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Metabolism-independent binding of toxic metals by *Ulva lactuca*: cadmium binds to oxygen-containing groups, as determined by NMR

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The metabolism-independent metal binding characteristics of *Ulva lactuca* were investigated using both freeze-dried thalli and cell walls stripped of intracellular material by incubation in Triton-X followed by methanol. Biosorption of Cd, Zn, Cu and Co by freeze-dried thallus was concentration-dependent and followed Freundlich and Langmuir isotherms. The Freundlich plot suggested that freeze-dried *U. lactuca* had the greatest binding affinity for Cu compared with Cd, Zn and Co. The BET (Brunauer–Emmett–Teller) plot, which indicates a more complex form of adsorption, and the Scatchard plot were not adequate models for Cu adsorption. The Scatchard plot of Cd suggested that two Cd binding sites were available on the freeze-dried thallus, with the second, lower affinity site only becoming available at Cd loading capacities greater than 4.9 μmol g^{-1} dry wt. ^{113}Cd nuclear magnetic resonance (NMR) studies confirmed that two binding sites were available for Cd on the freeze-dried algal powder, though only one was available on the cell wall, and that the affinity of the binding sites was greater for Cu than for Cd. The results of the NMR experiments suggested that Cd binds to oxygen-containing functional groups in the algal powder and on the cell wall. It is proposed that sulphate or hydroxyl groups attached to polysaccharide subunits are possible sites.

Keywords: BET, biosorption, cadmium, cobalt, copper, Freundlich, NMR, Scatchard, *Ulva lactuca*, zinc

Introduction

Biosorption describes the adsorption of metals to biomass which is dead or metabolically inactive, for example cell walls (Tsezos & Volesky 1981, Gadd 1988, 1990). Previous work has shown that microbial biomass (including microalgae) can act as an efficient system for accumulating metals, a phenomenon which has been applied to the removal of toxic metals or radionuclides from effluents to allow safe disposal into water courses (Greene & Darnall 1990, Reed & Gadd 1990, Volesky 1990). In addition, there is a growing need to recover metals of economic importance in an effort to conserve existing resources (Volesky 1990).

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Toxic metals bind to biomass as a result of covalent, electrostatic or redox reactions between the metal ion and sites on the adsorbate (Greene & Darnall 1990). Previous studies have indicated that algal cell walls can act as cation exchange systems, for example in the green macroalga *Enteromorpha intestinalis* (Ritchie & Larkum 1982) and in brown algae (Kloareg *et al.* 1987). Biosorption has been characterised by mathematical models such as Freundlich and Langmuir isotherms (Langmuir 1918, Freundlich 1926) which refer to single-layer adsorption, and the Brunauer–Emmett–Teller (BET) isotherm which relates to multi-layer adsorption, although it does reduce to the Langmuir model when the limit of adsorption is a monolayer (Brunauer *et al.* 1938). These isotherms were originally developed to describe the adsorption of gases by solids and are by necessity simplistic when applied to biomass; however, in spite of these limitations they have been used as models to describe

metal adsorption onto biomass (Weber 1972, De Rome & Gadd 1987, Garnham *et al.* 1992) and offer a means of comparing the metal adsorption potential of different adsorbates. Scatchard plots can also be used to model uptake systems which utilise multiple sites, and to identify any differences in affinity for the adsorbate which may become apparent as the concentration increases (Scatchard 1949, Huang *et al.* 1990, Garnham *et al.* 1992).

The structure of the cell wall in *Ulva lactuca* (Haug 1976, Percival 1979) presents a number of potential metal binding sites, including calcium binding sites (Haug 1976), and amino and carboxyl groups, each of which may have a different affinity for metals (Greene & Darnall 1990). In addition, the availability of metals for accumulation by biomass will depend on the speciation of the metal. This is influenced by the physico-chemical environment of the medium, such as pH (Babich & Stotzky 1978, Hughes & Poole 1991); metal biosorption has been described by several authors (Reed & Gadd 1990, Skowronski & Szubinska 1991) as a pH-dependent process. At the normal pH (pH 8) and salinity (35%) of seawater, cadmium exists mainly as $CdCl^+$ or $CdCl_2$; copper as $CuCO_3$ (Kester 1986); zinc as Zn^{2+}, $ZnOH^+$ or $ZnCO_3$; and cobalt as Co^{2+} and possibly $CoCO_3$ (Stumm & Morgan 1981).

Nuclear magnetic resonance (NMR) spectroscopy is a powerful, non-invasive method of examining metal binding to sites on the algal cell walls. This technique has been used by Majidi *et al.* (1990) to examine metal binding sites in *Stichococcus bacillaris*, to investigate competition between metals for the binding sites, and to investigate the effects of pH on metal binding. NMR was also used by Weich *et al.* (1989) to examine the role of calcium phosphates in the cell wall of *U. lactuca*, and by Lundberg *et al.* (1989) to investigate the uptake of phosphorus and nitrogen compounds by *U. lactuca*.

The present work is part of an investigation of the effects of toxic metals on algae, which are the primary producers of the marine environment. The aim of the current study was to characterise metabolism-independent metal uptake by *Ulva lactuca*. It is important to characterise metabolism-independent metal accumulation because (as discussed earlier) powdered algae may be used to remove metals from solution. In addition, the cell wall (through which the bathing medium must pass before it reaches the cell) makes up a large proportion of the algal biomass. By removing the metal from solution, the cell wall will reduce the metal concentration immediately exterior to the plasmalemma and influence uptake into the cell, in some cases acting as a detoxification mechanism (Ritchie & Larkum 1982, Mariani *et al.* 1990).

Materials and methods

Experimental organism, media and freeze-drying procedure

Ulva lactuca (Link) was collected from the East Rocks adjacent to St Andrews harbour, Fife, Scotland, UK (O.S. Grid Ref. 517168). The algal powder was prepared from algal thalli by briefly rinsing (< 15 sec) in distilled deionised water, then freezing (–10ºC), freeze-drying, and grinding to pass a 600 µm mesh. The cell wall preparation was prepared using the method of Ritchie & Larkum (1982). Discs of *U. lactuca* thallus (25 mm in diameter) were placed in 0.5% Triton-X 100 in filtered seawater for 12 h then in 100% methanol for 12 h alternately for 3 days. This procedure produced colourless, rubber-like discs with very little intracellular material evident when viewed under the light microscope. Seawater was collected from the same site and was filtered through Whatman No. 1 filter paper before use.

