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Evaluation of proton and europium (III) binding reactions in a sewage sludge humic acid[1]

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ABSTRACT

Recent increases in land application of sewage sludge have raised concerns over the potential introduction of contaminants into the human food chain. The establishment of sound regulation requires knowledge on the fate and distribution of contaminants in sludge amended soils. Humic substances play a crucial role in trace element bioavailability and transport; thus research is needed to elucidate their mechanisms of reaction. This paper reports the results of a study conducted with a sewage sludge humic acid. The objectives were to evaluate potential differences in composition between this humic acid and its soil analogs and to ascertain their impact on its reactivity. Higher nitrogen and sulfur contents, as well as lower oxygen levels were detected in the sewage sludge humic acid compared to those of its soil counterparts. Determinations of proton and Eu^{3+} affinity constants suggest that the ion binding reactions in this humic acid are controlled mainly by a class of functional groups possessing carboxyl-like reactivities. This finding contrasts with the general notion that ion binding reactions in humic substances are controlled by equivalent fractions of carboxyl and phenolic-type groups, and it confirms the heterogeneous nature of these compounds.

Key words: Humic acid, sewage sludge, affinity constants, europium

RESUMEN

Evaluación de las reacciones de enlace protón y europio en un ácido húmico de cieno sanitario

El aumento reciente en la cantidad de cieno sanitario aplicado a suelos agrícolas ha generado gran preocupación sobre la posible introducción de

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contaminantes en la cadena alimentaria humana. El establecimiento de una reglamentación adecuada requiere amplio conocimiento sobre la distribución y destino final de sustancias potencialmente contaminantes en suelos enmendados con este tipo de material. Los ácidos húmicos desempeñan un rol vital en la disponibilidad y el transporte de los elementos traza, y por lo tanto es necesario dilucidar sus mecanismos de reacción. Se realizó un estudio con un ácido húmico extraído de un cieno sanitario con el propósito de determinar posibles diferencias en composición entre este tipo de compuesto y compuestos extraídos del suelo. Además, evaluamos el efecto de dichas diferencias en la reactividad del compuesto hacia los iones protón y europio. En términos de composición los resultados revelaron un mayor porcentaje de nitrógeno y azufre, así como también menor cantidad de oxígeno en nuestro ácido húmico en comparación con sus análogos terrestres. Estimados de las constantes de enlace protón y europio sugieren que los grupos funcionales que controlan la reactividad en este ácido húmico exhiben capacidades de enlace similares a las del grupo carboxilo. Esto contrasta con la percepción general de que la reactividad de estos compuestos, irrespectivamente de su procedencia, es controlada por fracciones equivalentes de grupos carboxilo y fenol, y confirma la naturaleza heterogénea de estos compuestos.

INTRODUCTION

Land application is one of the most cost effective alternatives for sewage sludge disposal. At present, about 50% of the sewage sludge produced in Britain (approximately 1.2 million t/yr, dry wt.), and about 23% of that produced in the United States is applied to agricultural land (Wang et al., 1995). In Puerto Rico, the vast majority of it is landfilled (Alpert and Soto-López, 1997). Both the amount and proportion of sludge applied to land will continue to increase because of the recent banning of sea disposal (Wild and Jones, 1992). Concern has arisen for the possible negative repercussions of said situation. For instance, significant increases in the concentration of a number of potentially toxic metals (e.g., Cd, Cr, Pb) have frequently been observed as a result of applications of sewage sludge to soils (McBride, 1995). It is therefore necessary to regulate this practice in order to prevent those levels from reaching values that may constitute a hazard. For this regulation, a proper understanding is needed of the mechanisms that control trace element fate and distribution in sludge amended soils. Vital to this issue is the characterization of the role of humic substances (HS) in trace element chemistry.

Humic substances are polyacidic macromolecules that result from the degradation of plant and animal residues. They are structurally heterogeneous; hence their molecular features are usually described only in general terms (Shevchenko and Bailey, 1996). As organic polyelectrolytes they are important sinks for contaminant sorption, and are relevant in numerous physico-chemical processes such as metal solubility and transport, mineral precipitation and dissolution, redox equilibria and reaction kinetics, colloid stability, and transformation of organic contaminants (Magdoff et al., 1996).

Available evidence indicates that the chemical composition of humic substances obtained from sludge amended soils differs from that of compounds obtained from natural soil organic matter (Deiana et al., 1990). Generally, higher nitrogen and sulfur contents, as well as lower C:N ratios, are observed in humic substances obtained from sludge than in those obtained from unamended soils (Sposito and Holtzclaw, 1977). These differences are believed to significantly alter the affinity of these compounds for different ions. To elucidate this aspect, we evaluated the proton affinity, as well as the Eu^{3+} complexation capacity, of a humic acid (HA) extracted from sewage sludge. Emphasis was given to a mechanistic understanding of the underlying processes controlling those reactions in an attempt to correlate their overall nature to the molecular environment prevalent in the system.

MATERIALS AND METHODS

Humic acid extraction

A sample of anaerobically digested sewage sludge from Kenton, Ohio, was air dried and passed through a 2-mm sieve. Approximately 15 g of that material was placed in four polyethylene centrifuge bottles (250 ml). To each, 150 ml of a 0.5M HCl solution was added. This step is used as a pretreatment to remove floating plant debris and inorganic forms of C, N, P, and S prior to extraction with NaOH. The suspensions were stirred occasionally for one hour, after which time they were centrifuged at 10,000 rpm for half hour and the supernatants discarded. To each bottle, 150 ml of distilled deionized water was added. The suspensions were mixed once again, centrifuged, and the supernatants discarded. Then, 150 ml of a fresh 0.5M NaOH solution was added to each bottle. During this step, the head space of each bottle was flushed with 0_2-free N_2 gas and the cap quickly tightened. The bottles were shaken for 18 hours at room temperature and then centrifuged at 10,000 rpm for one hour. The supernatants were placed in a 1.0 L Erlenmeyer flask. The solutions were initially acidified to pH 5.0 with concentrated HCl and then slowly to pH 1.0 with a 1M HCl solution added dropwise. The acidified solutions were permitted to stand overnight at room temperature for HA coagulation. The suspensions were centrifuged at 10,000 rpm for one hour and then the supernatant (fulvic acid) was separated from the precipitate (humic acid).

Purification of the humic acid

Suspended clays were removed from the humic acid (HA) by dissolving the HA in a minimum volume of 0.1 M KOH under a N_2 atmosphere

(Schnitzer and Schuppli, 1989). Enough KCl was added to make the system 0.3 M. The suspension was left standing for four hours, after which time the solids were removed by centrifugation (10,000 rpm for half hour). The clear HA solution was acidified to pH 1, and allowed to coagulate overnight, and then the process was repeated. At this point the HA was separated by centrifugation and 300 ml of a HCl-HF solution (5 ml conc. HCl + 5 ml 52% HF + 990 ml distilled-deionized water) was added to further remove mineral constituents. The suspension was shaken for 24 hours at room temperature, and centrifuged (10,000 rpm for half hour) to separate the HA. This process was repeated several times until the percentage of ash content in the HA was less than 2%. There are indications that prolonged treatment (HCl + HF) can lead to significant losses of nitrogenous components and carbohydrates from humic acid. However, our main interest in this study was to characterize metal complexation reactions in a sludge derived humic acid; to that purpose the need for obtaining an ash-free material obviated the need to obtain a pristine sample from that particular sludge. After completing the treatment, the HA was separated by centrifugation, washed with distilled water, and subsequently freeze-dried.

