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Cadmium uptake from sediment by *Cylindrotheca closterium* and the effect of diatom presence on partitioning of cadmium between sediment and water: A laboratory study

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Abstract

Although it is well established that microalgae take up metals and other contaminants from water and it has been suggested that algae may play a significant role in mobilizing sediment bound contaminants, there has been little research on the uptake of sediment-associated contaminants by microalgae. This may be important for microphytobenthos, which is closely associated with sediments. We report on laboratory experiments investigating the uptake of cadmium (Cd) from sediment and water by *Cylindrotheca closterium* over 96 h. The role of microalgae in the partitioning of Cd between sediment and water was also investigated. While concentrations do not typically represent those in the natural environment, we showed *C. closterium* takes up Cd from sediment, and concentration in microalgae is affected by sediment organic matter content. *Cylindrotheca closterium* influenced Cd movement between sediment and water: transfer from water to sediment was slowed, while transfer from sediment to overlying water (all treatments) and interstitial water (unprocessed sediment treatments) was increased. This is the first article to describe Cd uptake by diatoms from intertidal sediment in relation to sediment properties and mobilization of Cd from sediment in the presence of diatoms. Microalgae may serve as a pathway for sediment-associated metals to enter into aquatic food webs, and their presence appears to increase metal concentrations in water potentially making any mobilized metals available for uptake by other species. Given this and their importance as the basis of the food chain, there may be implications for environmental and human health and potential impacts for the biological stability of the sediment.

Microphytobenthos, the mixed community of photosynthetic algae and cyanobacteria that lives in the top few millimeters of sediment in the muddy intertidal zone (MacIntyre et al. 1996), is important in the stabilization of the mud flat surface (Van de Koppel et al. 2001; Stal and de Brouwer 2003) and is the main primary producer in estuaries (Baillie and Welsh 1980; De Jonge and Van Beusekom 1995; Meleder et al. 2010), forming the base of the estuarine food chain (Kanaya et al. 2008; Yoshino et al. 2012; McCormick et al. 2014). It has long been established that metals and other contaminants are taken up from the water column by algae, making them an important factor influencing the fate and transport of contaminants in natural systems (Schmitt et al. 2001; He and Chen 2014; Beldowska et al. 2015).

Phytoplanktonic microalgae are well studied with respect to contaminant uptake from water (Heldal et al. 2001; Lee and Fisher 2016); however, there has been very little research to date involving the algae of the microphytobenthos (Stronkhorst et al. 1994). In particular, although it has long been suggested that algae may play a significant role in mobilizing sediment bound metals (Laube et al. 1979), there has been a dearth of research involving the uptake of contaminants from sediment to microphytobenthic algae (Stronkhorst et al. 1994; Absil and van Scheppingen 1996).

Metals are naturally ubiquitous in estuarine systems and while some are essential micronutrients but toxic in high doses, others serve no biological function. The only known biological role of cadmium (Cd) is as a zinc (Zn) replacement at the catalytic site of carbonic anhydrase, reported in the marine diatom *Thalassiosira weissflogii* (Lane and Morel 2000) in a study which also discovered a distinct Cd-specific form of carbonic anhydrase (CDCA). In a subsequent investigation, Park et al. (2007) observed the presence of CDCA in other diatom species and speculated that this may be an adaptation to the marine environment where Zn is in short supply. Otherwise, Cd is known for its toxicity and represents a significant public health concern as it enters the food

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chain (Maret and Moulis 2013). Specifically, it has been suggested that uptake by microalgae may cause bioaccumulation and biomagnification of Cd through the food chain (Moreno-Garrido et al. 2003). Cd is known to be damaging to the kidneys and to affect the bone metabolism and the cardiovascular system of mammals (Maret and Moulis 2013).

The Screening Quick Reference Table (SQuiRT) identifies the threshold effects level, the maximum level at which no biological effects are observed, for Cd in marine sediment as 0.68 mg kg^{-1} and the apparent effects threshold, the level at which biological indicator effects are always observed, as 3.0 mg kg^{-1} (Buchman 2008). According to the SQuiRT for inorganics in sediment, expected background levels of Cd in uncontaminated locations are 0.1–0.3 mg kg^{-1} (Buchman 2008). This figure is supported by Bryan and Langston (1992), who describe sediment concentrations in pristine locations in the UK as about 0.2 mg kg^{-1}. In contrast, in contaminated sediment in the Scheldt Estuary in the Netherlands, Cd has been measured as 5.24 ± 3.31 mg kg^{-1} (Stronkhorst et al. 1994). Absil and van Scheppingen (1996) measured a range of concentrations from 0.06 to 7.9 mg kg^{-1} at 11 locations in the Oosterschelde and Westerschelde estuaries in the Netherlands.

Uptake and toxicity of Cd to the freshwater diatom *Navicula pellicosa* (Irving et al. 2009), freshwater green algae *Pseudokirchneriella subcapitata* (Paquet et al. 2015), marine microalgae (Wang and Wang 2009), and the coastal diatom *Thalassiosira pseudonana* (Sunda and Huntsman 1996) have previously been investigated; however, these studies involve uptake from water only. There have been studies concerning the toxicity of Cd-contaminated sediment (Moreno-Garrido et al. 2003) to benthic microalgae, but no measurements of uptake were reported.

In this study, the potential for uptake of an example metal (Cd) from sediment and water to the diatom *Cylindrotheca closterium* is investigated in a laboratory experiment to determine how uptake varies depending on the media (sediment or water) contaminated and sediment properties (particle size and organic matter content). Additionally, partitioning of Cd between the sediment and overlying water in the presence and absence of diatoms is examined. The experiments presented here are a first step toward understanding contaminant uptake from sediment by microphytobenthic algae and the influence of those algae on metal cycling between sediment and water. The information obtained, combined with data from field and mesocosm studies, could ultimately be used in biogeochemical models to understand Cd cycling in estuarine environments and contribute to environmental and human health risk assessment.

Materials and methods

A single diatom species *C. closterium* (Ehrenberg) Lewin and Reimann isolated from Loch Roag, Scotland in 2004 (strain no. 1017/10) was purchased from the Culture Collection of Algae and Protozoa (CCAP, Oban, UK). *Cylindrotheca closterium* was selected due to its high growth rate (Tanaka 1984; Silva-Aciares and Riquelme 2008) and the fact that it has long been grown in culture and used in toxicology studies (Araujo et al. 2010). Cells were cultivated in f/2 media (Guillard and Ryther 1962) enriched with silicon dioxide (SiO_2) (f/2 + Si) with a base of artificial seawater (Tropic Marin®) of 22 salinity prepared with Milli-Q® water. Diatoms were held in colonial, unialgal cultures maintained in glass Erlenmeyer flasks (De Orte et al. 2014; Lee and Fisher 2016) on a substratum of purified sea sand (Fisher Scientific) at 15°C under a 12:12 h light:dark cycle at an irradiance of 60 μmol photons m^{-2} s^{-1} in a controlled environment facility. Diatom cells were harvested for experimental use during the exponential growth phase (this was established to be 3-d-old cultures in earlier work using cell counts to assess the growth conditions).

To avoid the chelating effects of ethylenediaminetetraacetic acid (EDTA) on Cd during the experimental period, the growth medium was amended by exclusion of the trace metals stock (following the method of Moreno-Garrido et al. 2003 and Pérez-Rama et al. 2010), this is hereafter referred to as light media. Satisfactory growth of control cultures in this media was checked prior to the study (unpublished data).

Surface (top 50 mm) intertidal sediment was obtained from Loch Fleet (a tidal basin), Scotland (57°56′N, 4°2′W) and stored in the dark at 4°C prior to processing. This sediment was selected due to the low levels of pollution at this location, which is remote from industrial activity, in an area with low human population. The sediment was analyzed for potential metal contaminants and nothing of concern was found. The range of concentrations for aluminum (Al), iron (Fe), copper (Cu), Zn, and silver (Ag) was as follows: Al: 1.15–3.42 mg kg^{-1}; Fe: 2.71–12.64 mg kg^{-1}; Cu: 0.016–0.021 mg kg^{-1}; Zn: 0.012–0.030 mg kg^{-1}; and Ag: 3.63–7.77 μg kg^{-1}. Background Cd concentration was measured as 0.11 ± 0.01 mg kg^{-1}.

Sediment was sieved to 2 mm to remove macrobenthos and homogenized. Half the sediment (hereafter referred to as unprocessed) received no further treatment and was stored at 4°C in the dark prior to use. The other half (hereafter referred to as processed) was autoclaved (121°C, 20 min) to destroy remaining meiobenthos and microbiota. It was then washed in distilled water, dried, and stored dry at room temperature prior to use.

Differences in the sediment properties of organic matter content and particle size due to different levels of processing were determined by Coulter LS 230 granulometer and the loss on ignition method (Dean 1974), respectively.

A 2 g L^{-1} Cd stock solution (anhydrous $CdCl_2$; Sigma Chemical, St Louis, MO, U.S.A.) was made up in 22 salinity artificial seawater and used to spike water and sediments. Cd stock solution (6.75 mL) was made up to 1 L with the addition of light media, resulting in a final contamination level of 11.28 ± 0.08 mg L^{-1}. Water pH was adjusted to pH 8.2 ± 0.2 with the addition of 1 mol L^{-1} NaOH solution, the pH of

natural seawater and that at which the algae culture was maintained.

Sediment manipulations were carried out in a glove box filled with oxygen free nitrogen as recommended by Simpson et al. (2004). Cd stock solution (6.75 mL) made up to either 375 mL with artificial seawater (salinity of 15) and added to 1 kg dry processed sediment or 63.25 mL with Milli-Q® water and added to 1131.75 g wet unprocessed sediment, to achieve an 8:3 sediment water ratio, resulting in a final contamination level of 10.31 ± 0.12 mg kg^{-1} for both sediment types, with an interstitial water of 22 salinity. It was not our intention to mimic natural Cd contamination levels with this experiment, but rather to have levels of contamination that it could be easily measured. This sediment contamination level was selected as a previous study (Moreno-Garrido et al. 2003) showed little growth inhibition of *C. closterium* by Cd up to a concentration of 20 mg kg^{-1}. For uncontaminated sediments, 375 mL artificial seawater (salinity 15) was added to 1 kg dry processed sediment and 63.25 mL Milli-Q® water was added to 1131.75 g wet unprocessed sediment. Sediment pH was adjusted to pH 8 ± 0.2 with the addition of 1 mol L^{-1} NaOH solution following the recommendations of Hutchins et al. (2007), and monitored and further adjusted over the following 3 d. Sediments were stored at room temperature (18–21°C) in an oxygen free atmosphere for a minimum of 40 d (Simpson et al. 2004) to allow equilibration of Cd between sediment and pore water.

Experimental treatments (Fig. 1) were run in triplicate, two containing a *C. closterium* culture (enabling measurement of both total and internal contaminant uptake) and one without diatoms.

Experiments were run in 250 mL circular plastic pots. Pots for treatments that included sediment, were filled to a depth of 8 mm with either processed or unprocessed sediment, which was either contaminated or uncontaminated. Pots for treatments without diatoms had 100 mL light media added, treatments with diatoms had 70 mL light media added and were topped up with 30 mL of *C. closterium* culture in growth media at a cell density of 10^6 cells mL^{-1}.

The experiment ran for a total period of 96 h (following U.S. Environmental Protection Agency guidelines for toxicity tests; USEPA 2002) in the controlled environment facility under the conditions described above. Sampling was carried out at 3, 24, and 96 h. The experiment was repeated three times for replication purposes.

At the end of each time period, 24 containers were removed from the controlled environment chamber. The components (overlying water, diatoms, pore water, and sediment) were then separated out for further processing and analysis.

Overlying water was syphoned from the sediment using a vacuum pump, on a low draw and fitted with a pipette tip. Diatoms were separated from the sediment using the lens tissue method (Eaton and Moss 1966). Pore water was extracted by centrifuging the sediment at 3100 relative centrifugal force (RCF) for 20 min at 4°C (USEPA 2001). Overlying and porewater samples were filtered through a 47 mm 0.45 μm cellulose nitrate filters (Whatman™) diluted to 1:5 with Milli-Q® water and acidified to 2% v/v with concentrated nitric acid (HNO_3, 70% w/w) in preparation for analysis by inductively coupled plasma mass spectrometry (ICP-MS). All the equipment was Decon90® washed and rinsed with Milli-Q® water before use.

