

Trace metal interactions in the macroalga *Enteromorpha prolifera* (O. F. Müller) J.Ag., grown in water of the Scheldt estuary (Belgium & SW Netherlands), in response to cadmium exposure

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The littoral macroalga *Enteromorpha prolifera* was sampled along the Scheldt estuary at Lillo (Belgium), Bath and Ellewoutsdijk (SW Netherlands). The algae were incubated in water from the same locations with salinities of 7, 14 and 23‰, at about $50 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ irradiance and 15°C . One series of cultures was exposed to 400 nM Cd , a second series served as a control. Temporal changes in cadmium, copper, iron, lead and zinc contents of *E. prolifera* were monitored with atomic absorption spectroscopy in a 21 day experiment. Both synergistic and antagonistic metal interactions became evident in response to cadmium uptake. During the first 8 days of incubation iron contents increased in both cadmium-exposed and control algae. However, iron contents were relatively higher in cadmium-exposed algae. Similar, but weaker synergisms were also found between lead/cadmium and copper/cadmium. Zinc contents decreased in controls and cadmium-exposed algae, but were significantly lower in cadmium-exposed algae. A distinct antagonism between cadmium and zinc was observed in cadmium-exposed algae.

Keywords: antagonism, cadmium, *Enteromorpha*, metal, synergism

Introduction

Green macroalgae occur frequently in the littoral zone of the Scheldt and its estuary (Figure 1). The euryhaline *Enteromorpha* spp. grow on the tidal mudflats, attached to hard substrates. Levels of dissolved metals, in particular copper and zinc, are high in the polluted Scheldt estuary: up to 150 and 240 nM, respectively (Van den berg *et al.* 1987). Other potentially toxic metals, cadmium and lead, occur at levels lower than 4 and 10 nM, respectively. Precise concentrations of bioavailable metal species cannot be given for natural waters, whereas macrophytes metal contents are a widely accepted index of availability (Seeliger & Cordazzo 1982, Haritonidis *et al.* 1983, Ho 1990, Say *et al.* 1990, Malea 1992). Algae may alter their internal metal balance by selective uptake: synergisms of copper with iron and

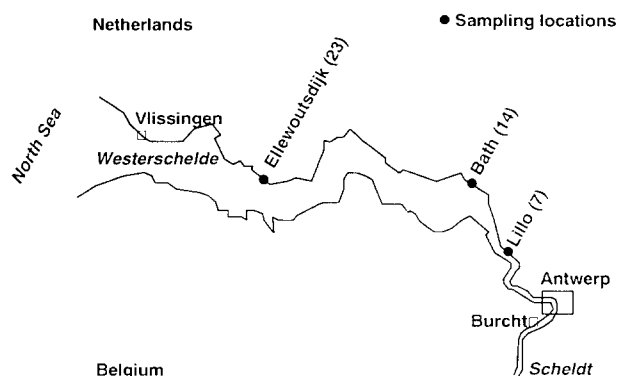


Figure 1. The river Scheldt and its estuary (Westerschelde) with sampling locations Lillo, Bath and Ellewoutsdijk. Salinities (‰) are given within brackets.

zinc in macroalgae from the Scheldt estuary were recently described by Rijstenbil *et al.* (1993).

Metal interactions in algae are attributed to differences in bioavailability in sediments and waters. Available metal species (e.g. ions) have

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been known to cause problems upon uptake by marine macrophyceae (Haritonidis & Nikolaidis 1989). As metal toxicity is a net result of all metal fluxes involved, it is important to monitor the effects of exposure to one metal on other algal metal pools (e.g. copper, lead and zinc in response to cadmium). Cadmium occurs at low concentrations in the Scheldt, but may increase upon incidental, local inputs. In this paper it was intended to search for cadmium-induced synergistic and antagonistic interactions of trace metals (copper, iron, lead and zinc) in *E. prolifera*. Algae were collected on from three locations along the Scheldt (Figure 1), and incubated in water sampled at the same locations, with or without an additional amount of cadmium ions.