Ash content determination

Three acid washed porcelain crucibles were heated at 750°C for four hours, and then stored in a dessicator over P_20_5 until analysis. We added 30 to 50 mg of dry HA to the previously weighed crucibles, which were then placed in a furnace and brought slowly to 750°C, and kept there for four hours. Subsequently, each sample was treated with 0.3 ml of 70% AG $HN0_3$. The samples were then dried on a hot plate slightly below their boiling point and placed once again in the furnace at 750°C for two hours. Then, they were removed and placed in the dessicator while still warm. After a couple of hours, the crucibles were reweighed.

Elemental Analysis

Carbon and nitrogen concentrations were determined in a Carlo Erba Nitrogen/Carbon analyzer (NA 1500 series 2). The analyses were performed in duplicate at the Soil Ecology Division of The Ohio State University. Sulfur was determined by a Combustion Iodometric procedure (A.S.T.M. E30-47) in a LECO Induction Furnace[4]. The percentages

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[4] Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

of oxygen and hydrogen in the humic acid were determined by difference, i.e., %[O + H] = 100 -%[C + N + S]. The results were corrected for ash content.

Infrared spectra

IR spectra were run on KBr pellets, consisting of approximately 0.2 mg of HA and 200 mg of dry KBr (IR grade). KBr-subtracted spectra were collected from 400 to 4000 cm^{-1} in a Polaris FT-IR Spectrometer (Mattson Instruments Inc.) operating at 2.0 cm^{-1}.

E_4:E_6 Ratios

E4:E6 ratios (ratio of optical density at 465 over 665 nm) were measured in a 0.1M $NaHCO_3$ solution in a Cary UV-Vis spectrophotometer. The average of three samples is reported.

Molecular weight determination

The number and weight averaged molecular weight of the HA were determined in the Aquatic Organic Geochemistry laboratory of The Ohio State University, Columbus, Ohio. A high-pressure size exclusion chromatography (HPSEC) procedure was used (Chin et al., 1994). The instrumentation consisted of a Waters 510 solvent pump, a Waters 486 variable wavelength detector, and a Rheodyne rotary injector valve equipped with a 20 µl sample loop (Waters Associates, Milford, MA). A Waters Protein-Pak 125 modified silica column was also used. The standards were sodium polystyrene sulfonates (PSS) (1.8 K, 5.4 K, 8 K, and 18 K) as well as acetone, which also served as the permeation volume probe.

Potentiometric titrations

Potentiometric titrations of the humic acid were performed at three ionic strengths (i.e., 0.01, 0.05, and 0.1M in a KCl background). The procedure used is described in detail elsewhere (Martínez et al., 1998a). Briefly, 0.02 g of HA was placed in a 100-ml polyethylene titration vessel. Approximately 50 g of a KCl solution of a specific ionic strength was added, followed by 0.25 ml of 0.1 M KOH. The total weight of the sample was recorded. The sample was sonified for approximately 10 minutes to dissolve the humic material. In all cases a sample twice as concentrated as the one being titrated was prepared in the same manner. The sample was placed in a constant temperature (25°C) vessel and titrated under N_2 from its original pH (approximately 10.3) to a pH of 3.5. During titration, 0.05 ml of the titrant (0.1M HCl in this case) was added at five-minute intervals in combination with a similar amount of the solution twice as concentrated as the one being titrated. The titrations were performed in triplicate. A formation function, n_H, was calculated for each point of the titration according to equation 1; n_H is a measure of the charge status of the ligand (humic acid in this case) along the titration curve. The term ($[A] - [H^+]$) refers to the total number of protons (H^+) bound to the ligand, whereas ($[B] - [OH^-]$) refers to the number of hydroxyls (OH^-) bound at each point of the titration.

$$n_H = \{([A] - [H^+]) - ([B] - [OH^-])\}/ L \qquad [1]$$

where n_H = equivalents (+) per gram of ligand, [A] = concentration (M) of acid added, [B] = concentration (M) of base added, L = g/L of ligand in the system, and [OH] and [H] are the concentrations of H^+ and OH^-, respectively (Martínez et al., 1998a).

A non linear curve fit of each pH vs. n_H plot was performed with the computer software program Table Curve (Jandel Scientific, version 3.18, San Rafael, CA). A user defined function was built for the Quasiparticle and the Gaussian distribution models (equations 3 and 4, respectively). An estimate for the fitting parameters was initially given. The program utilizes the Levenburg-Marquardt procedure to determine the global minimum of the squared sum of deviations. A convergence threshold requiring the R^2 value to remain unchanged in the 6th significant figure for five consecutive iterations was used.

Europium complexation studies

A humic acid stock solution was prepared by weighing 0.04 g of HA in a 150-ml polyethylene vessel. We added 100 ml of 0.1M $NaClO_4$ to the sample together with 0.30 ml of 0.1 M NaOH solution. The sample was sonified until complete dissolution of the HA. A series of experimental stock solutions of Eu^{3+} were prepared covering a range of 100 to 10,000 µM Eu^{3+}. Serial additions (1 to 7 g) of each solution were added to 30-ml polypropylene vials. The exact amount of Eu^{3+} added to each sample was recorded. Immediately, 1 ml of the HA stock solution was added, as well as enough $NaClO_4$ (concentrated enough to make the overall ionic strength in the samples 0.1M) to reach 10 g. The pH in the samples was adjusted to 5.5 with the aid of a diluted solution of either NaOH or HCl (prepared in 0.1M $NaClO_4$). A pH of 5.5 was chosen to avoid the possibility of HA precipitation. The samples were reweighed and stored in an incubator at 25°C until analyses (always performed within 24 hours).

The samples were analyzed in a SLM DF Aminco spectrofluorometer. Emission scans (550 to 675 nm) were collected. The excitation wavelength was set to 394 nm. Emission intensities were collected at 0.5 nm intervals. An average of 10 scans were collected per sample.

Free and complexed Eu^{3+} were calculated following the lanthanide luminescence procedure described elsewhere (Martínez et al., 1998b). A plot of free vs. complexed Eu^{3+} was built. Estimates of the pH-independent conditional Eu^{3+} affinity constants were obtained by curve fitting equation 2 to the experimental data (Table Curve software -Jandel Scientific, version 3.18, P.O. Box 7005, San Rafael, CA 94912-8920). Again, in this procedure a number of iterations are performed until a global minimum of the squared sum of deviations, $\sum([EuL]_{calc} - [EuL]_{obs})^2$ is reached. The value of μ_{Eui} that better describes the experimental data is then selected.

$$\sum_i [EuL_i] = \frac{C_L}{\sigma(2\pi)^{1/2}} \int_{-\infty}^{+\infty} \frac{K_{Eu_i}[Eu]}{1 + K_{Eu_i}[Eu] + K_{Hi}[H^+]} \exp\left[-\frac{1}{2}\left(\frac{\mu_{Eui} - \log K_{Eui}}{\sigma}\right)^2\right] d\log K_{Eui} \qquad [2]$$

RESULTS AND DISCUSSION

Characterization of HA

Elemental analyses, molecular weight and E4:E6 ratio

The nitrogen content of our sewage sludge humic acid is more than twice that commonly observed in humic acids obtained from soils (Table 1). In addition, the amount of sulfur is close to the highest values reported. As indicated previously, this is commonly observed in humic substances obtained from sewage sludge and is generally attributed to

TABLE 1.—*Elemental composition of a humic acid extracted from sewage sludge.*

	Sewage sludge HA	Soil HA[1]
C(%)	60.3	50-60
N (%)	8.25	2-4
S (%)	1.53	0-2
(O + H) (%)	29.92	34-41
C/N	7.31	
weight avg. M.W. (M_w)	6942	
number avg. M.W. (M_n)	1356	
M_w/M_n	5.12	
E_4/E_6	3.88	3.0-5.0
Ash (%)	1.40	

[1] Schnitzer and Khan, 1972.

