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Chemical and biological evaluation of sediments from the Wadden Sea, The Netherlands

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Abstract We describe the results of an evaluation of marine sediments using chemical measurements and bioassays. Four groups of chemicals, i.e., heavy metals, PAHs, chlorinated aromatic compounds, and tin compounds, were measured at 16 locations in the Wadden Sea, The Netherlands. Extractions of sediments from each location also were assessed using five bioassays. Our objective was to identify chemicals likely to pose biological risks, characterize the relation between bioassay results and particular classes of chemical(s) and determine ''clean'' reference sites on the basis of the chemical and biological evaluations. A multivariate technique (Principal Component Analysis; PCA) was used to meet these objectives. Results of the PCA indicated that the response of the Microtox Solid Phase bioassay had a positive, significant relationship with the levels of PAHs and organotin compounds. The responses of the other bioassays did not consistently relate to the concentrations of the other measured chemicals. Our findings indicate that the organotin compounds may still be a stressor for aquatic invertebrates in the Dutch Wadden Sea. On the basis of the chemical and biological evaluations, four sites (Dantziggat, Malzwin, Richel and Lauwers) can be considered to be ''clean'' reference sites.

Keywords Marine bioassays · Multivariate techniques · Marine sediments · Principal component analysis · Tributyltin · PAHs

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Introduction

The Wadden Sea stretches from Den Helder in the Netherlands, past the river estuaries of Germany to its northern boundary at Esbjerg in Denmark along a total length of some 500 km, and is Europe's largest wetland ecosystem (10,000 km^2). It is famous for the rich fauna, avifauna and flora and high productivity: its ecological importance extends far beyond its borders. The Wadden Sea ecosystem has been influenced by human activities such as diking, chemical pollution, recreation and fisheries. During the 1960s and 1970s, inputs of chlorinated compounds such as PCBs and pesticides were of concern (cf. Tougaard and Helweg Ovesen 1981; Essink and Wolff 1978). More recently, the levels of PCBs, chlorinated compounds and metals have decreased; polycyclic aromatic hydrocarbons (PAHs) presently are thought to pose negligible risk, hexachlorobenzene (HCB) levels are higher than the background and tributyltin (TBT) may now be the predominant chemical of concern in the Wadden Sea environment. These suppositions, though, are based purely on chemical measurements (Wolff 2000; Cadée et al. 1995; De Jong et al. 1999). Biological evaluations are needed to verify this supposition (Mensink et al. 1997).

Four main disadvantages are associated with the use of only chemical methods to characterize sediment quality. First, some compounds are difficult to measure due to the complex nature of the matrix; second, some important chemicals might be missed; and third, it is not possible to accurately assess the effects of exposure to a mixture of chemicals. Finally, the bioavailability of chemicals can vary markedly with environmental conditions, so chemical analyses alone cannot accurately estimate sediment's environmental hazards. The latter situation results because the toxicity of many chemicals is unknown, and because most chemicals have complex exposure pathways (e.g., Del Valls et al., 1997). To overcome these problems, chemical analyses should be coupled with bioassays. Bioassays provide essential information on the toxicological risks of sediments and are especially useful when a battery of tests is applied (Giesy and Graney, 1989).

We analysed four groups of chemicals (heavy metals, PAHs, chlorinated aromatic compounds, and tin compounds) in the sediment from 16 sites in the Wadden Sea. We also analyzed the sediment samples for toxicity, using five bioassays, to relate the response of the bioassays to different levels of these chemicals. The main goal of this study was to find clean reference sites in the Wadden Sea on the basis of a chemical and biological evaluation. With this study we were also able to identify:

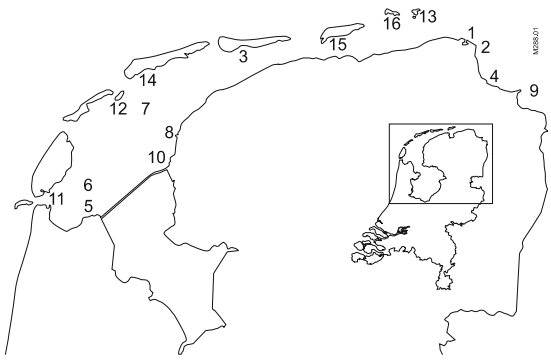
– (group of) chemicals indicated to pose biological risks,
– the correlation between the response of a certain bioassay and a certain (group of) chemical(s)

We used Principal Component Analysis (PCA) to relate the levels of the chemicals to the responses of the bioassays. This technique allowed us to create a two-dimensional summary of the relationship between the levels of the chemicals and the responses of the bioassays. PCA and similar statistical methods are used widely for creating summaries of this type (e.g., Bernard et al. 1997; Del Valls et al. 1997; Stephenson and Mackie 1988; Magnusson et al. 1996; Shaw and Manning 1996; Van den Brink et al. 2003).

