



## Cadmium tolerance and adsorption by the marine brown alga *Fucus vesiculosus* from the Irish Sea and the Bothnian Sea

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### ABSTRACT

Cadmium (Cd) uptake capacities and Cd tolerance of the marine alga *Fucus vesiculosus* from the Irish Sea (salinity 35 psu) and from the Bothnian Sea (northern Baltic, 5 psu) were quantified. These data were complemented by measurements of changes in maximal photosynthetic rate ( $P_{max}$ ), dark respiration rate and variable fluorescence vs. maximal fluorescence ( $F_v:F_m$ ). At concentrations between 0.01 and 1 mmol Cd l<sup>-1</sup>, *F. vesiculosus* from the Bothnian Sea adsorbed significantly more (about 98%) Cd compared with *F. vesiculosus* from the Irish Sea. The photosynthetic measurements showed that the Bothnian Sea *F. vesiculosus* were more sensitive to Cd exposure than the Irish Sea algae. The algae from the Irish Sea showed negative photosynthetic effects only at 1 mmol Cd l<sup>-1</sup>, which was expressed as a decreased  $P_{max}$  (-12.3%) and  $F_v:F_m$  (-4.6%). On the contrary, the algae from the Bothnian Sea were negatively affected already at Cd concentrations as low as 0.1 mmol Cd l<sup>-1</sup>. They exhibited increased dark respiration (+11.1%) and decreased  $F_v:F_m$  (-13.9%). The results show that *F. vesiculosus* from the Bothnian Sea may be an efficient sorption substrate for Cd removal from Cd contaminated seawater and this algae type may also have applications for wastewater treatment.

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### 1. Introduction

Conventional processes for the removal of heavy metals from wastewaters and soils have proven to be ineffective or expensive when heavy metals are present in low concentration (1–100 mg l<sup>-1</sup>) (Yun et al., 2001). The biosorption of metallic ions from solutions by living or non-living (dried) biomass offers an alternative to the remediation of industrial effluents as well as the recovery of metals (Gavrilescu, 2004; Volesky and Holan, 1995). Metal adsorption to a solid matrix (i.e., minerals or biological materials) is considered an important mechanism for Cd removal under seawater conditions (Volesky and Holan, 1995). A lot of chemical adsorbents have been considered for remediation, but only ion-exchange resins have been proven to be environmentally friendly options (Gupta et al., 2000; Horsfall and Spiff, 2005). In addition, abundant natural materials (i.e., bacteria, fungi and yeasts) are also considered good biosorbents for heavy metals recovery from wastewaters (Ahluwalia and Goyal, 2007; Chen and Mulchand, 2005; Gupta et al., 2000). Among these, marine

macroalgae are among the most promising types of biosorbents, because they are stable and fast growing (Brinza et al., 2005; Davis et al., 2003; Vijaepragavan et al., 2004). Cd<sup>2+</sup> is readily taken up by macroalgae, however the uptake mechanisms are not fully understood. What has been well established so far is that Cd uptake is partly light dependent (Hu et al., 1996) and that the algae require specific active protein to facilitate this process (McLean and Williamson, 1977).

Overall, biosorption is defined as the capacity of a substrate to retain metallic species, ionic forms and/or ligands from fluids in the molecular structure of the cell wall. The biosorption mechanisms include extracellular and intracellular bonds, as well as complex interactions that depend on the type of metal and the biosorbent structure (Davis et al., 2003).

Algal cell surfaces experience steady-state distribution processes with the surrounding water, mainly through ion-exchange equilibrium (Amer et al., 1997) and different surface functional groups contribute to the bio-sorptive metal binding, e.g., carboxyl, hydroxyl, carbonyl, sulfhydryl, thioether, sulfonate and phosphoester (Davis et al., 2003; Herrero et al., 2005). The role of each group depends mainly on the chemical state of the algal surface and the affinity between the functional groups and the metal (Davis et al., 2003). The accumulation of trace metals (i.e., As, Cd, Pb and Zn)

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seems to involve an ion exchange with cell wall polysaccharides, followed by an uptake into the algal vacuole. Although biosorption is a promising technique for heavy metal removal, specifically for Cd, the mechanisms behind the adsorption process are still poorly understood.

The aim of this study was to compare biosorption capacities and physiological stress responses to Cd exposure by *F. vesiculosus* algae from two different marine environments: the Bothnian Sea (northern Baltic Sea, 5 psu) and the Irish Sea (35 psu). Responses were quantified from the changes in photosynthetic parameters ( $O_2$ -evolution and fluorescence) at different Cd concentrations. Cd biosorption batch reactor studies were carried out to determine the maximum uptake capacities of Cd in seawater for the algae from both sites. In order to quantify the relevant metal binding functional groups involved in the biosorption process, the binding sites and acidic proprieties of the biosorbent materials were quantified via high-resolution potentiometric titration measurements. These data were complemented by geochemical modelling of the aqueous species involved in Cd uptake.

## 2. Methods

### 2.1. Samples and batch adsorption experiments

*F. vesiculosus* samples were collected from 1 m depth at the intertidal shore of the Irish Sea (Portaferry, Northern Ireland, 54°23'N; 5°34'E) at a salinity of 35 psu and in the non-tidal Bothnian Sea, northern Baltic Sea (Åstön, Sweden, 62°24'N; 17°45'E), at a salinity of 5 psu. The algae were transported back to lab and kept in outdoor cultivation tanks with aeration for 3 days before the experiment were carried out. Natural light conditions were used during cultivation and experiment, which reached a highest value of  $380 \mu\text{mol m}^{-2} \text{s}^{-1}$  (cloud condition: 5/8) at noon (March 2005).

