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Copper adsorption by esterified and unesterified fractions of Sphagnum peat moss and its different humic substances

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Abstract

The Cu(II) binding properties of Canadian Sphagnum peat moss, humic acid and humin extracted from the peat moss were investigated. Batch pH profile experiments indicated that the adsorption of Cu(II) is pH dependent. At pH 4.0 and 5.0 about 99% of Cu(II) was bound by all three biomasses. Time-dependent experiments showed that the binding of Cu(II) is very rapid. The Cu(II) binding capacities at pH 4.0 were 16.1 mg per gram peat moss, 28.2 mg per gram humic acid, and 17.9 mg per gram humin. More than 90% of Cu(II) bound to the biomasses was recovered by treatment with 0.1 *M* HCl. We showed that carboxyl groups on these humic substances are responsible for some of the Cu(II) binding by esterifying them with methanol in the presence of trimethoxymethane (trimethyl orthoformate) and observing a decrease in Cu(II) binding. Infrared analysis confirmed the esterification and base hydrolysis of the esterified biomasses corroborated that esterification (and not degradation) had occurred since the metal-binding ability was regained. Our results provide important information on the interaction of Cu(II) ions with Sphagnum peat moss and its humic fractions. This may have practical applications for the removal of hazardous copper ions from contaminated water supplies.

Keywords: Humic substances; Sphagnum; Peat moss; Humic acids; Humin; Copper(II) binding; Esterification

1. Introduction

Copper is a hazardous toxic metal ion that can cause stomach and intestinal distress, liver and kidney damage, and anemia. Persons with Wilson's disease may be at higher risk of adverse health effects due to copper than the general public. For these harmful health effects, copper is one of the heavy metal ions controlled by federal drinking water regulations. The United States Environmental Protection Agency

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has set standards for drinking water to have concentrations of copper of less than 1.3 parts per million (ppm) [1].

Humic substances comprise a general class of biogenic, refractory, yellow-black organic substances present throughout terrestrial and aquatic environments [2]. These substances are very important biomasses because they serve as a major reservoir of organic carbon for the global carbon cycle; furthermore, they are thought to be one of the major reasons for the transport of metal ions in the environment [3]. Humic substances have been shown to be heterogeneous consisting of numerous oxygen-containing functional groups and fractions (humic acids, fulvic acids and humin) with different molecular weights [4]. Their chemical functional groups include acidic (primarily carboxylic and phenolic), carbonyl, hydroxyl, and others [5]. Fulvic acids have low molecular weight, contain more acidic groups and higher oxygen content but less carbon. Some branches of these structures are similar to salicylic, phthalic, maleic, or glutaric acids [6]. In contrast to fulvic acids, humic acids have higher molecular weight and less oxygen content. Stevenson's structure of humic acids shows oxygen incorporated in aromatic carboxyl groups, phenols, quinones, and other bridging units [7]. Of all three fractions of humic substances, humin has the highest molecular weight and lowest oxygen content. Most of the oxygen is present in bridging units, or in quinones, esters, and other non-acidic structures [8].

Recently, humic substances have been studied because these materials can form stable complexes with heavy metal ions such as Cu(II), Cr(III), Cd(II), and Hg(II) and since traditional methods of removing these metals from contaminated waters are not always economical. Humic substances in peat land such as Sphagnum peat moss may be a potential technology substitute because of their high harvest and low price. For example Benedetti et al. determined that existence of humic substances in surface waters tend to lower the free Cu(II) ion concentration seven orders of magnitude below the total Cu(II) concentration [9]. Ardakani and Stevenson discovered that the formation of metal–humic complexes facilitated the mobilization, transport, segregation, and deposition of trace metals in soils, sediments, and sedimentary rocks [10].

Previous work by geologists, biologists, environment chemists, and engineers has established that the ability of humic substances to bind heavy metal ions can be attributed to their high content of oxygen-containing functional groups, including carboxyl, phenol, hydroxyl, enol, and carbonyl structures of various types [11]. Gamble and Schnitzer postulated that two types of reactions are involved in metal–humic interactions, the most important one involving both phenol and carboxyl groups [12]. Infrared spectroscopy studies confirmed that carboxyl groups, or more precisely carboxylates, play a prominent role in the complexing of metal ions by humic and fulvic acids [13, 14]. Rate and McLaren showed that changes in pH, Cu(II)–humic acid ratio, and ionic strength can greatly affect the dissociation kinetics of Cu(II)–humic acid complexes [15]. Various models of proton and metal binding to natural organic matter have been proposed. In these models, humic substances are represented as a combination of known ligands of similar structures and binding constants, and the binding constants and acidity dissociation constants (pK_a) were obtained for some humic substances [9]. When Y.J. Park and K.K. Park [16]

performed binding experiments using Eu(III) with soil fulvic acids, they found two types of carboxylate moieties binding metal ions in 1:1 and 1:2 complexes (EuL^{+} and EuL^{2+}). The weaker binding species, EuL^{2+} was quite abundant and increased as the pH was raised from 2.9 to 6.3 but it was susceptible to hydrolysis at a pH higher than 7.

No studies on the binding of Cu(II) ions to peat moss and humic compounds extracted from it have been reported. In addition, even though a great deal of research indicates that carboxyl groups are involved in the metal-binding process, no reports have appeared on chemical modifications of the carboxyl groups to prove metal binding. In this study, fractions of humic substances were extracted from Sphagnum peat moss on which different types of Cu(II) binding experiments such as pH profile, titration, time dependendency, and capacity experiments were performed. Various humic substances were also esterified. If the proposed Cu(II) binding occurs through interaction with carboxyl groups, conversion of the free carboxyl groups to methyl esters should diminish metal binding.

