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Sorption of Heavy Metals by Biological Materials

by

© Sameer Al-Asheh

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Dedication

This work is dedicated to my dear wife Nesreen and my children Acil, Sara and Yousef

Abstract

In this work the ability of some plant materials, as potentially new sorbents, to sorb heavy metals from aqueous solutions was investigated. In addition, a new microbial sorbent, that has not been tested before for the sorption of heavy metals, was also considered.

The microorganism *Aspergillus carbonarius* and the following plant materials were found to be able to sorb copper ions from aqueous solutions: canola meal (CM), pine bark, pine needles, pine cones, moss, oak leaves, oak cobs, maple leaves, birch leaves and cider needles. Of these sorbents *A. carbonarius*, CM, pine bark and moss were considered more extensively in this work. The uptake of metal ions per unit mass of sorbent decreased with an increase in the concentration of the sorbent. Increasing the initial metal concentration in the solution resulted in an increase in uptake per unit mass of sorbent. The initial pH and temperature of the solution also influenced the sorption process.

The kinetics of metal uptake by *A. carbonarius*, CM, bark and moss were studied. It was found that, for all of these sorbents, most of the metal ions were attached to the biosorbents within the first 10 minutes of the sorption process, and after that, the rate of metal uptake became very slow. This indicates that the sorption occurred mainly at the surface of the biosorbents. The slower rate of metal adsorption, which was more pronounced at low sorbent concentrations, indicates the involvement of intrapore diffusion in the sorption process.

Sorption studies using *A. carbonarius* showed that pre-heating of this biomass to higher temperatures prior to utilizing it in the biosorption tests decreased its metal sorption capacity. Preincubation of the active biomass with glucose enhanced metal sorption and the optimum glucose concentration in this process depended on the pH of the solution. The affinity of *A. carbonarius* for different metal ions followed the order (molar basis): $Cu^{2+} > Cr^{3+} > Cd^{2+}$.

It has been noticed in this study that utilization of the same ratios of CM to zinc concentrations, regardless of their levels, resulted in almost the same amount of metal sorbed per unit mass of CM. It was found that the metal sorption capacity of this sorbent decreased as the phytic acid content decreased. The uptake of metal ions was enhanced with decreasing CM particle sizes. The affinity of CM for the sorption of the tested metals is shown by the following order (molar basis): $Zn^{2+} > Cr^{3+} > Cu^{2+} > Cd^{2+} > Ni^{2+}$.

The ability of moss to adsorb various metal ions was examined in a batch sorption process. The affinity of this sorbent toward heavy metals was (molar basis): $Cr^{3+} > Cu^{2+} > Zn^{2+} > Ni^{2+} > Cd^{2+}$. The sorption of Cu^{2+} by moss was tested using various copper salts, and it was found that the copper uptake from $CuCl_2$ solution was higher than that from $CuSO_4.5H_2O$ followed by $Cu(NO_3)_2.3H_2O$ solution. Sulfuric, hydrochloric and nitric acids were good desorbents for copper ions from moss. The desorption process was affected by both the volume and the concentration of these desorbing agents.

The performance of moss for metal sorption was also examined in a continuous process using a column packed with this sorbent. Increases in the flow rate or the influent metal concentration resulted in shorter break-through times and higher copper uptakes per unit mass of moss. Sorption of copper was also carried out using two moss-packed columns connected either in parallel or in series. The results showed the same total metal loading in both cases. Desorption of copper from the moss-packed column was carried out using different concentrations of HCl. A solution of 0.025 M HCl was found the most suitable for this process. It has also been demonstrated that the efficiency of the moss-packed column did not change significantly after five sorption/desorption cycles.

Pine bark can be used for the sorption of the following metals from aqueous solutions with the following order of capacities (molar basis): $Cu^{2+} > Zn^{2+} > Cd^{2+} > Ni^{2+}$. It can be re-utilized several times for further adsorption processes without regeneration. The reuse of bark after regeneration resulted in a decrease of the absolute value of the newly adsorbed Cd^{2+} ions compared to the fresh bark, however the total loading still increased. The desorption of Cd^{2+} was more efficient with higher molarity HCl than with lower molarity. A high concentration of soft or hard ions strongly depressed the uptake of cadmium by pine bark. It was also found that bark pre-loaded with one metal ion responded better for the sorption of another metal ion.

The performance of CM, bark and moss for the removal of copper from industrial wastewater was tested. The copper concentration in the wastewater was decreased from 36.5 to 2.5, 4.1 and 5.2 ppm by bark, moss and CM, respectively, when the sorbents concentrations were 18.4 mg/mL. When the wastewater was treated with 9.2 mg/mL of bark, followed by treatment with 2.3 mg/mL of activated carbon, the copper concentration was decreased to the permissible limit in drinking water. This study also showed that the utilization of the same amount of sorbent in either single- or multi-stage sorption processes resulted in the same level of copper reduction. The wastewater was also treated in a column packed with bark, and after three sorption/desorption cycles, the effluent copper was decreased to 4 ppm.

Single, binary, ternary and quaternary metal systems of Cu^{2+}, Ni^{2+}, Cd^{2+} and Pb^{2+} were considered. In single metal sorption, the selectivity of the sorbents for each of these metal ions has the following order: Cu^{2+}: moss > CM > bark; Cd^{2+} and Ni^{2+}: CM > bark > moss; Pb^{2+}: CM = bark ≈ moss. It has been found that, in a mixture of metal ions, the adsorption of one metal was affected by the presence of the others. It was also noted that the uptake of Ni^{2+} by each of the three sorbents decreased significantly when Cu^{2+} was present with this metal, especially in ternary and quaternary metal systems. When the four metals existed together in a quaternary mixture, the following order (molar basis) of preference was obtained for bark and moss: $Cu^{2+} > Pb^{2+} > Cd^{2+} > Ni^{2+}$, while for CM the order was: $Cu^{2+} > Cd^{2+} > Ni^{2+} > Pb^{2+}$.

Two types of mathematical models were proposed in this work to represent the dynamics of the batch sorption process. The first model assumed that sorption occurred only at the surface of the sorbent. This model fit the experimental data reasonably well at high sorbent concentrations, but resulted in a significant deviation from the experimental data at low sorbent concentrations where intrapore diffusion was more significant. The

second model was a modification of the first model. It took into consideration the intrapore diffusion and, therefore, represented the experimental data very well for both high and low concentrations of the sorbent. The collected single metal equilibrium data were well represented by the linearized Freundlich and Langmuir isotherm models. Binary equilibrium data were also collected in this work using pine bark. These data were represented by three types of multicomponent equilibrium models which resulted in good fitting of the experimental data. The model of Bohart and Adams was used to analyze the data obtained from the packed sorption process. The model resulted in quantitative determination of the characteristic process parameters which can be used for performance comparison and process design.

The global compositional analysis showed that lignin and carbohydrates for bark and moss, and proteins, carbohydrates and phytic acid for CM, are the major components responsible for the sorption of heavy metals. The roles of the carboxyl and amine groups in the sorption process were also examined. It was found that the esterification of the carboxyl or the methylation of the amine groups in CM, bark and moss resulted in a lower metal uptake by these sorbents. The ion-exchange mechanism in the heavy metals sorption by CM, bark and moss was investigated. It was found that this mechanism is important in this sorption process for moss and bark but it was insignificant for CM. Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray (EDX) microanalyses revealed that metal ions were sorbed mainly at the cell wall and only a small amount of ions diffused into the cytoplasm. The EDX spectra also showed a release of Ca^{2+} ions, due to metal sorption, from the cell wall of bark and moss, while this was not noticed in CM tests. Electron Spin Resonance tests demonstrated that free-radicals from the sorbents play a significant role in the sorption processes. The groups with these free radicals directly coordinated with Cu^{2+} ions in the case of moss. In the case of CM and bark, the cell wall matrix opened up for metal uptake and the free radicals paired to each other rather than coordinating with the metal ions.

Résumé

Dans cette étude, on a examiné la capacité des matériaux dérivés des plantes, comme adsorbants nouveaux et potentiels, d'adsorber les métaux lourds des solutions aqueuses. Des plus, l'étude a été étendue pour inclure l'adsorption des métaux lourds par un nouveau adsorbant microbien.

On a trouvé que le microorganisme, *Aspergillus carbonarius*, et les matériaux dérivés des plants suivants: tourteaux de canola (CM), écorce de pin, aiguilles de pin, cônes de pin, mousse, feuilles de chêne, épis de chêne, feuilles d'èrable, feuilles de bouleau et aiguilles de cidre sont capables d'adsorber les ions de cuivre des solutions aqueuses. Dans ce travail, on a considèrablement étudié l'*A. carbonarius*, CM, l'écorce de pin et la mousse. L'adsorption des ions metalliques par unité de masse d'adsorbant a diminué quand la concentration de l'adsorbant a augmenté. L'augmentation de la concentration initiale du métal dans la solution a entrainé une augmentation de la prise par unité de masse d'adsorbant. On a aussi trouvé que le pH initial de la solution n'a pas sensiblement influencé le processus d'adsorption quand la concentration de la solution était élevée. Par contre, à de basses concentrations, on a remarqué une augmentation significative de la prise du métal causée par une augmentation du pH initial de la solution. De plus, on a remarqué que l'adsorption des métaux par CM, l'écorce de pin et la mousse a augmenté quand la température a augmenté indiquant la nature endothermique du processus.

Dans cet ouvrage, on a aussi étudié la cinétique de la prise du métal par l'*A. carbonarius*, CM, l'écorce de pin et la mousse. On a trouvé que la plupart des ions métalliques ont été attachés aux bio-adsorbants durant les premières 10 minutes du processus d'adsorption, ensuite, le taux de prise du métal est devenu très lent. Le taux lent d'adsorption, qui a été dominant à de basses concentrations d'adsorbant, indique qu'une diffusion intraporeuse a participé au processus d'adsorption.

Les études faites sur le processus d'adsorption, utilisant l'*A. carbonarius* comme adsorbant, ont montré que le préchauffage de la biomasse, avant de l'utiliser dans les analyses d'adsorption, a entrainé une diminution de sa capacité d'adsorber le métal. La pré-incubation de la biomasse active avec du glucose a augmenté l'adsorption du métal, et la concentration optimale du glucose dans ce processus dépendait du pH de la solution. L'affinité du *A. carbonarius* pour differents ions métalliques suivait l'ordre (base molaire): $Cu^{2+} > Cr^{3+} > Cd^{2+}$.

Dans cette étude, on a remarqué que l'utilisation des proportion égales de concentration de CM et de zinc, indépendamment du niveau d'égalité, a abouti à une adsorption presque ègale du zinc par unité de masse de CM. L'acide phytique jouait aussi un rôle très important dans le processus d'adsorption. La réduction de la quantité de cet acide a entraîné une réduction de la capacité d'adsorption du métal par le CM. L'adsorption des differents métaux par CM a suivi l'ordre suivant: $Zn^{2+} > Cu^{2+} > Cd^{2+} > Cr^{3+} > Ni^{2+}$.

Quand l'adsorption a été traitée par lots, on a examiné la capacité de la mousse d'adsorber une variété des métaux. L'affinité de cet ordre adsorbant pour les ions métalliques a suivi l'ordre suivant: $Cr^{3+} > Cu^{2+} >$

$Zn^{2+} > Zn^{2+} > Cd^{2+}$. Pour examiner l'adsorption du Cu^{2+} par la mousse on a utilisé une variété de sels de cuivre, et on a trouvé que la prise de la solution de $CuCl_2$ était plus élevée que celle de la solution de $CuSO_4.5H_2O$ qui, à son tour, était plus élevée que celle de la solution de $Cu(NO_3)_2.3H_2O$. Les acides sulfurique, chlorhydrique et nitrique étaient tous de bons désorbants des ions de cuivre de la mousse. La processus de désorption dépendait du volume et de la concentration des agents de désorption.

De plus, on a examiné l'adsorption des ions métalliques par la mousse dans un procédé continu en utilisant une colonne garnis d'adsorbant. L'augmentation du taux d'écoulement ou de la concentration du métal affluent ont causé une réduction du temps de precée et une augmentation de la prise du cuivre par unité de masse de mousse. L'adsorption du cuivre a été accompli en utilisant deux colonnes garnis connectées soit en paralléle ou en série. Les résultats ont montré que la charge totale du métal était la même dans les deux cas. La désorption du cuivre des colonnes garnise a été accomplie en utilisant du HCl à de différentes concentrations. Du point de vue économique, une solution de 0.025 M de HCl était le désorbant le plus convenable pour ce processus. De plus, l'efficacité de la colonne garnise n'a pas sensiblement changé après cinque cycles d'adsorption et de désorption.

La capacité d'adsorption des ions métalliques par l'écorce de pin a suivi l'ordre suivant (base molaire): $Cu^{2+} > Zn^{2+} > Cd^{2+} > Ni^{2+}$. On a trouvé que cet adsorbant pouvait être utilisé plusieurs fois sans régéneration. La réutilisation de l'écorce, après régéneration, a entraîné une diminution de la valeur absolue des ions de Cd^{2+} récemment adsorbés, comparée à celle de l'écorce fraîche, alors que la charge totale a continué d'augmenter. Une concentration élevée d'ions tendres ou dures a énormément diminuer l'adsorption du cadmium par l'écorce.

On a étudié des systèmes singuliers, binaires, ternaires et quaternaires de Cu^{2+}, Ni^{2+}, Cd^{2+} et Pb^{2+}. Dans une adsorption singulière, la sélectivité des trois adsorbants pour chacun de ces ions a suivi l'ordre suivant: Cu^{2+}: mousse > CM > écorce; Cd^{2+} et Ni^{2+}: CM > écorce > mousse; Pb^{2+}: CM = écorce ≈ mousse. Dans un mélange d'ions, on a remarqué que la présence d'un métal a généralement affecté la prise des autres métaux. On a aussi remarqué que la prise des ions Ni^{2+}, par chaque adsorbant, a sensiblement diminué quand Cu^{2+} était présent avec le métal, et surtout, dans les système ternaires et quaternaires. Quand les ions métalliques se trouvaient ensemble dans un systèmes quaternaire, l'ordre de préference suivant a été obtenu (base molaire): $Cu^{2+} > Pb^{2+} > Cd^{2+} > Ni^{2+}$ pour l'écorce et la mousse, alors que pour CM l'ordre était le suivant: $Cu^{2+} > Cd^{2+} > Ni^{2+} > Pb^{2+}$.

Dans cette ouvrage, on a proposé deux modèles mathématiques a fin de représenter les dynamiques de l'adsorption traitée par lots. Dans le premier modèle, on a supposé que l'adsorption a eu seulement lieu à la surface de l'adsorbant. A de basses concentrations d'adsorbants, ce modèle s'accordait bien avec les données experimentales, alors qu'à de basses concentrations, on a remarqué une grande déviation des données experimentales causée par une importante diffusion intraporeuse. Le second modèle était une modification du premier modèle où on a pris en considération la diffusion intraporeuse, et par conséquent, ce modèle a très

bien représenté les données experimentales pour toutes les concentrations. Les données, pour un métal singulier, collectées à l'equilibre sont représentées par les isothermes de Freundlich et Langmuir. On a aussi collecté les données à l'équilibre du système binaire, quand on a utilisé l'écorce de pin. Ces données sont représentées par trois types de modèles à multicomposants à l'équilibire La meilleure représentation des données expérimentales a été obtenue par le modèle de Langmuir-Freundlich.

Les analyses compositionnelles globales ont montré que la lignine et la carbohydrate de l'écorce et de la mousse, les protéines, la carbohydrate et l'acide phytique de CM sont les principaux composants responsables de l'adsorption des métaux lourds. On a aussi examiné les rôles des groupes carboxyles et aminés dans le processus d'adsorption. On a trouvé que l'estérification des groupes carboxyles et la méthylation des groupes aminés dans CM, l'écorce et la mousse ont entraîné une diminution de la prise du métal par ces adsorbants. On a examiné le mécanisme d'échange d'ions en mesurant le taux d'échappement de cations vers la solution avant et après l'adsorption des métaux lourds par CM, l'écorce et la mousse. On a trouvé que le nombre de cations relâchés par l'écorce et la mousse était plus élevé que le nombre de cations relâchés par CM. Ceci indiquait que le mécanisme d'échange d'ions représentait un des plus importants mécanisme impliqués dans le processus d'adsorption par l'écorce de pin et la mousse. Microscopiques du balayage électronique (SEM) et EDX ont révélé que les ions métalliques ont été surtout adsorbés sur la paroi de la cellule et qu'un petit nombre d'ions a diffusé dans le cytoplasme de la cellule des adsorbants. Les spectres EDX ont aussi montré que des ions Ca^{2+} ont été relâchés de la paroi de la cellule de mousse et d'écorce à cause de l'adsorption du métal. Par contre, ces spectres n'ont pas montré aucun relâchement net de cations après l'adsorption du métal par CM. Les tests ESR ont montré que les radicaux libres dérivant des adsorbants ont joué un rôle important dans le processus d'adsorption. La concentration de ces radicaux, dans le CM, l'écorce ou la mousse a diminué quand la concentration des ions Cu^{2+} a augmenté dans ces adsorbants. Dans les cellules de mousse, ces radicaux se sont coordonnés avec les ions de Cu^{2+}, alors que dans les cellules de CM et d'écorce de pin, les radicaux se sont regroupés en paires au lieu de se coordonner avec les ions de Cu^{2+}.

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Notation

Symbols

b	Langmuir constant, L/mmol;
b_j	Langmuir constant of the jth component in the extended Langmuir model, L/mmol;
b'	adsorption energy constant, L/mmol;
C_A	metal concentration in the bulk, mM;
C_A^*	metal concentration at sorbent surface, mM;
C_0	initial metal concentration, mM;
C_e	equilibrium metal concentration, mM;
C_{ej}	equilibrium concentration of the jth component, mM;
C_{in}	metal concentration in the influent of the packed bed, mM;
C_{out}	metal concentration in the effluent of the packed bed, mM;
F	influent flow rate, mL/min;
g	Lande's constant (separating factor);
K	linear equilibrium constant, g/L;
K_{ij}	selectivity index;
K_L	Langmuir constant, mmol/g;
k_F	Freundlich constant related to the sorbent capacity;
k_l	mass transfer coefficient, mL/(cm^2.min);
k_l'	mass transfer coefficient, mL/(mg.min);
k	rate constant for sorption of metals in a packed bed, (mM . min)$^{-1}$;
M	amount of sorbent, mg;
N	residual sorbate capacity of the sorbent in a packed bed, μmol/cm^3;
N_0	initial sorption capacity of the sorbent in the packed bed, μmol/cm^3;
n_i, n_j	constants for the multicomponent equilibrium model;
$1/n$	Freundlich constant related to the sorption intensity;
P	number of parameters in the model;
q	amount of adsorbed metal, mmol/g;
q_e	amount of adsorbed metal at equilibrium, mmol/g;

$q_{e,j}$ equilibrium solid phase concentration for the *j*th component, mmol/g;

q_m monolayer capacity of the sorbent for the solute, mmol/g;

q_{mix} amount of adsorbed metal in from a mixture of metals, mmol/g;

q_{exp} experimentally measured uptake, mmol/g;

$\overline{q_{exp}}$ mean of the total measured uptakes, mmol/g;

q_{calc} predicted uptake by the model, mmol/g;

S specific surface area of the sorbent, cm^2/mg;

T Temperature, °C;

t contact time, min;

t_b break-time for a given effluent concentration and influent flow rate, min;

V volume, mL;

X_0 sorbent concentration, mg/mL;

x_j mole fraction of the *j*th component in the bulk phase at equilibrium;

y_j mole fraction of the *j*th component in the sorbent at equilibrium;

Z depth of the packed bed, cm;

Z_{min} critical bed depth, cm.

Greek symbols

β rate constant contributed to intrapore diffusion, $mmol/(mg.min^2)$;

ε correction factor, mmol/(mg.min);

ΔG free energy change, kJ/mol;

ΔH apparent enthalpy change, kJ/mol;

ΔS entropy of adsorption, kJ/(kmol.K);

σ_0 free energy of the surface covered with pure solvent; kcal/g;

σ_1 free energy of the surface covered with a monolayer of solute; kcal/g.

Abbreviations

AAS Atomic absorption spectrophotometry

CM Canola meal;

CCME Canadian Council of Ministers of the Environment;

EDX Energy Dispersive X-ray;

ESP	Electron Spin Resonance;
HM	Heavy metal;
Me^{2+}	Metal cation;
MSC	Model Selection Criterion;
ppb	part per million (μg/mL);
ppm	part per billion (μg/L);
SEM	Scanning Electron Microscopy;
SSR	Sum Square of Residuals;
WHO	World Health Organization.

Part I

Introduction

Literature Review

Materials and Methods

and

Theoretical Aspects

Chapter 1

Introduction

1.1 Motivation

Heavy metals is the term that is generally applied to those metals of particular concern in the treatment of industrial wastewater: copper, silver, zinc, cadmium, mercury, lead, chromium, iron, and nickel (Lanouette, 1977). Other metals that may be included in this category are tin, arsenic, selenium, molybdenum, cobalt, manganese, and aluminum.

Recovery of heavy metals (HMs) from wastewater became a very important environmental issue. These metals are very harmful if they are discharged to the natural water resources. On the other hand, they find many applications in daily life.

Elevated levels of HMs may come from a variety of sources such as metal plating, metallurgical alloying, ceramics, photography, and from many other industries (Forstner and Wittmann, 1979). The amounts of HMs discharged to the environment varied from one industry to another, they range from few ppm (part per million or μg/mL) to thousands of ppm.

Keeping in mind the harmful effects and the applications of HMs, it is necessary to remove them from their sources at least to a limit accepted by National and International 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, especially when the concentration of metal ions in the discharge is very low.

Activated carbon and different types of ion-exchange resins are very often used in adsorption processes. High capital and regeneration cost of activated carbon and ion-exchange resins encouraged researchers to look for low cost adsorbents. This opens a new branch in the area of adsorption separation. The utilization of microbial biomass, either alive or dead, for the removal of metals from industrial wastewaters and polluted waters has already been recognized. The biomass has to satisfy the following requirements to be considered as an adsorbent (Volesky, 1987): (1) the efficient and rapid uptake and release of metals, (2) the low production cost of the biosorbent and possibility of its reutilization, (3) the efficient, rapid and cheap separation of the biosorbent from the solution, (4) a high selectivity of metal adsorption and desorption. If these requirements are fulfilled, the utilization of the biomass would make the biosorption process more attractive compared to processes which use other materials for adsorption. There are large numbers of microorganisms reported in literature for sorption

of metals: *Saccharomyces cerevisiae* was used for sorption of uranium (Shamate *et al.*, 1978; Tsezos and Volesky, 1981; Weidemann and Tanner, 1981), Sag and Kutsal (1989) used *Z. ramigera* for the uptake of chromium, and de Rome and Gadd (1987) used *Cladosporium resinae* and *Pencillium italicum* for the adsorption of copper. As an explanation of microbial metal ion binding capability, Kuyucak and Volesky (1988a) reported that cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids, offer particularly abundant metal-binding functional groups such as carboxylate, hydroxyl, sulphate, phosphate and amino groups. Muraleedharan and Venkobachar (1990) attributed the metal binding capacity of microbes to their proteineous component.

Researchers also studied the use of various types of agricultural and forestry products and by-products or wastes for the removal of heavy metals from aqueous solutions. Friedman and Waiss (1972) used peanut skin, wool, rice straw, peanut hulls, rice hulls and sugar cane bagasse for adsorption of Hg^{2+}, Ferro-Garcia *et al.* (1988) removed Pb^{2+}, Cd^{2+} and Zn^{2+} from their aqueous solutions using almond shells, olive stones and peach stones, Salim *et al.* (1992) used peech leaves for the adsorption of Cd^{2+}, and Gardea-Torresdey *et al.* (1996a) used *Sphagnum* peat moss and its different humic substances for the removal of Cu^{2+} ions.

Considering that the biosorption of heavy metals is still in its infancy, it has been decided, in this work, to use several biomaterials of various origins in order to study and to compare their capabilities for the sorption of metals. The microorganism *Aspergillus carbonarius* was used previously for the production of the enzyme phytase (Al-Asheh and Duvnjak, 1994). This biomass has never been studied before for the adsorption of heavy metals, therefore, it was considered in this work. Canola meal (CM) is a by-product of canola oil processing from canola seeds that is largely produced in Canada. The presence of undesirable materials in the meal, such as phytic acid and phenolic compounds, limited the utilization of this commodity. Therefore, CM was used in this work in order to study the possibility of using this material as a sorbent for heavy metals. Pine bark is a by-product from the pulp paper industry. The waste bark resulted from this industry is normally burned to produce energy. New applications for this material are sought as well. Therefore, pine bark was considered in this work to study its ability to remove heavy metals. Moss is widely distributed in the ecosystem and was also tested in this work for its capability to sorb heavy metals.

1.2 Research Objectives and Contributions

The primary objective of this work was to examine the possibility for the utilization of microbial biomass (*Aspergillus carbonarius*) and some forestry and agricultural materials for the

removal of metals, namely copper, zinc, cadmium, nickel and chromium, from aqueous solutions. Another main objective of this work was to compare the efficiencies of these new biosorbents for the removal of heavy metals. The effect of parameters such as sorbent and metal concentrations, temperature and pH of the sorbent-metal suspension on the metal adsorption have been studied. Single- and multi-metal systems were also considered in this work as well. Mathematical models that describe the dynamics of the batch sorption process were presented. The uptake of these metals using adsorption columns was evaluated. An attempt to describe the mechanisms involved in the sorption process was considered for the biosorbents studied in this work.

The main contributions of this work to the fields of bioseparation of heavy metals and environmental engineering can be summarized in the following points:

- The biomass of *Aspergillus carbonarius*, which has never been previously used for metal adsorption, was shown in this work to have a significant potential for this purpose.
- It was demonstrated that the agricultural and forestry materials, canola meal, moss, pine needle, pine cones, pine bark, oak leaves, oak cones, maple leaves, birch leaves and grass can also be used for adsorption of heavy metals. A literature survey showed that these materials either were not addressed in previous studies or the published data were very scarce.
- The effects of various operating conditions on batch process performance using both synthetic and industrial aqueous metal solutions were determined.
- Sorption of multi-metal ions by the tested biomaterials was examined.
- A continuos process for the adsorption of metals using these new adsorbents was developed.
- Mathematical models for the dynamics of metal uptake in the batch process were proposed.
- Bohart and Adams analysis was applied to some results of the continuous process.
- The plant components responsible for metal adsorption were identified.
- The mechanisms of heavy metal adsorption by plant materials were elucidated.

The thesis is divided into three parts. Part I includes the introduction, the literature review pertinent to this study, the materials and methods used during the course of the study, and the theoretical aspects to be used in this study. Part II includes the results and the discussion. The results for each sorbent are presented in separate chapters, followed by a separate chapter that describes the mechanism of metal removal based on the composition of the plant sorbents, the Scanning Electron Microscopy (SEM) and X-ray analyses, and the Electron Spin Resonance (ESR) analysis, and finally the conclusions and recommendations are outlined. Part III includes the references and the appendices.

Chapter 2

Literature Review

2.1 Heavy Metals in Industrial Wastes

The presence of heavy metals (HM) in the environment can be detrimental to a variety of living species. The most important features that distinguishe heavy metals from other toxic pollutants are their nonbiodegradability and tendency to accumulate in living materials (Orhan and Buyukgungor, 1993). Metal processing, finishing, and plating are obvious sources of metal wastes, and the nature of the waste directly reflects the particular combination of metals and manufacturing process utilized by a given plant. However, there are also many other processes from which heavy metals originate. Table 2.1 shows the type of metals released by various industries. Very often, these metals appear in industrial wastewaters. The concentration of heavy metals in these effluents depends on the type of industry and the nature of the process applied (Table 2.2).

Table 2.1: Heavy metals found in major industries (Dean *et al.*, 1972)

	Al	Ag	As	Cd	Cr	Cu	Fe	Hg	Pb	Ni	Sn	Zn
Pulp, paper mills, paperboard, building paper, board mills					●	●		●	●	●		●
Organic chemicals, petrochemicals	●		●	●	●		●	●	●		●	●
Alkalis, chlorine, inorganic chemicals	●		●	●	●		●	●	●		●	●
Fertilizers	●		●	●	●	●	●	●	●	●		●
Petroleum refining	●		●	●	●	●	●		●	●		●
Basic steel works, foundries			●	●	●	●	●	●	●	●	●	●
Basic non-ferrous metal-works, foundries	●	●	●	●	●	●	●		●			●
Motor vehicles, aircraft-plating, finishing	●	●		●	●	●		●		●		
Flat glass, cement, asbestos products, etc.					●							
Textile mill products					●							
Leather tanning, finishing					●							
Steam generation power plant					●							●

Table 2.2: Representative metal concentrations of untreated industrial waters (Patterson and Minear, 1975)

	Cd (ppm)	Cr (ppm)	Cu (ppm)	Fe (ppm)	Ni (ppm)	Zn (ppm)
Pickling-dipping			20-90	10-2,300		10-35
Automatic plating of zinc base die casting		5-10	20-30		45-55	3-8
Automatic plating on plastic base			10-15		30-40	
Automatic band plating and passivation: mixed barrel and rack	7-15	2-25	3-8		15-25	5-10
Vulcanized fiber						200-300
Cold steel finishing with galvanizing		1-6		60-150		2-88

2.2 Toxicity and Applications of Heavy Metals

Heavy metals are considered to be toxic materials due to the many diseases that result from their excess exposure. According to some surveys from public health services of different countries, significant numbers of people have been exposed to hazards of excess metals in the municipal water supplies (WHO, 1984). However, these metals are also very useful and they find many applications in the industrial life. The following paragraphs give brief description of the applications and harmful effects of heavy metals used in this study.

2.2.1 Copper

Copper is a reddish-brown metal which occurs free or in ores such as malachite, cuprite and chalcopyrite. It may form both mono- and divalent compounds. It is insoluble in water, but soluble in nitric acid and hot sulfuric acid. Metallic copper is an excellent conductor of electricity and is widely used in the electrical industry in all gauges of wire for circuitry, coils, armature windings, high conductivity tubes, and so forth. It is made into casts, sheets, rods, tubing and wire and is used in water and gas piping, roofing materials, cooking utensils, chemical and pharmaceutical equipment and coinage.

Considering the effect of copper on human health, it was demonstrated that its salts act as an irritant to intact skin causing itching and dermatitis. In the eyes, copper salts may cause conjunctivitis and even ulceration and turbidity of the cornea. Metallic copper may cause keratinization of the hands and soles of the feet, but it is not commonly associated with industrial dermatitis (Sitting, 1981). Intake

of excessively large doses of copper by man leads to hepatic and renal damage, and central nervous systems irritation followed by depression (WHO, 1984). Because of its wide utilization, it is also found in natural resources as a pollutant. For example, in 1960s, copper together with zinc, amonia phenols and cyanide, was considered as one of the commonest toxic pollutants of the UK rivers.

2.2.2 Cadmium

Cadmium is a bluish white metal. The only cadmium mineral, greenockite, is rare. However, small amounts of cadmium are found in zinc, copper and lead ores. It is generally produced as a by-product from the production of these metals, particularly of zinc. Cadmium is insoluble in water but soluble in acids.

Cadmium is highly corrosion resistant and is used as a protective coating for iron, steel, and copper. It is generally applied by electroplating, but hot dipping and spraying are possible. Cadmium may be alloyed with copper, nickel, gold, silver, bismuth, and aluminum to form easily fusible compounds. These alloys may be used as coatings for other materials, welding, electrodes and solders. It is also utilized in electrodes of alkaline storage batteries, as a neutron absorber in nuclear reactors, a stabilizer for polyvinyl chloride plastics, a deoxidizer in nickel plating, an amalgam in dentistry, in the manufacture of fluorescent lamps, semiconductors, photocells, and jewelry, in process engraving, and in the automobile and aircraft industries (Sitting, 1981).

Fifty years ago, cadmium was declared as toxic metal. This occurred in Japan where in 1947, 44 cases of a 'rheumatic' disease were recorded in villages on the Jinstu River (Mance, 1987). Health effects of cadmium have been widely demonstrated in industrial workers heavily exposed to cadmium oxide fumes and dusts (Commission of the European Communities, 1979). Bronchitis, emphysema, anaemia, and renal stones have been reported (National Research Council, 1977). The renal cortex is generally accepted to be the critical organ for cadmium accumulation in man (WHO, 1984; EPA, 1977). The classical renal effects of cadmium poisoning are associated with proteinuria, glucosuria, and aminoaciduria (EPA, 1980).

2.2.3 Chromium

Chromium may exist in one of three valence states in compounds: +2, +3 and +6. Chromium trioxide is produced from chromite ore by roasting with alkali or lime, leaching, crystallizing the soluble chromate or dichromate, followed by reaction with sulfuric acid. Chromium trioxide is used in

chrome plating, copper stripping, aluminum anodizing, as a catalyst, in organic synthesis, and photography (Sitting, 1981).

High doses of chromium have been implicated as the cause of digestive tract cancers in man (Guidelines for Canadian Drinking Water Quality, 1979), and it is firmly evidenced that there is an increased risk of lung cancer for workers exposed to high levels of chromium (Commission of the European Communities, 1979). Chromium can also result in cutaneous and nasal mucous-membrane ulcers and deramatitis from skin contact (National Research Council, 1977).

2.2.4 Nickel

Nickel is a hard, ductile, magnetic metal with silver-white color. It is insoluble in water and soluble in acids. It occurs free in meteorites and in ores combined with sulfur, antimony, or arsenic. Nickel forms alloys with copper, manganese, zinc, chromium, iron and molybdenum. Stainless steel is the most widely used nickel alloy. Nickel-copper alloy has excellent corrosion resistance properties. Elemental nickel is used in electroplating, anodizing aluminum, casting operations for machine parts, in coinage, in the manufacture of acid-resisting and magnetic alloys, magnetic tapes, nickel-cadmium batteries, nickel soap in crankcase oils, and glass. It is used as a catalyst in the hydrogenation of fats, oil, and other chemicals (Sitting, 1981).

Nickel is a relatively nontoxic element (WHO, 1984) as its concentrations in, e.g., water are below the toxic concentrations. The levels of nickel usually found in food and water are not considered a serious health hazard (WHO, 1984). Nickel can react with DNA, and at high concentrations can results in DNA damage as shown *in vitro* mutagenicity (WHO, 1984). Nickel and its compounds are also irritants to the conjunctiva of the eye and the mucous membranes of upper respiratory tract (Sitting, 1981).

2.2.5 Zinc

Zinc is used as a wood preservative, and as a soldering flux, for dry battery cells, as well as in textile finishing, vulcanized fiber, reclaiming rubber, manufacture of parchment paper, dyes, activated carbon, chemical synthesis, dentists cement, deodorants, and disinfection and embalming solutions.

Zinc is an essential element for both animals and man and is necessary for the functioning of various enzymatic systems such as alkaline phosphatase, carbonic anhydrase, and alcohol dehydrogenase (WHO, 1984). In some countries, an endemic zinc deficiency syndrome among young men had been reported (Halsted, 1972). However, if it is in high concentrations in drinking-water and

dietary sources it can show toxic effects (WHO, 1984). Symptoms of zinc toxicity in human include vomiting, dehydration, electrolyte imbalance, abdominal pain, nausea, lethargy, dizziness, and lack of muscular coordination (Prasad and Oberleas, 1976).

2.3 Permissible Levels of Heavy Metals

Many chemical elements are essential, or at least beneficial to human health, but they also can become toxic when taken in excess. However, there are elements, HM, which are not essential to human at all and even at low levels they are very dangerous for human beings. This fact may be expressed by the two curves presented in Figure 2.1 which shows the relationship between the concentration of the chemical element, essential and toxic, and the resulted health response.

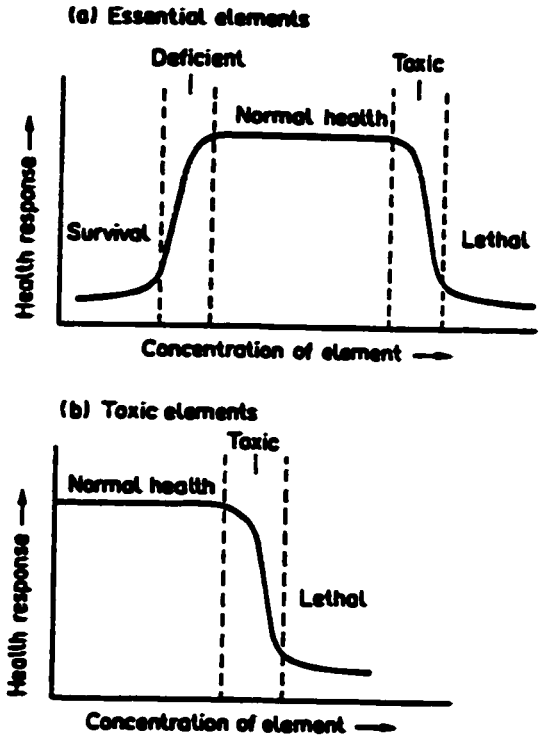

Figure 2.1: The relationship between health response and concentrations of the elements, essential (a) and toxic (b). Adopted from Fergusson (1990).

Environmental criteria for HM

In order to protect the environment from the adverse toxic effects of HM, environmental quality criteria has been set by National and International Agencies. Table 2.3 summarizes the water quality criteria settled by the Canadian Council of Ministers of the Environment for some HMs.

Table 2.3: Water quality for some heavy metals* (CCME, 1991)

Metal	Water Type			
	Fresh water aquatic life	Irrigation	Live stock watering	Drinking water
Cadmium	0.2-1.8	10	20	5
Chromium	2-20	100	1000	50
Copper	2-4	200-1000	500-5000	≤ 1000
Nickel	25-150	200	1000	–
Zinc	30	1000-5000	50000	≤ 5000

* All values in ppb.

World Health Organization, WHO, (1984) reported the exposure limit of some toxic metals on the average consumption of 2 liters of water per day. These values are shown in Table 2.4.

Table 2.4: Maximum limit of HM in drinking water per capita

Metal	µg/day
Cadmium	1-10
Chromium	10-40
Nickel	10-20

Table 2.5 presents comparison of U.S. primary drinking water regulations with Canadian, the European Economic Community (EEC) and WHO guidelines. Inspection of the table shows that there are differences among these regulations with respect to the level of metals in drinking water. According to the US regulations the maximum permissible concentrations of cadmium and mercury in drinking water are as twice as that outlined by the other guidelines.

Table 2.5: Comparison of U.S. primary drinking water regulations with Canadian, EEC, and WHO guidelines* (Van der Leeden *et al.*, 1990)

Metal	U.S.	Canadian	EEC	WHO guideline
Arsenic	0.05	0.05	0.05	0.05
Cadmium	0.01	0.005	0.005	0.005
Chromium	0.05	0.05	0.05	0.05
Lead	0.05	0.05	0.05	0.05
Mercury	0.002	0.001	0.001	0.001
Selenium	0.01	0.01	0.01	0.01
Silver	0.05	0.05	0.01	–

* All values in ppm

Emissions to the atmosphere

The biogeochemical cycling of a variety of trace metals has been changed with advanced technologies and industrialization. Table 2.6 shows the emission of some HMs to the atmosphere by different industrial and natural sources. It is seen that for many metals man-made emission to the air are significantly larger than natural emissions (Table 2.6). A measure of the industrial influence on metal global cycling is the *interference factor* (IF) which is defined as the ratio of the natural to the industrial emission (Lantzy and Mackenzie, 1979). For Cr^{3+} and Cu^{2+}, natural and industrial emissions are relatively close (IF $\approx$ 1). For Zn^{2+}, Cd^{2+}, and Ni^{2+} man's activity now dominates these metals' global atmospheric cycling.

Table 2.6: Estimate of global emissions of trace metals to the atmosphere from industrial and natural sources*

Industrial sources (Units: 10^6 kg/yr)	Cd	Cr	Cu	Ni	Zn
Coal combustion	0.53	11	5.2	14	11
Oil combustion	0.14	1.4	1.9	27	1.4
Nonferrous metal production	5.5		23	8.7	72
Steel and iron manufacturing	0.16	16	1.5	3.7	20
Refuse incineration	0.75	0.84	1.6	0.36	5.9
Phosphate fertilizers	0.17		0.41	0.41	4.1
Cement production	0.27			0.49	9.8
Wood combustion	0.12	1.3	0.9	1.2	3.6
Others					3.2
Total	7.6	30.5	34.5	55.9	131.0
Natural sources (Units: 10^6 kg/yr)	Cd	Cr	Cu	Ni	Zn
Windblown dust	0.20	27	8	11	19
Sea salt	0.06	0.7	3.6	1.3	0.44
Volcano	0.82	15	9.4	14	9.6
Wild forest fires	0.11	0.09	3.8	2.3	7.6
Continental biosphere particulates	0.15	1.0	2.6	0.51	2.6
Continental biosphere-volatile	0.04	0.05	0.32	0.10	2.5
Marine biosphere-volatile	0.05	0.06	0.39	0.12	3.0
Total	1.43	43.9	28.1	29.33	44.7
Interference factor (IF)	5.34	0.69	1.23	1.90	2.93

* Adopted from Salbu and Steinnes (1991).

2.4 Solubility and Speciation

The solubility of metal ions is pH dependent. The solubility of ion oxides, hydroxides, carbonates and hydroxide carbonates can be expressed by the following relationship:

$$\log[Me^{z+}] = \log K_{s_0} + zpK_w - zpH \qquad (2.1)$$

where K_{s_0} is the solubility constant of oxides, hydroxides, carbonates, and hydroxide carbonates, K_w is the ion product of water ($pK_w = -\log K_w$), and z is the ion charge. The values of K_{s_0} for several metal ions can be found in the classical reference of Stumm and Morgan (1981). Eq. (2.1) is represented in a graphical form for few oxides and hydroxides as shown in Figure 2.2.

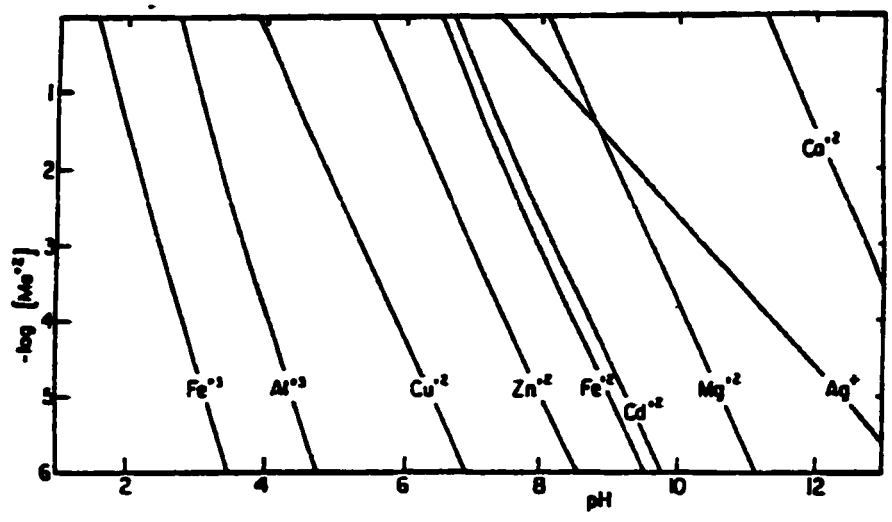

Figure 2.2: Theoretical solubility of oxides and hydroxides. Free metal ion concentration in equilibrium with solid oxides or hydroxides. Adopted from Stumm and Morgan (1981).

Solubility data for some metal hydroxides are also displayed in Figure 2.3 (Gopalratnam *et al.*, 1992). Examining the last two figures, it can be noticed that the reported solubilities for a particular metal slightly differs, probably indicating that solubility tests were carried out at different conditions.

For an aqueous system containing several metals and ligands, the total concentration of each metal is the sum of its free and complex ion concentrations. The speciation of metal ions in natural water depends on many factors including pH, redox, temperature, hardness, CO_2 concentration, the type and concentration of available ligands and chelating agents, as well as type and concentrations of metal ions (Gopalratnam *et al.*, 1992; Tunay and Kabdasli, 1994). It is generally observed that the free

ions are predominant at low pH. At high pH, the complex such as carbonates, oxides, and hydroxides are more stable and thus prevail. Their solubilities are usually much lower at higher pH values, and the increase in the pH is the usual method to precipitate metals from the solutions as complex compounds. The speciation of cadmium at various pH is presented in Figure 2.4 (Morel *et al.*, 1973). Cadmium is in the form of Cd^{2+} up to pH 6 and formation of $CdCO_3(s)$ begins at a pH close to 6. Stability diagram of $Cd(OH)_2(s)$ and $CdCO_3(s)$ as function of pH is shown in Figure 2.5 (Huang and Ostovic, 1978), at a total carbonate concentration of 10^{-3} M.

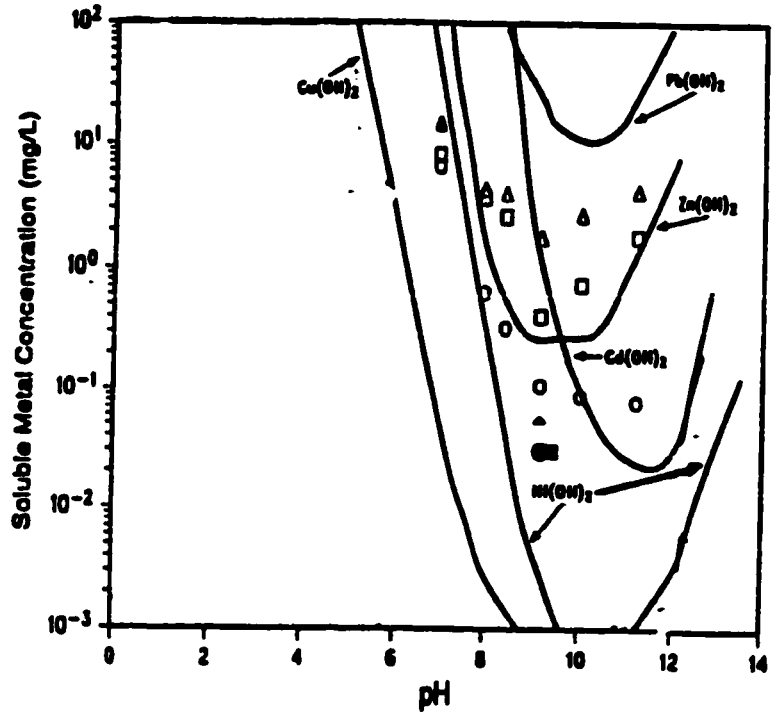

Figure 2.3: Solubility of metal hydroxides. Adopted from Coparatnam *et al.* (1992).

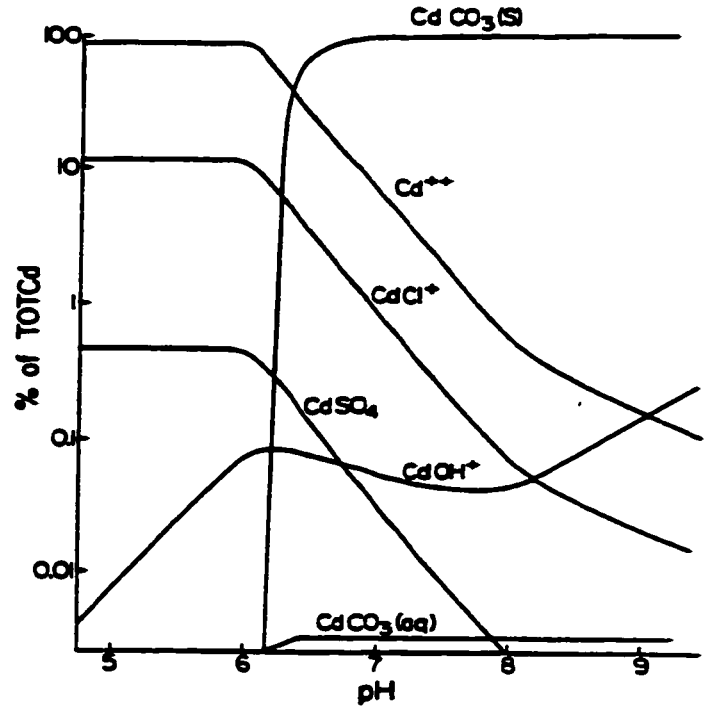

Figure 2.4: Speciation of cadmium as function of pH. Adopted from Morel *et al.* (1973).

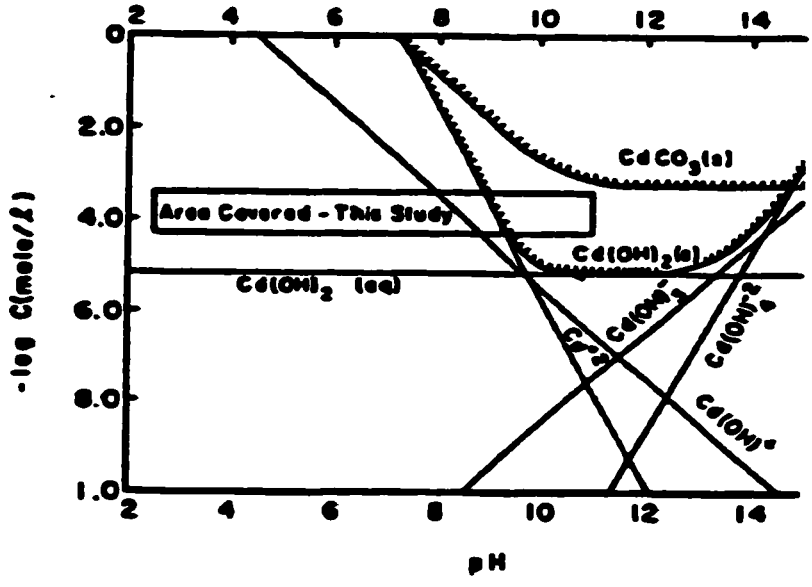

Figure 2.5: Stability diagram of $Cd(OH)_2(s)$ and $CdCO_3(s)$ as function of pH. Adopted from Huang and Ostovic (1978).

The speciation of copper as function of pH is shown in Figure 2.6 (Panday *et al.*, 1985). At low pH, Cu^{2+} is predominant, at mid pH (5-5.5) $CuOH^{+}$ is predominant, and at high pH $Cu(OH)_2^{o}$ become predominant. According to the data of Stumm and Morgan (1981) copper exist as free up to pH 6; $CuCO_3(aq)$ in the pH range 6-9.3; $Cu(CO_3)_2^{2-}$ (aq) in the pH range 9.3-10.7; $Cu(OH)_3^{-}$ and $Cu(OH)_4^{2-}$ above pH 10.7 and 12, respectively, in natural water at constant carbonates of 10^{-2} M.

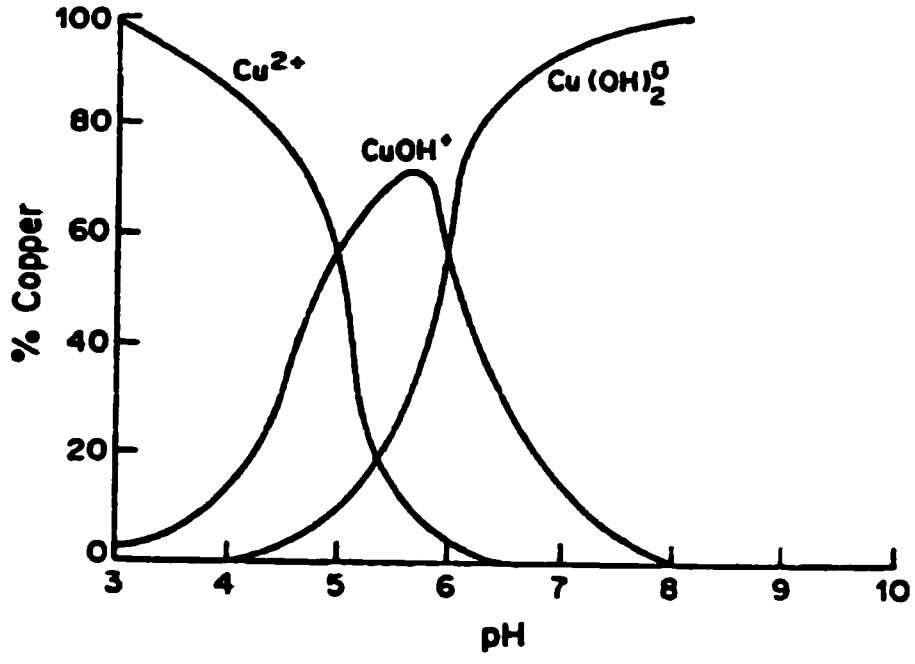

Figure 2.6: Speciation of copper as function of pH. Adopted from Panday *et al.* (1985).

The distribution of copper species in natural water buffered by the carbonate system is presented in Figure 2.7. The diagram is based on the presence of the copper precipitate tenorite, CuO (s). If the solution is not in equilibrium with this solid, the species distribution would not be quite the same; however, radical changes would not occur (Snoeyink and Jenkins, 1980). Therefore, the diagram can be used to determine regions where certain species are important. From Figure 2.7, it can be seen that only below pH 6.6 copper ion, Cu^{2+}, is free, the predominant copper-containing species. At higher pH values the carbonatocopper(II) complex, $CuCO_3^{o}$, and the hydroxycopper(II) complex, $Cu(OH)_3^{-}$, are the major copper-containing species. The diagram predicts that in the pH range 6.5 to 9.5 the major copper-containing species is $CuCO_3^{o}$.

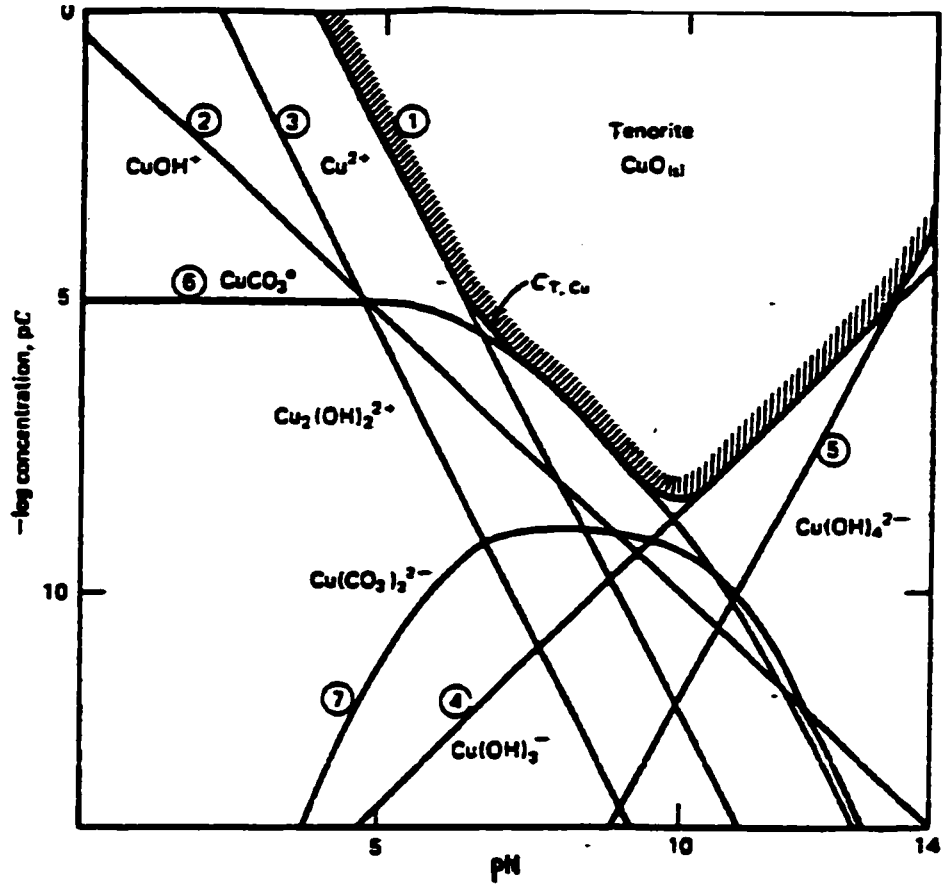

Figure 2.7: Distribution of copper as function of pH in a water containing total inorganic carbonate of 10^{-3} M. Adopted from Snoeyink and Jenkins (1980).

2.5 Methods of Removal

There are several methods used for the removal of metals from waste discharges. In practice, the choice of one type of treatment versus another depends on several factors, including the form and concentration of metals in the wastewater, other constituents present, the extent of removal desired, environmental regulations pertaining to the discharge of the treated wastewater, associated capital and operating costs and the amount of sludges or residues generated and their disposal costs (Beszedits and Netzer, 1986). Nowadays, chemical precipitation, electrolysis, membrane separation, ion exchange and adsorption are the widely used methods for removal of heavy metals. The following paragraphs will briefly describe each of these methods with some examples.

2.5.1 Chemical precipitation

Chemical precipitation involves the addition of a reagent which reacts with the dissolved metals and forms insoluble or sparingly soluble compounds; the more insoluble the compound, the less the metal remains in the solution. The precipitated metals in the form of suspended solid particles can be removed by settling, filtration or centrifugation. To enhance the settling characteristics and agglomeration of the suspended solids, coagulation agents such as alum ($Al_2(SO_4)_3 \cdot 14H_2O$), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), ferric chloride ($FeCl_3$) or polymers are often added (Beszedits and Netzer, 1986).

2.5.2 Electrolysis

Electrolysis is primary a recovery technique, normally yielding an effluent which requires subsequent treatment by precipitation or other means (Patterson and Minear, 1975). This process uses a pair of electrodes, cathode and anode, which are capable of maintaining an electric field in which cations move to the cathode, and anions move to the anode. The disadvantage of the process results from the presence of metal contaminants which may interfere by simultaneous or preferential plating at the surface (Patterson and Minear, 1975). The method is widely used for metal removal from soil. Acar *et al.* (1994) used this method for the removal of cadmium from saturated kaolinite.

2.5.3 Membrane processes

These processes involve application of a pressure differential across a semipermeable membrane to separate a solution into a concentrated and a more dilute (effluent) solution. A pilot study employing crossflow microfiltration unit to treat industrial wastewater containing mixed metals discharged by plating waste showed that this process was more efficient than other processes such as precipitation (Squires, 1992). In their study the concentrations of Cd, Cr, Cu, Pb, Hg, Ni, and Zn in the feed stream corresponding to 2.44, 7.24, 9.98, 4.88, 18.0, 13.0, and 71.2 ppm, respectively, were decreased to the level of 0.04, < 0.08, 1.48, 0.42, 0.08, 1.16, and 1.63 ppm, respectively, in the effluent stream.

A combination of membrane technology and metal bioaccumulation is also well known. Cross-flow microfiltration was shown to retain *Saccharomyces cerevisiae* biomass used for heavy metal bioaccumulation. The passage of metal-laden influent through a series of sequential bioaccumulation systems allowed for further reduction in the levels of copper, cadmium, and chromium in the final effluent than that afforded by a single bioaccumulation process (Brady *et al.*, 1994). A decrease of

71%, 31%, and 7% of Cr^{3+}, Cu^{2+} and Cd^{2+}, respectively, was obtained using this process, when the initial concentration of each metal ion was 0.2 mM.

Some advantages of membrane processes for the removal of metal ions, as reported by Huang and Koseoglu (1993), include low energy requirement, small volumes of retentate than needed to be handled and selective removal of pollutants with use of complexing agents and biocatalysts or by membrane surface modification. Some disadvantages include fouling of membranes by slightly soluble components in solution is possible, as well as fouling of membranes of wastewaters high in suspended solids (Guha *et al.*, 1994).

2.5.4 Adsorption

Adsorption is a separation process in which certain components of a fluid phase (metal ions in this case) are transferred and physically or chemically attached to the surface of a solid adsorbent. The adsorbent is characterized by its high porosity, where adsorption takes place primarily on the walls of the pores at specific site inside the particle. Because the pores are generally very small, the internal surface area is much greater than the external surface area. Differences in molecular weight or polarity cause some molecules to be held more strongly on the surface than others. This causes separation (McCabe *et al.*, 1985). In selective adsorption, the adsorbate is held strongly enough to permit its complete removal from the solution with very little adsorption of other components. Regeneration of the adsorbent can then be carried out to obtain the adsorbate in concentrated or nearly pure form.

Activated carbon and aluminosilicates are very often used as adsorbents for metal removal. Reed and Matsumoto (1993) used two powdered activated carbons (PAC), Darco HDB and Nuchar SN, for the removal of Cd^{2+} from solution. Filtrasorb-200 granular carbon was used to reduce the concentration of Cr^{3+} from 2 ppm to about 0.2 ppm using 25 mg carbon/mL (Abdel-Shafy and Abo-El-Wafa, 1987). Granular activated carbon (GAC) was employed in a fixed-bed adsorber for removing Pb^{2+} from aqueous solution (Kuennen *et al.*, 1992). Elliott and Huang (1981) investigated the adsorption of Cu^{2+} by aluminosilicates with varying Si/Al ratios. Mortazavi *et al.* (1993) used a combination of activated alumina adsorption and microfiltration to treat contaminated water samples from Deloro water treatment plant containing 100 ppm arsenic.

Adsorption is effective method for removal of low level metal ions. However, the regeneration, sometimes, might be expensive and could influence the performance of the adsorbent.

2.5.5 Ion exchange

Ion exchange is a special case of adsorption in which an ion exchange resin exchanges its ions for those in the solution. The process continues until the solution being treated exhausts the resin

exchange capacity. When this point is reached, the exhausted resin must be regenerated by another chemical which replaces the ions given up in the ion exchange operation, thus converting the resin back to its original composition, and yielding a concentrated regenerant brine. The regenerated brine must be treated; often more easily than the original volume of the solution. A typical example is the mineral zeolite (Treybal, 1984), $Na_2O{\cdot}Al_2O_3{\cdot}4SiO_2{\cdot}2H_2O$, where positively charged ions, e.g. Ca^{2+}, diffuse through the pores and exchange with Na^+ ions of zeolite, which is therefore called a cation exchanger,

$$Ca^{2+} + Na_2R \longrightarrow CaR + 2Na^+ \qquad (2.2)$$

where R represents the residual material of the zeolite. The reaction is reversible, and after saturation with Ca^{2+} the zeolite can be regenerated by contact with a solution of salt,

$$CaR + 2NaCl \longrightarrow Na_2R + CaCl_2 \qquad (2.3)$$

Different types of ion exchangers were used for removal of heavy metals from their solutions. Maliou *et al.* (1992) tested the ability of clinoptilolite for the removal of Cd^{2+} and Pb^{2+} from analytical solutions. Uptakes of 1.4 and 1.1 meq/g for Pb^{2+} and Cd^{2+}, respectively, were obtained using initial concentration of 10 mM of each of these metals. Amberlite IR 120 was used to remove Zn^{2+}, Cd^{2+}, and Cu^{2+} from analytical solutions (Sengupta and Paul, 1985).

Ion exchange is known to be attractive method for the removal of small amounts of impurities from dilute wastewaters. It also permits the recirculation of a high quality water for re-use, thus saving on water consumption (Patterson and Minear, 1975). However, the limited capacity of ion exchange system means that relatively large installations are necessary to provide the exchange capability needed between regeneration cycles.

2.6 Microbial Sorbents

Microorganisms have been commonly reported to be capable of sequestering heavy metals and concentrating them up to several thousand times above their environmental levels (Ting *et al.*, 1989). In the past, studies concerned with metal sorption biomass were conducted from a toxicological point of view for understanding of the microbial tolerance and resistance to heavy metals (Wood and Wang, 1983; Jernelov and Martin, 1975) by identification of uptake mechanisms. Since the beginning of last decade, interest has been focused on metal recovery (Tsezos and Volesky, 1982a, b; Strandberg and Shumate, 1982; Nakajima and Sakaguchi, 1986). Although this potential has only relatively recently been recognized, it is implicit in the literature concerned with biochemistry of heavy metals (Siegel *et al.*, 1990). The passive sorption and/or complexation of metal ions by the biomass was

termed "biosorption", and is dependent on the metabolic activity of the cell. The process takes place in many different ways and is dependent on the degree of affinity resulting in different types of binding between the metal ions and an "active site" of a particular molecular structure of the cell wall (Volesky, 1990). Biosorption is caused by a number of different mechanisms, depending on the type of particular binding sites responsible for metal sequestering. It can occur even when the cell is no longer active (dead cells). A number of microorganisms have been reported in literature for metal recovery. Table 2.4 reviews some biosorbents used with the amount of metals being taken up by them. The concept of using the biomass as sorbent materials opens a novel area in biotechnology for the removal of heavy metals from wastewaters. Biosorption processes can compete with other processes for the removal of heavy metals from wastewaters (Friis and Meyers-Keith, 1986) especially when the other processes are ineffective or extremely expensive and the discharge concentrations are required to be very low (Shumate *et al.*, 1978; Volesky, 1990). The source of the raw materials for the biosorbents is a waste material, as is the case in using by-product biomass types from large-scale fermentation processes (Volesky and Holan, 1995). Another inexpensive source of biomass, where it is produced in copious quantities, is in the oceans as seaweeds, representing many different types of marine macroalgae (Kuyucak and Volesky, 1990).

2.7 Plant Sorbents

Plant materials can also be considered as low cost adsorbents (Table 2.9). The use of these materials for metal removal from aqueous media including natural water and industrial wastewater have been highlighted recently (Tee and Khan, 1988). Activated carbons derived from plant materials have been used for heavy metal adsorption as well (Namasivayam and Periasamy, 1993). Utilization of plant materials, without any treatment, is also very well known (Kumar and Dara, 1980). New research shows the effective sorption of heavy metals from wastewater using many sorts of plant products and by-products such as walnut expeller meal, peanut skins, rice straw, lumpit shells, peanut hulls and sugar cane baggase (Ferro-Garcia *et al.*, 1988). The use of waste tea leaves and coffee powder for the removal of Hg^{2+} has been reported as well (Macchi *et al.*, 1986).

Table 2.7: Microbial sorbents used for metal removal

Biosorbent	Metal	Uptake (μg/mg)	Initial conditions				Reference
			pH	T (°C)	C_0 (ppm)	Biomass (mg/mL)	
Zoogloea ramigera	Cr^{6+}	27.5	2.0	25	125	1.0	Sag and Kutsal (1989)
Saccharomyces cerevisiae	Cu^{2+} Cd^{2+} Zn^{2+}	1.7 2.5 0.9	5.5	25	3.2 5.6 3.3	1.0	Avery and Tobin (1993)
Chlorella vulgaris	Cd^{2+}	1.4		25	1.0	0.575	Khummongkol *et al.* (1982)
Mucor miehei	UO_2^{+2}	180	5.0	20	100.0	0.50	Guibal *et al.* (1992)
Escherichia coli	Zn^{2+}	1.65	5.0	20	50.0	0.40	Baldry and Dean (1980)
Chlorella vulgaris	Pb^{2+}	83.0	5.0	25	100.0	0.75	Aksu and Kutsal (1991)
Pseudomanas stutzeri	Cr^{6+}	0.75	7.5	22	5.0	2.0	Guan and Petersen (1993)
Thiothrix strain A1	Ni^{2+} Zn^{2+}	7.6 7.8			3.1 2.9	0.197	Shuttleworth and Unz (1993)
Rhizopus arrhizos *Cladosporium resinae* *Pencillium italicum*	Cu^{2+}	9.5 16.5 6.67	5.5	25	22.8 22.3 26.7	1.07 1.16 0.96	De Rome and Gadd (1987)
Aspergillus niger *Mucor rouxii*	Ag^{+} Cu^{2+} La^{3+} Ag^{+} Cu^{2+} La^{3+}	22.4 1.78 7.5 17.4 2.6 5.7	4.0	5	107.8 63.5 138.8 107.8 63.5 138.8	3.0	Mullen *et al.* (1992)
Streptomyces iongwoodensis	UO_2^{+2} Pb^{2+}	380.0 92.0	3.0		200.0 170.0	0.3	Friis and Myers-Keith (1986)
Streptomyces noursei	Cd^{2+} Cr^{3+} Cu^{2+} Ni^{2+} Zn^{2+}	3.4 10.6 9.0 0.8 1.6	5-6	30	112.4 52.0 63.5 58.8 65.38	3.53	Mattuschka and Straube (1993)
Vaucheria sp.	Cu2+	25.4	4.5		250.0	5.0	Crist *et al.* (1990)
Sargassum fluitans	Cd^{2+}	125.0	4.5	25	400.0	2.0	Yang and Volesky (1996)
Ascophyllum nodosum	Cu^{2+} Cd^{2+} Zn^{2+}	50.0 58.0 30.0	4.5	25	150.0 175.0 120.0	2.0	Chong and Volesky (1995)
Ganoderma lucidum	Cu^{2+}	2.3	4.0		2.3	20.0	Muraleedharan and Venkobachara (1990)

Table 2.8: Plant sorbents used for heavy metals removal

Sorbent	Metal	Uptake (μg/mg)	Initial conditions				Reference
			pH	T (°C)	C_o (ppm)	sorbent (mg/mL)	
Raw rice husk Crushed coconut shells Activated rice husk	Cd^{2+}	0.30 0.40 0.47	6.6 6.6 6.6	27-28	0.5	1.0	Bhattacharya and Venkobachar (1984)
Hardwickia binata bark	Hg^{2+}	8.3	6.0	25	100.0	10.0	Deshkar *et al.* (1990)
Peanut skin Rice straw Peanut hulls Sugar cane bagasse	Hg^{2+}	820.0 280.0 220.0 180.0	3.4-3.7	25	20,000	10.0	Friedmann and Waiss (1972)
Accacia bark	Cu^{2+} Pb^{2+} Zn^{2+} Cd^{2+} Cr^{3+}	2.9 10.6 7.7 7.3 12.5	7.0 7.0 7.0 9.0 2.0	25	40.0 110.0 100.0 80.0 100.0	10.0 2.0	Kumar and Dara (1980) Singh *et al.* (1994)
Exhausted coffee ground	Hg^{2+}	9.5	10.0	25	10.0	1.0	Macchi *et al.* (1986)
Powder coffee Nut shell	Cr^{3+} Cd^{2+} Al^{3+} Cr^{3+} Cd^{2+} Al^{3+}	1.63 1.16 1.63 1.50 1.33 1.67			5.0	3.0	Orhan and Buyukgungor (1993)
Sphagnum peat moss	Cu^{2+} Ni^{2+} Zn^{2+}	5.6 4.0 4.0			50.0	2.5	Viraraghavan and Dronamraju (1993)
Irish peat Peanut shell Walnut shell	Cd^{2+}	4.40 3.40 2.31	5.2	23	100.0	20.0	Azab and Peterson (1989)
Decaying leaves	Pb^{2+}	0.6	5.0		30.0	33.3	Salim et al. (1994)
Rice bran	Cu^{2+} Cr^{3+} Ni^{2+} Zn^{2+}	8.24 8.25 5.28 9.37	5.8-6.0	20	100.0	10.0	Marshall et al. (1993)
Soybean hulls	Cu^{2+} Cr^{3+} Ni^{2+} Zn^{2+}	1.99 1.96 1.91 1.93	5.0	25	100.0	50.0	Marshall and Champagne (1995)
Beech leaves	Cd^{2+}	0.032	4.0	25	1.68	18.1	Salim *et al.* (1992)
Tea leaves	Pb^{2+}	43.62		25	100.0	1.0	Singh *et al.* (1993)

2.8 Mechanisms of Biosorption

Atoms and molecules are held together by cohesive forces that range in magnitude from strong valence bonds to weak Van der Waals forces. The atoms and molecules in the interior of a solid are completely surrounded by each other, thus, their attractive forces are satisfied on all sides. In contrast, atoms and molecules in the surface have unsatisfied orbitals extending outward into space. These attractive forces in the surface can be satisfied only if other atoms or molecules become attached to the surface giving rise to the phenomenon of adsorption (Wagner and Jula, 1981). The adsorbed species are considered to form a monolayer on the surface of the adsorbent. Adsorption is generally limited to molecules with molecular weight less than 2,000 since many of the surface sites may be of limited accessibility. Large molecules may be difficult to elute because of the accessibility of multiple sites of adsorptive interaction (Dechow, 1989).

There are many mechanisms suggested in the literature for the uptake of metal ions by biomaterials. These mechanisms claim that metal sequestration by different part of the cell can occur via

- Complexation
- Coordination
- Chelation of metal
- Ion exchange
- Adsorption
- Inorganic microprecipitation

Any or a combination of the above metal-binding mechanisms may be functional to various degrees in immobilizing one or more metallic species on the biosorbent.

Norris and Kelly (1977) stated that metal accumulation by microorganisms generally comprised of two phases: a rapid binding of cations to negatively charged groups at the cell surface, and progressive intracellular cation uptake. The latter stage was also described by Norberg and Persson (1984) as a gradual transport and accumulation of metal ions within the cytoplasm. Khummungkol *et al.* (1982) reported that the first stage is passive (that is, physical adsorption or ion exchange at the cell surface) and occurs in a short time after a biomaterial comes into contact with the metal, and the second stage, possibly active (that is, related to metabolic activity), is slow. Figure 2.8 shows some of the functional groups which contain these negatively charged species, that are found in the polymers of the fungal wall. In addition, the fungal wall contain proteins, lipids and pigments, and this diversity is reflected by the presence of a range of distinct potential complexation sites, such as carboxylate, phosphate, sulfhydryle and amine groups (Avery and Tobin, 1993). Amino acids, histidine and cysteine, are reported to have a strong affinity for metal ions (Ashmead *et al.*, 1985). Aspartic acid

and glutamic acid were also reported by Huang *et al.* (1991) as amino acid residues that might be responsible for metal adsorption.

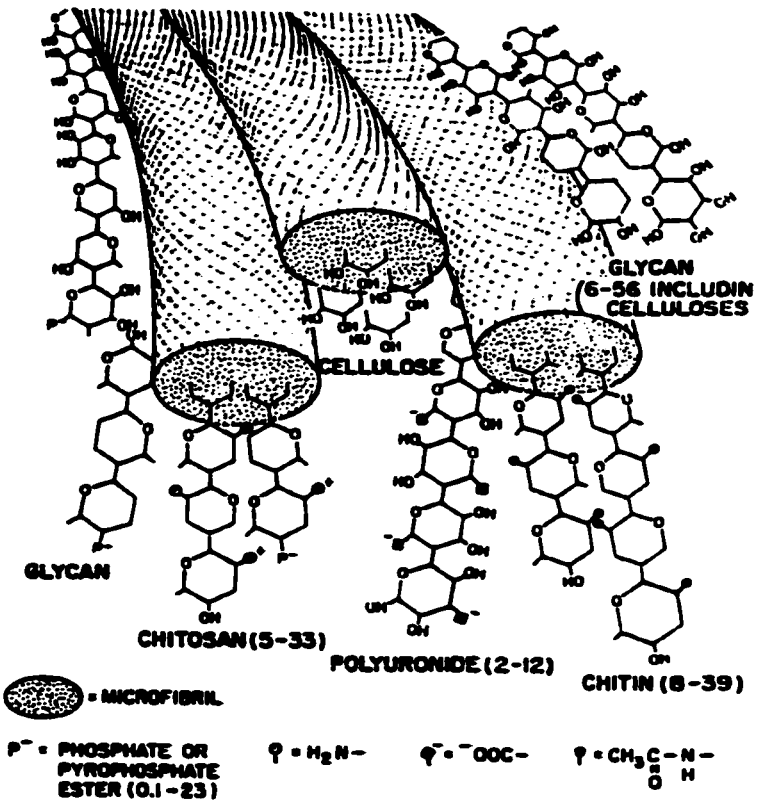

Figure 2.8: Diagrammatic representation of major fungal wall polysaccharides active in biosorption (Siegel *et al.*, 1990). Architecture is indicated only symbolically with many OH groups and small molecules omitted for clarity. Figures in parenthesis indicate percentage composition of walls. Not shown are protein (6-8), lipids (3-11) and melanin (0-5). The O group, indicated here as H_2N^+-, may be present as H_3N^+-, according to ambient pH.

According to Tsezos (1980), various steps in the sorption mechanism which deal with metal transfer through layers bordering the cell, are proposed (Figure 2.9):

1. metal transport from the bulk solution to the boundary film present around the cell wall, Figure 2.9 (a);
2. transport of metal from the boundary film to the cell surface, Figure 2.9 (a);
3. transport of metal from the cell surface to active sites of uptake, Figure 2.9 (b);
4. uptake phases: complexation, adsorption and intramembrane precipitation, Fig. 2.9 (c).

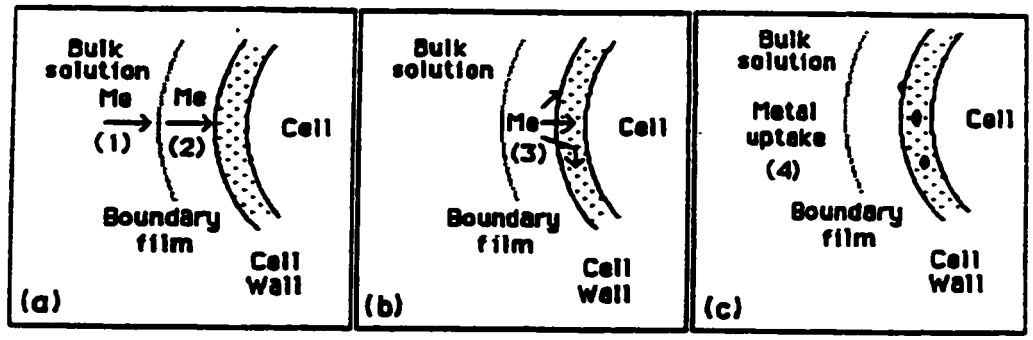

Figure 2.9: Various steps involved in sorption kinetics.

Shumate *et al.* (1978) reported that metal ions could react to form a precipitate, discretely settled within the cells or as a fine colloid entrapped by extracellular polymers. They also reported that metal ions may be transported into the cell and react to form a product that remains within the cell. They cited that metal cations may be complexed by negatively charged sugar units at the end of polysaccharide chain. Besides, they cited that extracellular polyphosphate groups, associated with sugar metabolism, complex metal ions by chelating through negatively charged oxygen atoms. The nature of these suggested binding units and other potential metal binding groups on the cell surface indicates that the binding may be dependent on the cell environment (i.e., the solution in intimate contact with the cell).

Tobin *et al.* (1990) suggested that, based on the non-linear Scatchard plots (i.e., uptake versus the ratio of uptake to equilibrium concentration), the binding of metal to the cell surface is multiple, non-equivalent binding sites, rather than single distinct type of binding sites. The potential metal-binding groups occurring in the biomass are phosphate, carboxylate, amine and hydroxyl, amongst others. These authors excluded the portion of the biomass phosphate groups from the metal-binding groups according to their tri-ethyle phosphite-nitromethane treatment. They also reported that the uptake may be enhanced by electrostatic attraction to negatively charged functional groups, but this is a secondary mechanism, while the primary interactions are due to a complexation mechanism.

Blocking of carboxyl groups (Tobin *et al*, 1990; Gardea-Torresdey *et al.*, 1996b), phosphate and amine groups (Tobin *et al.*, 1990) of the biosorbents resulted in a decrease of binding capacity of these sorbents compared to those of unblocked. This decrease was related to the degree of esterification, in the case of carboxyl groups (Gardea-Torresdey *et al.*, 1996b). The blocking confirmed the participation of these groups in the biosorption phenomena.

Tsezos (1983) and Muraleedharan and Venkobachar (1990) used electron Spin resonance (ESR) spectra to study the mechanism of biosorption. The authors obtained spectra for the biosorbents, before and after exposing them to the metal solutions. They observed a decrease in the concentration of free radicals with samples exposed to metal solution compared to the control. This, also, indicate the participation of the negatively charged groups in the biosorption process.

Chitin and chitosan, as a cell wall content of some biomaterials, have also been reported to play a role in the sequestration of metal ions from solutions (Muzzarelli and Tubertini, 1969; Tsezos, 1980; Muraleedharan and Venkobachar, 1990). However, they do not seem to be the major metal-binding compounds active in the complex sequestration of metal by biosorption (Volesky and Holan, 1995). Chitin and glucan belong to the main structural polysaccharides in fungi. Volesky and Holan (1995) reported that the metal uptake by these compounds might be due to not only the acetamido groups of chitin but also the entrapment of metals in the inter- and intrafibrillar capilarities of chitin and glucan.

Another important mechanism involved in biosorption is the ion-exchange, where the cell wall of the biomaterial is generally regarded as a complex ion exchanger similar to a commercial resin. It is reported (Remacle *et al.*, 1982) that the cell walls contain a mixture of monovalent and divalent cations and that the displacement of one cation by another (e.g. H^+) is dependent on the strength of the individual ligand complex, i.e. CN^-, RS^-, SH^-, NH_2^-, $RCOO^-$, OH^-, etc. Treen-Sears *et al.* (1984) attributed the uptake of uranil ion by the nonliving biomass of *Rhizopus oligosporous* to the ion exchange or complexation, since binding was reversed by the addition of complexing ligands, e.g. EDTA, or the reduction of pH to a value less than 2. Tobin *et al.* (1988) demonstrated that the metal uptake, by the biomass *Rhizopus arrhizus* is a reversible process, when, for example, equimolar UO_2^{2+} completely replaced adsorbed Cd^{2+} ions. Kuyucak and Volesky (1988a) reported that alginates of the marine algae usually occur as natural salts of K^+, Na^+, Ca^{2+} and/or Mg^{2+}, and these ions can exchange with the counter ion(s) such as Co^{2+} resulting in the biosorptive uptake of the metal. Crist *et al.* (1990) reported that adsorption of Sr^{2+} on the algae *Vaucheria* released equivalent amounts of Ca^{2+} and Mg^{2+}, thus adsorption can be viewed primarily as an ion-exchange equilibrium of cation character on the anionic algal surface, electrostatic bonding. The authors studied proton uptake by the algae, and found a release of 1 equivalent of $Ca^{2+} + Mg^{2+}$ per 2 equivalent of H^+ adsorbed. The authors also studied the uptake of Cu^{2+} by the algae, and observed that adsorption of Cu^{2+} released Ca^{2+}, Mg^{2+}, and H+ ions (covalent bonding). They suggested that the release of H^+ ions was due to the complex formation of Cu^{2+} with adjacent algal ammonium or carboxylic acid, according to the reactions:

$$Alg-(NH_3)_2^{2+} + Cu^{2+} \longrightarrow [Alg-(NH_2)_2Cu]^{2+} + 2H^+ \quad (2.4)$$

$$Alg-(CO_2H)_2 + Cu^{2+} \longrightarrow [Alg-(CO_2)_2Cu] + 2H^+ \quad (2.5)$$

Other reactions are possible also, e.g. ones involving sulfhydryl groups. Avery and Tobin (1993) also noticed displacement of Mg^{2+}, Ca^{2+}, and H^+ by sorption of Sr^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Cd^{2+} and Tl^{2+} on the surface of *S. cerevisiae*, and that the cation displacement increased at increasing metal concentration. The authors concluded that relative covalent bonding (H^+ displacement) of the metals was greater at low metal concentrations, while weaker electrostatic interactions (Mg^{2+} + Ca^{2+} displacement) become increasingly important at higher concentrations. Shuttleworth and Unz (1993) stated that the primary mechanism of metal sorption by *Thiothrix* strain A1 apparently was ion exchange, because 66 to 75% of Ni^{2+} or Zn^{2+} could be desorbed by placing metal-laden cells in a solution of 5 mM $CaCl_2$. Biosorption of the heavy metal ions Cd^{2+}, Cu^{2+}, and Zn^{2+} by previously protonated nonliving biomass of the marine algae *Sargassum fluitans* was observed to be coupled with a release of protons (Schiewer and Volesky, 1995). However, the ratio of protons released to metal ions binding was slightly smaller than two. The authors explained this deviation due to the presence of a small number of unprotonated groups that bound metal ions but did not release protons.

Electron microscopy analysis is another technique to investigate the mechanisms of biosorption. Tsezos and Volesky (1982b) obtained electron micrographs of thin section of *R. arrhizus* nonliving cells exposed to uranium, and they found electron-dense layers throughout the cell wall compared to the one which was not exposed to uranium. The electron microscopy of the cell interior did not indicate any concentration of electron-dense material anywhere else in the cell. They positively identified uranium as the electron-dense material by X-ray energy dispersion analysis; higher energy levels corresponding to the uranium spectral lines were obtained when the microprobe was focused on the electron-dense areas of the *R.arrhizus* cell walls. Kuyucak and Volesky (1988b) used electron microscopy to verify that gold and uranium were located in a crystalline form on both the inside and outside of the cell wall of the *Ascophyllum nodosum* biomass. In the study of Ag^+ sorption by the biomass of *Bacillus subtilis* (Mullen *et al.*, 1989), electron micrographs of metal-treated cells showed that Ag^+ was associated with the cell primarily as discrete particles at or near the cell walls of the bacteria. Energy dispersive X-ray analysis confirmed that the particles were silver ions. The same authors also studied the sorption of La^{3+} by the *Pseudomonas aeruginosa*. They found that La^{3+} bounded as needle-like precipitates deposited uniformly around the cell wall periphery. The identity of

the metal was confirmed by the energy-dispertive X-ray analysis. The authors did not notice any precipitate of Cu^{2+} or Cd^{2+} by the electron micrographs. However, the energy-dispersive X-ray analysis indicated that Cu^{2+} and Cd^{2+} were present mostly in the cell wall, although small amounts were also detected in the cytoplasm. The transmission electron micrograph of *Aspergillus niger* cell treated with Ag^{+} (Mullen *et al.*, 1992) showed small electron-dense aggregates accumulated at and within fungal cell walls. These aggregates have been identified as colloidal elemental silver through the use of energy dispersive X-ray spectroscopy.

Although there were some attempts to describe the attachment of metals to plant sorbents, the mechanism of adsorption is not well characterized yet. Friedman and Waiss (1977) studied the sorption of Hg^{2+} by various plant sorbents (Table 2.9). They found that the uptake of mercury was approximately proportional to the proteins content of these sorbents, and they claimed that in addition to proteins, tannins were mainly responsible for the sorption of this metal. However, they reported that the adsorption of the metal by carbohydrate-nature materials (cellulose, starch, rice straw, bagasse) was almost negligible. According to Tee and Khan (1988), waste tea leaves contain 37% cellulose and hemicellulose, 15% lignin and structural proteins which contain acidic functional groups that help in the metal removal process. Coupal and Lalancette (1976) reported that peat moss is a rather complex material containing lignin and cellulose as major constituents. They suggested that these constituents, especially lignin, bear polar functional groups, such as alcohols, aldehydes, ketones, acids, phenolic hydroxide and ethers that can be involved in chemical bonding. The authors also suggested that due to the very polar character of peat moss, the specific adsorption for heavy metals is quite high.

Bhattacharya and Venkobachar (1984) explained the mechanism of Cd^{2+} sorption, using crushed coconut shells, in terms of electrostatic forces and chemical interactions. According to their explanation, at low pH both sorbent and sorbate species are positively charged and therefore the interaction is that of electrostatic repulsion. Besides this, a higher concentration of H^{+} ions exists in the reaction mixture. H^{+} ions compete with the Cd^{2+} ions for the adsorption sites as indicated by a lack of adsorption at very low pH. As pH increases, the concentration of H^{+} ion decreases, whereas, the concentration of Cd^{2+} remains constant. The uptake in this range can be explained as an H^{+}-Cd^{2+} exchange reaction. At high pH, where adsorption rate becomes lower, the surface of the sorbent is negatively charged and the sorbate species are still positively charged. This implies that in addition to electrostatic forces, specific chemical interaction may also play an important role in metal adsorption.

