Bull. Environ. Contam. Toxicol. (1994) 52:818–824

Environmental
Contamination
and Toxicology

Trace Metals, PCBs, and PAHs in Benthic (Epipelic) Diatoms from Intertidal Sediments; a Pilot Study

J. Stronkhorst,[1] P. C. Vos,[2] R. Misdorp[1]

[1]National Institute for Marine and Coastal Management, P.O. Box 20907,
2500 EX The Hague, The Netherlands
[2]Geological Survey of The Netherlands, District North, P.O. Box 85,
Oosterwolde, The Netherlands

Received: 29 March 1993/Accepted: 20 October 1993

Intertidal sediments in many estuaries around the world have a history of contamination resulting from long term discharges of industrial, agricultural and domestic waste effluents. These contaminated sediments are now regarded as a major source of toxicants for bottom-related organisms (Adams *et al.*, 1992) which, in turn, may pass on certain contaminants (e.g. methylmercury, polychlorinated biphenyls (PCBs)) to organisms higher in the foodchain. Many studies have been conducted on the contamination of benthic macrofauna, estuarine fish and birds, but to our knowledge no research has yet been carried out on benthic diatoms which form the lowest trophic level of an intertidal ecosystem. Research on the effects of micro-contaminants on primary producers in marine ecosystems is mainly performed with phytoplankton (e.g. Biggs *et al.*, 1978).

In the estuaries of temperate regions, benthic diatoms make a significant contribution to primary production in the ecosystem and are predated especially by deposit feeding Polychaete and Mollusca (Plante-Cuny and Plante, 1986). Knowledge of the level of contamination in benthic diatoms is of major importance to recognize possible effects on growth rate and species composition of the benthic diatom populations and to understand the accumulation of toxicants into the foodchain.

For chemical analysis it is difficult to obtain 'pure' samples of benthic diatoms because they form part of the sediment. A similar problem occurs with the sampling of phytoplankton in turbid estuarine waters (Balls, 1990).

The aim of this pilot study was a) to improve a trap technique to collect pure samples of benthic diatoms of at least 2 gram dry weight for analysis of trace metals, PCBs and poly-aromatic hydrocarbons (PAHs) and b) to compare the concentrations in benthic diatoms with levels in sediment and some bottom-related organisms.

MATERIALS AND METHODS

De Jonge (1979) described a trap technique to separate benthic diatoms from inorganic sediment by density gradient centrifugation. To obtain sufficient quantities for chemical analysis, however, this method is very time-consuming and samples contain not only diatoms but detritus as well. Reinicke (1858) used fabric to harvest positively phototactic diatoms. The limitation of this method is that only epipelic (mobile) diatoms, which are

---

Correspondence to: J. Stronkhorst

generally found in high abundances on sheltered muddy sediments and cause a brown colouring of the surface, are collected. These diatoms migrate mainly in the upper centimeter of the sediment. Epipsammic diatoms, immobile taxa attached to sand grains which dominate the diatom populations of the sandy intertidal flats (Vos *et al.*, 1988), are not included. Eaton and Moss (1966) improved the method of Reinicke by the introduction of lens cleaning tissues instead of fabric. In our study, we applied a combination of lens cleaning tissues and plankton gauze.

At a sampling site 50-100 basal 'protection' lens cleaning tissues (Whatman, 46x27 cm) were spread out on the sediment and 'harvest' planktonic gauze (engineered sieve cloth Monodur-Polyamide, mesh opening 80 µm, 20x30 cm) was laid on top. Epipelic diatoms migrate through the protection tissue into the gauze by positive phototaxis and after 15-30 minutes the gauze was removed and taken to the laboratory. The diatoms were washed out in 5-10 L brackish water made of distillate water and super-filtrated seawater. The suspension was sieved over 120 µm gauze to remove any tissue threads. The diatoms settled out and were further concentrated by centrifugation. The application of plankton gauze as harvesting tissue has the advantage that epipelic diatoms can be washed out very easily. When lens-tissue or polyester tissue paper is used, it is difficult to remove the diatoms from it.

