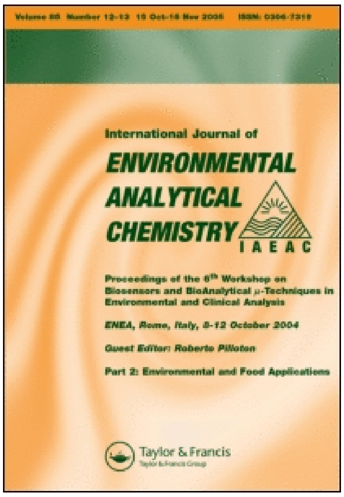

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COMPLEXATION PROPERTIES OF TYPICAL SOIL AND PEAT HUMIC ACIDS WITH COPPER(II) AND CADMIUM(II)

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Potentiometric titration with ion-selective electrodes was applied to determine characteristic parameters like the degree of complexation and the approximate molecular weight of humic acids from theoretical considerations and conditional stability constants for the complexes of Cu(II) and Cd(II) with humic acids obtained from sources such as garden soil, peat prepared by decomposing water hyacinth in soil and humified water hyacinth. Double-reciprocal and Scatchard plots were constructed to determine the conditional stability constants of the complexes formed. Cu(II) was found to have more affinity for the humic acids than Cd(II) and the stability of the metal complexes in aqueous medium was found to increase with increasing pH. The order of stability of the complexes was $M-HA$ (soil) > $M-HA$ (peat) > $M-HA$ (humified water hyacinth). where M and HA represent metal and humic acids, respectively.

KEY WORDS: Ion-selective electrodes, humic acid, complexation, double reciprocal plot, Scatchard plot.

INTRODUCTION

Humic acids are widely distributed in soils, sediments, animal wastes, lakes, streams and certain biogenic deposits, where they form water-soluble and water-insoluble complexes with metal ions.[1] The humic acids participate in complex formation with metal ions through their ionizable functional groups (—COOH, —OH, etc.) with a wide range of acidities.[2] The metal binding ability of soil organic matter influences their translocation and, thus, their bioavailability.[3] Therefore, it is important to characterize this behaviour by using suitable methods that may yield information about the actual role of humic acids in nature.

In the present study, we have investigated the complexation properties of humic acids originating from three different sources, viz. (i) garden soil, (ii) peat prepared by decomposition of water hyacinth (*Eichhornia crassipes*) in soil, and (iii) humified water hyacinth. Water hyacinth is a submerged aquatic weed found in most subtropical and tropical regions of the world. In freshwater ecosystems, the submerged plants contribute mostly to the total humus content.[4] In such ecosystems, the water hyacinth is mostly decomposed and humified in the aqueous medium and along with the water-logged soils. So there arises the need of studying

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the metal complexation properties of humic acids representing both aqueogenic (water-derived) and pedogenic (soil-derived) types. The metals chosen for the study were divalent copper and cadmium, because they play a significant role in aquatic systems in terms of their limiting nutrient value as well as toxicity. Also they form fairly stable complexes with many O- and N-containing chelating ligands.[5] Copper is classified as intermediate between 'class a' and 'class b' metals in its chemical interactions with donor atoms, preferring oxygen donors forming essentially electrovalent bonds and sulphur donor atoms forming covalent bonds. Cadmium has a medium 'class b' character which imparts moderate covalency in bonds and increasing bioaccumulation and toxicity.[6]

METHODOLOGY

The degree of complexation, α, is defined as:

$$\alpha=(M_t)/(M) \tag{1}$$

where (M_t) is the total concentration of the metal ion irrespective of the form in the medium, and (M) is the concentration of the metal present in the ionic form; α can be calculated by means of:

$$\alpha=(M_t)\times 10^{(E_0-E)/P} \tag{2}$$

where E is the electrode potential, E_0 is the standard electrode potential for an activity of 1, and P $(=RT/nF)$ is the slope factor for the electrode response under ideal conditions.[7,8] The percentage of M_t present in the ionic form, M, is calculated using Eqs. (1) and (2). In the present case M will be in the form of M^{2+}.