2. Methodology

2.1. Extraction of humic substances of Sphagnum peat moss

Canadian Sphagnum peat moss was purchased from Fisons Horticulture Inc., Vancouver, Canada. After drying, 100 g of the peat moss was ground and sieved through an 80-mesh screen. Humin and humic acids extraction methods are given in Fig. 1. After washing the Sphagnum peat moss, it was treated with NaOH which produced two fractions: a supernatant (humic and fulvic acids), and humin and other insoluble compounds. Subsequently, the supernatant was acidified with HCl to pH 0.5 with the humic acids precipitating. The fulvic acids stayed in solution and were not studied. All fractions were separated by centrifugation and finally lyophilized in a labconco freeze-dryer.

2.2. pH profile experiments for Cu(II) ion binding

A 250 mg sun-dried sample of each biomass (peat moss, humin and humic acids) was washed twice with 40 ml 0.01 *M* HCl to remove debris. In order to achieve a biomass suspension concentration of 5 mg/ml, 50 ml of 0.01 *M* HCl was added to each sample. While stirring, the pH of the suspensions was adjusted to 2.0 by adding NaOH solution and three 2 ml aliquots of each solution were transferred into plastic tubes. The aliquots were centrifuged at 3000 rpm for 5 min, and supernatants were transferred to clean test tubes with 2 ml 0.1 m*M* copper sulfate solution at pH 2.0 to see whether soluble materials would precipitate Cu(II) ions (pH control). Other three 2 ml 0.1 m*M* Cu(II) solutions at pH 2.0 were transferred into the pH 2.0 biomass pellets and equilibrated for 1 h. After centrifugation, the supernatants were transferred into three clean tubes. All of the final pHs were tested using a pH electrode (Orion) before analyzing the remaining Cu(II) concentration by flame

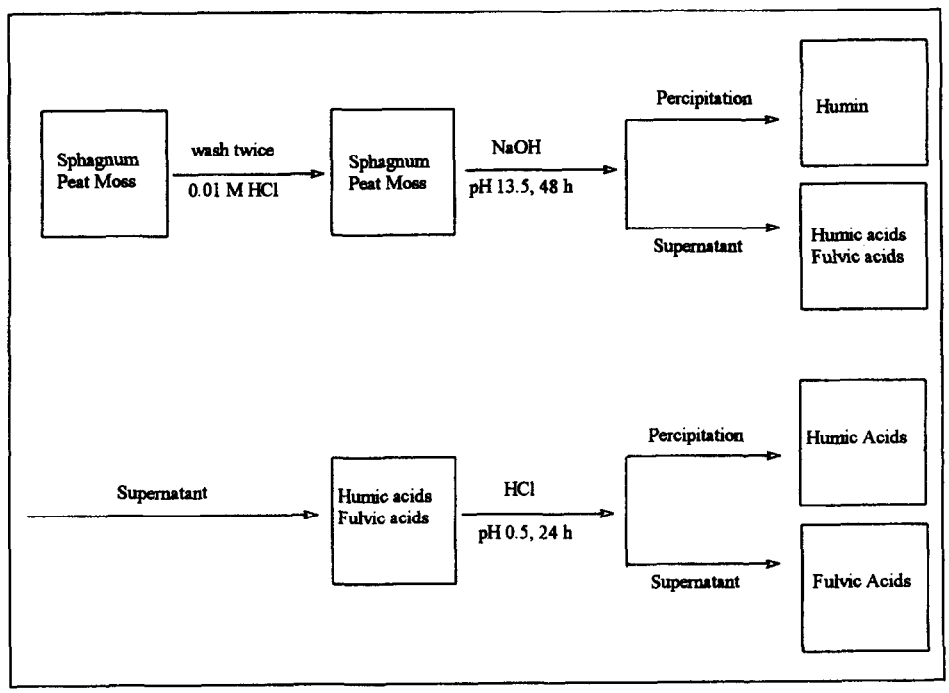

Fig. 1. Method for extraction of humic acids and humin from Sphagnum peat moss.

atomic absorption spectrometry. The adsorbed Cu(II) was obtained by calculating the difference between initial Cu(II) concentration and remaining Cu(II) concentration. The same procedures were performed at pH 3.0, 4.0, 5.0 and 6.0.

2.3. Titration experiment for the biomasses

A 150 mg sample of each biomass was washed with 40 ml 0.01 *M* HCl. After centrifugation the biomass was mixed with 30 ml 0.1 *M* HCl, and was then transferred into beakers. The pH of the biomass suspensions was adjusted to 1.0, then the suspensions were titrated from pH 1.0 to 9.0 with 0.100 *M* NaOH. A blank solution (30 ml deionized water) was titrated using the same procedure.

2.4. Time-dependent experiments for Cu(II) binding

A 250 mg sample of biomass was washed twice with 0.01 *M* HCl to remove any debris or soluble biomolecules that might interact with ions. The washings were collected, dried, and weighed to account for any biomass weight loss. Each biomass sample was resuspended in 50 ml 0.01 *M* HCl with biomass concentration approximately 5 mg/ml. The solution pH was then adjusted to 4.0 and allowed to equilibrate. Subsequently, 2 ml of the suspension was transferred to 18 tubes; 3 tubes for each time interval of 15, 30, 60, 120 and 180 min. After centrifugation, 2 ml 0.1 m*M*

Cu(II) solution was added to each of the tubes and controls. All the tubes were equilibrated by rocking and were removed at the appropriate time intervals. The samples were then transferred to clean tubes. Final pHs for all tubes were recorded and analysis for Cu(II) was performed by flame atomic absorption spectrometry.

