Ecotoxicity assessment of natural attenuation effects at a historical dumping site in the western Baltic Sea

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Abstract

During the late 1950s and early 1960s of the past century, industrial waste material highly enriched in various contaminants (heavy metals, PAHs) was dumped in the inner Mecklenburg Bay, western Baltic Sea. Large-scale shifts in the spatial distribution of heavy metals in surface sediments were mapped by geochemical monitoring in the mid-1980s and 12 years later in 1997. A further study in 2001 was designed to investigate the small-scale spatial distribution of contaminants inside, on top of, and around the historical dumping ground and to examine possible effects to benthic organisms (*Arctica islandica*, microbiological toxicity tests). The site is located within an area characterized by a discontinuous deposition of fine sediments, but net sedimentation rate of about 3 mm/y led ultimately to a gradual coverage of the dumped material. During the first decades after the dumping, about half of the originally dumped material was already spread over the surrounding area as a result of sediment dynamics and re-suspension processes. Recent sediments of the historical dump site are still significantly enriched in heavy metals and PAHs. Microbiological toxicity test results were positive, though uptake of contaminants in mussels was found to deviate only slightly from that of a non-contaminated reference station due to a gradual dilution and decrease in bioavailability of the contaminants.
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1. Introduction

During the mid-1980s of the past century, extensive seabed monitoring programmes were started to investigate the distribution of geochemical parameters in surface sediments of major basins of the western Baltic Sea (Leipe et al., 1998). One result was the discovery of a heavy metal hot spot in the inner part of the Mecklenburg Bay (Figs. 1 and 2, top) which was readily assumed not to be of natural origin. The very high concentrations of both heavy metals and PAHs in the centre of the anomaly clearly indicated an anthropogenic impact. Information on history and sequence of dumping activities in that area, as well as origin of the material is sparse. Reliable documentation is not available, and all information at hand is that industrial waste material from several industrial sources in Lübeck city was dumped in this area since the late 1950s, before dumping activities were stopped in mid-1960s.

Twenty years after dumping was stopped, a considerable part of the material was found to be spread over the whole inner part of the Mecklenburg Bay due to re-suspension and transport of surface sediments in the 20–25 m deep area (Leipe et al., 1998). In 1997, the

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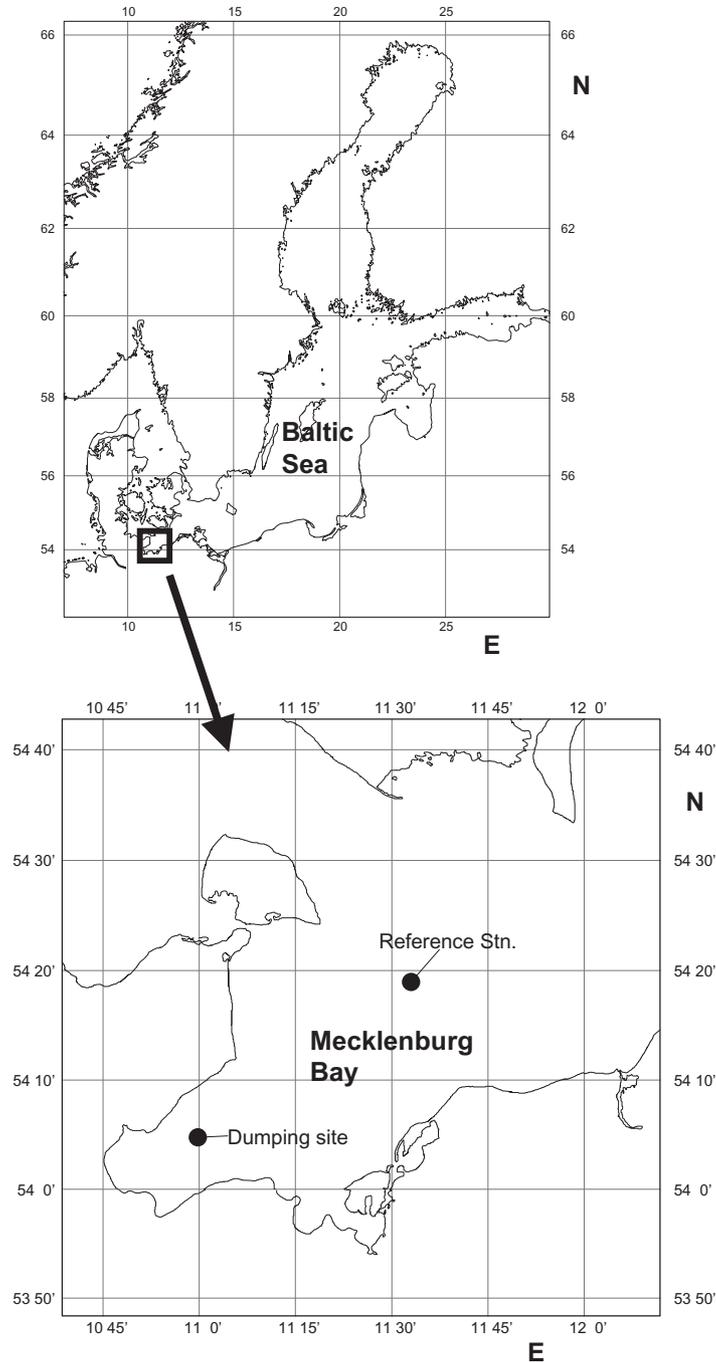 *E-mail address:* thomas.leipe@io-warnemuende.de (T. Leipe).

Fig. 1. Map of the study area in the Mecklenburg Bay, western Baltic Sea, Germany.

sediment mapping was therefore repeated again for quantifying the contaminant dynamics. Another survey of the hot spot at high spatial resolution was performed in August 2001 to investigate (i) its spatial extension, thickness, and effects of natural sediment capping; (ii) its contaminant inventory (heavy metals, organic pollutants) in comparison to that of regional background and capping sediments; (iii) biological effects on benthic molluscs (*Arctica islandica*) habitating the affected area; and (iv) ecotoxicological test response with both pol-luted sediments and material from a reference station outside.