Materials and methods

Sampling sites

Sediment samples were collected from 16 sites in the Wadden Sea during May 1998. Sites were selected on the basis of expectance to vary in degree of contamination. Figure 1 gives a map with sites and a short description of the site. Sediment samples were collected using a Van Veen grabber (two hinging bins with carrying arms) operated from a ship. Sediment was directly put into PVC barrels until a total of 10 l was obtained. These barrels



Sample site	Name sample sampling station	Site description
1	Bocht van Watum dijkvoet	shallow
2	Bocht van Watum oost	mud flat
3	Dantziggat	trench edge
4	Delfzijl	harbour
5	Den Oever	shallow
6	Doove Balg	trench
7	Griend	sludge-basin
8	Harlingen	harbour
9	Heringsplaat	mud flat
10	Kornwerderzand	shallow
11	Malzwin	shallow
12	Richel	mud flat
13	Rottumeroog	mud flat
14	Terschelling	harbour
15	Zoutkamperlaag	mud flat
16	Lauwers	trench edge

Fig. 1 Name of sampling stations and description

were transported to the laboratory and stored at 4°C. Before distributing the sediment for chemical measurement and bioassays, the content of each barrel was homogenized by firmly stirring it.

Chemical measurements

Within a period of 4 weeks a subsample (1 l) of each sampled sediment was analysed on its chemical composition, dry weight, grain size and organic carbon content. All samples were preprocessed according to standard procedures. Sediment was dried at ambient temperature and homogenised. Table 1 gives an overview of the chemicals measured in the homogenised soils samples. Metals were analysed using AES or AAS after dissolution with aqua regia. Poly Aromatic Hydrocarbons were extracted with hexane using Soxhlet and subsequently measured using HPLC. Chlorinated Aromatic Compounds and Tin compounds were extracted using acetone and subsequently measured using GC/MS.

Bioassays

Each sediment sample was analyzed for biological quality using five types of bioassays (Table 2). The bioassays were selected from the Dutch handbook on toxicity tests for marine sediments (National Institute for Coastal and Marine Management 1999). Each bioassay was conducted according to standard operating procedures described in the Dutch handbook (op cit., 1999). Details follow:

Table 1 Abbreviations and full names of chemicals measured in the sediments of the locations sampled ($N = 16$)

Abbreviation	Full name	Geom. conc.	Max. conc.
Elements			
As (mg/kg)	Arsenic	22	41
Cd (mg/kg)	Cadmium	0.7	1.1
Cr (mg/kg)	Chrome	90	105
Cu (mg/kg)	Copper	23	39
Hg (mg/kg)	Mercury	0.3	0.6
Ni (mg/kg)	Nickel	31	47
Pb (mg/kg)	Lead	58	89
Zn (mg/kg)	Zinc	169	261
Al (g/kg)	Aluminium	42	60
Fe (g/kg)	Iron	31	42
Mn (g/kg)	Manganese	0.6	1.3
Co (mg/kg)	Cobalt	11	14
Ca (g/kg)	Calcium	72	117
Mg (g/kg)	Magnesium	12	15
Na (g/kg)	Sodium	7.5	11
K (g/kg)	Potassium	12	16
Li (mg/kg)	Lithium	54	72
Ce (mg/kg)	Cerium	70	174
La (mg/kg)	Lanthanum	36	81
Nd (mg/kg)	Neodymium	33	70
PAH (μg/kg)			
Ant	Anthracene	25	62
BaA	Benzo(a)anthracene	64	159
BaP	Benzo(a)pyrene	78	173
BbF	Benzo(b)fluoranthene	115	226
BeP	Benzo(e)pyrene	73	149
BghiPe	Benzo(g,h,i)perylene	84	157
BkF	Benzo(k)fluoranthene	53	109
Chr	Chrysene	67	176
DBahA	Dibenzo(ah)antrhaceen	20	37
Fen	Phenanthrene	93	220
Flu	Fluoranthene	156	424
InP	Indeno(1,2,3-cd)pyrene	113	202
Pyr	Pyrene	116	298
Chlorinated aromatic compounds (μg/kg)			
HCB	Hexachlorobenzene	0.6	47.6
PCB18	2,2′,5-tri-CB*	0.3	0.6
PCB28	2,4,4′-tri-CB	1.5	2.4
PCB31	2,4′,5-tri-CB	1.0	1.6
PCB44	2,2′,3,5′-tetra-CB	0.3	0.9
PCB52	2,2′,5,5′-tetra-CB	0.4	1.5
PCB101	2,2′,4,5,5′-penta-CB	1.3	3.1
PCB105	2,3,3′,4,4′-penta-CB	0.3	1.0
PCB118	2,3′,4,4′,5-penta-CB	1.3	2.9
PCB138	2,2′,3,4,4′,5-hexa-CB	1.8	3.6
PCB153	2,2′,4,4′,5,5′-hexa-CB	2.4	4.4
PCB170	2,2′,3,3′,4,4′,5,-hepta-CB	0.4	1.0
PCB180	2,2′,3,4,4′,5,5′-hepta-CB	0.6	1.6
PCB187	2,2′,3,4′,5,5′,6-hepta-CB	0.9	1.6
Tin compounds (μg/kg)			
MBT	Monobutyltin	9.2	17
DBT	Dibutyltin	22	95
TBT	Tributyltin	40	614
MFT	Monophenyltin	0.7	6.0
DFT	Diphenyltin	1.1	17
TFT	Triphenyltin	5.3	37