2.5. Cu(II) binding capacities experiments

Samples of each biomass (5 mg/g, pH 5.0) were suspended in a solution containing 0.1 m*M* Cu(II) as copper sulfate at pH 5.0. The suspensions were shaken for 45 min, centrifuged and decanted. The supernatants were analyzed for the target metal as before. The same biomaterial was resuspended several more times in a fresh metal solution, repeating the procedure until the saturation capacity of the materials was attained (e.g., the metal concentration in the supernatant was the same as the initial solution). The samples were diluted as required and analyzed for Cu(II) content. The amount of metal ions bound to the humic substance was calculated from the total metal accumulated from the separate metal-containing solutions.

2.6. Recovery of Cu(II) adsorbed

To remove the bound metal ions, the pellets with adsorbed Cu(II) from the capacity studies were treated twice with 2 ml 0.1 *M* HCl, and equilibrated by shaking for 5 min and centrifuged. After centrifugation the supernatants were removed, diluted as required and analyzed for Cu(II) content by flame atomic absorption spectrometry.

2.7. Chemical esterification experiments

A 2.5 g sample of each biomass was added to a three-neck flask followed by 150 ml 99.9% methanol and 50 ml trimethoxymethane to make a biomass solution of 12.5 mg/ml. The temperature was increased to about 63 °C to maintain reflux. Then 3.3 ml of concentrated sulfuric acid was added drop wise to each reaction. After 15, 30, 60, 180, and 360 min, 20 ml of each sample (containing 50 mg biomass) was taken out and the reactions were terminated by (1) centrifuging the samples, (2) removing acidic supernant, and (3) finally washing the precipitate (sample) three times with 50 ml cold water. When all the samples were ready, 250 mg of unesterified and esterified samples were suspended in 50 ml 0.01 *M* HCl at various times to make suspensions of 5 mg/ml. Cu(II) binding experiments were done at pH 2.0 and 5.0 using 0.3 m*M* Cu(II) solution according to the method previously described.

2.8. Hydrolysis of esterified carboxyl groups

Upon determination of the Cu(II) binding ability of the methanol-esterified humic substances, the pH of esterified humic materials was adjusted to 13.0 using a sodium hydroxide solution in order to hydrolyze the esterified carboxyl groups. Following two hours of shaking, the biomasses were readjusted to pH 2.0 and 5.0 for humin

and pH 2.0 and 3.0 for humic acids, and the Cu(II) binding ability was determined as indicated before.

2.9. Metal ion analysis

All of the analyses for Cu(II) were performed by flame atomic absorption using a Perkin Elmer model 3110 atomic absorption spectrometer with deuterium background correction. Impact bead was utilized to improve the sensitivity at a wavelength of 327.4 nm. Samples were read three times and the mean value was computed. A calibration was performed in the range of the analyses and the correlation coefficients for the calibration curves were 0.98 or greater. Controls for the metal solutions were introduced to detect possible metal precipitation.

2.10. Infrared analysis of esterified and unesterified biomass

Freeze-dried modified and unmodified samples (0.035 each) were mixed with 0.3465 g of potassium bromide (KBr) to give a biomass concentration of 1% by weight. The KBr–biomass mixtures (0.065 g) were then pressed into solid disks. After the background was corrected, each pellet was analyzed using a Perkin Elmer 1600 Fourier transform infrared spectrometer (FTIR) with a scan speed of 16 scans/s from 600 to 4000 cm^{-1}.

3. Results and discussion

We investigated the pH dependence of Cu(II) binding to Sphagnum peat moss, humic acids, and humin. Fig. 2 shows the Cu(II) binding of all three biomasses from pH 2.0 to 6.0. In this figure, we see that the ability to bind Cu(II) increases with increase in pH. At pH 4.0, the binding abilities of humin and peat moss reach their maximum. It was very interesting to observe that over a wide range of pHs, even at pH 2, all of the biomasses bind Cu(II) quite well. The humic acid fraction adsorbs the best at pH 2.0 with almost 90% binding. This can be explained by the high carboxyl content of humic acids. However, since some of the humic acids are soluble at pHs higher than 4.0 (due to their high carboxylate content and low molecular weight), some Cu(II) was complexed into solution. Thus, this complexed Cu(II) also precipitated when the sample was centrifuged and this is the reason why in Fig. 2 the adsorption of Cu(II) by the humic acid fraction seems quite low at pHs higher than 4.0.

Titration experiments were performed in order to determine the acidity of each humic fraction. Compared to the blank solution, all of the three biomasses showed strong buffer capacity between pH 2.0 and 5.0 (see Fig. 3). Humic acids seem to have two buffer sites, one around pH 2.3 and the other at 4.0 (which can be considered as pK_a's). Humin had only one buffer site around pH 2.5, no strong buffer capacity was observed at another site, but it still buffered the solution. This indicates that there were various kinds of acids. Perhaps carboxyl groups are attached

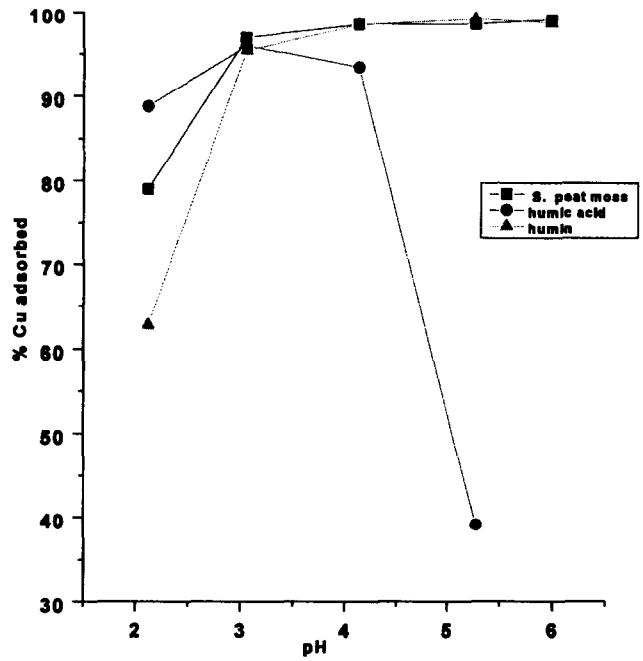

Fig. 2. Percent copper adsorbed as a function of pH by Sphagnum peat moss, humic acids, and humin. Each biomass (5 mg/ml) was reacted for 1 h at the appropriate pH with 0.1 m*M* Cu(II).

