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Binary Metal Sorption by Pine Bark: Study of Equilibria and Mechanisms

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ABSTRACT

Pine bark was able to sorb cadmium, copper, and nickel ions from aqueous solutions. Binary equilibrium data from the combination of these metals were collected in this work using this sorbent. These data were modeled using three types of binary component equilibrium isotherms, all of which resulted in good fitting of the experimental data, with the Langmuir–Freundlich model resulting in their best representation. In general, the capacity of bark for each metal in the binary system was lower than in the single metal systems. The study also examined the mechanisms of metal biosorption by bark. Scanning electron microscopy (SEM) and energy-dispersive x-ray (EDX) microanalyses revealed that metal ions were sorbed mainly at the cell wall of the bark and only a small amount of ions diffused into the cytoplasm. Both the EDX analysis and the atomic absorption spectrophotometry (AAS) measurements showed that ion exchange was an important mechanism in this sorption process. Electron spin resonance (ESR) tests demonstrated that free radicals from the sorbent also have a significant role in the sorption processes.

INTRODUCTION

The recovery of heavy metals from wastewater has become a very important environmental issue since these metals are very harmful if discharged into the natural water resources. On the other hand, they find many applications in

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daily life. Elevated levels of heavy metals may result from a variety of sources such as metal plating, metallurgical alloying, ceramics, photography, as well as many other industries (1). The amounts of heavy metals discharged to the environment vary from one industry to another, and can range from a few ppm to several thousand ppm.

Considering the harmful effects of heavy metals, it is necessary to remove them from liquid wastes at least to a limit accepted by national and international regulatory agencies. There are many processes that can be used for the removal of metals from wastewaters including chemical precipitation, coagulation, solvent extraction, electrolysis, membrane separation, ion exchange, and adsorption. Ion exchange and adsorption are the most common and effective processes for this purpose, and are more effective when the concentration of metal ions in the discharge is very low.

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Activated carbon and different types of ion-exchange resins are very often used in adsorption processes. The high capital and regeneration costs of activated carbon and ion-exchange resins has encouraged researchers to look for low cost adsorbents. Relatively recently, some agricultural and forestry products and wastes have been recognized as new adsorbents. The cost of these biomaterials is negligible compared with the cost of activated carbon or ion-exchange resins that are in the range of approximately \$2.0–\$4.0/kg. Ferro-Garcia et al. (2) produced several activated carbons from almond shells, olive stones, and peach stones, and used them for adsorption of Zn^{2+}, Cd^{2+}, and Cu^{2+}. Some Indian tree barks (*Accacia arabica*, *Pterocarpus masrupsium*, *Terminalia temetosa*, and *Techtona grands*) treated with formaldehyde and sulfuric acid were used for the removal of Zn^{2+}, Cd^{2+}, Pb^{2+}, and Cu^{2+} (3). Recently, canola meal has been used for the sorption of chromium from aqueous solutions (4).

The sorption of heavy metals by these materials might be attributed to their proteins, carbohydrates, and phenolic compounds (5) which have carboxyl, hydroxyl, sulfate, phosphate, and amino groups that can bind metal ions (6). Salim et al. (7) claimed that the loss of metal ions from aqueous solutions in the presence of agricultural materials may be due to complexation with the constituents of these materials.

In this work the possibility for the utilization of pine bark for the adsorption of different metals and its regeneration were investigated. Pine bark can be obtained as a waste material from the pulp and paper industry, and considering that it is often simply burned, it is advisable to find another utilization for the bark. The same bark was used before for the sorption of metals from single metal aqueous solutions, and the effects of different operating parameters were investigated (8). However, multimetal biosorption equilibria by plant materials and the mechanisms involved in the adsorption of metals using

these materials have not been considered extensively and, therefore, these subjects were treated in this study using pine bark.

MATERIAL AND METHODS

Batch Sorption Process

Pine bark collected from the Ottawa region was washed several times with deionized water and left to dry at room temperature. The dry material was disintegrated in a blender to a particle size between 0.07–0.71 mm and stored for further use in the sorption process.

The batch process was carried out by transferring 10 mL of a solution, either single metal or a mixture, into a plastic tube. The mixture was prepared by solubilizing a combination of either Cu^{2+} + Cd^{2+}, Cu^{2+} + Ni^{2+}, or Cd^{2+} + Ni^{2+} in the concentration range of 0 to 400 mg/L of each of the metals. Copper, cadmium, and nickel were in the form of $CuSO_4 \cdot 5H_2O$, $CdSO_4 \cdot 7H_2O$, and $NiSO_4 \cdot 6H_2O$, respectively. All of the above chemicals were of analytical grade (Sigma Chemical Company, Oakville, Ontario). The initial pH of the metal solutions was adjusted to 4 using NaOH. After the addition of 9.2 mg/mL of bark, it was noticed that the pH dropped to 3.4–3.6 depending on the metal system considered. To avoid precipitation of metals at higher concentrations, the initial pH of the solutions with 9.2 mg/mL of suspended bark were adjusted to 4 using 1 M NaOH. Although the kinetics study showed that after 6 hours of adsorption the uptake of metals by pine bark did not change (8), the above metal systems were agitated on a rotary shaker (Fisher Roto-Rack Model 340) for 24 hours to ensure equilibrium. The adsorbent from the samples was separated by vacuum filtration through a 0.45-μm cellulose filter paper (Millipore Corporation), and the filtrate was analyzed for the metals under consideration using an atomic absorption spectrophotometer (Varian AA-1475 series). The atomic absorption spectrophotometer was also used to measure Ca^{2+}, Mg^{2+}, and K^{+} during the ion-exchange tests. Metal solutions without sorbent were agitated in the tubes and then filtered to determine metal precipitation or its adsorption to the tube walls. The concentration before and after filtration was the same. Each test was carried out in duplicate, and the average results are presented in this work.

Scanning Electron Microscopy and X-Ray Analyses

Scanning electron microscopy (SEM) and energy-dispersive x-ray (EDX) analyses were used in this work to identify the areas on the bark cell for metal sorption. The procedure for these two techniques was described by Huang et al. (9). Fresh samples of bark and bark loaded with metal ions were trimmed as small as was convenient and mounted on stubs with Tissue-Tek

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(Miles Inc., Elkhart, IN). The stub and tissue were transferred to the chuck of a cryo-microtome (CR2000, Research and Manufacture, Inc., Tucson, AZ). The sample was planed with slow cutting strokes, first removing thick sections to clear away the tissue damaged in cutting the piece, and finally with 1 μm sections to produce a smooth surface. The planing extended as far into the specimen as was desired, and the stub and tissue were transferred under liquid nitrogen and then under vacuum to the cold block in a cryo-preparation chamber (CT1500, Oxford Instruments, Eynsham, Oxford, England) held at −180°C. From there, it was removed to the sample stage (−170°C) in the column of a scanning electron microscope (JEOL 6400, JEOL Ltd., Tokyo, Japan), and observed uncoated at 1 kV. EDX microanalysis was done at 15 kV, and images were recorded for the cell surface, cytoplasm, and vacuoles of each tested sample using Polaroid film.

Microanalysis was carried out with a Link eXL, LZ-4 system (Oxford Instruments, Eynsham, Oxford, England), using the Be-window at 15 kV, 35 mm working distance, 33° take-off angle, and probe current set at 1.0 nA with a Faraday cup. X-ray spectra were accumulated to a total of 80,000 counts.

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Electron Spin Resonance

Electron spin resonance (ESR) provides signals corresponding to free radicals and/or paramagnetic ions, which is a count of the number of unpaired electrons (spins) available in a sample. ESR measurements were made at an X-band microwave frequency of ~9.57 GHz using a Bruker ESR 200 DSRC spectrometer at room temperature. The sample, treated or untreated with metal ions or a diluted acid, was washed several times with deionized water, and placed in a 3-mm i.d., 180 mm long ESR tube, capped at the top. The height of bark sample in the tube was about 5 mm. All the tests were run at the following instrument settings: time constant 2 seconds; room temperature; microwave power 2 mW; modulator frequency 100 kHz; scan time 500 seconds; field modulation intensity 20 Gauss.

If all the instrumental conditions, including the sample weight, are identical, the variation in the strength of ESR signal may be taken to be proportional to the number of unpaired electrons and hence the strength of the free radicals. The number of spins of organic free radicals in the bark sample was evaluated by comparing the height of the free-radical ESR signal with the height determined with a standard sample of coke (supplied by Bruker) containing 10^{14} spins measured under similar conditions.