Marshall *et al.* (1993) studied the removal of heavy metals by rice hulls. They reported that the low levels of phytic acid and phenolic components, protein and hemicellulose of rice hulls possess

negatively charged groups under mildly acidic pH of 5.8-6.0. These groups would be considered most likely to attract metal ions through charge-charge interactions. Marshall and Champagne (1995) reported that cottonseeds and soybean hulls contain large amounts (74-90%) of lignocellulosic material (lignin, cellulose and hemicellulose) and this material is mainly responsible for the adsorption of Cr^{3+}, Co^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} by these sorbents.

Chapter 3

Materials and Methods

3.1 Microorganism, Media and Culture Conditions

Aspergillus carbonarius NRC 401124 was used to study the adsorption of heavy metals from their solutions. This microorganism was selected in this work since it was not used previously for adsorption of heavy metals. The microorganism was maintained on a solid medium composed of 4.5% malt agar, 0.5% glucose, 0.5% yeast extract and distilled water. The medium was sterilized at 115°C for 15 min, inoculated after cooling and incubated at 30°C. After the third or fourth day, spores formed. The slants were stored at 4°C for further use. *Aspergillus carbonarius* grew well on a rotary shaker (200 rpm) at 30°C in a liquid medium, composed of 0.8% nutrient broth, 0.5% glucose, 0.5% yeast extract and distilled water. The microorganism grew in the form of small pellets ranging from 0.5 mm to 5 mm in diameter. The pellets were separated by centrifugation (10000 × g, 15 min) and washed three times with deionized water. After that the cells were filtrated and kept at 4°C before their utilization in the adsorption experiments.

3.2 Canola Meal

3.2.1 Moisture content

Commercial canola meal (CM) of which 84% was in the particle size range of 0.18-1.4 mm was used to study the adsorption of Cu^{2+}, Zn^{2+}, Cr^{2+}, Ni^{2+} and Cd^{2+} from aqueous solutions. The moisture content of the meal was 8% but its dry basis was used for the calculation of the amount of copper adsorbed per unit mass of meal. The dry weight of CM was obtained by weighing after drying at 105°C for 48 h.

3.2.2 Protein

Approximately 2 g of wet CM was boiled gently with 33 mL of 1 N NaOH. After cooling and centrifuging (6000 × g, 15 min), the supernatant was diluted appropriately and the protein content was determined by the Lowry method.

3.2.3 Phytic acid

Phytic acid was extracted from approximately 2 g of wet CM using 33 mL of 250 mM HCl under continuous shaking (200 rev/min) for 1 h. After extraction, the suspension was centrifuged (6000 × g, 15 min), the supernatant was collected and the phytic acid content was determined by the method of Haug and Lantzsch (1983).

3.3 Agricultural and Forestry Adsorbents

Moss, pine needle, pine cones, pine bark, oak leaves, oak cobs, maple leaves, birch leaves, lilac leaves, fir tree needles, cider leaves and grass were collected in Gatineau Park, Quebec, and tested for heavy metals adsorption. They were washed several times with deionized water and dried. The dry matter of these materials were separately disintegrated in a blender (in the size range of 0.07-0.14 mm) and stored.

The moss used in this work was a mixture of several different varieties. The main species were identified by the Canadian Museum of Nature (Ottawa) as follows, in descending order of abundance with their approximate amounts in a representative sample: *Pleurozium schreberi* (Brid.) Mitt.-moss (about 20%), *Dicranum scoparium* Hedw.-moss (15-20%), *Dicranum fuscescens* Sm.-moss (15-20%), *Scapania nemorea* (L.) Grolle-hepatic (10-15%), *Dicranum polysetum* Sw.-moss (10-15%), *Lophozia ventricosa* (Dicks.) Dum.-hepatic (10-15%), *Polytrichum commune* Hedw.-moss (about 5%), *Ptilidium ciliare* (L.) Hampe-hepatic (about 5%), *Sphagnum* spp.-2, possibly 3, species (2-3%), *Hypnum* sp.-moss (trace) and *Cephaloziella* sp.-hepatic (trace).

3.4 Elemental Analysis

The plant materials used in this work were analyzed for their carbon, hydrogen and nitrogen contents. About 80-100 mg of the samples were transferred into tin foil cups (Leco Corporation), thoroughly rapped, and, then, were injected into a CHN-1000 Macrosample Elemental Analyzer (Leco Corporation). The analyzer automatically provides the composition of carbon, hydrogen, and nitrogen in percent basis. This analysis was carried out at National Research Council Canada (Ottawa, Ontario).

3.5 Proximate Analysis

Proximate analysis have been widely used in plant research. It emphasizes on the bulk ('proximate') components rather than individual compounds. Most dried plant materials have the following percentage composition (Allen, 1982): crude lipids: 0.5-5; crude protein: 3-15; soluble carbohydrates: 5-20; cellulose (holo): 15-40; cellulose (α): 5-15; cellulose (hemi): 10-25; lignin: 20-35; mineral ash: 1-8. The plant materials, used in this work, were analyzed for these constituents, and the following paragraphs describe the methods used for the analysis of each of these constituents.

3.5.1 Lipids and pigments, and soluble sugars

The following method was used for determination of lipids in microbial tissues (Bisseret *et al.*, 1984). The method was modified for the lipid determination in plant tissues (Kates, 1972) as follows: 2 g of dried sample was extracted in a blender containing 30 mL of isopropanol and 2 mL of deionized water. The extraction was carried for 2 min, and the homogenate was filtered in Büchner funnel with suction using number 2 filter paper (Whatman). The filter residue (which contains carbohydrates, proteins, lignin and ash) was re-blended with another 30 mL of isopropanol and 2 mL of deionized water, and the homogenate was filtrated again. The combined extracts were diluted with benzene (5-10 mL) and brought to dryness in rotary evaporator (Rotavapor Buchi 461, Brinkmann) and the residue was re-dissolved in 30 mL of chloroform/methanol (1:1, v/v) and 13.5 mL of deionized water was added to form a biphasic system. The clear chloroform phase (which contained lipids and pigments) was withdrawn, mixed with benzene, and brought to dryness by the rotary evaporator. The upper phase (which contained soluble sugars and some amino acids) was withdrawn, mixed with benzene and brought to dryness by the rotary evaporator. The two fractions were kept in a vacuum oven at 40°C for 2 hours, cooled in a desiccator and weighed for their lipids and pigments, and soluble sugars and amino acids constituents, respectively.

3.5.2 Crude proteins

The crude proteins were determined in terms of the total nitrogen content of the plant sample. The total nitrogen content determined by the elemental analyzer was multiplied by 6.25 to obtain the protein content of the sample. The factor 6.25 is based on the assumption that plant proteins on average contain about 16% nitrogen (Allen, 1982).

3.5.3 Cellulose and other carbohydrates

The following analysis for cellulose and other sugars was performed at IOGEN Incorporation (Ottawa). The method used was adopted from TAPPI (TAPPI, 1992) but modified by IOGEN

Incorporation. A sample (duplicate) of 0.2-0.5 g was placed in a test tube, and 5 mL of 70% H_2SO_4 was vortexly mixed with the sample. To solubilize cellulose the sample was incubated for 1 h at 50°C with vortexing at every 10 min intervals. During the 1 h incubation, 195 mL H_2O was transferred into a weighed flask for each tested sample. At the end of the incubation, the content of the test tube was poured into the flask with rinsing to insure that all the content from the test tube was transferred into the flask. Subsequently, the flask was weighed. After that, 5-7 mL (weighed amount) was placed into a weighed test tube, the tube was capped and autoclaved for 1 hour at 121°C, to hydrolyze the cellulose. At the end of autoclaving, the tube was cooled, and weighed to determine if there is any loss during the autoclaving period. A scoop of $BaCO_3$ was added to the test tube to neutralize the pH of the reaction mixture. Then the content of the tube was filtered, and diluted for the HPLC (High Performance Liquid Chromatography) analysis. The chromatographs for glucose, xylose, and arabinose were run on a Dionex HPLC using the column CarboPac™ PA1. The cellulose content of the sample was calculated from the following correlation (IOGEN Incorporation, Ottawa):

$$\text{Cellulose (\%)} = \left(\frac{\text{Glucose (mg / mL)} \times \text{Filtrate (mL)}}{\text{Sample weight (mg)} \times 1.11} \times 100 \right)$$

The factor 1.11 is to account for the hydrolysis of cellulose into glucose and water. Since to get this factor, the molecular weight of water was added to that of glucose and divided by the molecular weight of glucose, (180+18)/180 = 1.11.

3.5.4 Lignin determination

The analysis for lignin determination was adopted from the AOAC Official Method of Analysis, method number 949.04 (AOAC, 1990). The following steps show the procedure for this determination:

1. Extract 1 g (W_1) dry sample with alcohol/benzene (1:2, v/v) for 4 h in soxhlet extractor. Close top of the thimble with plug of cotton.
2. Wash sample in thimble with suction using 2 small portions alcohol followed by 2 small portions ether.
3. Heat at 45°C in vacuum oven to drive off ether.
4. Transfer the sample to 250 mL Erlenmeyer flask.
5. Add 40 mL 1% solution of pepsin in 0.1 M HCl.
6. Incubate at 40°C for overnight.
7. Add 20-30 mL hot water and filter, using filter stick.
8. Repeat washing twice by adding another 20-30 mL hot water.

9. Wash residue into flask by forcing 7-8 mL 5% (w/w) H_2SO_4 downward through filter stick, using air pressure.
10. Repeat washing with 5% H_2SO_4, and transfer the content into spherical flask.
11. Add enough 5% H_2SO_4 to bring total volume to 150 mL.
12. Reflux vigorously on hot plate for 1 h, adding H_2O occasionally to maintain original volume.
13. Filter off acid using filter stick.
14. Wash residue with 20-30 mL portions hot water, two 15-20 mL portions alcohol and two 15 portions ether.
15. Leave vacuum on few min to dry residue.
16. Heat in vacuum oven to drive off any residue ether.
17. Transfer the content from the stick to a spherical flask by tapping and brushing.
18. Add 20 mL 72% (w/w) H_2SO_4 at 20°C to residue and hold for 2 h at 20°C, stirring occasionally.
19. Add 125 mL H_2O.
20. Filter using filter stick.
21. Wash with 20 mL hot H_2O, and filter again.
22. Reflux for 2 h using 150 mL 3% (w/w) H_2SO_4.
23. Filter residue onto gooch with asbestos pad.
24. Wash with hot water until acid-free to pH paper.
25. Dry at 105-110°C and weigh (W_2).
26. Ignite the sample at 600°C and weigh (W_3).
27. Determine lignin as

$$\text{Lignin (\%)} = \frac{W_2 - W_3}{W_1} \times 100$$

3.5.5 Ash content

A crucible was pre-heated in a furnace (Thermolyne type 1500, Sybron Corporation) to about 550°C. The crucible was allowed to cool in a desiccator and weighed. One g of sample transferred to the crucible and weighed, the crucible and its content dried at 105°C for 24 h, cooled in a desiccator, and weighed. The crucible, containing the dry sample, was placed in the furnace and the temperature was allowed to rise to 550°C (Allen, 1982). After 2 h at 550°C, the crucible was removed from the furnace, allowed to cool, and then transferred to a desiccator. When cooled, the ash content was determined as:

$$\text{Ash (\%)} = \frac{\text{Ash weight (g)}}{\text{Oven dry weight (g)}} \times 100$$

3.6 Chemical Treatment of the Plant Sorbents

Some of the plant materials were treated during the course of this work either to enhance their ability for adsorption or to study the functional groups responsible for adsorption. These treatments are described in the following paragraphs.

3.6.1 Aldehyde treatment

Canola meal was treated with formaldehyde or glutaraldehyde before their use in metal adsorption, in order to immobilize the protein content of the meal. The treatment was carried out by suspending 10 g of the adsorbent in 100 mL of 5% (unless otherwise stated) of either formaldehyde or glutaraldehyde solution. The suspensions were left in a shaker (200 rev/min) overnight. The adsorbents were separated by centrifugation (10,000 g × 15 min) and washed three times with deionized water (centrifugation was followed after each washing step). After the treatment the adsorbents were dried at 70°C for 48 h and they were ready for use in adsorption tests.

3.6.2 Esterification of carboxyl groups

The method for the esterification of carboxyl groups was adopted from Gardea-Torresdey *et. al.* (1996b). These authors used this method to esterify the carboxyl groups of *Sphagnum* peat moss, therefore, it was considered that it could be applied in this work as well. One g of sample was transferred to an Erlenmeyer flask, followed by 60 mL methanol and 20 mL trimethoxymethane. The temperature was increased to 63°C to maintain reflux. Then, 1.3 mL concentrated H_2SO_4 was added dropwise. Refluxing was continued for 2 h, and after that the content of the flask was centrifuged (10,000 g × 15 min). The precipitate was washed three times with cold water, and dried at 65-70°C.

Hydrolysis of the esterified carboxyl groups was carried out by adjusting the pH of the material to 13 using 1 N NaOH solution. Following 2 h of shaking, the sample was adjusted to pH 5 using 1 M HCl and then it was washed several times with water before drying at 65-70°C.

3.6.3 Methylation of amine groups

The use of formaldehyde as a carbonyl component in the formaldehyde-formic acid treatment gives a good method of methylation of primary and secondary amines (Fieser and Fieser, 1961). The procedure for the methylation of amine groups was carried out by refluxing 1 g of sample with 15 mL of formaldehyde (HCHO) and 30 mL of formic acid (HCOOH) under heating for 4 h (Tobin *et al.*,

1990). After that, the sample was centrifuged (10,000 g × 15 min) and washed several times with deionized water before drying at 65-70°C.

3.7 Batch Sorption Processes

3.7.1 Kinetic experiments

Batch process adsorption was carried out by transferring a certain amount of either microbial or plant adsorbent into a 250 mL Erlenmeyer flask containing 49 mL of either deionized water or 5 mM 2{N-morpholino}-ethano sulphonic acid (MES) buffer, adjusted to a certain pH with 1 N NaOH. The latter buffer has negligible metal complexing properties (Good *et al.*, 1966).

One mL of 5000 ppm Cu^{2+}, or Zn^{2+}, or Cd^{2+}, or Cr^{3+} or Ni^{2+} (in the form of $CuSO_4 \cdot 5H_2O$, or $ZnSO_4 \cdot 7H_2O$, or $CdSO_4 \cdot 7H_2O$, or $[Cr(H_2O)_4Cl_2]Cl \cdot 2H_2O$ or $NiSO_4 \cdot 6H_2O$, respectively) solution was added to the sorbent suspension to make up a final metal concentration of 100 ppm (unless otherwise stated). All the above chemicals were of analytical grade (Sigma Chemical Company, Oakville, Ontario). The mixture was agitated on a shaker (Lab-Line orbit, Environ-shaker) and samples, about 2 mL, were taken at a certain period of time. Sorbent from the samples was separated by vacuum filtration through a 0.45 μm cellulose filter paper (Millipore Corporation), and the filtrate was analyzed for these metals using atomic absorption spectrophotometer (Varian AA-1475 series). The low metal concentrations (≤ 1 ppm) were analyzed using ICP-AES, Inductively Coupled Plasma-Atomic Emission Spectrometer (Thermo Jarell Ash AtomScan 25). Neither precipitate, nor metal ions adsorbed to the wall of the flasks were observed with the tested metals under the experimental conditions. When samples of metal solutions without sorbent, which served as blanks, were filtered, the concentrations of the metals were the same before and after filtration.

To determine the number of replicates that should be carried out in this study, a test with 6 identical systems containing 100 ppm of metal ions at a given pH and sorbent concentration was carried out. It was observed that the results of five of the six systems were within 7% of difference range, while one of them was significantly out of the range. Considering this, each experiment was carried out in duplicate and the average results are presented in this work.

3.7.2 Equilibrium experiments

Equilibrium experiments were performed by using metal solutions each at different initial concentration adjusted at certain pH. The experiments were carried out by transferring 10 mL of a metal solution into a plastic tube. Certain amount of adsorbent was added to the above solution and the

system was agitated on a rotary shaker (Fisher Roto-Rack Model 340) for 24 h, to ensure the attainment of equilibrium. The adsorbent from the samples was separated by vacuum filtration through 0.45 μm cellulose filter paper, and the filtrate was analyzed for the metal under consideration.

3.8 Metal desorption

Once adsorption equilibrium was achieved, metal-laden sorbents were separated from the solution and transferred into a plastic tube. Then, certain volume of desorbent, either HCl, or H_2SO_4 or HNO_3, at a specified molarity was added into these tubes. The tubes were agitated on a rotary shaker. Desorption batches reached desorption equilibrium in less than two hours, and after that, the liquid was filtered off using vacuum filtration through 0.45 μm cellulose filter paper. For reutilization of the sorbent for subsequent adsorption processes, the sorbent after desorption was washed several times with deionized water until the pH of the suspension reached that of water.

3.9 Continuous Sorption Process

Continuous sorption process was carried out in a column packed with biological sorbent. A 20 × 1.2 cm glass column was designed with four ports at a distance of 4 cm from each other. The diameter of each port was 5 mm. These ports were connected to filter discs. The two ends of the column, as well as the ports, were fitted with 0.45 μm cellulose filter paper, an O-ring, and plug stoppers. The column was packed with 5 g (unless otherwise stated) of adsorbent. A metal solution of a given concentration was pumped upward through the column at a set flowrate. Samples were collected, continuously, from the top of the column, as well as from the ports. This process was carried out until the column became saturated with the metal under consideration (i.e., when the effluent and the influent concentrations were equal). In some cases, columns without these ports along the length of the column were used.

After saturation of the column, desorption was carried out for recovery of the metal(s) under consideration using 25 mM HCl (unless otherwise stated). The process of desorption was continued until the metal concentration in the effluent stream became undetectable. When the adsorbent in the column was to be used for another adsorption process, the column was washed with deionized water after the desorption process until the pH of the effluent stream reached that of the deionized water. The metal solution was then pumped through the column for another adsorption process.

3.10 Industrial Wastewater

Wastewater from Noranda plant (Montreal) was obtained. The solution was filtered, in order to analyze its HMs content and to treat the filtrate with the sorbents used in this work for the decrease of its metal ions content. The pH was detected before and after filtration.

The sediment concentration of the suspension was measured by centrifugation (10,000 g × 15 min) of 10 mL of the suspension. The dry matter of the sediment was obtained by weighing after drying at 105°C for 48 h.

3.11 Scanning Electron Microscopy and X-ray Analyses

Scanning Electron Microscopy (SEM) combined with energy-dispersive X-ray (EDX) analyses were used in this work to identify the areas of the sorbent cell for the metal sorption. The procedure for these two techniques described by Huang *et al.* (1994) was followed. Fresh samples, loaded and unloaded with metal ions, of either pine bark, or CM or moss were trimmed as small as was convenient and mounted on stubs with Tissue-Tek (Miles Inc., Elkhart, IN). The aluminum stubs had been specially made with bases to fit into the chuck of the cryo-microtome, and with holes, slots or other appropriate receptors for the chosen tissue. The stub and tissue were immediately frozen in N_2-slush, transferred under liquid N_2 to the cold box and then to the chuck of a cryo-microtome (CR2000, Research and Manufacture, Inc., Tucson, AZ). The chamber and glass knife had been pre-cooled to −80°C. 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 tissue removed by the knife were brushed away with a cold, fine brush to keep the knife edge clean. The planning 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 the 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 the scanning electron microscope (JEOL 6400, JEOL Ltd., Tokyo, Japan), and observed uncoated at 1 kV. The surface was watched continuously while the stage warmed and the specimen was very lightly etched to reveal traces of the cell shapes. The temperature of the stage was set to −90°C for this etching. The etching was stopped (as this temperature was approached at a rate of 0.65°C per minute) when the first signs of cell outline and sequestered vacuolar salts became visible. The specimen was rapidly re-cooled, transferred back to the preparation chamber and returned to the sample stage at −170°C for observation or analysis. EDX

microanalysis was done at 15 kV and images were recorded for the cell 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 count of 1000 counts.

3.12 Electron Spin Resonance

Electron Spin Resonance (ESR) provides signals corresponding to free radicals, and/or paramagnetic ions in a sample, which is a count of the number of unpaired electrons (spins) available in the 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, after washing several times with deionized water, were placed in a 3 mm i.d., 180 mm long ESR tube, capped at the top. The height of moss sample in the tube was ~5 mm. All tests were run at the following instrument setting: time constant 2 s; temperature 27°C; microwave power 2 mW; modulator frequency 100 Khz; scan time 500 s; 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 as 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 plant sample was evaluated by comparing the height of the free radical ESR signal with the height determined with standard sample of coke (supplied by Bruker) containing 10^{14} spins measured under similar conditions.

Chapter 4

Theoretical Aspects

Adsorption processes are characterized by their kinetics and equilibrium isotherms. This chapter deals with mathematical models related to the kinetics and equilibria of batch process adsorption. The Bohart and Adams model will be used for the analysis of the packed bed adsorption process.

4.1 Isotherm Equilibrium Models

The adsorption isotherm is the starting point for any analysis of adsorption process. The isotherm is an essential part of modeling adsorption equilibrium and, thus, column or batch processing design, efficiency and economics (Dechow, 1989). The isotherm predicts the degree of the purification that might be achieved, the approximate amount of adsorbent required to reach that degree of purity and the sensitivity of the purification process to the concentration of the solute. Several mathematical models have been proposed in the literature in order to describe the equilibrium isotherm of adsorption process. Langmuir and Freundlich isotherm models, which are very well known models and often used in the biosorption of heavy metals, will be discussed in this section.

4.1.1 Langmuir model

The Langmuir model was originally developed to describe and quantify sorption on a set of distinct localized adsorption sites, and it has been used to describe both physical and chemical adsorption. This model was based upon the following assumption (Dechow, 1989):

- each active site interacts with only one adsorbate molecule,
- sorbate molecules are adsorbed on well defined localized sites,
- there is no interaction between adjacent adsorbed molecules, and
- the adsorption sites are all energetically equivalent.

The Langmuir equation relates solid phase adsorbate concentration (q_e), the uptake, to the equilibrium liquid concentration (C_e) as follows:

$$q_e = \frac{K_L b C_e}{1 + b C_e} \tag{4.1}$$

where K_L and b are the Langmuir constants, representing the maximum adsorption capacity for the solid phase loading and the energy constant related to the heat of adsorption, respectively. Equation (4.1) can be represented by the following linearized form:

$$\frac{1}{q_e} = \left(\frac{1}{K_L}\right) + \left(\frac{1}{bK_L}\right)\frac{1}{C_e} \tag{4.2}$$

Then, the constants K_L and b can be evaluated from the intercept and the slope of the linear plot of the experimental data of $1/q_e$ versus $1/C_e$, respectively.

The Langmuir constant b can be used to calculate the change in the apparent enthalpy (ΔH) and entropy (ΔS) of adsorption of metal by the adsorbent using the following thermodynamic equations (Panday *et al.* 1985):

$$\ln b = \ln b' - \frac{\Delta H}{RT} \tag{4.3}$$

$$\ln(1/b) = -\frac{\Delta G}{RT} \tag{4.4}$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \tag{4.5}$$

where b' is the adsorption energy constant, ΔG is the free energy change for adsorption of metal by the adsorbent and T is the absolute temperature of the metal solution. ΔH is determined from the slope of ln b vs. $1/T$, while ΔG and ΔS can be determined directly from Eq. (4.4) and Eq. (4.5), respectively, for a given temperature.

4.1.2 Freundlich model

This model is derived from the Gibbs adsorption equation combined with a mathematical description of the free energy of the surface (Dechow, 1989). Freundlich model describes adsorption in terms of the adsorbate concentration:

$$q_e = k_F C^{1/n} \tag{4.6}$$

where k_F is a Freundlich constant related to the sorption capacity, and $1/n$ is related to the sorption intensity that is defined as:

$$\frac{1}{n} = \frac{RTq_m}{\sigma_o - \sigma_1} \tag{4.7}$$

where q_m is the monolayer capacity of the sorbent for the solute, σ_0 and σ_1 are the free energies of the surface covered with pure solvent and with a monolayer of solute, respectively. The following linearized form of the Freundlich isotherm model can be used to obtain the values of the Freundlich constants:

$$\ln q_e = \ln k_F + \frac{1}{n} \ln C_e \tag{4.8}$$

The intercept and the slope of the linear plot of $\ln q_e$ vs $\ln C_e$ at a given experimental conditions provide the values of k_F and $1/n$, respectively.

4.2 Modeling of Metal Uptake Dynamics

In the development of this model in this work it is assumed that the metal uptake occurred through its attachment to the sorbent surface. Therefore, the rate of metal adsorption is assumed to be proportional to the sorbent concentration, its surface area and to the difference of metal concentration in the bulk solution and that at the sorbent surface. This can be written as:

$$-\frac{dC_A}{dt} = k_l S X_0 (C_A - C_A^*) \tag{4.9}$$

where C_A is the metal concentration in the bulk solution, C_A^* is the metal concentration at the sorbent surface, X_0 is the sorbent concentration, k_l is the mass transfer coefficient and S is the specific surface area per unit weight of sorbent. Since the surface area is difficult to measure, S can be combined with k_l and then Eq. (4.9) can be written as:

$$-\frac{dC_A}{dt} = k_l' X_0 (C_A - C_A^*) \tag{4.10}$$

where $k_l' = S k_l$. The metal concentration at the sorbent surface, C_A^*, is in equilibrium with the amount of adsorbed metal per unit weight of sorbent, q. For this process the following linear equilibrium relationship is assumed between C_A^* and q:

$$C_A^* = Kq \tag{4.11}$$

where K is the equilibrium constant. The assumption of linear equilibrium is good when applying the model to a low solute concentration. The mass balance for the metal is:

$$C_0 = C_A + X_0 q \tag{4.12}$$

where C_0 is the initial metal concentration in the solution. Combining Eqs. (4.10), (4.11) and (4.12) gives the following expression:

$$\frac{dC_A}{dt} = -k_l'\left[(X_0 + K)C_A - KC_0\right] \tag{4.13}$$

Solution of Eq. (4.13) is:

$$\frac{C_A}{C_0} = \frac{K}{K + X_0} + (1 - \frac{K}{K + X_0})e^{-k_l'(X_0+K)t} \tag{4.14}$$

Eq. (4.14) fits the experimental data well when the concentrations of the sorbent were high. However, at lower concentrations, some disagreement between experimental data and those obtained by the model was observed for the region which approaches the steady state. This could be due to the slow rate of intrapore diffusion which has not been considered in this model. This intrapore diffusion seems to be more important at low sorbent concentrations than that at higher sorbent concentrations. Therefore, a factor has to be added into Eq. (4.13) to take into consideration the intrapore diffusion. When this factor, as a function of time necessary for intrapore diffusion is taken into consideration, the discrepancy between the experimental results and those predicted by the equation is diminished.

When a correction factor as a function of time, $\varepsilon(t)$, is taken into consideration to address intrapore diffusion Eq. (4.13) becomes:

$$\frac{dC_A}{dt} = -k_l'\left[(X_0 + K)C_A - KC_0\right] + \varepsilon(t) \tag{4.15}$$

The general solution of Eq. (4.15) is

$$C(t) = C_0 e^{-k_l'(X_0+K)t} + \int_0^t e^{-k_l'(X_0+K)(t-\tau)}(\varepsilon(\tau) + k_l' K C_0)d\tau \tag{4.16}$$

Different forms of $\varepsilon(t)$ have been tested but the following one gave the best fit to the experimental data:

$$\varepsilon(t) = \beta t \tag{4.17}$$

where β can be considered as a rate constant of metal uptake contributed to intrapore diffusion. Substituting Eq. (4.17) into Eq. (4.16) and integrating this final equation resulted in the following expression:

$$C_A(t) = C_0 e^{-k_l'(X_0+K)t} + \frac{k_l'^2(X_0 + K)KC_0 - \beta}{k_l'^2(X_0 + K)^2}(1 - e^{-k_l'(X_0+K)t}) - \frac{\beta t}{k_l'(X_0 + K)} \tag{4.18}$$

The metal uptake, $q(t)$, was calculated using the following equation:

$$q(t) = \frac{C_0 - C_A(t)}{X_0} \tag{4.19}$$

The parameters k_1', K and β can be determined by fitting Eqs. (4.18) and (4.19) to the experimental data.

4.3 Selectivity index

The relative affinity of the sorbent for different metal ions in a mixed metal ions system may be described by the binary selectivity index K_{ij} between pair of ions i and j, that is often used to describe the selectivity of ion exchange resins, which is defined as

$$K_{ij} = \frac{y_i x_j}{y_j x_i} \tag{4.20}$$

where x is the equilibrium molar composition of adsorbate in solution and y is its equilibrium molar composition on sorbent.

The effect of ionic interaction, of metal ion on the other metal ion, on the adsorption process may be represented by the ratio of the adsorption capacity for one metal in the presence of other metal ions, q_{mix}, to the adsorption capacity for the same metal when it is alone in solution, q_0, (Tan *et al.*, 1985) such that for

$\frac{q_{mix}}{q_0} > 1$ adsorption is promoted by the presence of other metal ions

$\frac{q_{mix}}{q_0} = 1$ no observable net interaction effect

$\frac{q_{mix}}{q_0} < 1$ adsorption is suppressed by the presence of other metal ions

4.4 Bohart and Adams model

The following classical analysis of Bohart and Adams (1920) was applied to some of the results obtained from the packed columns. Although the analysis was done for the gas-charcoal absorption system, its overall approach based on reaction kinetics can be applied successfully in quantitative description of other systems, e.g., for the sorption of Cd^{2+} by *Ascophyllum nodosum* (Volesky and Prasetyo, 1994). Therefore, it is decided to apply this model for the column studies in this

work. The derivation of the model was based on a reaction between chlorine and charcoal. In this work chlorine and charcoal will be replaced by the sorbate metal and the plant sorbent, respectively.

Let C represent the concentration of sorbate at any distance of the bed, Z, N the residual sorbate capacity of the sorbent and F the rate supply of sorbate. Then, according to Bohart and Adams the rate of absorption reaction is $k \times C \times N$, where k is the reaction rate constant or the sorption rate constant, in this case. Considering a given portion of sorbing material, its residual capacity is diminished at the rate given by:

$$\frac{\partial N}{\partial t} = -kNC \tag{4.21}$$

and the solute concentration (C) is diminished in the solvent at a rate given by:

$$\frac{\partial C}{\partial Z} = -\frac{k}{F}NC \tag{4.22}$$

The initial and boundary conditions for the two partial differential equations of (4.21) and (4.22) are:

$$t = 0, \quad N = N_0$$

$$Z = 0, \quad C = C_{in}$$

where N_0 represents the initial sorption capacity of the sorbent material. Solution of the two equations results in the following expression:

$$\ln\left[\frac{C_{in}}{C} - 1\right] = \ln\left(e^{ZN_0k/F} - 1\right) - kC_{in}t \tag{4.23}$$

Since the exponential term in Eq. (4.23) is much larger than unity, the later can be omitted, resulting in the removal of the remaining logarithm and leading to the expression for t (Bed-Depth-Service-Time, BDST):

$$t = \left(\frac{N_0}{C_{in}F}\right)Z - \frac{1}{kC_{in}}\ln\left[\frac{C_{in}}{C_{out}} - 1\right] \tag{4.24}$$

The term $\frac{1}{kC_{in}}\ln[(C_{in}/C_{out}) - 1]$ represents the bed depth over which adsorption takes place and t represents the service time to breakthrough, time required to attain certain effluent concentration for a given set of conditions (flow rate, depth and influent concentration). The N_0 and k, which are the key comparative and process design parameters, can be determined from the slope and intercept, respectively, of the straight-line relationship between t and Z for a given C_{out} (break-through concentration).

In Eq. (4.24) at 50% service time ($C_{in}/C_{out} = 2$) the logarithmic term reduces to zero and the expression can be written as:

$$t_{50} = \frac{N_0}{C_{in}F} Z \tag{4.25}$$

Thus, a straight line of t_{50}/Z curve passing through the origin demonstrates that the adsorption data follow the model.

The minimum or critical bed depth (Z_{min}) is another useful comparative parameter which is defined as the theoretical minimum depth of the sorbent sufficient to prevent effluent solute concentration from exceeding the given value of C_{out} at time $t = 0$. It can be calculated from Eq. (4.24) by letting $t = 0$ and solving for Z;

$$Z_{min} = \left(\frac{F}{kN_0}\right) \ln\left[\frac{C_{in}}{C_{out,b}} - 1\right] \tag{4.26}$$

where $C_{out,b}$ is effluent concentration at the break-though.

4.5 Multicomponent Isotherm Models

This section describes the models used to represent the isotherms of multicomponent equilibrium data. In this work three different models were used to fit the binary equilibrium data. The first one is the extended-Langmuir model (Model 1) which can be written as:

$$q_{e,j} = \frac{K_j b_j C_{e,j}}{1 + \sum_{i=1}^{m} b_i C_{e,i}} \tag{4.27}$$

where $q_{e,j}$ is the equilibrium solid phase concentration for the jth component in an m component system, $C_{e,j}$ and $C_{e,i}$ are the equilibrium concentration of jth and ith component, respectively, K_j represents the maximum uptake for component j in a mixture of metal ions and b_j and b_i are the Langmuir constants for component j and i, respectively. Obviously, single Langmuir isotherm, Eq. (4.1), for component 1 or 2 can be obtained be setting $C_{e,2} = 0$ or $C_{e,1} = 0$ in Eqs. (4.27), for the case of binary system.

The second multicomponent isotherm model was proposed by Yon and Turnock (1971) and Fritz and Schluender (1974), and suggests extension of the three-parameter model (Eq. (4.27)), in the case of binary system, to five-parameter model, by introducing the exponent n_i in the denominator of the of the above model. This model (Model 2), some times called modified extended-Langmuir, can be written as:

$$q_{e,j} = \frac{K_j b_j C_{e,j}}{1 + \sum_{i=1}^{m} b_i C_{e,j}^{n_i}} \tag{4.28}$$

Although the above model is empirical, it is very often used to describe the equilibrium isotherm data of multicompnent systems (Fritz and Schlunder, 1974; Chong and Volesky, 1995).

The third model is a combination of the conventional single component Langmuir and Freundlich models (Fritz and Schlunder, 1974; Liapis

/.p and Rippin, 1977) and is called Langmuir-Freundlich model (Model 3):

$$q_{e,j} = \frac{K_j b_j C_{e,j}^{n_j}}{1 + \sum_{i=1}^{m} b_i C_{e,j}^{n_i}} \tag{4.29}$$

The parameters of Eqs. (4.27)-(4.29) can be determined by fitting these equations to the binary equilibrium data of component 1 and 2, using the Least Square algorithm.

Part II

Results and Discussion

and

Conclusions and Recommendations.

Chapter 1

Adsorption of Heavy Metals by *Aspergillus carbonarius*

1.1 Introduction

In this chapter, the ability of *Aspergillus carbonarius* to sorb Cu^{2+}, Cr^{3+} and Cd^{2+} from aqueous solutions is described. This microorganism has been previously used for the production of phytase and the decrease of the phytic acid content in canola meal during a solid- state fermentation process (Al-Asheh and Duvnjak, 1994). This microorganism has not been previously used for the removal of heavy metals. The effects of biomass and metal concentrations, pH, temperature and addition of glucose to the metal-containing solutions on the adsorption of metals by the above microorganism will be discussed.

1.2 Adsorption Kinetics

The kinetics of the biosorption of Cu^{2+}, Cr^{3+} and Cd^{2+} by the biomass of *A. carbonarius* was studied in a batch process and the experimental data are presented in Figure 1.1. The kinetics study is essential to establish the rates of metal uptake and release. A rapid uptake would allow for a shorter solution-biosorbent contact time and would result in the use of much shallower contact beds of sorbent materials in their column applications (Kuyucak and Volesky, 1988a). The results of Figure 1.1 show, as most of others in this work, that the largest amount of the adsorbed metal ions, in the case of Cu^{2+} and Cd^{2+}, were attached to the biomass within the first 10 min of the adsorption process, and after 30 min, the rate of metal uptake became very slow. A rapid uptake was also obtained by Mattuschka and Straube (1993) for the biosorption of Cu^{2+}, Cr^{3+} and Cd^{2+} by *Streptomyces noursei*, and by Guibal *et al.* (1992) for the uranium biosorption by a filamentous fungus *Mucor miehei*. It was also shown (Figure 1.1) that up to 60 min of adsorption the rate of Cu^{2+} uptake by *Aspergillus carbonarius* was higher than that of Cr^{3+} followed by Cd^{2+}. This might indicate that the sites at the cell surface, which are available for metal sorption, have a greater preference for certain metals than others.

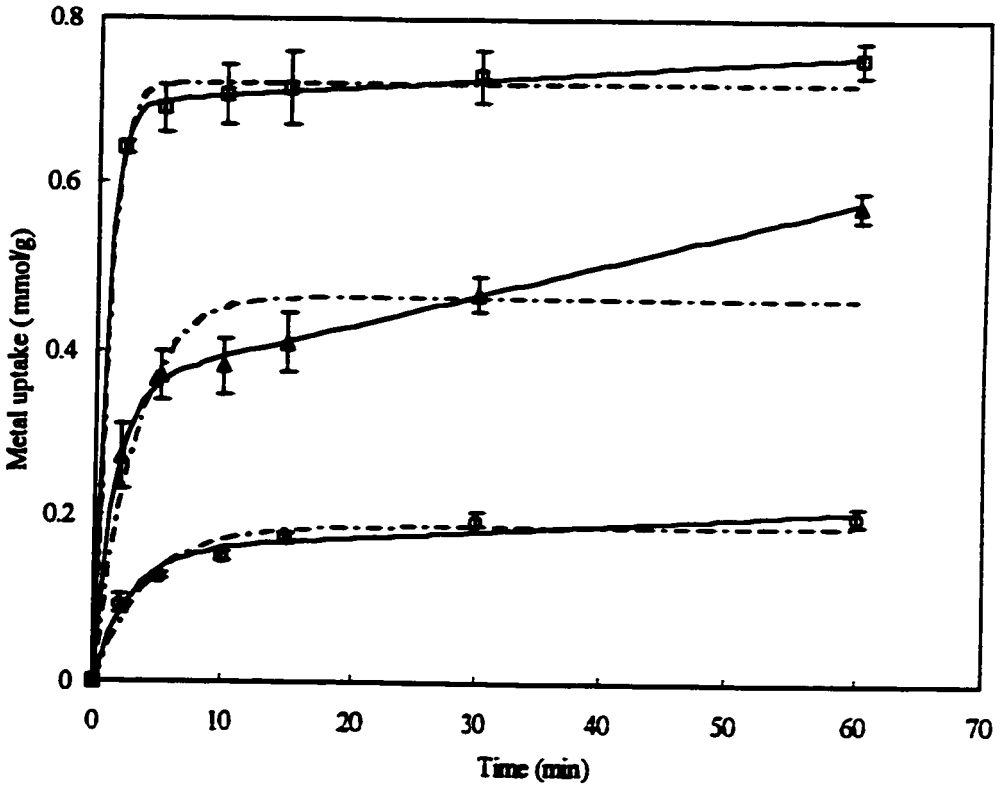

Figure 1.1: Kinetics of Cu^{2+}, Cr^{3+} and Cd^{2+} uptake by *A. carbonarius*. Symbols are experimental data (□-Cu^{2+}, Δ-Cr^{3+}, O-Cd^{2+}), interrupted lines and solid lines are fitting data using Eq. (4.14) and Eq. (4.18), respectively. Initial metal concentration: 100 ppm; initial biomass concentration: 1.15 mg/mL; initial pH: 5.0.

The high initial rate of metal uptake suggests that the sorption occurs mainly at the surface of the biomass. When the metal uptake results were plotted against square root of the sorption time (Figure 1.2), linear correlations were obtained. This indicates that the mechanism of intrapore diffusion was also involved in the sorption (Weber and Morris, 1963). However, the intrapore diffusion is not a controlling step in the sorption of copper by this biosorbent and this can be concluded from the plotted lines which do not pass through the origin (Singh *et al.*, 1989).

Equations (4.14), (4.18) and (4.19) were used to fit the data in Figure 1.1 using the least square technique. The values of the constants for these models were computed for each metal ion (Table 1.1) using the SCIENTIST software package (Micromath Scientist Software). Equation (4.18) represented the experimental data better than Eq. (4.14) (Figure 1.1). The reason for this is that the former equation takes into consideration the intrapore diffusion, the existence of which can be noticed from the figure after the first few minutes of the sorption process. However, this model is semi-empirical. It does not have a full physical meaning and therefore, it is suggested to use Eq. (4.14). As

it is expected, Table 1.1 shows that the mass transfer coefficient, k_t', for Cu^{2+} is higher than that of Cr^{3+} which is followed by Cd^{2+}. This is consistent with the previous conclusion concerning the order of the uptake of these three metals by the considered biomass. These models, Eqs. (4.14) and (4.18), can be applied for other biosorbents as well, to estimate the amount of metal uptake at any contact time for a given set of conditions and model parameters.

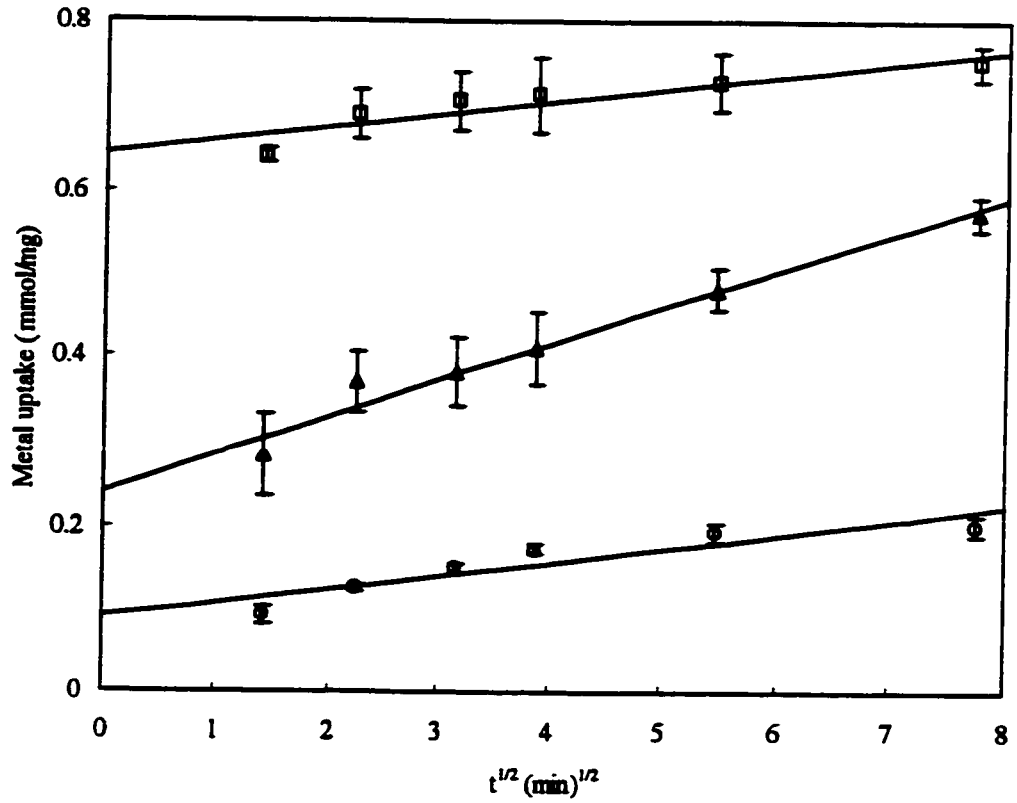

Figure 1.2: Plots of metal uptake versus square root of time for various metal ions. □-Cu^{2+}, Δ-Cr^{3+}, O-Cd^{2+}. Initial metal concentration: 100 ppm; initial biomass concentration: 1.15 mg/mL; initial pH: 5.0.

Table 1.1: Constants of Eq. (4.14) and (4.18) for various metal ions

Metal ion	k_t' ($\beta=0$) mL/(mg.min)	K ($\beta=0$) (mg/mL)	K (mg/mL)	k_t' mL/(mg.min)	β mmol/(g.min^2)
Cu^{2+}	0.5008	1.036	1.122	0.5539	0.00147
Cr^{3+}	0.0830	2.989	4.334	0.1253	0.00291
Cd^{2+}	0.0506	3.584	4.648	0.0639	0.00036

1.3 Effect of Biomass Concentration

Different amounts of metal ions can be removed from the solution depending on the amount of the biosorbent used in the sorption process. The effect of the biomass concentration on the amount of Cu^{2+}, Cr^{3+} and Cd^{2+} removal was studied using the biomass concentrations in the range of 1.15 to 9.20 mg per mL of a 100 ppm metal solution. The results after 60 min of adsorption are shown in Figure 1.3. The kinetics of the uptake at each biomass concentration and for each metal are shown in Appendix A (Figures A.1–A.3). Figure 1.3 shows that the total amount of the metal ion removal increased with an increase in the biomass concentration, but the uptake of each of these metal ions per unit of biomass increased with a decrease in the biomass concentration. Depending on the biomass concentration, 55-67, 34-48 and 25-38% of the initial amounts of Cu^{2+}, Cr^{3+}, and Cd^{2+}, respectively, were removed from the individual solution of these metal ions. The higher metal loading of biomass with a decrease in biomass concentration has also been noticed by Gadd and White (1989) for the uptake of thorium by *S. cerevisiae* and by Aksu and Kutsal (1991) for the removal of Pb^{2+} ions from wastewater by *Chlorella vulgaris*. The amounts of metal ions removed by the tested biosorbent in this work as displayed in Figure 1.3 are comparable to those obtained by other workers using different biosorbents (Table 2.8; Part I).

1.4 Equilibrium Isotherm

The equilibrium isotherms for the sorption of different metals by *A. carbonarius* were investigated in a batch process. The isotherms for Cu^{2+}, Cr^{3+} are Cd^{2+} were obtained using initial metal concentrations in the range 0.157-1.57, 0.48-7.69, and 0.089-0.89 mM, respectively. The results of the equilibrium concentration, C_e (mM), i.e. the metal concentration in the suspension after biosorption, and the metal uptake at equilibrium, q_e (mmol/g), are shown in Figure 1.4. The results showed that the increase in the metal uptake is associated with an increase in the initial metal concentration. This can be explained by the gradual increase in electrostatic interactions, relative to covalent interactions, with sites of progressively lower affinity for Me^{2+} when its initial concentrations increased (Van Cutsem *et al.*, 1984). The results were well described by the linearized Freundlich adsorption isotherm equation, Eq. (4.8), Chapter 4; Part I, (Figure 1.4). The Freundlich constants for the three metals are given in Table 1.2, together with some other Freundlich constants from the literature for the same metal ions and other biosorbents. The value $1/n$ indicates that the sorption intensity of Cu^{2+} by *A. carbonarius* is

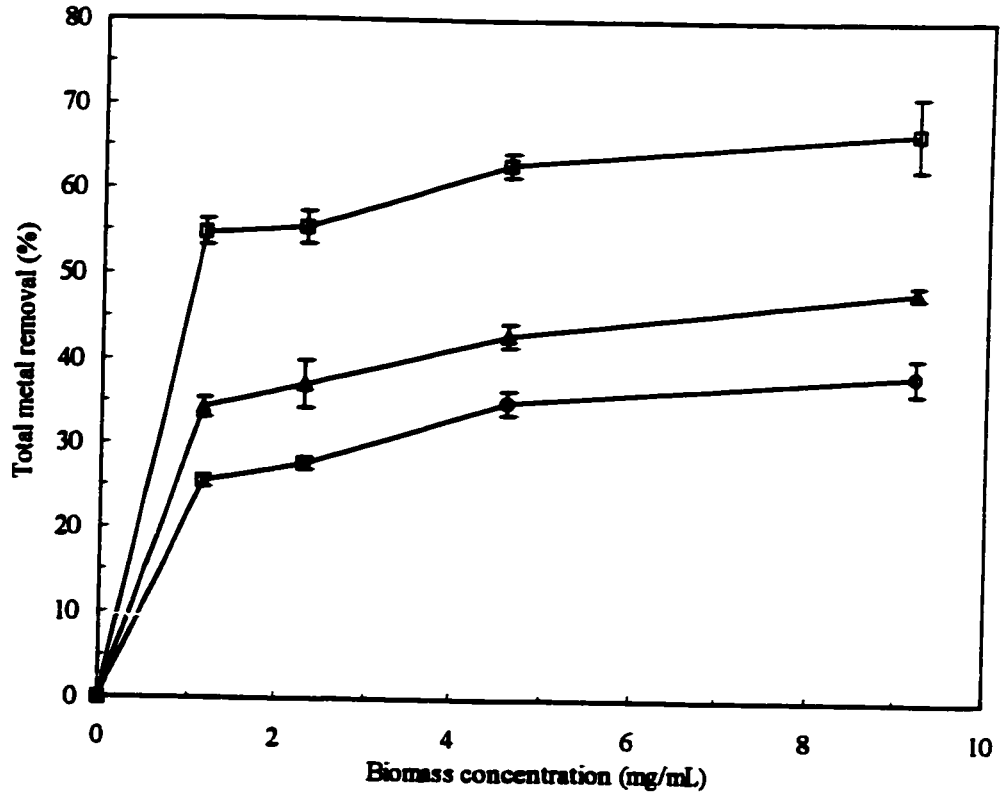

Figure 1.3: Effect of biomass concentration on metal removal. Metal: □-Cu^{2+}, Δ-Cr^{3+}, O-Cd^{2+}. Initial metal concentration: 100 ppm; initial pH: 5-5.3; sorption time: 60 min.

comparable to the other biosorbents cited in Table 1.2 and that of Cd^{2+} is higher than that of other biosorbents from the literature (Table 1.2). It is seen that the values of k_F and $1/n$ obtained in this work are in agreement with those obtained for the other types of microbial biomass. The results from Figure 1.4 showed that order of the uptake of the three metals is: $Cr^{3+} > Cu^{2+} > Cd^{2+}$ which is different than the order concluded previously ($Cu^{2+} > Cr^{3+} > Cd^{2+}$). The reason for this was that the isotherms were obtained using different biomass concentrations and the kinetics (Figure 1.1) were performed up to 60 min of adsorption.

One of the major disadvantages of the Freundlich equation is that it has no physical basis. However, since it applies to the isotherm data (Figure 1.4), this indicates the existence of multiple sites available for adsorption (Masel, 1996).

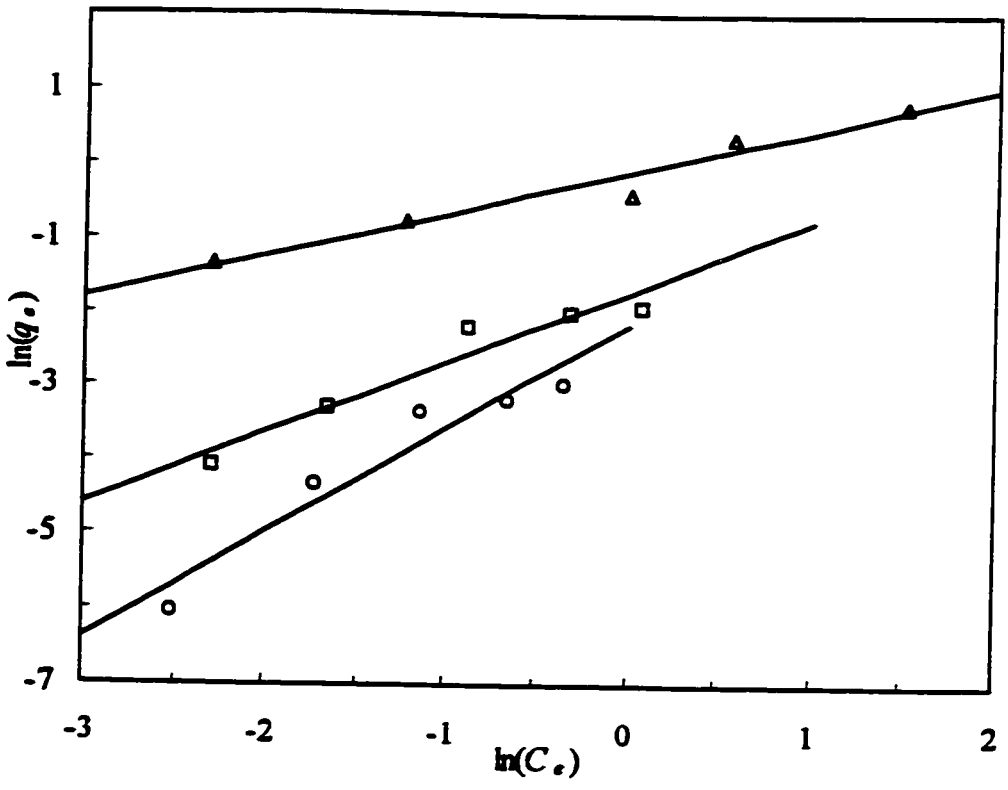

Figure 1.4: Freundlich isotherms for sorption of copper (□), chromium (Δ), and cadmium (O) by *A. carbonarius* using a biomass concentration of 3.36, 1.44, and 3.71 mg/mL, respectively, at pH 5.0.

Table 1.2: Freundlich constants for sorption of Cu^{2+}, Cr^{3+}, and Cd^{2+}

Metal	Biosorbent	k_F	$1/n$	R^2	Reference
Cu^{2+}	*Streptomyces noursei*	1.403	0.964		Mattuschka and Straube (1993)
	Cladosporium resinae	1.089	0.640		de Rome and Gadd (1987)
	Thiothrix Strain A1	1.38	0.650	0.999	Shuttleworth and Unz (1993)
	A. carbonarius	0.178	0.955	0.994	This work
Cr^{3+}	*Streptomyces noursei*	1.52	1.100		Mattuschka and Straube (1993)
	A. carbonarius	0.896	0.555	0.964	This work
Cd^{2+}	*Zoogloae ramigera*	0.3	0.75		Sag and Kutsal (1989)
	Escherichia coli	1.067	0.497	0.966	Mullen *et al.* (1989)
	Bacillus cereus	0.212	0.657	0.962	
	A. carbonarius	0.114	1.40	0.994	This work

1.5 Effect of the Initial pH

The adsorption of metals by microorganisms is highly pH sensitive. Aksu *et al.* (1991) noticed a very low uptake of Cu^{2+} at pH 2 and an increase in the uptake of these ions by activated sludge bacteria with an increase in pH up to 5. Almost no Cu^{2+} uptake by *Chlorella vulgaris* was noticed at pH 3, while the maximum uptake was obtained at pH 5 (Harris and Ramelow, 1990). A further increase in pH up to 7 resulted in a slight decrease in Cu^{2+} adsorption. Kuyucak and Volesky (1988b) studied the adsorption of Cr^{3+} by *Halimeda opuntia* and *Ascophyllum nodosum*. With *H. opuntia*, the uptake increased with increasing pH from 2 to 4, while there was no significant change in the Cr^{3+} uptake with a further increase in pH up to 11. In the case of *A. nodosum*, the authors obtained a steady increase in the metal uptake with an increase in pH from 2 to 8. However, with a further increase in pH up to 11, a slight decrease in the Cr^{3+} uptake was noticed. The effect of pH on Cd^{2+} removal was investigated by Pascucci (1993) using the algal biomass *Chlorella vulgaris*. The study covered the range of pH 2-7, and demonstrated an increase in Cd^{2+} uptake with increase in pH up to 7. From these literature data, it can be concluded that the optimum pH for metal adsorption depends on the type of biomass and the metal used for the adsorption studies.

Considering this, it was of interest to investigate the relationship between pH and the adsorption of metals using *A. carbonarius* biomass. The adsorption of Cu^{2+}, Cr^{3+} and Cd^{2+} was studied in deionized water, the pH of which was adjusted with 1 M NaOH or 1 M HCl. The results (Figure 1.5) showed an increase in the amount of metal removal with an increase in the initial pH of the suspension for all of the three tested metals. The results showed that the greatest adsorption occured at higher pH values, but because of the low solubility of some metals at high pH, especially Cu^{2+}, higher pH can not be considered. Therefore, the optimum (maximum) pH was not achieved because the study was restricted to pH values up to 5. The increase in biosorption by raising the pH may be, in general, ascribed to the increase in the number of (or the concentration of) negatively charged groups at the surface of the microbial cells as it was reported by Luef *et al.* (1991) who used *Aspergillus niger* for the sorption of Zn^{2+}.

1.6 Effect of Temperature

Temperature is another important parameter that influences the sorption process. Suspensions of 1.6 mg/mL biomass at pH 5 were prepared and transferred to water baths set at various temperatures. After 10 min, 1.57 mM Cu^{2+} was added, and the adsorption was followed for 15 min.

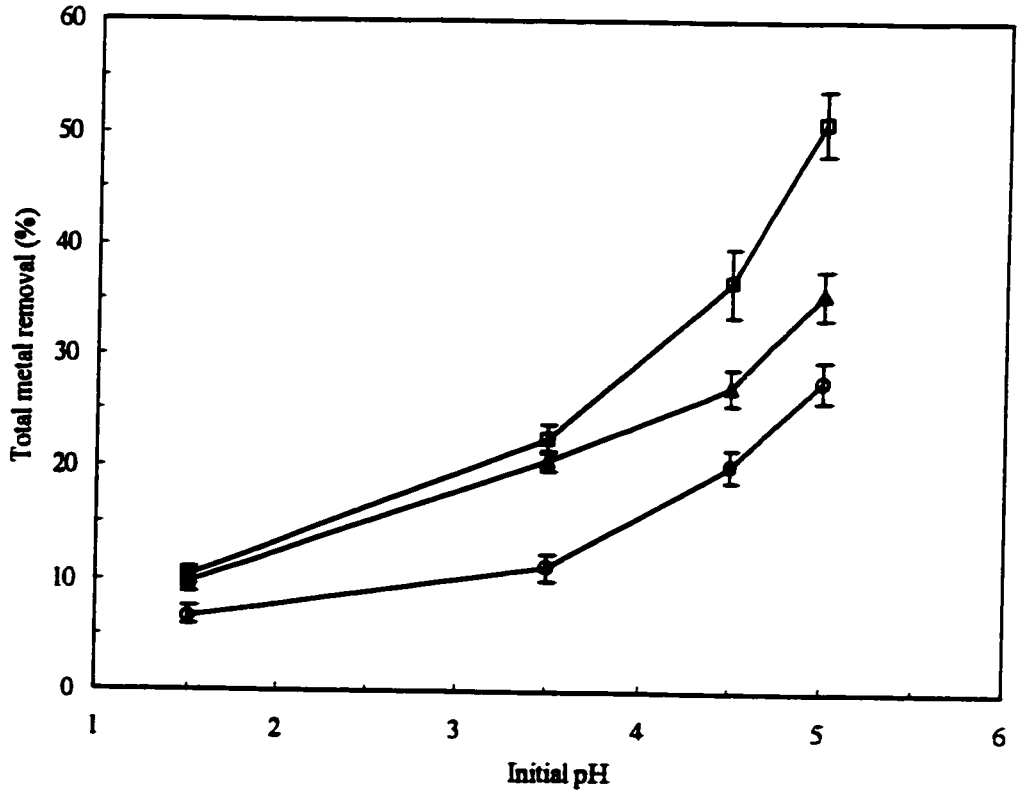

Figure 1.5: Effect of pH on the sorption of copper (□), chromium (Δ), and cadmium (O) by *A. carbonarius*. Biomass concentration: 2.3 mg/mL; initial metal concentration: 100 ppm; sorption time: 60 min.

The highest temperature for the uptake was 25°C (Figure 1.6). Similar trends were obtained for Zn^{2+} uptake by *Candida utilis* (Failla *et al.*, 1976). In their case, however, the optimum temperature was 30°C. The uptake was depressed with further increase in temperature. The increase in the uptake with temperature could be due to the increase in the energy of the system, which facilitates the attachment of Cu^{2+} to the surface of the cells, while the decrease with further increase in temperature is probably a result of the distortion of some sites of the cell surface available for metal sorption, or due to the increase in the desorption process that might occur at high temperatures.

The decrease in Cu^{2+} uptake by the microbial biomass at higher temperatures can be also related to the metabolic activity of the cell as reported by Norris and Kelly (1977). The authors reported that Cu^{2+} uptake by *S. cerevisiae* required an energized-membrane state. Therefore, the amount of metal transfer to the microbial cell of *A. carbonarius* increased with the increase in the temperature because of the increase in the energy of the cell membrane until the optimum temperature

(Figure 1.6). However, the further increase in the temperature may have resulted in the breakdown o the membrane-selective permeability barrier causing a decrease in the metal uptake.

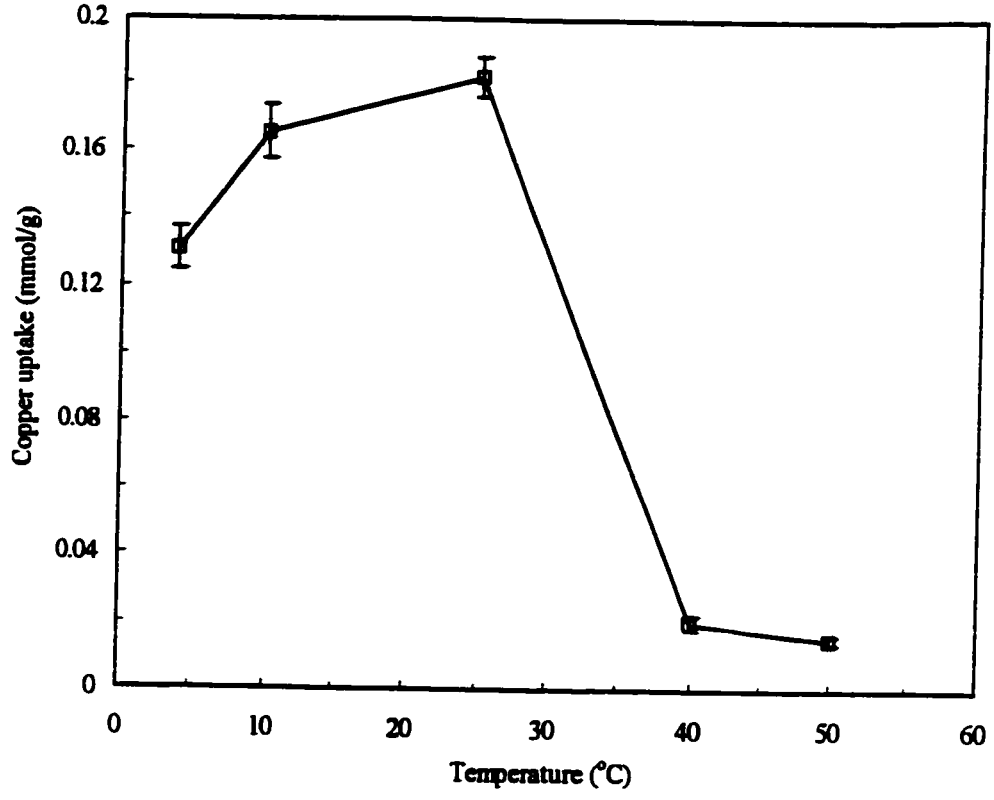

Figure 1.6: Effect of temperature on the sorption of copper by *A. carbonarius*. Biomass concentration: 1.6 mg/mL; initial metal concentration: 1.57 mM; initial pH: 5.0; sorption time: 15 min.

1.7 Preheating of Cells

To examine the ability of preheated cells to sorb Cu^{2+} ions, two pH 5 suspensions of cells, each containing 6.2 mg/mL dry biomass, were preheated at 50°C; the first and the second suspension were exposed to this temperature for 1 h and 2 h, respectively, and then allowed to cool down to the room temperature. A third suspension was used to serve as the control (without preheating). Each system was supplemented with Cu^{2+} to a concentration of 1.57 mM and the metal sorption was measured after 15 minutes. The data indicated that the preincubation of the cells at higher temperature had a negative effect on the sorption process. The Cu^{2+} uptakes of 0.0463 mmol/g and 0.0127 mmol/g were noticed in the systems preincubated for 1 h and 2 h, respectively. The sorption of 0.0545 mmol Cu^{2+} per g of biomass was observed in the control. Such an effect of the exposure of the cells to a higher temperature

was expected because of the heat sensitivity of protein and lipid constituents of microbial cells surfaces, which are found to be at least partially responsible for metal adsorption.

1.8 Effect of Glucose

The effect of glucose on the metal sorption by microbial cells has been reported. Norris and Kelly (1977) observed higher Co^{2+} and Cd^{2+} uptakes by *Saccharomyces cerevisiae* in the presence of 1 M glucose than without it. Fuhrmann and Rothstein (1968) studied the transport of Zn^{2+}, Co^{2+} and Ni^{2+} onto bakers' yeast cells in the presence of glucose as well as phosphate. They reported that the uptake was enhanced 5-20 fold in the presence of these compounds compared to the starved cells. In this study it was also noticed that glucose affected the amount and the rate of Cu^{2+} uptake by the cells of *A. carbonarius* (Table 1.3, Figure 1.7). A longer pre-exposure of the cells to glucose resulted in a higher Cu^{2+} uptake (Table 1.3). It was also noticed that, depending on the initial pH of the suspension, lower concentrations of glucose resulted in higher rates and amounts of Cu^{2+} sorption, while higher concentrations substantially reduced the ability of the cells to adsorb this metal. The increase in the sorption can be the result of an increase in the cells' activities, including the metal adsorption activity, caused by the increase in the energy available to the cells in the presence of glucose. At higher glucose concentrations, it may happen that glucose interferes with the cells' metal-active sites, thus preventing their interactions with metal ions. Therefore, it is necessary to find the optimum glucose concentration for the adsorption of metals by microbial biomass. In this study, the optimum was found to be pH dependent and it is 0.3%, 0.1% and 0.05% of glucose (w/v) at pH 4.3, 4.7 and 5.2, respectively (Figure 1.7). This means that the optimum glucose concentration is increasing with the decrease in the pH of the suspension.

Table 1.3: Effect of glucose on copper uptake by *A. carbonarius*. Initial Cu^{2+} concentration: 1.57 mM; biomass suspension: 3.23 mg/mL; initial pH 5.0.

System	Cu^{2+} uptake (mmol/g)
No glucose	0.109
0.1% glucose[1]	0.112
0.1% glucose[2]	0.134
0.1% glucose[3]	0.148

[1] Cu^{2+} added after shaking the suspension with glucose for 15 min.
[2] Cu^{2+} added after shaking the suspension with glucose for 1 h.
[3] Cu^{2+} added after shaking the suspension with glucose for 2 h.

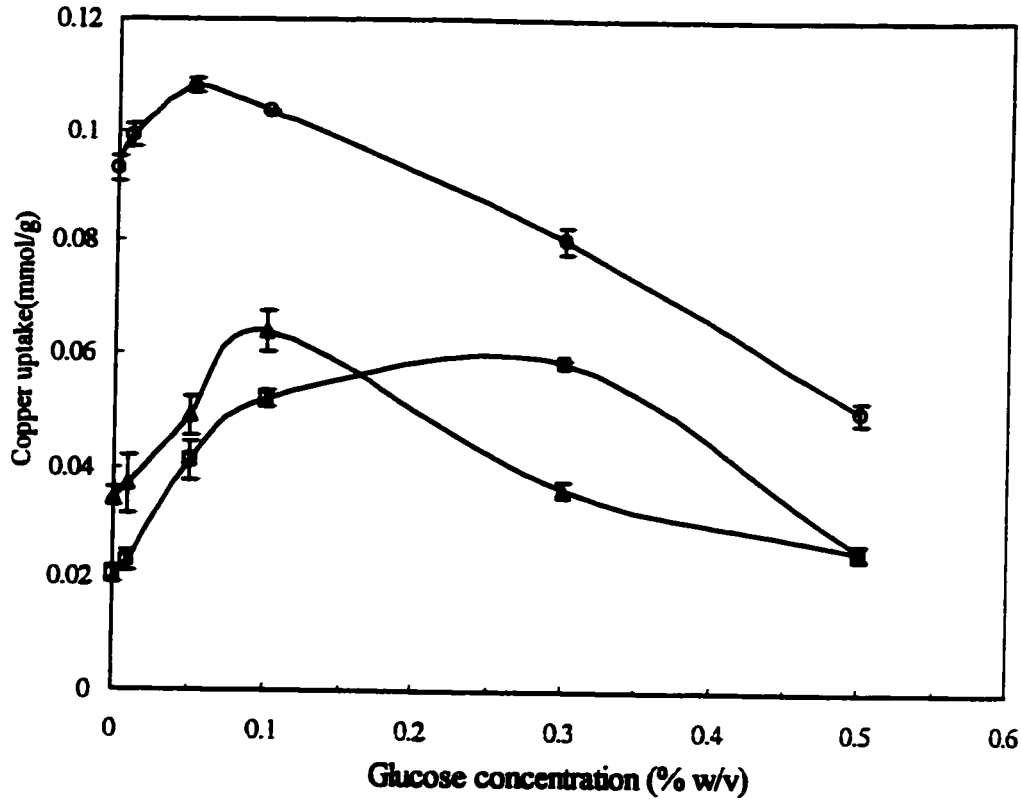

Figure 1.7: Effect of glucose concentration on Cu^{2+} uptake by *A. carbonarius* at different pH values. Initial Cu^{2+} concentration: 1.57 mM; biomass suspension: 3.2 mg/mL; pH: 4.2-□, 4.7-Δ, 5.2-O.

1.9 Effect of Other Metals on Copper Uptake

The presence of other metal ions in the solution could influence the uptake of the metal under consideration by the biosorbent. This was tested by the addition of different metal ions, namely Zn^{2+}, Mg^{2+}, Co^{2+}, and Ni^{2+}, individually in a range of 10-100 ppm, to a biomass suspension of 100 ppm initial Cu^{2+} concentration. The relationship between Cu^{2+} uptake and the initial concentration of these metal ions is displayed in Figure 1.8. Copper uptake was substantially inhibited by the presence of each of these metal ions, and the level of inhibition depends on the concentration of the added ion. At equal initial concentrations of Cu^{2+} and either Mg^{2+} or Zn^{2+} at 100 ppm, no Cu^{2+} uptake was observed. These results could indicate that there is competition between Cu^{2+} and each of these metal ions for the available adsorption sites at the surface of the biomass. Such a trend was also observed by Norris and Kelly (1977), where the uptake of Ni^{2+} by *Saccharomyces cerevisiae* was inhibited by the presence of Zn^{2+} or Mg^{2+}, individually. Failla *et al.* (1976) noticed an inhibition of Zn^{2+} by Cd^{2+}, and suggested that Cd^{2+} is accumulated by *S. cereviciae* as a result of its chemical similarity to Zn^{2+}.

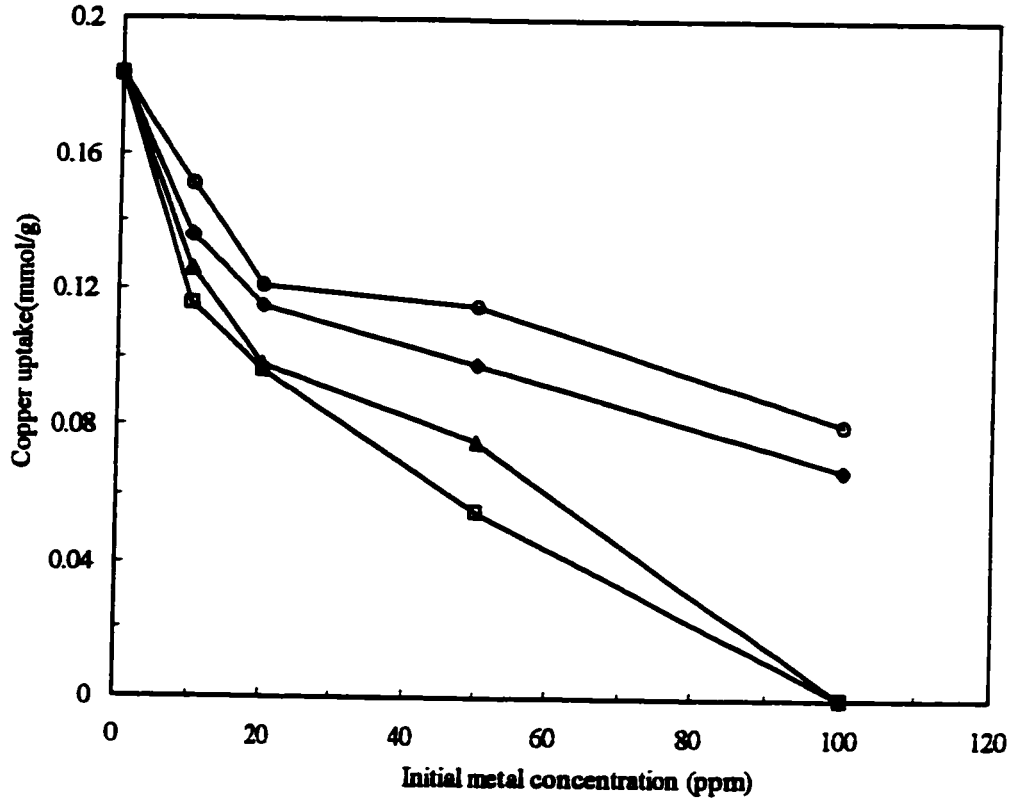

Figure 1.8: Effect of the concentration of Zn^{2+} (□), Mg^{2+} (Δ), Co^{2+} (◊), and Ni^{2+} (○) on copper uptake by *A. carbonarius*. Initial Cu^{2+} concentration: 1.57 mM; biomass: 3.2 mg/mL; initial pH: 4.7.

The data from this study showed that the biomass of *A. carbonarius* can be used for the selective adsorption of metals from aqueous solutions. More metal uptake per unit mass of the biomass was noticed at lower biomass concentrations than at higher ones. Freundlich-type model fitted the equilibrium isotherm data of Cu^{2+}, Cr^{3+} and Cd^{2+}. The highest temperature for copper uptake was 25°C. Preheating of the biomass suspension depressed copper adsorption compared to that of the control. It was also noticed that the addition of glucose enhanced copper adsorption and the optimum glucose concentration depended on the pH of the suspension.

Chapter 2

Adsorption of Heavy Metals by Canola Meal

2.1 Introduction

In this chapter, the results concerning the use of canola meal (CM) for metal sorption are discussed. In 1970's Canadian researchers developed new rapeseed varieties which were relatively low in glucosinolates and erucic acid and were called "Canola". Canada produced more than 5.487×10^6 metric tons of canola seeds in 1995/1996 (Statistics Canada, 1996). Canola seeds are very rich in oil (40-45%) and proteins (20-25%) (Harris, 1988). Canola meal is a by-product of canola oil production and its production in Canada in 1995/1996 was 1.724×10^6 metric tons (Statistics Canada, 1996). Canola meal contains 37-38% proteins and a number of vitamins (Clandinin, 1986). The amino acid composition of these proteins is considered to be of a good quality. This would qualify the commodity as a good feedstuff candidate, but, unfortunately, its glucosinolate, phenolic acid and phytic acid contents do not permit the utilization of the meal for animal feed to a larger extent. The utilization of this commodity for other purposes should also be considered. Taking into account that the protein content of microbial cell walls is one of the components responsible for metal adsorption (Kuyucak and Volesky, 1988a), and the fact that canola meal is rich in this component, it was decided to test the meal for the recovery of heavy metals from aqueous solutions. In addition, the phytic acid content (4.7%) in canola meal can also contribute to the metal binding capacity because of its ability to complex cations. This could lead to the opening of a potentially new market for this commodity as well as contribution to the solution of problems related to liquid wastes containing heavy metals. Canola meal costs about 15¢ per kg and, therefore, comparing it with other materials for the removal of heavy metals from wastewaters, e.g. activated carbon or ion exchange resins, it can be considered relatively inexpensive.

In this chapter, a study of the possible utilization of CM for the removal of copper, zinc, cadmium, chromium and nickel from aqueous solutions is reported. This is the first time that this material has been considered for this purpose. The effects of parameters such as the CM and metal concentrations, particle size of CM, its phytic acid content, and pH of the meal-metal suspensions on the metal adsorption were examined and the results are presented in the sections that follow.

2.2 Effect of CM Concentration

The effects of the meal concentration on the removal of metals from their aqueous solutions are shown in Figure 2.1. The metal uptake per unit weight of meal increased as the meal concentration decreased but the percentage of metal removal increased with an increase in the meal concentration. The results indicate that 45-69%, 48-75%, 42-95%, 37-65% and 11-40% of Cu^{2+}, Zn^{2+}, Cd^{2+}, Cr^{3+} and Ni^{2+}, respectively, were removed by various concentrations of CM from their individual solutions after 60 min of adsorption at initial pH 5-5.2 when the initial concentration of each of these metals was 100 ppm (Figure 2.2). Thus, the final metal concentration in the solution was higher in the presence of low meal concentrations than with higher meal concentrations. The affinity of CM for these metals (mass basis), when the concentration of CM was 9.2 mg/mL in the suspension, was in the following order: $Cd^{2+} > Zn^{2+} > Cu^{2+} > Cr^{3+} > Ni^{2+}$. High initial adsorption rates were noticed for Cu^{2+}, Zn^{2+}, Cr^{3+} and Cd^{+2} while the initial adsorption rate for Ni^{2+} was lower. In general, this characteristic of a biosorbent can be taken as a measure of its affinity for metal ions (Kuyucak and Volesky, 1988a). A rapid metal uptake was also observed by Shuttleworth and Unz (1993) in tests with Ni^{2+} and Zn^{2+} using *Thiothrix* Strain A1, and by Periasamy and Namasivayam (1994) for the adsorption of cadmium using activated carbon prepared from peanut hulls. The amounts of the metals adsorbed by CM in this work are comparable to the values obtained for other similar agricultural sorbents (Table 2.9; Part I).

The amount of metal ions removed from the solution depends on both the concentration of metal and the concentration of the sorbent. The relationship between the initial copper concentration and CM concentration, and the uptake of copper was studied using CM concentrations in the range of 1.38 to 9.2 mg/mL and each concentration was exposed to Cu^{2+} solutions of 0.394, 0.787 and 1.57 mM. The results (Figure 2.3) showed a higher copper uptake per unit mass of CM in the systems with the lower meal concentrations regardless of the initial copper concentration. It is also observed (Figure 2.3) that for a given CM concentration, Cu^{2+} uptake increased with an increase in its initial concentration. It is noticed (Figure 2.3) that for the initial copper concentrations up to approximately 1.57 mM (100 ppm), the uptake of copper per unit weight of CM tended to be fairly comparable values for the same meal to initial copper concentration ratios (Figure 2.3). Luef *et al.* (1991) studied the same dependence for the adsorption of zinc by *Claviceps paspali*, *Aspergillus niger* and *Pencillium chrysogenum*, and obtained results similar to those in Figure 2.3.

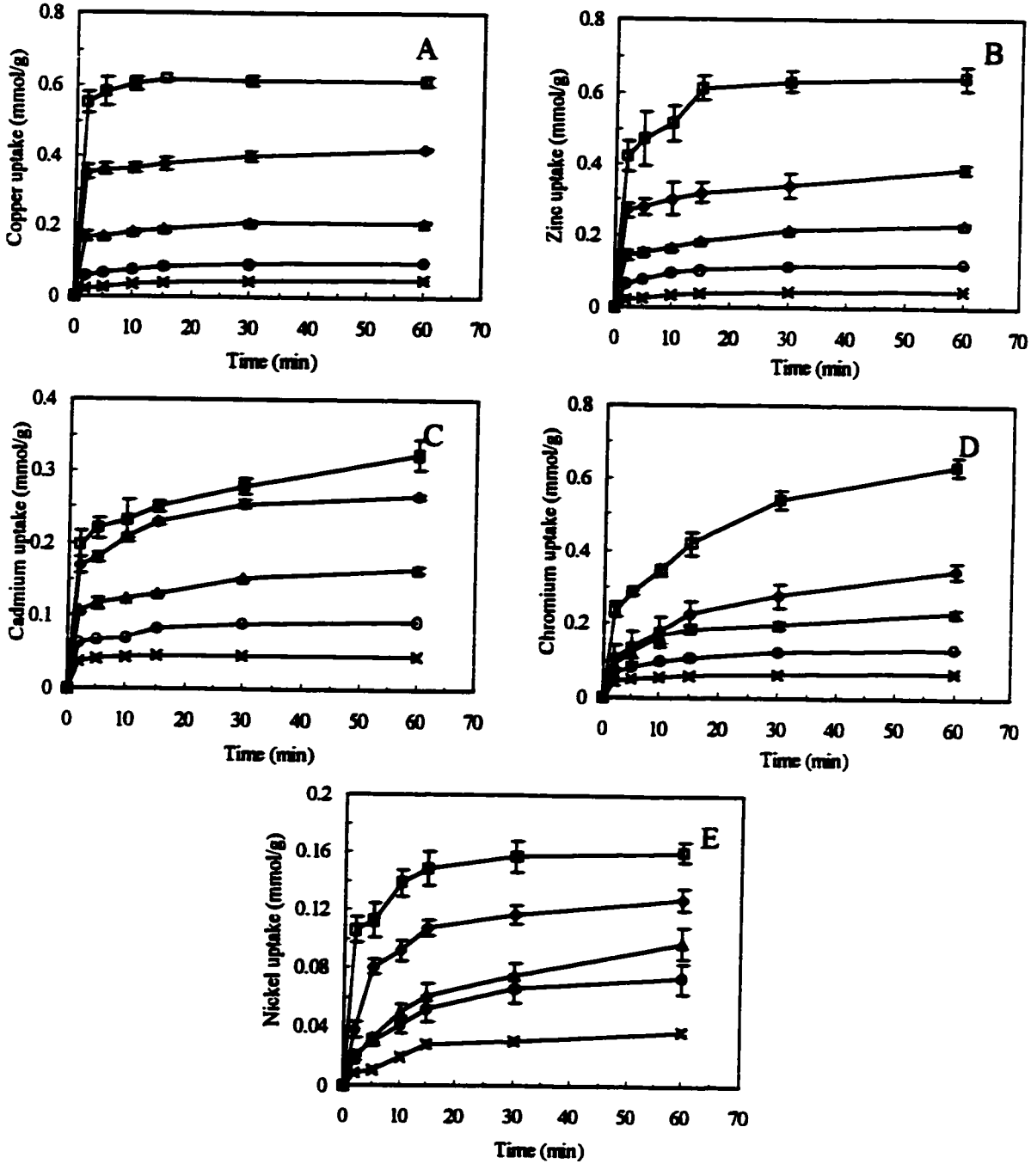

Figure 2.1: Effect of CM concentration on (A) Cu^{2+}, (B) Zn^{2+}, (C) Cd^{2+}, (D) Cr^{3+} and (E) Ni^{2+} uptake. Initial metal concentration: 100 ppm; initial pH: 5.0-5.2. CM (mg/mL): 1.15-□, 2.3-◊, 4.6-Δ, 9.2-O, 18.4-x.

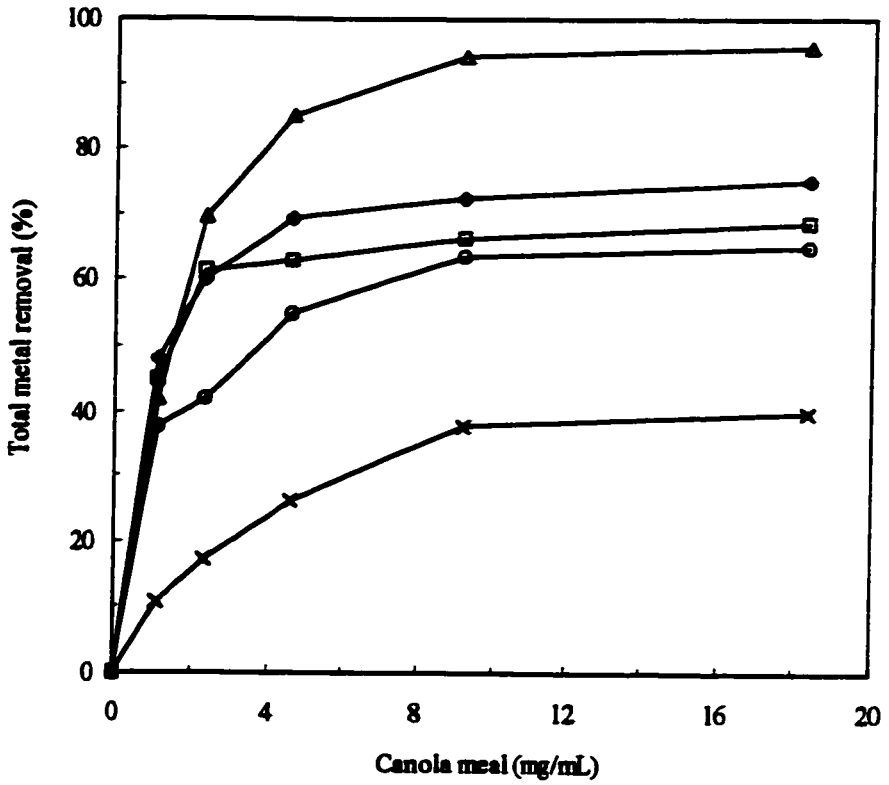

Figure 2.2: Relationship between the amount of metal removal and the CM concentration. Cu^{2+}-□, Zn^{2+}-◊, Cd^{2+}-Δ, Cr^{3+}-O, Ni^{2+}-×. Initial metal concentration: 100 ppm; initial pH: 5.0-5.2; sorption time: 60 min.