at different positions on aromatic rings. Like humin, peat moss has one strong buffer site at 2.5, and an unapparent site at pH 4.0. Basically, humin and peat moss have almost identical titration curves, which is explained by the fact that humin constitutes most of the Sphagnum peat moss (the total mass percentage of fulvic and humic acids is no more than 15%). The fact that humic acids require more NaOH to titrate corroborates the higher carboxyl content of this fraction.

Fig. 4 demonstrates that the Cu(II) adsorption by the three different biomasses is very rapid. Cu(II) adsorption occurred in less than 15 min and was relatively stable thereafter. These experiments were performed at pH 4 due to the solubility of humic acids at pH 5. Unlike living or fresh plant tissues, humic substances are decomposed organic matter with cell walls and other tissues destroyed after hundreds or thousands of years. The humication process has therefore exposed functional groups such as carboxyl, hydroxyl, and phenol groups to the surface of the biomasses. We believed that the exposure of these chemical groups to the surface accounts for the rapid adsorption of Cu(II) by the three biomasses.

Table 1 shows the amount of Cu(II) that was adsorbed from solution by Sphagnum peat moss, humic acids, and humin as the saturation point was reached. These studies were performed at pH 4.0. The binding capacities of the different populations ranged from 16.1 to 28.2 mg of bound Cu(II) per gram of biomass with humic acid having the highest adsorption capacity. We expected that humic acids would have the highest binding capacity since as shown in the titration experiments,

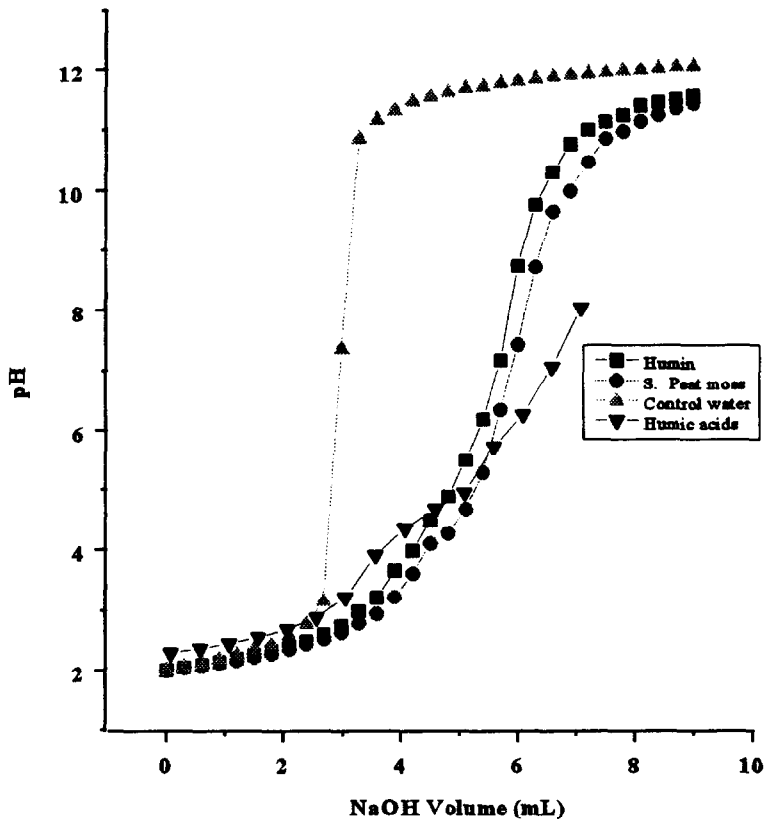

Fig. 3. Base titration of Sphagnum peat moss, humic acids, and humin. Each biomass (150 mg) was diluted with 30 ml of 0.1 *M* HCl and they were titrated with 0.1 *M* NaOH. The abscissa represents the volume of NaOH added.

humic acids had the highest buffering capacity and thus the highest carboxylate content.

We investigated the possibility of recovering the Cu(II) bound to capacity by the biomasses. We hypothesized that by protonating the carboxylate groups with HCl the adsorbed Cu(II) would be desorbed. Table 2 shows that between 95% and 100% of Cu(II) adsorbed was desorbed by treatment with 0.1 *M* HCl with humic acids having the lowest Cu(II) desorption (95%).

One of the main goals of this work was to understand the involvement of the carboxyl groups contained by the three different fractions in Cu(II) binding. Our first attempt to gain this information was to chemically block the biomass carboxyl groups by methanol esterification [17]. The three humic substances were not easily esterified by procedures previously used to modify algae [17]. After many attempts (e.g., increasing the acidity, the temperature, and methanol concentration) we decided to try to shift the equilibrium towards esterification by using trimethoxymethane ($(CH_3O)_3CH$) in methanol to remove water [18]. That trimethoxymethane does not