Buffers and chemicals used

Stock buffer solutions (100 mM) were prepared of 2-[*N*-morpholino]ethanesulfonic acid (MES), pK_a = 6.1 at 25ºC; piperazine-*N*, *N*^{1}-bis[2-ethanesulfonic acid](PIPES), pK_a = 6.8 at 25ºC; *N*[2-hydroxyethyl] piperazine-*N*^{1}-[2-ethanesulfonic acid] (HEPES), pK_a = 7.5 at 25ºC; and *N*-tris[hydroxymethyl]methyl-3-amino-propanesulfonic acid (TAPS), pK_a = 8.4 at 25ºC. All solutions were diluted to 10 mM using distilled, deionised water, and NaCl was added (to a final concentration of 0.5 M) prior to use. $3CdSO_4.8H_2O$, $Z_nSO_4.8H_2O$, $CuSO_4.8H_2O$ and $CoCl_2$ were used throughout the experiments. All chemicals from Merck Ltd, Lutterworth LE17 4XN, UK.

Metal uptake and desorption

Freeze-dried *U. lactuca* (2.5 g) was incubated in 800 µM Cd, Zn, Cu or Co in 400 ml filtered seawater (or buffer at the appropriate pH) at 25ºC on an orbital shaker (1500 *g*, 15 min). At 15 min intervals (uptake study) or after 2 h (desorption study) 5 ml aliquots of the suspension were removed and centrifuged (1500 *g*, 15 min), then rinsed with 5 ml filtered seawater (or the appropriate buffer) and centrifuged (twice) to remove unbound, extraneous metal. For the desorption study, the pellets of loaded biomass were resuspended in the appropriate washing solution (pH 2 or 3 HCl; filtered seawater or 10 mM $CaCl_2$) and mixed. After the appropriate time the rinsing/centrifugation steps were repeated (as before). Each experiment was repeated twice, and the results shown are typical of one experiment.

Determination of metal content

After rinsing and centrifugation, the pellets were digested in 1 ml concentrated HNO_3 at 90ºC for 3 h, diluted with 3 ml deionised water then analysed using a Pye Unicam SP9 atomic absorption spectrophotometer, with reference to appropriate standards.

Dry weight determination

Dry weights of the aliquots of freeze-dried alga were determined by centrifuging 5 ml of the required suspension in tared plastic test tubes. Pellets were rinsed (using distilled deionised water) and centrifuged (1500 *g*, 15 min) before freeze drying.

^{113}Cd nuclear magnetic resonance (NMR) spectroscopy

NMR spectra were acquired on a Bruker AM 300/WB FT NMR spectrometer fitted with a 10 mm Multinuclear Probehead. In this system, ^{1}H nuclei resonate at 300.03 MHz; ^{113}Cd at 66.56 MHz. ^{113}Cd parameters were: sweep width, 20 kHz; acquisition time, 0.4 sec; time domain, 16 k words; relaxation delay, 0.3 sec; pulse width, 8 μsec (16º) and total acquisition time was 50 min. The data were transformed using 25 Hz line broadening. In all cases $CdSO_4$ was used as reference.

(a) Freeze-dried powder. Samples were prepared by adding 1 g (dry weight) of freeze-dried *U. lactuca* powder to 100 ml 0.05 M Cd. A 10 mm sample tube was filled to a depth of 40 mm (4 cm^{-3}) with the suspension and D_2O added to provide a lock signal.

(b) Cell walls. Cell walls (discs of thallus stripped of cellular contents) of *U. lactuca* (0.4 g) were incubated at 25ºC for 18 h in 5 ml of 0.05 M Cd in 80% distilled deionised H_2O/20% D_2O (v/v). D_2O was added to each sample to provide a lock signal. For the competition experiments, a background spectrum was obtained, then 0.5 M Zn, Cu or Co was added (in 100 μl aliquots) to the preparation detailed above.

Results

Metal adsorption and desorption

Metal adsorption from buffers (pH 6–9) in the presence of 0.5 M NaCl, and from filtered seawater (pH 6 after the addition of the algal powder) was investigated. The results (Figure 1) show that for Cd, Zn and Co metal uptake increased with increasing pH, with the greatest uptake from pH 9, and the least from pH 6, whereas Cu adsorption was greatest from pH 7 PIPES, and least from pH 9 TAPS. In each case, the amount of metal desorbed increased as the pH decreased (Figure 2).

Metal uptake

Metal uptake by freeze-dried *U. lactuca* was plotted against the concentration of metal remaining at equilibrium in the bathing medium. The resulting isotherms for Cd, Zn and Co (Figure 3) all show a rapid uptake at lower concentrations with an equilibrium being reached at higher concentrations. This curvilinear relationship between the amount of metal adsorbed and the amount remaining in solution has been described by Weber (1972) as 'favourable' adsorption. The Cu adsorption isotherm (Figure 3) is linear, which indicates that Cu adsorption is proportional to the concentration of the metal in the medium for the range of concentrations used. The uptake data were used to construct Langmuir, Freundlich and BET plots, which can describe the deposition of an adsorbate as either a mono- or multi-layer on an absorbent (Weber 1972, De Rome & Gadd 1987, Garnham *et al.* 1992).

Langmuir and Freundlich isotherms

The Langmuir isotherms for Cd, Zn, Co and Cu (Figure 4) depict the data from Figure 3 as an inverse plot of metal uptake versus the metal concentration remaining in solution at equilibrium. For each metal this transformation of the data revealed a linear relationship between the amount of metal adsorbed and the amount remaining in solution. This indicates that for each metal, adsorption occurs as a single layer of adsorbate (the metal) onto the adsorbent (the algal cells walls). This was confirmed by constructing Freundlich isotherms for each of the metals, with the straight lines obtained again indicating single layer adsorption of each metal. The logarithmic form of the Freundlich equation is the one most commonly used and takes the form:

$$\log q_e = \log K_F + {}^{1}/_{n} \log C$$

where q_e is the amount of metal sorbed, and C is the amount remaining in solution at equilibrium. From this form of the equation, log K_F (the y intercept) indicates the sorption capacity (or the amount of metal which can be sorbed), and the slope ($^{1}/_{n}$) indicates the intensity of adsorption (or affinity) (Weber 1972). Table 1 gives a summary of this data for each metal and shows that Cu has the greatest affinity and sorption capacity of the metals used (Cu > Zn > Cd > Co).