2.3 Equilibrium Isotherm

To examine the effect of metal concentration on its uptake, which can provide an indication of the meal's sorption capacity, individual meal suspensions (9.2 mg/mL) were subjected to different initial concentrations of Zn^{2+}, Cd^{2+} and Cr^{3+} ranged between 20-400 ppm. Due to the low copper solubility, its concentration was in the range of 10-100 ppm. sorption was carried out for 24 h. The results (Figure 2.4) indicated that an increase in the initial metals concentration resulted in an increase in their sorption. An increase in metal uptake with an increase in the metal concentration was also noticed by other researchers such as by Marshall and Champagne (1995) for the adsorption of zinc by soybean hulls, cottonseed hulls, rice straw and sugar cane bagasse, and by Bhattacharya and Venkobachar (1984) for the removal of cadmium by crushed coconut shells. The results for the four metals can be well represented by the linearized Freundlich isotherm model (Figure 2.4). When the

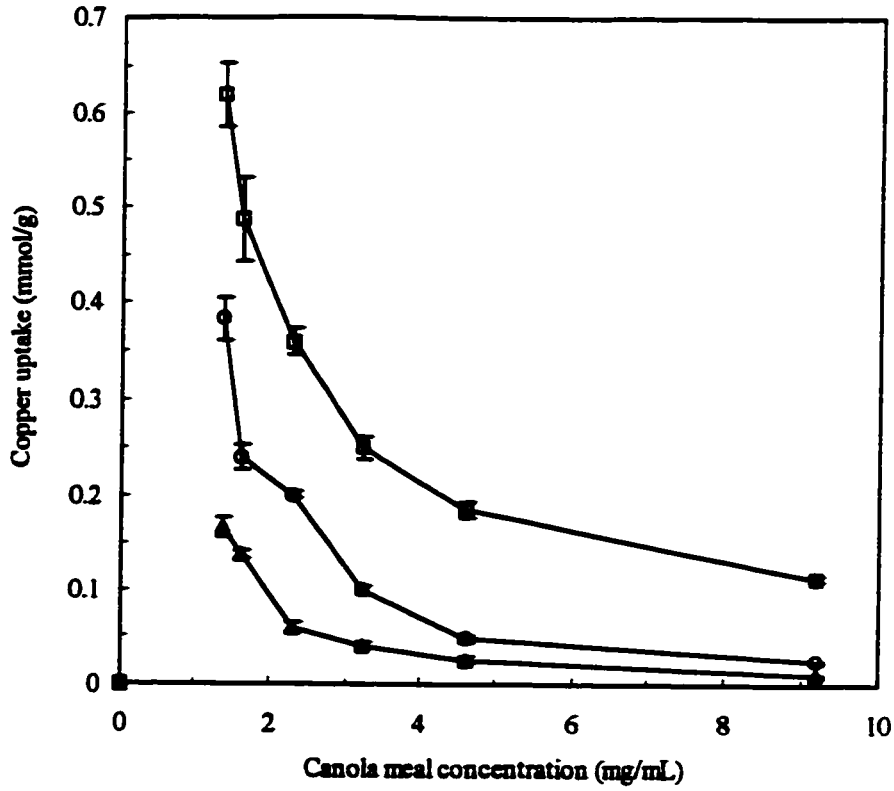

Figure 2.3: Effect of CM concentration on Cu^{2+} uptake using various Cu^{2+} concentrations. Initial pH: 5.0-5.2; sorption time: 60 min. Initial copper concentration (mM); 0.394-Δ, 0.787-O, 1.57-□.

Langmuir isotherm model was applied to the data of the four metals, a reasonable fit was also obtained for the equilibrium data of Cu^{2+}, Zn^{2+}, and Cr^{3+} (Figure 2.4). This model was not applicable to the equilibrium data of Cd^{2+}, i.e. it resulted in a large deviation from the experimental data and an unreasonable value of R^2, a measure of the applicability of the model. The constants for the two models for the considered metals were evaluated (Table 2.1) using the SCIENTIST package (Micromath Scientist Software). Comparing these constants with those obtained for other similar adsorbents of various origins, it can be concluded that CM has a significant adsorption capacity for the tested metals (Table 2.1).

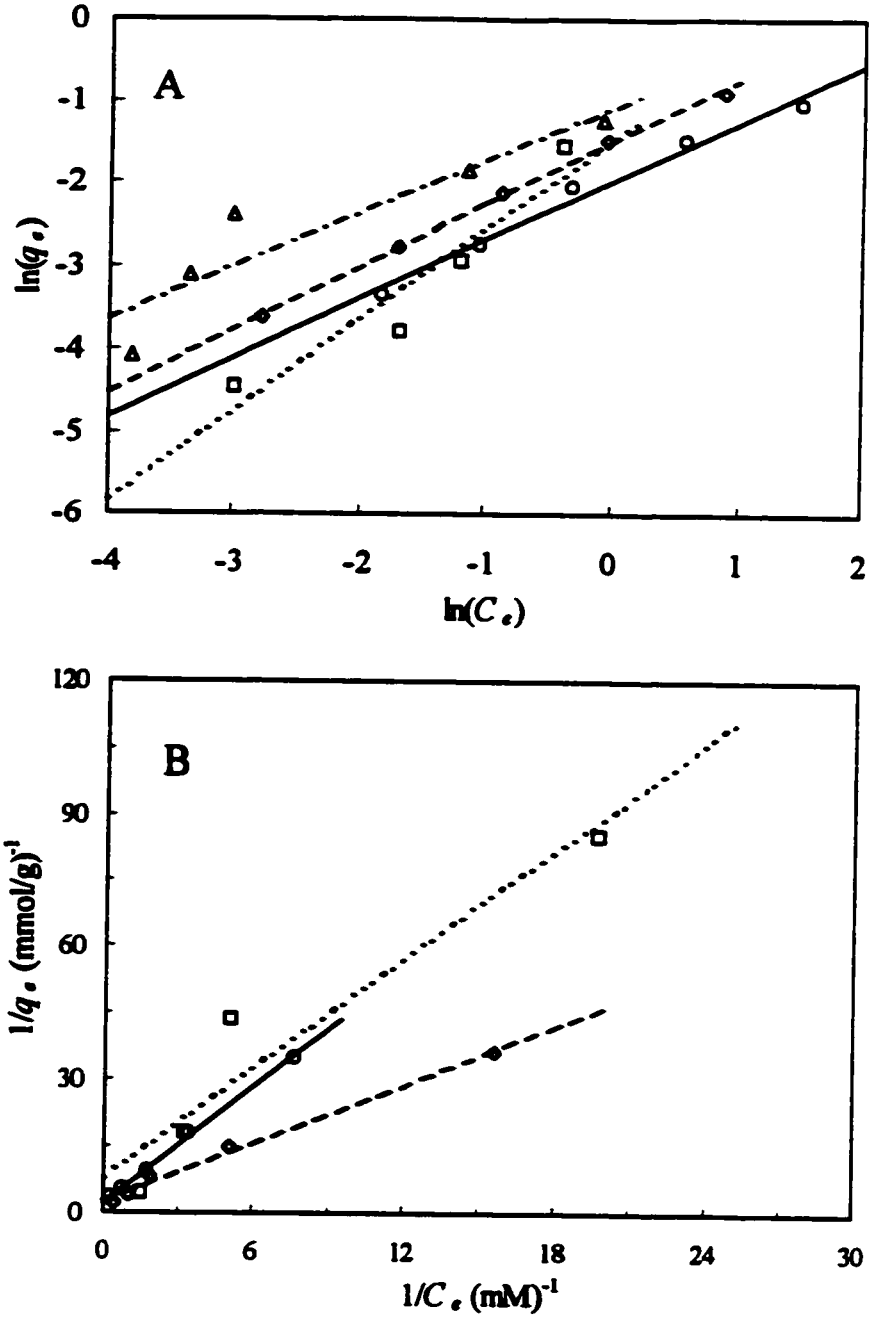

Figure 2.4: Freundlich (A) and Langmuir (B) isotherms for adsorption of various metals by CM. Symbols are experimental and lines are fitting data by the models. CM concentration: 9.2 mg/mL; initial pH: 5.2. Metal: Cu^{2+}-□, ·····; Zn^{2+}-◇, ——; Cd^{2+}-Δ, – – –; Cr^{3+}-O, –.

Table 2.1: Freundlich constants, k and $1/n$, and Langmuir constants, K_L and b, for Cu^{2+}, Zn^{2+}, Cd^{2+} and Cr^{3+} for various sorbents

Adsorbent	Metal	Freundlich model			Langmuir model			Referenc
		$1/n$	k_F	R^2	K_L (mmol/g)	b $(mM)^{-1}$	R^2	
Waste tea leaves	Cu^{2+} Zn^{2+}				0.430 0.179			Tee and Khan (1988)
Soybean hulls	Cu^{2+} Zn^{2+} Cr^{3+}				0.609 0.503 0.540	2.09 0.35 1.82		Marshall and Champagne (
Cottonseed hulls	Cu^{2+} Zn^{2+} Cr^{3+}				0.300 0.260 0.260	2.47 2.35 1.82		Marshall and Champagne (
Sphagnum peat	Cu^{2+} Zn^{2+}	0.895 0.961	0.195 0.257	0.898 0.956	0.055 0.014	7.30 2.16	0.927 0.959	Viraraghavan Dronamraja (1
Giridih coal Crushed coconut shells	Cd^{2+}	0.108 0.123	1.500 1.460					Bhattacharya a Venkobachar (1984)
Activated carbon from peanut hulls Commercial Activated carbon	Cd^{2+}				0.794 0.024	41.59 11.24		Periasamy and Namasivayam (1994)
Turkish coffee	Cd^{2+} Cr^{3+}	1.240 1.180	1.500 0.800					Orhan and Buyukgungor (1993)
Nut shell	Cd^{2+}	1.080	1.600					Orhan and Buyukgungor (
Walnut shell	Cr^{3+}	16.90	1.900					Orhan and Buyukgungor (
Beech leaves	Cd^{2+}	0.920	18.600					Salim et al. (19
Canola meal	Cu^{2+} Zn^{2+} Cd^{2+} Cr^{3+}	1.090 0.755 1.080 0.710	0.230 0.225 0.343 0.140	0.945 0.999 0.975 0.997	0.126 0.347 0.499	1.93 1.33 0.463	0.965 0.996 0.998	This work

2.4 CM to Metal Concentration Ratio

In this section, the results of the effects of CM to metal concentration ratios on the metal uptake are reported. Zinc was used in this study and three different ratios of CM to Zn^{2+} concentrations were considered. Three various metal concentration levels were selected for each of the three ratios and the adsorption was followed in all nine systems separately. The results (Figure 2.5) showed that the utilization of the same ratio of canola meal to metal concentrations, regardless of their initial levels, resulted in approximately the same amount of Zn^{2+} adsorbed per unit weight of meal. The results also indicated that for lower CM to Zn^{2+} ratios, more Zn^{2+} was adsorbed per unit mass of meal than at higher ratios (Table 2.2). Systems with higher CM concentrations showed higher amounts of Zn^{2+}

removal. These results are in agreement with those presented in Figures 2.1 and 2.3. These results also indicate that one of the problems concerning the utilization of CM as feed is associated with the high affinity that this commodity has for heavy metals.

Table 2.2: Adsorption of zinc by CM after 1 h in systems with various CM : zinc ratios

CM : Zn^{2+} ratio	Initial Zn^{2+} concentration (ppm)	Final Zn^{2+} concentration (ppm)	Zn^{2+} uptake (mmol/g)	Zn^{2+} uptake* (mmol/g)	Amount of Zn^{2+} removed (%)
23×10^{-3}	50	24.5	0.338	0.363(0.024)	51.0
	100	41.7	0.387		58.3
	200	90.0	0.365		55.0
11.5×10^{-3}	100	51.5	0.642	0.658(0.035)	48.3
	200	95.7	0.692		52.1
	400	212.3	0.624		46.9
5.75×10^{-3}	200	107.1	1.235	1.205(0.033)	46.4
	400	218.0	1.210		45.5
	800	448.0	1.170		44.0

*Average values for three tests within the same CM : Zn^{2+} ratio. Standard deviation shown in brackets.

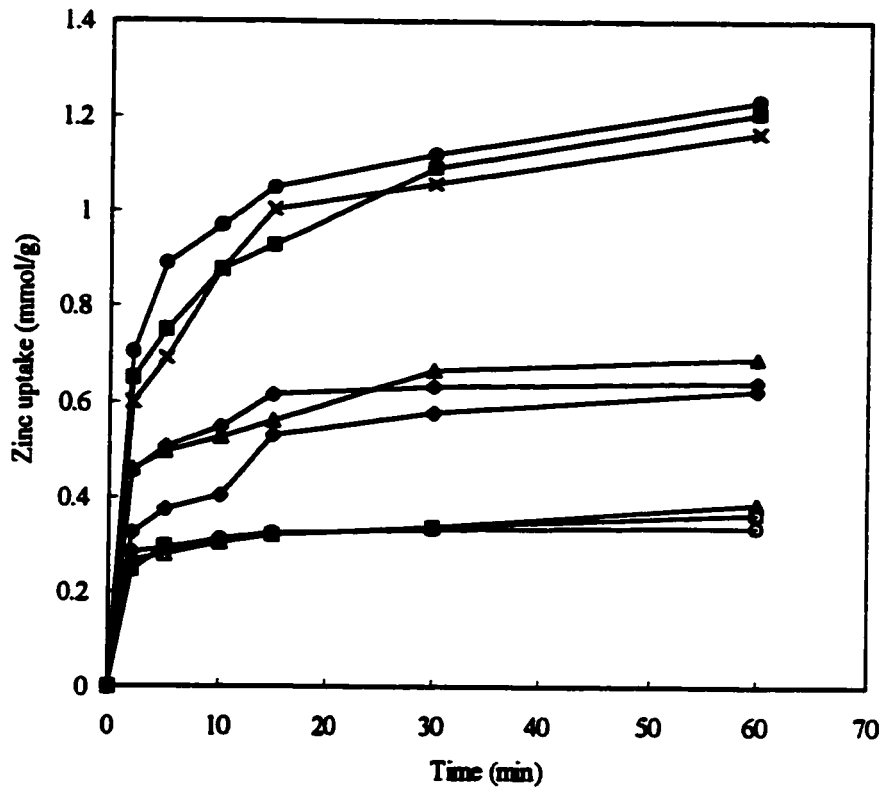

Figure 2.5: Effect of CM : zinc concentration ratios on zinc uptake. CM (mg/mL) and zinc (ppm) concentrations, respectively: O-1.15 and 50, Δ-2.3 and 100, □-4.6 and 200, ◊-1.15 and 100, ▲-2.3 and 200, ◆-4.6 and 400, ●-1.15 and 200, ■-2.3 and 400, ×-4.6 and 800.

2.5 Effect of Particle Size

The particle size of CM is expected to influence the adsorption process because adsorption is a surface phenomenon. As the particle size decreases, the surface area per unit weight increases and so does the adsorption capacity of the adsorbent. To investigate this, CM was screened through sieves and the following size distribution fractions were obtained: one smaller than 0.18 mm, four fractions with sizes between 0.18 and 1.4 mm, and one fraction with particle size bigger than 1.4 mm. The results of the screen analysis are presented in Table 2.3, with the amount of copper uptake obtained by the fraction retained on each screen. As was expected, very fine particles showed the maximum amount of Cu^{2+} uptake (Table 2.3). However, the utilization of very small sorbent particles could result in difficulties relating to their separation from the liquid. In addition, in a packed column very small particles may result in blocking of the column, causing a high pressure drop. Moreover, size reduction might require a large amount of energy, resulting in high operating costs. Nevertheless, large particles reduce these effects but have a lower sorption capacity. Therefore, the optimum particle size of the sorbent for any adsorption process should be determined. Based on the above discussion, utilization of CM in the particle size range of 0.18-0.71 mm could be considered. A similar trend was also observed by Singh *et al.* (1994) for the sorption of Cr^{6+} by *Acacia arabica* bark and by Tiwari *et al.* (1995) for adsorption of Hg^{2+} from aqueous solutions using rice-husk ash.

Table 2.3: Particle size of CM and its influence on Cu^{2+} removal by the meal. Initial Cu(II) concentration: 1.57 mM; CM concentration 9.2 mg/mL; initial pH: 5.2; sorption time 60 min

Particle size (mm)	Mass fraction	Total copper removal* (%)	Copper uptake* (mmol/g)
Smaller than 0.180	0.0978	66.3 ± 3.10	0.113 ± 0.0086
0.180-0.425	0.2706	64.0 ± 5.37	0.109 ± 0.0091
0.425-0.710	0.2166	54.9 ± 2.54	0.094 ± 0.0042
0.710-1.000	0.1829	48.4 ± 1.98	0.083 ± 0.0033
1.000-1.400	0.1680	47.0 ± 3.04	0.080 ± 0.0052
Bigger than 1.400	0.0638	45.5 ± 2.12	0.077 ± 0.0036

* Average values of duplicate ± standard deviation.

2.6 Effect of Phytic Acid

The CM used in this study contained about 34% proteins and 4.7% phytic acid. It can be presumed that the sorption of metal by this material was largely influenced by these components. To test the effect of phytic acid on the adsorption process, the meal was treated with 2.4% HCl for various

times in order to extract different amounts of phytic acid. After the treatment, the meal was dried and used for Cu^{2+} uptake. The results indicate (Figure 2.6) a substantial decrease in the copper adsorption capability of the meal with the extraction of phytic acid. After 60 min of sorption, there was about 33.7 and 74.7% less Cu^{2+} uptake by CM when 76.5 and 100% of the initial phytic acid content in CM was reduced, respectively. However, some proteins were also extracted and, therefore, the loss of copper adsorption capability should be ascribed to them as well. Since CM contains larger amounts of proteins than phytic acid, it can be assumed that the proteins are one of the important set of ligands responsible for the binding of metal ions. An additional test indicated that the sorption of Cu^{2+} by the meal was very low when most of the proteins were extracted; the uptake of Cu^{2+} by CM was 0.006 mmol/g in this case, compared to 0.047 mmol/g without proteins removal.

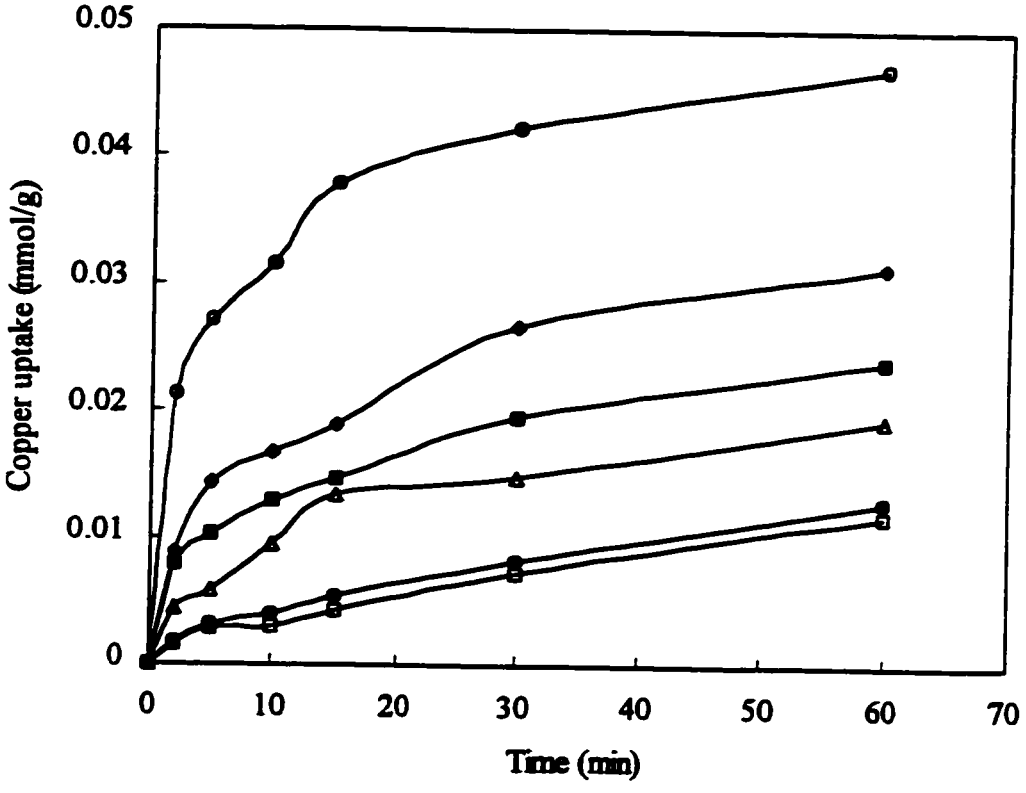

Figure 2.6: Effect of phytic acid and protein removal from CM, using 2.4% HCl, on copper uptake from a 1.57 mM copper and a 18.4 mg/mL meal suspension at pH 5.2. Phytic acid and protein removal (%); O-0.0 and 0.0, ◆-76.5 and 15.4, ■-81.5 and 16.0, Δ-87.2 and 16.5, ●-93.6 and 17.3, □-100.0 and 17.7.

2.7 Effect of pH

It has also been reported that the pH of sorbent suspensions affects the adsorption process when agricultural sorbents are used. Singh *et al.* (1993) studied the binding of Pb^{2+} to chemically treated tea leaves and observed that the uptake of Pb^{2+} increased from 5.5 mg/g at pH 2 to 77 mg/g at pH 6. Bhattacharya and Venkobachar (1984) obtained no cadmium uptake by crushed coconut shells at pH 3.0, while the uptake reached its maximum value at pH 10. On the other hand, some researchers reported a decrease in metal uptake with an increase of the initial pH of the suspension. Sorption of Cr^{6+} by *Acacia arabica* bark decreased from 9.25 mg/g at pH 2 to 2.5 mg/g at pH 6.8. The higher sorption was attributed to chlorochromate complex anion $(CrO_3Cl)^-$ formation below pH 3 in the presence of hydrochloric acid (Singh *et al.*, 1994). Increasing the pH increased both the amount and rate of cadmium removal from a suspension of beech leaves, until a maximum at pH 5, then cadmium uptake decreased with an increase in pH (Salim *et al.*, 1992). The results from the present study (Table 2.4, kinetics are presented in Appendix A, Figures A.4-A.7) showed that the uptake of the metals increased with an increase in pH up to 5.2 but the differences in the increases from one pH to another was not very large, with the largest effect of pH being noticed for the adsorption of Ni^{2+} (Table 2.4). The influence of pH on Cu^{2+} uptake by CM is similar to that in the case of *A. carbonarius*, as presented in the previous chapter, however the level of the increase in the uptake is not as pronounced as that in the case of *A. carbonarius*. Canola meal, as previously mentioned, contains a large amount of proteins which act as a buffer in the meal-metal suspension; this may be a reason why a greater difference in the uptake of these metals from aqueous solutions was not observed with the pH increase. An increase in biosorption when the pH was raised was explained by Luef *et al.* (1991) by an increase in the amount of negatively charged groups at the surface of the microbial cells. This explanation can also be applied to CM, since by raising the pH of the suspension, the number of negatively charged groups of the proteineous component is enhanced.

Table 2.4: Effect of initial pH on metals uptake by CM. Initial metal concentration: 100 ppm; CM concentration: 9.2 mg/mL; sorption time: 60 min

pH	Metal			
	Cu^{2+}	Zn^{2+}	Cd^{2+}	Ni^{2+}
	Metal uptake (mmol/g)*			
3.8	0.088 ± 0.0062	0.125 ± 0.0023	0.083 ± 0.0021	0.062 ± 0.0013
4.7	0.092 ± 0.0049	0.134 ± 0.0011	0.085 ± 0.0004	0.071 ± 0.0022
5.2	0.098 ± 0.0016	0.146 ± 0.0005	0.092 ± 0.00	0.076 ± 0.0014

* Average uptake of duplicate ± standard deviation.

2.8 Effect of Temperature

The removal of metals from aqueous solutions by biosorption was also affected by the temperature. The tests were carried out with suspensions of 9.2 mg/mL of CM at pH 5.2 that were incubated in water baths set at various temperatures. After 10 min, thermal equilibrium was achieved and amounts of Zn^{2+}, Cd^{2+}, Cr^{3+} or Ni^{2+} were individually added to these suspensions to give a final concentrations of 100 ppm. The lowest uptake for these metals was observed at 4°C (Table 2.5, kinetics are presented in Appendix A, Figures A.8-A.11), and then at 25°C followed by 50°C. However, the differences in the uptakes between 25°C and 50°C, expressed in percentages, were much larger in the case of Zn^{2+} than with the other three metals (Table 2.5). The increase in the metal uptake with temperature could be due to the dissociation of some compounds available at the surface of the meal which respond to metal adsorption, and thus, providing more sites for metal adsorption. Many other authors have reported an increase in metal sorption by biological materials with an increase in temperature, e.g., Singh *et al.* (1993) noticed a higher lead sorption by tea leaves at 70°C than at 25°C; Viraraghavan and Dronamraju (1993) recovered more Cu^{2+}, Ni^{2+} and Zn^{2+} from their solutions at 21°C than at 5°C using the sorbent of *sphagnum* peat; Singh *et al.* (1994) obtained an increase in Cr^{6+} uptake by *Acacia arabica* bark with a temperature increase up to 70°C.

It is seen that the influence of temperature on the metal sorption by plant materials, i.e. CM, is different than that of microbial materials, i.e. *A. carbonarius*. In the previous chapter an optimum temperature of 25°C was reported for the biomass of *A. carbonarius*. It was also shown that the uptake of Cu^{2+} decreased substantially when tests were carried out above this temperature. However, in the case of CM, the uptake of metals is increasing with the further increase in temperature from 25 to 50°C. Therefore, it can be concluded that plant materials are more resistant to the temperature effects than that of microbial materials.

Table 2.5: Effect of temperature on metal uptake by CM. Initial metal concentration: 100 ppm; CM concentration: 9.2 mg/mL; initial pH: 5.2; sorption time: 60 min

Temperature (°C)	Metal			
	Zn^{2+}	Cd^{2+}	Cr^{3+}	Ni^{2+}
	Metal uptake(mmol/g)*			
4	0.122 ± 0.0079	0.085 ± 0.0034	0.094 ± 0.0010	0.061 ± 0.0065
25	0.133 ± 0.0012	0.087 ± 0.0043	0.128 ± 0.0017	0.073 ± 0.0025
50	0.153 ± 0.0012	0.093 ± 0.0002	0.136 ± 0.0012	0.076 ± 0.0013

* Average uptake of duplicate ± standard deviation.

2.9 Treatment with Chemical Agents

The adsorption capacity of biological materials can be enhanced by the treatment of these materials before their utilization in the sorption processes. Treatment of CM with formaldehyde and glutaraldehyde prior to the sorption process was considered in this work. It has been reported (Deshkar *et al.*, 1990; Friedman and Waiss, 1972) that proteins and tannins could be responsible for adsorption of metals by agricultural sorbents. These compounds are soluble in aqueous solution and once the sorbents are suspended in the media some proteins and tannins might be lost from the sorbent surface to the solution, thus reducing adsorption capacity. To avoid this, the solubilization must be prevented or reduced. Deshkar *et al.* (1990) reported that formaldehyde (HCHO) polymerized and insolubilized the colored, water-soluble tannin from bark, which was used for adsorption of Hg^{2+} from aqueous solution. Glutaraldehyde is another chemical agent used for the immobilization of enzymes and proteins in general. These two agents were used in this work for treatment of CM before its utilization in metal adsorption. The results show (Table 2.6) that the copper uptake by CM increased by about 52% when CM was treated by these reagents. Although the treatment of CM with glutaraldehyde or formaldehyde increased the adsorption of copper by this sorbent, the increase was much lower than in some cases reported in the literature, e.g. sorption of Pb^{2+} by tea leaves treated with formaldehyde (Singh *et al.*, 1993).

Table 2.6: Treatment of CM with 5% formaldehyde and glutaraldehyde. Initial copper concentration: 1.57 mM; CM concentration: 9.2 mg/mL; initial pH: 5.0; sorption time: 60 min

System	Total copper removal (%)	Copper uptake (mmol/g)
Control	57.2 ± 0.707	0.098 ± 0.0012
Formaldehyde treated-CM	83.6 ± 0.848	0.143 ± 0.0014
Glutaraldehyde treated-CM	84.4 ± 2.20	0.144 ± 0.0037

It is also expected that the concentration of the reagent for the treatment of CM has an effect on the adsorption of metals. This was investigated by the treatment of CM with various concentrations of glutaraldehyde, with the treated CM being used for copper adsorption. The results show (Figure 2.7) that the highest metal adsorption was achieved when the concentration of glutaraldehyde was 1.0%. Although further increase in the concentration of this reagent (up to 10%) resulted in lower copper binding than when the concentration of glutaraldehyde was 1%, the amounts of metal adsorbed were still higher than when the untreated meal was used for adsorption of copper. From the presented

results, it can be concluded that preventing the solubilization of proteins enhanced metal binding to CM.

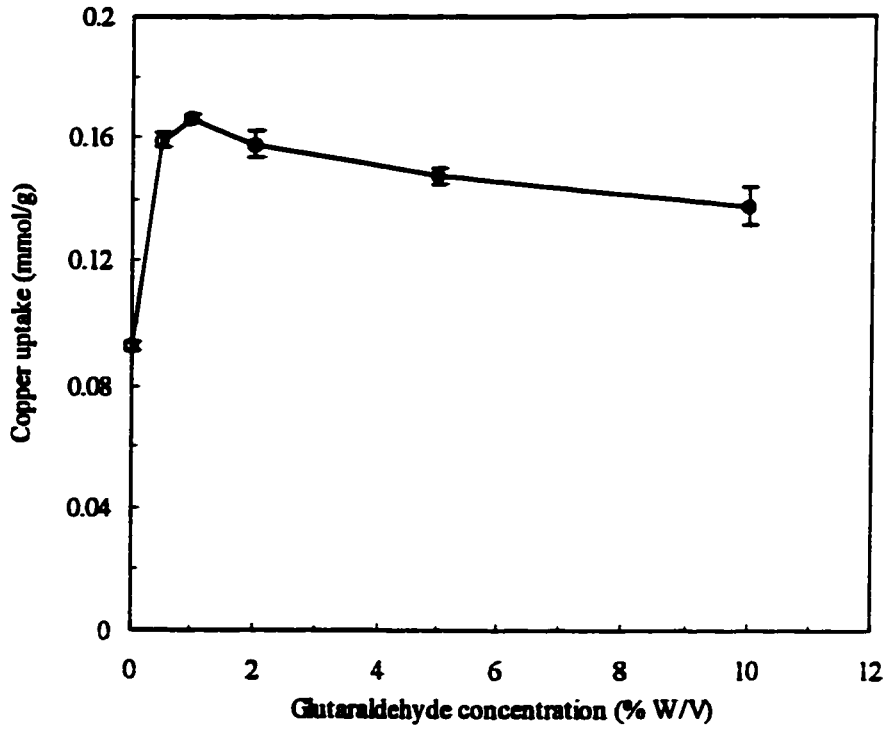

Figure 2.7: Adsorption of copper by CM previously treated with different concentration of glutaraldehyde. Initial Cu(II) concentration: 100 ppm; CM concentration: 9.2 mg/mL; initial pH: 5.0.

2.10 Removal of Low Concentrations of Cadmium

The permissible level of cadmium, one of the most dangerous heavy metals besides mercury and lead in potable water, as outlined by the Canadian Council of Ministers of Environment (CCME, 1991) is 0.005 ppm in drinking water and 0.02 ppm in livestock watering. In order to test the possibility of the utilization of CM for the decrease of Cd^{2+} concentration to these levels, tests were performed using an initial cadmium concentrations of 0.8, 3.5, and 7.0 ppm, and each of these concentrations was exposed to three CM concentrations (Table 2.7).

The usual trend of increasing the amount of metal removal with the increase in the sorbent concentration was noticed. Although the results showed that CM, at the concentrations tested in this study, did not reduce the cadmium concentration to the permissible limit set by the Environmental Agencies, they suggested that the removal of cadmium to a lower level could be accomplished in a multiple stage adsorption process where the effluent from one stage would be fed to the following stage

and, in such a way, the concentration of cadmium could be further decreased. This was tested by transferring 4.6 mg/mL of CM into a Cd^{2+} solution with an initial concentration of 0.78 ppm. After 24 h of adsorption, CM was separated and a Cd^{2+} concentration of 0.062 ppm was detected in the filtrate. When the filtrate was treated with 4.6 mg/mL of fresh CM, a Cd^{2+} concentration of 0.0092 ppm was measured in the aqueous phase. Another adsorption cycle was repeated with 4.6 mg/mL of fresh meal and the Cd^{2+} was decreased to a level of <0.008 ppm. The process was terminated because of the lowest detectable limit of the used ICP-AES spectrometer for metal measurements. These results indicate that CM is able to decrease Cd^{2+} concentration to a level which is close to the permissible limit for drinking water and is suitable for other types of waters such as irrigation and livestock watering (Table 2.3 ; Part I).

Table 2.7: Removal of low levels of cadmium by CM. Sorption time: 24 h.

Initial Cd(II) concentration (ppm)	CM concentration (mg/mL)		
	1.15	2.3	4.6
	Residual concentration (ppm)*		
0.80	0.485 ± 0.049	0.275 ± 0.021	0.061 ± 0.0035
3.5	0.680 ± 0.056	0.355 ± 0.0165	0.175 ± 0.0085
7.0	1.230 ± 0.0282	0.720 ± 0.0042	0.220 ± 0.0150

* Average values ± standard deviation of the duplicate.

The results of this Chapter showed that canola meal can be used as a biosorbent for heavy metals. When Zn^{2+}, Cd^{2+}, Cu^{2+}, Cr^{3+} and Ni^{2+} adsorption were studied the following order of the uptake for these metals by the meal was noticed (molar basis): $Zn^{2+} > Cr^{3+} > Cu^{2+} > Cd^{2+} > Ni^{2+}$. This order is different when expressed in terms of total metal removed (mass basis): $Cd^{2+} > Zn^{2+} > Cu^{2+} > Cr^{3+} > Ni^{2+}$. Increasing the CM concentration in the suspension resulted in a decrease of the metal uptake per unit weight of the meal. With increases in the metal concentration, pH and temperature, an increase in the metal uptake was observed. The same CM to Zn^{2+} concentration ratios resulted in approximately the same amount of the metal being adsorbed by the meal regardless of the concentration levels. Phytic acid and protein contents of CM are important components for the sorption process. Formaldehyde and glutaraldehyde enhanced the metal sorption by the meal, and the optimum glutaraldehyde concentration of 1.0% was found. The proposed Freundlich and Langmuir isotherms models gave a good representation of the experimental equilibrium concentration data.

Chapter 3

Adsorption of Metals by Moss

3.1 Introduction

In the previous chapter, the utilization of CM for metal removal was performed. The use of other plant materials for this purpose is necessary in order to find suitable adsorbents. In the present chapter, moss, which is relatively widely distributed in the ecosystems, was tested for sorption of heavy metals. It contains lignin and cellulose as major constituents (Souci, 1938), which bear polar functional groups, such as alcohols, aldehydes, ketones, acids, phenolic hydroxides and ethers that can be involved in chemical bonding (Adler and Lundquist, 1963). The dynamics of metal adsorption by this material, isotherms at various pH and temperatures, and some desorption tests will be considered in this chapter. Copper was selected, as an example, to test the ability of binding heavy metals by this plant sorbent.

3.2 Effect of Moss Concentration

The effect of the moss concentration on the copper uptake is shown in Figure 3.1. It was noticed that the uptake per unit mass of the sorbent was higher when its concentration was lower (Figure 3.1). The reason for such a performance of the sorbent is the higher concentration of the metal ions per binding site of the sorbent when its concentration was lower. However, the results also showed that the total amount of the copper ions removed increased with increasing the concentration of the sorbent, because of the increase in the number of binding sites available to the metal ions. The relationship between loading of the moss with copper and the concentration of this sorbent is in agreement with already discussed data in this work and with the data published for other plant adsorbents such as for beech leaves for the adsorption of Cd^{2+} (Salim *et al.*, 1992) and for waste tea leaves for the removal of Pb^{2+}, Cd^{2+} and Zn^{2+} from their solutions (Tee and Khan, 1988). Similar to the previous results, the high initial rate of Cu^{2+} uptake suggests that the adsorption occurs mainly at the moss surface. When the metal uptake results were plotted against square root of the adsorption time (Figure 3.2), linear correlations were obtained. This indicates that the mechanism of intrapore diffusion was also involved in the adsorption (Weber and Morris, 1963) and it is represented by the slower adsorption rate which was noticed after the first five minutes of the fast sorption (Figure 3.1).

However, the plotted lines (Figure 3.2), which do not pass through the origin, indicate that, according to Singh *et al.* (1989), the intrapore diffusion is not a controlling step in the adsorption of copper by the moss.

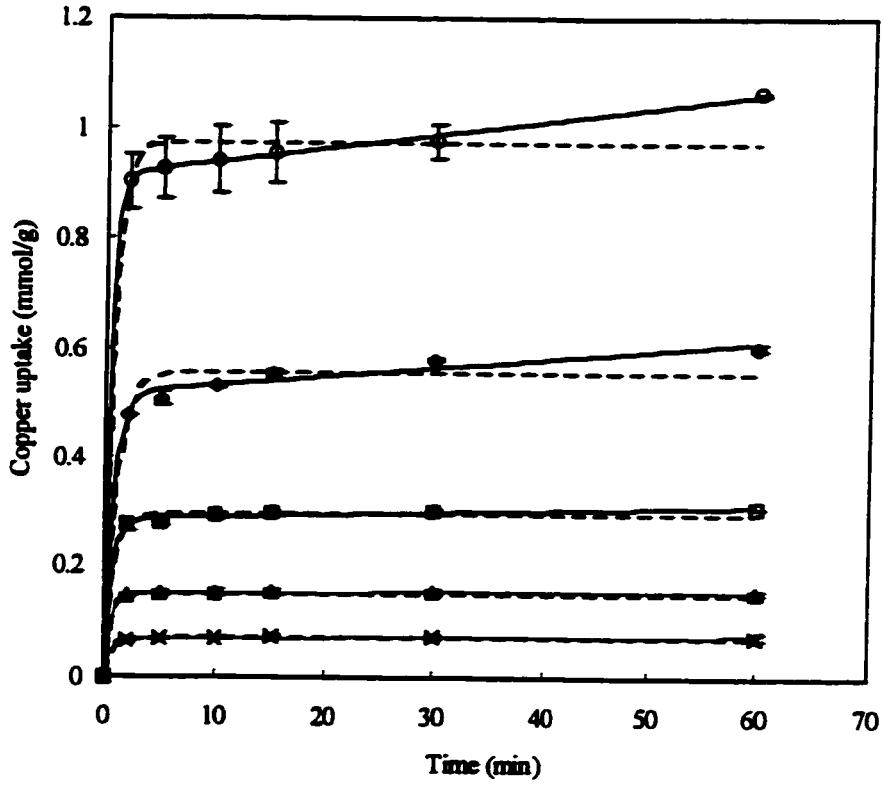

Figure 3.1: Effect of moss concentration on copper uptake. Symbols are experimental, solid lines and interrupted lines are fitting data using Eq. (4.18) and Eq. (4.14), respectively. Initial Cu^{2+} concentration: 100 ppm; initial pH: 4.9-5.2. Moss concentration (mg/mL); O-1.15, ◊-2.3, □-4.6, Δ-9.2., ×-18.4.

Equations (4.14), (4.18) and (4.19) were used to fit the data in Figure 3.1. The values of the constants for different moss concentrations for these models were computed (Table 3.1) using the SCIENTIST package (Micromath Scientist Software). Equation (4.14) gives very good representation of the experimental data for copper adsorption in the systems with higher moss concentration. With a decrease in the moss concentrations, the deviations between the experimental results and those predicted by Eq. (4.14) increased. The reason for such a disagreement is probably due to the intrapore diffusion which is more significant for the systems with lower moss concentrations, because they have a smaller surface area than for the systems with higher sorbent concentrations, if the other conditions are the same.

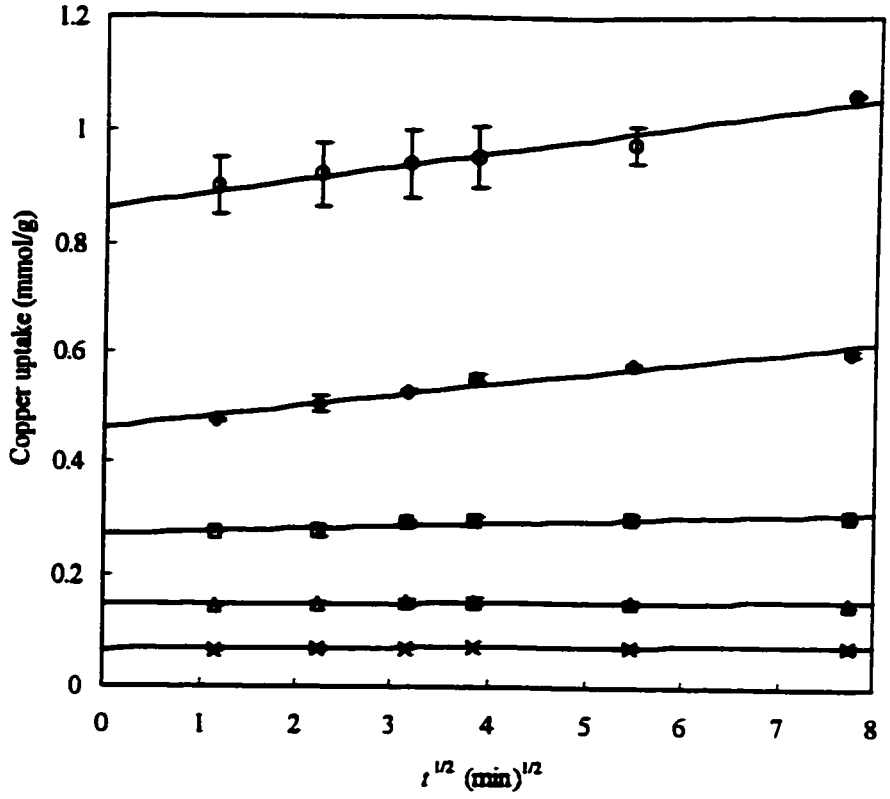

Figure 3.2: Plots of copper uptake versus square root of time for various moss concentration. Initial Cu^{2+} concentration: 100 ppm; initial pH: 4.9-5.2. Moss concentration (mg/mL); O-1.15, ◊-2.3, □-4.6, Δ-9.2., ×-18.4.

Table 3.1: Constants of Eq. (4.14) and (4.18) for various moss concentrations

X_0 (mg/mL)	k_1' ($\beta=0$) mL/(mg.min)	K ($\beta=0$) (mg/mL)	K (mg/mL)	k_1' mL/(mg.min)	$\beta \times 10^3$ mmol/(g.min^2)
1.15	0.803	0.469	0.576	1.183	5.73
2.30	0.325	0.528	0.760	0.400	4.53
4.60	0.238	0.733	0.928	0.278	2.94
9.20	0.153	1.173	1.271	0.162	0.89
18.4	0.058	3.592	3.989	0.095	0.80

To take into consideration the above mentioned discrepancies, Eq. (4.18), which is a modification of Eq. (4.14), was used to represent the experimental data (Figure 3.1). This equation fits all the data very well but, unfortunately, it is semi-empirical and does not have a full physical meaning.

It is noticed (Table 3.1) that the k_1' values decreased as the moss concentration increased, indicating that there is a correlation between k_1' and sorbent concentration. The differences in the

values of the two kinds of K and k_1' parameters are probably due to the intrapore diffusion which was not addressed by Eq. (4.14). It is also seen (Table 3.1) that the rate constant β decreased with the increase in the moss concentration. This might indicate that the fraction of copper bound inside the pores decreased with the increase in the sorbent concentration.

3.3 Effect of Metal Concentration and Temperature

To investigate the effects of the initial metal concentration and the temperature on metal uptake, the process was carried out at initial copper concentrations between 10-100 ppm at 8°C, 15°C, 25°C and 35°C. The sorption was carried out for 24 h to ensure that equilibrium was attained. The results show (Figure 3.3) that the metal uptake increased with temperature. This indicates that the process is endothermic. The increase in the uptake of metal ions with temperature (Knocke and Hemhill 1981) is caused by an enhanced ion exchange and by a change in the size of adsorbents' pores. Ferro-Garcia *et al.* (1988) studied adsorption of copper using several agricultural by-products as adsorbents. They obtained results similar to those shown in Figure 3.3. The results also indicate that the increase in the copper uptake is associated with an increase in its concentration. An increase in the metal uptake with an increase in the metal concentration is a common phenomenon observed with a variety of biosorbents such as with beech leaves, tea leaves and *Hardwicia Binata* bark used for the adsorption of Cd^{2+} (Salim *et al.*, 1992), Pb^{2+} (Singh *et al.*, 1993) and Hg^{2+} (Deshkar *et al.*, 1990), respectively.

The four equilibrium curves that are obtained at the four temperatures in this study are well represented by the Freundlich isotherm model (Figure 3.3). When the Langmuir isotherm model, Eq. (4.2), was applied to these data, a good fit was not obtained for the low metal concentrations. However, when the data for these low metal concentrations were disregarded in the fitting, this isotherm model was also applicable (Figure 3.4). The Freundlich constants, k_F and $1/n$, and the Langmuir constants, K_L and b, at various temperatures are displayed in Table 3.2 and 3.3, respectively.

Table 3.2: Freundlich constants at various temperatures

Temperature (°C)	k_F (mmol/g)	$1/n$	R^2
8	0.538	0.673	0.996
15	0.621	0.618	0.987
25	0.790	0.595	0.992
35	0.935	0.616	0.982

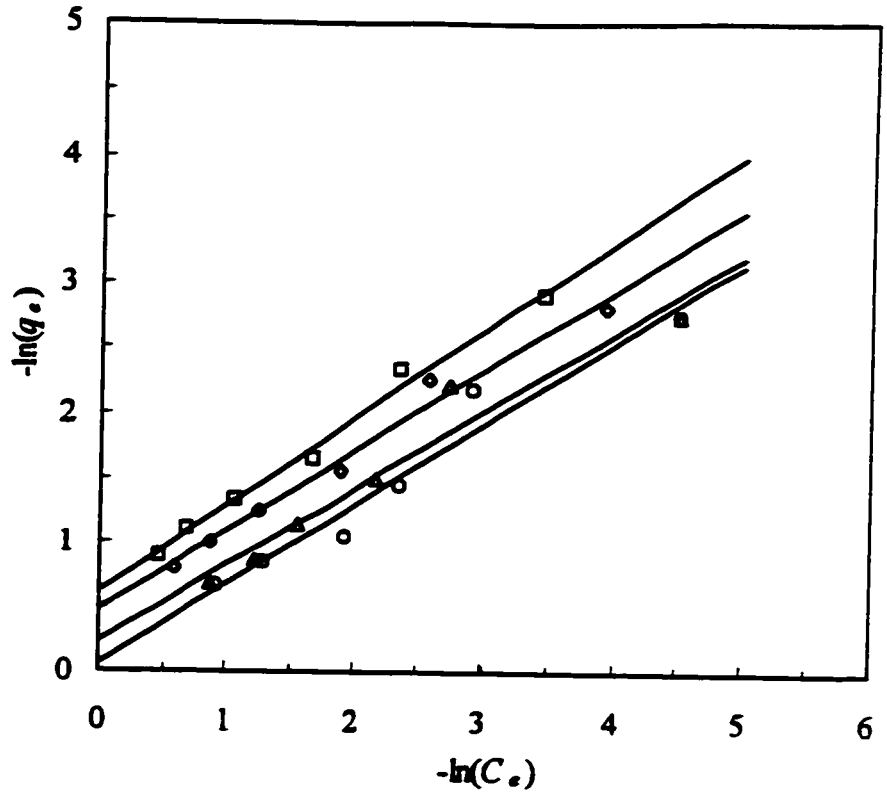

Figure 3.3: Relationship between the equilibrium copper concentration and its uptake at various temperatures. Moss concentration: 2.3 mg/mL: initial pH: 4.8. Symbols are experimental data and lines are fitting data using Freundlich model. Temperature (°C); □-8, ◊-15, Δ-25, O-35.

Table 3.3: Langmuir constants and thermodynamic parameters at various temperatures

Temperature (°C)	K_L (mmol/g)	b (mmol^{-1}.L)	R^2	ΔG (kJ/mol)	ΔS (kJ/kmol.K)
8	0.864	1.34	0.986	0.683	108.9
15	0.913	1.72	0.987	1.27	104.2
25	0.887	2.89	0.994	2.48	96.7
35	0.804	4.48	0.991	3.50	89.6

The Langmuir constant b can be used to calculate the dimensionless constant separation factor $1/(1+bC_0)$. This constant was found to be less than 1 for the range of temperatures and copper concentrations used in this study. This indicates that the type of adsorption is favorable (Weber and Chakravorti, 1974). The K_L values for all temperatures, except the one at 35°C, are relatively close to each other, which means that the sorption capacity of moss for Cu^{2+} is almost the same regardless of the temperature. This can also be concluded from Figure 3.4 since all of the temperatures have the same intercept. However, utilization of the Freundlich model did not result in the same intercept for

each temperature; the k_F value increased with the increase in temperature, indicating the increase of the sorbent capacity for Cu^{2+} with the increase in the temperature of the system. According to McKay *et al.* (1982), the values of the Freundlich constant $1/n$ in this study, which were between 0.1 and 1.0, again indicate that the type of Cu^{2+} adsorption by moss is favorable. Therefore, in this case both of the models resulted in different conclusions concerning the sorption capacity, however, both models suggested that the adsorption is favorable.

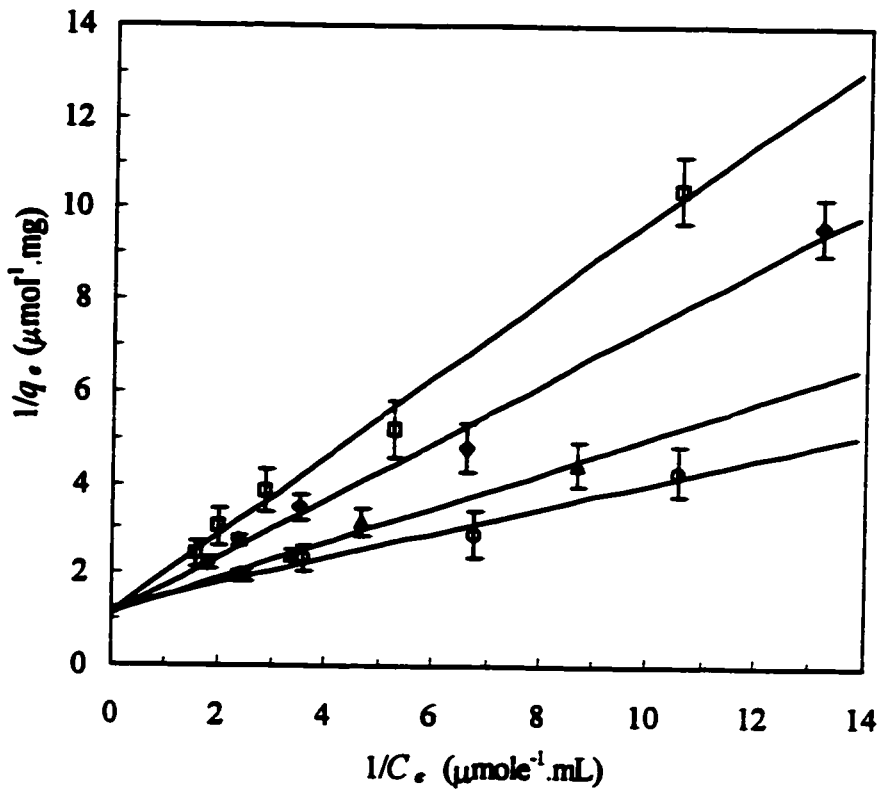

Figure 3.4: Relationship between equilibrium copper concentration and its uptake at various temperatures. Moss concentration: 2.3 mg/mL; initial pH 4.8. Symbols are experimental and lines are fitting data using Langmuir model. Temperature (°C); □-8, ◊-15, Δ-25, O-35.

The enthalpy change of the sorption, ΔH, was calculated from the slope of the straight line which is obtained by plotting ln*b* (Langmuir constant) versus $1/T$ (Figure 3.5) and found to be 31.3 kJ/mol. The positive ΔH value confirms the endothermic nature of the sorption process (Singh *et al.* 1993). The ΔH value from this study is in the range of those reported for the adsorption of Cu^{2+} by fly ash, 63.2 kJ/mol (Panday *et al.* 1985), and the sorption of Pb^{2+} by chemically treated tea leaves, 10.0 kJ/mol (Singh *et al.* 1993). The values of free energy change, ΔG, and the entropy change, ΔS, at

different temperatures are listed in Table 3.3. The ΔG and ΔS values reported by Panday *et al.* (1985) and Singh *et al.* (1993) are also similar to those obtained from this study.

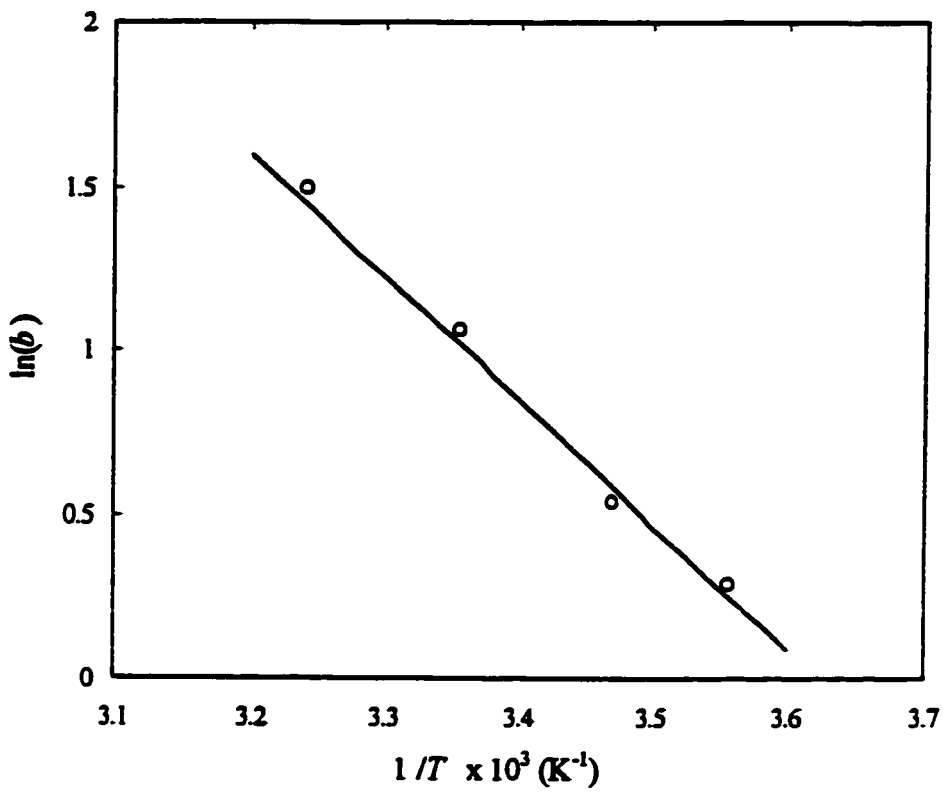

Figure 3.5: Plot of ln*b* (Langmuir constant) vs 1/*T* for adsorption of copper by moss.

3.4 Effect of pH

The effect of the initial pH of the metal solution on the adsorption of copper by moss was investigated. The results are displayed in Figure 3.6. The plotted equilibrium data, in the form of a sorption isotherm as a function of pH, followed the linearized Freundlich isotherm model. The values of the Freundlich constants are given in Table 3.4. A significant increase in the copper uptake per unit weight of moss is noticed as the pH increased from 3.5 to 5 (Figure 3.6). The effect of pH on Cu^{2+} sorption by moss can be ascribed to the electrostatic repulsion between the cations and the positively charged surface of moss which took place at the low pH values, while with an increase in pH, the metal ions replaced the hydrogen ions from the moss surface and, therefore, the extent of adsorption increased. This was also stated by Ferro-Garcia *et al.* (1988) who studied the adsorption of Zn^{2+}, Cd^{2+} and Cu^{2+} onto activated carbon obtained from agricultural by-products. They reported that at low pH

levels the adsorption of these metals was negligible. However, between pH 3 and 5 this process was enhanced substantially attaining values that stayed almost constant for higher pH levels. They attributed this increase to the change in the charge of the carbon surface with the change of pH. An increase in metal uptake with an increase in pH was also noticed by other researchers, e.g. by Namasivayam and Periasamy (1993) for the adsorption of Hg^{2+} by peanut hull carbon, and by Kumar and Dara (1980), for the uptake of Cu^{2+}, Pb^{2+} and Cd^{2+} by *Accacia* bark.

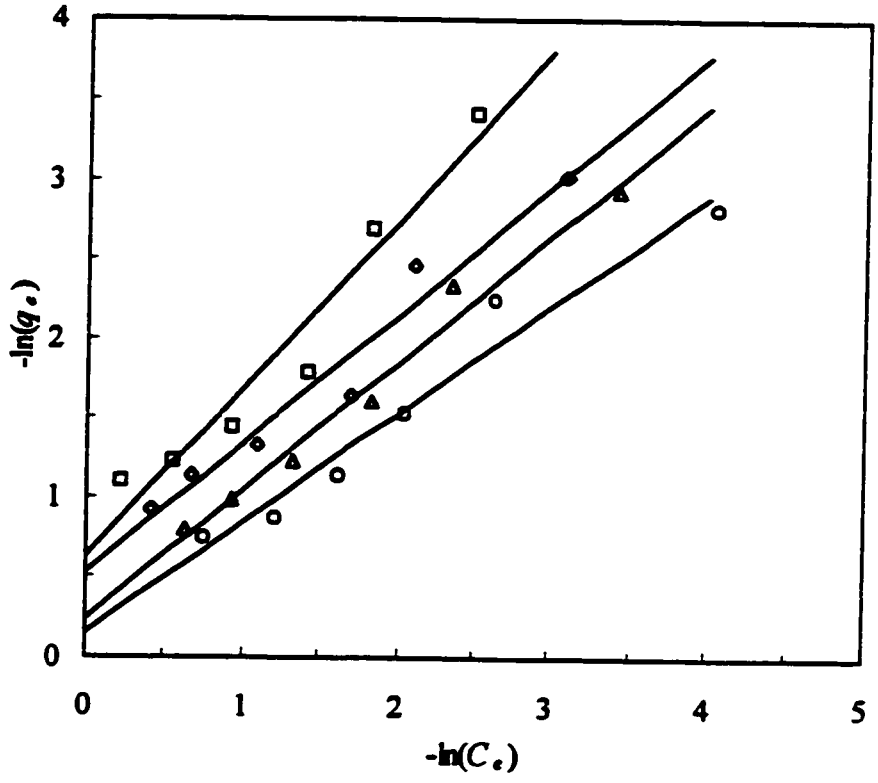

Figure 3.6: Relationship between the equilibrium copper concentration and its uptake at various pH. Moss concentration: 2.3 mg/mL. Symbols are experimental and lines are fitting data using Freundlich model. pH: □-3.5, ◊-4.0, Δ-4.5, O-5.0.

Table 3.4: Freundlich constants at various pH

pH	k_F (mmol/g)	1/*n*	R^2
3.5	0.532	1.054	0.987
4.0	0.589	0.811	0.991
4.5	0.786	0.805	0.981
5.0	0.860	0.687	0.975

3.5 Adsorption of Other Metals by Moss

To examine the possibility for the utilization of moss for the adsorption of other heavy metals, adsorption tests were carried out with Zn^{2+}, Cd^{2+}, Ni^{2+} and Cr^{3+}. The results obtained indicated that moss showed a high affinity for all of the tested metals. The results (Table 3.5) indicate that after 60 min of adsorption, the order of uptake of these metals, expressed on a molar bases, was as follows: $Cr^{3+}>Cu^{2+}>Zn^{2+}>Ni^{2+}>Cd^{2+}$. However, when the uptake was expressed in terms of the weight amount of metals removed from the solutions, the following order was obtained (Table 3.5): $Cd^{2+} > Zn^{2+} > Cu^{2+} > Cr^{3+} > Ni^{2+}$. The percent removal of these metals by the moss in this study is comparable with those reported for other adsorbents (Table 2.8 and 2.9; Part I). These results also compare favorably with those obtained from some activated carbon tests carried in this study. Powder and granular activated carbon removed 93% and 58% of the initial copper concentration, respectively, when the initial copper and activated carbon concentrations were 100 ppm and 9.2 mg/mL, respectively. The latter comparison also shows the benefits that can be drawn from using plant materials for the removal and recovery of heavy metals.

Table 3.5: Adsorption of various metals from their individual solutions. Moss concentration: 9.2 mg/mL; initial metal concentration: 100 ppm; initial pH: 5.0; sorption time: 60

Metal	Metal uptake (mmol/g)	Amount removal (%)
Cr^{3+}	0.179±0.0085	85.7±0.42
Ni^{2+}	0.149±0.0078	80.9±0.62
Cu^{2+}	0.156±0.0049	90.1±0.69
Cd^{2+}	0.089±0.0004	92.3±0.41
Zn^{2+}	0.151±0.0009	91.1±0.39

3.6 Adsorption/Desorption of Metal Ions from Moss

In addition to studying the adsorption of heavy metals from aqueous solutions, it is also important to take into consideration their removal from the sorbents. The purpose of the desorption of the metal is either for their recovery or for the regeneration of sorbent or both. The metal ions sorbed by bio-materials can be released using various kinds of desorbents. In this work, H_2SO_4, HNO_3 and HCl were used for the desorption of Cu^{2+} ions from moss. This was carried out after subjecting several samples of 4.6 mg/mL of moss to different copper salt aqueous solutions ($CuSO_4.5H_2O$, $CuCl_2$, and $Cu(NO_3)_2.3H_2O$), each having an initial copper concentration of 100 ppm. When adsorption

equilibrium was attained, the sorbents were separated and treated with 4 mL of one of the above desorbents (25 mM). The results (Table 3.6) indicate that the adsorption of copper from $CuCl_2$ solution was higher than that from $CuSO_4.5H_2O$ or $Cu(NO_3)_2.3H_2O$ solutions. The desorption tests showed that, among the three acids tested, sulfuric acid was the strongest desorbent (Table 3.6).

Tobin *et al.* (1987) suggested that the interaction between metal ions, complexing ligands (anions), and adsorbents may be divided into three categories: (1) metal-ligand complexes may form that are non-adsorbing or weakly adsorbing, resulting in a decrease in metal adsorption due to the presence of ligand; (2) the ligands (anions) may interact with the adsorbent so as to enhance or decrease the metal crystal uptake potential; and (3) metal-ligand complexes may form that are more strongly adsorbing than the free metal so that the presence of ligand results in enhanced metal uptake. Therefore, the higher Cu^{2+} uptake in the presence of chloride could indicate that the sorption of this metal by moss falls into the third category, while the lower uptake of Cu^{2+} in the presence of nitrate could indicate, in this case, that this sorption falls into the first category.

Table 3.6: Adsorption/desorption of copper by moss using various copper salts and desorbents of 25 mM concentration; sorption time: 60 min

Salt	Average uptake (mmol/g)	Desorbent	Amount desorbed (%)
$CuSO_4.5H_2O$	0.191 ± 0.0041	HCl	81.15 ± 3.8
		H_2SO_4	80.95 ± 2.3
		HNO_3	70.85 ± 2.2
$CuCl_2$	0.234 ± 0.0063	HCl	49.35 ± 0.35
		H_2SO_4	55.30 ±2.40
		HNO_3	47.0 ± 0.56
$Cu(NO_3)_2.3H_2O$	0.174 ± 0.0110	HCl	76.3 ± 0.141
		H_2SO_4	84.5 ± 0.98
		HNO_3	77.28 ± 1.72

Tan *et al.* (1985) found that the adsorption capacity of human hair for Cu^{2+} and Cd^{2+} was not affected by the type of anion (chloride, nitrate or sulphate) present in the solution for similar initial concentrations of the adsorbate. In a column study for the removal of metal ions by dyed cellulosic materials, copper in the form of nitrate was decreased from 1300 to 910 ppm while copper in the form of chloride was reduced from 1300 to 960 ppm (Shukla and Sakhardande, 1992). The maximum sorption of mercury by *Hardwickia Binata* bark was when it was in the form of nitrate (97%), whereas in the case of sulphate it was 92% and was minimum for chloride (75.5%) (Deshkar *et al.*, 1990). These differences show that each sorbent behaves in a specific way and the conclusions drawn from

one sorbent can not be directly applied to others. This is also one of the reasons why it is necessary to study the characteristics of each sorbent.

Tests were also carried out to determine the relationship between the amount of copper desorbed and the volume and the concentration of each of these three desorbents. The results indicate (Figure 3.7) that the metal desorption process is influenced by both the volume of the desorbent and its strength. A greater level of desorption can be attained with higher volumes of the desorbents. If a greater concentration of the desorbent is used, a smaller volume can be used for the desorption process to attain the same results as a larger volume of a less concentrated desorbent. These results also showed that although H_2SO_4 is the best desorbing agent of the three tested in this work, when their concentrations were relatively low, but at higher concentrations (e.g. 50 mM) their desorption capabilities were approximately the same.

3.7 Removal of Low Concentrations of Cadmium

Adsorption of low levels of cadmium by moss was also examined. The tests were performed using aqueous solutions with the initial cadmium concentrations of 0.8, 2.4, 3.8 and 6.5 ppm. Each of these solutions was treated with a different amount of moss, and the residual cadmium concentration was measured (Table 3.7).

Table 3.7: Removal of low levels of cadmium using different concentrations of moss

Initial Cd^{2+} concentration (ppm)	Moss concentration (mg/mL)		
	1.15	2.3	4.6
	Residual concentration (ppm)*		
0.80	0.365 ± 0.021	0.29 ± 0.028	0.096 ± 0.0028
2.4	0.341 ± 0.029	0.26 ± 0.007	0.131 ± 0.0042
3.8	0.368 ± 0.003	0.25 ± 0.004	0.154 ± 0.0056
6.5	0.487 ± 0.032	0.37 ± 0.028	0.238 ± 0.0035

* Average values ± standard deviation of the duplicate.

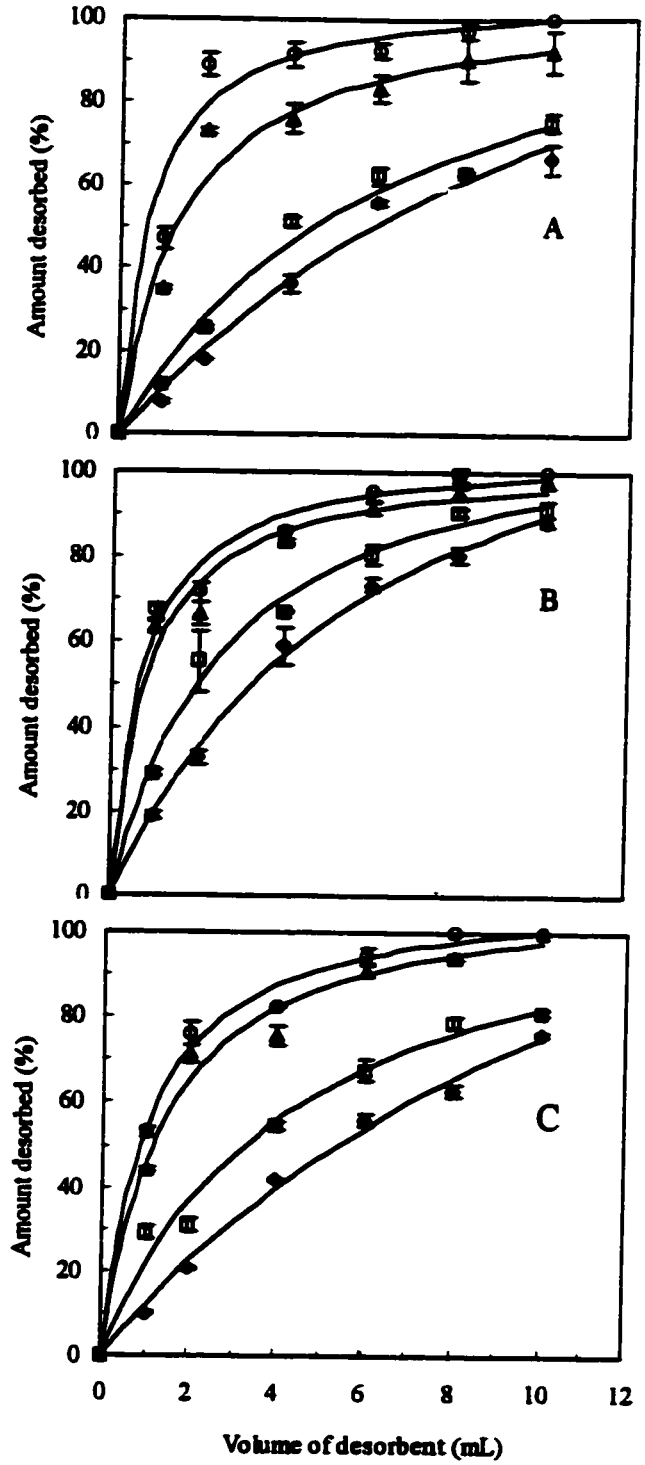

Figure 3.7: Effect of the volume and concentration of (A) HCl, (B) H_2SO_4 and (C) HNO_3 on the desorption of copper from moss. Average loaded copper is 0.19 mmol/g. Desorbent concentration (mM): ◊-2.5, □-5, Δ-25, O-50.

Under the experimental conditions, the lowest residual concentration of Cd^{2+} that was achieved by moss was 0.096 ppm. This concentration is still higher than the permissible limit set by the Environmental Agencies for drinking water, to lower it a multiple stage adsorption process, as mentioned in Section 2.10, was carried out using an initial Cd^{2+} concentration of about 0.74 ppm and 4.6 mg/mL of moss suspension. After 24 h of adsorption, moss was separated and a Cd^{2+} concentration of 0.090 ppm was detected in the filtrate. When the filtrate was treated with 4.6 mg/mL of fresh moss a Cd^{2+} concentration of 0.040 ppm was measured in the aqueous phase. Another adsorption cycle was carried out again with 4.6 mg/mL of fresh moss and the Cd^{2+} was brought to a level of <0.008 ppm. The process was terminated because of the lowest detectable limit of the ICP-AES, as mentioned previously. These results indicate that moss is effective for the removal of low levels of cadmium ions.

This study showed that moss is a good sorbent for metal ions in aqueous solutions. This biosorbent is widely available on the shores of swamps and lakes, and therefore, can serve as an environmental refiner. When the temperature of the adsorption process increased from 8°C to 35°C as well as when the pH increased from 3.5 to 5, substantial increases in copper adsorption were noticed. The mathematical models proposed in this work for the dynamics of metal uptake fitted the experimental data reasonably well. In addition, both the Langmuir and Freundlich models can be used to fit the experimental equilibrium data. Moss was able to sorb the following metals from their aqueous solutions in the indicated order (molar basis): $Cr^{3+} > Cu^{2+} > Zn^{2+} > Ni^{2+} > Cd^{2+}$. The results indicated that the type of the anion of copper salt used for the preparation of the copper solution also influenced the adsorption process. Sulfuric, hydrochloric and nitric acid were good desorbents for copper ions from moss. The desorption process was affected both by the volume and the concentration of these desorbing agents. Finally, moss efficiently sorbed the low concentrations of cadmium and a Cd^{2+} concentration of 0.74 ppm was decreased to <0.008 in a three stage adsorption.

Chapter 4

Continuous Recovery of Metals by Moss-Packed Column

4.1 Introduction

Packed column operation, as a continuous process, is often considered to be more efficient and economical to operate than a batch operation. Therefore, the practical application of the adsorption of heavy metals is most commonly carried out in a packed-column (Volesky, 1987; Wagner and Jula, 1981). The objective of this chapter is to present some of the results obtained from a column packed with moss as sorbent for heavy metals.

Packed columns that used plant and microbial sorbents have already been studied by several researchers. For example: columns packed, separately, with ground redwood bark, walnut expeller meal, and ground formaldehyde-treated peanut shells were used to remove Cu^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+} and Ag^{+} from waste solutions (Randall and Hautala, 1975). The length of these columns ranged from 20-45 cm with an inner diameter (ID) of 20 mm. Volesky and Prasetyo (1994) used columns of 4 mm ID and varying bed depth (70, 90, and 120 cm) packed with the marine alga *Ascophyllum nodosum* to remove Cd^{2+}. Macchi *et al.* (1986) used a bed volume of 12 mL with ID 1.34 cm, packed with two grams of exhausted coffee grounds, to remove mercury ions from a water solution. *Sphagnum*- moss peat was packed in two columns, 1 m length and 5 cm ID, for the removal of Cr^{6+} from aqueous solutions (Sharma and Forster, 1995).

The main parameters that influence the performance of the column are: the length of the column, and the flow rate and initial concentration of the influent. These parameters are important for determination of some design parameters of the column (Volesky and Prasetyo, 1994). Therefore, in this chapter, the effect of these parameters on the break-through curves, with some desorption studies, will be investigated. Bohart and Adams analysis for the performance of the packed-bed will be considered.

4.2 Concentration Profile

To study the concentration profile, i.e. the change of metal concentration along the bed, a solution of 100 ppm (1.57 mM) initial copper concentration (using deionized water) was allowed to flow upwards at a rate of 8.4 mL/min through a 20 cm × 1.2 cm ID column packed with 5 g moss.

Samples were collected from ports located in the column at 4, 8, 12, 16 and 20 cm (top of the column) from its bottom. The process continued until the metal concentration from the top of the column became the same as the influent concentration. The results are presented in Figure 4.1, as the ratio of the effluent to the influent concentrations (C/C_0) versus time of the process. Steady-state was attained after 320 min. During that time 2.5 L of copper solution flowed through the column. The results indicated that the higher ports required more time to achieve saturation and that the break-through is faster in the lower ports than in the higher ones. The copper concentration in the total collected volume, the reservoir for the effluent from the top of the column, was found to be 60.4 ppm (0.95 mM), giving a capacity of 19.8 mg of Cu^{2+} per g of moss (0.312 mmol/g) for the packed moss column. This is comparable to the result of Deshkar *et al.* (1990) when they obtained a capacity of 21 mg Hg^{2+} per g of *Hardwickia binata* bark packed in a column.

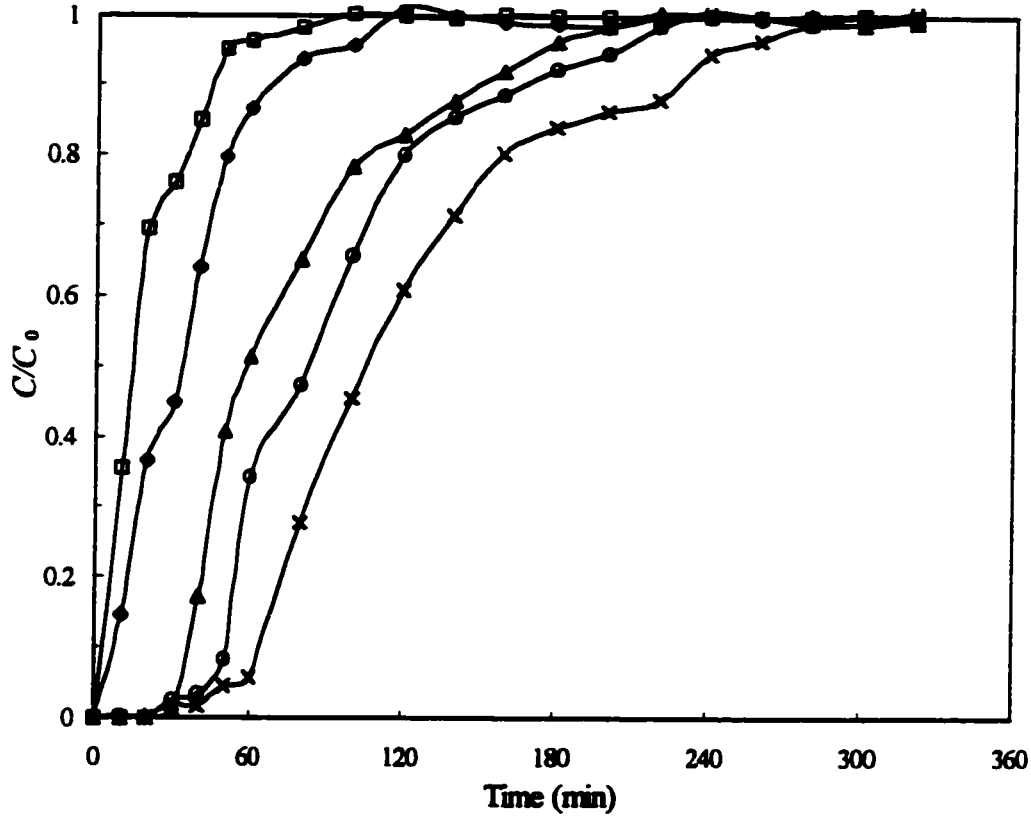

Figure 4.1: Adsorption of copper through a packed column of 20 cm length and 1.2 cm I.D. Port location (cm): □-4, ◊-8, Δ-12, O-16, ×-20.

4.3 Effect of Influent Flow Rate

The flow rate of the solution can affect the performance of a packed bed column. It has a significant influence on the break-through curve (Sharma and Forster, 1995; Randall and Hautala, 1975). To determine the effect of the solution flow rate on the performance of the column, experiments were carried out at constant flowrates of 2.5, 8.6, 16.6 and 26.6 mL/min using an influent copper concentration of 100 ppm (1.57 mM). Break-through curves at different locations in the column (4, 8, 12, 16, and 20 cm) were obtained for each of these flow rates (Figures 4.2-4.6). At the top of the column (Figure 4.6), break-through occurred after 180 min at the flow rate of 2.5 mL/min; however, when the flow rate increased to 8.6 mL/min, only 60 min was required for the break-through to occur. Almost 45 min were required when the flow rates were 16.6 and 26.6 mL/min. Therefore, larger flow rates resulted in a shorter break-though times. This was valid for each port along the column. However, at higher ports, the influence of the flow rate on the break-through curves was more obvious than when lower ports were considered (e.g. at 4 cm and 20 cm of Figures 4.2 and 4.6). It is also shown (Figures 4.2-4.6) that, within experimental error, the break-through curves at the flow rates 16.6 and 26.6 mL/min were pretty close.

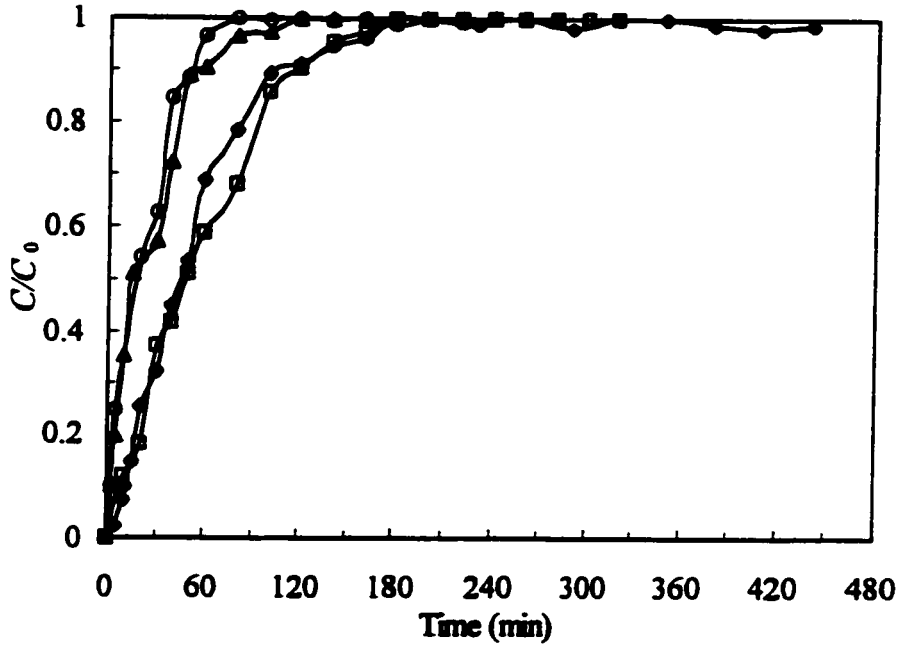

Figure 4.2: Break-through curves for Cu^{2+} sorption by moss at 4 cm. Influent copper concentration: 100 ppm; pH: 4.5; T: 25°C; flow rate (mL/min): □-2.5, ◊-8.6, Δ-16.6, O-26.6.

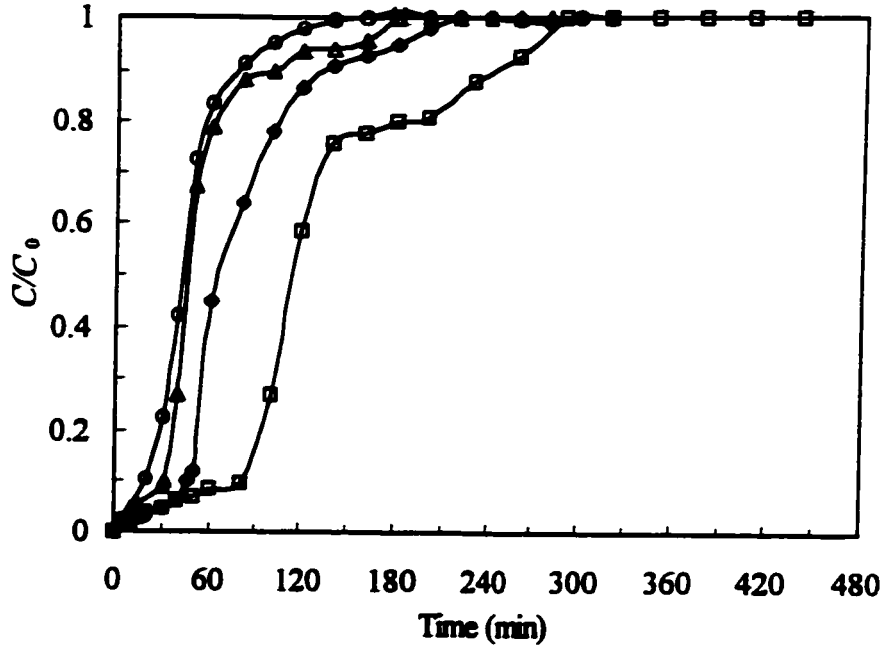

Figure 4.3: Break-through curves for Cu^{2+} sorption by moss at 8 cm. Influent copper concentration: 100 ppm; pH: 4.5; T: 25°C; flow rate (mL/min): □-2.5, ◊-8.6, Δ-16.6, O-26.6.

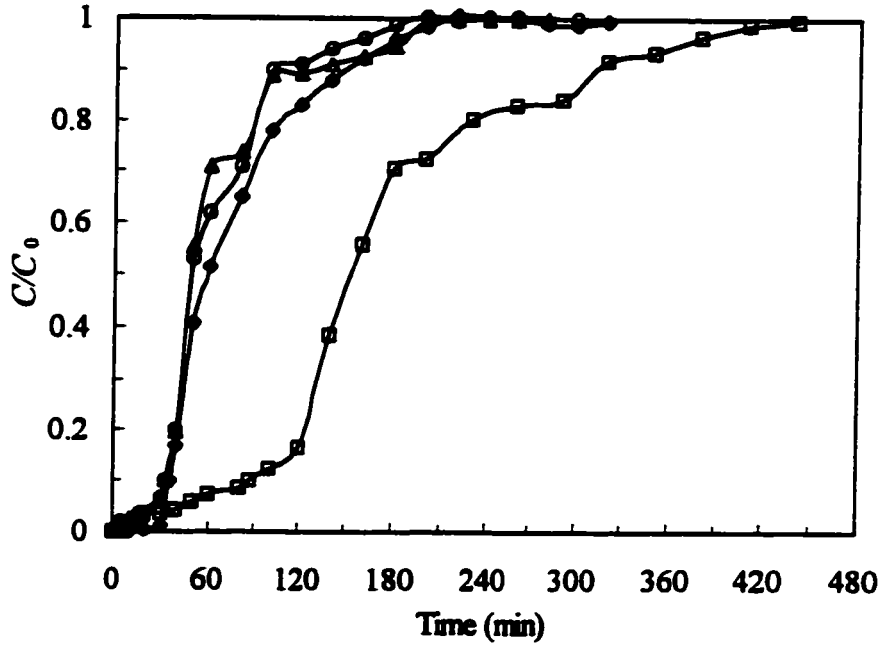

Figure 4.4: Break-through curves for Cu^{2+} sorption by moss at 12 cm. Influent copper concentration: 100 ppm; pH: 4.5; T: 25°C; flow rate (mL/min): □-2.5, ◊-8.6, Δ-16.6, O-26.6.

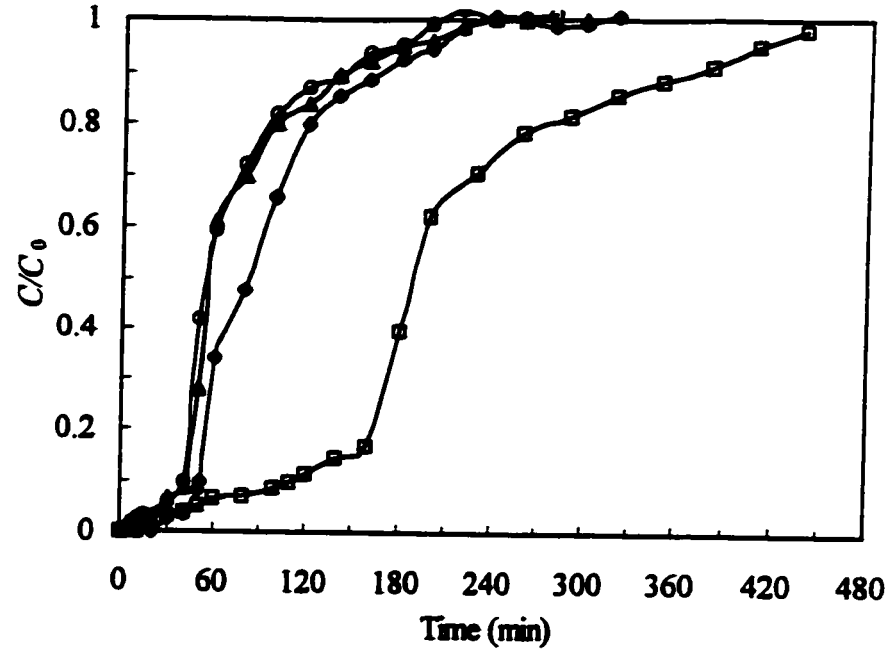

Figure 4.5: Break-through curves for Cu^{2+} sorption by moss at 16 cm. Influent copper concentration: 100 ppm; pH: 4.5; T: 25°C; flow rate (mL/min): □-2.5, ◊-8.6, Δ-16.6, O-26.6.

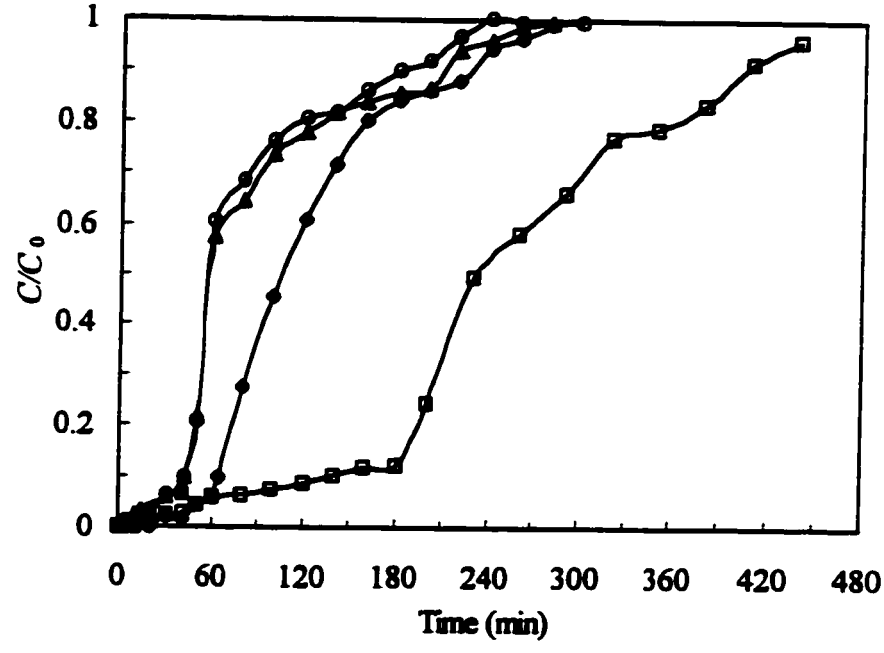

Figure 4.6: Break-through curves for Cu^{2+} sorption by moss at 20 cm. Influent copper concentration: 100 ppm; pH: 4.5; T: 25°C; flow rate (mL/min): □-2.5, ◊-8.6, Δ-16.6, O-26.6.

