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Metal accumulation kinetics by the estuarine macroalga, *Fucus ceranoides*



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ABSTRACT

The kinetics of Cu, Cd and Pb accumulation by the macroalga, *Fucus ceranoides*, was studied under simulated estuarine conditions. Accumulation of Cu and Pb proceeded via a pseudo-first-order reaction that was reversible, suggesting desorption or efflux of accumulated metal, with forward rate constants on the order of 0.1 h^{-1} . For both metals, reaction reversibility increased and the equilibrium constant decreased with increasing salinity (from 1 to 33.5) and system response times were $<10 \text{ h}$ throughout. Accumulation of Cd proceeded via a first-order reaction that was irreversible, suggesting little desorption or efflux of metal, with rate constants that decreased with increasing salinity (from 0.023 to 0.015 h^{-1}) and reaction half-lives ranging from approximately 30–50 h. Inorganic equilibrium speciation calculations suggest that interactions of Cu, Cd and Pb principally involve the respective free ions, but that additional ions (e.g. CdCl^+) and biotic processes may also be significant.

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1. Introduction

The accumulation of metals by aquatic macrophytes is well established (Phillips, 1994). Resulting concentrations can be several orders of magnitude greater than corresponding concentrations in ambient water and are often taken to reflect the net metal accumulation from the dissolved fraction in the water column integrated over time of exposure (Ali et al., 1999; Zhou et al., 2008; Materazzi et al., 2012). Brown seaweeds belonging to the order Fucales (e.g. *Fucus* spp., *Ascophyllum nodosum*) are efficient accumulators of metals in the marine environment due, partly, to the abundance and strength of complexants associated with cell walls and the intercellular matrix (Davis et al., 2003). In particular, *F. vesiculosus* has been employed extensively in metal bioaccumulation studies of estuaries and coastal waters throughout its geographical range in northwest Europe and is used as a bio-monitor of metal contamination (Rönnerberg et al., 1990; Struck et al., 1997; Barreiro et al., 2002; Rainbow et al., 2011; Ryan et al., 2012).

Compared with many other marine organisms, there exists little information on the mechanisms and kinetics by which macroalgae accumulate metals (Vasconcelos and Leal, 2001;

Cosden et al., 2003; Costa et al., 2011). It is generally assumed that metabolically-independent accumulation occurs through binding at polysaccharide-rich cell surfaces, and that subsequent (or simultaneous) transport across the cell membrane is accompanied by complexation with intracellular ligands such as polyphenols and thiol peptides (Wang and Dei, 1999; Garcia-Rios et al., 2007). The precise mechanisms of adsorption and uptake, however, are dependent on a number of extrinsic and intrinsic factors, including the speciation of aqueous metal, the chemistry of the algal cell walls and the kinetics of interactions of the metal with the algal surface and intracellular ligands (Brown and Depledge, 1998).

In the present study, and in order to better understand both the mechanisms and kinetics of metal interactions with macroalgae in estuaries, we examine the rates of accumulation of Cu, Cd and Pb by *Fucus ceranoides*, a furoid that is able to withstand the full estuarine range of salinities within its North Atlantic distribution (Lein, 1984; Barreiro et al., 1993). Specifically, we exposed *F. ceranoides* to different salinities under controlled conditions (i.e. mixtures of nutrient amended synthetic sea water and distilled water) and couple our observations with the computed inorganic equilibrium speciation of Cu, Cd and Pb. The metals selected for study are important contaminants of estuaries and coastal waters and exhibit different chemical and biological characteristics in the aquatic environment (e.g. speciation, reactivity, essentiality, toxicity).

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2. Methods

2.1. Sampling and sample treatment

Samples of *Fucus ceranoides* were collected from the upper, freshwater reaches of the Yealm, a small, macrotidal estuary whose catchment is dominated by agricultural land and granitic moorland. An earlier study revealed that metal concentrations in *F. ceranoides* from the Yealm were among the lowest of samples analysed from a number of sites in south west England (Varma et al., 2011). Approximately 25 whole individuals of between 10 and 15 cm in length were collected by hand at low tide and from near the high water mark, where *F. ceranoides* was the dominant seaweed present. Samples were stored in zip-lock polyethylene bags and transported to the laboratory where visible epiphytes and organic and animal debris were washed off with filtered English Channel sea water (salinity = 33.5). Actively growing apical tips were used in the experiment (Young et al., 2007; Baumann et al., 2009) and were cut to a length of 2.5 cm from individuals using a pair of stainless steel scissors and stored in zip-lock bags at 4 °C until required.

2.2. Culture medium

Experiments were performed in various dilutions of nutrient-amended synthetic sea water, whose formulation was based on that of the culture medium, Aquil, but without the complexing agent, EDTA (Morel et al., 1979). Thus, full strength synthetic sea water was prepared by appropriate dilution of various anhydrous salts (NaCl, Na₂SO₄, KBr, KCl, H₃BO₃, NaHCO₃) and hydrated salts (CaCl₂·2H₂O, MgCl₂·6H₂O), purchased from VWR, Fisher or Sigma, in 5 L of Millipore Milli-Q water (MQW). A concentrated nutrient solution was prepared by appropriate dilution of various salts (SrCl₂·6H₂O, NaF, KI, NaNO₃, NaH₂PO₄) in 1 L of MQW. Five, 1 L working solutions were then prepared in different MQW dilutions of synthetic sea water, each amended with 1 ml of nutrient solution. The salinities of the working solutions were 33.5, 20, 10, 5 and 1, and pH was 7.7 with the exception of the most dilute solution (pH = 6.7). The pH of the latter was raised to 7.7 by the dropwise addition of 1 M NaOH (BDH AnalaR) and the subsequent addition of about 1.5 ml of HEPES, a non-complexing buffer that is widely used in plant culture experiments (Stoll and Blanchard, 1990).