TABLE 2.—*Assignment of major absorption bands in the IR spectra of humic acids (from Schnitzer and Khan, 1972).*

Wavenumbers cm^{-1}	Assignments
3400	O-H or N-H stretch
2900	aliphatic C-H stretch
1725	C=O stretch of COOH and Ketones
1630	aromatic C=C, H-bonded C=O, double bond conjugated with carbonyl, asymmetric stretching of COO^-
1560-1540	aromatic C=C or NH deformation in amide II
1450	aliphatic C-H
1400	COO-, aliphatic C-H
1200	C-O stretch and OH deformation of COOH
1050-1020	O-H and C-O stretching in various groups

the preponderance of proteinaceous materials as well as S- containing surfactants and amino acids in sludge (Holtzclaw et al., 1976). Such difference in composition could have important implications in trace element movement and bioavailability. For instance, it is well known that some N- and S-containing functional groups exhibit a very high affinity toward ions such as Cu, and Hg, respectively. The introduction of high concentrations of metals and humics into agricultural lands from sewage sludge applications may bring about the transport of these contaminants into water sources through metal-ligand complexes. Thus, it is imperative to consider the role of humic substances in trace element chemistry in the regulation of sewage sludge application to agricultural lands.

The high carbon content, as well as the relatively low E4:E6 ratio, suggests that our humic acid is well humified. On the other hand, oxygen levels were lower than expected. This finding would indicate that some O-containing functional groups (e.g., carboxyl, phenol, alcohol) would be in lesser amounts in this humic acid than in those derived from soils. This indication will be discussed further in the next section. The large polydispersity (M_w/M_n), is indicative of a complex mixture of species covering a wide range of molecular weights (values close to one would indicate a homogeneous mixture of compounds).

IR spectra

The complex nature of humic substances has led to considerable disagreement on the assignment of their IR bands. Usually poorly resolved broad bands result from the overlapping of different structural moieties. However, considerable agreement exists as to the group of entities that may contribute to a specific band in the spectra (Deiana et al., 1990). Strong bands are observed at 2924, 1662, 1527, 1450 and 1224 cm^{-1} (Table 2, Figure 1). This is typical of humic substances as it reflects the contribution from aliphatic and aromatic moieties, as well as those from carboxylic groups. In this case, a strong contribution from nitrogen containing groups (e.g., amides) is also observed in agreement with its elemental composition and in accord with spectra of humic acids of similar origin (Deiana et al., 1990).

Potentiometric titrations

Proton affinity curves have traditionally been used to make inferences on the overall binding capacity of humic substances, the nature and distribution of their functional groups, their molecular configura-

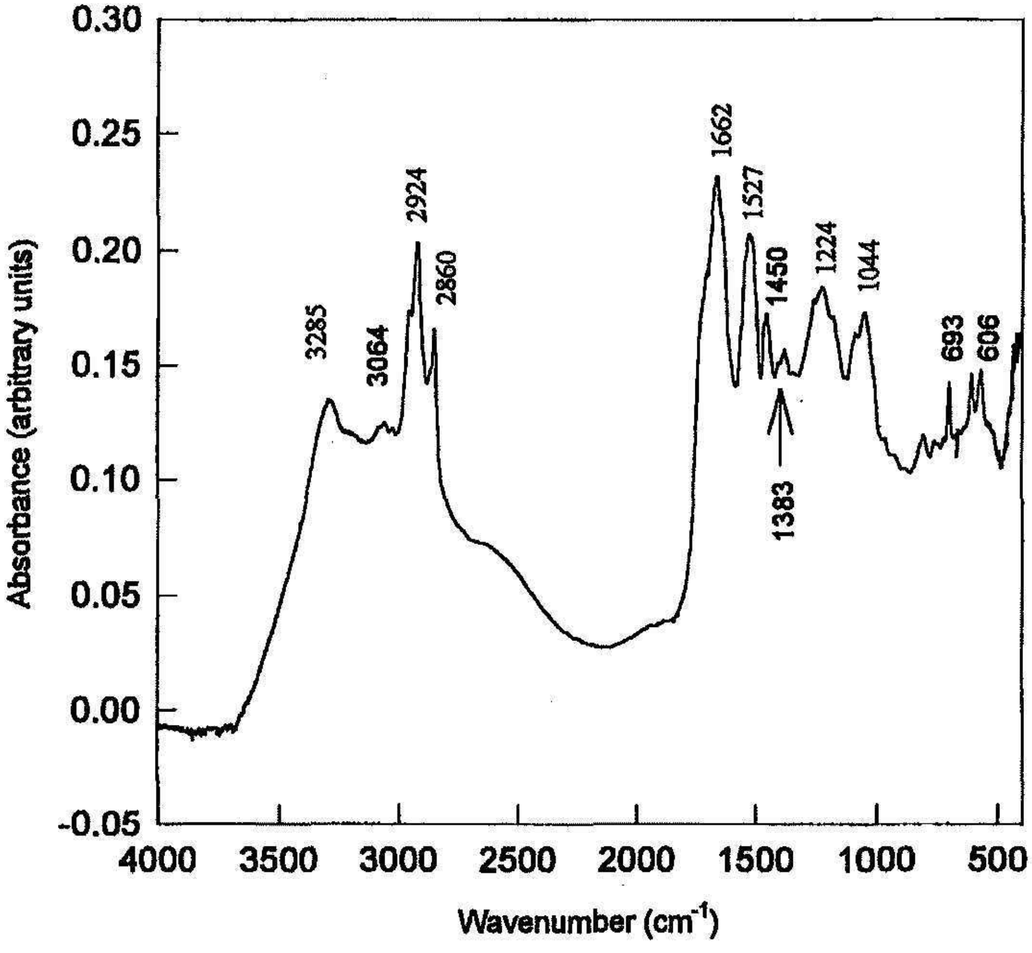

FIGURE 1. Infrared spectra of the sewage sludge humic acid.

tion, and their electrochemical properties (Sposito et al., 1977). Conflicting views in terms of the most appropriate empirical portrayal of these compounds have led to numerous interpretations of similar experimental evidence. In this study we employed two of the most widespread utilized models in the characterization of affinity reactions by humic substances. The models represent contrasting views on the characterization of humic substances, and were selected for comparison purposes.

The Scatchard Quasiparticle model (QM) (equation 3) views humic substances as a set of non-interacting discrete classes of functional groups (Figure 2), each described exclusively by an affinity constant and its binding capacity (Sposito and Blaser, 1992). The Gaussian distribution model (GM) (equation 4), represents a continuous distribution approach. Similar to the QM, the GM considers HS to be composed of different classes of functional groups. In this case, however, each class is characterized by a range of affinity constants, which are distributed around a mean value following a Gaussian distribution (Perdue and Lytle, 1983) (Figure 2).

$$\mathrm{n}_H([H^+], \mathrm{C}_L) = \sum_i \frac{n_{Hi}\,{}^{c}K_{HLi}[H^+]}{1 + {}^{c}K_{HLi}[H^+]} \qquad [3]$$

$$\sum_i [\mathrm{HL}_i] = \frac{\mathrm{C_L}}{\sigma(2\pi)^{1/2}} \int_{-\infty}^{+\infty} \frac{\mathrm{K}_{HLi}[H^+]}{1 + K_{HLi}[H^+]} \exp\left[-\tfrac{1}{2}\left(\frac{\mu - \log K_{HLi}}{\sigma}\right)^2\right] d\log K_{HLi} \qquad [4]$$

Recently, we evaluated the effectiveness of these two models to describe different proton affinity curves in well characterized polyelectrolyte systems (Martínez et al., 1998a). This study builds upon those findings to evaluate the much more complex sewage sludge humic acid system.