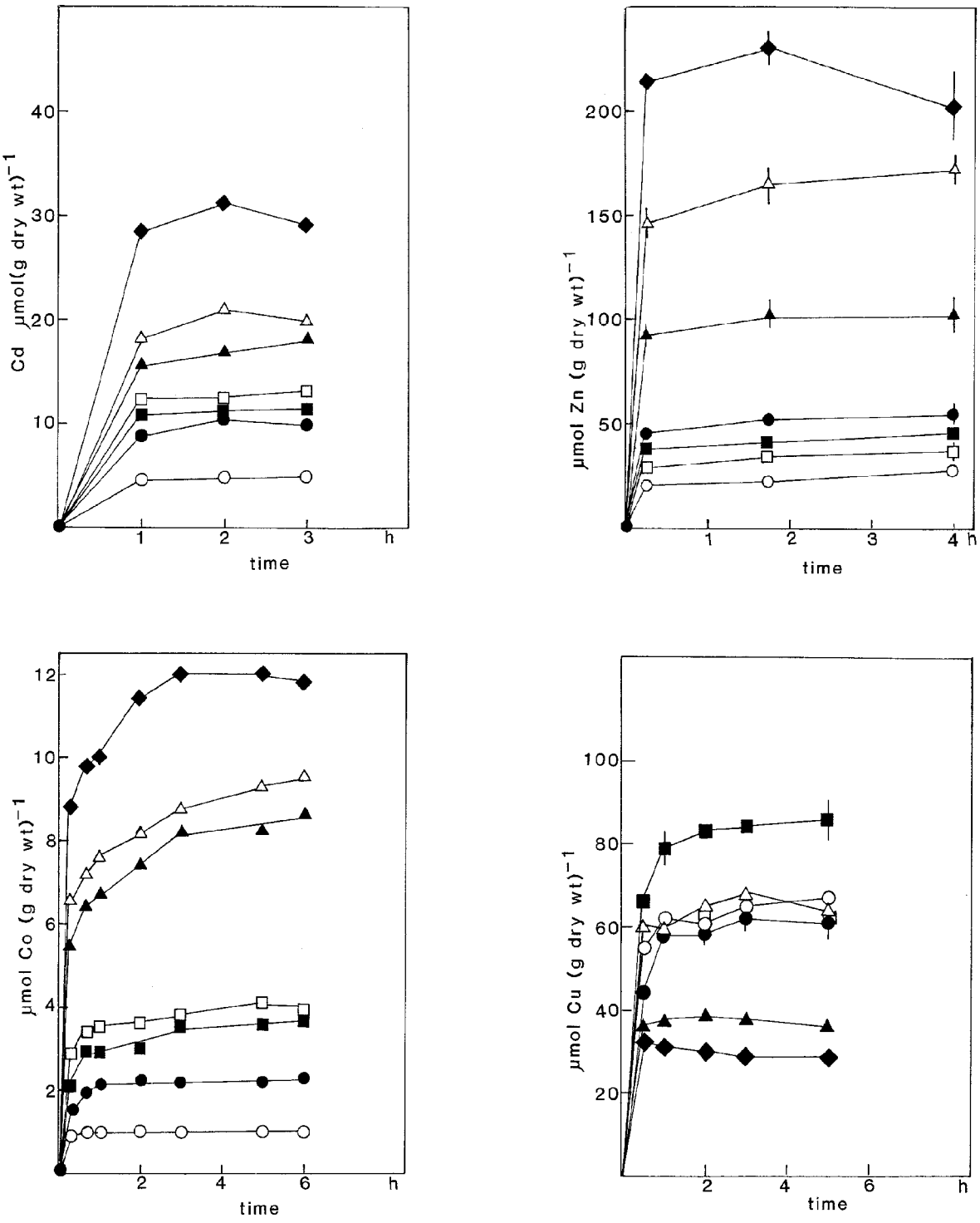

Figure 1. Adsorption of Cd, Zn, Co and Cu, from buffers at pH 6–9, in the presence of 0.5 M NaCl. MES pH 6 (○); filtered seawater pH 8 (●); HEPES pH 7 (□); PIPES pH 7 (■); TAPS pH 8 (▲); HEPES pH 8 (△); TAPS pH 9 (◆) Cd, Co and Cu concentrations were 800 µM; the Zn concentration was 5 mM. Each point represents four replicates ± 95% confidence limits.