2.3. Experimental approach

Since the algae we used contained variable concentrations of pre-existent Cu, Cd and Pb, we monitored the accumulation of added metal by determining the change in metal concentrations in the aqueous phase (Vasconcelos and Leal, 2001; Cosden et al., 2003). Thus, 200 ml of each working solution was added, in quadruplicate, to individual polypropylene containers (Magenta GA 7 culture vessels). Three randomly selected tips of *Fucus ceranoides* were weighed wet and then added to each of three of the replicate solutions, while the fourth solution served as an algal-free control. The contents of each container were then placed in a culture room at a controlled temperature of 15 ± 1 °C under a light regime of 250 μmol m⁻² s⁻¹ photosynthetic active radiation on a 12 h light–dark cycle; here, containers were individually aerated using the tapered end of a Pasteur pipette connected to an air cylinder via plastic tubing. After an acclimation period of 36 h to allow for recovery of the algae from any potential stress, 0.4 ml of a mixed metal solution of 10 mg L⁻¹ of Cu, Cd and Pb in 0.01 M HNO₃, derived from dilution of Spectrosol metal standard solutions, was added to each container, resulting in a nominal concentration of 20 μg L⁻¹ of each metal. The contents of each container were then incubated for 120 h. Five ml of the

culture medium was pipetted into a 10 ml centrifuge tube or 25 ml volumetric flask (salinities 20 and 33.5) and diluted to mark with 0.1 M HNO₃ after 0, 0.25, 0.5, 1, 2, 5, 12, 24, 48 and 120 h. At the end of the experiment (120 h), the three apical tips were retrieved from each container and reweighed (note that the increase in mass of the combined tips from each container was <10% of the original weight). Tips were subsequently frozen and freeze-dried for 48 h before 70 mg of material from each container, triplicate freeze-dried tips of unexposed samples (i.e. those not used in the experiment), and triplicate 100 mg aliquots of a certified reference alga (*Fucus* spp.; IAEA-140) were weighed into individual screw-capped 5 ml Teflon digestion vessels. Three ml of concentrated HNO₃ (BDH Aristar) was then added to each vessel and the tightly capped contents placed in a CEM-MDS 2000 microwave digester on medium power for 45 min. Cooled digests were washed into 10 ml volumetric flasks, where they were diluted to mark with MQW, and subsequently transferred to individual 25 ml polypropylene containers pending analysis.

2.4. Analysis

Algal digests were analysed for Cd, Cu and Pb by inductively coupled plasma-optical emission spectrometry (ICP-OES) using a Varian 725 ES with a V-groove nebuliser and Sturman-Masters spray chamber. Forward power was set at 1.4 kW, and plasma, nebuliser and auxiliary gas flows were 15, 0.68 and 1.5 L min⁻¹, respectively. Measurements were made at a viewing height of 8 mm above the load coil at resonance lines of 327.395 nm for Cu, 214.439 nm for Cd and 220.353 nm for Pb with a replicate ($n = 3$) read time of 4 s. Calibration was achieved using five mixed standards (and a blank) in 0.3 M HNO₃. Measured metal concentrations in the certified alga were within 15% of certified values in each case.

Acidified water samples were analysed by inductively coupled plasma-mass spectrometry (ICP-MS) using a Thermo Scientific X Series II quadrupole bench top instrument with a concentric glass nebuliser and conical spray chamber with impact bead. Power was set at 1.4 kW, and coolant, nebuliser and auxiliary gas flows were 13, 0.86 and 0.7 L min⁻¹, respectively. The isotopes analysed were ⁶⁵Cu, ¹¹¹Cd and ²⁰⁸Pb, with a dwell time of 10 ms, 50 sweeps and 3 replicates. External calibration was achieved using matrix-matched standards (in proportions of Aquil and HNO₃ identical to those in the samples) and internal calibration was achieved by the addition of 10 μg L⁻¹ of ¹¹⁵In and ¹⁹³Ir to all samples and standards.

2.5. Speciation calculations

The inorganic equilibrium speciation of Cu, Cd and Pb under the experimental conditions was calculated using the Windermere Humic Aqueous Model (WHAM, v6; Tipping, 1998) and the stability constants in its default database. The chemical composition of synthetic sea water and MQW dilutions thereof was used to define the ionic composition of the samples, temperature was set at 288 K and pH at 7.7, and equilibrium with the atmosphere was assumed ($p\text{CO}_2 = 3.5 \times 10^{-4}$ atm). The Davies equation was employed to calculate ion activity coefficients.