Three classes of functional groups are required to describe the proton affinity data of the humic acid with the QM (Figure 3). The need to adopt three distinct classes of binding sites is commonly observed in applications of this model to binding isotherms in polyelectrolytes; thus skepticism arises as to the accuracy of chemical implications of the fitting parameters employed. The dissociation constants obtained in our study closely resemble those reported by Sposito et al. (1977) for a fulvic acid extracted from sewage sludge (i.e., 3.9, 6.5, and 9.4). Sposito and Holtzclaw (1977) stated that those classes would likely correspond to: 1) carboxylic groups, 2) a mixture of weakly acidic carboxyl groups and nitrogen containing groups, and 3) phenolic and SH groups. However, our previous evaluation of this model revealed that its fitting parameters

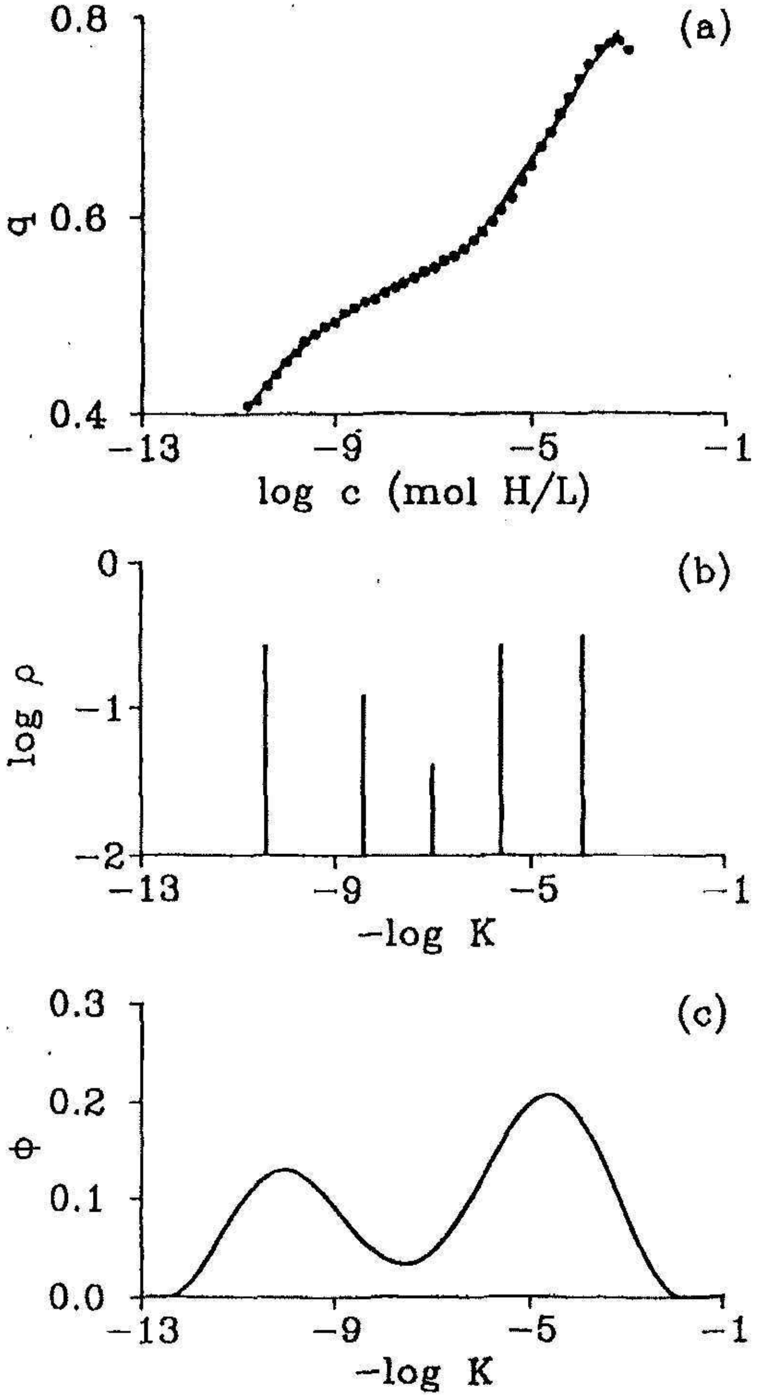

FIGURE 2. Conceptual basis for the empirical description of humic acid binding reactions. A) Proton affinity curve. B) Schatchard Quasiparticle model (discrete site approach) portrayal of the experimental data. C) Gaussian distribution model (continuous site approach) description. Adapted from Cernik et al. (1995).

did not necessarily hold any relation to the chemical nature of the systems (Martínez et al., 1998a). Specifically, the proton affinity constants obtained with this model for polyacrylic acid did not match the known reactivity of its functional group (i.e., carboxyl). Thus, we do not feel confident about making assumptions on the molecular significance (e.g., composition and relative distribution of functional groups) of the values obtained in the curve-fitting procedure with this model.

Results with the GM attest to the conceptual discrepancy created by these opposing empirical constructs. In this case, an adequate description of the experimental data is attained by assuming only one class of functional group (Figure 4). Thus only those groups with reactivities

