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Copper adsorption kinetics of cultured algal cells and freshwater phytoplankton with emphasis on cell surface characteristics

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Abstract

Comparative studies were carried out on the adsorption of copper by a range of laboratory-cultured algae and freshwater phytoplankton samples. The level of surface mucilage associated with the cultured algae ranged from high (*Anabaena spiroides*, *Eudorina elegans*) to moderate (*Anabaena cylindrica*, *Microcystis aeruginosa*) to complete absence (*Chlorella vulgaris*, *Asterionella formosa*, *Aulacoseira varians*, *Ceratium hirundinella*). With laboratory cultures, the rapid uptake, EDTA release and quantitative similarity between living and dead (glutaraldehyde-fixed) algae were consistent with physical binding of Cu at the cell surface. The higher Cu adsorption per unit surface area and longer adsorption time of mucilaginous algae in the time-course study, and the relatively high level of Cu bound to mucilage found by X-ray microanalysis suggest that mucilage played an important role in metal binding. For all species examined, Cu adsorption kinetics (external Cu concentrations 1 to 1000 mg L^{-1}) showed a clear fit to the Freundlich, but not the Langmuir isotherm, indicating a monolayer adsorption model with heterogenous binding sites. The Freundlich adsorption capacity constant (K_f) was higher in mucilaginous (3.96–12.62) compared to nonmucilaginous (0.36–3.63) species, but binding intensity (Freundlich constant $1/n$) did not differ between the two cell types. The results suggest that mucilaginous algal species may have potential as biosorbents for treatment of industrial effluents containing heavy metals. Investigation of the Cu adsorption behavior of four mixed phytoplankton samples also revealed a good fit to the Freundlich, but not the Langmuir, isotherm. Freundlich constants were in the range 2.3–3.2 for samples dominated by Chlorophyta, Bacillariophyta and Cyanophyta, but recorded a value of 7.4 in the sample dominated by Dinophyta. Comparison with data from laboratory monocultures suggested that the adsorption kinetics of mixed environmental phytoplankton samples cannot be predicted simply in terms of the major algal species.

Introduction

Extensive use of heavy metals in a wide range of agricultural and industrial activities results in direct release into the aquatic environment which poses an adverse effect to aquatic organisms. Thus, removal of metal pollutants has become a major research topic in the last decade. Because algae have the advantages of cheap availability, relatively high surface area and high binding affinity, most studies have focused on finding best algal biosorbents for use in wastewater treatment (Xue et al., 1988; Radway et al., 2001). However, little attention has been given to metal removal and detoxification by environmental phytoplankton and other algae in the natural environment. As the growth of large amounts of algae due to eutrophication were commonly seen in most of water bodies, such eutrophic algae may help to eliminate the toxicity of heavy metals and exert a major influence on the behavior and fate of trace metals entering freshwaters (Mcknight & Morel, 1979; Holan et al., 1993). Therefore, understanding the biosorption kinetics of

eutrophic algal species is important for water quality assessment.

The uptake of heavy-metal ions by biologically active algae was found to occur in two principal ways: passive uptake due to surface adsorption followed by cellular uptake (absorption) via intracellular transport and chelation (Khummongkol et al., 1982; Cho et al., 1994). Adsorption occurs directly onto the cell wall (e.g. Darnall et al., 1986) in some algae but the presence of various amounts of mucilage or extracellular polymeric substances (EPS) (Leppard, 1995; Lee, 1997) in others (e.g. Cyanophyta) may play a key role in metal binding (Weckesser et al., 1988). Parker et al. (2000) and Li et al. (2001) have found mucilage sheaths isolated from *Microcystis aeruginosa* and *Aphanothece halophytica* exhibited strong affinity for metal ions. A high copper uptake by two mucilaginous cyanobacteria, *Cyanospira capsulata* and *Nostoc* PCC7936 was also shown by De Philippis et al. (2003) in comparison with the other microbial biomass. In addition, Pistocchi et al. (2000) found increased production of extracellular polysaccharides by phytoplankton to be a general response to the presence of heavy metals. However, Tien (2002) found non-mucilaginous *Chlorella vulgaris* showed higher metal binding activity than mucilaginous *Anabena spiroides* and *Eudorina elegans* and indicated that the surface area/dry weight ratio was a major parameter determining the sorption activities and mechanisms of algae. From the investigation of Tien (2002), *Oscillatoria limnetica* did not produce large amounts of surface mucilage, but showed the highest Cu and Pb sorption capacity per unit mucilage volume suggesting that its diffuse mucilage structure may contain more metal binding sites than the dense and thick structure of mucilage produced by *Anabena spiroides* and *Eudorina elegans*. Thus, the metal binding activity of surface mucilage may depend on the mucilage structure, but not on the amount of surface mucilage produced. As mucilaginous algal species normally dominate in the eutrophic ocean and lakes (Reynolds, 1993), the detailed investigation on the metal binding properties of eutrophic algal species, particularly mucilaginous ones, is therefore needed.

Inter-specific differences in the affinity and specificity of metal adsorption has been conducted in laboratory-cultured algae and was attributed to differences in the presence of polyfunctional groups (e.g. carboxylate, sulphydryl and amino acid) on both the cell wall and mucilage (Greene & Darnall, 1990; Tien, 2002). However, seldom researches studied adsorption activity of environmental mix phytoplankton and the possibility to predict metal binding capacity of environmental phytoplankton by the cultured ones.

