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Competitive Metal Ion Binding to a Silicate-Immobilized *Datura innoxia* Biomaterial

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Abstract: Metal ion binding with a flowing system to a biosorbent comprised of cultured cell-wall fragment within a polysilicate matrix has been investigated. Solutions containing 0.10 mM Pb^{2+}, Cu^{2+}, Ni^{2+}, Cd^{2+}, and Zn^{2+} were exposed to the material in combinations of two, three, and five metals while simultaneously monitoring the concentration of all metals in the effluent stream. A relative affinity order of $Pb^{2+} > Cu^{2+} >> Zn^{2+} \approx Cd^{2+} > Ni^{2+}$ was determined when all five metal ions were exposed to the material. Lower-affinity metal ions were exposed to the material sequentially. Both metal-specific and common binding sites were observed for each metal ion. The presence of both binding sites that are common to all metal ions investigated and sites that appear to be unique for each metal ion could significantly impact the utility of single-metal ion studies on the application of such biosorbents for the selective removal of metal ions from natural water.

Keywords: biosorption, competitive binding, *Datura innoxia*, metal ions

INTRODUCTION

The chemical heterogeneity of biomaterials is an attractive property for their use as sorbents for metals (1). Unfortunately, this same heterogeneity complicates their chemical characterization, limiting their practical

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application. To enhance the utility of these materials, numerous studies have been described to elucidate the chemical interactions responsible for the binding of specific metal ions to biogenic materials such as algae (2), alfalfa (3), and creosote (4).

Other studies involving a biomaterial derived from *D. Innoxia* have attempted to characterize metal-ion interactions using single metal systems (5–7). This has typically involved measuring the equilibration of the material with a solution of the targeted metal ion and determining the amount of metal ion bound using a mass balance condition. Unfortunately, binding of metal ions under batch exposure conditions can result in the displacement of protons and a decreased solution pH. This can negatively impact metal binding to biomaterials (8,9). Efforts to control the pH of the solution through the use of a buffer have necessitated the addition of other species that can either coordinate with the metal ions (e.g., the use of an acetate or phosphate buffers) or compete for binding sites (5,10).

Lin et al. (10) addressed these problems using a variation of frontal affinity chromatography. Breakthrough curves were generated as the biomaterial was exposed to a known amount of the metal ion. Through the direct interfacing of the column effluent to the nebulizer of a direct reading inductively coupled plasma atomic emission spectrometer (ICP-AES), real-time multi-element breakthrough curves for both bound and released metal ions were recorded (11). It should be noted that the spectrometer used throughout these studies was a Jarrel-Ash AtomCom 800 configured with 31 separate exit slits positioned on a Rowland Circle with individual photomultiplier tube (PMT) detectors and signal collection electronics. Because of this configuration, it was not possible to select alternate wavelengths for the monitoring of each metal in the column effluent.

Incorporation of such biogenic materials into a flowing system requires their encapsulation within a mechanically robust matrix (9,12). In the present studies, a biosorbent derived from cell-wall fragments of cultured cells of the plant *Datura innoxia* were immobilized within a polysilicate matrix (13). Earlier studies reported this matrix to exhibit negligible binding of heavy metal ions (13).

EXPERIMENTAL

Biomaterial Preparation

Cell-wall fragments resulting from lysed cultured cells from the anther of the flower of *D. innoxia* were used. This plant was selected for metal ion

binding studies because of its possible use in phytoremediation and phytofiltration treatment schemes (6). The plant is native to arid regions of northern Mexico and southwestern United States and has been observed to grow near heavy metal rich mine tailings. Because the present studies are limited to the cell walls of lysed cells, metal binding by intra-cellular molecular species was not a factor. To minimize other tissue-dependent variations in the plant cells, cells from the anther of the flower were cultured and studied (14).

The procedure for the cultivation of the anther cells has been described in detail elsewhere (15). Briefly, the cells were isolated and grown in modified Gamborg's 1B5 growth media with supplemental vitamins. Harvested cells were then washed twice with 95% ethanol and dehydrated by heating at 42°C. Dehydration of the lysed cells was determined complete with the attainment of a constant mass. Before further use, the cell fragments were washed in a pH-2 sulfuric acid solution (5% v/v). They were then rinsed with >16 MΩ distilled deionized water to remove any excess sulfate ions until an aliquot failed to produce a precipitate in a barium test. The biomass was then freeze-dried and stored.

Biomaterial Immobilization

Native biomasses typically exhibit poor mechanical strength, low density, and a smaller than desirable particle sizes which can result in column clogging (12). These factors often prohibit their general use as sorbent materials in a column-based treatment scheme. The *D. innoxia* biomaterial was therefore immobilized in a polysilicate matrix (16). Particle sizes of 423–635 μm (40–60-mesh) were used as column packing material.

The procedure used for immobilizing the biomaterial was adapted from the method described in detail elsewhere (16). Briefly, 300 milliliters of 5% v/v sulfuric acid were adjusted to pH 2.0 using a 6% (wt/v) solution of $Na_2SiO_3{\cdot}5H_2O$. A 20 g suspension of the biomaterial (100–200-mesh size) was generated and stirred for 1 hour. The pH of the solution was then slowly raised to 7.0 using the 6% $Na_2SiO_3{\cdot}5H_2O$ solution. Under these conditions, the silica polymer was subsequently formed. The solution was then covered and stored at ~ 4°C overnight to allow settling. The aqueous layer was then decanted and excess sulfate ions were removed using distilled deionized water until an aliquot of the washings failed a barium test for sulfate. After a final wash to ensure complete removal of sulfate ions, the polymer was baked at approximately 100°C overnight. The resulting material was then ground and sieved. All subsequent determinations were performed using triplicate samples of this material.