Sediment aliquots for Cd concentration analysis were ground to 125 μm with a pestle and mortar and 0.25 g was weighed into Teflon™ tubes to which 2 mL HNO_3 (70% w/w) was added prior to microwave digestion (USEPA 2007). Samples were gravity-filtered and diluted to 100 mL with Milli-Q®

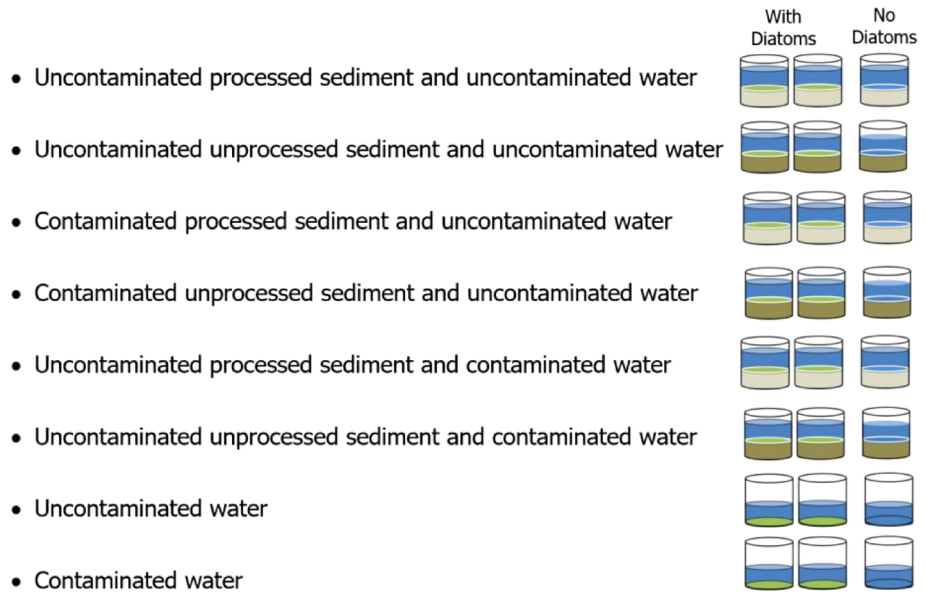

Fig. 1. Experimental treatments.

water to give a 2% v/v acid matrix in preparation for analysis by ICP-MS.

Three fractions of removed Cd were obtained: total, intracellular, and bioadsorbed (or extracellular). Following separation from the sediment, total Cd in the cells was determined by filtration through two preweighed superposed 47 mm, 0.45 μm cellulose nitrate filters (Mucha et al. 2003; Zidour et al. 2019). Filters were dried and reweighed to determine the dry weight of the diatom sample before being separately digested for 48 h with 1 mL HNO_3 (70% w/w) and 0.5 mL perchloric acid ($HClO_4$, 72% w/w). Cd was measured on both filters and the lower filter used as a blank.

Intracellular Cd was measured by suspending the diatom pellet in 0.02 mol L^{-1} EDTA solution for 20 min. This was followed by centrifuging and washing twice in artificial seawater (salinity 22). EDTA washing removed Cd adsorbed to the cell surface allowing intracellular Cd to be measured (Pérez-Rama et al. 2010). The washed pellet was filtered, weighed, and digested in the same way as in total Cd determination. Extracellular Cd was determined by subtracting the intracellular Cd concentration from the total.

All samples (algae, sediment, pore, and overlying water) were analyzed for Cd concentration using an ICP-MS (XSERIES 2, Thermo Scientific, Germany) using collision cell technology to reduce potential polyatomic interferences. Multielement standards (Merck, Germany) certified by the National Institute of Standards and Technology (NIST), internal standards (scandium [Sc] and rhodium [Rh]), and blanks were included in each run, limits of detection (LOD) plus the certified and measured values of the standard are given in Table 1. All samples were run in triplicate and reference samples included in each run to check accuracy and consistency. All samples from treatments containing Cd had measured values at least one order of magnitude greater than the LOD.

The bioconcentration factor (BCF) is used to describe the distribution of Cd between *C. closterium* and sediment (BCF_{Sed}) or overlying water (BCF_{Wat}), while the concentration factor (CF) is applied to describe the Cd distribution between sediment and water. BCF was calculated from the total Cd accumulated by the algae as follows:

$$BCF_{Sed} = Cd_{Alg}\left(\text{mg kg}^{-1}\right)/Cd_{Sed}\left(\text{mg kg}^{-1}\right) \quad (1)$$

$$BCF_{Wat} = Cd_{Alg}\left(\text{mg kg}^{-1}\right)/Cd_{Wat}\left(\text{mg L}^{-1}\right) \quad (2)$$

where Cd_{Alg} is Cd concentration in *C. closterium*, Cd_{Sed} is concentration in sediment, and Cd_{Wat} is concentration in overlying water.

CF was calculated from Cd concentration in the sediment or water as follows:

$$CF_{Sed} = Cd_{Sed}\left(\text{mg kg}^{-1}\right)/Cd_{Wat}\left(\text{mg L}^{-1}\right) \quad (3)$$

$$CF_{Wat} = Cd_{Wat}\left(\text{mg L}^{-1}\right)/Cd_{Sed}\left(\text{mg kg}^{-1}\right) \quad (4)$$

Statistical analysis was carried out using R (3.2.3) in RStudio. The lme4 package (Bates et al. 2015) was used to perform linear mixed effects analysis of the relationship between Cd concentrations and experimental treatments. As fixed effects, the contaminated compartment, time, sediment type, and diatom presence were entered into the model. As a random effect, there was an intercept for the effect of experiment run.

$$\text{CdPPM} \sim \text{Contamination} + \text{Time} + \text{Sediment} + \text{Diatom} + (1|\text{Run}) \quad (5)$$

p values and χ^2 were calculated using likelihood ratio tests. A p value of ≤ 0.05 was used to determine statistical significance.

Table 1. Table of detection limits and certified and measured values for Merck multielement standard.

Element (isotope)	LOD (μg kg^{-1})	Certified value (μg kg^{-1})	Measured value (μg kg^{-1})	σ	Relative standard deviation (%)
Al (27)	2.24E+01	10	9.498	0.320	3.395
V (51)	5.84E−03	10	9.894	0.035	0.355
Cr (52)	7.95E−02	10	10.000	0.123	1.229
Mn (55)	1.44E−01	10	9.941	0.124	1.245
Fe (56)	7.31E−01	10	9.871	0.195	1.971
Co (59)	4.40E−03	10	9.952	0.096	0.962
Ni (60)	2.01E−01	10	10.010	0.127	1.270
Cu (65)	7.59E+00	10	9.981	0.115	1.153
Zn (66)	8.32E−01	10	9.929	0.222	2.236
Ga (69)	3.74E−01	10	9.957	0.031	0.315
As (75)	3.74E−01	10	9.876	0.119	1.205
Ag (107)	8.44E−03	10	9.961	0.120	1.208
Cd (111)	1.33E−01	10	9.900	0.093	0.943
Pb (208)	1.19E−01	10	9.782	0.085	0.873

Results

Sediment properties

Sediment properties prior to the start of the experiment are shown in Table 2.

At the end of the experimental period, both sediment types, processed and unprocessed, had very low organic matter content. Mean organic matter content of the processed sediment was 0.34% lower than the unprocessed sediment ($p = 2.6 \times 10^{-7}$). Unprocessed sediment has a greater percentage of particles in the smaller size fractions, clay ($p = 7.4 \times 10^{-3}$) and silt ($p = 1.1 \times 10^{-4}$), while the processed sediment has a higher percentage of particles in the sand size fraction ($p = 1.3 \times 10^{-4}$). These differences are likely due to the sediment processing, organic matter is likely to have been flushed out of the sediment during the washing phase and smaller sediment particles lost during the washing and drying of the processed sediment.

Diatom growth

The mass of diatoms retrieved was used as a proxy for diatom growth. The only treatment in which there was consistent growth over the 96-h experimental period was that in which the water was uncontaminated and there was no sediment present. In all other configurations, the mass of diatoms retrieved either remained the same or decreased over the course of the experiment (Fig. 2).

There was an effect of Cd contamination on treatments without sediment, with higher growth when there was no Cd added ($\chi^2 = 6.4$, $p = 1.2 \times 10^{-2}$, $r^2 = 0.61$).

Diatom growth appears to be restricted in the presence of sediment. The mass of diatoms collected from uncontaminated, processed sediment decreased from 4.72 ± 2.75 mg after 3 h to 2.02 ± 0.50 mg after 96 h, while that collected from uncontaminated, unprocessed sediment decreased from 6.08 ± 2.45 mg after 3 h to 3.75 ± 0.82 mg after 96 h.

There was greater recovery of *C. closterium* from containers with unprocessed (4.48 ± 2.69 mg) as opposed to processed sediment (2.68 ± 2.37 mg), whether these were contaminated (sediment or water) or uncontaminated ($\chi^2 = 7.8$, $p = 5.3 \times 10^{-3}$, $r^2 = 0.33$). There was no difference in diatom growth in treatments containing sediment whether or not this was Cd contaminated ($\chi^2 = 1.6 \times 10^{-1}$, $p = 9.2 \times 10^{-1}$). Possible reasons for this are addressed in the "Discussion" section.

Table 2. Properties and cadmium concentration of sediments prior to the addition of cadmium (values given as mean ± standard deviation, $n = 3$). As the processed sediment is dried, water content does not apply.

Properties	Sediment	
Particle size (%)	**Processed**	**Unprocessed**
Clay (<4 μm) (%)	4.40 ± 0.42	5.18 ± 0.19
Very fine silt (4–8 μm) (%)	3.75 ± 0.45	4.36 ± 0.26
Fine silt (8–16 μm) (%)	5.93 ± 0.76	6.38 ± 0.10
Medium silt (16–32 μm) (%)	8.05 ± 1.01	7.86 ± 0.03
Coarse silt (32–64 μm) (%)	11.10 ± 1.03	9.77 ± 0.31
Very fine sand (64–128 μm) (%)	9.71 ± 0.76	8.94 ± 0.13
Fine sand (128–256 μm) (%)	42.01 ± 2.57	40.84 ± 0.91
Medium sand (256–512 μm) (%)	15.05 ± 1.88	16.67 ± 0.13
Coarse sand (512–1024 μm) (%)	0.00 ± 0.00	0.00 ± 0.00
Very coarse sand (1024–2000 μm) (%)	0.00 ± 0.00	0.00 ± 0.00
Water in wet sediment (%)	N/A	22.70 ± 0.07
Organic matter (%)	1.20 ± 0.09	1.54 ± 0.10
Cadmium (mg kg^{-1})	0.10 ± 0.04	0.12 ± 0.03

Uptake of cadmium to *C. closterium* from water and sediment

Uptake of Cd to *C. closterium* was greater when water was initially contaminated ($\chi^2 = 73.5$, $p < 2.2 \times 10^{-16}$, $r^2 = 0.87$) irrespective of whether sediment was processed or unprocessed (Fig. 3). When water was initially contaminated (Fig. 3a), there was an indication of a difference in Cd uptake by *C. closterium* between sediment treatments ($\chi^2 = 5.9$, $p = 5.2 \times 10^{-2}$, $r^2 = 0.32$). A comparison of the three treatments (processed, unprocessed, and no sediment, Fig. 3a–c) using Tukey's honest significant difference (HSD) test revealed that uptake of Cd to *C. closterium* was greater from water only (no sediment) than when the sediment was processed ($p = 3.6 \times 10^{-2}$). There was no difference in uptake from contaminated water between unprocessed sediment and water only ($p = 3.8 \times 10^{-1}$) or between processed and unprocessed sediment ($p = 4.8 \times 10^{-1}$). There was no change in Cd concentration in *C. closterium* over time ($\chi^2 = 7.9 \times 10^{-1}$, $p = 6.7 \times 10^{-1}$).

When sediment was initially contaminated (Fig. 3d,e), there was greater uptake of Cd to *C. closterium* from processed than from unprocessed sediment ($\chi^2 = 20.7$, $p = 5.5 \times 10^{-6}$, $r^2 = 0.86$). After 96 h, the mean Cd concentration in *C. closterium* from processed sediment was 201 ± 34 mg kg^{-1}, about four times the concentration in diatoms from unprocessed sediment, 52 ± 17 mg kg^{-1}. Again there was no change in Cd concentration in the diatoms over time ($\chi^2 = 4.6$, $p = 1.0 \times 10^{-1}$).

Bioconcentration factor

The total (both externally adsorbed and internally absorbed) BCF of Cd in *C. closterium* related to both sediment and water is shown in Table 3 as the range of BCF values at each time point for each of the treatments related to either sediment (BCF_{Sed}) or water (BCF_{Wat}) Cd concentrations ($n = 3$). If BCF is more than 1, then Cd is bioconcentrated (i.e., higher in the algae than the contaminated compartment).