The aim of our investigations was to examine the copper-binding kinetics of environmental phytoplankton and related cultured algae with particular reference to the role of surface mucilage. Quantitative studies, using Freundlich and Langmuir isotherms, were carried out to determine whether cultured algae and phytoplankton samples conformed to a particular adsorption model, and whether that adsorption parameters of the mixed phytoplankton samples could be related to the constituent algae. Copper was selected for study as this metal binds efficiently to algal surfaces (Zhang & Majidi, 1994; Kiefer et al., 1997) and is an important potential pollutant (Förstner & Wittmann, 1981). Phytoplankton samples were collected from a eutrophic lake at intervals during the summer and autumn.

Materials and methods

Culturing

Details of species nomenclature, growth media and origin of cultured algae are given in Table 1. Algae were routinely grown in 100 mL sterile growth medium in 250-mL Ehrlenmeyer flasks. Cultures were typically incubated at 23 °C under fluorescent light

Table 1. Presence or absence of mucilage in eight laboratory-cultured species, together with the medium used and source of the culture.

Taxon	Surface mucilage	Growth medium	Source
Cyanophyta			
Anabaena cylindrica	+	BG-11	PCC 7122
Anabaena spiroides	+	CT	NIES-76
Microcystis aeruginosa	+	BG-11	Sciento LA 905
Chlorophyta			
Eudorina elegans	+	BBM	Sciento LA 85
Chlorella vulgaris	–	BBM	Sciento LA 155
Bacillariophyta			
Asterionella formosa	–	DM	CCAP 1005/8
Aulacoseira varians	–	BM	Sciento LA 750
Dinophyta			
Ceratium hirundinella	–	MWC	CCAP 1110/4

PCC: Pasteur Culture Collection, Paris, France; NIES: National Institute for Environmental Studies, Tsukuba, Japan; Sciento: Sciento Educational Services, Manchester, UK; CCAP: Culture Collection of Algae and Protozoa, Centre for Ecology and Hydrology, UK.

(22 μmol photon m^{-2} s^{-1}, 15 h light/ 9 h dark) on a shaker at 90 rpm. *Microcystis aeruginosa* was cultured without shaking and *Ceratium hirundinella* was grown in ambient daylight. As algal surface polyfunctional groups varied with the growth phase and more functional groups appeared during the exponential growth phase (Tien, 1999), in order to obtained comparable results and better metal binding activity, cultured algae were harvested in the exponential growth phase.

Cu uptake by living and dead (glutaraldehyde-fixed) Anabaena cylindrica

The relative degree of binding of copper to living and dead *Anabaena cylindrica* was compared to establish whether uptake of copper by algae was a wholly passive process. Living and dead (fixed in 2.5% glutaraldehyde solution for 15 min) cells in the exponential growth phase were respectively washed with distilled water and resuspended in 30 mL 450 μg L^{-1} Cu for 30 min, 1, 2 and 4 h. Controls containing 450 μg L^{-1} Cu solution but without algae were also run. Following exposure, treatments were centrifuged and the clear supernatant analyzed for Cu using atomic absorption spectrophotometry. The algal pellets were resuspended, sonicated in 30 mL 10 mM EDTA and centrifuged. The supernatant was then analyzed for Cu as above.

Time course of copper uptake by four laboratory-cultured algae

Four species of algae secreting different amounts of surface mucilage were examined: *Anabaena cylindrica, Anabaena spiroides*, *Eudorina elegans* and *Chlorella vulgaris* (Table 1). Algae, grown in 100 mL sterile medium and in exponential growth phase, were harvested by centrifugation and suspended in distilled water containing 250 μg L^{-1} Cu. One hundred mL aliquots of the suspension were then placed in three (replicate) 150-mL plastic beakers. Controls (three replicates) containing 250 μg L^{-1} Cu, but no alga, were also run. After incubation for 15 min, 45 min, 75 min, 2 h, and 4 hr, 5 mL of the suspension from each beaker was filtered through a 0.4 μm Cyclopore^{TM} (Whatman, Kent) polycarbonate membrane and the Cu concentration of the filtrate measured.

Immediately prior to commencement of the experiment, 10 mL aliquots of culture were taken for determination of dry weight. A further 5-mL aliquot was then fixed with 0.5 mL of 25% glutaraldehyde solution prior to determination of algal surface area and mucilage volume using the methods described by Bellinger (1974) and Reynolds (1993). A final 5 mL aliquot was fixed with Lugol's iodine solution for cell counting following standard procedures.

Localization of copper adsorption on the surface of laboratory-cultured Anabaena spiroides *using Scanning Electron Microscopy X-ray Microanalysis*

Anabaena spiroides was collected during exponential growth phase by centrifugation. Cells were resuspended with 100 mL distilled water containing 50 mg L^{-1} of copper in a 150-mL plastic beaker. After incubating at 25 °C with gentle magnetic stirring for 30 min, 5 mL were filtered through 0.4 μm Cyclopore^{TM} polycarbonate membranes (Whatman) and then washed with distilled water to remove excess copper on the filter membrane. The filter membrane with deposited *Anabaena spiroides* was immediately frozen in nitrogen slush, freeze-dried for 12 hours in an Edwards tissue-drier at -60 °C, mounted on aluminum SEM stubs using double sided sellotape and carbon DAG, and carbon coated. Carbon-coated samples were analysed using a Cambridge 360 Stereoscan Scanning Electron Microscope associated with a Link AN10000 analyser. For X-ray microanalysis, the probe current was adjusted to give a count rate of approximately one thousand counts per second. X-ray emission spectra were obtained from the cell, mucilage and the filter membrane (to check if there was residual copper present on the filters) at an accelerating voltage of 25 kV and 100 s live time. Quantification of the elements in the samples was carried out using the Link ZAF/PB program, with cobalt as reference element because this metal has a well defined peak of known energy. The 'fit index' which is a measure of the quality of fit of the stored standard peak profiles to the unknown spectrum was calculated and was kept between 0 and 2. Within this range of 'fit index', copper concentration was obtained as elemental mass fractions (g/100 g dry weight).