Biosorption of Cd (II) to *F. vesiculosus* from the two sites was studied in batch systems. Approximately 5 g fresh weight (FW) algae (vegetative tips) were placed in beakers that contained natural seawater from the two collection sites. The algae were equilibrated for 4 h with different concentrations of Cd (0.01, 0.1, 1 and 10  $\text{mmol l}^{-1}$ ). Only the growing tips of the algae were used, since they have the highest Cd uptake (Amer et al., 1997). Five replicate experiments (with algae from different thallus) and at each Cd concentration were carried out and controls ( $n = 5$ ) without addition of Cd were run in parallel. Air was bubbled through each beaker to provide mixing and the experiments were carried out at 8 °C (outside ambient conditions). Aliquots of the supernatant solutions were removed after 60, 120 and 240 min for aqueous Cd analysis (after filtration through a 0.2  $\mu\text{m}$  filters). The concentrations of Cd as well as other heavy (Cu, Cr, Fe, Ni, Pb, Zn, Mn) and light metals (K, Ca, Mg) were quantified using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Thermo Elemental-IRIR intrepid Duo HR 27 Forge Parkway) with a 0.002 ppm detection limit for Cd. The dry weights (DW) of *F. vesiculosus* were obtained after drying a sample at 50 °C for 24 h. The Cd solutions used in the experiments were prepared from a stock solution of 10  $\text{mmol Cd l}^{-1}$  using  $\text{CdSO}_4 \cdot \text{H}_2\text{O}$  (PRONASLYS®AR). Temperature and pH were determined using a pHep® meter (HANNA HI 98128) and HANNA standard buffer solutions. Initial pH was set as the pH of the sampling site (8.0 for the Irish Sea and 8.2 for the Bothnian Sea).

From the measurements of aqueous Cd in the batch experiments, the uptake capacities for Cd were obtained from the mass balance using Eq. (1) (Volesky and Holan, 1995):

$$q = \frac{V(C_i - C_e)}{m} \quad (1)$$

Where  $V$  is the water volume (L),  $m$  is the DW algae (g),  $C_i$  is the initial concentration of Cd ( $\text{mg l}^{-1}$ ),  $C_e$  is the equilibrium concentration of Cd ( $\text{mmol l}^{-1}$ ) and  $q$  is the Cd adsorbed ( $\text{mmol Cd g}^{-1}$  DW algae).

### 2.2. Photosynthetic and dark respiration

Photosynthesis was measured as oxygen evolution (net oxygen evolution and dark respiration) and fluorescence (variable fluorescence vs. maximum fluorescence,  $F_v:F_m$ ). The values for  $F_v:F_m$ , which provide a measure of the maximal photochemical efficiency of photosystem II, were monitored with a Pulse Amplitude Fluorometer (PAM-2000, Walz, Effeltrich, Germany). The oxygen evolution was obtained with a Clark  $O_2$ -electrode in a temperature controlled (10 °C) cuvette (volume 5 ml), where the alga samples were placed in a vertical position facing a light projector (PAR, 400–700 nm), above a magnetic stirrer. The  $O_2$ -electrode was calibrated to zero in water containing sodium dithionite crystals, and to 100% (saturation) by bubbling the water with air for 20 min prior to the measurement. The oxygen content of air equilibrated seawater was obtained from (Green and Carritt, 1967). Prior to the onset of the experiments, photosynthetic rate vs. irradiance curves ( $P:E$ -curves) were produced by using neutral density glass filters (10 min at each filter with dark periods between) in front of the light projector to obtain different irradiances (10, 20, 50, 100, 200, 300, 400, 500, 600, 700 and 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). From the  $P:E$ -curves, the irradiance that generated the maximum net photosynthetic rate without any signs of photoinhibition ( $P_{\text{max}}$ ;  $\text{nmol O}_2 \text{g}^{-1} \text{FW s}^{-1}$ ) was obtained. An irradiance of 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was found to be sufficient to generate  $P_{\text{max}}$  for both populations.  $P_{\text{max}}$  as well as dark respiration and  $F_v:F_m$  were measured before and after exposure to natural seawater (from each collection site) spiked with different concentrations of Cd and only discs from the thallus (distal parts from plants without receptacles) with a diameter of 1.5 cm were used ( $n = 5$ ). Dark respiration and  $F_v:F_m$  were measured after 10 min dark adaptation, followed by measurements of  $P_{\text{max}}$  after 10 min exposure to 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Oxygen evolution and dark respiration are expressed on a fresh weight (FW) basis, where FW was obtained by blotting the alga between two papers for 30 s directly after the photosynthetic measurements.

### 2.3. High-resolution potentiometric titrations

Forward and reverse titrations of live algae thallus were carried out in order to derive information about protonation and deprotonation of the active sites of the algal surface. All titration experiments were carried out with a Man-Tech auto-titration system equipped with an automatic burette, using about 2.5  $\text{g l}^{-1}$  of algae. Prior to each titration the pH electrode was manually calibrated using NIST certified pH standard solutions. The temperature was maintained constant at  $25 \pm 0.5$  °C and for each titration, fresh alga tips (about 3 cm; 0.261 g for the Baltic and 0.264 g for the Irish algae) were washed three times with distilled water (18 M $\Omega$ ) excess charge. The algae were thereafter equilibrated in 100 ml 0.1 M NaCl (which served as electrolyte background) and the algae and the control (electrolyte only) titrations were carried out from high pH to low pH and vice versa to study hysteresis effects (data not shown). Titrations from acidic to basic conditions (A–B) were done with 0.1 M NaOH, while the reverse titrations (B–A) were done with 0.1 M HCl. The titrants were injected stepwise at a rate of 0.10–0.25 ml with a 3 s stabilisation time between steps.

Titration data were evaluated using Gran's method (Gran 1952; Naja et al., 2005; Rosotti and Rosotti, 1965). From the shape of the A–B titration curves information about the neutralization capacity of the acidic groups on the algae surface were extracted.

#### 2.4. Geochemical modelling, microscopy and diffraction

The geochemical modelling package Geochemist's Workbench® (Bethke, 2002) was used to predict the stability of aqueous and mineral Cd species at the conditions of the experiments. The subprogram Act2 was used to generate diagrams of Cd activity vs. pH, taking into account the basic chemical composition of the two marine systems (Bothnian and Irish Sea). Models were run under standard conditions of temperature (25 °C), pressure (1.01325 bar), O<sub>2</sub> concentration/fugacity (0.003) and basic seawaters composition (Cl and Na, Ca, K, Mg). The algae exposed to Cd were imaged and analysed using a CANSCAN Scanning Electron Microscopy (SEM) at 20 keV with the elemental analyses being carried out using an Energy Dispersive Spectrometer (EDS). A PHILIPS X-ray diffractometer (XRD) (Cu K $\alpha$  radiation at 40 kV, and 30 mA) was used to identify the mineral precipitates formed in the experiments with the highest Cd concentration. Evaluation of patterns and identification of precipitated phases was done using the JCPDF database.