2. Material and methods

2.1. Small scale survey of the dumping site

A high resolution geoacoustic sediment profiling was performed on basis of the assumption, that the dumped
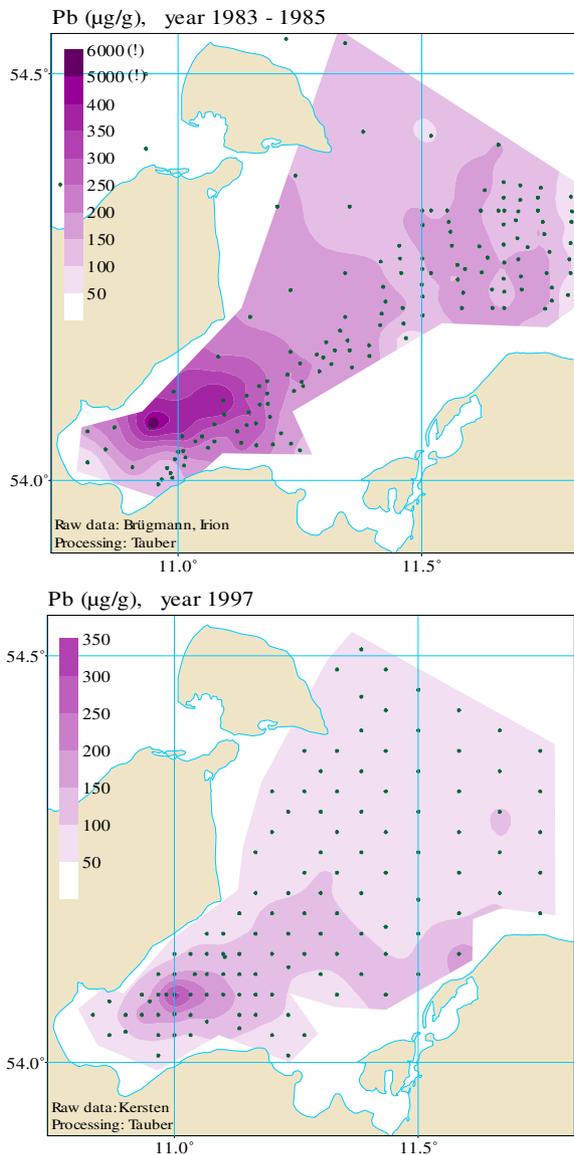
Pb (µg/g),   year 1983 - 1985

Raw data: Brügmann, Irion
Processing: Tauber

Pb (µg/g),   year 1997

Raw data: Kersten
Processing: Tauber

Fig. 2. Regional distribution of Pb (µg/g) in surface sediments (0–2 cm; <20 µm sediment fraction) in Mecklenburg Bay, based on two monitoring surveys in the mid-1980 (top) and 1997 (bottom). The historical dump site appears as a hot spot in the south-western part of the bay.
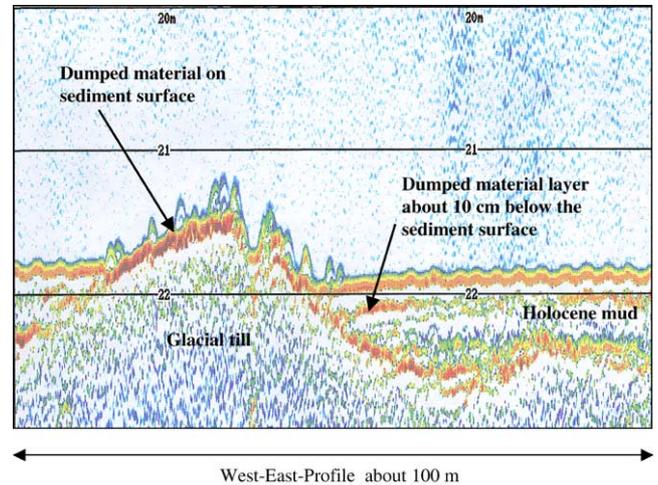

West-East-Profile about 100 m

Fig. 3. High resolution sediment acoustic profile across the centre of the dump site. Vertical scale is from 20 to 23 m below the sea surface.

these cores were selected for detailed chemical analyses of major and trace elements. One core (#237550) was selected for PAH analysis because of its striking odour. All cores were sliced into 2 cm segments down to 30 cm depth. A box-corer was used additionally for sub-sampling larger amounts of pure dumped material. A dredge was used for collecting mussels (*Arctica islandica*). All sediment sub-samples for chemical analyses were freeze dried and subsequently homogenised. No sieving or other normalization approach was deemed necessary because >95 wt.% of the material was of a grain size <63 µm.

For the ecotoxicity tier test approach, sub-samples were selected from 3 stations (A, B, C, see Fig. 4) within the old dumping site, and one surface sample (0–2 cm) from the reference station (R). At the dumping site, layers of conspicuous colour (black and red) or consistency were sub-sampled. Since about 50 cm^3 were needed for the ecotoxicity tier test, segments of 5 cm thickness were pooled, homogenized and stored at 4 °C until the tests were carried out in the laboratory.

2.2. Laboratory analyses

X-ray fluorescence (XRF) was used for major and trace element analysis of sediment core sub-samples after fusion with Li-metaborate. ICP-AES and AAS were used for analyses of As, Pb, Zn, and Cd in selected sediment samples upon pressure digestion by a strong acid mixture (HNO_3/HF/$HClO_4$). All data were corrected for loss-on-ignition, and for salt content based on the XRF-determined Cl content. For the analyses of Cd, Pb, Cu, and Zn in *Arctica islandica* mussel soft tissue, samples were digested by pressure wet-ashing with 1.5 mL HNO_3 (subboiled) and 100 µL $HClO_4$ (suprapur) for 2 h at 180 °C, evaporated to dryness, and re-dissolved in 1 mL diluted HNO_3. The final acidic

material is characterised by a higher density than the surrounding natural muddy sediment. This technique enabled us to follow the sediment layers in the topmost tens of cm of the sediment column for selecting suitable core sampling positions (Figs. 3 and 4). On basis of this geophysical screening, 41 stations were sampled in total with help of both a multi-corer and single-tube gravity corer device. In addition, a reference station was chosen in the outer Mecklenburg Bay about 25 nautical miles NE of the hot spot (see Fig. 1), for comparison with less impacted adjacent areas.

On board of the ship, the cores were optically examined for the presence of dumped material. Nine out of
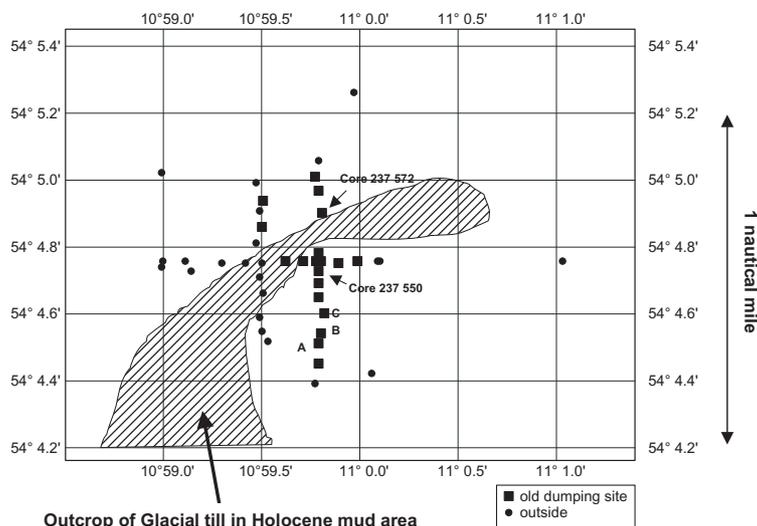
Fig. 4. Results of a small scale survey of the dump site in August 2001, based on sediment acoustic profiling and sediment coring (dots and squares). A, B, C: Location of the stations which are used for ecotoxicological testing, PAHs analyses and sampling of *Arctica* mussels. Core #237550 was analyzed for PAHs (see Fig. 6) and the element analyses results from core #237572 is seen in Fig. 5.

solutions were analysed by atomic absorption spectro-photometry (AAS system Analyst 800 of Perkin-Elmer, with Zeeman correction). For the analyses of Hg in biota and sediment, samples were digested by pressure wet-ashing in Quartz-tubes with 3 mL HNO_3 for 2 h at 130 °C. The final acidic solutions were analysed by using atomic fluorescence spectrometry (AFS system Merlin of PS Analytical) upon reduction with $SnCl_2$ and amalgamation on a gold net (Hatch and Ott, 1968).