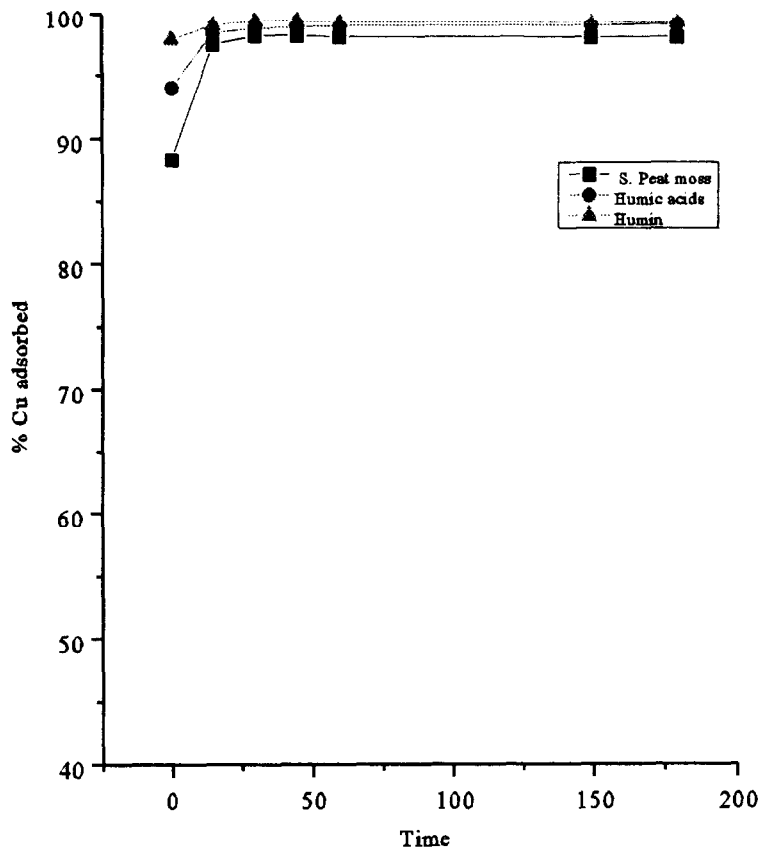

Fig. 4. Time-dependent experiment for Cu(II) adsorption by Sphagnum peat moss, humic acids, and humin. Each biomass (5 mg/ml) was reacted at the appropriate time with 0.1 m*M* Cu(II) at pH 4.0.

Table 1
Capacities of Cu(II) binding for Sphagnum peat moss, humic acid and humin[a]

Biomasses	Capacities (mg/g)
Sphagnum peat moss	16.1 ± 1.40
Humic acid	28.2 ± 1.04
Humin	17.9 ± 0.39

[a] The experiments were performed at pH 4.0.

react in the absence of methanol indicates that trimethoxymethane does not react directly with humic substances and therefore is not formylating hydroxyl groups in the humic substances. The proposed esterification reaction is shown below:

$$R-\overset{O}{\overset{\|}{C}}-O-H + HO-CH_3 \underset{63\,^{\circ}C}{\overset{(CH_3O)_3CH}{\rightleftharpoons}} R-\overset{O}{\overset{\|}{C}}-O-CH_3 + CH_3-O-\overset{O}{\overset{\|}{C}}-H + 2HO-CH_3 \qquad (1)$$

Table 2
Percent of copper removal by Sphagnum peat moss, humic acid and humin by treatment with 0.1 *M* HCl

Biomasses	Percentage of Cu(II) recovered
Sphagnum peat moss	102.9% ± 2.5%
Humic acid	95.5% ± 1.1%
Humin	102.0% ± 0.14%

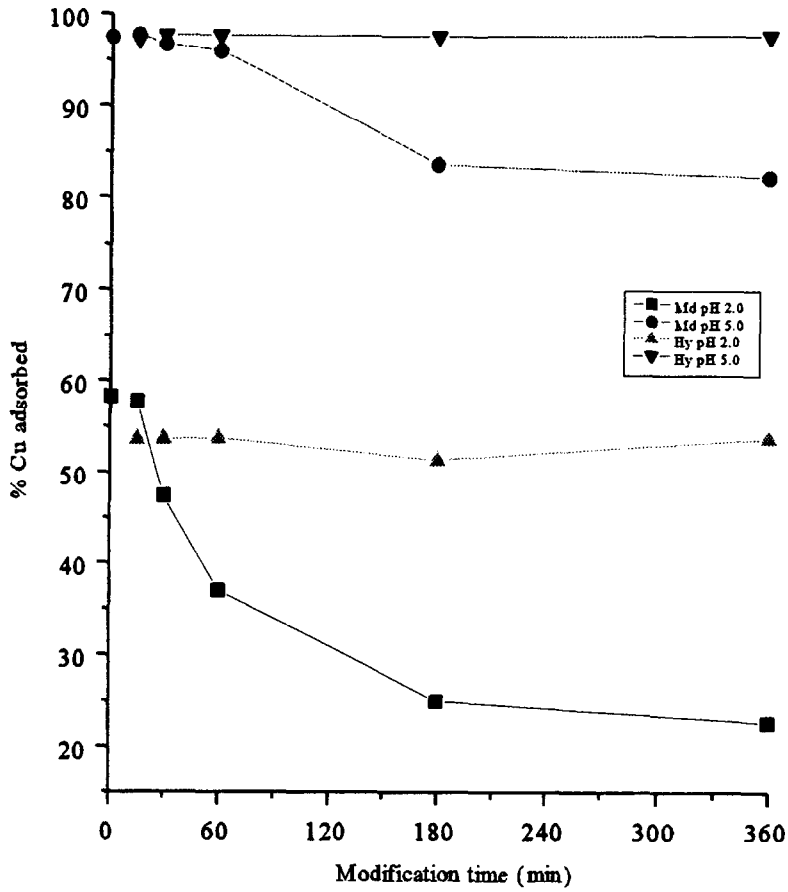

Fig. 5. Percent copper adsorption by humin at different reaction times after methanol esterification and percent copper adsorbed after base hydrolysis of the esterified humin. Humin esterified biomass (5 mg/ml) was reacted at the appropriate time with 0.3 m*M* Cu(II) at pH 2.0 and 5.0. The Md in the figure represents the modified (esterified) biomass. Also, the esterified biomass (5 mg/ml) after base hydrolysis was reacted at the appropriate time with 0.3 m*M* Cu(II) at pH 2.0 and 5.0. The Hy in the figure represents the biomass after hydrolysis.

