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Concentrations of Selected Heavy Metals in Benthic Diatoms and Sediment in the Westerschelde Estuary

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In recent years considerable data have been compiled on heavy metal levels in biota in marine and estuarine environments. With respect to the fauna, much information is available on accumulation and effects of heavy metals in birds, fish and benthic macrofauna (e.g., Salomons and Förstner 1984; Bryan and Langston 1992). Accumulation of heavy metals in aquatic flora has been studied mostly in benthic macroalgae, in particular in relation to the use as a biological monitor (Phillips 1990).

The response of planktonic algal species to heavy metals has been studied extensively in cultured populations (Thomas et al. 1977; Romeo and Gnassia-Barelli 1985). Also, heavy metal concentrations in natural plankton have been studied (Knauer and Martin 1973). As far as we know, very few data are available on the concentrations of heavy metals in the lowest benthic trophic level, the benthic microflora. It is a major food supply for numerous intertidal species, so it is obvious that microflora might play an important role in the accumulation of contaminants through coastal food chains.

The aim of this research was to adjust a recently developed collection technique for benthic diatoms so that it is suitable for large-scale field studies. The method was then used to assess the concentration of the heavy metals Cd, Cu, Pb and Zn in benthic diatoms and sediments along an estuarine gradient.

MATERIALS AND METHODS

The collection of samples took place on intertidal mudflats in the Scheldt Estuary (also called Westerschelde) in Belgium and the Netherlands and the Oosterschelde Sea Arm in the Netherlands (Fig. 1). The diatoms were collected between May and August 1991. The Scheldt Estuary is a heavily polluted area, receiving domestic and industrial wastewater from cities such as Brussels, Antwerp and Ghent (for a review, see van Eck et al. 1991).

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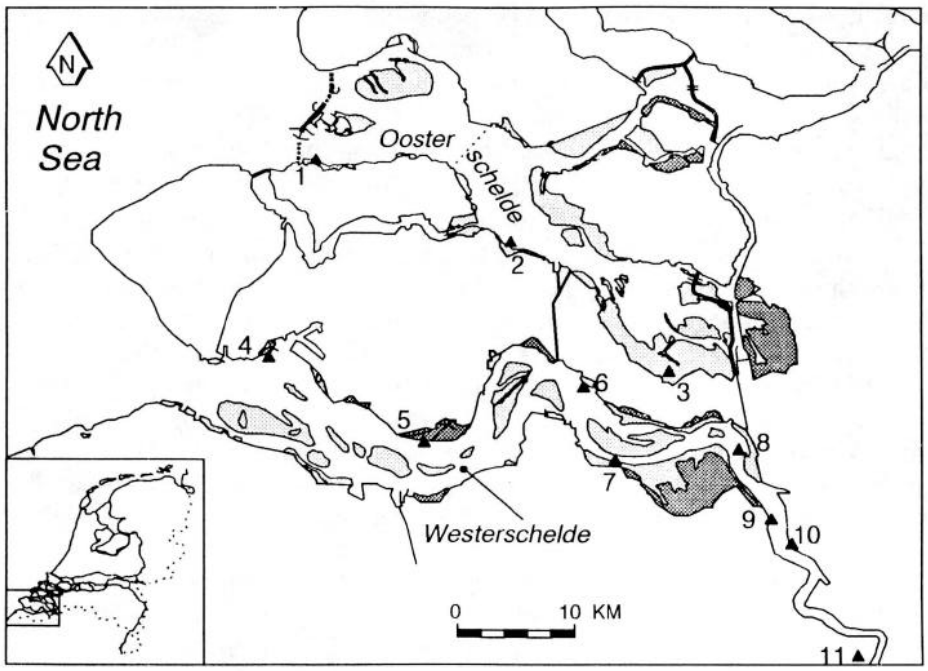

Figure 1. Map of Delta Area in the Netherlands with sampling stations. **In *Oosterschelde* 1:** Jacobahaven **2:** Kattendijke **3:** Stroodorpepolder. **In *Westerschelde* 4:** Rammekes **5:** Ellewoutsdijk **6:** Kruiningen Veerhaven **7:** Baalhoek **8:** Appelzak **9:** Doel **10:** Lillo **11:** Burcht. The shaded areas are saltmarshes (dark) and intertidal mudflats (light).

It is a very dynamic area with tidal differences of 4 to 5 meters and a salinity gradient from the North Sea to the river Scheldt. The Oosterschelde is considered a relatively clean area. It is hardly mixed with fresh water and salinity levels range from 28.6-32.3‰. The sea arm stays in direct contact with the North Sea through a storm surge barrier.