Materials and methods

E. prolifera (O. F. Müller) J.Ag. was collected along the shores of the Scheldt as indicated in Figure 1, between mean and low tidal level (17 June 1991). The thalli of algae were rinsed and stored in estuarine water, previously sieved over 20 μm mesh gauze, and transported cool. At each location 20 l of water was sampled in metal-cleaned polyethylene containers (soaked in HNO_3), and sieved over 20 μm mesh gauze. Portions of 5 l of this water, stored in metal-cleaned flasks, were placed for 1 h in a Bransonic 52 ultrasonic bath. After sonication a precipitate of detritus and microorganisms was formed. To eliminate these particles, that may interfere with algal metal uptake, 2 l portions of estuarine water were decanted into (metal-cleaned) 3 l erlenmeyer flasks and served as incubation media for the algae. Some of this water was used for cleaning of about 10 g of wet algal biomass that were added to each flask with water of its corresponding salinity (Figure 1). There is no effective washing procedure to separate adsorbed metal from incorporated (cytosolic) metal. With phytoplankton cells, we noticed that the commonly used EDTA at normal pH (8) stripped only part of the metals (copper and zinc) from the cell wall, but also extracted part of the cytosolic metal. For this reason we do not trust washing procedures. However, because we have established that purely chemical sorption of metals is not a matter of days, but of hours, we are convinced that what we observe is real uptake in algal tissue. For each station, one culture was enriched with 400 nM $\text{Cd}(\text{NO}_3)_2$, a second culture served as a control. Algae were incubated at 15 °C and illuminated (Philips TL 33) at 50 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$ (QSL-100 quantum meter) in a 12:12 h light:dark cycle. Salinity was measured using a WTW LFCNX2000 conductivity meter. The pH of the estuarine Westerschelde (control) water was 7.9 and reached a value of 7.8 in the 400 nM cadmium series.

Ammonium, nitrate and phosphate were analyzed (Strickland & Parsons 1968) to check for nutrient limitations. Nutrients concentrations remained well above

limiting values (0.7–7.8 μM $\text{NH}_4\text{-N}$; 60.7–202.1 μM $\text{NO}_3\text{-N}$; 1.0–5.5 μM $\text{PO}_4\text{-P}$) throughout the experiment and are therefore not discussed in the Results section.

As incorporated metals give a better insight in availability than total dissolved metal levels, only algal metal contents were considered. Algal subsamples (about 1.5 g wet weight) were taken on days 2, 4, 7, 14 and 21, instantly dried on metal-clean filter paper, and stored at -70°C . For metal analyses, algae were freeze-dried and transported frozen to the Institute of Plant Physiology, University of Giessen (Germany). Algae [0.1 g dry weight (dwt) per subsample] were digested in HNO_3 , and analyzed as described earlier (Haritonidis *et al.* 1983): for iron and zinc the flame atomic absorption spectroscopy (AAS) method (Perkin-Elmer 360) was used, and for cadmium, copper and lead, the graphite-furnace-AAS method (GF-72) with Zeeman correction.

Results

After adding 400 nM Cd to the media, a marked increase of this metal in *E. prolifera* was evident on day 2 (Figure 2): from 0.09 to 0.81 $\mu\text{mol Cd g}^{-1}$ dwt at 7% salinity (Lillo), from 0.03 to 0.84 $\mu\text{mol Cd g}^{-1}$ dwt at 14% salinity (Bath) and from 0.02 to 0.57 $\mu\text{mol Cd g}^{-1}$ dwt at 23% salinity (Ellewoutsdijk). Cadmium contents remained very low in the control algae, and kept increasing in the cadmium-exposed algae throughout the experiment. This rather gradual, continuous cadmium-uptake may imply that its dosage was low relative to the exposed biomass and that the additional amount of cadmium incorporated probably was not critical to *E. prolifera*'s metabolism.