Assessment of copper adsorption using Freundlich and Langmuir Models

Adsorption models were applied to cultured algal and environmental phytoplankton samples. Eight species of laboratory-culture algae (Table 1) were harvested during exponential growth phase and resuspended in 300 mL distilled water. A 105-mL aliquot of cell suspension was freeze-dried and resuspended with the same amount of distilled water. Five milliliter cell

suspension (either a living or freeze-dried suspension of each species) was added to 5 mL copper solution, to give final copper concentrations of 1, 5, 10, 50, 100, 500 and 1000 mg L^{-1} in a polycarbonate tube and incubated on a 100 rpm shaker for 1 h at 25°C. Five milliliter distilled water was added to 5 mL of each Cu concentration as controls. As pH is considered to be the most important parameter influencing the biosorption process and in order to obtain comparable results, the pH of each tube was adjusted within the optimum ranges of pH 4 and 5 for copper binding (Özer et al., 1994). All samples were filtered through a 0.4 μm Cyclopore™ polycarbonate membrane (Whatman) and the filtrate analyzed for copper using atomic absorption spectrophotometry.

Concentrated mixed phytoplankton samples were collected by trawling a phytoplankton net across the surface of Rostherne Mere (a eutrophic lake with National Grid Reference SJ 745843, in the south of Manchester, U.K.) for 3 min during July, August, September and November 1997. Lake water containing the concentrated algal cells was filtered through a 0.45 μm cellulose nitrate membrane filter and the cells resuspended in 300 mL of distilled water. Five-milliliter cell suspension was added to 5 mL copper solution to give the same range of concentrations as specified for the cultured algal cells. All experiments were conducted as previously.

All glass and plastic containers used were acid-washed beforehand and all chemicals were of analytical-grade ('Analar': BDH, Poole, Dorset). Triplicate measurements were made in all cases. Dry weight, surface area, mucilage volume and cell counts of each algal species were determined as previously.

Statistical analysis was carried out using the non-parametric Kruskal–Wallis one-way ANOVA and Mann–Whitney U-Wilcoxon Rank Sum W test (with a significance level of $p < 0.05$) to compare differences between different treatments, and linear regression and correlation analysis for fitting the experimental data to the adsorption models.

The Langmuir model (Langmuir, 1918) is a single-layer adsorption model applicable to homogenous surfaces. The model defines the amount of metal adsorbed per unit cell dry weight (x/m) by

$$x/m = QbC(1 + bC)^{-1}, \tag{1}$$

where Q is the adsorption capacity (mg g^{-1}) in forming a complete surface monolayer; b is a constant related to the adsorption intensity of the metal associated with the surface (Holan et al., 1993) and C is the metal concentration (mg L^{-1}) remaining in solution at equilibrium.

A linear form of the Langmuir equation (Equation 2) was used to calculate values of Q and b, given the linear relationship between C and $C/(x/m)$ (Weber, 1972).

$$C(x/m)^{-1} = (bQ)^{-1} + C(Q)^{-1} \tag{2}$$

The Freundlich model (Freundlich, 1926) is also applicable to monolayer adsorption, but only for a heterogeneous surface. The Freundlich model assumes that the energy of adsorption is variable and that the heat of adsorption varies with surface binding site. Metal adsorption is defined as

$$x/m = K_f C^{1/n}, \tag{3}$$

where K_f and $1/n$ ($n > 1$) are constants and indicators of adsorption capacity and adsorption intensity, respectively (Adamson, 1967; Weber, 1972). The logarithmic form of Equation (4) gives a straight line with the slope equal to $1/n$ and the intercept equal to log K_f.

$$\log(x/m) = \log K_f + (1/n) \log C \tag{4}$$

Results

Cu uptake by living and dead Anabaena cyclindrica

Exposure of living and dead (glutaraldehyde-fixed) *Anabaena cylindrica* to distilled water containing 450 μg L^{-1} Cu resulted in a significant ($p < 0.05$) extraction of the cation within 30 min, reaching maximum removal of approximately 50% (compared to the control without algae) by 60 min, with no significant difference ($p > 0.05$) between living and dead (glutaraldehyde-fixed) cells (Figure 1A). Addition of 10-mM EDTA to algae (Figure 1B) resulted in a corresponding and equal release of copper by living and dead cells ($p > 0.05$). The time of maximum copper adsorption (60 min, see Figure 1A) corresponded to the time of maximum copper release (Figure 1B) from the algal cells. The maximum concentration of EDTA-released Cu from algal biomass previously exposed to 450 μg L^{-1} Cu reached 160 μg L^{-1} (Figure 1B). The amount of copper released from the cell surface was approximately 70% of that removed from the surrounding medium.