In the sediment, both 'free-living' (epipelic) and 'immobile' (epipsamnic) benthic diatoms can be distinguished (Round 1971). Generally, epipelic diatoms are rather large (> 20 µm), whereas epipsamnic diatoms are mostly smaller species (5-20 µm). The latter are firmly attached to sand grains and, consequently, are hardly able to move or migrate through the sediment. They can be found mostly in sandy, exposed locations. The epipelic species dominate in sheltered habitats where they are not easily suspended (Sabbe and Vijverman 1991). On intertidal mudflats, epipelic diatoms tend to move to the surface when the water withdraws. The diatoms were collected by allowing them to move into planktonic-gauze (80–µm mesh) tissues (20 x 30 cm) that were spread on the sediment. The planktonic-gauze was separated from the sediment by two layers of lens tissue paper. This method of collection is described in detail by Stronkhorst et al (1994). A major advantage of this method is that diatoms can be collected without contamination

from the sediment. Immediately after collection, they were washed from the gauze with 0.45µm filtered water from the sampling site. Further, they were concentrated by low-speed centrifugation. The diatoms were prepared for metal analysis by filtering the concentrated suspension over pre-weighed, acid-washed, cellulose-acetate filters (0.45 µm, Sartorius). The content of one algal concentrate was distributed over several filters and the filters were stored at -20 °C. After freeze-drying and dry-weight assessment, the filters were destroyed in a low temperature asher and redissolved in 65% HNO_3 (Merck Suprapur). This method of concentrating and destroying the algae was different from the method described by Stronkhorst et al (1994). Metals were measured with graphite furnace atomic absorption spectroscopy (GFAAS) furnished with a Zeeman background correction using graphite tubes with L'vov platforms. By concentrating the diatoms on cellulose-acetate filters, we were able to analyze trace metals in samples of only a few milligrams. This reduced the number of plankton gauze sheets needed to less than 10 per analysis. To account for local variation at the sampling site, we used a minimum of 10 sheets per location.

For metal analysis of the sediment, the upper 2 mm of the sediment in between the sheets was scraped with a bone spatula. The upper 10-cm layer was collected with a corer (diameter 20 mm; 10 cores per sample). The sediment was freeze-fried within 24 hr after collection. After separation of clay and sand particles and removal of organic material and carbonates, the grain size distribution was assessed with a laser diffraction technique (Malvern Particle Sizer type 3600 Ec). Particulate organic carbon (POC) and total nitrogen (N_{tot}) were measured on a Carlo Erba Nitrogen/Carbon analyzer, type NA 1500. Calciumcarbonate was measured volumetrically. For the metals analysis, the freeze-dried samples were ground in agate bowls. Five hundred mg of sediment was destructed with *aqua regia* in closed PTFE beakers in a microwave oven (Nieuwenhuize et al. 1991). The samples were measured with AAS as described above. As reference material estuarine sediment (BCR 277) was used. The recovery was 93.8 % (Pb), 98.6 % (Cu), 98.3 % (Cd) and 95.8 % (Zn). The levels of detection were 2.0 µg kg^{-1} (Pb), 3.0 µg kg^{-1} (Cu), 0.1 µg kg^{-1}, (Cd) and 0.1 mg kg^{-1} (Zn).

RESULTS AND DISCUSSION

The sediments in the Westerschelde had a high percentage of small particles, whereas in the Oosterschelde sediments were typically more sandy (see Table 1). Although the sediments were not normalized for a certain standard fraction, a distinct metal pollution gradient was recognized, going from the mouth of the estuary to the more riverine part of the Scheldt Estuary (location 4 to 11, Fig. 1).
As expected, metal concentrations in sediments from the Oosterschelde locations were lower than in Westerschelde samples (location 1 to 3). The more sandy sediment texture also contributed to the lower metal concentrations.