Shortly after adding cadmium, copper contents became slightly higher than those of the controls, but did not increase further from day 2 to 21 (Figure 2). This phenomenon was most clearly observed in the samples from Lillo and Bath (Figure 2a and b). In spite of the relatively high initial copper content of the algae from Ellewoutsdijk, differences between cadmium-exposed and control algae were of minor importance (Figure 2c).

A cadmium-induced increase of lead was noticed in *E. prolifera* sampled at Lillo (Table 1). Here, initial lead contents were twice as high as those from Bath and Ellewoutsdijk. In algae from Bath, lead showed a weak response to cadmium addition. In the Ellewoutsdijk samples, lead contents were almost duplicates. The lead co-uptake appeared to be inversely related to salinity.

Non-toxic iron was co-transported with cadmium into the algal tissue, whilst there was a considerable loss of algal zinc in the cultures from all locations (Figure 3). This appeared to have been an immedi-

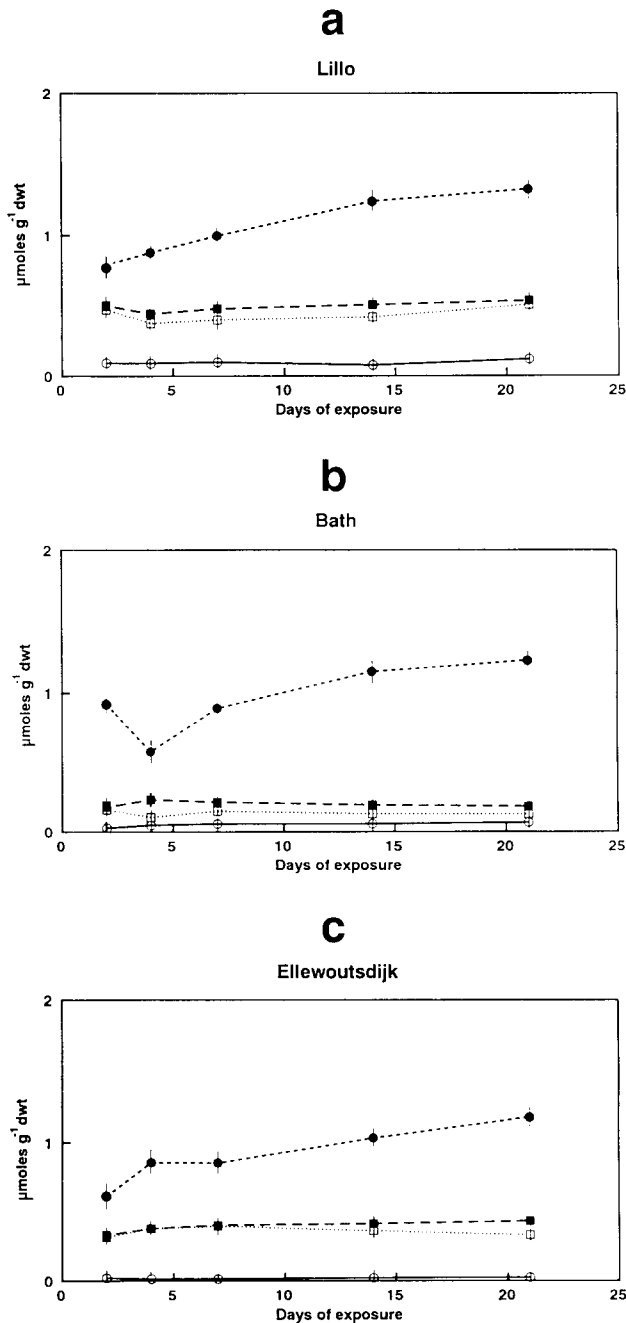


Figure 2. Cadmium and copper contents of *E. prolifera* versus time, after a 400 nM Cd dosage (+ series), relative to controls (– series). Figure headings refer to incubations of algae from the sampling locations: (a) Lillo, (b) Bath and (c) Ellewoutsdijk. Data points are the mean of a triplicate AAS analysis, with the standard error indicated as a vertical line. ○, Cd–; ●, Cd+; □, Cu–; ■, Cu+.

ate loss, as most zinc desorbed during the first 2 days of incubation. Further loss of zinc was negligible, whereas in the control algae a more gradual, minor loss of zinc occurred with time.