BCF_{Sed} was higher in processed than unprocessed sediment when sediment was initially contaminated. When the water was initially contaminated, BCF_{Wat} was higher when sediment was present. When there was no sediment present,

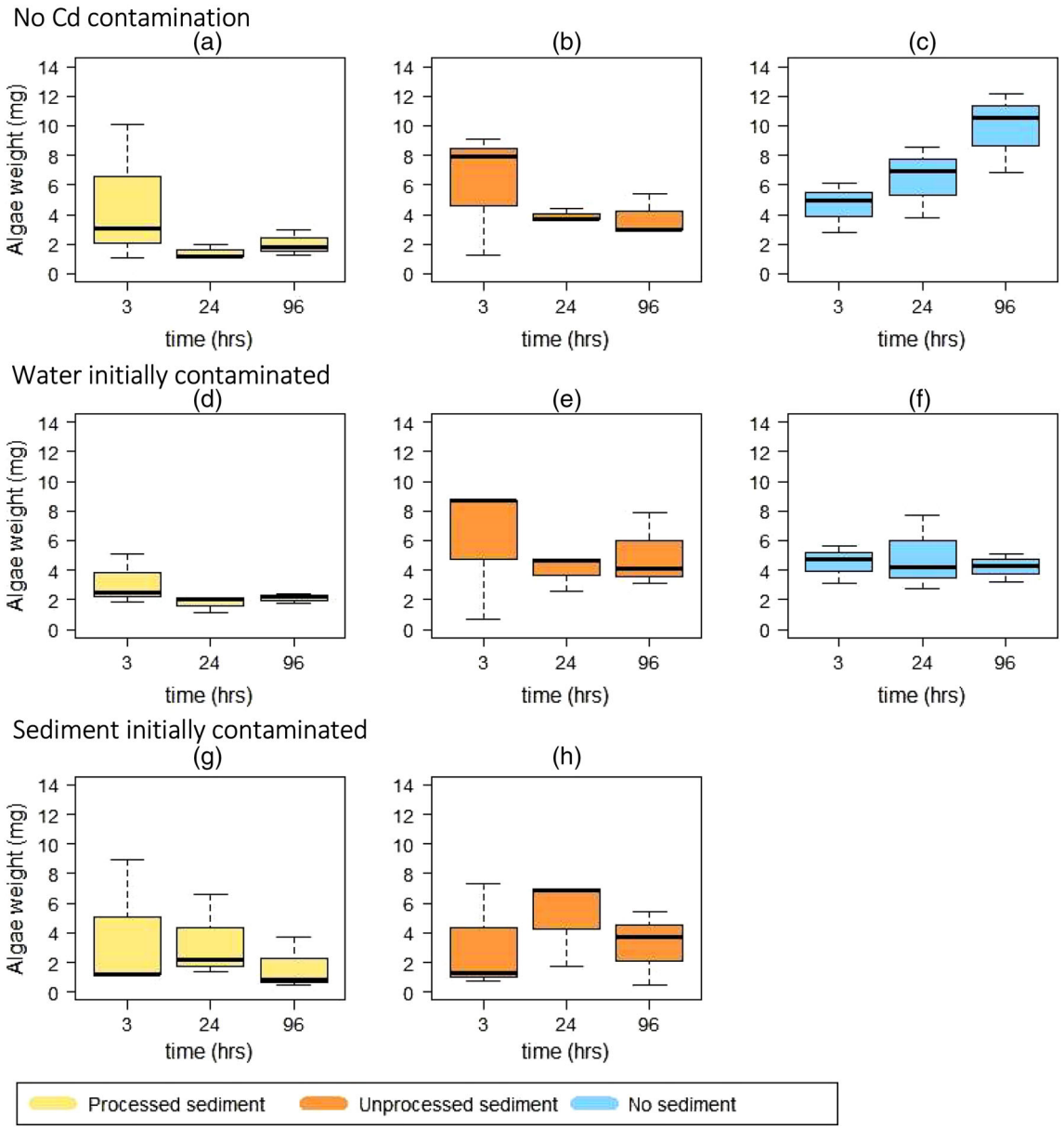

Fig. 2. Mass of *C. closterium* (mg DW) retrieved at three time points. In (**a**–**c**), there is no contamination; (**d**–**f**) water is initially contaminated; and (**g**, **h**) sediment is initially contaminated.

BCF_{Wat} remained fairly constant for the duration of the experiment.

External and internal cadmium in *C. closterium*

Internal Cd concentration was consistently higher than external concentration (Fig. 4), i.e., absorption into the diatom cell was greater than external adsorption, for all treatments at all three time points (χ^2 = 19.7, $p = 9.0 \times 10^{-6}$, r^2 = 0.46), except at 24 h when the processed sediment was initially contaminated (Fig. 4d). The range of values for Cd concentration was quite variable, probably due to the small quantities of algae extracted. This resulted in the calculation of extracellular concentration (obtained by subtracting intracellular concentration from the total) occasionally having a negative value, when the measured total concentration was lower than the internal concentration. The resulting negative values for external adsorption at 3 and 24 h have been excluded from Fig. 4.

Cadmium in sediment and water when water is initially contaminated

When water was initially contaminated, Cd concentration in the sediment increases over time (χ^2 = 30.2, $p = 2.8 \times 10^{-7}$, r^2 = 0.59) (Fig. 5a–d). There was no difference in Cd uptake to sediment from water due to the different sediment types (χ^2 = 2.5, $p = 1.1 \times 10^{-1}$). The presence of diatoms slowed

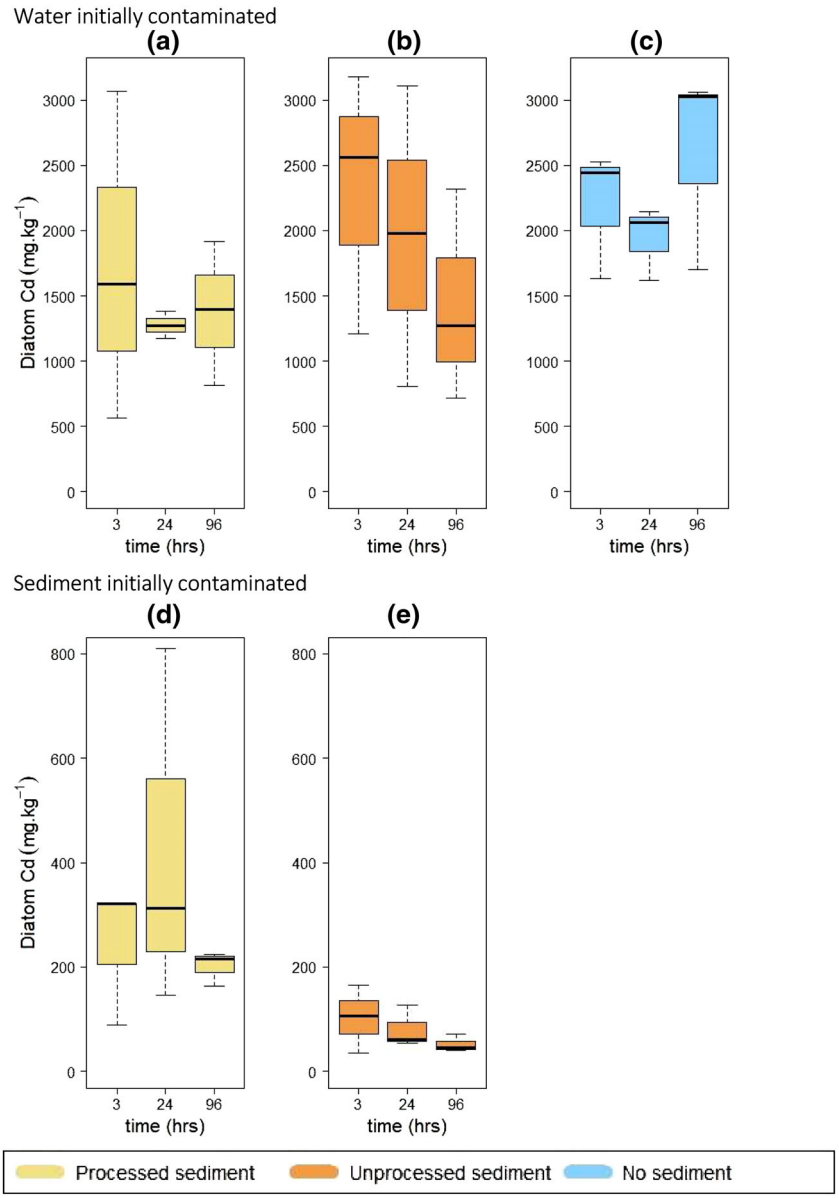

Fig. 3. Total cadmium uptake (internal plus external, mg kg^{-1} DW) to *C. closterium* when (**a**–**c**) water is initially contaminated and (**d**, **e**) sediment is initially contaminated. Note difference in the *y*-axis scale in plots (**d**, **e**).

the uptake of Cd from the overlying water to the sediment for both processed and unprocessed sediment (χ^2 = 4.2, $p = 4.1 \times 10^{-2}$).

Cd concentration in water decreases over time (χ^2 = 30.6, $p = 2.3 \times 10^{-7}$, r^2 = 0.77) (Fig. 5e–h). For both sediment types, this decrease happened more quickly where *C. closterium* was present (χ^2 = 6.7, $p = 9.7 \times 10^{-3}$), when there is biological adsorption and uptake, as well as transfer to sediment. Mean Cd concentrations in the water were higher when the sediment was unprocessed (χ^2 = 17.5, $p = 2.9 \times 10^{-5}$), this was particularly noticeable at 3 h especially where there were no algae present (Fig. 5h).

Cadmium in sediment and water when sediment is initially contaminated

When sediment was initially contaminated, Cd concentration in the sediment did not change over time (χ^2 = 7.0, $p = 7.3 \times 10^{-2}$, r^2 = 0.52), due to the presence of *C. closterium* (χ^2 = 1.8, $p = 1.8 \times 10^{-1}$) or due to sediment type (χ^2 = 3.6, $p = 5.7 \times 10^{-2}$, Fig. 6a–d). Cd concentrations in the water increased over time (χ^2 = 50.7, $p = 9.6 \times 10^{-12}$, r^2 = 0.77, Fig. 6e–h). Cd concentrations in water were greater in the presence of *C. closterium* (χ^2 = 5.9, $p = 1.5 \times 10^{-2}$); however, there was no difference in contamination due to the different sediment treatments (χ^2 = 1.8, $p = 1.8 \times 10^{-1}$).

Table 3. Bioconcentration factor from contaminated sediment (BCF_{Sed}) and water (BCF_{Wat}) to *C. closterium* given as the range (n = 3) to two significant figures.

Experimental treatment		Time (h)		
Initially contaminated	**Sediment type**	**3**	**24**	**96**
BCF_{Sed} (Cd_{Alg}/Cd_{Sed})				
Sediment	Processed	8.0–37	14–71	15–25
Sediment	Unprocessed	3.0–22	5.0–13	4.0–9.0
BCF_{Wat} (Cd_{Alg}/Cd_{Wat})				
Water	Processed	130–18,000	900–11,000	2200–7600
Water	Unprocessed	420–2200	390–6900	710–3600
Water	No sediment	190–300	210–240	200–380

Concentration factor

CF_{Sed} and CF_{Wat} related to sediment and water contamination are shown in Table 4 as the range of CF values at each time point for all the treatments that included both sediment and water. If CF is more than 1, then Cd is concentrated in that compartment.

The CF_{Sed} was more than 1 at all three time points and for all treatments, except at 3 h where water was the initially contaminated component. The inverse was true of CF_{Wat}, which was only greater than 1 after 3 h, when water was initially contaminated. CF_{Sed} was higher than CF_{Wat} across all treatments at all time points. When sediment was initially contaminated, CF_{Sed} was higher in treatments without *C. closterium*. When water was initially contaminated, CF_{Sed} was higher when *C. closterium* was present except for unprocessed sediment at 96 h.

That concentration of Cd was always higher in the sediment than the water (i.e., CF_{Wat} was always < 1) except at 3 h when the water was initially contaminated, shows there was quick equilibration of Cd between water and sediment; however, this was mediated by *C. closterium* (Figs. 5–6).