Samples of epipelic diatoms were harvested at low tide on June 20 and 21, 1989 at four muddy intertidal sites in the brackish zone of the Scheldt estuary, SW-Netherlands (Fig. 1). Sites A and B are located approximately 10 km and sites C and D about 30 km downstream of the port and industrial areas of Antwerp (Belgium). The salinity of the surface water is approximately 13 °/oo and 20 °/oo, respectively. At these sites the sediment surface showed a strong brown colouring, indicating the presence of a high biomass of epipelic diatoms. At each site a sediment sample was taken from the top 0-5 cm. Pooled samples of Baltic tellin (*Macoma balitica*), ragworm (*Nereis diversicolor*), shrimp (*Crangon crangon*) and the livers of ten flounders (*Platichthys flesus*) were collected near site B and C within 2 weeks time of the diatom sampling.

The sediment samples were sieved through a nylon sieve to obtain the fraction < 63 µm and freeze dried in a Virtis Unitrap 2. Samples of biota were homogenized with an Ultra Turrax TP18/10 and freeze dried. Dry weight and ash weight of the sample were determined according to the Dutch standard NEN 3235.
Fat was isolated from the sample of biota by extraction with hexane/acetone (3:1) in a hot soxhlet for four hours. The extract was used to determine 1. total lipid content (evaporation with a Kurdena Danish apparatus, drying at 103 °C and weighing the residue) and 2. what will be called 'apolar lipid', i.e. the lipid content after eluting the acetone extract over a florisil column according to the method of Schneider (1982).
Extraction of the samples for analysis of PCBs and PAHs was performed as for fat. The cleanup for PCB analysis of the extracts of diatoms and sediment was performed by eluting with hexane over a column containing SiO_2 and desulphating reagent made of Na_2SO_3, NaOH, Al_2O_3 and H_2O. For PAHs only desulphating reagent was used. The cleanup for PCB analysis of fish and benthic fauna extracts was performed by eluting with hexane/acetone over a florisil column followed by eluting with hexane over a $Si0_2$ column. For PAHs only the latter was used.

For PCB analysis iso-octane was added to all elutes and the extracts were evaporated by nitrogen to 1 mL iso-octane. PCBs were determined on a 5880 Hewlett Packard gaschromatograph equipped with two columns (SE54 50 m x 0.32 mm, 0.2 µm and CPSil 19CB 50 m x 0.32 mm, 0.2 µm) and two Electron Capture Detectors. For PAHs analysis the extract is brought on a column containing Al_20_3 using pentane. The column is dried by purging it with nitrogen and eluted with methanol. PAHs were determined on a HP1090 HPLC equipped with two HP 1046A programmable fluorescence detectors and a Vydac 201

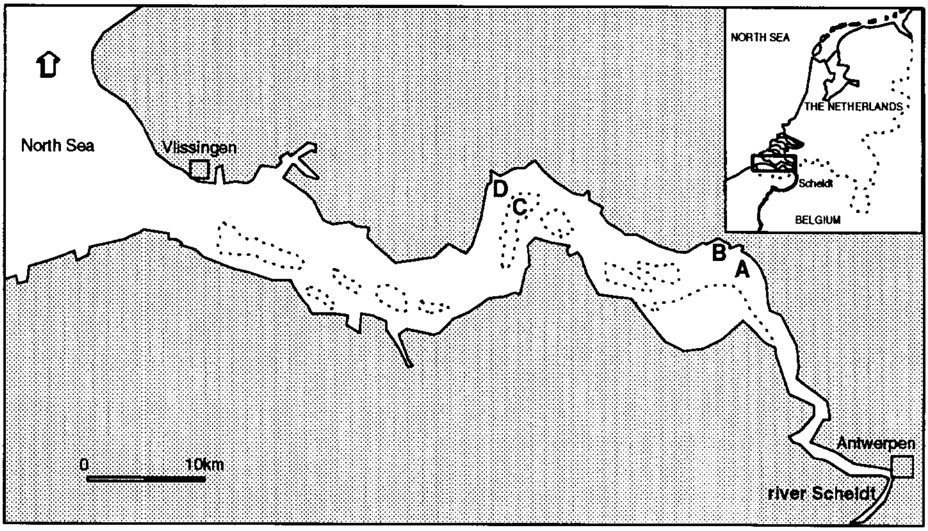

Figure 1. Sampling sites in the brackish zone of the Scheldt estuary.