The metal uptake of the column at each flow rate, as estimated from the material balance at steady-state (when the effluent concentration from the top of the column became the same as the influent concentration), is summarized in Table 4.1. Columns with high flow rates require large volumes of metal solution to attain equilibrium ($C_{out}/C_{in} \approx 1$), and so, the metal concentration in the effluent reservoir is higher. Therefore, the metal uptake by the column, and the absolute amount of copper removal (mg), with high flow rates was higher than those with low flow rates (Table 4.1). However, the percentage of metal removal at low flow rates was higher than that at higher flow rate, and so the efficiency of the column was higher.

Table 4.1: Copper uptake and copper removal at different flow rates. Inlet metal concentration: 100 ppm (1.57 mM); amount of packed sorbent: 5 g

Flow rate (mL/min)	Total volume pumped (L)	Concentration of metal in the effluent reservoir (mM)	Column uptake (mmol Cu^{2+}/g moss)	Amount of metal removal	
				(mg)	(%)
2.5	0.9	0.674	0.16	50.7	57.0
8.6	3.2	0.986	0.37	117.3	37.2
16.6	4.2	1.106	0.39	123.6	29.5
26.6	7.7	1.260	0.47	149.0	19.7

Application of the Bohart and Adams analysis (Chapter 4; Part I) requires specification of the break-through time. In this study, the break-through time was arbitrary specified as the time corresponding to when the metal concentration in the effluent was 5 ppm, that is $C/C_0 = 0.05$ in this case. The relationship between the break-through time (t_b), or the time which corresponds to $C/C_0 = 0.05$, and the bed depth (Z) for the four flowrates is shown in Figure 4.7. This relationship can be represented by the following straight lines, according to Eq. (4.24), for each flow rate, respectively:

flow rate 2.5 mL/min: $t_b = 3.37\,Z - 0.90$

flow rate 8.6 mL/min: $t_b = 3.06\,Z - 6.20$

flow rate 16.6 mL/min: $t_b = 2.08\,Z - 5.76$

flow rate 26.6 mL/min: $t_b = 2.01\,Z - 7.82$

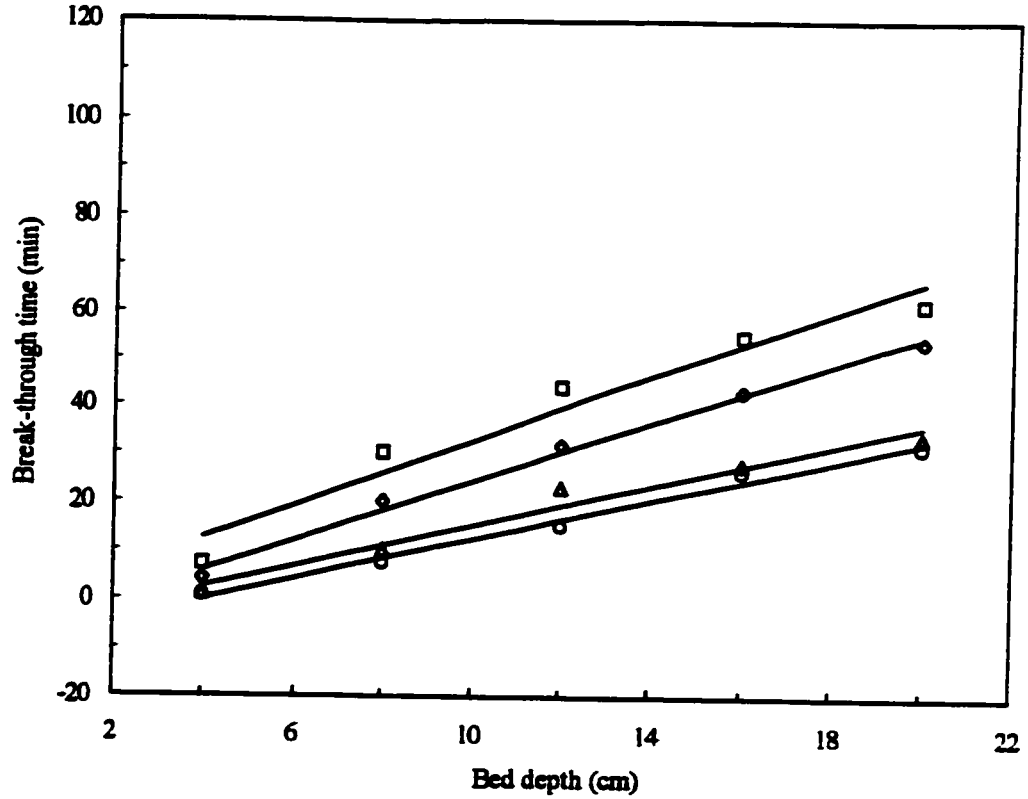

Figure 4.7: Relationship between bed depth and bed break-through time. Flow rate (mL/min): □-2.5, ◊-8.6, Δ-16.6, O-26.6. Symbols are experimental and lines represent the fitting of Eq. (4.24).

The values of the parameters Z_{min}, the minimum bed depth, were determined from the above equations by solving each equation for Z with $t_b = 0$. The values of N_0, the average sorption capacity of moss at a given influent concentration and flow rate, and k, the sorption rate constant, were determined from the intercept and the slope of these equations, respectively, according to Eq. (4.24), found in Part I. The values of these parameters, which can be used for the evaluation of the sorbent parameters, are shown for each flow rate in Table 4.2.

Table 4.2: Performance parameters of the moss column for various flow rates. Inlet copper concentration: 100 ppm

Flow rate (mL/min)	k (mM . min)$^{-1}$	N_0 (μmol/cm^3)*	Z_{min} (cm)
2.5	2.080	12.2	0.27
8.6	0.332	36.6	2.03
16.6	0.302	48.1	2.77
26.6	0.239	74.6	3.88

* μmol of Cu^{2+} per cm^3 of moss.

Unlike the values of N_0 determined by Volesky and Prasetyo (1994), who found a slight decrease in the values of N_0 with increasing flow rate for the removal of cadmium by *A. nodosum*, Table 4.2 shows a significant increase of this parameter with increasing the flow rate. Bearing in mind that N_0 is related to the sorbent capacity, its values in Table 4.2 are consistent with the values of the copper uptake displayed in Table 4.1. This increase in the sorbent capacity with the increase in the flow rate can be explained in terms of the driving force. At low flow rates, the contact time between the solute and the sorbent at the bottom portion of the column is longer than that at higher flow rates, which enable the solute to pass faster through the column. Therefore, the driving force at high flow rates is greater than that at low flow rates for a given influent concentration, which resulted in the increase of the sorbent capacity for the copper ions.

The term $(1/kC_{in})\ln[(C_{in}/C_{out})-1]$ in Eq. (4.24), Chapter 4 (Part I), represents the depth over which sorption takes place (Bohart and Adams, 1920). When $C_{in}/C_{out} = 2$, this term in Equation. (4.24) reduced to zero, and the corresponding time is called t_{50}; the time required for the effluent concentration to attain half of the influent concentration. Figure 4.8 shows plots of t_{50} against the depth of the column for the removal of copper by moss at flow rates of 2.5, 8.6 and 26.6 mL/min. Theoretically, the 50% curve must pass through the origin, but Figure 4.8 shows that these lines deviate slightly from the origin. Such a deviation is similar to that reported by Tan *et al.* (1993) for the adsorption of Cr(IV) by coconut-husk fibers and palm pressed-fibers, and to that obtained by Sharma and Forster (1995) for adsorption of Cr(VI) using sphagnum moss peat, while t_{50} versus bed depth curves for sorption of Hg(II) from aqueous solutions using rice-husk ash (Tiwari *et al.*, 1995) passed through the origin. Poots *et al.* (1976), when they studied adsorption of acid dye by *sphagnum*-moss peat, attributed the deviation of t_{50} curves from the origin to the existence of more than one rate-limiting step. In this work, the failure of the t_{50} versus bed depth line to pass through the origin, together with the kinetics study in the previous chapter for the batch sorption of Cu^{2+} by moss (uptake versus $t^{1/2}$ curves, Figure 3.2), indicate that the adsorption of copper by moss could also involve more than one rate-limiting step.

4.4 Effect of Influent Concentration

To study the effect of the influent copper concentration on the break-through curves, copper solutions of different concentrations were pumped through the column at an average flow rate of 8.5 mL/min. Break-through curves were obtained at different locations along the column for different influent concentrations of 25, 50, 75 and 100 ppm. The results are shown in Figures 4.9-4.13 for the

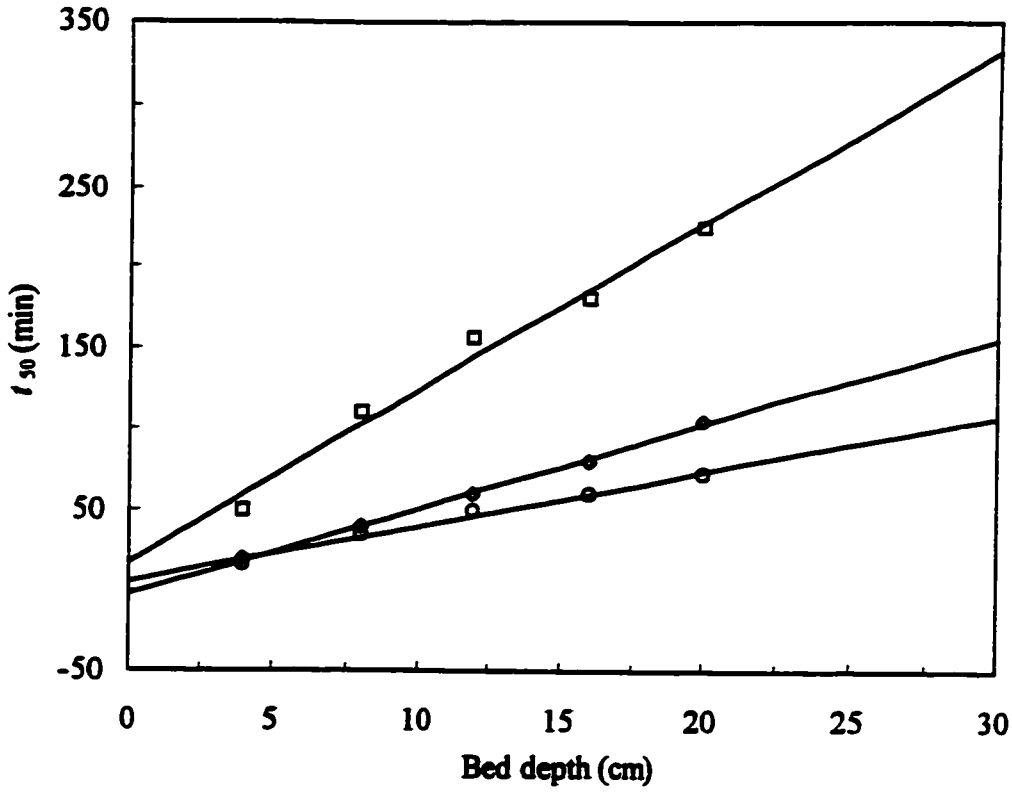

Figure 4.8: BDST curves at 50% (t_{50}) versus bed depth for copper removal by moss at various flow rates. Influent copper concentration: 100 ppm; pH: 4.5; T: 25°C; flow rate (mL/min): □-2.5, ◊-8.6, O-26.6.

different ports along the column. Generally, at all locations, a decrease in the influent concentration resulted in a delay in the occurrence of the break-through. This delay became longer when going from lower ports to higher ports. For example, at the top of the column (20 cm), Figure (4.13), the influent copper concentration of 25 ppm required about 300 min to reach break-through, while the influent copper concentration of 100 ppm required only about 50 min. Based on the results from the previous tests, it was expected that break-through would occur sooner at lower ports than at higher ports, regardless of the influent concentration. It is also seen (Figures 4.9-4.13) that, at different influent concentrations, the break-through curves are closer to each other at lower locations than at higher locations, e.g. at 4 cm (Figure 4.9) the curves are very close to each other while at 20 cm (Figure 4.13) the break-throughs are very far apart from each other. This is because the lower layers of the column were saturated with a certain amount of metal ions and the increase in the metal concentration did not influence this saturation. However, when going towards the top of the column, the layers were less

saturated with metal ions. This is because most of the metal ions were being adsorbed by the lower layers. Therefore, the increase in the metal concentration resulted in more saturation of these layers.

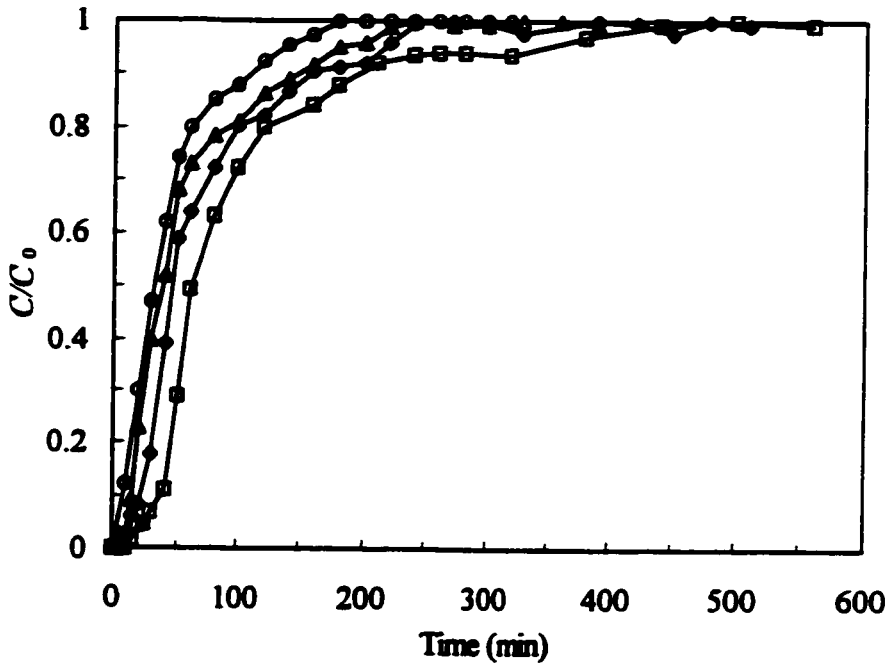

Figure 4.9: Effect of influent Cu^{2+} concentration on the break-through curves at 4 cm. Flow rate: 8.5 mL/min; pH: 4.5; T: 25°C; influent concentration (ppm): □-25, ◊-50, Δ-75, O-100.

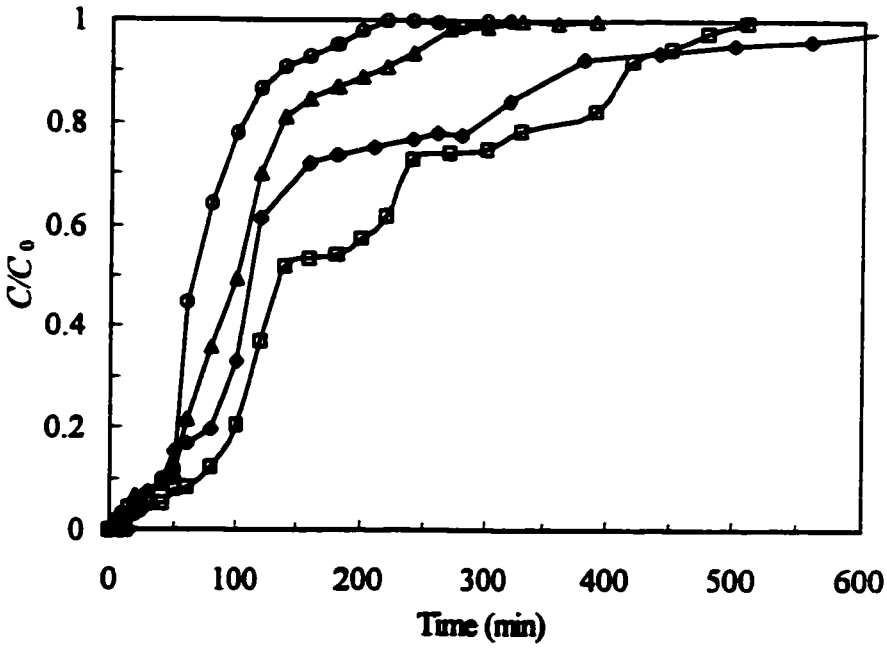

Figure 4.10: Effect of influent Cu^{2+} concentration on the break-through curves at 8 cm. Flow rate: 8.5 mL/min; pH: 4.5; T: 25°C; influent concentration (ppm): □-25, ◊-50, Δ-75, O-100.

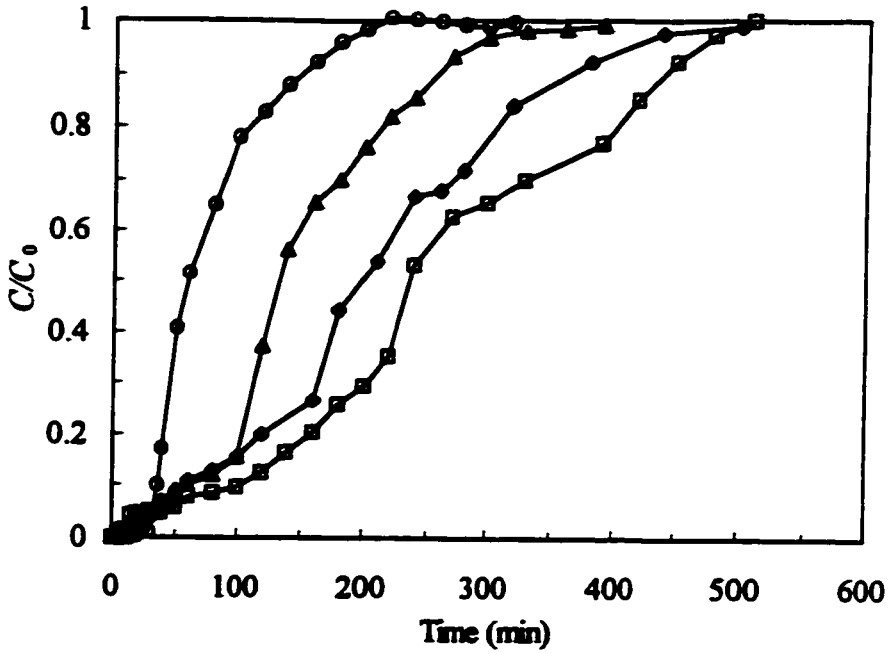

Figure 4.11: Effect of influent Cu^{2+} concentration on the break-through curves at 12 cm. Flow rate: 8.5 mL/min; pH: 4.5; T: 25°C; influent concentration (ppm): □-25, ◊-50, Δ-75, O-100.

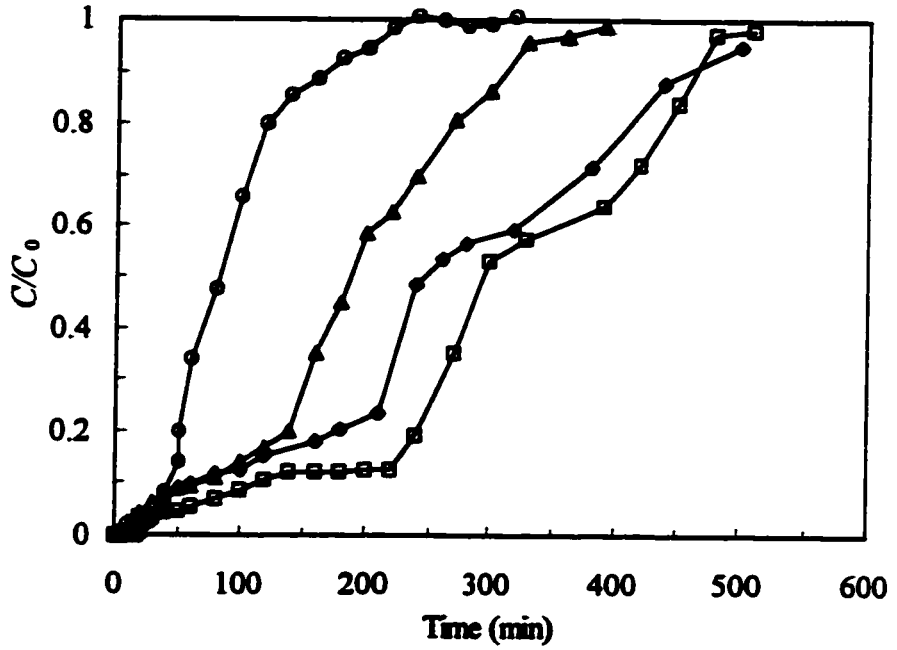

Figure 4.12: Effect of influent Cu^{2+} concentration on the break-through curves at 16 cm. Flow rate: 8.5 mL/min; pH: 4.5; T: 25°C; influent concentration (ppm): □-25, ◊-50, Δ-75, O-100.

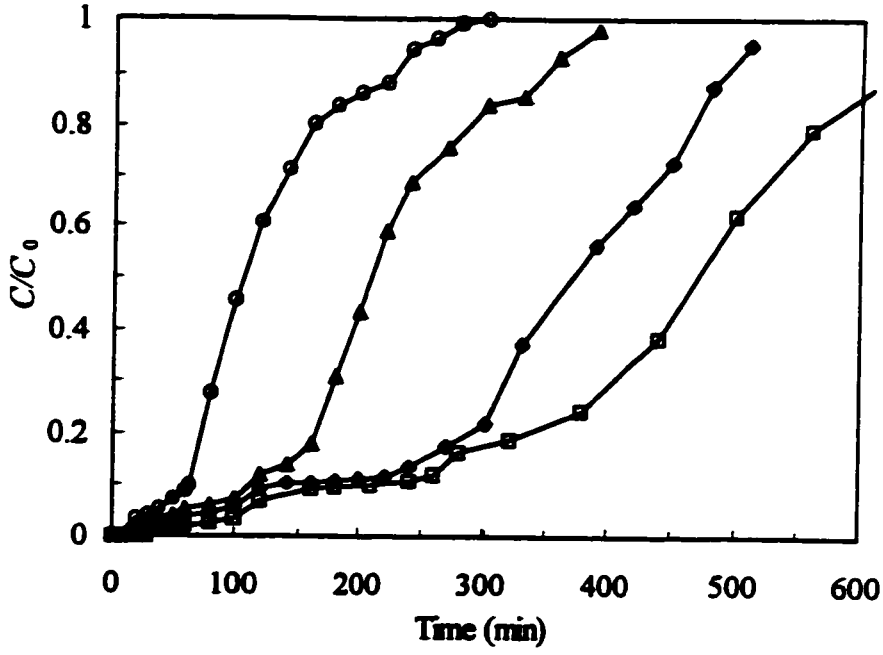

Figure 4.13: Effect of influent Cu^{2+} concentration on the break-through curves at 20 cm. Flow rate: 8.5 mL/min; pH: 4.5; T: 25°C; influent concentration (ppm): □-25, ◊-50, Δ-75, O-100.

The relationships between the break-through time (time corresponding to $C/C_0 \approx 0.05$) and the bed depth, at different influent copper concentrations are shown in Figure 4.14. It is obvious that these data are not linear, especially at low influent concentration, and therefore Equation. (4.24) is not applicable for these set of data. At high influent concentrations, the bed was saturated faster with copper ions than in the case of low influent concentration. This resulted in a larger concentration gradient along the bed in the case of the low influent concentration. This might be the reason for the longer time required to reach break-through at the upper part of the bed when the influent concentration is low.

The results of the material balance and the calculations for the sorbate capacity in the bed is shown in Table 4.3. It is seen that the amount of metal removal (%) increased with the decrease in the influent concentration, indicating that the bed is more efficient for the treatment of lower metal concentrations than of higher metal concentrations. However, the uptake of copper ions by the packed-moss column, and the absolute amount of copper removed (mg), increased with the increase in the influent concentration. The results of Table 4.3 suggest the utilization of more than one column for the removal of metal ions for a given initial concentration. For example, the influent concentration of 1.57 mM can be decreased to 0.94 mM in the first column where the reservoir from the effluent of this column can be used as a feed for another column for further reduction in the copper concentration.

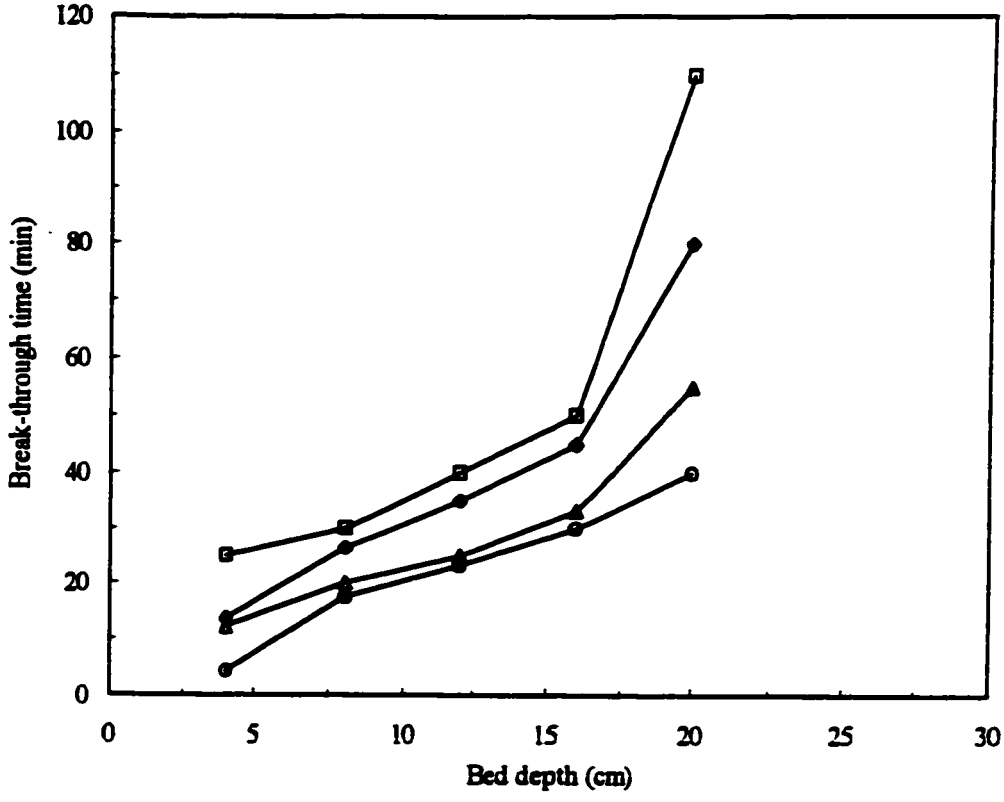

Figure 4.14: Relationship between bed depth and bed break-through times at various influent concentrations. Influent concentration (ppm): □-25, ◊-50, Δ-75, O-100.

Table 4.3: Relationship between copper uptake, copper removal and influent concentration. Average influent flow rate: 8.5 mL/min; amount of packed sorbent: 5 g

Influent concent-ration (mM)	Total collected volume (L)	Concentration of metal in the collected volume (mM)	Column uptake (mmol Cu^{2+}/g moss)	Amount of metal removal	
				(mg)	(%)
0.393	5.1	0.097	0.301	95.4	77.1
0.785	4.0	0.270	0.410	130.0	65.6
1.170	3.6	0.560	0.431	137.0	52.1
1.570	3.5	0.940	0.441	140.0	40.1

The relationship between the influent concentration (C_{in}) and the uptake by the bed at saturation (q_B) follows (Hines and Maddox, 1985) the Langmuir equation:

$$q_B = \frac{QK'C_{in}}{1+K'C_{in}} \tag{4.1}$$

where Q and K' are the Langmuir constants, related to the maximum uptake by the bed and the energy of adsorption, respectively. Figure 4.15 displays the linearized plot of the Langmuir equation, from which values of Q and K' were found to be 0.547 mmol/g and 3.2 mM, respectively. These constants are related to the capacity and affinity of moss for copper ions, respectively. It is seen that the value of K' obtained here is close to the value of b (2.89) obtained previously in the batch process for the sorption of copper by moss.

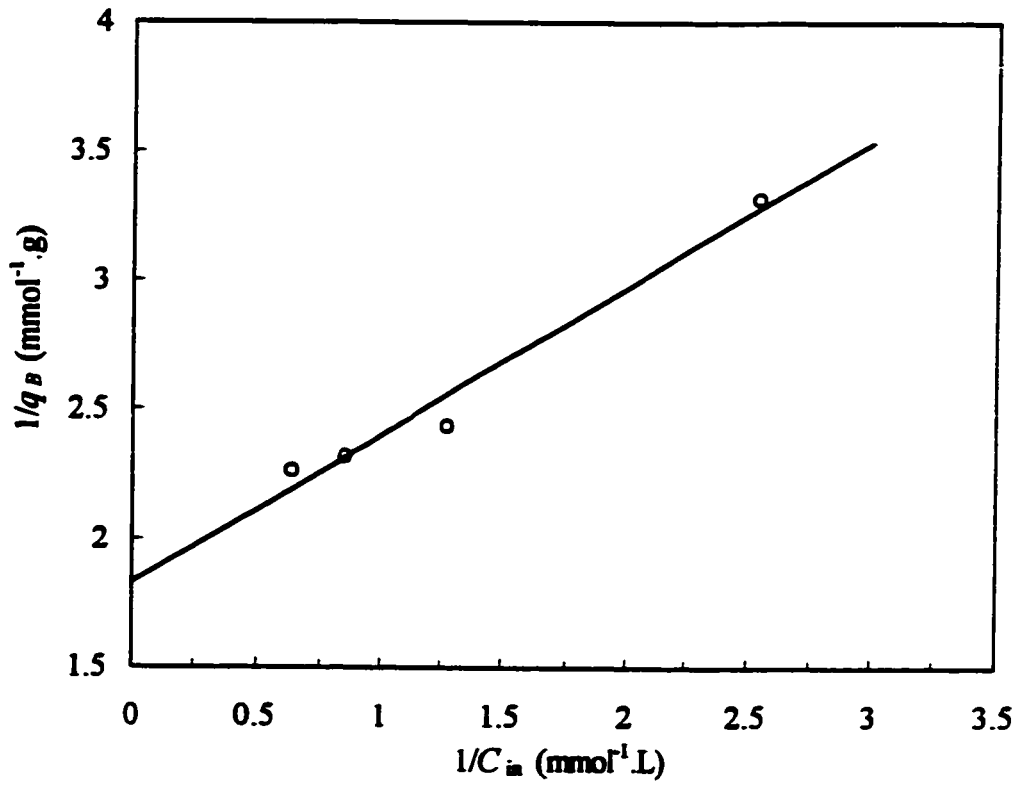

Figure 4.15: Langmuir isotherm of the packed bed for the sorption of copper at an average flow rate of 8.5 mL/min. Symbols are experimental data and line is the Langmuir fit.

4.5 Kinetics of Loading

The break-through curves show the changes in the effluent metal concentration with time. They do not give any information about the changes in the metal uptake by the moss- column with time. To determine this, a copper solution of 100 ppm was pumped at about 8.0 mL/min through the column, and samples were taken from the top of the column as well as from the reservoir of the effluent stream, at various periods of time. The uptake of the column per unit weight of moss was calculated as a function of time by applying a material balance on the column using the following relation:

$$q = \frac{(C_{in} - C_T)V}{M} \tag{4.2}$$

where C_T is the metal concentration in the total volume (V) passed through the column and M is the amount of moss (grams) packed in the column.

The loading curve (Figure 4.16) can be considered as consisting of two regions, the first where the loading increased linearly with time and the second which was characterized by slow rate of uptake. The reason for this is that, at first most of the mass transfer took place near the inlet of the bed, where the metal solution contacted fresh moss which sorbed metal ions until saturation. After that, the mass transfer zone moved towards the top of the column by contacting fresh sorbent. This trend continued till the saturation point, where all of the moss material in the bed was saturated with copper ions. This corresponded, approximately, to halfway of the break-through curve. At this point the first region lost its linearity and the second stage began. This stage approached the asymptote as the effluent concentration attained that of the influent, or when a complete saturation was established, where the column can not load any more metal ions.

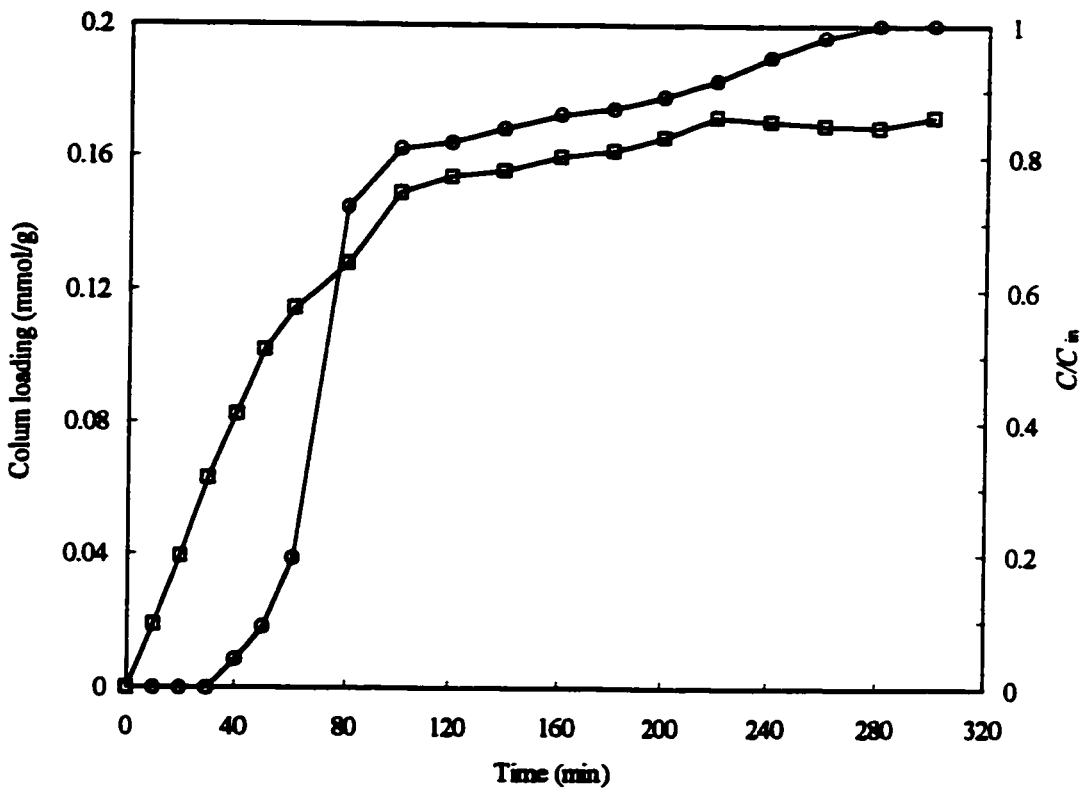

Figure 4.16: Kinetic of loading moss column with copper. Influent concentration: 100 ppm; Average flow rate: 8.0 mL/min. □-loading curve; O-break-through curve.

4.6 Parallel and Series Connection

In industrial practice very often adsorption systems with more than one column are used. The multi-column units are applied when a large amount of liquid waste effluents should be treated continuously. The columns can be connected either in series or in parallel (Wagner and Jula, 1981; Sharma and Forster, 1995). This practice enables a better utilization of the sorbents and flexibility of the process units. In this study, a set-up of two 20 cm × 1.2 cm ID columns, without ports, were used to investigate the removal of copper from solution by moss. The columns were connected either in parallel or in series. Each of them was packed with 5 grams of moss and a solution of 100 ppm (1.57 mM) copper was pumped through them. The results are shown in Figure 4.17A and B.

When columns were connected in parallel (Figure 4.17A), they were fed from the same line and the break-through curve pattern was the same. In both columns the break-through appeared after 100 min of the process, and saturation was attained after about 250 min. The similarity between the two break-through curves, which were run at the same conditions, indicates a satisfactory reproducibility in column preparation and operation. After the complete saturation of the columns with the metal, which was reached after 340 min of operation, the volumes of the reservoirs for the effluents of the first and second column were measured to be 1.5 and 1.7 L, respectively. The copper concentrations in the first and second reservoir were 0.94 and 0.89 mM, respectively, giving capacities of 0.19 and 0.23 mmol/g, respectively, to the moss columns. The slight differences in the columns performances were probably due to experimental error or due to blocking or channeling that could occur in one or both of the columns during the pumping period.

When the columns were in series (Figure 4.17B), in which the effluent from the first column was the feed for the second column, the break-through in the first column occurred earlier, while in the second column, it occurred at the time when the first column became almost saturated, $C/C_{in} = 1.0$. In this configuration, 3.6 L of copper solution were passed in order to achieve saturation in both of the columns. The saturation was attained after 400 min, a longer period than in the case of parallel configuration. The total metal loading in both columns was 0.47 mmol/g, a value close to the total obtained by the two columns connected in parallel (0.42 mmol/g).

The amount of copper removal in the parallel and series connections was almost the same, about 42%. In parallel connection, the break-through was faster than that of series connection. In multi-columns operation, series connection is more practical in terms of regeneration (Wagner and Jula, 1981; Volesky, 1987). Once the first column is saturated, its regeneration, by acid followed by washing, can be carried out while the other columns used for sorption, and the process proceeds

alternatively. However, in parallel connection both of the columns are used at the same time, either for sorption or regeneration.

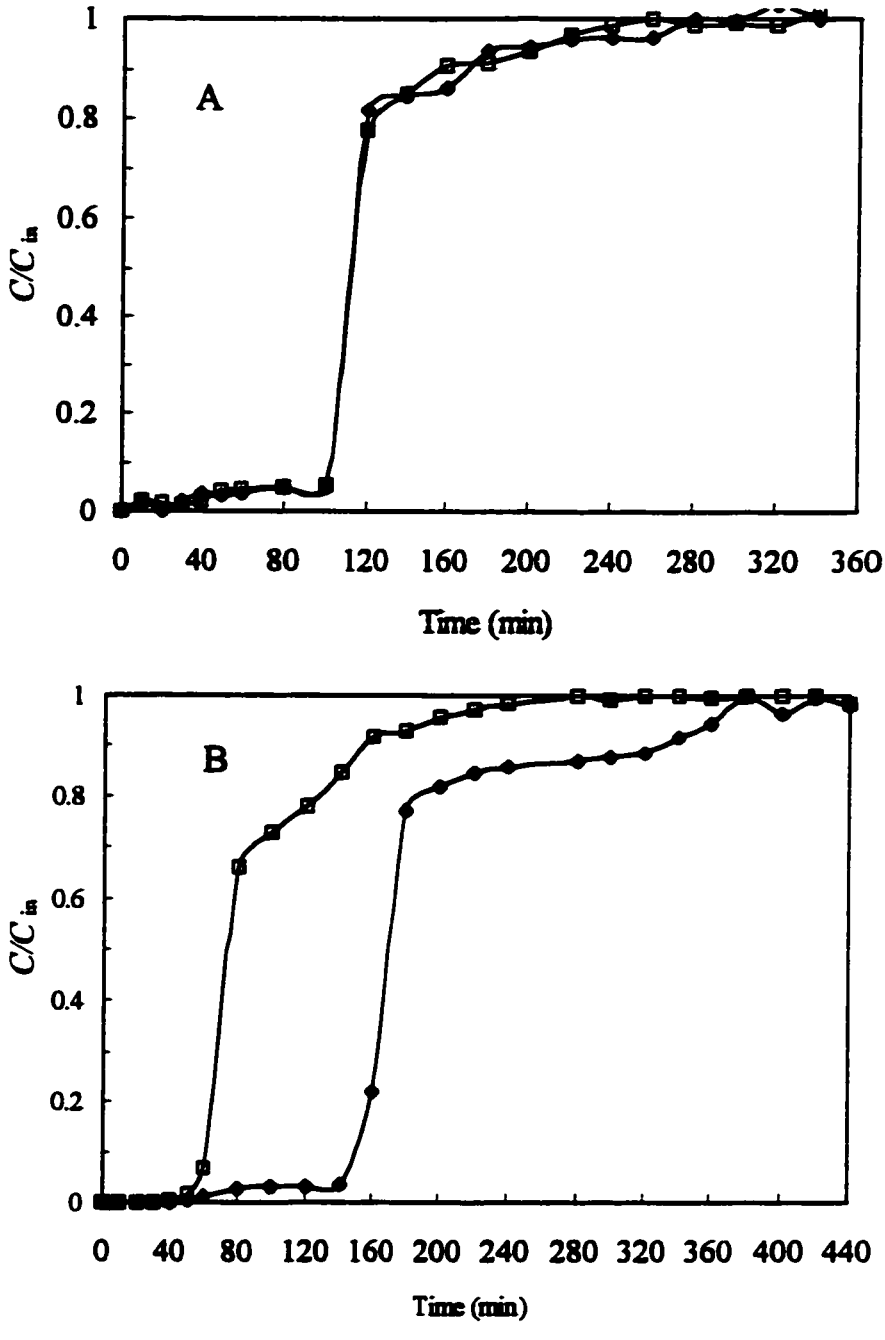

Figure 4.17: Parallel (A) and series (B) connection of two columns, each packed with 5 g of moss subjected to flow of 100 ppm copper solution. Column: □-first, ◊-second.

4.7 Desorption

The desorption of metal ions from the moss was also studied. Once the column is laden with the metal, the metal should be recovered. The most common method for the release of metal ions from the sorbent is desorption by acid solution (Volesky, 1987). Burning of the sorbent would be another alternative if the sorbent material is inexpensive and available in large amounts. In this study, hydrochloric acid (HCl) was used to desorb copper ions from the moss-packed column. Several moss-packed columns were first subjected to a 100 ppm (1.57 mM) influent copper solution, giving a load of about 0.31 mmol/g to each of these columns. Hydrochloric acid of a particular concentration was pumped through a specified column, at a rate of about 8.4 mL/min, for the desorption of the metal. The concentration of copper was measured from the top of each of these columns until the concentration dropped to zero.

The results (Figure 4.18) indicated that in all the cases, the effluent metal was concentrated at the beginning of the desorption process, and then dropped quickly to the zero level after a certain period of time. Eluants of a high HCl concentration resulted in a rapid increase and decrease in the metal concentration from the column effluent of the bed. Eluants of 0.25 M and 0.50 M HCl behave similarly and, from an economical point of view, 0.25 M HCl is preferable. In addition, a system with 0.5 N HCl could cause more deterioration to the biological sorbent.

Desorbents with high concentrations might be advantageous because small volumes of acid, and shorter periods of time, are required to flush the whole contents of the column, resulting in a higher concentration of metal in the effluent (Figure 4.18). The major disadvantage of using a high desorbent concentration is that it results in damage to the column contents, especially when dealing with biological materials such as a plant sorbent as moss. On the other hand, desorbents with low concentrations cause less damage to the column contents, but, unfortunately, require larger volumes of desorbent and longer periods of time, and do not result in a high effluent concentration in a short period of time. Therefore, in a desorption study, it is important to find a desorbent that results in a high effluent concentration with a small volume of eluant, short periods of time and does not damage the sorbent material.

While systems with 0.25 and 0.5 M HCl required about 160 mL to flush the column, the system with 0.025 M HCl required 260 mL of eluant. In addition, 10 more minutes were required for desorption when the more diluted desorbent was used. However, it can be assumed that it caused less deterioration, and, therefore, makes the life of the sorbent longer. The other, lower strength eluants,

0.01 and 0.005 M HCl, might cause less deterioration but require a greater volume of eluant, and a longer flushing time. Therefore, 0.025 M HCl was considered suitable for elution.

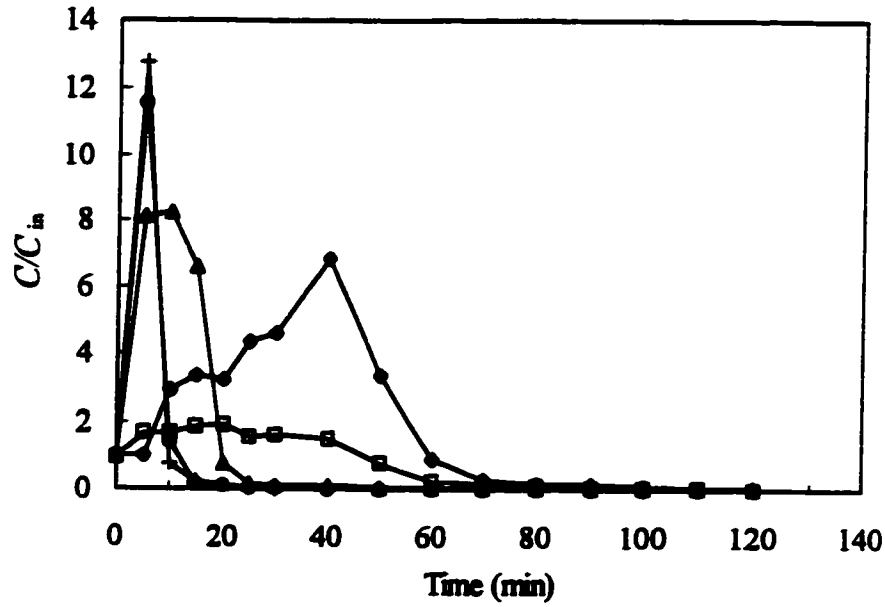

Figure 4.18: Effect of the desorbent strength on copper desorption by HCl. Amount of packed moss: 5 g moss; average load: 0.315 mmol/g. Desorbent strength (M HCl): □-0.005, ◊-0.01, Δ-0.025, O-0.25, +-0.5.

The concentration of metal released from the column by pumping 1 L of eluant was almost the same for all of the HCl solutions: about 1.48 ± 0.05 mM. This means about 1.48 mmol of copper was released from the column. Keeping in mind, that on average, the total amount of copper loaded in the column was 1.55 mmol (0.31 mmol/g × 5 g), this gave an average copper recovery of 95.5% for this desorption process. An incomplete recovery of copper might be attributed to the strong complexation/coordination of copper ions with the moss' functional groups. In conclusion, desorption of copper from the moss was influenced by the volume and concentration of the desorbent and a solution of 0.025 M HCl would be the most suitable desorbent for this process.

4.8 Regeneration of the Moss-Packed Column

If the sorbent is expensive or if it is not always available in large amounts, it is necessary to regenerate it. However, sometimes when the sorbent is so cheap and abundant, recycling is not considered worthwhile. It can be combusted and the metal can be recovered from the ash. From an economical point of view, the sorbent is considered effective if it can be easily regenerated and re-

utilized as much as possible without alteration of its performance. Taking this into account, it was of interest to study the possibility of regenerating the moss used in this research. A column packed with 5 g of moss was used in this test and a copper solution of 100 ppm was pumped at 7.5 mL/min until saturation. After that, the adsorbed metal was desorbed using 0.025 M HCl. One liter of the eluant was used to ensure maximum recovery of copper ions from the column. Following desorption, the bed was washed with water till the pH of the effluent stream corresponded to that of the water. This cycle (sorption-desorption-washing) was repeated five times. The break-through curves and the pattern of desorption curves were similar for all of the cycles and they followed the same trends as those presented in the previous sections. The total (absolute) amounts of copper loaded and desorbed in each cycle are displayed in Figure 4.19.

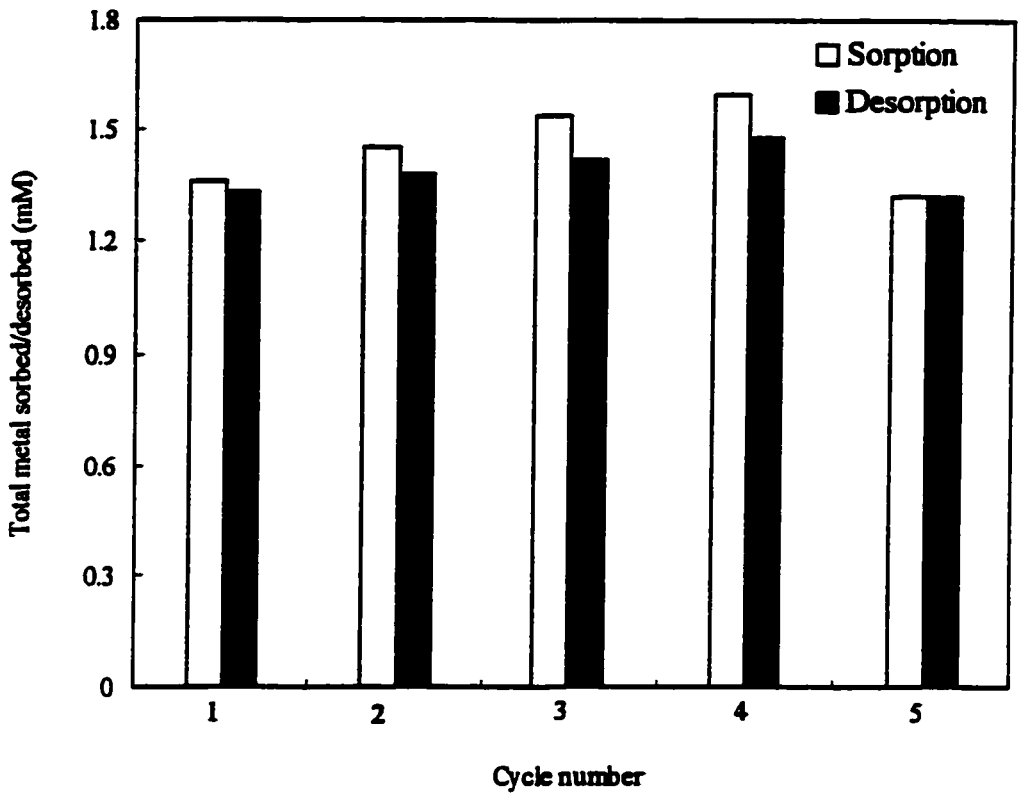

Figure 4.19: Regeneration of moss column for sorption of copper ions. Average flow rate: 7.5 mL/min; influent copper concentration: 1.57 mM; desorbent: 0.025 M HCl.

The results show slight differences in the amount of copper sorbed/desorbed from one cycle to another. However, within the experimental errors, the values are comparable (Figure 4.19) and relatively close to each other. This indicates that the moss-packed column is still effective after five sorption/desorption cycles. The incomplete removal of copper ions from the bed could be due to ions

that diffused into the pores of the moss, which are difficult to be released under these conditions, or are strongly complexed by the sorbent constituents, as previously mentioned.

4.9 Continuous Recovery of Other Metals

The ability of the moss-packed column to sorb other metal ions was also considered. Individual solutions of cadmium, nickel or copper solutions pumped through the packed bed for the purpose of comparison. The flow rate and the initial concentration of each metal ion were 8.2 mL/min and 100 ppm, respectively. Samples were obtained from different locations (4, 8, 12, 16 and 20 cm) along the column, and the break-through curves at each location were obtained for the three metals. The break-through curve at 20 cm depth and the relationship between the break-through time and the depth of the bed, for the three metal ions, are shown in Figures 4.20 and 4.21, respectively. The break-through of Ni^{2+} occurred near the very beginning of the process, then copper and followed by cadmium (Figure 4.20). This trend gave a loading of 0.07, 0.221 and 0.31 mmol/g for Ni^{2+}, Cd^{2+} and Cu^{2+}, respectively. This means that the uptake per unit weight of moss for these three metals was in the following order: $Cu^{2+} > Cd^{2+} > Ni^{2+}$. However, the total amount of removal followed the following order: Cd^{2+} (49.7%) $> Cu^{2+}$ (35%) $> Ni^{2+}$ (9.5%). These results are consistent with the results obtained from the batch process (Table 2.5). The earlier occurrence of the break-through of the Ni^{2+} ions indicates that the removal of Ni^{2+} by the moss column was rather ineffective.

The relationship between the break-through time and the bed depth for the three metals (Figure 4.21) can be well correlated linearly by using Eq. (4.24). This gives the following relations:

$$Cu^{2+}: \quad t_b = 2.22\,Z - 4.6$$

$$Cd^{2+}: \quad t_b = 3.82\,Z - 15.4$$

$$Ni^{2+}: \quad t_b = 1.20\,Z - 6.7$$

The minimum depths of the bed, Z_{min}, sufficient to prevent the effluent Cu^{2+}, Cd^{2+} and Ni^{2+} concentrations to exceed $C/C_0 \approx 0.05$ are 2.07, 4.03, and 5.6 cm, respectively. These values were obtained by setting $t_b = 0$ in the above equations. These results indicate that the performance of the column is different from one metal to another.

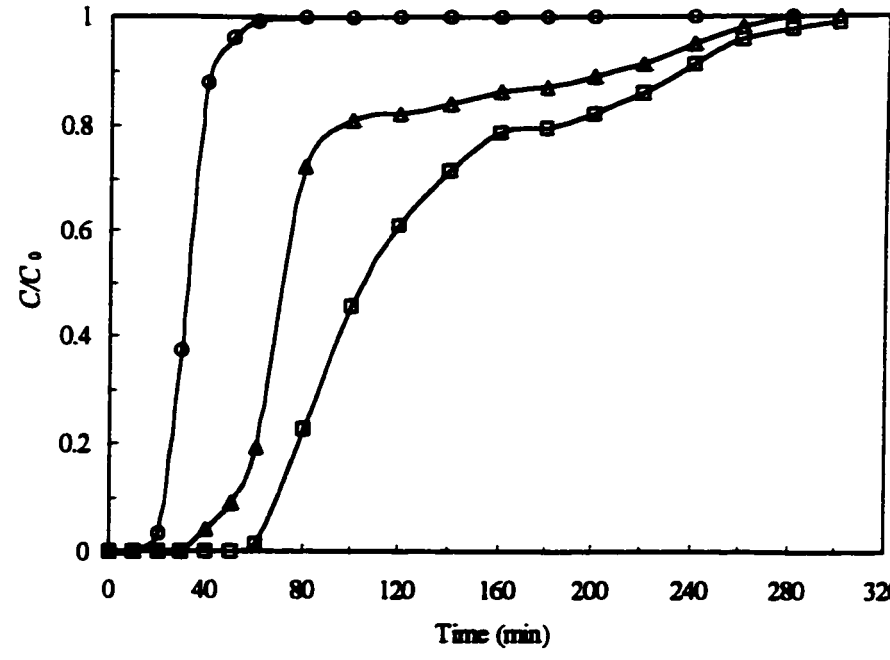

Figure 4.20: Break-through curves for the sorption of cadmium (□), copper (Δ), and nickel (O) by moss column. Average flow rate: 8.2 mL/min; influent metal concentration: 100 ppm.

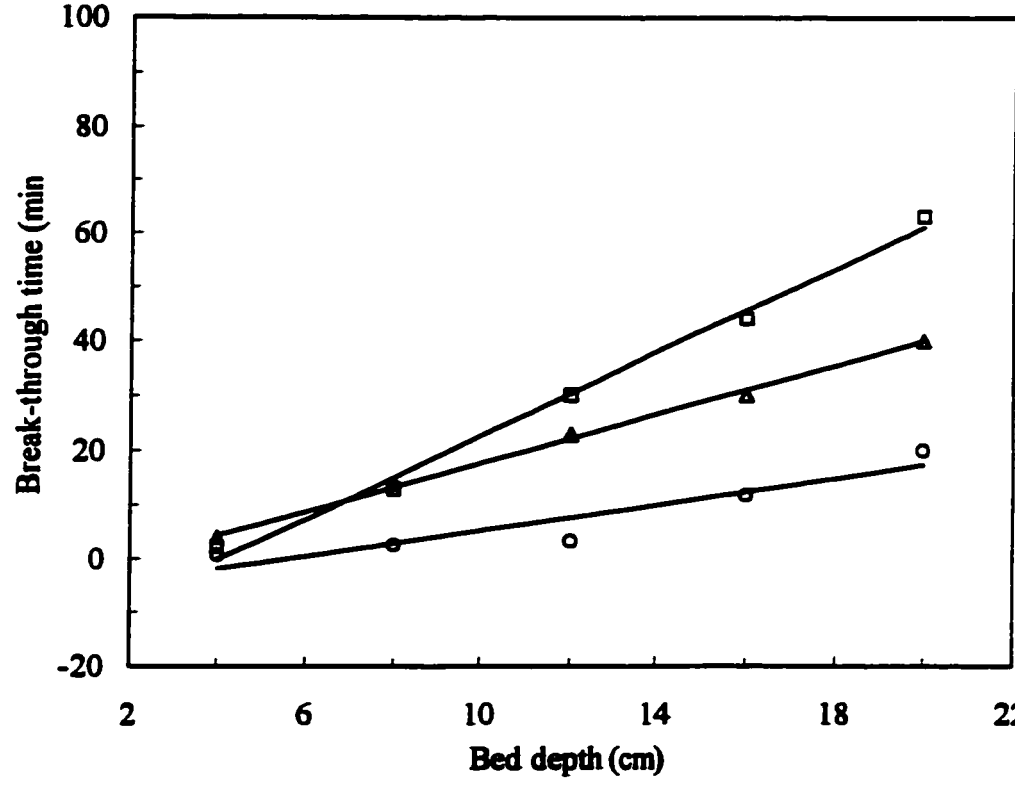

Figure 4.21: Relationship between bed depth and bed breakthrough time for cadmium (□), copper (Δ) and nickel (O). Symbols are experimental and lines represent the fitting to Eq. (4.24).

To conclude, it may be said that the inexpensive moss material, which has been shown to sorb different metal cations in the batch experiments, can be used efficiently in packed columns to make a continuous adsorption process. The results obtained indicate the high performance of the moss column process featuring short contact time, short critical bed depth and good sorptive capacity of the moss. The key process parameters derived serve as a basis for the sorption process evaluation and design, offering the possibility of an effective and relatively accurate sorption process scale-up. The results showed that the desorption of copper from the moss was influenced by the volume and concentration of the desorbent and a solution of 0.025 M HCl would be the most suitable for this process. The regeneration experiments have proved to be quite encouraging, further enhancing the economics of the process.

Chapter 5

Adsorption of Metals by Pine Bark

5.1 Introduction

Other forestry and agricultural materials, in addition to those already discussed, could also be used for the removal of heavy metals. 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+} (Kumar and Dara, 1980).

The objective of this chapter is to study the possibility of the utilization of pine bark for the adsorption of cadmium and other metals. The effect of bark and metal concentrations, pH, temperature, particle size of sorbent, and soft ions concentration on the removal of cadmium from its aqueous solutions was considered. The recovery of this metal from bark was also considered.

Pine bark was selected in this work because it has not been used before for metal removal. Although some other types of bark were tested by some researchers, the tests were very limited and did not consider the mechanism of the sorption process and the influence of various parameters on the metal uptake. Moreover, pine bark is widely available and can be obtained as a waste from the pulp and paper industry. In many cases, this waste is simply burned, and so it is advisable to find another utilization of this waste material. If the material is suitable for metal adsorption it would also be necessary to consider the ability of its regeneration.

5.2 Adsorption by Different Parts of Pine

Different constituents of pine, namely bark, needles and cones, were tested for their ability to sorb HMs. When they were tested for their ability to sorb Cd^{2+} ions from aqueous solution, it was noted that these materials were comparable to other biosorbents when the tests were carried out at similar conditions (Table 5.1); *Accacia* bark (Kumar and Dara, 1980), Irish peat (Azab and Peterson, 1989) and tea leaves (Tee and Khan, 1988) removed about 91% of Cd^{2+}, 88% of Cd^{2+} and 65% of Cd^{2+} from the solution, respectively. Since Cd^{2+} uptake by pine bark is higher than that of cones and needles (Table 5.1), bark was used in the further tests in this study for the sorption of this metal ion. In addition, pine bark is more abundant (as an industrial waste material) than the other two pine materials.

Table 5.1: Adsorption of cadmium by different parts of pine. Initial cadmium concentration: 100 ppm; sorbent concentration: 9.2 mg/mL; pH 5.0; sorption time: 60 min

Adsorbent	Cd^{2+} uptake (mmol/g)	Amount removal (%)
Pine bark	0.082 ± 0.0046	84.7
Pine cones	0.067 ± 0.0042	69.0
Pine needles	0.063 ± 0.0036	65.3

5.3 Dynamics of Cadmium Uptake

The dynamics of Cd^{2+} uptake was studied in batch experiments using a 9.2 mg/mL bark suspension and an initial Cd^{2+} concentration of 1000 ppm. The results (Figure 5.1) showed that the uptake occurred in two stages. In the beginning, a steep increase in the rate of the uptake was noted. This is probably due to the rapid attachment of Cd^{2+} ions to the surface of the bark by physical adsorption. A slower rate of uptake then followed, indicating the existence of an intrapore diffusion mechanism, however, this did not appear to be the rate controlling step. This was concluded from the

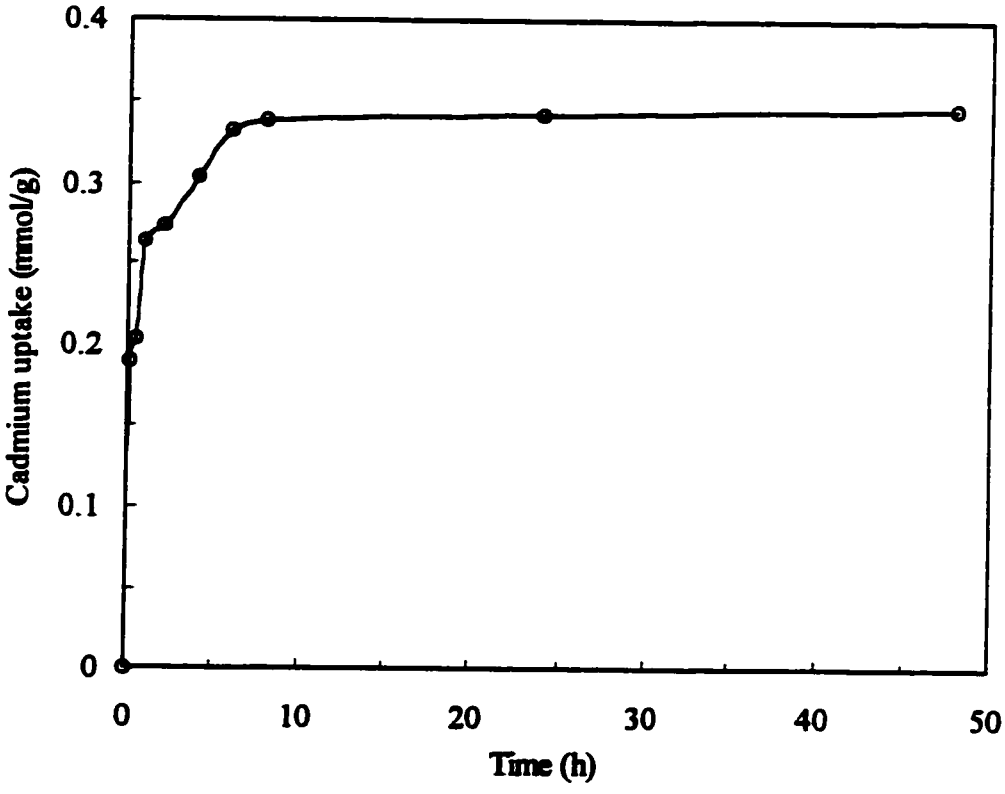

Figure 5.1: Dynamics of cadmium uptake by pine bark. Initial Cd^{2+} concentration: 8.89 mM; bark concentration: 9.2 (mg/mL); initial pH 5.0.

plot of the uptake rate versus square root of the time (data not shown) which did not pass through the origin (Weber and Morris, 1963). During this stage, cadmium ions may complex with the functional groups of the sorbent, possibly due to chemisorption. Similar dynamic curves were also obtained for the sorption of Hg^{2+} by *Hardwickia binata* bark (Deshkar *et al.*, 1990) and for the uptake of Cd^{2+} by beech leaves (Salim *et al.*, 1992).

Generally, these results indicate that a large amount of cadmium was sorbed by the bark and that the rate of uptake of this metal was fast. Although sorption equilibrium was attained after 6 hours (Figure 5.1), all subsequent experiments with this sorbent were carried out for 24 hours.

5.4 Effect of Bark and Cadmium Concentrations

To investigate the effects of the Cd^{2+} and bark concentrations on the uptake of this metal, the process was carried out with initial Cd^{2+} concentrations between 20-1000 ppm and bark concentrations between 1.15-9.2 mg/mL. At equilibrium (24 hours contact time), the Cd^{2+} concentration in each system was measured and the isotherms as functions of bark concentration were displayed in Figure 5.2. It can be noted that the Cd^{2+} uptake per unit weight of the sorbent increased with a decrease in the bark concentration, while the total amount of the metal removed increased with an increase in the adsorbent concentration. Such a trend is similar to the results presented in the previous Chapters (Chapters 1-3; Part II). The results in Figure 5.2 and those in Figures 5.3, 5.4 and 5.5 also indicate that the Cd^{2+} uptake was increased with an increase in its concentration. These results can be represented by the linearized Freundlich model (Eq. (4.8), Chapter 4; Part I). The linear plots of $\ln q_e$ vs $\ln C_e$ for different sorbent concentrations (Figure 5.2) suggests the formation of a monolayer coverage of the outer surface of the bark by Cd^{2+} ions. The values of k_F and $1/n$ for various bark concentrations were evaluated from the intercepts and the slopes of the plots, respectively (Table 5.2). The values of R^2, which is a measure of goodness-of-fit, shown in Table 5.2 confirm that the experimental data at the various bark concentrations are well represented by the Freundlich model. The slopes of all the plots ($1/n$) indicate that the intensity of sorption was virtually the same regardless of the sorbent concentration, while the sorbent capacity (k_F values) increased as the sorbent concentration decreased. The reason for this could be, as mentioned previously, the large number of cadmium ions per binding site at low concentrations of bark.

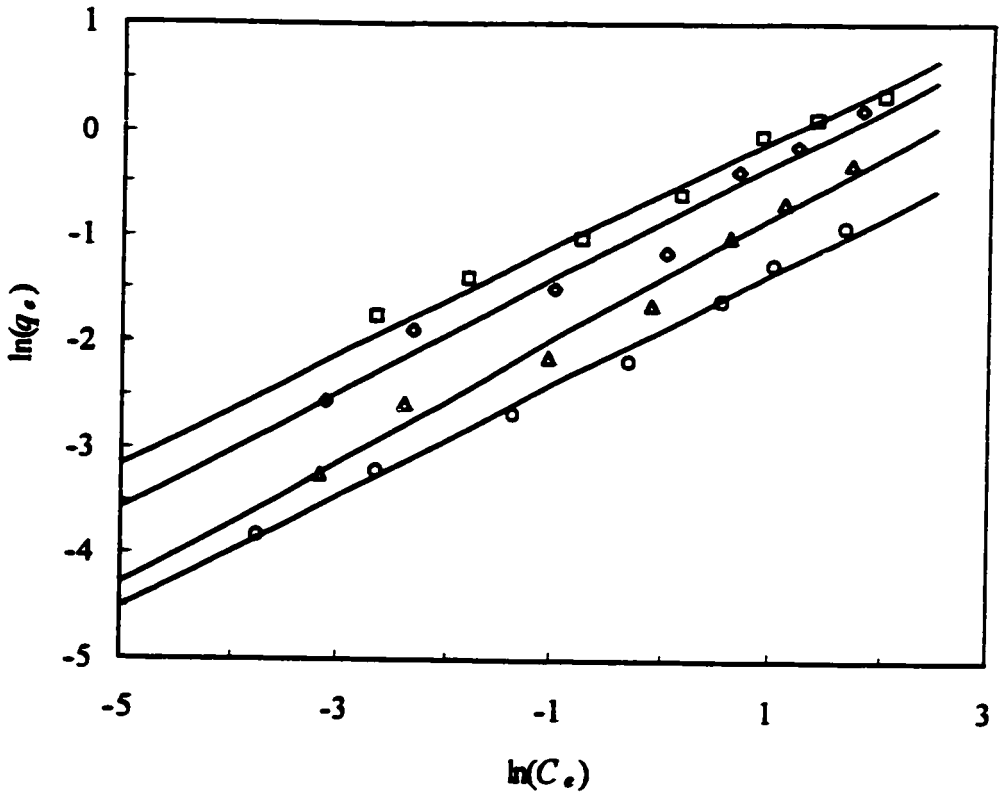

Figure 5.2: Relationship between the equilibrium cadmium concentration and its uptake (molar basis) at various bark concentrations at pH 5.0. Symbols are experimental and lines are fitting data using the Freundlich isotherm model. Bark concentration (mg/mL): □-1.15, ◊-2.3, Δ-4.6, O-9.2.

Table 5.2: Freundlich constants at various bark concentrations at pH 5.0

Bark concentration (mg/mL)	k_F	$1/n$	R^2
1.15	0.551	0.51	0.987
2.30	0.424	0.54	0.987
4.60	0.249	0.58	0.990
9.20	0.154	0.53	0.991

5.5 Effect of pH

As previously discussed, many researchers have demonstrated that the binding of heavy metal ions onto biomaterials is pH dependent. In this study, it was also shown, as in the case of the other biosorbents in this study (*A. carbonarius*, CM and moss), that pH plays a significant role in the metal sorption process. The effect of pH on the sorption of Cd^{2+} was studied in systems with initial Cd^{2+} concentrations in the range from 20-1000 ppm. At equilibrium, the isotherms were obtained as a

function of pH. A significant increase in the Cd^{2+} uptake per unit weight of bark was noticed as the pH increased from 4 to 5.5 (Figure 5.3). This can also be attributed to the increase of the number of negatively charge groups at the surface of bark, as mentioned in the previous chapters. The Freundlich constants, k_F and $1/n$, calculated using these data (Table 5.3) showed that the sorption capacity of this biosorbent increased with the pH of the aqueous metal solutions.

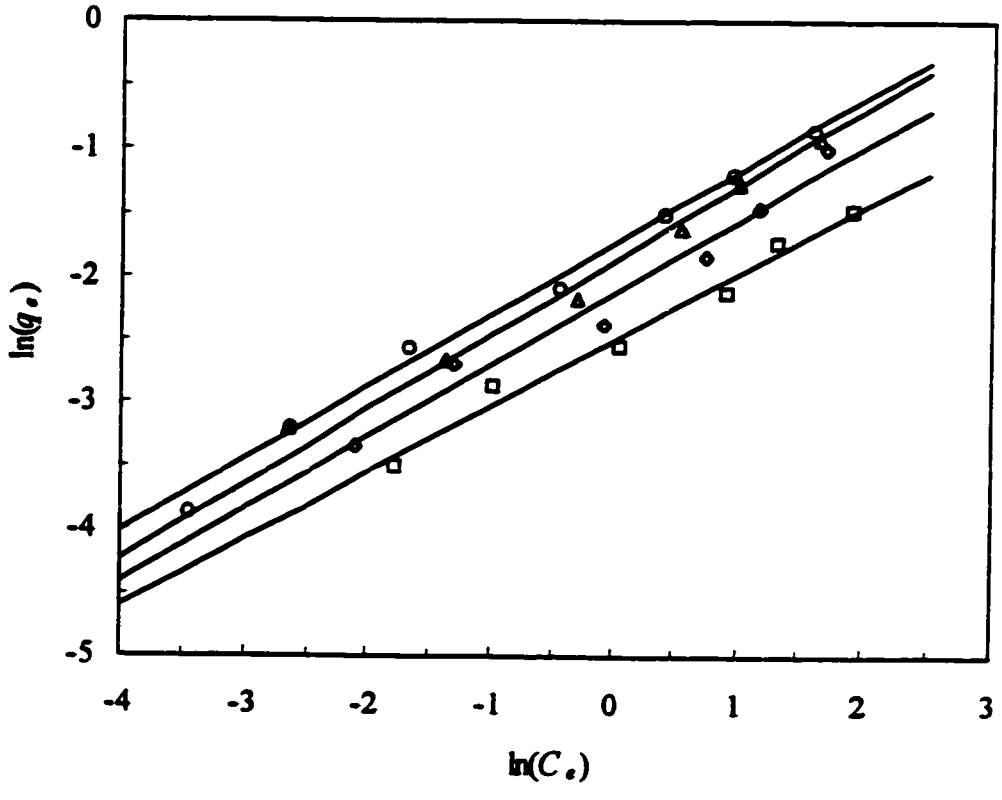

Figure 5.3: Relationship between the equilibrium cadmium concentration and its uptake at various pHs. Symbols are experimental and solid lines are fitting data using the Freundlich isotherm model. Bark concentration: 9.2 mg/mL. pH: □-4.0, ◊-4.5, Δ-5.0, O-5.5.

Table 5.3: Freundlich constants at various pH values and 9.2 mg/mL bark concentration

pH	k_F	$1/n$	R^2
4.0	0.082	0.53	0.982
4.5	0.118	0.57	0.968
5.0	0.153	0.59	0.989
5.5	0.176	0.57	0.985

5.6 Effect of Temperature

Temperature can influence adsorption of heavy metals. The results found in the literature, as well as in this work, showed that there is no general trend regarding the effect of temperature on sorption of heavy metals by bio-materials. When the effect of temperature on the sorption of Cu^{2+} by moss was tested, it was found that the process was endothermic and that greater sorption was achieved at higher temperatures. However, in certain cases, as in the case of the biosorbent *A. carbonarius* (Chapter 1; Part II), the sorption at high temperatures was lower than that at low temperatures. The endothermic nature of the adsorption process was also shown by Singh *et al.* (1993) who reported that the adsorption of Pb^{2+} by tea leaves increased as the temperature increased from 20°C up to 70°C. However, Ferro-Garcia *et al.* (1988) reported that the sorption of Cd^{2+} by almond shells, olive stones and peach stones were exothermic processes, since the isotherms were lower at 40°C than at 20°C. When the effect of temperature on the sorption of Cd^{2+} by pine bark was tested, the isotherm curves showed (Figure 5.4) that the process, in this case, was endothermic. The intrapore diffusion rate

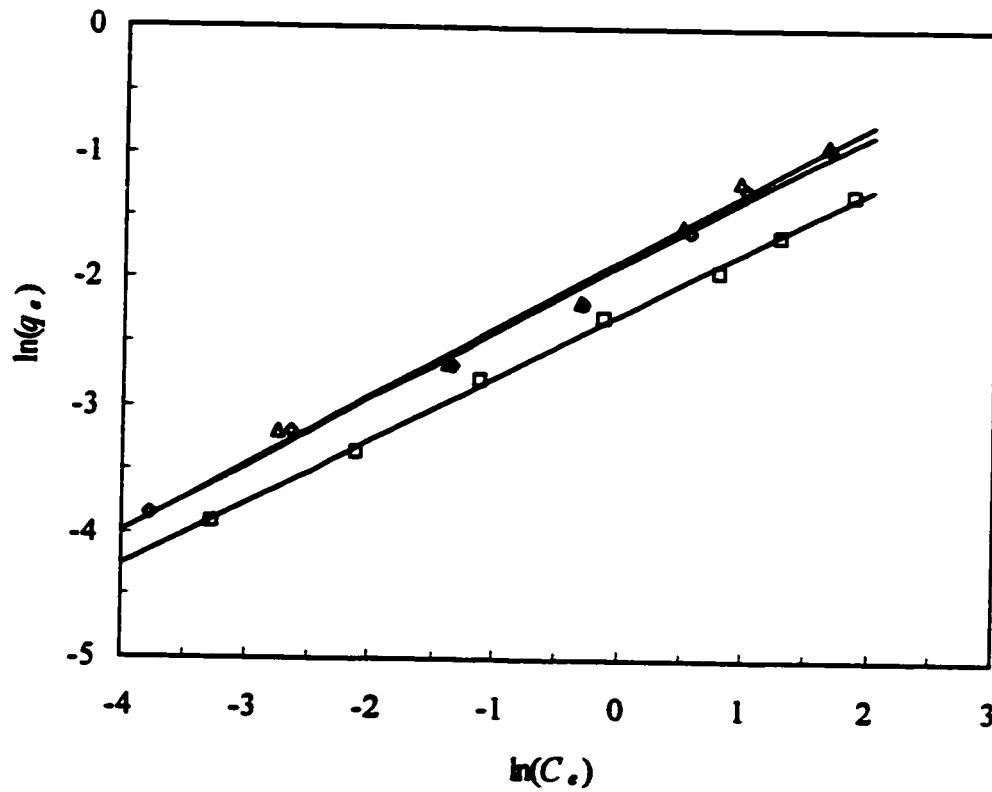

Figure 5.4: Relationship between the equilibrium cadmium concentration and its uptake at various temperatures. Symbols are experimental and solid lines are fitting data using the Freundlich isotherm model. Bark concentration: 9.2 mg/mL; initial pH 5.0. Temperature (°C): □-9, ◊-25, Δ-37.

increased with the increase in temperature, because of the opening of the pores, and so, the total uptake increased (Figure 5.4). The typical plots of Freundlich isotherms and Freundlich constants for the three temperatures tested in this study are shown in Figure 5.4 and Table 5.4, respectively. The results show (Table 5.4) that the sorption capacity, as well as the sorption intensity, are almost the same for 25°C and 37°C.

Table 5.4: Freundlich constants at various temperatures

Temperature (°C)	k_F	$1/n$	R^2
9	0.104	0.51	0.994
25	0.153	0.53	0.978
37	0.1604	0.54	0.982

5.7 Effect of Particle Size of the Bark

It was already mentioned (Section 2.5; Part II) that particle size of the sorbent is an important parameter in the design of adsorption operations. To investigate the effect of particle size on the sorption process, bark was screened through sieves and the following size distribution fractions were obtained: one smaller than 0.075 mm, four fractions with sizes between 0.075 and 0.71 mm, and one fraction with a particle size bigger than 0.71 mm. Each of these fractions were exposed to a series of Cd^{2+} solutions in the concentration range between 20-1000 ppm. At equilibrium, the isotherms were obtained as a function of the particle size (Figure 5.5). The results indicated that the particle size influenced the sorption process, due to an increase in the surface area per unit weight of the sorbent with a decrease in its particle size. The linear Freundlich isotherm model was fitted to the experimental data (Figure 5.5) and the constants for each particle size are shown in Table 5.5. Although this model showed some deviation, it still represented the experimental data better than other equilibrium models (not shown), such as the Langmuir model. A slight decrease in the $1/n$ values with the decrease of the particle size indicated a decrease of the sorption intensity. Jha *et al.* (1989) also obtained an increase in the metal uptake with a decrease in the particle size for the sorption of Cd^{2+} by chitosan but, in their case, the sorption intensity increased with a decrease in the particle size.

The adsorption of Cd^{2+} by pine bark can be considered favorable because the $1/n$ values obtained in this work (Table 5.2-5.5) were between 0.1 and 1 (McKay *et al.*, 1982).

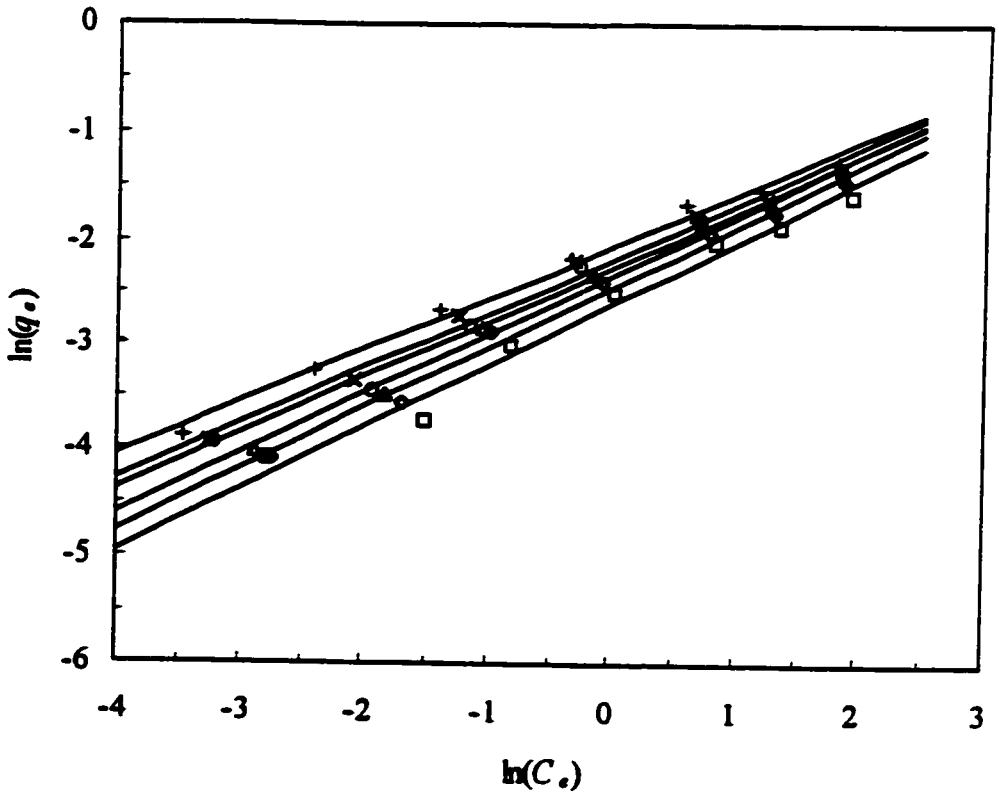

Figure 5.5 Relationship between the equilibrium cadmium concentration and its uptake by pine bark of various particle sizes. Bark concentration: 9.2 mg/mL; pH 5.0; Particle size (mm); (+) smaller than 0.075, (×) between 0.075 and 0.14, (O) between 0.14 and 0.212, (Δ) between 0.212 and 0.425, (◊) between 0.425 and 0.71, (□) bigger than 0.71.

Table 5.5: Freundlich constants at different particle sizes

Particle size (mm)	k_F	$1/n$	R^2
Smaller than 0.075	0.128	0.49	0.980
0.075-0.140	0.114	0.52	0.988
0.140-0.212	0.105	0.53	0.988
0.212-0.425	0.097	0.57	0.987
0.425-0.710	0.087	0.58	0.988
Bigger than 0.710	0.074	0.59	0.991

5.8 Reutilization of Bark without Regeneration

The recovery of metal from the sorbent after each sorption stage could result in the distortion of the biomaterial by the acid used for desorption. Regeneration of the sorbent might not be economical if the desorbent is inexpensive or if the regeneration process requires large amounts of energy and

materials. Therefore, it is advantageous to utilize the sorbent as many times as possible before its regeneration. Bark was tested for its ability to be reutilized without regeneration. Two series of tests were performed using initial Cd^{2+} concentrations of 100 ppm (0.89 mM) and 1000 ppm (8.89), respectively, in a 9.2 mg/mL bark suspension. Another two series of tests were also performed using Cu^{2+} of 100 ppm (1.57 mM) initial concentration and Ni^{2+} of 100 ppm (1.70 mM). After 24 h of sorption, the bark was separated and transferred to another metal solution having the same initial metal concentration. The process was repeated 5 times. The results (Figures 5.6 and 5.7) showed that each time the bark was able to sorb additional metal ions. As was expected, the largest amount of Cd^{2+}, Cu^{2+} and Ni^{2+} (Figures 5.6A, 5.7A and 5.7B, respectively) were removed from the solution with fresh bark. With each of the subsequent reutilizations, the bark performance decreased (Figure 5.6 and 5.7). Comparing the results of Figure 5.6A and 5.6B, it is seen that bark with a high initial Cd^{2+} concentration (8.89 mM) can not be re-utilized as much as that with the lower Cd^{+2} concentration. This is because the bark was almost completely saturated after the second re-utilization. Bearing in mind that the maximum load of bark is about 0.49 mmol of Cd^{2+} per g of bark (Figure 5.6B), it can be concluded that the adsorbent, if each time suspended in an initial Cd^{2+} concentration of 0.89 mM, could be used even more than 5 times without regeneration. These results could suggest the possibility of applying slightly saturated bark to fresh metal solutions, while the metal solutions that still contain residual metal ions could be treated with a fresh bark. These results also indicate that the amount (weight basis) of cadmium removal by bark is higher than that of copper or nickel.

5.9 Desorption

The desorption of sorbed metal ions from bark was considered in this work. The bark, which contained on the average 0.302 mmol/g of Cd^{2+}, was treated with HCl of different molarity to desorb the metal. Higher molarities of HCl were more efficient in the release of Cd^{2+} ions (Figure 5.8), however, a complete desorption of Cd^{2+} ions was not obtained even with 500 mM HCl. This might be due to Cd^{2+} ions that could have been intrapped into the pores and, therefore, difficult to release.

Desorption has also been studied by treating bark samples containing various initial Cd^{2+} concentrations with 10 mM HCl. Desorption from samples with 0.022 and 0.053 mmol of Cd^{2+} per g of bark, resulted in a 100% and 97% release of Cd^{2+} ions from the bark, respectively (Figure 5.9). The greater extent of desorption in these two cases could mean that the surface adsorption was predominant in the case of low metal ion concentrations in this sorbent. The desorption efficiency decreased to about 70% when the metal concentration was above 0.24 mmol of Cd^{2+} per g of bark.