Cadmium in pore water

It was not possible to extract pore water from all experimental treatments due to the small (if any) quantities collected and the properties of the sediments (processed and unprocessed) which affected their ability to hold water. From a potential total of 162 pore-water samples, 102 were successfully extracted. Of these, 67 were from contaminated treatments (34 where sediment was initially contaminated and 33 where water was initially contaminated). Fewer samples were extracted from processed (n = 14) than unprocessed (n = 53) sediment and fewer were from samples without diatoms (n = 26) than with diatoms (n = 41). Across the time periods, there were 22 samples extracted at 3 h, 22 at 24 h, and 23 at 96 h.

The issues with extraction have implications in terms of replication. While graphical indications show that there may be a difference in pore-water concentrations due to sediment type (Fig. 7), the fact that there is only one replicate for processed sediment with *C. closterium* when water was initially contaminated (Fig. 7c) makes meaningful comparison difficult. With this in mind, there was no statistical test made between Cd concentrations in pore water for different sediment types.

There was higher Cd concentration in pore water when the overlying water was initially contaminated (χ^2 = 20.1, $p = 7.2 \times 10^{-6}$, r^2 = 0.59) and higher concentration, regardless of whether sediment or water was initially contaminated, when *C. closterium* was present (χ^2 = 32, $p = 1.9 \times 10^{-8}$, Fig. 8). There is some indication that contamination of pore water may increase over time (χ^2 = 5.9, $p = 5.3 \times 10^{-2}$).

Discussion

This is the first report to describe differences in Cd uptake by diatoms from sediment, dependent on sediment properties (particle size and organic matter content), and the first to describe the mobilization of Cd from sediment in the presence of diatoms. While there are many studies which have examined Cd uptake by diatoms from water, the authors were only able to find two field studies (Stronkhorst et al. 1994; Absil and van Scheppingen 1996) describing Cd uptake from intertidal sediment to diatoms and were unable to identify any reports on effects of sediment associated diatoms on Cd partitioning between sediment and water.

Effect of cadmium and sediment on *C. closterium* growth

Despite previous studies (Moreno-Garrido et al. 2003) reporting no effect of Cd on *C. closterium* at concentrations in sediment below 20 mg kg^{-1}, the presence of Cd does appear to restrict diatom growth. The mass of diatoms retrieved from the contaminated and uncontaminated treatments without sediment was similar at the 3-h time point; however, at 24 and 96 h, diatom mass in the contaminated water treatment remained stable, while in the uncontaminated water treatment diatom mass increased over time. This indicates some toxic effect of Cd at concentrations in water of about 10 mg L^{-1}.

There was also an effect of the presence of sediment on diatom growth. Over the course of the experiment, there was no increase (and in some cases, a slight decrease; Fig. 2b) in the

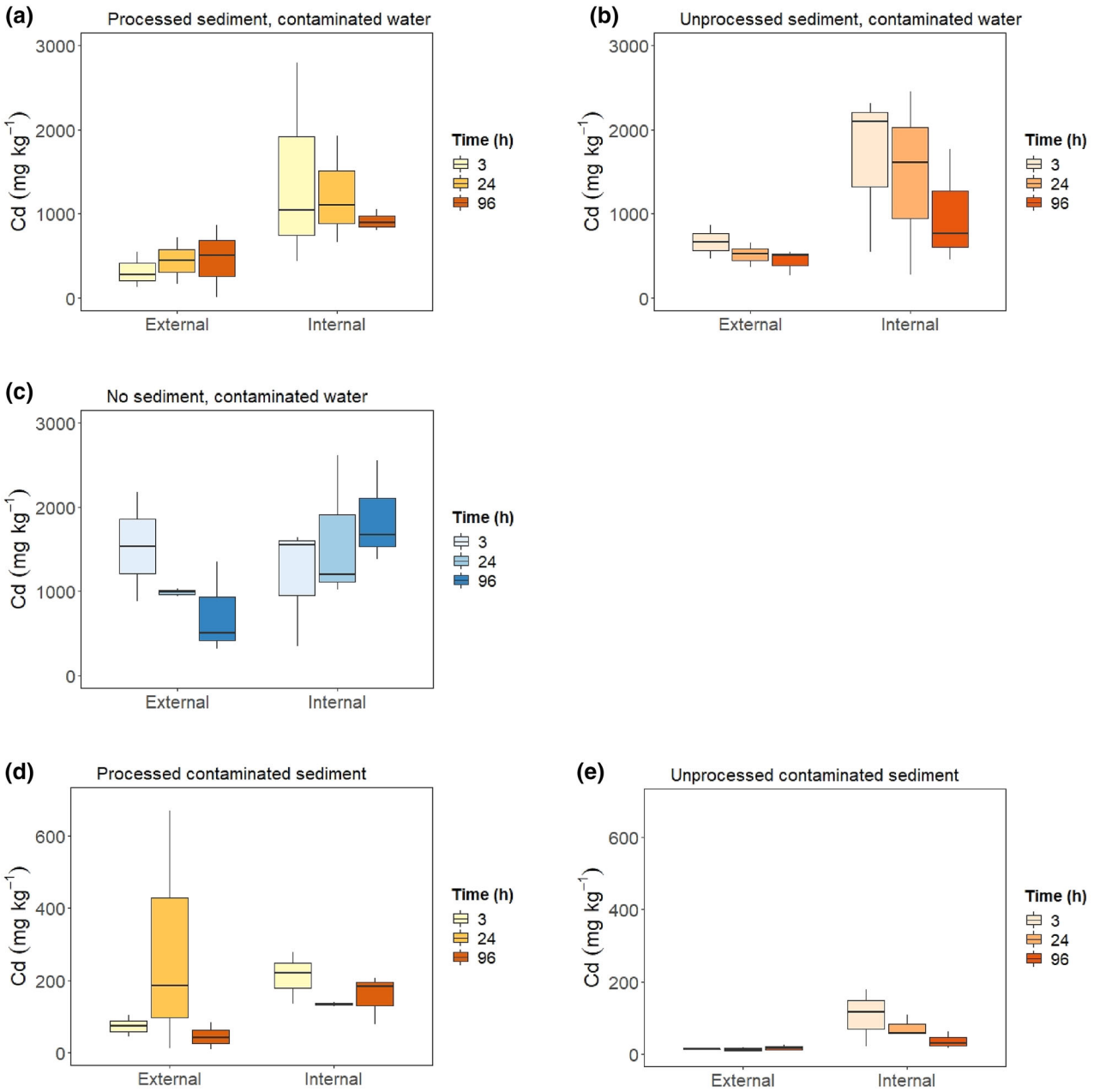

Fig. 4. Cadmium concentrations (external and internal, mg kg^{-1} DW) in *C. closterium* when (**a**) sediment is processed and water is initially contaminated, (**b**) sediment is unprocessed and water is initially contaminated, (**c**) no sediment and water is initially contaminated, (**d**) sediment is processed and initially contaminated, and (**e**) sediment is unprocessed and initially contaminated. Note difference in the *y*-axis scale in plots (**d**, **e**).

mass of algae recovered from treatments, which included sediment. As there were no other potentially toxic metals at high concentrations found in the sediment, it is thought that the impact on growth was due either to an effect of sediment on the ability of the diatoms to migrate into the lens tissue or a reduction in the *C. closterium* growth in the presence of sediment. A previous study using artificial sediments for toxicity testing (Moreno-Garrido et al. 2007), reported a negative effect of sediments containing more than 15% silt sized (< 63 μm) particles (with the remainder of the sediment consisting of sand particles) on *C. closterium* growth. This was thought to be due to algae becoming buried beneath fine sediment. In the current study, diatoms were the final addition to the experiment and sediment was not disturbed during the 96 h period; therefore, diatoms would not be buried by sediment unless they migrated. It seems more likely that growth was affected by the presence of sediment, although the reasons for this are unknown.

A further theory is that diatoms may be subject to predation by autochthonous organisms (Mauffret et al. 2010); however, a lower mass of diatoms was recovered from the processed

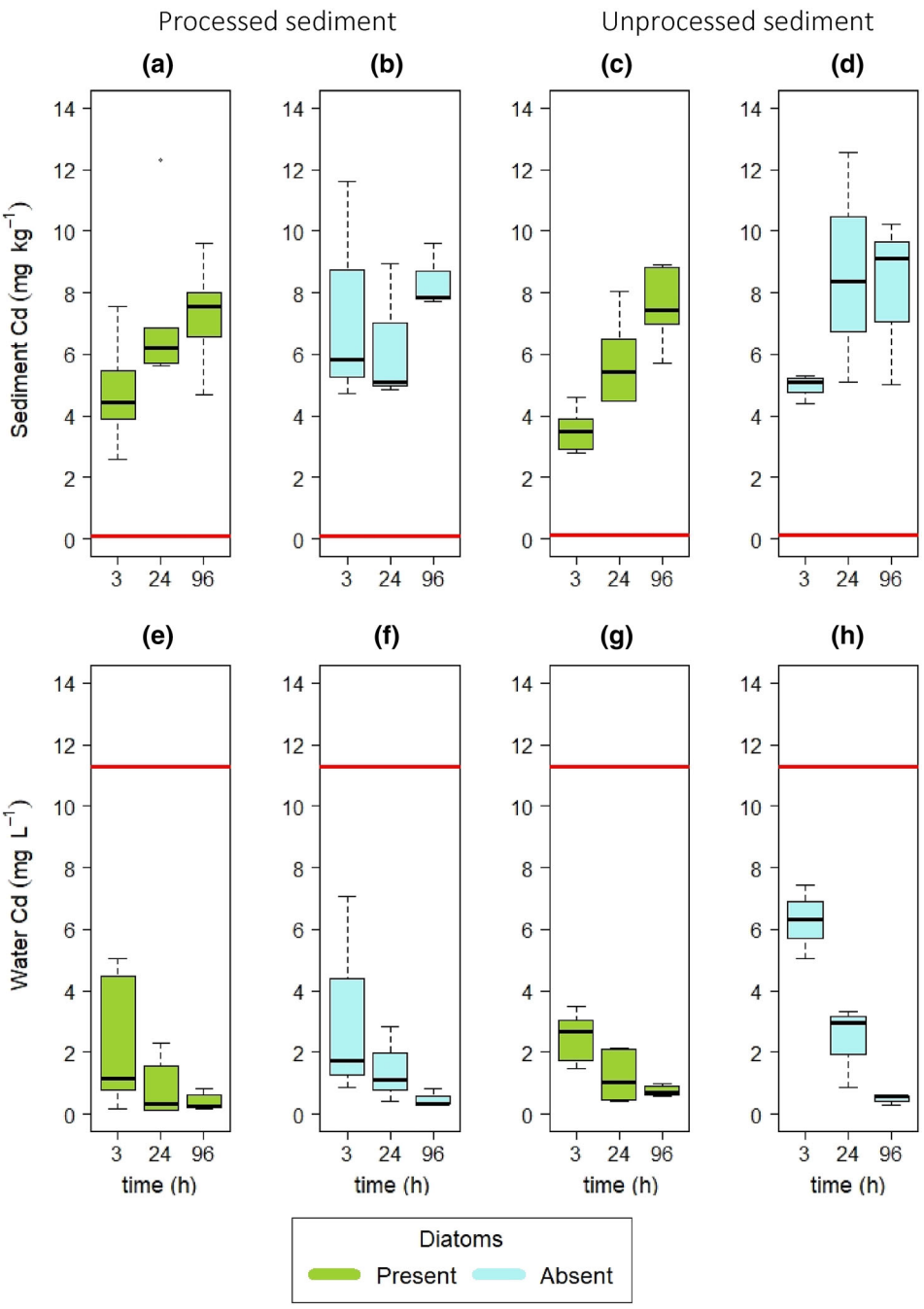

Fig. 5. Cadmium concentrations in (**a**–**d**) sediment (mg kg^{-1} DW) and (**e**–**h**) water (mg L^{-1}) when water is initially contaminated and (**a**, **b**, **e**, **f**) sediment is processed and (**c**, **d**, **g**, **h**) sediment is unprocessed, outliers (°) calculated as Q1 − 1.5IQR and Q3 + 1.5IQR (where IQR is interquartile range). Line indicates Cd concentration at $t = 0$.