Table 1. Surface areas for each material using the BET method

	Surface areas/$m^2\,g^{-1}$		
Method BET	Native cell material	Polymer	Immobilized cell material
Average	0.98	468.86	268.54
Std.Dev.	0.39	45.72	2.18
% RSD	39.19	9.75	0.81

Composition of Material

The composition of this material was determined thermo-gravimetrically. Briefly, each of three 35-mg samples of the immobilized biomaterial was placed in a muffle furnace with continuous airflow and heated to 600°C until constant mass was achieved. The weight percent biomass was found to be $66 \pm 5\%$.

Pore-size distributions and surface areas were determined for each of three materials: the native *D. innoxia* cell wall material; the immobilized *D. innoxia*; and the blank silicate polymer. These measurements were performed on a Coulter SA 3100. The Brunauer, Emmett, and Teller (BET (17)) method used to calculate the surface areas. Pore size distributions were calculated based on the method described by Barrett, et al. (BJH (18)). These results are listed in Tables 1 and 2 respectively.

Solution and Column Preparation

Single metal, 2.0 mM stock solutions of cadmium, nickel, and zinc were prepared from their respective nitrate salts dissolved in distilled-deionized water. A 2.0 mM multi-element stock solution containing Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, and $Zn^{2+}$was similarly prepared. Column influent solutions were prepared by serial dilution using distilled-deionized water.

The columns used in these studies were constructed from 2.5 cm plastic tubing (3.0 mm i.d.). These were each packed with 98 mg of the immobilized *D. innoxia* material. Teflon tubing (0.8-mm internal diameter) was used for all influent and effluent connections. The outlet of the column was connected to the nebulizer and spray chamber assembly of the ICP-AES using a minimal length of Teflon tubing (15 cm). All solution flow rates were calibrated at 1.0 mL min^{-1} using a peristaltic pump (Rainin, Miniplus2). This configuration resulted in minimal elution times with imperceptible memory effects between sample solutions.

Table 2. Pore volume distributions for each material using desorption and adsorption isotherm data

Sample pore diameter range (nm)	Pore volumes (%RSD)								
	Under 6	6–8	8–10	10–12	12–16	16–20	20–80	Over 80	Total
Desorption pore size distribution/mL g^{-1}									
Native cell material	0.000933	0.000987	0.00139	0.001573	0.001863	0.001337	0.005483	0.001857	0.015423
	(173%)	(152%)	(59%)	(44%)	(40%)	(51%)	(25%)	(18%)	(41%)
Immobilized cell material	0.215	0.304	0.0053	0.00249	0.00226	0.00142	0.00480	0.00139	0.537
	(1.37%)	(2.44%)	(6.37%)	(3.27%)	(6.90%)	(5.59%)	(8.22%)	(2.16%)	(1.00%)
Polymer	0.511	0.366	0.0185	0.00430	0.00350	0.00183	0.00588	0.00121	0.912
Adsorption pore size distribution/mL g^{-1}									
Native cell material	0.00409	0.001853	0.00132	0.001047	0.001137	0.000727	0.003193	0.00157	0.014937
	(36%)	(45%)	(43%)	(61%)	(48%)	(54%)	(31%)	(15%)	(37%)
Immobilized cell material	0.133	0.138	0.124	0.0745	0.0239	0.00326	0.00383	0.00130	0.502
	(0.21%)	(2.80%)	(0.18%)	(2.59%)	(12.6%)	(13.0%)	(6.79%)	(15.2%)	(1.00%)
Polymer	0.212	0.245	0.198	0.0530	0.0154	0.00249	0.00433	0.0011	0.731

Each packed column was first pretreated with a solution of 1.0 M HCl for 20 minutes to remove any remaining metals from the immobilized biomaterial (31). This was followed with a 5 mL wash with distilled-deionized water to return the column pH to its natural state (pH = 6.3–6.7). Each subsequent metal ion solution was then pumped through the column for 75 minutes while continuously monitoring the concentration of each metal ion in 0.78 mL increments (i.e., an acquisition rate of 1.6 s^{-1}). An example of the resulting breakthrough curves for a solution containing 0.1 mM of each of five metal ions (Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) is shown in Fig. 1a. Any remaining metals bound to the material were removed by passing 5.0 mL of 1.0 M HCl through the column and monitoring the effluent (Fig. 1b). As evidenced by the baseline signals recorded early in these stripping profiles, memory effects resulting from metals adsorbed onto the walls of the transfer tubing were not observed.