Table 1. Lead contents ($\mu\text{mol Pb g}^{-1} \text{dwt}$) of cadmium-exposed (+) and control (–) thalli of *E. prolifera* versus time (algae were sampled from the Scheldt at Lillo, Bath and Ellewoutsdijk and incubated during 21 days in estuarine water of the same locations)

	Days				
	2	4	7	14	21
Lillo (–)	0.33	0.32	0.29	0.28	0.26
Lillo (+)	0.35	0.38	0.37	0.41	0.44
Bath (–)	0.16	0.14	0.14	0.14	0.13
Bath (+)	0.16	0.17	0.17	0.17	0.17
Elle (–)	0.15	0.13	0.16	0.16	0.17
Elle (+)	0.16	0.12	0.15	0.16	0.17

Iron/cadmium co-uptake (Figure 4) was more evident than lead/cadmium (Table 1) and copper/cadmium synergisms (Figure 2). Existence of a true cadmium/zinc antagonism is demonstrated in Figure 5.

Discussion

E. prolifera biomass dramatically diminished, moving along the river Scheldt into the direction of Antwerp. This species occurred up to Burcht near Antwerp (Figure 1). A combination of decreasing salinity and increasing pollution may have limited its distribution. Copper, cadmium, iron and lead contents in *E. prolifera* were highest in algae from Lillo (Figures 2 and 3; Table 1), close to Antwerp's industrial areas. While cadmium and iron contents decreased with salinity, zinc contents were highest in algae sampled at the location with the highest salinities and the lowest dissolved zinc levels (Van den Berg *et al.* 1987). The data of Van den Berg *et al.* (1987) concerning dissolved metals in the Scheldt versus salinity are confirmed by recent monitoring data of the Dutch Water Board (unpublished). The above data indicate that the ambient metal composition may be entirely different from the metal composition of algal tissues. As the balance of tissue metals determines toxicity, our experiments with *E. prolifera* (this work and Rijstenbil *et al.* 1993) support the conclusion of others (Seeliger & Cordazzo 1982, Ho 1990, Say *et al.* 1990), that algal metal contents are more valuable parameters for toxicity than dissolved metals in seawater. Moreover, it was reported that algal metal contents (e.g. copper) often correlate better with metal contents of marine sediments or suspended matter, than with dissolved metals in seawater (Luoma *et al.*

