

SELECTIVE REMOVAL OF TOXIC HEAVY METALS
FROM CONTAMINATED WATER SUPPLIES USING
IMMOBILIZED HUMIC SUBSTANCES

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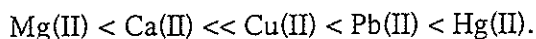
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ABSTRACT

The investigation of the competitive binding of metal ions to a biogenic material is described. The material was comprised of organic peat immobilized in a polysilicate matrix and packed into a 5.0 mL bed-volume column. Two separate mixtures of metal ions were studied regarding the concentration of each metal in the effluent as a function of the volume of influent introduced to the material. These mixtures contained 0.157 mM Ca(II), Mg(II), and Cu(II) and 0.05 mM Cu(II), Hg(II), and Pb(II), respectively. A general order of binding affinities was determined to be



Investigations of binding affinities and capacities for Pb(II) binding to this material indicated the presence of two separate types of interactions. The first, a low-affinity site with a mean affinity of ~ 501 and a maximum capacity of $73 \mu\text{mol/gram}$ sorbent was attributed to an electrostatic-driven ion-exchange process. The second distribution of binding sites, with a mean affinity of $56,000$ (capacity of $50 \mu\text{mol/gram}$ sorbent) was proposed to involve the formation of a metal-ligand complex between the Pb(II) and the immobilized humic material.

Key Words: Humic Acids, Chemical Treatment, Biomass, Chelating Agents, Heavy Metals

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JUSTIFICATION OF WORK PERFORMED

There has been increasing public attention focused upon the pollution of municipal and industrial water supplies (Peavy et al. 1985; Federal Register 1975). Prior waste disposal practices have caused serious contamination of resources (Darnall, et.al 1989). Heavy metal pollution of waters from landfills and abandoned mines challenge current remediation technologies (United States Department of the Interior 1988). It has become necessary for several industries and municipalities to install treatment facilities to remove heavy metals from waste streams prior to their discharge into the environment. The chemical and energy costs associated with the various components of treatment processes currently available can be substantial, making innovative, low-cost remediation processes desirable. One possible source of potential inexpensive sorbents for the removal of toxic heavy metals is biologically generated materials, that is, biomaterials.

The accumulation of environmentally significant inorganic metal ions from aqueous solution by a variety of microorganisms including algae, bacteria, fungi and yeast has been investigated for heavy metal reclamation and waste remediation. (Greene and Darnall 1986; Zhou and Kiff 1991; Mitani, et al. 1992; Scott 1992; Nakajima and Sakaguchi 1993). There are two distinct approaches to the application of such materials: the use of living organisms (Greene, et al. 1986a). and the use of non-living (nonviable) materials (Volesky 1990b; Darnall 1991; Greene, et al. 1986b). The mechanisms responsible for metal ion binding to living cells has been reported to occur through surface adsorption and through various types of intracellular accumulation or sequestering processes (Volesky 1990b). The limited success of this approach has been reported to be the result of the death and subsequent microbial decay of the biomaterial after exposing the organisms to a "critical" concentration of metal ions (Volesky 1990a). Alternatively, metal ion binding by nonliving cells has been proposed to occur exclusively through surface adsorption, independent of active metabolic processes (Darnall, et al. 1986b). Methods employing nonliving biomaterials represent a more viable approach because preservation of cell growth and maintenance of metabolic processes under adverse conditions is not required.

The term biosorption has been applied to the use of dead organisms for the "passive" absorptive binding of metals in aqueous solution (Volesky 1990b). This occurs through interaction of the metal ions with functional groups found on or in the cell wall biopolymers

(Darnall 1991; Greene, et al. 1986b). Metal uptake by different parts of the biosorbent cell chemical functionalities, depending upon the biomaterial, can occur through any number of mechanisms including complexation, (Darnall, et al. 1988; Watkins, et al. 1987). coordination, (Greene, et al. 1986a). chelation, (Darnall 1991). ion exchange, (Watkins, et al. 1987; Greene and Darnall 1988; Mahan and Holcombe 1992a). adsorption, (e.g., Sakaguchi and Nakajima 1987). and inorganic microprecipitation processes (Volesky 1990b). Others (Watkins et al. 1987). report, for metal binding to the green algae *Chlorella vulgaris*, that biosorption occurs through both complexation and ion exchange processes. They proposed the replacement of a ligand bound to the metal by a ligating moiety from the biomass and the interaction of the charged metal complexes with charged or polar functional groups on the surface of the biosorbent. This diversity of possible mechanisms necessitates the evaluation of various biomaterials regarding their ability to bind representative heavy metal ions.

This study's main objective was to develop the use of immobilized organic peat, an inexpensive material, for the removal of heavy metal ions from contaminated waters. Only a few studies had been previously undertaken to ascertain the metal-binding properties specifically of immobilized humics. Organic peat has been indicated to preferentially adsorb some heavy metal ions even in the presence of the common cations and anions present in hard water. Sodium, calcium and magnesium ions commonly present in hard water do not seem to interfere significantly with its adsorption properties and capacity for copper and lead ions. The identification of this material was the result of earlier studies directed, primarily, toward screening metal-binding capabilities of numerous biologically-derived materials. In preliminary studies, organic peat immobilized in a polysilicate matrix (i.e., a sol gel) demonstrated favorable binding characteristics for ions such as lead and copper. The material's low cost clearly justified a more careful and detailed evaluation of this biogenic material's binding characteristics.

REVIEW OF METHODS USED

Immobilization Procedure

Inherent problems pertain to the use of biomaterials for industrial process and wastewater remediation. In their native state, they frequently suffer from poor mechanical strength, low density, and small particle size (Brierley 1990). These limitations can be problematic with

regard to the design of systems capable of performing large-scale metal extractions. Strategies to overcome these limitations often involve the chemical transformation through the immobilization or “entrapment” of the biomaterial in an inert polymer. The primary justification of an immobilization scheme includes: using the biomaterial in fixed column, batch, or fluidized bed reactors; enhancing the stability of the biomaterial; and improving the sorbent’s metal adsorption characteristics. One approach used to achieve this objective is the cross-linking of polymers to create rigid pores to promote better metal loading and elution (Holbein 1990).

Several immobilization schemes have been reported. These include the use of silica-based (Darnall, et al. 1989; Darnall et al. 1986b; Mahan and Holcombe 1992b), calcium alginate-based (Nakajima et al. 1982), polyacrylamide-based polymers (Darnall, et al. 1986b; Nakajima, et al. 1982), and the use of porous polysulfone beads (Ferguson, et al. 1989; 1991). Each of these materials was reported to exhibit superior mechanical and physical properties with enhanced metal binding capabilities compared to the native material. In each study, supportive evidence indicated the increases in metal binding were not the result of metal binding to the polymeric material but to improvements in the biosorbents, for example, the porosity, chemical resistivity, and structural integrity of the materials.

Chemical transformations or immobilization techniques are generally performed to improve the material characteristics of the biomaterial to enhance the porosity, chemical resistivity, and structural integrity of the material. The immobilization procedure used throughout this work is based upon the method described elsewhere (Darnall et al. 1991). for the encapsulation of various biomaterials within a polysilicate matrix for the removal of target contaminants in aqueous waste streams. A principle advantage of using a silica-based immobilization scheme is that the final product is a recyclable material which is robust and relatively resistant to chemical attack under acidic conditions.

The humic materials were immobilized within this polysilicate matrix by the following procedure. The oven-dried material was ground and sieved to obtain a sample of a 100/200 mesh fraction of the material. A 5% (v/v) sulfuric acid solution was adjusted to pH = 2.0 by the slow addition of a 6% (w/v) solution of sodium silicate pentahydrate (Fisher Scientific #S407-3). The biomass was then added to this solution. The mixture was allowed to equilibrate for approximately one hour to enable the complete coverage of the biomass by the silicate monomer.

The solution's pH was then adjusted to pH 7.0 by adding the 6% Na₂SiO₄ solution. Under these conditions, a gel rapidly formed.

The resulting biomass-containing silica gel subsequently was washed several times to remove excess sulfate. After approximately eight washings, the supernatant was tested with 2-3 drops of a 1.0 M BaCl₂ (technical grade or better) solution in approximately 5 milliliters of the supernatant. The lack of a BaSO₄ precipitate indicated excess sulfate had been adequately removed. After being baked in an oven at 80 °C, the immobilized biomaterial was then ground and sieved to the desired particle size. For these investigations a 40/60 M fraction was used.