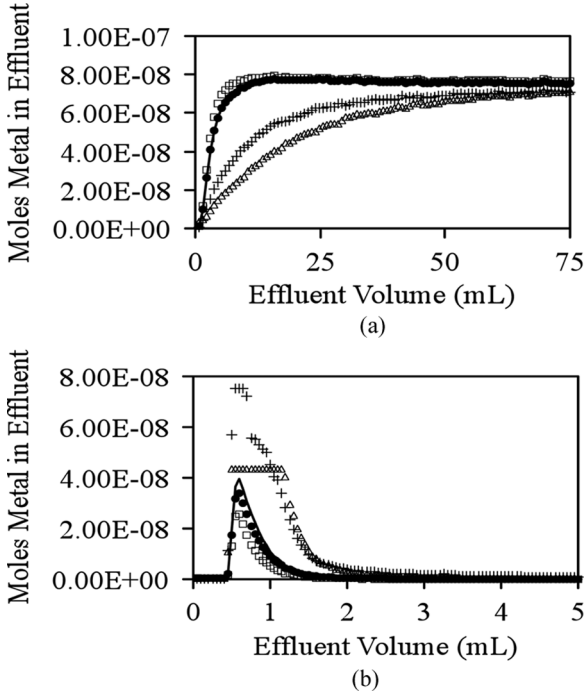


Figure 1. (a) Simultaneous multielement exposure to 0.1 mM Cd^{2+}(●), Cu^{2+} (+), Ni^{2+} (□), Pb^{2+} (Δ), and Zn^{2+} (–), and (b) the amounts of each metal ion in the effluent of a subsequent stripping procedute using 1.0 M HCl.

RESULTS AND DISCUSSION

Material Characterization

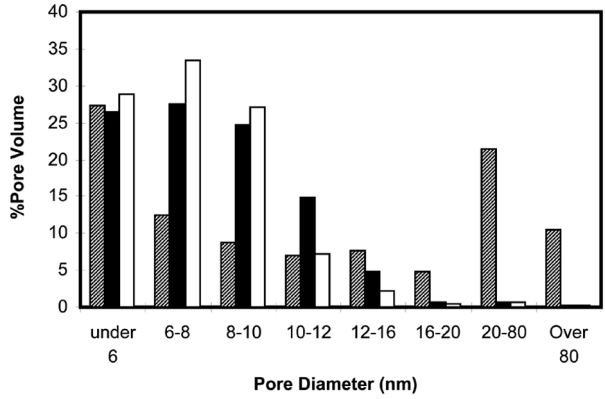
Comparison of the calculated surface areas of the three materials (cell material, silicate polymer, and biomaterial within the polysilicate matrix) indicated a significant decrease (>42%) in surface area with the inclusion of the plant cell material. The free cell fragments exhibited a surface area less than 0.8% of the silicate polymer material. A simple calculation reveals 42% of the surface area of the final material to result from the plant biomaterial. This suggests the inclusion of the biomaterial within the polysilicate matrix results in a material consisting of the low-surface area cell material within the high-area silicate polymer.

Analysis of the pore volume distribution data for the three materials revealed pores with diameter less than 10 nm to dominate for the silicate polymer both with and without the plant cell material. This is further revealed in the calculation of the mean pore diameters for each of these materials to be 8.9 and 8.0 nm, respectively. Conversely, the cell material was observed to significantly contribute to the total pore volume of that material with both small (i.e., <6 nm) and relatively large (i.e., >20 nm) diameter pores. This contribution resulted in a mean pore diameter of 28.3 nm (see Fig. 2). If the cell material is present as larger aggregates of the cell-wall fragments, the pore size distribution measurement would reveal a greater fraction of larger diameter "pores" but such clumping would not be expected to significantly affect the surface area of the material.

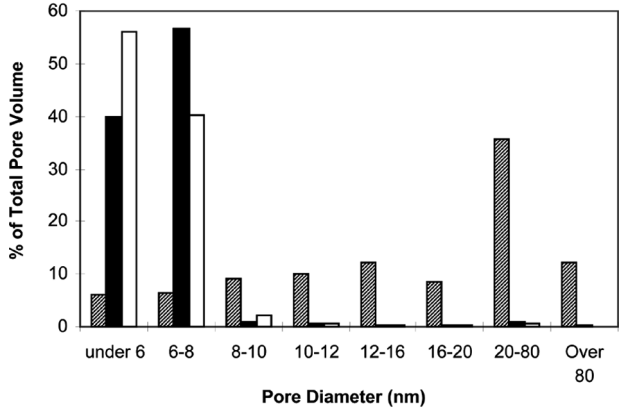
Interestingly, an assumption of a linear combination of pore volumes from each component of the material yields a calculated contribution of 41.9% and 31.9% from the plant cell material using adsorption and desorption data, respectively. This would suggest that the immobilization process enables the relatively uniform separation of the heterogeneous cell-wall material within the polysilicate matrix. This would be predicted to enable more complete access to the cell wall surface by metal ions.