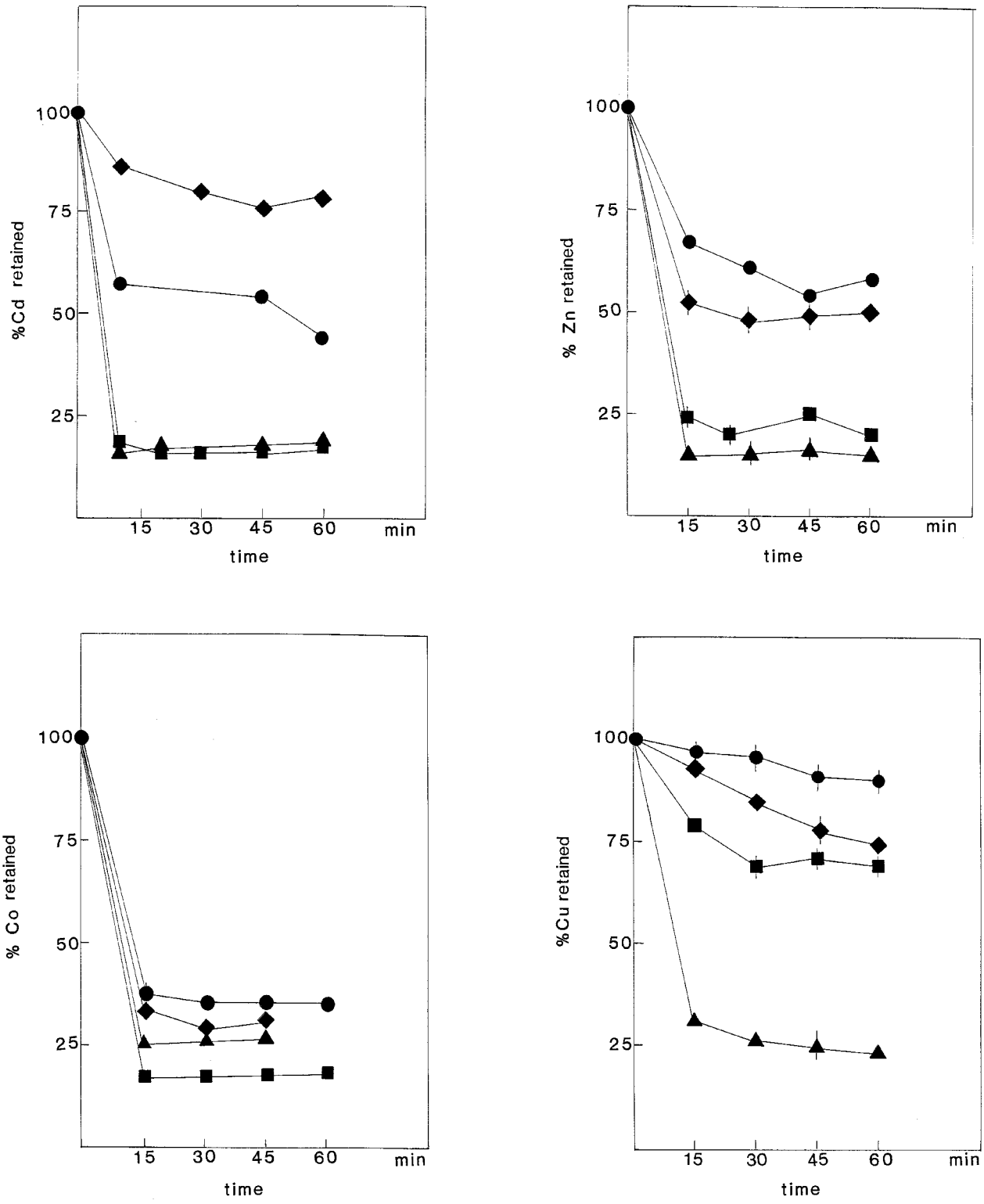

Figure 2. Desorption of Cd, Zn, Co and Cu from freeze-dried *U. lactuca*. The freeze-dried powder was incubated in 800 μM Cd, Zn, Co or Cu in filtered seawater for 2 h. pH 2 wash (▲); pH 3 wash (■); filtered seawater (●); 10 mM Ca wash (◆). Metal adsorbed (100%) was: Cd, 19 μmol g^{-1} dry wt; Zn, 48 μmol g^{-1} dry wt; Co, 14 μmol g^{-1} dry wt; Cu, 87 μmol g^{-1} dry wt. Each point represents four replicates ± 95% confidence limits.

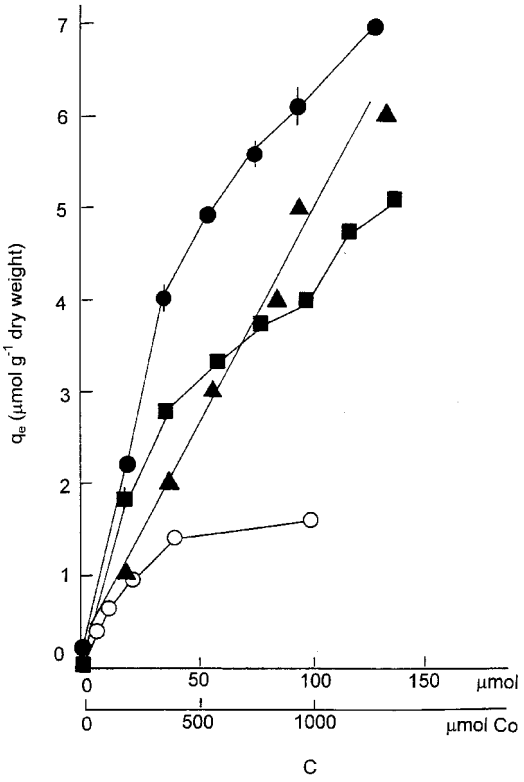

Figure 3. Adsorption isotherms for freeze-dried *U. lactuca*. Metal adsorption (q_e) from filtered seawater after 2 h incubation was plotted against the metal concentration remaining in solution at equilibrium (C). Cd (■), Zn (●), Cu (▲), Co (○). Each point represents four replicates ± 95% confidence limits. The line for Cu adsorption was fitted by linear regression and the correlation coefficient was 0.98.

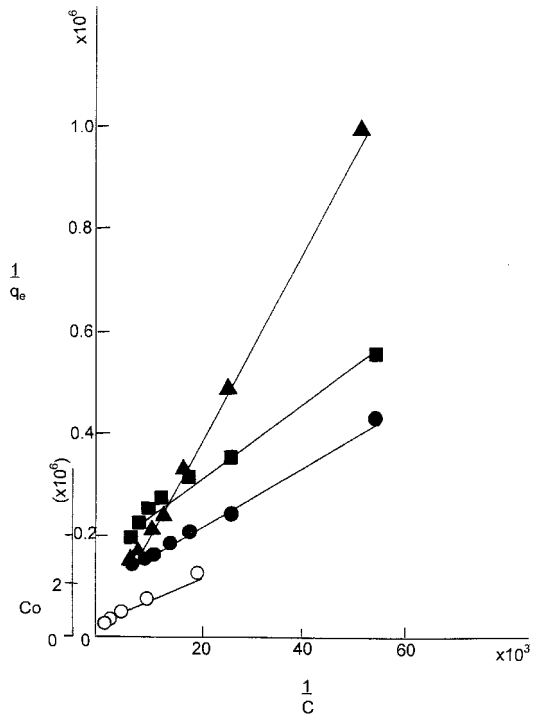

Figure 4. Langmuir isotherms for metal adsorption by freeze-dried *U. lactuca* from filtered seawater (data derived from Figure 1). The inverse of adsorbed metal ($^1/q_e$) is plotted against the inverse of the metal concentration remaining in solution ($^1/C$). Cd (■), Zn (●), Cu (▲), Co (○). The lines were fitted by linear regression and the correlation coefficients were 0.99 for each metal.

Scatchard plots

There are several potential metal binding sites on algal cell walls (Sunda 1989, Greene & Darnall 1990) and each site will have its own binding constant and saturation capacity. The Scatchard plot (Figure 5), which offers a means of analysing the data to give an average binding constant for all available binding sites and a total saturation density for each metal, is the ratio of the amount of metal adsorbed at equilibrium (Γ) to the equilibrium concentration of the metal in solution (C_e) plotted against the amount of metal adsorbed (Γ) (i.e. Γ/C_e versus Γ (Huang *et al.* 1990). The curved plot for Cd indicates that binding sites with a strong affinity for Cd are available at lower Cd uptake values (4.9 µmol g^{-1} dry wt) and above this capacity, lower affinity sites become available. The plot for Zn was a straight line indicating that there was no change in affinity of the binding sites for Zn over the range of concentrations used. The situation was similar for Co, with the straight line obtained again indicating no change in the affinity of the binding sites for Co over the range of concentrations used. The data obtained for Cu fitted neither a curve nor a straight line, suggesting that the Scatchard plot is not an adequate model for

Table 1. Summary of data obtained from the Freundlich isotherms for Cd, Zn, Co and Cu biosorption by *U. lactuca*

Metal	Adsorption intensity (slope)	Sorption capacity (y intercept) (mol g^{-1} dry weight)	Correlation coefficient
Cd	0.49	340×10^{-6}	0.99
Zn	0.54	960×10^{-6}	0.99
Co	0.45	40×10^{-6}	0.97
Cu	0.95	30×10^{-3}	0.99