Humic Material

The organic peat used in these studies was obtained from a peat bog located in Salida, Colorado (Paul Brinkerhoff, Salida, CO). It is a black, organic soil with few rocks and grasses, with only a moderate mineral content, as described by the supplier. However, it contained a significant amount of iron that was removed by washing the sifted material in a 1.0 M HNO₃ solution for approximately eight hours. The remaining native minerals within the material were found to be calcium and magnesium.

Chromatographic Probe

Two models have been proposed for the metal binding process on algal biomass: adsorption processes (including complexation, coordination, chelation) and ion exchange processes. In the adsorption process, a metal becomes bound to one of several unoccupied sites with no additional changes at that site, while in the ion exchange process, a metal displaces another ion. Previous batch studies of *D. innoxia* cell material by using Pb²⁺ metal ion indicated that the combination of these two processes occur concurrently when the metal concentration was high. Conversely, ion exchange processes were determined in these studies to dominate when the metal concentration was low. It is very useful to determine experimentally whether the fundamental process is one primarily of adsorption or ion exchange for different metal ions. Some conclusions of the structural effects on the binding may then be drawn.

The typical approach used in biomaterial metal complexation studies is to systematically vary the metal to biomaterial ratio. Laboratory batch and titration techniques are most frequently used (Buffle, et al. 1990). Basically, in the batch study the biomaterial of a constant mass concentration, {S_i}, is contacted with a metal containing solution of

known initial pH, ionic strength and concentration, C_M . The system is allowed adequate time for equilibration, then the supernatant is analyzed for the concentration of free metal ion, $[M]$. The difference between initial and final concentration of free metal ion is attributed to metal sorption, $[M]_b$, by the biomaterial (Stark 1994). By this method, the binding isotherm is the only possible experimental datum used to derive binding constants and capacities to be achieved.

Similarly, in the titration technique, a titration curve is first obtained by measuring the molar concentration of the unbound cation, $[M]$, for various distinct states of the system expressed by the ratio, $C_M/\{S_t\}$. Then by defining the total bound metal concentration as $[M]_b = C_M - [M]$, the titration curve can be transformed to the corresponding binding isotherm. Simplicity is the major advantage of these two methods. However, they each have two disadvantages: (1) Variations in the pH and ionic strength of the contact solution can occur during binding processes. It then becomes necessary to monitor these parameters and make the appropriate additions to control those parameters (2) The displacement of metal ions native to the biomaterial can significantly alter the composition of the contact solution. Marc et al. (1995) reported that uncontrolled variations in these parameters resulted in significantly impacting the amount of metal bound. Moreover, such unregulated variables affecting metal binding can greatly inhibit the applicability of any type of mathematical algorithm to describe that process. Therefore, an alternate experimental approach is required.

Affinity chromatography is a promising alternative technique for the studies of metal/biomaterial interactions. Affinity chromatography has been demonstrated to be very useful in the investigation and characterization of specific interactions between biomolecules (Ken-ichi and Yuko 1986 and Arnold, et al. 1986). Frontal analysis (FA) in affinity chromatography is advantageous from both theoretical and experimental viewpoints (Ken-ichi and Yuko 1986). For the analysis of frontal chromatography, a solution containing interacting substance(s) (namely, metal ion(s) for our system), M , is applied continuously at constant concentration, $[M]_o$, to a column packed with an adsorbent, the immobilized biomaterial in our case. The resultant elution profile, or the breakthrough curve, is composed of an elution front and a plateau. The elution volume of the front, V , or V_o , is measured, where V_o is dead volume. For determining binding isotherms by this technique,

the simplest model is assumed, that is, merely the equilibrium state or the extent of retardation is considered, not the shape of the elution front (Ken-ichi and Yuko 1986). Thus, the dynamic aspect of chromatography is neglected. The column is thought of as a cell for equilibrium dialysis. The chromatographic pattern is used only to determine the accurate amount of adsorbed target substance, which is equal to $[M]_o \cdot (V - V_o)$.

There are several advantages of frontal analysis over the traditional batch and titration techniques: (1) Concentration of free metal ion is constant and equal to $[M]_o$ once dynamic equilibrium is reached in the column (2) Effluent solution composition (e.g., pH and ionic strength) are known and are the same as the influent solution when the equilibrium is established. (3) Flame atomic absorption spectrometry (FAAS) can easily be interfaced to a column as a sensitive and selective detector for metal species. Detector calibration is unnecessary in FA. (4) Theoretical treatment is very simple. If only one binding site is present, the double reciprocal plot of the following equation can be used to determine the binding constant K_b , and binding capacity B_t :

$$\frac{1}{[M]_o (V - V_o)} = \frac{1}{K_b \cdot B_t} \frac{1}{[M]_o} + \frac{1}{B_t}$$

Disadvantages of frontal analysis include: (1) Large amounts of influent solution are needed to equilibrate the column when metal concentration is low. (2) Several folds of dilution factors occur when the column is interfaced to FAAS. The latter restricts the detection limit from going down to the concentration range in which we are interested. To overcome this drawback, a slight modification of frontal analysis has been made in our research.

Rather than monitoring the effluent concentration for the breakthrough curve, all of the effluent is collected in a single container. After the equilibrium in the column is established (i.e., the plateau region has been reached), the amount of metal bound under the influent condition can be obtained by taking the difference between the total metal amount in the influent and the effluent solutions. In this way, binding isotherms with wider concentrations can be constructed.

Once a set of raw experimental data has been obtained, a mathematical procedure for determining sound fundamental parameters, particularly the binding constants and capacities, is needed. For adsorption processes on the heterogeneous ligand system of the biomaterial, several models have been proposed. They include one-site models (Marinsky, et al. 1986; Bartschat, et al. 1992; Dzombak, et al. 1986). multi-discrete site models, and continuous site models (Susetyo, et al. 1990; Tipping 1993; Perdue and Lytle 1983). When the solution composition is held constant during the binding process, the ion exchange process can be mathematically treated in the same manner as an adsorption process.

One site models are based on the assumption that the biomaterial surface contains only one type of binding site. The differences among binding strength are ascribed to the different electrostatic interactions on the surface. While intrinsic binding constants can be extracted from experimental data by this model, the resulting constants are of limited use in predictions, because no general theory exists to enable calculation of the surface potential of the biomaterial.

The binding of a metal species, M, to a heterogeneous ligand system may be considered as the sum of the contributions of the different site types present. For one such site type i, the binding of M can be expressed as



where S_i is the free site and S_iM the complexed site. The corresponding binding (affinity) constant K_i may be defined as

$$K_i = \frac{\{S_iM\}}{\{S_i\}[M]} \quad (2)$$

where $[M]$ is the concentration of the M in solution and $\{S_iM\}$ and $\{S_i\}$ are the site concentrations of complexed and free sites, respectively. The fraction of sites of type i occupied by M, θ_i , equals

$$\theta_i = \frac{\{S_iM\}}{\{S_i\} + \{S_iM\}} \quad (3)$$

It follows from equation (2) and (3) that

$$\theta_i = \frac{K_i[M]}{1 + K_i[M]} \quad (4)$$

Equation (4) is called the local isotherm or local binding function and is equivalent to the well-known Langmuir equation. If we have m classes of independent sites, each class, i , having site capacity $S_{t,i}$ with a binding constant, K_i , then we can generalize Equation 4 and write

$$r = \sum_{i=1}^m \frac{S_{t,i} K_i [M]}{1 + K_i [M]} \quad (5)$$

where r is the total amount of metal bound. Starting from equation (5), we may write

$$\frac{1}{r} = \left[\sum_{i=1}^m \frac{S_{t,i} K_i [M]}{1 + K_i [M]} \right]^{-1} = \left[\sum_{i=1}^m \frac{S_{t,i} K_i}{\frac{1}{[M]} + K_i} \right]^{-1} \quad (6)$$

This equation is the basis of multiple, discrete-site models.

When the differences between the K_i , or rather the $\log K_i$, values become infinitesimal, the basic integral equation for continuous site models is obtained as follows:

$$\theta_r = \int \theta(K, [M]) f(\log K) d \log K \quad (7)$$

where $\theta(K, [M])$ is the local binding function and $f(\log K)$ is the distribution function which indicates the probability of finding sites with an affinity in the range $\log K + d \log K$. The above integral equation is a Fredholm of the first kind, which is difficult to solve numerically for $f(\log K)$. Several kinds of approximation methods have been proposed (Stark 1994; Marc, et al. 1995). Among them, local isotherm approximation (LIA) methods have been proven to be better methods, especially the condensation approximation (CA) and logarithmic symmetrical approximation (LOGA). These two algorithms have been applied to the present system.