We performed experiments with methanol-modified humin to determine if the esterification had changed the Cu(II) binding ability of the biomass. Fig. 5 shows that after modification for 360 min, the humin Cu(II) binding ability had decreased

from almost 100% to 80% at pH 5.0, and from 60% to 20% at pH 2.0. This means that even though carboxyl groups are involved in Cu(II) binding, other groups such as phenol and hydroxyl groups may still also be binding. We also carried out Cu(II) binding experiments with the esterified humin after base hydrolysis to prove that the esterification of the groups had really occurred and that the decreased Cu(II) binding was not a result of chemical or thermal degradation. Fig. 5 also shows that after base hydrolysis of the esterified humin, the Cu(II) binding ability was completely regained. Similar results were obtained for humic acids and peat moss on Cu(II) binding (Figs. 6 and 7, respectively). Base hydrolysis of the modified humic substances also indicates that esters and not acetals are being formed with trimethoxymethane [18].

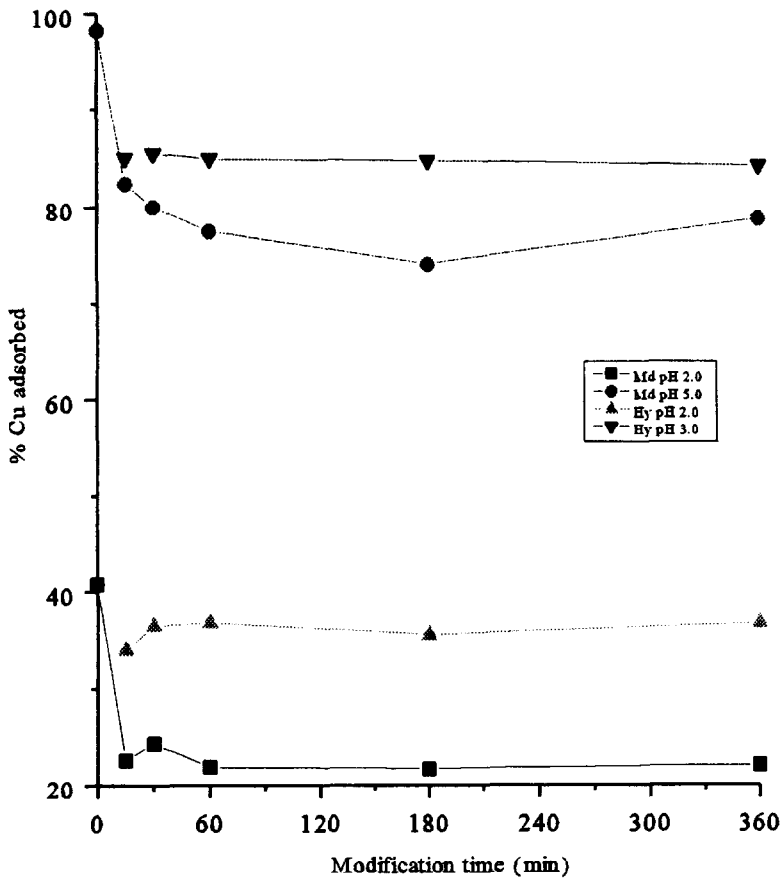

Fig. 6. Percent copper adsorption by humic acids at different reaction times after methanol esterification and percent copper adsorbed after base hydrolysis of esterified humic acids. Humic acids esterified biomass (5 mg/ml) was reacted at the appropriate time with 0.3 m*M* Cu(II) at pH 2.0 and 5.0. The Md in the figure represents the modified (esterified) biomass. Also, the esterified biomass after base hydrolysis was reacted at the appropriate time with 0.3 m*M* Cu(II) at pH 2.0 and 5.0. The Hy in the figure represents the biomass after hydrolysis.

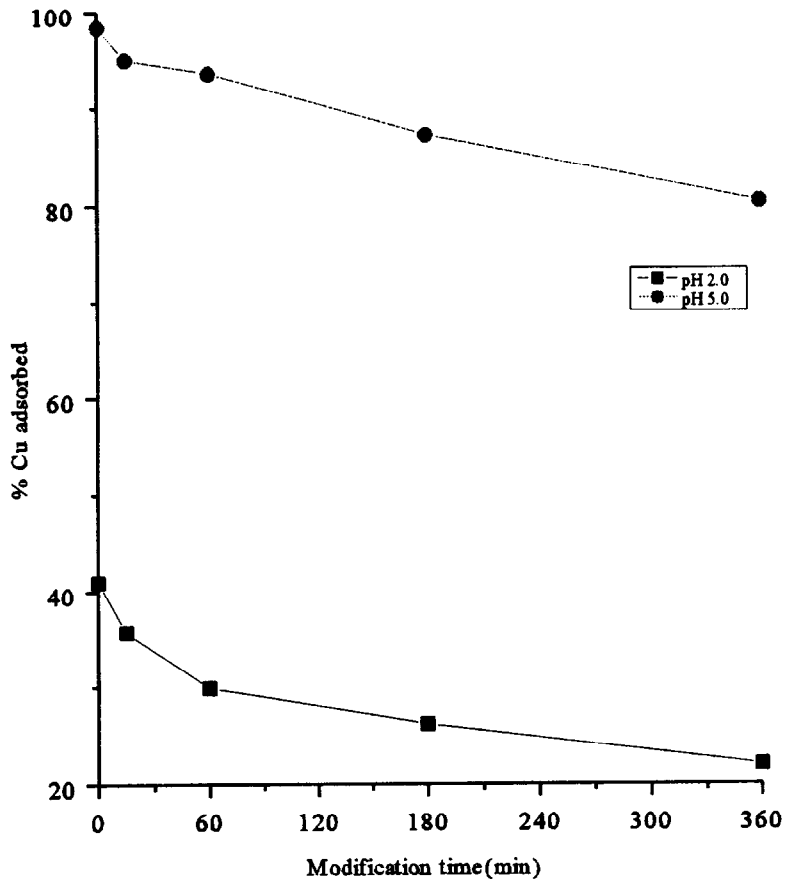

Fig. 7. Percent copper adsorption by Sphagnum peat moss at different reaction times after methanol esterification. Sphagnum peat moss esterified biomass (5 mg/ml) was reacted at the appropriate time with 0.3 m*M* Cu(II) at pH 2.0 and 5.0.