RESULTS AND DISCUSSION

Single-Metal Isotherm

Conventional single-metal sorption isotherms for Cu^{2+}, Cd^{2+}, and Ni^{2+} were obtained in order to determine the capacity of bark for these metals individ-

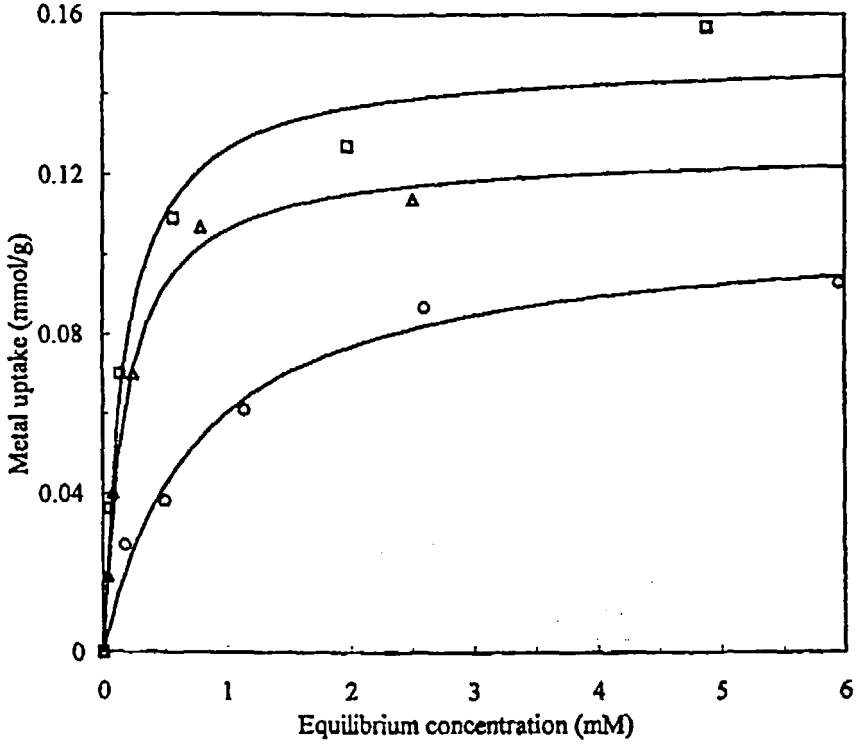

FIG. 1 Sorption isotherms of Cu^{2+} (□), Cd^{2+} (△), and Ni^{2+} (○) using bark. Solid lines represent predicted data by the Langmuir model, and the symbols are the experimental data. Bark concentration: 9.2 mg/mL; initial pH: 4.

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ually. The results (Fig. 1) indicated that an increase in the metal concentration resulted in an increase in the amount adsorbed and that the capacity of the bark for these metals followed the order $Cu^{2+} > Cd^{2+} > Ni^{2+}$. The same effect of metal concentration was also noticed by other researchers who studied, e.g., the adsorption of zinc by soybean hulls, cottonseed hulls, rice straw, and sugar cane bagasse (10) and the removal of cadmium by crushed coconut shells (11). The results for the three metals can be represented reasonably well by the following Langmuir-type adsorption isotherm model (Fig. 1):

$$q = \frac{q_{max} b C_e}{1 + b C_e} \quad (1)$$

where q is the metal uptake per unit weight of bark (mmol/g) at the equilibrium metal concentration, C_e (mM), in the aqueous phase, q_{max} is the maximum metal uptake (mmol/g), and b is the Langmuir constant related to the energy

TABLE 1
Langmuir Parameters for Single-Metal Uptake

Metal ion	q_{max} (mmol/g)	b (mM)$^{-1}$	K_d (mM)
Cu^{2+}	0.149	5.50	0.182
Cd^{2+}	0.126	5.34	0.187
Ni^{2+}	0.107	1.27	0.787

of adsorption (L/mmol). The model parameters, q_{max} and b (Table 1), were determined by fitting the model to the experimental data using the SCIENTIST package. The applicability of the Langmuir model to the experimental data of Fig. 1 indicates monolayer coverage on the bark surface by each of these metals, individually. Table 1 also contains the apparent dissociation constant for the sorption system (K_d) which is the ratio of the desorption rate constant to the adsorption rate constant. This constant is the inverse of the Langmuir constant b. The K_d values indicate that Ni^{2+} has a much higher "net" desorption rate than Cd^{2+} and Cu^{2+}.

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Binary-Metal Isotherm

The collected equilibrium data from the binary systems, Cu^{2+}–Cd^{2+}, Cu^{2+}–Ni^{2+}, and Cd^{2+}–Ni^{2+}, were represented by three-dimensional (3-D) sorption plots (Fig. 2) whereby the metal uptake was plotted as a function of the equilibrium concentrations of the two metals from the same system. To represent the isotherms of the binary-component equilibrium data mathematically (Fig. 2), three different models were used. The first one is the extended-Langmuir model (Model 1) which can be written as

$$q_{e,1} = \frac{q_{max,1} b_1 C_{e,1}}{1 + b_1 C_{e,1} + b_2 C_{e,2}} \tag{2}$$

$$q_{e,2} = \frac{q_{max,2} b_2 C_{e,2}}{1 + b_1 C_{e,1} + b_2 C_{e,2}} \tag{3}$$

where $q_{e,1}$ and $q_{e,2}$ are the equilibrium solid phase concentrations (mmol/g) for the first and the second component, respectively; $C_{e,1}$ and $C_{e,2}$ are the equilibrium concentrations (mM) of these components; $q_{max,1}$ and $q_{max,2}$ represent the maximum uptakes (mmol/g) for the first and second components, respectively, in a mixture of metal ions; and b_1 and b_2 are the Langmuir constants (L/mmol) for the first and second components, respectively.

The second multicomponent isotherm model used in this study is an extension of the three-parameter model, Eqs. (2) and (3), to a five-parameter model

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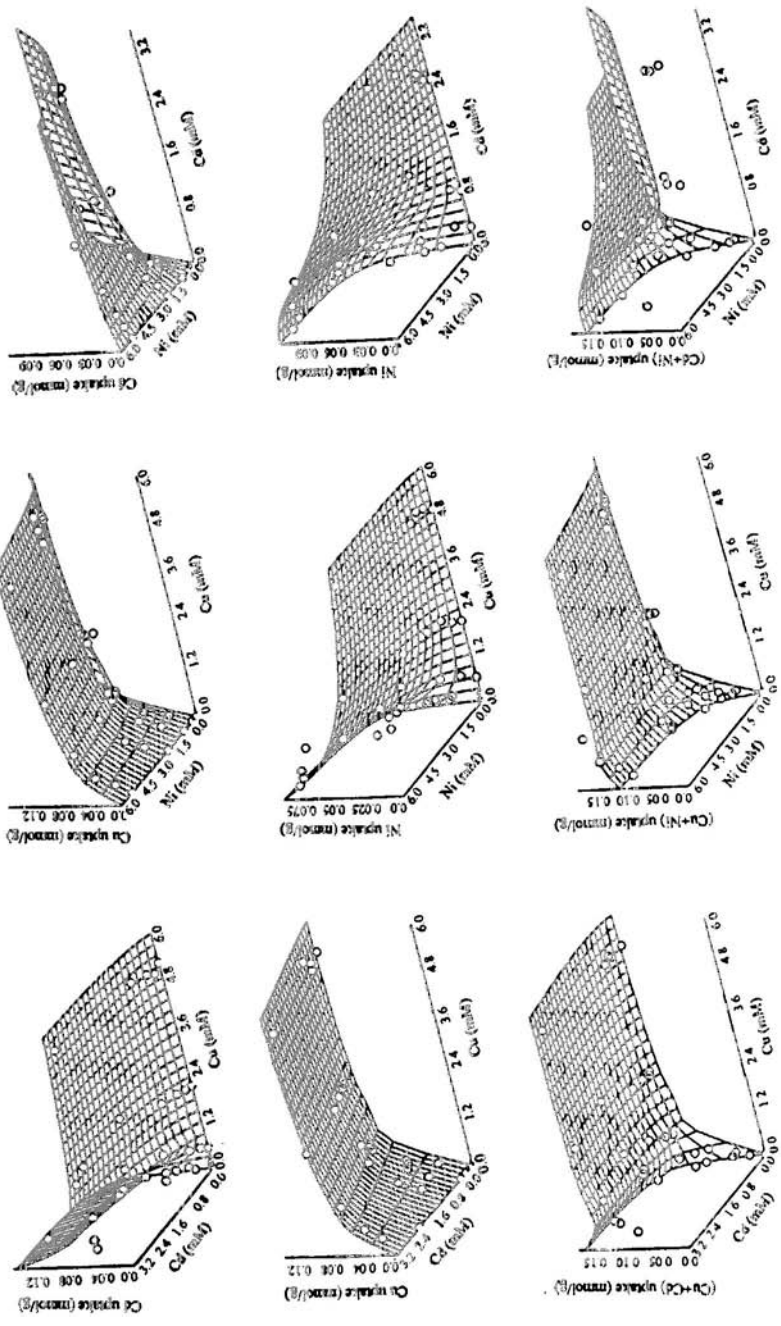

FIG. 2 Binary sorption isotherms of (A) Cu^{2+}–Cd^{2+}, (B) Cu^{2+}–Ni^{2+}, and (C) Cd^{2+}–Ni^{2+} using pine bark. Symbols are experimental data, and the surfaces are predicted by the Langmuir–Freundlich model.

by introducing the exponent n_i in the denominator of the above model (12, 13). This model (Model 2) can be written as

$$q_{e,1} = \frac{q_{max,1} b_1 C_{e,1}}{1 + b_1 C_{e,1}^{n_1} + b_2 C_{e,2}^{n_2}} \quad (4)$$

$$q_{e,2} = \frac{q_{max,2} b_2 C_{e,2}}{1 + b_1 C_{e,1}^{n_1} + b_2 C_{e,2}^{n_2}} \quad (5)$$

where n_1 and n_2 are empirical constants. Although the above model is empirical, it is very often used to describe the equilibrium isotherm data of multicomponent systems (13, 14).