DISCUSSION OF RESULTS AND THEIR SIGNIFICANCE

Competitive Metal-Ion Binding Studies

Studies were performed using a column of immobilized organic peat (5.0 mL bed volume) with an influent containing equimolar concentrations of three different metals. The metals were each selected for their environmental significance, practical utility, and varying Lewis acidity. These studies involved two different three-metal influent solutions. The first part of the study concerned the binding by immobilized Colorado organic peat of a .157 millimolar contacting solution containing Ca(II), Mg(II), and Cu(II) adjusted to pH 5.0. This particular matrix was chosen to evaluate those chemical functionalities possessing hard to intermediate

Lewis acid character. The impact of alkaline earth metals on the overall copper binding ability of this material also was investigated.

The second part of the study similarly examined the simultaneous binding by this same material of 0.05 millimolar contacting solution containing Cu(II), Hg(II), and Pb(II) also adjusted to an initial pH of 5.0. This particular matrix was chosen to evaluate and compare further the binding characteristics of immobilized Colorado peat to additional environmentally significant metal ions. Additional information pertinent to the quantity and nature of those chemical functionalities responsible for binding these metals of intermediate to hard Lewis acidity was sought.

The premise behind these experiments was that hard metals will preferentially bind to hard Lewis acid groups while the soft metal ions would exhibit greater affinity for those functionalities soft in Lewis acid character. With the inclusion of the intermediate Cu(II) ion to both matrices, the binding exhibited by groups possessing intermediate Lewis acid values should be occupied by these ions. The primary rationale in performing investigations in this fashion was that it allowed further evaluation of the binding behavior the material exhibits from which selectivity information could be obtained. Additionally, relative binding affinity as well as capacity data can be extracted. Combined with the pH behavior, this information can offer further insight regarding the metal-biosorbent interactions. Table 1 is a summary of the experimental conditions employed for each of the three-metal studies.

	<u>Study 1</u>	<u>Study 2</u>
Metals investigated	Ca, Mg, Cu	Cu, Hg, Pb
Influent concentration	0.157 mM	0.05 mM
Hydraulic flow	1/6 bvm (0.83 mL/min)	1/6 bvm (0.83 mL/min)
Biosorbent volume	5.0 mL	5.0 mL
Biosorbent mass	1.58 g	1.67 g
Influent pH	5.0	5.0
Total volume	7.5 L	19.04 L

Prior to performing the actual competitive binding studies it was necessary to independently evaluate the binding of each of the respective metals separately by the immobilized Colorado organic peat substrate. Table 2 lists the experimental conditions and results obtained from those baseline experiments.

Table 2. Results from Separate Column Experiments Towards Metals Under Investigation Performed Under Noncompetitive Conditions.

	<u>Ca</u>	<u>Mg</u>	<u>Cu</u>	<u>Hg</u>	<u>Pb</u>
Influent concentration (ppm)	10.	10.	10.	10.	10.
Hydraulic flow (bvm)	1/6	1/6	1/6	1/6	1/6
Biosorbent volume (mL)	5.	5.	5.	5.	5.
Biosorbent mass (g)	1.33	1.33	1.54	1.87	1.59
Breakthrough volume (bv)	160	48	480	1856	1568
Breakthrough volume (L)	0.80	0.24	2.40	9.28	7.84
Breakthrough binding (millimoles)	0.200	0.099	0.378	0.463	0.378
Total Binding (millimoles)	0.230	0.174	0.453	0.693	0.435
Effective binding capacity (mmole/g)	0.150	0.074	0.245	0.247	0.238
Apparent binding capacity (mmole/g)	0.173	0.131	0.294	0.371	0.274

Figure 1 shows the effective and apparent binding capacities resulting from these independently performed metal binding experiments. Immediately observable is a general trend for increased overall binding capacity as the Lewis acidity decreases for the respective metals under investigation. These studies indicated the chemical functionalities responsible for the observed copper binding prior to breakthrough (effective binding capacity) to be the same sites responsible for mercury and lead binding. Also evident from these data is that this material exhibits some propensity toward binding alkaline earth metals, but evidently not to the same

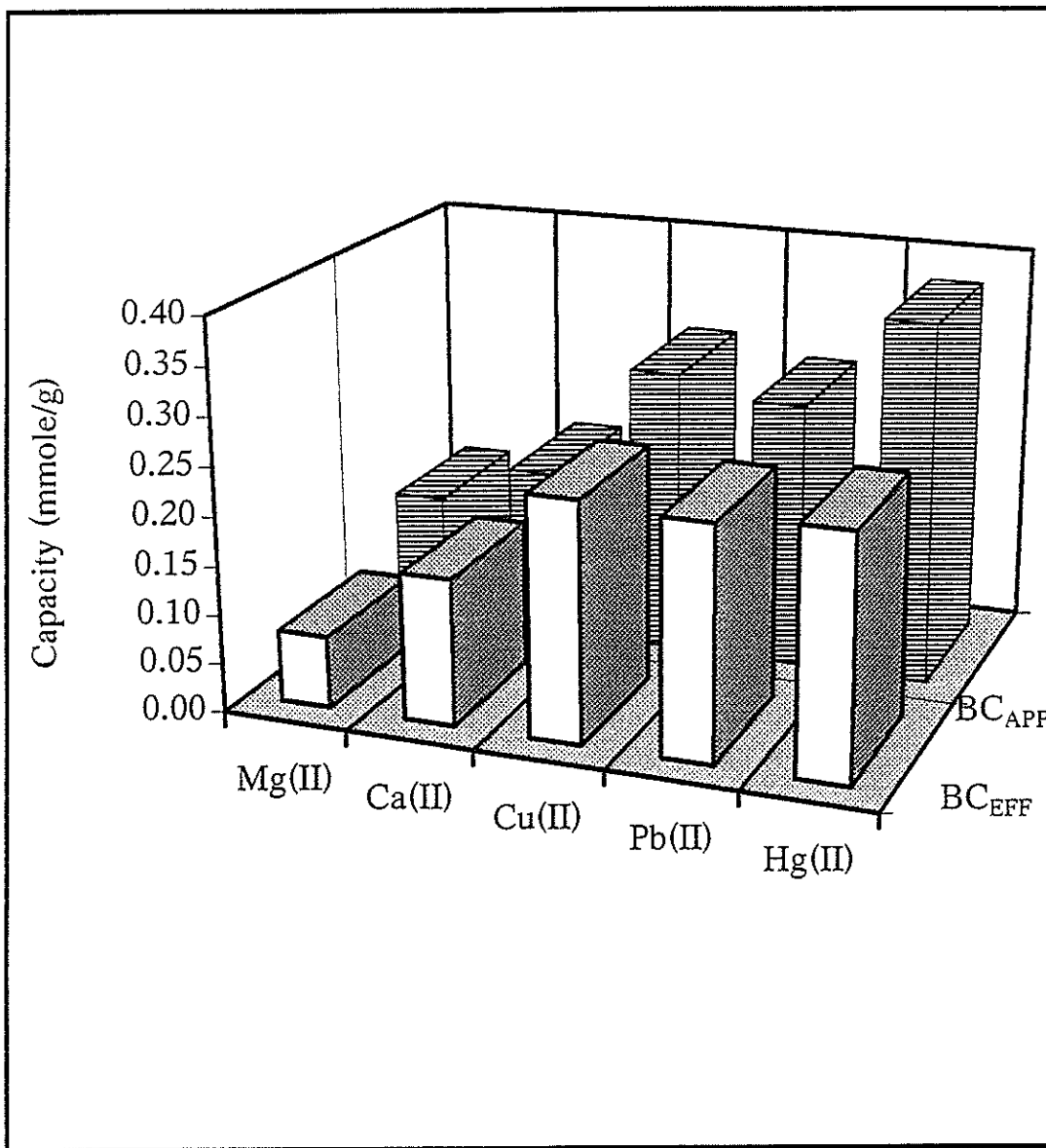


Figure 1. Effective and apparent binding capacities resulting from independent evaluation of these metals performed under noncompetitive conditions. (Experimental conditions as described in Table 2.)

degree as for the transition and heavy metals. What is not clear from these experiments is the relative binding strengths exhibited by those chemical functionalities present on the peat for these particular metals under investigation. By subsequently performing these equimolar competitive binding experiments, insight regarding the classification and characterization of the respective types of sites participating in the observed binding becomes available.

