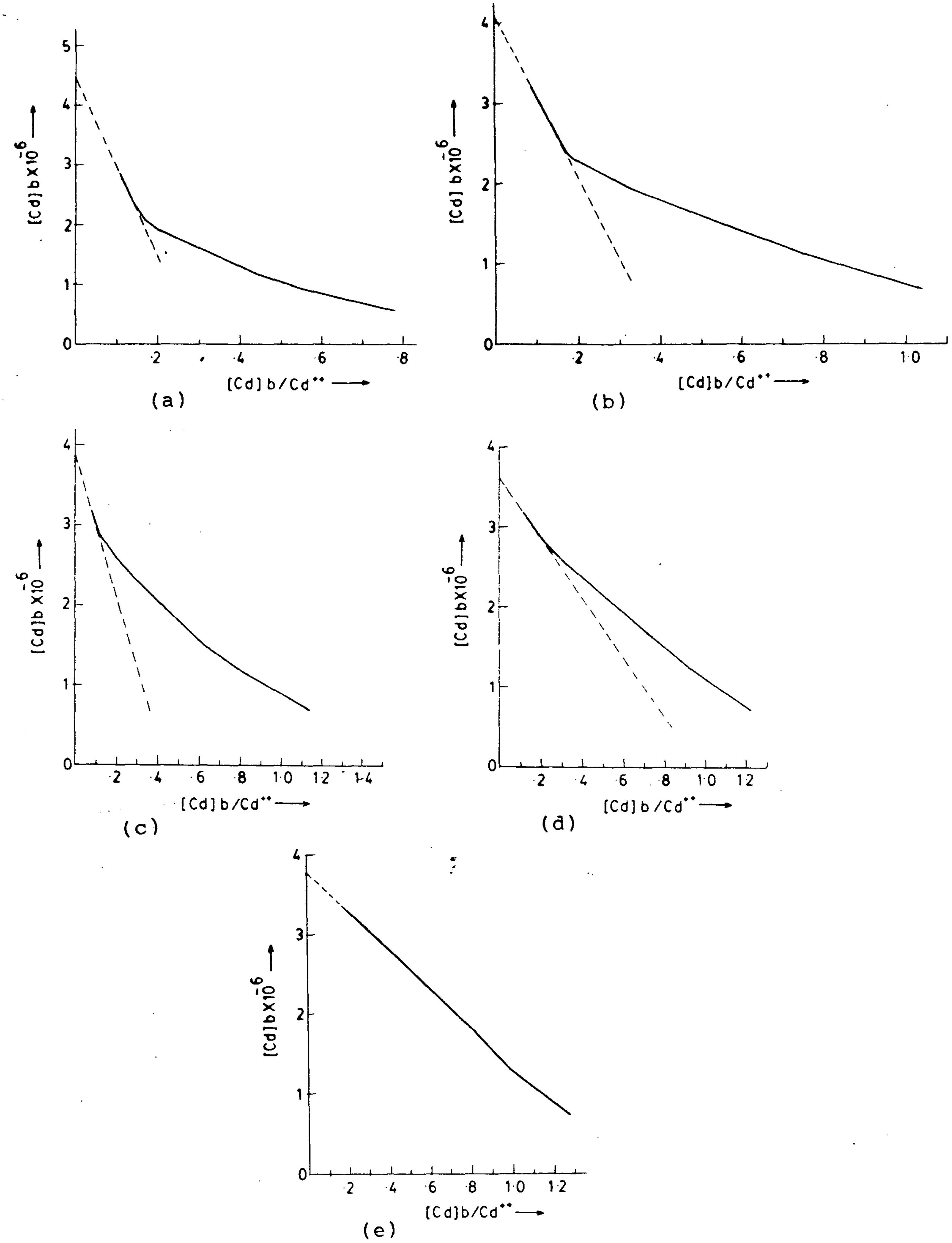


Fig.12. Double Surface Langmuir Plots of Cd(II) binding by HA (soil) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d), 5.8(e).

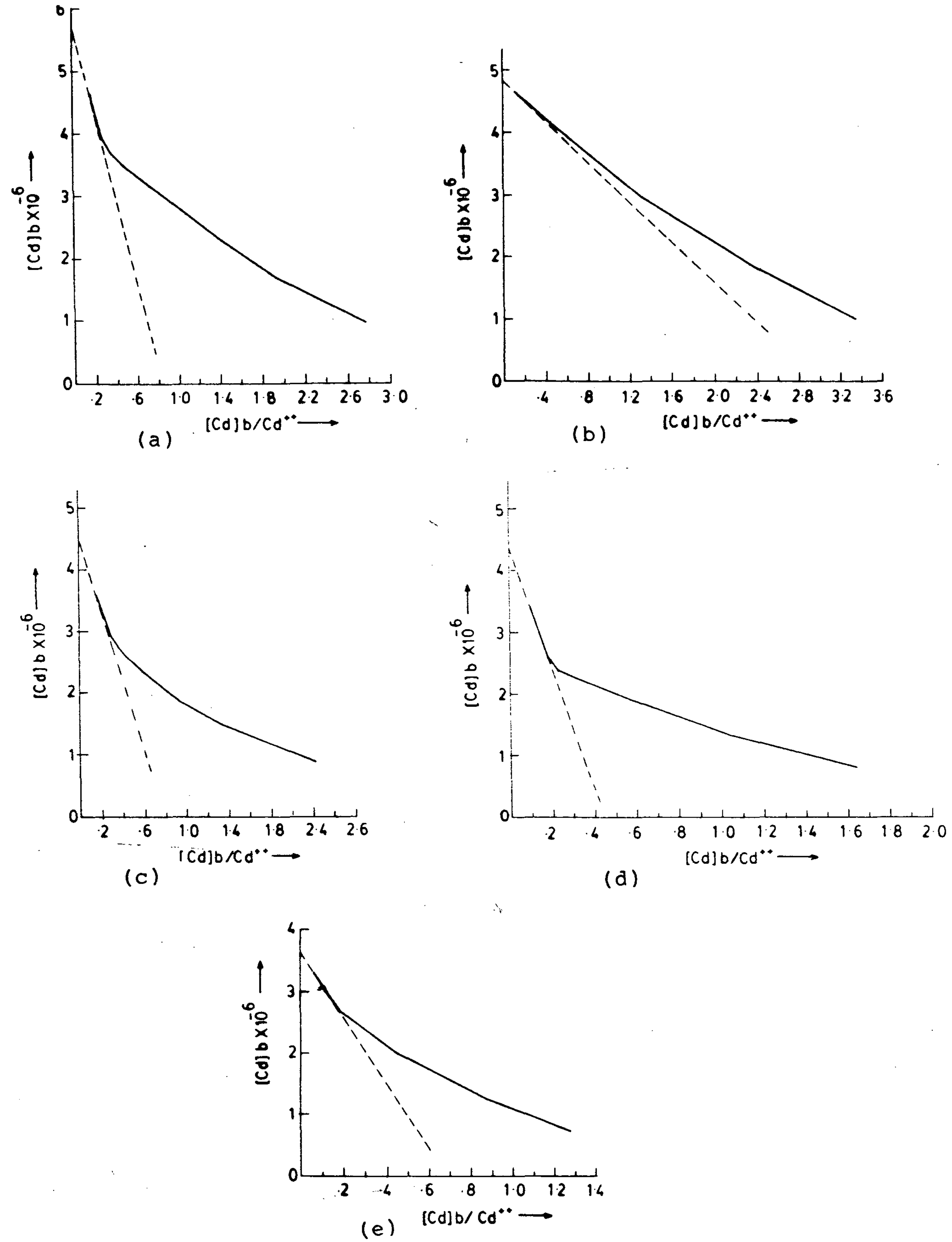
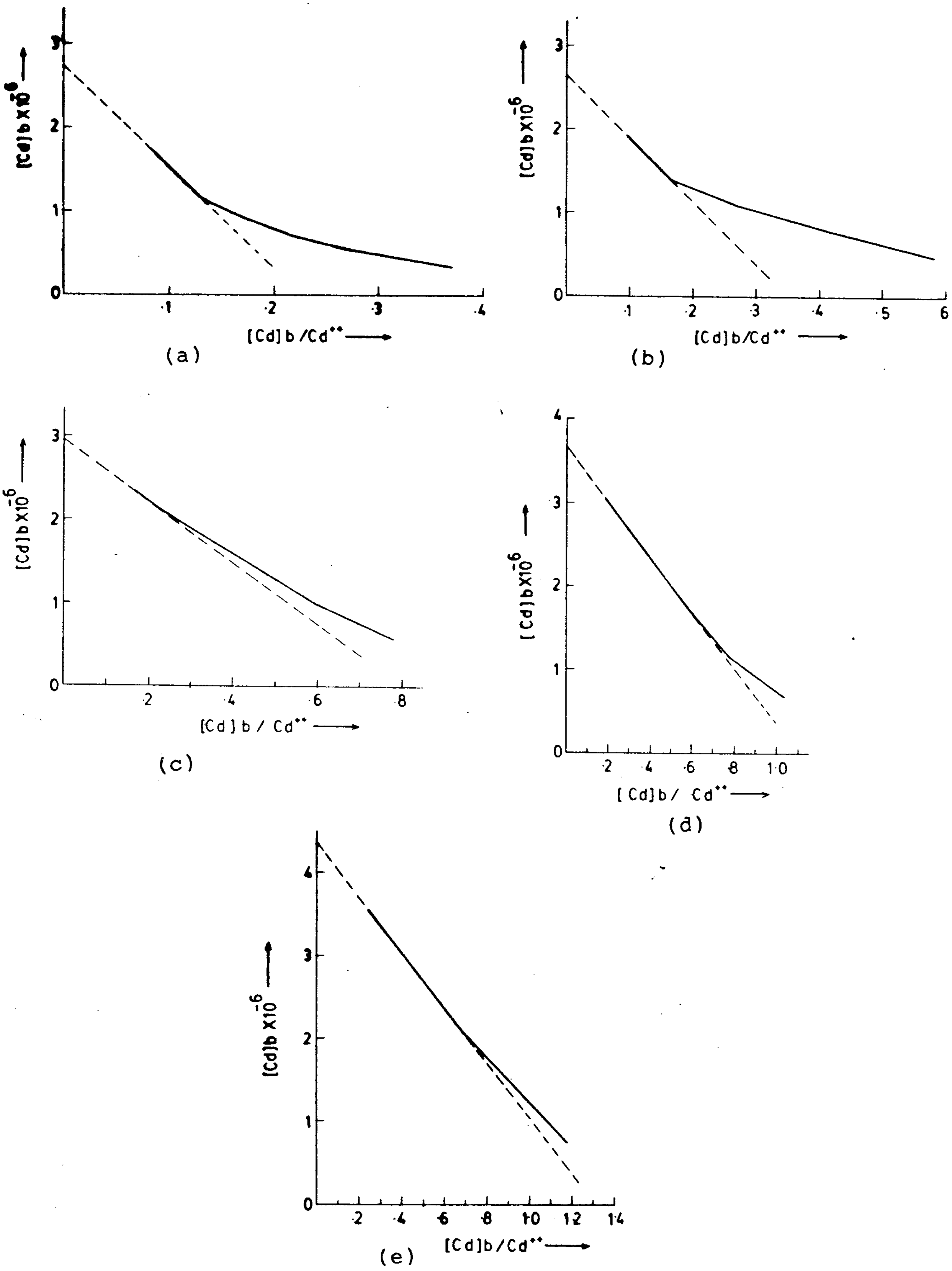


Fig. 13. Double surface Langmuir plots of Cd(II) binding by HA (peat) at pH : 3.0 (a), 3.5 (b), 4.0 (c), 4.5(d),



(a)

(b)

(c)

(d)

(e)

Fig.14. Double Surface langmuir Plots of Cd(II) binding by HA (humified water hyacinth) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d), 5.8(e)

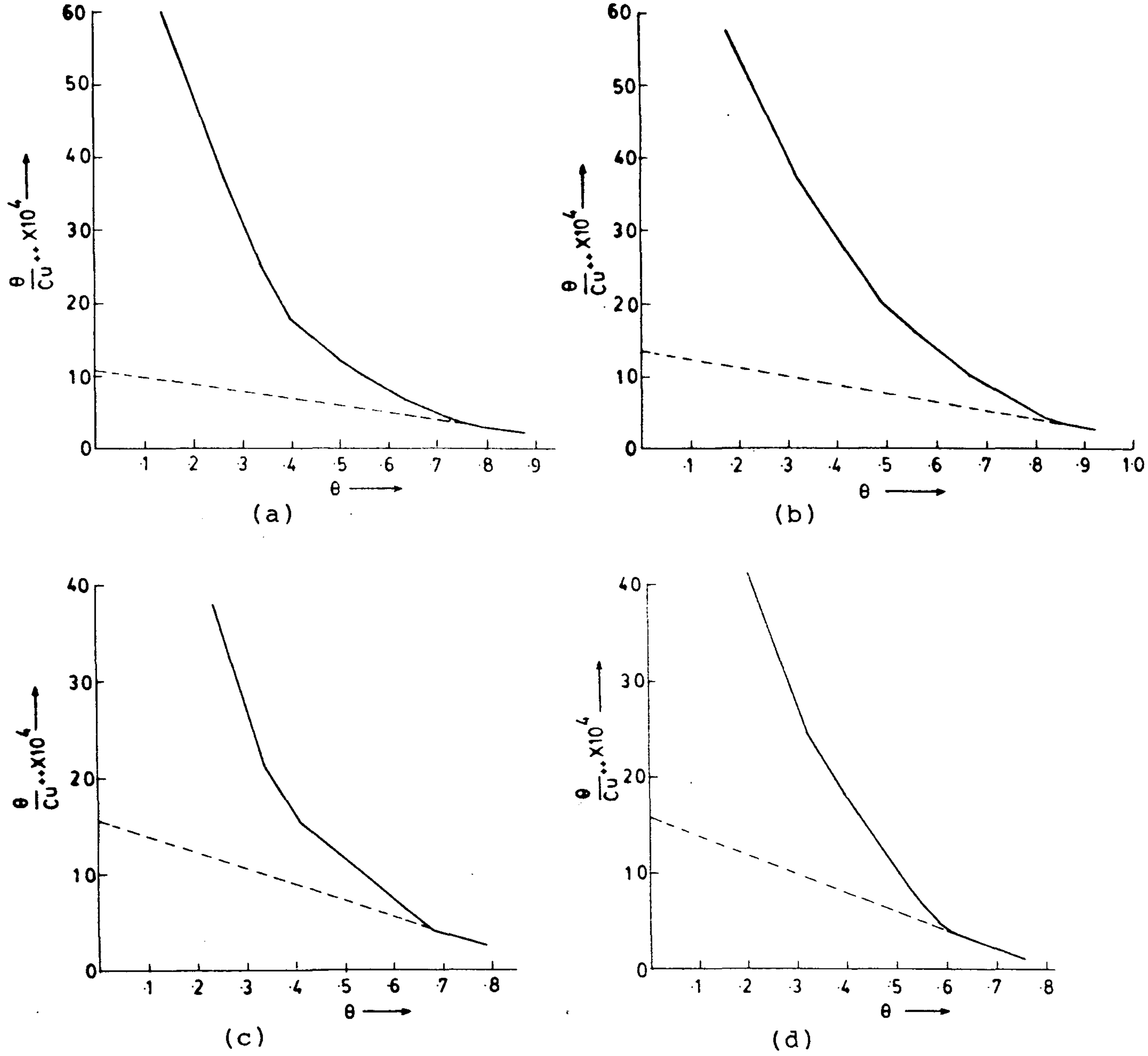


Fig.15. Scatchard Plots of Cu(II) binding by HA (soil) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d).