3. Results

3.1. Aqueous metal speciation

The calculated equilibrium speciation of Cu, Cd and Pb is shown as a function of salinity in Fig. 1. Note that only inorganic components are shown and that possible complexes with undefined organic ligands derived from the macroalga have not been considered. In all cases, the percentage of total aqueous metal as

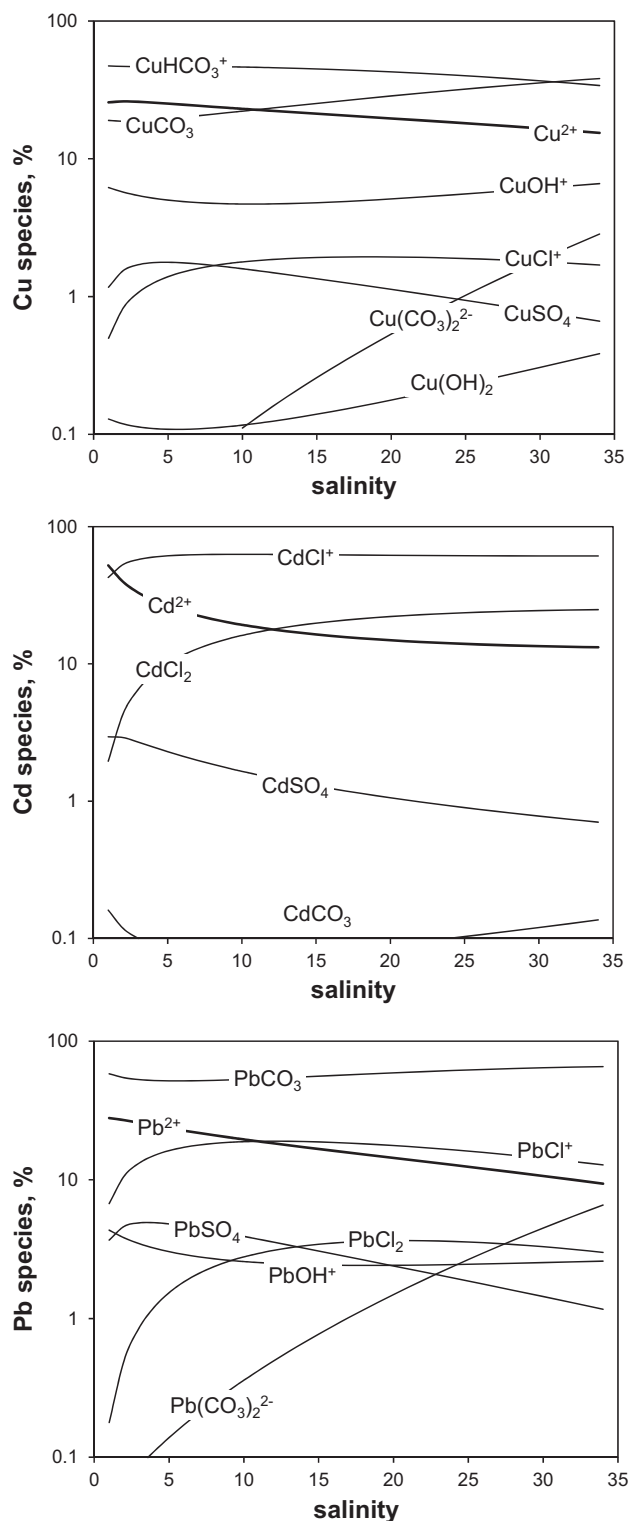


Fig. 1. Inorganic equilibrium speciation of Cu, Cd and Pb as a function of salinity, calculated using WHAM v6 and the default stability constants in its database (temperature = 288 K; pH = 7.7).

the free ion and the free ion activity (not shown) both decrease across the salinity gradient. With respect to Cd, the majority of remaining metal is complexed by chloride, principally as CdCl^+ and CdCl_2 since both CdCl_3^- and CdCl_4^{2-} comprised <0.1% of total Cd. Regarding Cu, most bound metal exists as the carbonate complexes, CuCO_3 and CuHCO_3^+ , with the abundance of the former increasing

and the latter decreasing with increasing salinity. Lead also exhibits increasing chloro-complexation with increasing salinity but the dominant species throughout is PbCO_3 .

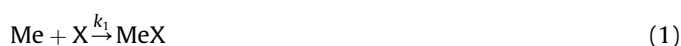
3.2. Metal accumulation time-courses

Figs. 2, 3 and 4 show the concentrations of aqueous Cu, Cd and Pb, respectively, as a function of time for the incubations in which *Fucus ceranoides* was suspended in media of different salinities. Note that aqueous metal may include undefined complexes with algal exudates (Gledhill et al., 1999) as well as the species computed in Fig. 1. Corresponding controls, in which algae and, therefore, exudates, were absent, revealed no measurable loss of metal with the exception of Cu at the two lowest salinities; here, up to 20% of metal was lost by the end of the time course, presumably through the adsorption of Cu ions to the container walls. With the exception of these cases, it is reasonable to assume that loss of metal in the presence of *F. ceranoides* is entirely the result of various metal accumulation mechanisms by the alga.

The time-dependent accumulation of dissolved Cd appears to be exponential over the timeframe of the experiment (Fig. 3), attaining relatively low concentrations after 5 days at all salinities. Removal of aqueous Cu and Pb by *Fucus ceranoides* (Figs. 2 and 4, respectively) appears to be biphasic in that a period of rapid accumulation over a timescale of about 5 h is succeeded by a more protracted period of slower accumulation in which equilibrium is eventually approached or attained. In the case of Cu at $S = 1$ and $S = 5$, aqueous metal appears to be almost completely consumed by 40 h, potentially due to its loss to the walls of the reactor as mentioned above. For Pb at salinities of 5 and 10 there appears to be an intermediate period of about 30 h in duration in which metal is released back into the aqueous phase.