The third model is a combination of the conventional Langmuir and Freundlich models (13, 15) and is called the Langmuir–Freundlich model (Model 3):

$$q_{e,1} = \frac{q_{max,1} b_1 C_{e^1,1}^{n_1}}{1 + b_1 C_{e,1}^{n_1} + b_2 C_{e,2}^{n_2}} \quad (6)$$

$$q_{e,2} = \frac{q_{max,2} b_2 C_{e,2}^{n_2}}{1 + b_1 C_{e,1}^{n_1} + b_2 C_{e,2}^{n_2}} \quad (7)$$

The parameters of these models are presented in Tables 2, 3, and 4. They were obtained by fitting each of Eqs. (2) and (3), Eqs. (4) and (5), and Eqs. (6) and (7) to the experimental data of Fig. 2 using the SCIENTIST package. Based on the extended-Langmuir model, the q_{max} values of Cu^{2+} in the binary Cu^{2+}–Cd^{2+} and Cu^{2+}–Ni^{2+} systems were lower (Table 2) than that obtained from the single Langmuir isotherm (Table 1). This is also the case for Cd^{2+} in the binary Cd^{2+}–Ni^{2+}. The q_{max} value for Ni^{2+} in the binary Cu^{2+}–Ni^{2+} system, obtained from the extended-Langmuir model, is the same as that for

TABLE 2
Estimated Parameters of the Binary Systems Using Model 1

System	Component	Estimated parameters			Statistical parameters	
		q_{max}	b_1	b_2	SSR	MSC
Cu–Cd	Cu	0.130	13.53		0.0132	1.36
	Cd	0.126		3.39	0.0118	
Cu–Ni	Cu	0.132	6.28		0.0051	2.53
	Ni	0.107		1.57	0.0047	
Cd–Ni	Cd	0.103	8.18		0.0050	2.05
	Ni	0.097		3.67	0.0055	

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TABLE 3
Estimated Parameters of the Binary Systems Using Model 2

System	Component	Estimated parameters					Statistical parameters	
		q_{max}	b_1	b_2	n_1	n_2	SSR	MSC
Cu–Cd	Cu	0.095	12.6		0.844		0.0076	1.89
	Cd	0.521		0.56		2.436	0.0071	
Cu–Ni	Cu	0.132	8.27		0.990		0.0044	2.69
	Ni	0.083		2.68		0.835	0.0031	
Cd–Ni	Cd	0.112	7.80		1.181		0.0042	2.15
	Ni	0.082		4.44		0.873	0.0044	

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the single system, while in the Cd^{2+}–Ni^{2+} system it is slightly lower than that of the single isotherm Langmuir model. It should also be emphasized that the extended-Langmuir model gives reasonable estimates of multicomponent equilibrium data provided that the q_{max} values determined from the multicomponent models agree closely with the single-component q_{max} values. This is the basic assumption of the extended-Langmuir model: each species maintains its own molecular area (16). The unequal q_{max} values for single metal sorption (Table 1) and those estimated for the binary systems (Table 2) indicate that the solutes occupy different amounts of surface area (17). Comparison of the b values obtained from the binary systems (Table 2) with those of the single-metal systems indicates that the presence of one metal could decrease or increase the bark's affinity for the other metal depending on the system under consideration. For example, the value of b for Cd^{2+} decreased from 5.34 mM^{-1} in the single-metal system to 3.39 mM^{-1} in the binary system of

TABLE 4
Estimated Parameters of the Binary Systems Using Model 3

System	Component	Estimated parameters					Statistical parameters	
		q_{max}	b_1	b_2	n_1	n_2	SSR	MSC
Cu–Cd	Cu	0.123	13.84		0.637		0.0078	1.98
	Cd	0.173		1.21		0.659	0.0069	
Cu–Ni	Cu	0.139	4.64		0.872		0.0037	2.96
	Ni	0.112		1.59		0.674	0.0043	
Cd–Ni	Cd	0.098	13.84		1.131		0.0023	2.56
	Ni	0.092		4.91		1.067	0.0032	

Cu^{2+}–Cd^{2+}, while it is increased to 8.18 mM^{-1} in the Cd^{2+}–Ni^{2+} system. This indicates that the presence of Cu^{2+} decreases and the presence of Ni^{2+} increases the affinity of bark for Cd^{2+} sorption. This was also observed for the sorption of Cd^{2+} from the Zn^{2+}–Cd^{2+} and Cu^{2+}–Cd^{2+} systems using *Ascophyllum nodosum* seaweed biomass (14).

The comparison between the experimental data and those predicted by the three models, Eqs. (2)–(7), can be presented in terms of the x–y diagrams, where x is the molar composition of the first component in the liquid phase and y is the molar composition of the same component in the sorbent, as shown in Figs. 3A, 3B, and 3C for the Cu^{2+}–Cd^{2+}, Cu^{2+}–Ni^{2+}, and Cd^{2+}–Ni^{2+} systems, respectively. Examining the figures, in general it can be said that all three models can be used to represent these experimental data. The extended-Langmuir model (Model 1) represented the experimental data at higher copper concentrations reasonably well but exhibited considerable deviation at low equilibrium concentrations of this metal (Fig. 3A). This type of deviation of the Langmuir-like model from the experimental data in biosorption studies has also been reported by other researchers (18, 19). The other two models represented the experimental data for lower concentrations better than the extended-Langmuir model. However, at higher copper concentrations there was a significant discrepancy between the experimental and predicted data. When these three models were applied to the experimental data for the Cu^{2+}–Ni^{2+} system, it appeared that Models 2 and 3 represented the experimental data better than Model 1 (Fig. 3B), while for the Cd^{2+}–Ni^{2+} system Models 2 and 3 exhibited more deviation at low Cd^{2+} concentrations than that of Model 1 (Fig. 3C).

Discrimination among the three models can be performed based on the minimum sum square of residuals (SSR). Application of this technique to Model 2 and 3 resulted in lower SSR values for each binary system (Tables 3 and 4) than those obtained using Model 1 (Table 2). This is understandable because of the extra two parameters, n_1 and n_2, in Models 2 and 3. Another criterion that can be used to measure the improvement achieved by an extended model is the use of the model selection criterion (MSC) which is defined (20) as