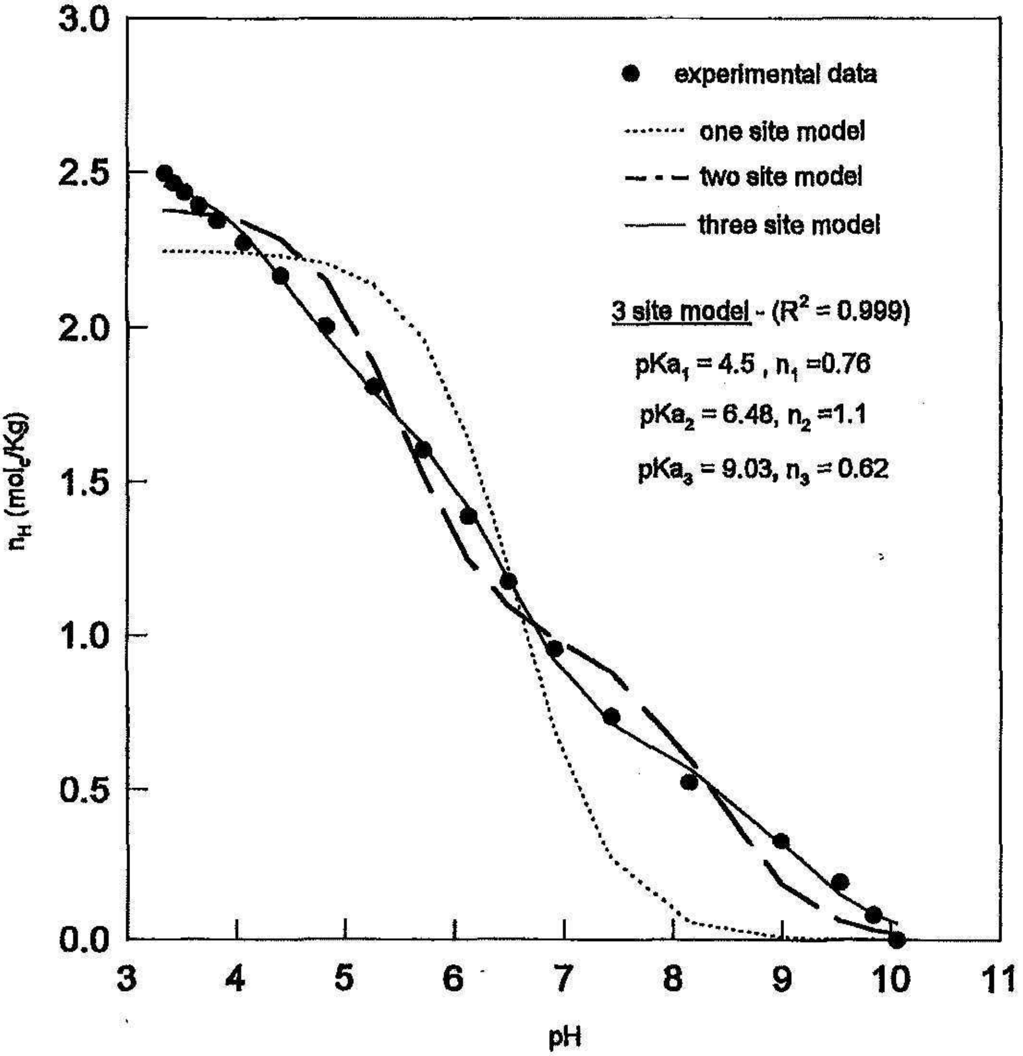

FIGURE 3. Schatchard Quasiparticle model description of the sewage sludge humic acid proton affinity isotherm.

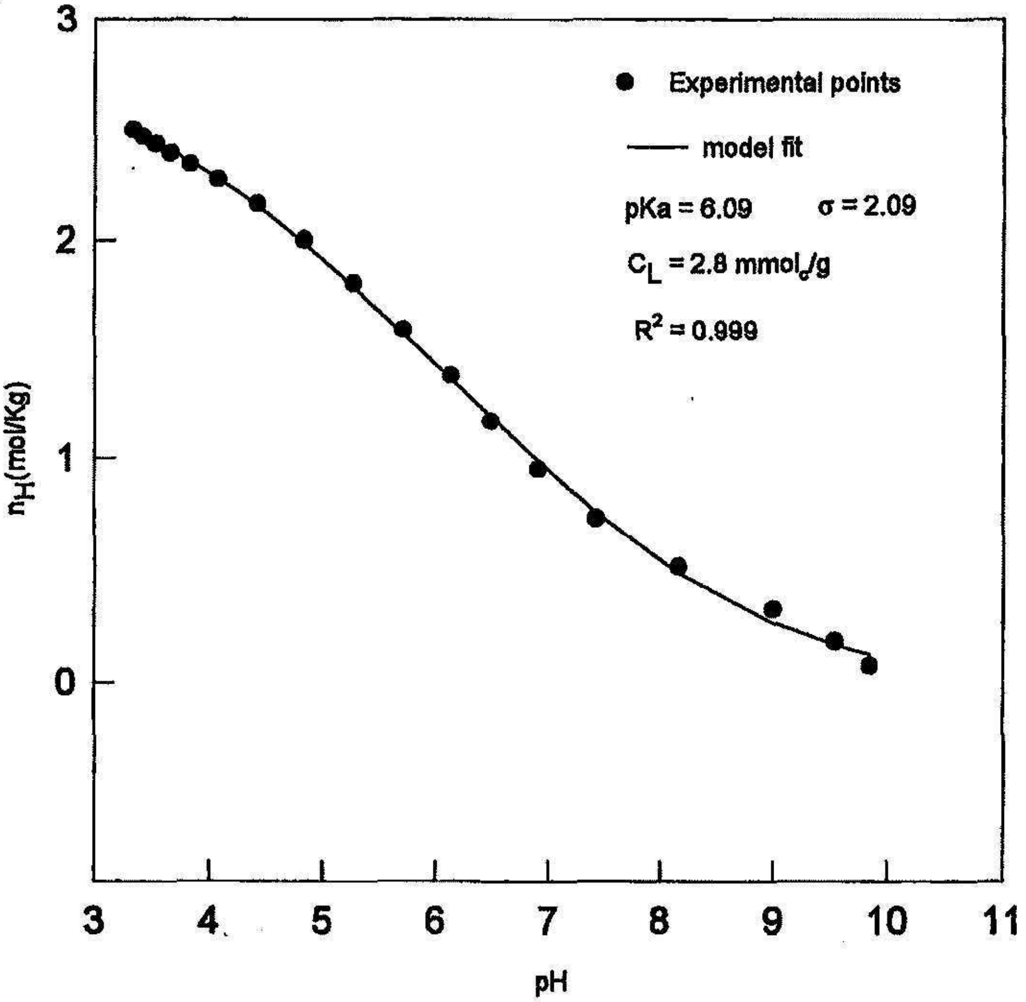

FIGURE 4. Gaussian distribution model description of the sewage sludge humic acid proton affinity curve.

similar to that of a carboxyl-type group (pKa 4 to 8) are relevant under the pH range utilized in our conditions (pH 3 to 10). Reports on applications of this model to other humic substances are limited exclusively to its bimodal version; that is, two binding classes are assumed to describe the experimental data set (Perdue and Lytle, 1983; Manunza et al., 1992; Manunza et al., 1995). It is uncertain whether this is simply due to a perceived need for conforming to the accepted model of humic acids as being a mixture of carboxyl- and phenolic-type functional groups, or whether the one-site model actually could not describe the experimental data. In any case, if we compare the results of a bimodal description of our experimental data with others (Figure 5, Table 3), it seems that the functional groups that comprise our humic acid exhibit a narrower range in reactivities than those reported by others. That is,