3.3. Metal accumulation kinetics

The time-dependent Cd concentrations observed in the experiment are consistent with a first-order, unidirectional reaction mechanism (Turner et al., 2007). Kinetic data were, therefore, modelled using an irreversible first-order reaction:



where Me is total aqueous metal, X represents binding sites on the algal cell surfaces, MeX is bound or accumulated metal and k_1 is a removal or accumulation rate constant that is conditional to the concentration of alga present. The rate equation for this reaction:

$$\frac{d[\text{Me}]}{dt} = -k_1[\text{Me}]_t \quad (2)$$

may be integrated to yield:

$$\ln[\text{Me}]_t = \ln[\text{Me}]_0 - k_1 t \quad (3)$$

where $[\text{Me}]_0$ is the initial concentration of aqueous metal. First-order rate constants for Cd were obtained from the slopes of $\ln([\text{Me}]_t/[\text{Me}]_0)$ versus time, where $[\text{Me}]_0$ was assigned as the first measured concentration in the time-course, and the last point in the time course, whose concentration was always close to the detection limit, was omitted. Constants for all salinities are shown in Table 1, along with the respective reaction half-lives, calculated from $\ln 2/k_1$ and which range from about 30 to 50 h. Model fits to the data based on these rate constants are annotated on Fig. 3.

Time-dependent aqueous Cu concentrations at $S = 1$ and $S = 5$ also conformed to an irreversible first-order reaction, although loss

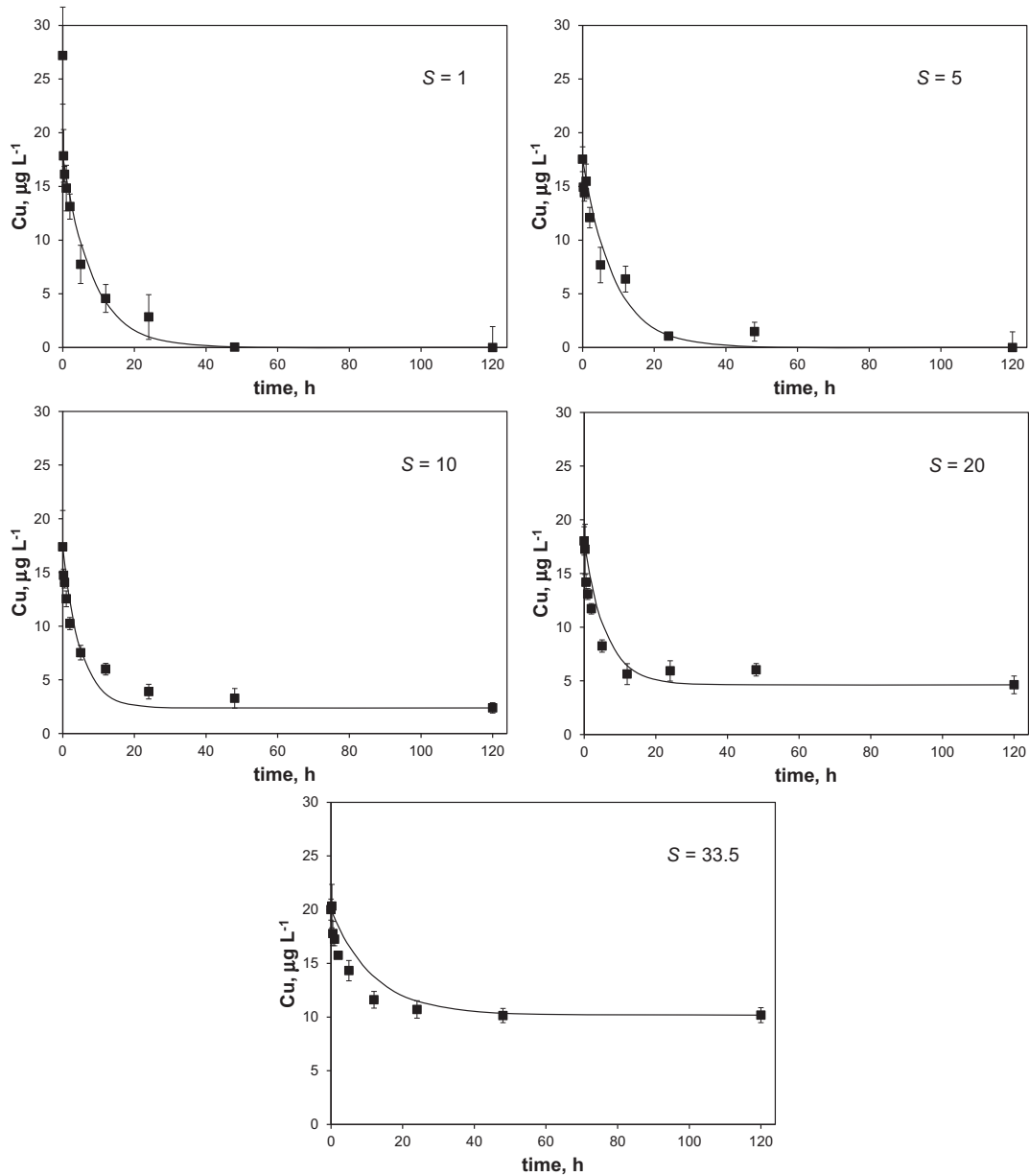


Fig. 2. Concentrations of dissolved Cu as a function of time in culture containers with different dilutions of synthetic sea water (whose salinities are annotated) and tips of *Fucus ceranoides*. Errors denote the standard deviation about the mean of three independent measurements and lines represent fits according to Equation (8) and the rate constants given in Table 2.