Figures 2 and 3 show the results from each of the three metal competitive binding studies. The data are presented in these plots as differential moles bound as a function of bed volumes of influent passed through the material. This presentation allows inspection of the extraction efficiency, in terms of the total number of moles of each of the respective metals, as the chemical functionalities present on the biomaterial become occupied. Also included in these plots is a series of data points representing the ratio of total moles of hydrogen ions released from the immobilized peat to the total number of metal ions bound by the biosorbent.

Figure 2 pertains to the first three-metal equilibrium study. Significant copper(II) binding was observed. Initial copper breakthrough occurred after approximately 464 bed volumes (2.320 L). Conversely, inspection of the calcium and magnesium curves in this figure indicated only initial binding by the peat of these metals. Breakthrough occurred rapidly in both cases with a subsequent loss of some of those bound calcium and magnesium ions indicated by negative values of the moles bound by each of these metals by the biomass. These negative values are interpreted as indicative of replacement by copper(II) ions at sites previously bound to calcium and magnesium ions. As the extraction of these three metals from the influent solution progresses, some binding by the immobilized peat to the calcium and magnesium ions can be observed. The mechanism responsible for this binding is apparently relatively inefficient (as evidenced by the low number of differential moles bound as compared to the influent concentration).

The most significant calcium and magnesium binding occurs after copper(II) breakthrough where competition by these three metals for the lower affinity sites on the peat-based biosorbent is greatest. Inspection of the released-proton to bound-metal ratio curve reveals this value initially starts out at two protons released for every metal bound but declines steadily until, by the end of the extraction, is seen to stabilize at one proton released per metal bound. Multiple mechanisms for the observed binding by this biomaterial toward these metals are certainly a possibility and apparently these data confirm this assertion, with initially an ion

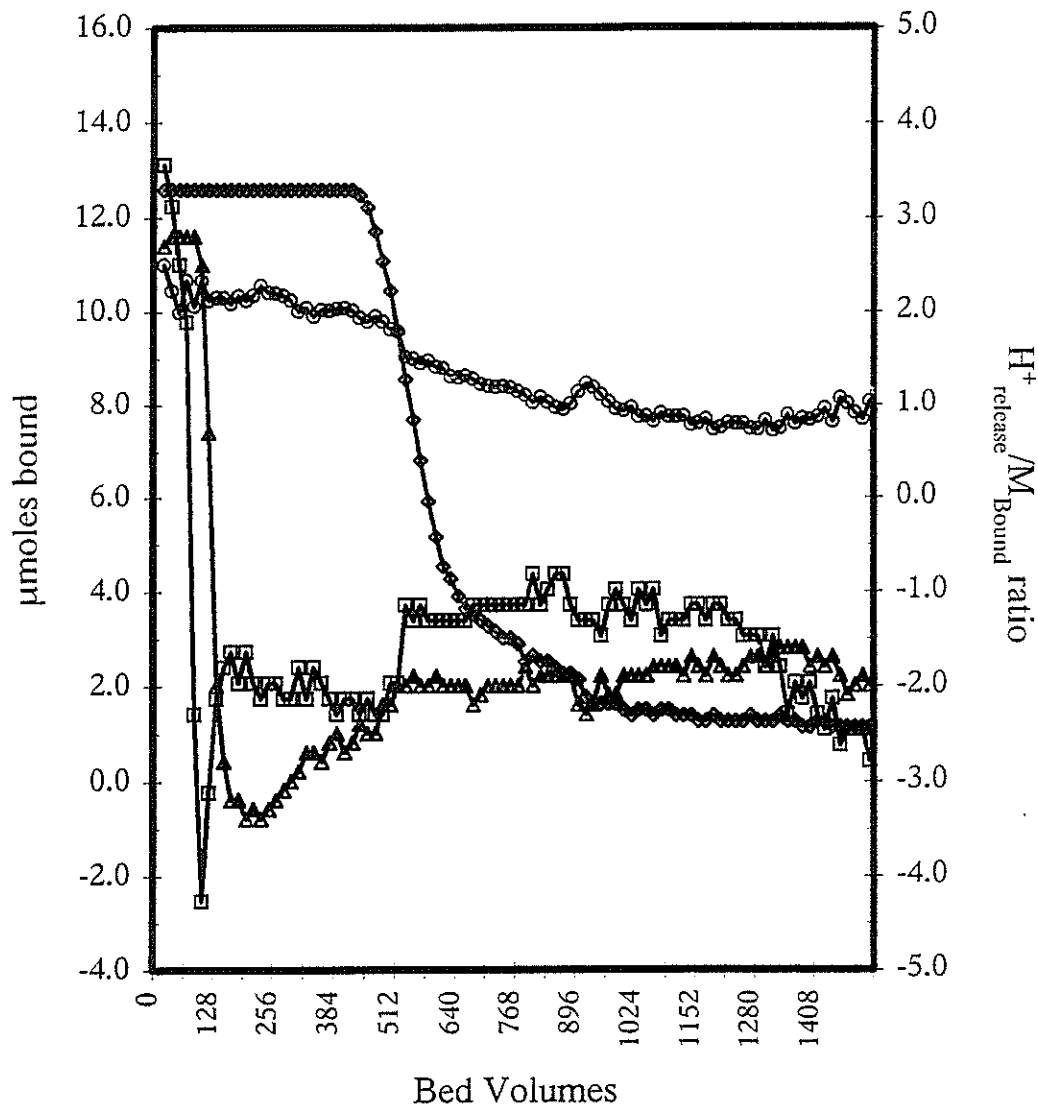


Figure 2. Differential moles bound and released proton to bound metal ratio (○) obtained from equimolar competitive binding to $\text{Ca}(\text{II})$ (Δ), $\text{Mg}(\text{II})$ (\square), $\text{Cu}(\text{II})$ (\diamond) by immobilized Colorado organic peat. Influent was 0.157 mM in each metal ion with the influent pH adjusted to 5.0; the hydraulic flow through the column was 1/6 bed volumes per minute (bvm).