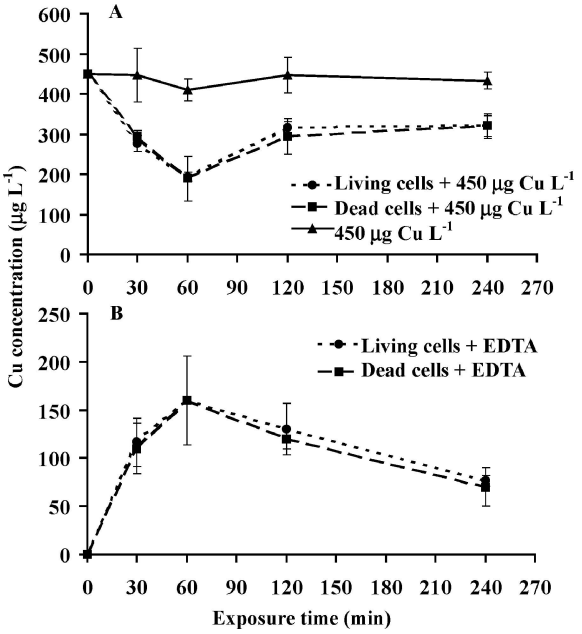

Figure 1. Copper concentrations (A) remaining in distilled water after exposure living and dead *Anabaena cylindrica* to 450 μg L^{-1} added copper for up to 240 min, and (B) released into the surrounding medium following treatment of living and dead *Anabaena cylindrica* with 10 mM EDTA solution. $n = 3$; error bars indicate SD.

Copper sorption characteristics of four laboratory-cultured algae differing in surface mucilage content

Adsorption of Cu by *Anabaena cylindrica*, *A. spiroides*, *Eudorina elegans* and *Chlorella vulgaris* exposed to 250 μg L^{-1} added metal over the 240-min experimental period was determined per unit dry weight, per unit surface area and per unit mucilage volume (for algae with mucilage only) (Figure 2). Cu adsorption per gram dry weight reached a maximum value by 45 min in *Chlorella vulgaris* and by 75 min in the other three algae (Figure 2A). None of the subsequent changes in amounts of associated metal were significant ($p > 0.05$).

Anabaena spiroides showed the highest Cu adsorption per unit surface area with a maximum value of 0.16 μg cm^{-2} (Figure 2B). Differences in copper adsorption per unit surface area were significant between all species ($p < 0.05$) except between *Anabaena cylindrica* and *Eudorina elegans* ($p > 0.05$). Of the three laboratory-cultured algae with cell-associated mucilage, *Anabaena cylindrica* had the highest copper concentration per cubic centimeter mucilage volume ($p < 0.05$) with a maximum of 180 μg Cu cm^{-3}. There was no significant difference ($p > 0.05$) in copper concentration per cubic centimeter mucilage volume between *Anabaena spiroides* and *Eudorina elegans* which had maximum adsorption values of 28 and 21 μg cm^{-3}, respectively (Figure 2C).

Localization of copper adsorption on the surface of laboratory-cultured Anabaena spiroides

Anabaena spiroides was selected as this alga produces large amounts of mucilage to determine whether copper was directly bound to surface mucilage. A scanning electron micrograph, measured areas and their copper concentrations of *Anabaena spiroides* treated with 50 mg L^{-1} Cu for 30 min are shown in Figure 3. Owing to dehydration, the part of mucilage was reduced to strands of fibrillar material attaching the cells to the filter membrane. The mean mass fraction of copper in mucilage strands was 2.4 mg g^{-1} dry weight with 24% coefficient of variation (CV). The mean copper mass fraction in cells with surface mucilage was 9.6 mg g^{-1} dry weight with a 13% CV. Approximately 20% of adsorbed copper was bound to the mucilage strands. Presuming the cell surface mucilage also bound the same amount (2.4 mg g^{-1}) of copper with mucilage strands, there would be about 40% of adsorbed copper bound to mucilage. No copper peak was detected on the filter membranes.

Mathematical adsorption models

Copper adsorption to a range of living and dead (freeze-dried) laboratory-cultured algae (Table 2) was examined using Freundlich and Langmuir isotherms. The experimental data closely fitted the Freundlich model for all living and freeze-dried samples over the whole range (1–1000 mg L^{-1}) of Cu concentrations ($p < 0.01$ for Student's t-test of goodness of fit of the regression line). There was a strong positive correlation (coefficient of determination $R^2 > 0.90$) between the Cu concentration remaining in solution at equilibrium and the amount of Cu adsorbed per unit cell weight both in logarithmic form. In no case did adsorption data fit the Langmuir model over the whole range of exposure concentration (1–1000 mg L^{-1}). The Langmuir and Freundlich plots shown for *Anabaena cylindrica* (Figure 4) were typical of all the cultured algae.