Infrared (IR) analyses were performed on unesterified and esterfied biomasses of Sphagnum peat moss, humic acids, and humin. We intended to gain further evidence that the esterification of the carboxyl groups had occurred by observing differences in the IR absorption before and after modification. Unlike pure compounds, which have sharp and typical absorption peaks, humic substances contain only a relatively few broad bands [19, 20]. These broad IR bands result from the overlap of absorptions of all kinds of similar functional groups [20, 21]. Two important IR regions related to our work are 3200–3600 cm^{-1} and 1100–1450 cm^{-1}. Absorption in the first region is from OH stretching vibrations in hydroxyl, phenol, and carboxyl groups. The second absorption region (1100–1450 cm^{-1}) is due to C–O stretching vibrations. Fig. 8 shows the IR spectra of esterified Sphagnum peat moss (Fig. 8(a)) and of unesterified Sphagnum peat moss (Fig. 8(b)). Higher absorption is observed in the region around 3400 cm^{-1} for the unmodified Sphagnum peat moss over the

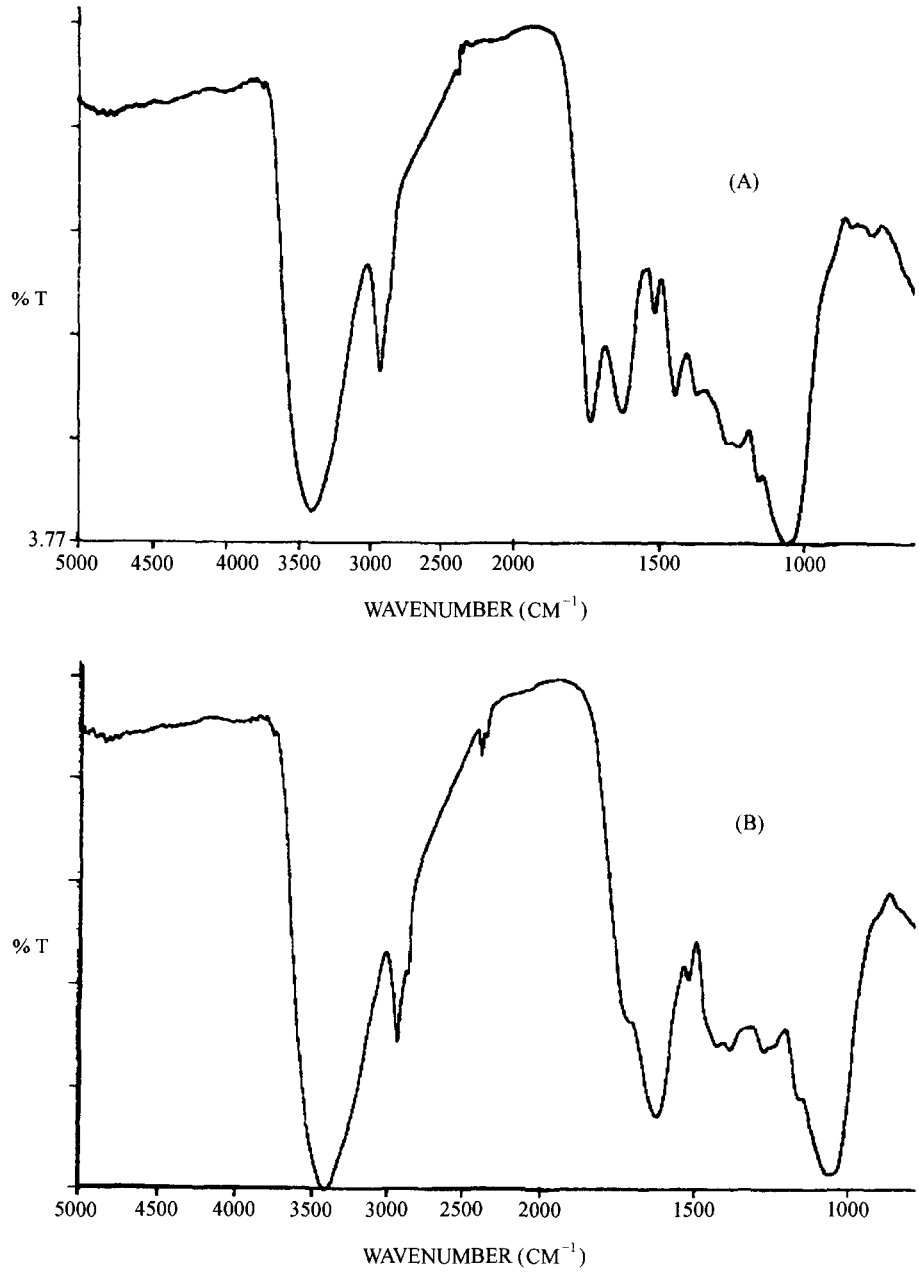

Fig. 8. Infrared analysis of Sphagnum peat moss: (a) esterified biomass; (b) unesterified biomass.

