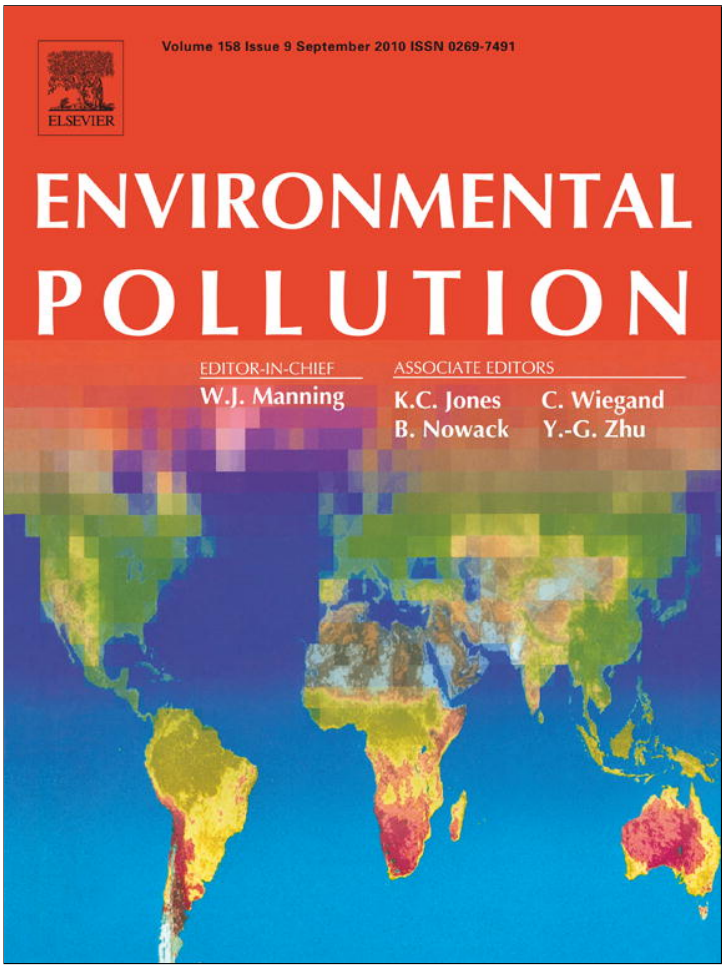

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Variability of sediment-contact tests in freshwater sediments with low-level anthropogenic contamination – Determination of toxicity thresholds

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Sediment-contact tests require toxicity thresholds based on their variability in native sediments with low-level contamination.

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ABSTRACT

Freshwater sediments with low levels of anthropogenic contamination and a broad range of geochemical properties were investigated using various sediment-contact tests in order to study the natural variability and to define toxicity thresholds for the various toxicity endpoints. Tests were performed with bacteria (*Arthrobacter globiformis*), yeast (*Saccharomyces cerevisiae*), nematodes (*Caenorhabditis elegans*), oligochaetes (*Lumbriculus variegatus*), higher plants (*Myriophyllum aquaticum*), and the eggs of zebrafish (*Danio rerio*). The variability in the response of some of the contact tests could be explained by particle size distribution and organic content. Only for two native sediments could a pollution effect not be excluded. Based on the minimal detectable difference (MDD) and the maximal tolerable inhibition (MTI), toxicity thresholds (% inhibition compared to the control) were derived for each toxicity parameter: >20% for plant growth and fish-egg survival, >25% for nematode growth and oligochaete reproduction, >50% for nematode reproduction and >60% for bacterial enzyme activity.

1. Introduction

The ambitious aim of the European Water Framework Directive (WFD) is to achieve a good ecological status of surface waters in all European river basins by the year 2015 (European Community, 2000). However, the presence of contaminated sediments is one of several obstacles potentially hindering the achievement of this goal (De Zwart et al., 2009). Sediments are often highly contaminated by chemicals that have been introduced into the water body, where they tend to bind to particles and thus accumulate as these particles settle in the sediments (Power et al., 1992). Ignoring this functional aspect of sediments, as sink and source of contaminants, can lead to erroneous conclusions concerning the ecotoxicological status thus far achieved (Förstner, 2002). Therefore, sediment quality assessment is an important component of environmental risk assessment. Accordingly, sediment toxicity tests, in which benthic organisms are exposed to bulk sediment (sediment-contact tests) are appropriate tools for assessing the potential hazard of contaminated sediments, as they consider more realistic exposure conditions than aqueous toxicity tests (Chapman and Anderson, 2005; Ingersoll et al., 1997, 1995).