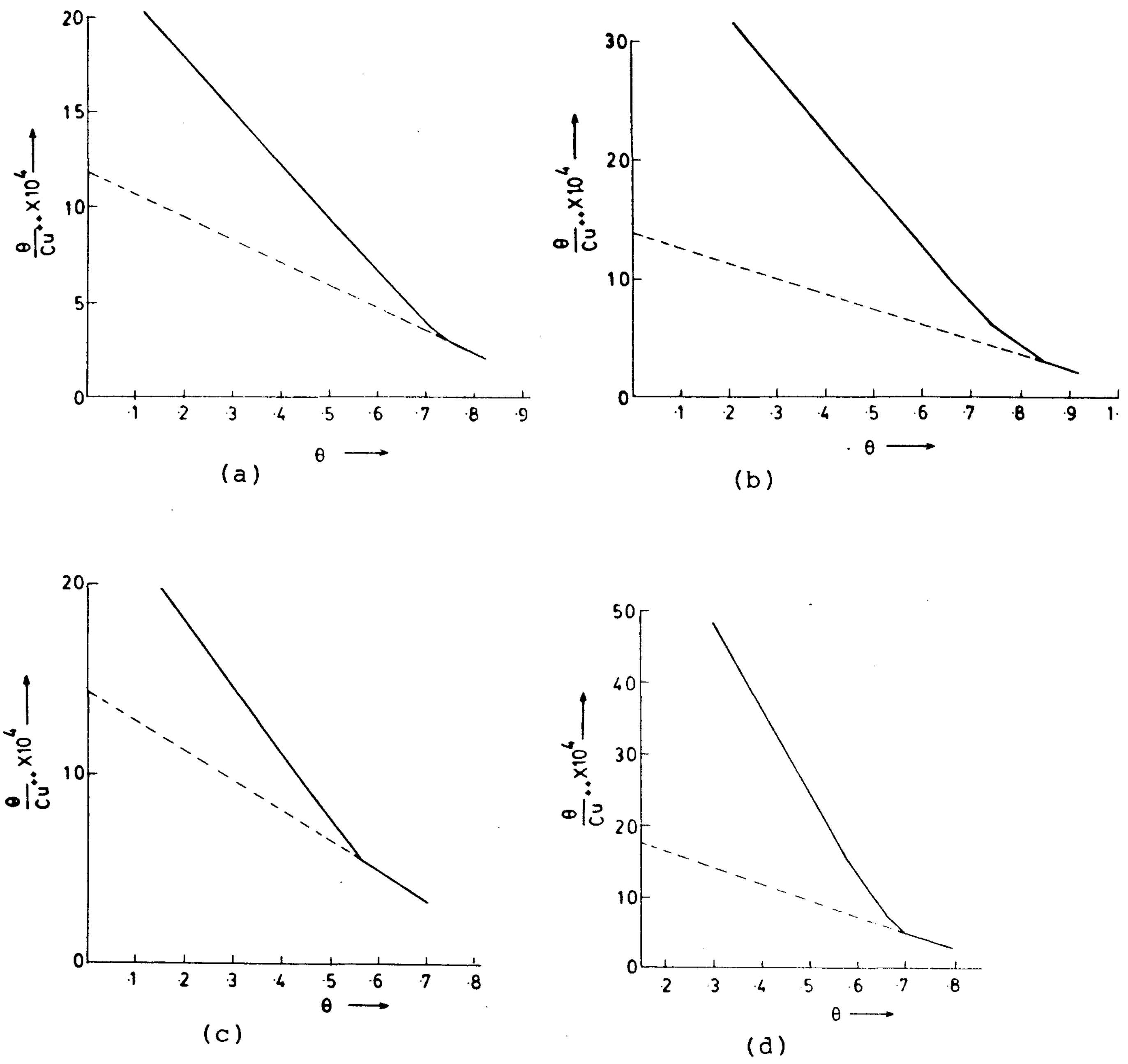


Fig.16. Scatchard Plots of Cu(II) binding by HA (peat) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d).

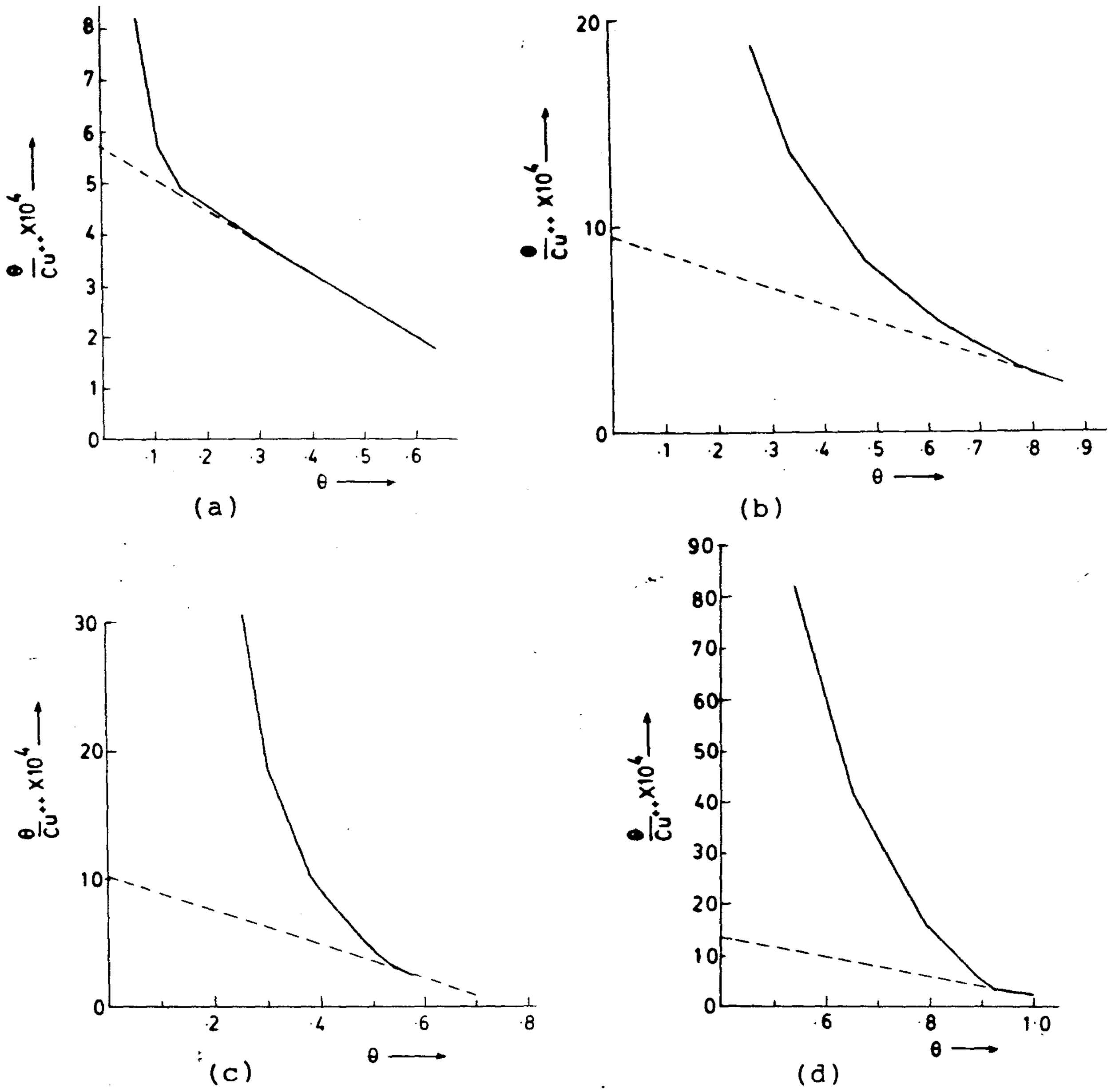


Fig.17. Scatchard Plots of Cu(II) binding by HA (humified water hyacinth) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d).

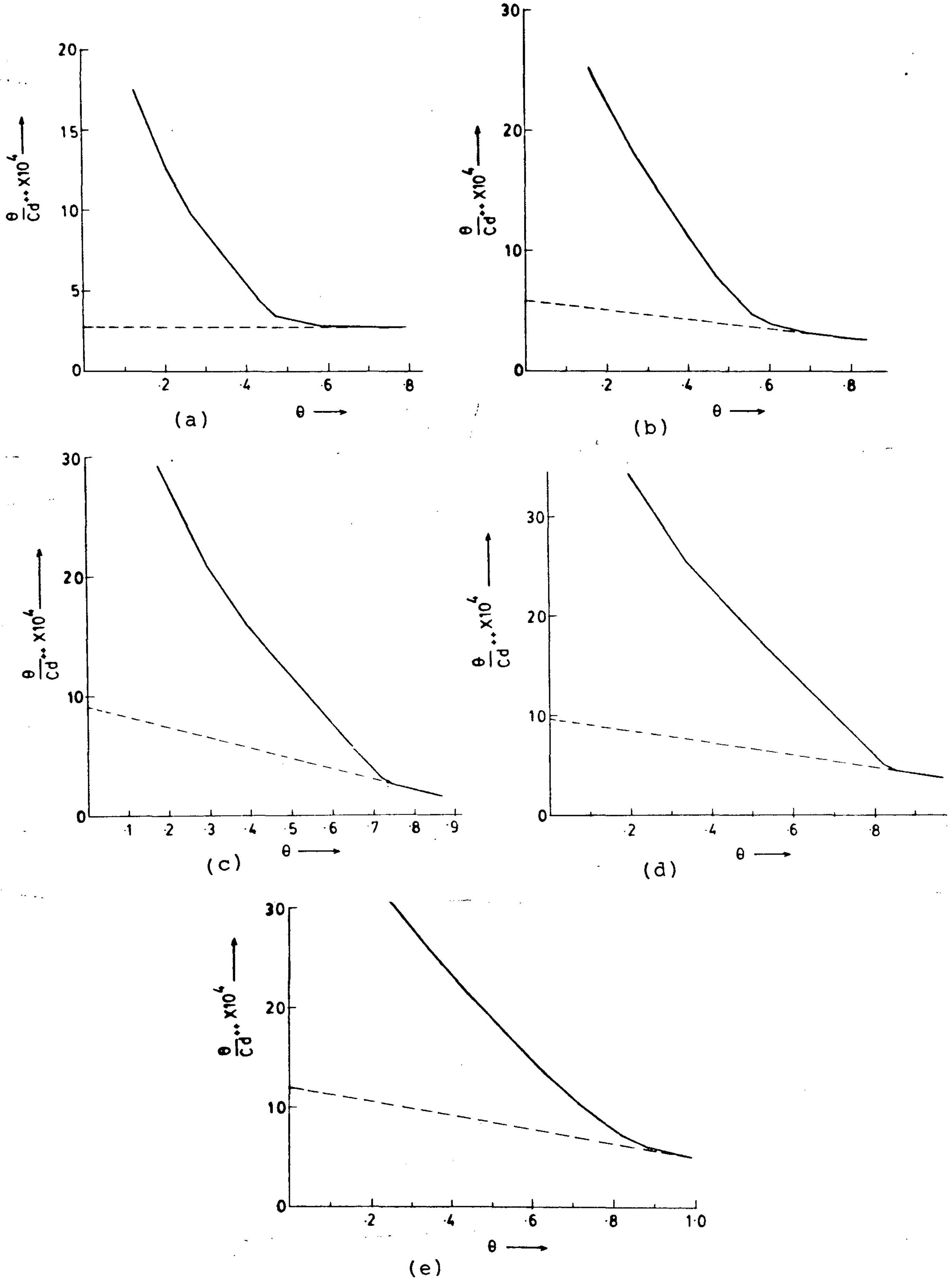


Fig.18. Scatchard Plots of Cd(II) binding by HA (soil) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d), 5.8(e).

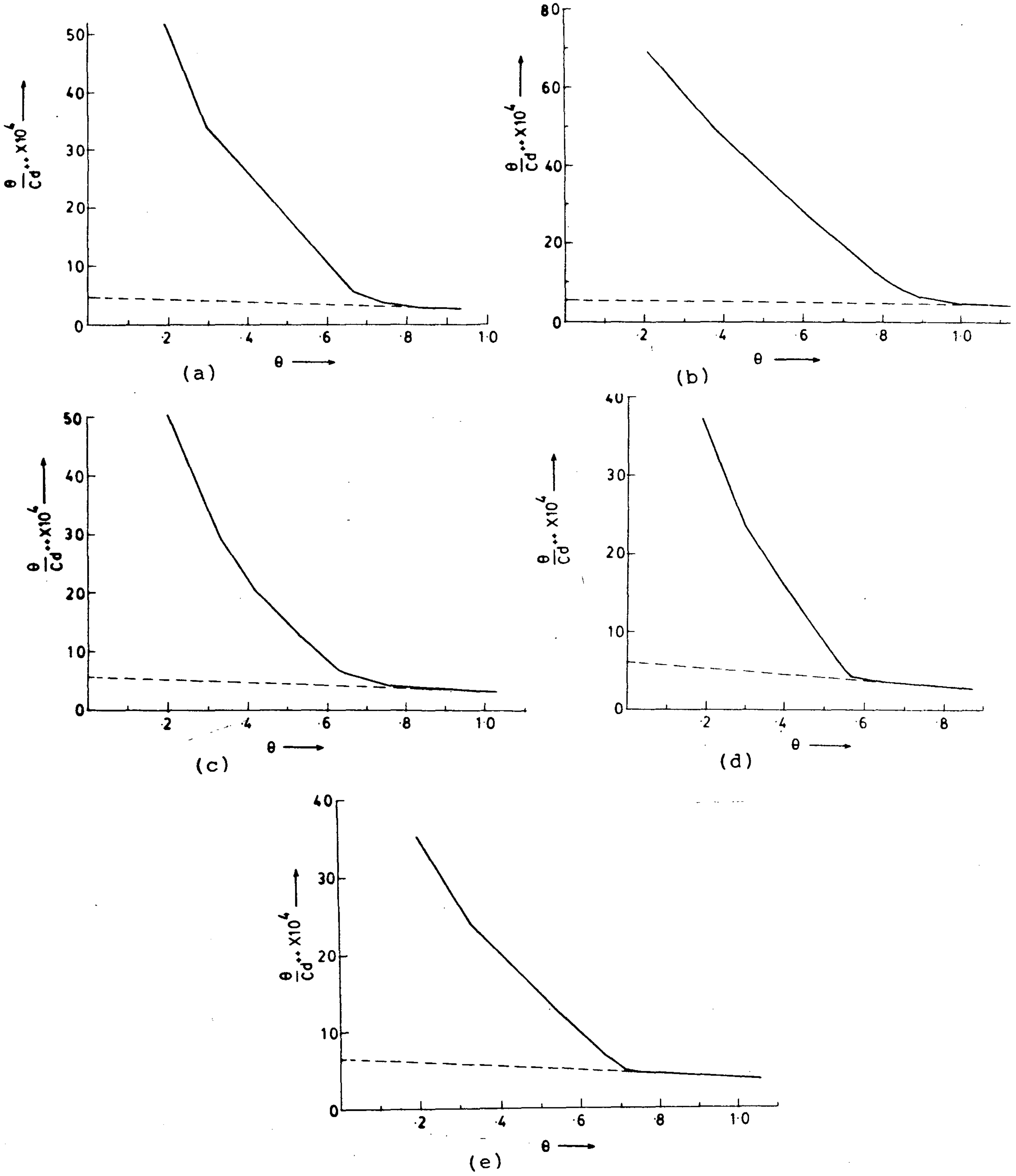


Fig.19. Scatchard Plots of Cd(II) binding by HA (peat) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d), 5.8(e).