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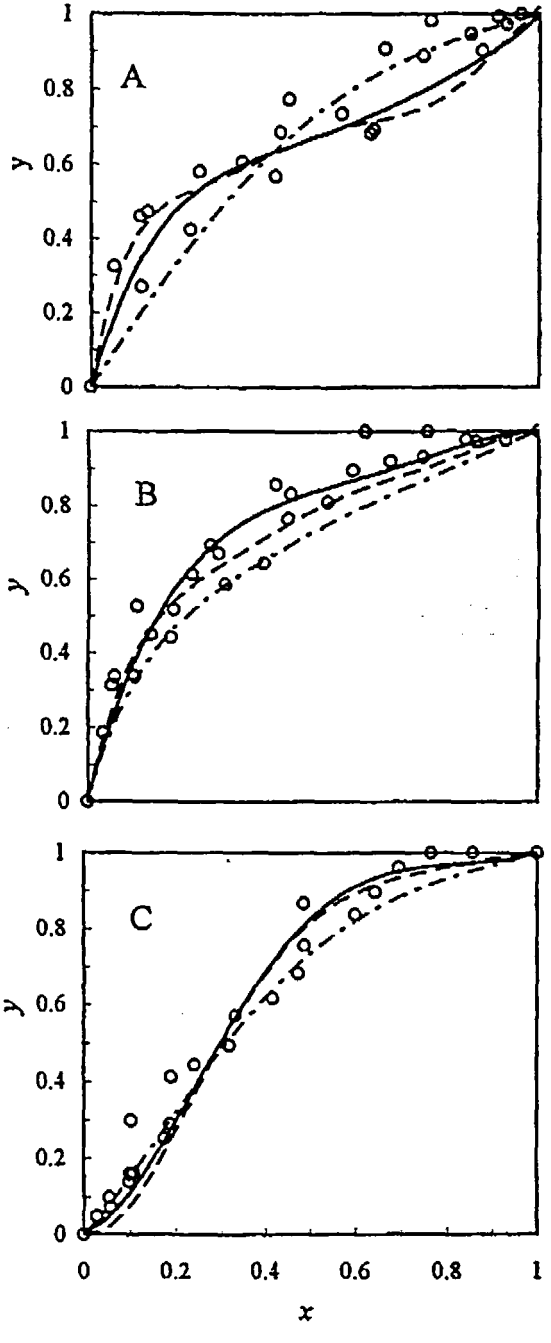
FIG. 3 Comparison between experimental and predicted data for the sorption of (A) Cu^{2+} from Cu^{2+}–Cd^{2+}, (B) Cu^{2+} from Cu^{2+}–Ni^{2+}, and (C) Cd^{2+} from Cd^{2+}–Ni^{2+} by pine bark. Symbols represent the experimental data and solid lines are predicted by Model 1 (–·–·–), Model 2 (– – –), and Model 3 (——). x: molar composition of the adsorbate in the liquid phase; y: molar composition of the adsorbate in the sorbent.



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$$\mathrm{MSC} = \ln\left[\frac{\sum_{i=1}^{n}(q_{\mathrm{exp},i} - \bar{q}_{\mathrm{exp}})^2}{\sum_{i=1}^{n}(q_{\mathrm{exp},i} - q_{\mathrm{calc},i})^2}\right] - \frac{2P}{m} \quad (8)$$

where q_{exp} is the experimentally measured uptake (mmol/g), q_{calc} is the predicted uptake (mml/g) by the model, $\bar{q}_{\mathrm{exp}}$ is the mean of the total measured uptakes (mmol/g), m is the number of experimental data points, and P is the number of parameters in the model. According to this criterion, the most appropriate model is the one with the largest MSC. The MSC values were calculated during the fitting of these models to the experimental data by the SCIENTIST program as one of the outputs from the regression analysis and are also shown in Tables 2, 3, and 4. On the basis of the SSR and MSR data it can be concluded that all three models can be used to represent the experimental binary data from this study. However, Model 2, and especially Model 3, gave a better fitting than Model 1.

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Since Model 3 was the most accurate in representing the experimental data, this model was selected to show the three-dimensional (3-D) sorption isotherm surfaces for the systems studied (Fig. 2). These 3-D isotherm surfaces represent a summary of the two-metal equilibrium results but are difficult to examine. However, to facilitate analysis of the results, two-dimensional (2-D) sorption isotherm curves for one metal at constant equilibrium concentration(s) of the other metal were produced (Fig. 4) by cutting the 3-D sorption isotherm surface at a constant second-metal concentration plane (iso-concentration). The selected cuts through the 3-D diagrams reveal better the quantitative trends observed in the two-metal sorption systems.

From Fig. 4 it can be seen that in each binary system, for a given equilibrium concentration of one metal, the uptake of the other increased with an increase in its concentration. At the same time, with an increase in the uptake of this metal, the adsorption of the metal with a given equilibrium concentration decreased. It can also be noticed that for a given equilibrium concentration of one metal, the total uptake of adsorbed metals either increased with an increase in the concentration of the other metal or remained at almost a constant value as that of the metal when it was alone (e.g., last segments of Figs. 4A–C). The latter might be related to the specificity of adsorption sites and to their affinity for the considered metals.

The effect of the secondary metal on the uptake of the primary metal is displayed in Fig. 5 which has been derived from Fig. 4. For the following discussion, two concentrations of the primary metal ions have been arbitrary chosen to represent "low" (0.5 mM) and "high" (2.5 mM) equilibrium concentrations. Figure 5A shows that at low or high equilibrium concentra-

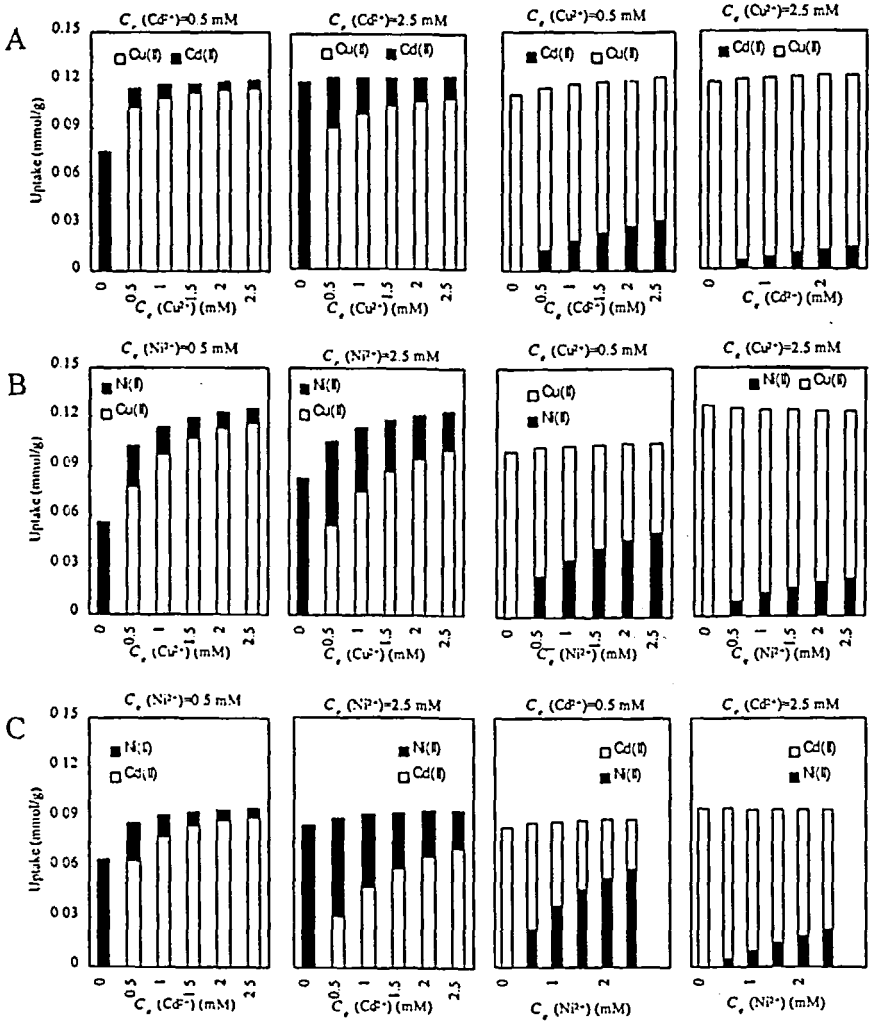

FIG. 4 Iso-concentration cuts of the binary-systems sorption isotherm surfaces shown in Fig. 2 A: Cu^{2+}–Cd^{2+}; B: Cu^{2+}–Ni^{2+}; C: Cd^{2+}–Ni^{2+}.

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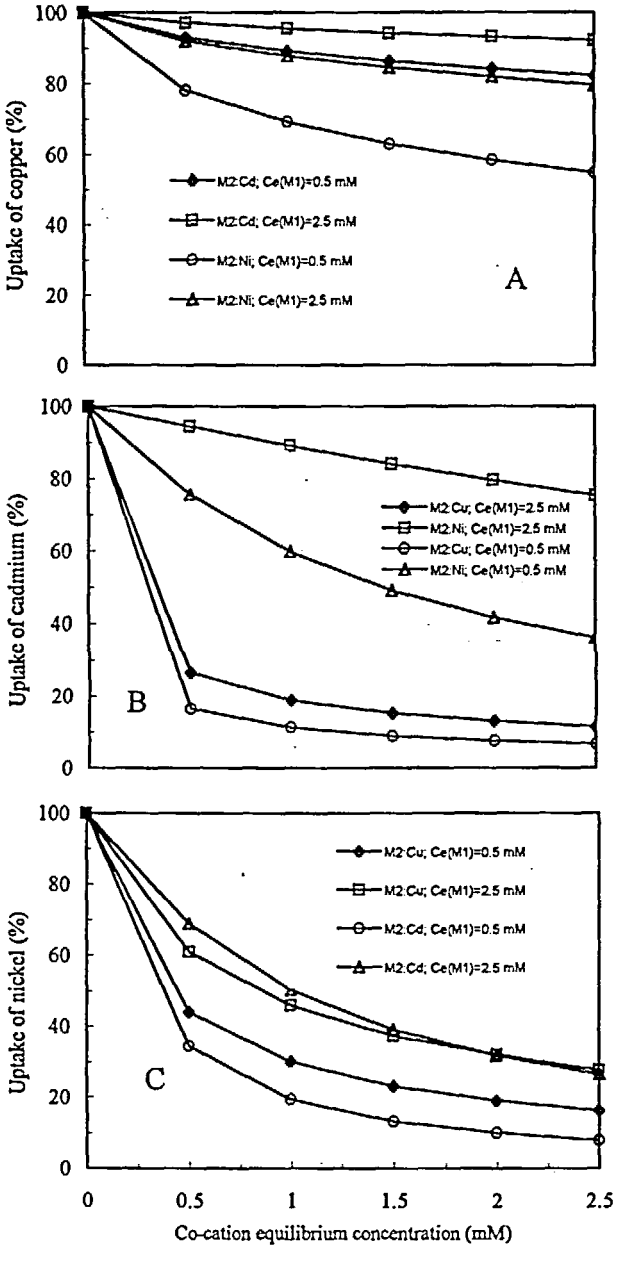