Metal Binding

The focus of this study was the interactions of each of three metal ions, Cd, Ni, and Zn, with the immobilized *D. innoxia* biomaterial. These three transition metals were selected for study because of their environmental significance and the similarities in their respective binding to this material (Fig. 1) (11) (Williams and Rayson, 2003). Although the concentrations of Ni^{2+}, Cd^{2+}, and Zn^{2+} in the column effluent were observed to rapidly



(a)



(b)

Figure 2. Percentages of total volume associated with pores of varied diameters for (■) the native cell wall material, (■) the immobilized cell material, and (□) the silicate polymer material as determined from desorption (a) and adsorption (b) data.

reach the influent concentrations (i.e., 7.8×10^{-8} moles metal in each sampled volume), exposure of the same columns required considerably larger amounts of either Cu^{2+} or Pb^{2+} to reach that condition. These data suggest finite available sites with varied affinities for each metal ion. Metals are predicted to bind to a collection of sites with similar chemical functionalities. Weakly bound metal ions (i.e., lower affinities) are predicted to be displaced by metal ions with greater affinity. Under this hypothesis, the affinity order of these metal ions would be

$Pb^{2+} > Cu^{2+} >> Zn^{2+} \cong Cd^{2+} > Ni^{2+}$. This hypothesis predicts displacement of each of the lower affinity metals (i.e., Ni^{2+}, Cd^{2+}, and Zn^{2+}) by either of the other two similar metals.

It has been proposed that the dominant mechanism for metal ion binding to biological materials is ion-exchange (1). If this is true for the present system, displacement of similarly charged ions would be predicted to occur with a molar ratio of 1.0. To test this hypothesis for the binding of Cu^{2+} and Cd^{2+} ions to this *D. innoxia* derived material, the material was initially exposed to a flowing solution of 0.1 mM Cd^{2+} until a steady-state metal ion concentration was observed in the effluent. This material was then exposed to an equi-molar solution of Cu^{2+} and the concentration of each metal ion (i.e., Cd^{2+} and Cu^{2+}) in the effluent was monitored (Fig. 3a). The molar ratio of the amount of Cu^{2+} bound

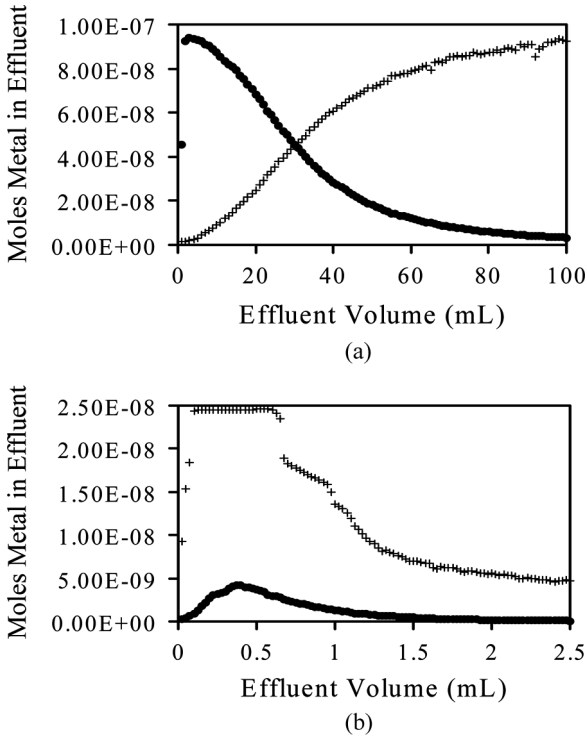


Figure 3. Effluent metal ion content during exposure of a Cd^{2+}(•)-containing column to a 0.1 mM Cu^{2+} (+) solution (a) and the amounts of each metal ion in the effluent during a subsequent exposure to 1.0 M HCl (b).

to the amount of Cd^{2+} released was determined to be 1.40. This indicated more binding of Cu^{2+} then release of Cd^{2+}. This could be explained by

1. the presence of Cu^{2+} binding sites that were not occupied by Cd^{2+} ions (inferring the existence of metal-specific binding, or coordination sites) or
2. the involvement of mechanisms other then simple ion-exchange.

To further investigate the relative binding of Cu^{2+} and Cd^{2+}, the material was exposed to 1.0 M solution of HCl to quantitatively remove all metal ions from the material (11). The effluent was monitored for each metal ion and the result is shown in Fig. 3b. Several observations can be made from these data. First, the amount of Cu^{2+} released early in this acid treatment was sufficient to saturate the detection system with an instantaneous concentration of greater the 1.0 mM (~64 ppm). Second, the time-dependent elution of the Cu^{2+} exhibited at least three discernable features (i.e., 1) the peak between 0.1 and 0.6 mL, 2) the shoulder at about 1 mL, and 3) the "tail" region beyond 1 minute). Finally, a significant additional amount of Cd^{2+} ion was removed from the material. These observations would support both the presence of multiple mechanisms of Cu^{2+} binding to this material and the existence of metal-ion specific sites. This is consistent with related studies conducted in our laboratory pertaining to metal ion binding to this material that have revealed distributions of sites with varied affinities attributed to both ion-exchange and metal chelation mechanisms (5).

To test the hypothesis that these effects could be a result of differences in the sizes of these two ions, a similar experiment was conducted using equi-molar solutions of Ni^{2+} and Zn^{2+} was undertaken (Fig. 4). If ion exchange was indeed the primary mechanism of metal ion binding and the result of the Cd^{2+}–Cu^{2+} experiment were due to differences in site availability, a molar ratio of 1.0 should be observe. When a Zn^{2+}-containing solution was exposed to material containing bound Ni^{2+}, a ratio of 1.29 was observed. Although this is lower than the 1.40 observed earlier, it is significantly different than the value of 1.0 predicted for only ion-exchange processes.