For each chemical, we show the geometric mean and maximum concentration (μg, mg, or g per kg dry weight of fraction <63 μm) of all sediments * -CB means chlorobiphenyl

Table 2 Summary of the bioassays executed on the different sediments

Name testorganism	Abbreviation	Phylum of test organism	Duration (days)	Endpoint	Parameter
Vibrio fisheri	MSP (Microtox Solid Phase)	Proteobacteria	0.014	Luminicence	TU50 (dry-weight)
Corophium volutator	Coroph	Crustacea	10	Survival	Percentage survival
Crassostrea gigas	Crasso	Mollusca	2	Larval survival and deformation	Percentage total effect
Brachionus plicatilis	Rotox	Rotifera	1	Survival	NOEC
Echinocardium cordatum	Echin Sur Echin TE	Echinodermata	14	Survival and re-burying behaviour	Percentage survival (Sur) Total effect (TE)

- *Corophium volutator* is an amphipod and widely used for in-vivo bioassays (Lourens et al. 1995; OSPARCOM 1997; Thain et al. 2000; Heijerick et al. 2000; Peters et al. 2002). For the application on the Wadden Sea sediments organisms unable to pass a 500 um sieve were collected from a clean site (Oesterput, Eastern Scheldt, The Netherlands). After an acclimation period of 2–4 days, in which the animals were not fed, *Corophium* was exposed to the Wadden Sea sediments. In this bioassay animals are exposed in a steady state system. Fore each sediment, five glass beakers are filled with 200 ml sediment and 800 ml sea water. After 24 h 20 *Corophium* are added to each beaker. During a period of 10 days bioassays are kept at a temperature of 15°C, and abiotic parameters are measured regularly. Each bioassay is accompanied by several controls. After ten days the number of surviving *Corophium* is counted.
- *Echinocardium cordatum* is a sea urchin belonging to the family of echinoderms. The animals used were obtained from the Eastern Scheldt and kept in the laboratory, sometimes for months, until they were used. The bioassay with *Echinocardium cordatum* is performed in a flow-through system, in witch 10 animals (10–30 g) are exposed in a sediment layer of 4.5 l and a water volume of 2 l which is refreshed wit a speed of 10 l/d. The bioassay is performed in quadruple. During a period of ten days all bioassays are kept at a temperature of 15°C, and abiotic parameters are measured regularly. Each bioassay is accompanied by several controls. After ten days the number of surviving *Echinocardium* is counted and the reburial behaviour of the surviving animals is studied.
- The sediment bioassay with the bacteria *Vibrio fisheri* (Microtox Solid Phase) is based on measuring the light emission from the fluorescent bacterium. The sediment (7 g) used for this test is brought into suspension. During the bioassay a suspension of the sample is incubated with *V. fisheri* for 20 min, after which the pore water is extracted. The light emission of the bacteria is the pore water is measured, using photospectrometry, and measured by the Microtox Analyzer bought at Azur (Germany). The concentration of the sample that reduced the light emission of the bacteria 50% in relation to the control was calculated for each sediment. This value was corrected for the percentage dry weight of the wet sediments and inverted to the number of toxic units causing 50% effect (TU50). This test was also used by e.g., Pedersen et al. (1998) to characterize sediments from the Copenhagen harbour.
- In the bioassay with larvae of the oyster *Crassostrea gigas*, the larvae are exposed to an elutriate (15 g of sediment in 750 ml sea water) of the sediments. *C. gigas* larvae are between 70 and 300 μm large and feed on pelagic algae, and can die or deform when exposed to contaminated sediments. To obtain larvae, adult oysters are stripped and gametes are divided over 10 replicates. These are kept at 15°C and abiotic parameters are measured regularly. After 48 h, the mortality of the larvae is recorded and the amount of deformation is estimated.
- The pore water bioassay with the rotifer *Brachionus plicatilis*, is a bioassay in which rotifers are exposed to a dilution range of pore water. *B. plicatilis* is between 200 and300 μm in size and feeds on unicellular algae. These bioassays can be purchased under the name ROTOXkitM. Pore water is collected by centrifugation of the sediment. The dilution range is made with filtered sea water. The rotifer is exposed in a Mulitwell test plates, each dilution has five replicates containing 5 rotifers each. After 24 h at 25°C in darkness the mortality is determined. From these data a NOEC (expressed in % pore water) is calculated.