modified peat moss since more hydroxyl groups are present. On the other hand, the C–O absorption increases in modified peat moss as expected (region 1100 cm^{-1}) due to the formation of methyl esters. The same result is observed for humic acids

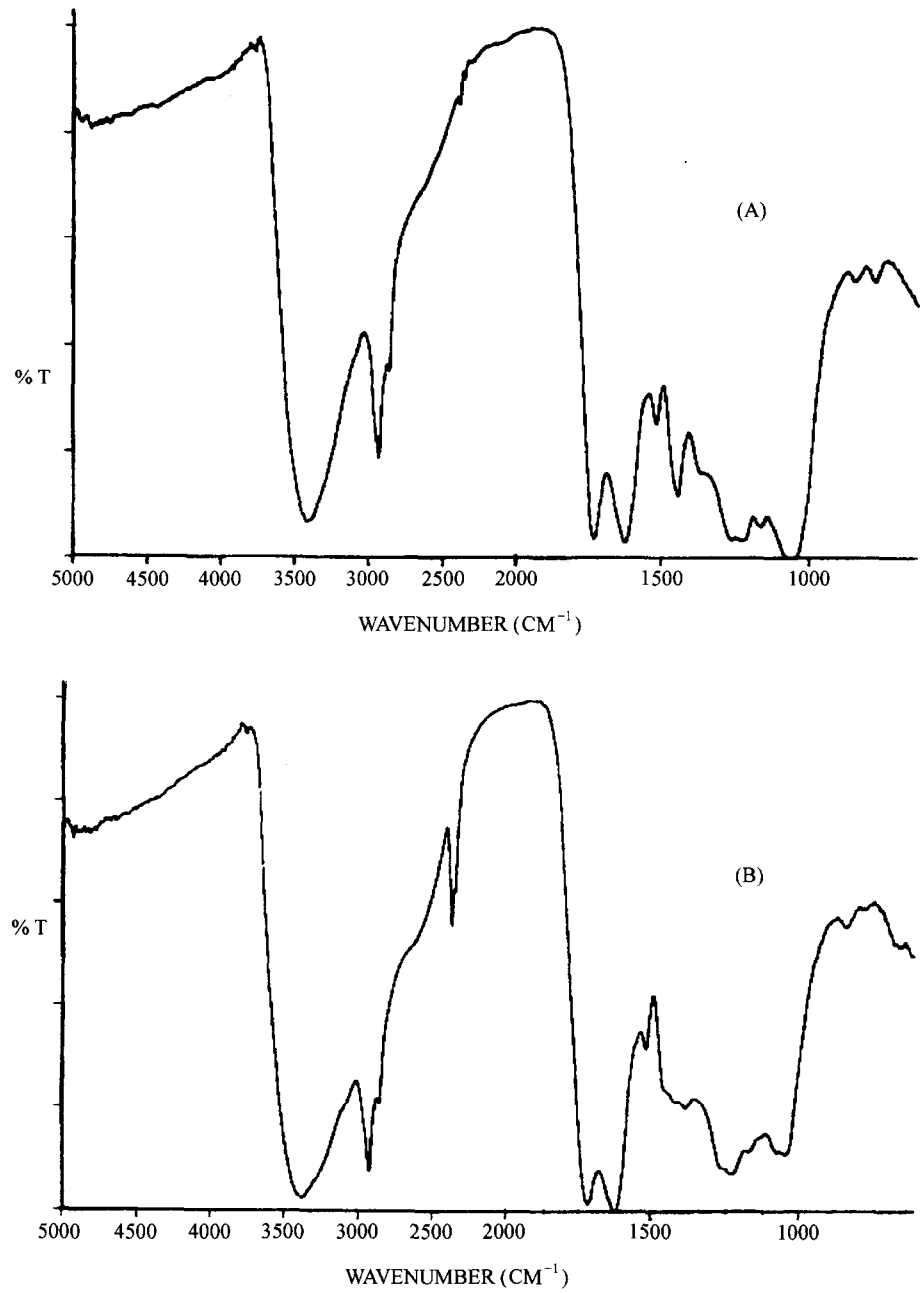

Fig. 9. Infrared analysis of humic acids: (a) esterified biomass; (b) unesterified biomass.

(Fig. 9), although the reduction of the hydroxyl absorption and the increase of the ester absorption is much more pronounced. This is expected since humic acids contain more carboxyl groups. The IR data for humin (not shown) was very similar to that of Sphagnum peat moss.

4. Summary and conclusions

Our work provides valuable information on the binding of Cu(II) ions by Sphagnum peat moss and two humic substances extracted from the peat moss (humic acids and humin). The adsorption of Cu(II) was pH dependent, with increasing adsorption as the pH increased from 2 to 5. However, there is still excellent Cu(II) adsorption at pH 2.0. Humic acids had the highest Cu(II) binding capacities, followed by humin. The higher Cu(II) binding capacity of humic acids is directly related to the higher content of acidic functionalities as shown from titration experiments. The three humic fractions adsorbed Cu(II) rapidly.

Methanol esterification of the three biomasses (driven by trimethoxymethane) showed that carboxyl groups play a role in Cu(II) binding. IR spectroscopic analysis confirmed that carboxyl groups had been esterified. In addition, base hydrolysis of the esterified biomasses corroborated that indeed esterification and not degradation had occurred since the metal-binding ability was regained.

Our results provide important information on the interaction of Cu(II) ions with Sphagnum peat moss and its humic fractions, and demonstrate that not only are carboxyl groups involved in metal binding but that other groups (i.e., phenol) must also be involved. This may have practical applications for the removal of hazardous copper ions from contaminated water supplies. Since most drinking water supplies have pHs ranging from 4 to 7, Sphagnum peat moss and its humic fractions may prove quite helpful to remove copper ions from contaminated waters.

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