PB-5 250 mm x 4.6 mm column.
For analysis of trace metals, samples are digested with nitric acid (65 %) in a teflon bomb according to the Dutch standard NEN 6439. Zn is measured with FAAS and Cd, Cr and Cu by GFAAS. Hg is measured by cold vapour AAS after reduction with $SnCl_2$, concentrating on a gold trap.

In the analytical procedure for PCBs the congeners IUPAC no. 29 and no.155 were added to the sample to control the recovery and Benz(b)chrysene in the case of PAHs. The recovery of PCB-29 and PCB-155 ranged from 109 % in samples of sediment to 88 % in fish-liver and the recovery of Benz(b)chrysene was *c.* 98%. Relative standard deviations of reference material of mussels (*Mytilus edulis*) were 1-4% for Cd, Cu and Zn, 30% for Cr, 11% for Hg, less than 10 % for PCBs except for congeners #18, #28 and #170 (20-25 %) and *c.* 10 % for PAHs. The standard deviation in duplo measurements of trace metals is less than 10 % and for PCBs less than 5 % .

Differences between concentrations in benthic diatoms and sediment and between concentrations in benthic diatoms and other bottom-related organisms mentioned above were tested with an analysis of variance (ANOVA) according to a block design with sampling sites as blocks (Wilkinson, 1990).

For each site bioconcentration factors (BCFs) were calculated as C_d/C_s were C_d is the concentration in diatoms and C_s the concentration in sediment. For trace metals the concentrations are expressed on a dry weight basis and for PCBs and PAHs on an organic carbon basis.

RESULTS AND DISCUSSION

The samples of benthic diatoms had a percentage dry weight (DW) of 8.4 ± 2.2 %. Samples from sites A, B, C and D weighed 10, 7, 1.5 and 4 g DW, respectively. The harvest of epipelic diatoms depends on the biomass as well as on their migration strategy which may be influenced by factors like light intensity, water saturation of the sediment

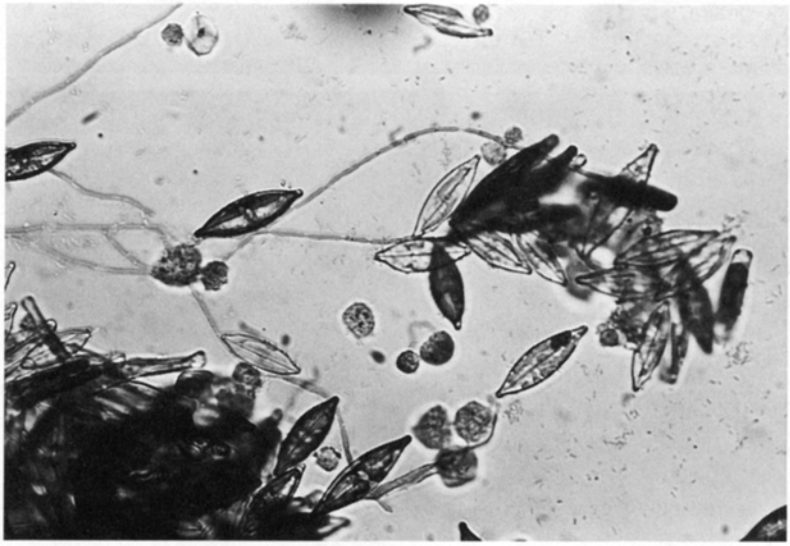

Figure 2. A suspension of coagulated epiphetic diatoms (500 x) dominated by *Navicula phyllepta*. Some filamentous cyanobacteria and amoeba are also present.

and tidal rhythm (Palmer and Round, 1965). The samples were obtained under good weather conditions. Rain during sampling tended to cause a disturbing mud-film on the harvest gauze and even a low wind velocity made it difficult to place the tissues correctly. Microscopic observations of the diatoms showed that the samples contain hardly any sediment particles. The dominant species was the epipelic diatoms *Navicula phyllepta* (Fig. 2).