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Sediment-contact tests aim to assess the toxicity of anthropogenic contaminants that have been introduced into freshwater ecosystems. However, environmental samples do not only differ in their quantity and quality of contamination, but also in terms of their geochemical properties, such as grain size distribution or content of organic matter. These sediment properties might also affect the test organisms and thus impede the interpretation of toxicity data. This has already been shown for various benthic organisms in freshwater sediments (Ankley et al., 1994, 1993; Höss et al., 1999; Sibley et al., 1998; Suedel and Rodgers, 1994) and estuarine or marine sediments (DeWitt et al., 1988, 1989; Nipper and Roper, 1995; Swartz et al., 1985). Due to the different ways in which the various benthic organisms interact with sediment (e.g. epibenthic, endobenthic, and tube-dwelling organisms), it is not possible to generalize the influence of sediment properties on organisms. Instead, whether or not a certain sediment property is able to bias the output of a toxicity test and to which degree it might do so, strongly depend on the type of test organism and toxicity endpoint.

In toxicity tests, organismal effects can only be detected by comparing the response of a certain toxicity endpoint, such as survival, growth or reproduction, to a test sediment with the response to a negative control, in which, by definition, no toxic effect occurs. This negative control can be a formulated sediment that is composed of commercially available, mineral and organic particles without chemical contamination or a field-collected natural control sediment (ASTM, 2005; Kemble et al., 1999; Suedel et al., 1996). In both cases, the sediment's inherent properties rarely exactly match those of the test sediment. Consequently, the observed difference in the organism's response to the contaminated vs. the control sediment might be due to differences in these inherent properties, rather than to the contaminants in the test sediment. This inherent variability among uncontaminated sediments, produces a background noise that has to be considered in toxicity tests and thus in the criteria used to define toxicity.

For acute tests, sediments that inhibit a toxicity endpoint by more than 20% compared to the control or reference sediment are often regarded as toxic, regardless of the test organism. However, Chapman and Anderson (2005) concluded that this 20% threshold might not be appropriate for chronic toxicity tests. Instead, it is necessary to identify the variability of single toxicity endpoints in reference sediments in order to be able to define the appropriate toxicity threshold, thus distinguishing between "natural variability" among sediments and the "toxic effects" of anthropogenic contaminants (Ahlf and Heise, 2005). Comparable approaches were published by Hunt et al. (2001) and Reynoldson et al. (2002) who set up test-specific tolerance limits or effect classes based on the response of benthic invertebrates to reference sediments, with the goal of determining elevated toxicity relative to reference conditions.

In the present study, six different standardized sediment contact tests were compared in terms of their variability among natural sediments characterized by low to moderate anthropogenic contamination and a wide range of geochemical properties. The test battery consisted of organisms from various trophic and organizational levels (bacteria, fungi, plants, invertebrates and vertebrates) with different uptake routes for contaminants. This approach allowed us to consider, on the one hand, the variety of mode of actions of sediment-associated contaminants and, on the other hand, the different exposure routes in sediments (dissolved and particulate phases). Tests were performed with *Arthrobacter globiformis* (decomposer; bacteria; Neumann-Hensel and Melbye, 2006; Rönnpagel et al., 1995), *Saccharomyces cerevisiae* (decomposer; fungi; Weber et al., 2006), *Myriophyllum aquaticum* (primary producer; higher plants; Feiler et al., 2004), *Caenorhabditis elegans* (primary consumer; nematode; Traunspurger et al., 1997), *Lumbriculus variegatus* (primary consumer; oligochaete; Phipps et al., 1993), and *Danio rerio* (secondary consumer; fish; Hollert et al., 2003). The choice of the appropriate organisms took into account the degree of standardization. As the ecologically most relevant organisms are in most cases not the easiest to culture, standardized toxicity test often use model organisms that represent relevant organism groups. Accordingly, in the present study, model organisms, including the yeast *S. cerevisiae*, the nematode *C. elegans* and the zebra fish *D. rerio* were used; however, most of these organisms are abundant (Crocker et al., 2000; Hussner, 2009; Talwar and Jhingran, 1991; Wachs, 1967), or at least occur in freshwater ecosystems (Zullini, 1988). Moreover, all of the tests carried out in the present study were already used in previous studies assessing the toxicity of freshwater sediments (Ahlf and Heise, 2005; Keiter et al., 2006; Phipps et al., 1993; Stesevic et al., 2007; Traunspurger et al., 1997).