Analysis quality control and assurance (QA/QC) were checked by using the appropriate GMP/GLP procedures such as using internationally certified and in-house reference materials (TORT1 for heavy metals in marine biota, and MESS-2 for sediments), as well as successful performance in international intercomparison exercises such as in the framework of the QUASI-MEME program, both for sediments and biota (Pohl, 1997; Pohl et al., 2001).

Accelerated Soxhlet extraction was used for PAH analysis (DIONEX ASE system). Details of sample preparation, PAH isolation and QA/QC were discussed earlier (Witt, 1995; Witt and Trost, 1999). In brief, extraction was first carried out twice with a mixture of *n*-hexane and acetone (9:1). These extractants were pre-cleaned using a 5 g Al_2O_3 SPE column, eluted with 10 ml 50% *n*-hexane/CH_2Cl_2. After separation of the final extract and subsequent evaporation to about 200 μL, the PAH fraction was isolated by HPLC using a silicagel column (MERCK, LiChrospher Si 100-5). For quantification an ion-trap GC/MS system (GCQ system from THERMO QUEST) was used. All samples were spiked with internal standards prior to the extraction (naphthalene d-8, phenanthrene d-10, fluoranthene-d-10, chrysene d-12 and perylene d-12,

benzo(*g,h,i*)perylene-d-12), which yielded in successful quantification of 18 parent PAHs as well as some methylated homologues. The methylated PAHs were quantified with external standards. Blank procedure were systematically carried out and showed no detectable interferences. Analytical quality control was carried out with a certified reference material for PAHs in marine sediments (PROMOCHEM, SRM 1941 A). The mean recovery values ranged between 41% and 102%.

An ecotoxicological tier test approach was performed using five different microbiological biotest methods, in order to assess potentially harmful effects of the dump wastes on marine organisms. Using a variety of biotests rather than just one bioassay has been recommended for the unequivocal detection of potential adverse effects of complex admixtures of contaminants (Ahlf et al., 2002; Keddy et al., 1995). A variety of organisms with different sensitivities towards chemicals is warranted to minimize false negative responses which may be otherwise biased by high specific tolerances of a single test species. Microorganisms (three bacteria and one algae species) were used in this tier test approach because of (i) the similarity of complex biochemical functions (e.g. photosynthesis, cellular respiration) with higher organisms (Cairns et al., 1992; Liss, 1996; Liss and Ahlf, 1997), (ii) a short generation time, allowing effects to be measured over 2–3 generations, (iii) small sample volume requirements, facilitating higher toxicity resolution, (iv) high number of test organisms, increasing reproducibility and accuracy of the test, and (v) the potential for miniaturization, allowing for higher number of samples to be measured with more replicates and dilution steps. These points were all of high advantage in this study, as the distribution of contaminants at the disposal site was

assumed to be heterogeneous and different between individual sediment layers.

The biotest tier ultimately chosen addressed different exposure pathways. The sediment elutriate approach has frequently been recommended for toxicity assessment of water-soluble substances that can be remobilized and enter the water column following resuspension events, e.g. dredging or disposal (Ankley et al., 1992). Elutriates were produced from mixing one volume of sediment with four volumes of de-ionized or saline (20‰) water. After 24 h of mixing and aeration, the supernatant was separated by centrifugation (10 min at 10,000g), stored at 4 °C and tested within two days. Sediment direct tests, that address particle-bound toxicity, were carried out by mixing a suspension (1:1 sediment/de-ionized or saline water) with bacteria (1:1 sediment/bacteria), and incubation on an end-over-end-shaker for 2 h. A resazurine solution was added, and the substrate reduced by respiratory chain activity to resorufin. The resorufin was measured fluorometrically after 20 min of incubation. Quenching effect variations by sediments on the fluorescence yield were corrected for by determining resorufin fluorescence-concentration curves. Additionally, methanol was used to extract more lipophilic substances of potential toxicity. Sediment sub-samples were mixed with methanol at a 1:4 volume ratio for 1 h, followed by centrifugation, and diluted to a 3% solution before testing.

Another biotest combination in the tier approach comprises an algae growth inhibition test on elutriates according to DIN 38412 Part 33 with the ISO-standardized organism *Pseudokirchneriella subcapitata*, a 30-min bioluminescence inhibition test of *Vibrio fischeri* (DIN 38412 Part 34) performed both on elutriates and methanol extracts, and two sediment contact test with *Bacillus cereus* (reduction of dehydrogenase activity as endpoint, carried out according to DIN 38412, part 48 as discussed previously by Liss and Ahlf, 1997; Rönnpagel et al., 1995), and *Vibrio proteolyticus*.

Although there is a variety of test protocols recommended for marine bioassays (e.g. from EPA, OSPAR, ICES, etc.), only two ecotoxicity tests are standardized for the aqueous phase according to ISO standards, which are (i) the Luminescent Bacteria Acute Toxicity (LBAT) test with species *Vibrio fischeri* (ISO/FDIS 11 348), and (ii) the Algae Acute (72 h) Toxicity (AAT) test using *Skeletonema costatum* or *Phaeodactylum tricornutum* species (ISO 10 253). The marine diatom species *Phaeodactylum tricornutum* sensitive in single-substance toxicity tests (Bjoernestad et al., 1993) showed no simulation effects in our elutriate testing. It has therefore not been considered for inclusion into our biotest tier approach.

A critical point might be that our biotest tier approach for brackish water conditions comprised both freshwater (*B. cereus*, *P. subcapitata*) and marine organisms (*V. fischeri*, *V. proteolyticus*). However, the species

B. cereus did not show any negative response to salinities up to 20‰, neither in growth nor in sensitivity towards the positive control compound paranitrophenol. *P. subcapitata* was used to test elutriates that derived from a 1:16 sediment/water ratio (corresponding to 25% of the initial elutriate), in order to ensure a salinity below 1.5‰ (controlled before the test). *P. subcapitata* shows salinity-induced toxicity of more than 30% above 3‰. Consequently, only inhibition of more than 30% was counted for as a toxic effect. All tests included the measurement of a dilution series, whereby elutriates were diluted with de-ionized and saline (20‰) water for freshwater and marine organisms, respectively, or with fine quartz sand (average grain size: 65 μm) in the direct contact tests. Effects are expressed as percent inhibition of the respective physiological activity in the sample compared to uncontaminated fine quartz sand.