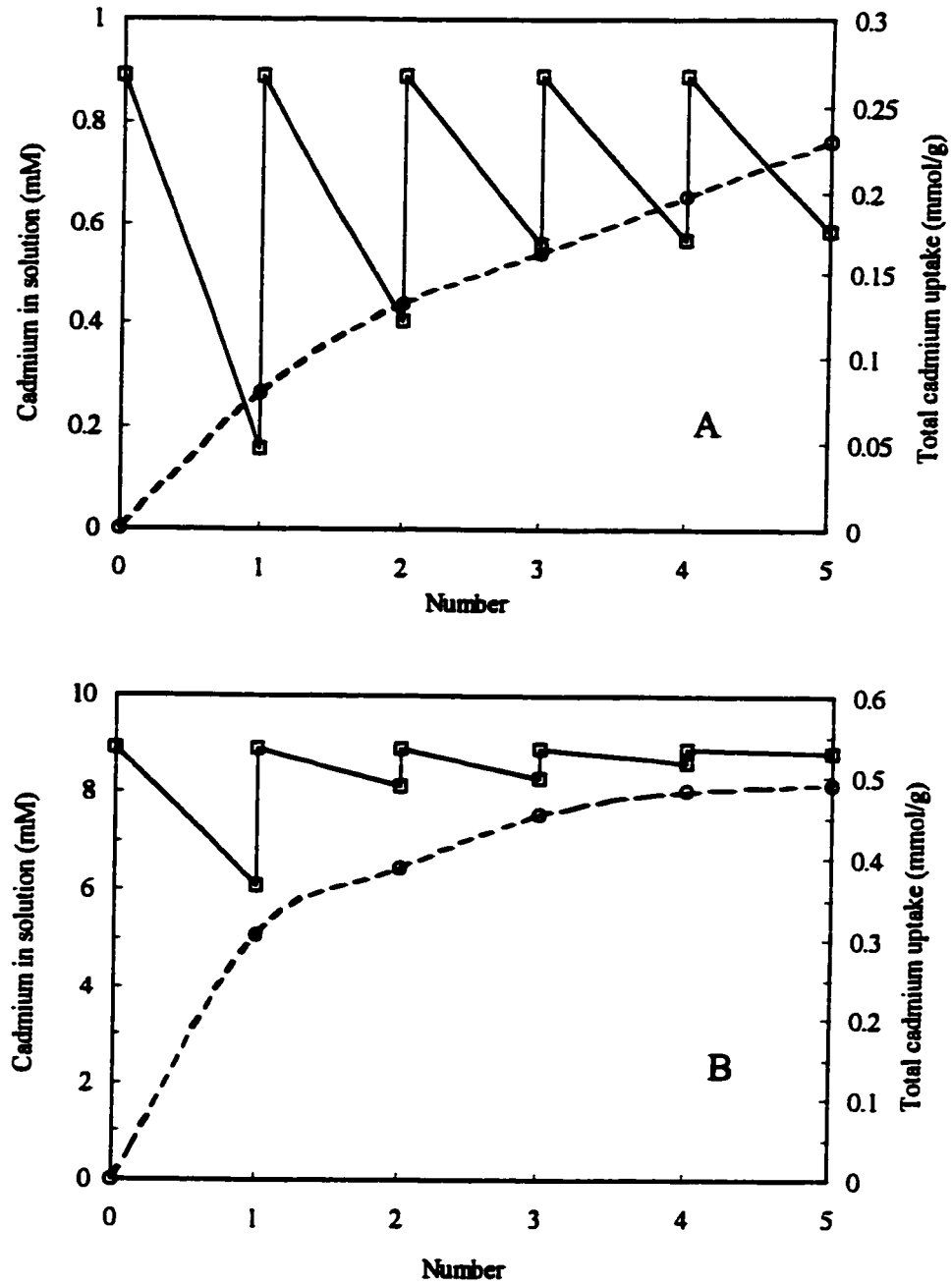

Figure 5.6: Re-utilization of pine bark for Cd^{2+} adsorption. Bark concentration: 9.2 mg/mL; initial Cd^{2+} concentration: 100 ppm (A) and 1000 ppm (B): pH 5.0. Solid lines represent the amount of Cd^{2+} in solution and interrupted lines represent the uptake curves.

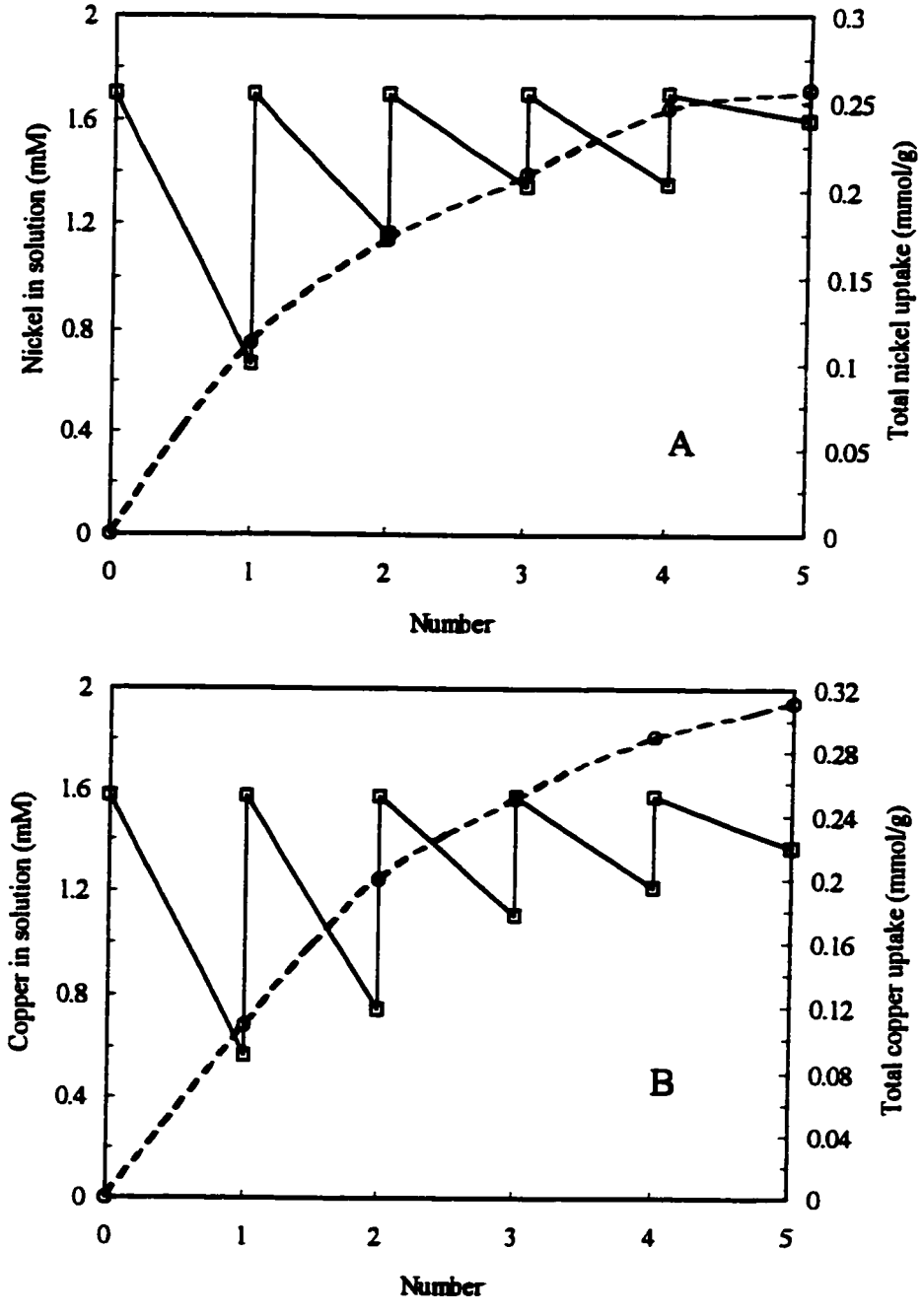

Figure 5.7: Re-utilization of pine bark for Cu^{2+} (A) and Ni^{2+} (B) adsorption. Bark concentration: 9.2 mg/mL; initial metal concentration: 100 ppm: pH 5.0. Solid lines represent the amount of metal in solution and interrupted lines represent the uptake curves.

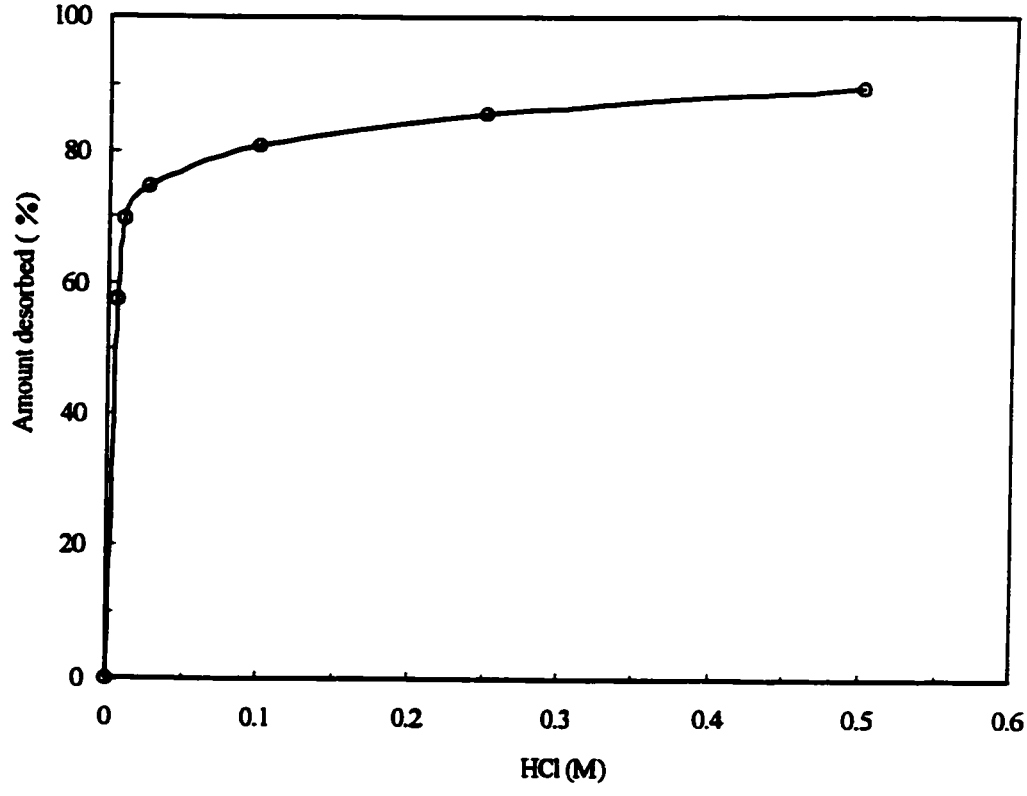

Figure 5.8: Effect of the HCl concentration on the desorption of Cd^{2+} from bark. Amount of Cd^{2+} on bark: 0.302 mmol/g. The volume of the desorbent and the bark concentration were 10 mL and 9.2 mg/mL, respectively.

5.10 Reuse of the Bark after Regeneration

The reuse of bark after regeneration with HCl was investigated. In these tests, Cd^{2+}, which was exposed to a concentration of 100 ppm (0.889 mM) of this metal, was desorbed from the bark with 10 mM HCl solution. The bark was separated by filtration, washed several times with deionized water until the pH of the filtrate corresponded to that of the water, and then reused for further Cd^{2+} adsorption. This process was repeated 5 times. The results showed (Figure 5.10) that with each subsequent reutilization of the bark, the amount of the newly sorbed Cd^{2+} decreased, but the total amount of this metal adsorbed , i.e. the sum of the newly sorbed and that retained after desorption increased. It was logical that the amount of the newly adsorbed Cd^{2+} was decreasing because some adsorption sites were already occupied by previously adsorbed ions of this metal which resisted desorption treatment.

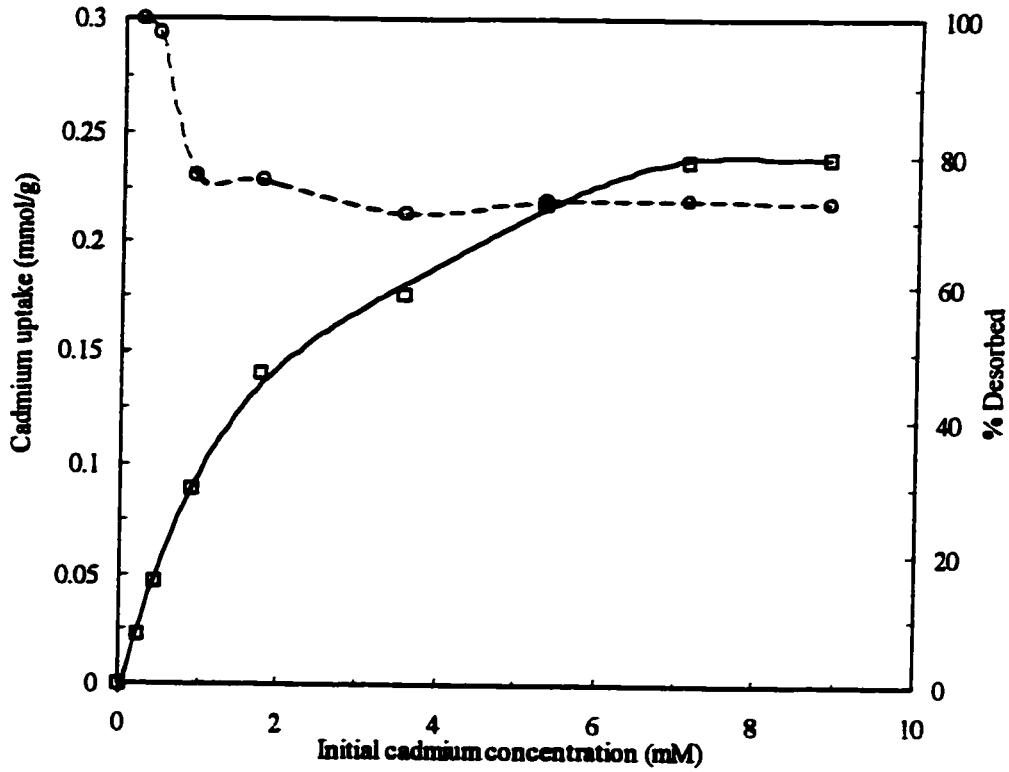

Figure 5.9: Adsorption/desorption of Cd^{2+} by/from pine bark using different initial Cd^{2+} concentrations, and 0.01 M HCl as desorbent. Thick line: the Cd^{2+} uptake vs its initial concentration. Thin line: the percent of Cd^{2+} desorbed from the bark.

5.11 Bark to Metal Concentrations Ratio

The response of pine bark with the same ratio of sorbent to metal concentrations at various levels of metal concentrations was investigated. To study this response, three different ratios of bark to Cd^{2+} concentrations were considered. Three various concentration levels were selected for each of the three ratios and the adsorption was followed in all nine systems separately. The results are shown in Table 5.6. It can be said that, within the experimental errors, the utilization of the same ratio of bark to metal concentrations, regardless of the initial metal levels, resulted in approximately the same amount of Cd^{2+} adsorbed per unit weight of bark. The results also indicated that, for lower bark to Cd^{2+} ratios, more Cd^{2+} was adsorbed per unit weight of bark than for higher ones (Table 5.6). Sorbent to metal ratio also determined the level of metal removal. With an increase in the sorbent to metal ratio, the total amount of metal removal increases. These results are similar to the previous results when the various ratios of CM to Zn^{2+} concentration (Section 2.4; Part II) were used.

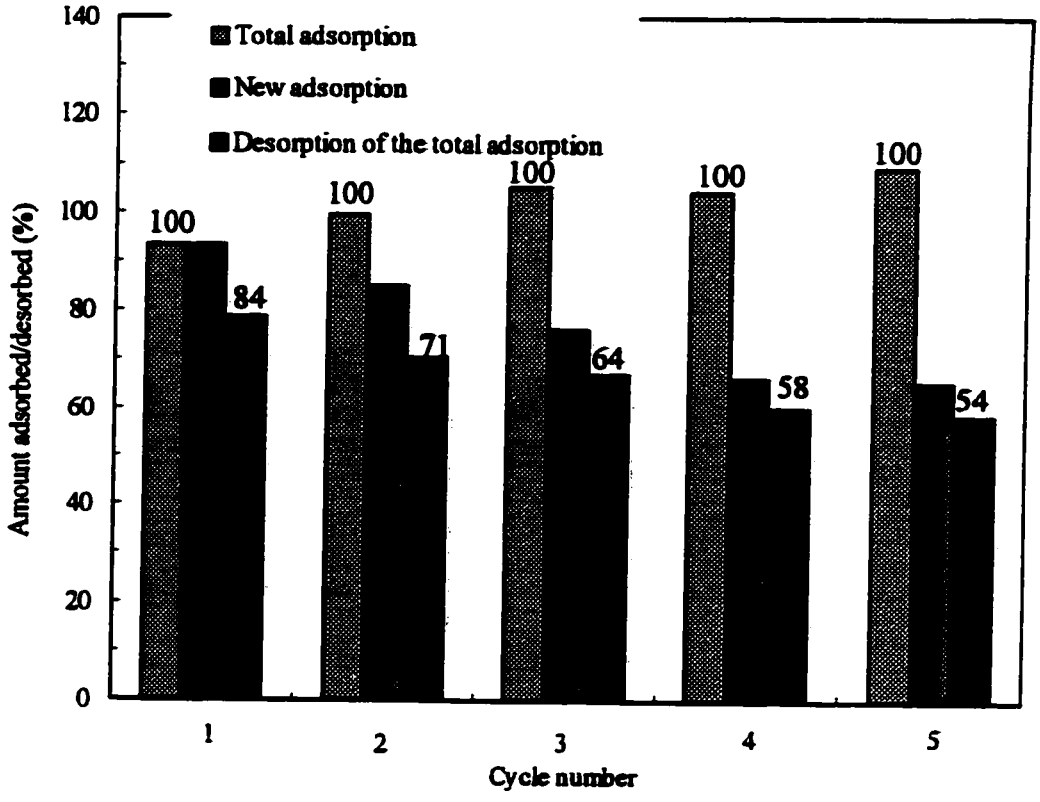

Figure 5.10: Reuse of pine bark for adsorption/desorption of Cd^{2+}. Initial Cd^{2+} concentration: 0.889 mM; bark concentration: 9.2 mg/mL; pH 5.0; 10 mL of 0.01 M HCl for desorption.

Table 5.6: Adsorption of Cd^{2+} by bark in systems with various bark to Cd^{2+} ratio

Bark : Cd^{2+} ratio	Initial Cd^{2+} concentration (mM)	Final Cd^{2+} concentration (mM)	Cd^{2+} uptake (mmol/g)	Cd^{2+} uptake* (mmol/mg)	Amount of Cd^{2+} removed (%)
0.092	0.222	0.044	0.077	0.072 (0.0043)	80.0
	0.445	0.121	0.070		73.0
	0.889	0.252	0.069		71.6
0.046	0.445	0.099	0.149	0.126 (0.0196)	77.6
	0.889	0.353	0.116		60.3
	1.78	0.734	0.114		58.7
0.023	0.889	0.375	0.224	0.205 (0.0166)	57.8
	1.78	0.894	0.193		49.7
	3.56	1.734	0.198		51.3

* Average values for three tests within the same bark : Cd^{2+} ratio. Standard deviation shown in brackets

5.12 Effect of Soft and Hard Ions

Soft and hard ions are among those agents which interfere with the sorption of metal ions by biomaterials. There are some data concerning the influence of these ions on the sorption of heavy metals using other type of materials. For example, Norris and Kelly (1977) reported that the initial rate of Cd^{2+} uptake by the cells of *Saccharomyces cerevisiae* was strongly depressed by Ca^{2+} ions when the initial concentrations of Cd^{2+} and Ca^{2+} in the solution were 22.5 and 8 ppm, respectively. Muzzarelli and Tubertini (1969) showed that K^+, Na^+ and Mg^{2+} did not interfere with the removal of some metal ions from solutions using chitin. The results of Jha *et al.* (1989) indicated that in the presence of 100 ppm of Ca^{2+} ions and 5 ppm of Cd^{2+} ions, the initial rate of Cd^{2+} removal by chitosan was decreased. However, there is not much data in the literature related to the effect of these ions on the sorption of heavy metals by plant materials. Therefore, it was decided to study the effects of soft and hard ions on the sorption of Cd^{2+} by pine bark. The results of this work showed (Figure 5.11) that the presence of 100 ppm of Na^+, K^+, Mg^{2+} and Ca^{2+}, individually, in a 100 ppm (0.889 mM) Cd^{2+} solution containing

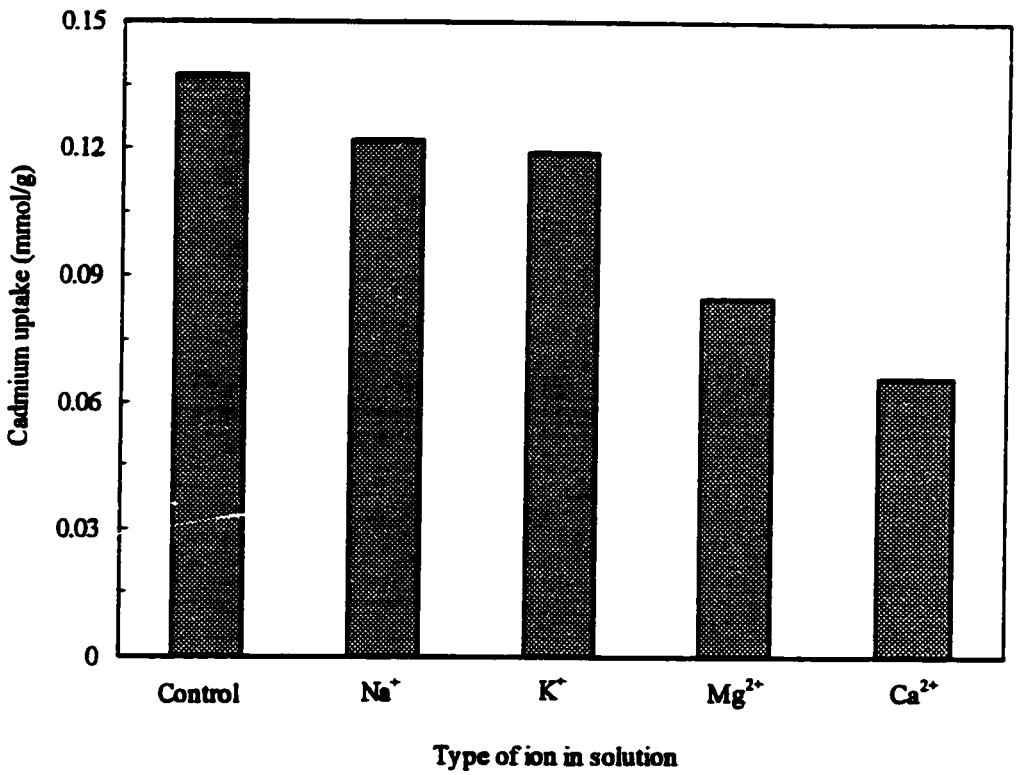

Figure 5.11: Effect of soft and hard ions on Cd^{2+} uptake by pine bark. Initial Cd^{2+} and soft ions concentrations: 100 ppm of each; bark concentration: 9.2 mg/mL: pH 5.0.

9.2 mg/mL of bark, partially depressed the uptake of Cd^{2+} by this sorbent. Magnesium and calcium exhibited a stronger inhibition of Cd^{2+} sorption than Na^{+} and K^{+}. This is in agreement with already published data for other biosorbents which showed that the inhibition was stronger with higher valent ions.

The concentration of soft and hard ions plays an important role in this respect. Deshkar *et al.* (1990) reported a negative effect of the soft and hard ions on the sorption of Hg^{2+} by *Hardwickia binata* bark when the concentrations of Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} were from 40 to 200 ppm. However, there are also data that showed an increase in Hg^{2+} sorption by activated carbon with an increase of Ca^{2+} concentration (Thiem *et. al.*, 1976). In this study, it was noticed that lower concentrations (10 ppm) of Na^{+} and Mg^{2+} resulted in an increase of Cd^{2+} uptake by bark (Figure 5.12). With a further increase in the concentration of these soft ions, between 40 ppm and 400 ppm, they started inhibiting Cd^{2+} adsorption. The effects of Mg^{2+} and Ca^{2+} on this process were much more pronounced than those of K^{+} and Na^{+}. The latter is consistent with the results of Deshkar *et al.* (1990).

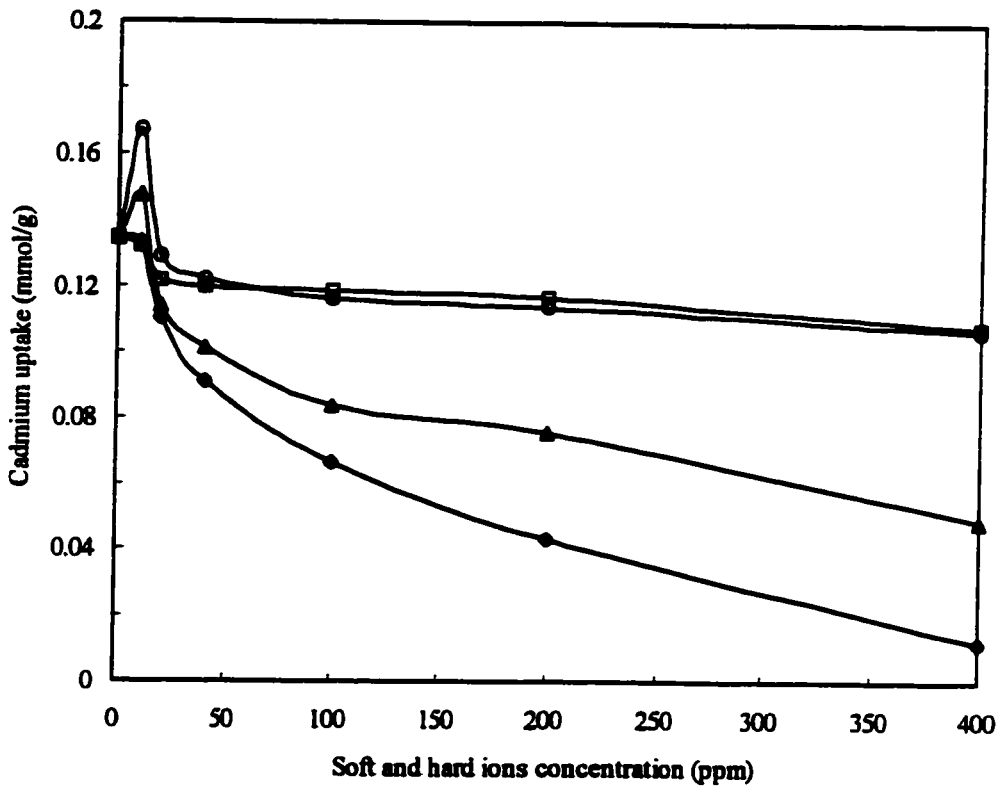

Figure 5.12: Effect of soft and hard ions concentration on Cd^{2+} uptake. Initial Cd^{2+} concentration: 0.889 mM; bark concentration: 9.2 mg/mL: pH 5.0. Soft metal ions: □-Na^{+}, O-K^{+}, Δ-Mg^{2+}, ◊-Ca^{2+}.

5.13 Pre-loading of Bark with Other Metal Ions

It is of interest to determine the performance of pine bark for its sorption capacity for heavy metals when it is pre-loaded with other metal ions. The effect of pre-loading was studied by Rowly *et al.* (1984) for the sorption of Cd^{2+}, Pb^{2+} and Hg^{2+} by waste tire rubber pre-loaded with Zn^{2+} ions. The authors found a marked increase in capacity for Cd^{2+}, a lesser increase in capacity for Hg^{2+} and no change in capacity for Pb^{2+} by Zn^{2+} pre-loaded rubber compared to the capacity for these metals by untreated rubber. This increase was explained due to the replacement of Zn^{2+} by Cd^{2+} and Hg^{2+} ions, since some Zn^{2+} ions were released from the sorbents. To examine the response of the bark, preloaded with a metal ion, for the sorption of another metal ion, the bark was pre-loaded, individually, with Cu^{2+}, Ni^{2+} or Cd^{2+} using sorbent and metal concentrations of 9.2 mg/mL and 100 ppm, respectively, at an initial pH of 4. Sorption was carried out until equilibrium (24 h). After that, the sorbent was separated and then transferred to a solution containing another metal ion. It was shown that, for example, the capacity of bark for Ni^{2+} was higher by about 41% and 37% when it was pre-loaded with Cu^{2+} or Cd^{2+} ions, respectively (Table 5.7). It was also observed that some pre-loaded Ni^{2+} and Cd^{2+} ions were released from the bark during the second stage of adsorption (Table 5.7), while almost no pre-loaded Cu^{2+} was released from the sorbent. This indicates a stronger binding of Cu^{2+} onto the bark than that of Ni^{2+} and Cd^{2+}. This might also indicate the possibility of formation of complexes between Cu^{2+} and Ni^{2+} and Cu^{2+} and Cd^{2+} on pine bark.

Table 5.7: Metal adsorption using pine bark previously loaded with other metals. Initial metal concentration: 100 ppm; bark concentration: 9.2 mg/mL; pH 4; sorption time: 24 h

Metal preloaded	Amount preloaded (mmol/g)	Metal used for second adsorption	Amount of metal uptaken (mmol/g)	Amount of preloaded metal i solution (%)
Cu^{2+}	0.090	Ni^{2+}	0.086	2.8
	0.093	Cd^{2+}	0.062	1.1
Ni^{2+}	0.065	Cu^{2+}	0.104	84.4
	0.069	Cd^{2+}	0.069	31.3
Cd^{2+}	0.068	Cu^{2+}	0.102	29.5
	0.065	Ni^{2+}	0.096	36.0

5.14 Removal of Low Concentrations of Cadmium

Bark was tested for its ability to remove the low levels of Cd^{2+} from aqueous solutions. Multiple stage adsorption process, as mentioned in Section 2.10, was performed using an initial Cd^{2+} concentration of about 0.74 ppm and 4.6 mg/mL of bark suspension. After 24 h of adsorption, bark

was separated and a Cd^{2+} concentration of 0.220 ppm was detected in the filtrate. Another 4.6 mg/mL of fresh bark was added to the filtrate and then after adsorption, bark was separated and a Cd^{2+} concentration of 0.062 ppm was measured in the new filtrate. Another adsorption cycle was carried out with fresh bark (4.6 mg/mL) and the Cd^{2+} was brought to a level of <0.008 ppm. The process was terminated because of the lowest detectable limit in the metal measurement. These results indicate that pine bark can also be considered to be effective sorbent for the removal of low levels of cadmium ions.

5.15 Other Plant Sorbents

The ability of other plant sorbents to adsorb copper ions, for their removal from their solutions and their recovery, was also tested. It is shown (Table 5.8) that the copper uptake by these sorbents is comparable to the previously discussed sorbents from this study and to those used by other researchers (Table 2.9; Part I).

Table 5.8: Adsorption of copper by several plant adsorbents. Initial copper concentration: 1.57 mM; sorbent concentration: 9.2 mg/mL; initial pH: 5.0 -5.2; sorption time: 60 min

Adsorbent	Copper uptake (mmol/g)	Copper removal (%)
Pine cones	0.147	85.7
Pine needles	0.138	80.5
Oak cobs	0.138	80.5
Oak leaves	0.126	73.5
Cider needles	0.122	71.3
Maple leaves	0.109	63.7
Birch leaves	0.104	61.0
Fir tree needles	0.104	56.5
Grass	0.082	48.9
Lilac leaves	0.078	46.0

The results concerning the effect of pine needle, pine cones, oak leaf and oak cob concentrations on copper uptake after one adsorption stage are summarized in Table 5.9. Again, more copper uptake per unit of sorbent was obtained with lower sorbent concentrations, while more copper was removed when using high sorbent concentrations. The kinetics of these results are presented in Appendix A (Figures A.12-A.15).

The effect of the initial copper concentration on its uptake by pine needles and pine cones, as well as oak cobs and oak leaves were studied using initial copper concentrations of 0.394, 0.787, and 1.57 mM. The relationship between copper uptake at equilibrium and its initial concentration, for these

sorbents, is shown in Table 5.10. It can be seen that the copper uptake also increased with an increase in its initial concentration.

Table 5.9: Effect of sorbent concentration on copper uptake. Initial copper concentration: 1.57 mM; initial pH 5.0-5.2; sorption time: 60 min

Adsorbent	Sorbent concentration (mg/mL)				
	1.15	2.3	4.6	9.2	18.4
	Copper uptake (mmol/g)				
Pine needles	1.072	0.573	0.282	0.138	0.072
Pine cones	1.116	0.480	0.275	0.147	0.075
Oak cobs	0.910	0.576	0.277	0.136	0.074
Oak leaves	1.034	0.467	0.274	0.126	0.065

Table 5.10: Effect of the initial copper concentration on copper uptake by different sorbents. Sorbent concentration: 9.2 mg/mL; initial pH 5.0; sorption time: 60 min

Adsorbent	Copper concentration (mM)		
	0.394	0.787	1.57
	Copper uptake (mmol/g)		
Pine needles	0.031	0.056	0.140
Pine cones	0.042	0.076	0.148
Oak cobs	0.027	0.067	0.137
Oak leaves	0.025	0.061	0.128

The response of these sorbents for the uptake of other metal ions, namely Zn^{2+}, Cd^{2+}, Cr^{3+}, and Ni^{2+}, is shown in Table 5.11. The selectivity order (in molar basis) of the sorbents for these metal ions was as follows:

Pine needles: $Cu^{2+} > Zn^{2+} > Cr^{3+} > Ni^{2+} > Cd^{2+}$

Pine cones: $Cu^{2+} > Cr^{3+} > Zn^{2+} > Ni^{2+} > Cd^{2+}$

Oak cobs: $Cu^{2+} > Zn^{2+} = Ni^{2+} > Cr^{3+} > Cd^{2+}$

Oak leaves: $Zn^{2+} > Cu^{2+} > Cr^{3+} > Ni^{2+} > Cd^{2+}$

Table 5.11: Adsorption of different metals by various sorbents. Sorbent concentration: 9.2 mg/mL; initial pH 5.0; initial metal concentration: 100 ppm; sorption time: 60 min

Adsorbent	Metal				
	Cu^{2+}	Zn^{2+}	Cd^{2+}	Cr^{3+}	Ni^{2+}
	Metal uptake (mmol/g)				
Pine needles	0.136	0.125	0.063	0.091	0.069
Pine cones	0.148	0.134	0.067	0.135	0.087
Oak cobs	0.136	0.126	0.071	0.112	0.126
Oak leaves	0.126	0.135	0.073	0.122	0.121

These results show that these materials can also be considered as good sorbents for Zn^{2+}, Cu^{2+}, Cr^{3+}, Ni^{2+} or Cd^{2+}. Sorbent and metal concentrations positively influence the metal sorption by these materials.

In conclusion, pine bark is a better sorbent for Cd^{2+} ions than pine cones or needles. Decreasing the bark concentration and increasing the Cd^{2+} concentration resulted in an increase in cadmium uptake per unit weight of bark. An increase in pH significantly increased the cadmium uptake per unit weight of bark over the range of pH 4 to 5.5. The uptake of cadmium ions was enhanced by decreasing the pine bark particle sizes. The Freundlich model fitted the equilibrium data reasonably well, and the obtained *1/n* values indicated favorable adsorption. Bark can be re-utilized several times for further adsorption processes without regeneration. The desorption of Cd^{2+} was more efficient with higher molarity HCl than with lower molarity solutions. A complete release of Cd^{2+} ions could not be achieved at high concentrations of this metal on the bark. The reuse of the bark after regeneration resulted in a decrease in the absolute value of the newly adsorbed Cd^{2+} ions compared to the fresh bark, however, the total uptake increased. Magnesium and calcium were more efficient in suppressing Cd^{2+} uptake than sodium or potassium, particularly at higher concentrations. The results also indicated that the sorption capacity of the bark, pre-loaded with Cu^{2+} or Cd^{2+}, for Ni^{2+} ions was higher than the fresh bark. It has also shown that bark was able to decrease Cd^{2+} concentration to <0.008. Plant materials, other than pine, showed an ability to sorb heavy metals as well.

Chapter 6

Sorption of Copper from Industrial Wastewater

6.1 Introduction

In this chapter the results concerning the utilization of CM, bark and moss for the adsorption of copper from a real industrial wastewater are presented. The wastewater was obtained from the effluent of a copper smelting and refining plant (Noranda, Montreal, Quebec). It should be emphasized that the characteristics and composition of this type of wastewater vary from one batch to another depending on the raw materials and the process under consideration for each batch. The results of this chapter will be expressed in mass basis (metal concentration in ppm and the uptake in μg/mg), since only one metal ion is considered, and in addition, most of the industries express metal concentrations on a mass basis (ppm).

6.2 Characteristics of the Wastewater

The color of the wastewater was yellowish to beige. Its sediment concentration was 2.04 ± 0.018 mg/mL, and the pH was 4.2. The water was filtered through a 0.45 μm filter paper, and the filtrate and sediment were analyzed. The clean, colorless filtrate had a pH of 5.2 and contained only copper ions in a concentration of 36.5 ± 0.984 ppm. The sediment was beige to brown in color.

The objective of this research was to decrease the concentration of copper in the filtrate of this wastewater by using the new adsorbents tested in this work. Removal of copper from the wastewater was tested in batch and continuous processes.

6.3 Removal of Copper by CM, Moss and Bark

Three plant sorbents, CM, moss and bark, were considered for the removal of copper from the filtrate of this wastewater. The effect of the concentration of these sorbents on copper removal is shown in Figure 6.1. It is seen that at low sorbent concentrations, CM removed more Cu^{2+} ions followed by moss then bark, while at high sorbent concentrations this order was different: the most Cu^{2+} ions were removed by bark followed by moss then CM. The latter order resulted in residual Cu^{2+} concentrations of about 2.3, 4.1 and 5.2 ppm, respectively. These residual concentrations are still higher than the

maximum permissible limit of Cu^{2+} in drinking water as outlined by the Canadian Council of Ministers of Environment (CCME, 1991), which is 1 ppm or lower, but it is within the limit for livestock watering that is 0.5-5 ppm. Therefore, this removal process is suitable for livestock watering but requires further treatment for drinking water.

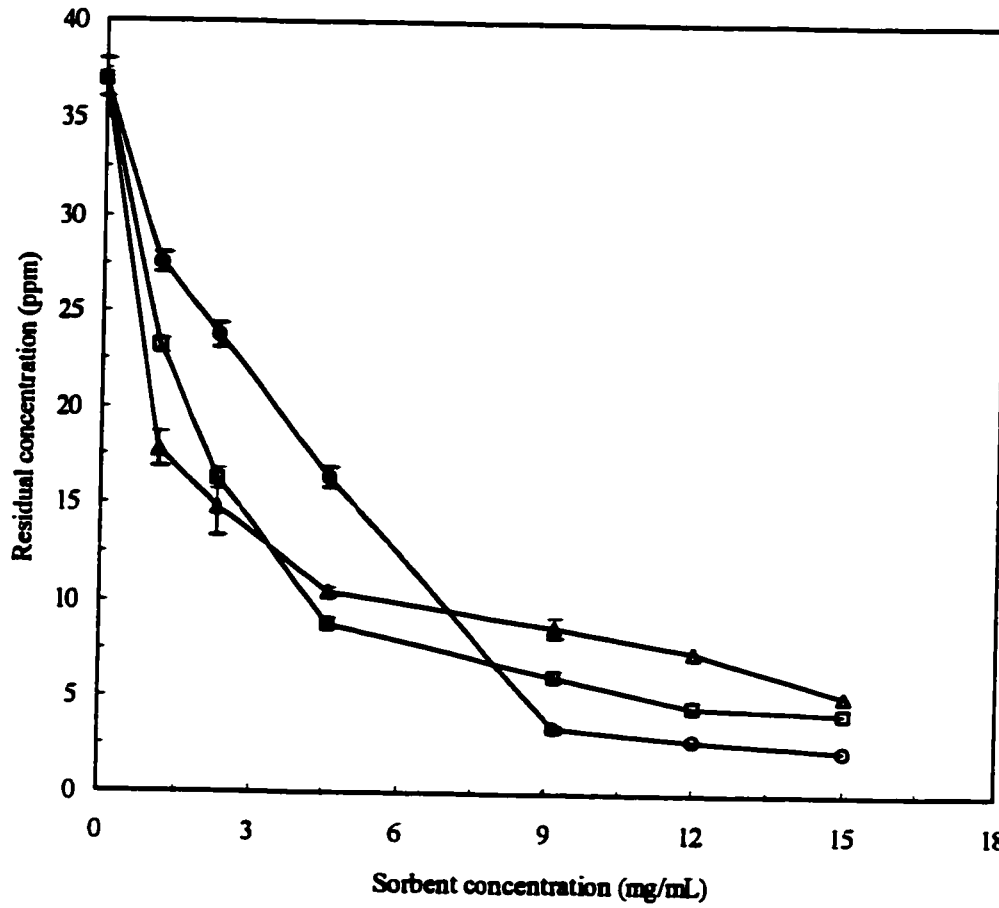

Figure 6.1: Effect of sorbent concentration on the removal of copper from industrial water using CM (Δ), bark (O) and moss (□).

The effect of the initial pH of the wastewater on the copper removal was considered. Three samples of the water were adjusted to pH of 4.5, 5.2 and 5.6. The concentration of Cu^{2+} at these pH values was 36.5 ppm. Various amounts of bark were added to each of these systems. The relationship between pH and bark concentration, and the residual Cu^{2+} concentration is shown in Figure 6.2. It is seen that more Cu^{2+} ions were removed as the initial pH of the wastewater increased. This was especially pronounced for lower sorbent concentrations, whereas at higher concentrations, the effect of pH was less important. This might be

due to the abundant number of sites available for adsorption at high sorbent concentrations. This is also the reason why, at a bark concentration of 18.2 mg/mL, the residual concentration was the same (about 2.5 ppm) regardless of the initial pH of the solutions.

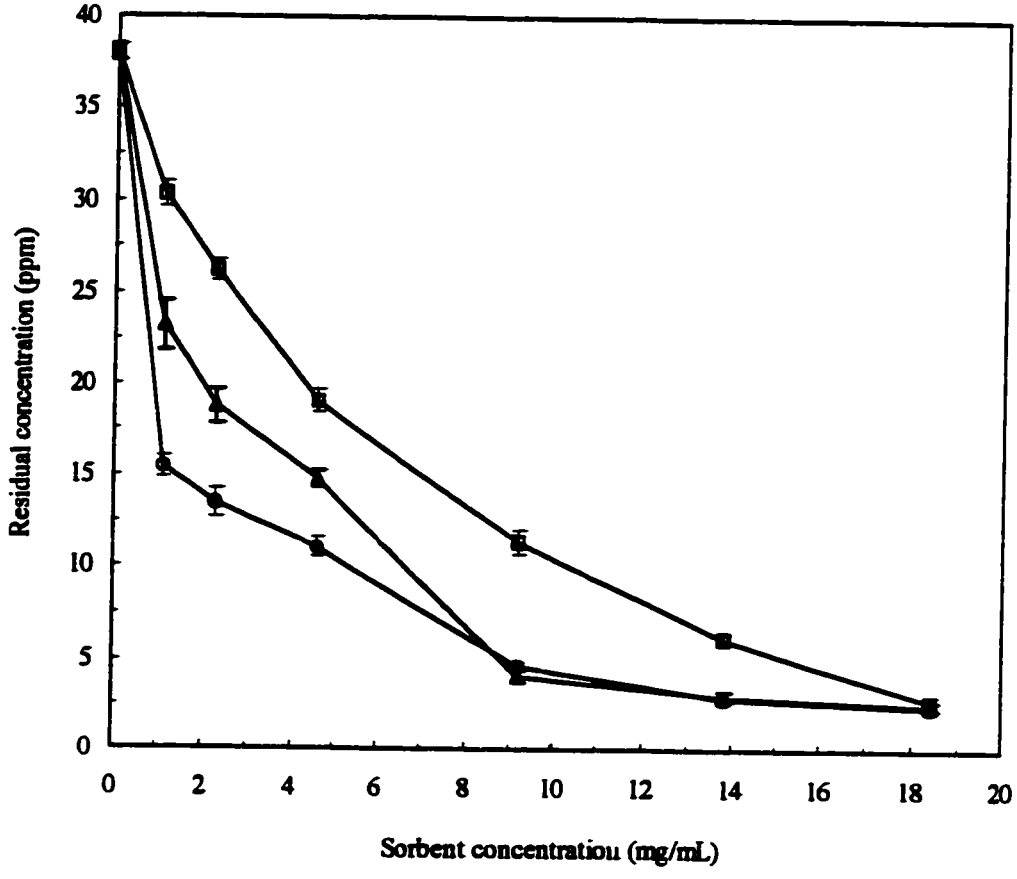

Figure 6.2: Effect of the initial pH of the industrial water on the copper removal by bark. Initial pH: □-4.5; Δ-5.2; O-5.6.

Although the initial pH of the wastewater was adjusted to a certain value, it was noted that this pH was reduced after the addition of the sorbent to the wastewater. At equilibrium, the pH, in the case of moss and bark, attained a values of 3-3.5 regardless of the initial pH of the solution. However, in the case of CM, the pH was not reduced significantly, which could be due to its large proteins content. Therefore, another set of sorption experiments were carried out using various concentrations of CM, bark and moss, where the pH was kept constant at a value of 5.2, by adding few droplets of 1 N NaOH, which corresponded to the pH of the filtrate of the wastewater.

The results of data plotted in Figure 6.3 showed that, in the case of bark and moss at low sorbent concentrations, more Cu^{2+} was removed when the pH was controlled at pH 5.2 than without pH

control (Figure 6.1). At high sorbents concentrations, no improvement was obtained using pH controlled tests. Tests with different concentrations of pulverized activated carbon (AC) were also conducted without pH control. It was noted that 10 mg/mL of activated carbon reduced Cu^{2+} concentration to about 0.6 ppm, which was much lower than in the case of biological sorbents (Figure 6.2). The addition of activated carbon to the wastewater (as well as to the distilled water without metal) resulted in an increase in the pH of the solution. The level of this increase depended on the concentration of activated carbon, for example, at 10 mg/mL of activated carbon the final pH was about 6.2. This might be one of the reasons for the higher removal of Cu^{2+} by activated carbon compare to the plant sorbents.

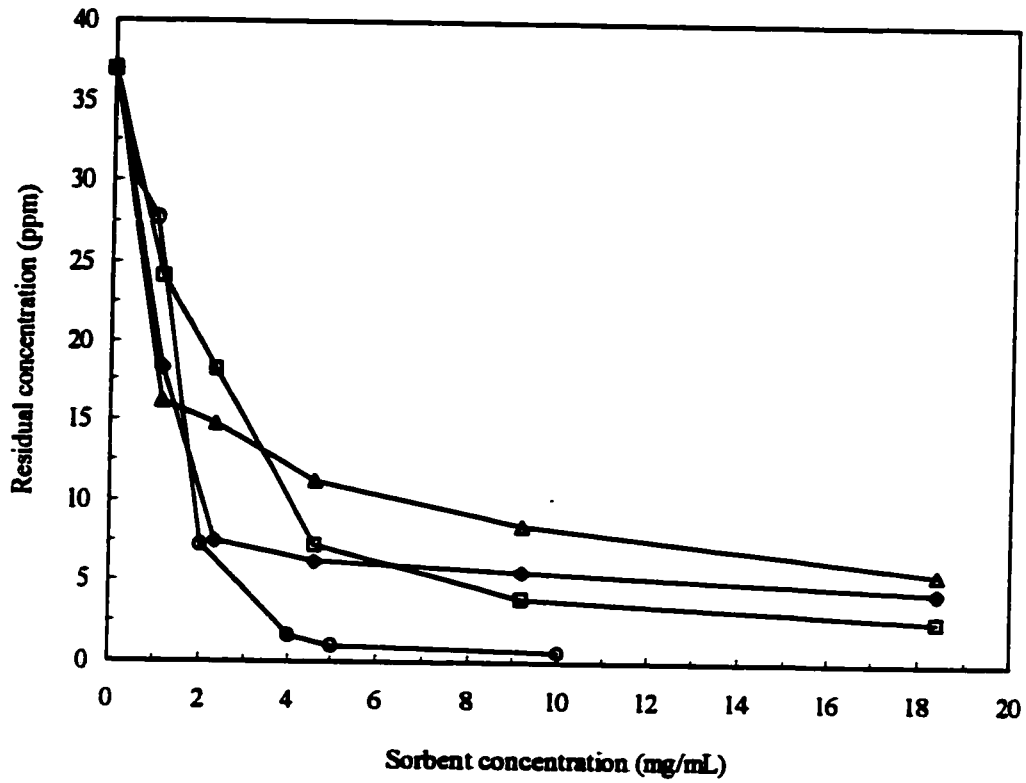

Figure 6.3: Removal of copper from wastewater using various concentrations of CM (Δ), moss (◊), bark (□) and activated carbon (O). pH of agricultural sorbents controlled at 5.2; pH of activated carbon was not controlled.

From these data, it could be concluded that the removal of Cu^{2+} can be carried out (up to certain limit) by the tested biomaterials without pH adjustment if high concentrations of sorbents are used.

6.4 Combination of Bark and Activated Carbon

In the previous sections, it was noticed that the utilization of the plant sorbents did not decrease the Cu^{2+} concentration in the tested wastewater to the allowable limit outlined by the environmental agencies. It was also shown that 10 mg/mL of activated carbon can reduce the copper level to the permissible level. From an economic point of view, the use of 10 mg/mL of activated carbon is expensive. Therefore, a combination of activated carbon with one of the tested low cost sorbents (CM, bark or moss) in this study might be advantageous. To explore such a possibility, several tests were carried out with activated carbon and pine bark. The concentrations of activated carbon and bark in wastewater were 2.3 and 9.2 mg/mL, respectively. The study was performed with four different alternatives as described in the following paragraphs:

Alternative I: Activated carbon and bark were mixed together, and sorption was carried out until equilibrium (1 hour) at a constant pH of 5.2 (requiring 62 μL of 1 N NaOH). The solution was filtered and the residual Cu^{2+} concentration was found to be 3 ppm.

Alternative II: Sorption with 9.2 mg/mL of bark was carried out until equilibrium at constant pH of 5.2 (requiring 80 μL of 1 N NaOH). After equilibrium, at pH 5.2, activated carbon was added to make a final concentration of 2.3 mg AC/mL in the suspension. The solution was continuously maintained at a pH of 5.2 until equilibrium was again attained. The solution was then filtered, and the Cu^{2+} concentration was found to be 3.4 ppm.

Alternative III: Sorption with 9.2 mg/mL of bark was carried out until equilibrium at constant pH of 5.2 (requiring 85 μL of 1 N NaOH). The bark was then separated from the solution by filtration and activated carbon was added to make a final concentration of 2.3 mg AC/mL in the filtrate. Equilibrium was attained at a constant pH value of 5.2. After filtering the activated carbon from the solution, the Cu^{2+} concentration in the filtrate was 3.3 ppm.

Alternative IV: Sorption with 9.2 mg/mL of bark was carried out until equilibrium at constant pH of 5.2 (requiring 86 μL of 1 N NaOH). The bark was then separated from the solution by filtration. The copper concentration in this solution was measured to be 5.1 ppm. After that, activated carbon was added to make a final concentration of 2.3 mg AC/mL in this bark-free solution and equilibrium was attained without pH adjustment (the final pH was 5.6). After filtering the activated carbon, the Cu^{2+} concentration in the filtrate was 0.6 ppm.

The above results indicate that only the last alternative (Alternative IV) produced the treated wastewater with the permissible level of copper. But, generally speaking, the combination of both of

the adsorbents enhances the process of recovery and also reduces the cost of the sorbent, since bark is less expensive and more available than activated carbon.

6.5 Multi-Stage Sorption

The removal of Cu^{2+} ions from the wastewater was carried out using different stage of sorption. In a single stage sorption, 4.6 mg/mL of sorbent was utilized. When a two stage sorption was carried out, 2.3 mg/mL of fresh sorbent was utilized in each stage. The filtrate from the first stage was used in the second stage for the sorption process. In the four-stage sorption, 1.15 mg/mL of fresh sorbent was used in each stage and the filtrate from the preceding stage was used for the subsequent stage. Each of these alternatives is characterized by an equal amount of total sorbent used and the same initial Cu^{2+} concentration. These tests were carried out with moss, CM and bark, and the results are presented in Tables 6.1, 6.2 and 6.3, respectively.

Table 6.1: Removal of Cu^{2+} from wastewater by moss in a multi-stage process. Initial pH: 5.2; initial Cu^{2+} concentration: 36.5 ppm.

Moss Concentration per stage (mg/mL)	Stage number			
	I	II	III	IV
	Residual Cu^{2+} concentration (ppm)			
4.6	8.9±0.282			
2.3	16.3±0.565	10.5±1.838		
1.15	23.15±0.353	16.9±0.070	13.3±0.141	10.75±0.353

Table 6.2: Removal of Cu^{2+} from wastewater by CM in a multi-stages process. Initial pH: 5.2; initial Cu^{2+} concentration: 36.5 ppm.

CM Concentration per stage (mg/mL)	Stage number			
	I	II	III	IV
	Residual Cu^{2+} concentration (ppm)			
4.6	10.55±0.212			
2.3	14.8±1.414	10.95±0.494		
1.15	17.8±0.848	13.1±0.424	12.4±0.231	10.85±0.495

Table 6.3: Removal of Cu^{2+} from wastewater by bark in multi-stages process. Initial pH: 5.2; initial Cu^{2+} concentration: 36.5 ppm.

Bark Concentration per stage (mg/mL)	Stage number			
	I	II	III	IV
	Residual Cu^{2+} concentration (ppm)			
4.6	16.4±0.565			
2.3	23.75±0.636	17.7±0.707		
1.15	27.55±0.495	22.2±0.283	19.0±0.283	15.9±0.141

It was shown (Tables 6.1-6.3) that the utilization of the same amount of sorbent resulted in almost the same level of copper reduction, whether this amount was used in a single- or multi-stage sorption process. All three sorbents showed the same trend. When multi-stage sorption was used, the sorbent in the preceding stage removed more metal ions from the wastewater than the same amount of sorbent in the subsequent stages. The reason for the lower amount of metal ions removed in the subsequent stages by the same amount of biosorbent is due to a decrease in the metal ions to sorbent ratios (i.e. a lower concentration gradient).

6.6 Sorption by Packed Columns

As has been discussed previously, a continuous sorption process is more practical for industrial operations. Therefore, this type of process was also applied to the study of the treatment of the wastewater from Noranda. A column of 10 cm length and 8 mm ID was designed for this purpose. The column was packed with 2 g of bark. Six hundreds mL of this wastewater, with an initial copper concentration of 36.5 ppm, was pumped (upward) through the column at a rate of 2.5 mL/min. Samples were obtained from the effluent of the column, as well as from the reservoir, for the purpose of calculating the metal uptake by the column. The process continued until steady-state, i.e. when the concentration in the effluent and influent streams were the same. The concentration of copper in the reservoir of the effluent at steady-state was 18.6 ppm. This indicates that almost half of the initial copper was removed from the wastewater during this cycle. To further decrease the concentration of copper in the treated water, the column was regenerated by pumping 50 mM of HCl through it. During the regeneration process, the concentration of copper in the effluent of the column was measured until it dropped to zero, at which point, the column was washed with deionized water until the pH from the effluent of the column became the same as the pH of water. The sorption was repeated using the effluent from the first sorption (which contained 18.6 ppm of copper). In the second stage, the

concentration of copper was decreased to a level of 8.6 ppm. Again, another desorption-washing-sorption cycle was conducted and a residual copper concentration of 4.0 ppm was attained in the final collected volume. A further (fourth) sorption was not very efficient. The concentration of copper in the effluent after the fourth treatment was only 3.5 ppm, consequently, the process was terminated.

The break-through curves and the uptake of the column at various times for each of the first three adsorption cycles are shown in Figures 6.4A and B, respectively, while the desorption results for the three desorption cycles are represented by Figures 6.5A and B, respectively. The break-through in the first cycle occurred earlier than the second or third cycles (Figure 6.4A). This was expected, since the concentrations of the solute in the feed of the second and third cycles were lower than that in the first cycle. It was also noted (Figure 6.4A) that the copper concentration in the effluent from the column during the third cycle did not attain the influent concentration of the column. This was due to the low metal concentration in the influent of this cycle and, therefore, the column required more time and solution to attain saturation (steady-state). However, the process was limited by the initial volume of 600 mL of wastewater. The uptake of copper per unit mass of bark in the first cycle was higher than that of the second or the third cycles (Figure 6.4B). This was also expected since most of the metal ions were recovered in the first cycle and the initial concentrations of the metal in the subsequent cycles were lower.

Following the desorption results, it is seen (Figure 6.5A) that the eluant concentrated more Cu^{2+} ion in the effluent of the column in the third cycle than the second followed by the first. This is because of the low concentration of copper in the bark in the third cycle, bearing in mind that the ratio C_{out}/C_{in} in Figure 6.5A represents the effluent to the influent concentrations of the same column and not to the original solution. The ratio $Q_{release}/Q_{load}$ in Figure 6.5 B represents the ratio between the amount of copper eluted from the column to the amount of total copper uptaken in the sorption process for each cycle at steady-state. It is seen that almost complete release of Cu^{2+} ions was achieved in each cycle.

These results indicate that bark-packed column was efficient at removing copper ions from the wastewater and could decrease the copper concentration to 4 ppm after three sorption/desorption cycles.

The above results showed that CM, bark and moss were able to decrease the initial copper concentration in the industrial wastewater from a copper refining/smelting plant from 36.5 to 2.5, 4.1 and 5.2 ppm, respectively, when the sorbents concentrations were 18.4 mg/mL. When the pH of the wastewater was controlled at 5.2 the enhancement in the Cu^{2+} removal, compared to that without pH control, was higher at low sorbent concentrations than at higher concentrations. The copper

concentration was decreased to below the permissible limit, 0.6 ppm, when the wastewater was treated with 9.2 mg/mL of bark and the filtrate of this process was treated with 2.3 mg/mL of activated carbon in a system without pH control. This combination is considered cheaper than using only activated carbon, since it was found that higher concentrations of activated carbon were required to reduce the copper concentration to the same level. As a result, substituting activated carbon by the much less

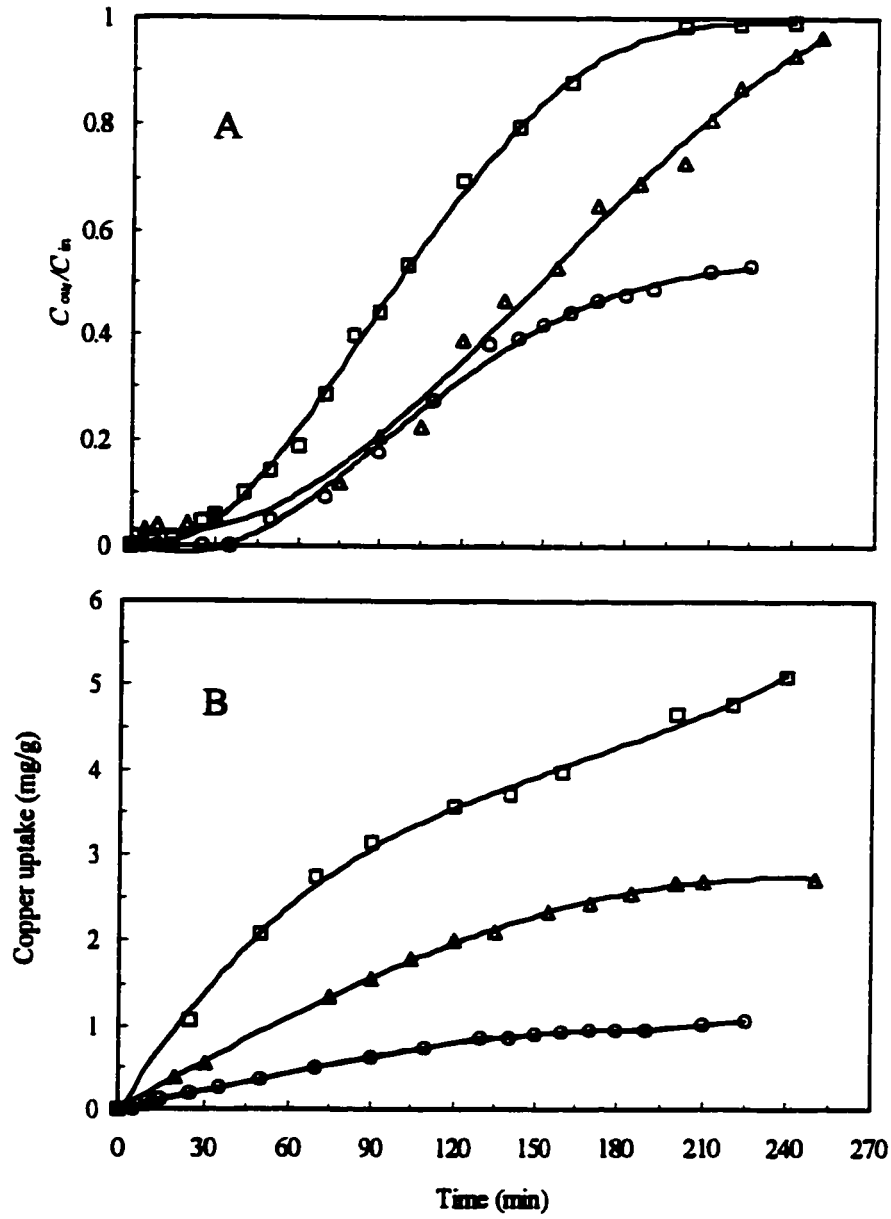

Figure 6.4: Break-through curves (A) and uptake curves (B) for the sorption of Cu^{2+} from wastewater. □-first cycle, Δ-second cycle, O-third cycle.

expensive biological sorbents could amount to greater savings when treating these wastewaters. This study also showed that the utilization of the same amount of sorbent either in a single- or multi-stage sorption process resulted in the same level of copper reduction. Copper ions were reduced to a low level when the wastewater was treated in a column packed with bark. After three sorption/desorption cycles, copper was decreased to 4 ppm.

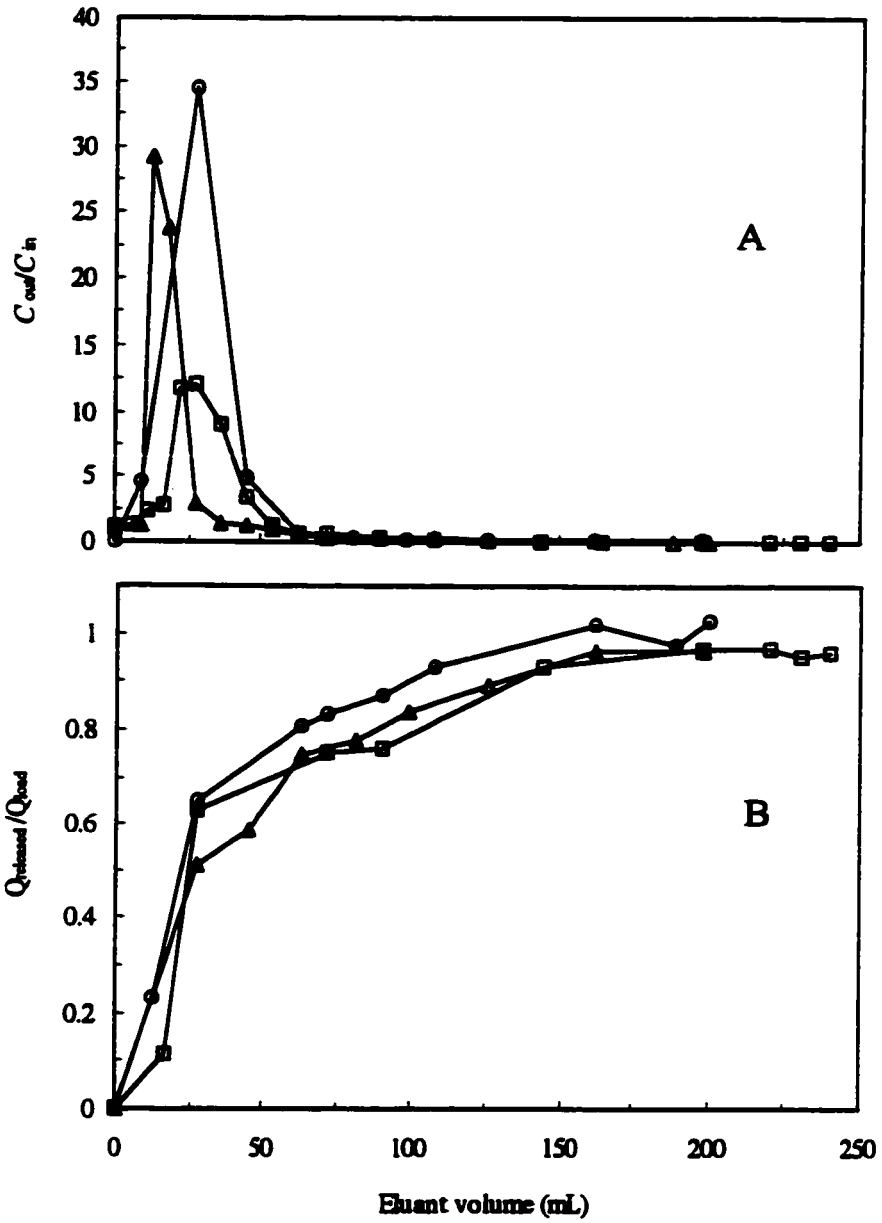

Figure 6.5: Desorption of Cu^{2+} ions from the bark-laden column using 50 mM HCl, concentration curves (A), and material balance curves (B). □-first cycle, Δ-second cycle, O-third cycle.

Chapter 7

Multi-metal Biosorption

7.1 Introduction

Most of the previous studies in biosorption dealt with one metal solutions (Fourest and Roux, 1994; Schiewer and Volesky, 1995; Singh and Lal, 1992). However, industrial effluents often contain more than one metal. Biosorption from multi-metal solutions was not extensively considered in the literature. In some cases, even when the influence of one metal on another was examined, the results can not be extrapolated and no predictive conclusions can be drawn (Chong and Volesky, 1995).

Therefore, it is necessary to study the sorption from a mixture of metal ions, since the degree of metals removal from their solutions depends on the multi-metal competition interactions in solution with the sorbent material. In this study, it was decided to take into consideration systems containing two or more metals in solution and the results are reported in this chapter. Three plant sorbents, CM, moss and bark, were considered in this work to compare their performances in the multi-metal sorption processes.

7.2 Single, Binary, Ternary and Quaternary Metal Systems

Adsorption of metals from solutions containing two, three or four various metals by CM, bark and moss were studied. The Cu^{2+}, Ni^{2+}, Cd^{2+} and Pb^{2+} ions were selected and used in various combinations in binary, ternary and quaternary systems for adsorption experiments. Tables 7.1, 7.2, and 7.3 give the adsorption capacity of CM, pine bark and moss, respectively in the tested metal systems.

Single metal sorption

The results of single metal sorption show the following order of preference for the sorbents CM and bark, on a molar basis, Tables 7.1 and 7.2, respectively: $Cu^{2+} > Cd^{2+} > Ni^{2+} > Pb^{2+}$. For moss the order was: $Cu^{2+} > Ni^{2+} > Cd^{2+} > Pb^{2+}$. However, when the comparison is expressed in terms of the amount of metal removal, mass basis, the order: $Pb^{2+} > Cd^{2+} > Cu^{2+} > Ni^{2+}$ was obtained, for the three sorbents. Comparing the selectivity of the three sorbents for each of these metal ions, the following

order can be concluded for each metal: Cu^{2+} and Ni^{2+}: moss > CM > bark; Cd^{2+}: CM > moss > bark; Pb^{2+}: CM = bark ≈ moss.

Table 7.1: Adsorption capacity of CM for metal ions from ion mixtures. Initial concentration of each ion: 100 ppm; CM concentration: 9.2 mg/mL; initial pH 4; sorption time: 24 h

System	q_{mix} (mmol/g)	q_0 (mmol/g)	x	y	K_{ij}	q_{mix}/q_0
(A) Cu^{2+}	0.124 (72.4)	0.115 (67.2)	0.77	0.60	(AB) 0.45	1.08
(B) Cd^{2+}	0.082 (85.2)	0.086 (89.6)	0.23	0.40		0.95
(A) Cu^{2+}	0.126 (73.7)	0.115 (67.2)	0.26	0.69	(AB) 9.35	1.09
(B) Ni^{2+}	0.057 (31.1)	0.075 (40.3)	0.74	0.21		0.76
(A) Cu^{2+}	0.107 (62.8)	0.115 (67.2)	0.88	0.71	(AB) 0.33	0.93
(B) Pb^{2+}	0.044 (84.1)	0.048 (92.3)	0.12	0.29		0.92
(A) Cd^{2+}	0.088 (91.5)	0.086 (89.6)	0.07	0.54	(AB) 15.6	1.02
(B) Ni^{2+}	0.075 (40.4)	0.075 (40.3)	0.93	0.46		1.00
(A) Cd^{2+}	0.090 (93.1)	0.086 (89.6)	0.48	0.67	(AB) 2.20	1.05
(B) Pb^{2+}	0.045 (86.5)	0.048 (92.3)	0.52	0.33		0.94
(A) Ni^{2+}	0.071 (38.4)	0.075 (40.3)	0.95	0.60	(AB) 0.08	0.95
(B) Pb^{2+}	0.046 (88.3	0.048 (92.3)	0.05	0.40		0.96
(A) Cu^{2+}	0.115 (66.7)	0.115 (67.2)	0.23	0.54	(AB) 0.43	1.00
(B) Cd^{2+}	0.080 (83.1)	0.086 (89.6)	0.07	0.38	(BC) 47.5	0.93
(C) Ni^{2+}	0.016 (8.5)	0.075 (40.3)	0.70	0.08	(AC) 20.5	0.21
(A) Cu^{2+}	0.122 (71.4)	0.115 (67.2)	0.66	0.50	(AB) 0.57	1.06
(B) Cd^{2+}	0.079 (81.4)	0.086 (89.6)	0.24	0.32	(BC) 0.74	0.92
(C) Pb^{2+}	0.045 (86.4)	0.048 (92.3)	0.10	0.18	(AC) 0.42	0.94
(A) Cd^{2+}	0.080 (82.5)	0.086 (89.6)	0.09	0.40	(AB) 10.21	0.93
(B) Ni^{2+}	0.073 (39.6)	0.075 (40.3)	0.85	0.37	(BC) 0.11	0.97
(C) Pb^{2+}	0.045 (85.8)	0.048 (92.3)	0.06	0.23	(AC) 1.16	0.94
(A) Ni^{2+}	0.008 (4.8)	0.075 (40.3)	0.76	0.04	(AB) 0.02	0.10
(B) Cu^{2+}	0.122 (71.4)	0.115 (67.2)	0.21	0.70	(BC) 0.38	1.06
(C) Pb^{2+}	0.045 (85.8)	0.048 (92.3)	0.03	0.26	(AC) 0.01	0.94
(A) Cu^{2+}	0.119 (69.5)	0.115 (67.2)	0.4	0.50	(AB) 0.80	1.03
(B) Cd^{2+}	0.072 (74.7)	0.086 (89.6)	0.19	0.30	(BD) 0.41	0.83
(C) Ni^{2+}	0.002 (0.84)	0.075 (40.3)	0.36	0.01	(CD) 0.01	0.02
(D) Pb^{2+}	0.046 (87.1)	0.048 (92.3)	0.05	0.19	(AD) 0.33	0.96

q_{mix} = adsorption of a metal ion in mixture; q_0 = adsorption of a metal ion in single ion system; x = equilibrium molar composition of adsorbate in solution; y = equilibrium molar adsorbate composition on CM; K_{ij} = selectivity index (i refers to the first symbol in brackets while j refers to the second one). Values in brackets represent the amount of metal removal (%).

Binary system

Considering the metal sorption by CM from different binary systems, Cu^{2+} was the most adsorbed metal. The uptake of metals by CM in binary systems containing Cu^{2+} as a second component followed the order: Cd^{2+}> Ni^{2+}> Pb^{2+} (Table 7.1). The same order of affinity for these ions was also

observed when adsorption was carried out from single metal solutions. Based on this finding, it was logical to expect the following order of metal uptake by CM in the binary systems containing a combination of these metals: Cd^{2+}> Ni^{2+}, Cd^{2+}> Pb^{2+} and Ni^{2+}> Pb^{2+}. The experimental results confirmed this hypothesis (Table 7.1).

Table 7.2: Adsorption capacity of bark for metal ions from ion mixtures. Initial concentration of each ion: 100 ppm; bark concentration: 9.2 mg/mL; initial pH 4; sorption time: 24 h

System	q_{mix} (mmol/g)	q_0 (mmol/g)	x	y	K_{ij}	q_{mix}/q_0
(A) Cu^{2+}	0.088 (51.6)	0.108 (63.3)	0.57	0.70	(AB) 1.79	0.82
(B) Cd^{2+}	0.034 (35.2)	0.068 (70.4)	0.43	0.30		0.50
(A) Cu^{2+}	0.082 (48.0)	0.108 (63.3)	0.40	0.60	(AB) 2.25	0.76
(B) Ni^{2+}	0.054 (29.2)	0.060 (32.4)	0.60	0.40		0.90
(A) Cu^{2+}	0.072 (41.6)	0.108 (63.3)	0.88	0.64	(AB) 0.20	0.66
(B) Pb^{2+}	0.039 (74.4)	0.048 (92.9)	0.12	0.44		0.81
(A) Cd^{2+}	0.048 (49.7)	0.068 (70.4)	0.28	0.43	(AB) 1.94	0.70
(B) Ni^{2+}	0.063 (34.0)	0.060 (32.4)	0.72	0.57		1.05
(A) Cd^{2+}	0.032 (33.1)	0.068 (70.4)	0.92	0.40	(AB) 0.06	0.47
(B) Pb^{2+}	0.047 (89.7)	0.048 (92.9)	0.08	0.60		0.98
(A) Ni^{2+}	0.077 (41.6)	0.060 (32.4)	0.94	0.63	(AB) 0.12	1.28
(B) Pb^{2+}	0.045 (85.9)	0.048 (92.9)	0.06	0.33		0.94
(A) Cu^{2+}	0.096 (56.2)	0.108 (63.3)	0.24	0.67	(AB) 1.58	0.89
(B) Cd^{2+}	0.043 (44.5)	0.068 (70.4)	0.17	0.30	(BC) 34.7	0.63
(C) Ni^{2+}	0.005 (2.7)	0.060 (32.4)	0.59	0.03	(AC) 54.9	0.08
(A) Cu^{2+}	0.067 (39.3)	0.108 (63.3)	0.54	0.50	(AB) 2.05	0.62
(B) Cd^{2+}	0.023 (23.8)	0.068 (70.4)	0.40	0.18	(BC) 0.08	0.34
(C) Pb^{2+}	0.040 (76.3)	0.048 (92.9)	0.06	0.32	(AC) 0.17	0.83
(A) Cd^{2+}	0.027 (27.4)	0.068 (70.4)	0.33	0.21	(AB) 0.84	0.40
(B) Ni^{2+}	0.058 (31.3)	0.060 (32.4)	0.61	0.46	(BC) 0.14	0.97
(C) Pb^{2+}	0.041 (78.2)	0.048 (92.9)	0.06	0.33	(AC) 0.11	0.85
(A) Ni^{2+}	0.003 (1.6)	0.060 (32.4)	0.65	0.02	(AB) 0.01	0.05
(B) Cu^{2+}	0.083 (48.7)	0.108 (63.3)	0.31	0.65	(BC) 0.25	0.77
(C) Pb^{2+}	0.042 (80.2)	0.048 (92.9)	0.04	0.33	(AC) 0.004	0.87
(A) Cu^{2+}	0.066 (38.7)	0.108 (63.3)	0.29	0.46	(AB) 1.17	0.61
(B) Cd^{2+}	0.034 (35.2)	0.068 (70.4)	0.17	0.23	(BD) 0.13	0.50
(C) Ni^{2+}	0.001 (0.5)	0.060 (32.4)	0.51	0.007	(CD) 0.001	0.02
(D) Pb^{2+}	0.043 (82.1)	0.048 (92.9)	0.03	0.303	(AD) 0.16	0.089

q_{mix} = adsorption of a metal ion in mixture; q_0 = adsorption of a metal ion in single ion system; x = equilibrium molar composition of adsorbate in solution; y = equilibrium molar adsorbate composition on bark; K_{ij} = selectivity index (i refers to the first symbol in brackets while j refers to the second one). Values in brackets represent the amount of metal removal (%).

Table 7.3: Adsorption capacity of moss for metal ions from ion mixtures. Initial concentration of each ion: 100 ppm; moss concentration: 9.2 mg/mL; initial pH 4; sorption time: 24 h

System	q_{mix} (mmol/g)	q_0 (mmol/g)	x	y	K_{ij}	$q_{mix}/$
(A) Cu^{2+}	0.131 (76.5)	0.118 (68.7)	0.48	0.71	(AB) 2.65	1.1
(B) Cd^{2+}	0.053 (55.6)	0.080 (83.3)	0.52	0.29		0.6
(A) Cu^{2+}	0.116 (68.0)	0.118 (68.7)	0.30	0.68	(AB) 4.95	0.9
(B) Ni^{2+}	0.055 (30.0)	0.113 (61.0)	0.70	0.32		0.4
(A) Cu^{2+}	0.126 (73.8)	0.118 (68.7)	0.89	0.73	(AB) 0.33	1.0
(B) Pb^{2+}	0.047 (89.3)	0.047 (89.3)	0.11	0.27		0.9
(A) Cd^{2+}	0.065 (67.6)	0.080 (83.3)	0.25	0.43	(AB) 2.26	0.8
(B) Ni^{2+}	0.089 (48.2)	0.113 (61.0)	0.75	0.57		0.7
(A) Cd^{2+}	0.077 (79.5)	0.080 (83.3)	0.79	0.62	(AB) 0.43	0.95
(B) Pb^{2+}	0.047 (90.0)	0.047 (89.3)	0.21	0.38		0.92
(A) Ni^{2+}	0.104 (56.3)	0.113 (61.0)	0.97	0.67	(AB) 0.06	0.92
(B) Pb^{2+}	0.050 (95.9)	0.047 (89.3)	0.03	0.33		0.98
(A) Cu^{2+}	0.124 (72.5)	0.118 (68.7)	0.18	0.63	(AB) 3.85	1.05
(B) Cd^{2+}	0.039 (40.6)	0.080 (83.3)	0.22	0.20	(BC) 3.21	0.48
(C) Ni^{2+}	0.033 (18.0)	0.113 (61.0)	0.60	0.17	(AC) 12.35	0.29
(A) Cu^{2+}	0.118 (69.1)	0.118 (68.7)	0.43	0.59	(AB) 3.89	1.00
(B) Cd^{2+}	0.035 (36.1)	0.080 (83.3)	0.51	0.18	(BC) 0.09	0.43
(C) Pb^{2+}	0.045 (86.3)	0.047 (89.3)	0.06	0.23	(AC) 0.35	0.88
(A) Cd^{2+}	0.048 (50.1)	0.080 (83.3)	0.33	0.25	(AB) 0.97	0.60
(B) Ni^{2+}	0.092 (50.0)	0.113 (61.0)	0.63	0.49	(BC) 0.12	0.81
(C) Pb^{2+}	0.047 (89.4)	0.047 (89.3)	0.04	0.26	(AC) 0.12	0.92
(A) Ni^{2+}	0.047 (25.5)	0.113 (61.0)	0.73	0.21	(AB) 0.11	0.42
(B) Cu^{2+}	0.127 (73.9)	0.118 (68.7)	0.23	0.58	(BC) 0.48	1.08
(C) Pb^{2+}	0.046 (88.5)	0.047 (89.3)	0.04	0.21	(AC) 0.05	0.90
(A) Cu^{2+}	0.110 (64.5)	0.118 (68.7)	0.19	0.57	(AB) 3.53	0.93
(B) Cd^{2+}	0.032 (33.2)	0.080 (83.3)	0.20	0.17	(BD) 0.14	0.40
(C) Ni^{2+}	0.005 (2.9)	0.113 (61.0)	0.57	0.02	(CD) 0.006	0.04
(D) Pb^{2+}	0.044 (84.0)	0.047 (89.3)	0.04	0.24	(AD) 0.50	0.86

q_{mix} = adsorption of a metal ion in mixture; q_0 = adsorption of a metal ion in single ion system; x = equilibrium molar composition of adsorbate in solution; y = equilibrium molar adsorbate composition on moss; K_{ij} = selectivity index (i refers to the first symbol in brackets while j refers to the second one). Values in brackets represent the amount of metal removal (%).

When the metals were sorbed from the same systems using bark, Cu^{2+} was again the most adsorbed metal (Table 7.2). Similarly, in the systems containing Cu^{2+} ions, the uptake of the other metals by bark followed the order: Ni^{2+}> Pb^{2+}> Cd^{2+} (Table 7.2). Experimental data also showed that the order of the uptake in a combination of these metals was: Ni^{2+}> Pb^{2+}, Ni^{2+}> Cd^{2+} and Pb^{2+}> Cd^{2+} (Table 7.2). In addition, the presence of Pb^{2+} caused an increase in the uptake of Ni^{2+} by bark to a level higher than that when this metal was sorbed from its individual solution.

Similar conclusions can also be drawn for the sorption of metals by moss from their binary systems (Table 7.3). Bearing in mind that Cu^{2+} is the most sorbed metal by this sorbent in these binary systems, the order of the other binary systems was: $Ni^{2+} > Cd^{2+} > Pb^{2+}$.

Ternary systems

Cu^{2+}–Cd^{2+}–Ni^{2+}: When comparing the uptake of each metal in this ternary system by CM to the uptake when each of them was sorbed from their individual solutions, it was noticed (Table 7.1) that the uptake of Ni^{2+} decreased significantly, while the uptake of Cd^{2+} changed only slightly. The order of metal uptake in this case was: $Cu^{2+} > Cd^{2+} > Ni^{2+}$, which is consistent with the orders of binary systems.

The suppression of Ni^{2+} was also noticed when bark and moss were used as sorbents in this ternary system (Tables 7.2 and 7.3). However, the uptake of Cd^{2+} by these sorbents was also considerably suppressed, but not as much as that of Ni^{2+}. These results indicate that CM is more selective for Cd^{2+} than bark or moss.

Cu^{2+}–Cd^{2+}–Pb^{2+}: The sorption of each metal in this system by CM did not differ significantly from the single metal sorption. The order of the uptake was: $Cu^{2+} > Cd^{2+} > Pb^{2+}$, which is the same as when the metals were sorbed individually by CM and is the same as the one determined in the binary systems.

The sorption of these metals by bark was different than that of CM. The uptakes of Cu^{2+} and Cd^{2+} were decreased and the order of preference was different than that of single metal sorption: $Cu^{2+} > Pb^{2+} > Cd^{2+}$ (Table 7.2). However, this order is consistent with the results of the binary sorption by this sorbent.

Moss also showed a decrease in the uptake of Cd^{2+} in this ternary system, but Cu^{2+} and Pb^{2+} remained at almost the same level of uptake (Table 7.3). These results indicate that CM and moss are more selective for Cu^{2+} than bark.

Cd^{2+}–Ni^{2+}–Pb^{2+}: Similarly, the uptakes by CM did not change significantly, keeping the same order as that of single metal sorption (Table 7.1). For bark and moss, a significant reduction in the Cd^{2+} uptake can be noticed (Table 7.2 and 7.3), while the other two metals remained at almost the same level of sorption in this ternary system.

Ni^{2+}–Cu^{2+}–Pb^{2+}: The uptake of Ni^{2+} in this system by the three sorbents, CM, bark and moss, was significantly suppressed by the presence of Cu^{2+} and Pb^{2+} (Tables 7.1-7.3).

Quaternary system

When the four metals exist together in a quaternary mixture, the following order (molar basis) of preference was found for bark and moss: $Cu^{2+} > Pb^{2+} > Cd^{2+} > Ni^{2+}$, while for CM, the order was: $Cu^{2+} > Cd^{2+} > Pb^{2+} > Ni^{2+}$. However, on a mass basis, these orders became: $Pb^{2+} > Cu^{2+} > Cd^{2+} > Ni^{2+}$, for bark and moss, while for CM, the order was: $Pb^{2+} > Cd^{2+} > Cu^{2+} > Ni^{2+}$.

These results showed that the metal sorption capacity varied from one sorbent to another and depended on the presence of other metal(s) in the solution. Generally, the uptake of Ni^{2+} by any of these sorbents was always suppressed when Cu^{2+} ions were present in the Ni^{2+} solution. Canola meal can be considered to be more selective for Cd^{2+} than bark and moss. The sorption of Pb^{2+} by the different systems was not strongly influenced by the presence of the other metal ions.

The effect of ionic interactions on the sorption process, as represented by the ratio q_{mix}/q_o, can be seen from Tables 7.1-7.3. From the ratios given for the different metal systems, the various suppressers and promoters can be identified for each of the ions studied.

As an explanation for the trends for the uptake of single metal by the biosorbents, it has been demonstrated, using the biomass of *R. arrhizus*, that the uptake (in molar basis) of metals having larger ionic radii was higher than that of smaller ionic radii (Tobin *et al.*, 1984). In this work, for single metal sorption, this relationship was not valid for the three sorbents under consideration, when the comparison was carried out on molar basis; the ionic radius of Cu^{2+} is smaller than that of Pb^{2+} and Cd^{2+} (Table 7.4). However, the relationship is valid when comparing the uptake of the four metals by the three sorbents (Table 7.1-7.3) based on the amount of metal removal (mass basis). The results of this work (Table 7.1-7.3) also showed that, when comparing the uptakes of single component sorption based on mass basis, the uptake of metals having high atomic weight was higher than that of low atomic weight (Table 7.4).

Table 7.4: Some physical and chemical properties of metal ions[1].

Properties	Cu^{2+}	Cd^{2+}	Zn^{2+}	Ni^{2+}	Pb^{2+}
Ionic radius ($\mathring{A}$)	0.72	0.97	0.74	0.69	1.21
Atomic weight	63.4	112.4	65.5	57.8	207.2
Coordination number	2,4	4	4, 5, 6	4, 5	4, 5, 6
Electron configuration	[Ar] $3d^9$	[Kr] $4d^{10}$	[Ar] $3d^{10}$	[Ar]$3d^8$	[Xe]$5d^{10}$
Electronegativity of the atom	1.90	1.69	1.65	1.91	2.33

[1] Adopted from Lagowski (1973) and Purcell and Kotz (1980).

Copper has one unpaired electron (Table 7.4) and is paramagnetic (Purcell and Kotz, 1980). Thus, it can be attracted by a magnetic field possibly originating from the sorbent (Chong and Volesky, 1996). 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 7.4), if the system forms complexes in the sorbent phase, then Cu^{2+} (the least stable among the other metal ions) only requires two more electrons to coordinate with the sorbent constituents as compared to Cd^{2+}, Pb^{2+} and Ni^{2+} which require at least four electrons. That might be a reason why the sorption of Cu^{2+} was superior comparing to the other ions both in single metal sorption and in a mixture of metals.

When comparing the results of Table 7.1-7.3 on a mass basis, it is seen that the uptake of Pb^{2+} by each of the tested sorbents was superior to the other metals. This could be due to the larger atomic radius and higher electronegativity of Pb^{2+} than the other metals (Table 7.4). Chong and Volesky (1996) stated that as the electronegativity of the atom increases, its ionic forms seem to be more easily sorbed by the biosorbent. This explanation might also apply to Pb^{2+} which has the highest electronegativity value. However, the explanation failed when comparing the uptake of Cd^{2+} and Ni^{2+}, either in single or a mixture of them; Ni^{2+} has higher electronegativity than Cd^{2+} (Table 7.4), but its uptake was always lower than that of Cd^{2+}.

The suppression of Ni^{2+} and Cd^{2+} by Cu^{2+} in the different kind of systems (Tables 7.1-7.3) could be explained by the physical and chemical properties of these metal ions. The electronegativities of Cu^{2+} and Ni^{2+} are higher than that of Cd^{2+} (Table 7.4), which makes the former two ions capable of competing with Cd^{2+} for the sites available for metal sorption. Thus, because of the lower coordination number of Cu^{2+}, it could be easier for Cu^{2+} to complex or to coordinate with certain groups originating from the sorbent than Ni^{2+}.

It is seen that there are more than one factor that might play a role in this sorption process, and these factors might interact with each other. It should also be emphasized that the previous explanations, based on the physical and chemical properties of metal ions, are just hypotheses. Moreover, the nature of the sorbent surface could also play a role; some functional groups might have more preference for certain metal ions than others.