(autoclaved and dried) sediment, in which predators are unlikely to have survived, than the unprocessed sediment. Mauffret et al. (2010) also suggested diatoms would be adversely affected by nutrients released from the sediment, although this seems unlikely. It seems more plausible that increased nutrients would stimulate growth and potentially mask toxic effects (Adams and Stauber 2004). Indeed this may have occurred in the current study in which the presence of sediment and Cd does not have a cumulative adverse effect on the mass of diatoms retrieved. Additionally, higher availability of nutrients in unprocessed sediment (nutrients are likely to have been flushed out of the processed sediment during washing) may account for the higher mass of diatoms retrieved from that sediment compared to the processed sediment, although

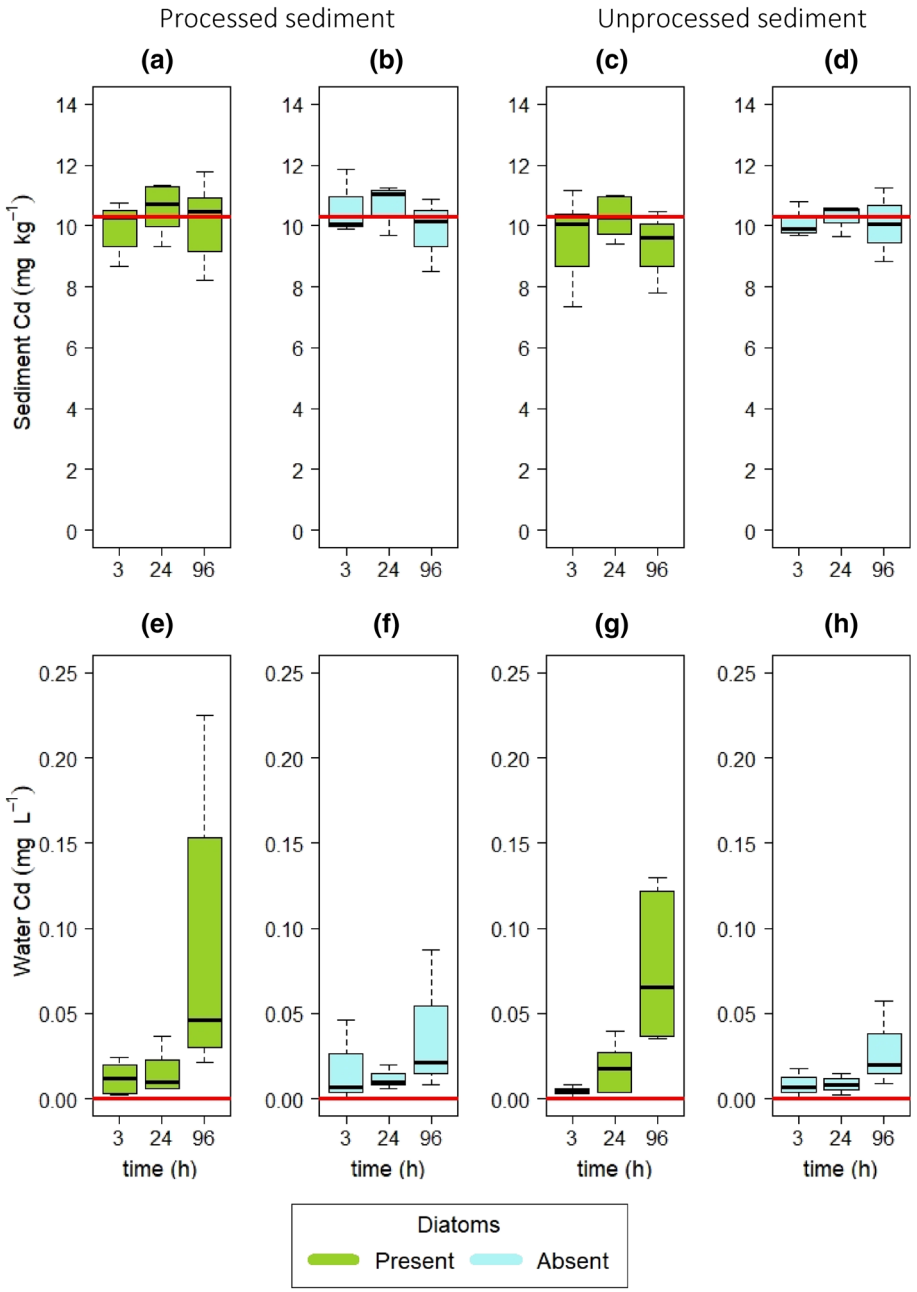

Fig. 6. Cadmium concentrations in (**a**–**d**) sediment (mg kg^{-1} DW) and (**e**–**h**) water (mg L^{-1}) when sediment is initially contaminated and (**a**, **b**, **e**, **f**) sediment is processed and (**c**, **d**, **g**, **h**) sediment is unprocessed. Line indicates Cd concentration at $t = 0$.

this is also potentially due to the survival and growth of autochthonous algae in addition to *C. closterium*.

The effect of the presence of sediment on diatom growth has implications for toxicity testing and future studies measuring contaminant uptake by microalgae from sediment and would warrant further investigation.

Cadmium accumulation by *C. closterium*

The total concentration (adsorption plus absorption) of Cd to *C. closterium* was an order of magnitude greater when water was the initially contaminated component. While no previous studies of marine or estuarine systems which directly compared uptake of Cd to microalgae from either sediment or water were found, there was previous a study (Laube et al. 1979) which compared uptake of Cd from either contaminated water or sediment in a freshwater system. Laube et al. (1979) found uptake from Cd contaminated water to *Anabaena* and *Ankistrodesmus braunii* to be ~ 50 and ~ 650 times greater, respectively, than from contaminated sediment.

Table 4. Concentration factor from contaminated sediment and water to sediment (CF_{Sed}) and water (CF_{Wat}) given as the range (n = 3) to two significant figures.

Experimental treatment			Time (h)		
Initially contaminated	**Sedimenttype**	***C. closterium***	**3**	**24**	**96**
CF_{Sed} (Cd_{Sed}/Cd_{Wat})					
Sediment	Processed	Present	450–570	480–1200	77–400
		Absent	260–13,000	550–1500	120–1100
	Unprocessed	Present	1800–3700	240–2600	77–230
		Absent	550–8500	690–5200	200–1000
Water	Processed	Present	7.7E−1 to 9.6	2.4–100	9.5–38
		Absent	6.6E−1 to 13	1.7–20	10–27
	Unprocessed	Present	8.0E−1 to 1.9	3.0–11	8.5–12
		Absent	7.1E−1 to 8.7E−1	2.8–5.7	15–18
CF_{Wat} (Cd_{Wat}/Cd_{Sed})					
Sediment	Processed	Present	1.7E−3 to 2.2E−3	8.3E−4 to 2.1E−3	2.5E−3 to 1.3E−2
		Absent	8.0E−5 to 3.8E−3	6.7E−4 to 1.8E−3	8.9E−4 to 8.5E−3
	Unprocessed	Present	2.7E−4 to 5.5E−4	3.8E−4 to 4.1E−3	4.3E−3 to 1.3E−2
		Absent	1.2E−4 to 1.8E−3	1.9E−4 to 1.4E−3	9.7E−4 to 5.1E−3
Water	Processed	Present	1.0E−1 to 1.3	1.0E−2 to 4.1E−1	2.6E−2 to 1.1E−1
		Absent	7.5E−2 to 1.5	4.9E−2 to 5.9E−1	3.6E−2 to 1.0E−1
	Unprocessed	Present	5.3E−1 to 1.3	9.4E−2 to 3.3E−1	8.2E−2 to 1.2E−1
		Absent	1.2–1.4	1.7E−1 to 3.5E−1	5.7E−2 to 6.7E−2

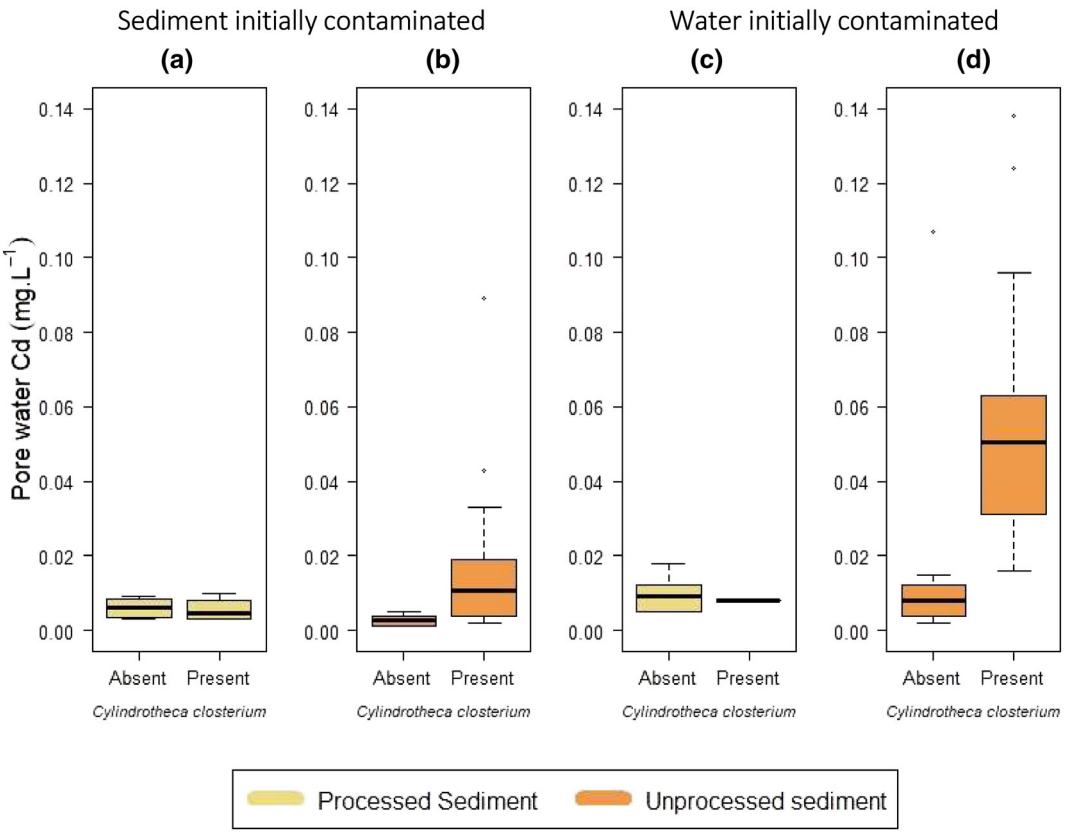

Fig. 7. Cadmium concentrations in pore water (mg L^{-1}), collected at all three time points, when (**a**, **b**) sediment is initially contaminated and (**c**, **d**) water is initially contaminated, outliers (°) calculated as Q1 − 1.5IQR and Q3 + 1.5IQR.

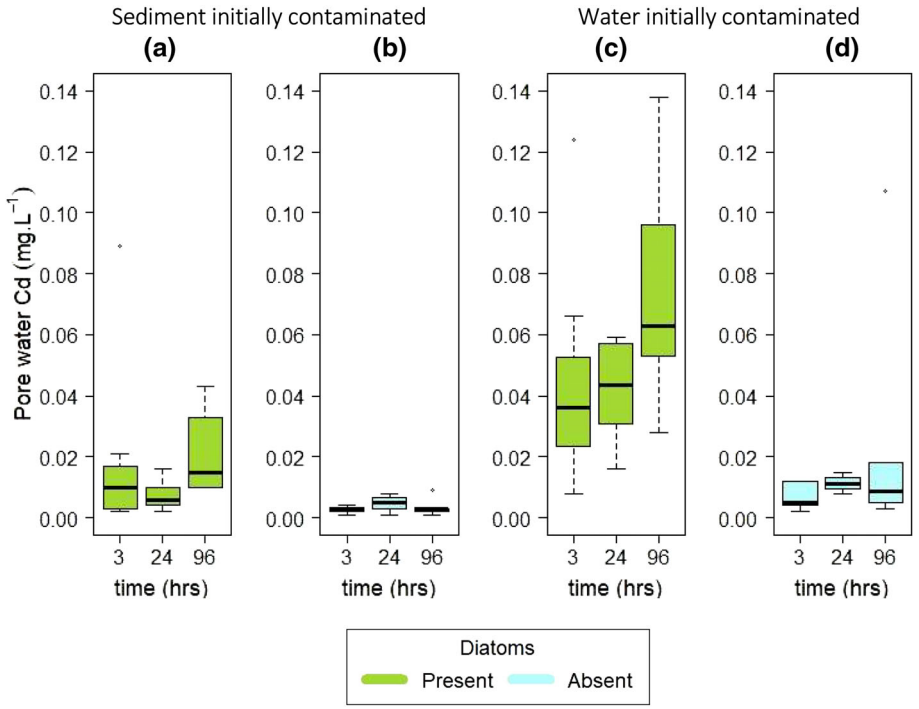

Fig. 8. Cadmium concentrations in pore water (mg L^{-1}), in both processed and unprocessed sediment, when (**a**, **b**) sediment is initially contaminated and (**c**, **d**) water is initially contaminated, outliers (°) calculated as Q1 − 1.5IQR and Q3 + 1.5IQR.