Data analysis

Multivariate techniques are used to link field concentrations of chemicals with bioassay responses (Shaw and Manning 1996; Del Valls et al. 1997). We used ordination methods to reduce the data-set to a two-dimensional summary, represented by an ordination diagram. This diagram provides an overview of mutual relationships between concentrations of chemicals on the one hand and their

relationship with sample sites and bioassay responses on the other hand. PCA is one of the most frequently used ordination technique (Ter Braak 1995); it is based on a linear or monotonic response model similar to that used in linear regression analysis. However, in regression analysis the explanatory variables are measured (manifest), but in PCA, explanatory variables are latent: that is, the explanatory variables are calculated from the data set so as to best explain the variation in concentrations of chemicals in the sediments of the different sample sites. After the construction of a diagram using the first and second latent variables, several characteristics of the sample sites can be superposed on the diagram, i.e., they can be regressed on the axes using the sample site points. In this paper the characteristics are bioassay responses, and the diagram shows the (cor)relationship between these bioassay and the arrangement of the sample sites and chemicals.

In this section the interpretation of the biplots resulting from a PCA analysis is provided; for a more elaborate description is referred to Ter Braak (1995). Figure 2 gives an example of a triplot representing the chemicals and sample sites by points and the bioassay variables by arrows. If an (imaginary) line is drawn through a chemical point and the origin of the plot, the relative concentration of this chemical in the sediments of the different sample sites can be derived by perpendicularly projecting of the sample site points on this (imaginary) line. For the site point projecting on the 'chemical line' far away from the origin, but on the same side of the origin as the chemical point, a relatively high concentration of this chemical is indicated. The greater the distance between the projection of a sample site point and the origin, the higher the indicated concentration of this chemical is in the sediment of this site. If a site point projects on the other side of the origin, compared to the chemical point, the indicated concentration of this chemical is relatively low in the sediment of this site. The distance of the chemical point from the origin indicates the magnitude of the differences in sediment concentrations between the different sites. The PCA triplot also indicates the relationship between the bioassay responses and the concentrations of chemicals in the sediment. The variables are represented by an arrow, which points to the sample sites with the highest bioassay response. Redundancy Analysis (RDA), the constrained form of PCA can be followed by a Monte Carlo permutation tests to test the significance of the correlation between the response of the bioassays and the differences in chemical concentrations between the sample sites (Ter Braak 1995).

The following statistical analyses were performed to meet the objectives of the paper:

– A PCA, to characterize the correlations between the concentrations of all chemicals and the concentrations at all sample sites, using bioassay responses as passive variables. This to identify clean reference sites and identify chemical classes that may pose biological risks.
– Monte Carlo permutation tests to identify the significance of the correlation between the bioassays and