FIG. 5 Effect of the cocation (M2) equilibrium concentration on the uptake of the primary cation (M1) at its equilibrium concentration C_e (M1).

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TABLE 5
Some Physical and Chemical Properties of Metal Ions[a]

Properties	Cu^{2+}	Cd^{2+}	Ni^{2+}
Ionic radius (Å)	0.72	0.97	0.69
Atomic weight	63.4	112.4	57.8
Coordination number	2, 4	4	4, 5
Electron configuration	[Ar] $3d^9$	[Kr] $4d^{10}$	[Ar] $3d^8$
Electronegativity of the atom	1.90	1.69	1.91

[a] Adopted from Lagowski (21) and Purcell and Kotz (22).

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tions of Cu^{2+}, its uptake was reduced by 7 or 5%, respectively, of the initial value when the equilibrium concentration of Cd^{2+} was 0.5 mM. However, this reduction was increased to 18 and 8% when the equilibrium Cd^{2+} concentration reached a value of 2.5 mM for the low and high Cu^{2+} concentrations, respectively. Similar trends can be seen for the adsorption of cadmium and nickel at equilibrium concentrations of cocations (Figs. 5B and 5C).

The previous results showed that the selectivity of bark is of the order $Cu^{2+} > Cd^{2+} > Ni^{2+}$. Table 5 shows some properties of the three metal ions. Tobin et al. (23) reported that the molar uptake of metals, by the biomass of *R. arrhizus*, was higher for metals having larger ionic radii than smaller ionic radii. This correlation was not valid for the results in this work; Cu^{2+} has the smallest ionic radius and was the most sorbed ion among the three ions examined. However, Cu^{2+} has one unpaired electron (Table 5) and is paramagnetic (22). Thus, it can be attracted by a magnetic field possibly originating from the sorbent (24). The other metal ions are very stable (no unpaired electron) and so they are slightly repelled by a magnetic field. Moreover, comparing the coordination numbers of the considered metal ions (Table 5), if the system forms complexes in the sorbent phase, then Cu^{2+} (the least stable among the other metal ions) only requires two additional electrons to coordinate with the sorbent constituents, compared to Cd^{2+} and Ni^{2+} which require at least four electrons. That might be a reason why the sorption of Cu^{2+} was superior compared to the other ions, both in single metal sorption and in a mixture of metals.

Ion-Exchange Mechanism

Ion exchange is considered one of the most predominant mechanisms involved in the biosorption processes (25–27). The existence of this mechanism during metal sorption by bark was investigated in this work by following the release of Ca^{2+}, Mg^{2+}, K^+, and H^+ from this sorbent after the sorption of Cu^{2+}, Cd^{2+}, and Ni^{2+} from single, binary, and ternary systems. The release of cations

from the control, consisting of the sorbent and deionized water, was also measured. The net release of metals from the different metal sorption systems (Table 6) represents the differences between the metal cations measured in the supernatant of the metal system after the sorption process and that of the control.

The results of Table 6 show a significant release of Ca^{2+}, Mg^{2+}, K^+, and H^+ from bark due to the uptake of Cu^{2+}, Cd^{2+}, and Ni^{2+}. This might indicate the displacement of these cations by the considered heavy metals. It appears that there was more Ca^{2+} released whenever Cu^{2+} existed in the systems (Table 6). The displacement of H^+ indicates covalent bonding of the metals (25), while the displacement of Ca^{2+}, Mg^{2+}, and K^+ indicates ionic bonding with the metal (27). The relative amounts of these two groups of cations released from the sorbent would provide information about the strength of the two kinds of bonding. Consequently, the release of larger amounts of Ca^{2+}, Mg^{2+}, and K^+ than H^+ from the bark indicates that the ionic bonding involved in this sorption process is much more significant than the covalent bonding. Avery and Tobin (27) reported that for the release of cations from a biosorbent due to metal uptake, the stoichiometric ratio of Ca^{2+} or Mg^{2+} to H^+ was always equal to 2. Taking into consideration the release of K^+ from the bark, the same ratio should exist between Ca^{2+} or Mg^{2+} and this metal ion. Therefore, the release of one Ca^{2+} or Mg^{2+} ion would be equivalent to a release of two ions of H^+ or K^+.

Crist et al. (25) and Tobin et al. (28) stated that pure ion exchange occurs at equimolar concentrations. This implies that the ratio of the metal(s) bound to the metals released ($R_{b/r}$) is equal to unity. In this study it was noticed that $R_{b/r}$ values (Table 6) are close to 1 for the binary $Cu^{2+} - Ni^{2+}$ and the ternary $Cu^{2+} - Cd^{2+} - Ni^{2+}$ systems. However, the values of $R_{b/r}$ are less than 1 for

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TABLE 6
Release of Ca^{2+}, Mg^{2+}, K^+, and H^+ Due to Sorption of Cu^{2+}, Cd^{2+}, and Ni^{2+} by Bark. Initial Concentration of Each Heavy Metal: 100 ppm; Bark Concentration: 9.2 mg/mL

System	Total metal bound (mM)			Amount of cation released[a] (mM)				$R_{b/r}$[c]
	Cu^{2+}	Cd^{2+}	Ni^{2+}	Ca^{2+}	Mg^{2+}	K^+	H^+	
Control[b]	—	—	—	0.057	0.022	0.097	0.040	
Cu^{2+}	0.795	—	—	0.558	0.248	0.348	0.200	0.74
Cd^{2+}	—	0.608	—	0.301	0.337	0.476	0.194	0.63
Ni^{2+}	—	—	0.651	0.275	0.215	0.361	0.169	0.86
Cu^{2+}–Ni^{2+}	0.865	—	0.200	0.514	0.256	0.358	0.200	1.02
Cu^{2+}–Cd^{2+}	0.649	0.283	—	0.528	0.079	0.061	0.179	1.28
Cu^{2+}–Cd^{2+}–Ni^{2+}	0.461	0.208	0.183	0.604	0.064	0.069	0.189	1.07

[a] Difference between metal released by system and that by the control.
[b] Bark and water.
[c] $R_{b/r}$: ratio of metal bound to metal released.

single metal sorption by bark, indicating that the sum of the cations released ($Cu^{2+} + Mg^{2+} + K^{+}/2 + H^{+}/2$) was greater than the amount of metal bound. These results indicate that ion exchange could be an important mechanism for the binding of the metal(s) by bark.

Scanning Electron Microscopy and X-Ray Analyses

Scanning electron microscopy analysis is another technique to investigate the mechanisms of biosorption. This technique has been used by several researchers to study the sorption of heavy metals by microbial sorbents, but to the best of our knowledge it has not been used for the sorption of metals by plant materials. Therefore, it was decided to use the technique in order to have additional insight into the sorption mechanisms by the plant material considered in this work. In this study, electron microscopy examination of bark before and after metal ion sorption was carried out to locate the active sorptive areas of this sorbent. Energy-dispersive x-ray analysis was used to confirm the identity of the metal in different parts of the cell.

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The electron micrographs of bark before and after Cd^{2+} sorption are shown in Fig. 6. It is noticed that in the electron micrograph the cells after Cd^{2+} sorption (Fig. 6B) are more dense than the cells which were not in contact with these ions (Fig. 6A). The change in the structure of the cell material was probably influenced by the cadmium ions. Thus, it was believed that the dense areas contain most of the sorbed metal. To confirm this assumption, energy-dispersive x-ray microanalysis was carried out. The EDX spectra for the untreated cells of the bark show (Figs. 7A–C) that the energy level of the cadmium remained at the same level as the background when the microprobe was focused on the cell wall, cytoplasm, and vacuoles areas. The cell wall of the bark was characterized by the appearance of Ca^{2+} (Fig. 7A). This element was not detected by EDX analyses in the cytoplasm or the vacuoles. The energy spectrum of phosphorus was always used as an indicator of cytoplasm areas (Fig. 7B). The vacuoles were completely evacuated; no components were detected.