The aim of the joint research project, SeKT (funded by the German Ferderal Ministry of Education and Research), is to validate a battery of sediment contact tests for assessing the toxicity of native freshwater sediments (Feiler et al., 2005). This study, which represents the first part of SeKT, investigated the variability in the response of the individual sediment-contact test organisms arising from natural sediment properties, i.e. properties distinct from anthropogenic contamination. The following hypotheses were tested: (1) The test organisms differ in their responses to the native sediments with low-level anthropogenic contamination. (2) The different responses can be explained by the measured sediment properties and considered as reflecting the natural variability of the contact tests. A further aim of the study was to set up toxicity thresholds for each endpoint to distinguish toxic (undesirable adverse) effects from natural variability.

2. Materials and methods

2.1. Sediment sampling

Sediment samples were taken from ten sampling sites (Table 1; Fig. 1). The sediments were selected according to the following criteria: (1) low-level anthropogenic contamination, (2) variation in their geochemical properties (mainly grain size and organic content), (3) derived from lotic (rivers) and lentic (lakes) systems or (4) from different river basins. Some of the sediments were obtained as part of routine monitoring programs in Germany (Federal Institute of Hydrology, Germany) and the Netherlands (Lahr et al., 2003). Surface sediments (0–10 cm) were collected in winter 2005/2006 with a stainless steel Van Veen grab sampler, homogenized, and stored in plastic jars in the dark at a temperature below 4 °C until further use.

2.2. Sediment analysis

The sediments were characterized with respect to their geochemical properties, nutrient content and concentrations of priority pollutants and analyzed according to standard procedures. Pore water was obtained by centrifuging the samples for 20 min at 17,000 *g*. Dry weight was determined after drying the material at 105 °C until a constant weight was reached (DIN 38414 S2). Grain size distribution was analyzed by sieving dry sediments for the sand fractions (DIN 18123) and by pipette analysis for the fine fractions (DIN ISO 11277). In whole-sediment samples, organic matter content was analyzed as loss on ignition (LOI; DIN EN 12879 S3a) and total organic carbon (TOC; DIN ISO 10694). Nitrogen, phosphorous, sulfur, and mineral contents were analyzed according to DIN ISO 11261, DIN 38414 S12, DIN ISO 15178, and DIN ISO 11466, respectively. In pore water, dissolved organic carbon (DOC) was determined according to DIN 38409 H3. Total nitrogen and phosphorous were analyzed in the pore water fraction using the methods described for whole sediment analyses.

Concentrations of pollutants were analyzed in freeze-dried sediments that had been sieved to achieve a size <2 mm. The list of investigated parameters included anthropogenic contaminants that are typically enriched in sediments, such as heavy metals and persistent organic pollutants. The concentrations of the analyzed contaminants were normalized to dry weight of the sediments. In order to compare the concentrations with sediment quality guidelines (MacDonald et al., 2000), concentrations of selected organic chemicals were also normalized to 1% TOC. Heavy metals and minerals were analyzed from aqua regia extracts (DIN ISO 11466) using atomic absorption spectroscopy. Polycyclic aromatic hydrocarbons (PAH; EPA list of

Table 1
Investigated native freshwater sediments; R = River, L = Lake.