The relative affinity of the three sorbents for different metal ion systems may also be described by the binary selectivity index K_{ij} (Eq. (4.20); Part I), between pairs of ions i and j, that is often used to describe the selectivity of ion exchange resins. The K_{ij} values for various pairs of metal ions are given

in Tables 7.1-7.3. These allow the various affinities series to be identified depending on the system and the sorbent under consideration.

The inhibition of metal ion adsorption in the presence of other metal ions was also observed by other researchers. Norris and Kelly (1977) studied the accumulation of Zn^{2+}, Ni^{2+} and Co^{2+} on *S. cerevisiae*. They noticed that Ni^{2+} uptake was inhibited by Zn^{2+} and Mg^{2+} and also that the inhibition of Co^{2+} uptake by Ni^{2+} or Mg^{2+} was higher than that by Zn^{2+}. Shuttleworth and Unz (1993) found that the sorption of Zn^{2+} and Ni^{2+} in a mixture of the two metals by *Thiothrix* strain A1 was lower than when each of the metal ions were sorbed from individual solutions. They concluded that both of the metals mutually compete for the same sorption sites. Baldry and Dean (1980) reported that the presence of Fe^{3+} and Al^{3+} in the microbial suspension of an *Escherichia coli* strain almost completely prevented the subsequent uptake of Cu^{2+}. The authors also found that Ce^{3+} and La^{3+} were very effective in the reduction of Cu^{2+} sorption by the strain, but their action was significantly lower than that of Fe^{3+} or Al^{3+}, and next in the order of decreasing effectiveness came Y^{3+} and the divalent cations Cd^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Ni^{2+}, while the monovalent cations K^{+} and Na^{+} had a negligible effect. A recent study by Chong and Volesky (1996) for the sorption of the ternary system Cu^{2+}-Cd^{2+}-Zn^{2+} by *Ascophyllum nodosum* seaweed biomass, indicated a higher affinity of the sorbent for Cu^{2+} followed by Cd^{2+} and Zn^{2+}, however, the presence of one metal always decreased the uptake of the other, with respect to single metal sorption. The uptake of chromium and gold by the biomass of a green alga, *Halimeda opuntia*, and a brown alga, *Sargassum natans*, was not affected by the presence of Cu^{2+}, UO_2^{2+}, Pb^{2+} or Ni^{2+} (Kuyacak and Volesky, 1988b). The authors attributed this trend to the extraordinary selectivity of the biomass for the former metal ions.

To examine the interaction between Cu^{2+} and Ni^{2+} in the biosorption processes, other systems were also considered. Table 7.4 shows that Cu^{2+}, Zn^{2+} and Ni^{2+} have similar ionic radii. Therefore, it was interesting to see the sorption behavior of a combination of these three metal ions. The results obtained in the binary and ternary systems containing these metal ions using CM, bark and moss are presented in Tables 7.5-7.7, respectively.

The results obtained (Tables 7.5-7.7) by the three sorbents are consistent. Copper suppressed the uptake of Zn^{2+} and Ni^{2+}, however, the suppression of Ni^{2+} by Cu^{2+} seems to be stronger in the case of moss (Table 7.7) than in the case of bark (Table 7.6). This might be due to the higher electronegativity, lower coordination number and to the electron configuration of Cu^{2+} compared to Zn^{2+} and Ni^{2+} (Table 7.4). Tables 7.5-7.6 show that when Zn^{2+} and Ni^{2+} were in solution together, their uptakes were always lower than their uptakes when they were separate. This indicates that both ions

compete for the same adsorption sites. However, the nature of suppression was different than when Ni^{2+} was suppressed by Cu^{2+}; the uptake of both Ni^{2+} and Zn^{2+} were decreased in the binary system Zn^{2+}–Ni^{2+} while the uptake of Cu^{2+} was not influenced by the presence of Ni^{2+} in the binary system Cu^{2+}–Ni^{2+}.

In the ternary system, Cu^{2+}–Zn^{2+}–Ni^{2+}, no Ni^{2+} was sorbed by any of the three sorbents, and a much lower amount of Zn^{2+} uptake was observed using moss and bark than by using CM. The latter might be due to the high affinity of CM for Zn^{2+} (Table 7.5). It was also noted that the trend of the results from the ternary system of the three sorbents was consistent with the results of the three binaries for each sorbent (Tables 7.5-7.7), regardless of the level of the uptakes.

Table 7.5: Adsorption capacity of CM for metal ions from ion mixtures (Cu^{2+}, Ni^{2+} and Zn^{2+}). Initial concentration of each ion: 100 ppm; CM concentration: 9.2 mg/mL; initial pH 4; sorption time: 24 h

System	q_{mix} (mmol/g)	q_0 (mmol/g)	q_{mix}/q_0
(A) Cu^{2+}	0.125 (72.9)*	0.115 (67.2)	1.17
(B) Zn^{2+}	0.107 (64.3)	0.138 (82.9)	0.77
(A) Cu^{2+}	0.126 (73.7)	0.115 (67.2)	1.09
(B) Ni^{2+}	0.057 (31.1)	0.075 (40.3)	0.76
(A) Ni^{2+}	0.053 (28.5)	0.075 (40.3)	0.71
(B) Zn^{2+}	0.118 (70.8)	0.138 (82.9)	0.85
(A) Cu^{2+}	0.120 (72.2)	0.115 (67.2)	1.04
(B) Zn^{2+}	0.089 (53.3)	0.138 (82.9)	0.64
(C) Ni^{2+}	0.00 (0.0)	0.075 (40.3)	0.00

*Values in brackets represent the amount of metal removal (%).

Table 7.6: Adsorption capacity of bark for metal ions from ion mixtures (Cu^{2+}, Ni^{2+} and Zn^{2+}). Initial concentration of each ion: 100 ppm; bark concentration: 9.2 mg/mL; initial pH 4; sorption time: 24 h

System	q_{mix} (mmol/g)	q_0 (mmol/g)	q_{mix}/q_0
(A) Cu^{2+}	0.092 (46.0)*	0.108 (63.3)	0.85
(B) Zn^{2+}	0.021 (12.7)	0.077 (46.4)	0.27
(A) Cu^{2+}	0.082 (48.0)	0.108 (63.3)	0.76
(B) Ni^{2+}	0.054 (29.2)	0.060 (32.4)	0.90
(A) Ni^{2+}	0.029 (15.7)	0.060 (32.4)	0.48
(B) Zn^{2+}	0.047 (28.3)	0.077 (46.4)	0.61
(A) Cu^{2+}	0.072 (41.8)	0.108 (63.3)	0.67
(B) Zn^{2+}	0.01 (5.9)	0.077 (46.4)	0.13
(C) Ni^{2+}	0.00 (00.0)	0.060 (32.4)	0.00

*Values in brackets represent the amount of metal removal (%).

Table 7.7: Adsorption capacity of moss for metal ions from ion mixtures (Cu^{2+}, Ni^{2+} and Zn^{2+}). Initial concentration of each ion: 100 ppm; moss concentration: 9.2 mg/mL; initial pH 4; sorption time: 24 h

System	q_{mix} (mmol/g)	q_0 (mmol/g)	q_{mix}/q_0
(A) Cu^{2+}	0.121 (70.6)*	0.118 (68.7)	1.02
(B) Zn^{2+}	0.032 (19.6)	0.105 (63.7)	0.30
(A) Cu^{2+}	0.116 (68.0)	0.118 (68.7)	0.98
(B) Ni^{2+}	0.055 (30.0)	0.113 (61.0)	0.49
(A) Zn^{2+}	0.056 (34.2)	0.105 (63.7)	0.53
(B) Ni^{2+}	0.052 (28.5)	0.113 (61.0)	0.46
(A) Cu^{2+}	0.117 (68.5)	0.118 (68.7)	0.99
(B) Zn^{2+}	0.028 (17.0)	0.105 (63.7)	0.27
(C) Ni^{2+}	0.000 (00.0)	0.113 (61.0)	0.00

*Values in brackets represent the amount of metal removal (%).

7.3 Binary Equilibrium Isotherm

In the field of ion exchange, multicomponent systems have been studied extensively (Sengupta and Paul, 1985; Shallcross *et al.*, 1988). However, the only biosorption equilibrium study involving multi-ionic components is the one carried out by Chong and Volesky (1995, 1996) using *Ascophyllum nodosum* seaweed biomass. Therefore, binary component equilibria will be considered in this section using the three binary systems Cu^{2+}–Cd^{2+}, Cu^{2+}–Ni^{2+} and Cd^{2+}–Ni^{2+} and the sorbent pine bark. The binary data were obtained in batch equilibria sorption experiments using the standard batch equilibrium procedure described in Chapter 3 (Part I). Metal solutions containing combinations of either Cu^{2+}+Cd^{2+}, or Cu^{2+}+Ni^{2+}, or Cd^{2+}+Ni^{2+}, in the concentration range between 0 to 400 ppm of each metal ion, were prepared. An initial pH of 4 and a bark concentration of 9.2 mg/mL were used. For convenience, the equilibrium concentration of metal 1 and 2 will be designated as $C_e(M_1)$ and $C_e(M_2)$, respectively, which correspond to a final uptakes of $q(M_1)$ and $q(M_2)$, respectively.

Conventional single-metal sorption isotherms for Cu^{2+}, Cd^{2+} and Ni^{2+}, are presented in Figure 7.1. The Langmuir isotherm model (Eq. (4.1), Chapter 4; Part I), was used to fit the equilibrium data of each metal and the Langmuir parameters, K_L and b, were determined by fitting the model to the experimental data using the SCIENTIST package (Table 7.8). The model (Figure 7.1) fitted the experimental data of the three metal reasonably well. Table 7.8 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. It is obvious that the capacity of the bark for these metals follows the order: $Cu^{2+} > Cd^{2+} > Ni^{2+}$. Moreover, the K_d values indicate that

Ni^{2+} has much higher rate of desorption than Cd^{2+} and Cu^{2+}. This means that the latter two metals bind stronger to the sorbent than Ni^{2+}.

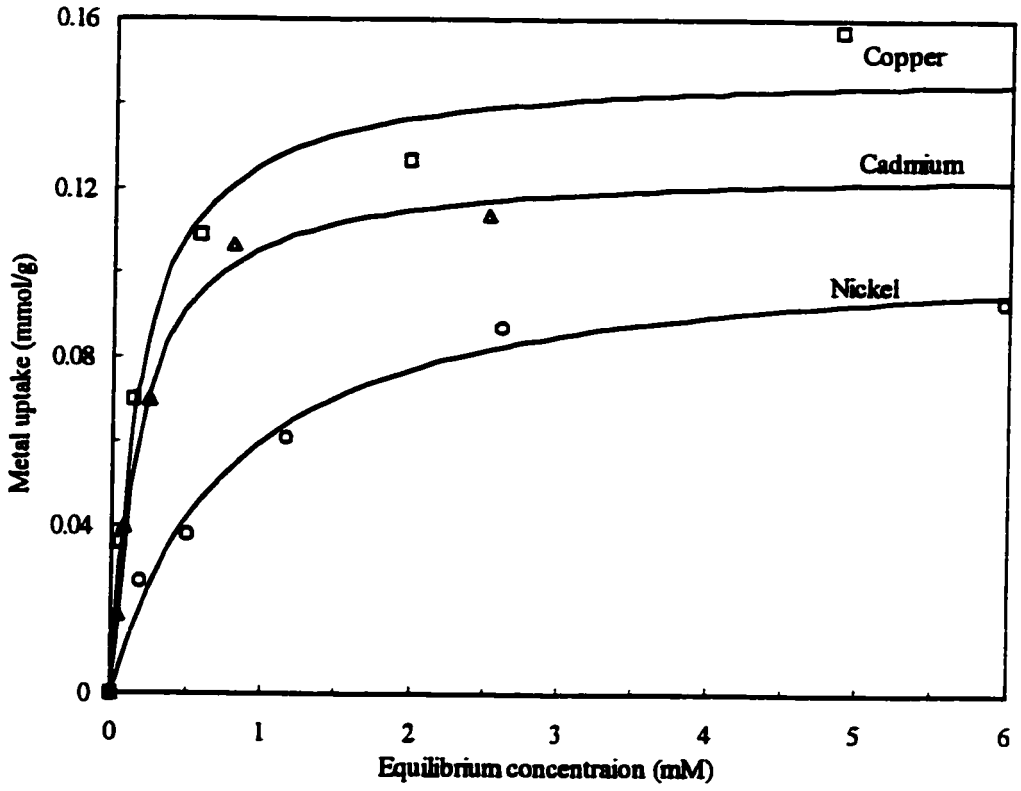

Figure 7.1: Sorption isotherms of Cu^{2+} (□), Cd^{2+} (Δ) and Ni^{2+} (O) by bark. Solid lines represent fitting data by the Langmuir model and the symbols are the experimental data. Bark concentration: 9.2 mg/mL; initial pH: 4.

Table 7.8: Langmuir constants for single-metal uptake.

Metal ion	K (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

The collected equilibrium data of the binary systems, Cu^{2+}–Cd^{2+}, Cu^{2+}–Ni^{2+} and Cd^{2+}–Ni^{2+}, was represented by three dimensional (3-D) sorption plots whereby the metal uptake was plotted as a function of the final equilibrium concentrations of the two metals. These 3-D plots for the uptake of metal 1, metal 2 and the sum of metal 1 + metal 2 are presented in Figures 7.2, 7.3 and 7.4 for the systems Cu^{2+}–Cd^{2+}, Cu^{2+}–Ni^{2+} and Cd^{2+}–Ni^{2+}, respectively.

The three binary component isotherm models, Eqs. (4.27)-(4.29), Chapter 4; Part I, were used to represent the equilibrium data of Figures 7.2-7.4. The parameters of these models were obtained by fitting Eqs. (4.27), (4.28) and (4.29) to the experimental data using the SCIENTIST package and are presented in Tables 7.9, 7.10, and 7.11, respectively. Based on the extended-Langmuir model, the K values of Cu^{2+} in the binaries Cu^{2+}–Cd^{2+} and Cu^{2+}–Ni^{2+} systems are lower (Table 7.9) than that obtained from the single Langmuir isotherm (Table 7.8). This is also the case for Cd^{2+} in the binary Cd^{2+}–Ni^{2+}. The K value for Ni^{2+} in the binary Cu^{2+}–Ni^{2+} system, obtained from extended-Langmuir model, is the same as that for the single system, while in the Cd^{2+}–Ni^{2+} system it is lower than that of the single Langmuir model. It should also be emphasized that the extended-Langmuir model gives reasonable estimates of multicomponent equilibrium data when the single-solute K values agree closely with the K values determined from the multicomponent models. The unequal K values for single metal sorption (Table 7.8) and those estimated for different binary systems (Tables 7.9-7.11) indicate that the solute occupied different amounts of surface area (Perry and Green, 1984). Comparison of the b values obtained from the binary systems (Table 7.9) with those of the single-metal systems indicates that the presence of one metal could decrease or increase the affinity of bark 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 Cu^{2+}–Cd^{2+}, while it is increased to 8.18 mM^{-1} in the binary system Cd^{2+}–Ni^{2+}. This indicates that the presence of Cu^{2+} decreases the affinity of bark for Cd^{2+} sorption, however, the presence of Ni^{2+} (instead of Cu^{2+}) increases the affinity of bark for Cd^{2+} sorption.

Table 7.9: Estimated parameters of the binary systems using Model 1, extended-Langmuir model, Eq. (4.27)

System	Component	Estimated parameters			Statistical parameters	
		K	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	

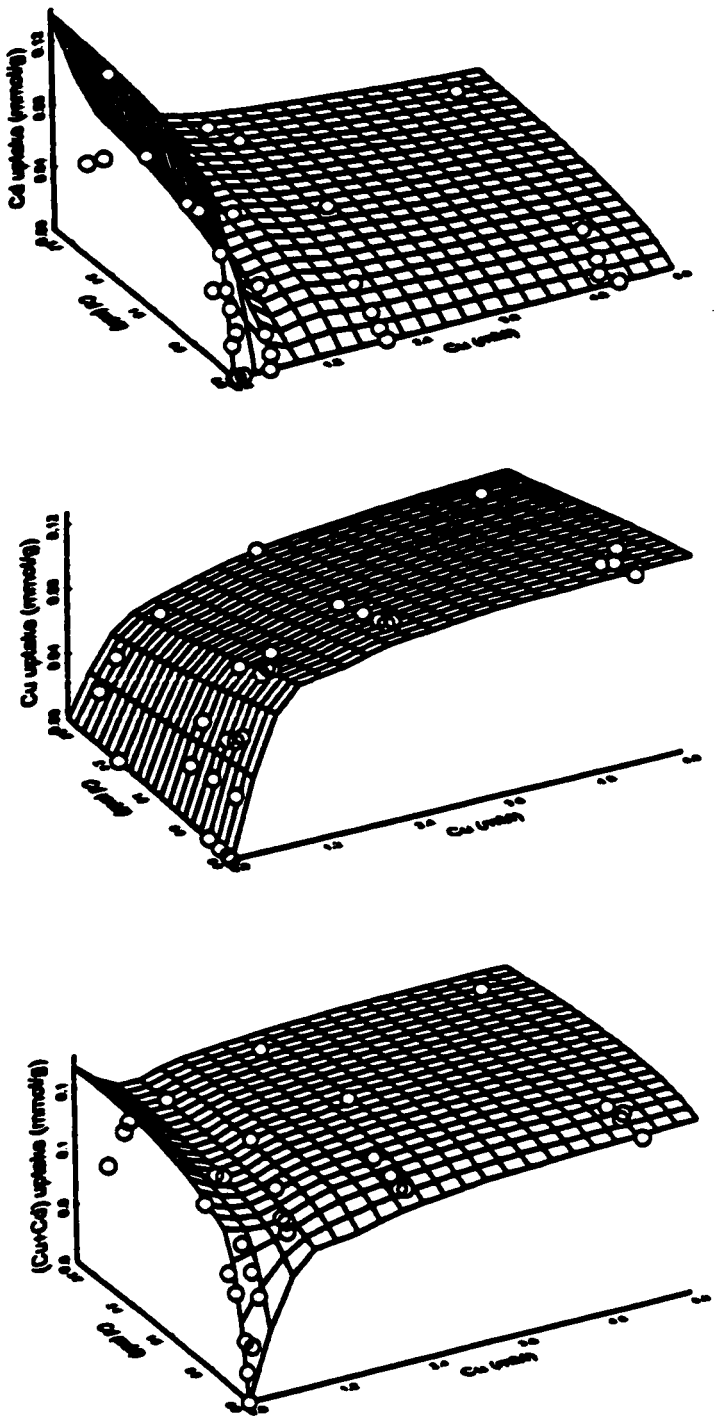

Figure 7.2: Binary sorption isotherms of Cu^{2+}–Cd^{2+} using pine bark. Symbols are experimental and the surfaces are fitting data by using the Langmuir-Freundlich model.

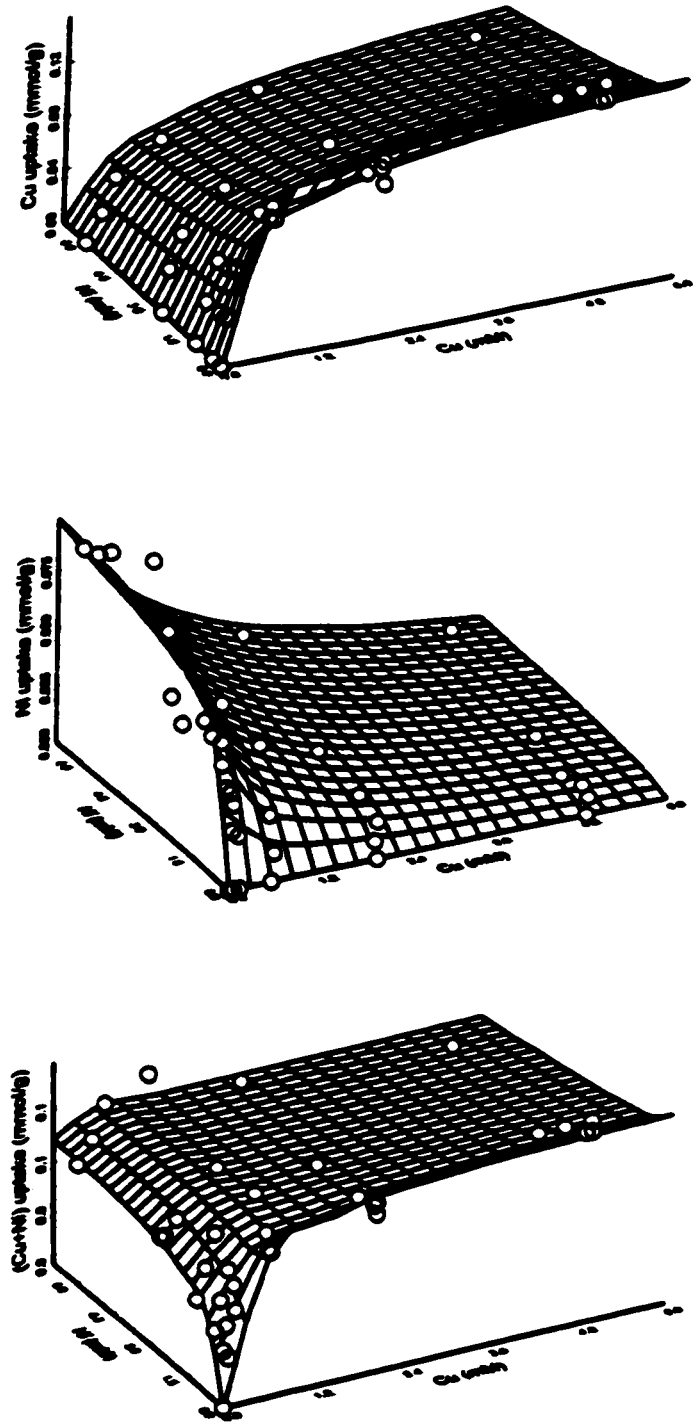

Figure 7.3: Binary sorption isotherms of Cu^{2+}–Ni^{2+} using pine bark. Symbols are experimental and the surfaces are fitting data by using the Langmuir-Freundlich model.

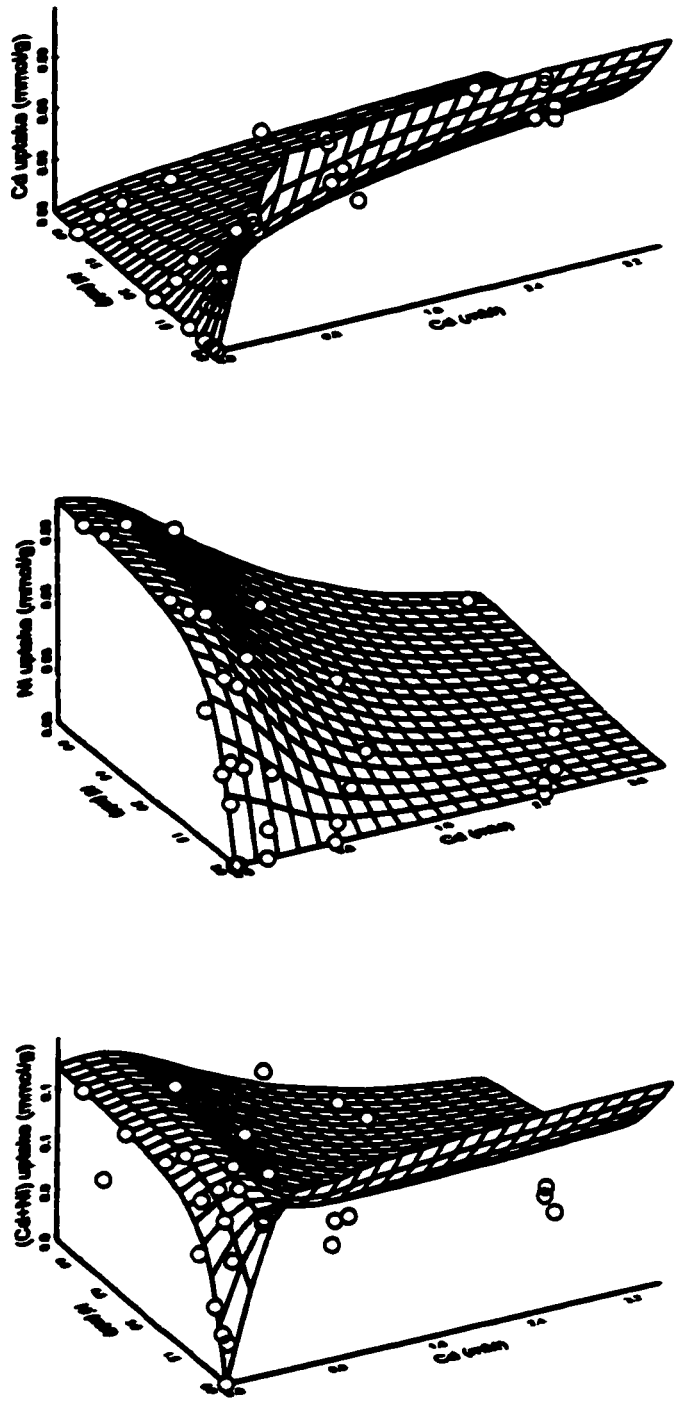

Figure 7.4: Binary sorption isotherms of Cd^{2+}–Ni^{2+} using pine bark. Symbols are experimental and the surfaces are fitting data by using the Langmuir-Freundlich model.

Table 7.10: Estimated parameters of the binary systems using Model 2, Eq. (4.28)

System	Component	Estimated parameters					Statistical parameters	
		K	b_1	b_2	n_1	n_2	SSR	MSC
Cu–Cd	Cu	0.094	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	

Table 7.11: Estimated parameters of the binary systems using Model 3, Eq. (4.29)

System	Component	Estimated parameters					Statistical parameters	
		K	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	

The comparison between the experimental data and those predicted by the three models (Eqs. (4.27)-(4.29), Chapter 4; Part I) can be presented in terms of the x-y diagrams as shown in Figure 7.5, 7.6 and 7.7 for the three systems Cu^{2+}–Cd^{2+}, Cu^{2+}–Ni^{2+} and Cd^{2+}–Ni^{2+}, respectively. Although some of the experimental data are scattered, generally, the three models can be used to represent these experimental data. In Figure 7.5, it is seen that 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 copper. This deviation of the Langmuir-like model from the experimental data in biosorption studies has been reported (Crist *et al.*, 1994; Ferguson and Bubela, 1974). Considering the other two models, it can be said that they represented the experimental data for lower concentrations better than the extended-Langmuir model. However, at higher concentrations of copper 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+} and Cd^{2+}–Ni^{2+} binary systems, Model 2 and Model 3, the modified extended-Langmuir and the Langmuir-Freundlich, respectively, represented the experimental data better than the extended Langmuir model (Figures 7.6 and 7.7). However, for the binary system Cd^{2+}–Ni^{2+} Models 2 and 3 exhibited more deviation at high concentrations than that of extended-Langmuir model (Figure 7.7).

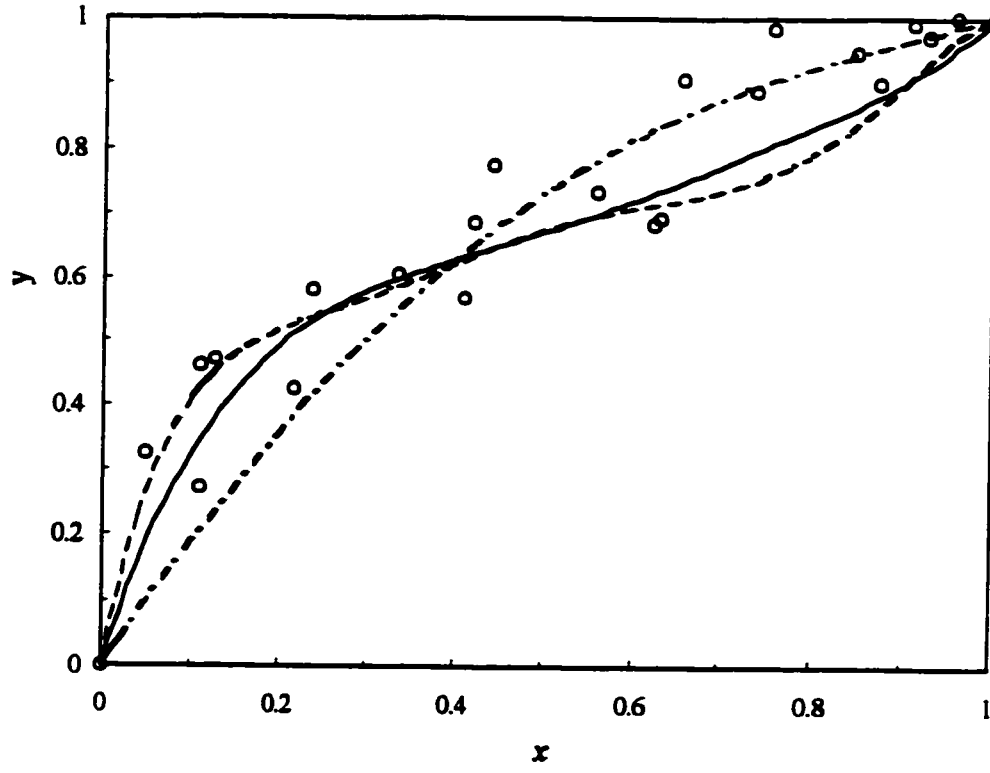

Figure 7.5: *x-y* diagram for the sorption of Cu^{2+} in the binary system Cu^{2+}-Cd^{2+} by pine bark. Symbols represent the experimental data and solid lines are fitting data by Model 1 (----), Model 2 (– – –) and Model 3 (—).

Discrimination among the three models can be performed based on the minimum Sum Square of Residuals (SSR). Application of Model 2 and Model 3 resulted in almost the same SSR for each binary system (Table 7.10 and 7.11), and these values were always less than those obtained by using extended-Langmuir model (Table 7.9). This is because of the extra two parameters, n_1 and n_2, in Model 2 and 3. Therefore, based on the SSR, Model 2 and Model 3 represent the experimental data of the binary systems better than the extended-Langmuir model (Model 1). Another criterion that can be used to measure the improvement achieved by an extended model is the use of the Model Selection Criterion (MSC). This factor (MSC) is defined by Micromath Scientist (1995) as:

$$\mathrm{MSC} = \ln\left[\frac{\sum_{i=1}^{n}\left(q_{exp,i} - \bar{q}_{exp}\right)^2}{\sum_{i=1}^{n}\left(q_{exp,i} - q_{calc,i}\right)^2}\right] - \frac{2P}{n} \qquad (7.1)$$

where q_{exp} is the experimentally measured uptake, q_{calc} is the predicted uptake by the model, $\overline{q_{exp}}$ is the mean of the total measured uptakes, n is the number of experimental data point and P is the number of parameters in the model. According to this criterion, the most appropriate model will be the one with the largest MSC. The MSC value was provided by the SCIENTIST program as one of the outputs from the regression analysis and are also shown for each system in Tables 7.9, 7.10 and 7.11 for Model 1, Model 2 and Model 3, respectively.

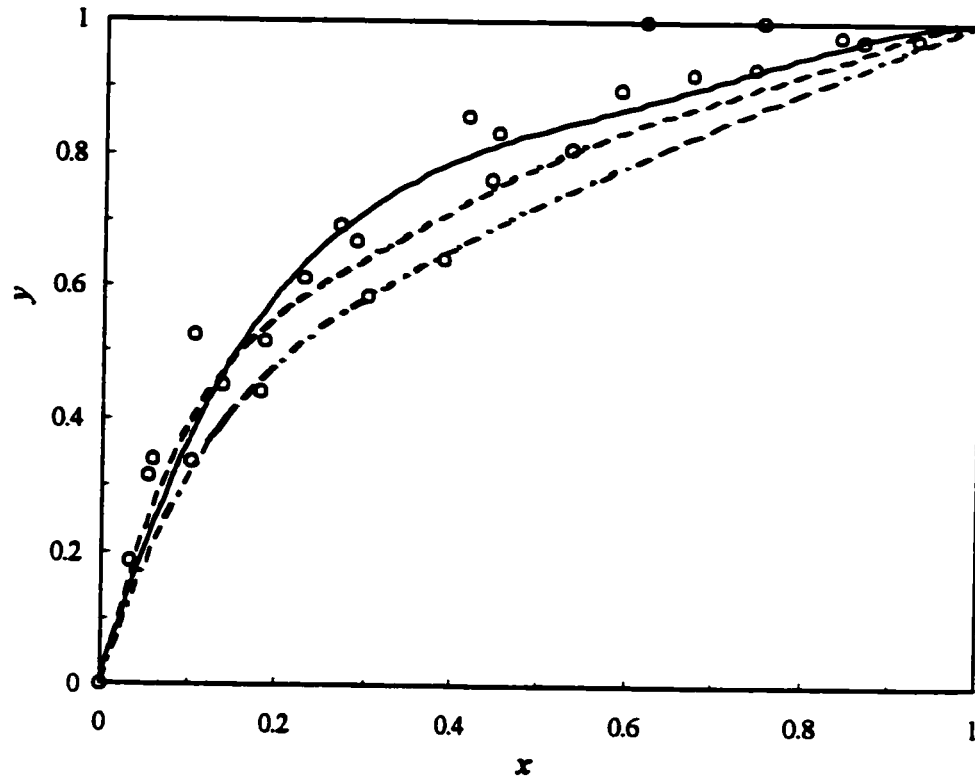

Figure 7.6: *x-y* diagram for the sorption of Cu^{2+} in the binary system Cu^{2+}-Ni^{2+} by pine bark. Symbols represent the experimental data and solid lines are fitting data by Model 1 (----), Model 2 (– – –) and Model 3 (—).

The results in Tables 7.9-7.11 indicate that the most improvement was achieved by using Model 2 and Model 3, compared to Model 1, when fitting these models to the experimental data of both components of the binary systems. Therefore, based on the MSC values in Tables 7.9-7.11, it can be also said that the utilization of five-parameter models is worthwhile for fitting the experimental data of the binary systems. It is also seen that the MSC values obtained by using Model 3 are always higher than those obtained by using Model 2. Therefore, utilization of Model 3 provided more improvement than that of Model 2.

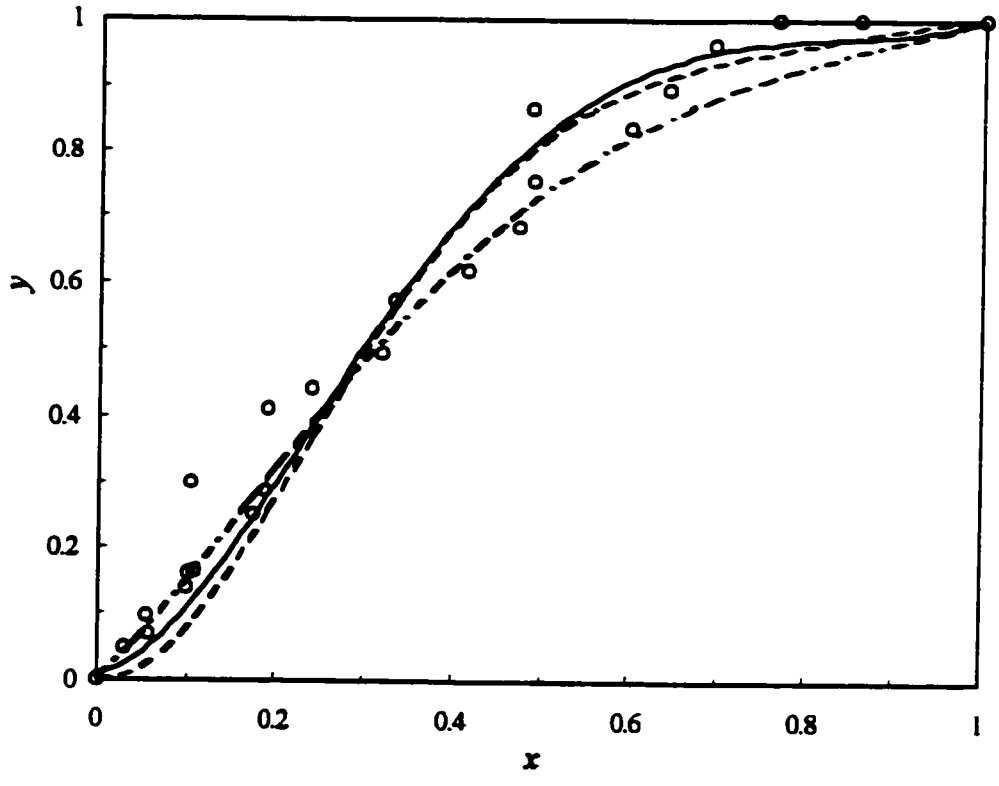

Figure 7.7: *x-y* **diagram for the sorption of Cd^{2+} in the binary system Cd^{2+}-Ni^{2+} by pine bark. Symbols represent the experimental data and solid lines are fitting data by Model 1 (----), Model 2 (– – –) and Model 3 (—).**

Based on the above statistical considerations, any of these three models can be selected to perform the 3-D sorption isotherm surfaces. However, Model 3 was selected to perform these surfaces, as presented in Figures 7.2-7.4, since it showed the largest improvement over the other models. These 3-D isotherm surfaces represent a summary of the two-metal equilibrium results, but are difficult to be examined. However, to obtain two-dimensional (2-D) sorption isotherm curves for one metal at constant final concentration(s) of the other metal, the 3-D sorption isotherm surface can be cut by the constant second-metal concentration plane (iso-concentration). Therefore, the selected cuts through the 3-D diagrams, as presented in Figure 7.8, better reveal the quantitative trends observed in the two-metal sorption systems. Moreover, the effect of the secondary metal on the uptake of the primary metal (Figure 7.9) can also be derived from Figure 7.8. The following discussion describes the trend of each binary system based on the results of Figure 7.8.

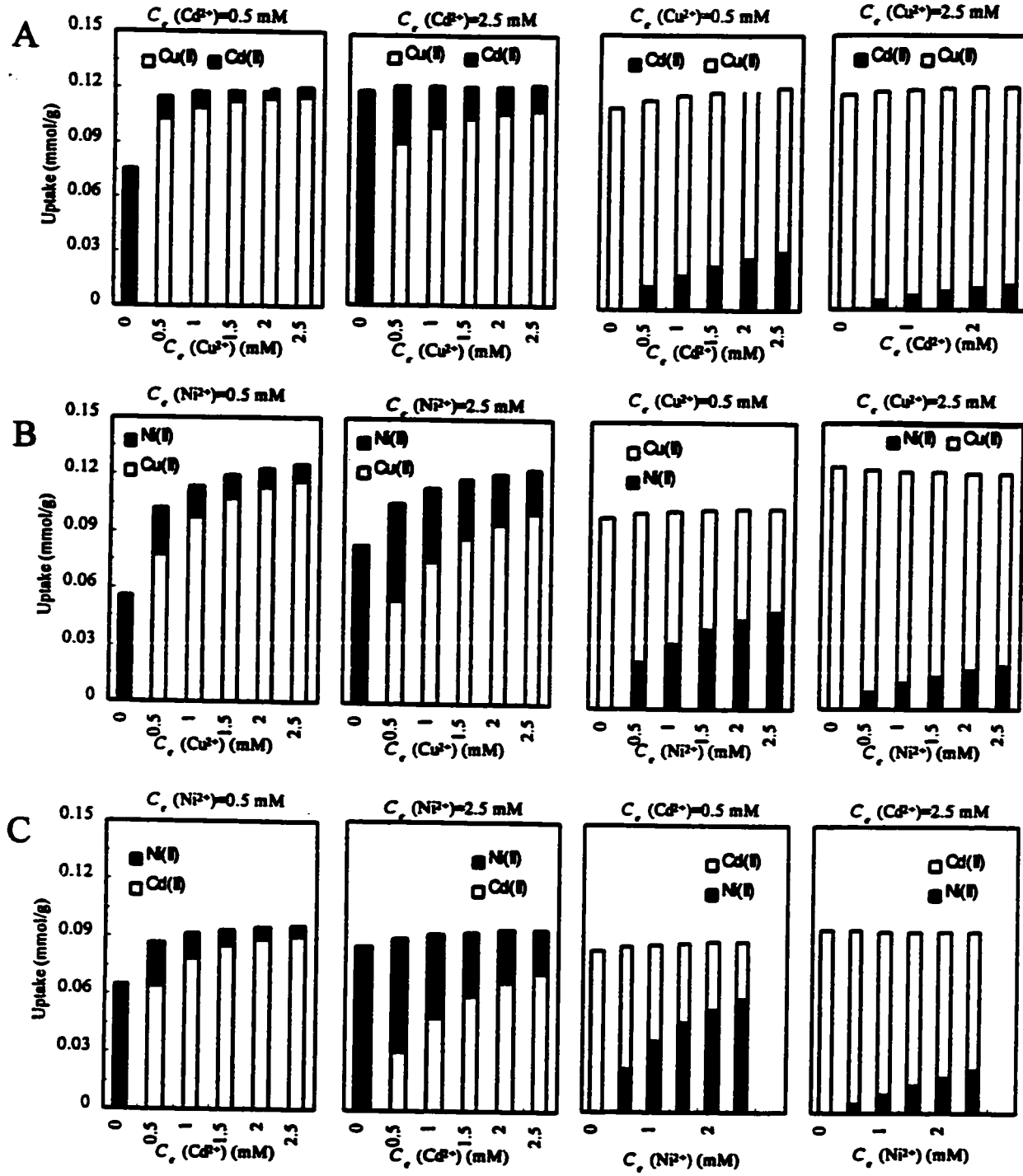

Figure 7.8: Iso-concentration cuts of the binary-systems sorption isotherm surfaces for bark. A: Cu^{2+}–Cd^{2+}; B: Cu^{2+}–Ni^{2+}; C: Cd^{2+}–Ni^{2+}.

Cu–Cd system

For a given equilibrium cadmium concentration, $C_e(Cd^{2+})$, as copper equilibrium concentration, $C_e(Cu^{2+})$, increased, the uptake of Cu^{2+} also increased (Figure 7.8 A). However, this increase became less noticeable at higher Cu^{2+} concentrations, possibly due to the saturation of the sites within the biosorbent. The total uptake of metals did not change significantly with increasing C_e of one metal when the C_e of the other metal was held constant. In general, the application of the Langmuir-Freundlich model indicated that the presence of one metal in the sorption system lowered the sorption capacity for the other metal. This is an apparent case of sorption competition.

At low equilibrium concentrations of Cu^{2+} and Cd^{2+} (0.5 mM of each), bark demonstrated a preference for sorbing Cu^{2+} over Cd^{2+}, whereby, 89% of the total metal uptake of 0.115 mmol/g, was contributed by Cu^{2+}. At high concentrations (2.5 mM of each), the total metal uptake increased to 0.122 mmol/g, of which 89% was contributed by the uptake of Cu^{2+}. This indicates that the contribution to Cu^{2+} uptake is the same at low and high Cd^{2+} concentrations. At high concentration of Cu^{2+} ($C_e(Cu^{2+})$=2.5 mM), although the inhibition of Cu^{2+} sorption was being compensated by the Cd^{2+} uptake, the total uptake did not increase significantly. Overall, the preference of the bark for sorbing Cu^{2+} over Cd^{2+} was apparent over the entire concentration range.

Cu–Ni system

The equilibrium data (iso-concentration cuts) of the Cu^{2+}–Ni^{2+} system are presented in Figure 7.8B. As the equilibrium concentration of one of the metal ions increased, both the total metal uptake and the uptake of that metal increased when C_e of the other metal held constant (Figure 7.8 B). The exception is clearly seen when $C_e(Cu^{2+})$=2.5 mM (last segment of Figure 7.8 B), where the increase in the $C_e(Ni^{2+})$ did not increase the total uptake. This might be due to much higher affinity of Cu^{2+} to bark compared to Ni^{2+}. The last segment of Figure 7.8B also shows that the total uptake of metals was the same as the uptake of Cu^{2+} when it was alone. At $C_e(Cu^{2+})$=0.5 mM, the increase in the Ni^{2+} concentration almost did not change the total uptake of metals; the total uptakes are almost the same as when Cu^{2+} was alone. The reason could be due to the interference of the Ni^{2+} ions, since the increase in Ni^{2+} concentration decreased the possibility that Cu^{2+} ions (at low concentration) could come into contact with sorption sites.

When the equilibrium concentrations of Cu^{2+} and Ni^{2+} were 0.5 mM (first segment of Figure 7.8B), about 75% of the total metal uptake was due to Cu^{2+} uptake; while when the equilibrium concentrations were 2.5 mM (second segment of Figure 7.8B), about 82% of the total metal uptake was contributed by Cu^{2+} uptake. Again, bark exhibited a net preference for the Cu^{2+} ions over Ni^{2+}

ions. However, when comparing the Cu^{2+}–Cd^{2+} and Cu^{2+}–Ni^{2+} systems, the uptake of Cu^{2+} was more susceptible to interference from Ni^{2+} than from Cd^{2+}.

Cd–Ni system

The equilibrium data (iso-concentration cuts) of the Cd^{2+}–Ni^{2+} system are shown in Figure 7.8C. The total uptake of this system was the lowest among the three systems examined, since each metal inhibited the sorption of the other. At $C_e(Ni^{2+})$=0.5 mM, the total uptake increased when the final Cd^{2+} concentration increased, and also at $C_e(Ni^{2+})$=2.5 mM, the total uptake slightly increased when $C_e(Cd^{2+})$ increased. However, at $C_e(Cd^{2+})$=0.5 and 2.5 mM, with an increase in the equilibrium concentration, the total uptake was almost the same as the uptake of Cd^{2+} when it was alone. When the equilibrium concentrations were both 2.5 mM (second segment of Figure 7.8C), Cd^{2+} uptake accounted for 76% of the total metal uptake.

The metal preference exhibited by bark can be also exemplified by Figure 7.9. For the following discussion, two concentrations of metal ions have been arbitrary chosen to represent "low" (0.5 mM) and "high" (2.5 mM) equilibrium concentrations. Figure 7.9A shows that at low or high equilibrium concentrations of Cu^{2+}, its uptake was reduced by 7% or 3%, respectively, of the initial value when the equilibrium concentration of Cd^{2+} was 0.5 mM. However, this reduction was increased to 18% and 8.0% when the equilibrium Cd^{2+} concentration reached a value of 2.5 mM for the low and high Cu^{2+} concentrations, respectively. About 45% of the initial Cu^{2+} uptake was decreased by the presence of 2.5 mM of Ni^{2+} at low concentration of Cu^{2+}.

The uptake of Cd^{2+} at low concentration of Cd^{2+} was much more sensitive to the presence of Cu^{2+} than Ni^{2+}. At low Cd^{2+} concentration (Figure 7.9B), about 83% of the initial uptake of Cd^{2+} was decreased when the final Cu^{2+} concentration exceeded 0.5 mM, while only about 25% of the initial Cd^{2+} uptake was reduced by the presence of 0.5 mM of final Ni^{2+} concentration.

Nickel exhibited the highest reduction of its initial uptake due to the presence of co-cations (Figure 7.9C), compared to the other metal ions (Figures 7.9A and B). The highest reduction of 92% was noticed in the presence of 2.5 mM of Cd^{2+} concentration, at a low Ni^{2+} concentration, and about 73% reduction due to the presence of 2.5 mM of Cd^{2+} at high Ni^{2+} concentration.

The influences of co-cations shown above could be related to the mechanism of metal biosorption. The complexity of the mechanism of biosorption, which could be any of, or a combination of, these processes: complexation, ion exchange, physical adsorption, or organic microprecipitation of metal, makes it difficult to explain such influences. However, it could also be related to the physical or

chemical properties of each of the considered metal ions, which, as mentioned previously, are difficult to standardize.

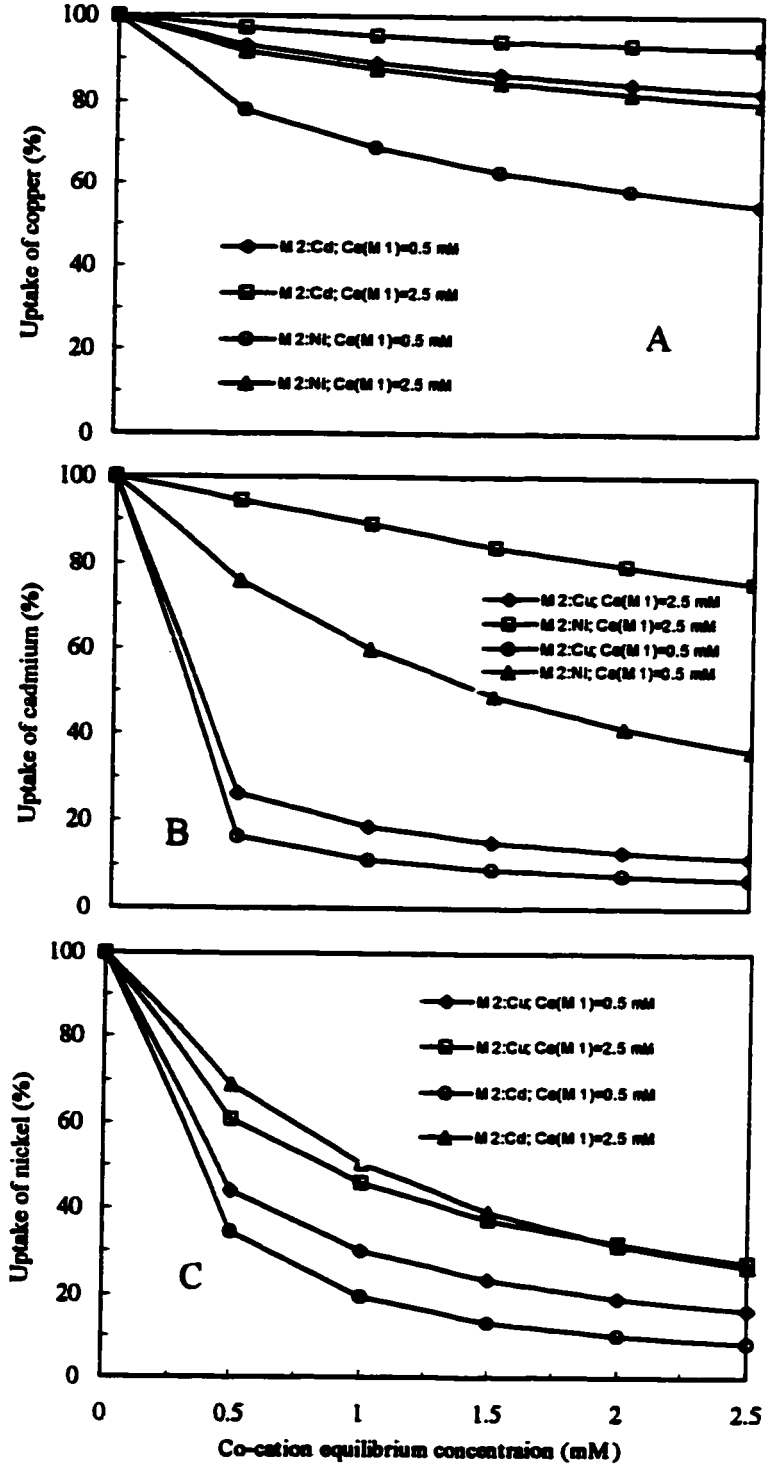

Figure 7.9: Effect of co-cation (M2) concentration on the uptake of the primary cation (M1). C_e(M1) and C_e(M2) represent the equilibrium concentrations of the primary cation and co-cation, respectively.

It can be concluded from the results of this chapter that the selectivity for single metal sorption of CM, bark and moss for Cu^{2+}, Ni^{2+}, Cd^{2+} and Pb^{2+} was as follows: Cu^{2+} and Ni^{2+}: moss > CM > bark; Cd^{2+}: CM > moss > bark; Pb^{2+}: CM = bark ≈ moss. In a mixture of metal ions, the presence of one metal influenced, competing or excluding, the uptake of the other metal, depending on the chemical and physical properties of each metal in the solution. The uptake of Ni^{2+}, by each of the three sorbents, decreased significantly when Cu^{2+} was present in the solution, especially in ternary and quaternary metal systems. When the four metals existed together in a quaternary mixture, the following order (molar basis) of preference was obtained: $Cu^{2+} > Pb^{2+} > Cd^{2+} > Ni^{2+}$, for bark and moss, while for CM the order was; $Cu^{2+} > Cd^{2+} > Pb^{2+} > Ni^{2+}$. The collected binary equilibrium data, using pine bark, were represented by the extended-Langmuir, the modified extended-Langmuir and the Langmuir-Freundlich multicomponent equilibrium models. Although all of the three models resulted in a good fitting of the experimental data, the Langmuir-Freundlich model showed the best representation.

Chapter 8

Investigation of the Mechanisms of Sorption

8.1 Introduction

The removal of metal ions from aqueous solutions by biological materials involves several mechanisms. In some cases only one mechanism may be involved, while in other cases, two or more mechanisms may take place in the metal removal process. Since, in general, biosorbents are complex materials, it can be assumed that in the majority of cases several mechanisms of sorption participate in this process.

In this chapter, it will be attempted to interpret the mechanisms of the removal of metal ions by the plant materials used in this work. The following will be taken into consideration: the composition of the materials and the sorption fraction contributed to each constituent, the blocking of some functional groups, the release of ions to the solution (exchange mechanism), the electron microscopy and X-ray analyses, and the electron spin resonance spectra. Moss, canola meal and pine bark were used for this study because they are the sorbents that were used extensively during the course of this work.

8.2 Compositional Analyses

The analysis of the composition of the sorbents helps to identify the contribution of each constituent for its metal binding capacity. The elemental and proximate analyses of CM, moss and bark are presented in Table 8.1 along with the amount of copper uptake by each of these sorbents. The elemental and proximate (protein, carbohydrates, lipids and pigments, and ash) analyses for other plant sorbents considered in this work are given in Appendix B.

The elemental analysis of the three materials shows (Table 8.1) that the carbon content of these materials is very high, which is a characteristic of organic matters. Bark contains more carbon than CM or moss. The hydrogen content is almost the same in the three sorbents. It is also seen that canola meal contains a much higher amount of nitrogen than moss or bark. The remaining elements in the materials could be contributed by oxygen, phosphorus, sulfur and various minerals. The sum of the components in the proximate analysis did not equal to 100%. This might be due to the incomplete extraction of some of the components from the material or due to the contents of some other components in the material that were not considered in the analysis presented in Table 8.1. Canola

meal, for example, contains phytic acid (about 4.7%), crude fibre (11-15%) and some phenolic acids which were not considered in the proximate analysis (Table 8.1).

Table 8.1: Elemental and approximate analyses of CM, moss and bark

Sor-bent	Elemental analysis			Proximate analysis								[1]Cu upta
	C (%)	H (%)	N (%)	Prot-eins (%)	Carbohydrates			Lipids &pig-ments (%)	Lign-in (%)	Ash (%)	Solu-ble suga-rs (%)	(mg/
					cellu-lose (%)	xylose (%)	arab-inose (%)					
CM	[2]43.6 ±0.00	5.83± 0.021	5.94± 0.035	37.2± 0.219	11.4± 0.127	3.60± 0.042	4.00± 0.028	3.52± 0.256	3.1	2.51± 0.049	0.45	9.7 0.32
Moss	43.1± 0.283	5.43± 0.028	1.18± 0.127	7.37± 0.799	24.7± 0.346	6.37± 0.052	2.71± 0.081	1.92± 0.078	42.2	6.96± 0.294	0.38	9.5 0.28
Bark	51.8± 0.495	5.72± 0.026	0.29± 0.026	1.80± 0.162	22.8± 0.063	2.84± 0.111	2.78± 0.106	6.13± 0.297	38.0	1.84± 0.077	0.28	8.4 0.29

[1] Uptake using initial Cu^{2+} concentration of 100 ppm; sorbent concentration: 4.6 mg/mL; initial pH: 4.5.
[2] Values represent average of duplicates ± standard deviation

The compositional analyses of the moss and bark (Table 8.1) show that these materials contain mainly lignin and cellulose which possess negatively charged groups (Marshall *et al.*, 1993; Friedman and Waiss, 1972). Therefore, the binding of copper to these two materials would be mainly contributed to their lignocellulosic constituents. However, CM is a proteineous material (37.2% proteins) and also contains considerable amounts of carbohydrates. The binding of copper to CM might be attributed to its proteins and carbohydrates contents. In addition, its 4.7% phytic acid content should not be ignored since this compound has a strong metal binding ability (Clandinin, 1986). Moss binds more Cu^{2+} ions than bark (Table 8.1); this might be due to its higher protein content than that of bark, because it has been already reported that proteineous matters participate in the sorption process.

Considering the relatively high content of lipids and pigments in bark (Table 8.1), it was expected that the participation of these compounds in the sorption process would be higher in this material than in CM or moss. The contribution of lipids and pigments to metal sorption was verified experimentally by loading each of the sorbent materials with copper ions and fractionating the loaded material into two parts, using isopropanol, according to the procedure for lipids analysis (Part I: Section 3.5.1). The first fraction, the solid fraction, contained proteins, lignin, carbohydrates and ash, while the second fraction, the filtrate, contains lipids, pigments, amino acids, soluble sugars and soluble salts. The filtrate was further fractionated, using a mixture of chloroform and methanol, into another two fractions: the first containing lipids and pigments and the second containing soluble sugars, amino acids and salts. The latter two fractions were evaporated and the residues were treated with 0.1 M HCl

to release the Cu^{2+} ions content. The fraction containing proteins, carbohydrates and lignin was also treated with 0.1 M HCl to release its copper content. The copper content in each fraction was determined for each sorbent, CM, moss and bark (Table 8.2).

Table 8.2: Contribution of the different constituents of CM, moss and bark to copper sorption, based on lipid analysis[1]

Component	CM[2]		Moss[3]		Bark[4]	
	Composition (%)	Cu^{2+} sorption (%)[5]	Composition (%)	Cu^{2+} sorption (%)	Composition (%)	Cu^{2+} sorption (%)
Protein + lignin + carbohydrates + ash	95.85	97.9	98.04	98.4	93.8	98.0
Lipids and pigments	3.70	0.42	1.58	0.11	5.9	1.85
Soluble sugars, amino acids and salts	0.45	0.10	0.38	0.04	0.28	0.11

[1] Sorption at initial pH of 4.5 and 4 mg/mL sorbent concentration. [2] Copper uptake by pure CM was 17.6 mg/g. [3] Copper uptake by pure moss was 11.2 mg/g. [4] Copper uptake by pure bark was 9.6 mg/g. [5] Percent of the uptake of pure material.

The results of Table 8.2 show that the contribution of each constituent to copper sorption varies from sorbent to another. In the case of CM, most of the copper sorption is contributed to the protein and lignocellulosic constituents of the meal, followed by lipids and pigments, and then soluble sugars, amino acids and salts. The participation of soluble sugars and amino acids in the sorption of copper by CM is very limited, due to their low content in the meal. Moss and bark follow the same trend as that of CM. However, the contribution of lipids and pigments to copper uptake in bark is greater than that of CM, followed by moss. This is due to the higher content of these compounds in bark (5.9%) than in CM (3.7%) or moss (1.6%). Therefore, it can be said that the contribution of copper sorption to lipids and pigments increases with an increase in the content of these compounds in the sorbent.

The contribution of the proteins, insoluble carbohydrates, lignin and ash, individually, to metal sorption was investigated by hydrolyzing the sorbents into each of these components following the lignin analysis described previously (Part I: Section 3.5.4). This procedure is illustrated in Figure 8.1, where the different constituents of the sorbent were separated at different stages. The sorption of copper was carried out with the residual fraction from each stage, and the difference in the uptake between each treatment was taken as the amount of the uptake contributed to the constituent that was separated. The uptake by pure material was designated as A; the difference between the uptake of fractions B and A was the uptake contributed to the lipids and pigments; the difference between the

uptake of fractions C and B was the uptake due to proteins; the difference between the uptake of fractions D and C was the uptake due to carbohydrates; the difference between the uptake of fractions E and D was the uptake due to lignin; and finally E represented the uptake by ash. This procedure was applied to the sorbents CM, moss and bark. The results of the copper uptake contributed by the different constituents of these three sorbents are presented in Tables 8.3, 8.4 and 8.5, respectively, (sample calculation is presented in Appendix C).

The results of Tables 8.3-8.5 show some disagreements with the previous results (Tables 8.1 and 8.2). It should be emphasized that the components separated following the procedures of Figure 8.I, could be associated with the separation of some other components. This procedure is normally used for lignin determination and not for the determination of the other components listed in Tables 8.3-8.5. However, the procedure was used in this work to estimate, approximately, the degree of participation of lipids and pigments, proteins, carbohydrates, and lignin in the sorption process. That was the reason for the discrepancies between the results of Tables 8.3-8.5 and Tables 8.1 and 8.2.

Table 8.3: Amount of copper sorption contributed to the different constituents of CM following lignin analysis[1]

Component separated (A)	Amount of A in sorbent (%)	Cu^{2+} uptake	
		(mg/g_{CM})	(mg/g_A)
Lipids and pigments	3.4	0.34	10.0
Components released by pepsin treatment	73.5	7.47	10.2
Carbohydrates	18.2	2.03	11.1
Lignin	3.1	0.38	12.2
Ash	2.1	0.095	4.4

[1] Calculations using a basis of 1 g CM. Sorption using sorbent concentration: 4.6 mg/mL; initial copper concentration: 50 ppm; pH controlled at 4.5. Uptake of pure CM: 9.7 mg/g.

Table 8.4: Amount of copper sorption contributed to the different constituents of moss following lignin analysis[1]

Component separated (A)	Amount of A in sorbent (%)	Cu^{2+} uptake	
		(mg/g_{moss})	(mg/g_A)
Lipids and pigments	2.47	0.49	19.8
Components released by pepsin treatment	14.6	0.16	1.10
Carbohydrates	34.4	3.78	11.0
Lignin	42.2	2.90	6.90
Ash	6.20	0.58	9.40

[1] Calculations using a basis of 1 g moss. Sorption using sorbent concentration: 4.6 mg/mL; initial copper concentration: 50 ppm; pH controlled at 4.5. Uptake of pure moss: 9.5 mg/g.

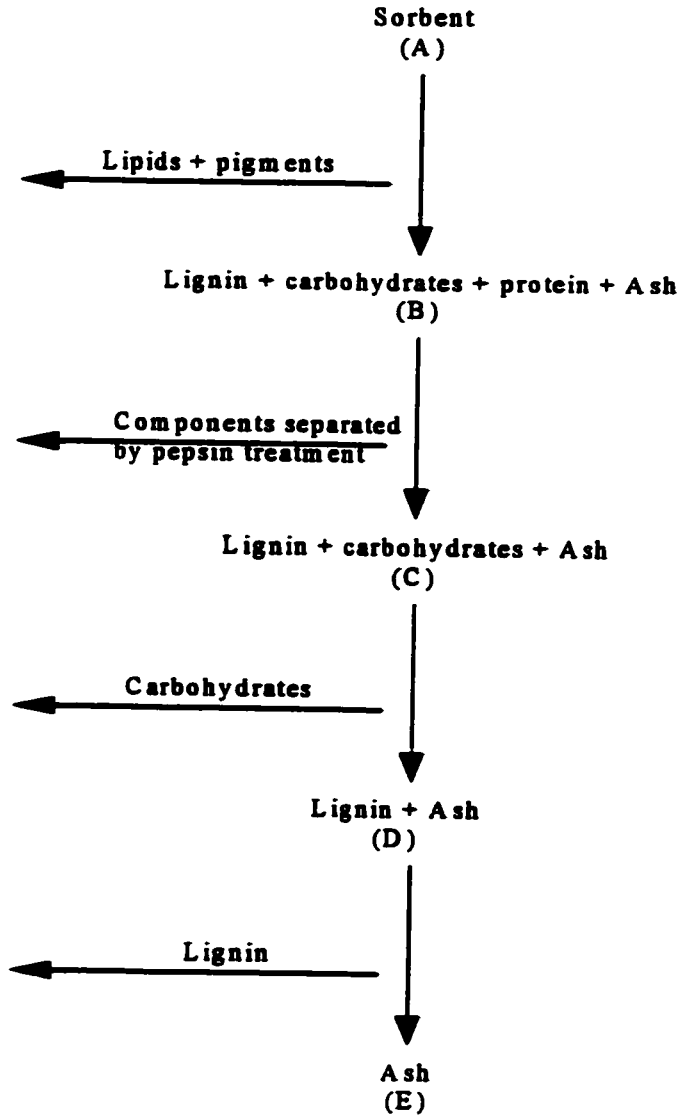

Figure 8.1: Flow diagram for the hydrolysis of different constituents of the plant sorbents.

Table 8.5: Amount of copper sorption contributed to the different constituents of bark following lignin analysis[1]

Component separated (A)	Amount of A in sorbent (%)	Cu^{2+} uptake	
		(mg/g_{bark})	(mg/g_A)
Lipids and pigments	7.3	0.70	9.6
Components released by pepsin treatment	6.5	0.26	4.0
Carbohydrates	46.0	3.38	7.3
Lignin	38.0	3.30	8.7
Ash	2.1	0.09	4.3

[1] Calculations using a basis of 1 g bark. Sorption using sorbent concentration: 4.6 mg/mL; initial copper concentration: 50 ppm; pH controlled at 4.5. Uptake of pure bark: 8.4 mg/g.

The amounts of metal uptake (Tables 8.3-8.5) contributed by each constituent was expressed on two bases. The first base considered the amount of metal uptake by the separated component expressed per gram of sorbent, mg/g_{CM}, in order to compare the uptake due to each component on the same basis. The other base considered the metal uptake by the separated component expressed per gram of that component, mg/g_A, in order to compare the adsorption capacities of the constituents.

Examination of the results of CM showed that most of the meal constituents were released during the pepsin treatment (Table 8.3). This treatment is supposed to release only proteins, however, because of the high solubility of CM, some other components were also released under the acidic condition (0.1 M HCl) of this treatment. As a result, the amount of 73.5% should be referred to as proteins and some other contents of the meal. The amount of carbohydrates (18.2%) in CM is in a closer agreement with the amount of carbohydrates (19.0%) determined previously (Table 8.1). Comparing the amount of Cu^{2+} uptake per unit weight of CM, it is seen (Table 8.3) that most of the sorption was due to proteins, and other components released in the pepsin treatment, and carbohydrates. This was expected, because these components are the most abundant meal constituents. It was also noted (Table 8.3) that the amount of Cu^{2+} sorption contributed by the proteins was higher than that of carbohydrates, which was due to the higher contents of the former component in the meal. The other components, lipids and pigments and lignin, contribute to almost the same level of Cu^{2+} sorption. However, per unit weight of the component, all of the components, except ash, showed almost the same sorption capacities. This might indicate that the environment for metal sorption was the same in each constituent.

In the case of moss, most of the Cu^{2+} sorption was contributed by the carbohydrates and lignin, which are the main components of the material, when the uptake was expressed per gram of moss (Table 8.4). The results also showed that the amount of Cu^{2+} sorption due to carbohydrates was higher than that of lignin, even though the material contains more lignin than carbohydrates. This could indicate that the environment for copper sorption was different for each component, since when the metal uptake was expressed per unit weight of each component, it was found that the uptake per gram of carbohydrates was higher than that per gram of lignin (Table 8.4). The lipids and pigments content in moss is lower than that of CM. The capacity of Cu^{2+} sorption (per unit weight of lipids and pigments) in moss was higher than that per unit weight of the same fraction in CM. This indicates that the chemical composition of this fraction in these two sorbents is different. The results of Table 8.4

also show that the proteins and other components separated during pepsin treatment of moss did not have a significant contribution to this sorption process.

Analysis of the results for bark (Table 8.5) showed that the uptake by each component per unit weight of the pure sorbent followed a similar trend to that noticed in moss. This material is also mainly composed of carbohydrates and lignin, and therefore, most of the Cu^{2+} uptake was contributed by these components. It is also seen (Table 8.5) that the carbohydrates and lignin adsorbed approximately the same amounts of copper. However, when the results were expressed per unit weight of the separated component (Table 8.5), the lipids and pigments showed the highest uptake followed by lignin, carbohydrates, ash and then proteins.

It can be concluded from the previous results that proteins and carbohydrates in the case of CM, and carbohydrates and lignin in the case of moss and bark are the main components responsible for metal sorption. It can also be said that the sorption capacity of each component depends on its chemical composition, which can vary from one sorbent to another.

8.3 Blocking of Functional Groups

It has been mentioned that the sorbents used in this work contain mainly proteins, carbohydrates and lignin in addition to lipids and pigments. This diversity is reflected by the presence of distinct potential metal complexation sites, e.g., carboxyl, sulphate, phosphate and amine groups. The roles of the carboxyl and amine groups in the sorption process were considered in this work.

To understand the involvement of the carboxyl groups in the sorption by the plant materials, the sorbent's carboxyl groups were chemically blocked by methanol esterification. If binding of metal occurs through interaction with carboxyl groups, a chemical modification rendering the carboxyl groups unavailable for metal binding should cause a marked reduction in the metal binding. Esterification was carried out in the presence of trimethoxymethane to force the equilibrium towards esterification with methanol (Gardea-Torresdey *et al.*, 1996b). The esterification reaction is shown below:

$$R\text{-}COOH + HO\text{-}CH_3 \xrightarrow[63^{\circ}C]{(CH_3O)_3CH} R\text{-}COOCH_3 + CH_3COOH + 2HO\text{-}CH_3 \qquad (8.1)$$

Experiments were conducted on esterified CM, moss and bark to determine whether the modification of the sorbents could alter Cu^{2+} binding characteristics. These studies were performed

using Noranda wastewater, having a copper concentration of 36.5 ppm, and sorbent concentration of 4.6 mg/mL. The pH was controlled at pH 5.2, during the 2 hour sorption process by addition of microdroplets of 1 N NaOH, as required. The results (Table 8.6) showed that the binding of Cu^{2+} by CM, moss and bark after the chemical modification of the carboxyl groups was reduced (32.5, 22.2 and 16.4%, respectively) by this treatment. However, after esterification there was still 67.5, 77.8, and 83.6% of Cu^{2+} bound by CM, moss and bark, respectively. This implies, assuming that all the carboxyl groups were esterified, that the carboxyl groups were not completely responsible for metal binding, and other groups such as amine and hydroxyl groups may also be involved in metal binding. This could also imply that mechanisms other than the complexation with the functional groups could be involved in the sorption process. The results also indicated that the carboxyl groups of CM bound more Cu^{2+} ions than those of moss and bark. The reduction in the metal uptake due to the esterification of the carboxyl groups was also reported by other researchers using different type of biosorbents. Gardea-Torresdey *et al.* (1996a) obtained a 25-30% reduction in Cu^{2+} binding by the esterified biomass of *Mucor rouxii*. Gardea-Torresdey *et al.* (1996b) investigated the copper binding capacity by esterified *Sphagnum* peat moss, and esterified humic acid and humin extracted from the peat moss. They noticed a decrease in the Cu^{2+} binding ability of these esterified materials by 20%. Fourest *et al.* (1996) demonstrated that the blocking of the carboxyl groups of the algal *Sagassum fluitans* resulted in an 80% reduction of the metal ion binding. However, Tobin *et al.* (1990) reported only a 5% reduction in the uptake of La^{3+} and Zn^{2+} by the esterified *R. arrhizus*, compared to the un-esterified biomass.

Table 8.6: Adsorption of copper from industrial wastewater* by esterified moss, bark, and CM using methanol and trimethoxymethane

Sorbent	Control		Esterified sorbent	
	Copper uptake (mmol/g)	Final pH	Copper uptake (mmol/g)	Final pH
Moss	0.101	5.2	0.078	5.2
Bark	0.100	5.2	0.083	5.2
CM	0.080	5.2	0.054	5.2

*Initial copper concentration: 36.5 ppm; sorbent concentration: 4.6 mg/mL; pH controlled at 5.2.

To demonstrate that the modification of the carboxyl groups had indeed occurred and that the decreased copper binding by the plant sorbents was not due to the destruction of the sorbents during esterification, copper binding was studied after base hydrolysis of the esterified groups. The role of the carboxyl groups in the sorption process would be confirmed if the Cu^{2+} binding ability of the modified sorbents is regained after base hydrolysis of the esters and reconstitution of the carboxyl groups. Table

8.7 shows that the total binding capacity for copper by each of the tested sorbent at pH 5.2 was recovered after ester hydrolysis, since, within experimental error, the uptake of copper by each sorbent was identical to that of the untreated material, i.e. control, (Table 8.6). The full recovery of the copper binding ability of the sorbents after de-esterification of carboxyl groups verified the role of these functional groups in the metal adsorption process.

Table 8.7: Adsorption of copper from industrial wastewater[1] using de-esterified sorbent with 0.1 N NaOH (pH of suspension 13)

Sorbent	Copper uptake (mmol/g)	Final pH
Moss	0.103	5.2
Bark	0.102	5.2
CM	0.083	5.2

[1]Initial copper concentration: 36.5 ppm; sorbent concentration: 4.6 mg/mL; pH controlled at 5.2.

The involvement of the amine groups in the sorption process was also investigated by blocking of these functional groups. The use of formaldehyde as a carbonyl component in the formaldehyde-formic acid treatment provides a good method of methylation of primary and secondary amines (Fieser and Fieser, 1961). The general reaction scheme is:

$$R\text{-}CH_2NH_2 \xrightarrow{CH_2O,\ HCOOH} R\text{-}CH_2N(CH_3)_2 + CO_2 + H_2O \qquad (8.2)$$

If the amine groups do participate in the sorption process, the binding capacity of the sorbent treated in this way should be reduced after the treatment.

Experiments were performed with synthetic solutions containing 50 ppm of copper and sorption was carried out with the methylated and unmethylated (untreated) CM, moss and bark at a constant pH 4.5. The results (Table 8.8) indicated that amine groups also contributed to the uptake of copper by the tested sorbents, especially by CM and moss. The small reduction (5.3%) in the uptake of Cu^{2+} by bark due to methylation might be attributed to its low proteins content (1.8%) compared to CM and moss (37.2 and 7.4%, respectively), which are abundant in amine groups. Again, the incomplete reduction in the uptake by the methylated sorbents implies that other functional groups or mechanisms are also involve in the sorption process. These results are consistent with those reported by Tobin *et al.* (1990) regarding the inhibition of La^{3+} and Zn^{2+} uptake by *R. arrhizus* treated with formaldehyde-formic acid.

Table 8.8: Adsorption of copper[1] by methylated moss, bark and CM using formic acid and formaldehyde

Sorbent	Control		Methylated sorbent		Sorption Reduction (%)
	Copper uptake (mmol/g)	Final pH	Copper uptake (mmol/g)	Final pH	
Moss	0.149	4.5	0.120	4.5	19.9
Bark	0.133	4.5	0.125	4.5	5.3
CM	0.152	4.5	0.128	4.5	16.1

[1]Initial copper concentration: 50 ppm; sorbent concentration: 4.6 mg/mL; initial pH: 4.5.

8.4 Ion-Exchange Mechanism

Ion-exchange is considered one of the most predominant mechanisms involved in the biosorption processes (Crist *et al.*, 1990; Mullen *et al.*, 1992; Avery and Tobin, 1993). Ion-exchange is important since, if it is readily reversible, the biosorbent may be used as an ion exchange material. The existence of this mechanism in the sorbents used was investigated in this work by following the release of Ca^{2+}, Mg^{2+}, K^{+} and H^{+} cations from bark, moss and CM, after the sorption of Cu^{2+}, Cd^{2+} and Ni^{2+} from their single, binary and ternary systems. The release of cations from the control, consisting of the sorbent and deionized water, was measured as well. The net release of metals from the different metal sorption systems presented in Tables 8.9-8.11 represent the differences between the metal cations measured in the supernatant of the metal system after the sorption process and that of the control.

Sorption by pine bark

The results of Table 8.9 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. Table 8.9 also shows that there was more cations released in the case of the single metal system than that of the binary and ternary systems. It also appears that there was more Ca^{2+} released whenever Cu^{2+} existed in the systems (Table 8.9). This might be related to the physical and chemical properties of Cu^{2+}, namely the coordination number and the electronegativity, as discussed in the previous chapter.

It has also been reported that the displacement of H^{+} indicates covalent bonding of the metals (Crist *et al.*, 1990), while the displacement of Ca^{2+}, Mg^{2+} and K^{+} indicates ionic bonding with the metal (Avery and Tobin, 1993). 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 (1993) reported that the release of one equivalent of either Ca^{2+} or Mg^{2+}, due to metal uptake, was also associated with a release of two equivalents of H^+. Taking into consideration the release of K^+ from the bark, which was not accounted by these authors, this could also be associated with the release of two equivalents of K^+. Therefore, the release of one equivalent of either Ca^{2+} or Mg^{2+} would be associated with a release of two equivalents of H^+ or K^+. However, examination of the values of Table 8.9 shows that the release of Ca^{2+} and Mg^{2+} is always greater than the release of H^+ and K^+. This indicates that the exchange is not on an equivalent basis.

Table 8.9: 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* (mM)				$R_{b/r}$
	Cu^{2+}	Cd^{2+}	Ni^{2+}	Ca^{2+}	Mg^{2+}	K^+	H^+	
Control**	–	–	–	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

* Difference between metal released by system and that by the control.

** Bark and water.

$R_{b/r}$: the ratio of metal bound to metal released.

Crist *et al.* (1990) and Tobin *et al.* (1988) stated that pure ion-exchange occurs at equimolar concentrations. This implies that the ratio $R_{b/r}$, which is the ratio of the metal(s) bound to metals released, should be equal to unity. The values of $R_{b/r}$ (Table 8.9) are less than one for single metal sorption by bark, indicating that the sum of the cations released (Ca^{2+} + Mg^{2+} + $K^+/2$ + $H^+/2$) was greater than the amount of metal bound. However, they are closer to one for the binary system Cu^{2+}–Ni^{2+} and the ternary system Cu^{2+}–Cd^{2+}–Ni^{2+}. These values indicate that ion-exchange could be the most predominant mechanism for the binding of the metal(s) by bark. The greater release of cations, compared to the amount of metal bound, might be attributed to the decrease in the pH due to the metal sorption, which results in the desorption of these cations from the bark. Therefore, one can imagine the occurrence of two simultaneous processes: the displacement of cations by metal sorption and the desorption of cations due to the release of H^+.

Sorption by moss

The removal of metals from the solutions by moss was always associated with a release of Ca^{2+}, Mg^{2+}, K^{+} and H^{+} (Table 8.10). Calcium was released in the greatest amounts in all cases, and again there was more Ca^{2+} ions released during Cu^{2+} sorption than during Cd^{2+} or Ni^{2+} sorption. The ratio $R_{b/r}$ was always greater than 1.0, indicating less cations were released than the amount of metal(s) bound. This implies that ion-exchange is not the only binding mechanism and that some other mechanisms are involved. The lower release of $Ca^{2+} + Mg^{2+} + H^{+}/2$ than the amount of Cu^{2+} or Cd^{2+} bound was also reported by Avery and Tobin (1993) for the sorption of these heavy metals by *Saccharomyces cerevisiae*. The amounts of $Ca^{2+} + Mg^{2+} + K^{+}/2$ released by bark are always higher than that of $H^{+}/2$ (Table 8.10). This indicates that ionic bonding of the metal is more significant than covalent bonding.

Table 8.10: Release of Ca^{2+}, Mg^{2+}, K^{+} and H^{+} due to sorption of Cu^{2+}, Cd^{2+} and Ni^{2+} by moss. Initial concentration of each heavy metal: 100 ppm; moss concentration: 9.2 mg/mL

System	Total metal bound (mM)			Amount of cation released* (mM)				$R_{b/r}$
	Cu^{2+}	Cd^{2+}	Ni^{2+}	Ca^{2+}	Mg^{2+}	K^{+}	H^{+}	
Control**	–	–	–	0.072	0.033	0.529	0.050	
Cu^{2+}	1.17	–	–	0.574	0.267	0.121	0.201	1.17
Cd^{2+}	–	0.666	–	0.298	0.171	0.131	0.174	1.07
Ni^{2+}	–	–	0.920	0.324	0.212	0.125	0.179	1.34
Cu^{2+}- Ni^{2+}	0.982	–	0.391	0.586	0.342	0.213	0.190	1.17
Cu^{2+}- Cd^{2+}	1.106	0.389	–	0.678	0.262	0.279	0.195	1.27
Cu^{2+}-Cd^{2+}-Ni^{2+}	0.997	0.221	0.543	0.650	0.247	0.255	0.184	1.58

* Difference between metal released by system and that by the control.
** Moss and water.
$R_{b/r}$: the ratio of metal bound to metal released.

Sorption by CM

Canola meal, as previously mentioned, is a very soluble material. When the meal was dissolved in deionized water, large amounts of Ca^{2+}, Mg^{2+}, and K^{+} were released (Table 8.11) compared to bark (Table 8.9) and moss (Table 8.10). Therefore, in the metal systems, when subtracting the amount of cations released after the sorption process from that of the control (CM and water), the net amounts of metals released due to heavy metal adsorption were very small, and sometimes more cations were released in the control than in the metal solution (Table 8.11). However, CM always exhibited the release of Ca^{2+} whenever Cu^{2+} ions were present in the sorption system, while there was no net release of Ca^{2+} when Cd^{2+} or Ni^{2+} were sorbed separately by CM. This is relatively consistent with the

previous observations for the two sorbents, bark and moss, when Cu^{2+} is available in the solution. Therefore, it can be concluded that Cu^{2+} is always associated with a release of Ca^{2+}.

The low amount of H^+ released from CM compared to bark and moss could be attributed to the large proteins content of the meal. These proteins act as buffer in the meal, thus preventing the pH from dropping significantly, either in the deionized water or in the metal solutions. The high values of $R_{b/r}$ presented in Table 8.11 indicate that the ion-exchange mechanism is not a significant contributor to the sorption of metals, especially Cd^{2+} and Ni^{2+}, by CM.

Table 8.11: Release of Ca^{2+}, Mg^{2+}, K^+ and H^+ due to sorption of Cu^{2+}, Cd^{2+} and Ni^{2+} by CM. Initial concentration of each heavy metal: 100 ppm; CM concentration: 9.2 mg/mL

System	Total metal bound (mM)			Amount of cation released* (mM)				$R_{b/r}$
	Cu^{2+}	Cd^{2+}	Ni^{2+}	Ca^{2+}	Mg^{2+}	K^+	H^+	
Control**	–	–	–	0.357	0.929	2.44	0.025	
Cu^{2+}	1.060	–	–	0.167	0.483	0.010	0.084	1.52
Cd^{2+}	–	0.771	–	0.000 (-0.094)	0.000 (-0.247)	0.000 (-0.664)	0.070	22.03
Ni^{2+}	–	–	0.545	0.000 (-0.067)	0.000 (-0.119)	0.000 (-0.682)	0.064	17.03
Cu^{2+}- Ni^{2+}	1.110	–	0.425	0.327	0.349	0.000 (-0.213)	0.075	2.15
Cu^{2+}- Cd^{2+}	1.140	0.788	–	0.111	0.412	0.000 (-0.133)	0.070	3.45
Cu^{2+}-Cd^{2+}-Ni^{2+}	1.090	0.560	0.237	0.496	0.569	0.360	0.068	1.47

* Difference between metal released by system and that by the control.

** CM and water.

$R_{b/r}$: the ratio of metal bound to metal released.

Values in brackets represent the actual difference between metal released by system and that by the control.

The previous results demonstrated that ion exchange is an important mechanism in the biosorption process by the considered plant materials and cannot be ignored. This exchange mechanism was also investigated by examining the uptake of Ni^{2+} by sorbents pre-loaded with other metal ions. Bark, moss and CM were loaded separately with either Cu^{2+} or Cd^{2+}. After equilibrium, the solutions were separated, and the loaded sorbents were resuspended in Ni^{2+} solutions. Nickel and the preloaded metal were measured in the solutions, after attaining equilibrium with respect to Ni^{2+} uptake. The results (Table 8.12) indicated the following:

Bark

The uptake of Ni^{2+} by preloaded bark with Cu^{2+} or Cd^{2+} was always higher than the uptake of Ni^{2+} by pure bark (Table 8.12). However, the previous results from Chapter 7, showed that the uptake of Ni^{2+} was always suppressed when combined with either Cu^{2+} or Cd^{2+}. This implies that there are more sites available on the preloaded bark for Ni^{2+} uptake that that of the pure bark. The amount of Cd^{2+} released due to Ni^{2+} uptake was much higher that that of Cu^{2+} released (Table 8.12). This would indicate the strong bonding of Cu^{2+} to bark. Also, the uptake of Ni^{2+} by bark preloaded with Cd^{2+} was higher than bark preloaded with Cu^{2+}. This is attributed to more Cd^{2+} being released than Cu^{2+}. Generally, there is a partial ion-exchange between Cd^{2+} and Ni^{2+} and to a lesser extent between Cu^{2+} and Ni^{2+}.

Table 8.12: Nickel sorption using bark, moss and CM previously loaded with other metals. Initial metal concentration: 100 ppm; sorbent concentration: 9.2 mg/mL; pH 4.5

Metal Preloaded/ sorbent	Amount preloaded (mmol/g)	Nickel uptake with pure sorbent (mmol/g)	Amount of Ni^{2+} uptake* (mmol/g)	Amount of preloaded metal in solution (%)
Cu^{2+}/bark	0.0899 ± 0.0015	0.067 ± 0.0088	0.0860 ± 0.0014	2.8 ± 0.32
Cd^{2+}/bark	0.0620 ± 0.0014		0.0960 ± 0.0014	36.0 ± 2.03
Cu^{2+}/moss	0.1230 ± 0.0028	0.095 ± 0.0014	0.0670 ± 0.0014	9.5 ± 0.78
Cd^{2+}/moss	0.0800 ± 0.0012		0.1025 ± 0.0063	42.3 ± 2.6
Cu^{2+}/CM	0.0930 ± 0.0042	0.065 ± 0.0014	0.0755 ± 0.0021	3.3 ± 0.42
Cd^{2+}/CM	0.0825 ± 0.0007		0.0915 ± 0.0021	5.8 ± 0.68

* Nickel uptake by preloaded sorbent

Moss

The results for moss preloaded with other heavy metals showed that the amount of Ni^{2+} uptake by moss preloaded with Cu^{2+} was lower than that preloaded with Cd^{2+} (Table 8.12). This can be explained by the larger amounts of metal being released after Ni^{2+} sorption with the moss preloaded with Cd^{2+} than when the moss was preloaded with Cu^{2+}. This again indicates the stronger bonding of Cu^{2+} with the moss constituents than that of Cd^{2+}.

Canola meal

The uptake of Ni^{2+} by CM preloaded with either Cu^{2+} or Cd^{2+} was higher than that by pure CM (Table 8.12). In accordance with the results determined using bark and moss, the uptake of Ni^{2+} by CM preloaded with Cd^{2+} was higher than that preloaded with Cu^{2+}. However, in this case, the amount of Cd^{2+} released was less than that released from moss or bark, after Ni^{2+} uptake with the preloaded

sorbents. This could be due to the high affinity of the meal for Cd^{2+} ions or to the strong binding of Cd^{2+} to CM, compared to moss and bark.

In general, the binding of Ni^{2+} by preloaded sorbents could be attributed to the sites available in the sorbents for Ni^{2+} binding and to the ion-exchange mechanism. These results also show a partial participation of this type of ion-exchange in the sorption mechanism, since a complete release of the preloaded metal was not achieved.