Table 1 presents basic parameters, mean concentrations of trace metals, PCBs and PAHs in benthic diatoms and sediments expressed on a dry weight basis, as well as ranges in BCFs. Approximately 40% of the dry weight of the diatoms consists of ash. The ash probably consists of both silica cell-walls of diatoms and salt crystals from the centrifugated diatom-suspension. Approximately 6.5 % of the dry weight is lipid, half of which is regarded as apolar lipids.

In general, the sediment concentrations are some what higher than those of the Wadden Sea and in nearshore sediments of the North Sea (Kramer *et al.*, 1988) but cadmium concentrations are strongly elevated and, for instance, higher than levels reported for estuaries of the United Kingdom (Bryan and Langston, 1992).

In benthic diatoms concentrations of cadmium, mercury, chromium, zinc and PAHs (except pyrene) are significantly lower than in sediment. No significant differences could be established for copper and PCBs (except congener #52).

A literature search conducted in January 1993 (on: BIOSIS, ASFA and Pollution Abstracts over the last decade and Current Content over the previous 6 months) uncovered no publications on contaminants in benthic diatoms. Therefore, a comparison with our observations is restricted to estimated trace metal levels in pelagic diatoms in the Scheldt estuary (Zwolsman & van Eck, 1993). Concentrations in pelagic diatoms from the brackish zone (presumably *Coscinodiscus commutatis*) had concentrations of zinc, chromium,

Table 1. Basic parameters and mean concentrations (± standard deviation) of PCBs, PAHs (ng/g DW) and trace metals (μg/g DW) in epipelic benthic diatoms and sediment fraction <63 μm from four sites in the brackish zone of the Scheldt estuary.

	Diatoms		Sediment		BCF
	mean	sd.	mean	sd.	range
org. carbon	30.7	0.6	2.9	0.4	
% ash	38.5	1.2	89.3	2.9	
% total fat	6.5	4.6	-	-	
% 'apolar' fat	3.1	1.3	-	-	
PCB-18	3.3	0.8	2.0	0.7	0.07 - 0.28
PCB-28	4.1	1.2	2.9	0.9	0.06 - 0.23
PCB-52[a]	9.5	3.0	4.4	2.6	0.12 - 0.31
PCB-49	5.1	1.4	4.5	2.1	0.06 - 0.15
PCB-44	3.2	1.2	2.4	1.4	0.06 - 0.20
PCB-101	16.7	9.8	9.6	5.7	0.10 - 0.23
PCB-118	4.1	1.3	6.4	3.0	0.04 - 0.08
PCB-153	25.8	15.0	15.4	8.9	0.11 - 0.22
PCB-105	5.3	4.4	5.3	3.9	0.05 - 0.14
PCB-138	12.7	6.8	13.2	7.8	0.07 - 0.12
PCB-187	6.7	3.5	5.5	3.3	0.08 - 0.15
PCB-180	10.9	6.1	10.1	6.7	0.08 - 0.14
PCB-170	3.6	2.0	5.2	3.3	0.05 - 0.08
sum PCB congeners	110.9	52.1	86.8	50.2	0.08 - 0.16
Phenanthrene[a]	109	41	462	174	0.01 - 0.04
Anthracene[a]	8	7	105	56	0.00 - 0.01
Fluoranthene[a]	83	68	538	257	0.00 - 0.03
Pyrene	80	40	453	234	0.00 - 0.04
Benz(a)anthracene[a]	28	20	272	141	0.00 - 0.02
Chrysene[a]	35	24	303	146	0.00 - 0.03
Benz(e)pyrene[a]	19	23	289	136	0.01 - 0.01
Benz(b)fluoranthene[a]	30	15	433	197	0.00 - 0.01
Benz(k)fluoranthene[a]	16	25	206	96	0.00 - 0.02
Benz(a)pyrene[a]	25	25	340	174	0.00 - 0.01
Benz(ghi)perylene[a]	21	25	283	129	0.01 - 0.02
Ind.(123cd)pyrene[a]	ND		339	147	0.00 - 0.00
sum PAH	454	285	4084	1919	0.00 - 0.02
Cd[b]	0.43	0.26	5.24	3.31	0.01 - 0.30
Hg[a]	0.11	0.05	0.66	0.24	0.06 - 0.34
Cr[a]	9	8	116	28	0.01 - 0.18
Cu	32	15	61	32	0.19 - 1.49
Zn[a]	89	37	319	109	0.10 - 0.54