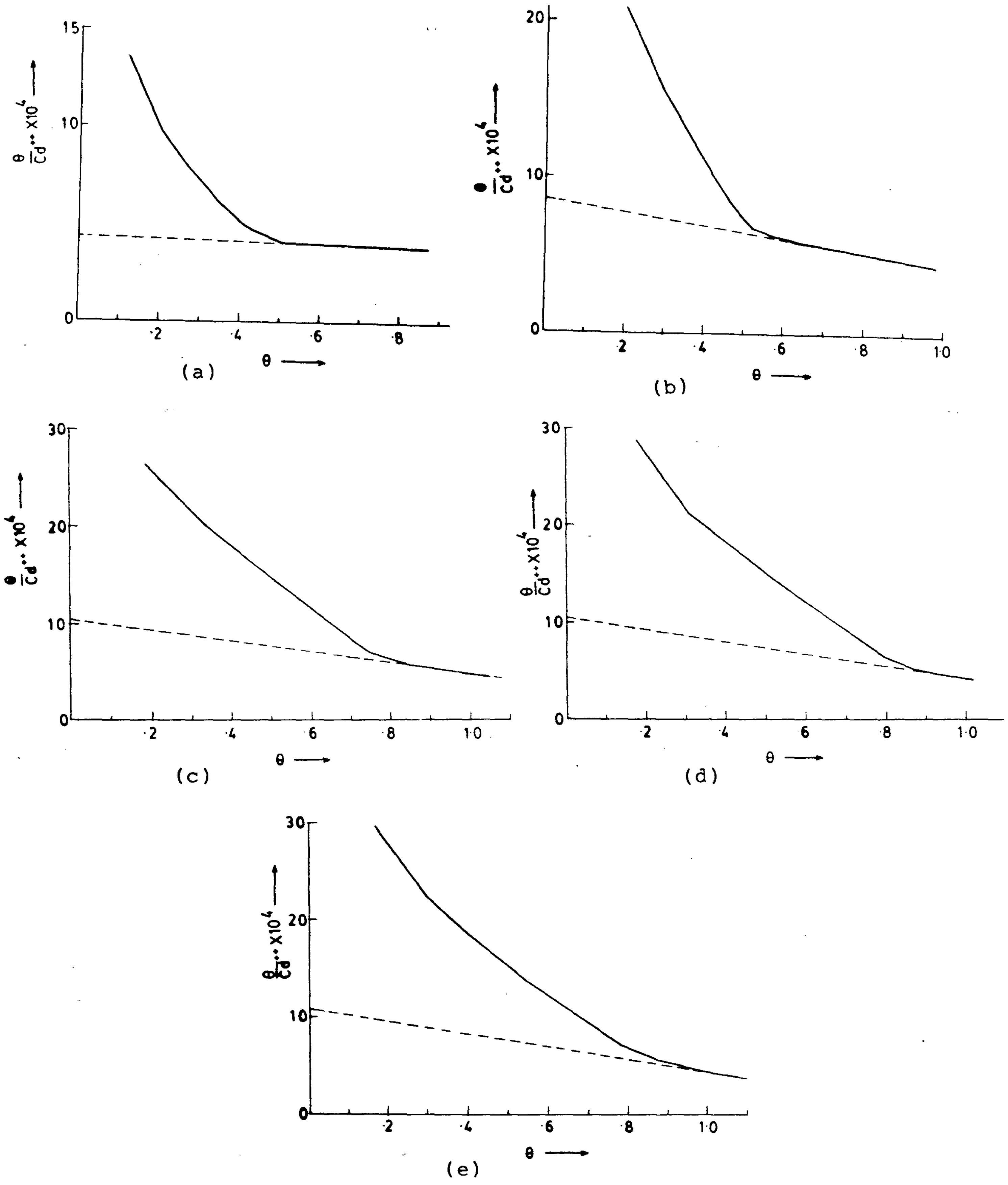


Fig.20. Scatchard Plots of Cd(II) binding by HA (humified water hyacinth) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d), 5.8(e).

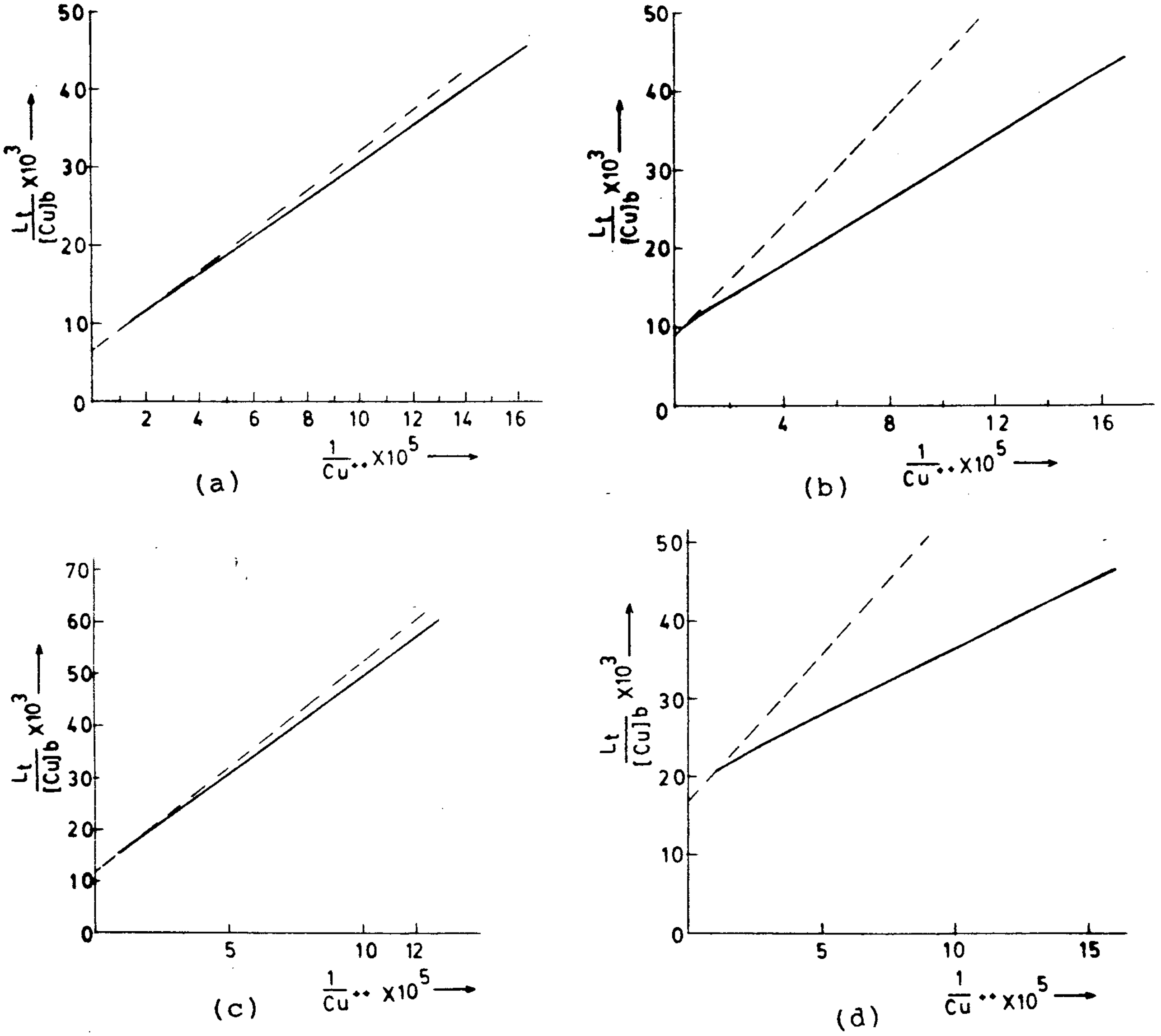


Fig.21. Double Reciprocal Plots of Cu(II) binding by HA (soil) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d).

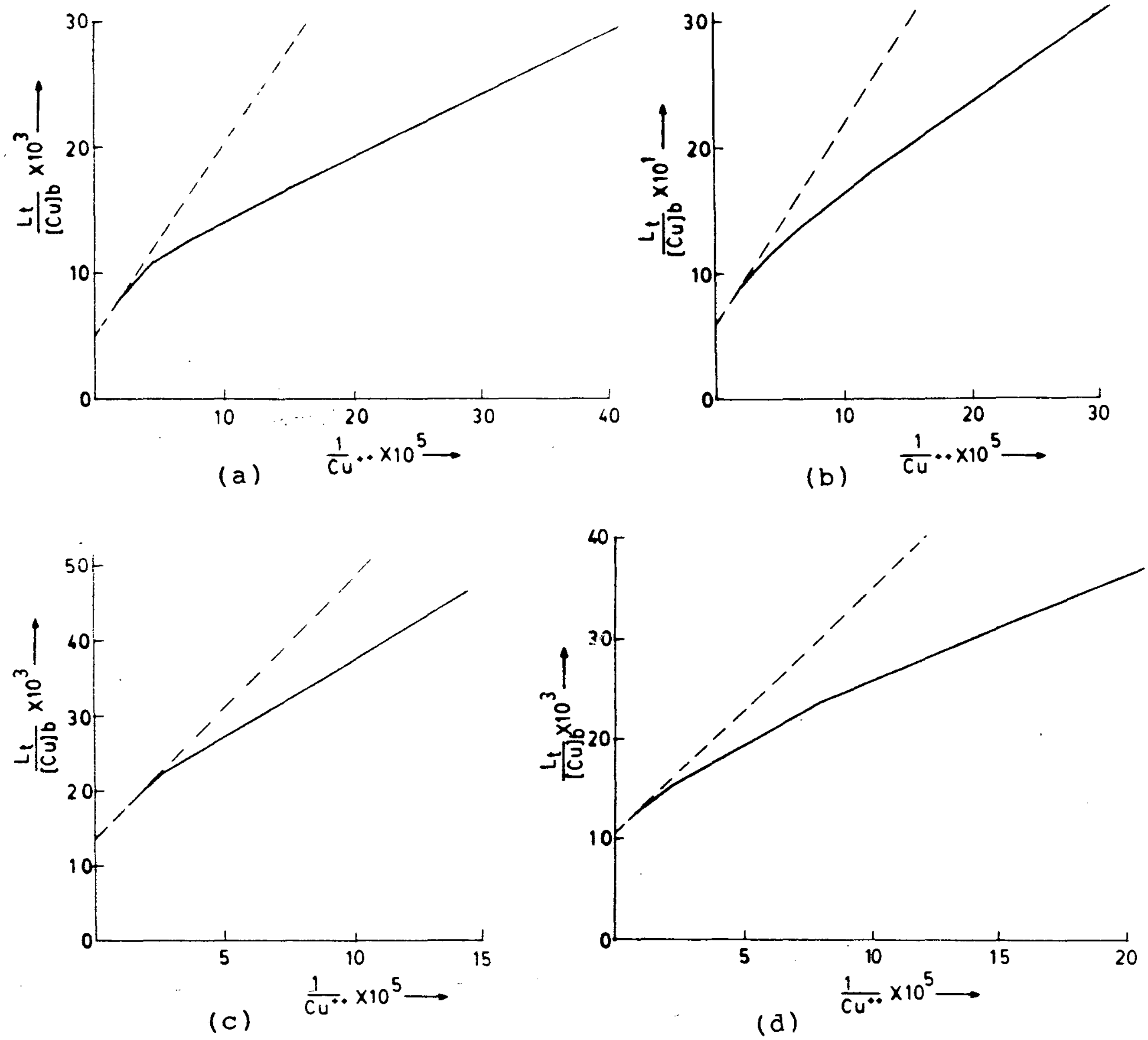


Fig.22. Double Reciprocal Plots of Cu(II) binding by HA (peat) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d).

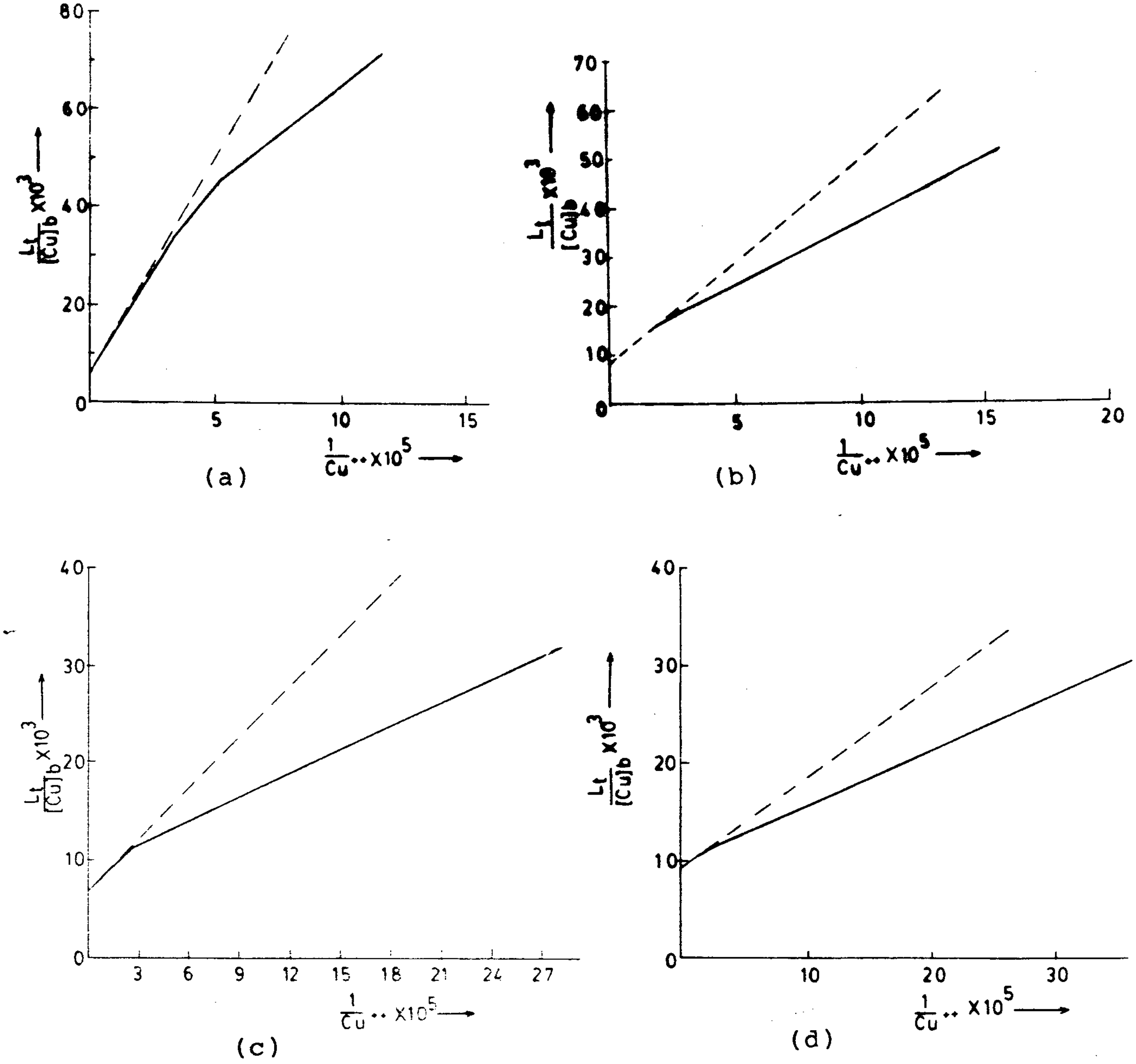
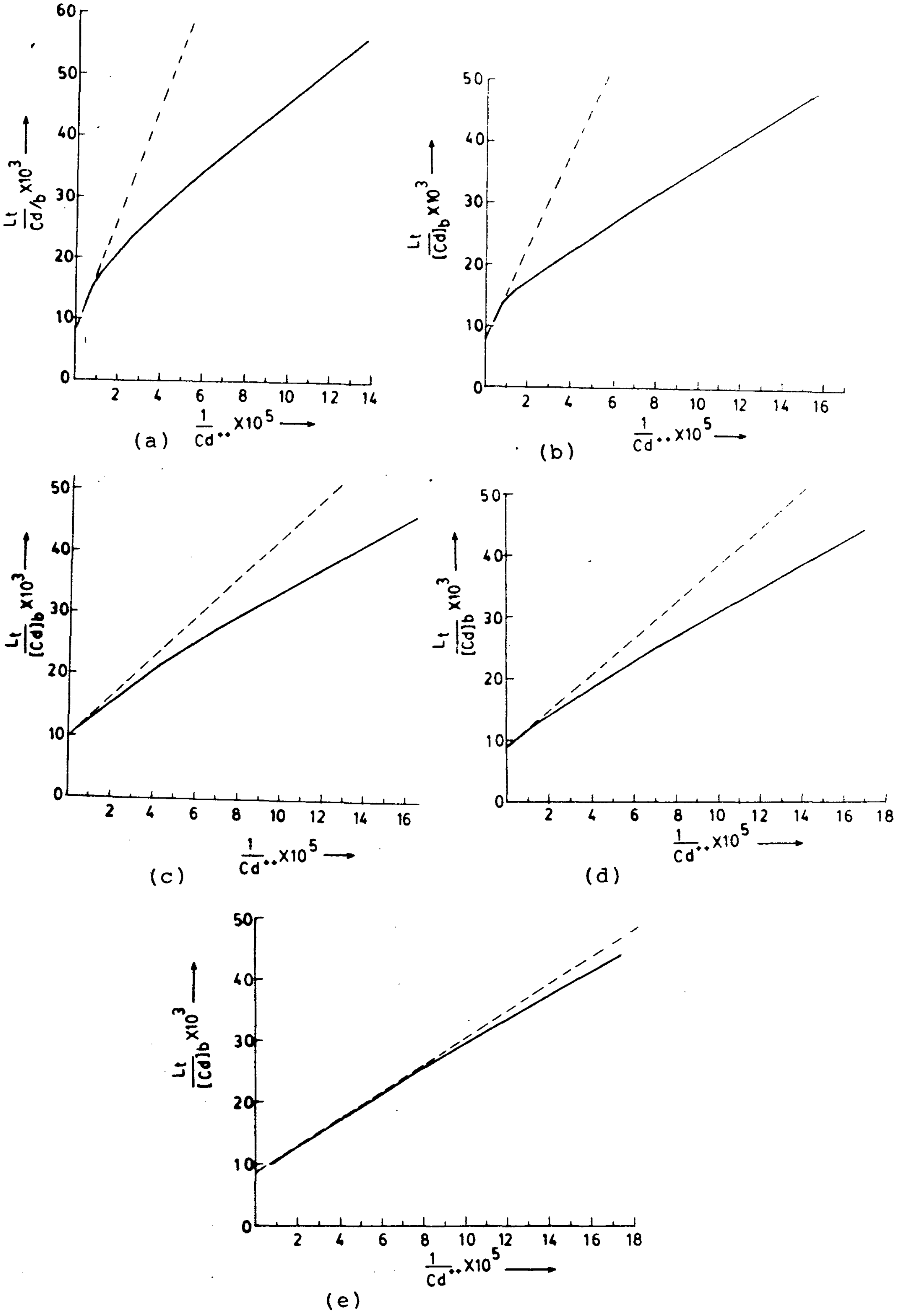