Acronym	Site	Coordinates/River km	River catchment	Type
PA-R	Müritz-Elde-Wasserstrasse (channel; Parchim)	53°25′ N, 11°50′ O / 72.3 km	Elbe	River
PO-L	Starnberger See (littoral zone; Possenhofen)	47°58′ N, 11°19′ O	Donau	Lake
ST-L	Starnberger See (profundal zone; Starnberg)	48°0′ N, 11°20′ O	Donau	Lake
BA-R	Donau (back water; Bad Abbach)	48°56′ N, 12°3′ O / 2402.6 km	Donau	River
JO-R	Donau (barrage; Jochenstein)	48°26′ N, 8°30′ O / 2203.5 km	Donau	River
DM-L	Drontermeer (Netherlands)	52° 30′ N, 5°51′ O	Rhein	Lake
LO-L	Lohmer See	53°41′ N, 12°5′ O	Warnow-Peene	Lake
N1-L	Stechlin See (littoral zone; Neuglobsow)	53°9′ N, 13°3′ O	Elbe	Lake
AA-R	Rhein (back water; Altrip)	49°26′ N, 8°30′ O / 416.9 km	Rhein	River
N2-L	Stechlin See (profundal zone; Neuglobsow)	53°9′ N, 13°3′ O	Elbe	Lake

16 compounds) were analyzed from extracts using HPLC and fluorescence detection (DIN 38414 S21). Polychlorinated biphenyls (PCB; 7 congeners), hexachlorocyclohexane (α-, β-, γ-HCH), hexachlorobenzne (HCB), and p-p′-DDT and its homologues were analyzed from extracts using gas chromatography (GC) separation and electron capture detection, according to DIN 38414 S20. Mineral oil content (petroleum-derived hydrocarbons) was determined by GC using a flame ionization detector, according to ISO TR 11046. Alkylphenols were detected after solid–liquid extraction using GC/mass selective detection. Organotin was alkylated, extracted with hexane, and analyzed using GC/atomic emission detection. For each sample, two replicates (independent subsamples) were analyzed, with two injections for each replicate analysis. To monitor methodological analyte losses, certified reference or external control standard material was used. Procedural blanks were carried out, covering the total analytical procedure.

2.3. Sediment-contact tests

All sediment contact tests were carried out according to standard procedures (bacteria: ISO/CD 10871, ISO, 2009; nematodes: ISO/FDIS 10872, ISO, 2010; oligochaetes: OECD 225, OECD, 2007), or published test protocols (yeast: Weber et al., 2006; fish eggs: Hollert et al., 2003; plants: Feiler et al., 2004). Table 2 summarizes all of the relevant test conditions and criteria. Sediments were pre-treated according to test specific methods to assure aerobic conditions during the test. Each test system made use of the appropriate artificial control sediment, according to the specific needs of the test organisms to achieve optimal test performance. For the nematode and yeast contact tests, all ten native sediments were studied in a single experiment. For all other contact tests, two test series were carried out (first series: PA-R, PO-L, ST-L, BA-R, JO-R, DM-L; second series: LO-L, N1-L, AA-R, N2-L), in which the toxicity endpoints in the various sediments were compared to those in the respective artificial control sediment. The control sediments of the two test series were called C1 and C2.

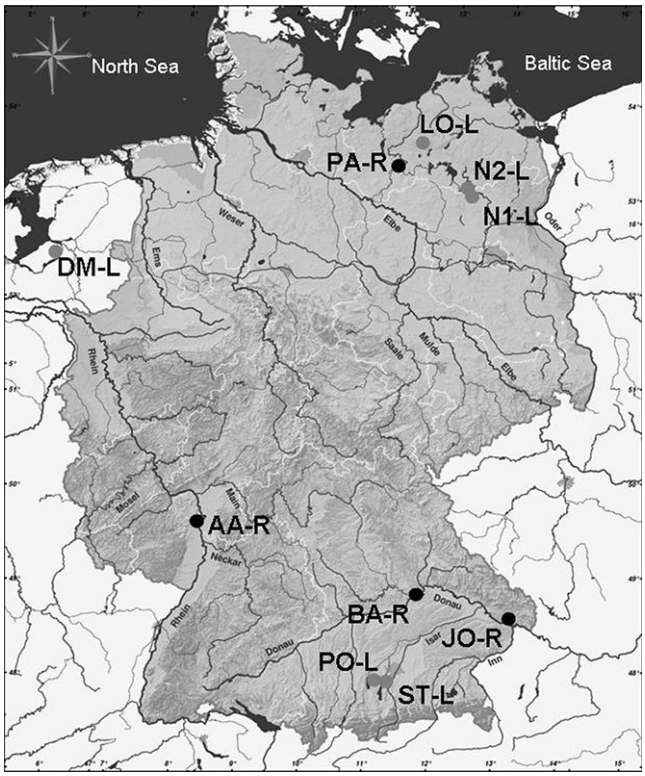

Fig. 1. Map of the sampling sites in Germany (9) and the Netherlands (1); for definitions of abbreviations, see Table 1.