The adsorption constants K_f and $1/n$, indicators of adsorption capacity and adsorption intensity, respectively, were derived from the Freundlich regression

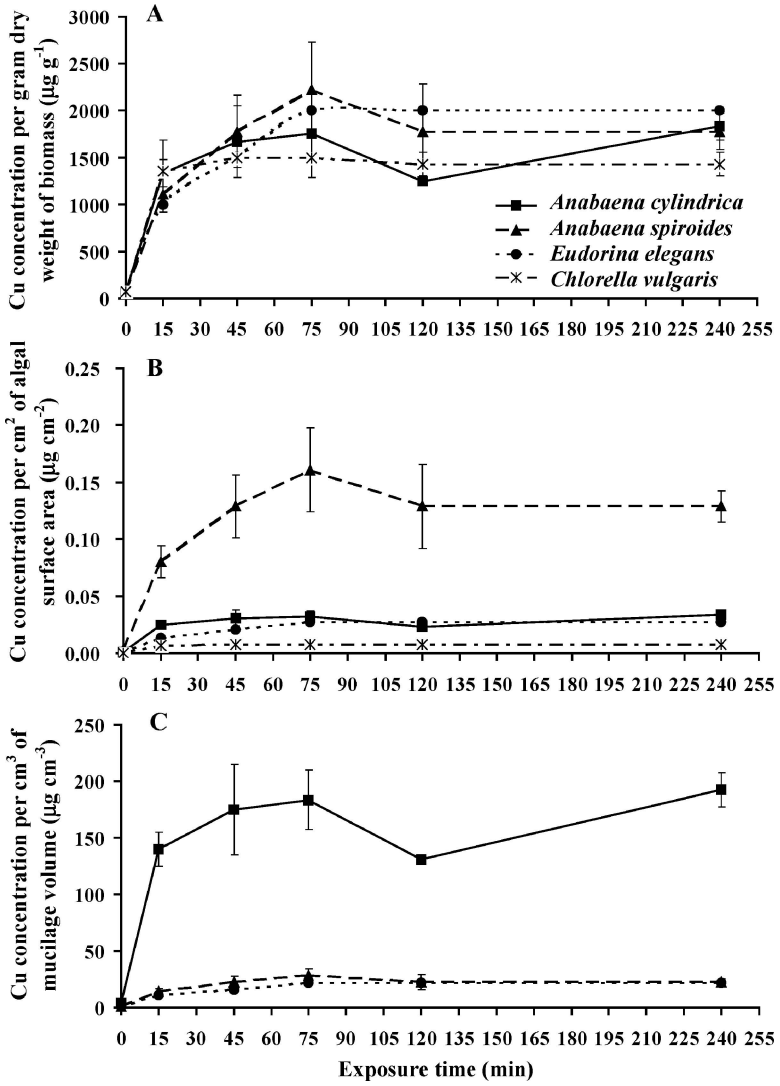

Figure 2. Copper concentrations (A) per gram dry weight, (B) per cm^2 surface area and (C) per cm^3 mucilage volume in laboratory-cultured *Anabaena cylindrica*, *Anabaena spiroides*, *Eudorina elegans* and *Chlorella vulgaris* after exposure to 250 μg L^{-1} added copper for up to 240 min. $n = 3$; error bars indicate SD.

plots and are shown for cultured algae in Table 2. The adsorption capacity (K_f) of live cells ranged from 0.36 (*Asterionella formosa*) to 12.62 (*Anabaena cyclindrica*) mg Cu g^{-1}. K_f values for freeze-dried algal samples were typically quite different from values obtained for living cells. K_f values for the freeze-dried mucilaginous algae *Microcystis aeruginosa* (2.47 mg Cu g^{-1}) and *Eudorina elegans* (2.13 mg Cu g^{-1}) were substantially lower than for live cells, while values for the freeze-dried nonmucilaginous algae *Chlorella vulgaris* (4.26 mg Cu g^{-1}), *Asterionella formosa* (1.21 mg Cu g^{-1}), *Aulacoseira varians* (3.03 mg Cu g^{-1}) and *Ceratium hirundinella* (5.75 mg Cu g^{-1}) were typically higher.

The Freundlich K_f values represent the predicted amount of Cu bound to algal cells (mg Cu g dry wt^{-1}) at an equilibrium concentration of 1 mg L^{-1} Cu. From these data, the predicted metal adsorption per unit surface area can be obtained by dividing the K_f value by the surface area/dry weight ratio, and the predicted amount of Cu per unit mucilage volume by the mucilage volume/dry weight ratio (Table 2). The amount of Cu bound per unit surface area ranged from 0.03 (*Microcystis aeruginosa*) to 0.79 μg cm^{-2} (*Aulacoseira*

Table 2. Freundlich adsorption constants (K_f and $1/n$) and derived parameters for eight living and freeze-dried cultured algae exposed to copper for 1 h.

Species	Cell stage	K_f (mg Cu g^{-1})	$1/n$	R^2	Adsorption per unit surface area (μg Cu cm^{-2})	Adsorption per unit mucilage volume (μg Cu mm^{-3})
Anabaena cylindrica	Living	12.62	0.35	0.97	0.41	5.12
Anabaena spiroides	Living	8.73	0.36	0.94	0.30	3.00
Microcystis aeruginosa	Living	8.21	0.50	0.93	0.11	1.93
	Freeze-dried	2.47	0.68	0.95	0.03	0.58
Eudorina elegans	Living	3.96	0.45	0.97	0.34	0.46
	Freeze-dried	2.13	0.55	0.92	0.18	0.25
Chlorella vulgaris	Living	3.63	0.45	0.99	0.11	–
	Freeze-dried	4.26	0.53	0.90	0.13	–
Asterionella formosa	Living	0.36	1.10	0.98	0.10	–
	Freeze-dried	1.21	0.53	0.93	0.34	–
Aulacoseira varians	Living	2.29	0.42	0.91	0.60	–
	Freeze-dried	3.03	0.16	0.93	0.79	–
Ceratium hirundinella	Living	2.30	0.37	0.99	0.09	–
	Freeze-dried	5.75	0.63	0.93	0.22	–

R^2 (coefficient of determination) represents degree of fit to the Freundlich equation.

varians). *Anabaena cylindrica* and *Anabaena spiroides* had the highest predicted Cu concentration per unit mucilage volume of the four mucilage-producing species.