Fig.23. Double Reciprocal Plots of Cu(II) binding by HA (humified water hyacinth) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d).



(a) (b) (c) (d) (e)

Fig.24. Double Reciprocal Plots of Cd(II) binding by HA (soil) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d) 5.0(e)

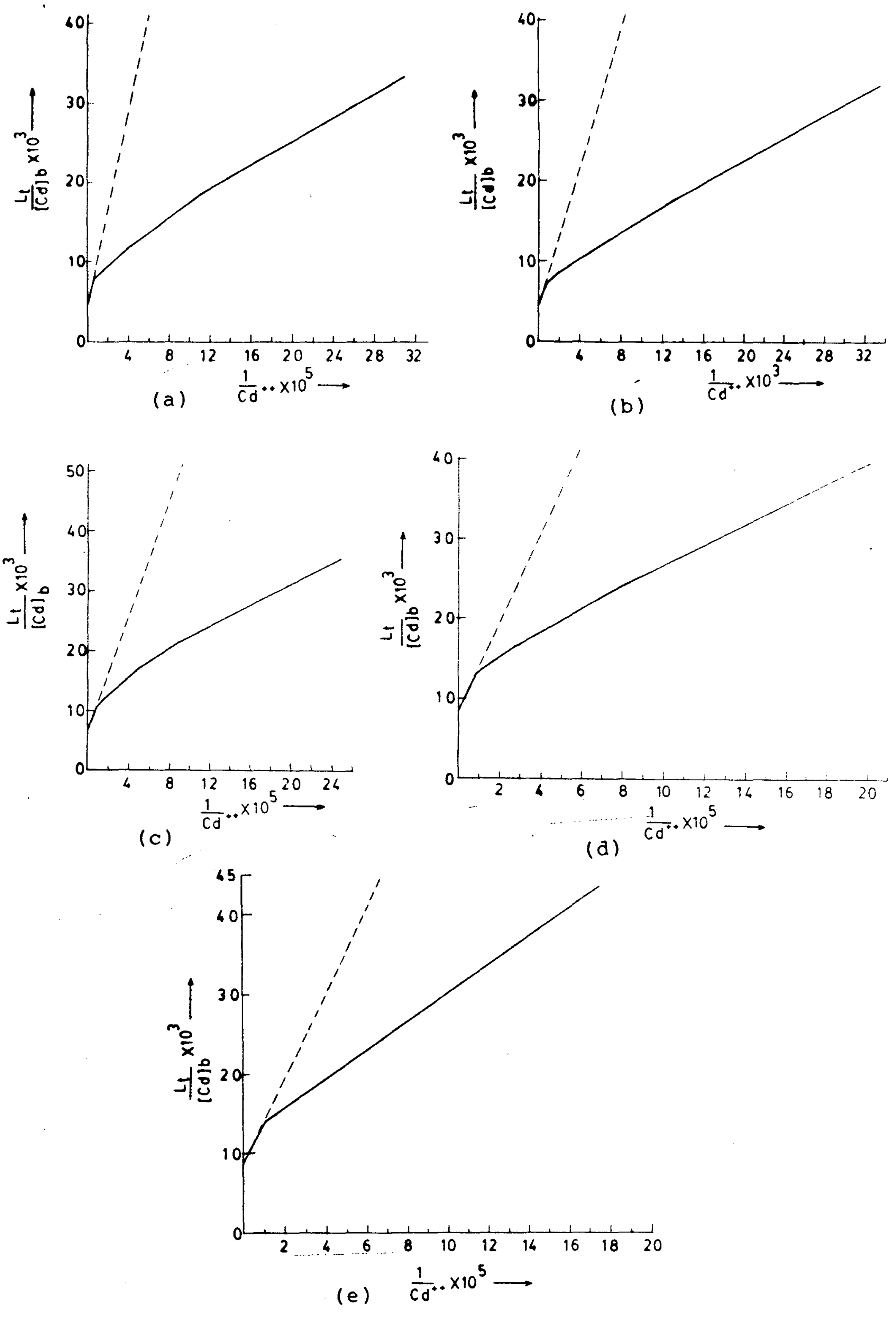
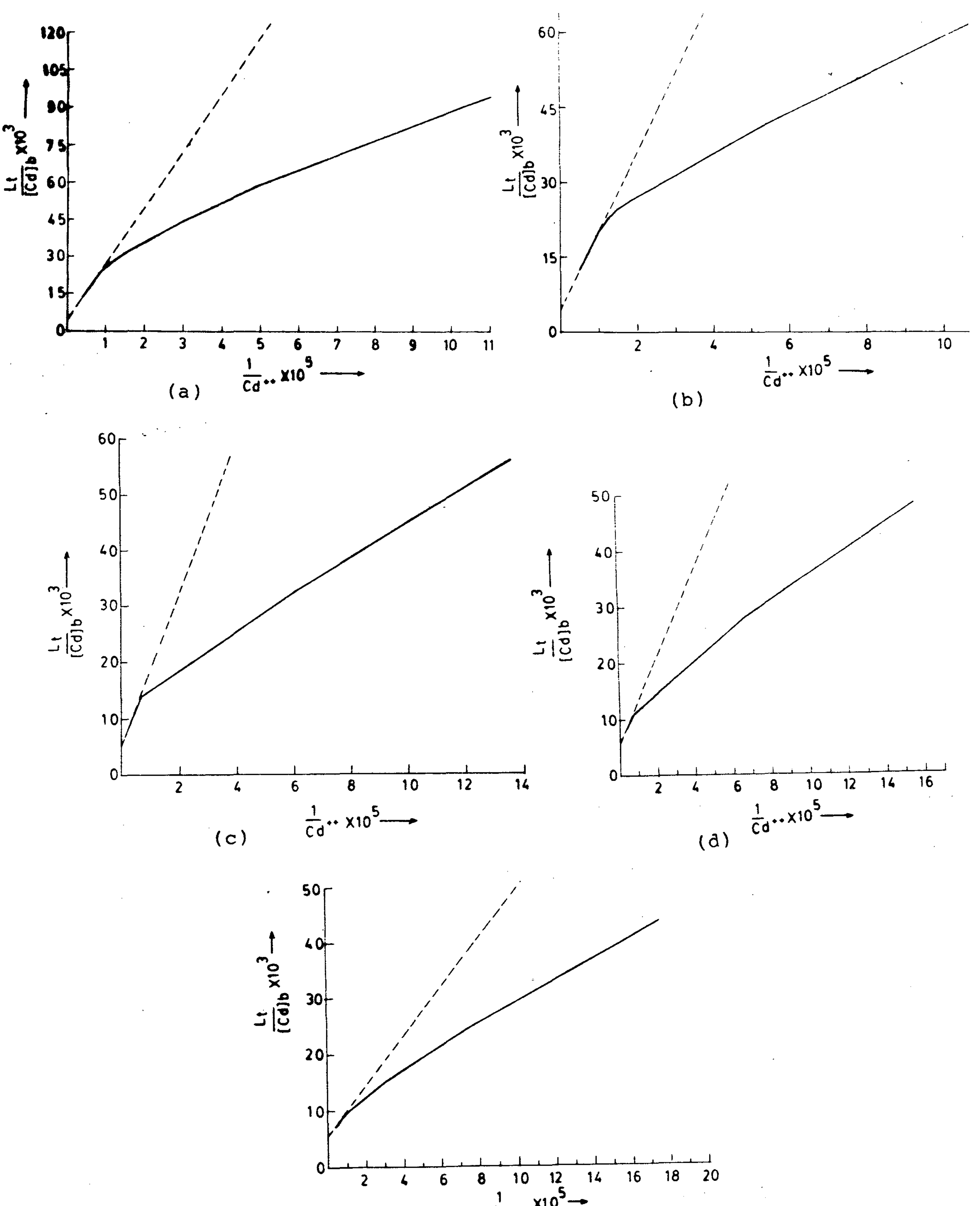


Fig.25. Double Reciprocal Plots of Cd(II) binding by HA (peat) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d) 5.8(e).



(a) (b) (c) (d) (e)

Fig.26. Double Reciprocal Plots of Cd(II) binding by HA 3.0 (a), 3.5(b), 4.0(c), 4.5(d), 5.8(e).

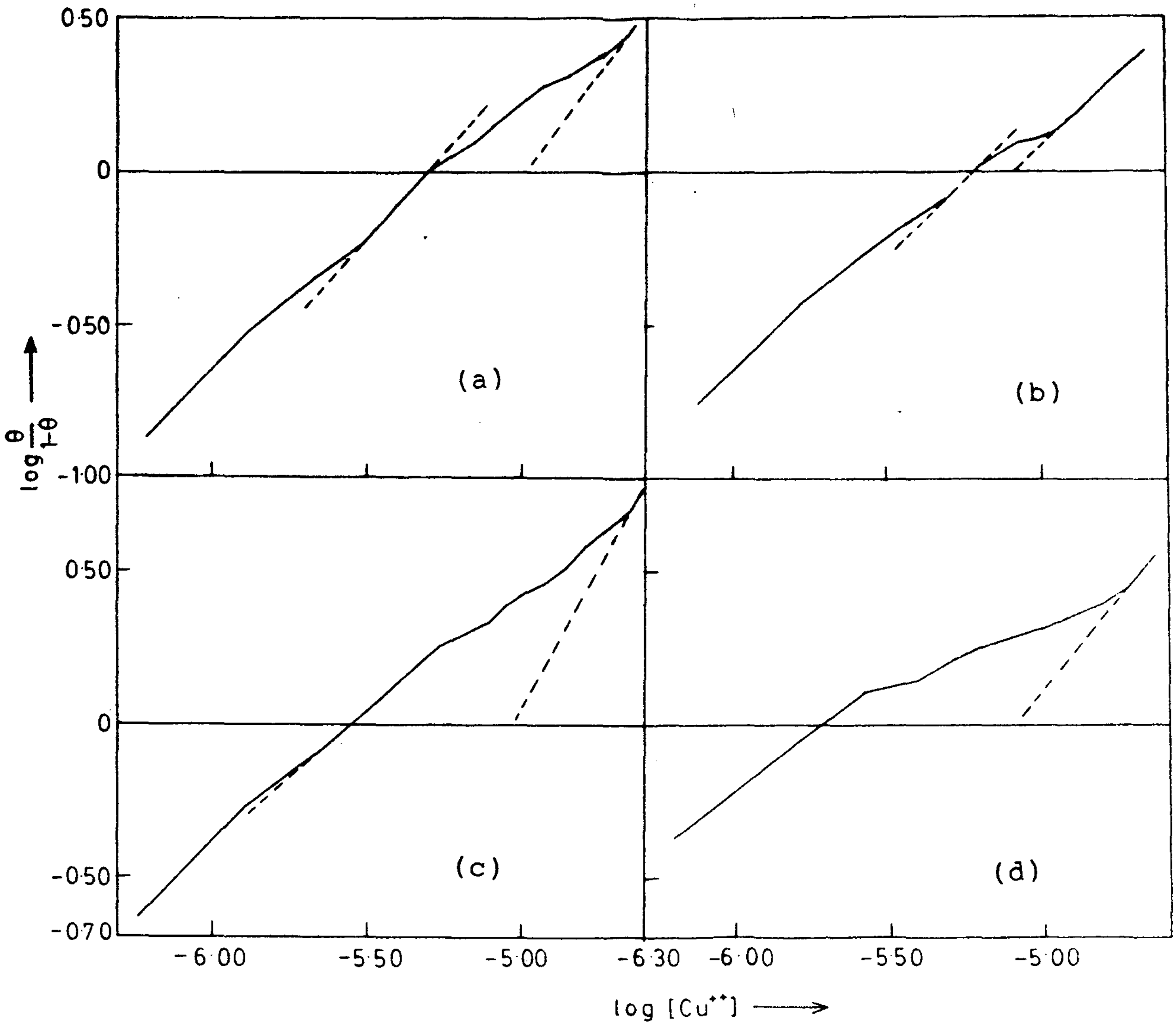


Fig.27. Hill Plots of Cu(II) binding by HA (soil) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d).