Figures 7D–F present EDX spectra of the bark cells after Cd^{2+} sorption. When the microprobe was focused on the dense areas of the cell walls, higher cadmium levels were observed, corresponding to the α- and β-cadmium spectral lines (Fig. 7D). Interestingly, a comparison between Figs. 7A and 7D shows that the Ca^{2+} in the bark cell wall decreased significantly after Cd^{2+} binding. This is consistent with the results of the AAS presented previously (Table 6), and confirms that ion exchange is one of the mechanisms involved in this sorption process.

The microprobe focused on the cytoplasm of the bark cells revealed small Cd^{2+} peaks (Fig. 7E), indicating that a small amount of this metal was transferred into this part of the cell. However, on the basis of the energy level spectral lines corresponding to Cd^{2+} for the cell wall and the cytoplasm, it can be con-

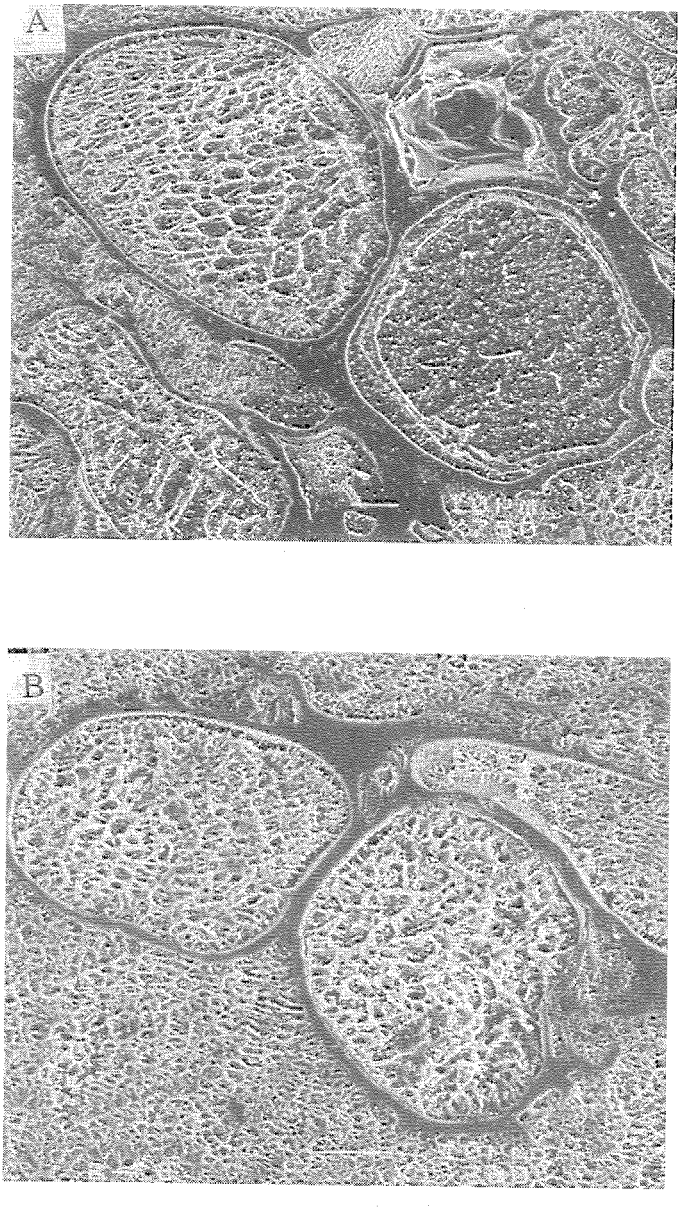

FIG. 6 Scanning electron micrographs (750×) of untreated bark cells (A) and treated bark cells with 100 ppm of Cd^{2+} (B). Cadmium uptake: 0.058 mmol/g.

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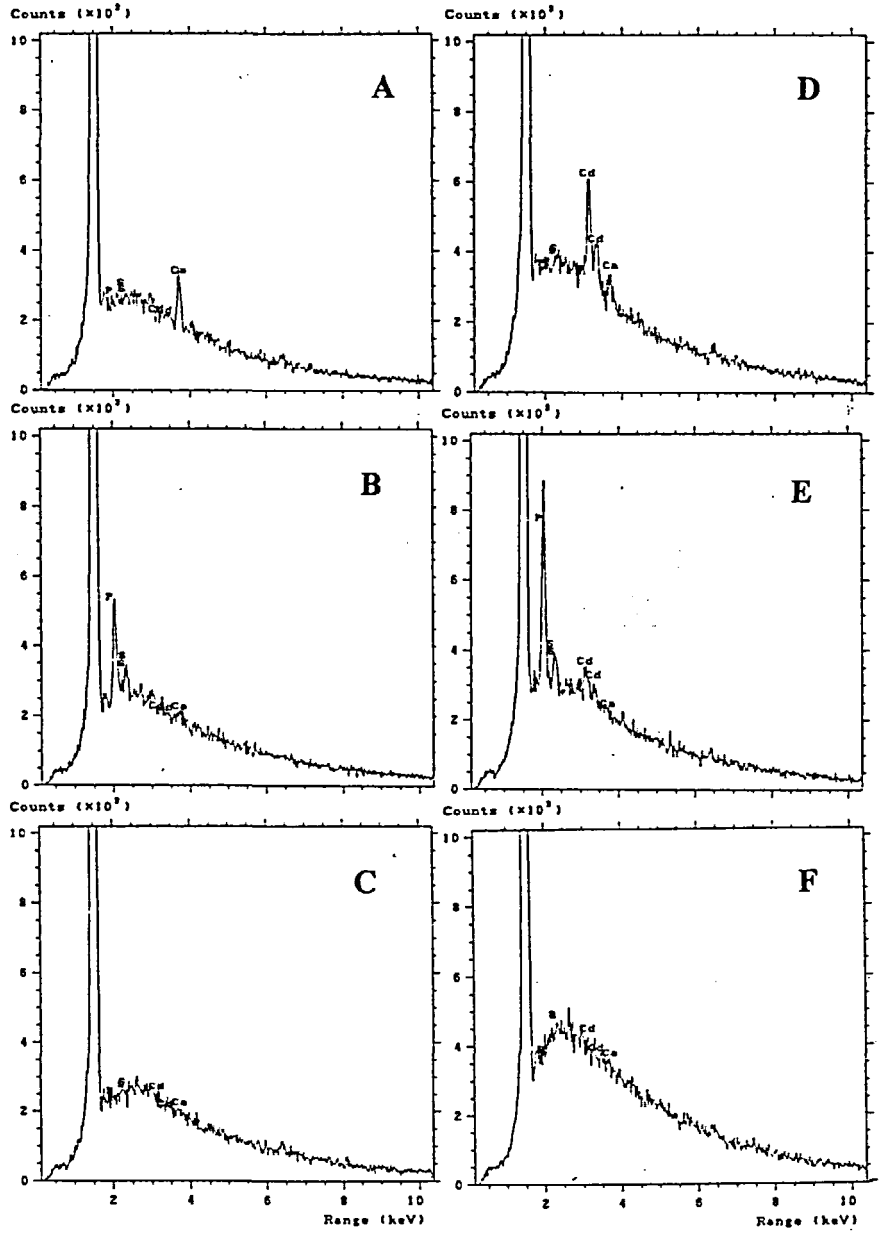

FIG. 7 Energy-dispersive x-ray of bark before Cd^{2+} uptake (A, cell wall; B, cytoplasm; C, vacuoles) and after Cd^{2+} uptake (D, cell wall; E, cytoplasm; F, vacuole).

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cluded that the majority of this metal was associated with the former cellular structure. Most researchers who studied the areas of metal sorption on microbial biomass observed that heavy metals were deposited at the cell walls (26, 29–32). However, electron microscopy examination for the uptake of gold by the algal biomass *Ascophyllum nodosum* (6) revealed that in addition to a considerable amount of this precious metal associated with the cell wall, a small amount of gold was detected inside the cell when they were exposed to high initial metal concentrations for long periods of time.

It was observed that the energy level spectral lines of Cd^{2+} remained in the background when the microprobe was focused on the vacuoles of the bark cells (Fig. 7F). This means that the vacuoles were not involved in the sorption process. This could be due to the low cadmium concentration in the cytoplasm area and, thus, the driving force was not high enough to bring the metal ions into the vacuoles. It might happen that with a higher metal concentration and a longer contact time, cadmium could penetrate into the vacuoles areas as well.