The double-reciprocal plotting method as proposed by Buffle *et al.*[7] and Fitch *et al.*[9] is used to calculate the mean molecular weight of humic acids and the conditional stability constants of metal–humate complexes. The equation representing this plot is expressed as:

$$(L_t)/(M_b)=mw/n+\{mw/n\}\{(H^+)^x/\beta_1^*\} \tag{3}$$

where (L_t) is the total concentration of the macromolecule in mg/ml, mw is the molecular weight, n is the total number of binding sites, (M_b) is the bound metal concentration which is taken as the difference between the total and free metal ion concentrations, i.e.,

$$(M_b)=(M_t)-(M) \tag{4}$$

β_1^* is the formation constant and is related to (H^+) by the equation:

$$K_0=\beta_1^*/(H^+)^x \tag{5}$$

where K_0 is the conditional stability constant.

Rearranging Eq. (3), we obtain

$$(L_t)/(M_b) = mw/n + \{mw/n\}\{1/K_0(M)\}. \tag{6}$$

The Scatchard plot method is used to determine conditional stability constants. The equation representing a Scratchard plot is expressed as:

$$\Theta/(M) = K_0 - \Theta K_0 \tag{7}$$

where Θ is the number of sites bound/total number of reactive sites and the other terms have their usual significance. Θ is expressed as:

$$\Theta = (M_b)/n(L_t) \tag{8}$$

where $n(L_t)$ is a measure of the maximum binding ability, MBA.[9] MBA can be obtained by using the double-surface Langmuir equation:

$$(M) = [b_1 - (M_b)/K_1(M)] + [b_2 - (M_b)/K_2(M)] \tag{9}$$

where b_1 and b_2 are site concentrations of the metal and K_1 and K_2 are constants related to the binding energy. A plot of (M) vs. $(M_b)/(M)$ yields straight-line segments from which b_1 and b_2 can be obtained by extrapolation. MBA can be obtained by extrapolating the straight-line segment corresponding to binding at high metal ion saturation.[9,10]

With the help of Eq. (7), the Scatchard plots of $\Theta/(M)$ vs. Θ can be drawn. The intercept on the Y-axis of the tangent to the curve towards higher concentration of metal gives K_0, the conditional stability constant.

EXPERIMENTAL

Samples of humic acids were isolated from three different sources: (a) garden soil (from J.N.U. Nursery), (b) peat soil, and (c) humified water hyacinth (*Eichhornia crassipes*). Three rectangular glass vessels containing 4 kg of garden soil, 2 kg of the same soil thoroughly mixed with 2 kg of crushed water hyacinth (1:1 ratio) and 4 kg of thoroughly crushed water hyacinth, respectively, moistened with enough distilled water were kept for 3 months; the average room temperature varied between 30 and 35 °C. The contents of the vessels were thoroughly mixed periodically in order to enable aerobic decomposition. The extraction and purification of humic acids was carried out by the method proposed by Takamatsu *et al.*[11] The samples (soil, peat and humified water hyacinth) were air-dried and sieved (2 mm). 250 g of each sample were extracted with 1 litre of 0.1 M NaOH for 1 h at 95 °C with stirring. Each extract was filtered through a G-4 glass filter and centrifuged to remove suspended flocculants. The pH of the solution was adjusted to slightly lower than 2 with HCl to precipitate humic acid.

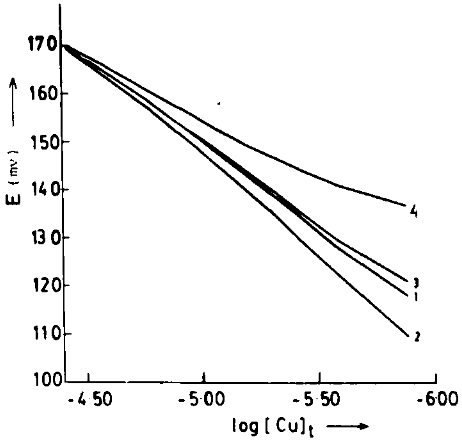

Figure 1 ISE response curves for Cu(II)-humic acid interactions at pH 3.5. 1, HA (soil); 2, HA (peat); 3, HA (humified water hyacinth); 4, blank.

The precipitate of HA was collected by centrifugation and washed three times with 0.1 M HCl and twice with distilled water and then dissolved with 0.1 M NaOH. After the purification procedure of dissolution and precipitation repeated three times, the HA precipitate was washed repeatedly with water until dissolution was no longer observed. The prepared HA was then freeze-dried prior to use.