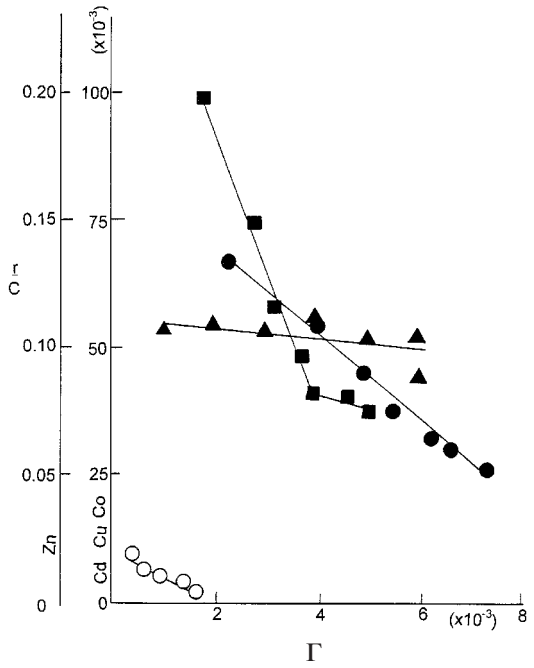

Figure 5. Scatchard plots for Cd (■), Zn (●), Cu (▲), Co (○) adsorption from filtered seawater (data derived from Figure 1). Γ = the amount of metal adsorbed at equilibrium, and C_e = the equilibrium concentration of metal in solution. The Scatchard model suggests that the metal will bind strongly to Y_t (the x intercept), while K_t (the gradient of the line) is the binding constant. The lines were fitted by linear regression.

	K_t ($\times 10^{-3}$)	Y_t ($\times 10^{-6}$)	r^2	y intercept ($\times 10^{-3}$)
Cd (20–100 μM)	–27.5	5.1	0.99	149
Cd (100–140 μM)	–2.9	18.5	0.51	53.2
Zn	–16.9	10.3	0.97	174
Co	–5.6	1.9	0.97	10.7
Cu	–1.0	54.0	0.32	56.4

adsorption of Cu by *U. lactuca*. Thus, for Cd, Zn or Co the x intercept of the Scatchard plot will give the sum of the saturation capacities of the binding sites, and the slope will give the average binding constant (Huang *et al.* 1990); these values suggest that of the metals Cd, Zn or Co, the algal powder has the greatest affinity for Zn.

Brunauer–Emmett–Teller (BET) isotherms

The adsorption data were also used to construct BET isotherms, which are generally assumed to provide a model for multi-layer adsorption (Figure 6). The BET isotherms for Cd, Zn and Co each took the form of a straight line, apparently indicating that the metals are adsorbed as a series of layers (Weber 1972), whereas for Cu, the data could not be fitted to a straight line (r^2 = 0.38).

Competitive binding (NMR)

Algal powder. The metal binding characteristics of freeze-dried *U. lactuca* were also investigated using solution ^{113}Cd NMR. The spectrum obtained for Cd in solution (Figure 7a) appears as a single resonance, and the spectrum for freeze-dried algal powder suspended in Cd solution (Figure 7b) as two resonances (at –3 and –9 ppm) corresponding to ^{113}Cd adsorbed onto the algal powder. The peaks at –3 and –9 ppm are broader because the Cd is bound to a solid substrate (Majidi *et al.* 1990). The addition

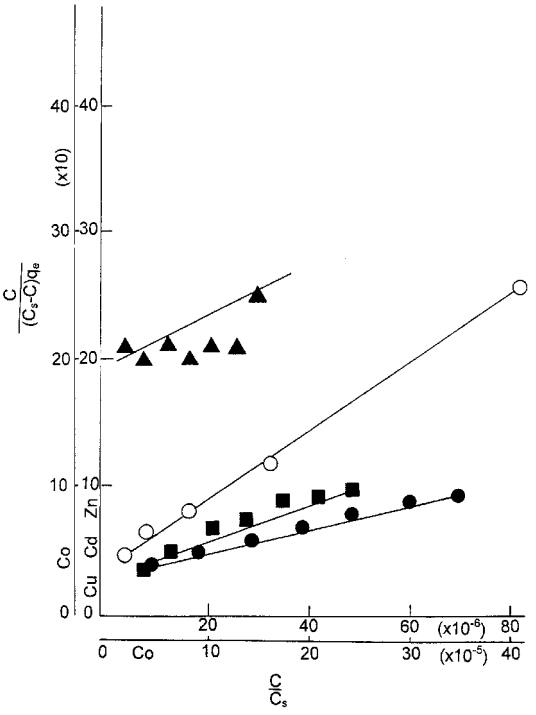

Figure 6. BET plots for metal adsorption from filtered seawater (data derived from Figure 1). Cd (■), Zn (●), Cu (▲), Co (○). The lines were fitted by linear regression. q_e is the amount of metal adsorbed per gram dry weight, C is the amount of metal remaining in the solution at equilibrium, and C_s is the saturation concentration of the solute. The equations of the lines are: Cd, $y = 3.1 + (0.145 \times 10^{-6})x^2 = 0.96$; Zn, $y = 3.1 + (0.1 \times 10^{-6})x^2 = 0.99$; Co, $y = 37.1 + (5.4 \times 10^{-5})x^2 = 0.99$; Cu, $y = 19.9 + (20.2 \times 10^{-3})x^2 = 0.38$.

of Cu to the Cd/algal suspension (Figure 8) caused a reduction in the resonances due to bound Cd as the concentration of Cu increased, until the resonances for bound Cd disappeared completely with Cu presumably replacing Cd.

Cell wall preparation. ^{113}Cd NMR spectroscopy was also used to investigate competition between metals for the Cd binding sites on the whole cell wall. Figure 9a shows the spectrum obtained for the Cd solution, and Figure 9b the spectrum obtained for the cell wall preparation in Cd solution. The latter shows a sharp peak to 0 ppm, which corresponded to the Cd in solution (similar to that in Figure 9a), while the Cd bound to the cell wall was represented by a broader peak at –15 ppm. The resonance due to bound Cd is expected to be broader when the metal is bound to a solid substrate (Majidi *et al.* 1990). The addition of Zn reduced the amount of Cd bound to the cell wall, but did not abolish it (Figure 10). Thus, Zn did not replace Cd completely in spite of the increased concentration of Zn used.