Removal of bound metal ions using a 1.0 M HCl solution (Fig. 4b) again revealed several features. First is the intense signal measured for Zn emission from the effluent corresponding to an instantaneous concentration greater then 80 mM (520 ppm Zn). Second is the temporal location of these observed peaks relative to those for the Cd^{2+}–Cu^{2+} system (Fig. 3b). A third feature is an apparent discontinuity in the Zn elution profile at ~0.6 mL. Finally, a significant amount of Ni^{2+} was observed to elute from the material during this acid treatment. These

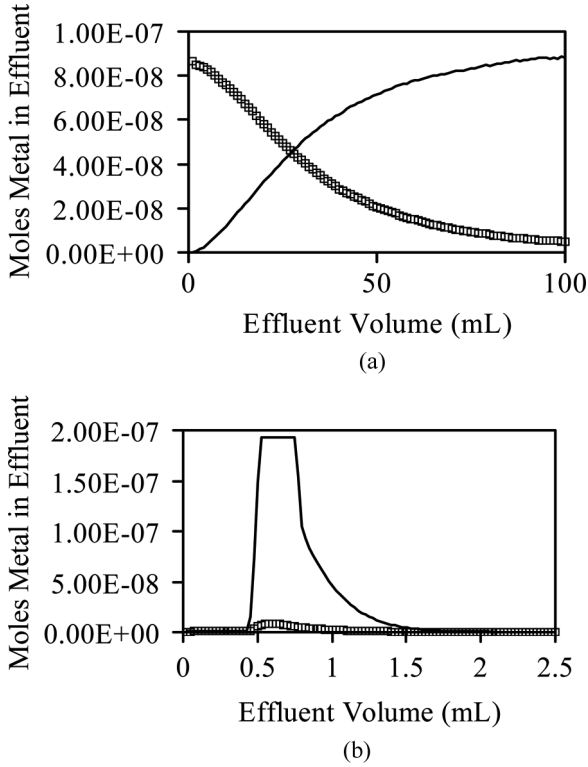


Figure 4. (a) Displacing of bound Ni^{2+} (□) with 0.1 mM Zn^{2+} z(–). (b) Subsequent metal removal from the *D. innoxia* column packing with 1.0 M HCl.

observations are again consistent with both the presence of multiple (i.e., more than one) mechanisms of metal ion binding to this material and the existence of metal-specific binding sites.

In an effort to combine these two experiments, a three-metal ion experiment was designed using the metal ions Cd^{2+}, Ni^{2+}, and Zn^{2+}. This consisted of a sequential exposure study involving these three metal ions with similar binding to the *D. innoxia* biomaterial. With a three metal sequence, six exposure order combinations were possible: $Cd^{2+} \Rightarrow Ni^{2+} \Rightarrow Zn^{2+}$, $Cd^{2+} \Rightarrow Zn^{2+} \Rightarrow Ni^{2+}$, $Ni^{2+} \Rightarrow Cd^{2+} \Rightarrow Zn^{2+}$, $Ni^{2+} \Rightarrow Zn^{2+} \Rightarrow Cd^{2+}$, $Zn^{2+} \Rightarrow Cd^{2+} \Rightarrow Ni^{2+}$, and $Zn^{2+} \Rightarrow Ni^{2+} \Rightarrow Cd^{2+}$. The symbol, $\Rightarrow$, designates exposure of the biosorbent to a solution of the previous metal ion "followed by" an equimolar solution of the next metal ion listed.

Monitoring the amounts of each metal bound, displaced, and subsequently recovered during the acid wash enables the investigation of metal ion interactions occurring with the biomaterial. It should be noted that

exposure to each metal laden material to deionized water at ambient pH (~6.3–6.7) did not result in the elution of a detectable amount of metal ion.

As described above, an exposure of approximately 1.0×10^{-5} moles of metal-ion to the biomaterial was sufficient to reach a steady state condition with the *D. innoxia* material within each column (i.e., 100.0 mL of a 0.10 mM solution of each metal ion flowing at 1.0 mL min^{-1}). For the three-metal ion studies, the time was reduced to 50 minutes (i.e., $\sim 5.0 \times 10^{-6}$ mole metal ion). Although this resulted in each column only achieving 65% of steady state capacity for each individual metal, it enabled the qualitative investigation of metal ion binding to this material.

Effluent profiles from each of the six three-metal sequential exposures are represented in Fig. 5. For each exposure (A, B, and C), the concentration of the effluent is shown as a function of the 0.1 mM influent solution volumes. Prior to exposure to the first metal ion in each sequence, the material in each column was first "stripped" of any metals by exposure to 20 mL of a 1.0 M hydrochloric acid solution followed by a 5.0 mL rinse using distilled-deionized water (11).

Binding of these metal ions through an ion-exchange mechanism involving negatively charged sites would result in the displacement of each metal by ions exhibiting a higher affinity. The molar ratio of the amount of metal ion bound to that of the metal ion(s) released would then be predicted to have a value of 1.0. If high-affinity binding sites unique to the biosorption of a metal ion were present, exposure of the material to metal ions would be predicted to result in its initial binding to those sites with subsequent displacement of any bound metal ions. Multiple types of metal-ion binding sites would yield final elution profiles (when exposed to a 1.0 M HCl solution) containing features corresponding to sites with varied affinities for the different metal ions.