The potentiometric titration method using ion-selective electrodes (ISE)[7-9] was adopted in the present study. The instrument used was an Orion Ionalyzer 901 with Orion Cu- and Cd-ion-selective electrodes. 3.2 mg of each of the three humic acids obtained as mentioned above were dissolved in 100 ml of 0.1 M NaOH. These solutions were prepared freshly in each case before titration. The solutions were adjusted to the required pH levels with the help of 0.1 M NaOH and 0.1 M NHO_3 and the total ionic strength was adjusted to 0.1 using 2 ml of 5 M $NaNO_3$ solution. 0.2 ml of the corresponding metal ion solution (10^{-3} M $Cu(NO_3)_2$ or $Cd(NO_3)_2$) was added stepwise. After each step, the pH of the solution was readjusted to within ±0.05 units of the original value in order to maintain a constancy throughout the titration. The electrode potential, *E*, at each step was directly recorded from the instrument.

Correlations and variability between different parameters were computed and plotted for both Cu(II) and Cd(II). However, in the following discussions only representative figures have been given.

RESULTS AND DISCUSSION

With blank titrations in the absence of humic acids, a more or less linear relationship between the electrode potential, *E*, and $\log(Cu)_t$ (or $\log(Cd)_t$) was observed over a range of 1.31×10^{-6} M to 3.77×10^{-5} M (Figure 1). It has been

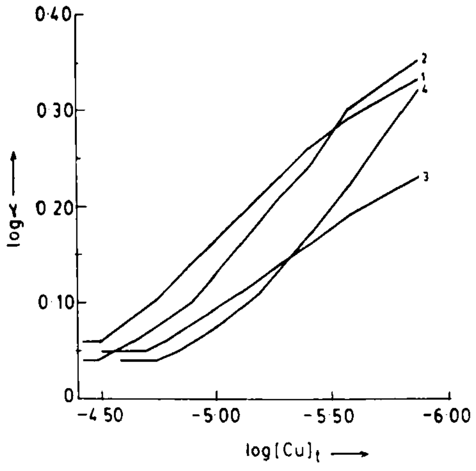

Figure 2 Log α vs. log(Cu)$_t$ for soil humic acid at pH 3.0 (1), 3.5 (2), 4.0 (3) and 4.5 (4).

reported earlier[12,13] that in a non-complexing medium there can be a range of nonlinearity of the calibration curve as has also been seen in the present study. However, the working range for complexation measurements should be the linear portion of the calibration curve (loc. cit.).

In the presence of humic acids, both the shape and the slope of the curve was affected by pH, as reported also by earlier workers.[7,8] This non-linear relationship between electrode potential and log(Cu)$_t$ or log(Cd)$_t$ is due to complexation of the added copper or cadmium.[8] The present study showed that more Cu(II) or Cd(II) was generally bound to the soil humic acid at low pH levels, such as 3.0 and 3.5 (Figure 2). This may be due to relatively high ionization of the acidic functional groups (—COOH) at low pH levels.[7] The complexation was found to continue up to a point when about 90% of the added copper or cadmium was present as free Cu^{2+} or Cd^{2+}, respectively (Figure 3). The saturation effect of humic acids seemed to occur here because any increase in the total metal concentration did not alter the degree of complexation. This is concurrent with the saturation of almost all the complexing sites in the humic acid. Copper and cadmium added further will remain fully in the ionic form. The degree of complexation was computed for the three HA's with respect to Cu(II) and Cd(II) at different pH values. It was found to follow the order: HA (peat) > HA (soil) > HA (humified water hyacinth). A comparison of these values revealed that the degree of complexation with Cd(II) was always substantially lower than with Cu(II).