2.4. Data analysis

Principal Component Analysis (PCA; Hotelling, 1933) maps information from a large number of variables onto a smaller number of linear combinations, thereby simplifying the data interpretation. Variables are sorted in descending order with respect to their variability. This quantifies the relevance of variables with respect to the extracted patterns. PCA was calculated by use of CANOCO for Windows Ver. 4.53 (Microcomputer Power) (Ter Braak and Šmilauer, 2002). Sediment characteristics were standardized by variables standard deviation (PCA based on correlation matrix, centering by species). Multivariate correlations between the variables in PCA were calculated as the cosine of the angle between the vectors in the 2-dimensional ordination space formed by the first two ordination axes.

Hierarchical agglomerative classification (cluster analysis) is a frequently used method to group a large number of objects in a smaller number of clusters. Calculation of cluster analysis was performed by use of PC-Ord Ver. 5 (MjM Software Design) (McCune and Mefford, 1999). Euclidean distance was used in combination with Ward's method.

Linear models were fitted using the routine lm (Chambers and Hastie, 1992) in package stats from the statistical software environment R (R Development Core Team, 2009). A typical model has the form 'response ~ terms' where 'response' is the (numeric) response vector and 'terms' is a series of terms which specifies a linear predictor for 'response'.

Model selection techniques attempt to find the model that best explains the data with a minimum of free parameters. Adding additional parameters to the model increases the likelihood but may result in overfitting. Model selection based on the Akaike Information Criterion (AIC) was performed by use of the routine AIC (Sakamoto et al., 1986), also from the stats package in R. The preferred model was the one with the lowest AIC.

Coefficients describing the variability of every single toxicity endpoint and to defining the appropriate toxicity threshold for each endpoint were calculated. A test's inherent coefficient of variation, CVi, considers the variability of a test parameter regardless of any environmental factor and is calculated from the variance of a test parameter within each of the investigated sediments (artificial control sediment, native sediments).

$$CVi_x = SD_x/Mean_x \times 100, \quad (1)$$

where $Mean_x$ and SD_x are, respectively, the mean and standard deviation of a test parameter as calculated from replicates of the respective control or reference sediment x. For native sediments the mean CVi_x over all ten sediments was calculated (CVi_S). For the artificial control sediment, a separate CVi was calculated (CVi_C).

The coefficient of variation between different native sediments, CVs, considers the influence of sediment characteristics (besides pollution) and was calculated from the variance of a test parameter x between the various investigated native sediments.

$$CVs = SD_{S\text{-}RV}/Mean_{S\text{-}RV} \times 100 \quad (2)$$

where $Mean_{S\text{-}RV}$ and $SD_{S\text{-}RV}$ are, respectively, the mean and standard deviation of the test parameter expressed as relative values (RV) with respect to the control sediment (% of control response) of all investigated native sediments.

For estimating the appropriate toxicity threshold for the different sublethal toxicity endpoints, the potential minimal detectable difference (MDD) and the maximal tolerable inhibition (MTI) were calculated.

Table 2
Description and test conditions for the applied test systems.