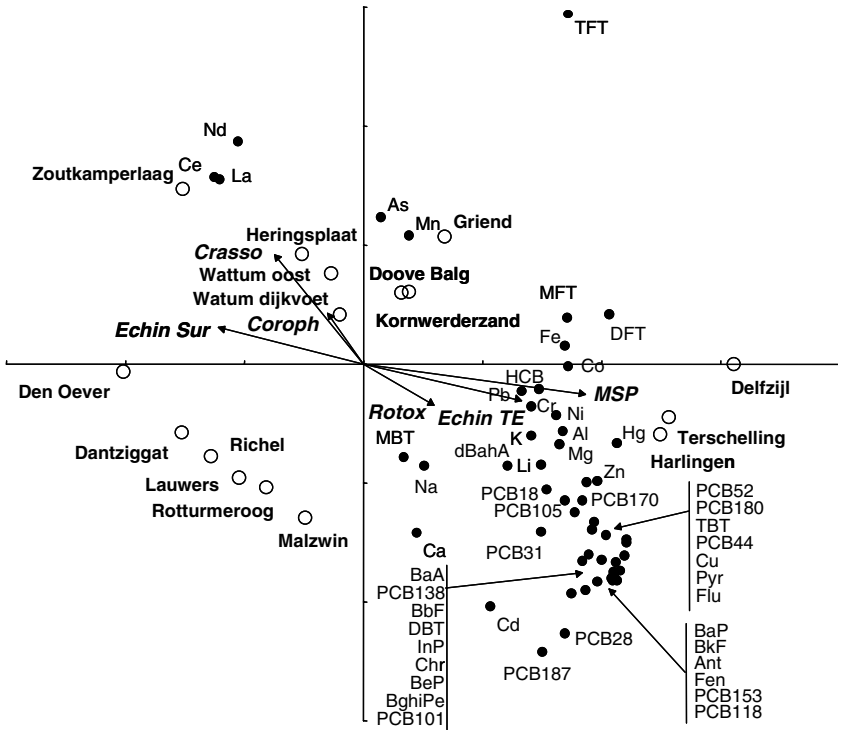


Fig. 2 PCA triplot showing the relationship between the variation in concentrations of chemicals in Wadden Sea sediment samples, and the response of five bioassays on (extracts of) these sediments. The first and second axes account for 56% and 17% of the variation in chemical composition between sample sites, respectively. The six endpoints of the 5 bioassays explain 62% of this variation (54% on the first axis, and 18% on the second axis). Abbreviations used to denote the types of bioassays are given in Table 2

chemical classes. When significant, the sub-data set containing only the chemical class is also analysed by PCA to provide an graphical overview on the correlations

In all analyses chemical concentrations were ln (ax + 1) transformed. The factor a was determined for each chemical separately according to the procedure described in Van den Brink et al. (2000). Concentrations of chemicals lower than the detection limit were set to one half of the detection limit; this technique standardizes chemicals with respect to their lowest concentration (often the detection limit). The Toxic Units of the MSP bioassay of two samples were above the highest tested concentration, they were replaced by twice the highest concentration. The analyses were performed using the computer program Canoco for Windows 4, running on a PC (Ter Braak and Smilauer 1998).

Results

The results of the five bioassays performed on the 16 sediments are summarized in Table 3. Microtox Solid Phase shows a high response for sediments from six sites; *B. plicatilis* responses were evident for samples from only 2 sites, while *E. cordatum. C. volutator* only showed a large response at the sample of 1 site. *C. gigas* responses were intermediate for 2 sites, and large for 12 sites. Only 2 sites did not appear to adversely affect any of the five species tested.

Figure 2 shows the triplot of the PCA analysis on the chemical data set using bioassays as passive variables. The PCA triplot is a way to ''map'' sites with respect to concentrations of pollutants. The sediment concentrations of PAHs, PCBs, tin compounds and some of the metals correlate strongly in this study (Fig. 2). Contaminant concentrations were greatest in Delfzijn, Harlingen and Terschelling, and the lowest at the sandbars Zoutkamperlaag, Heringsplaat and Watum oost and the shallows Den Oever and Watum dijkvoet. The concentrations of the elements show more variability than the other chemical groups; for some elements (Ce, Nd and La) high concentrations are indicated for Zoutkamperlaag while for two others (As and Mn) high concentrations are associated with Griend and some other sample sites (Fig. 2). The phenyl tin compounds are in a different place of the diagram compared to the other chemicals. All tin compounds are associated with the harbour sample sites, but this relationship is stronger for the chemicals belonging to the butyl class compared to those belonging to the phenyl class. High concentrations of TFT are especially associated with Griend (Fig. 2).

The direction of the toxicity, as appointed by the bioassays, is generally from left to right in Fig. 2. Only the bioassays performed with *B. plicatilis* and *C. gigas* indicate an opposite direction. Their individually explained variance, however, is very low and the Monte Carlo permutation tests also indicate no significant relationship between their response and the concentration of the chemicals at the different sample sites (Table 4). Both the PCA triplot as the Monte Carlo permutation test indicate a high, statistically significant relationship between the sediment concentrations of the chemicals and the Microtox bioassay on suspensions of these sediments (Table 4). The response of

Table 3 Results of the six endpoints measured in five bioassays performed on the 16 sediments. For the names of the sample sites is referred to Figure 1