### 3. Results

#### 3.1. Cd adsorption kinetics

The changes in biosorption uptake rates with time (Fig. 1A; calculated from Eq. (1)) are reflected in the initial slopes of the curves. At all concentrations the algae from the Bothnian Sea reveal a Cd uptake capacity about 98% higher than those from the Irish Sea (not shown). In most experiments, Cd uptake was highest during the first hour with a maximum reached after 2 h, after which no more changes were observed (Fig. 1A). The maximum amount of Cd adsorbed per g DW of algae was proportional to the initial concentration of Cd (0.01–1 mmol Cd l<sup>-1</sup>) (Fig. 1B). From these results sorption capacity,  $q$ , (values in mmol g<sup>-1</sup> DW) for the Bothnian *F. vesiculosus* of 0.002, 0.018 and 0.230 mmol g<sup>-1</sup>, respectively, were calculated. The algae from the Irish Sea adsorbed on average two orders of magnitude less Cd (0.00007, 0.00044 and 0.00500 mmol g<sup>-1</sup> DW for the corresponding concentrations of 0.01, 0.1 and 1 mmol Cd l<sup>-1</sup>) (Fig. 1B).

Experiments were also carried out at 10 mmol l<sup>-1</sup> Cd but at this concentration the precipitation of a Cd-carbonate phase were observed (see below) and therefore, biosorption calculations were not feasible.

#### 3.2. Cd tolerance

The initial  $P_{\max}$  was significantly ( $p < 0.001$ ) higher for *F. vesiculosus* from the Irish Sea (10.61 nmol O<sub>2</sub> g<sup>-1</sup>FW s<sup>-1</sup>) as compared to

*F. vesiculosus* from the Bothnian Sea (4.08 nmol O<sub>2</sub> g<sup>-1</sup>FW s<sup>-1</sup>; Table 1). No significant differences in initial dark respiration or initial  $F_v:F_m$  were found between the two populations (Tables 2 and 3) and neither population was affected by the lowest concentration of Cd used, 0.01 mmol l<sup>-1</sup> (Tables 1–3). The algae from the Irish Sea showed negative photosynthetic effects at 1 mmol Cd l<sup>-1</sup>, which was expressed as a decrease in  $P_{\max}$  (–12.3%; Table 1) and in  $F_v:F_m$  (–4.6%; Table 3). The Bothnian Sea algae showed negative effects already at 0.1 mmol Cd l<sup>-1</sup>, with increased dark respiration (+11.1%; Table 2) and decreased  $F_v:F_m$  (–13.9%; Table 3). At 1 mmol Cd l<sup>-1</sup> they showed negative effects in all the measured photosynthetic parameters, with a decrease in  $P_{\max}$  (–25.4%; Table 1) and  $F_v:F_m$  (–25.5%; Table 3) but an increased dark respiration (+25.7%; Table 2).

#### 3.3. Algae surface charge characterisation

The base into acid titration curves (Fig. 2) revealed a faster response for *F. vesiculosus* from the Bothnian Sea when compared to the Irish Sea *F. vesiculosus* and the difference in the volumes of base added ( $x$ -axis in Fig. 2) were used to derive the deprotonation ability of the different algae surfaces. The added hydroxyl groups and the buffering capacities of functional groups on the surface of the thallus indicate that the deprotonation of the acidity groups for the algae in the Irish Sea was lower than for those from the Bothnian Sea (Fig. 2).

Using the Gran's function approach (Naja et al., 2005) and the Origin 6.1 software (OriginLab, 2000) the ionic exchange capacities (me acidity groups per g dry algae) for each algae were calculated. The results (Table 4) showed that the Bothnian *F. vesiculosus* contained 0.5314 me total acidity groups g<sup>-1</sup> dry algae ( $A_{TO}$ ), while *F. vesiculosus* from the Irish Sea only contained 0.2382 me total acidity groups g<sup>-1</sup> dry algae. The surface charges and points of zero net proton charge were derived from the basic potentiometric data and from the first derivative ( $\delta pH/\delta Vol$  plotted against the volume added) the end point pH value, which corresponds to the point of zero net proton charge (PZNPC) was derived. The pH values corresponding to the two end points were 6.84 for the Bothnian Sea algae and 6.75 for the Irish Sea algae, showing that the algae surfaces in both cases were positively charged below pH 6.8. These results, in combination with the geochemical modelling (see below), also revealed that the in the batch experiments conducted during this study (pH = 8.2 and 8.0, respectively), Cd<sup>2+</sup> had a high binding affinity to the algae surfaces.

Using the Gran's approach (Naja et al., 2005) the values of the  $pK_a$  for the three major acidity classes (weak and very weak

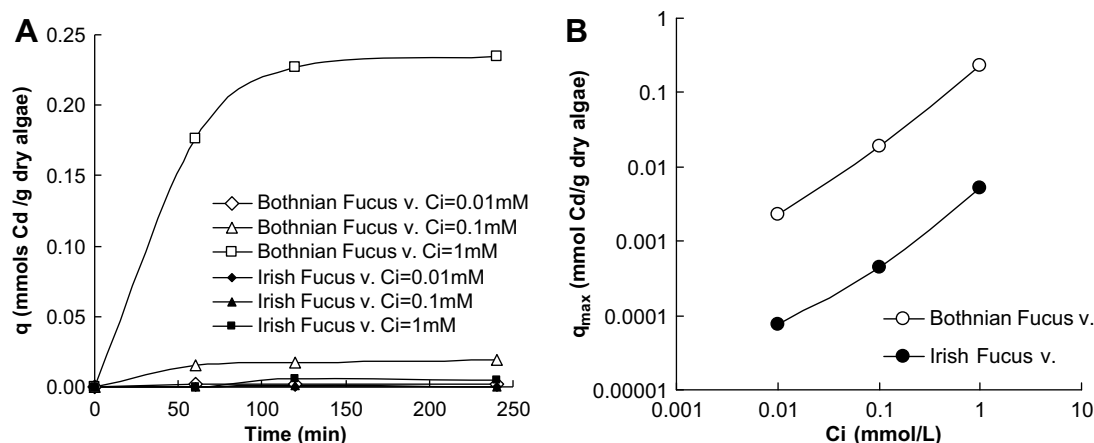


Fig. 1. (A) The kinetics of Cd biosorption for *F. vesiculosus* ( $n = 5$ ) from the Bothnian Sea and the Irish Sea in natural seawater at various Cd concentrations and (B) comparison between the maximum uptake capacities of *F. vesiculosus* from the Bothnian Sea and the Irish Sea vs. initial Cd concentration. All experiments were carried out at 8 °C.