Significant difference between benthic diatoms and sediment are indicated by [a] ($p<0.05$) and [b] ($p=0.06$). Bioconcentration factors (BCFs) for trace metals are on a DW basis and for PCBs and PAHs on a OC basis. (ND=not detected)

cadmium and copper of 105 ± 61, 19 ± 6, 1.2 ± 0.8 and 7.1 μg/g DW respectively. These concentrations are in the same ranges as for benthic diatoms reported here (table 1). Zwolsman and van Eck (1993) suggest that there is an uptake by phytoplankton of zinc and cadmium but not of copper. Nevertheless, all metal concentrations in phytoplankton were lower than in inorganic suspended matter, just as is observed for benthic diatoms and sediment.

Table 2. Concentrations of PCBs, PAHs (ng/g AFDW), Cd, Cu and Zn (μg/g AFDW) in epipelic benthic diatoms, shrimp, ragworm, Baltic tellin and the livers of flounders from two sites in the brackish zone of the Scheldt estuary.

	Diatoms		Schrimp		Ragworm		B.tellin		Flounder	
	mean	sd.	mean	sd.	mean	sd.	mean	sd.	mean	sd.
% AFDW[a]	61.4	2.1	78.3	1.3	73.5	2.1	75.0	2.8	92.0	1.8
% ap.lipid[a]	3.4	1.8	0.9	0.1	-	-	1.6	0.3	27.3	3.0
Sum PCBs[b]	216	120	243	6	389	164	188	38	6115	3315
Sum PAHs	884	525	410	285	785	409	947	449	872	34
Cd	0.90	0.24	0.78	0.21	1.37	0.70	1.46	0.69	0.92	0.10
Cu	50	4	83	9	25	8	43	6	60	19
Zn[a]	153	23	112	23	244	54	647	20	119	30

Significant differences in concentrations between biota are indicated with [a] ($p<0.001$) and [b] ($p=0.05$).

For all compounds BCFs are below one (table 1). This is probably the result of the low bioavailability of the micro-contaminants from the sediment particles and, alternatively, may be caused by the fast growth and short lifespan of the benthic diatoms so no equilibrium with ambient (pore) water is reached.

Minimum BCFs are all found for site A while maximum BCFs were calculated for site C and D. The bioavailability of a toxicant can differ markedly between sediments and depends on, for instance, the organic carbon content of sediment in the case of organic micro-contaminants and acid volatile sulphide (AVS) in the case of trace metals (Adams *et al.*, 1992). However, no data are available on AVS in the sediments investigated and the observed percentages of organic carbon showed little variation.

For phenanthrene, chrysene and benz(a)pyrene in the infaunal *Macoma inquinata* from high organic sediments BCFs are 0.1, 0.31 and 0.06 respectively (Augenfeld *et al.*, 1982) which are comparable with the ratios found here. Lake *et al.* (1990) reported a concentration factor for PCB-153 between the concentrations in lipid of infaunal organisms and organic carbon of sediment of 4.58 (CV = 53%). This is significant higher than the ratio of 0.6 (CV = 40%) calculated for lipid in benthic diatoms and sediment organic carbon.