The adsorption intensity ($1/n$, the slope of the regression line) ranged from 0.35 (*Anabaena cylindrica*) to 1.1 (*Asterionella formosa*) for living cells and from 0.16 (*Aulacoseira varians*) to 0.68 (*Microcystis aeruginosa*) for freeze-dried cells. The adsorption intensity was greater in freeze-dried than in living *Microcystis*, *Eudorina*, *Chlorella* and *Ceratium*, but Bacillariophyta (diatoms). The four phytoplankton samples analyzed in reference to Freundlich and Langmuir isotherms provide a useful comparison between different algal groups in terms of Cu-binding characteristics. The cell/colony counts presented in Table 3 are per milliliter of concentrated sample (not per milliliter of lake water) and give information on the

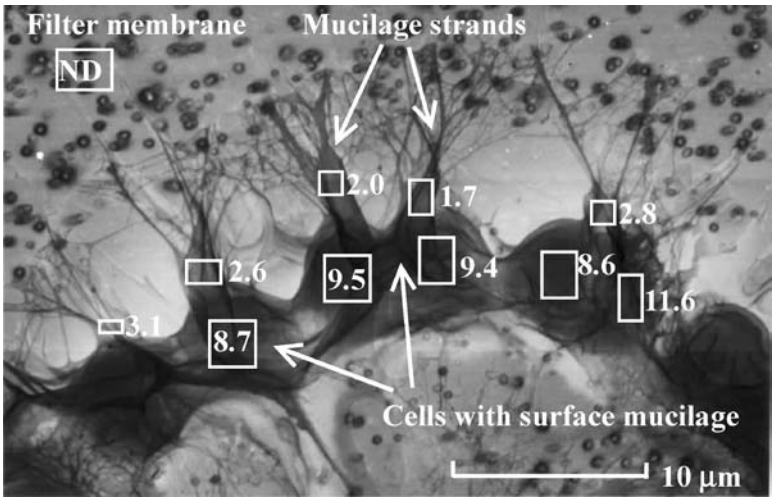

Figure 3. A Scanning Electron Microscopical view of *Anabaena spiroides* exposed to 50 mg L^{-1} copper for 30 min. White box and number show measured area and copper concentration (mg g^{-1}) within the area. ND, not detectable.

Table 3. Algal density (cells or colonies mL^{-1}) and Freundlich copper adsorption constants of environmental mixed phytoplankton samples from Rostherne Mere on different sampling dates.

	July 9	August 5	September 2	November 27
Cyanophyta				
Anabaena	100	5,200	950	0
Microcystis	400	800	1,300	4200
Oscillatoria	300	1,800	50	0
Chlorophyta				
Eudorina	1200	200	370	0
Staurastrum	1000	5,100	230	1000
Bacillariophyta				
Asterionella	0	31,000	1,000	200
Aulacoseira	0	7,200	3,200	400
Dinophyta				
Ceratium	500	5,400	14,000	0
Freundlich constants				
K_f	2.29	2.37	7.36	3.20
$1/n$	0.89	0.54	0.59	0.67
Coefficient of determination				
R^2	0.99	0.97	0.92	0.97

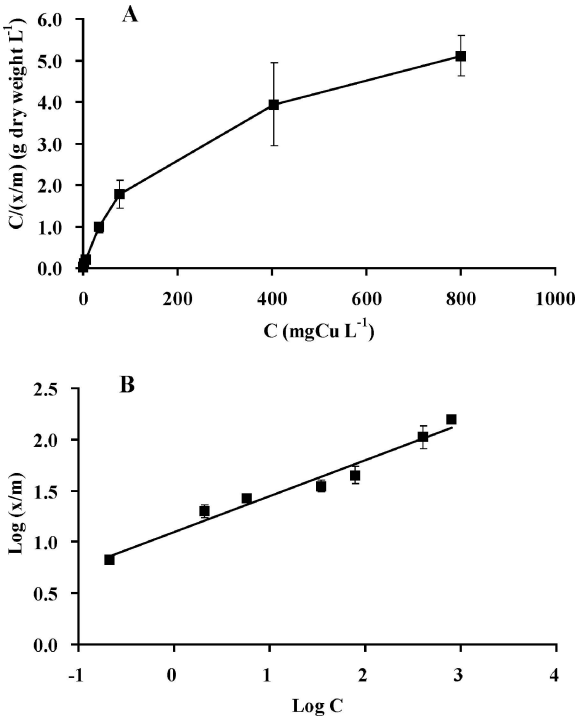

Figure 4. (A) Langmuir and (B) Freundlich isotherm plots for copper adsorption by cultured *Anabae/na cylindrica* after exposure of the same biomass to between 1 and 1000 mg L^{-1} of copper for 1 h at 25°C. C, copper concentration remaining in the medium; x/m, amount of copper adsorbed per unit dry weight. $n = 3$; error bars indicate SD.

relative proportions of different taxa within the mixed phytoplankton samples.