Sample location Endpoint	*Corophium volutator* % survival	*Crassostrea gigas* % survival	*Echinocardium cordatum* % survival	*Echinocardium cordatum* Total effect	*Vibrio fisheri* (MSP) TU50	*Brachionus plicatilis* (Rotox) NOEC
1	95	13.0	100	0	2,398	100
2	95	99.2	100	0	179	100
3	88	12.2	90	10	161*	100
4	92	10.6	100	5	3,362	100
5	92	18.8	100	0	1,047	12.5
6	93	46.6	95	5	130	100
7	63	17.8	100	0	2,231	25
8	71	12.6	25	75	7,454	100
9	99	14.2	95	5	314	100
10	81	16.8	100	0	339*	100
11	91	3.6	100	0	609	100
12	93	-11.0	100	0	637	100
13	34	37.8	100	0	1,176	100
14	93	3.0	100	0	655	100
15	98	99.2	100	0	140	100
16	95	24.2	100	0	428	100

*The Toxic Units of these samples were above the highest tested concentration, they were replaced by twice the highest concentration

Table 4 Results of RDA analyses and Monte Carlo permutation tests (percentage explained variance and *P*-values, respectively) of the relationship between bioassay response and the concentrations of the chemicals

		Coroph	Crasso	Echin Sur	Echin TE	MSP	Rotox
All chemicals	% explained	3	9	13	14	22	6
	P-value	>0.10	>0.10	>0.10	>0.10	0.028	>0.10
Elements (including metals)	% explained	9	10	6	7	12	18
	P-value	>0.10	>0.10	>0.10	>0.10	>0.10	0.055
PAHs	% explained	5	17	16	19	34	9
	P-value	>0.10	>0.10	>0.10	>0.10	0.015	>0.10
Chlorinated aromatic compounds	% explained	2	10	10	11	16	7
	P-value	>0.10	>0.10	>0.10	>0.10	>0.10	>0.10
Tin compounds	% explained	2	5	14	16	24	4
	P-value	>0.10	>0.10	>0.10	0.078	0.016	>0.10

These analyses were performed for all substances and for each chemical class separately (elements, PAHs, chlorinated aromatic substances, and tin compounds) *P* values deemed to be statistically significant ($P < 0.05$) are underlined; moderately significant *P* values ($0.05 < P < 0.10$) are not underlined. For explanation of the abbreviations of the bioassays, refer to Table 2

all other bioassays did not relate significantly with the sediment concentrations of the chemicals. However, the position of the bioassay results for *E. cordatum* in the triplot, and the amount of variance explained by the results of the E. cordatum tests (>10%) indicated a positive correlation.

When chemical classes are analysed separately, the *B. plicatilis* assay related significantly ($P = 0.055$) to concentrations of the elements (including metals). Stronger relationships with other chemical classes were found the Microtox bioassay (Table 4). Microtox SP was the only bioassay yielding significant responses to sediment concentrations of PAHs. Microtox bioassays also had a significant relationship with sediment concentrations of tin compounds. The *E. cordatum* bioassay had moderately significant relationship ($P = 0.078$) with the tin compounds when total effect (survival and reburying behaviour) is used as endpoint (Table 4).

The Monte Carlo permutation tests indicated significant relationships between the sediment concentrations of PAHs, tin compounds, and bioassay responses (Table 4). For this reason, we also used PCA to examine relationships among these three sets of parameters separately. The results from these PCAs are shown in Figs. 3 and 4. The concentrations of PAHs, except dBahA, correlate strongly with each other (Fig. 3). The deviating position of dBahA in the triplot can be explained by relatively low concentrations of this PAH in sediments from Zoutkamperlaag, Harlingen and Richel. When interpreting the triplot, one should remember that relationships indicated by the first axis are much more important than those comprising the second axis (Fig. 3). The Monte Carlo permutation tests