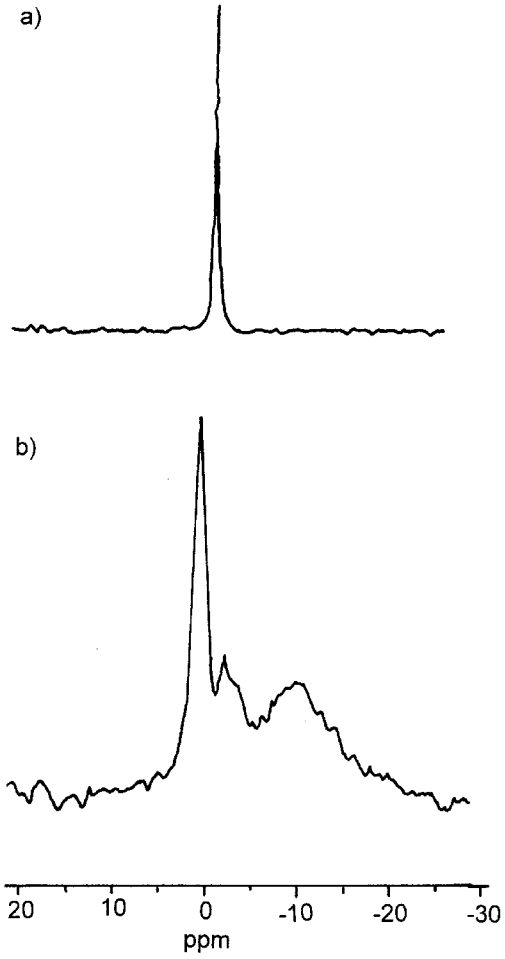

Figure 7. ^{113}Cd NMR spectrum for: (a) 0.5 M $CdSO_4$ solution; and (b) 0.5 M $CdSO_4$ solution + freeze-dried *U. lactuca* powder. All chemical shifts are with reference to $3CdSO_4 \cdot 8H_2O$ solution, which has a chemical shift of –45 with reference to $Cd(ClO_4)_2$ (Armitage and Boulanger, 1983).

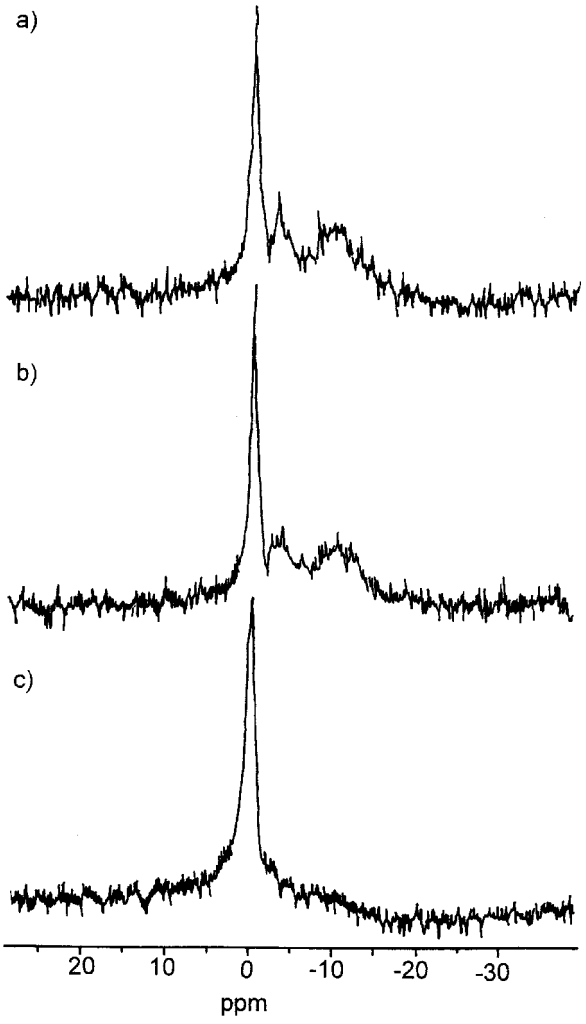

Figure 8. NMR spectrum for displacement by Cu of Cd adsorbed onto freeze-dried *U. lactuca* powder. (a) Freeze-dried algal powder incubated in Cd solution; (b) freeze-dried algal powder incubated in Cd solution plus Cu (100 µl 0.5 M $CuSO_4$); (c) freeze-dried algal powder incubated in Cd solution plus Cu (400 µl 0.5 M $CuSO_4$).

Discussion

Freeze-dried algal powder has previously been used to study biosorption (Crist *et al.* 1981, Kuyucak & Volesky 1990). Thallus particles in the powder have a greater surface to volume ratio than the intact thallus which means that more binding sites will be available; consequently, metal uptake is generally greater than for intact thallus (Kester 1986). Freeze-drying the alga will increase the efficiency of the biomass as a potential agent for the removal of toxic metals from solution, while the use of adsorption isotherms allows comparisons to be made with other adsorbents (Volesky 1990).

In the present study, the uptake of Cd, Zn and Co from buffers (pH range 6–9) supplemented with NaCl was pH-dependent; uptake increased with increasing pH, which is consistent with observations using other organisms, including microalgae (Gadd 1990, Huang *et al.* 1990, Majidi *et al.* 1990, Skowronski & Szubinska 1991). Copper uptake from buffers was exceptional in that the greatest uptake was from pH 7 PIPES, and least from pH 9 TAPS.

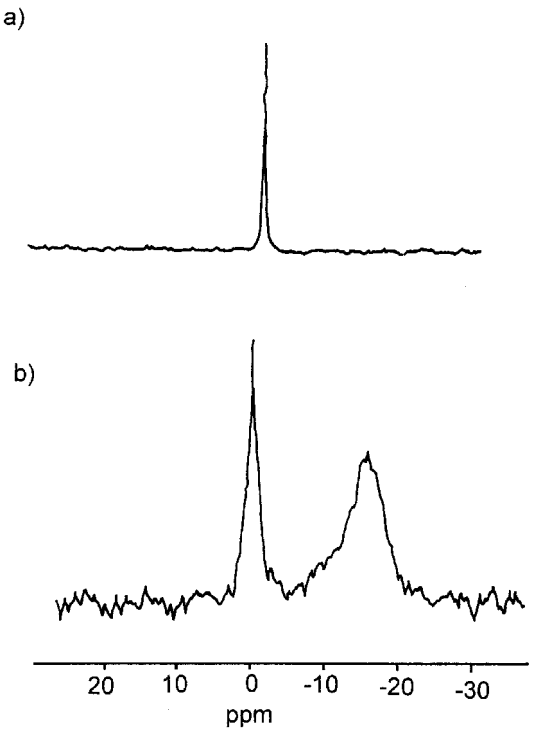

Figure 9. NMR spectrum for *U. lactuca* cell wall preparation incubated in Cd solution. (a) 0.5 M $CdSO_4$ solution (used as reference); (b) *U. lactuca* cell wall preparation in 0.5 M $CdSO_4$ solution.