To assess the autochthonous bacterial activity, dimethylsulfoxide (DMSO) reduction rates were determined. DMSO is reduced by enzymes of the electron transport chain under oxic as well as anoxic conditions to dimethylsulfide, the production of which was measured by gas chromatography (Ahlf and Gratzer, 1999; Griebler, 1996, 1997).

3. Results and discussion

3.1. Natural attenuation by sediment capping

A comparison of the Pb maps for the entire Mecklenburg Bay shows remarkable changes in between the monitoring campaigns undertaken in 1983–1985 and 1997 (Fig. 2). Thirty-two years after the abandoning of dumping activities, a clear trend is obvious (e.g.) for the Pb concentrations which fell towards ambient background values in the outer part of the bay, while the hot spot anomaly is still recognizable at the historical dumping site. Fresh material and new sedimentation have been diluting the contaminated layers and covered the old deposits. This natural attenuation process was advanced by the general decline in atmospheric Pb input as a result of gasoline lead regulations (Von Storch et al., 2003) and the overall success in environmental control policies and technologies.

The natural sedimentation rate in the bay varies between 1 and 3 mm/y (Niedermeyer and Lange, 1989). ^{210}Pb radionuclide dating of the sediment core (#237660) from the reference station by the Gamma Dating Centre, Copenhagen (H. Kunzendorf, personal communication), resulted in a rate of 1 mm/y, with a mass accumulation rate of about 300 g/m^2/y. This dating technique, however, is challenging due to the highly dynamic sediment transport regime at this site (Kersten et al., in press). Since a distinct maximum contamination horizon is visible in sediment cores of the dumping site, it could

be used for a simple estimation of the net sediment capping rate. Peaks of heavy metal concentrations in sediment cores collected at the same spot just two nautical miles east of the dumping site in 1985 (Leipe et al., 1998) and 2000 (unpublished) were found at sediment depths of 8 and 12 cm, respectively, which corresponds to a recent sediment burial rate of about 3 mm/y.

3.2. Localisation and composition of the buried waste layer

Use of geophysical methods for localisation of buried waste or contaminated sediment layers is possible, if properties of the material leads to a geophysical contrast to surrounding natural sediment. Chan et al. (2001) gave an example for the correlation of magnetic properties to heavy metal contamination. In our case, the dumped material has both a higher density and a higher magnetic susceptibility, as could be confirmed by multi-sensor core logging. Fig. 3 shows an example of a high resolution sediment acoustic section across the centre of the historical dumping ground to demonstrate the distinctive geological feature of the area. On an outcrop of Glacial till bedrock, the old dumped material is still present at the sea floor as we could prove by application of a video-camera equipped remote operating vehicle (ROV). Fig. 4 shows a sketch of the actual situation at the dumping ground area. The squares indicate the coring stations where we found a thin layer of buried waste material, macroscopically visible as reddish coloured layers (Fe-oxides) at sediment depths between 5 and 15 cm. Dots are coring stations without any visible indication of waste layers.

Fig. 5 gives the vertical profiles of selected major and trace elements (including heavy metals) in a sediment core representative for the centre of the dump site (core #237572). In total, we analyzed a series of nine cores across the dump ground. This enabled us to assess the
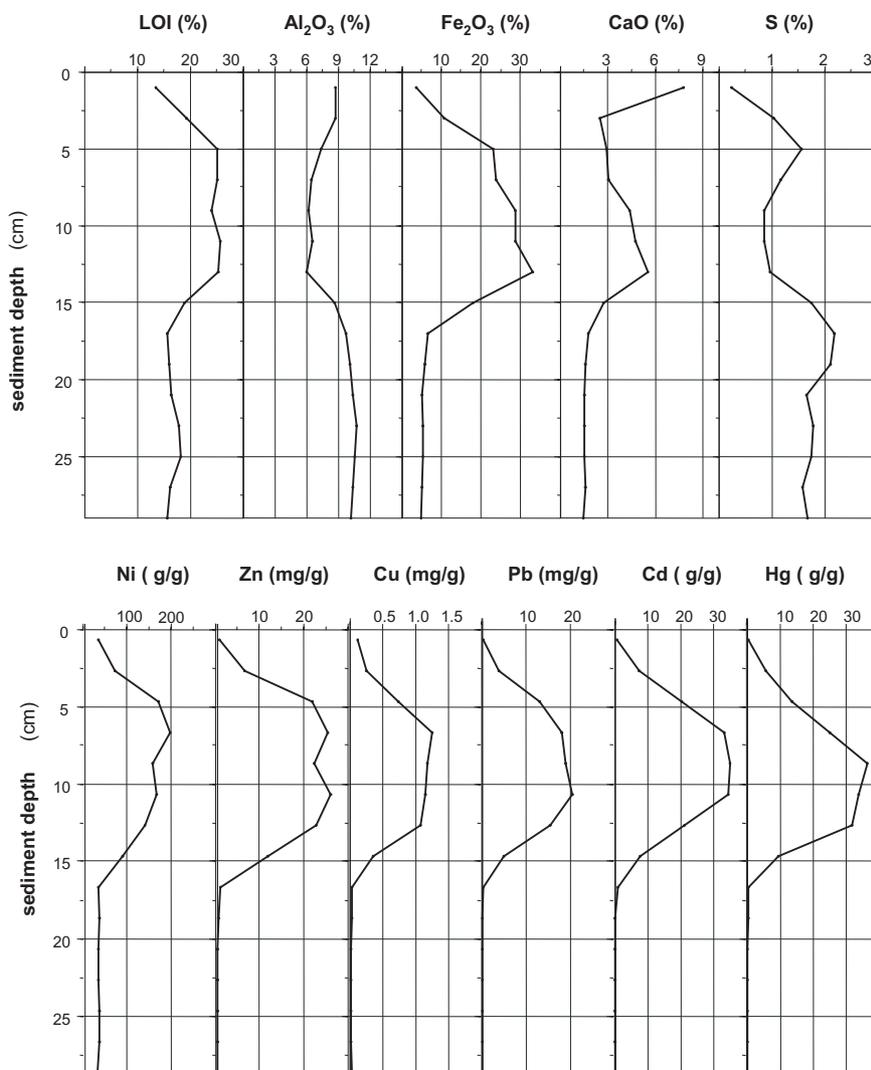

Fig. 5. Major and trace element (heavy metal) profiles of a 30 cm long sediment core (#237572) from the historical dump site.

mass of buried waste on base of lateral and vertical extension of the contaminated horizon. The contamination could be detected to a maximum depth of 17 cm, and reaches maximum pollutant concentration values between 5 and 13 cm. Towards the sediment surface, the concentrations decreased, but do not reach background values (which cannot be seen in Fig. 5 because of the extreme broad scale of element concentrations). Some elements (e.g., Pb and Zn) reach maximum concentrations of even 1–2% (dry-wt).