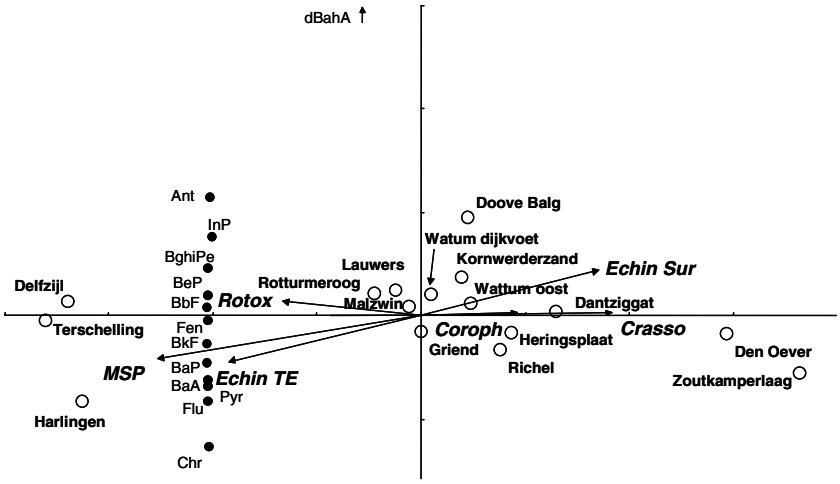


Fig. 3 PCA triplot showing the relationship between the variation in concentrations of chemicals belonging to group of PAHs, of the Wadden Sea sediments and the response of five bioassays on (extracts of) these sediments. The first and second axes display 95 and 4% of the variation, respectively, in chemical composition among sampling sites. The bioassays explain 63% of this variation (97% of this is expressed along the first axis, and 2% is expressed along the second axis). Abbreviations of the bioassays are given in Table 2

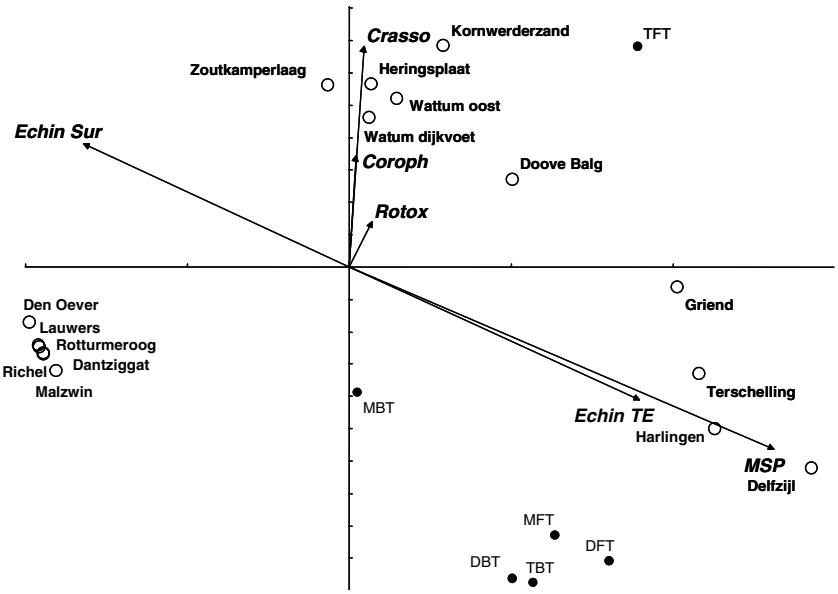


Fig. 4 PCA triplot showing the relationship between the variation in concentrations of chemicals belonging to the tin compounds of the Wadden Sea sediments and the response of five bioassays on (extracts of) these sediments. The first and second axis displays 70 and 18% of the variation in chemical composition between sample sites, respectively. The six endpoints of the five bioassays explain 48% of this variation of which 61% is displayed on the first axis and another 20% on the second one. For explanation of the abbreviations of the bioassays is referred to Table 2

also showed that the response of the Microtox bioassay had the strongest relationship with PAH levels. A relatively strong (but not statistically significant; $P > 0.10$; see Table 4) correlation was detected between the concentrations of PAHs and the responses of *C. gigas* and *E. cordatum* (Fig. 3).

The data sets containing only the concentrations of the tin compounds and only the PAHs were very one-dimensional. This condition occurred because the percentage of the total variance explained by the first axis was very high (70% for the tin compounds, 95% for the PAHs; Fig. 4). For both of these classes of toxicants, the harbour sample sites separated well from the other sample sites. However, as a result of its relatively high concentrations at Griend and Kornwerderzand, TFT separated from the other chemicals. The differences expressed on the second axis explained only a small amount of the total variance.