The double-reciprocal plots were found to be nonlinear (Figure 4). The linearity of such plots is attributed to the formation of 1:1 complexes, whereas the nonlinear nature may be attributed to the formation of 1:2 complexes.[7] In the present case, both for Cd and Cu, nonlinear curves are obtained which indicate the formation of 1:2 complexes. Since the nonlinearity of these curves was not very explicit, it was also cross-checked for the formation of 1:1 complexes which, as

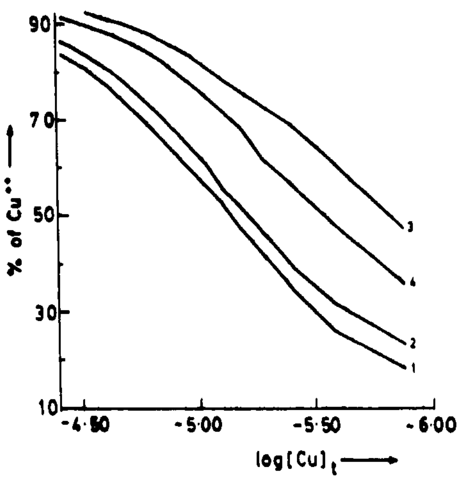

Figure 3 Per cent (Cu^{2+}) vs. log(Cu)$_t$ for peat humic acid at pH 3.0 (1), 3.5 (2), 4.0 (3) and 4.5 (4).

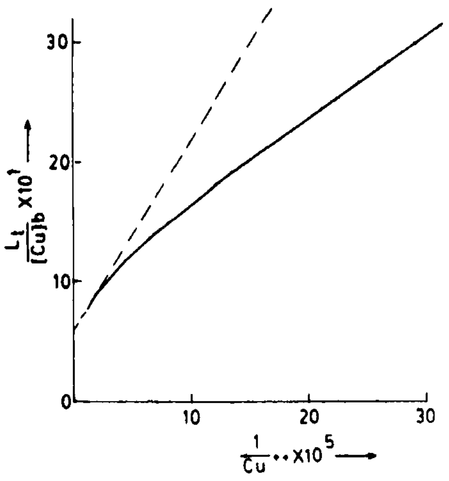

Figure 4 Double-reciprocal plot of Cu(II) binding by peat humic acid at pH 3.5.

stated before, is associated with linear curves. This was done by calculating the molecular weights of the humic acids from the slopes of the curves, following Buffle's method. From the Cu–HA interaction curves, the values worked 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. The corresponding values for the Cd–HA interaction curves were 8.75×10^3, 6.52×10^3 and 4.93×10^3, respectively. These anomalous differences in the approximate molecular weight confirm that the assumption of only 1:1 complex formation is not valid. On the other hand, 1:2 and 2:1 complexes can also be formed along with 1:1 complexes.

The log *K* values (Table 1) calculated by this method increased with an increase

Table 1 Conditional stability constants of Cu(II)- and Cd(II)-humic acid complexes as determined by the double-reciprocal plot method[a]

	pH	$\log K_n$		
		HA (soil)	*HA (peat)*	*HA (humified water hyacinth)*
Cu–HA interactions	3.0	5.40	5.53	4.78
	3.5	5.42	5.58	5.33
	4.0	5.46	5.60	5.70
	4.5	5.66	5.63	6.00
Cd–HA interactions	3.0	4.94	4.88	4.21
	3.5	4.98	5.01	4.49
	4.0	5.46	5.12	4.55
	4.5	5.48	5.17	4.87
	5.8	5.60	5.21	5.11

[a] $I = 0.1$.

in pH, similar to the previously reported results.[14] The degree of condensation and, thus, the molecular weight of the HA molecules tend to increase with an increase in humification.[15] The soil HA has high molecular weight probably due to its longer period of humification than the other two HA's. Similarly, the decomposition and humification processes in peats would be more rapid than those in water hyacinth in aqueous medium. Condensation of molecules in humification is attained in many ways. Plant residues including polysaccharides, lignins and proteins are attacked by micro-organisms and then degraded to smaller organic molecules through a series of biochemical reactions.[16] For instance, lignin degradation products have a carboxyl group and are phenol derivatives with different numbers of methoxyl groups.[17] Some of the phenols are further degraded by many organisms and are used for energy and cell synthesis, whereas others undergo enzymatic reactions in the presence of phenoloxidases to form highly active radicals or hydroxybenzoquinones which link with other phenolic units to form the large humic acid molecules.[16,18] Some of these humic acid molecules may undergo chemical and/or enzymatic oxidative degradation into fulvic acid.[19]