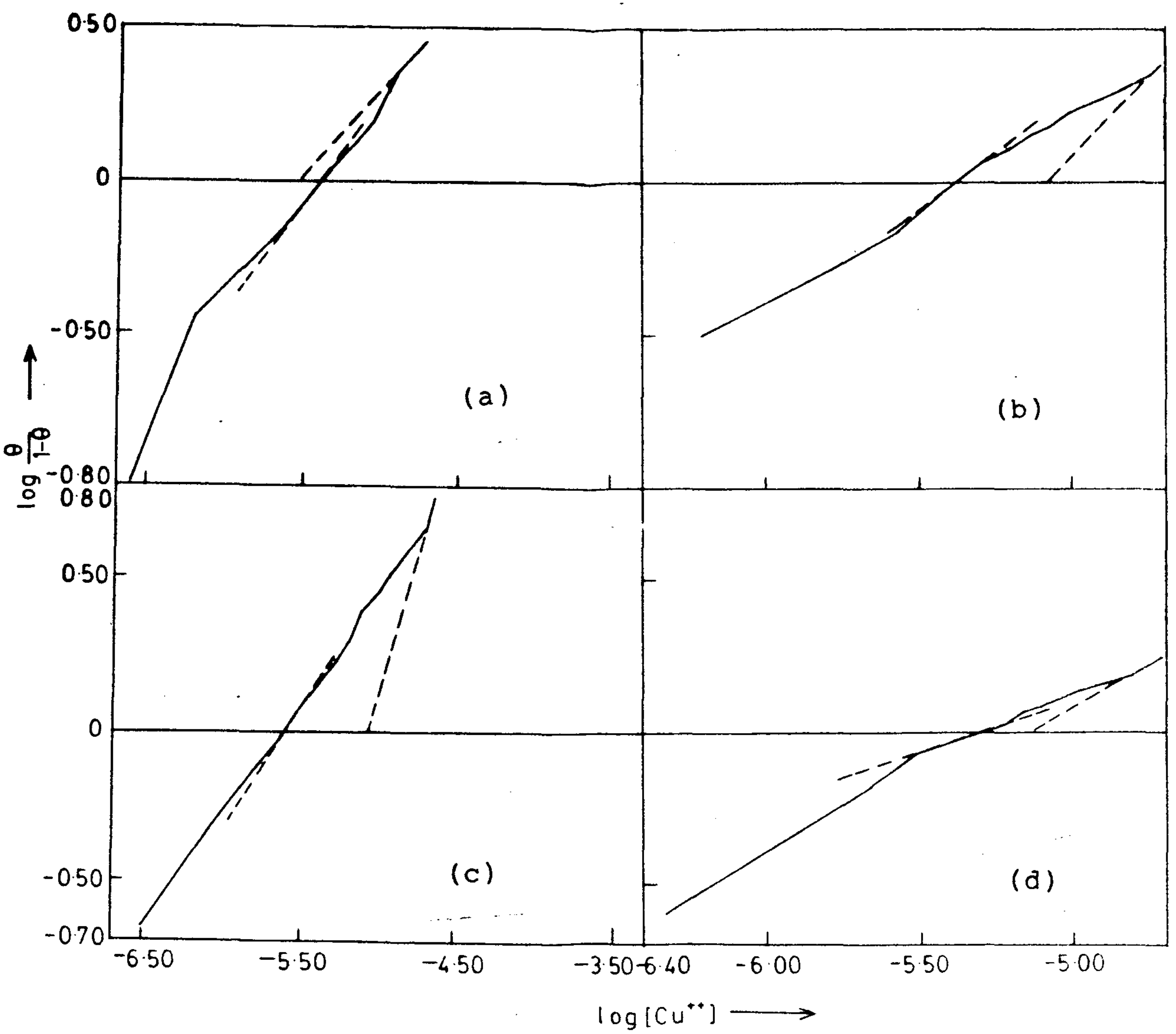


Fig.28. Hill Plots of Cu(II) binding by HA (peat) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d).

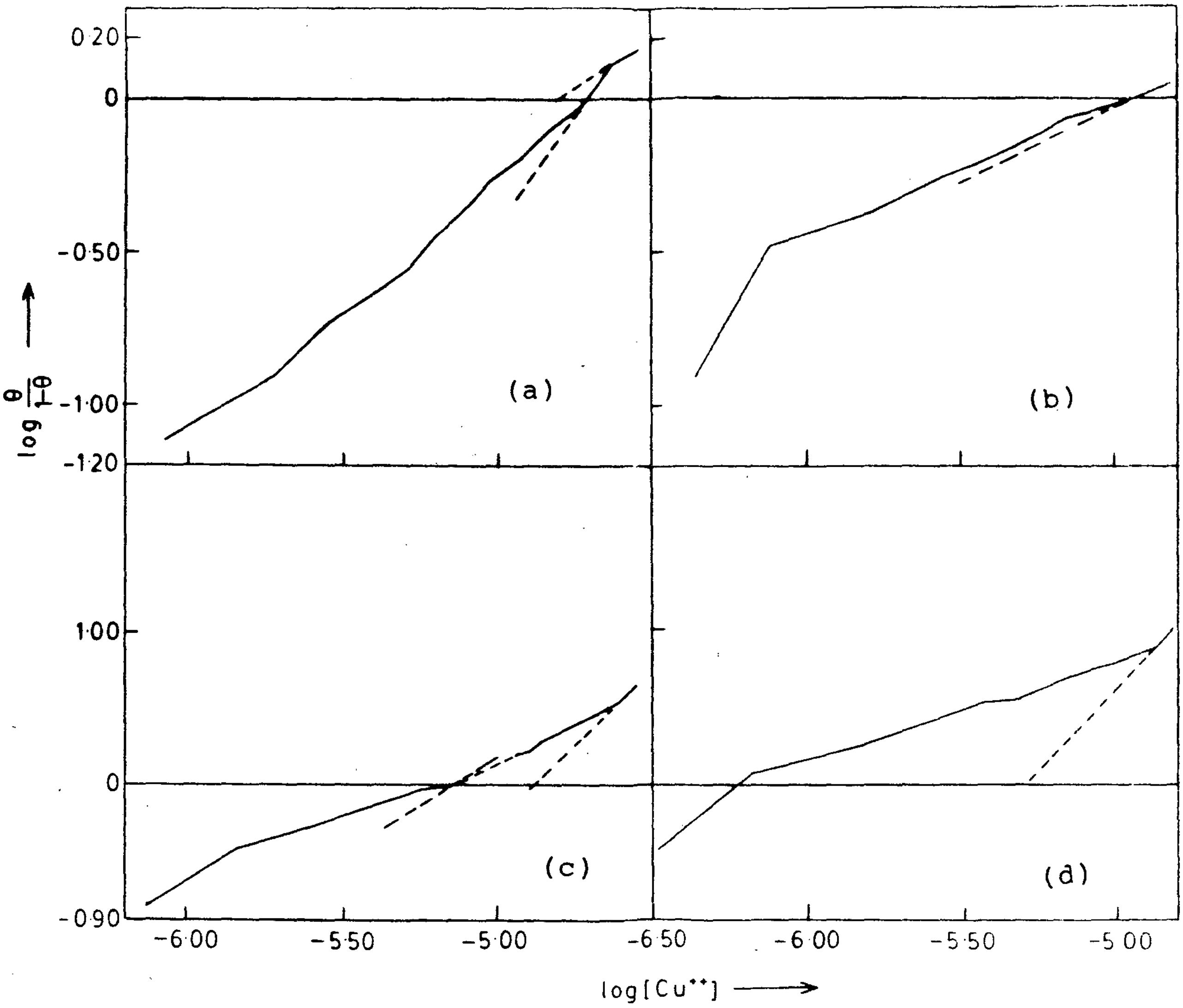


Fig.29. Hill Plots of Cu(II) binding by HA (huminified water hyacinth) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d).

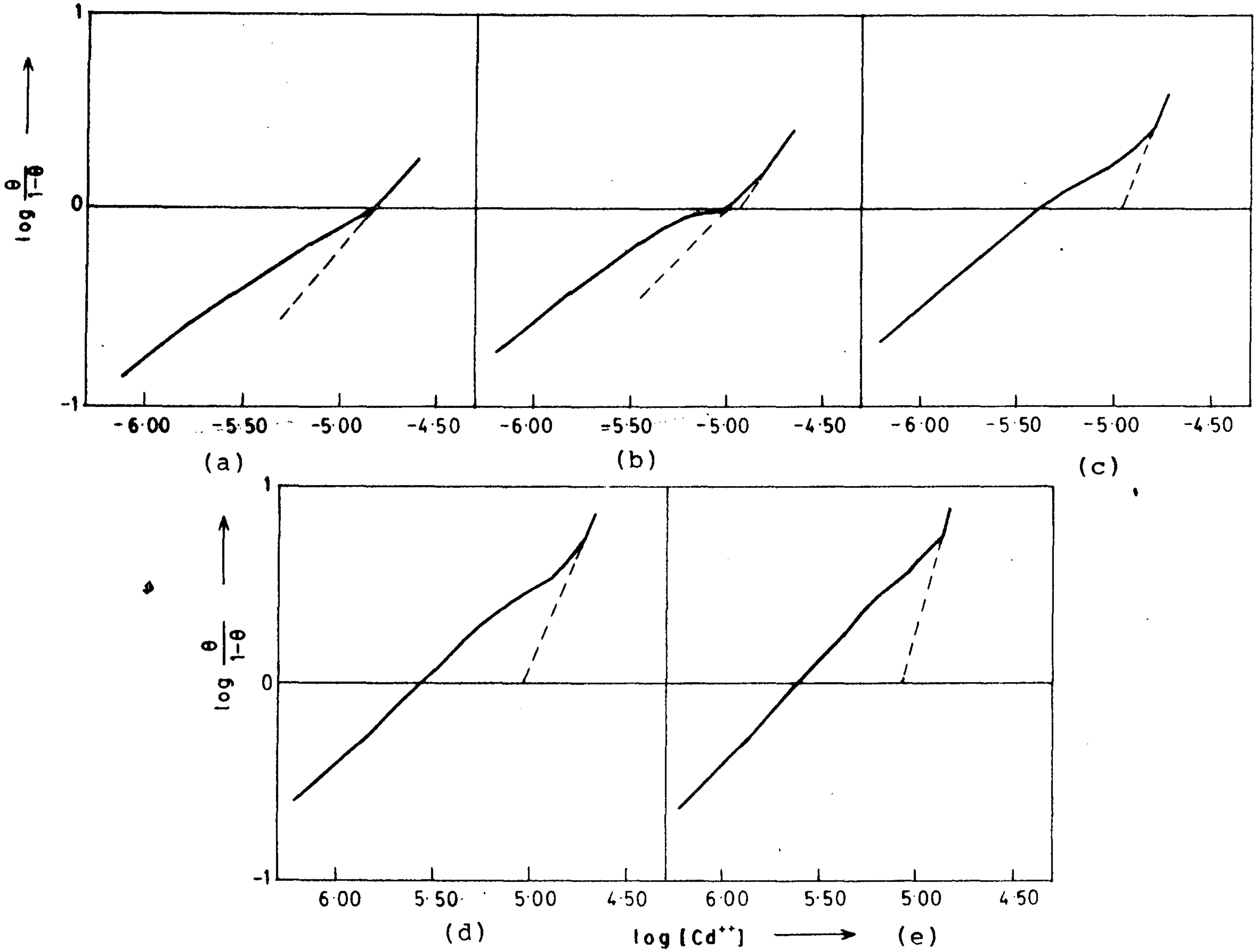


Fig.30. Hill Plots of Cd(II) binding by HA (soil) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d), 5.8(e).

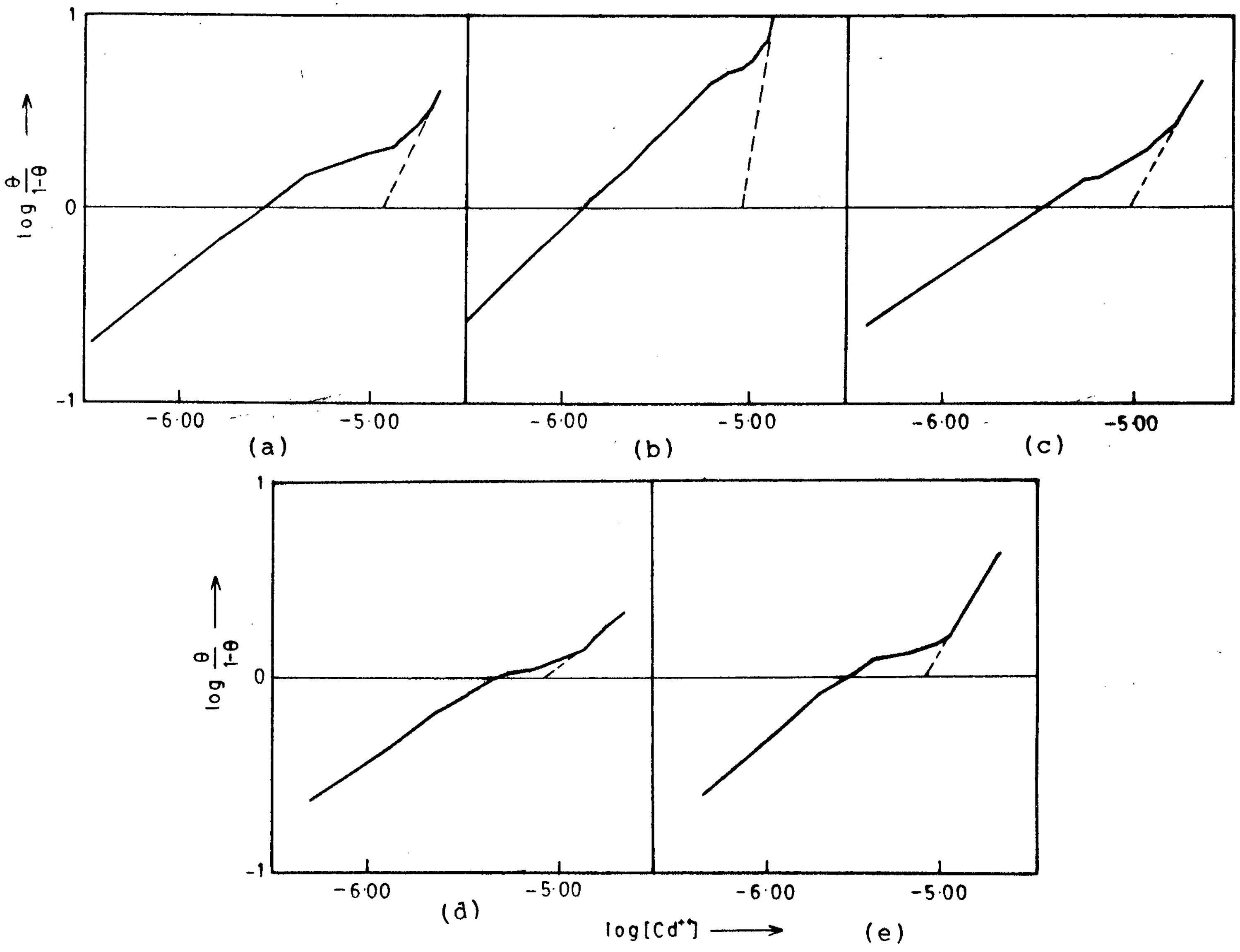


Fig.31. Hill Plots of Cd(II) binding by HA (peat) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d), 5.8(e).