**Table 1**

Maximum net photosynthetic rate ( $P_{\max}$ ;  $\text{nmol O}_2 \text{g}^{-1} \text{FW s}^{-1}$ ) of *F. vesiculosus* from the Bothnian Sea (5 psu) and the Irish Sea (35 psu)

Algae	Cd ( $\text{mmol l}^{-1}$ )	Initial $P_{\max}$	$P_{\max}$ after 4 h	Change (%)	<i>p</i>
Bothnian Sea	0.01	3.84	3.84	-0.1	n.s.
	0.1	3.82	3.54	-7.5	n.s.
	1.0	4.19	3.13	-25.4	*
	10.0	4.11	1.32	-67.9	***
	Control	4.08	4.27	4.7	n.s.
Irish Sea	0.01	10.61	10.24	-3.5	n.s.
	0.1	10.01	9.52	-4.9	n.s.
	1.0	10.54	9.24	-12.3	*
	10.0	10.29	6.83	-33.6	**
	Control	10.61	10.46	-1.4	n.s.

Measurements ( $n = 5$ ) were performed at an irradiance of  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  and a temperature of  $8^\circ\text{C}$ , prior and after exposure to Cd at different concentrations (0.01, 0.1, 1.0 and  $10.0 \text{ mmol l}^{-1}$ ) for 4 h. Controls ( $n = 5$ ) were kept in natural seawater without addition of Cd. The change (%) between initial  $P_{\max}$  and  $P_{\max}$  after 4 h exposure to Cd is shown with the significance expressed as *p*-value.

n.s., non significant.

\*  $p \leq 0.05$ .

\*\*  $p \leq 0.01$ .

\*\*\*  $p \leq 0.001$ .

**Table 2**

Dark respiration rate (DR;  $\text{nmol O}_2 \text{g}^{-1} \text{FW s}^{-1}$ ) of *F. vesiculosus* from the Bothnian Sea (northern Baltic, salinity 5 psu) and the Irish Sea (salinity 35 psu)

Algae	Cd ( $\text{mmol l}^{-1}$ )	Initial DR	DR after 4 h	Change (%)	<i>p</i>
Bothnian Sea	0.01	-1.56	-1.57	5.1	n.s.
	0.1	-1.87	-2.07	11.1	*
	1.0	-1.88	-2.36	25.7	*
	10.0	-1.89	-2.94	55.6	**
	Control	-1.32	-1.29	-1.6	n.s.
Irish Sea	0.01	-1.30	-1.23	-4.9	n.s.
	0.1	-1.35	-1.38	2.3	n.s.
	1.0	-1.36	-1.32	-2.9	n.s.
	10.0	-1.61	-2.09	29.9	*
	Control	-1.44	-1.46	1.3	n.s.

Measurements ( $n = 5$ ) were performed after 10 min dark adaptation, prior and after exposure to Cd at different concentrations (0.01, 0.1, 1.0 and  $10.0 \text{ mmol l}^{-1}$ ) for 4 h ( $8^\circ\text{C}$ ). Controls ( $n = 5$ ) were kept in natural seawater without addition of Cd. The change (%) between initial DR and DR after 4 h exposure to Cd is shown with the significance expressed as *p*-value.

n.s., non significant.

\*  $p \leq 0.05$ .

\*\*  $p \leq 0.01$ .

\*\*\*  $p \leq 0.001$ .

**Table 3**

Variable fluorescence vs. maximum fluorescence ( $F_v:F_m$ ) of *F. vesiculosus* from the Bothnian Sea (northern Baltic, salinity 5 psu) and the Irish Sea (salinity 35 psu)

Algae	Cd ( $\text{mmol l}^{-1}$ )	Initial $F_v:F_m$	$F_v:F_m$ after 4 h	Change (%)	<i>p</i>
Bothnian Sea	0.01	0.74	0.74	0.7	n.s.
	0.1	0.73	0.63	-13.9	*
	1.0	0.73	0.54	-25.5	*
	10.0	0.74	0.41	-43.8	***
	Control	0.74	0.74	0.2	n.s.
Irish Sea	0.01	0.74	0.74	0.3	n.s.
	0.1	0.74	0.73	-1.0	n.s.
	1.0	0.75	0.71	-4.6	*
	10.0	0.75	0.62	-17.0	**
	Control	0.74	0.74	-0.4	n.s.

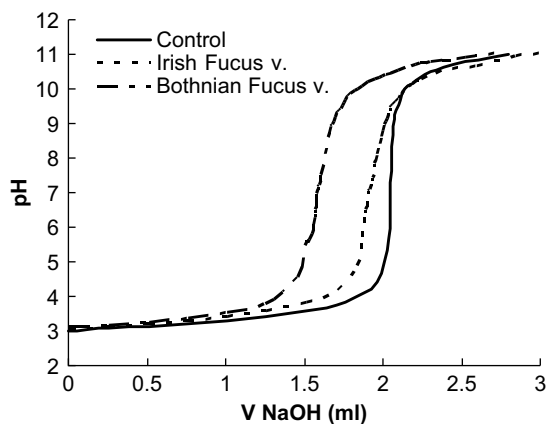
Measurements ( $n = 5$ ) were performed after 10 min dark adaptation, prior and after exposure to Cd at different concentrations (0.01, 0.1, 1.0 and  $10.0 \text{ mmol l}^{-1}$ ) for 4 h ( $8^\circ\text{C}$ ). Controls ( $n = 5$ ) were kept in natural seawater without addition of Cd. The change (%) between initial  $F_v:F_m$  and  $F_v:F_m$  after 4 h exposure to Cd is shown with the significance expressed as *p*-value.

n.s., non significant.

\*  $p \leq 0.05$ .

\*\*  $p \leq 0.01$ .