The Scatchard plots (Figure 5) of $\Theta/(M)$ vs. Θ were also nonlinear, indicating metal-binding at two or more classes of sites.[9,14,20–23] The conditional stability constants for binding of Cu(II) and Cd(II) with the three humic acids were calculated from the Scatchard plots (Table 2). The conditional stability constants obtained from the Scatchard plots were found to increase with an increase in pH which has been reported by many other workers.[24,25] The $\log K_0$ values obtained from the double-reciprocal plots were, in most cases, higher than those from the Scatchard plots. Fitch *et al.*[9] have stated that for accurate experimental data, the Scatchard plot is preferred for calculating conditional stability constants, K_n, because the values for high free metal concentrations are not compressed into a narrow zone. This is probably the reason why the $\log K_n$ values obtained from

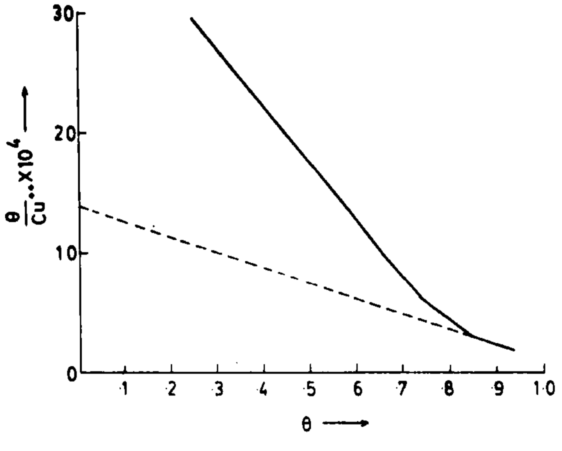

Figure 5 Scatchard plot of Cu(II) binding by peat humic acid at pH 3.5.

Table 2 Conditional stability constants of Cu(II)- and Cd(II)-humic acid complexes as determined by the Scatchard plot method[a]

	pH	$\log K_n$		
		HA (soil)	*HA (peat)*	*HA (humified water hyaccinth)*
Cu–HA interactions	3.0	5.07	5.03	4.76
	3.5	5.14	5.14	4.98
	4.0	5.21	5.19	5.01
	4.5	5.25	5.20	5.13
Cd–HA interactions	3.0	4.74	4.67	4.63
	3.5	4.77	4.74	4.93
	4.0	4.96	4.75	5.02
	4.5	4.99	4.78	5.02
	5.8	5.08	4.81	5.03

[a] $I = 0.1$.

double-reciprocal plots were found to be higher than the $\log K_n$ values obtained from Scatchard plots.

The $\log K_n$ values obtained from the double-reciprocal plots often show fluctuations. They do not show a clear trend at any particular pH level for any of the three humic acids. This may be due to the fact that the molecular weights could be calculated from the intercepts of the curves only by considering the formation of 1:1 complexes, as explained before. This could give rise to fluctuating values of the conditional stability constants. From Scatchard plots, it was found that the stability constants for Cu–HA (soil), Cu–HA (peat) and Cu–HA (humified water hyacinth) steadily increased from 5.07 to 5.25, 5.03 to 5.20 and 4.76 to 5.13, respectively, if the pH was increased from 3.0 to 4.5. A higher value of $\log K_n$ for soil HA than peat HA and HA derived from water hyacinth was always indicated. The corresponding figures for Cd–HA complexes between the same pH limits were 4.74 to 4.99, 4.67 to 4.78 and 4.63 to 5.02, respectively. In this case, the Cd–HA (humified water hyacinth) complexes had somewhat higher values of $\log K_n$ than the cadmium complexes of soil and peat humic acids up to pH 3.5. Above this pH, the value stabilized at 5.02, whereas the complexes with soil and peat humic acids showed a steadily increasing stability constant value with increase in pH (Table 2).

Because of the appreciable values of their stability constants, the complexes are believed to have a chelate structure and the complexing groups are assumed to be phenolic or carboxylic.[7] The phenolic groups generally dissociate at higher pH levels (7.0), while the carboxylic groups dissociate at pH levels lower than 7.0. In the present study, because of the pH levels used in the experiments, the complexation reactions can be expected to occur predominantly in an environment of —COOH group dissociation. Mantoura *et al.*[26] assumed the existence of several complexing sites on the same organic molecule for the humic acid extracted from soil. So the curvilinear nature of the double-reciprocal and the Scatchard plots do not allow one to distinguish between ML ($L>1$) complexes, complexes which ligands having several complexing sites and, simply, the existence of several 1:1 complexes with different stability constants in the reaction mixture.[7,27]

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