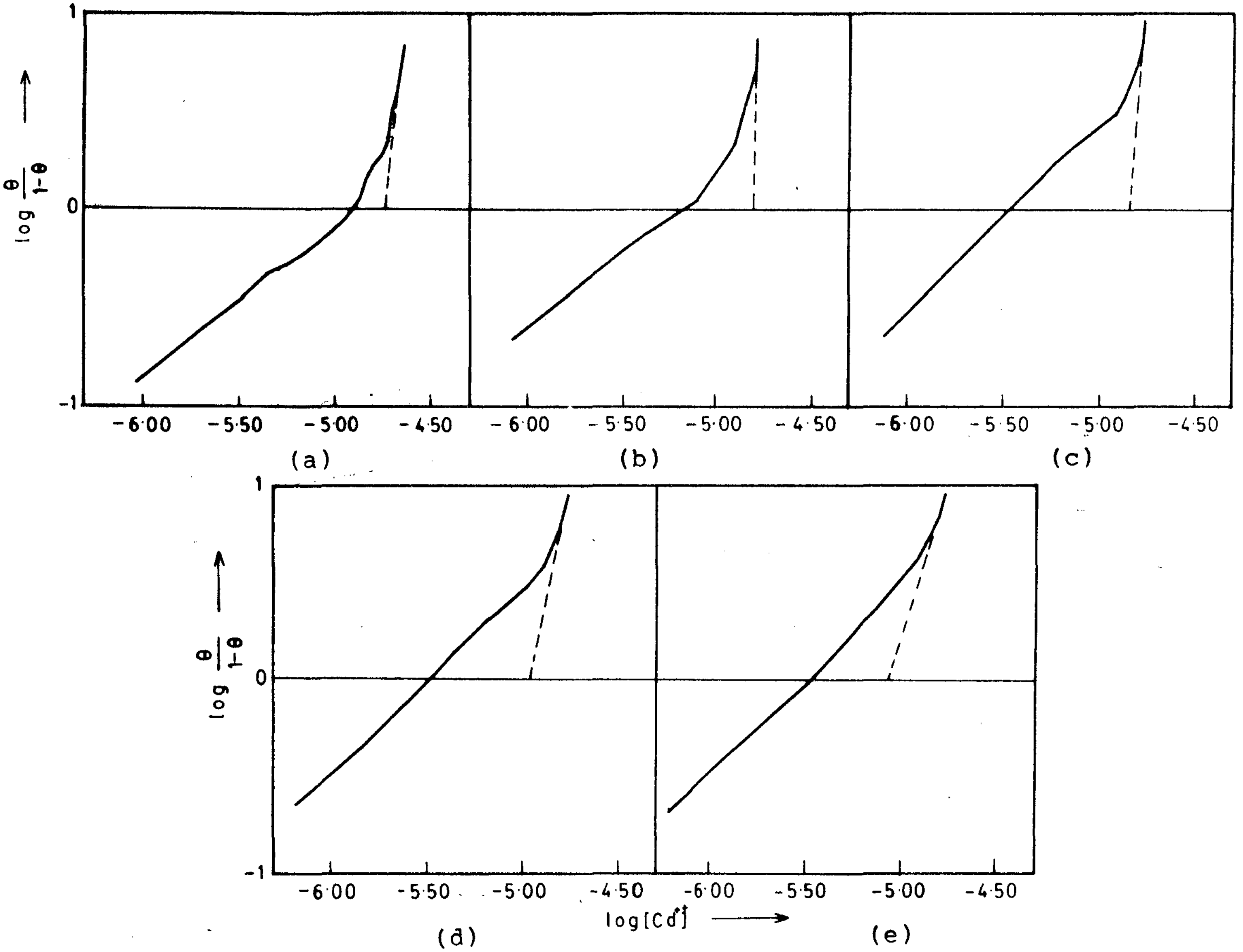


Fig.32. Hill Plots of Cd(II) binding by HA (humified water hyscinth) at pH : 3.0(a), 3.5(b), 4.0(c), 4.5(d), 5.8(e).

CHAPTER - V

DISCUSSION

The humic acid samples which were isolated for the present study represent three different sources of unique characteristics. The sources are (a) garden soil, (b) a peat prepared in the laboratory by mixing the same garden soil and crushed water hyacinth (Eichhornia Crassipes) in 1:1 ratio under aerobic conditions for a period of three months and (c) humified water hyacinth under aerobic conditions for the same period as above. Since the humic acids had been originated from diverse sources they would obviously differ from one another with respect to their structure and contents of acidic functional groups such as : Carboxylic (-COOH), Phenolic and alcoholic (-OH) groups which are mainly responsible for complexation reactions with heavy metals.

The first source, i.e., the garden soil represents the upper 6 cms. of the soil surface and would contain HA originated from the vegetable cover in the nursery. In this case, the process of humification might have occured for a longer period thus resulting in the formation of humic acids of more condensed and complete nature than the other two sources (Schnitzer, et al 1972). The second source, i.e., the peat consisted of decomposition and humification products of fresh plant materials (water hyacinth) along with soil and its original humic fractions. Under aerobic conditions, the decomposition and humification processes would be rapid enough in this case in the presence of sufficient amount of soil microorganisms. But since the duration of these processes is far less than that in the soil itself, it would contain humic acids of less condensed and complete nature than the former. The third source, i.e. the humified water hyacinth would probably contain less number of microorganisms for decomposition processes to be completed, thus, in turn, retarding the process of humification.

It is well known that microorganisms present in soil and water systems are mainly responsible for the process of decomposition of various plant-derived materials such as : proteins, polysaccharides, carbohydrates, resins, celluloses, hemicelluloses and lignis (Flaig, et al 1975). Flaig, (1972) also observed that the lignins constitute a major part of the plant materials besides the other decomposable products as mentioned above. The lignin is far less degradable than the other compounds but contributes most to the humic acids of soils and waters. Therefore, the posibility exists that lignin degradation products participate directly in the formation of HA's. Martin, et al (1971) after a series of investigations concluded that lignins are phenolic polymers and are relatively resistant to microbial decomposition. They are considered to be a major, if not the primary source of phenolic units of HA's. This view is also supported by the works of many researchers such as : Prakash, et.al.(1972). Serra, et.al(1972); Roletto.et.al. (1985); Ingrid kogel,et.al.(1987); Kyuma(1987); Neue (1987); Fillip, et.al.(1987); Saij-Jimenez (1987); etc.

In view of the above facts, the humic acids used in the present study can be expected to show the following properties. (a) the order of degree of humification would be HA(soil) > HA(peat) > HA(humified water hyacinth). (b) the order of degree of condensation of HA molecules would be HA (soil) > HA (peat) > HA(humified water hyacinth).

The interactions between Cu(II) and Cd(II) and humic acids were studied using Copper- and Cadmium- ion selective electrodes. This method had earlier been used in a similar way by many workers (Buffle, et. al. 1977; Fitch, et.al. 1984) in order to calculate the degree of complexation and the molar concentrations of heavy metals in metal-humic acid systems.

Response of ISE (Ion Selective Electrodes) to added copper and cadmium in the presence and absence of each of the three humic acids (duplicate titrations) respectively are illustrated in figres 1 and 2. An initial lag in the ISE response (more with Cu(II) and less with Cd(II)) was observed and was followed by a consistent increase in potential as more of Cu(II) and Cd(II) respectively were added to the system. The initial portion of the ISE response curves corresponded to concentration of free Cu(II) and Cd(II) ions near the lower limit of detection of the respective ISE's. Therefore the values in this portion of the response curve were not given much importance in the interpretations of experimental data. Also the values in the upper region were not used in the different plotting procedures since precipitation of humic acids occured frequently in this region (Fitch, et.al.1984).

With blank titrations of solutions in the absence of HA's adjusted to a total ionic strength of 0.1M with $NaNO_3$, a somewhat linear relationship between electrode potential (E) and log $[Cu]_t$, log$[Cd]_t$ respectively (fig. 1 and 2) was observed over the range $1.31x10^{-6}$M to $3.77x10^{-5}$M for both

Cu(II) and Cd(II). This was found to be in agreement with the works of Kerven, et.al.(1984) who observed such a linear relationship over the range 5×10^{-7}M to 10^{-3}M. But with various humic acids, this relationship was markedly non-linear (especially with Cu(II) and both the slope and shape of the curves were affected by pH. ISE response curves for all the three HA's with respect to Cu(II) and Cd(II) at various pH levels such as 3.0, 3.5, 4.0, 4.5 (for Cu(II) and 3.0, 3.5, 4.0, 4.5, 5.8 [for Cd(II)] are shown in the figures 1 and 2 Kerven, et.at. (1984) explained that this non-linear relationship between electrode potential (E) and $\log[Cu]_t$ & $\log[Cd]_t$ respectively was due to complexation of the added copper and Cadmium during various steps of titration. It was also observed that the non-linear nature of these curves was much pronounced at low metal ion concentrations.

The concentrations of ionic copper $[Cu^{2+}]$ and ionic cadmium $[Cd^{2+}]$ present in Cu-HA and Cd-HA solutions respectively at various pH levels were calculated (Tables 1 - 6) using equation (1). Similarly the concentrations of brown copper and brown cadmium were calculated with the help of equation(4). From the data, it was observed that in Cu^{2+} - HA(soil) solutions, at a definite total Cu(II) concentration, $[Cu]_t$, the concentration of ionic copper,

$[Cu^{2+}]$ gradually increases with incrase in pH from 3.0 to 4.5 and is spread over a wide range of total copper(II) concentrations in the medium : 5.20×10^{-6}M to 3.77×10^{-5}M. This revealed that more Cu(II) was generally bound to the soil humic acid under study at low pH levels such as 3.0 and 3.5. This may be due to relatively high ionization of the acidic functional groups (-COOH) of soil HA at low pH levels of higher acidity range (Buffle, et.al.1977). For peat humic acid the values of Cu^{2+} indicated an increasing trend only upto a pH of 4.0 and at higher pH(4.5) it decreased slightly over the whole range of total copper concentrations used in the titration. In case of HA derived from humified water hyacinth, although of increasing order, the values showed fluctuations from the trend very often. From a comparative study of the data, it was observed that the peat humic acid could bind more copper(II) at a particular pH level which is followed by soil HA and HA derived from humified water hyacinth. The reason of this behaviour might be that the peat HA contained more number of functional groups than the soil HA followed by the HA isolated from humified water hyacinth.

There was no appreciable increase in $[Cd^{2+}]$ concentrations in soil and peat HA solutions with the increase in pH even upto 5.8. Even a decreasing trend was observed in case of the humic acid derived from humified water hyacinth. A comparism between the three HA's revealed the same trend as observed in Cu(II)-HA binding data, that is, the peat HA has the capacity to bind more Cd(II) than the soil HA followed by HA derived from humified water hyacinth.

Comparing the relative binding of Cu(II) and Cd(II), it was observed that the amount of Cd(II) bound to HA was substantially lower than that of Cu(II) at all the ph levels showing that Cd(II) has very low afinity for HA's than Cu(II). Thus the affinity was least with the HA derived from humified water hyacinth, intermediate with soil-derived HA and most pronounced with peat humic acid (Moore, et.al.1984).

The proportions of $[Cu]_t$ & $[Cd]_t$ present as $[Cu^{2+}]$ & $[Cd^{2+}]$ (Tables 7,8) and the degree of comlexation of HA's (Tables 8,10) were calculated with the help of equations (1), (2) and (3). The various plots such as : log x vs. $\log[Cu]_t$ log x vs. $\log[Cd]_t$, $\%[Cu^{++}]$ vs. $\log [Cu]_t$ and $\%[Cd^{++}]$ vs. $\log [Cd]_t$ are illustrated in figures 3,4,5 and 6 respectively. From the above mentioned data and figures, the following observations were drawn :

(a) The percentages of Cu^{++} in the three HA solutions showed somewhat similar trends as their counterparts in Cd(II) - HA solutions at respective pH levels. With the data both of Cu(II) and Cd(II) in mind, it can be concluded that, the percentage $[Cu^{++}]$ and $[Cd^{++}]$ in the three HA solutions follow the order :

> %$[Cu^{++}]$ and %$[Cd^{++}]$ in HA (humified water hyacinth) solution > %$[Cu^{++}]$ and %$[Cd^{++}]$ in HA (soil) solution > %$[Cu^{++}]$ and %$[Cd^{++}]$ in HA(peat) solution.

(b) According to Kerven, et.al. (1984), the HA which contains high amount of soluble organic carbon can complex more metal ions (leaving less amounts in the solution) than the one having less soluble organic carbon content. In view of this fact and considering the percentage of Cu^{++} and Cd^{++} ions in solution as well as bound copper and cadmium, it can be concluded that the peat HA contains the highest amount of soluble organic carbon which is followed by soil HA and HA isolated from humified water hyacinth containing least amount of soluble organic carbon.

For all the three HA's, the degree of complexation was found to decrease substantially with the addition of Cu(II) and Cd(II) to the system at various pH levels although quite differently from one another. The decrease of the degree of complexation continued upto a point when about 90% of the added copper and cadmium was present at free Cu^{2+} respectively. This is concurrent with the saturation of almost all complexing sites resulting in further copper and cadmium additions remaining fully in the ionic form. These observations were in agreement with earlier works of Buffle,et.al. (1977) and Kerven, et.al.(1984). Although the degree of complexation values were found to be very much closer to one another towards the higher metal ion concentration range. They showed marked differences at low metal ion concentrations in HA solutions. The degree of complexation (Tables - 9,10) of the three HA's was found to follow the order :

HA(peat) > HA(soil) > HA(humified water hyacinth).

The high degree of complexation present in the peat HA can be readily exlained by its high soluble organic carbon content (Kerven, et.al.1984). A companion between the degree of complexation values of HA's with respect to Cu(II) and Cd(II) revealed that Cd(II) always had substantially lower value than that of copper.

Fitch,et.al. (1984) in their review discussed various models for determining conditional stability constants, maximum binding abilities, total number of binding sites, sites bound per total number of reactive sites, macroscopic binding constants, etc. of metal-humate complexes. Use of these modelling techniques for calculating the above parameters of metal complexes with HA's had led to confusion in interpreting published data. However, several of these recent models were shown to have a common basis in that they represent modifications of Adair's equation. In the present study, the graphical approaches of Adair's equation were compared using a single set of experimental data for the binding at Cu(II) and Cd(II) to all the three HA's originating from diverse sources.

Graphical approaches based on Adair's equation(Fitch, et. al.1984) is based on the assumption that the macromolecule(L), i.e. HA in the central group to which some small molecules (e.g., a metal ion M) are bound (formation of LM, LM_2 ..... LM_n complexes). The reaction can be described by n stepwise stability constants :

$$k_1 = \frac{(LM)}{(L)\ (M)}, \qquad k_2 = \frac{(LM_2)}{(LM)\ (M)},$$

$$k_n = \frac{(L\ M_n)}{(LM_{n-1}\ (M)} \qquad \ldots(14)$$

Where the parenthesis designate concentrations. The extent of binding is expressed in terms of a formation function, v, defined as :

$$v = \frac{\text{Sites bound}}{\text{Polymer concentration}} = \frac{(Mb)}{(Lt)}$$

$$= \frac{(LM) + 2\ (LM_2) + \ldots\ldots\ldots\ n(LM_n)}{L + (LM) + \ldots\ldots\ldots\ (LM_n)} \qquad \ldots\ldots (15)$$

Where (Lt) and (Mb) are the concentrations of the total ligand and bound metal respectively. The later was taken as the difference between the total and free metal ion concentration, i.e. (Mb) = (Mt) - (M).

In terms of the formation constants, eqn. (15) becomes

$$v = \frac{k_1(M) + 2\ k_1k_2\ (M)^2 + \ \ldots\ldots\ n\ k_1k_2\ \ldots\ldots\ kn(M)^n}{1 + k_1(M) + k_1k_2(M)^2 + \ \ldots\ldots\ k_1k_2\ \ldots\ldots\ kn(M)^n} \qquad \ldots(16)$$

The equation (16) is sometimes referred to as Adair's equation.

Polynomial expansion of Adair's eqn (16) for binding at identical and independent sites results in

$$v = nKo\,(M) / [1 + Ko(M)] \quad .... \quad (17)$$

Where Ko is the intrinsic or microscopic binding constant.