\*\*\*  $p \leq 0.001$ .



**Fig. 2.** Potentiometric titration curves for *F. vesiculosus* from the Bothnian Sea and the Irish Sea.

acidities) were determined (Table 4). The results showed a good correlation between the higher deprotonation capacity and the resulting abundance of acidity groups obtained from the Gran's function evaluation when compared with the results from the batch adsorption measurements. Both measurements showed a higher Cd uptake capacity for Bothnian Sea algae in comparison to the algae from the Irish Sea.

### 3.4. *Thallus characterization and Cd chemistry*

The comparison between the control samples from the Irish Sea and the Bothnian Sea showed differences in the morphology of the dry thallus, namely individual  $\alpha$ -like shape features for the Irish Sea alga and spiral shape features for the Bothnian Sea alga, respectively (data not shown). Interestingly, both algae types even when exposed to cadmium concentrations as high as  $10 \text{ mmol l}^{-1}$  showed no visual changes in surface morphology. However, in all experiments at  $10 \text{ mmol l}^{-1}$  Cd SEM imaging revealed spherical to slightly isometric mineral precipitates on the thallus surfaces, which were characterized by C, Cd and O peaks in the qualitative EDS analyses (not shown). The mineralogical composition of the precipitates was determined by XRD to be the Cd carbonate mineral, Otavite. Scanning large areas of both algae surfaces, at various magnifications, showed that the Otavite precipitate was predominantly deposited along the edges of the thallus.

Higher resolution imaging of algae (not shown) of the *F. vesiculosus* from the Irish Sea exposed to low Cd concentrations ( $<0.1 \text{ mmol Cd l}^{-1}$  for 4 h) showed no change in cell structure when compared with the controls, while the algae from the Bothnian Sea showed an increased content of electron translucent compounds after the same treatment. In addition, the Bothnian Sea control samples revealed a higher density of presumably physode globules, which are common in brown algae and usually contain phenolic compounds (Schoenwaelder and Wiencke, 2000), yet, the exposure to Cd did not effect of the number or shape of these globules.

The high-resolution images of the controls showed differences in the overall morphological aspect of the *F. vesiculosus* from the two sites. This difference is reflected by the dissimilarity in salinity (ionic strength) between the two sites and this was also supported by the 1.5–4 times higher concentration of light metals (i.e., Mg, K and Ca) in the Irish Sea when compared with the Bothnian Sea (Table 5).

Geochemical modelling based on both seawater compositions equilibrated with the Cd concentrations used in the batch experiments ( $0.01$ – $10 \text{ mmol l}^{-1}$ ), showed that the higher salinity in the Irish Sea lead to a lower saturation index for Cd species at the relevant pH values (i.e., 8.0 and 8.2, respectively, Fig. 3A and B). In

**Table 4**

Acidity values (weak, very weak, and total,  $A_w$ ,  $A_{vw}$  and  $A_{TO}$ ) calculated per fresh weight (FW) and dried weight (DW) and  $pK_a$  values derived for *F. vesiculosus* from the Bothnian and the Irish Sea

Algae	$A_w$		$A_{vw}$		$A_{TO}$		$pK_a$
	me/g (FW)	me/g (DW)	me/g (FW)	me/g (DW)	me/g (FW)	me/g (DW)	
Bothnian Sea	0.045	0.225	0.061	0.306	0.106	0.531	8.33 ± 0.01 6.24 ± 0.12
Irish Sea	0.016	0.132	0.012	0.106	0.028	0.238	6.91 ± 0.00 6.25 ± 0.05 5.49 ± 0.03

**Table 5**

Light element concentrations (in ppm) in the seawater from the Bothnian Sea (5 psu) and the Irish Sea (35 psu). Heavy metal concentrations in both water samples were below the detection limits of our analyses

Element	Ca	K	Mg
Bothnian Sea	61.4	102.7	29.6
Irish Sea	265.9	320.6	41.0

both cases, at seawater pH values, Otavite was oversaturated and was predicted to precipitate.

For the Bothnian Sea (Fig. 3A) the predominant Cd complex at pH values below ~9 was  $CdCl_2(aq)$ , while the predominant complex of Cd in the Irish Sea (Fig. 3B) at pH values below ~10 was  $CdCl_3^-$ . Above these pH values Cd in both cases was present as a  $Cd(CO_3)_2^-$  complex.

#### 4. Discussion

The data presented above showed that the Bothnian Sea algae have a much higher Cd uptake capacity than the Irish Sea algae. It has been previously shown that during biosorption Cd displaces Ca and Na on dealginate via an ion-exchange mechanism (Romero-Gonzalez et al., 2001). It has also been previously shown that Atlantic *F. vesiculosus* contain higher ion (K, Na and Cl) and mannitol levels than Baltic *F. vesiculosus* (Bäck et al., 1992) and that K was the major cation and KCl the main inorganic solute responsible for the osmotic balance, while mannitol played a less important role in the Baltic algae, where ion effects had a greater effect

on sorption (Bäck et al., 1992). The displacement of light metals by Cd might therefore affect the osmotic balance in this species.

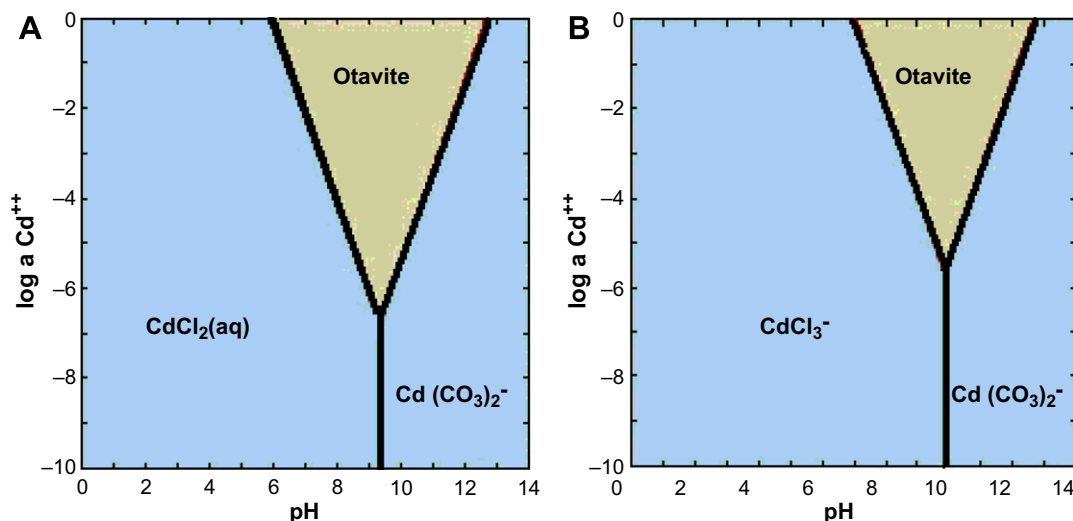
#### 4.1. Cd uptake and algae surface charge