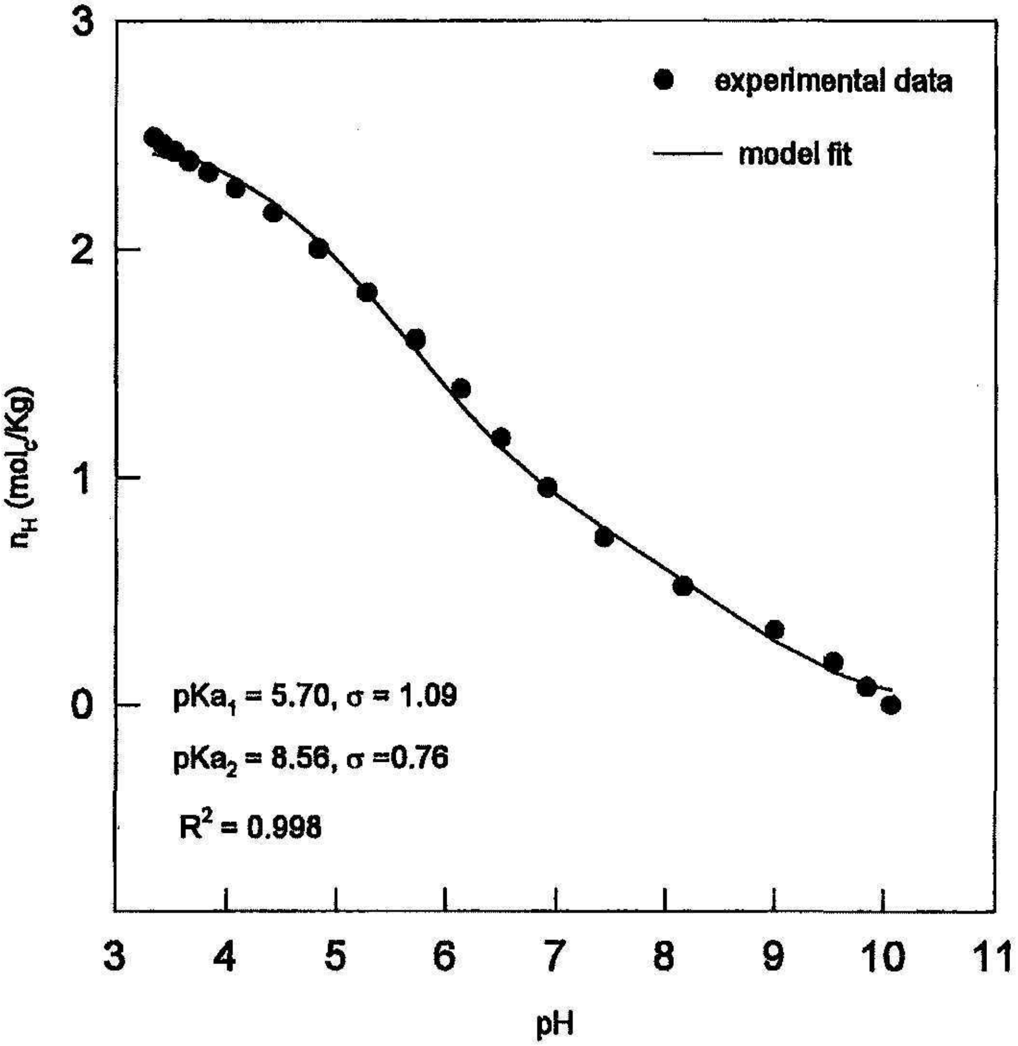

FIGURE 5. Gaussian distribution model (bimodal version) description of the sewage sludge humic acid proton affinity curve.

instead of being composed of similar fractions of carboxyl (pKa 3 to 7) and phenolic-type (pKa 7 to 12) groups (which would lead to two very distinct pKa values), our humic acid might be composed of a mixture of functional groups with carboxyl-type reactivities, e.g., carboxyl, amides (pKa 3 to 9). This would agree with our elemental composition data (i.e., low oxygen content) and would allude to a humic acid with a reactivity significantly different from those obtained from soil. However, we must emphasize that establishing the nature of the functional groups based on indirect evidence such as this can be misleading.

As expected, the values obtained for the conditional dissociation constant decrease as the ionic strength increases (Table 4). However,

TABLE 3.—*Proton affinity constants of humic acids obtained from different sources. Experimental parameters determined with the Gaussian distribution model*

Sample	pKa_1	σ_1	pKa_2	σ_2
SSHA[1]	5.70	1.09	8.56	0.76
WO[2]	4.04	1.06		
SO[2]	3.69	1.46		
SHA[3]	4.50	0.98	10.89	3.31
AHA[4]	3.62	2.31	12.3	1.68

[1] Sewage sludge humic acid of present study.

[2] Humic acid extracted from worm compost (WO), and sewage sludge (SO). Data obtained from Manunza et al., 1992. The authors were unable to determine the binding parameters for the second class of functional groups because of the unreliability of their experimental values in the highest pH region (> 10.5).

[3] Humic acid extracted from soil (Manunza et al., 1995).

[4] Aquatic humic acid (Perdue and Lytle, 1983).

contrary to what was observed with synthetic polymers (Martínez et al., 1998a), there is no noticeable effect of ionic strength on the variance parameter (σ) that accounts for the overall heterogeneity of the system. This observation suggests that, as indicated by its high polydispersity (Table 1), intrinsic structural heterogeneity is the primary contributor to this parameter.

Eu^{3+} luminescence studies

Europium has been used for decades as a surrogate of calcium in biological studies. In recent years, its luminescence properties have turned it into an ideal molecular probe for environmental studies (Lee

TABLE 4.—*Application of the Gaussian Distribution model to the potentiometric titration data obtained with humic acid extracted from sewage sludge.*

I.S. (M)	pK_a	σ	C_L (mmol$_c$g^{-1})	R^2
0.01	6.48	2.12	2.75	0.997
0.01	6.38	1.99	2.50	0.997
0.01	6.47	1.98	2.60	0.997
0.05	6.09	2.09	2.78	0.999
0.05	5.97	2.11	2.60	0.999
0.05	6.05	2.34	2.87	0.998
0.1	6.03	1.82	2.60	0.999
0.1	5.96	1.94	2.36	0.999
0.1	5.56	2.53	3.20	0.999

et al., 1997). In this study, we employed Eu^{3+} luminescence to determine the binding strength of Eu^{3+} in the sludge humic acid (Susetyo and Carreira, 1990). The log K_{Eu} values obtained (Figure 6) compare relatively well with those reported by Dobbs et al. (1989) for Suwannee River fulvic acid (5.2 vs. 4.5, respectively). In addition, the results are similar to those obtained with polyacrylic acid {$[CH_2CH(CO_2H)-]_n$-PAA}, (5.8), all of which probably reflects the strong carboxylic character of the humic material (Martínez et al., 1998b).

Competitive metal study

A preliminary study was conducted to determine the effects of metal competition on the spectroscopic behavior of Eu^{3+} in the humic acid. As explained in detail in a previous paper (Martínez et al., 1998b), the Eu^{3+}

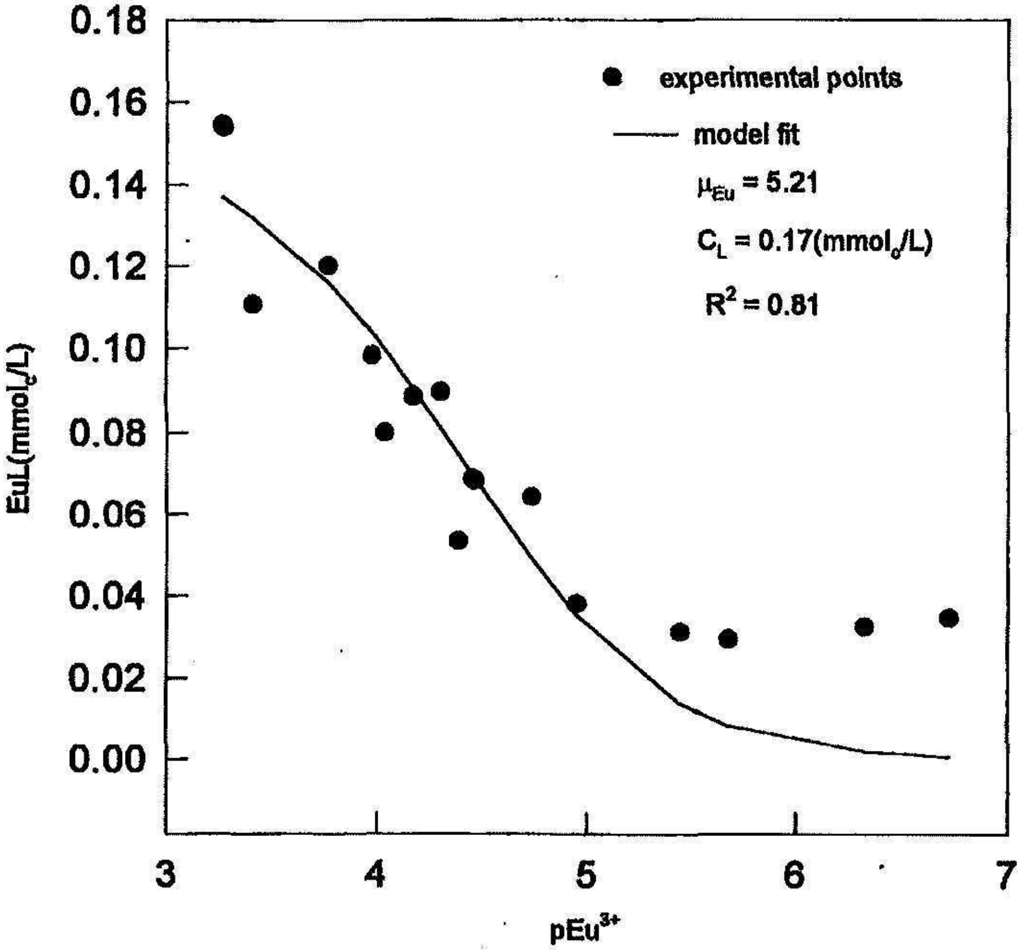

FIGURE 6. Empirical description (Gaussian distribution model) of the Eu^{3+} - humic acid binding isotherm.