The effluent concentration of each metal throughout each step in the sequential three-metal ion exposure experiment is represented in Fig. 5 for the three metal ions. As the initial metal was exposed to the biosorbent, the effluent concentration was observed to increase as involved binding sites became filled (Fig. 5a). During the second step in the procedure, both the increasing concentration of the influent metal and the presence of the displaced metal ion were recorded (Fig. 5b). Further exposure of the metal-laden sorbent to a solution containing the third metal ion resulted in the presence of each of the three metal ions in the effluent (Fig. 5c).

If the only (or predominate) mechanism of metal ion sorption involved electrostatic attraction forces, exposure of the biomaterial to either the second or third metal ion in the sequence would have been predicted to result in complete displacement of the previous metal ion. This was not observed for any sequence of these three metal ions. Each sequence of

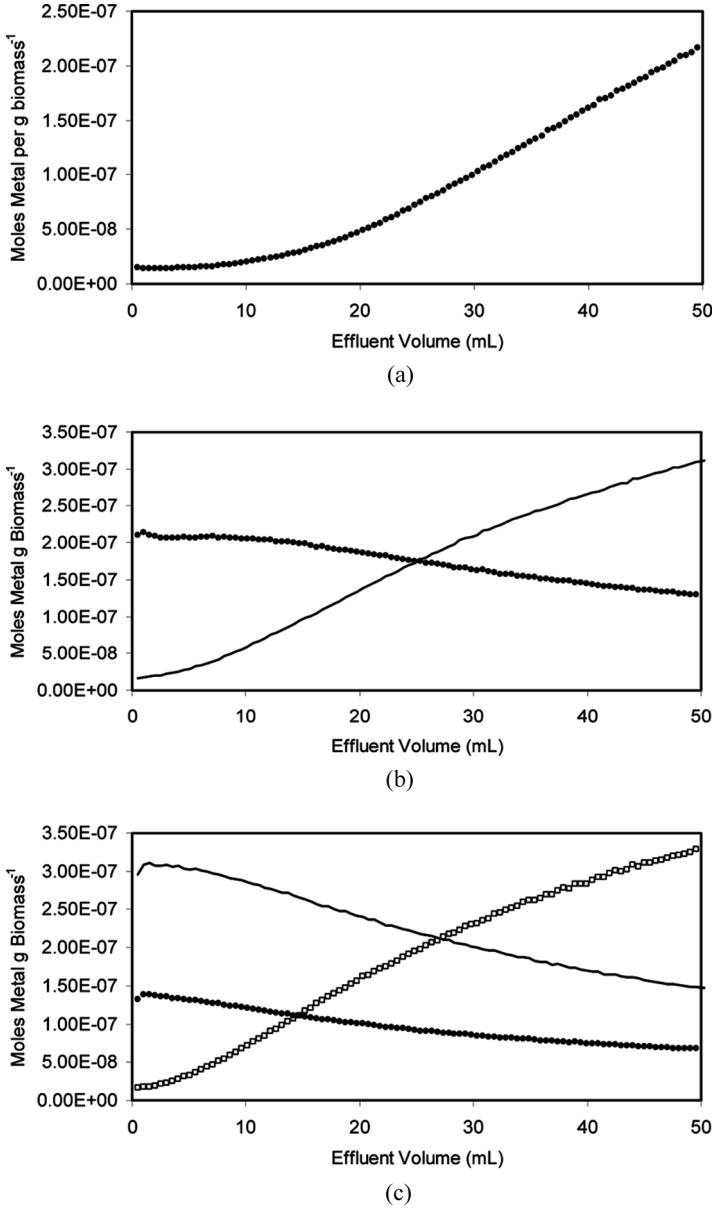
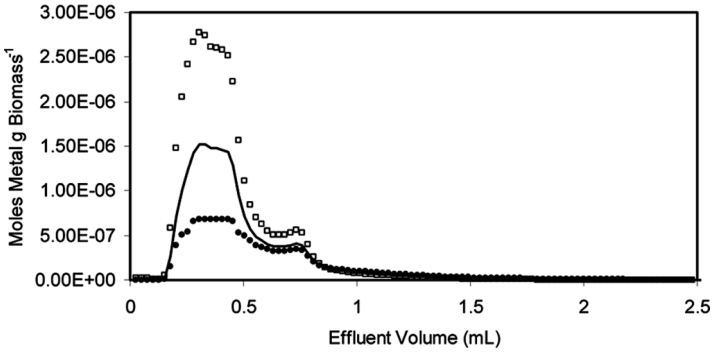


Figure 5. Column effluent content during (a) initial exposure to 1.0 mM Cd^{2+} (●) and subsequent exposure to 1.0 mM Zn^{2+} (—) (b) and 1.0 mM Ni^{2+} (□) (c) followed by a solution of 1.0 M HCl (d).



(d)

Figure 5. (Continued)

metal ion exposure resulted in both incomplete displacement of previously bound metals and additional release of the first metal ion during exposure of the material to the third metal ion in the sequence (Fig. 5c).

When the biosorbent was then exposed to a 1.-0 M HCl solution, multiple features were observed in the effluent as each metal ion was removed form the biosorbent (Figs. 5d and 6).