The microscopic and spectral analysis of the algae surfaces and the precipitates combined with the data from the adsorption studies revealed that in the batch experiments Cd was removed through sorption (at Cd concentrations below the saturation with respect to Cd carbonate; i.e., 0.01–1 mmol l<sup>-1</sup>) and through surface precipitation of a Cd-carbonate phase, Otavite, at the high Cd concentrations (10 mmol l<sup>-1</sup>). The formed precipitate was predicted from the thermodynamic modelling and was confirmed by XRD and SEM analyses.

High resolution imaging confirmed the well known difference in thallus morphology, cells wall size and cell content and these differences was interpreted as stemming from the differences in salinity of the two seawater environments (Bäck et al., 1992; Nygård and Ekelund, 2006).

From the kinetic data for Cd removal by Bothnian Sea and Irish Sea *F. vesiculosus* the significantly higher maximum rates and extent of Cd removal for the Bothnian Sea *F. vesiculosus* (0.23 mmol g<sup>-1</sup> DW) than for the Irish Sea *F. vesiculosus* (0.0059 mmol g<sup>-1</sup> DW) was derived revealing that increasing ionic strength strongly affected the Cd uptake capacity.

Adsorption studies of Cd by *F. vesiculosus* with fresh algae or under seawater-simulated conditions are so far not available in the literature. However, Herrero et al. (2006) carried out Cd sorption studies with *F. vesiculosus* (Coruña coast, Galicia, Spain) but with dried algae and under freshwater conditions. Comparing their data



**Fig. 3.** Cd speciation plotted in a pH- $\log a Cd^{2+}$  diagram for (A) the Bothnian Sea and (B) the Irish Sea; Conditions:  $T = 25$  °C,  $p = 1.01325$  bar and  $f_{CO_2} = 0.003$ .

(maximum Cd uptake capacity  $0.79 \text{ mmol g}^{-1} \text{ DW}$ , pH 4.5, IS = 0.05 M) with the results from this study (maximum Cd uptake capacity  $0.23 \text{ mmol g}^{-1} \text{ DW}$ , pH 8, IS = 0.1 M, fresh algae) showed that fresh *F. vesiculosus* equilibrated with low salinity seawater (Bothnian algae) take up three times less Cd than dried algae in freshwater.

Only few studies used live algae for metals decontamination purposes (Mamboya, 2007; Mellor, 2002; Vasconcelos and Leal, 2001). The one study that also used fresh algae for Cd sorption (Vasconcelos and Leal, 2001) used *Porphyra* spp. and *Enteromorpha* spp. (collected from the Oporto Coast, Portugal) and showed an even lower maximum uptake capacity for Cd ( $0.050 \text{ mmol g}^{-1} \text{ DW}$ ). Thus, under similar seawater conditions the Bothnian *F. vesiculosus* is a far better sorbent than other live algae species.

Although many other algae types have been evaluated for metal decontamination purposes, most of them used treated materials (i.e., dried, powdered, etc.). For example, Romera et al. (2007) evaluated the maximum Cd uptake capacities of six species of treated algae (*Codmium vermilara*, *Spirogyra insignis*, *Asparagopsis armata*, *Chodrus crispus*, *Ascophillum nodosum* and *F. spiralis*) by equilibrating the algae for 120 min at pH = 6 with Cd concentrations between 0.089 and  $1.334 \text{ mmol l}^{-1}$ . Their results showed that Cd was adsorbed by all algae, but that *F. spiralis* had the highest sorption capacity ( $1.022 \text{ mmol g}^{-1}$ ) followed in decreasing order by *Ascophillum nodosum* ( $0.780 \text{ mmol g}^{-1}$ ), *Chodrus crispus* ( $0.668 \text{ mmol g}^{-1}$ ), *Asparagopsis armata* ( $0.287 \text{ mmol g}^{-1}$ ), *Spirogyra insignis* ( $0.203 \text{ mmol g}^{-1}$ ) and *Codmium vermilara* ( $0.193 \text{ mmol g}^{-1}$ ). As the pH is the most important parameter controlling metals sorption (influencing the algae surface charge as well as the metal speciation in solution), it is indispensable for this comparison noting that the above-cited adsorption studies (which all used dried algae) were all carried out at the optimum pH (6).

The ionic strength, another important parameter which influences metal sorption revealed that for the algae from the higher salinity Irish Sea (compared with the Bothnian Sea) more energy was required for bond dissociation of functional groups. In low ionic strength solutions (i.e., Bothnian Sea) the diffuse layer becomes broader and exchanges between ions are more facilitated thus leading to higher sorption capacities. This higher adsorption in low seawater salinity might also be the reason for the observed higher photosynthetic sensitivity of the Bothnian Sea *F. vesiculosus*. In addition, another supporting line of evidence is the fact that the potentiometer titration data and Gran's function analyses suggested that the surfaces of the Bothnian Sea *F. vesiculosus* algae contained more total acidity groups ( $A_{\text{TO}} = 0.5314 \text{ me g}^{-1} \text{ DW}$ ) than the Irish Sea *F. vesiculosus* ( $A_{\text{TO}} = 0.2382 \text{ me g}^{-1} \text{ DW}$ ). These results are in line with earlier findings by Möhlenberg and Jensen (1980), who showed an increased adsorption of Cd onto particulate matter with decreasing salinity.