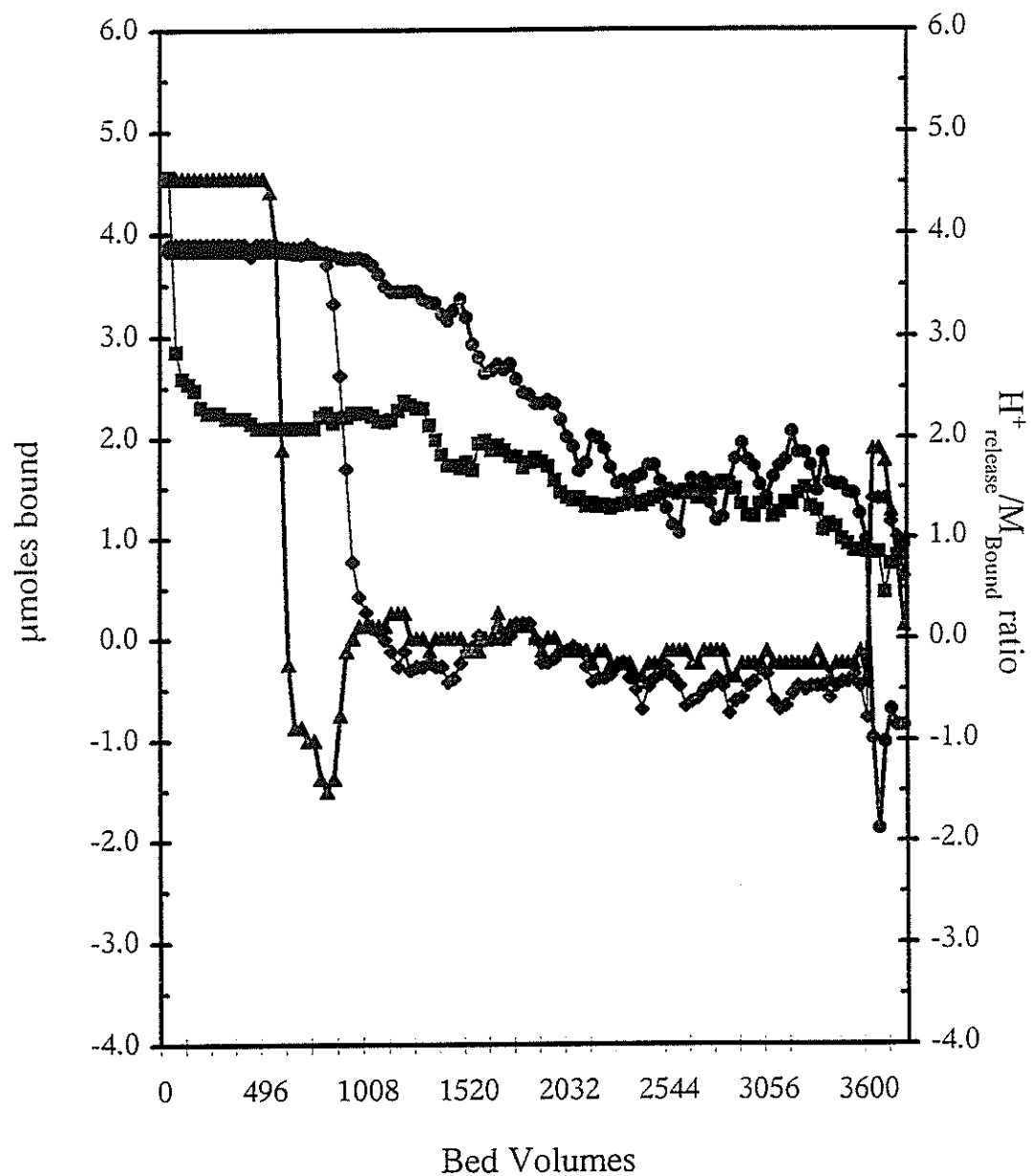


Figure 3. Differential moles bound and released proton to bound metal ratio (■) obtained from equimolar competitive binding to Cu(II) (Δ), Hg(II) (●), and Pb(II) (◆) by immobilized Colorado organic peat. Influent was 0.05 mM in each metal ion with the influent pH adjusted to 5.0; the hydraulic flow through the column was 1/6 bed volumes per minute.

exchange type mechanism responsible for the high affinity binding with the later binding due to a complexiometric based mechanism.

Figure 2 shows a similar presentation of the binding data as it relates to the second equimolar binding study. Recall that this study is concerned with the simultaneous binding by the immobilized organic peat to metals of Lewis acidity varying from intermediate to soft in character. Initially the data indicate significant binding by the immobilized peat to all three of the metals under investigation. The Cu(II) and Pb(II) ions breakthrough rather sharply after approximately 624 and 880 bed volumes (3.120 and 4.400 L), respectively, with Hg(II) breakthrough occurring at approximately 1136 bedvolumes (5.680 L) with a gradual tailing off of the observed Hg(II) binding occurring until the end of the extraction. This plot indicates a preponderance of the participating binding sites on the peat for the metals softer in Lewis acid character. Inspection of the copper binding curve in this figure reveals that after initial copper binding, there is subsequent replacement of a portion of these bound ions by the Pb(II) and Hg(II) ions. This is similar to the first equimolar study observations for the calcium and magnesium metals. Also, as in the first study, the released proton to bound metal ratio starts at an initial value of around two protons released per metal bound and gradually decreases until a value of one proton per bound metal is observed by the end of the experiment. Again this is interpreted as possibly indicating a dual mechanism of metal binding by this material, with initially ion exchange responsible for the high affinity (pre-breakthrough) binding followed by complexation by lower affinity sites.

Figures 4 and 5 offer an alternative representation of how the metal binding proceeded throughout each of the respective equimolar investigations. These figures show the cumulative binding (normalized to the total amount bound) of each metal under study by the immobilized peat as a function of treated influent volume. The depictions allow further evaluation as to where on the binding curve the most efficient binding occurs with respect to each of the metal ions studied. Changes in the slope of the individual curves indicate changes in the biosorbent binding behavior. A positive slope represents uptake by the biosorbent for a particular metal species whereas a negative slope indicates a release of previously bound metal.

Figure 4 shows data from the three-metal equimolar binding study one, where the metals of hard to intermediate Lewis acid character were investigated. At the onset of this particular

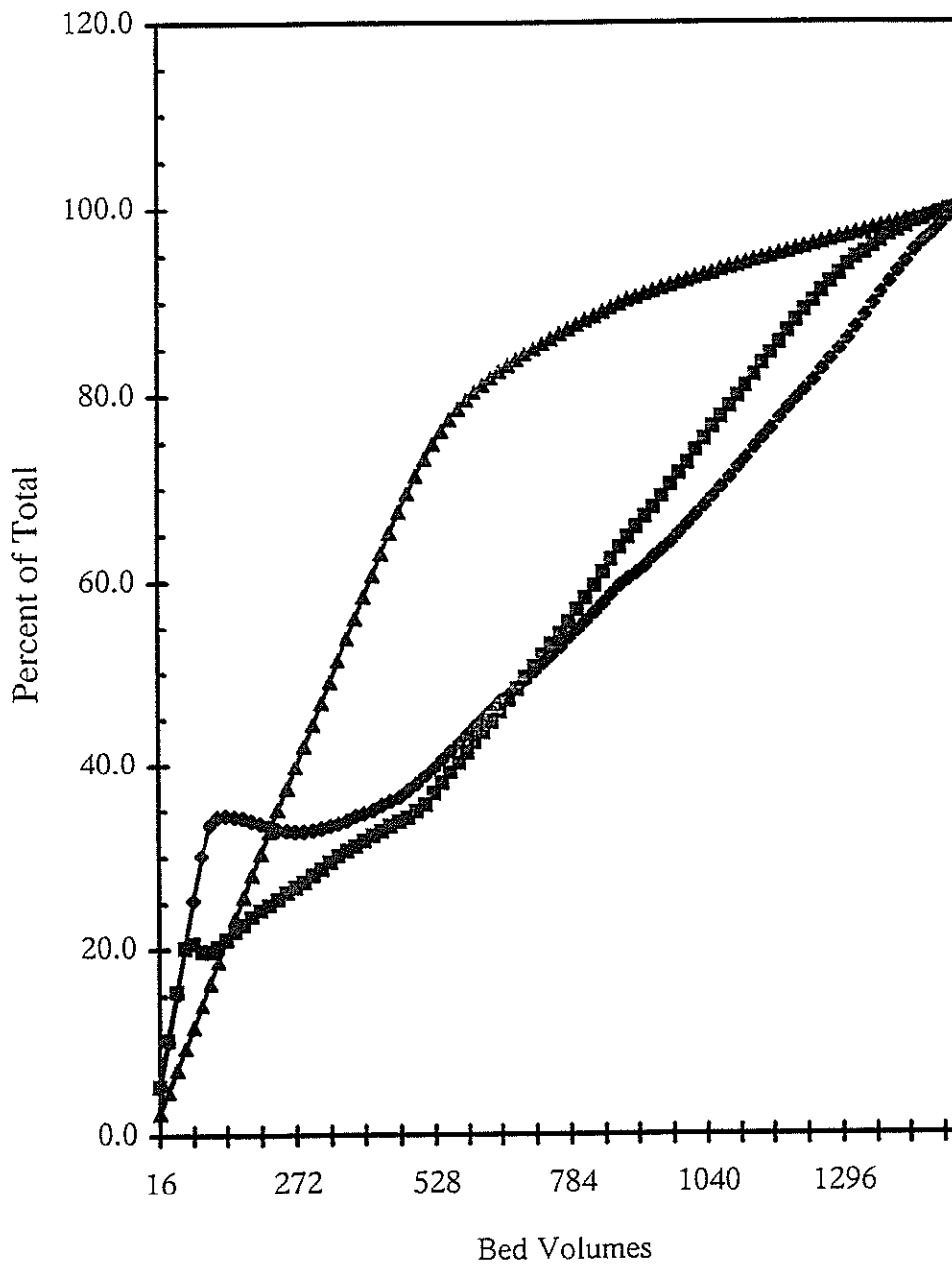


Figure 4. Cumulative moles bound as the percent of the total obtained from competitive binding to Ca(II) (◆), Mg(II) (□), and Cu(II) (△) by immobilized Colorado organic peat. Influent was 0.16 mM in each metal ion with the influent pH adjusted to 5.0; the hydraulic flow through the column was 1/6 bed volumes per minute.