Chlorophyta were the dominants in Rostherne Mere in the July sample (*Eudorina elegans*, 1200 colonies mL^{-1}; *Staurastrum planctonicum*, 1000 cells mL^{-1}) with substantial numbers of Cyanophyta (mainly *Microcystis aeruginosa* and *Oscillatoria* spp.) and *Ceratium hirundinella* also present. Bacillariophyta became abundant in August, dominated by *Asterionella formosa* (31000 cells mL^{-1}) and *Aulacoseira* (7200 filaments mL^{-1}). The September sample contained a large number of dinoflagellates (*Ceratium hirundinella*, 14000 cells mL^{-1}), changing to a cyanophyte-dominated population in November, with large amounts of *Microcystis aeruginosa* (4200 colonies mL^{-1}) plus smaller quantities of *Staurastrum*, *Asterionella* and *Aulacoseira*.

Freundlich plots for living environmental phytoplankton preparations showed a good fit to experimental data (in all cases, $p < 0.01$ and $R^2 > 0.92$, Table 3). In no case did the adsorption data fit the Langmuir model over the whole range of copper concentration (1–1000 mg L^{-1}). Typical linear Freundlich plots and nonlinear Langmuir plots for fresh phytoplankton samples from August are shown in Figure 5. The adsorption capacity (K_f) of the live phytoplankton samples ranged from 2.29 (July) to 7.36 mg Cu g^{-1} (September), with adsorption intensity ($1/n$) ranging from 0.54 to 0.89 (Table 3).

Discussion

Initial studies on Cu uptake by laboratory monocultures demonstrated the importance of surface mucilage to the adsorption process and provided a useful reference for subsequent phytoplankton analyses. The rapid uptake, EDTA release and quantitative similarity between living and dead (glutaraldehyde-fixed) cells indicated a clear adsorption (physical sequestration at the cell surface) rather than an absorption (metabolic uptake) process. The results are consistent with data obtained by other workers for various cations and microorganisms. For example, rapid adsorption of Cu by algal cells has been demonstrated by Harris and Ramelow (1990), who showed that 90% of Cu uptake by dead *Chlorella* occurred within 15 min. Similarities in Cu adsorption by living and dead microorganisms have been demonstrated by He and Tebo (1998) for the marine

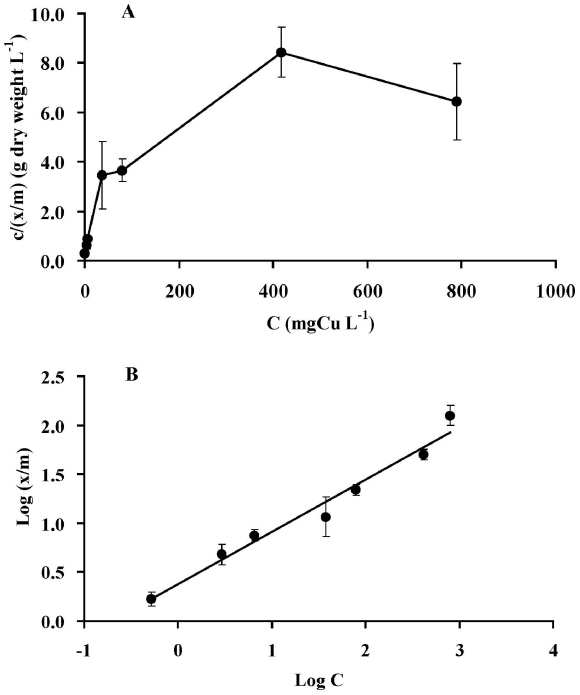

Figure 5. (A) Langmuir and (B) Freundlich isotherm plots for copper adsorption by mixed phytoplankton cells from Rostherne Mere on 05 August 1997 after exposure of the same biomass to between 1 and 1000 mg L^{-1} copper for 1 h at 25 °C. (C) copper concentration remaining in the medium; x/m, amount of copper adsorbed per unit dry weight. $n = 3$; error bar indicates SD.

bacterium *Bacillus* sp., and by Parker et al. (1998) for cells of *Microcystis aeruginosa*, where heat-killed, formaldehyde-treated and air-dried cells adsorbed similar levels of Cu to viable cells. Other workers have shown, however, that formalin-treated and heat-killed cells adsorbed higher levels of mercury (Glooschenko, 1969; Darnall et al., 1986), suggesting that such treatments may result in artifactual changes in algal surface chemistry that may affect adsorption of some cations. Mucilaginous algal species (e.g. *Anabaena* spp. and *Eudorina elegans*) showed rapid copper sorption kinetics in comparison with nonmucilaginous one (*Chlorella vulgaris*), suggesting this gelatinous layer might slow down the diffusion rate of the copper ions into the chelating matrix (De Philippis et al., 2003).