Dependence of occupied binding sites on only the sorted metal ion would be predicted to yield final elution profiles that were simply dependent on the final metal ion in the sequence (Fig. 5d for Ni^{2+}). Visual comparison of these profiles fails to confirm that prediction. Because the greatest amount of the final metal was bound to the material before acid treatment, it was postulated that the higher instantaneous metal ion concentrations might saturate the emission detection electronics the spectrometer. This condition was observed for only one condition: the elution of Zn^{2+} in the sequence $Ni^{2+} \Rightarrow Cd^{2+} \Rightarrow Zn^{2+}$ (Fig. 6).

The presence of a single type of binding site unique for each metal ion would yield secondary acid elution profiles dependent on the metal ion species. Again, inspection of the corresponding data does not support this hypothesis.

Binding of each of these metal ions through similar interactions with common chemical functionalities would result in one-to-one displacement of each metal ion by subsequent ions. This can be investigated by comparing the respective effluent metal ions concentrations during each stage of the se metal-binding sequences. Table 3 lists results for these data analyses. Listed are the slopes and intercepts of the resulting regression lines and the corresponding correlation coefficients, indicating the degree to which these quantities are correlated. If displacement occurs, a high

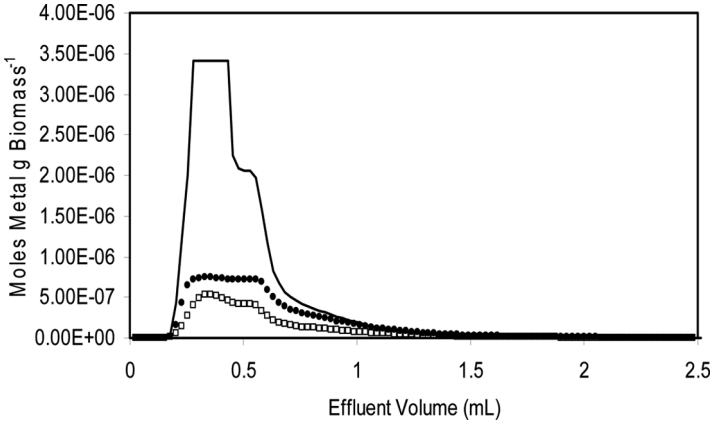


Figure 6. Column effluent content during exposure to a 1.0 M HCl solution following initial exposure to 1.0 mM Ni^{2+} (□) with subsequent sequential exposure to 1.0 mM Cd^{2+} (●) and 1.0 mM Zn^{2+} (—).

degree of correlation should exist with a slope near unity in units of mole metal released per mole metal bound. If only such common sites are present on the sorbent, the intercepts of those regression lines should be near zero. A positive intercept would be interpreted as the continual presence of an excess of metal released relative to the amount bound during each interval of time. A negative intercept would then be indicative of the binding of the exposed metal ion to additional sites.

Review of the results listed in Table 3 reveals several conclusions pertaining to the binding of these three metal ions. The slopes of the regression lines reveal the displacement of metal ions in a one-to-one ratio with the following metal for the conditions of Zn^{2+} followed by Cd^{2+} and the inverted order. Both Zn^{2+} and Cd^{2+} were displaced by Ni^{2+}. Additionally, Zn^{2+} was observed to displace Ni^{2+} after it had displaced some Cd^{2+}-laden sites. More metal ion was released than bound when a Ni^{2+}-containing solution was exposed to the material containing either Cd^{2+} or Zn^{2+} (i.e., a slope > 1.00). Conversely, more metal ion was observed to bind to the material than was displace (i.e., a slope < 0.90) when the material had been initially exposed to the Ni^{2+}-containing solution. Alternatively, variability in the affinities of each metal ion to specific sites could result in a slope greater than unity. However, similarities in Lewis acidities among these metal ions and the probability of ion-exchange mechanisms (5) place some doubt on this interpretation.

These results are supportive of the presence of a majority of binding sites that are common to all three metal ions. However, these results are

Table 3. Linear Regression parameters for the amount of metal released (ordinate) a function of the amount of metal bound (abscissa) for each step in each sequence of sequential exposure of the immobilized biomaterial to 1.0 mM solutions of each metal ion

Sequence	Slope (moles/moles)	Intercept	R^2	Conditions
Cd ⇒ Zn ⇒ Ni	0.9753	-7×10^{-9}	0.9996	Zn Bound: Cd Released
	0.9315	-9×10^{-9}	0.9997	Ni Bound: Zn Released
	0.0966	-1×10^{-9}	0.9888	Ni Bound: Cd Released
	1.0281	-1×10^{-8}	0.9997	Ni Bound: Total Released
Cd ⇒ Ni ⇒ Zn	1.1652	-2×10^{-8}	0.9988	Ni Bound: Cd Released
	0.8723	-1×10^{-8}	0.9997	Zn Bound: Ni Released
	0.0665	$+1 \times 10^{-8}$	0.9266	Zn Bound: Cd Released
	0.9439	-1×10^{-8}	0.9986	Zn Bound: Total Released
Zn ⇒ Cd ⇒ Ni	1.0476	-2×10^{-8}	0.9967	Cd Bound: Zn Released
	0.9366	-1×10^{-8}	0.9983	Ni Bound: Cd Released
	0.0719	-1×10^{-9}	0.9952	Ni Bound: Zn Released
	1.0086	-1×10^{-8}	0.9983	Ni Bound: Total Released
Zn ⇒ Ni ⇒ Cd	1.079	-2×10^{-8}	0.9998	Ni Bound: Zn Released
	0.8342	-1×10^{-8}	0.9998	Cd Bound: Ni Released
	0.0604	$+7 \times 10^{-10}$	0.9779	Cd Bound: Zn Released
	0.896	-9×10^{-9}	0.9996	Cd Bound: Total Released
Ni ⇒ Zn ⇒ Cd	0.8639	-9×10^{-9}	0.9993	Zn Bound: Ni Released
	1.0395	-1×10^{-8}	0.9982	Cd Bound: Zn Released
	0.0329	-3×10^{-10}	0.9473	Cd Bound: Ni Released