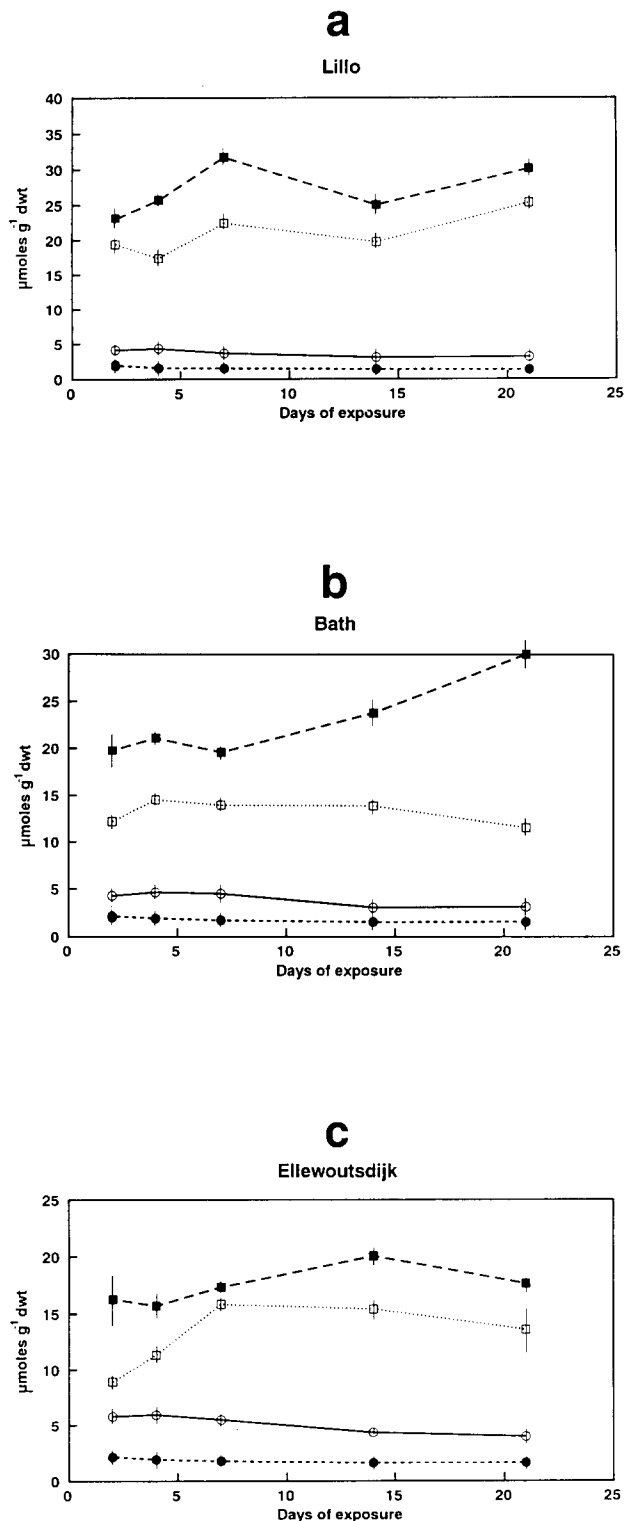


Figure 3. Iron and zinc contents of *E. prolifera* versus time, after a 400 nM Cd dosage (+ series), relative to control (- series). Figure headings refer to incubations of algae from the sampling locations: (a) Lillo, (b) Bath and (c) Ellewoutsdijk. Data points are the mean of a triplicate AAS analysis, with the standard error indicated as a vertical line. ○, Zn-; ●, Zn+; □, Fe-; ■, Fe+.

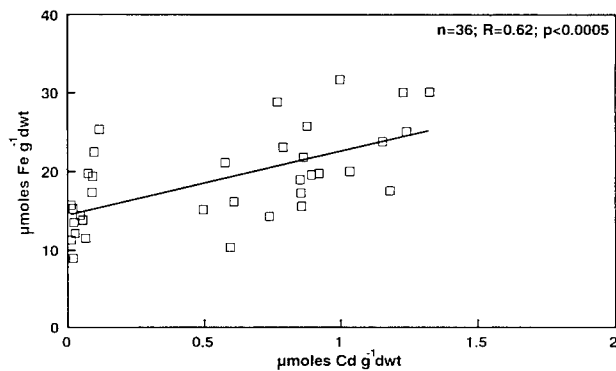


Figure 4. Iron versus cadmium in *E. prolifera*; data include metal contents of cadmium-exposed and control algae from the locations Lillo, Bath and Ellewoutsdijk.