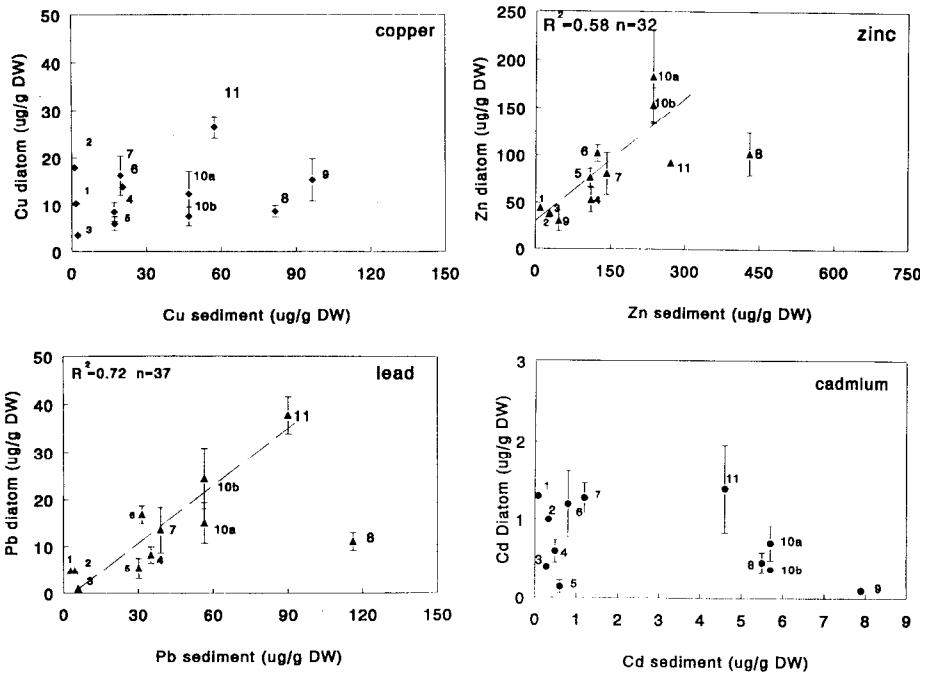

Figure 2. Relationship between metals in the sediment (0-2mm) and metals in benthic diatoms. The error bars indicate the standard error of the filter samples.

The observed heavy metal concentrations in sediments agree with previous reports (Wollast et al. 1985), be it that at location 9 markedly high Pb levels were found in the top as well as in the 0 to 10-cm layer (not shown). The high Pb concentrations are attributed to the industrial activity along the estuary.

Cadmium concentrations in the diatoms varied between less than 0.1 and 2 µg.g D W^{-1} (Fig. 2). Although Cd concentrations in the sediment amounted to 7.9 µg.g D W^{-1} (location 9), levels in the diatoms were very low and the bioconcentration factor was < 1. At the less polluted sites, however, the bioconcentration factor (Cd diatom/Cd sediment) was > 1.

Low concentration factors were found also for Pb; although concentrations in the sediment were rather high, levels in the diatoms were not more than 24.4 µg.g D W^{-1}. Yet, if location 9 is not taken into account, an almost linear relation was found between Pb in the sediment and Pb in the diatoms. At location 9 (not shown in Fig. 2), Pb concentration in the diatoms was only 7.25 (± 3.59) µg.g DW^{-1}, whereas sediment levels were more than 350 µg.g DW^{-1}.

Table 1. Characteristics and concentrations of Cd, Pb, Cu and Zn in the sediment. For each location, the bioconcentration factor (BCF; metals in diatoms/metals in sediment) is given. The locations are marked in Figure 1.

Location	No.	Layer	(%) POC	(%) Ntot	(%) CaCO3	(µg/g) Cd	BCF	(µg/g) Pb	BCF	(µg/g) Cu	BCF	(µg/g) Zn	BCF	(%) <63µm	(%) <16.3µm
Jacobahaven	**1**	0-10 cm	0.19		21.1	0.06	21.7	3.2	1.50	3.1	3.3	10	4.4		
	1	0-2 mm	0.2		14.4	0.08	16.3	2.8	1.71	1.7	6.1	10	4.4		
Kattendijke	**2**	0-10 cm	0.26		6.1	0.33	3	4.6	1.04	1.3	13.8	27	1.4		
	2	0-2 mm	0.57	0.03	6	0.33	3	4.4	1.09	1.2	14.9	27	1.4		
Stroodorp	**3**	0-10 cm	1.02	0.06	9.4	0.27	1.5	9.6	0.09	2.3	1.5	34	1.1		
	3	0-2 mm	0.96	0.07	7.3	0.28	1.4	5.8	0.16	2.4	1.4	29	1.3		
Rammekes	**4**	0-10 cm	1.16	0.06	7.5	0.09	6.67	16.2	0.51	6.7	1.28	41	1.28		
	4	0-2 mm	0.21		14.3	0.49	1.23	35.2	0.23	17	0.50	111	0.47		
Ellewoutsdijk	**5**	0-10 cm	1.17	0.11	16.4	0.82	0.17	35.7	0.15	20.4	0.30	123	0.62	61	28
	5	0-2 mm	1.26	0.15	215	0.61	0.23	30.2	0.18	17.2	0.35	109	0.70	88	52
Kruiningen-haven	**6**	0-10 cm	0.95	0.07	15.9	0.65	1.85	20.6	0.63	13.6	1.02	78	1.30	82	53
	6	0-2 mm	1.08	0.18	22	0.8	1.50	31.6	0.41	20	0.69	123	0.83	86	57
Baalhoek	**7**	0-10 cm	0.49	0.06	12.5	0.8	1.60	20.4	0.62	12.2	1.23	94	0.86	55.6	19
	7	0-2 mm	1.05	0.1	16.8	1.2	1.07	39.1	0.33	19.5	0.77	142	0.57	62	21
Appelzak	**8**	0-10 cm	1.83	0.1	5.1	2	0.23	40.6	0.27	20.9	0.42	126	0.81		
	8	0-2 mm	1.17	0.02	15.7	5.5	0.08	116	0.10	81.6	0.11	430	0.24		
Doel	**9**	0-10 cm	2.37	0.15	9.9	6.9	0.02	775	0.01	195	0.08	56	0.55		
	9	0-2 mm	2.29	0.06	16.2	7.9	0.01	353	0.02	96.3	0.16	47	0.65		
Lillo	**10**	0-10 cm	1.54	0.22	15	7.8	0.09	88.5	0.17	88.5	0.14	350	0.52	86.6	45.3
	10	0-2 mm	1.81	0.18	15.7	5.7	0.13	56.5	0.27	47	0.26	234	0.78	78.9	36.7
Burcht	**11**	0-10 cm	1.44	0.12	15.6	3.5	0.40	55.6	0.68	51	0.52	206	0.45	64.4	25.6
	11	0-2 mm	0.57	0.05	13.4	4.6	0.30	90	0.42	57.3	0.46	270	0.34		