of metal to the container surfaces was evident in these treatments. However, as more generally the removal of Cu and Pb appeared to approach equilibrium within the timeframe of the experiment kinetic data for these metals were modelled using a pseudo-first-order reversible reaction (Turner et al., 2007):



where k_1 and k_{-1} are the rate constants for the forward and reverse reactions, respectively. The rate equation for this reaction:

$$\frac{d[\text{Me}]}{dt} = -k_1[\text{Me}]_t + k_{-1}[\text{MeX}]_t \quad (5)$$

may be integrated to give the following expression:

$$([\text{Me}]_t - [\text{Me}]_e) = ([\text{Me}]_0 - [\text{Me}]_e) \exp[-(k_1 + k_{-1})t] \quad (6)$$

where $[\text{Me}]_e$ is aqueous metal concentration at equilibrium and $[\text{MeX}]_t = [\text{Me}]_0 - [\text{Me}]_t$. Given that, under equilibrium conditions, $d[\text{Me}]/dt = 0$, we may define an equilibrium constant, K , and rewrite Equation (5) as follows:

$$K = \frac{k_1}{k_{-1}} = \frac{[\text{Me}]_0 - [\text{Me}]_e}{[\text{Me}]_e} \quad (7)$$

Rearranging Equation (7) in terms of k_1 and substituting this into Equation (6) results in the following:

$$k_1 t = \left(\frac{[\text{Me}]_0 - [\text{Me}]_e}{[\text{Me}]_0} \right) \ln \left(\frac{[\text{Me}]_0 - [\text{Me}]_e}{[\text{Me}]_t - [\text{Me}]_e} \right) \quad (8)$$

Assuming that $[\text{Me}]_e$ is approximated by the last measurement in the time-course, and neglecting data points whose concentrations

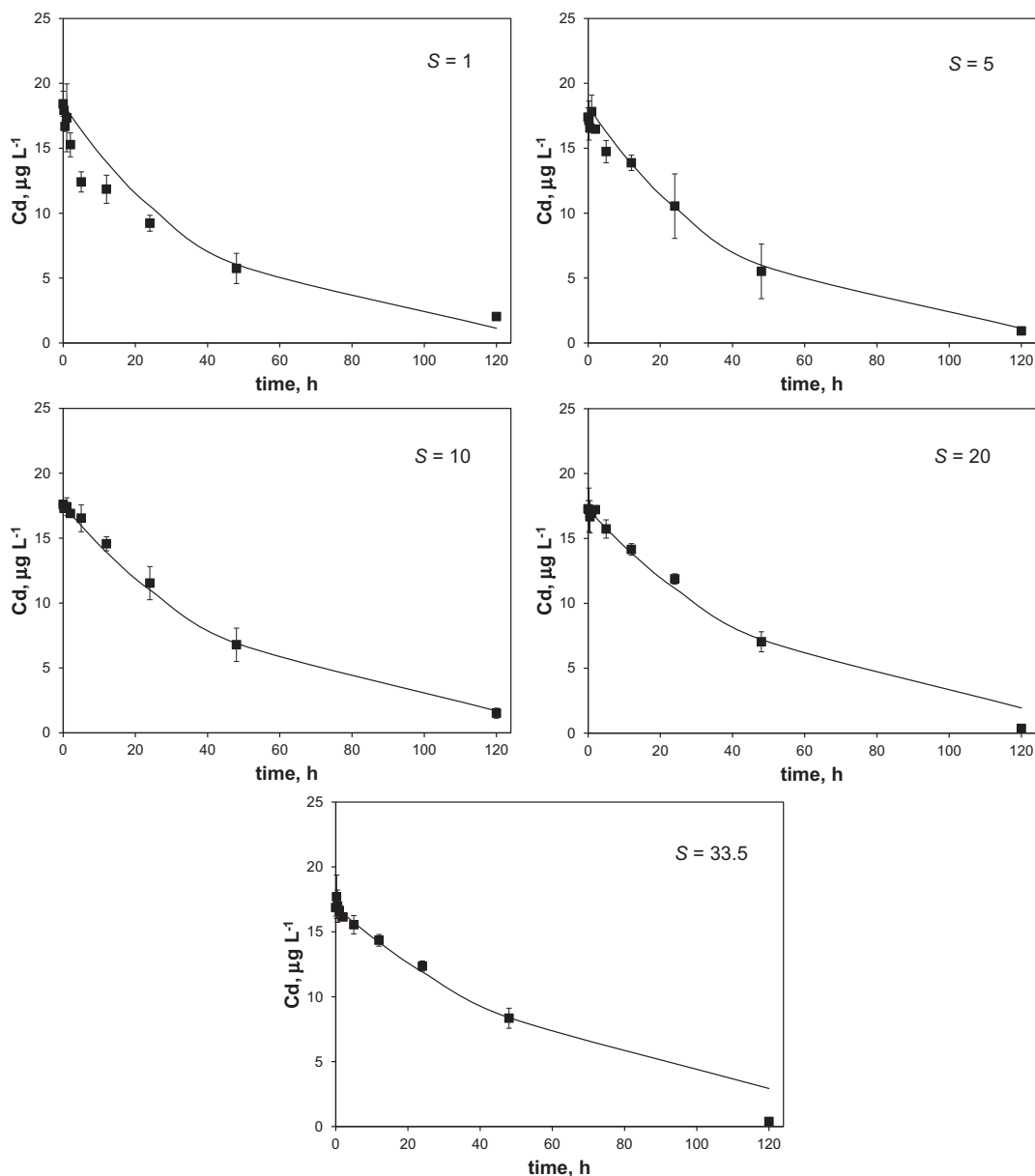


Fig. 3. Concentrations of dissolved Cd as a function of time in culture containers with different dilutions of synthetic sea water (whose salinities are annotated) and tips of *Fucus ceranoides*. Errors denote the standard deviation about the mean of three independent measurements and lines represent fits according to Equation (3) and the rate constants given in Table 1.