	Bacteria	Yeast	Nematodes	Oligochaetes	Plants	Fish egg
Test organism	*Arthrobacter globiformis* (strain ATCC 8010)	*Saccharomyces cerevisiae* (isolate N 06.98)	*Caenorhabditis elegans* (wild type; strain N2)	*Lumbriculus variegatus* (MÜLLER)	*Myriophyllum aquaticum*	*Danio rerio* ("Westaquarium" strain)
Source	German collection of microorganisms (DSM)	Nordum GmbH & Co. KG, Germany	Caenorhabditis Genetic Center, MN, USA	Co. Etzbach, Mechernich-Bergheim, Germany	University of Jena, Germany	German Federal Environment Agency
Standardization	ISO/DIS 10871	–	ISO 10872	OECD 225	ISO NWIP	Based on DIN 38415-6
Reference	Rönnpagel et al., 1995; Neumann-Hensel and Melbye, 2006	Weber et al., 2006	Traunspurger et al., 1997; Höss et al., 1999	Phipps et al., 1993; Egeler et al., 2005	Feiler et al., 2004	Hollert et al., 2003
Toxicity parameter	Enzyme activity (resorufin formation)	Fermentation (ml CO_2 h^{-1})	Growth (based on body length); Reproduction (Number of offspring per test organism)	Reproduction (Total number of organisms)	Growth rate (based on fresh weight)	Survival
Temperature	30 °C	16 h at 28 °C; 6 h at 40 °C	20 ± 0.5 °C	18–22 °C	24 ± 0.5 °C	27 °C
Test period	6 h	22 h	96 h	28 d	10 d	48 h
Food	–	–	*Escherichia coli* OP50 (10^9 cells ml^{-1})	Fish food (Tetramin; 0.5–0.75 g per worm and day)	–	–
Amount of tested sediment (wet weight)	0.6 g	40 g	0.5 g	60–90 g	200 g	3 g
Control sediment	Quartz sand	Quartz sand	ISO 10872[a]	OECD 218[b]	OECD 207[c]	Quartz sand
Replicates	3	3	4 (control: 9)	6	3 (control: 6)	1
Validity Criteria	5fold increase in fluorescence	>25 ml CO_2 h^{-1}	≥80% fertility; ≥ 30 offspring per test organism	Reproduction: ≥ 18 organisms	Growth rate ≥ 0.075	Survival ≥ 90 %

[a] 40% Coarse quartz sand (0.1–0.4 mm); 30% fine quartz sand (0.1 mm); 20% Al_2O_3; 4.5% Fe_2O_3; 0.5% Dolomit; 1% $CaCO_3$; 4% peat.
[b] 75% Sand; 20% kaolin; 5% peat.
[c] 70% Sand; 20% kaolin; 10% peat.

Table 3
Geochemical properties of investigated sediments.

Parameter	Acronym	Unit	PA-R	PO-L	ST-L	BA-R	JO-R	DM-L	LO-L	N1-L	AA-R	N2-L
Dry weight	DW	%	17	56	29	31	57	22	24	38	37	20
Loss on ignition	LOI	%	28	4.3	9.1	11	4.5	15	17	6.7	13	27
Total carbon	TC	%	14	11	14	8.4	4.0	7.7	10	8.2	6.9	14
Total organic carbon	TOC	%	14	4.2	8.5	4.3	3.4	6.9	6.2	5.9	3.4	8.2
Nitrogen	N	mg kg^{-1}	11	1.6	3.6	3.4	1.3	6.2	6.8	2.6	2.9	6.4
Phosphor	P	g kg^{-1}	1.6	0.2	0.3	1.0	0.7	0.6	2.7	0.1	0.7	0.2
Sulfur	S	%	1.6	< 0.06	< 0.07	< 0.09	< 0.07	1.3	0.15	0.06	0.05	0.11
Aluminum	Al	%	1.1	0.18	0.32	2.0	1.6	1.3	0.24	0.06	2.0	0.08
Iron	Fe	%	2.9	0.19	0.26	2.1	2.5	1.9	0.48	0.23	2.2	0.3
Magnesium	Mg	%	0.26	1.6	1.1	2.1	2.4	0.44	0.16	0.08	1.2	0.11
Calcium	Ca	%	2.3	25.8	20.7	12.6	6.6	3.9	19.4	24.4	12.7	35.6
Lithium	Li	mg kg^{-1}	4.1	0.40	0.60	6.9	7.5	5.4	0.80	0.60	15.0	0.80
Grain size distribution												
>2000 μm	Gravel	%	0.80	1.2	0.0	0.30	0.0	0.0	1.2	1.1	0.80	0.20
630–2000 μm	Sa630	%	0.5	1.8	0.20	0.40	0.0	0.1	1.1	3.9	0.40	2.3
200–630 μm	Sa200	%	5.9	14	1.0	0.50	0.10	3.4	3.6	38.2	0.40	8.5
63–200 μm	Sa63	%	19	40	3.9	2.5	16	26	27	20	0.90	15
63–2000 μm	Sand	%	26	55	5.1	3.4	16	29	32	62	1.7	25
20–63 μm	Si20	%	30	20	39	15	47	33	33	15	4.5	25
2–20 μm	Si2	%	33	17	46	59	28	24	27	16	70	38
2–63 μm	Silt	%	63	37	85	74	75	56	60	31	74	63
<2 μm	Clay	%	12	7.9	10	23	9.0	15	6.5	5.9	23	11
Pore water												
Dissolved organic carbon	DOC	mg l^{-1}	10	19	14	14	23	15	22	19	11	8.7
Total nitrogen	TN	mg l^{-1}	2.6	16.6	3.8	2.3	14	7.1	13	2.5	5.3	3.1
Total phosphor	TP	mg l^{-1}	0.74	0.14	0.37	1.5	0.15	0.43	1.3	0.12	0.55	0.28