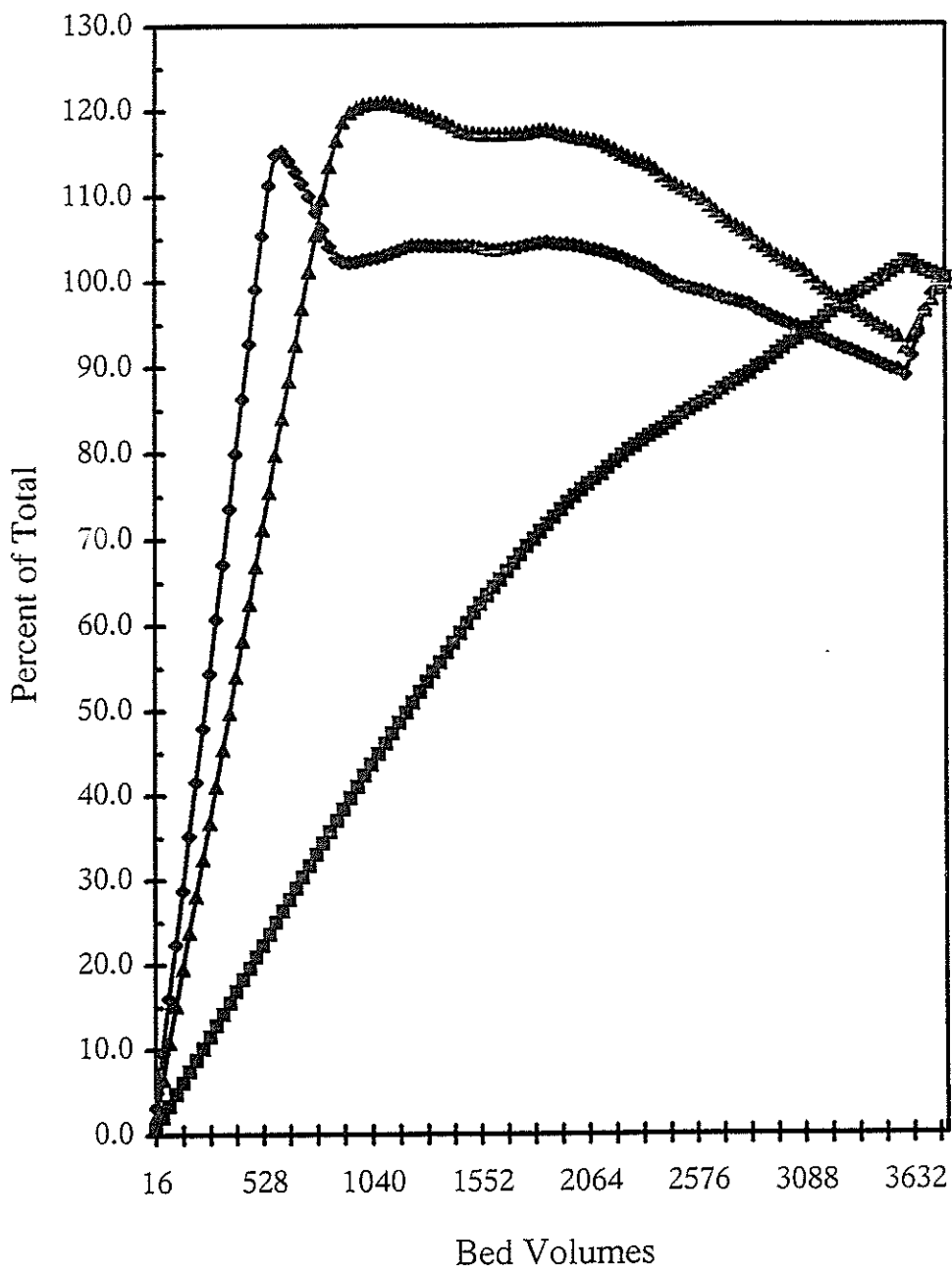


Figure 5. Cumulative moles bound as the percent of the total obtained from competitive binding to Cu(II) (◆), Hg(II) (■), and Pb(II) (Δ) by immobilized Colorado Organic peat. Influent was 0.05 mM in each metal ion with the influent pH adjusted to 5.0; the hydraulic flow through the column was 1/6 bed volumes per minute.

extraction process all three cumulative binding curves exhibit a positive slope. This is indicative of the differential binding curve's region (Figure 2) where the number density of available binding sites on the peat is sufficient to accommodate each of the metals in the influent. This behavior was observed independent of their respective Lewis acidities.

As the extraction process was continued, most of the high affinity sites were occupied. It is at this point where competition among the remaining sites would be predicted to intensify and result in changes in the slopes of the individual cumulative binding curves. It is evident from this figure that the initial calcium and magnesium binding occurs as a result of binding to these high affinity sites and subsequently is displaced by copper ions. After copper breakthrough, an indication of the consumption of the higher affinity sites, apparently all three ions compete somewhat equally for the remaining lower affinity sites. Both the calcium and magnesium curves in this plot exhibit multiple slopes and regions where their slopes are negative, indicating release of previously bound metal. Conversely, prior to copper breakthrough, its corresponding cumulative binding curve displays a nonvarying slope. These observations lead to the conclusion that, for this system under study, the majority of available binding sites present on this biomaterial are more intermediate in Lewis acidity than hard.

Figure 5 similarly conveys the respective cumulative binding curves for the case of the equimolar competitive binding study two, where the copper, lead, and mercury ions were evaluated. Recall from the separate binding experiments to each of these metals, that roughly the same number of moles of each were bound prior to breakthrough occurring. This plot shows a definite preponderance of those sites participating in the observed binding toward metals softer in Lewis acidity than the copper(II) ion as evidenced by negative slope values for the copper and lead curves.

Tables 3 and 4 list the numerical results obtained from mass balance calculations performed on the flow data obtained from each of the respective equimolar competitive binding experiments. These tables list the total millimoles bound, occurring both at breakthrough and at the completion of the experiment, for each of the metals under investigation. Additionally, both effective and apparent binding capacities are reported for each metal under these experimental conditions.

Table 3. Results from Three-Metal Equimolar Study 1.

	<u>Ca</u>	<u>Mg</u>	<u>Cu</u>
Influent Concentration (mM)	0.157	0.157	0.157
Biosorbent Mass (g)	1.58	1.58	1.58
Breakthrough Volume (bv)	96	48	448
Breakthrough Volume (L)	0.48	0.24	2.24
Breakthrough Metal Binding (millimoles)	0.075	0.038	0.352
Total Metal Binding (millimoles)	0.210	0.276	0.590
Effective Binding Capacity (mmole/g)	0.048	0.024	0.223
Total Binding Capacity (mmole/g)	0.133	0.175	0.373

Table 4. Results from Three-Metal Equimolar Study 2.

	<u>Cu</u>	<u>Hg</u>	<u>Pb</u>
Influent Concentration (mM)	0.05	0.05	0.05
Biosorbent Mass (g)	1.67	1.67	1.67
Breakthrough Volume (bv)	560	1088	816
Breakthrough Volume (L)	2.8	5.44	4.08
Breakthrough Metal Binding (millimoles)	0.140	0.272	0.204
Total Metal Binding (millimoles)	0.129	0.563	0.177
Effective Binding Capacity (mmole/g)	0.084	0.163	0.122
Total Binding Capacity (mmole/g)	0.077	0.337	0.106

Figure 6 is a graphical representation of the calculated effective and apparent binding capacity data for each of the respective equimolar competitive binding studies. This depiction allows inspection of the total molar binding occurring at breakthrough, and at the end of the experiment, normalized to one gram of the immobilized peat biosorbent.

Plot A in this figure pertains to

the first equimolar study (Ca²⁺, Mg²⁺, and Cu²⁺), which was involved with the evaluation of