Concentrations of PCBs, PAHs, Cd, Cu and Zn in benthic diatoms and the other estuarine organisms are presented in table 2 on an ash-free dry weight (AFDW) basis.
Despite the limited number of data, some preliminary conclusions can be drawn regarding the differences between these biota. Levels of sum-PCBs in benthic diatoms are in the range of infaunal organisms. Higher concentrations are found in livers of flounders which can be ascribed partly to the higher lipid content of the liver. In the case of zinc, relatively high concentrations are observed in Baltic tellin. Sum-PAHs, Cd and Cu do not show significant differences between the biota investigated.

The relatively simple method described here, can provide sufficient quantities of pure benthic (epipelic) diatoms to perform chemical analysis. In spite of their short lifespan and high turn-over rate concentrations of several micro-contaminants in benthic diatoms are surprisingly high. The method provides a way to investigate the contribution of benthic diatoms in the uptake of toxicants by, for instance, Mollusca (eg. *Macoma balthica*) or fish (eg. *Mugil ramada*).

Acknowledgment. We thank Mr. F. Smedes (National Institute for Marine and Coastal Management, Haren, The Netherlands) for his advice regarding the chemical methods.

REFERENCES

Adams WJ, Kimerle RA, Barnett JW (1992) Sediment quality and aquatic life assessment. Environ Sci Technol 26: 1864-1875.

Augenfeld JM, Anderson JW, Riley RG, Thomas BL (1982) The fate of polyaromatic hydrocarbons in an intertidal sediment exposure system: bioavailability to *Macoma inquinata* (Mollusca: Pelecypoda) and *Abarenicola pacifica* (Annaelida: Polychaeta) Mar Environ Res 7: 31-50.

Balls PW (1990) Distribution and composition of suspended particulate material in the Clyde estuary and associated sea lochs. Estuar Coast Shelf Sci 30, 475-487.

Bryan GW, Langston WJ (1992) Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. Environ Poll 76: 89-131.

Biggs DC, Rowland RG, O'Connors HB, Powers CD, Wurster CF (1978) A comparison of the effects of chlordane and polychlorinated biphenyls on the growth photosynthesis and cell size of estuarine phytoplankton. Environ Pollut 15: 253-264.

Eaton JW, Moss B (1966) The estimation of numbers and pigment content in epipelic algal populations. Limnol Oceanog 11: 548-595.

Jonge de VN (1979) Quantitative seperation of benthic diatoms from sediments using density gradient centrifugation in the colloidal silica Ludox-TM. Mar Biol 51: 267-278.

Kramer KJM, Misdorp R, Duyts R (1989) Contaminants in sediments of the North Sea and Wadden Sea. Rijkswaterstaat, Tidal Waters Division, The Hague. Report GWWS-90.011.

Lake JL, Rubinstein NI, Lee II H, Lake CA, Heltshe J, Pavignano S (1990) Equilibrium partitioning and bioaccumulation of sediment-associated contaminants by infaunal organisms. Environ Toxicol Chem 9: 1095-1106.

Palmer J, Round FE (1965) Persistent, vertical migration rhytms in benthic microflora. J Mar Biol Assess U.K. 45: 567-582.

Plante-Cuny MR, Plante R (1986) Benthic marine diatoms as food for benthic marine animals. In: M. Richard (ed.) Proc. eighth intern. symp. on recent and fossil diatoms, Paris, 1984.

Reinicke F (1858) Das einsammeln und präparieren der bacillaren. Beitr Mikroskopie 1: 47-57.

Schneider R (1982) Polychlorinated biphenyls (PCBs) in cod tissues from the Western Baltic: significance of equilibrium partitioning and lipid composition in the bioaccumulation of lipophilic pollutants in gill-breathing animals. Meeresforsch. 29: 69-79.

Vos PC, de Boer PL, Misdorp R (1988) Sediment stabilization by benthic diatoms in intertidal sandy shoals. In: de Boer *et al.*. Tide-influenced sedimentary environments and faches, pp 511-526.

Wilkinson L (1990) Systat: The system for statistics. Evanston, IL: Systat Inc.

Zwolsman JJG, van Eck GTM (1993) Geochemistry of suspended matter in the Scheldt estuary. Geochimica et Cosmochimica Acta (in press).