Discussion

The response of the Microtox bioassay had a positive, significant relationship with the levels of PAHs and tin compounds. The PCA triplot of the overall analysis (Fig. 2) showed that the levels of all PAHs correlate strongly with each other as well with PCBs, some metals and tin compounds such as TBT. The TBT levels measured in this study (geometric mean 40, maximum 614 μg TBT/kg; Table 1) are similar to those reported by Wade et al. (1990). Wade et al. (1990) reported average values of 54 μg TBT/kg for the coastal areas, and a maximum value of 457 μg TBT/kg. Argese et al. (1998) and Davoren et al. (2005) reported that the Microtox bioassay was a good screening tool for the toxicity of tin compounds in water. Argese et al. (1998) also found a strong correlation between the response of the Microtox bioassay and toxicity data for aquatic organisms, with the highest correlations reported for invertebrates followed by algae and fishes. A recent review (Doherty 2001) also reported that Microtox is a good predictor for the toxicity of tin compounds to aquatic invertebrates. Collectively, these reports, plus our findings, indicate that organotin compounds should not be excluded as risk factors to aquatic invertebrates in the Dutch Wadden Sea. However, this assessment is based on correlations only, and it is possible that effects observed in the Microtox bioassay may be a result of (unknown) co-occurring compounds (cf. Batley et al. 2002). The possibility of toxicity due to tin compounds in the Wadden Sea is supported by Mensink et al. (1997), who reported imposex frequency >95% for the common whelk on sediments in the eastern Scheldt (The Netherlands); these sediments contained maximally 3.4 μg TBT/kg. Alzieu (2000) reported bivalve imposex at concentrations as low as 1 ng TBT/L, which corresponds with 0.6 μg TBT/kg assuming a sediment-water partition coefficient of 550 (cf. Tas 1993). Using the same partition coefficient, Alzieu (2000) reported that reproduction of bivalves was affected by a TBT concentration of 11 μg/kg; a concentration that is much lower than the concentrations reported in our study.

For effects on mortality, Stronkhorst et al. (1999) reported NOEC values of 2.8 mg TBT/kg for the urchin *E. cordatum* and the amphipod *C. volutator*. In our study the response of both species in the bioassays also showed no relationship between mortality and the levels of tin-compounds (Table 4). The moderate significant relationship of the total effect (including reburying) on *E. cordatum* and the levels of tin-compounds indicate the risk of sub-lethal effects. Potential adverse effects of the levels of

TBT are not indicated for the oyster *C. gigas*. The toxic effects of TBT on the shell thickness in *C. gigas* starts at concentrations of about 2 ng/l which corresponds with a sediment concentration of 1.1 μg TBT/kg when the above mentioned partition coefficient is used (Tanguy et al., 1999). Tanguy et al. (1999) observed direct lethal effects at a concentration of 150 ng TBT/L after 5 days of exposure. In accordance with these findings we also did not observe mortality of *B. plicatilis* in our study which could be attributed to tin-compounds (Table 4). These comparisons can suffer from differences in bioavailability of the compounds in the sediments and differences in analytical techniques used between the different studies

No apparent relationship between HCB and the responses of the bioassays were present. So based on the battery of bioassays performed in this study these HCB levels are not indicated to pose an ecotoxicological risk.

The Monte Carlo permutation tests indicate a moderate significant relationship between the response of the bioassay performed with *B. plicatilis* and the levels of several elements (among which metals). This could be a result of the occurrence of occasionally high levels of metals (e.g., cadmium at Den Oever). It is difficult to assign this toxicity to a (combination of) metals because the rotifers where exposed to pore water in which the concentrations of these elements are unfortunately unknown (Mowat and Bundy 2001).

In terms of ecological relevance the results of the in-vivo bioassays performed with *E. cordatum* and *C. volutator* can most easily be extrapolated to actual risks because they are performed with relevant species using a realistic exposure. The results of in vivo bioassays using pore water (*B. plicatilis* and *C. gigas*) are more difficult to translate to ecotoxicological risks because pore water is most likely not a very good descriptor for the concentration of pollutants in the overlying water to which the species in the field will be exposed. In vitro bioassays like Microtox have very limited ecological value because their endpoints are opaque, but can be a very cost-effective tool when their predictive value is validated for a number of compounds using results of other bioassays or field studies (see Doherty 2001 for a review of the Microtox bioassay).

The results of the chemical and biological evaluation of the sediments are in good general agreement, but differences are apparent, as well (Fig. 2). From a chemical perspective, sediments in the three harbour sites (locations 4, 8 and 14) were clearly the most polluted (Fig. 2), and from a biological perspective, sediment from locations 4, 5, 7, 8 and 13 (Table 3) appeared problematic. On the basis of chemical measurements, locations 3, 5, 11, 12, 13, and 16 appeared to be relatively ''clean'' (Fig. 2). Based on the results of the bioassays, sediments from locations 1, 3, 6, 9, 10, 11, 12, 14, 16 also were relatively clean. Thus, on the basis of both chemical and biological evaluations, the sample sites Dantziggat (3), Malzwin (11), Richel (12) and Lauwers (16) should be suitable as ''clean'' reference sites.

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