This constant is related to the macroscopic constants (k_1, k_2 ... kn through a stastical factor and is given by :

$$k_1 = Ko\,(n - i + 1)/i \quad .... \quad (18)$$

Where n is the number of LMn forms and i is the ith form. When more than one class of sites occurs on the macromolecule, v is the sum of the v values for each site :

$$v = \frac{n_1 K_1 (M)}{1+K_1(M)} + \frac{n_2 K_2 (M)}{1+K_2(M)} \quad ... \quad \frac{n_j K_j (M)}{1+K_j(M)}$$

$$= \frac{n_j K_j (M)}{1+K_j(M)} \quad .... \quad (19)$$

Where n_j is the number of sites of class j, and K_j is the stability constant for class j.

In the absence of a known molecular weight, which is often the case for humic acids, the formation function can be expressed in terms of binding site concentration, $n(L_t)$, where n is the total number of binding sites (n =

$n_1+n_2+\ldots$). The value of $n(L_t)$ can be obtained from metal ion data with the help of langmuir adsorption equations.

We can define

$$\theta = \frac{\text{Sites bound}}{\text{Total number of reactive sites}} = \frac{Mb}{n(L_t)} = \frac{v}{n}$$

Which by substitution into eqn. (17) yields :

$$\theta = Ko(M)/[1+Ko(M)] \quad \ldots\ldots\ldots\ldots \quad (20)$$

Adair's equation (16) which can be expressed as eqn (17) and its modified form (20) can be arranged in a number of ways to yeild information about various complexation properties of metal-HA systems. Plotting can be done as a Scatchard plot , a reciprocal plot , a Double reciprocal plot and in some cases as a Hill plot.

The model proposed independently by Buffle, et.al. (1977) to study the binding of Cu(II) to FA is based on the equation (3) (ch.II). In equation (3), α /(Mt) (α -1) can be shown to be 1/(Mb) and α/(Mt) is shown to be 1/(M). Thus equation (3) reduces to :

$$\frac{(Lt)}{(Mb)} = \frac{mw}{n} + \frac{mw}{n} \cdot \frac{(H^+)^x}{B_1} \cdot \frac{1}{(M)} \quad \ldots\ldots (21)$$

In this case, (Lt)/(Mb) is plotted against 1/(M) from which $(H^+)^x/B_1$ is calculatd from the slope and intercept. Since (Lt/(Mb) is equal to 1/v, equation (21) as well as (3) has the same form as the double reciprocal plot.

When binding at one site greatly enhances binding at subsequent sites, binding of all n metal ions can be considered to occur in one step (Fitch, et.al. 1984) :

$$L + nM \xrightarrow{K^*} MnL \quad \ldots\ldots \quad (22)$$

In this case equation (4) becomes

$$v = \frac{n\ K^{*}\ (M)^{n}}{1+K^{*}(M)^{n}} \qquad \ldots\ldots\ (23)$$

Expression of equation (23) in terms of yields

$$[\theta/(1-\theta)] = K^{*}\ (M)^{n} \quad \ldots\ldots\ (24)$$

$$\text{or} \quad \log\ [\theta/(1-\theta)] = \log\ K^{*} + n\ \log(M)$$

Which is the equation for the Hill plot (equation (9) ch.II).

It is important to note that $K^{*} = k_1 k_2 k_3 \ \ldots\ldots\ k_n$, where k_1, k_2, k_n are defined by equation (14).
Zunino and Martin (1977) also derived the equation (10) (ch.II) to determine conditional stability constants for the reaction of Cu(II) with a soil Humic acid . To obtain log K a plot was made of the left side of the equation vs. log M. Incidentally the expression (Mb)/[MBA-(Mb)] is identical to $\theta/(1-\theta)$ and thus the approach used by Zunino and Martin was seen to be a Hill plot.

Application of the plotting procedures required that estimates be obtained for total binding sites [n(Lt) or

MBA]. Application of the Langmuir equation (single surface) produced non-linear curves (Fig. 7,8). This observation was found to be in agreement with the results of Fitch, et.al. (1984), who explained that this non-linearity was expected since this equation has the same form as the reciprocal plot and previous works using the Scatchard plot method has implicated two or more sites for the binding of Cu(II) and Cd(II) to humic acids (Bresnahan, et.al. 1978). Thus the linear treatment of langmuir plots to obtain estimates for MBA, as done by Zunino, et.al. (1977) would appear to be of questinable value for most studies of the binding of metal ions to humic acids (Fitch, et.al. 1984). Fitch, et.al. therefore suggested the use of Double surface longmuir equation (11) (Ch.II) in order to calculate MBA [n(Lt)].

The b_2 (MBA) values were recorded in the tables 11(Cu(II)-HA) and 12 (Cd(II) - HA). These values include binding at all sites ($n_1 + n_2 + \ldots\ldots n_j$) of equation (6) (Cornish-Bowden, et. al. 1975; Fitch, et.al. 1984). The MBA values for Cd(II) - HA interactions were seen to be always less than those of Cu(II) - HA interactions.

The values of 0 (sites bound/total number of reactive sites) of humic acids with respect to Cu(II) & Cd(II) are given in tables 13 and 14. It was obsered that :

(a) θ increased from near zero to almost one with the increase in metal ion concentration [both Cu(II) and Cd(II)] indicating progressive saturation of the binding sites on humic acid molecules. $\theta \simeq 1$ indicated near-total saturation.

(b) θ was found to increse in the order :

HA (peat) > HA (soil) > HA (humified water hyacinth) at a particular total Cu(11) or total Cd(11) concentration in the solution.

The Scatchard plots of the experimental data were recorded in figures 15 to 20. The plot for both Cu(II) and Cd(II) binding with the three humic acids at all the pH levels under consideration were seen to be non-linear which was in agreement with previous works (Bresnahan, et.al. 1978; Buffle, et.al. 1977; Perdue, et.al. 1983; Saar, et.al. 1980b, 1980c. Sposite et.al. 1979; Fitch, etal. 1984), indicating binding at two or more classes of sites. In a similar way, the double reciprocal plots (Fig. 21 - 26) were also found to be of non-linear in nature supporting the above fact. Buffle, etal. (1977) suggested that this non-linearity in the curves may be attributed to the formation of 1:2 complexes. In view of the common origin of the two plotting methods, the curvilinear nature of the double reciprocal plot was obviously expected (Fitch, et.al. 1984). With the help of these methods, the conditional stability constants for binding of Cu(II) and Cd(II) with the three humic acids were calculated from the slope and intercept (Tables 15, 16, 17, 18). The following observations were made from the stability constant data :

(a) the conditional stability constants (Kn) calculated from both the plots were seen to be increasing with the increase in pH,

(b) the conditional stability constant values from double reciprocal plots were seen to be always higher than those obtained from the Scatchard plots, and

(c) the conditional stability constants of Cu(II) - HA complexes were always found to be higher than Cd(II) - HA complexes.

The increase in stability constants with increase in pH has been reported by many investigators (Saar, et.al. 1979 and 1980; Bresnahan, et.al. 1978; Schnitzer, et.al. 1967; Stevenson, 1966; Ardrakani, et.al. 1972; Fitch, etal 1984, etc.). The second observation can be explained from the experimental results of Fitch, et.al. (1984). According to them provided that the experimental data are accurate, the Scatchard plot is preferred for establishing Kn because values at high free (M) are not compressed into a narrow zone which is generally seen in case of double reciprocal plots. Thirdly the fact that the stability constants of Cu(II) - humate complexes were more than the Cd(II) - humate complexes was in agreement with the Irving-William's order :

$$\mathrm{Mg} < \mathrm{Ca} < \mathrm{Cd} \sim \mathrm{Mn} < \mathrm{Co} < \mathrm{Zn} \sim \mathrm{Ni} < \mathrm{Cu} < \mathrm{Hg}.$$

Bresnahan, et.al.(1978) postulated that differences in two clases of sites for the Cu(II) and Cd(II) complexes of some fulvic acids were due to the geometry of the site rather than the type of donor atoms. Also it should be noted that non-linear Scatchard plots would be expected for the case where 2:1 complexes are formed (e.g., where the metal ion is the central group).

Non-linear curves of Scatchard and double reciprocal plots have been used by many researchers to obtain stability constants for binding at two classes of sites (log K_1, and log K_2). But the division of these plots into two straight line segments is somewhat arbitary and additional sites can be found by assigning additional linear segments to these non-linear plots. (Fitch, etal. 1984). In practice, a continuum of binding sites may be present (Saar, et.al. 1979). Thus, in the present study, it can be suggested that all the three HA's might contain a series of binding sites with which Cu(II) has greater affinity than Cd(II) as revealed from the stability constant data. It was observed that for Cu(II) - humate complexes at pH 3.0, 3.5 & 4.0 the incresing order of stability constants with respect to the HA's was :

HA (soil) > HA (peat) > HA (humified water hyacinth) where as at pH 4.5 the peat HA slightly exceeds that of soil HA. In case of Cd-humate complexes, although at pH 3.0 the above order was observed, the stability constants of HA (humified water hyacinth) increases quite rapidly than soil HA and peat HA respectively with the increase in pH upto about 4.0 where it remained almost constant even the pH was increased upto a value of 5.8. In all the cases, the soil HA was found to be of higher value than the peat HA.

The average molecular weight per total number of binding sites on the HA molecule was calculated for each of the humic acids from the double reciprocal plot (Tables 19,20). Assuming the total number of binding sites on the HA molecule to be 1 (formation of 1:1 complexes), the molecular weights were found out to be 10.96×10^3 for soil HA; 8.69×10^3 for peat HA and 7.56×10^3 for HA derived from humified water hyacinth (determined from Cu(ll) - HA interactions). The values obtained from Cd(II) - HA interactions were 8.57×10^3 for soil HA; 6.52×10^3 for peat HA and 4.93×10^3 for HA derived from humified water hyacinth. From the above data, the soil HA fraction was found to be of higher molecular weight than the peat HA, which in turn, higher than the HA derived from humified water hyacinth. This is usually expected since the degree of condensation and thus molecular weight of HA molecule tend to increse with increse in humifation (Schnitzer, et.al. 1972). The soil HA has high molecular weight probably due to its longer period of humification than the other two sources. Similarly, the decomposition and humification processes would be more rapid in peats than those of water hyacinth only in aqueous medium, thus being of the lowest molecular weight.

Finally the data were plotted according to the Hill plot (Fig. 27 to 32). The Hill plots were found to be perceptively curved, whichwere usually the case for Hill plots of binding data and apparat straightness of then plots was often an illusion (Cornish-Bouden, et.al. 1975) Intercepts & the asymptotes at higher metal ion concentration range on the ordinate axis {log[θ/(1-θ)]=0} provide approximations for stepwise stability constant for binding of the last metal (kn) (Fitch, et.al. 1984). The log kn values for both Cu (II) and Cd(II) are shown in tables 21 & 22. These constants are, in most cases, lower than those attained by the Scatchard and double reciprocal plot methods. This is explained in that extrapolated values from a Hill plot are macroscopic constants whereas those obtained from a Scatchard plot are intrinsic or microscopic site constants (Fitch, etal.1984). It should also be noted that stability constants obtained from the Hill plot are only valid when the slopes of the asymptotes are unity, which was not usually the case in the present study. Another point is that stbility constants based on the Hill plot are only valid when binding at one site enhances binding at subsequent sites (slope > 1) in regions $[\theta/(1-\theta)] = 0$ which is referred to as positive cooperative binding. If the slope & the line in the vicinity of $\log[\theta-/(1-\theta) = 0] < 1$, it indicates negative cooperativity or the presence of independent binding sites (Fitch, et.al.1984).

"n" values were calculated for each titration and shown in tables 21 and 22. For Cu(II), the data showed positive cooperativity at pH 3.0 and 3.5 but negative cooperativity at pH 4.0 and 4.5. Similarly in case of Cd(11), it was found that little positive cooperativity occured at pH 3.0 but almost zero cooperativity at pH's 3.5, 4.5 and 5.8 was observed for soil HA. For peat HA, with Cd(II), negative was found to be prevalent over the pH ranges 3.0, 4.0, 4.5 & 5.8. For the HA isolated from humified water hyacinth, positive cooperative binding was seen at pH 3.0 whereas negative cooperative binding at pH's 3.5 and 4.0 and zero cooperativity at pH 5.8.

The negative cooperative binding obtained by Hill plot are generally unexpected since Hill plot is based on the assumption that binding at one site enhances binding at subsequent sites (Fitch, et.al. 1984; Cornish-Bowden, et.al. 1975). Since negative and zero cooperative binding were observed in the Hill plot results in most of the cases along with a few instances of positive cooperative binding, the Hill plot is less reliable for calculating stability constants than Scatchard and Double reciprocal plots.

CONCLUSIONS

The present study was undertaken with the intention of obtaining informations regarding the interaction of two heavy metals, viz Cu(II) and Cd(II) with humic acids derived from different sources. The metal - HA interactions were examined in the light of some recently suggested models. The ion selective electrods (ISE) were used for analysis. The conclusions drawn from this study are summarized as follows:

1. The non-linearity of ISE response curves for all the three HA's with respect to Cu(II) and Cd(II) at various pH levels of low acidity range suggested the comlexation of the added copper and cadmium at different concentrations. The non-linearity was more pronounced with Cu(II) than that with Cd(II).

2. From the metal ion binding data, it was observed that the amount of Cd(II) bound to HA's was substantially lower than that of Cu(II) at the pH levels of low acidity range indicating low affinity of Cd(II) for HA's as compared to that of Cu(II).

3. The peat HA was found to get complexed with more of Cu(II) and Cd(II) respectively than that of soil HA followed by the HA isolated from humified water hyacinth.

4. The degree of complexation (α) values of the HA's were found to be of the following order :

   HA (peat) > HA (soil) > HA (humified water hyacinth).

5. In view of the above observations (3,4), it was concluded that the peat HA contained more soluble organic carbon than the soil HA followed by the HA derived from humified water hyacinth.

6. The number of sites bound per total number of reactive sites (θ) was found to be increasing from nearly zero to almost 1 with the increase in metal ion concentration indicating progressive saturation of all the binding sites in the HA molecule.

7. 'θ' was found to increase in the order :

   HA (peat) > HA (soil) > HA (humified water hyacinth).

8. The average molecular weights of three HA's (assuming 1:1 complex formation) were found to be of the following order :

   HA (soil) > HA (peat) > HA (humified water hyacinth).

9. The single surface langmuir plots were found to be less reliable in order to calculate maximum binding abilities of HA's because of their non-linear nature.

10. The non-linear nature of the Scatchard and Double reciprocal plots led to the conclusion that there were more than one classes of binding sites in HA molecules thus revealing the possibility of 1:2 & 2:1 complex formations along with 1:1 complexes.

11. The conditional stability constants (Kn) were seen to be increasing with the increase in pH.

12. The stability of the metal complexes of the HA's were found to be of the order :

HA(soil) > HA (peat) > HA (humified water hyacinth).

13. The double reciprocal plots and Hill plots were found to be less reliable for calculating stability constants than the Scatrchard plots supporting the conclusions drawn by Fitch, et.al. (1984).

14. The Hill plots indicated mostly negative and zero cooperative binding and less positive cooperative binding only at low pH levels in few cases.

15. The conditional stability constants of Cu(II) - HA complexes were found to be in the higher range than those of Cd(II) - HA complexes which was in arrangement with the Irving-William's order :

$\mathrm{Mg} < \mathrm{Ca} < \mathrm{Cd} \sim \mathrm{Mn} < \mathrm{Co} < \mathrm{Zn} \sim \mathrm{Ni} < \mathrm{Cu} < \mathrm{Hg}$.