luminescence technique allows an assessment of the amount of free and complexed Eu in a system by means of the ratio of two emission bands at 592 and 616 nm. Briefly, the 616 nm emission band is a hypersensitive transition, or a transition that is more intense when an ion (in this case Eu^{3+}) is complexed by ligands other than water. The 592 nm line represents a nonhypersensitive transition ($^7F_0$-$^5D_0$), and thus its intensity is insensitive to the environment surrounding the ion. As the amount of metal complexed by a ligand increases, the ratio of the

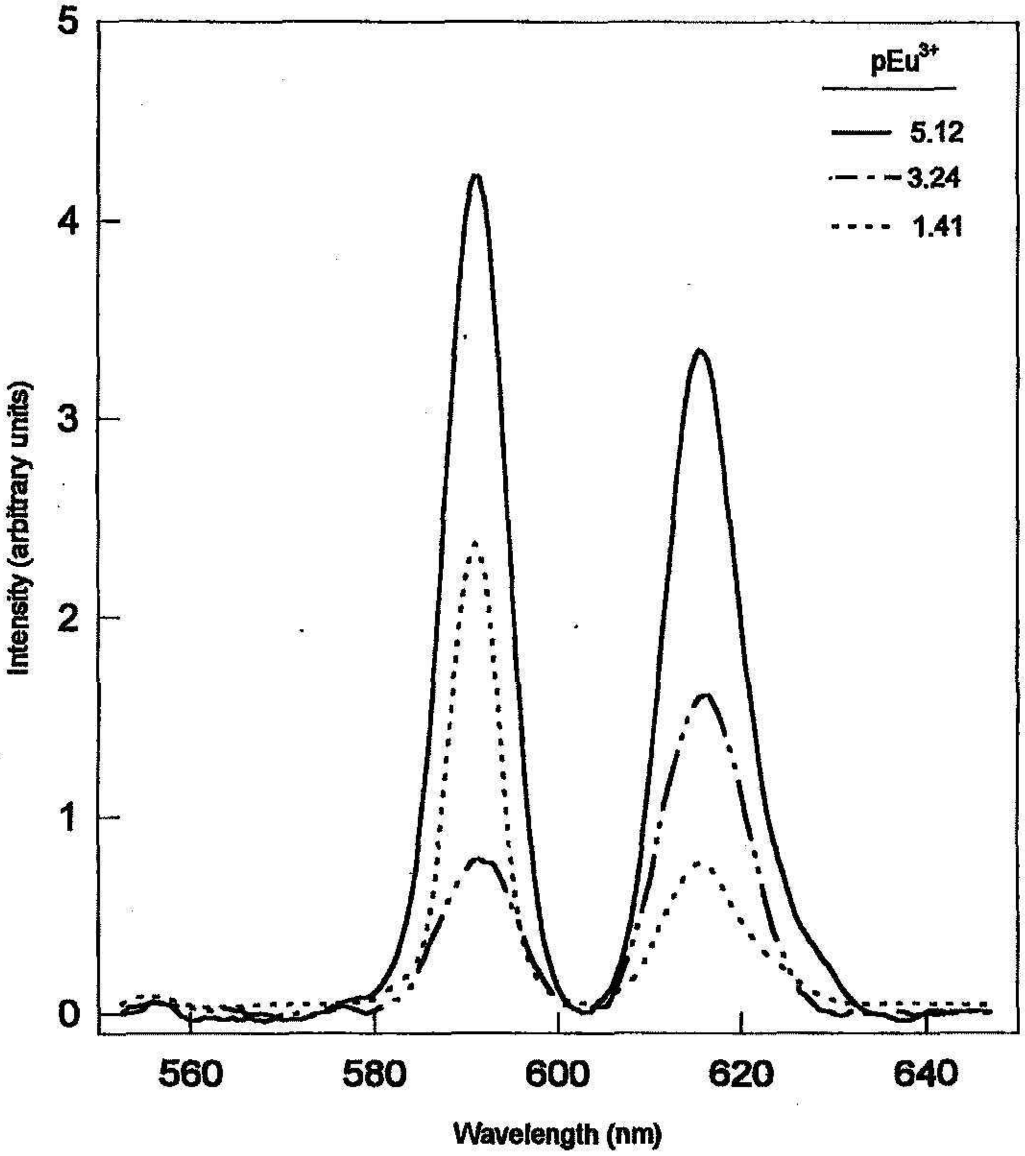

FIGURE 7. Effect of total Eu^{3+} concentration on the Eu - polyacrylic acid (PAA) spectra (PAA = 1e-5M).

592:616 bands decreases; and as the amount of free metal increases so does the ratio of the two bands (Figure 7).

We evaluated the effect of additions of different levels of Cu^{2+} and Pb^{2+} on the ratio of the two emission bands. Three levels of Cu^{2+} and four levels of Pb^{2+} were added to a system containing 0.195 μM Eu^{3+} (Figure 8). Both metals compete effectively for the ligand sites in the humic acid, as indicated by an increase in the I_{594}:I_{616} ratio. This finding once again confirms the strong binding capacity of these compounds for different trace metals and affirms their role in contaminant transport and distribution in the environment.

CONCLUSIONS

The composition of humic substances obtained from sewage sludge can be significantly different from that of their soil counterparts. In our