The only observed statistically significant difference in uptake of Cd to *C. closterium* from water occurred between treatments without sediment, ~ 2500 mg kg^{-1}, and those with processed sediment, ~ 1500 mg kg^{-1} (Fig. 3a,c). There was no difference in Cd uptake by *C. closterium* between treatments without sediment and those containing unprocessed sediment (Fig. 3b,c), or between treatments containing either unprocessed or processed sediment (Fig. 3a,b). This was unexpected in that processed sediment had a lower organic matter content and larger particle size than unprocessed sediment and was, therefore, expected to act in a more similar way to treatments without sediment.

In contrast, when sediment was the initially contaminated component, there was a difference in the total concentration of Cd in *C. closterium* between the sediment treatments, with greater uptake of Cd from processed than from unprocessed sediment (Fig. 3d,e). This may be due to two factors; first, unprocessed sediment has a higher organic matter content than processed sediment; and, second, a greater percentage of particles in unprocessed sediment were in the smaller size class compared to the processed sediment, this is due to both organic matter and small sediment particles being removed in the washing and drying process. Particle size, surface area, and composition all influence metal binding (Turner and Millward 2002). Metals preferentially attach to organic matter and smaller particle sizes, due to the availability of binding sites and high surface area (Fan et al. 2017). It is therefore suggested that a higher availability of binding sites in the unprocessed sediment results in lower Cd concentrations in *C. closterium*. This is in line with the findings of Eimers et al. (2002) that increasing sediment organic matter content increased Cd partitioning to sediment, reduced Cd in the overlying water and accumulation in the benthic detritivore *Asellus racovitzai*. There was an indication ($p = 5.7 \times 10^{-2}$) in the current study that there may be a corresponding difference in sediment concentration due to sediment type (Fig. 5a–d). The small size of this difference is probably due to the relative volume of sediment and algae.

There was no change in uptake of Cd to algae over time when either water or sediment was initially contaminated, although results for unprocessed sediment (Fig. 3b) suggest that, were the experiment to run for longer, there might be a reduction in Cd concentration over time. This result is in contrast to a study by Irving et al. (2009) which found a linear increase in Cd uptake over time to mats of the freshwater diatom *Navicula pelliculosa* from water contaminated to 7 μg L^{-1}. Morin et al. (2008) also reported a linear uptake of Cd to a diatom biofilm in an experiment that ran over 6 weeks with initial Cd concentrations in water of 10 and 100 μg L^{-1}. The fact that a linear increase uptake was not seen in the current study may be due to the inclusion of sediment. The only treatment which saw an increase in diatom Cd concentration from 3 to 96 h was the one in which there was no sediment present (Fig. 3c). Similarly, Cleveland et al. (2012), in an estuarine

mesocosm study, which included sediment, sand, and biota, did not report a linear increase in uptake of silver to biofilms.

The potential for benthic diatoms to take up metal contaminants from sediment is important as they represent the base of the food chain in estuarine locations, which are often associated with contaminated sediments (de Souza Machado et al. 2016). Although uptake is shown in this study, there was no increase in concentration in algae over time, which may indicate that uptake is limited. Alternatively, the limited uptake may be due to the static conditions of the laboratory study and it is suggested that the outcome may differ under field conditions.

Bioconcentration of cadmium in diatoms

Bioconcentration of Cd to *C. closterium* occurred across all treatments, whether the initial contamination was in the water or sediment.

There have been very few studies reporting transfer of metals from sediment to algae. A pilot study, carried out in the Scheldt estuary (Stronkhorst et al. 1994), found a BCF_{sed} for Cd in diatoms of 0.01–0.30 for sediment with a mean dry weight (DW) Cd concentration of 5.24 ± 3.31 mg kg^{-1} DW, which indicates no bioconcentration. However, a follow up study (Absil and van Scheppingen 1996) in the Westerschelde estuary found BCF_{sed} ranging from 0.01 to 16.3 for sediment with a Cd concentrations ranging from 0.08 to 7.9 mg kg^{-1} DW, with higher concentration factors occurring at sites with sediment Cd concentrations < 1.5 mg kg^{-1}. The second study shows greater agreement with results reported here, where a BCF_{sed} range of 3.0–71 across all time points when sediment was the initially contaminated component was observed. Previously reported BCF_{sed} may differ from those in the current study because the two cited studies (Stronkhorst et al. 1994; Absil and van Scheppingen 1996) were conducted in the field, where uptake is likely to be affected by the very different and variable environmental conditions. In particular, there is no exchange of water during the laboratory experiment and the volume of water relative to the volume of sediment is much smaller than in the field. The lack of a tidal cycle in the laboratory makes it possible for equilibrium between sediment and water to be achieved; while in the field, there will be a constant cycle between reductive and oxidative conditions. Additionally, in the field, resuspension of sediment by the tide and waves will cause remobilization of sediment bound metals (Eggleton and Thomas 2004). Partitioning and bioavailability of metals in the field is also affected by the variable salinity in estuarine location (Atkinson et al. 2007; Chapman et al. 2013), this in turn may affect bioconcentration (Mountouris et al. 2002).

Bioconcentration of Cd from water (in treatments without sediment) was an order of magnitude lower in this study (190–380, Table 3) than previously reported values (e.g., 1000; Fowler 2002). A study undertaken in Daya Bay, South China (Qiu 2015) reported BCF_{Wat} in phytoplankton of 4440 and in the macro algae *Ulva fasciata* of 5870 for concentrations in seawater of 0.12 ± 0.04 μg L^{-1} Cd. These differences may be due to the species studied, salinity, or variation between laboratory and field conditions and warrant further investigation. It has also been suggested that an increase in ambient nutrient concentration enhances metal uptake of Cd by algae (Wang and Dei 2001).

Alternatively, it is possible that the difference in BCF_{Wat} between this and other studies is due to the high initial Cd concentration in water used in this experiment. Although uptake is highest from water (Fig. 3), BCF_{Wat} is low (Table 3), suggesting a possible upper limit to the biosorption capacity of *C. closterium* for Cd. Torres et al. (2014) found a biosorption capacity for living cells of *Phaeodactylum tricornutum* from water of 67.1 ± 3.2 g kg^{-1} over 72 h. They also found that the highest BCF_{Wat} was obtained in cultures exposed to the lowest Cd concentration (1 mg L^{-1}), as Cd increased, BCF_{Wat} decreased to a minimum value of 419.3 at the highest (100 mg L^{-1}) water concentration. Reasons for this are first that at high concentrations, the ratio of binding sites to Cd is low and they are quickly saturated. Second, the toxicity of Cd at higher concentrations, which is likely to reduce the amount of Cd removed intracellularly. This may also explain why BCF_{Wat} values obtained from treatments with sediment were higher than in treatments without sediment (Table 3). Throughout the experimental period in treatments in which water was initially contaminated, sediment concentrations increased, while water concentrations decreased (Fig. 5). This reduced the water concentration to which *C. closterium* was exposed, reducing saturation of binding sites and potentially reducing toxic effect.

External and internal cadmium in *C. closterium*

Internal absorption into *C. closterium* cells was observed to be higher than adsorption to the cell surface for all but one treatment, which contained processed contaminated sediment at 24 h. This differs from other studies of Cd uptake by microalgae. A study of the planktonic marine microalgae *Tetraselmis suecica* (Pérez-Rama et al. 2010) found that, after 72 h, at initial water concentrations ≤ 6 mg L^{-1} Cd, the intracellular concentration was higher than the extracellular concentration, but at initial water concentrations of ≥ 15 mg L^{-1} Cd, extracellular concentration was higher. This was thought to be due to the toxic effect of Cd on algae. The same study found that at 24 h, all external Cd concentrations were higher than internal, probably because active uptake and incorporation to the interior cell occurs more slowly than external adsorption. Additionally, a study of Cd uptake from freshwater to a biofilm (Morin et al. 2008) found internal Cd was always lower than externally adsorbed Cd although it increased over the 6 week duration of the study.

Conversely, in line with the current findings, a study with *P. tricornutum* (Torres et al. 2014) found that at low water concentrations (≤ 10 mg L^{-1}), ~ 90% of Cd was internal to the diatom cell; however, this decreased over time and with increasing Cd concentrations. This was also in agreement with an earlier study (Torres et al. 1998) in which intracellular Cd was greater

than extracellular throughout the 96 h experiment. Difference in uptake between the current study and others is likely to be due to algal type (*T. suecica* is a green algae), as uptake differs with genus and species (Suresh Kumar et al. 2015), and salinity as well as initial Cd concentration. Other studies which examined diatoms in seawater (Torres et al. 1998, 2014) found results similar to those from the current study.

Effect of *C. closterium* on cadmium partitioning

Change in Cd concentration in sediment and water and uptake by diatoms for different treatments is illustrated in Fig. 9.

Cadmium in sediment and water when water is initially contaminated

When water was the initially contaminated component, water Cd concentration decreased over time, with a corresponding increase in the sediment Cd concentration (Fig. 5). This is to be expected as although sediment was stored for 40 d in an oxygen free environment (Simpson et al. 2004) following contamination (to allow equilibration between sediment and pore water, ensure Cd was bound to sediment, and ensure porewater concentrations were low), there was necessarily no equilibration between sediment and overlying water prior to the start of the experiment. However, it was also observed that, in the presence of *C. closterium*, Cd concentrations in the water decreased more quickly and concentrations in the sediment increased more slowly. This indicates that algae takes up metal contamination from water more readily and over shorter timescales than sediment. This was previously observed in a study of metal sorption to a range of sediment components in which Cd had a stronger affinity to the green algae, *Scenedesmus quadricauda*, than the mineral constituents, bentonite, aluminum oxide, goethite, and quartz (Calmano et al. 1988). Following initial differences in sediment and water concentrations in treatments with and without *C. closterium*, sediment concentrations increased and water concentrations reduced to similar values across all treatments by 96 h. This indicates a potential two-stage process in treatments with *C. closterium*, in which Cd is first taken up by the diatoms and then transferred to the sediment.

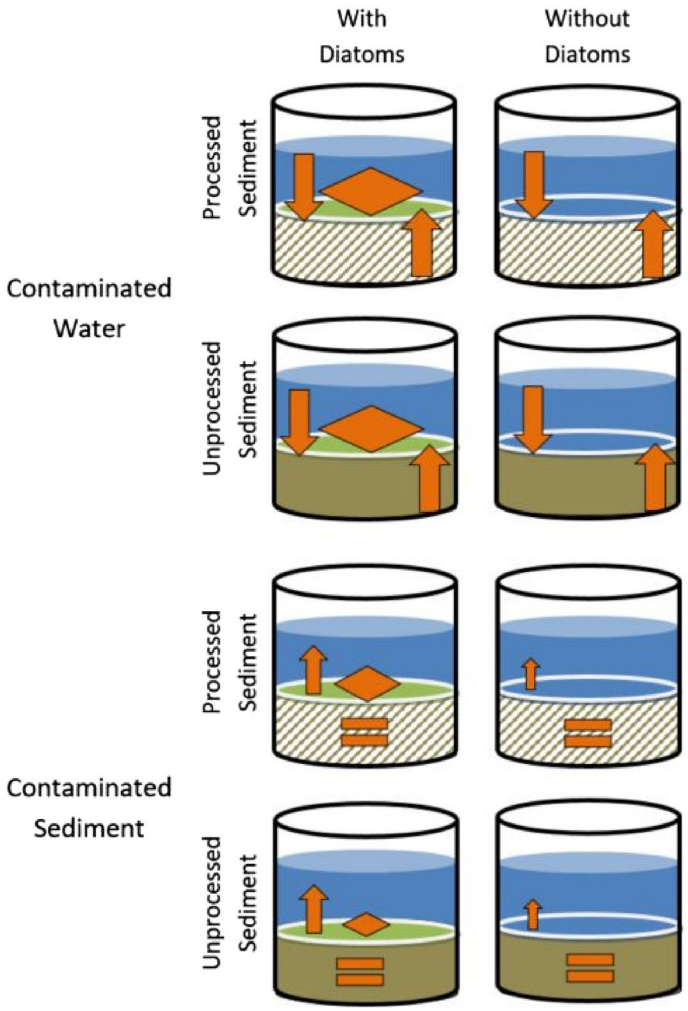

Fig. 9. Movement of cadmium between water and sediment and uptake by diatoms under different contaminant and sediment types. Symbols indicate ↑ increase or ↓ decrease or = no change in Cd in compartment, ♦ indicates Cd in diatoms. Size relates Cd concentration.