were less than $[Me]_e$, forward rate constants for Cu and Pb were derived from the slope of the right-hand side of Equation (8) versus time, and reverse rate constants were subsequently computed using Equation (7). Rate constants for each set of conditions are shown in Table 2, together with corresponding equilibrium constants and estimates of system response time, t_{resp} , defined as the time required to attain 63% of equilibrium conditions and calculated from the reciprocal of the summed rate constants ($=1/(k_1 + k_{-1})$). Model fits to the Cu and Pb data using these rate constants are annotated on Figs. 2 and 4, respectively.

The results of our kinetic analysis reveal that reactions involving Cu and Pb proceed considerably more rapidly than those involving Cd. Thus, forward rate constants for Cu and Pb are an order of magnitude greater than those for Cd, and T_{resp} , averaging about 6 h for Cu and 3 h for Pb, is significantly lower than the reaction half-lives for Cd (~ 30 – 50 h). Note also that the magnitude of the reverse reaction constants for Cu and Pb relative to the

corresponding forward constants (or $1/K$) increases with increasing salinity; thus, although forward or backward rate constants display no clear relationship with salinity, the reactions for Cu and Pb become increasingly reversible from fresh water to sea water.

4. Discussion

4.1. Reaction mechanisms

The differential, time-dependent behaviour of the metals (Figs. 2–4) suggests that there are dissimilarities in the mechanisms and kinetics of accumulation by the macroalga, *Fucus ceranoides*. Regarding Cd, rate constants exhibit a decrease with increasing salinity and percentages remaining in solution after a 5 day exposure, predicted from the kinetic analysis, exhibit a reduction with increasing salinity. Both observations are qualitatively