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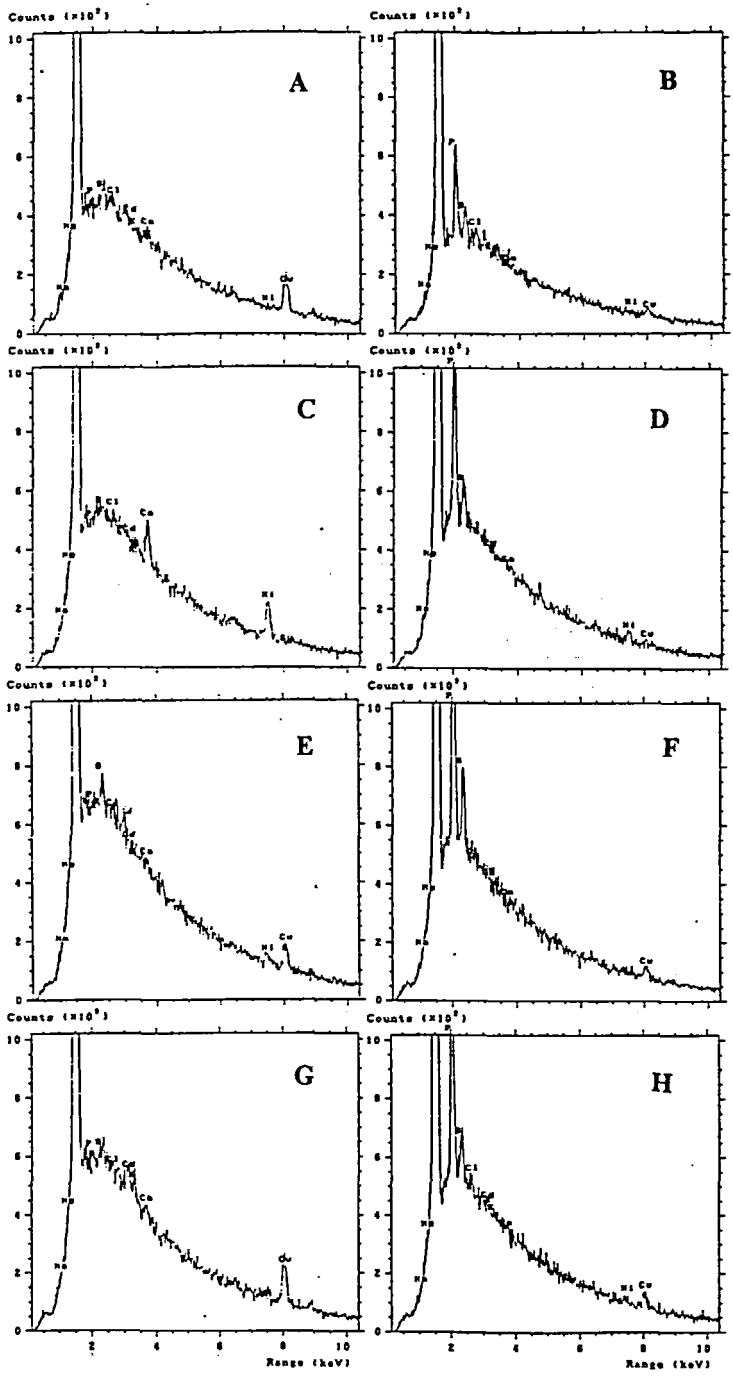
These results indicate that the cell wall of the bark represents the main area for Cd^{2+} binding and only a small amount of Cd^{2+} ions penetrated into the cytoplasm. The precipitation of Cd^{2+} at the bark cell wall was not observed (Fig. 6B). This is contrary to the findings of Tsezos and Volesky (31, 32) and Mullen et al. (29) who studied the adsorption of thorium and uranium at the cell wall of *Rhizopus arrhizus*, and the adsorption of silver and lanthanum at the cell wall of *Bacillus subtilis* and *Pseudomonas aeruginosa*, respectively. They reported precipitation of these elements at the cell walls of the microorganisms. In some cases needle-like deposits of metals were noticed at the cell wall area (29). However, the observations from this study are in agreement with those reported by Mullen et al. (29) who detected, using x-ray analysis, adsorbed copper and cadmium ions on the microbial cell wall but did not notice the metal precipitates on electron micrographs.

Samples of bark loaded with other metal systems, namely Cu^{2+}, Ni^{2+}, Cu^{2+}–Ni^{2+}, and Cu^{2+}–Ni^{2+}–Cd^{2+}, were also tested using electron transmission microscopy. As mentioned for the adsorption of Cd^{2+}, the electron micrographs of these metal systems (not presented) also showed that the bark cells exposed to each metal system were more dense than the untreated cells. The EDX spectra of the bark cell wall and cytoplasm for these metal systems are presented in Fig. 8. At the cell wall of the Cu^{2+}-laden and Ni^{2+}-laden

FIG. 8 Energy-dispersive x-ray of bark exposed to Cu^{2+} (uptake: 0.082 mmol/g; A, cell wall; B, cytoplasm), Ni^{2+} (uptake: 0.064 mmol/g; C, cell wall; D, cytoplasm), Cu^{2+}–Ni^{2+} [uptake (mmol/g): Cu^{2+}, 0.084; Ni^{2+}, 0.02; E, cell wall; F, cytoplasm] and Cu^{2+}–Ni^{2+}–Cd^{2+} [uptake (mmol/g): Cu^{2+}, 0.051; Ni^{2+}, 0.01; Cd^{2+}, 0.02; G, cell wall; H, cytoplasm].



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bark, EDX spectra show higher levels, with respect to the background, corresponding to copper (Fig. 8A) and nickel (Fig. 8C) spectral lines, respectively. It is also interesting to note that the Ca^{2+} peak disappeared from the spectra of cell walls of the bark that were exposed to copper ions while the peak still remained in the spectra of bark cells exposed to nickel ions. This confirms the fact that the uptake of copper was higher than that of nickel, and could indicate that ion exchange with copper is more significant than with nickel. These results are also consistent with the results of Table 6 which show that more Ca^{2+} cations were released to the solution due to copper binding than due to nickel binding. EDX microanalysis of the cytoplasm of bark cells exposed to copper or nickel detected small peaks for both of the metals (Figs. 8B and 8D). This shows that only small amounts of these metals diffused into the cytoplasm.

When the microprobe was focused at the bark cell wall that was exposed to the Cu^{2+}–Ni^{2+} system, Cu^{2+} and Ni^{2+} were detected (Fig. 8E). The smaller peak due to Ni^{2+} was expected because of the suppression of Ni^{2+} binding by Cu^{2+} in a mixture of the two. Calcium also disappeared from the bark cell wall (Fig. 8E). Its disappearance can be attributed solely to the copper uptake, since nickel alone did not affect the Ca^{2+} peak significantly (Fig. 8C). The EDX spectra of the cytoplasm of the bark cells loaded with Cu^{2+} and Ni^{2+} revealed a small Cu^{2+} peak relative to the background (Fig. 8F). Nickel was not observed in the cytoplasm, probably due to the suppression of its adsorption by copper. Figures 8G and 8H show EDX analyses for the ternary metal system composed of Cu^{2+}, Cd^{2+}, and Ni^{2+}. The microanalysis detected only Cu^{2+} and Cd^{2+} at the bark cell wall (Fig. 8G). However, the peak for Cd^{2+} is lower than that for Cu^{2+} because of the lower uptake of cadmium in this system. Nickel was not detected in the bark cell because of its negligible uptake in this ternary system due to suppression by Cu^{2+} and probably by Cd^{2+} ions as well. Only a small Cu^{2+} peak was detected in the cytoplasm of the bark cells loaded with these three metal ions (Fig. 8H). Cadmium was not able to diffuse into the cytoplasm, possibly due to its low concentration on the bark cell wall in this ternary system.

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Electron Spin Resonance

The electron spin resonance technique was used to study the mechanism of the sorption of uranium and copper by *R. arrhizus* (32) and that of copper by *Ganoderma lucidum* (34). ESR was applied in this work to examine the participation of free radicals in copper sorption by bark.

Samples of bark were exposed to 25, 50, 100, and 200 ppm initial copper concentrations. At equilibrium, the amount of copper adsorbed was measured. The bark was separated, dried, and then the ESR spectra were obtained for

samples loaded with different amounts of copper as well as for the pure sample (the spectra are not shown here). Each of these spectra showed an ESR line that corresponds to a g value (Landes constant) of 2.0079 ± (4.17 × 10^{-4}), indicating the presence of free radicals in the bark. It was noticed that the intensity of these lines decreased in the samples of bark exposed to copper ions with an increase in copper concentration. This could indicate that Cu^{2+} is chemically coordinated in the sorbent media. This hypothesis is supported by the ESR signals from the bark after Cu^{2+} sorption which show an ESR line at a g value of 2.0785 ± (3.46 × 10^{-4}) which is due to Cu^{2+} ions (35). The results were used to calculate the relationship between copper uptake and free radical concentration in the bark (Fig. 9).