8.5 Scanning Electron Microscope and X-Ray Analyses

Scanning Electron Microscopy (SEM) analysis is another technique to investigate the mechanisms of biosorption. This technique was used before by several researchers to study the sorption of heavy metals by microbial sorbents, but 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 more insight into the sorption mechanisms by the plant materials considered in this work. In this study, electron microscopy examination of bark, moss and CM, before and after metal ion sorption, was carried out to locate the active sorptive areas of these sorbents. Energy-Dispersive X-ray (EDX) analysis was also used to confirm the identity of the metal in the different parts of the cell.

The electron micrographs of bark before and after Cd^{2+} sorption are shown in Figure 8.2. It is noticed that the electron micrograph of the cells after Cd^{2+} sorption (Figure 8.2B) is more dense than the cells which were not in contact with Cd^{2+} (Figure 8.2A). The change in the structure of the cell material was probably influenced by the cadmium ions. Thus, it can be assumed that the dense areas contain most of the sorbed metal.

To confirm the existence of Cd^{2+} at the cell wall of the bark, energy-dispersive X-ray (EDX) microanalysis was carried out. The EDX spectra for the untreated cells of the bark are presented in Figure 8.3. The microprobe was focused on the cell wall (Figure 8.3A), the cytoplasm (Figure 8.3B) and the vacuoles (Figure 8.3C), in order to examine the existence of Cd^{2+} at these areas. Figure 8.3 indicates that the energy level of the cadmium remained at the same level as the background for each of the cell wall, cytoplasm and vacuoles areas. The cell wall of the bark was characterized by the appearance of Ca^{2+} (Figure 8.3A). This element was not detected by the EDX analyses in the cytoplasm or the vacuoles. The energy spectrum of phosphorus was always used as an indicator of cytoplasm areas (Figure 8.3B). The vacuoles were completely evacuated; no components were detected.

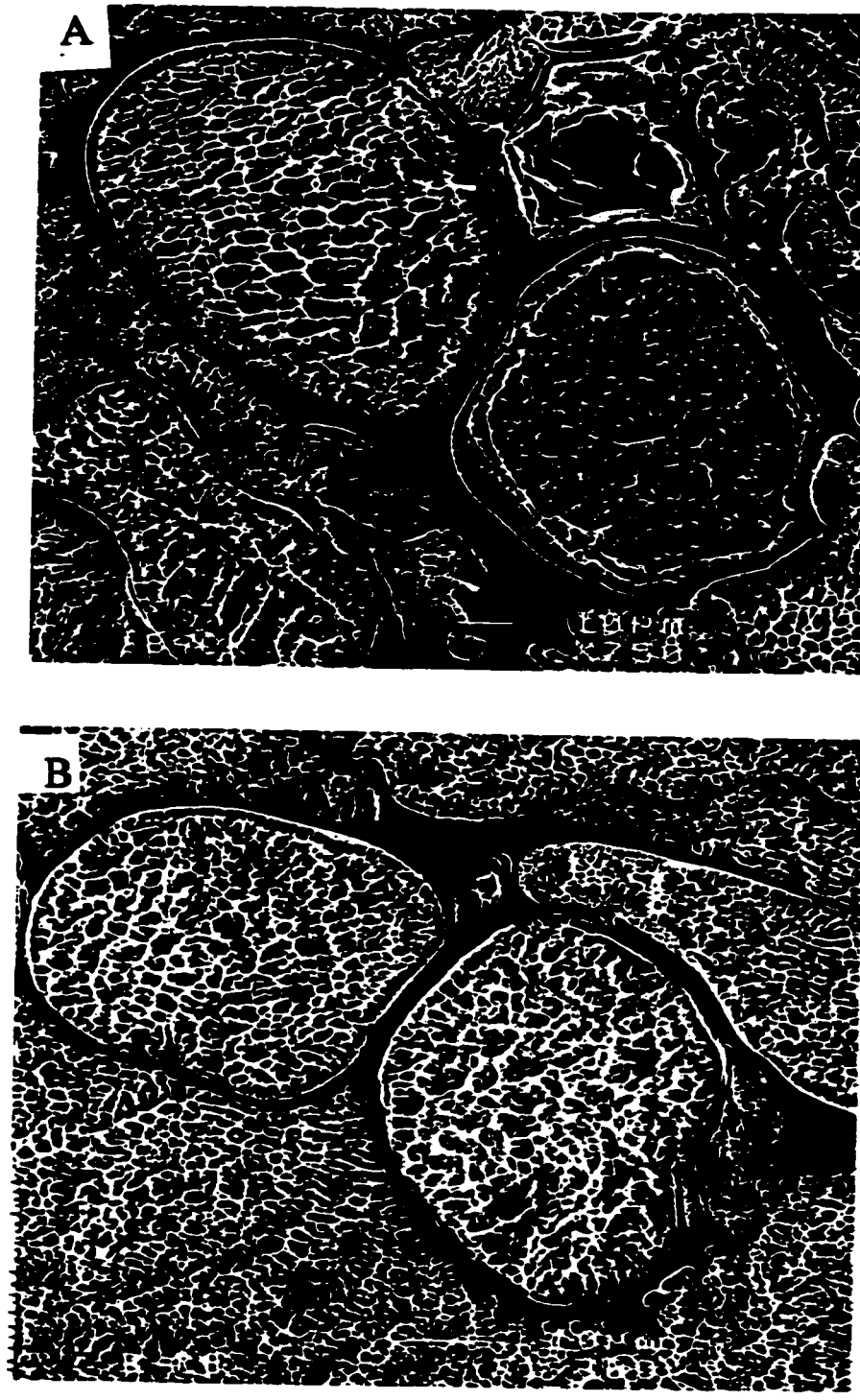

Figure 8.2: Scanning electron micrographs (Mag. 750 x) of untreated bark cells (A) and treated bark cells with 100 ppm of Cd^{2+} (B). Cadmium uptake: 0.058 mmol/g.

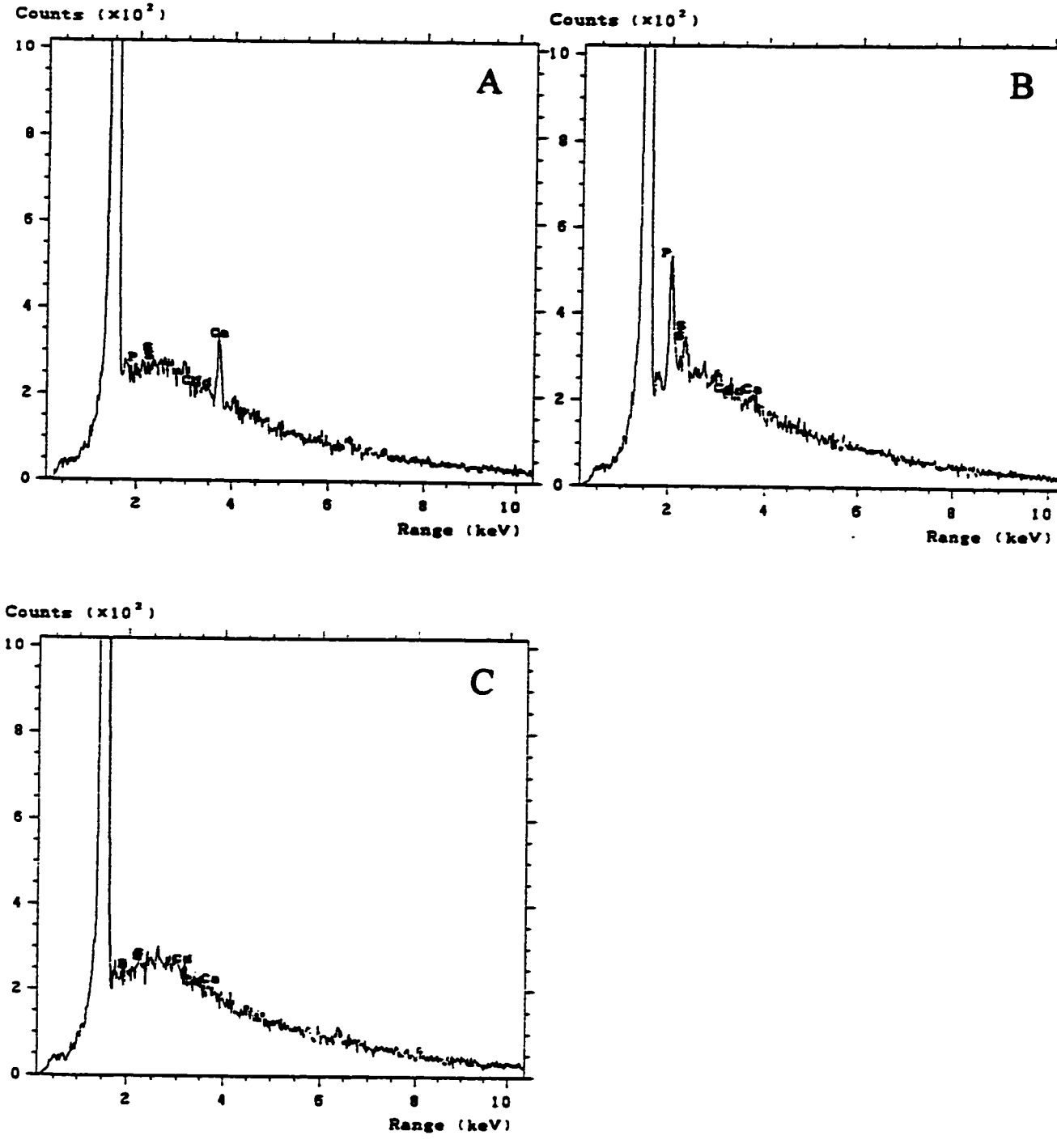

Figure 8.3: Energy-dispersive X-ray of bark before cadmium uptake. A) cell wall; B) cytoplasm; C) vacuole.

Figure 8.4 presents the 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 (Figure 8.4 A). Interestingly, a comparison between Figure 8.2A and 8.3A shows that the Ca^{2+} in the bark cell wall decreased significantly after Cd^{2+} binding. This is consistent with the results of the atomic absorption spectrophotometry presented in the previous section (Table 8.9), and confirms that ion-exchange is one of the mechanisms involved in this sorption process.

The microprobe, when focused on the cytoplasm of the bark cells, revealed small peaks of Cd^{2+} (Figure 8.4B) 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 concluded that majority of this metal was associated with the former cellular structure. Most of the researchers who studied the areas of metal sorption on microbial biomass observed that heavy metals were deposited at the cell walls (Mullen *et al.*, 1989, 1992; Tsezos and Volesky, 1982a, b; Shuttleworth and Unz, 1993). However, the electron microscopy examination for the uptake of gold by the algal biomass *Ascophyllum nodosum* (Kuyucak and Volesky, 1988a) revealed that in addition to a considerable amount of this precious metal which was 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+} remain in the background when the microprobe was focused on the vacuoles of the bark cells (Figure 8.4C). 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.

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 was penetrated into the cytoplasm. The precipitation of Cd^{2+} at the bark cell wall was not observed (Figure 8.2B). This is contrary to the findings of Tsezos and Volesky (1982a, b) and Mullen *et al.* (1989) 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. However, the observations from this study are in agreement with those reported by

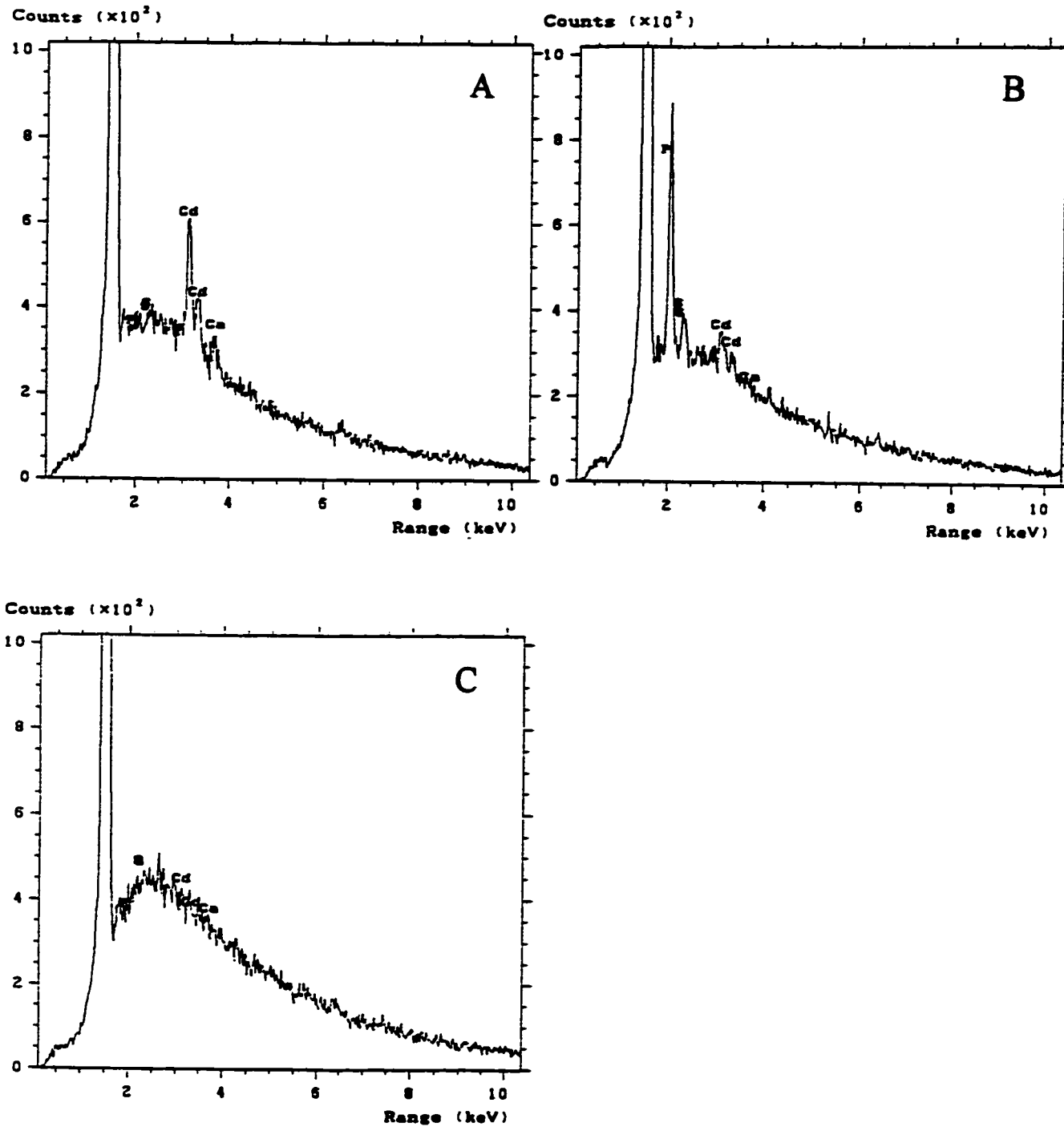

Figure 8.4: Energy-dispersive X-ray of bark after cadmium uptake (0.058 mmol/g). A) cell wall; B) cytoplasm; C) vacuole.

Mullen *et al.* (1989) who detected, using X-ray analysis, adsorbed copper and cadmium ions on the microbial cell wall but did not notice the metal precipitates using the electron microscopy.

Samples of bark loaded with other metal systems were also tested using electron transmission microscopy (Figure 8.5A,B and Figure 8.6A,B). The electron micrographs showed that bark cells exposed to copper (Figure 8.5A) were more dense than cells exposed to nickel ions (Figure 8.5B) and that there were more dense areas at the cell wall of Figure 8.5A than that of Figure 8.5B. This was expected because of the higher copper uptake by bark compared to nickel. At the cell wall of Figure 8.5A and 8.5B, the EDX spectra show higher levels, above the background, corresponding to copper (Figure 8.7A) and nickel (Figure 8.8A) spectral lines. It is also interesting to notice that the Ca^{2+} peak disappeared from the spectra of bark cells 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 is 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 8.9, 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 (Figure 8.5A) and nickel (Figure 8.5B), detected small peaks for both of the metals, Figure 8.7B and 8.8B, respectively. This shows that only small amounts of these metals diffused into the cytoplasm. The microanalysis did not detect any of these metals in the vacuoles of the bark cells (Figure 8.7C and 8.8C), indicating again that the vacuoles do not participate in the sorption process.

Figures 8.6A and 8.6B show the electron micrographs for bark cells loaded with a mixture of metal ions, Cu^{2+}–Ni^{2+} and Cu^{2+}–Ni^{2+}–Cd^{2+}, respectively. Again, the dense areas at the cell walls were confirmed by the EDX analyses. When the microprobe was focused at the bark cell wall (Figure 8.9A), it was detected that the dense areas at the cell surface of Figure 8.6A are Cu^{2+} and Ni^{2+}. The small peak due to Ni^{2+} was expected because of the suppression of Ni^{2+} binding by Cu^{2+} when in a mixture of the two. Calcium also disappeared from the bark cell wall (Figure 8.9A). This disappearance can be attributed to the copper uptake, since nickel alone did not affect the Ca^{2+} peak (Figure 8.8A). 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 (Figure 8.9B). Nickel was not observed in the cytoplasm, probably due to suppression of its adsorption by copper. Figure 8.9C shows that neither Cu^{2+} nor Ni^{2+} could reach the vacuole areas.

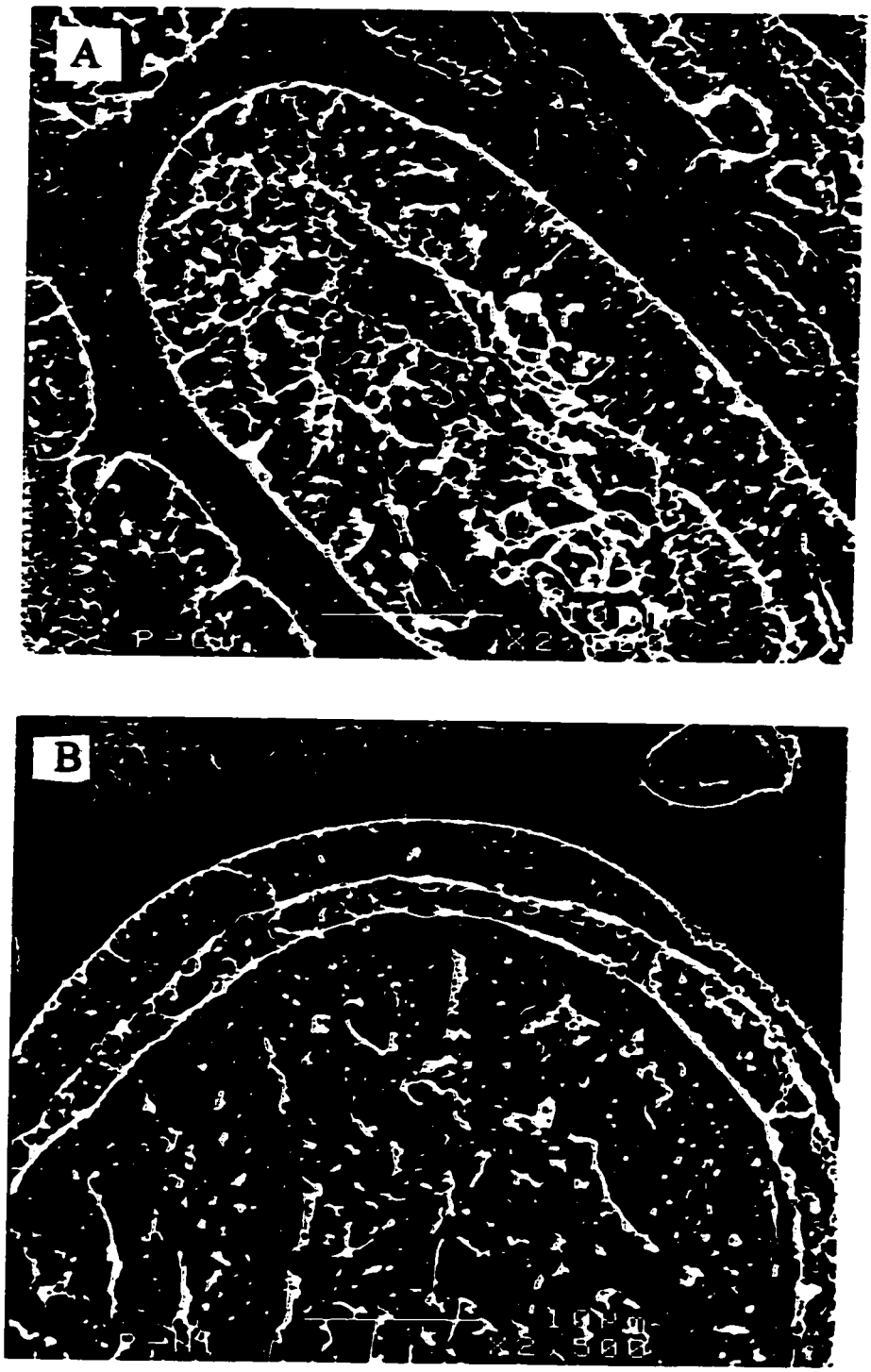

Figure 8.5: Scanning electron micrographs (Mag. 2500 ×) of bark cells exposed to (A) Cu^{2+} (0.082 mmol/g) and (B) Ni^{2+} (0.064 mmol/g).

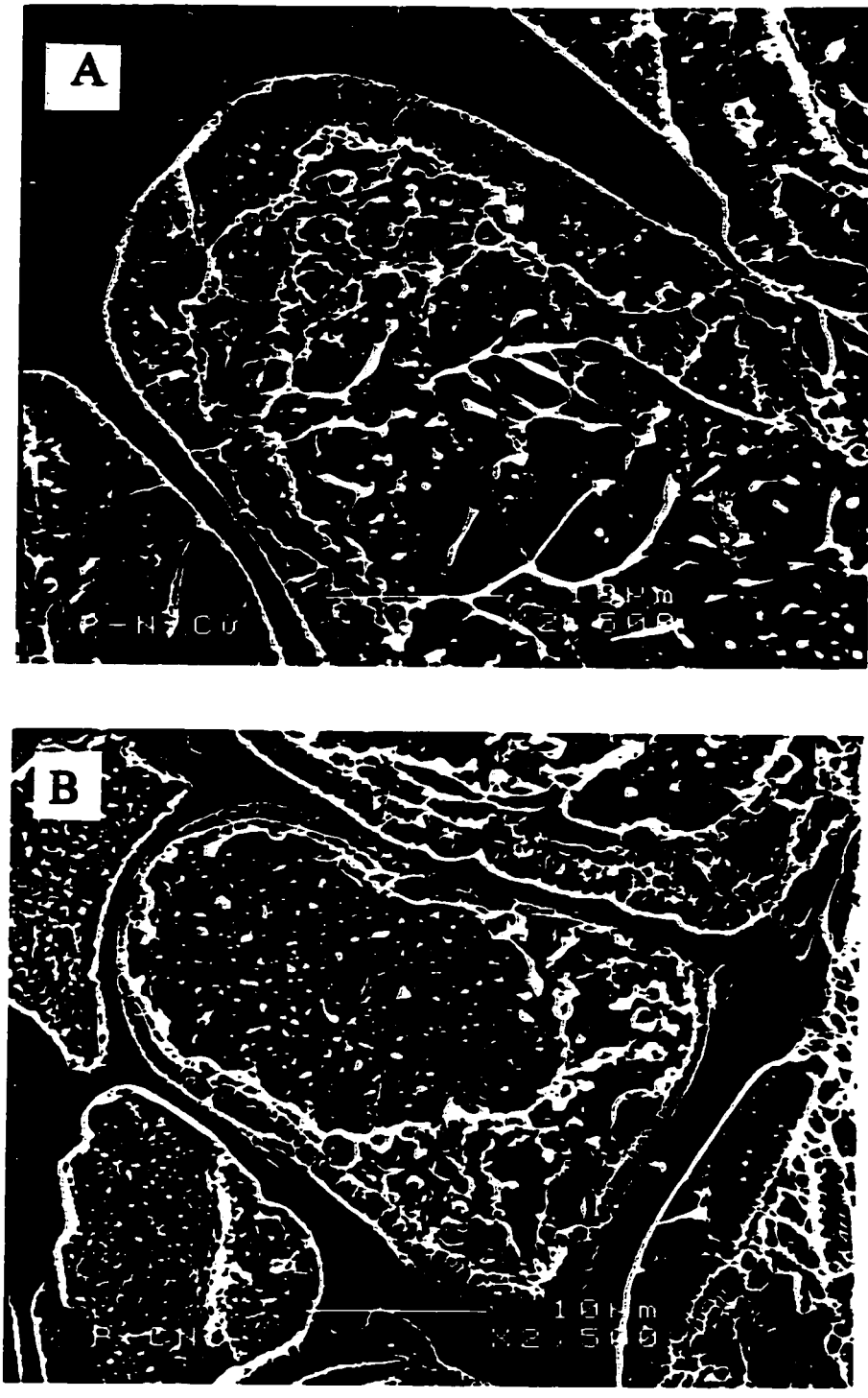

Figure 8.6: Scanning electron micrographs (Mag. 2500 ×) of bark cells exposed to (A) Cu^{2+}-Ni^{2+} metal system (uptake (mmol/g); Cu^{2+}: 0.084, Ni^{2+}: 0.02) and (B) Cu^{2+}-Ni^{2+}-Cd^{2+} metal system (uptake (mmol/g); Cu^{2+}: 0.051, Ni^{2+}: 0.01, Cd^{2+}: 0.02).

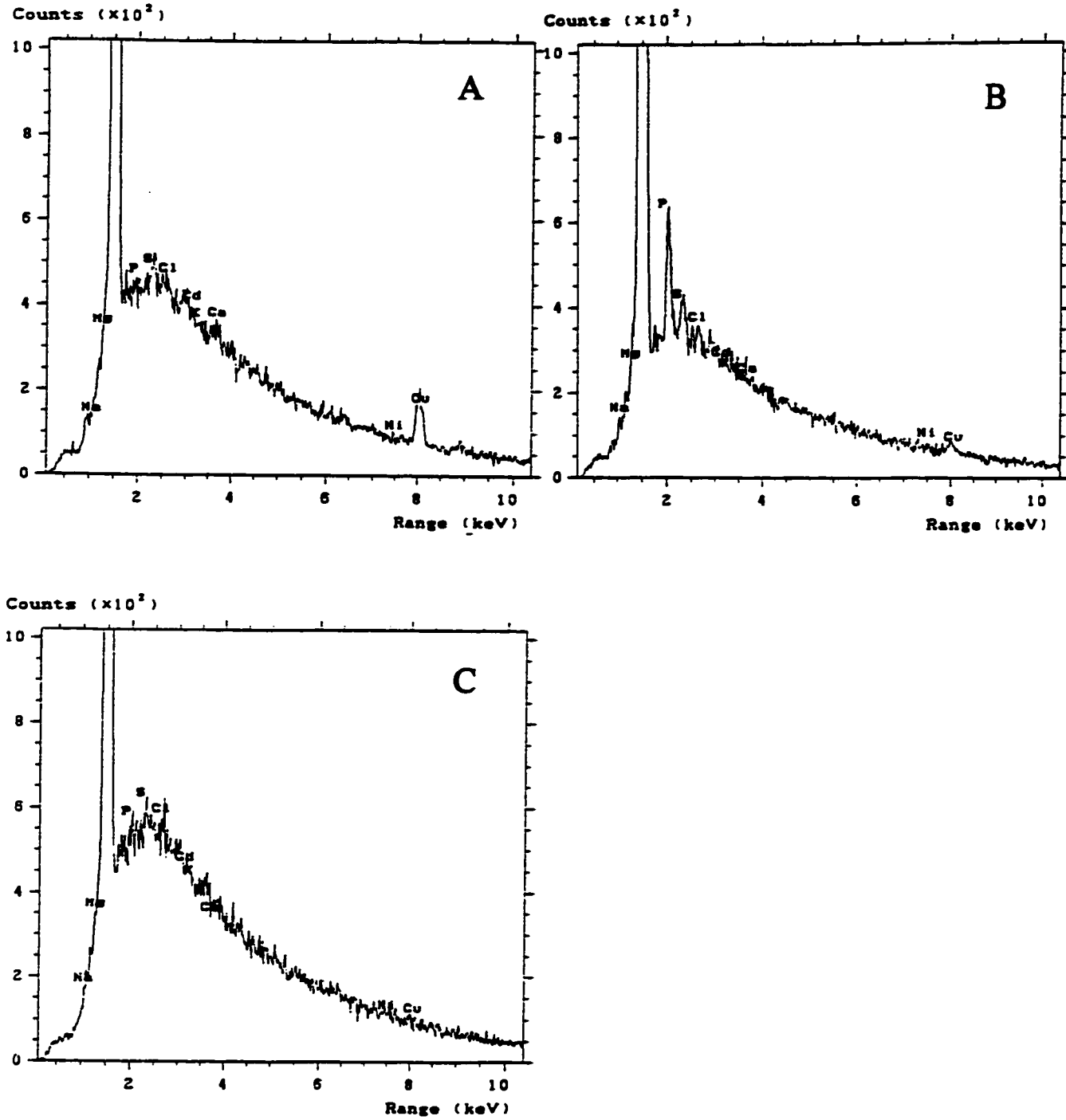

Figure 8.7: Energy-dispersive X-ray of bark after copper uptake. A) cell wall; B) cytoplasm; C) vacuole.

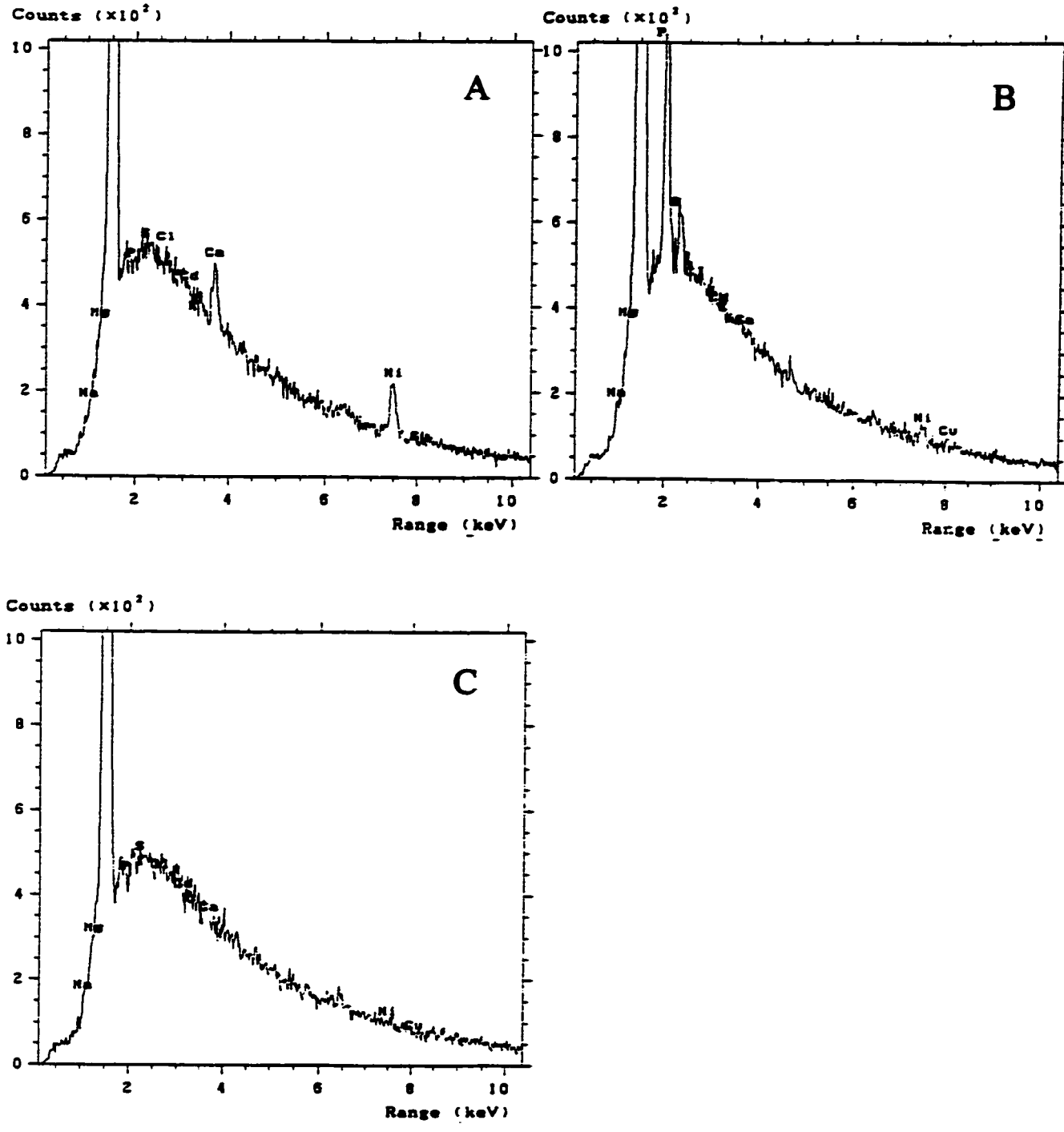

Figure 8.8: Energy-dispersive X-ray of bark after nickel uptake. A) cell wall; B) cytoplasm; C) vacuole.

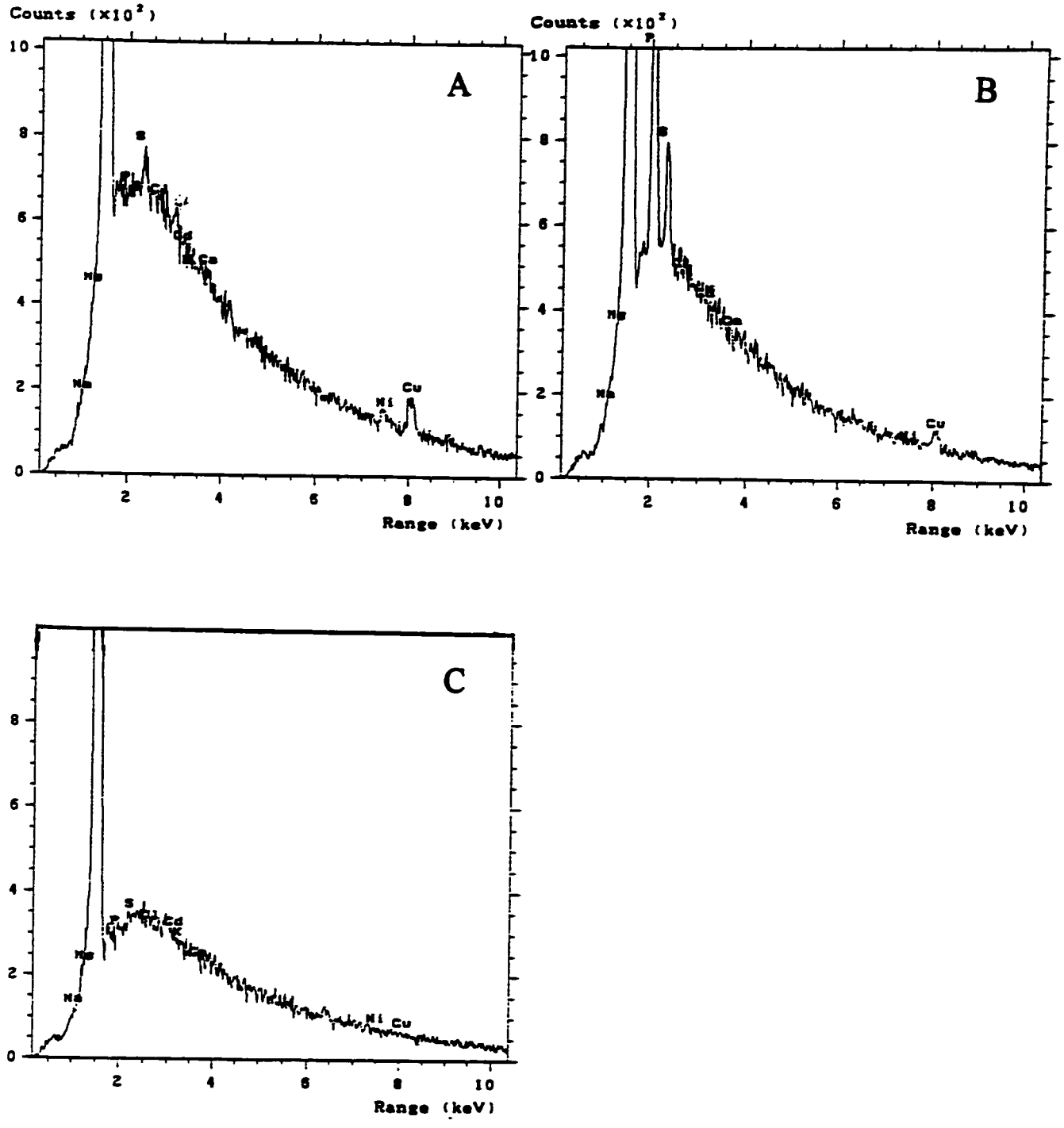

Figure 8.9: Energy-dispersive X-ray of bark after Cu^{2+}–Ni^{2+} uptake. A) cell wall; B) cytoplasm; C) vacuole.

Figure 8.10 shows the 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. However, the peak for Cd^{2+} spectrum 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 due to its negligible uptake in this ternary system, which was 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 (Figure 8.10B). Cadmium was not able to diffuse into the cytoplasm, because of its low concentration on the bark cell wall in this ternary system. Again, none of the three metals were able to diffuse into the vacuoles of the bark cells under the experimental conditions.

The same analyses for the detection of the areas of metal adsorption, from Figure 8.2 to 8.10, were also applied to moss loaded with different metal systems similar to those described previously for bark. The electron micrograph of moss loaded with Cu^{2+} ions (Figure 8.11B) seems to be more dense compared to that which was not exposed to the metal solution (Figure 8.11A). The dense areas were also confirmed by the EDX analysis. The high Cu^{2+} peak (Figure 8.13A), relative to the background level, indicates the existence of a significant amount of copper ions at the cell wall of the metal-laden moss compared to the control where such a peak did not exist (Figure 8.12A). The spectra of the cell walls in Figures 8.12A and 8.13A also show a release of Ca^{2+} ions after binding of Cu^{2+} to moss. As was previously mentioned regarding using bark as a sorbent, these results also indicate that most of the copper ions were dispersed at the moss' cell wall and only a small amount entered into the cytoplasm (Figure 8.13B). The EDX microanalysis of the vacuoles of the Cu^{2+}-laden moss showed (Figure 8.13C) that the vacuoles did not participate in the binding process.

Moss loaded with Cd^{2+}, Ni^{2+}, Cu^{2+}–Ni^{2+} or Cu^{2+}–Ni^{2+}–Cd^{2+} were also examined by the electron microscopy analysis. The results of the electron micrographs and the EDX analyses for these systems are presented in Appendix D. Generally, the electron micrographs of these systems showed more dense areas at the cell wall of moss after metal binding. The dense areas were confirmed, by the EDX analyses, to be the metals which were sorbed.

Canola meal samples, treated and untreated with cadmium ions, were also subjected to the microscopy examination. The electron micrograph of CM loaded with Cd^{2+} ions (Figure 8.14B) showed more dense areas at the cell wall compared to that which was not exposed to metal solution (Figure 8.14A). These areas were confirmed by the EDX analyses as well. The higher levels corresponding to the α and β cadmium spectral lines, relative to the background, in the cell wall

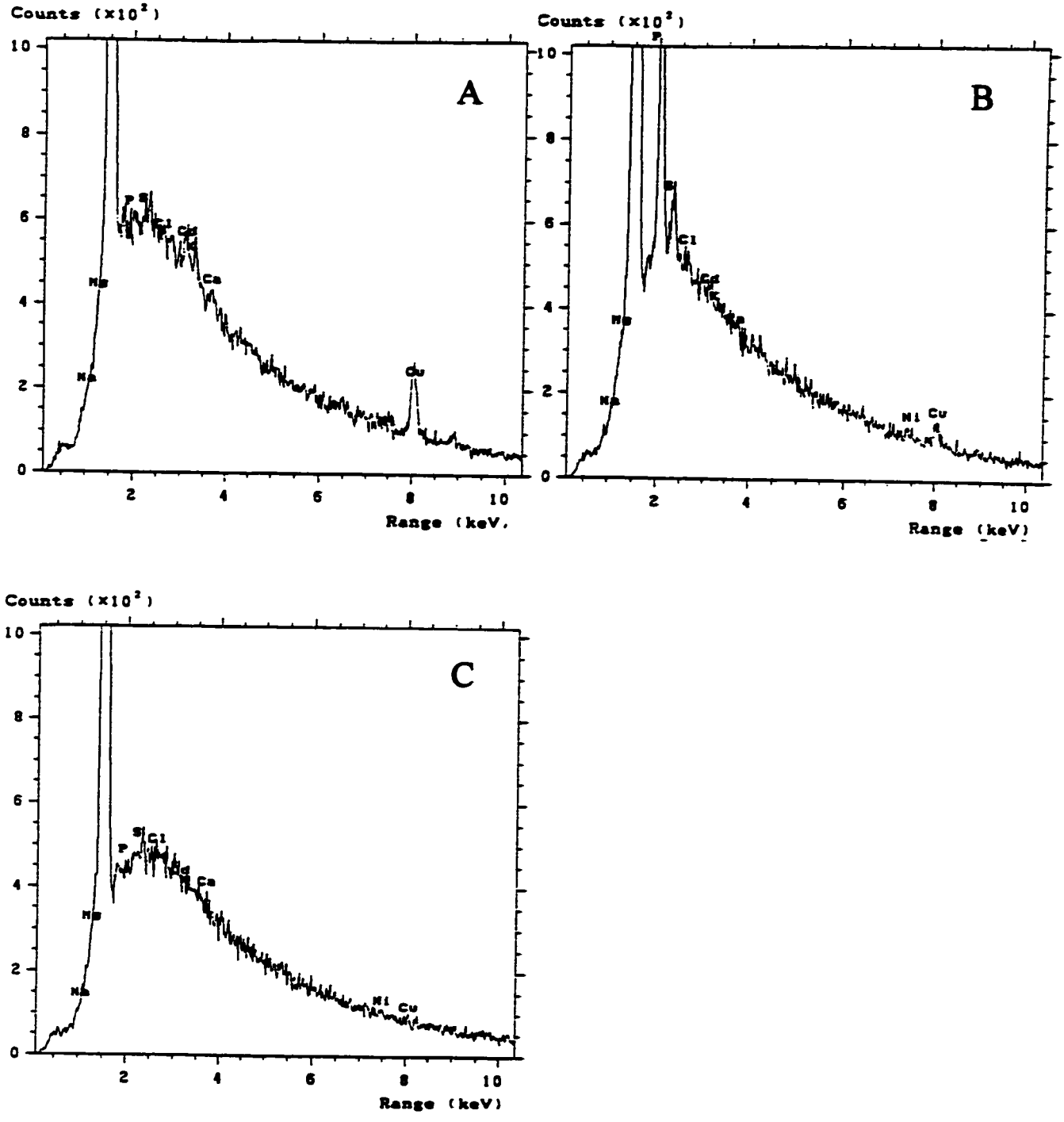

Figure 8.10: Energy-dispersive X-ray of bark after Cu^{2+}–Ni^{2+}–Cd^{2+} uptake. A) cell wall; B) cytoplasm; C) vacuole.

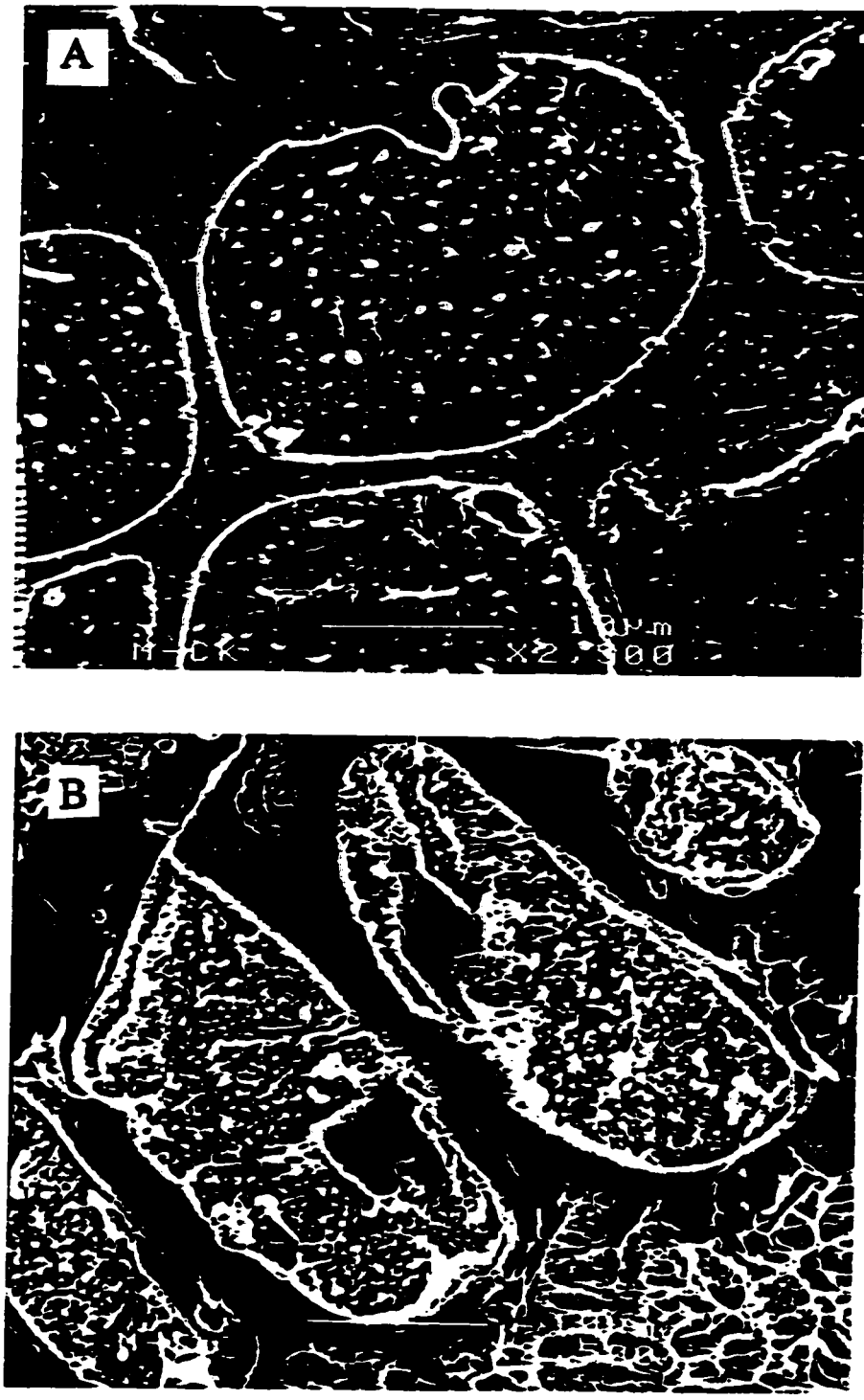

Figure 8.11: Scanning electron micrographs (Mag. 2500 ×) of untreated moss cells (A) and treated moss cells with 100 ppm of Cu^{2+} (B). Copper uptake: 0.135 mmol/g.

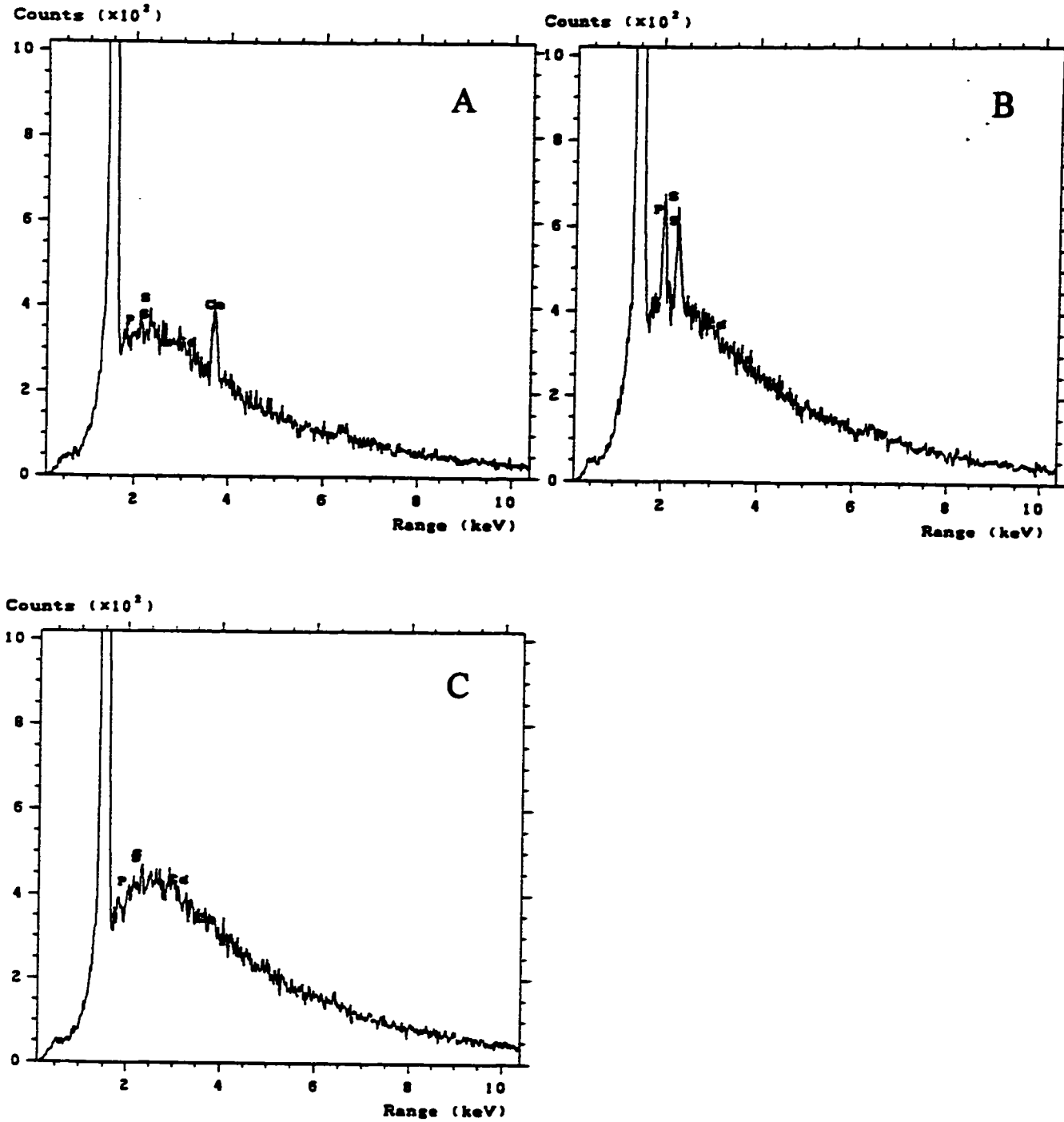

Figure 8.12: Energy-dispersive X-ray of moss before copper uptake. A) cell wall; B) cytoplasm; C) vacuole.

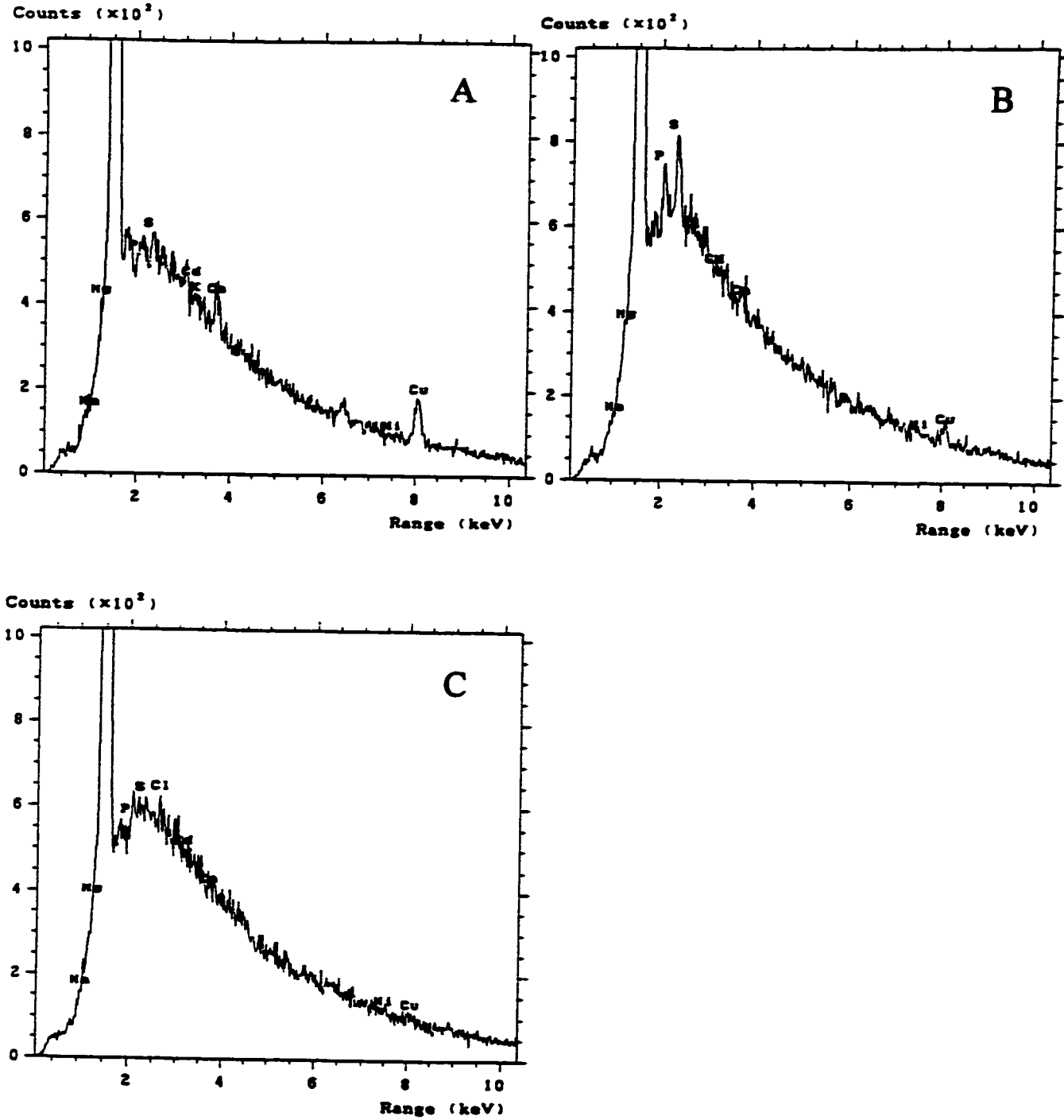

Figure 8.13: Energy-dispersive X-ray of moss after copper uptake (0.135 mmol/g). A) cell wall; B) cytoplasm; C) vacuole.

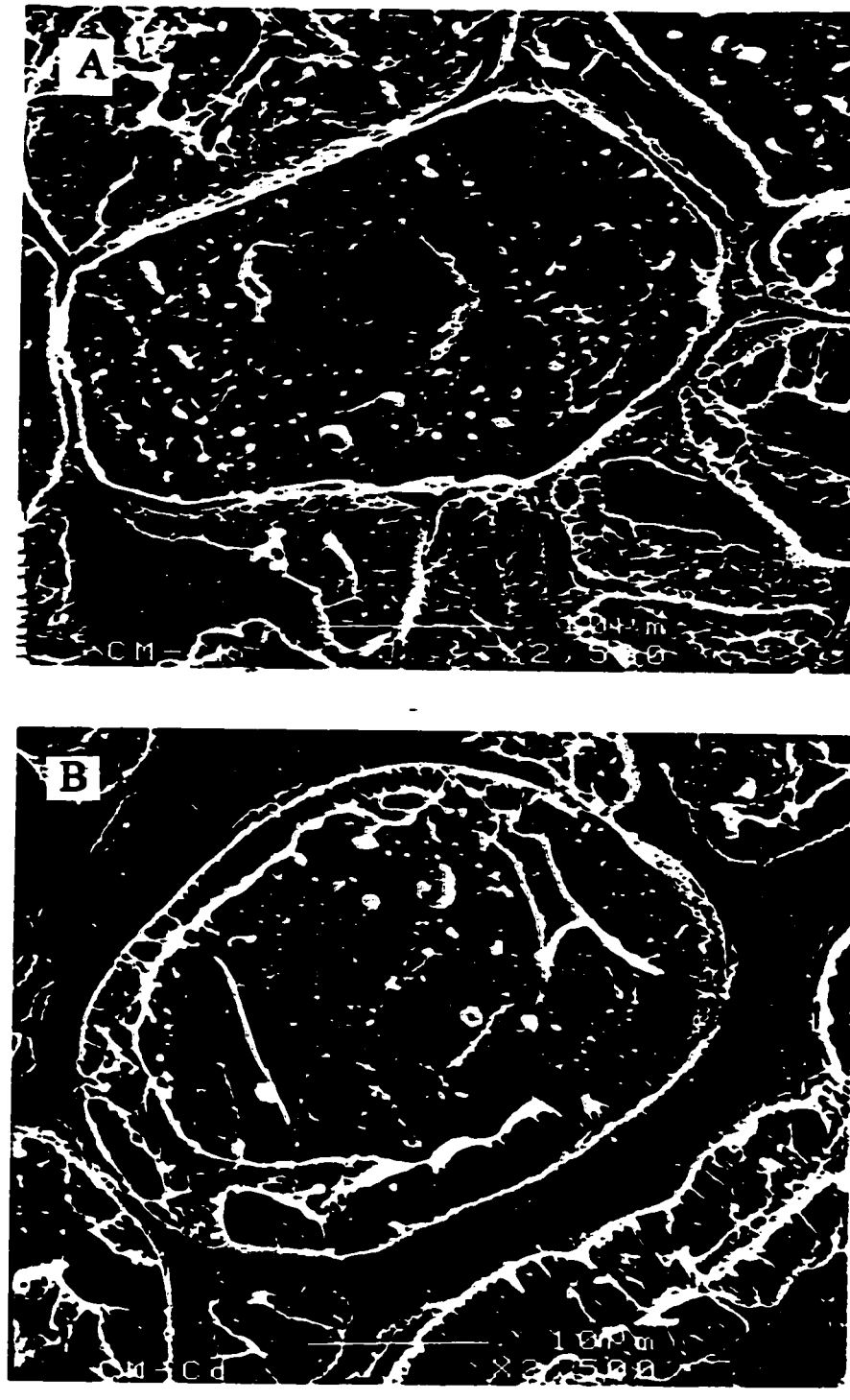

Figure 8.14: Scanning electron micrographs (Mag. 2500 ×) of untreated CM cells (A) and treated CM cells with 100 ppm of Cd^{2+} (B). Cadmium uptake: 0.08 mmol/g.

(Figure 8.16A), indicate that the dense areas at the cell wall of Figure 8.14B represent the loaded Cd^{2+} ions. This is factual because such lines did not emerge in the control (Figure 8.15A) which was not exposed to the metal solution. Unlike the previous EDX analyses, the comparison between Figures 8.15A and 8.16A indicated no net release of Ca^{2+} ions due to Cd^{2+} adsorption. This implies that ion-exchange does not seem to have a significant contribution in the sorption of Cd^{2+} by CM. In fact, this observation was consistent with the AAS measurements, which found that more Ca^{2+} was measured in the solution after exposing CM to water than when the sorbent was exposed to a Cd^{2+} solution (Table 8.11). Similarly, a small Cd^{2+} peak was detected in the cytoplasm of Cd^{2+}-laden CM (Figure 8.16) indicating the diffusion of a small amount of Cd^{2+} into the cytoplasm of CM. This peak was not detected in the cytoplasm of untreated CM (Figure 8.15A). In accordance with the observations noted for the vacuoles of bark and moss, the vacuoles of CM did not participate in the sorption process as well (Figures 8.15C and 8.16C).

It can be concluded from the previous SEM and EDX analyses that the cell walls of the plant sorbents, bark, moss and CM, were the main active areas for metal sorption, and only a small amount of metal penetrated into the cytoplasm. However, all of the results showed that vacuoles of the plant cells were not involved in the sorption process. It has also been demonstrated by these analyses that ion-exchange was an important mechanism of the metal sorption by the cell walls of bark and moss, but was not significant in CM when exposed to a single metal solution.

8.6 Electron Spin Resonance Analysis

Electron Spin Resonance (ESR) provides information about the concentration of free radicals, and/or the paramagnetic ions, in the material. This technique was used to study the mechanism of the sorption of uranium and copper by *R. arrhizus* (Tsezos, 1983) and that of copper by *Ganoderma lucidum* (Muraleedharan and Venkobachar, 1990). Electron Spin Resonance can also provide supportive evidence concerning the coordination/complexation of metal ions to the functional groups of the sorbents. This technique was also applied in this work to examine the participation of free radicals in the copper sorption by the plant materials, namely moss, bark and CM.

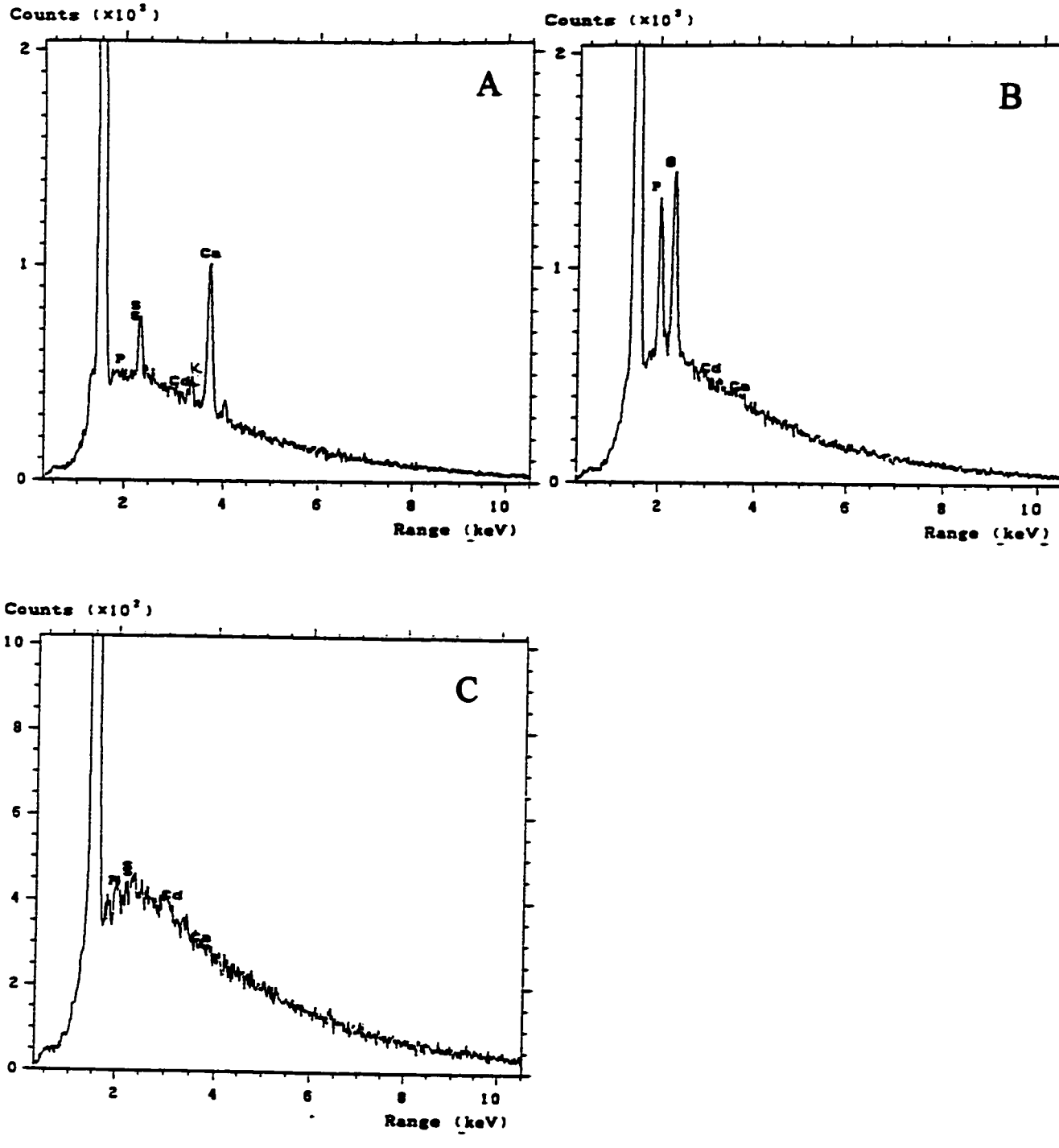

Figure 8.15: Energy-dispersive X-ray of CM before cadmium uptake. A) cell wall; B) cytoplasm; C) vacuole.

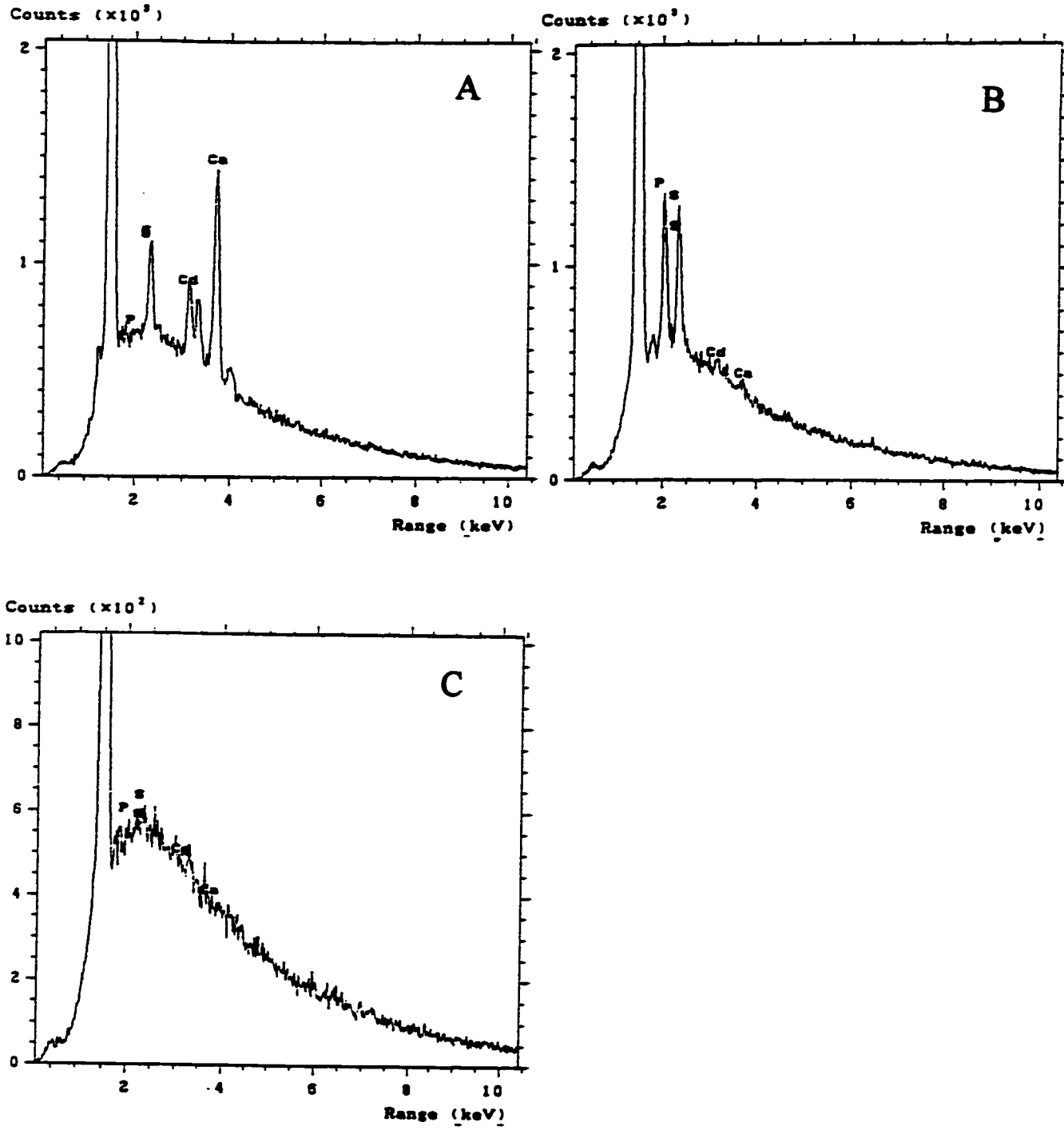

Figure 8.16: Energy-dispersive X-ray of CM after cadmium uptake (0.08 mmol/g). A) cell wall; B) cytoplasm; C) vacuole.

Samples of moss, bark and CM were treated with different initial copper concentrations, namely 25, 50,100 and 200 ppm. At equilibrium, the sorbents were separated, dried, and then the ESR spectra were obtained for both the samples loaded with the various amounts of copper as well as for the untreated sorbent samples. The spectra are presented in Figures 8.17, 8.18 and 8.19 for moss, bark and CM, respectively. Figures 8.17A, 8.18A and 8.19A show the ESR spectra of the untreated moss, bark and CM, respectively. In each sample an ESR line was detected that corresponds to g (Lande's constant) equal to $2.0079\pm4.17\times10^{-4}$, indicating the presence of free radicals in the untreated sample. The g values obtained in this study were the same for all cases (Figures 8.17-8.19, A-D), however, the concentration of free radicals associated with these spectra varied from one sorbent to another; moss: 1.92×10^{16} spins/g, bark: 5.7×10^{16} spins/g and CM: 5.4×10^{16} spins/g.

Following the uptake of Cu^{2+} ions, the intensity of the ESR spectra of the free radicals decreased significantly, and the decrease was proportional to the amount of the metal adsorbed (Figures 8.17-8.19, B-E). This could indicate that Cu^{2+} was chemically coordinated with the sorbent media. This hypothesis was supported by the ESR signals of the materials after sorption of Cu^{2+}, which showed an ESR line at a g value of $2.0785\pm3.46\times10^{-4}$ which was due to Cu^{2+} ions (Khulbe *et al.*, 1979). The four parallel components of the hyperfine interaction of Cu^{2+} were very weak in these spectra (Figures 8.17-8.19, B-E). The lack of hyperfine interaction indicates the restricted mobility of the copper species (Khulbe and Mann, 1978) within the sorbents. It was also reported that the occurrence of the Cu^{2+} hyperfine structures depends on the copper complexes in the material (Gersmann and Swalen, 1962) and the concentration of Cu^{2+} in the sorbent (Van Cutsem *et al.*, 1984). The results (Figures 8.17-8.19) showed that the copper peak was increased with the increase in the copper concentration. This confirmed the hypothesis that the binding of the metal to the sorbent increased with an increase in the initial metal concentration.

It is also observed that the level of the reduction in the free radicals concentration was different from one sorbent to another. In the case of moss, the free radical concentration decreased sharply with the increase in copper concentration and almost disappeared when the initial copper concentration was 200 ppm (Figure 8.17E). The reason for this is the low concentration of free radicals in the pure moss, compared to that of bark and CM. It was also noted (Figure 8.19) that the majority of the free radicals in CM participated in the copper sorption when the initial Cu^{2+} concentration was 200 ppm. This is in agreement with the previous observation, which found that ion exchange was the predominant mechanism for bark and moss sorption, but was not significant for CM. Therefore, it can be postulated that complexation with the sorbent' media may be an important mechanism in the case of CM.

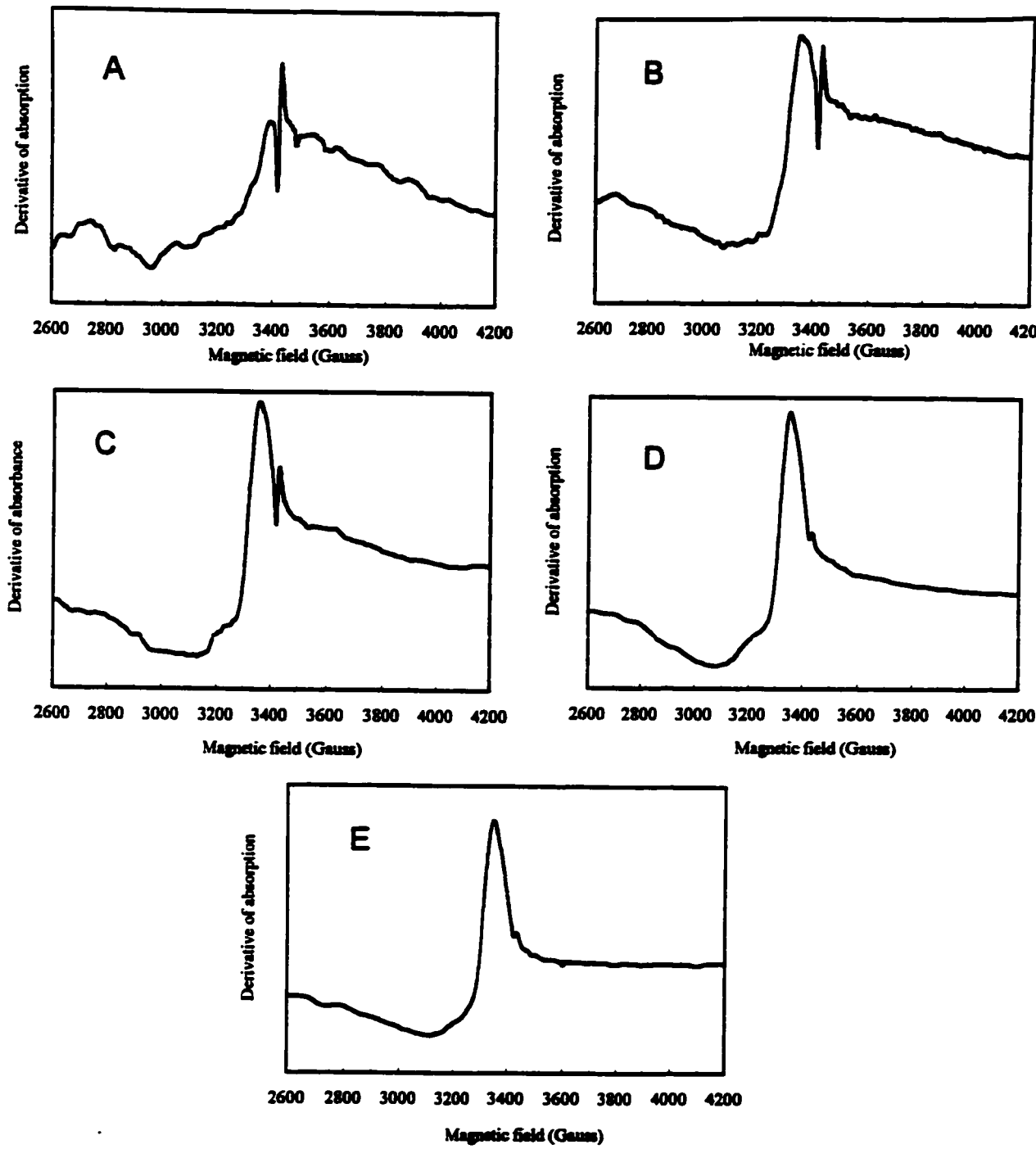

Figure 8.17: ESR spectra for moss exposed to various initial copper concentrations. Gain=4×10^4; moss concentration: 9.2 mg/mL; initial pH: 4.5: initial copper concentration (ppm): (A) 0.0, (B) 25, (C) 50, (D) 100, (E) 200.

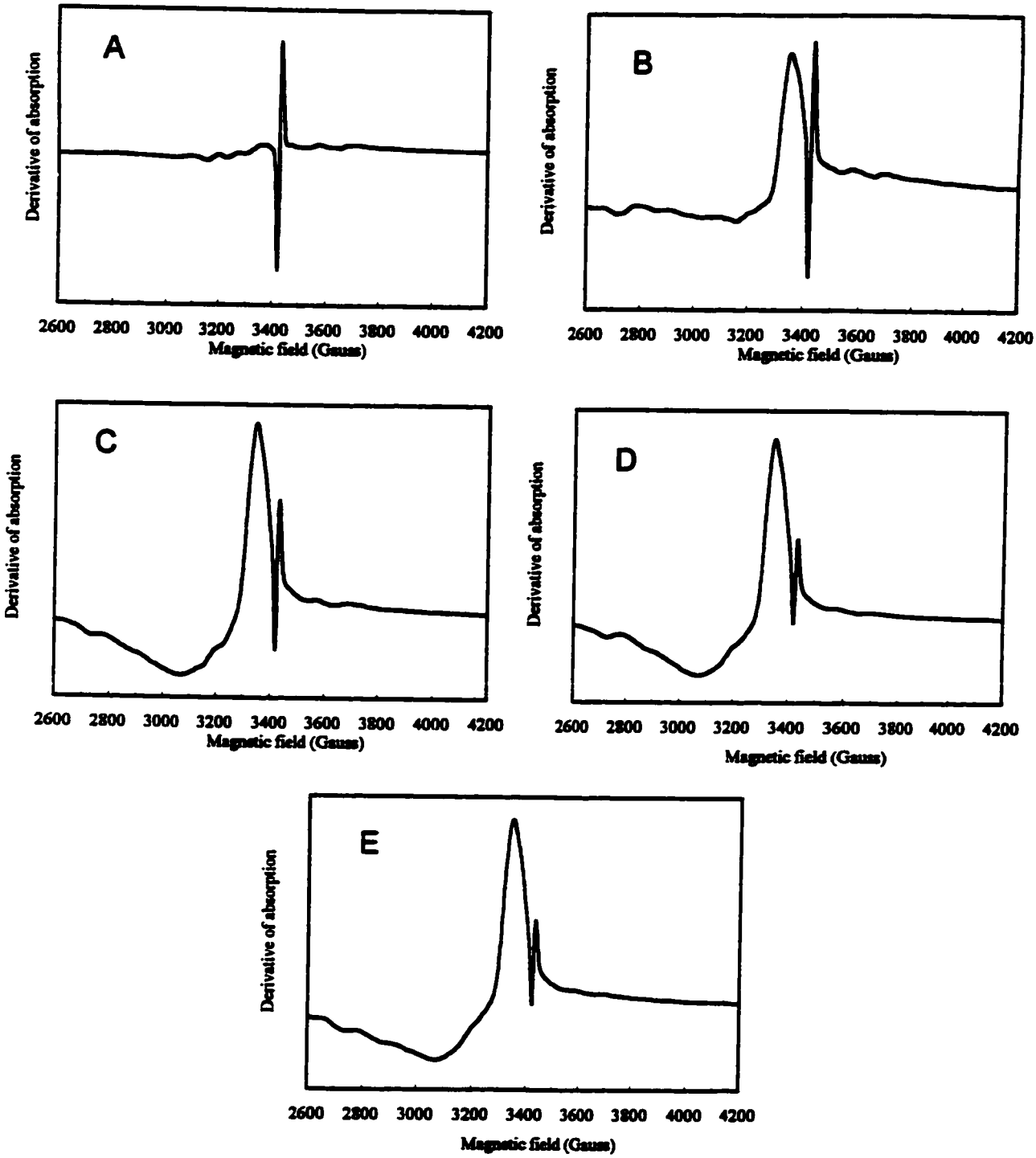

Figure 8.18: ESR spectra for bark exposed to various initial copper concentrations. Gain=4×10^{4}; bark concentration: 9.2 mg/mL; initial pH: 4.5: initial copper concentration (ppm): (A) 0.0, (B) 25, (C) 50, (D) 100, (E) 200.

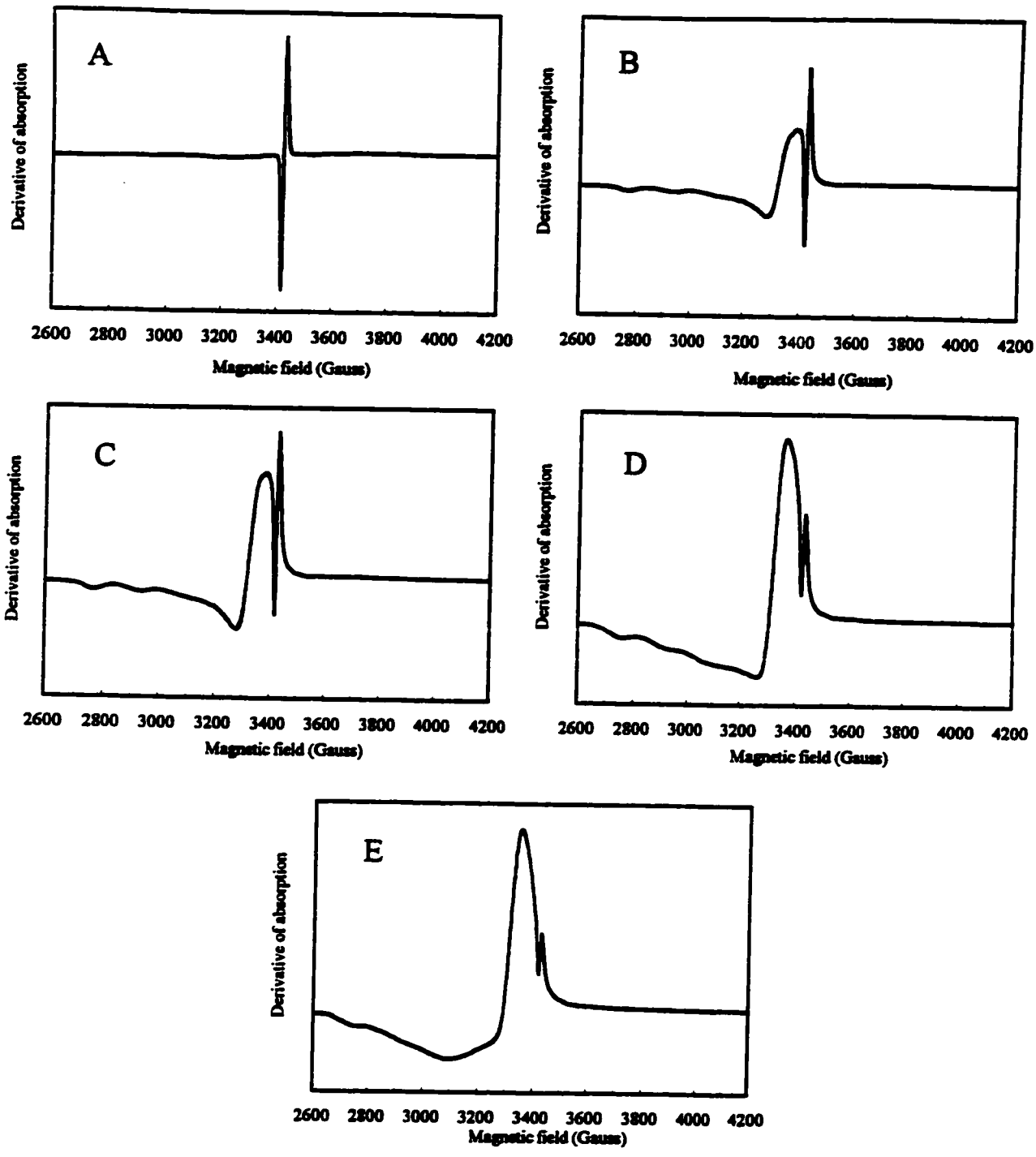

Figure 8.19: ESR spectra for CM exposed to various initial copper concentrations. Gain=1.25×10^4; CM concentration: 9.2 mg/mL; initial pH: 4.5: initial copper concentration (ppm): (A) 0.0, (B) 25, (C) 50, (D) 100, (E) 200.

The relationship between the copper uptake and the free radical concentration is shown in Figure 8.20. It is seen that the amount of free radicals decreased with an increase in the concentration of sorbed copper in an exponential pattern for the case of bark and moss, however, in the case of CM, there is a linear proportionality between copper uptake and free radicals (Figure 8.20).

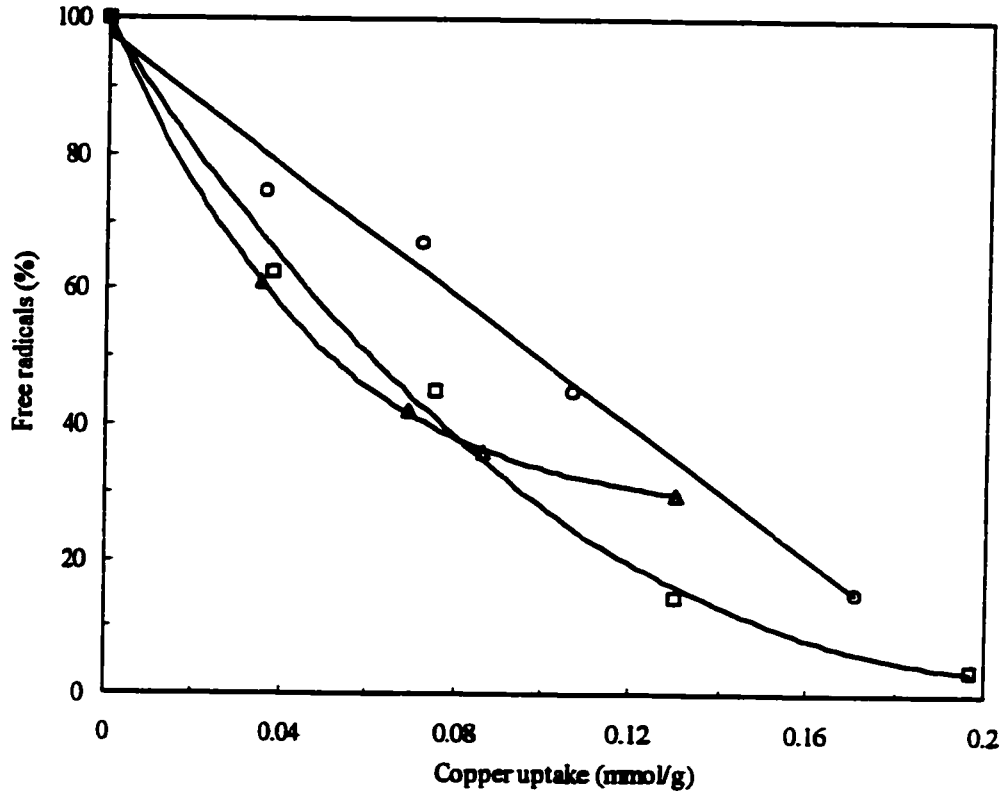

Figure 8.20: Relationship between the copper uptake and the free radical concentration. Sorbent and initial free radical concentration (spins/g): □-moss, 1.92×10^{16}; Δ-bark, 5.7×10^{16}; O-CM, 5.4×10^{16}.

It is reported (Muraleedharan and Venkobachar, 1990) that the reduction in the signal strength of the free radicals after sorption would lead to two inferences:

1. The group having these free radicals are coordinating with the metal, causing the pairing of electrons;
2. The cellular matrix embedding the free radicals opens up upon metal sorption, whereby the free radicals pair their electrons, without direct interaction with the metal.