The obtained  $\text{pK}_a$  values obtained in this study (Table 2), when combined with literature data (Naja et al., 2005), revealed that the carboxylic groups dissociation constants ( $\text{pK}_a \sim 5.5$ ) and the dissociation constant of *cis*- $\text{CO}_2\text{H}$  isomer or phosphorous protonated sites on aromatic chains ( $\text{Ph}_3\text{P}^+\text{CH}_2\text{COPh}$ ) at pH values of  $\sim 6.25$  were the dominant functional groups aiding the adsorption. Protonated nitrogen groups like  $\text{H}_2\text{N-N}^+\text{H}_3$ , may also be present as the associated  $\text{pK}_a$  values are 8.1–9.6 (Naja et al., 2005). Lastly, the  $\text{pK}_a$  value of 6.9 may identify heterocyclic amino protonated groups or isomers of amino groups from aliphatic chains. These results can be related to the number of binding sites, which might explain the better uptake capacity for the Bothnian Sea *F. vesiculosus*.

Finally, when considering the differences in seawater pH, salinity (ionic strength), surface deprotonation capacity, abundance of surface acidity groups, and Cd speciation it can be concluded that these factors all play important roles in regulating and controlling

the higher uptake capacitance of *F. vesiculosus* from the Bothnian Sea when compared with *F. vesiculosus* from the Irish Sea. Our data showed that following an initial ion exchange mechanism, biosorption occurred while at higher concentrations a secondary process lead to precipitation. However, the results derived from this study are based mainly on a macroscopic approach and an understanding of the molecular level mechanisms would require high-resolution spectroscopic methods that were beyond the purpose of this study. True chemical mechanisms and ion binding behaviour remain difficult to ascertain but important surface characteristics and adsorption respectively precipitation processes as well as the main differences between the two populations of algae have been documented.

#### 4.2. Photosynthesis and cell morphology

The photosynthetic responses to Cd exposure showed that at all Cd concentrations *F. vesiculosus* from the Bothnian Sea were more sensitive (more negative effects) than those from the Irish Sea. It is well known that Cd is an effective inhibitor of photosynthesis in higher plants (Krupa et al., 1993). For example, in *Ectocarpus siliculosus*, heavy metals ( $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ ) have been found to substitute  $\text{Mg}^{2+}$  in the chlorophyll molecule, that are primarily found in the light harvest complex of photosystem II (Küpper et al., 2002). Similar effects were observed for the green alga *Scenedesmus quadricauda*, but only under low light conditions (Krupa et al., 1993) while studies with the red macroalga *Gracilaria tenuistipitata*, (Hu et al., 1996) revealed that at least part of the uptake mechanisms of Cd was light dependent. During this study with *F. vesiculosus* under natural light conditions, the irradiance was relatively low, with the highest value of  $380 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Thus, the observed photosynthetic inhibition was likely due to substitution of  $\text{Mg}^{2+}$  by Cd in the chlorophyll molecule, but further work is needed before any unambiguous conclusions can be drawn.

The results from this study also revealed that  $F_v:F_m$  and dark respiration were the more sensitive parameters in the Bothnian Sea *F. vesiculosus* while  $P_{\text{max}}$  was not affected at  $0.1 \text{ mmol Cd l}^{-1}$ , but was affected at  $1 \text{ mmol Cd l}^{-1}$ . Similar results (increased dark respiration before any decrease in  $P_{\text{max}}$ ) have earlier been shown for Bothnian Sea *F. vesiculosus* exposed to different concentrations of Pb (Nygård and Ekelund, 1999). The same trend could not be seen for the Irish Sea *F. vesiculosus*. The physiological differences in response to a heavy metal might therefore be an effect of the low salinity in the Baltic Sea. The significantly lower initial  $P_{\text{max}}$  in the Bothnian Sea *F. vesiculosus* might cause a direct response exhibited by an increased dark respiration in a stress situation. At even higher stress, even the  $P_{\text{max}}$  showed negative effects (decreases), while the dark respiration continued to increase. However, a situation with increased dark respiration and decreased  $P_{\text{max}}$  will be unsustainable in the long run. Interestingly, even  $F_v:F_m$  showed negative effects at the same concentrations as the dark respiration (once again, only applies to the Bothnian Sea algae). Since the  $F_v:F_m$  parameter indicates the efficiency of the PSII system, expressed as the quantum yield per absorbed photon, the reduced value indicates that less of the absorbed energy is used for photochemistry (Maxwell and Johnson, 2000).

Finally, upon exposure of algae to toxic metals morphological changes would be most expected in the outer cell layers, both since they are in direct contact with the surrounding seawater, but also since the absorption of Cd is higher in the cell walls and the physodes of the outer cell layer (Lignell et al., 1982). However, in this study, no distinct morphological changes were observed in the *F. vesiculosus* from the Irish Sea, whereas the algae from the Bothnian Sea showed increased content of electron translucent materials.

## 5. Conclusions

*F. vesiculosus* from the Bothnian Sea were more sensitive to Cd exposure than those from the Irish Sea, with decreased  $P_{\max}$ ,  $F_v:F_m$  and increased dark respiration. Complementary to this, the adsorption of Cd was higher onto the algae from the Bothnian Sea, which might be in part explained by its surface charge characteristics such faster deprotonation capacity and higher number of total acidity groups. The results further indicate that seawater salinity plays the most dominant role with respect to metal adsorption and photosynthetic response of the algae. The higher adsorption capacity for *F. vesiculosus* from the Bothnian Sea revealed that these algae may be the more suitable biosorbent for removing Cd in waste-water treatments systems in industrially polluted estuarine environments. In addition, such environments could be remediated with a higher efficiency if the clean-up efforts would focus on a lower salinity system because the removal capacity of the toxic metals seems to be inversely proportional to ionic strength (i.e., salinity). Further work is needed to ascertain if the algae can be used in more than one treatment cycle. However, overall the results from this study showed that *F. vesiculosus* from the Bothnian Sea is a good and efficient biosorbent for Cd from wastewaters and thus it may constitute a cheap and renewable natural marine resource of biosorbent that can be used in water treatment technology applications.