Table 1 shows the concentrations of selected elements and contaminants in four different sediment layers or units which allow a numerical comparison of the degree of contamination in the area over the time. The sediment horizon at the bottom of the analysed sediment cores (25–30 cm) can be used to assess the regional background values, which are equal for both the dumping area and the reference station. The dumped material is represented in the table by a pure waste layer (maximum found values) before the onset of sediment dilution and capping. In the topmost 2 cm layers above the waste material, and at the reference station, the elements Al, Fe, Cr, Ni, and organic carbon show comparable concentration levels. The contents for Cu (factor ~2), Pb (factor ~4), Zn (factor ~3), Cd (factor ~3) As (factor ~2), and Hg (factor ~5), are still higher in the recent layer of the dumping area in comparison to those of the reference station. The organic carbon content of the dumped material was about 20% (dry wt). In some cases more than 50% of black substances were measured. In comparison, values of 4–5% of organic carbon are normally found in unaffected sediments of this area.

Among the analyzed PAHs, Phenanthrene, Fluoranthene, and Pyrene are the most abundant, with extreme concentrations (each 20–30 μg/g dry-wt) measured in the red and black material found at stations B and C of the historical dump site. The sum of all PAHs in the buried waste layers was up to 140 μg/g, which

means a factor of 100 higher than in the sediment of the reference station. A vertical profile for the sum of PAHs in sediment core #237550 from the centre of the dumping area is shown in Fig. 6. Highest concentrations of up to 80 μg/g were found at a depth of 16–18 cm. In comparison to the highly contaminated slices, about 0.5 μg/g were measured as a regional background value at the bottom of the core, and 2 μg/g in the topmost sediment at those sites (see also Table 1).

For source apportionment of the dumped material, the distribution patterns of the parent PAHs vs. their
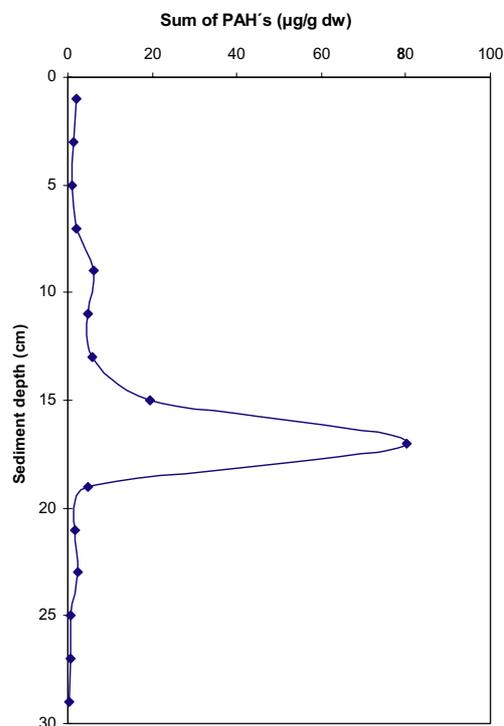

Fig. 6. Vertical profile of the sum of all parent and methylated PAHs in core #237550 from the historical dump site.

Table 1
Comparison of selected elements and contaminants in different sediment horizons of the study area

	Natural background (whole area)		Dumped material	Recent sediment dumping site		Recent sediment reference station	
	Mean	Stand. Dev.	Max. values	Mean	Stand. Dev.	Mean	Stand. Dev.
C-org. (%)	3.50	0.62	20	4.20	0.58	4.36	0.38
Al (%)	5.20	0.40	3.55	5.04	0.34	4.98	0.52
Fe (%)	3.80	0.20	18.70	4.11	0.48	3.51	0.20
Cr (μg/g)	60	10	102	75	6	80	3
Ni (μg/g)	25	3	436	38	4	36	2
Cu (μg/g)	23	5	2700	88	36	44	10
Pb (μg/g)	25	5	33,000	450	250	95	39
Zn (μg/g)	70	8	38,800	900	340	278	150
Cd (μg/g)	0.15	0.05	75	1.87	0.76	0.63	0.21
As (μg/g)	10	3	50	48	23	22	3
Hg (μg/g)	0.05	0.02	48	0.95	0.48	0.18	0.04
Sum PAHs (μg/g)	0.5		140	2		1	

methylated homologues were analyzed. While parent PAHs dominate in soot samples, crude oil contains primarily methylated PAHs. Naphthalene's are the most abundant PAH group in crude oil, whereas the fluoranthene/pyrenes are the most abundant in soot. Therefore the analyzed PAH series are a sensitive tool to distinguish between petrogenic and pyrogenic sources (Sporstøl et al., 1983). For all hot spot samples, the parent PAHs dominated which indicates pyrolytic processes as the probable source. Pyrolytic source is also evident from the extremely high concentrations of three and four-ring aromatics, especially for phenanthrene, fluoranthene, and pyrene, and the prevalent ratio between the components (fluoranthene > pyrene > benzo(*a*)pyrene) typical for combustion products.

To sum up, the composition of the waste material dumped in the past can be characterized as a mixture of (i) black carbon substances, partly greasy in form of heavy oil, tar, soot, and (ii) reddish coloured dusts or fine-grained material with Fe-oxides, together with some admixed ashes from various high temperature industrial processes (e.g., smelter and steel works, or chemical factories). Sediments of similar contaminant compound admixture are typically found in urban and harbour environments (e.g. Belzunce et al., 2001; Fatoki and Mathabatha, 2001; Cicero et al., 2000), or mining areas (Daka et al., 2003), but not in such high concentrations in the open marine environment which complicates an ecological impact assessment.

3.3. Mass budget for the hot spot plume

Based on these results, we calculated the total mass for selected heavy metals nowadays still present at the dump site, as well as the amount of material which was dispersed since cessation of the dumping activities (Table 2). Buried waste was determined to be spread over an area of 0.5 km^2 with an average thickness of 0.1 m and a dry bulk density (DBD) of 0.4 t/m^3. The total dry matter (TDM) of the volume, multiplied with the concentrations of heavy metals (μg/g = g/t), yields in to-

tal amount of these elements (in metric tons). For calculations of the contaminated plume around and off the dumping site we used the results from the 1985 mapping of the whole bay, which gave reliable data for at least two elements (Pb and Zn) at more than 150 stations. At this time, the contaminated surface sediment layer (samples were taken from 0 to 2 cm = 0.02 m) covered an area of about 7 × 15 nautical miles (361.4 km^2) with an average DBD of 0.3 t/m^3. The TDM of this volume was estimated at about two million tons. To calculate for the "additional" amount of contamination in this layer caused by the dispersion of material from the dump site, we used the mean concentration differences between stations inside the contaminated area and outside towards the NE part of the Mecklenburg Bay. The results show that the contaminated areas in and around the historical dump site contain nearly equal amounts of both Pb and Zn. This means that about half of the original dumped material was already dispersed in this highly dynamical bay area.