Therefore, if the free radicals are coordinating with the metal, they should be relieved when the metal is desorbed, unless pairing again takes place with the diluted acid used for desorption. This was investigated in this work using moss, bark or CM, by obtaining the ESR spectra for the pure sorbents before sorption, after sorption with 100 ppm Cu^{2+} solution, after desorption of Cu^{2+} ions using 0.1 M

HCl and, finally, sorbents treated only with 0.1 M HCl. The results of ESR spectra for these four kinds of samples are shown in Figures 8.21, 8.22 and 8.23 for moss, bark and CM, respectively, and the concentration of free radicals for each case are presented in Table 8.13 for these three sorbents.

Table 8.13: Concentrations of free radicals before and after Cu^{2+} sorption[1] by moss, bark and CM, as well as after desorption and after treatment with HCl

Sorbent	Sorbent		
	Moss	Bark	CM
	Free radical concentration (spins/g) $\times 10^{-16}$		
Untreated	2.07 ± 0.297	5.68 ± 0.167	5.45 ± 0.495
After Cu^{2+} sorption[2]	0.20 ± 0.007	1.89 ± 0.212	2.56 ± 0.219
After Cu^{2+} desorption with HCl[3]	2.04 ± 0.049	2.50 ± 0.132	1.80 ± 0.134
Treated with HCl only	1.89 ± 0.141	3.20 ± 0.18	1.25 ± 0.141

[1] Initial copper concentration: 100 ppm; sorbent concentration: 9.2 mg/mL; initial pH: 4.5.
[2] Copper uptake (mmol/g): moss- 0.13, bark- 0.082, CM- 0.106.
[3] Recovery of Cu^{2+} after desorption (%): moss- 96, bark- 96.5, CM- 94.2.

The numeric values of Table 8.13, as well as the results from Figures 8.21-8.23, show that the sorbents' free radicals responded differently to desorption and acid treatment. In the following paragraphs, the behaviours of each sorbent following such treatments are discussed.

Moss

Figures 8.21A and 8.21C show that the free radicals strength is restored upon desorption of copper. It can be concluded that the dilute acid (0.1 M HCl) used for desorption has no interaction with the free radicals (Figure 8.21D). This can also be concluded from the values of free radical concentrations calculated from the ESR spectra (Table 8.13). Therefore, the groups having these free radicals are directly coordinating with copper ions, causing the pairing of electrons (Figure 8.21B) and they are freed when copper is released.

When the initial copper concentration was 100 ppm, the uptake of copper by moss was 0.13 mmol/g, and this was coordinated to 1.86×10^{16} spins/g. In terms of the number of unpaired electrons of copper, the uptake of Cu^{2+} corresponded to 7.83×10^{19} electrons per gram of moss. This indicates that the number of unpaired electrons of copper is much higher than the number of unpaired electrons available for copper binding originating from the free radicals of the sorbents. This could mean that the coordination with free radicals or with functional groups is not the only mechanism responsible for copper sorption.

The small ESR peak at $g = 2.081$, presented in the moss sample after desorption (Figure 8.21C), indicated an incomplete recovery of copper from moss under the experimental conditions. This peak was also noticed in bark and CM, after desorption treatment (Figures 8.22C and 8.23C) because of incomplete recovery of copper ions.

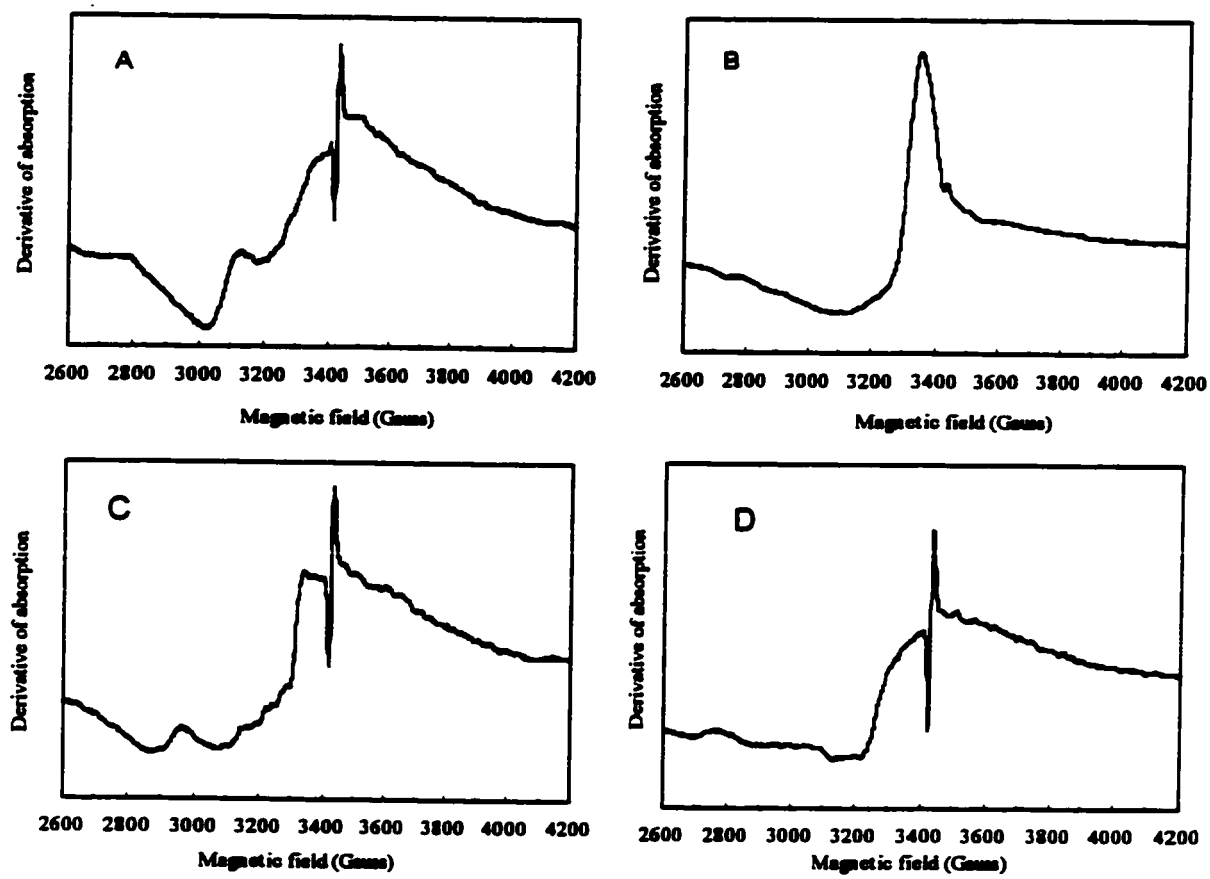

Figure 8.21: ESR spectra for pure moss (A), moss exposed to 100 ppm Cu^{2+} solution (B), regenerated moss after Cu^{2+} desorption using 0.1 M HCl (C) and moss treated with 0.1 M HCl (D). Gain=2×10^4; moss concentration: 9.2 mg/mL; initial pH: 4.5.

Bark

Figures 8.22A and 8.22C as well as Table 8.13 show that the complete restoration of the free radicals after the release of copper from bark was not attained, and only small numbers (about 16%) of the free radicals were restored upon the desorption process. The number of spins of free radicals in bark after the treatment with 0.1 M HCl (Figure 8.22D) was lower than that of pure bark (Figure 8.22A, Table 8.13). This illustrates the interaction of the diluted acid with the free radicals. This also explains the incomplete restoring of the free radicals upon desorption process, since the diluted acid that was used as metal desorbent enabled the free radicals to pair their electrons which resulted in a

lower number of free radicals. Therefore, it can be imagined that the cell wall matrix comprises of two parts: a small part containing those free radicals (16%) that are directly coordinated with metal ions, and the major part, which encompassed and trapped the free radicals and opens up upon metal uptake.

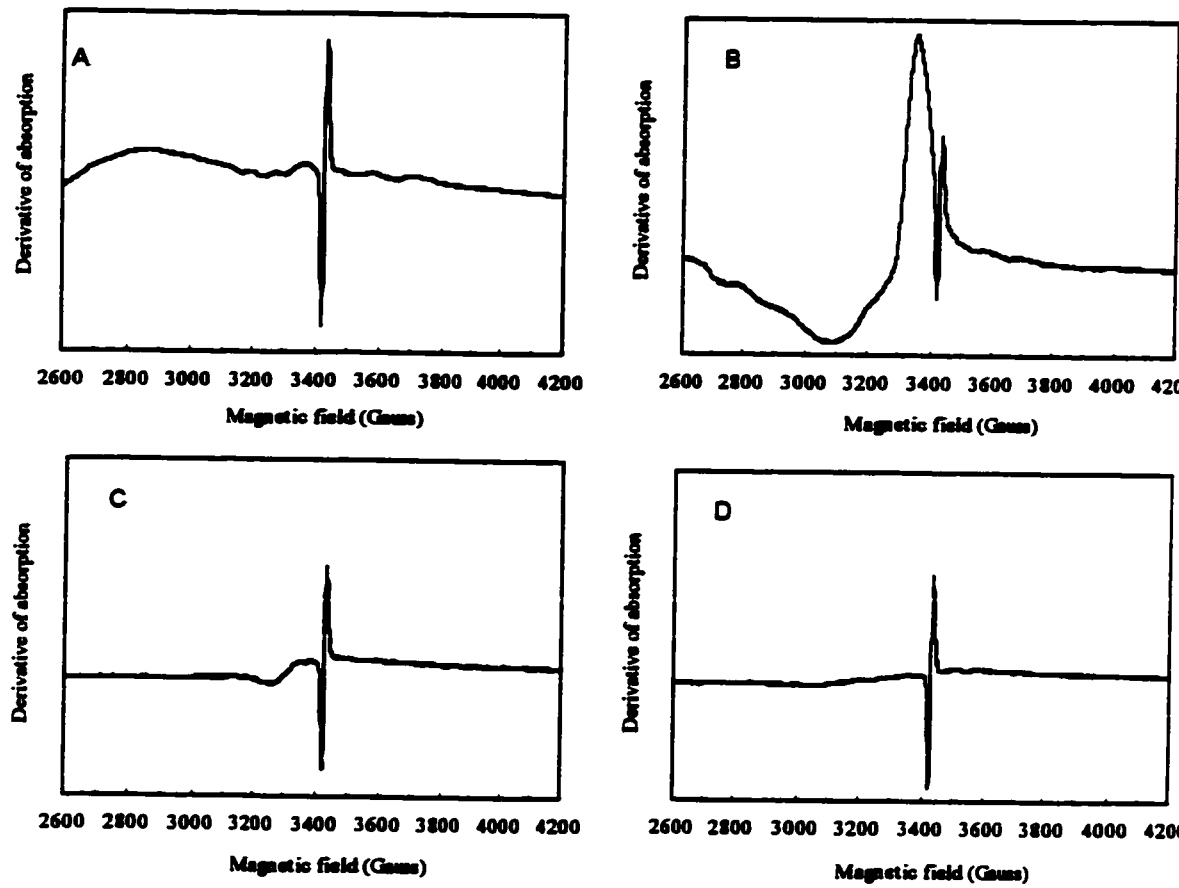

Figure 8.22: ESR spectra for pure bark (A), bark exposed to 100 ppm Cu^{2+} solution (B), regenerated bark after Cu^{2+} desorption using 0.1 M HCl (C) and bark treated with 0.1 M HCl (D). Gain=2×10^4; bark concentration: 9.2 mg/mL; initial pH: 4.5.

Canola meal

The behaviour of CM with respect to the free radicals was completely different. The comparison of the spectra before copper adsorption (Figure 8.23A) and after desorption (Figure 8.23C) showed that free radicals did not recover at all; there was actually a decrease in the number of free radicals after desorption (Figure 8.23B, Table 8.13). The ESR of CM treated with 0.1 M HCl showed a strong interaction of the acid with the free radicals (Figure 8.23D and Table 8.13). This explains the reduction of the free radical concentration after the desorption process. In this case, the acid acts as a strong agent that helps the free radicals to pair their electrons. Therefore, the free radicals present in

CM were not directly taking part in the metal uptake. However, the cell wall matrix containing the free radicals, opened up upon metal uptake, indicating its role in this sorption process. The exposed cell wall matrix of CM thus freely interacts with the metal, resulting in the uptake of the metal.

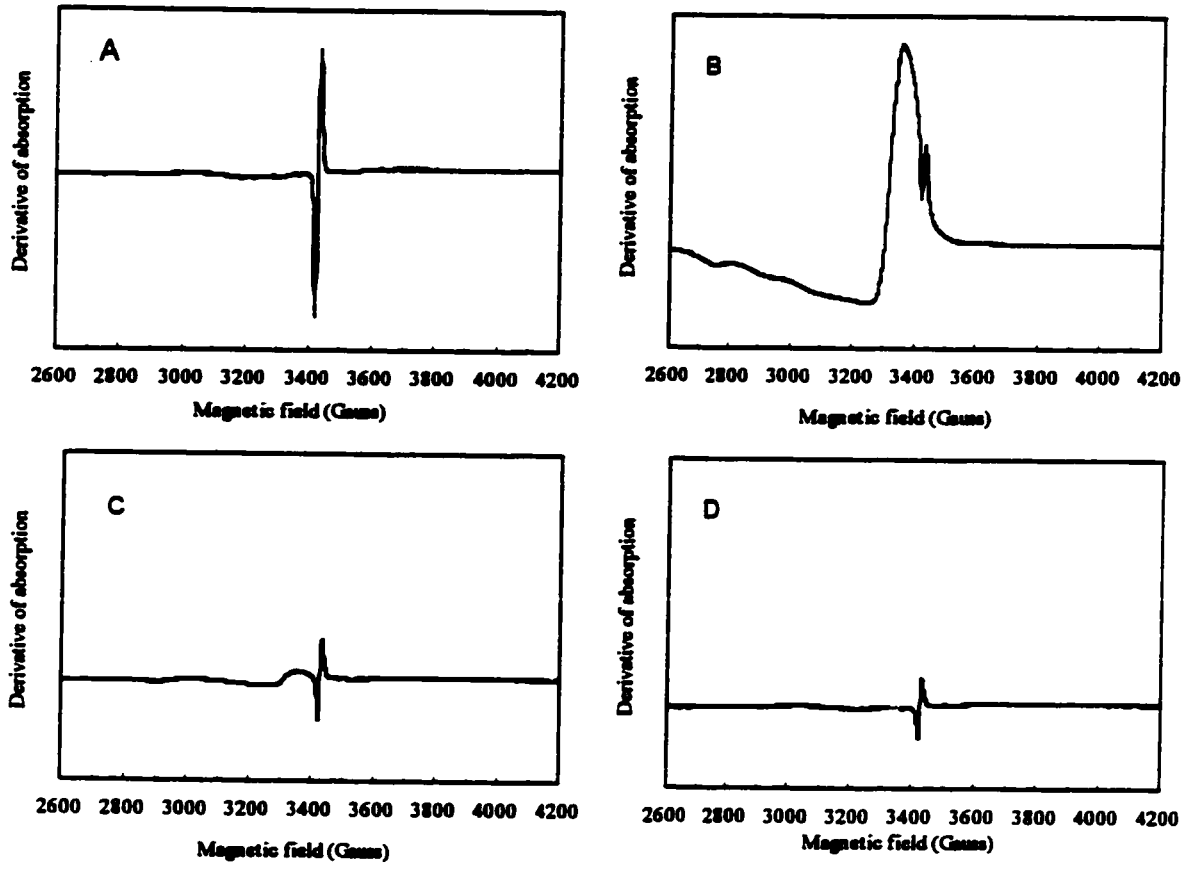

Figure 8.23: ESR spectra for pure CM (A), CM exposed to 100 ppm Cu^{2+} solution (B), regenerated CM after Cu^{2+} desorption using 0.1 M HCl (C) and CM treated with 0.1 M HCl (D). Gain=2×10^4; CM concentration: 9.2 mg/mL; initial pH: 4.5.

In conclusion, each material behaves differently upon complexation of the metal ions with the cell wall matrix encompassing the free radicals. Some free radicals are directly coordinated to metal ions during the sorption process. It might happen that, during the metal sorption, the cell wall matrix opens up for metal uptake, while the free radicals pair with each other rather than coordinating with the metal ions.

From the results of this chapter, it can be concluded that proteins, carbohydrates and phytic acid in CM, or carbohydrates and lignin in moss and bark are the main components responsible for metal sorption. The sorption capacity contributed by each component depends on its chemical composition which can vary from one sorbent to another. It was also showed that the carboxyl groups

and the amine groups in CM, bark and moss are involved in the metal uptake. Energy-dispersive X-ray spectra and atomic absorption spectrophotometric analyses demonstrated that ion-exchange represented one of the important mechanisms involved in the sorption process for moss and bark, but was insignificant for CM.

The SEM and EDX analyses showed that the cell walls of the three plant sorbents were the main active areas for metal sorption, and only a small amount of metal penetrated into the cytoplasm. However, all of the results showed that the vacuoles of the plant cells were not involved in the sorption process.

Electron Spin Resonance demonstrated that the free-radicals from the sorbents play a significant role in the sorption processes. The free radical concentrations, in each of the sorbents decreased with an increase in the adsorbed Cu^{2+} concentration. The groups of these free radicals were directly coordinating with the Cu^{2+} ions in the case of moss, while in the case of CM and bark, the cell wall matrix opens up for metal uptake and the free radicals pair to each other rather than coordinating with metal ions.

Chapter 9

Conclusions and Recommendations

9.1 Conclusions

The following conclusions can be drawn from this work:

- The biomass of *Aspergillus carbonarius* and the plant materials canola meal, pine bark, moss, pine needles, pine cones, moss, oak leaves, oak cobs, maple leaves, birch leaves and cider needles are good sorbents for heavy metals.
- Increasing the sorbent concentration in the suspension resulted in a decrease of the metal uptake per unit weight of the tested sorbents.
- An increase in the metal concentration, pH and temperature resulted in an increase of metal sorption.
- Preheating of the biomass suspension of *A carbonarius* decreased copper adsorption.
- The addition of glucose to the suspension of *A. carbonarius* enhanced copper adsorption, and the optimum glucose concentration in this process depended on the pH of the solution
- The same sorbent to metal concentration ratios resulted in approximately the same amount of the metal adsorbed by the sorbent, regardless of the concentration levels.
- The three sorbents, CM, bark and moss, were each able to decrease the Cd^{2+} concentration to a level of less than 0.008 ppm.
- The Linearized Langmuir and Freundlich isotherm models fit the equilibrium isotherm data from batch process adsorption reasonably well.
- Two mathematical models were proposed in this work to represent the dynamics of the batch sorption process. The first model, which assumed that sorption occurred only at the surface of the sorbent, fitted the experimental data reasonably well at high sorbent concentrations, but resulted in a significant deviation from the experimental data at low sorbent concentrations where intrapore diffusion was more significant. The second model, which is a modification of the first model, took into consideration the intrapore diffusion, and represented the experimental data very well for both high and low concentrations of the sorbent.

- The tested sorbents were able to adsorb several heavy metals with the following order (molar basis) when the initial concentration of each metal was 100 ppm in single metal systems:

A. carbonarius:	$Cu^{2+} > Cr^{3+} > Cd^{2+}$
Canola meal:	$Zn^{2+} > Cu^{2+} > Cd^{2+} > Ni^{2+} > Pb^{2+}$
Pine bark:	$Cu^{2+} > Zn^{2+} > Cd^{2+} > Ni^{2+} > Pb^{2+}$
Moss:	$Cu^{2+} > Ni^{2+} > Zn^{2+} > Cd^{2+} > Pb^{2+}$

When the uptake was expressed on mass basis (amount of metal removed), the above orders became:

A. carbonarius:	$Cu^{2+} > Cr^{3+} > Cd^{2+}$
Canola meal:	$Pb^{2+} > Cd^{2+} > Zn^{2+} > Cu^{2+} > Ni^{2+}$
Pine bark:	$Pb^{2+} > Cd^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+}$
Moss:	$Pb^{2+} > Cd^{2+} > Zn^{2+} > Cu^{2+} > Ni^{2+}$

- In a mixture of metal ions, the uptake of one metal was influenced by the presence of other metal(s). The nature of this effect depended on the physical and chemical characteristics of each metal, as well as the type of the sorbent used in the process.
- The Freundlich-Langmuir and modified extended-Langmuir binary component equilibrium models resulted in a better representation of the experimental data, for the sorption of different binary metal systems by pine bark, compared to the extended-Langmuir model.
- Metal ions can also be recovered in a continuous process using a bed packed with plant material. Application of the Bohart and Adams model as well as the experimental results of Cu^{2+} sorption by the moss-packed column showed that an increase in the influent flow rate or the influent concentration resulted in an increase of the sorbent capacity for the sorbate.
- Desorption of copper from the moss-packed column was influenced by the volume and concentration of the desorbent. A solution of 0.025 M of HCl was suitable for Cu^{2+} ions desorption from the packed moss column. The efficiency of the moss-packed column did not change significantly after five sorption/desorption cycles.
- Proteins, phytic acid and carbohydrates in CM, and carbohydrates and lignin in moss and bark are the main components responsible for metal sorption.
- The carboxyl and amine groups in the plant sorbents participated in the metal adsorption.

- Ion-exchange can be considered as an important mechanism for the sorption of metals by bark or moss but it is insignificant in CM.
- SEM and EDX analyses showed that the cell wall of the plant sorbents, bark, moss and CM, was the main active area for metal sorption, and only a small amount of metal penetrated into the cytoplasm. However, all of the results showed that the vacuoles of the plant cells were not involved in the sorption process.
- ESR tests demonstrated that free-radicals from the plant sorbents play a significant role in the sorption process. The groups with these free radicals were directly coordinated with Cu^{2+} ions in moss, and to certain limit in bark. In the case of CM and bark, the cell wall matrix opened up for metal uptake and the free radicals paired to each other rather than coordinating with the metal ions.

9.2 Recommendations

Based on the results obtained in this work, the following recommendations are suggested:

- Design a more sophisticated reactor to study the fast kinetics of the biosorption process. It would be also of interest to combine the biosorption process with the microfiltration process for the separation of the solution after adsorption either in batch or continuous processes.
- Extending the equilibrium study to the sorption of a ternary metal systems.
- Sorption of a mixture of ions in a continuous process using a packed column.
- Use of packed columns for the sorption of low metal concentrations (less than 1 ppm).
- SEM and EDX analyses of sorbents treated with high concentrations of heavy metals for longer periods of time to see the response of the cytoplasm and the vacuoles of the plant cells for these concentrations.
- Study of the sorption of other heavy metals, such as lanthanum, thorium and uranium, by the plant sorbents used in this work. Also determine if these metals form precipitates or crystals at the cell surface of the plant sorbents.
- ESR analyses at low copper concentrations to see if the hyperfine structures are more obvious in the sorbents under these conditions.

Part III

References

and

Appendices

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Appendix A

Experimental results of the dynamics of metals uptake

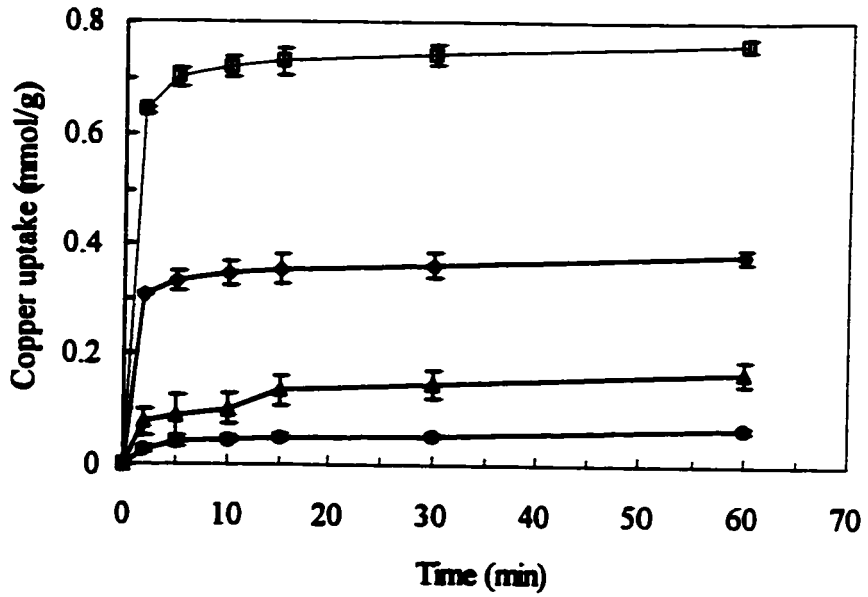


Figure A.1: Kinetics of Cu^{2+} uptake by *A. carbonarius* using various biomass concentrations. Initial Cu^{2+} concentration: 100 ppm; initial pH: 5.0; biomass concentration: □-1.15, ◊-2.30, Δ-4.60, O-9.20.

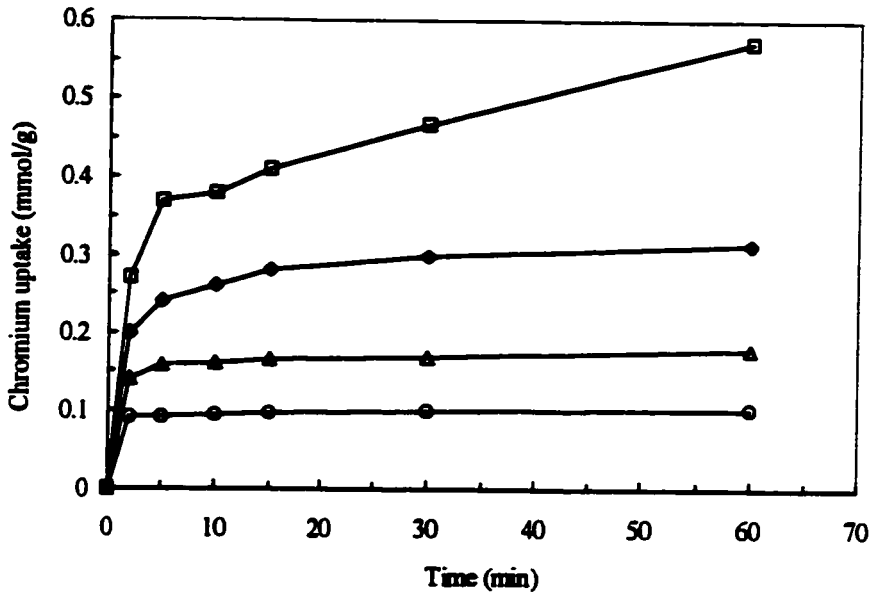


Figure A.2: Kinetics of Cr^{3+} uptake by *A. carbonarius* using various biomass concentrations. Initial Cr^{3+} concentration: 100 ppm; initial pH: 5.0; biomass concentration: □-1.15, ◊-2.30, Δ-4.60, O-9.20.

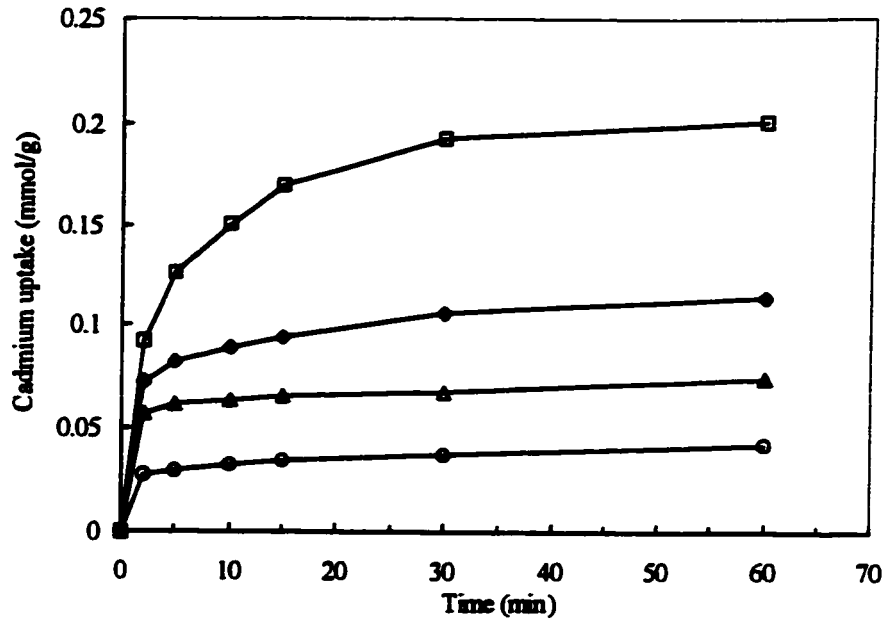


Figure A.3: Kinetics of Cd^{2+} uptake by *A. carbonarius* using various biomass concentrations. Initial Cd^{2+} concentration: 100 ppm; initial pH: 5.0-5.3; biomass concentration: □-1.15, ◊-2.30, Δ-4.60, O-9.20.

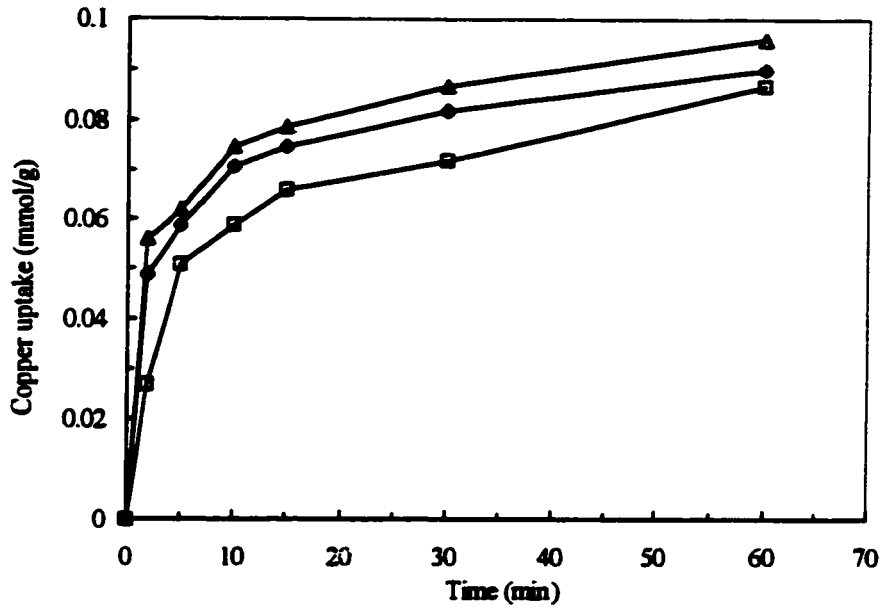


Figure A.4: Effect of pH on Cu^{2+} uptake by CM. CM concentration: 9.2 mg/mL; initial Cu^{2+} concentration: 100 ppm; initial pH: □-3.8, ◊-4.7, Δ-5.2.

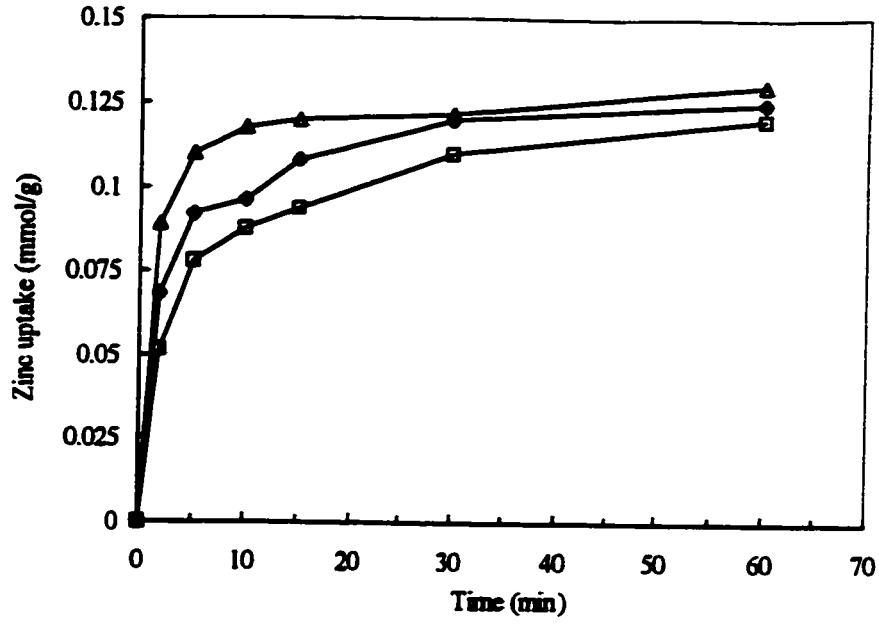


Figure A.5: Effect of pH on Zn^{2+} uptake by CM. CM concentration: 9.2 mg/mL; initial Zn^{2+} concentration: 100 ppm; initial pH: □-3.8, ◊-4.7, Δ-5.2.

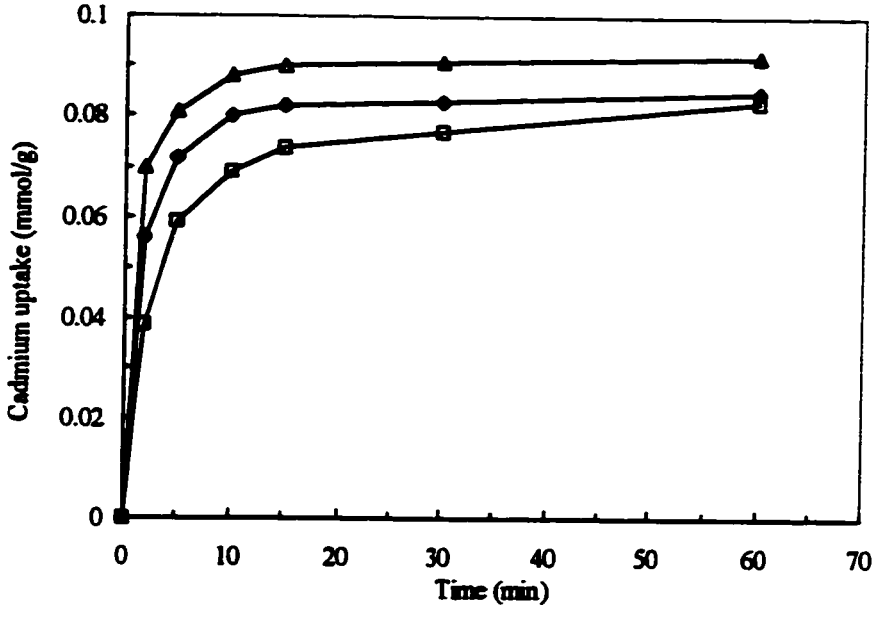


Figure A.6: Effect of pH on Cd^{2+} uptake by CM. CM concentration: 9.2 mg/mL; initial Cd^{2+} concentration: 100 ppm; initial pH: □-3.8, ◊-4.7, Δ-5.2.

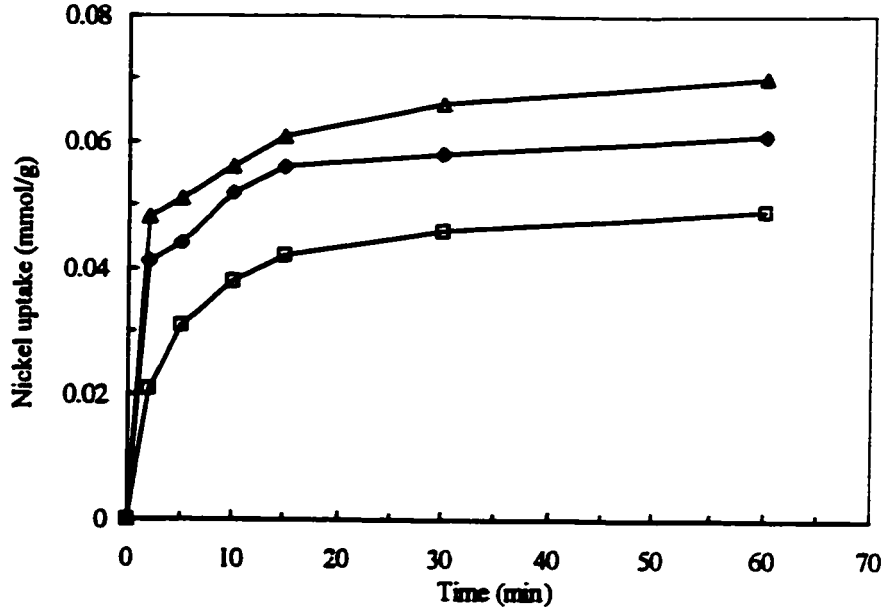


Figure A.7: Effect of pH on Ni^{2+} uptake by CM. CM concentrations: 9.2 mg/mL; initial Ni^{2+} concentration: 100 ppm; initial pH: □-3.8, ◊-4.7, Δ-5.2.

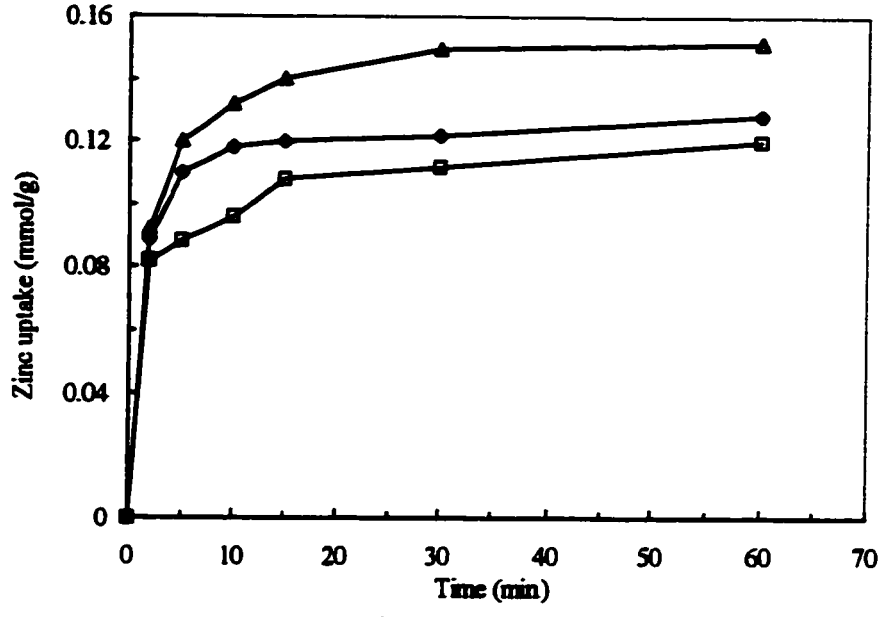


Figure A.8: Effect of temperature on Zn^{2+} uptake by CM. CM concentrations: 9.2 mg/mL; initial Zn^{2+} concentration: 100 ppm; initial pH: 5.2; temperature (°C): □-4, ◊-25, Δ-50.

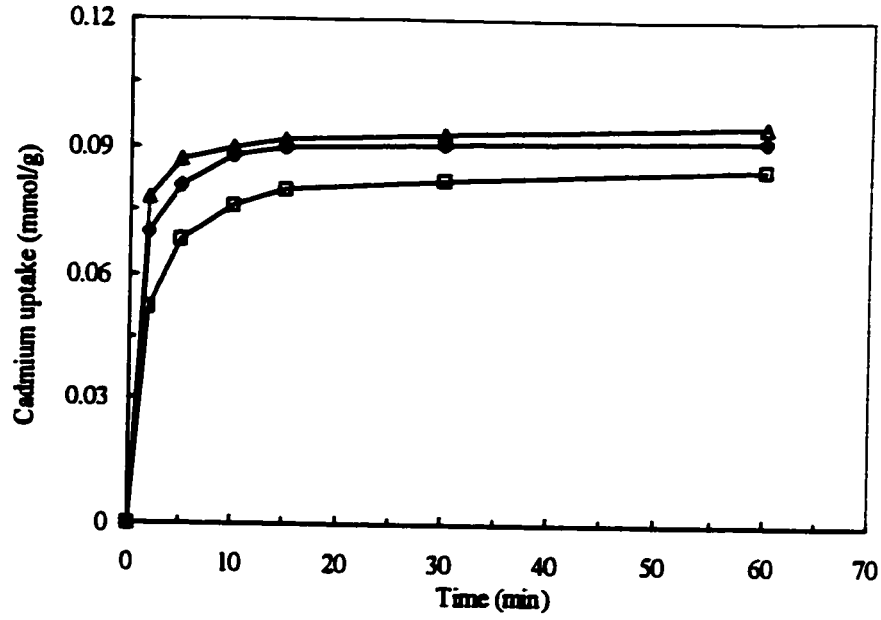


Figure A.9: Effect of temperature on Cd^{2+} uptake by CM. CM concentrations: 9.2 mg/mL; initial Cd^{2+} concentration: 100 ppm; initial pH: 5.2; temperature (°C): □-4, ◊-25, Δ-50.

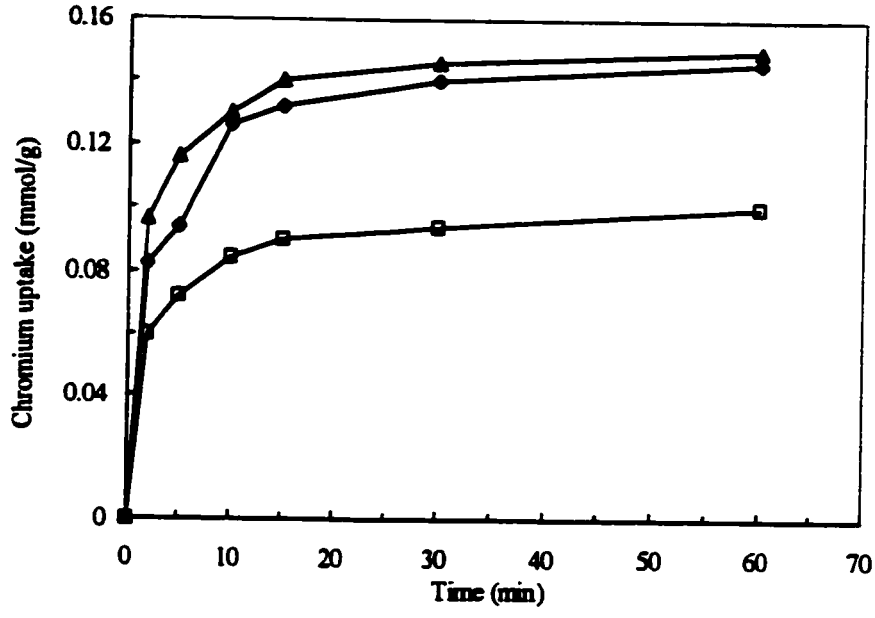


Figure A.10: Effect of temperature on Cr^{3+} uptake by CM. CM concentrations: 9.2 mg/mL; initial Cr^{3+} concentration: 100 ppm; initial pH: 5.2; temperature (°C): □-4, ◊-25, Δ-50.

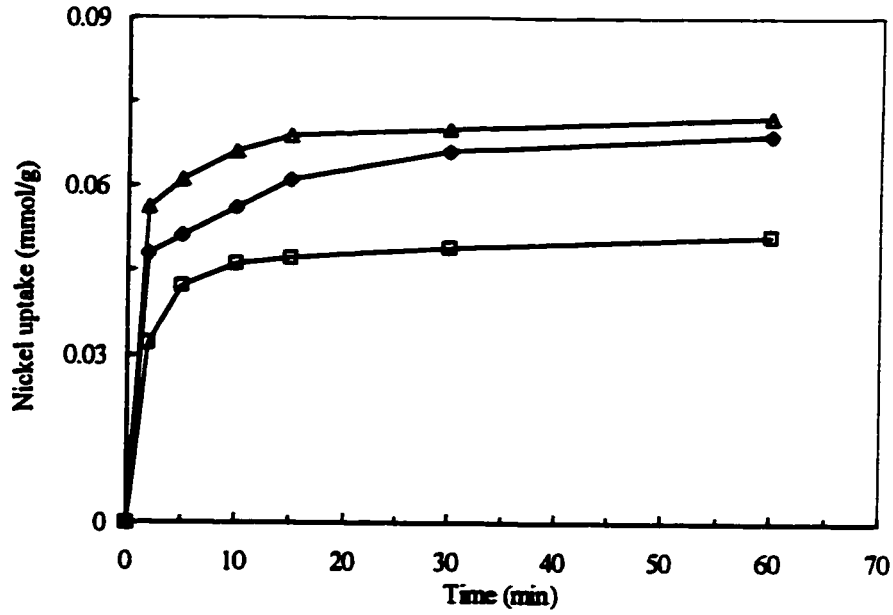


Figure A.11: Effect of temperature on Ni^{2+} uptake by CM. CM concentrations: 9.2 mg/mL; initial Ni^{2+} concentration: 100 ppm; initial pH: 5.2; temperature (°C): □-4, ◊-25, Δ-50.

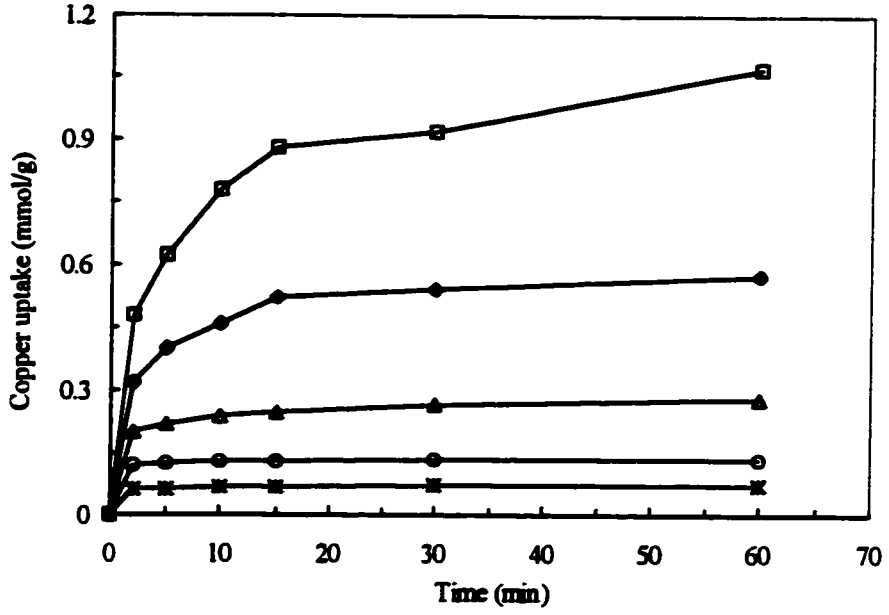


Figure A.12: Effect of sorbent concentration on Cu^{2+} uptake by pine needles. Initial Cu^{2+} concentration: 100 ppm; initial pH: 5.2-5.4; sorbent concentrations (mg/mL): □-1.15, ◊-2.3, Δ-4.6, O-9.2, ✳-18.4.

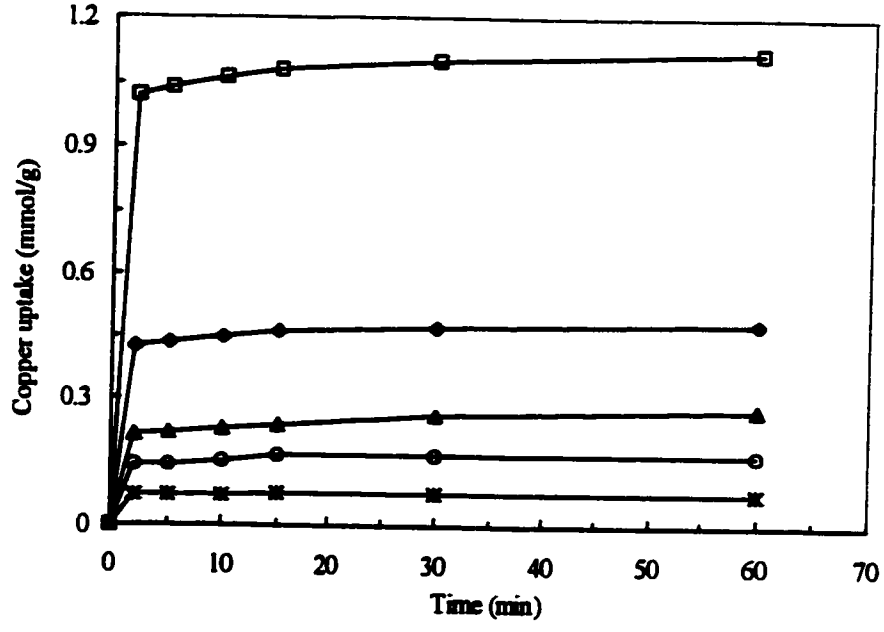


Figure A.13: Effect of sorbent concentration on Cu^{2+} uptake by pine cones. Initial Cu^{2+} concentration: 100 ppm; initial pH: 5.2-5.4; sorbent concentrations (mg/mL): □-1.15, ◊-2.3, Δ-4.6, O-9.2, ✳-18.4.

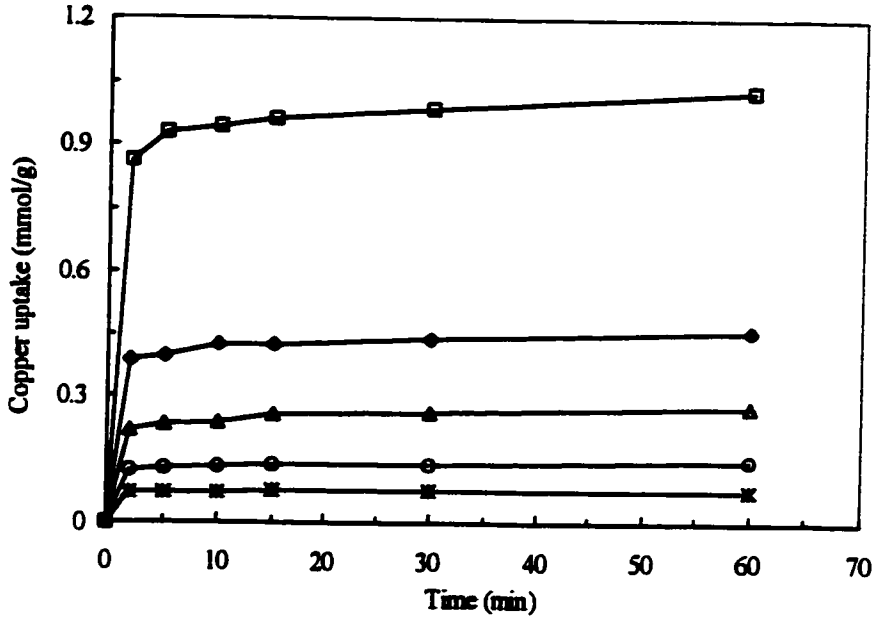


Figure A.14: Effect of sorbent concentration on Cu^{2+} uptake by oak cobs. Initial Cu^{2+} concentration: 100 ppm; initial pH: 5.2-5.4; sorbent concentrations (mg/mL): □-1.15, ◊-2.3, Δ-4.6, O-9.2, ✳-18.4.

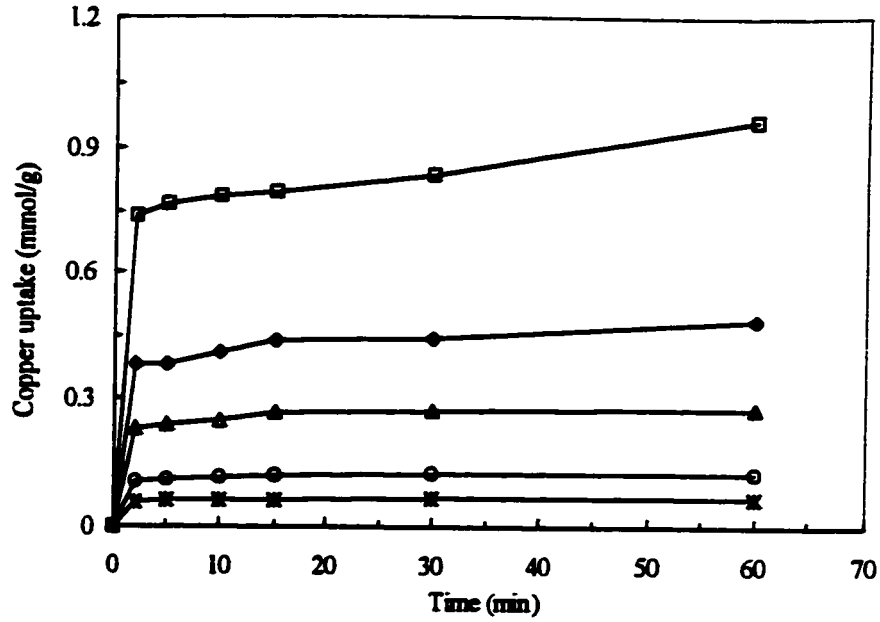


Figure A.15: Effect of sorbent concentration on Cu^{2+} uptake by oak leaves. Initial Cu^{2+} concentration: 100 ppm; initial pH: 5.2-5.4; sorbent concentrations (mg/mL): □-1.15, ◊-2.3, Δ-4.6, O-9.2, ✱-18.4.

Appendix B

Composition of Plant Sorbents

Table B.1: Elemental (C, H and N) analysis of the plant sorbents*.

Sorbent	C (%)	H (%)	N (%)
Pine needles	51.2 ± 0.141	6.01 ± 0.056	0.326 ± 0.018
Pine cones	50.5 ± 1.131	5.91 ± 0.056	0.786 ± 0.018
Oak cobs	47.3 ± 0.070	5.39 ± 0.014	0.181 ± 0.026
Oak leaves	49.2 ± 0.070	5.84 ± 0.035	0.781 ± 0.044
Corn cobs	44.1 ± 0.070	5.49 ± 0.127	0.339 ± 0.003
Corn straw	44.4 ± 0.636	5.28 ± 0.092	0.686 ± 0.090
Corn leaves	39.3 ± 0.141	4.92 ± 0.014	4.12 ± 0.0424
Maple leaves + stem	46.6 ± 0.141	4.94 ± 0.014	0.344 ± 0.010
Maple leaves only	46.6 ± 0.141	4.90 ± 0.042	0.384 ± 0.050
Maple stem only	43.9 ± 0.070	5.21 ± 0.028	0.142 ± 0.040
Birch leaves + stem	47.5 ± 0.212	5.85 ± 0.099	0.627 ± 0.011
Birch leaves only	47.3 ± 0.212	5.80 ± 0.184	0.656 ± 0.003
Birch stem only	44.2 ± 0.070	5.35 ± 0.007	0.349 ± 0.006
Lilac	46.0 ± 0.000	5.19 ± 0.007	1.010 ± 0.000

* Values represent average of duplicates ± standard deviation.

Table B.2: Lipids and pigments, proteins, cellulose and ash contents of plant sorbents*.

Sorbent	Lipids and pigments (%)	proteins (%)	Cellulose (%)	Ash (%)
Pine needles	7.190 ± 0.240	2.04 ± 0.113	28.21 ± 2.610	1.65 ± 0.010
Pine cones	6.650 ± 0.636	4.91 ± 0.113	28.85 ± 1.740	1.26 ± 0.077
Oak cobs	0.975 ± 0.015	1.13 ± 0.162	22.16 ± 0.198	1.86 ± 0.012
Oak leaves	4.340± 0.367	4.87 ± 0.275	13.48 ± 0.219	2.89 ± 0.024
Corn cobs	0.667 ± 0.054	2.12 ± 0.014	34.47 ± 0.283	1.27 ± 0.044
Corn straw	0.907 ± 0.096	4.28 ± 0.572	37.83 ± 0.410	
Corn leaves	2.457 ± 0.365	25.75 ± 0.268	19.52 ± 0.523	
Maple leaves + stem	3.094 ± 0.009	2.15 ± 0.056	13.02 ± 0.155	6.78 ± 0.003
Maple leaves only	3.450 ± 0.155	2.41 ± 0.296	12.27 ± 0.212	
Maple stem only	1.085 ± 0.064	0.89 ± 0.252	22.03 ± 0.084	
Birch leaves + stem		3.92 ± 0.070	17.39 ± 0.042	
Birch leaves only		4.10 ± 0.014	16.68 ± 0.021	
Birch stem only		2.17 ± 0.035	17.98 ± 0.290	
Lilac		6.31 ± 0.000	15.33 ± 0.558	

* Values represent average of duplicates ± standard deviation

Appendix C

Sample calculations for the uptake of copper by different constituents of moss following lignin analysis.

Basis: 1 g moss

Conditions of copper sorption; initial copper concentration: 50 ppm, initial pH: 4.5, sorbent concentration: 4.6 mg/mL.

Uptake of copper by pure moss = 9.48 mg/g_{moss} (0.149 mmol/g).

I. Separation of lipids and pigments:

Amount of lipids and pigment separated = 2.47%

Copper uptake by the residual after lipids and pigment removal = 9.22 $mg/g_{residual}$ (0.145 mmol/g).

Correction for lipids and pigments = 1 – 0.0247 = 0.9753

Amount of copper sorbed per gram of moss = 9.22 × 0.9753 = 8.99 mg/g_{moss} (0.149 mmol/g).

Amount of copper sorbed by lipids and pigments = 9.48 – 8.99 = 0.49 mg/g_{moss} (0.0077 mmol/g).

Amount of copper sorbed by lipids and pigments per gram of lipids and pigments =

$$\frac{0.49\ mg}{0.0247\ g} = 19.83\ mg/g_{lipids\ and\ pigments}\ (0.312\ mmol/g).$$

II. Separation of proteins by pepsin:

Amount of proteins separated = 14.63%

Copper uptake by the residual after proteins removal = 10.65 $mg/g_{residual}$ (0.167 mmol/g).

Correction due to lipids and pigments and proteins = 1 – 0.0247 – 0.146 = 0.829

Amount of copper sorbed per gram of moss = 9.22 × 0.829 = 8.83 mg/g_{moss} (0.139 mmo/g).

Amount of copper sorbed by protein = 8.99 – 8.83 = 0.16 mg/g_{moss} (0.00252 mmol/g).

Amount of copper sorbed by proteins per gram of proteins = $\frac{0.160\ mg}{0.146\ g}$

= 1.1 $mg/g_{proteins}$ (0.0173 mmol/g).

III. Separation of carbohydrates:

Amount of carbohydrates separated = 34.4%

Copper uptake by the residual after carbohydrates removal = 10.41 $mg/g_{residual}$ (0.164 mmol/g).

Correction due to lipids and pigments, proteins and carbohydrates =

1 – 0.0247 – 0.146 – 0.344 = 0.485

Amount of copper sorbed per gram of moss = $10.41 \times 0.485 = 5.046$ mg/g_{moss} (0.079 mmol/g).

Amount of copper sorbed by carbohydrates = $8.83 - 5.046 = 3.78$ mg/g_{moss} (0.059 mmol/g).

Amount of copper sorbed by carbohydrates per gram of carbohydrates = $\frac{3.78 \text{ mg}}{0.344 \text{ g}}$

= 11.0 mg/$g_{carbohydrates}$ (0.173 mmol/g).

IV. Separation of lignin:

Amount of lignin separated = 42.2 %

Copper uptake by the residual after lignin removal (ash) = 9.34 mg/g_{ash} (0.147 mmol/g).

Correction due to lipids and pigments, proteins, carbohydrates and ash =

$1 - 0.0247 - 0.146 - 0.344 - 0.422 = 0.0633$

Amount of copper sorbed per gram of moss = $9.34 \times 0.0633 = 0.591$ mg/g_{moss} (0.0093 mmol/g).

Amount of copper sorbed by lignin = $3.78 - 0.572 = 3.20$ mg/g_{moss} (0.0503 mmol/g).

Amount of copper sorbed by carbohydrates per gram of carbohydrates = $\frac{3.20 \text{ mg}}{0.422 \text{ g}}$

= 7.55 mg/g_{lignin} (0.118 mmol/g).

Appendix D

Electron Microscopy and X-ray spectra

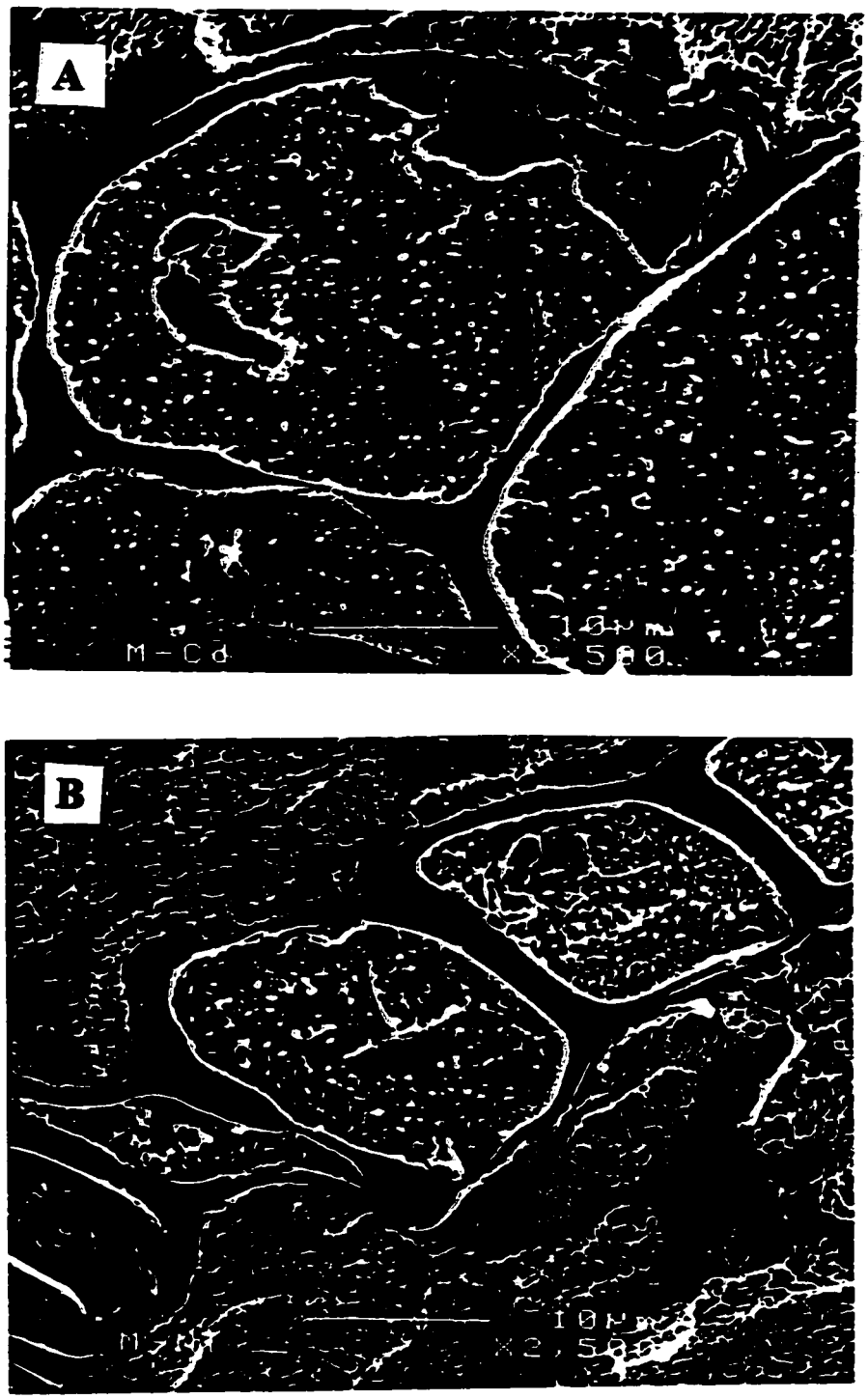


Figure D.1: Scanning electron micrographs (Mag. 2500 ×) of moss cells exposed to (A) Cd^{2+} (0.076 mmol/g) and (B) Ni^{2+} (0.108 mmol/g).

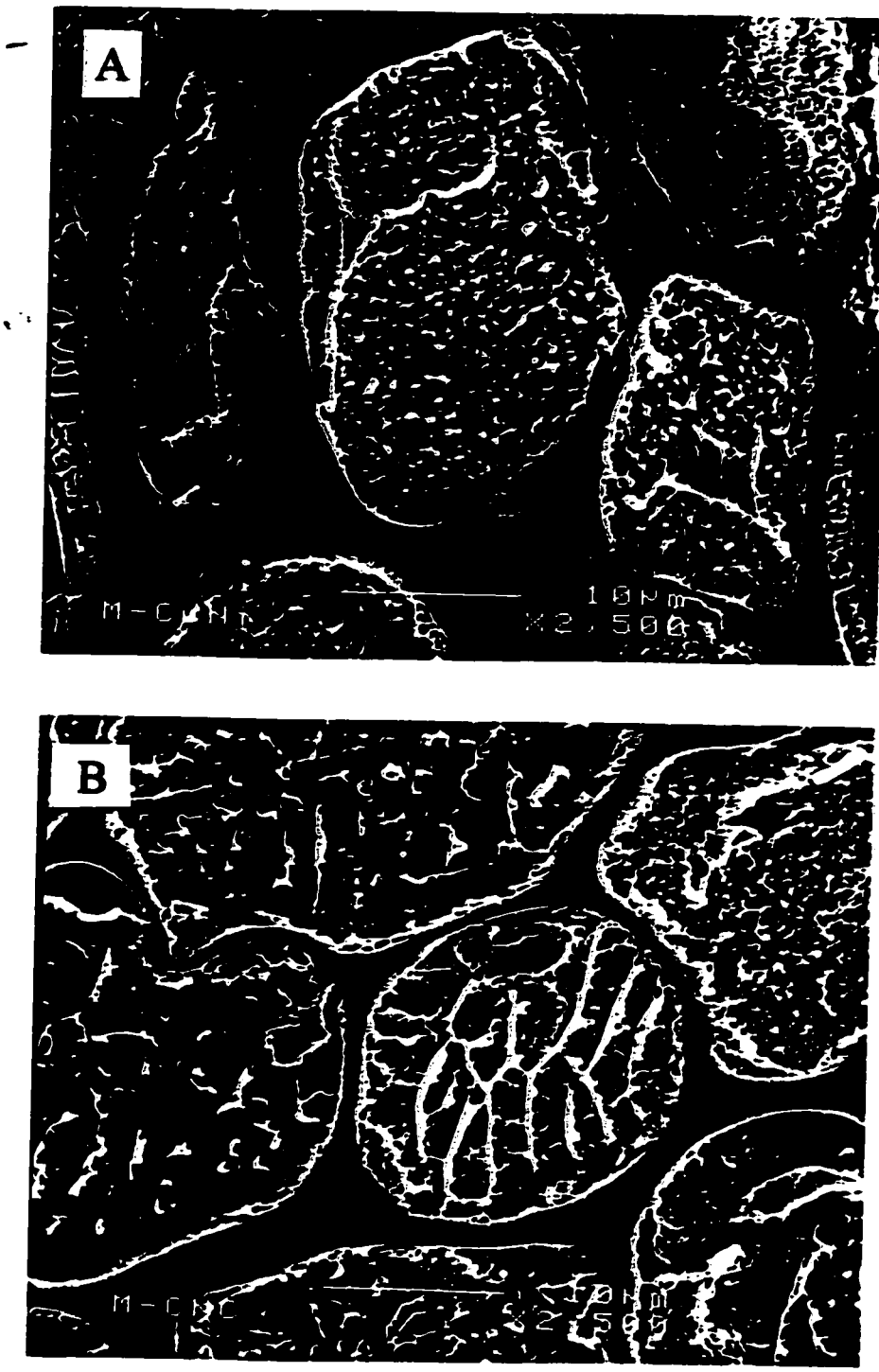


Figure D.2: Scanning electron micrographs (Mag. 2500 ×) of moss cells exposed to (A) Cu^{2+}-Ni^{2+} metal system (uptake (mmol/g); Cu^{2+}: 0.165, Ni^{2+}: 0.034) and (B) Cu^{2+}-Ni^{2+}-Cd^{2+} metal system (uptake (mmol/g); Cu^{2+}: 0.150, Ni^{2+}: 0.025, Cd^{2+}: 0.041).

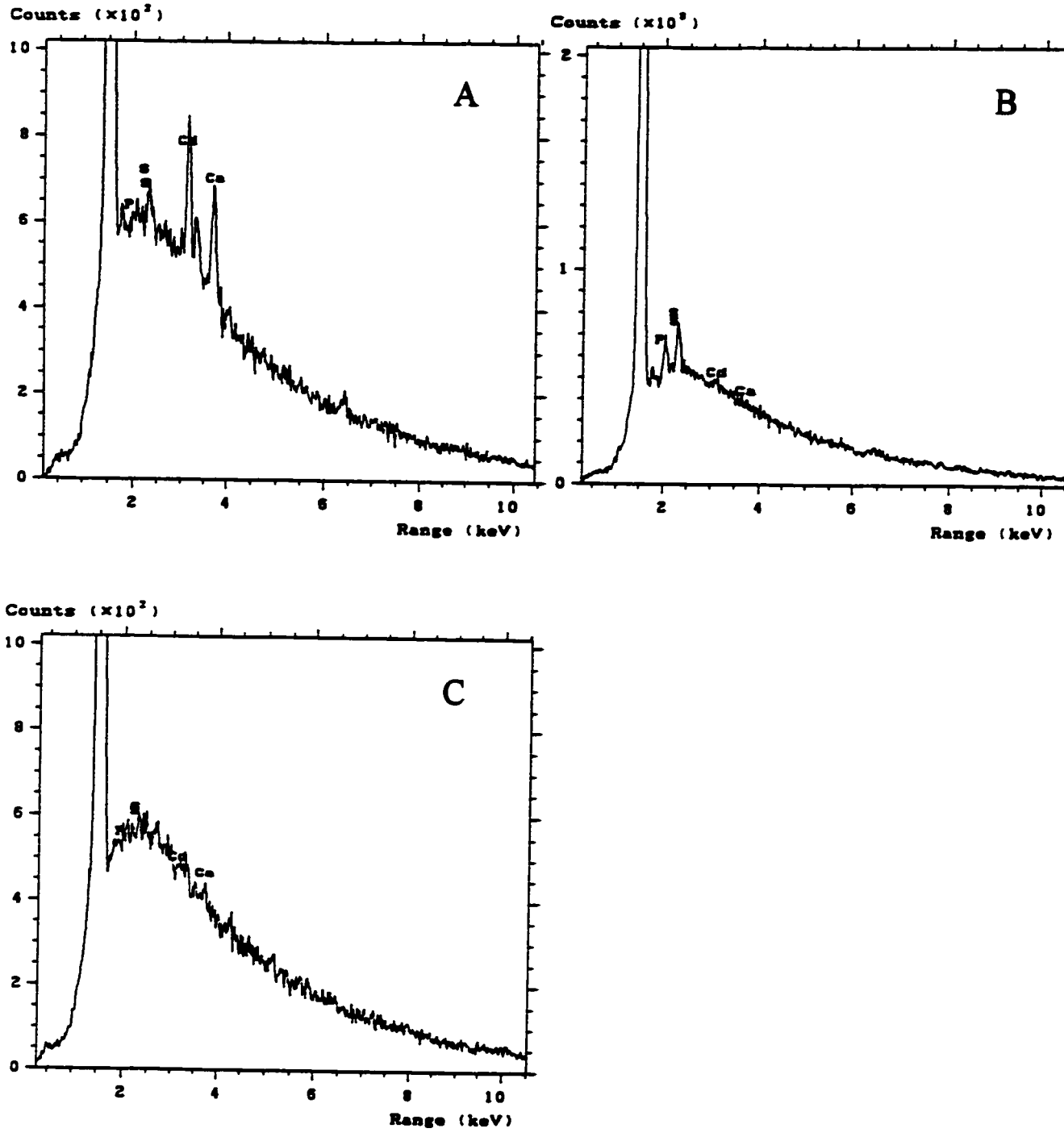


Figure D.3: Energy-dispersive X-ray of moss after Cd^{2+} uptake. A) cell wall; B) cytoplasm; C) vacuole.

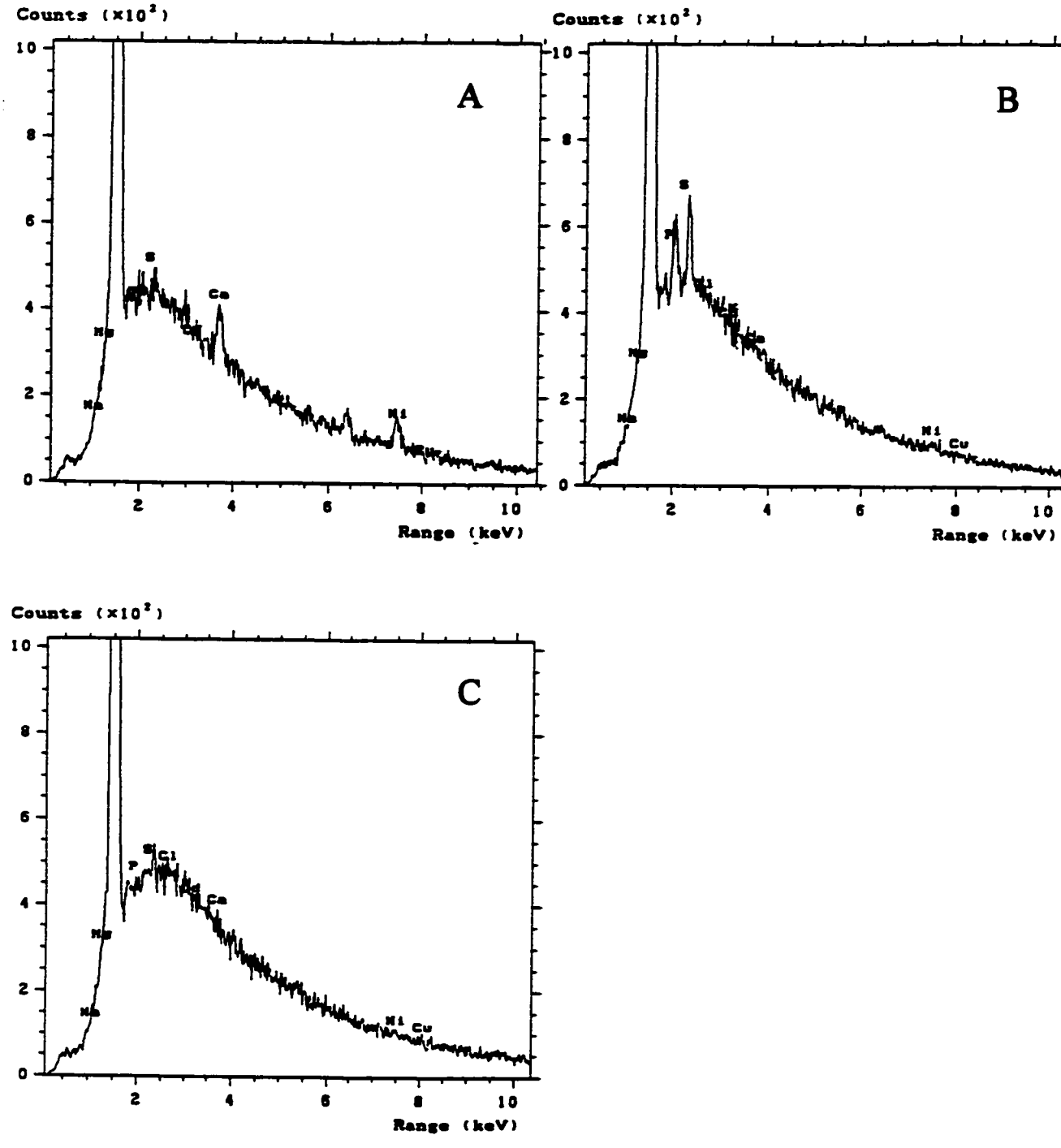


Figure D.4: Energy-dispersive X-ray of moss after Ni^{2+} uptake. A) cell wall; B) cytoplasm; C) vacuole.

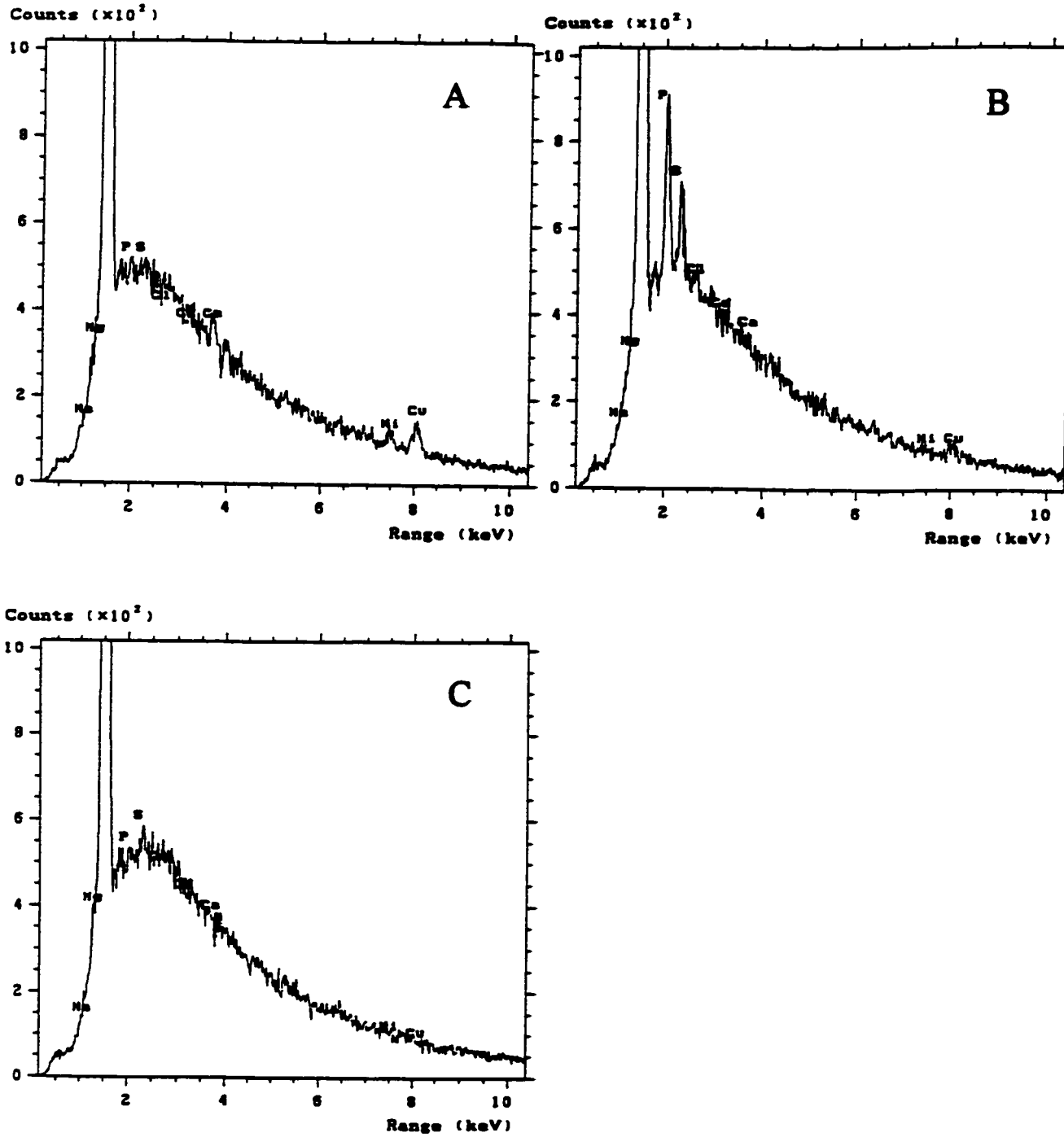


Figure D.5: Energy-dispersive X-ray of moss after Cu^{2+}–Ni^{2+} uptake. A) cell wall; B) cytoplasm; C) vacuole.

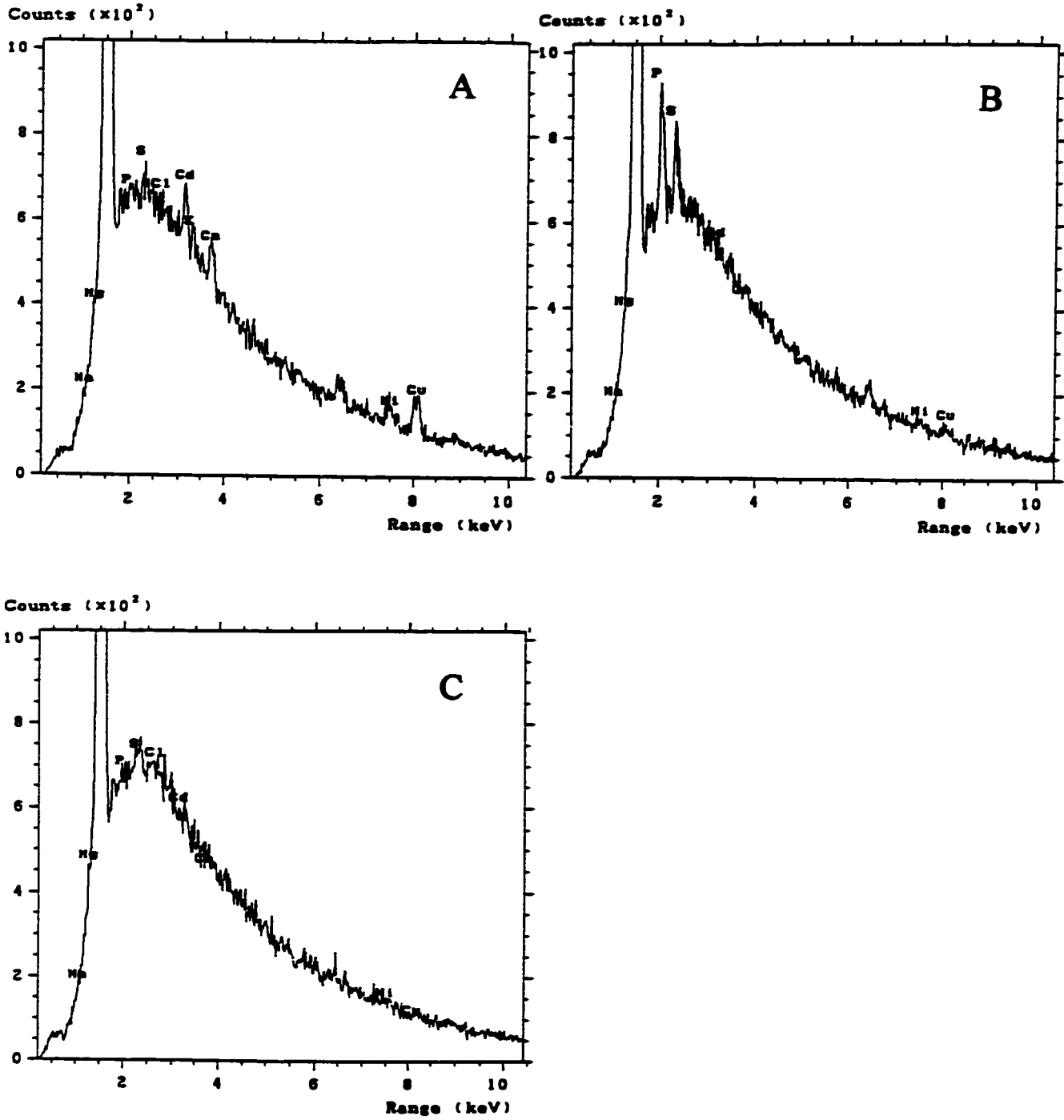
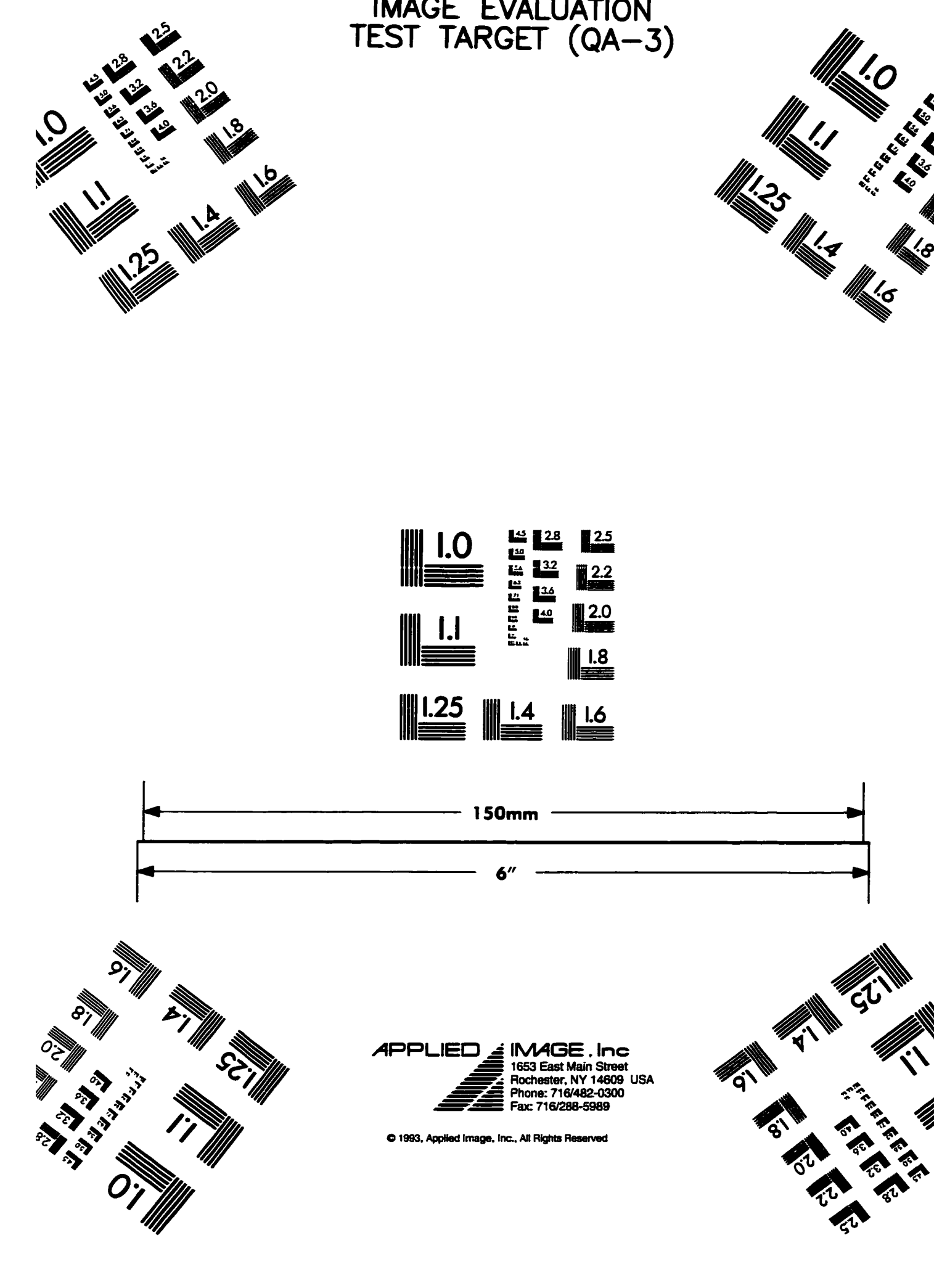


Figure D.6: Energy-dispersive X-ray of moss after Cu^{2+}–Ni^{2+}–Cd^{2+} uptake. A) cell wall; B) cytoplasm; C) vacuole.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4.5
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