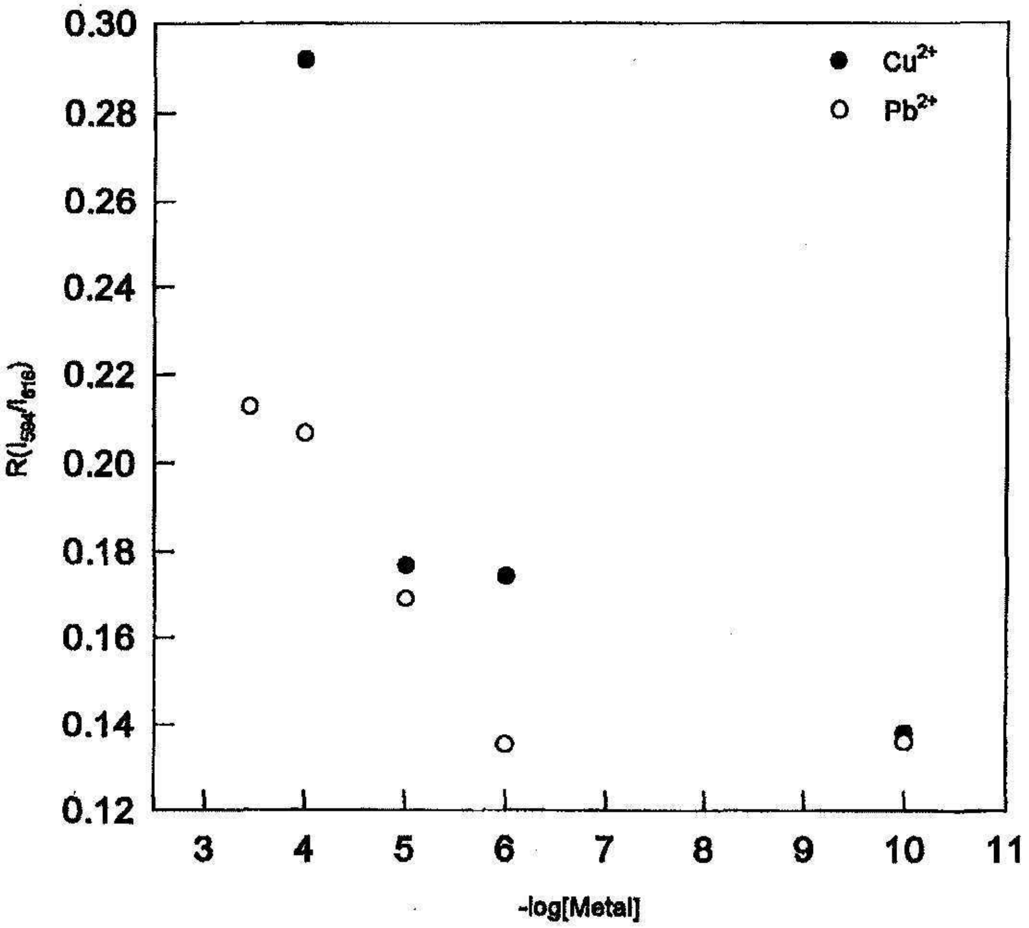

FIGURE 8. Metal competition effects on the complexation of Eu^{3+} by a sewage sludge humic acid.

case, higher nitrogen and sulfur contents as well as lower oxygen levels were observed in a humic acid extracted from sludge. This finding suggests important differences in terms of functional group composition and reactivities, which need to be evaluated in order to describe contaminant transport in sludge amended soils.

Proton affinity curves obtained at different ionic strengths were described by two empirical models to gain some insight into the reactivity of our humic acid. The models selected represent contrasting philosophies on the mathematical portrayal of the reactivity of humic substances in the environment. Each model was capable of describing the experimental data appropriately. However, the number of fitting parameters required, as well as their chemical implications, differed greatly. A determination of the Eu^{3+} binding constant yielded values similar to those obtained for polyacrylic acid in a separate study. This finding, together with the proton titration data and the elemental composition results, suggests that the majority of functional groups in this humic acid exhibit carboxyl-like behavior. This suggestion contrasts to what is generally believed for soil humic substances whose reactivities are thought to be governed by an equal population of carboxyl and phenolic type groups.

Land application represents one of the most feasible alternatives for the adequate disposal of sewage sludge. However, to gain full advantage of all the benefits that this practice offers, it is important to further evaluate the mechanisms controlling contaminant fate and bioavailability in sludge amended systems.

LITERATURE CITED

Alpert, J. E. and Y. Soto-López, 1997. Privatizing co-composting facilities in Puerto Rico. *Biocycle.* June issue. pp. 66-70.

Cernik, M., M. Borkovec and J. C. Westall, 1995. Regularized least-squares methods for the calculation of discrete and continuous affinity distributions for heterogeneous sorbents. *Environ. Sci. Technol.* 29:13-425.

Chin, Y. P., G. Aiken and E. O'Loughlin, 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. *Environ. Sci. Technol.* 28 (1):1853-1858.

Deiana, S., C. Gessa, B. Manunza, R. Rausa and R. Seeber, 1990. Analytical and spectroscopic characterization of humic acids extracted from sewage sludge, manure, and worm compost. *Soil Sci.* 150(1):419-424.

Dobbs, J. C., W. Susetyo and L. A. Carreira, 1989. Competitive binding of protons and metal ions in humic substances by lanthanide ion probe spectroscopy. *Anal. Chem.* 61:1519-1524.

Holtzclaw, K. M., G. Sposito and G. R. Bradford, 1996. Analytical properties of the soluble metal complexing fractions in sludge-soil mixtures. I. Extraction and purification of fulvic acid. *Soil Sci. Soc. Am. J.* 40:254-258.

Lee, B. H., K. H. Chung, H. S. Shin, Y. J. Park and H. Moon, 1997. Europium (III) complexes of polyfunctional carboxylic acids: Luminescence probes of possible binding sites in fulvic acid. *J. Colloid Interface Sci.* 188:439-443.

Magdoff, F. R., M. A. Tabatabai and E. A. Hanlon, Jr., eds, 1996. Soil Organic Matter: Analysis and Interpretation. SSSA Special Publication No. 46. Madison, Wisconsin, USA.

Manunza, B., S. Deinana, V. Maddau, C. Gessa and R. Seeber, 1995. Stability constants of metal-humate complexes: titration data analyzed by bimodal gaussian distribution. *Soil Sci. Soc. Am. J.* 59:1570-1574.

Manunza, B., C. Gessa, S. Deinana and R. Rausa, 1992. A normal distribution model for the titration curves of humic acids. *J. Soil Sci.* 43:127-131.

Martínez, G. A., S. J. Traina and T. J. Logan, 1998a. Characterization of proton affinity reactions in polyelectrolytes: discrete vs. continuous distribution of sites approach. *J. Colloid Interface Sci.* 199:53-62.

Martínez, G. A., S. J. Traina and T. J. Logan, 1998b. Characterization of metal complexation in multiligand systems with lanthanide spectroscopy. *J. Colloid Interface Sci.* 204:33-44.

McBride, M. B., 1995. Toxic metal accumulation from agricultural use of sludge: Are USEPA regulations protective? *J. Environ. Qual.* 24:5-18.

Perdue, E. M. and C. R. Lytle, 1983. Distribution model for binding protons and metal ions by humic substances. *Environ. Sci. Technol.* 17:654-660.

Schnitzer, M. and S. U. Khan, 1972. Humic substances in the environment. Marcel Dekker, Inc., N.Y.

Schnitzer, M. and P. Schuppli, 1989. The extraction of organic matter from selected soils and particle size fractions with 0.5M NaOH and 0.1M $Na_4P_2O_7$, solutions. *Canadian J. Soil Sci.* 69:253-262.

Shevchenko, S. M. and G. W. Bailey, 1996. Life after death: lignin-humic relationships reexamined. *Critical Rev. in Environ. Sci. Technol.* 26:95-153.

Sposito, G. and P. Blaser, 1992. Revised quasiparticle model of protonation and metal complexation reactions. *Soil Sci. Soc. Am. J.* 56:1095-1099.

Sposito, G., K. W. Holtzclaw and D. A. Keech, 1977. Proton binding in fulvic acid extracted from sewage sludge-soil mixture. *Soil Sci. Soc. Am. J.* 41:1119-1125.

Sposito, G. and K. W. Holtzclaw, 1977. Titration studies on the polynuclear polyacidic nature of fulvic acid extracted from sewage sludge soil mixtures. *Soil Sci. Soc. Am. J.* 41:330-336.

Susetyo, W., J. C. Dobbs and L. A. Carreira, 1990. Development of a statistical model for metal-humic interactions. *Anal. Chem.* 62:1215-1221.

Wang, M. J., S. P. McGrath and K. C. Jones, 1995. Chlorobenzenes in field soil with a history of multiple sewage sludge applications. *Environ. Sci. Technol.* 29:356-362.

Wild, S. R. and K. C. Jones, 1992. Polynuclear aromatic hydrocarbon uptake by carrots grown in sludge-amended soil. *J. Environ. Qual.* 21:217-225.