For Zn, concentrations in diatoms varied from 20 to more than 200 µg.g DW^{-1}. Here also, a linear relation was found between metals in diatoms and metals in the sediment. As for Cd, the variation in Cu concentrations in the diatoms was not as large as the variation in the sediment; it varied between 3 and 29 µg.g DW^{-1}. Only at the Oosterschelde locations was the bioconcentration factor > 1. These concentrations are in the range that has been found for the pelagic species *Dytilum brightwellii* (Rijstenbil and Poortvliet 1992). The relationship with sediment concentrations was less obvious than for Pb or Zn.

Except for Cu, which was lower in our samples, the observations made by Stronkhorst et al. (1994) in the Westerschelde estuary were within the range of the present measurements. Thus, we assume that the modification of the analytical part of the collection method (concentrating the diatoms on filters and destroying these in a low temperature asher) did not influence the results. Reports on metal concentrations in pelagic phytoplankton samples were also in the range of the benthic diatom measurements presented here (Knauer and Martin 1973).

Generally, metal concentrations in diatoms from the most upstream location (11) were highest, while the highest metal concentrations in the sediment were found in other locations. The higher metal concentrations in the sediment at locations 8, 9 and 10 can be explained by sedimentation of particulate matter in the estuarine mixing zone. For the high metal concentrations in diatoms at location 11 (the most upstream location), several explanations can be given. Firstly, it may indicate a difference in algal composition, which is likely because of the riverine character. Alternatively, high metal concentrations in the overlying and pore water could have caused the higher concentrations in the diatoms. Due to anoxia in the summer period, levels of dissolved metals are very low in this part of the river because of precipitation of metal sulphides. However, oxidation of reduced metals during low tide may result in locally elevated dissolved metal concentrations.

A disadvantage of the collection method used in this study is that only epipelic diatom species were collected, so the data represent only a part of the benthic microalgal community. However, epipelic diatom species form a substantial part of the diet of common intertidal deposit feeders like *Macoma balthica* (Hummel 1985).

The actual role of heavy metals from food for the overall accumulation by bivalves is subject to discussion: for mussels, food is considered to contribute a minor fraction (less than 5%) to the metal levels in this bivalve (Riisgård et al. 1987; Amiard-Triquet et al. 1988). On the other hand, for deposit feeding bivalves like *Macoma balthica,* food might contribute to a large extent to the overall metal uptake (Absil et al. 1994). This is likely, because filtration rates are much lower; deposit feeders select from a concentrated source, whereas suspension feeders have to concentrate a very dilute source (Gilbert 1977) and are therefore likely to accumulate more metals through the dissolved phase. If a *M. balthica* individual

(30 mg) would consume 3 mg benthic diatoms (10% of body weight) per day with an average Cu concentration of 15 µg.g DW^{-1}, then metal intake rate would be 0.045 $\mu g.day^{-1}$ (= 1.5 ppm increase per day; around 8% of the tissue metal content in clams from unpolluted regions), assuming that the diatom-associated Cu would be 100% biologically available. In reality, metal availability will not be 100%, and the clam would also lose Cu through elimination. A substantial metal contribution via food is nevertheless conceivable.

Because of its simplicity, the method described in this paper provides a valuable tool to monitor availability of heavy metals for biota in intertidal areas.

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