This assessment is only valid for the particulate phase. Nothing is known about the solubility of the original dumped waste, and hence how much of the heavy metals or PAHs may have been remobilized in dissolved or colloidal form. Monitoring surveys between 1994 and 1998 demonstrated elevated Pb and Zn concentrations in the water column of the Western Baltic Sea, as compared to the Baltic Proper (Schneider et al., 2002). Beside of the atmospheric input of these elements to the western Baltic Sea (as predicted recently from an atmospheric deposition model: Schneider, personal communication), wind induced admixing and bioturbation by marine organisms may be a continuing source for (at least particulate) trace metal contamination of the water column (Kersten et al., in press).

3.4. Biological and ecotoxicological effects

To evaluate biological effects of contaminated sediments, modern strategies not only focus on analyses of single species of organisms and concentrations of

Table 2
Mass calculations of total dry matter (TDM) and selected heavy metals in sediments of the dumping area and the surrounded contaminated area of the Mecklenburg Bay

	Area (km^2)	Thickness (m)	Volume (m^3)	DBD (t/m^3)	TDM (t)
Dumping ground	0.5	0.10	50,000	0.4	20,000
Contaminated area	361.4	0.02	7,228,000	0.3	2,168,400
Element concentration	Ni (μg/g)	Pb (μg/g)	Zn (μg/g)	Cu (μg/g)	
Dumping ground	167	6700	17,000	980	
Contaminant area (+)		80	210		
Masses	Ni (t)	Pb (t)	Zn (t)	Cu (t)	
Dumping ground	3.3	134.0	340.0	19.6	
Contaminant area (+)		173.5	455.4		

Values for contaminated area (+) means = total concentration − natural background.

contaminants, but also test the impact on benthic populations, e.g. with help of microcosm experiments (see Solomon and Sibley, 2002; Burton et al., 2000; Bonnet et al., 2000). Since *Arctica islandica* mussels can become more than 50 years old, growth rims in shells of this species could be used for retrospective monitoring. Richardson et al. (2001) could reconstruct the history of metal contamination in the North Sea by laser-ablation ICP-MS analysis of horse mussel shells. However, no clams older than 14 years were found at the dumping site. At the reference station, the oldest *Arctica islandica* was found to be 35 years old. Unfortunately, our measurements with the same LA-ICP-MS approach did not reveal any significant metal concentration trends along profiles through the growth rims of *Arctica islandica* shells. Therefore only total concentrations of Cu, Pb, Zn, and Cd in both the whole soft bodies and carbonate shells of 10 individual *Arctica* mussels were analyzed each from the dumping site and the reference station, at least to calculate bioconcentration factors.

In contrast to the distinct differences in the sediment values, only minor differences in heavy metal concentrations could be observed in the mussels from both areas (Fig. 7). Cu and Pb were only slightly enriched in softbodies and shells from the dump site in comparison to those of the reference station, and only for Cd a significant enrichment in the carbonate shells in comparison to the soft bodies and sediments was found.

Swaileh (1996) investigated the seasonal variation of heavy metals in dried soft tissues of *Arctica islandica*

from Kiel Bay not far away from our study area. All heavy metal concentrations except of Pb (factor 5 higher) and Cu (factor 2 higher) are near or in between the seasonal variation reported by Swaileh (1996). Compared to the studies of Richardson et al. (2001) with *Modiolus modiolus* mussel soft tissue from both a contaminated and uncontaminated site in the North Sea, we found higher contents for Zn (factor 5) and Pb (factor 3) at the dump site, while for Cu the trace metal contents were in the same range. For the reference stations of both studies, Zn showed elevated levels (factor 5) at our site, while those for Cu and Pb were comparable.

A remarkable result is the higher Cd content in the calcium carbonate shells. Substitution for Ca by Cd has been observed in human bones, where Cd is influencing the Ca metabolism during osteogenesis (Stoeppler, 1984). Carriker et al. (1980) reported higher Cd contents in shells of the American oyster *Crassostrea virginica* cultured in mesocosms. Hence it is not surprising to find concentrations of about 4 µg/g Cd in the shells, more than twice of those in the underlying unaffected sediment layers, and ten times higher than those in the soft bodies.

We suppose that most of the other heavy metals in recent sediments on top of the dumping site are present now in non-bioavailable forms. This could be due to a geochemical effect of immobilization by early diagenesis and ageing or even transformation of binding forms, accompanied by an overall natural attenuation due to sediment capping. It is difficult to speculate about acute
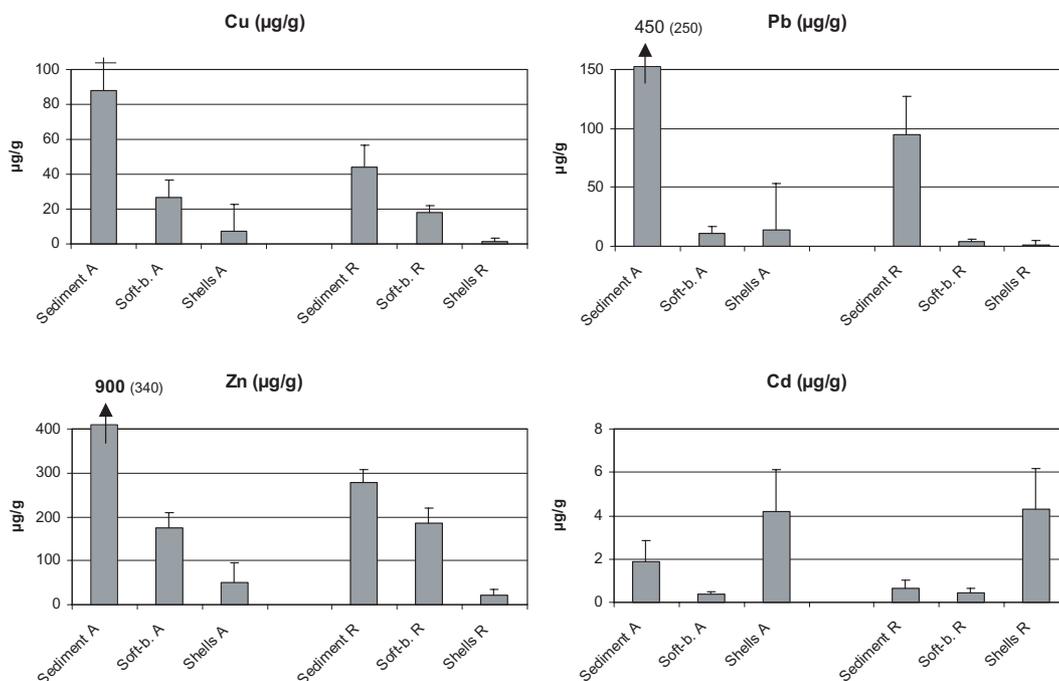

Fig. 7. Comparison of heavy metal concentrations in samples of mud sediments and *Arctica islandica* soft-tissues and shells from the dump site (A) and from the reference station (R) in Mecklenburg Bay.

toxicity effects to the benthic environment during the dumping activities. The absence of old mussels could be a hint to an impact on biota during the 1960s in the dumping area.