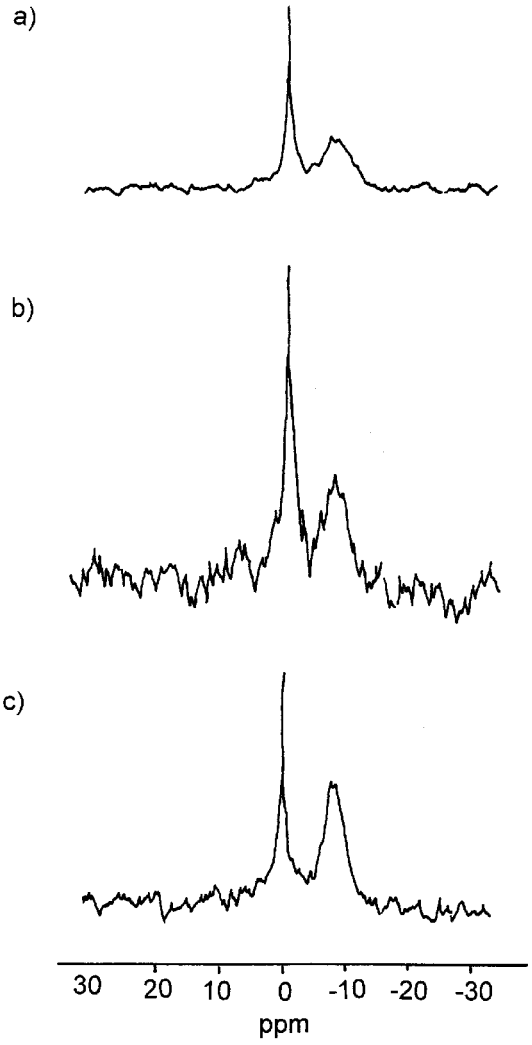

Figure 10. NMR spectrum for partial displacement by Zn of Cd from *U. lactuca* cell wall preparation incubated in Cd solution; ratio of Cd in solution to bound Cd as estimated by integrating the area under the peaks, shown in parentheses. (a) *U. lactuca* cell wall preparation in 0.5 M $CdSO_4$ solution (0.8); (b) *U. lactuca* cell wall preparation in 0.5 M $CdSO_4$ solution plus Zn (400 µl 0.5 M $ZnSO_4$) (1.2); (c) *U. lactuca* cell wall preparation in 0.5 M $CdSO_4$ solution plus Zn (200 µl 0.5 M $ZnSO_4$ (1.0).

This could be a characteristic of the buffers used. Good *et al.* (1966) have described the Cu complexing capability of MES, PIPES and HEPES as being negligible, although TAPS was not included in their investigation. Any change in pH will affect metal uptake by influencing the availability of either the metal in solution and/or the binding sites on the algal powder because competition between protons and

metal species increases as the pH decreases. In this study, desorption studies on each of the metals showed that the medium with the lowest pH was the most efficient desorbent, which is consistent with work by other authors (De Rome & Gadd 1987, Skowronski & Szubinska 1991, Garnham *et al.* 1992). The desorption results, and those from the pH studies, suggest that metal ions may bind to sites which become deprotonated as the pH decreases; unprotonated carboxyl-oxygen or sulphate groups (Crist *et al.* 1981) and carboxylate or amine groups (Greene & Darnall 1990) are possible binding sites. The observed decrease in pH when algal powder was suspended in filtered seawater indicated that protons were released from the algal powder (Crist *et al.* 1981) providing further evidence of the role of protonation in metal binding.

Weber (1972) defined adsorption as the accumulation of an adsorbate onto an adsorbent, which occurs at any interface between phases. In this study, the metal (adsorbate) was sorbed onto a solid adsorbent (algal powder) from a liquid phase. The uptake isotherms which model adsorption show that for Cd, Zn and Co the relationship between the amount of metal sorbed and the amount remaining at equilibrium in solution was curvilinear, which indicates a favourable pattern of separation between the two phases. This form of adsorption is advantageous if the adsorbent is being considered for the removal of metals from solution, because adsorption is efficient from low concentrations ('unfavourable' adsorption is the term applied to an adsorption isotherm which shows increased adsorption at higher concentrations, compared to low concentrations (Weber 1972). Cu adsorption showed a linear relationship, which theoretically is typical of a combination of adsorption onto the solid surface, and absorption into the biomass, with the molecules of each phase interpenetrating uniformly. Such an interpretation should be made with caution because, while it may be possible to make the distinction between adsorption and absorption for well defined substrates used in water quality control, this distinction may be too simplistic for a complex substrate such as algal cell walls (Volesky 1990). The linear isotherm observed for Cu may be in response to the range of concentrations used, and a different form of isotherm may become evident at higher Cu concentrations. When the data were transformed using the Freundlich equation, the 'adsorption intensity', i.e. the affinity of the adsorbent for Cu, was seen to be nearly twice that for Cd, Zn or Co, and the 'sorption capacity' was at least two orders of magnitude greater than that for Cd, Zn or Co, showing that the powdered *U. lactuca* cell walls had the greatest capacity to bind Cu. Thus, at the concentrations used for Cu the potential binding sites may not be fully occupied and equilibrium will not have been reached. The linear Freundlich isotherms which were obtained for Cd, Zn, Co and Cu suggest that the metals were adsorbed as a monolayer, and this was confirmed by an examination of the Langmuir isotherms. The use of Langmuir and Freundlich isotherms to describe biosorption may be limited because the fact that the data fit the models does not necessarily imply that 'pure' adsorption has occurred (Volesky 1990), nor that the theoretical basis of the model is met (Stumm & Morgan 1981). De Rome & Gadd (1987) also advocate caution in the use of adsorption models for biological systems. Most adsorption models were developed for use with defined, ideal systems, and make a number of assumptions. For the Langmuir model, these are that there is a constant energy of adsorption applicable to all sites on the surface of the adsorbent, that the adsorbed molecules do not migrate within the surface of the adsorbent, and that maximum adsorption has occurred when the adsorbate molecules have formed a saturated monolayer on the surface of the adsorbent. The Freundlich model makes similar assumptions but is applicable to situations in which the surface energies are not uniform, but vary as a function of the amount of metal adsorbed (q_e) (Weber 1972); any discrepancy between these plots for any two metals would suggest a difference in the energy requirements for adsorption of those metals. From the data presented in the current study, this is not the case and the Langmuir and Freundlich plots give similar results, suggesting that these models are adequate for basic analysis of metal adsorption by *U. lactuca.*

BET transformation of the data confirmed that Cu adsorption was apparently as a monolayer. By contrast, the BET plots for Cd, Zn and Co indicated that adsorption was multilayered, which apparently contradicts the evidence from the Langmuir and Freundlich isotherms. One explanation for this may be that the BET isotherm is an inappropriate model for adsorption to algal cell walls because of the multiplicity of potential binding sites, and because of the heterogeneous nature of the cell walls. De Rome & Gadd (1987) used BET isotherms in conjunction with Freundlich and Langmuir isotherms, to describe metal adsorption by filamentous fungi and to indicate differences in adsorption between different morphological types.