APPENDIX

TABLE - 23

Volume and concentration of $Cu(NO_3)_2$ & $Cd(NO_3)_2$ solutions used in the titration

Steps	Volume (ml.)	Molar Concentration (Mt)	Log(Mt)
1.	0.2	1.31×10^{-6}	-5.88
2	0.4	2.61×10^{-6}	-5.58
3	0.6	3.91×10^{-6}	-5.41
4	0.8	5.20×10^{-6}	-5.28
5	1.0	6.49×10^{-6}	-5.19
6	1.2	7.78×10^{-6}	-5.11
7	1.4	9.07×10^{-6}	-5.04
8	1.6	1.03×10^{-5}	-4.98
9	1.8	1.16×10^{-5}	-4.93
10	2.0	1.29×10^{-5}	-4.89
11	2.2	1.42×10^{-5}	-4.85
12	2.4	1.54×10^{-5}	-4.81
13	2.6	1.67×10^{-5}	-4.78
14	2.8	1.80×10^{-5}	-4.74
15	3.0	1.92×10^{-5}	-4.72
16	3.2	2.05×10^{-5}	-4.69
17	3.4	2.17×10^{-5}	-4.66
18	3.6	2.30×10^{-5}	-4.64
19	3.8	2.42×10^{-5}	-4.61
20	4.0	2.55×10^{-5}	-4.59
21	4.2	2.67×10^{-5}	-4.57
22	4.4	2.79×10^{-5}	-4.55
23	4.6	2.92×10^{-5}	-4.53
24	4.8	3.04×10^{-5}	-4.51
25	5.0	3.16×10^{-5}	-4.50
26	5.2	3.28×10^{-5}	-4.48
27	5.4	3.41×10^{-5}	-4.46
28	5.6	3.53×10^{-5}	-4.45
29	5.8	3.65×10^{-5}	-4.43
30	6.0	3.77×10^{-5}	-4.42

TABLE - 24

Electrode Potentials (in MV) in the absence of Humic Acids with $Cu(No_3)_2$ and $Cd(No_3)_2$ solutions respectively.

	$Cu(NO_3)_2$				$Cd(NO_3)_2$				
PH Steps	3.0	3.5	4.0	4.5	3.0	3.5	4.0	4.5	5.8
1	146.9	136.7	131.2	131.8	-201.7	-202.6	-204.1	-206.3	-210.3
2	149.2	140.9	136.3	137.3	-193.7	-194.7	-195.8	-197.7	-201.0
3	151.0	144.4	140.3	141.2	-189.8	-190.4	-191.3	-192.6	-195.9
4	152.7	147.4	143.4	144.1	-186.0	-186.8	-187.6	-189.4	-191.4
5	154.3	149.6	146.1	146.5	-183.7	-184.7	-185.5	-186.8	-189.2
6	155.5	151.5	148.2	148.6	-182.1	-182.4	-183.3	-184.7	-186.3
7	156.7	153.8	150.0	150.3	-180.0	-180.5	-181.7	-182.8	-184.4
8	157.7	155.0	151.7	152.0	-178.3	-179.1	-179.5	-181.2	-182.5
9	158.7	156.3	153.1	153.4	-177.3	-177.7	-178.5	-179.8	-181.6
10	159.5	157.5	154.5	154.7	-176.2	-176.5	-177.3	-178.4	-179.7
11	160.3	158.5	155.7	155.9	-175.0	-175.6	-176.3	-177.2	-178.5
12	161.1	159.6	156.8	157.0	-174.1	-174.7	-175.2	-176.1	-177.3
13	161.8	160.6	157.8	158.0	-173.3	-173.6	-174.3	-175.1	-176.4
14	162.6	161.6	158.8	159.0	-172.2	-172.5	-173.5	-174.2	-175.2
15	163.3	162.3	159.8	159.9	-171.6	-172.1	-172.9	-173.4	-174.6
16	163.9	163.2	160.6	160.7	-171.0	-171.4	-172.2	-172.7	-173.7
17	164.5	164.0	161.4	161.5	-170.4	-170.7	-171.4	-172.0	-172.8
18.	165.1	164.7	162.2	162.2	-169.6	-170.1	-170.7	-171.3	-172.3
19	165.6	165.3	162.9	162.9	-169.1	-169.3	-170.0	-170.7	-171.5
20	166.1	165.9	163.6	163.5	-168.4	-168.9	-169.5	-170.0	-170.6
21	166.6	166.3	164.2	164.2	-167.9	-168.3	-169.0	-169.4	-170.0
22	167.0	166.8	164.8	164.9	-167.3	-167.7	-168.4	-168.9	-169.6
23	167.5	167.3	165.4	165.5	-166.8	-167.2	-168.0	-168.2	-169.0
24	168.0	167.7	166.0	166.0	-166.4	-166.8	-167.4	-167.8	-168.4
25	168.4	168.2	166.6	166.6	-166.1	-166.2	-166.9	-167.2	-167.9
26	168.8	168.7	167.1	167.1	-165.6	-165.9	-166.5	-166.7	-167.3
27	169.2	169.1	167.6	167.6	-165.1	-165.4	-166.1	-166.3	-166.7
28	169.5	169.5	168.1	168.1	-164.7	-164.6	-165.7	-165.8	-166.3
29	169.9	169.8	168.5	168.5	-164.2	-164.3	-165.2	-165.5	-165.9
30	170.3	170.2	169.0	169.0	-163.9	-164.1	-164.9	-165.0	-165.5

TABLE - 25

Electrode Potentials (in MV) in the presence of HA(Soil) with respect to Cu (II).

PH →	3.0	3.5	4.0	4.5
1	119.7	117.8	119.0	116.4
2	129.6	127.9	128.8	128.1
3	135.8	135.0	135.0	134.9
4	140.5	139.6	139.3	139.8
5	143.7	143.3	142.7	143.3
6	146.5	146.2	145.4	146.1
7	149.2	148.8	147.6	148.4
8	151.2	150.9	149.5	150.3
9	153.2	152.7	151.4	152.1
10	154.7	154.3	152.9	153.6
11	156.3	155.8	154.3	155.0
12	157.6	157.1	155.4	156.1
13	158.9	158.3	156.6	157.3
14	160.1	159.4	157.6	158.3
15	161.0	160.3	158.5	159.2
16	162.0	161.3	159.4	160.1
17	162.9	162.1	160.2	160.9
18	163.8	162.9	161.0	161.7
19	164.8	163.7	161.8	162.4
20	165.3	164.4	162.5	163.1
21	166.0	165.1		
22	166.6	165.7		
23	167.3	166.4		
24	167.9	166.9		
25	168.4	167.5		
26	169.0	168.0		
27	169.5	168.5		
28	170.0	169.0		
29	170.5	169.5		
30	171.0	169.9		

TABLE - 26

Electrode Potentials (in MV) in the presence of HA(Peat) with resepect to Cu (II).

PH →	3.0	3.5	4.0	4.5
1	107.7	109.5	116.1	112.7
2	121.1	122.3	128.4	125.4
3	129.9	130.1	134.9	132.6
4	136.2	135.9	139.3	137.5
5	140.4	140.1	142.7	141.5
6	144.0	143.3	145.5	144.5
7	146.9	146.5	147.8	147.0
8	149.8	148.6	149.8	149.0
9	151.2	150.6	151.5	150.9
10	153.2	152.5	153.0	152.5
11	154.8	154.1	154.5	154.0
12	156.0	155.4	155.6	155.2
13	157.5	156.8	156.8	156.4
14	158.0	158.0	157.8	157.5
15	159.8	159.0	158.7	158.5
16	160.9	160.1	159.7	159.4
17	161.8	161.0	160.4	160.2
18	162.8	161.9	161.2	161.0
19	163.6	162.7	161.9	161.7
20	164.6	163.5	162.7	162.5
21	165.1	164.2	163.3	163.1
22	165.8	164.8	163.9	163.7
23	166.5	165.5	164.5	164.4
24	167.1	166.1	165.1	164.9
25	167.7	166.7	165.6	165.4
26	168.2	167.2	166.1	166.0
27	168.8	167.8	166.6	166.5
28	169.3	168.3	167.1	167.0
29	169.8	168.7	167.5	167.4
30	170.3	169.2	168.0	167.9

TABLE - 27

Electrode Potentials (in MV) in the presence of HA (humified water hyacinth) with respect to Cu (II).

PH	3.0	3.5	4.0	4.5
1	124.1	120.8	112.0	108.1
2	134.3	129.3	118.6	117.1
3	140.0	136.1	128.4	128.1
4	143.8	140.6	134.3	134.6
5	146.9	144.1	138.9	139.2
6	149.2	146.9	142.2	142.9
7	151.4	149.4	144.8	145.7
8	153.1	151.0	146.9	147.9
9	154.7	152.9	149.1	149.9
10	156.2	154.4	150.8	151.7
11	157.6	155.9	152.4	153.3
12	158.8	157.1	153.7	154.6
13	159.8	158.3	155.0	155.9
14	160.9	159.3	156.2	157.0
15	161.8	160.2	157.2	158.0
16	162.7	161.2	158.2	159.0
17	163.5	162.0	159.1	159.8
18	164.3	162.9	159.9	160.7
19	165.0	163.6	160.0	161.5
20	165.7	164.3	161.4	162.2
21	166.3	165.0	162.0	162.9
22	166.9	165.6	162.6	163.5
23	167.6	166.2	163.3	164.2
24	168.2	166.8	163.9	164.8
25	168.7	167.3	164.4	165.3
26	169.2	167.9	164.9	165.8
27	169.8	168.4	165.5	166.4
28	170.3	169.3	166.0	166.8
29	170.7	169.3	166.4	167.3
30	171.2	169.8	166.9	167.8

TABLE - 28

Electrode Potentials (in MV) in the presence of HA(Soil) with respect fo Cd (II).

PH	3.0	3.5	4.0	4.5	5.8
1	- 212.7	- 214.9	- 216.3	- 217.7	- 219.2
2	- 202.2	- 204.2	- 205.4	- 207.0	- 208.7
3	- 196.1	- 197.8	- 198.9	- 200.6	- 202.5
4	- 191.8	- 193.3	- 194.5	- 196.0	- 198.0
5	- 188.6	- 189.8	- 191.0	- 192.6	- 194.2
6	- 185.9	- 186.9	- 188.2	- 189.6	- 191.4
7	- 183.7	- 184.5	- 185.7	- 187.2	- 188.9
8	- 181.8	- 182.4	- 183.8	- 185.0	- 186.8
9	- 180.0	- 180.6	- 181.9	- 183.1	- 184.8
10	- 178.6	- 179.1	- 180.4	- 181.4	- 183.2
11	- 177.2	- 177.9	- 179.0	- 180.0	- 181.6
12	- 176.0	- 176.8	- 177.7	- 178.7	- 180.2
13	- 174.9	- 175.6	- 176.4	- 177.5	- 179.0
14	- 173.9	- 174.5	- 175.3	- 176.4	- 177.8
15	- 173.0	- 173.7	- 174.3	- 175.6	- 176.8
16	- 172.2	- 172.8	- 173.4	- 174.6	- 175.9
17	- 171.3	- 172.0	- 172.6	- 173.8	- 174.9
18	- 170.6	- 171.1	- 171.8	- 173.0	- 174.0
19	- 169.8	- 170.4	- 171.1	- 172.3	- 173.4
20	- 169.2	- 169.8	- 170.4	- 171.6	- 172.6
21	- 168.6	- 169.1	- 169.9	- 170.9	- 172.0
22	- 168.0	- 168.6	- 169.2	- 170.3	- 171.4
23	- 167.4	- 167.9	- 168.6	- 169.7	- 170.8
24	- 167.0	- 167.4	- 168.0	- 169.1	- 170.3
25	- 166.5	- 166.9	- 167.5	- 165.6	- 169.7
26	- 166.0	- 166.4	- 167.0	- 168.0	- 169.1
27	- 165.5	- 165.8	- 166.5	- 167.5	- 168.6

TABLE - 29

Electrode Potentials (in MV) in the presence of HA (Peat) with respect to Cd(II).

PH	3.0	3.5	4.0	4.5	5.8
Steps					
1	-222.3	-224.6	-221.5	-219.7	-219.3
2	-210.2	-212.6	-208.5	-207.7	-208.0
3	-202.8	-204.8	-201.1	-200.8	-201.6
4	-197.4	-199.1	-196.0	-195.8	-196.8
5	-193.3	-194.8	-192.1	-192.3	-193.1
6	-190.0	-191.3	-189.2	-189.1	-190.0
7	-187.0	-188.2	-186.5	-186.5	-187.5
8	-184.5	-185.8	-184.4	-184.5	-185.5
9	-182.5	-183.6	-182.5	-182.7	-183.7
10	-180.7	-181.7	-180.9	-181.1	-182.2
11	-179.0	-180.0	-179.4	-179.6	-180.8
12	-177.7	-178.7	-178.2	-178.4	-179.6
13	-176.4	-177.4	-177.0	-177.3	-178.6
14	-175.3	-176.1	-175.9	-176.4	-177.6
15	-174.3	-175.1	-175.0	-175.3	-176.7
16	-173.3	-174.1	-174.0	-174.4	-175.7
17	-172.4	-173.3	-173.3	-173.7	-175.0
18	-171.6	-172.4	-172.5	-172.9	-174.2
19	-170.9	-171.7	-171.8	-172.3	-173.6
20	-170.0	-170.9	-171.1	-171.6	-172.9
21	-169.4	-170.1	-170.3	-170.9	-172.2
22	-168.8	-169.5	-169.8	-170.4	-171.6
23	-168.1	-168.9	-169.2	-169.7	-171.0
24	-167.6	-168.3	-168.6	-169.2	-170.5
25	-167.0	-167.7	-168.3	-168.6	-170.0
26	-166.5	-167.2	-167.6	-168.1	-169.5
27	-166.0	-166.6	-167.1	-167.6	-169.0

TABLE - 30

Electrode Potentials (in MV) in the presence of HA(humified water hyacinth) with respect to Cd(II).

PH	3.0	3.5	4.0	4.5	5.8
Steps					
1	-210.0	-211.7	-213.9	-216.5	-219.2
2	-199.7	-201.6	-203.9	-206.0	-208.6
3	-194.1	-195.7	-197.9	-199.9	-202.4
4	-190.2	-191.4	-193.6	-195.5	-197.8
5	-187.1	-188.2	-190.2	-192.1	-194.3
6	-184.6	-185.6	-187.6	-189.3	-191.4
7	-182.5	-183.4	-185.2	-186.9	-189.0
8	-180.8	-181.7	-183.3	-184.9	-187.0
9	-179.1	-180.1	-181.5	-183.1	-185.2
10	-177.7	-178.7	-179.9	-181.4	-183.5
11	-176.5	-177.4	-178.5	-180.0	-182.0
12	-175.5	-176.4	-177.4	-178.8	-180.8
13	-174.5	-175.3	-176.4	-177.7	-179.5
14	-173.4	-174.3	-175.3	-176.6	-178.4
15	-172.6	-173.5	-174.4	-175.6	-177.5
16	-171.7	-172.5	-173.5	-174.7	-176.5
17	-171.1	-171.8	-172.8	-173.9	-175.7
18	-170.3	-171.0	-171.9	-173.1	-174.9
19	-169.7	-170.3	-171.3	-172.4	-174.1
20	-169.0	-169.6	-170.6	-171.7	-173.4
21	-168.4	-169.0	-170.0	-171.1	-172.7
22	-167.8	-168.5	-169.4	-170.5	-172.1
23	-167.3	-167.9	-168.8	-169.8	-171.4
24	-166.7	-167.3	-168.2	-169.3	-170.9
25	-166.2	-166.8	-167.7	-168.7	-170.4
26	-165.7	-166.3	-167.2	-168.3	-169.9
27	-165.2	-165.8	-166.7	-167.7	-169.3

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