(*Continued*)

Table 3. Continued

Sequence	Slope (moles/moles)	Intercept	R^2	Conditions
	1.0725	-1×10^{-8}	0.9984	Cd Bound: Total Released
Ni ⇒ Cd ⇒ Zn	0.8802	$+5 \times 10^{-10}$	0.9997	Cd Bound: Ni Released
	0.9444	$+4 \times 10^{-11}$	0.9998	Zn Bound: Cd Released
	0.0316	-3×10^{-10}	0.9239	Zn Bound: Ni Released
	0.976	-3×10^{-10}	0.9996	Zn Bound: Total Released

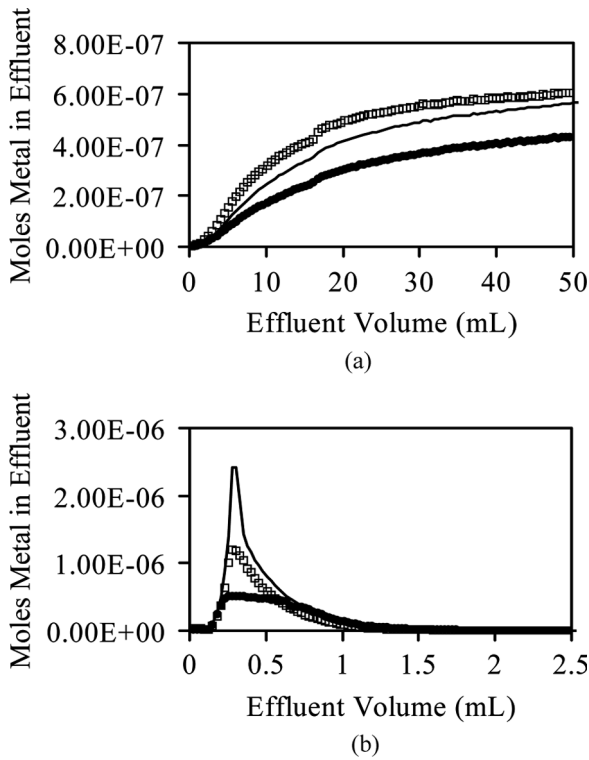


Figure 7. Simultaneous three metal exposure of 0.1 mM Cd^{2+}, Ni^{2+}, and Zn^{2+} for 50 minutes at a rate of 1.0 mL per minute.1.0 M hydrochloric acid wash following the simultaneous three metal exposure. Cd^{2+} (•), Ni^{2+} (□), and Zn^{2+} (—).

also indicative of the presence of significant fraction of binding sites that are unique to each metal ion.

To further investigate the inter-relationship of these metal-ion binding sites, a simultaneous exposure of the *D. innoxia* biomaterial to all three metal ions (0.1 mM each) was also undertaken (Fig. 7a). Interestingly, even with tripling of the total metal concentration, the observed steady-state values for all three metals did not reach the calculated influent concentrations. Specifically, the final effluent concentrations were 0.093, 0.083, and 0.079 mM in Ni, Zn, and Cd, respectively. The average total metal bound for the simultaneous exposure (mass balance condition) was calculated to be 72.9 μmol g^{-1} (24.9 μmol Cd^{2+}, 19.8 μmol Ni^{2+}, and 28.2 μmol Zn^{2+}). Figure 7b shows the effluent profile resulting from the subsequent 1.0 M HCl wash of the sorbent. All three metals exhibited a time of constant signal at the respective peak maxima. The average total metal recovered from both acid washes was 58.9 μmol g^{-1}, 14.6 μmol Cd, 17.9 μmol Ni, and 26.4 μmol Zn. Interestingly, the temporal behavior of these elution peaks was significantly different from those recorded following the sequential exposure of the same material to each of the same metal ions (Fig. 5d). The asymmetry in these elution peaks would suggest the presence of multiple sites for each metal ion. Specifically, the existence of a relatively weakly bound site from which each metal was quickly displaced by the acid front and a higher affinity site(s) that required greater volumes of acid (i.e., longer time) to elute from the column.

CONCLUSIONS

A study has been undertaken pertaining to competitive binding of chemically similar metal ions to an immobilized biosorbent. Specifically, metal ion binding to a biosorbent derived from cell wall fragments immobilized in a polysilicate matrix has been shown to involve numerous sites that are both common to each of the metal ions investigated and sites unique for each metal ion. The greatest commonality in binding sites was observed for the metal ions Zn^{2+} and Cd^{2+}. Early studies of Cd^{2+} binding to the material revealed the involvement of carboxylate-containing sites (19). The present studies suggest similar binding sites for the retention of Zn^{2+} and Ni^{2+}.

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