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## References

- Ahluwalia, S.S., Goyal, D., 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresource Technology* 98, 2243–2257.
- Amer, H., Emons, H., Ostapezuk, P., 1997. Application of multielement techniques for the fingerprinting of elemental contents in *Fucus vesiculosus* from the North Sea. *Chemosphere* 34, 2123–2131.
- Bäck, S., Collins, J.C., Russell, G., 1992. Comparative ecophysiology of Baltic and Atlantic *Fucus vesiculosus*. *Marine Ecology Progress Series* 84, 71–82.
- Bethke, C.M., 2002. The Geochemist's Workbench – Release 4.0 – A User's Guide to Rxn, Act2, React, and Gtplot, University of Illinois.
- Brinza, L., Dring, M.J., Gavrilescu, M., 2005. Ability of different algal species to take up heavy metals from wastewater: a review. *The Phycologist* 68, 30–31.
- Chen, W., Mulchandani, A., 2005. Environmental Biotechnology: challenges and opportunities for chemical engineers. *American Institute of Chemical Engineers* 51, 690–695.
- Davis, T.A., Volesky, B., Mucci, A., 2003. A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research* 37, 4311–4330.
- Gavrilescu, M., 2004. Removal of heavy metals from the environment by biosorption. *Engineering in Life Sciences* 3, 219–232.
- Gran, G., 1952. Determination of the equivalence point in potentiometric titration, Part II. *The Analyst* 77, 661–671.
- Green, E.J., Carritt, D.E., 1967. New tables for oxygen saturation of seawater. *Journal of Marine Research* 25, 140–147.
- Gupta, R., Ahuja, P., Khan, S., Saxena, R.K., Mohapatra, H., 2000. Microbial biosorbents: meeting challenges of heavy metal pollution in aqueous solutions. *Current Science* 78, 967–973.
- Herrero, R., Lodeiro, P., Rey-Castro, C., Vilarino, T., Vicente, M.E.S.D., 2005. Removal of inorganic mercury from aqueous solutions by biomass of the marine macroalga *Cystoseira baccata*. *Water Research* 39, 3199–3210.
- Herrero, R., Cordero, B., Lodeiro, P., Rey-Castro, C., Vicente, M.E.S.D., 2006. Interactions of cadmium (II) and protons with dead biomass of marine algae *Fucus sp.* *Marine Chemistry* 99, 106–116.
- Horsfall, M., Spiff, A.I., 2005. Effect of metal ion concentration on the biosorption of  $Pb^{2+}$  and  $Cd^{2+}$  by *Caladium bicolor* (wild cocoyam). *African Journal of Biotechnology* 4, 191–196.
- Hu, S., Tang, C.H., Wu, M., 1996. Cadmium accumulation by several seaweeds. *The Science of the Total Environment* 187, 65–71.
- Krupa, Z., Öquist, G., Huner, N.P.A., 1993. The effects of cadmium on photosynthesis of *Phaeoelus vulgaris* – a fluorescence analysis. *Physiologia Plantarum* 88, 626–630.
- Küpper, H., Šetlík, I., Spiller, M., Küpper, F.C., Prášil, O.J., 2002. Heavy metal-induced inhibition of photosynthesis: targets of in vivo heavy metal chlorophyll formation. *Phycology* 38, 429–441.
- Lignell, Å., Roomans, G.M., Pedersén, M., 1982. Localization of absorbed cadmium in *Fucus vesiculosus* L. by X-ray microanalysis. *Zeitschrift Fur Pflanzenphysiologie* 105, 103–109.
- Mamboya, F.A., 2007. Heavy Metal Contamination and Toxicity. Studies of Macroalgae from the Tanzanian Coast. PhD Thesis. University of Stockholm, Stockholm, 48pp.
- Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* 51, 659–668.
- McLean, M.W., Williamson, F.B., 1977. Cadmium accumulation by the marine red alga *Porphyra umbilicalis*. *Physiologia Plantarum* 41, 268–272.
- Mellor, A., 2002. The Uptake of Metals by Marine Macroalgae. PhD Thesis. Queens University of Belfast, Belfast, 172pp.
- Möhlenberg, F., Jensen, A., 1980. The ecotoxicology of cadmium in fresh and sea water and water pollution with cadmium in Denmark. The National Agency of Environmental Protection, Denmark.
- Naja, G., Mustin, C., Volesky, B., Berthelin, J., 2005. A high-resolution titrator: a new approach to studying binding sites of microbial biosorbents. *Water Research* 39, 579–588.
- Nygård, C., Ekelund, N.G.A., 1999. Effects of lead ( $PbCl_2$ ) on photosynthesis and respiration of the bladderwrack, *Fucus vesiculosus*, in relation to different salinities. *Water, Air and Soil Pollution* 116, 549–565.
- Nygård, C.A., Ekelund, N.G.A., 2006. Photosynthesis and UV-B tolerance of the marine alga *Fucus vesiculosus* at different sea water salinities. *Journal of Applied Phycology* 18, 461–467.
- OriginLab, 2000. Origin 6.1.
- Romera, E., Gonzalez, F., Ballester, A., Blazquez, M.L., Munoz, J.A., 2007. Comparative study of biosorption of heavy metals using different types of algae. *Bioresource Technology* 98, 3344–3353.
- Romero-Gonzalez, M.E., Williams, C.J., Gardiner, P.H., 2001. Study of the mechanisms of cadmium biosorption by dealginated seaweed waste. *Environmental Sciences and Technology* 15, 3025–3030.
- Rosotti, F., Rosotti, C., 1965. Determinations of Stability Constants and Other Equilibrium Constants in Solutions. Mir Publishers, Moscow.
- Schoenwaelder, M.E.A., Wiencke, C., 2000. Phenolic compounds in the embryo development of several Northern Hemisphere Fucoids. *Plant Biology* 2, 24–33.
- Vasconcelos, M.T.S.D., Leal, M.F.C., 2001. Seasonal variability in the kinetics of Cu, Pb, Cd and Hg accumulation by macroalgae. *Marine Chemistry* 74, 65–85.
- Vijaapraghavan, K., Jegan, J.R., Palanivelu, K., Velan, M., 2004. Cooper removal from aqueous solutions by marine green alga *Ulva reticulata*. *Electronic Journal of Biotechnology* 7, 61–71.
- Volesky, B., Holan, Z.R., 1995. Biosorption of heavy metals. *Biotechnology Progress* 11, 235–250.
- Yun, Y.S., Parck, D., Park, J.M., Volesky, B., 2001. Biosorption of trivalent chromium on the brown seaweed biomass. *Environmental Sciences and Technology* 35, 4353–4358.