It has been reported (34) that the reduction in the signal strength of free radicals after sorption would lead to two inferences:

1. The group having the free radicals coordinates with the metal causing the pairing of electrons.

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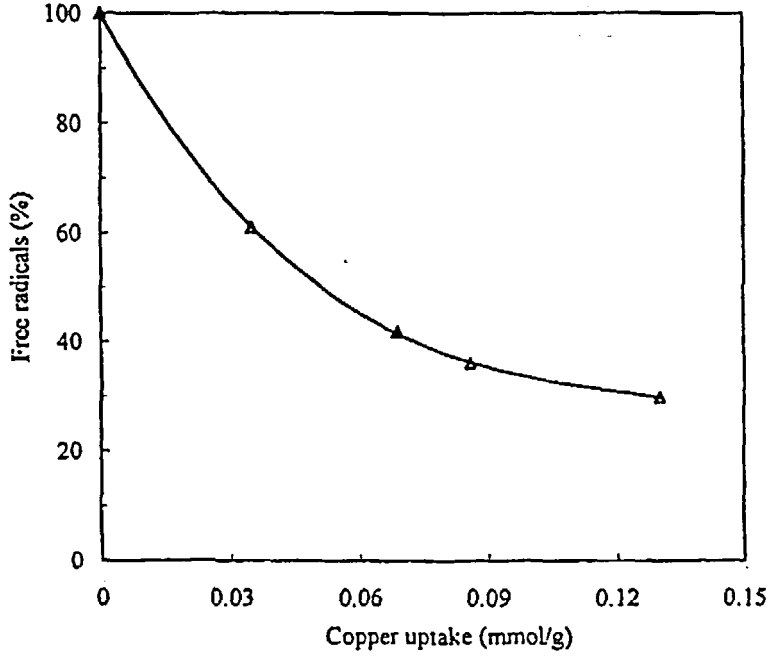

FIG. 9 Relationship between the copper uptake and the free radical concentration in bark. Initial free radical concentration: 5.7 × 10^{16} spins/g.

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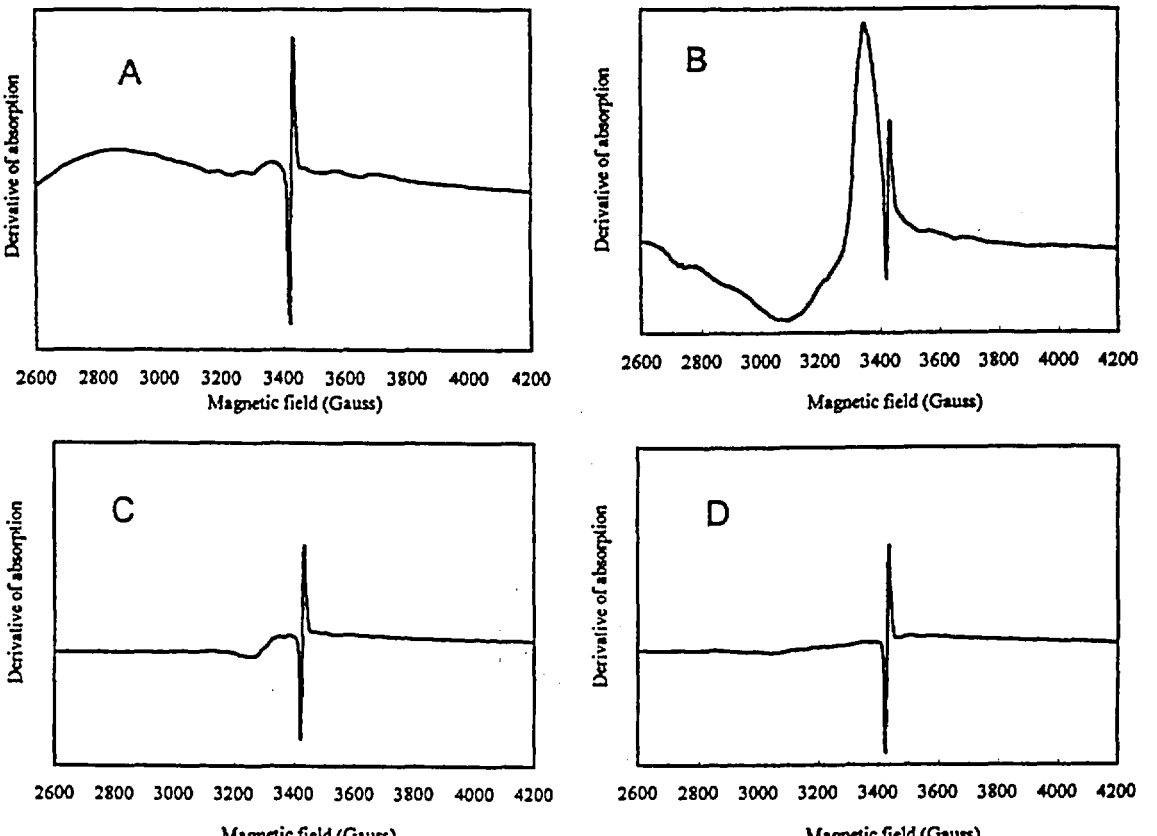

FIG. 10 ESR spectra for pure bark (A), bark exposed to 100 ppm Cu^{2+} solution (B), regenerated bark after Cu^{2+} desorption using 0.1 M HCl (C), and bark treated with 0.1 M HCl (D). Gain = 2×10^4; bark concentration: *9.2 mg/mL; initial pH: 4.5.*

TABLE 7
Concentration of Free Radical Before and After Cu^{2+} Sorption[a] by Bark, After Desorption, and After Treatment with HCl

Sorbent	Free radicals concentration (spins/g) $\times 10^{-16}$
Untreated	5.68 ± 0.495
After Cu^{2+} sorption[b]	1.89 ± 0.219
After Cu^{2+} desorption with HCl[c]	2.50 ± 0.134
Treated with HCl	3.20 ± 0.141

[a] Initial copper concentration: 100 ppm; bark concentration: 9.2 mg/mL; initial pH: 4.5.
[b] Copper uptake: 0.082 mmol/g.
[c] Recovery of Cu^{2+} after desorption: 96.5%.

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2. The cellular matrix embedding the free radical opens up upon metal sorption, whereby the free radical pairs its electrons without direct interaction with the metal.

Therefore, if the free radicals contained in the bark are coordinating with the metal, they should be restored when the metal is desorbed, unless pairing again takes place with the dilute acid used for desorption. This was investigated in this work for bark by obtaining the ESR spectra for pure sorbent before sorption, after sorption with 100 ppm Cu^{2+} solution, after desorption of Cu^{2+}-laden bark using 0.1 M HCl, and for bark treated only with 0.1 M HCl. The results of ESR spectra for these four kinds of samples are shown in Figs. 10A–D, with the concentrations of free radicals for each of them displayed in Table 7. Figure 10B also shows an ESR line at a g value of 2.0785 ± (3.46×10^{-4}) which is due to Cu^{2+} ions.

Figures 10A and 10C as well as Table 7 show only partial restoration (about 16%) of the free radicals after the desorption of copper from bark. Despite this, it can be considered that the free radicals coordinated with copper. The incomplete recovery of free radicals could be due to acid activity which may have caused opening of the cellular matrix and enabled free radicals to pair their electrons. This conclusion is supported by the observed decrease in the number of free radicals after treatment of the bark with the acid (Fig. 10D).

CONCLUSIONS

The following conclusions can be drawn from the above results.

1. The selectivity of bark for single metal sorption was as follows: $Cu^{2+} > Cd^{2+} > Ni^{2+}$.

2. In a mixture of metal ions, the preference of one metal influenced, competing or excluding, the uptake of other metal in the solution.
3. The collected binary equilibrium data, using pine bark, were well represented by the three models used.
4. Energy-dispersive x-ray spectra and AAS analyses demonstrated that ion exchange represented one of the important mechanisms involved in the sorption process using pine bark.
5. The SEM and EDX analyses showed that the cell wall of the bark was the main active area for metal sorption, and only a small amount of metal penetrated into the cytoplasm. However, the results showed that the vacuoles of the bark cell were not involved in the sorption process.
6. Electron spin resonance demonstrated that free radicals from the bark play a significant role in the sorption process. The free radical concentration in the bark decreased with an increase in the adsorbed Cu^{2+} concentration.

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