Freundlich and Langmuir mathematical models have been widely used to describe the adsorption of heavy metal ions by microorganisms (Wehrheim & Wettern, 1994; Tien, 2002). The application of these models to the present study provided both qualitative (binding site heterogeneity) and quantitative information (differences between mucilaginous and nonmucilaginous surfaces) on Cu adsorption by cultured algal cells. Close fit to the Freundlich, but not the Langmuir, isotherm in all cultured algae in this study is consistent with Cu binding to a heterogeneous rather than a homogeneous assembly of binding sites at the cell surface. Application of these isotherms to other microorganisms has brought a variety of results. Xue et al. (1988) and Zhou et al. (1998) found that copper and cadmium binding to micro- and macroalgal surfaces did not fit the Langmuir model over a wide range of metal concentrations, suggesting complex metal binding sites. Wehrheim and Wettern (1994) found both the Langmuir and Freundlich models were suitable for describing the short-term adsorption of Cd, Cu and Pb by the cell wall, and Cd and Cu adsorption by the whole *Chlorella fusca* cell. De Rome and Gadd (1987) showed that Cu adsorption by the fungi *Cladosporium resinae* and *Penicillium italicum* obeyed the Freundlich and Langmuir isotherms for single-layer adsorption but this may be due to the narrow range of concentrations (0.3–25 mg L^{-1}) used in these studies.

Quantitative information obtained from the Freundlich plots provides a useful comparison between mucilaginous and nonmucilaginous algae. The higher binding capacity (K_f) of mucilaginous species is consistent with Cu binding to sites throughout the mucilage layer in these algae and has been confirmed by Scanning Electron Microscopy X-ray microanalysis showing about 40% of adsorbed copper bound to mucilage. The longer adsorption time and higher copper adsorption per unit surface area of mucilaginous algae (compared to *Chlorella*) in the time-course experiments also supports this conclusion. No general differences between mucilaginous and nonmucilaginous cells were observed, however, in relation to binding intensity (Freundlich constant $1/n$), which relates to the proportion of binding sites occupied by the cation. Other studies have also shown a connection between high levels of mucilage and high adsorption capacity. Su et al. (1995) found a higher adsorption capacity in activated sludge enriched with the EPS-producing bacteria, *Zoogloea ramigera*, and suggested that the EPS provides more sorption sites for metal uptake. Scott et al. (1988) also found capsular bacteria removed more Cd than did noncapsular species.

Among the four species with cell-associated mucilage, *Eudorina elegans* has the firm mucilage structure but showed the least copper adsorption per unit mucilage volume compared to the other three species having the diffuse mucilage structure, suggesting the

firm mucilage structure may contain less copper binding sites than the diffuse one. After freeze-drying, the adsorption capacity (K_f) of mucilage decreased, but that of cell wall increased. This may be due to loss of many of the metal binding sites on mucilage after freeze-drying. The results also indicated that changes in cell surface chemistry occurred following freeze-drying.

The wide range of adsorption capacities (K_f, 0.36–12.62 mg g^{-1}) shown by the eight cultured algae indicates considerable diversity in surface properties. Similar results were also found by Tien (2002) who showed K_f values of 0.2–23.96 mg g^{-1} for Cu by four algae. Zhou et al. (1998) found relatively higher K_f values with 24.86 and 26.69 mg g^{-1} for Cu by two brown macroalgae. Studies on other groups of microorganisms have generally shown less variation. Mullen et al. (1989), for example, found K_f values from 0.14 to 0.26 mg g^{-1} for adsorption of Cu and Cd by four bacteria. K_f values of 0.047 and 0.055 mg g^{-1} were recorded for Cu sorption by the two fungi *Mucor rouxii* and *Aspergillus niger*, respectively (Mullen et al., 1992), indicating substantially less adsorption capacity than bacteria (Mullen et al., 1989) and algae in this study.

The chemical nature of the Cu-binding sites has been suggested by Kiefer et al. (1997) who found that ligands with a high affinity for Cu are likely to contain N or S donor atoms which form stronger complexes with this metal than carboxylic groups. Fehrmann and Pohl (1993), studying Cd uptake, have suggested that interspecific differences of cation adsorption by algae were due to differences in the ratio of high affinity (e.g. amino acids, proteins) to low affinity binding sites (e.g. negatively charged carboxylic and hydroxy-carboxylic groups). Lectin-binding studies (Tien, 1999) have demonstrated different patterns of surface sugars (including mannose, glucose, galactose and *N*-acetyl galactosamine) in these algae, which may contribute towards differences in Cu adsorption between species. Similar results with cyanobacteria were also found by De Philippis et al. (2001). The environmental samples analyzed in this study were a complex mixture of different algal species, and would also contain bacteria and fine particulate matter. All four environmental samples showed clear similarity to the cultured algal samples in conforming to the Freundlich but not the Langmuir isotherm, indicating similar heterogeneous monolayer Cu-binding dynamics in both situations.

A more detailed comparison between environmental and laboratory samples in terms of adsorption capacity (K_f values) also showed some similarities. The July and August samples, dominated by Chlorophyta and Bacillariophyta, respectively, had low adsorption capacities ($K_f < 4$), in agreement with the algal monocultures. The September sample, however, dominated by *Ceratium* had a much higher adsorption capacity than would be predicted from the cultured samples, while the November sample, dominated by mucilaginous Cyanophyta, had a much lower K_f value than predicted. The results showed that the overall adsorption capacity of environmental phytoplankton samples may be quite different from that expected by the dominant algal species. The reasons for this are not clear, but complicating environmental factors such as the presence of bacterial cells, fine debris and existing saturation of binding sites on algal surfaces may be important. As bacteria have been known to have a high surface area-to-volume ratio (Zouboulis et al., 2004), occurrence of numbers of planktonic bacteria during the autumn phytoplankton blooms (Booth, 1988) may provide a large contact interface to bind with metals from the surrounding environment.

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