Cadmium in sediment and water when sediment is initially contaminated

There was no difference in sediment Cd concentration due to the presence of diatoms when sediment was the initially contaminated component (Fig. 6a–d). As previously stated, this may be due to the comparative volume of sediment and algae, with a greater volume of sediment providing a greater number of binding sites, so although there is uptake by algae, sediment Cd concentrations are not observably reduced.

When sediment was the initially contaminated component, the Cd concentration in overlying water increased over time (Fig. 6e–h). It was further observed that concentrations in water were greater in the presence of *C. closterium* than when there were no diatoms present, suggesting that the microalgae facilitate the release of Cd from the sediment to the overlying water. This release may be due to three mechanisms: first, an alteration of redox conditions at the sediment surface in the presence of diatoms; second, the excretion of extracellular polymeric substances (EPSs) by diatoms; and finally, Cd efflux from cells.

Release of metals from sediment is known to take place due to changes in the properties of overlying water, such as pH, dissolved oxygen concentration, and salinity (Simpson et al. 2004). These properties are altered at the sediment water interface by the presence of benthic algae, which increases oxygen through photosynthesis (Yamamoto et al. 2008). It has been observed that, in Hiroshima Bay, Japan, in sites where sediment was seeded with microphytobenthos redox potential increased and acid-volatile sulfides decreased (Yamamoto et al. 2008). However, a study in France, using benthic chambers at the

sediment surface, found there was a positive flux of Cd from the sediment to the water column with increasing respiration, i.e., when available oxygen was reduced (Amouroux et al. 2003). Properties at the sediment water interface are important in contaminant flux and these changes may account for releases to the overlying water in this study.

EPS is a term, which encompasses a wide variety of microbially secreted molecules, comprised primarily of polysaccharides, with physical states ranging from a thick gel to dissolved organic carbon in solution and has a variety of chemical properties and many biological roles (Decho 2000). Secretion of EPS by diatoms is closely related to motility (Smith and Underwood 1998), with production occurring in response to changing light, nutrient availability, and physiological condition (De Brouwer and Stal 2002). A study of *C. closterium* (De Brouwer and Stal 2002) distinguished two EPS fractions, a soluble fraction (present in the culture supernatant) and a bound fraction (which could be extracted using water at 30°C). It is hypothesized that the soluble fraction may contribute contaminants to the overlying water. Adsorption of Cd to inorganic particles is reduced in the presence of dissolved organic matter (DOM) (Simpson 1981). An increase of DOM due to the presence of EPS could reduce adsorption of Cd to sediment and increase concentrations in the water column. However, a previous study (Schlekat et al. 1998) showed that bacterial EPS increased the binding of Cd to estuarine sediment. Differences in the availability of Cd in the presence of EPS may be due different properties of bacterial and diatom produced EPS, as well as pH and salinity (Gutierrez et al. 2012).

Production of phytochelatin is induced by the presence of certain metals, of which Cd has been shown to be the best activator (Cobbett 2000). In marine diatoms, phytochelatins complex with potentially harmful metals and are transported to vacuoles, whereby they are rendered inert (Nikinmaa 2014). It has been shown that under high concentrations of Cd, there is an efflux of Cd and phytochelatins from diatoms to growth media (Lee et al. 1996). This effect has also been seen with mercury (Deng et al. 2013) and, although the mechanism is not fully understood (Masmoudi et al. 2013), it is speculated that there may be export of phytochelatin-metal complex as a detoxification mechanism (Lee et al. 1996; Deng et al. 2013).

The observed release of Cd from the sediment to overlying water in the presence of microalgae has implications for potential exposure of other intertidal organisms. Further study is required to better understand both this and the role of benthic diatoms in biogeochemical cycling.

Change in cadmium concentration of pore water

There was difficulty in extracting pore water from treatments containing processed sediment, the properties of which (lower organic matter content and larger particle size) affected its ability to hold water. In treatments which contained *C. closterium*, the sediment held a greater volume of water than those without. This had implications in terms of replication and meant that no statistical test of Cd concentrations between sediment types could be carried out. However, it was possible to compare Cd concentrations in pore water between treatments in which either sediment or water was initially contaminated and between those with and without *C. closterium*. It was observed that Cd concentrations in pore water were greater when water was initially contaminated and when *C. closterium* was present in the treatments, regardless of whether water or sediment was initially contaminated (Fig. 8).

Higher Cd concentrations in pore water when water is initially contaminated are probably because there was no equilibration between sediment and water prior to the start of the experiment. Reasons for higher pore-water Cd concentrations in the presence of diatoms are probably related to oxygen availability and EPS excretion as described for overlying water, but would require further investigation.

Conclusions

Through this study, it has been established that the diatom *C. closterium* takes up a significant quantity of Cd from the sediment, as well as the water column, and that this differs with sediment properties. It has also been shown that the presence of diatoms affects partitioning of Cd between sediment and water in that when sediment is initially contaminated its presence increases Cd concentrations in overlying and pore water. Conversely, when water is initially contaminated, diatoms slow the transfer of Cd to the sediment.

These findings are important to our understanding of metal bioavailability in the estuarine system. Diatoms at the sediment surface will quickly sequester metal contaminants from the water and sediment with the potential that these will be passed up the food chain. Second, by increasing Cd concentrations in the overlying and pore-water diatoms may increase bioavailability to other organisms.

Further work is required to establish the controls on contaminant uptake from sediment and differences that may occur due to other environmental conditions such as pH, salinity, light, and nutrient levels. Additionally, there is more work to be carried out in understanding why the presence of diatoms increases Cd in the dissolved phase, in particular, whether this is due to EPS production, increased oxygen at the sediment water interface, or efflux of phytochelatin-Cd complexes. This study has shown that, using the described methods under controlled laboratory conditions, it is possible to study metal uptake from sediment to benthic microalgae, paving the way for these further questions to be investigated.

References

Absil, M. C. P., and Y. van Scheppingen. 1996. Concentrations of selected heavy metals in benthic diatoms and sediment in the Westerschelde Estuary. Bull. Environ. Contam. Toxicol. **56**: 1008–1015. doi:10.1007/s001289900145

Adams, M., and J. Stauber. 2004. Development of a whole-sediment toxicity test using a benthic marine microalga. Environ. Toxicol. Chem. **23**: 1957–1968. doi:10.1897/03-232

Amouroux, D., and others. 2003. Transfer of metallic contaminants at the sediment-water interface in a coastal lagoon: Role of the biological and microbial activity. J. Phys. IV **107**: 41–44. doi:10.1051/jp4:20030238

Araujo, C. V. M., J. Blasco, and I. Moreno-Garrido. 2010. Microphytobenthos in ecotoxicology: A review of the use of marine benthic diatoms in bioassays. Environ. Int. **36**: 637–646. doi:10.1016/j.envint.2010.04.017

Atkinson, C. A., D. F. Jolley, and S. L. Simpson. 2007. Effect of overlying water pH, dissolved oxygen, salinity and sediment disturbances on metal release and sequestration from metal contaminated marine sediments. Chemosphere **69**: 1428–1437. doi:10.1016/j.chemosphere.2007.04.068

Baillie, P. W., and B. L. Welsh. 1980. The effect of tidal resuspension on the distribution of intertidal epipelic algae in an estuary. Estuar. Coast. Mar. Sci. **10**: 165–180. doi:10.1016/S0302-3524(80)80056-9

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. **67**: 1–48. doi:18637/jss.v067.i01

Beldowska, M., A. Jedruch, J. Slupkowska, D. Saniewska, and M. Saniewski. 2015. Macrophyta as a vector of contemporary and historical mercury from the marine environment to the trophic web. Environ. Sci. Pollut. Res. **22**: 5228–5240. doi:10.1007/s11356-014-4003-4

Bryan, G. W., and W. J. Langston. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: A review. Environ. Pollut. **76**: 89–131. doi:10.1016/0269-7491(92)90099-V

Buchman, M. F. 2008. NOAA screening quick reference tables, p. 1–34. NOAA OR&R report 08–1. Office of Response and Restoration Division, National Oceanic and Atmospheric Administration, Seattle, WA.

Calmano, W., W. Ahlf, and U. Forstner. 1988. Study of metal sorption desorption processes on competing sediment components with a multichamber device. Environ. Geol. Water Sci. **11**: 77–84. doi:10.1007/BF02587766

Chapman, P. M., F. Wang, and S. S. Caeiro. 2013. Assessing and managing sediment contamination in transitional waters. Environ. Int. **55**: 71–91. doi:10.1016/j.envint.2013.02.009

Cleveland, D., and others. 2012. Pilot estuarine mesocosm study on the environmental fate of silver nanomaterials leached from consumer products. Sci. Total Environ. **421–422**: 267–272. doi:101016/j.scitotenv.2012.01.025

Cobbett, C. S. 2000. Phytochelatins and their roles in heavy metal detoxification. Plant Physiol. **123**: 825–832. doi:10.1104/pp.123.3.825

De Brouwer, J. F. C., and L. J. Stal. 2002. Daily fluctuations of exopolymers in cultures of the benthic diatoms *Cylindrotheca closterium* and *Nitzschia* sp. (Bacillariophyceae). J. Phycol. **38**: 464–472. doi:10.1046/j.1529-8817.2002.01164.x

De Jonge, V., and J. Van Beusekom. 1995. Wind-and tide-induced resuspension of sediment and microphytobenthos from tidal flats in the Ems Estuary. Limnol. Oceanogr. **40**: 766–778. doi:10.4319/lo.1995.40.4.0776

De Orte, M. R., A. T. Lombardi, A. M. Sarmiento, M. D. Basallote, A. Rodriguez-Romero, I. Riba, and A. Del Valls. 2014. Metal mobility and toxicity to microalgae associated with acidification of sediments: CO_2 and acid comparison. Mar. Environ. Res. **96**: 136–144. doi:10.1016/j.marenvres.2013.10.003

de Souza Machado, A. A., K. Spencer, W. Kloas, M. Toffolon, and C. Zarfl. 2016. Metal fate and effects in estuaries: A review and conceptual model for better understanding of toxicity. Sci. Total Environ. **541**: 268–281. doi:10.1016/j.scitotenv.2015.09.045

Dean, W. E., Jr. 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: Comparison with other methods. J. Sediment. Petrol. **44**: 242–248. doi:10.1306/74D729D2-2B21-11D7-8648000102C1865D

Decho, A. 2000. Microbial biofilms in intertidal systems: An overview. Cont. Shelf Res. **20**: 1257–1273. doi:10.1016/S0278-4343(00)00022-4

Deng, G. F., T. W. Zhang, L. M. Yang, and Q. Q. Wang. 2013. Studies of biouptake and transformation of mercury by a typical unicellular diatom *Phaeodactylum tricornutum*. Chin. Sci. Bull. **58**: 256–265. doi:10.1007/s11434-012-5514-3

Eaton, J. W., and B. Moss. 1966. The estimation of numbers and pigment content in epipelic algal populations. Limnol. Oceanogr. **11**: 584–595. doi:10.4319/lo.1966.11.4.0584

Eggleton, J., and K. Thomas. 2004. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environ. Int. **30**: 973–980. doi:10.1016/j.envint.2004.03.001

Eimers, M. C., R. Douglas Evans, and P. M. Welbourn. 2002. Partitioning and bioaccumulation of cadmium in artificial sediment systems: Application of a stable isotope tracer technique. Chemosphere **46**: 543–551. doi:10.1016/S0045-6535(01)00156-4

Fan, J., G. Zhao, and J. Sun. 2017. Binary component sorption of cadmium and copper ions onto Yangtze River sediments with different particle sizes. Sustainability **9**: 2089. doi:10.3390/su9112089

Fowler, S. W. 2002. Role of plankton in controlling fluxes and residence times of metals and radionuclides in the sea, p. 63–69. *In* F. Briand [ed.], Metal and radionuclides bioaccumulation in marine organisms. CIESM.