The Scatchard plot (Scatchard 1949) recognises that there will be a number of potential binding sites

and allows an average binding constant to be calculated. In addition, if a curved plot is obtained, this suggests that there are two binding constants, signifying a strong binding up to Y_t (the x-intercept) followed by weaker binding which occurs at higher concentrations. Gadd & Mowll (1985) used the Scatchard plot to obtain a dissociation constant for high affinity sites, and to derive the capacity of fungal biomass to bind Cu. The data from this study were fitted to the Scatchard transformation, but the scattered points suggested that this is not a suitable model for copper adsorption by *U. lactuca*. The Freundlich isotherm indicated that *U. lactuca* cell walls had a high sorption capacity for Cu; considering the complex structure of the algal cell wall, it is likely that this high capacity was achieved via a multiplicity of binding sites, which, in contrast to our findings, suggests that the Scatchard model should be appropriate. However, such a scattered plot may be a consequence of the range of concentrations used. Huang *et al*. (1990) used Scatchard plots to model uptake of Cu by *Saccharomyces cerevisiae*, and found that this plot provided a useful model for adsorption by that organism. In the current study, when the data for Cd, Zn or Co adsorption were fitted to Scatchard plots, only Cd showed any change in the apparent affinity of binding sites with increasing concentration, with Zn and Co showing no change in affinity with increasing concentration. This indicates the existence of two types of binding site for Cd; a primary site which binds at lower Cd concentrations, with a secondary site only becoming available at higher Cd concentrations.

The experiments which investigated competition between metals for binding sites on freeze-dried algal powder, carried out using ^{113}Cd NMR, indicated that two binding sites may be available for Cd, and that Cu could replace Cd completely, suggesting that although both metals could bind to the same sites, the affinity of the sites for Cu was greater than for Cd. The fact that two resonances (corresponding to bound ^{113}Cd) are present in addition to the resonance for Cd in solution, suggests that there are two separate binding sites for Cd, which confirms the results obtained from the Scatchard plot of the uptake data. The curved Scatchard plot for Cd suggested that there were Cd binding sites with two different affinities for the metal. The weaker binding sites would become available at higher metal uptake values (above 4.9 μmol g^{-1} dry weight), which will occur at media metal concentrations above 140 μM. This is considerably lower than the concentration used in the ^{113}C NMR study.

Freeze-dried algal powder can adsorb Cd, Cu and Zn efficiently. Transformation of the uptake data allowed the adsorption characteristics of the algal powder to be defined, enabling these characteristics to be compared with other metal sorbents. As discussed earlier, the algal powder offers a greater surface to volume ratio, resulting in a greater sorption capacity, than would be the case for whole thalli. Consequently, it must be stressed that the metal binding characteristics of freeze-dried thalli probably differ substantially from those of whole thalli and may be of little relevance to the physiology of the whole alga. For this reason, NMR was also used to investigate the metal binding characteristics of whole *U. lactuca* cell walls. To investigate metal binding to biologically inactive algal cell walls, it is necessary to inhibit or abolish cellular processes. Some algae are insensitive to metabolic inhibitors (Raven 1984, Webster & Gadd 1992, 1996a), so whole thalli stripped of intracellular contents were used to investigate metabolism-independent Cd binding. Previous work has shown that Cd uptake by discs of cell wall preparation was similar to rapid, short term (metabolism-independent) uptake of Cd by live *U. lactuca* (results not shown). The ^{113}Cd NMR spectra obtained for cell wall preparation incubated in Cd solution showed that the resonance due to bound Cd was broader and appeared at –15 ppm, whereas the resonance for Cd bound to algal powder appeared as two narrower peaks (–3 and –9 ppm). Algal powder is freeze-dried whole thallus, which will include intracellular contents as well as cell wall, and this may be the reason for the appearance of two resonances and the interpretation that there are two Cd binding sites available on the algal powder. It is suggested that there is one binding site on the cell wall component of the freeze-dried algal powder and a second binding site available on the component of the freeze-dried algal powder derived from the intracellular contents. The results obtained using ^{113}Cd NMR and cell wall preparation confirmed that one Cd binding site was available on whole cell walls, and that Zn partially displaced bound Cd.

Resonances for ^{113}Cd bound to oxygen groups are found in the region of 0 ppm to –125 ppm, whereas those consisting of nitrogen or thiolate ligands appear between 0 and 300 ppm, and 350 and 750 ppm, respectively (Armitage and Boulanger 1983). Thus, the chemical shifts observed for bound Cd (Cd bound to algal powder or to cell walls) indicate that Cd is probably bound to oxygen. The cell walls of algae such as *U. lactuca* carry a net negative charge (Percival 1979, Mariani *et al.* 1990) and algal cell walls can therefore function as cation exchange

systems (Mariani *et al*. 1990). The cell wall of *U. lactuca* is a complicated structure consisting of branched polysaccharides with sulphate groups substituting for some of the hydroxyl groups on the saccharide subunits (Percival 1979). Previous work using electron microscopy in conjunction with X-ray microanalysis suggests that Cd binds to sulphate groups in the cell wall of whole thallus (Webster & Gadd 1996b). It is suggested that the Cd binding sites are associated with oxygen, and that sulphate or hydroxyl groups are possible sites.

In conclusion, this work has described the uptake characteristics of Cd, Cu, Zn and Co by freeze-dried *U. lactuca* powder and indicates that the algal powder could act as a sorbent for these metals. Although adsorption isotherms should be used with appropriate caution, the results presented suggest that the simpler adsorption models (Freundlich and Langmuir) are adequate for metal adsorption, whereas the BET and Scatchard models were more limited in their application. This may be a reflection of the range of concentrations used and the complexity of the algal cell wall.

The results of the NMR studies showed that two Cd binding sites were available on the algal powder and that Cu could be bound preferentially. In addition, to relate this work to Cd binding by *U. lactuca* under more physiologically meaningful conditions, Cd binding characteristics of cell walls stripped of the intracellular contents were investigated. One Cd binding site on the cell wall was indicated, and it was confirmed that the Cd binding site may include an oxygen-containing functional group. Sulphate or hydroxyl groups are therefore suggested as possible sites for Cd binding.

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