Measurements of chlorinated hydrocarbons in the soft bodies of our mussel samples revealed that their concentrations in the dumping area (first value) are twice as high as in those from the reference site (second
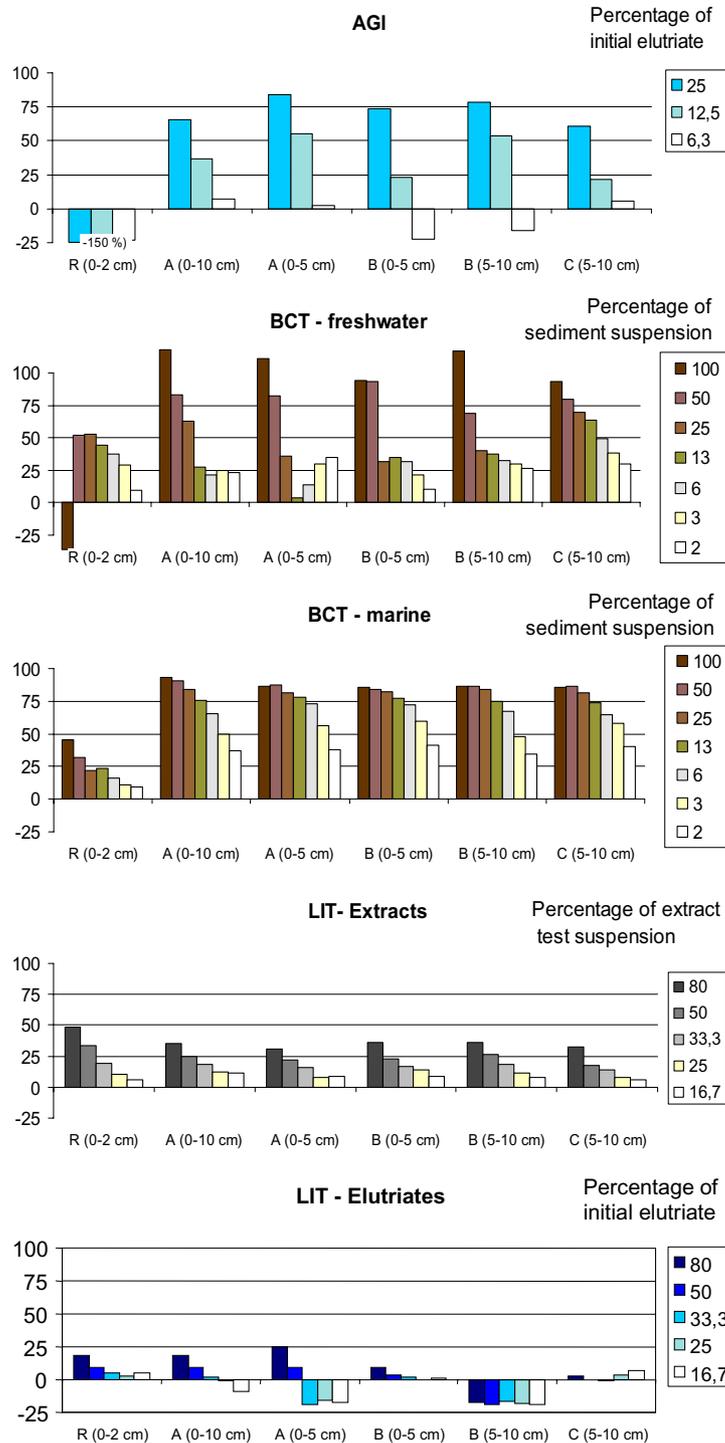

Fig. 8. Results of ecotoxicological testing of sediments from the reference station (R) and from the dump site (A, B, C). Samples were taken from different sediment layers (depth in cm) and the effects measured in a series of dilutions, as percentages of the processed test material (legend on the right side). Results are given in percent inhibition compared to an uncontaminated control. AGI = algae growth inhibition test with *Pseudokirchneriella subcapitata* (elutriates); LIT = bacterial luminescence inhibition test with *Vibrio fischeri* (elutriates, methanol-extracts); BCT = bacterial sediment contact tests with *Bacillus cereus* (freshwater) and *Vibrio proteolyticus* (marine).

value): Sum of CBs = 25.7 ng/g; 10.6 ng/g and sum of DDT products = 13.4 ng/g; 7.6 ng/g. Unfortunately we do not have sufficient data for these contaminants, especially for the sediments, but it seems that these organic pollutants kept their bioavailability over a long time in contrast to the heavy metals.

In addition to the studies on autochthonous benthic mussels, an ecotoxicity tier test was performed in order to assess bioavailability of the contaminated material to different organisms under controlled laboratory conditions. Interpretation of our biotest results followed a weight of evidence approach by which several individual positive tests in the tier add to an overall conclusion that a risk for the environment may in fact exist (Ahlf et al., 2002; Doherty, 2001). Fig. 8 depicts the results of five biotests with four organisms for the reference station (R) and for the contaminated site locations A, B, and C. While the elutriate tests with *P. subcapitata* species, and the contact tests with *B. cereus* and *V. proteolyticus*, were less inhibited by the sediment of the reference station, no difference by the bioluminescence inhibition test with *V. fischeri* was measurable for the two sites—neither in the elutriate nor in the extract test despite the high heavy metal and PAH concentrations. Responses to the elutriates were very low and—given a threshold of 10% inhibition above which effects can be regarded as adverse—even indicate toxicities at the reference station and also at location A. Inhibition effects resulting from addition of the extract of the reference station was also high. *V. fisheri* is relatively tolerant towards heavy metal contamination, but it has been regarded as very sensitive to organic pollutants (Adams and Stauber, 2004; Ferrari et al., 1999; Rönnpagel et al., 1995; Froehner et al., 2000). There are two explanations for the low response despite high PAH concentrations in the sediment, namely (i) low concentrations of PAHs in the methanol extracts and water elutriates, and (ii) the biologically less effective PAH distribution pattern in these sediments. Loibner et al. (2004) examined the effect of 16 PAHs in soils, prioritized by the US Environment Protection Agency, and found an insufficient contaminant concentration in water elutriates. Our measurements of PAH concentrations in methanol sediment extracts from the contaminated sites showed PAH concentrations of 0.008 µg/mL in the final test suspension, which were below the sensitivity limit of 0.05–0.08 µg/mL calculated for the Microtox test (Salizzato et al., 1998). Additionally, *V. fischeri* shows no toxicity to PAHs with more than three aromatic rings (Loibner et al., 2004), which make up most of the PAH contamination at the dump site locations in this study. Our results also confirm the conclusions of Ricking et al. (2002) who could not measure any contribution of PAHs to *V. fischeri* when applied in a solid phase test with concentrations in sediments up to 184 µg/g (dry-wt) EPA-relevant PAHs mostly comprising hydrocar-

bons with more than three aromatic rings. Hence, *V. fischeri* assay should not be applied as the only test system when assessing sites contaminated with combustion products as frequently encountered in navigational channels. This conclusion is of particular importance as the only test organisms required in German legislation for assessment of dredged material is the Microtox test.