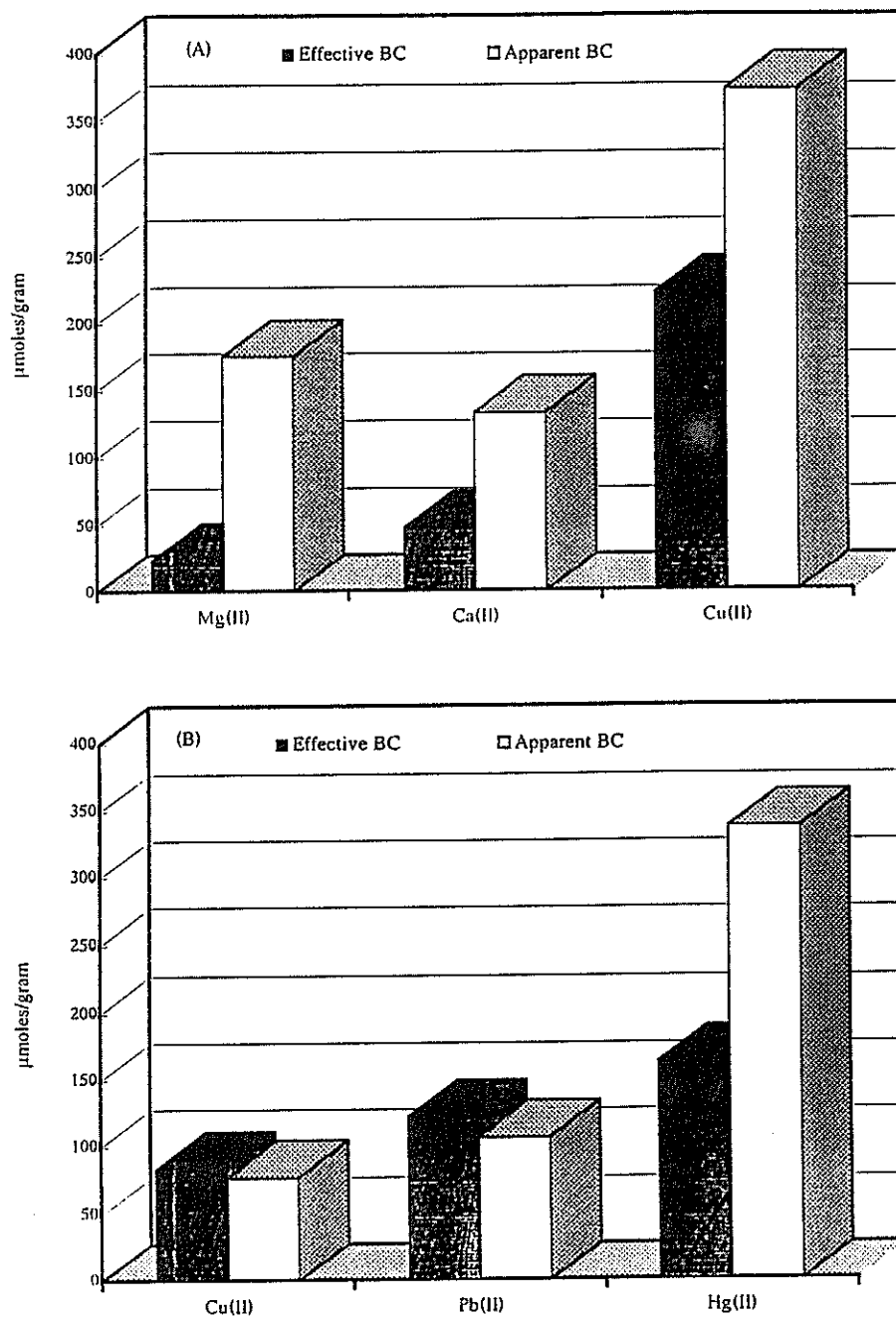


Figure 6. Effective and apparent binding capacities resulting from competitive binding of (A) .157 mM Ca(II), Mg(II), and Cu(II), and (B) .05 mM Cu(II), Pb(II), and Hg(II) to immobilized Colorado organic peat. Experimental conditions were as described in Table 1.

metals hard to intermediate in terms of Lewis acidity. Likewise, plot B relates the information relevant to the second equimolar study involving metals intermediate to soft in Lewis acid character (Cu^{2+} , Pb^{2+} , and Hg^{2+}). Both plots show similar trends for increased molar binding as the Lewis acid character is varied from hard to soft for the metals under investigation. The only anomaly in this trend occurs for the case of overall calcium binding. A slight decrease is observed relative to the magnesium binding. With respect to interference in the effective binding of toxic metals by the less innocuous alkaline earth metals, plot A also indicates effective treatment would only be hindered minimally in water containing these metals- significant for potential use of this biomaterial for toxic metals remediation in hard water.

Plot B in Figure 6 displays a slight decrease in the apparent binding capacities for copper and lead compared to their respective effective binding capacities. This indicates a replacement of these ions by metals softer in Lewis acid character as the extraction process progresses. Apparently competition for those soft Lewis acid sites increases near the end of the experiment when competition among these metals for available binding sites is greatest.

Figure 7 conveys the observed molar binding as percent of total occurring at initial metal breakthrough, and at the experiment's completion, for both equimolar competitive binding studies. In plot A of this figure, for binding that occurs prior to breakthrough, of metals hard to intermediate in Lewis acidity, approximately 24% of the total distribution of sites can be attributed to sites intermediate to hard in Lewis acid character. Likewise in plot B, for binding occurring in this same region of the binding curve, the data indicates roughly 44% of the pre-breakthrough binding can be attributed to sites soft to intermediate in Lewis acidity.

Data interpretation in this manner allows a grouping of the functional sites responsible for the high affinity binding (binding occurring prior to breakthrough) into classifications based upon their Lewis acidity characteristics. Although these groupings have been obtained through phenomenological based observations, and they rely somewhat on data interpretation, nevertheless this information begins to provide a chemical-based description from which the binding behavior of this biomaterial can be explained.

Figure 8A depicts the distribution of functional sites, as a percentage of the total, into groupings based upon their observed Lewis acidity behavior. This plot indicates that greater than 76% of the functional groups responsible for the pre-breakthrough binding by this material have Lewis acid characteristics soft to intermediate in nature. This is corroborated by the inefficient

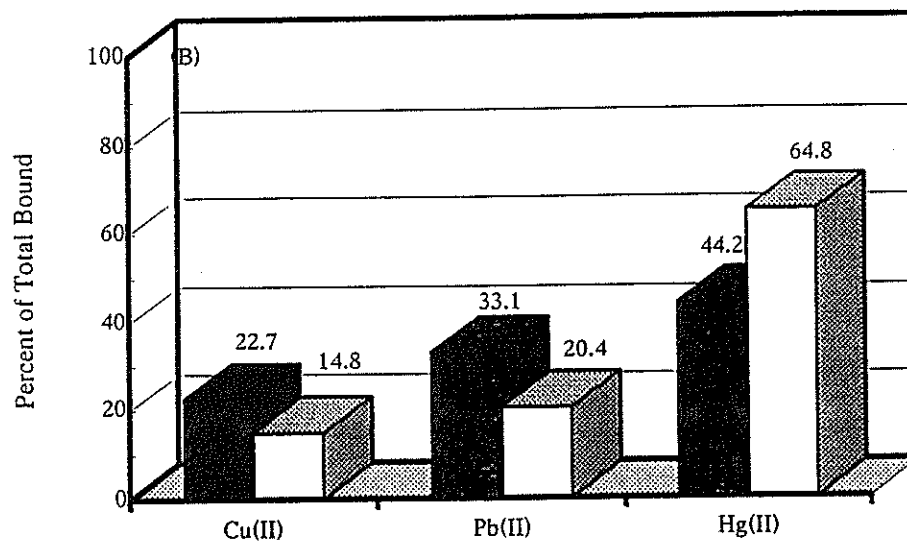
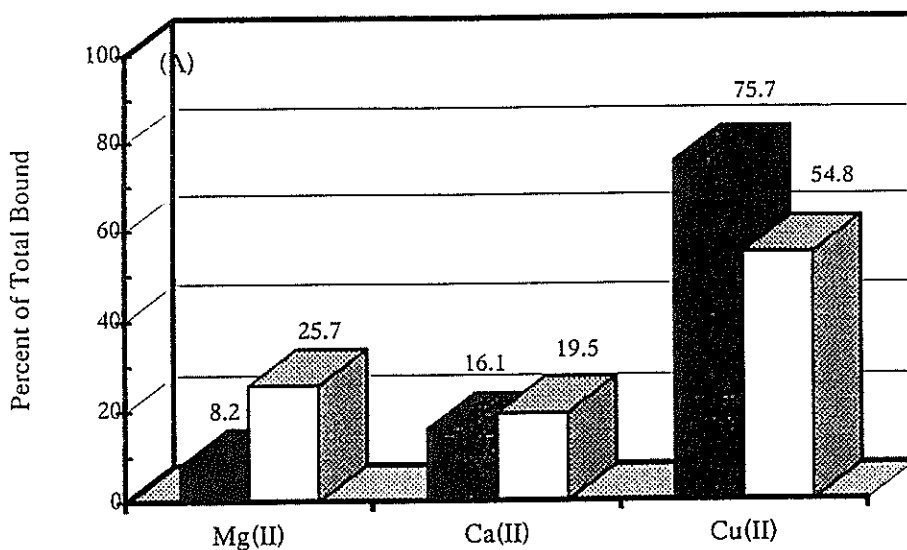


Figure 7. Percent distribution of total molar binding occurring at breakthrough and at the completion of the experiment for competitive binding to (A) .157 mM Ca(II), Mg(II), and Cu(II), and (B) .05 mM Cu(II), Pb(II), and Hg(II) to immobilized Colorado organic peat. Experimental conditions were as described in Table 1.