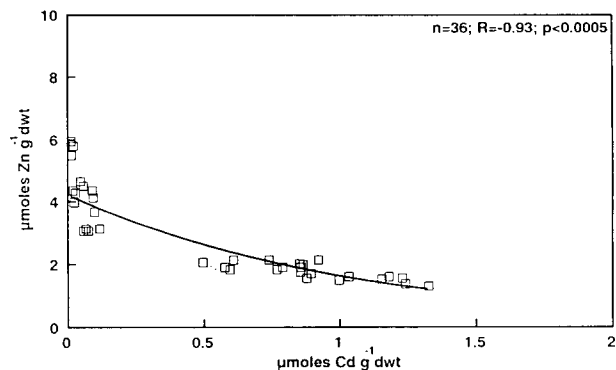


Figure 5. Zinc versus cadmium contents of *E. prolifera*, including cadmium-exposed and control algae from Lillo, Bath and Ellewoutsdijk. An exponential regression curve gave the best fit to these data. As a whole, both copper and lead in algae were not significantly influenced by cadmium ($P > 0.05$, ns).

1982, Bryan & Langston 1992). This was confirmed by similar measurements in marine macrophyceae and marine phanerogams from different Greek biotopes (Haritonidis & Nikolaidis 1989, Malea 1992, Malea and Haritonidis 1993).

The decrease of zinc in the control (Figure 3) in association with time has been quite characteristic. It can be explained by *E. prolifera*'s ability to eliminate this metal, which is in accordance with former studies (Haritonidis *et al.* 1993). However, it may also be interpreted as zinc ions binding to (extracellular) dissolved organic ligands.

The synergistic interaction between copper and cadmium in our experiment may be confirmed by similar interactions in the seagrass *Halophila stipulacea* (Malea 1992). Kabata-Pendias & Pendias

(1985) found that in terrestrial plants this interaction can be either synergistic or antagonistic.

According to other measurements, iron uptake presented certain peculiarities (Malea 1992). This was particularly true in the case of *Cymodocea nodosa*, in which iron showed relatively great ranges; 349–2325 $\mu\text{g Fe g}^{-1}\text{dwt}$, with a standard error of 102 $\mu\text{g Fe g}^{-1}\text{dwt}$ (Malea & Haritonidis 1993). In the above situation the biotope is rich in iron due to the presence of bauxites in the area and the discharge of an aluminum factory's wastes. It is well known that during the summer months macroalgal photosynthesis and respiration are more intense, thus enhancing algal iron uptake (Wahbeh 1984). Increasing iron contents as shown mainly in the control of Figure 3(a and c) might indicate such an impulse of these metabolic process under experimental conditions.

In this experiment algal lead contents were hardly affected by cadmium exposure, whereas in the copper experiment of Rijstenbil *et al.* (1993) lead contents increased slightly (20%) upon copper exposure. Synergistic interactions of copper and zinc were observed in the latter experiment, where zinc contents in copper-exposed algae became higher from the start, and remained so during 21 days. Copper/zinc co-uptake occurred rapidly, whereas iron became gradually higher in copper-exposed algae (Rijstenbil *et al.* 1993). Co-uptake of iron with copper was less significant ($P < 0.005$) than co-uptake of iron with cadmium in this experiment (Figure 4). The loss of algal zinc in the copper experiment could not have been due to copper exposure ($P > 0.05$, NS) as it occurred equally in controls, but may have resulted from a new equilibrium of algal (iron and) zinc with the environment. This was entirely different for the cadmium experiment (this work), where a cadmium–zinc antagonism was highly significant (Figure 5). As a whole, both copper and lead in algae were not significantly influenced by cadmium ($P > 0.05$ NS). A similar antagonism was reported by Haritonidis & Nikolaidis (1989). The absorption of zinc and cadmium associated with the same metabolic process (Eide *et al.* 1980). Cadmium-induced loss of zinc may result from a passive, physico-chemical exchange on metal-binding sites of the cell walls.

Finally, cadmium uptake (and toxicity) may be due to both (i) interference with transport of other metals across the cell membranes and (ii) competition with these metals for binding sites on, for example, metallo-enzymes; co-uptake of iron and loss of zinc may as well result from such internal reactions.

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