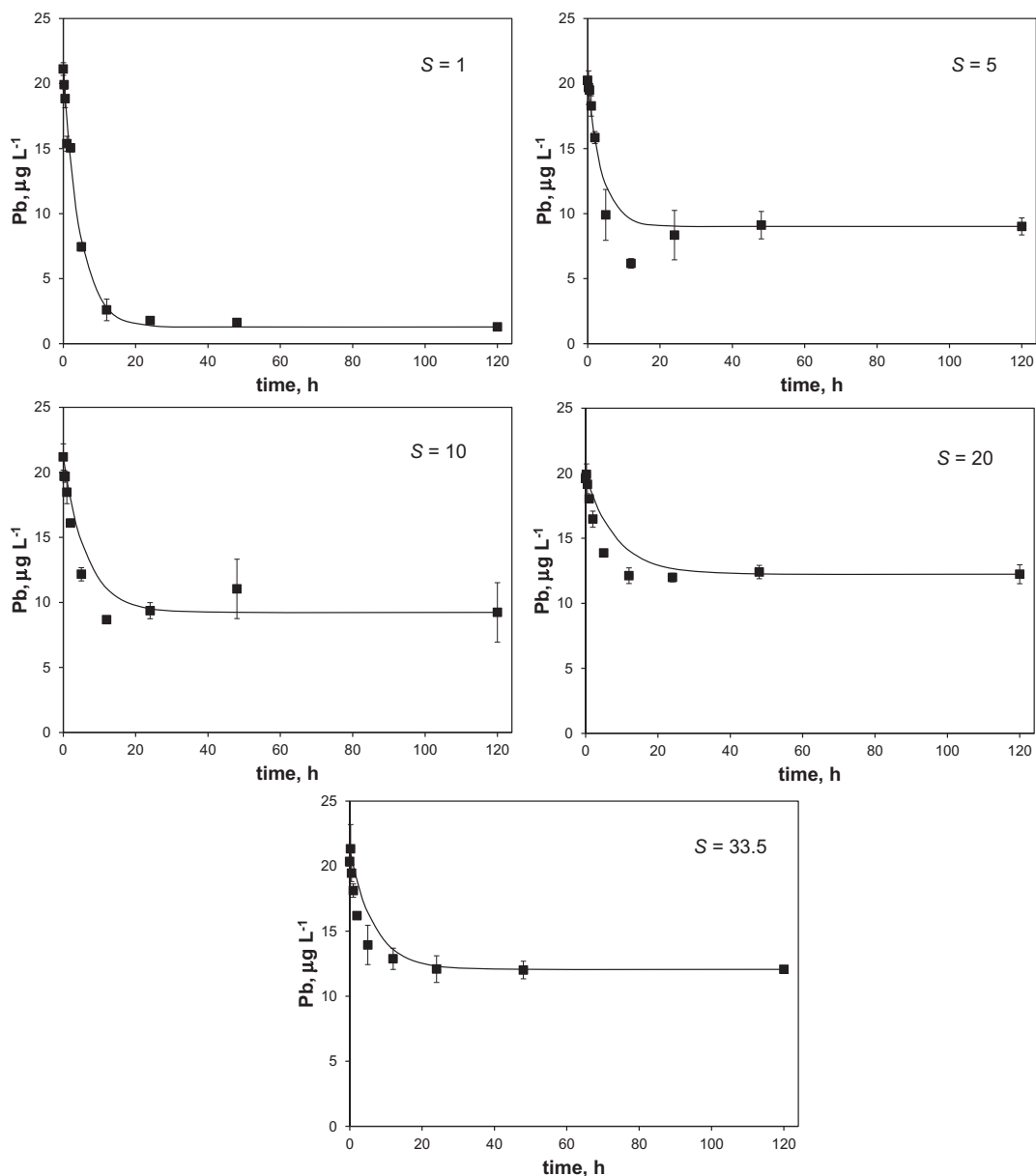


Fig. 4. Concentrations of dissolved Pb as a function of time in culture containers with different dilutions of synthetic sea water (whose salinities are annotated) and tips of *Fucus ceranoides*. Errors denote the standard deviation about the mean of three independent measurements and lines represent fits according to Equation (8) and the rate constants given in Table 2.

consistent with the computed distribution and activity of the free ion (Fig. 1).

That the removal of dissolved Cd proceeds exponentially and reaction curves become asymptotic (as a consequence of the near-complete consumption of the reactant) suggests desorption is not important but that unidirectional accumulation, including

Table 1
First-order rate constants and reaction half-lives for Cd accumulation by *Fucus ceranoides*.

Salinity	k_1, h^{-1}	r^2	$t_{1/2}, \text{h}$
1	0.0232	0.949	29.9
5	0.0232	0.988	29.9
10	0.0195	0.994	35.5
20	0.0182	0.991	38.1
33.5	0.0146	0.991	47.5

internalisation, is more significant. Other studies on the accumulation and subsequent depuration of radioactive ^{109}Cd by *U. lactuca* and *F. vesiculosus* observed virtually no loss of the metal when the organism was placed in clean sea water (Boisson et al., 1997; Wang and Dei, 1999), consistent with our assertion. We surmise that Cd is able to enter the cell through specific channels and subsequently combine, irreversibly, with sulphur-containing compounds (Vairavamurthy et al., 2000). For example, Cd is considered to be the most effective inducer of phytochelatins (PCs), enzymatically-synthesised thiol-containing peptides, in microalgae (Lee et al., 1996). Moreover, PCs have been identified in natural populations of *F. vesiculosus* and their induction in this species upon exposure to Cd has been demonstrated experimentally (Pawlik-Skowronska et al., 2007). It is possible, therefore, that exposure of *Fucus ceranoides* to the concentrations of Cd employed in our experiment induces the synthesis of PCs that act as a detoxification mechanism and thereby maintain the concentration gradient that

Table 2Pseudo-first-order forward and reverse rate constants, equilibrium constants and reaction response times for Cu and Pb accumulation by *Fucus ceranoides*.

Salinity	Cu					Pb				
	k_1, h^{-1}	k_{-1}, h^{-1}	r^2	K	$t_{\text{resp}}, \text{h}$	k_1, h^{-1}	k_{-1}, h^{-1}	r^2	K	$t_{\text{resp}}, \text{h}$
1	0.120	6.7×10^{-6}	0.954	17,900	8.33	0.216	0.014	0.996	15.43	4.35
5	0.114	6.5×10^{-6}	0.958	17,000	8.77	0.252	0.202	0.931	1.25	2.20
10	0.204	0.032	0.861	6.34	4.23	0.156	0.121	0.997	1.29	3.62
20	0.168	0.058	0.963	2.90	4.42	0.114	0.182	0.994	0.63	3.38
33.5	0.084	0.087	0.955	0.97	5.85	0.150	0.195	0.889	0.77	2.90

supports entry of further metal. Although PCs are also able to release Cd back into bulk solution as a complex, our observations suggest that this mechanism is not significant for *F. ceranoides*.

The percentages of dissolved Cu and Pb remaining at the end of the incubations (Figs. 3 and 4, respectively) exhibit a direct dependency on salinity which is qualitatively consistent with the computed distribution of the respective free ions and their activities. While the forward rate constants exhibit no clear dependence on salinity, the reversibility of the reactions (or equilibrium constant) also increases with increasing salinity. These observations suggest that desorption or efflux plays a more important role for Cu and Pb than it does for Cd. Thus, we propose a mechanism whereby Cu and Pb bind to the polysaccharide-rich cell surfaces and cross the cell membrane, and the rate of the back reaction(s) (desorption and efflux) increases with increasing salinity. As it is valid to assume that interactions with the alga are largely restricted to Cu^{2+} and Pb^{2+} , whose abundance and activity both decline with increasing salinity, desorption may be attributed to an exchange mechanism with divalent sea water cations (mainly Ca^{2+} and Mg^{2+}) at cell surfaces. On this basis, therefore, and relative to Cd, it appears that Cu and Pb are either externally located on the alga to a greater extent, and/or efflux from the intracellular environment more readily.

4.2. Bioconcentration factors

Concentrations of Cu, Cd and Pb in the apical tips of *Fucus ceranoides* retrieved at the end of the experiment were also measured in this study. Dry weight-normalised concentrations of Cu, Cd and Pb were significantly greater ($p < 0.05$ according to one-way ANOVA) in all tips exposed to $20 \mu\text{g L}^{-1}$ of each metal than in unexposed tips. Concentrations in exposed samples were therefore corrected for pre-existent metal by subtracting mean concentrations in the corresponding controls. Consistent with the observations of aqueous metal distributions above, concentrations of algal-bound Cu, Cd and Pb in exposed samples were significantly greater at the lowest salinity (31.8 ± 4.0 , 32.0 ± 5.0 and $31.4 \pm 4.4 \mu\text{g g}^{-1}$, respectively) than the corresponding concentrations in synthetic sea water (22.4 ± 2.1 , 22.0 ± 3.1 and $6.7 \pm 3.7 \mu\text{g g}^{-1}$, respectively).