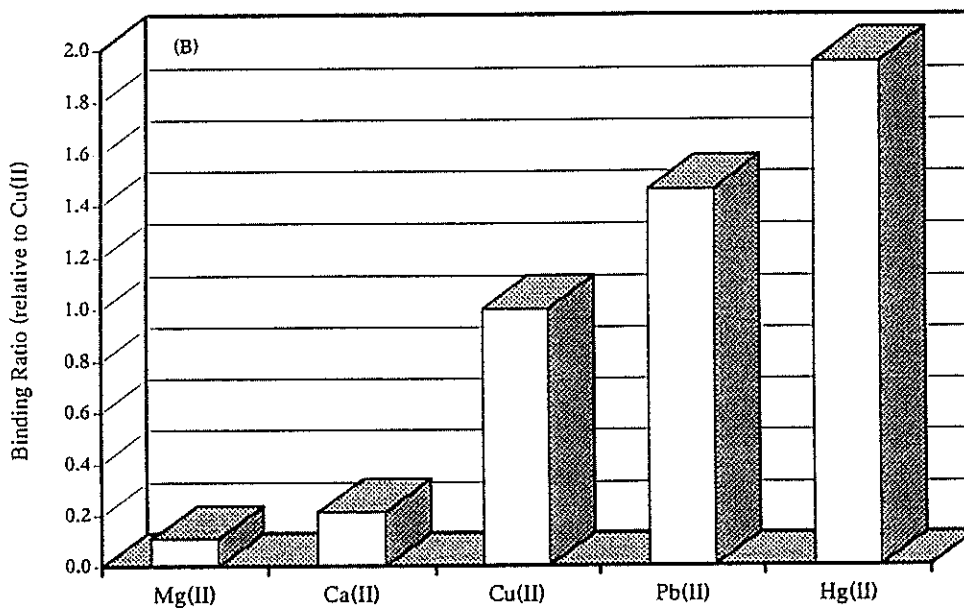
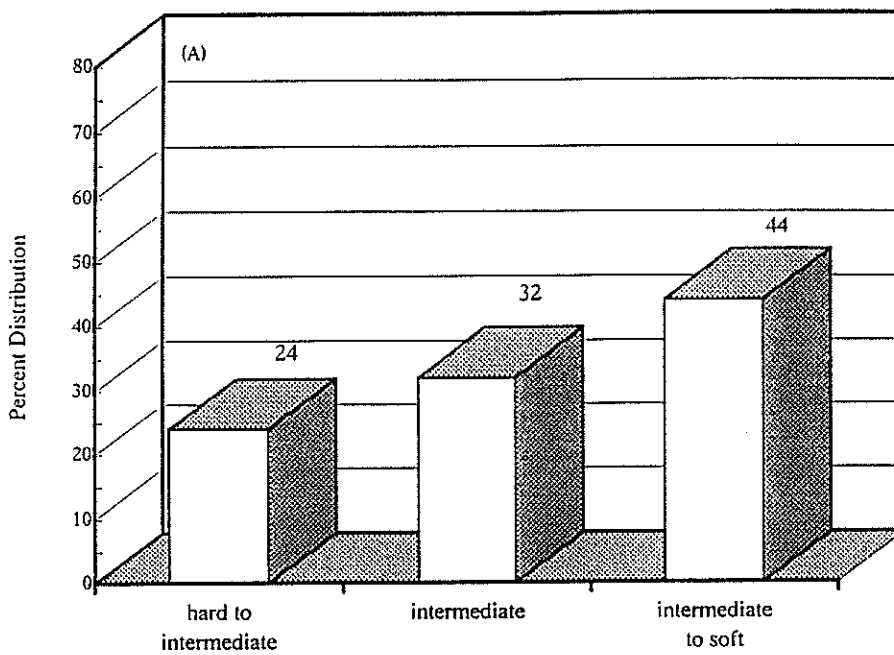
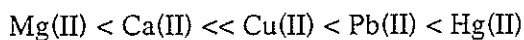


Figure 8. (A) Estimated grouping based on Lewis acidity of high affinity functional sites found on immobilized Colorado organic peat. (B) Relative binding affinity normalized to Cu(II) binding of metals included in equimolar competitive binding studies.

calcium and magnesium binding demonstrated by this material and also from the poor aluminum binding observed previously.

Figure 8B illustrates the relative binding affinity (normalized to the observed Cu(II) binding) by the immobilized Colorado peat to the metals included in these equimolar competitive binding studies. The data from these experiments have indicated that the binding affinity by the peat increases in the following order:



This plot again shows the trend for increased binding as the metals vary from hard Lewis acid character to soft.

These experiments have provided additional information pertinent to describing how and why the immobilized Colorado organic peat binds metals from aqueous solutions. The multitude of chemical functionalities known to be contained in this material have been categorized into groups based upon their Lewis acidity behavior useful for predicting which types of metals might be amenable to extraction by this material. The released-proton to bound-metal ratio information has indicated primarily an ion-exchange mechanism is responsible for the initial high-affinity binding, which is the region of the respective binding curves applicable for practical use of this material. Also, due to the chemical composition of the chemical functionalities responsible for the high affinity binding, little interference by waters containing appreciable amounts of calcium and magnesium should be anticipated. Overall these studies have indicated that divalent transition and heavy metals with soft to intermediate Lewis acidity characteristics are the best candidates for extraction from aqueous systems by this material.

Application of Chromatographic Approach

Results of Pb binding

The application of a continuum-site model to the binding data from a modified frontal affinity chromatography experimental design yielded a plot (Figure 9) displaying two separate maxima. These describe the distribution of binding sites for Pb^{2+} at a solution $\text{pH} = 5.5$ with ionic strength of the contact solution controlled with the non-complexing species, the sodium form of 2(N-morpholino) ethane sulfonic acid (MES). The presence of a collection of low-affinity sites ($\log K \approx 2.8$) is indicative of binding sites exhibiting electrostatic interactions. Such interactions are typified by ion-exchange processes. The second, high-affinity, distribution of Pb^{2+} binding sites to the immobilized organic peat are more demonstrative of binding

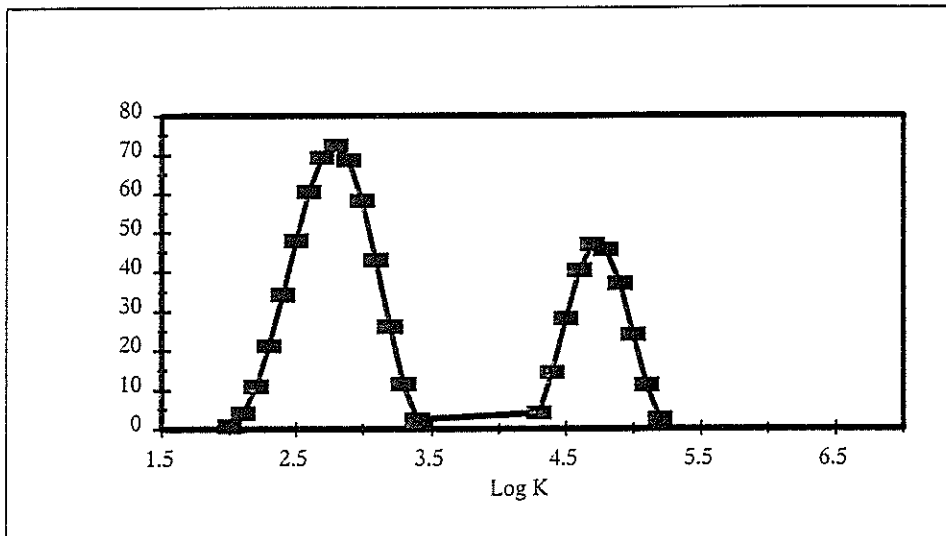


Figure 9. Distribution of Pb(II) binding sites on immobilized Colorado organic peat.

interactions involving the formation of a metal-ligand complex involving a coordination of the ligand (i.e., the immobilized humic material) and the metal ion).

SUMMARY

These results have demonstrated the applicability of this relatively inexpensive material for the selective binding of toxic heavy metal ions. The immobilization of this material within a polysilicate matrix has enabled the use of this substrate within a flowing system while maintaining adequate gross metal-binding affinities and capacities. The mechanisms of metal binding have been determined to involve both electrostatic and complexation interactions. The observation will have significant ramifications regarding the application of this biogenic material and the selection of operating conditions and any chemical modification schemes in the future.

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