In all other three tests, organisms showed elevated toxic responses when exposed to the contaminated material. The green algae *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*, *Raphidocelis subcapitata*) has been found to be generally more sensitive across the range of chemicals tested than a number of other bioassays, e.g. Microtox® (Thomas et al., 1986). Higher sensitivity of *P. subcapitata* towards heavy metals in elutriates as compared to *V. fischeri* were found by Ferrari et al. (1999) and Lambolez et al. (1994), although Sloterdijk et al. (1989) found the Microtox® assay to be more sensitive. *B. cereus* in contact with sediment was also found to be of higher sensitivity than Microtox solid phase test with regard to Cu (Rönnpagel et al., 1995), while *V. proteolyticus* was shown to be similarly or more sensitive (Cu, pNP) than *B. cereus*.

The influence of confounding factors in these biotests is considered to be low. All tests are carried out in buffered systems. However, the pH in the algae growth inhibition test still increases due to CO_2 production over the three days of test duration up to three units, albeit in the range tolerated by the German standard. It has been suggested, that this pH increase may lead to formation of toxic ammonia in sediment elutriates (Maaß and Bülow, 2003). However, any influence of ammonia was excluded, because elutriates in the present study were produced by diluting sediments with water at a ratio of 1:16. *P. subcapitata* does not show inhibition in biomass production up to 0.15 mmol/L ammonia while in sediment porewaters no concentrations above 1 mmol/L have ever been found in the top 10 cm sediment layers (unpublished monitoring data). Adverse effects due to dissolved hydrogen sulphide were prevented by a 24–48 h oxidation step prior to analysis, during which the sediments were mixed with air-saturated seawater. The redox potential was measured to check for the achievement of oxidized conditions. The influence of sediment organic matter and grain size on resorufin fluorescence in the contact assays were accounted for by control plates which mimicked the increase of fluorescence with increasing resorufin concentration.

However, three out of five biotests clearly show the increased toxicity of the contaminated site compared to that of the reference site, expressed in elutriate as well as in sediment contact assay, and under high as well as under low saline conditions. The unresponsiveness of the *V. fischeri* test can be explained by the little

sensitivity of this species towards combustion-derived PAHs and heavy metals which are the predominant contaminants at the study site.

4. Conclusions

Industrial waste material dumped in the Bay of Mecklenburg, western Baltic Sea, during the late 1950s and early 1960s is now mainly buried by a 5–10 cm thick recent muddy sediment layer. But, burial in the area is not necessarily mediated by undisturbed sedimentation. The highly dynamic environment of the 20 m deep seafloor of the Bay includes various forms of sedimentation and mixing processes caused primarily by wave and current induced hydroturbation and anthropogenic impacts (shellfish dredging) to the sediment surface. Thus contaminated material from the dump site was transported to the surrounding area over the decades. In 1985 an area of about 7×15 square nautical miles was found to be strongly influenced by dispersal of the heavy metal-laden waste material around the hot spot. In 1997, and later on in 2001, still high concentrations of heavy metals in surface sediments of the area were found, but a trend back towards ambient background values is already visible.

In contrast to sediment geochemistry, only minor differences were found in heavy metal concentrations for both soft-tissues and shells of *Arctica islandica* mussels from the dump ground and from the reference site. Chlorinated hydrocarbons in soft tissues were slightly elevated. Ecotoxicity data in three out of five different biotests clearly reflect the different degree of contamination at both sites. As conspicious sediment layers at the dump site were used for the ecotoxicity testing, these results point to still existing hot spots of high potential toxicity now buried in sediment. The suspension feeder *Arctica islandica* pumps in water from above the surface of the sea bottom, and uptake and direct contact with contaminated sediment and porewater is limited. This may be the reason for the low accumulation rate in mussels indicating a low bioavailability of heavy metals for *Arctica islandica*. Microorganisms are affected by the

contaminants, both by exposure to elutriate and by direct exposure to sediment. However, sediment and elutriates are aerated for 24 h prior to testing, and heavy metals, that may have been unavailable in their reduced form, could theoretically become bio-effective upon resuspension and oxidation in the water column. In addition, microorganisms are known to actively render adsorbed substances bioavailable (Wick et al., 2001). The results of the DMSO-reduction measurements (Fig. 9) show, that the authochtonous microbial activity is very low. The bacterial community appears to be severely altered compared to that in the sediment from the reference station. The evidence, very high chemical concentrations, very low bacterial activity in some layers, strong toxic effects in three out of five biotests, indicates the existence of an environmental risk. This risk seems to be decreasing, however, as is shown with the increasing acceptance of the site by autochthonous mussel population of relatively young age. So it seems that part of the bio-available contaminant proportions are being attenuated naturally over the decades that followed the dumping activities. In deeper sediment layers, potentially remobilizable metal fractions may turn to more stable sulphidic binding forms, due to the strongly anoxic milieu in those organic matter rich muddy sediments. Both this biogeochemical effect and the sediment burial result in a natural attenuation process by which the waste is immobilized and capped. It may render the dump site less harmful for biota in the future, at least as far as the climate will not change towards more frequent severe events. However, bioassays showed that toxicity in aerated buried material is still high upon dilution. The current risk for re-suspension and further distribution of the buried toxic waste is therefore not to be neglected (see Bonnet et al., 2000), and will be discussed in a subsequent paper (Kersten et al., in press). Another problem currently to be solved represents some glacial till outcrops at the hot spot site, where the dumped material is still exposed without effective sediment burial. A solution may be seen in an artificial capping of those sites with uncontaminated sediment material from offshore dredging grounds which is less mobile under the ambient current conditions.
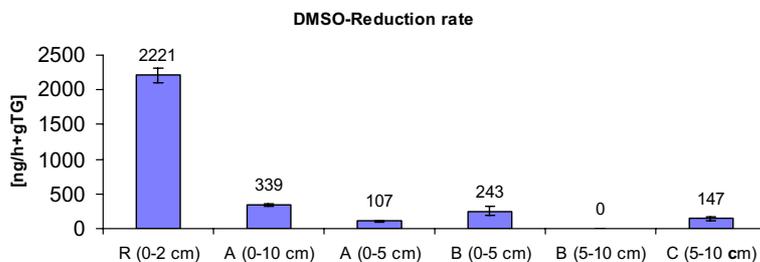

DMSO-Reduction rate

Fig. 9. Dimethylsulfoxide (DMSO) reduction rates of the samples from stations R (reference station) and A, B, C (dump site) for estimation of autochthonous bacterial activity.

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