Metal concentrations measured in *Fucus ceranoides* and corrected for pre-existent metal ($[\text{MeX}]$) and measured (Cu and Pb) or predicted (Cd) concentrations in the aqueous phase at the

termination of the experiment enable bioconcentration factors, BCF, to be calculated. (Note that while factors for Cu and Pb represent quasi-equilibrium values, those for Cd are clearly underestimates of such.) Thus, a BCF based on measured concentrations in both seaweed and water ($=[\text{MeX}]/[\text{Me}]$) represents the ratio of total metal accumulated (adsorbed plus internalised) to total metal remaining in solution, whereas a BCF whose denominator is either the computed concentration or activity of the free ion ($=[\text{MeX}]/[\text{Me}^{2+}]$ or $[\text{MeX}]/\{\text{Me}^{2+}\}$) represents the ratio of accumulated metal to available metal, assuming that the free ion is the only bioactive species in the experiment.

Bioconcentration factors, based on the three different denominators, are shown for Cu, Cd and Pb in Table 3. For Cu and Pb, a net reduction in BCF based on total aqueous metal occurs across the salinity gradient that is only partly offset when factors are normalised with respect to the free ion concentration or activity. Whichever denominator is selected, factors are greater for Cu than for Pb at any given salinity (although those for Cu at $S = 1$ and $S = 5$ may have been overestimated because of adsorption to the container surfaces). Regarding Cd, a reduction in BCF based on total aqueous metal occurs with increasing salinity; factors are relatively constant when normalised to free ion concentration and exhibit an increase with salinity when based on free ion activity. While the free ion is likely to represent the most bioactive species in each case, these observations suggest that additional chemical and biotic factors may be significant in the accumulation of metals by *Fucus ceranoides*. These include the presence of additional reactive aqueous species (e.g. CdCl^+ ; Lopez-Chuken et al., 2010) and salinity-dependent biochemical, anatomical or physiological changes to the macroalga. With respect to the latter, changes in external salinity produce a number of plastic responses in fucooids in both the short-term (e.g. changes in cell volume, membrane permeability, ion transport) and longer-term (e.g. thickness and composition of cell walls, synthesis and loss of organic osmolytes) (Kirst, 1989; Lobban and Harrison, 1994).

4.3. Implications for monitoring and modelling

To our knowledge, this is the first study to examine the mechanisms and kinetics of metal accumulation by a single macroalgal species over abiotic conditions encompassing the (near-) full estuarine gradient. Unlike many other studies (e.g. Wang and Dei,

Table 3Bioconcentration factors (in ml g^{-1}) defining the accumulation of Cu, Cd and Pb by *F. ceranoides* at different salinities; the three values presented in each case are based on different measures of aqueous metal ($[\text{Me}]$ = total metal concentration, $[\text{Me}^{2+}]$ = free ion concentration, $\{\text{Me}^{2+}\}$ = free ion activity).

Salinity	Cu			Cd			Pb		
	$[\text{Me}]$	$[\text{Me}^{2+}]$	$\{\text{Me}^{2+}\}$	$[\text{Me}]$	$[\text{Me}^{2+}]$	$\{\text{Me}^{2+}\}$	$[\text{Me}]$	$[\text{Me}^{2+}]$	$\{\text{Me}^{2+}\}$
1	3.18×10^7	1.24×10^8	2.66×10^8	28,100	53,900	94,500	24,300	87,100	217,000
5	1.36×10^7	5.4×10^7	1.90×10^8	19,800	19,800	240,000	755	3200	12,300
10	7100	30,900	143,000	17,400	90,200	424,000	1260	6420	31,100
20	3200	17,400	100,000	11,000	74,100	584,000	752	5220	30,600
33.5	2200	14,300	93,500	7500	57,000	674,000	414	4410	23,900

1999; Lodeiro et al., 2006), it has involved little modification of the organism and has employed experimental conditions (e.g. pH, irradiance, photoperiod) that are environmentally realistic. Moreover, rather than relying on data-fitting, we have also provided mechanistic evidence to support our kinetic analysis.

Our results have important implications for the use of *Fucus ceranoides* as a biomonitor. Specifically, within a given estuary, and neglecting other biotic and abiotic factors, one might expect the accumulation of Cu, Cd and Pb to reflect a reduction in the availabilities of these metals with increasing salinity. The limited information available on intra-estuarine distributions of metals in *F. ceranoides* reveals that concentrations of Cd decrease seaward along the axis of several estuaries, but that Cu and Pb exhibit no clear variations (Varma et al., 2011). With respect to Cu and Pb, experimental evidence in the present study suggests that these metals may also, at least partly, be regulated through desorption or efflux from the alga. Given the possibility of metal regulation and that biomonitoring encapsulates much longer periods of exposure than in our experiment, the precise rate constants themselves are likely to be of more direct, practical importance for modelling and evaluating shorter-term effects. These include the use of macroalgae as indicators of aqueous metal removal from the water column in the event of an accidental spillage or discharge.

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