Guillard, R. R., and J. H. Ryther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (cleve) Gran. Can. J. Microbiol. **8**: 229–239. doi:10.1139/m62-029

Gutierrez, T., D. V. Biller, T. Shimmield, and D. H. Green. 2012. Metal binding properties of the EPS produced by *Halomonas* sp. TG39 and its potential in enhancing trace element

bioavailability to eukaryotic phytoplankton. Biometals **25**: 1185–1194. doi:10.1007/s10534-012-9581-3

He, J., and J. P. Chen. 2014. A comprehensive review on biosorption of heavy metals by algal biomass: Materials, performances, chemistry, and modelling simulation tools. Bioresour. Technol. **160**: 67–78. doi:10.1016/j.biortech.2014.01.068

Heldal, H., I. Stupakoff, and N. Fisher. 2001. Bioaccumulation of ^{137}Cs and ^{57}Co by five marine phytoplankton species. J. Environ. Radioact. **57**: 231–236. doi:10.1016/S0265-931X(01)00020-0

Hutchins, C. M., P. R. Teasdale, J. Lee, and S. L. Simpson. 2007. The effect of manipulating sediment pH on the porewater chemistry of copper- and zinc-spiked sediments. Chemosphere **69**: 1089–1099. doi:10.1016/j.chemosphere.2007.04.029

Irving, E. C., D. J. Baird, and J. M. Culp. 2009. Cadmium toxicity and uptake by mats of the freshwater diatom: *Navicula pelliculosa* (Bréb) Hilse. Arch. Environ. Contam. Toxicol. **57**: 524–530. doi:10.1007/s00244-009-9299-6

Kanaya, G., S. Takagi, and E. Kikuchi. 2008. Dietary contribution of the microphytobenthos to infaunal deposit feeders in an estuarine mudflat in Japan. Mar. Biol. **155**: 543–553. doi:10.1007/s00227-008-1053-5

Lane, T. W., and F. M. M. Morel. 2000. A biological function for cadmium in marine diatoms. Proc. Natl. Acad. Sci. USA **97**: 4627–4631. doi:10.1073/pnas.090091397

Laube, V., S. Ramamoorthy, and D. J. Kushner. 1979. Mobilization and accumulation of sediment bound heavy metals by algae. Bull. Environ. Contam. Toxicol. **21**: 763–770. doi:10.1007/BF01685502

Lee, C., and N. S. Fisher. 2016. Methylmercury uptake by diverse marine phytoplankton. Limnol. Oceanogr. **61**: 1626–1639. doi:10.1002/lno.10318

Lee, J. G., B. A. Ahner, and F. M. M. Morel. 1996. Export of cadmium and phytochelatin by the marine diatom *Thalassiosira weissflogii*. Environ. Sci. Technol. **30**: 1814–1821. doi:10.1021/es950331p

MacIntyre, H. L., R. J. Geider, and D. C. Miller. 1996. Microphytobenthos: The ecological role of the 'secret garden' of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production. Estuaries **19**: 186–201. doi:10.2307/1352224

Maret, W., and J. M. Moulis. 2013. The bioinorganic chemistry of cadmium in the context of its toxicity. Met. Ions Life Sci. **11**: 1–29.doi:10.1515/9783110434330-001

Masmoudi, S., N. Nguyen-Deroche, A. Caruso, H. Ayadi, A. Morant-Manceau, G. Tremblin, M. Bertrand, and B. Schoefs. 2013. Cadmium, copper, sodium and zinc effects on diatoms: From heaven to hell – a review. Cryptogam. Algol. **34**: 185–225. doi:10.7872/crya.v34.iss2.2013.185

Mauffret, A., I. Moreno-Garrido, and J. Blasco. 2010. The use of marine benthic diatoms in a growth inhibition test with spiked whole-sediment. Ecotoxicol. Environ. Saf. **73**: 262–269. doi:10.1016/j.ecoenv.2009.12.001

McCormick, J., C. T. S. Clair, and L. I. Bendell. 2014. Concentration and partitioning of metals in intertidal biofilms: Implications for metal bioavailability to shorebirds. Ecotoxicology **23**: 229–235. doi:10.1007/s10646-013-1166-6

Meleder, V., P. Launeau, L. Barille, J. Combe, V. Carrere, B. Jesus, and C. Verpoorter. 2010. Hyperspectral imaging for mapping microphytobenthos in coastal areas, p. 71–139. *In* M. Maanan and M. Robin [eds.], Geomatic solutions for coastal environments. Nova Science Publishers.

Moreno-Garrido, I., M. Hampel, L. M. Lubián, and J. Blasco. 2003. Sediment toxicity tests using benthic marine microalgae *Cylindrotheca closterium* (Ehremberg) Lewin and Reimann (Bacillariophyceae). Ecotoxicol. Environ. Saf. **54**: 290–295. doi:10.1016/S0147-6513(02)00077-5

Moreno-Garrido, I., L. M. Lubián, B. Jiménez, A. M. V. M. Soares, and J. Blasco. 2007. Estuarine sediment toxicity tests on diatoms: Sensitivity comparison for three species. Estuar. Coast. Shelf Sci. **71**: 278–286. doi:10.1016/j.ecss.2006.08.003

Morin, S., T. T. Duong, O. Herlory, A. Feurtet-Mazel, and M. Coste. 2008. Cadmium toxicity and bioaccumulation in freshwater biofilms. Arch. Environ. Contam. Toxicol. **54**: 173–186. doi:10.1007/s00244-007-9022-4

Mountouris, A., E. Voutsas, and D. Tassios. 2002. Bioconcentration of heavy metals in aquatic environments: The importance of bioavailability. Mar. Pollut. Bull. **44**: 1136–1141. doi:10.1016/S0025-326X(02)00168-6

Mucha, A. P., M. F. C. Leal, A. A. Bordalo, and M. T. S. D. Vasconcelos. 2003. Comparison of the response of three microalgae species exposed to elutriates of estuarine sediments based on growth and chemical speciation. Environ. Toxicol. Chem. **22**: 576–585. doi:10.1002/etc.5620220316

Nikinmaa, N. 2014. Detoxification, p. 87–89. *In* M. Nikinmaa [ed.], An introduction to aquatic toxicology. Academic Press.

Paquet, N., M. Lavoie, F. Maloney, J. F. L. Duval, P. G. C. Campbell, and C. Fortin. 2015. Cadmium accumulation and toxicity in the unicellular alga *Pseudokirchneriella subcapitata*: Influence of metal-binding exudates and exposure time. Environ. Toxicol. Chem. **34**: 1524–1532. doi:10.1002/etc.2927

Park, H., B. Song, and F. M. M. Morel. 2007. Diversity of cadmium-containing carbonic anhydrase in marine diatoms and natural waters. Environ. Microbiol. **9**: 403–413. doi:10.1111/j.1462-2920.2006.01151.x

Pérez-Rama, M., E. Torres, C. Suárez, C. Herrero, and J. Abalde. 2010. Sorption isotherm studies of Cd(II) ions using living cells of the marine microalga *Tetraselmis suecica* (Kylin) Butch. J. Environ. Manage. **91**: 2045–2050. doi:10.1016/j.jenvman.2010.05.014

Qiu, Y.-W. 2015. Bioaccumulation of heavy metals both in wild and mariculture food chains in Daya Bay, South China. Estuar. Coast. Shelf Sci. **163**: 7–14. doi:10.1016/j.ecss.2015.05.036

Schlekat, C. E., A. W. Decho, and G. T. Chandler. 1998. Sorption of cadmium to bacterial extracellular polymeric sediment coatings under estuarine conditions. Environ. Toxicol. Chem. **17**: 1867–1874. doi:10.1002/etc.5620170930

Schmitt, D., A. Muller, Z. Csogor, F. Frimmel, and C. Posten. 2001. The adsorption kinetics of metal ions onto different microalgae and siliceous earth. Water Res. **35**: 779–785. doi:10.1016/S0043-1354(00)00317-1

Silva-Aciares, F. R., and C. E. Riquelme. 2008. Comparisons of the growth of six diatom species between two configurations of photobioreactors. Aquac. Eng. **38**: 26–35. doi:10.1016/j.aquaeng.2007.10.005

Simpson, S., B. Angel, and D. Jolley. 2004. Metal equilibration in laboratory-contaminated (spiked) sediments used for the development of whole-sediment toxicity tests. Chemosphere **54**: 597–609. doi:10.1016/j.chemosphere.2003.08.007

Simpson, W. R. 1981. A critical review of cadmium in the marine environment. Prog. Oceanogr. **10**: 1–70. doi:10.1016/0079-6611(81)90007-0

Smith, D. J., and G. J. C. Underwood. 1998. Exopolymer production by intertidal epipelic diatoms. Limnol. Oceanogr. **43**: 1578–1591. doi:10.4319/lo.1998.43.7.1578

Stal, L. J., and J. F. C. de Brouwer. 2003. Biofilm formation by benthic diatoms and their influence on the stabilization of intertidal mudflat, p. 109–111. *In* J. Rullkötter [ed.], Proceedings of a Workshop at the Hanse Institute of Advanced Study, May 14–17, Delmenhorst, Germany. Forschungszentrum Terramare.

Stronkhorst, J., P. C. Vos, and R. Misdorp. 1994. Trace metals, PCBs, and PAHs in benthic (epipelic) diatoms from intertidal sediments: A pilot study. Bull. Environ. Contam. Toxicol. **52**: 818–824. doi:10.1007/BF00200689

Sunda, W. G., and S. A. Huntsman. 1996. Antagonisms between cadmium and zinc toxicity and manganese limitation in a coastal diatom. Limnol. Oceanogr. **41**: 373–387. doi:10.4319/lo.1996.41.3.0373

Suresh Kumar, K., H.-U. Dahms, E.-J. Won, J.-S. Lee, and K.-H. Shin. 2015. Microalgae – a promising tool for heavy metal remediation. Ecotoxicol. Environ. Saf. **113**: 329–352. doi:10.1016/j.ecoenv.2014.12.019

Tanaka, N. 1984. The cell division rates of ten species of attaching diatoms in natural seawater. Bull. Jpn. Soc. Sci. Fish. **50**: 969–972. doi:10.2331/suisan.50.969

Torres, E., A. Cid, C. Herrero, and J. Abalde. 1998. Removal of cadmium ions by the marine diatom *Phaeodactylum tricornutum* Bohlin accumulation and long-term kinetics of uptake. Bioresour. Technol. **63**: 213–220. doi:10.1016/S0960-8524(97)00143-0

Torres, E., R. Mera, C. Herrero, and J. Abalde. 2014. Isotherm studies for the determination of Cd(II) ions removal capacity in living biomass of a microalga with high tolerance to cadmium toxicity. Environ. Sci. Pollut. Res. **21**: 12616–12628. doi:10.1007/s11356-014-3207-y

Turner, A., and G. E. Millward. 2002. Suspended particles: Their role in estuarine biogeochemical cycles. Estuar. Coast. Shelf Sci. **55**: 857–883. doi:10.1006/ecss.2002.1033

USEPA. 2001. Method for assessing the chronic toxicity of marine and estuarine sediment associated contaminants with the amphipod *Leptocheirus plumulosus*. EPA-600-R-01-020. USA Environmental Protection Agency, Washington, DC.

USEPA. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA-821-R-02-013. USA Environmental Protection Agency, Washington, DC.

USEPA. 2007. Method 3051A, Microwave assisted acid digestion of sediments, sludges, soils and oils. Revision 1, p. 30. USA Environmental Protection Agency, Washington, DC.

Van de Koppel, J., P. M. J. Herman, P. Thoolen, and C. H. R. Heip. 2001. Do alternate stable states occur in natural ecosystems? Evidence from a tidal flat. Ecology **82**: 3449–3461. doi:10.1890/0012-9658(2001)082[3449:DASSOI]2.0.CO;2

Wang, M.-J., and W.-X. Wang. 2009. Cadmium in three marine phytoplankton: Accumulation, subcellular fate and thiol induction. Aquat. Toxicol. **95**: 99–107. doi:10.1016/j.aquatox.2009.08.006

Wang, W.-X., and R. C. H. Dei. 2001. Metal uptake in a coastal diatom influenced by major nutrients (N, P and Si). Water Res. **35**: 315–321. doi:10.1016/S0043-1354(00)00256-6

Yamamoto, T., I. Goto, O. Kawaguchi, K. Minagawa, E. Ariyoshi, and O. Matsuda. 2008. Phytoremediation of shallow organically enriched marine sediments using benthic microalgae. Mar. Pollut. Bull. **57**: 108–115. doi:10.1016/j.marpolbul.2007.10.006

Yoshino, K., N. K. Tsugeki, Y. Amano, Y. Hayami, H. Hamaoka, and K. Omori. 2012. Intertidal bare mudflats subsidize subtidal production through outwelling of benthic microalgae. Estuar. Coast. Shelf Sci. **109**: 138–143. doi:10.1016/j.ecss.2012.05.021

Zidour, M., and others. 2019. Population response of the estuarine copepod *Eurytemora affinis* to its bioaccumulation of trace metals. Chemosphere **220**: 505–513. doi:10.1016/j.chemosphere.2018.12.148

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Conflict of Interest

None declared.

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