The MDD is based on the test inherent variability of a test parameter and was determined for each investigated sediment:

$$\%\mathrm{MDD}_{Sx} = \frac{100t\sqrt{\frac{\mathrm{SD}_C^2}{n_C} + \frac{\mathrm{SD}_{Sx}^2}{n_{Sx}}}}{\mathrm{Mean}_C} \quad (3)$$

where t is the tabulated value of the Student's t distribution (alpha = 0.05, one-sided, df = $n_C + n_{RS} - 2$), SD_C^2, SD_{Sx}^2 and Mean_C are, respectively, the variances or mean of the test parameter for the control sediment (C) and the native sediment × (Sx) and n_C and n_{Sx} are the numbers of replicates for the control sediment (C) and the investigated native sediment x (Sx), respectively. The calculated MDDs were expressed as a percentage of the control response. Finally, an average MDD was calculated over all single MDDs.

The MTI (maximal inhibition compared to the control that is still within the natural variability) refers to a specific control sediment (in this case the test-specific artificial sediment) and was based on the variability caused by natural sediment characteristics:

$$\%\mathrm{MTI} = \mathrm{Mean}\ (\%I_S) + \mathrm{SD}\ (\%I_S) \quad (4)$$

where Mean ($\%I_S$) and SD ($\%I_S$) are, respectively, mean and standard deviation of percent inhibition of a certain toxicity endpoint in a native sediment S compared to the respective control sediment. Percent inhibition was defined as follows:

$$\%I_S = 100 - X_S/X_C \times 100 \quad (5)$$

where X_S and X_C are, respectively, the mean values of a certain toxicity endpoint X in a native sediment S and the respective control sediment (C).

Thus, the MTI is dependent on the difference between the response to the control sediment and to all native sediments and on the variability in native sediments, as expressed by the standard deviation SD ($\%I_S$).

In contrast to sublethal toxicity parameters, for the test with fish eggs a mortality > 20% in native sediments was considered as not tolerable. Thus, it was not necessary to use MDD and MTI to define the toxicity threshold.

One-way ANOVAs were used to determine statistical differences between the responses in natural sediments vs. control sediment and treatments were compared with a post-hoc Dunnett test ($\alpha = 0.05$, two-sided).

3. Results

3.1. Sediment properties

In terms of their geochemical sediment properties, the investigated sediments varied considerably (Table 3), with dry weights ranging from 17 to 57%, total organic carbon (TOC) from 3.4 to 14.3%, and contents of sand, silt and clay ranging from 2 to 62%, 31 to 85%, and 6 to 23%, respectively. Sediments N1-L and PO-L can be described as silty sand, PA-R, DM-L, LO-L and N2-L as sandy silt, and ST-L, BA-R, JO-R and AA-R as clayey silt. Sediments PA-R and N2-L showed the highest contents of organic matter with 27.5 and 26.7% loss on ignition (LOI) and 14.3 and 8.2% TOC, respectively. Sediments PO-L, JO-R, and N1-L had the lowest contents of organic matter with 4.3, 4.5 and 6.7% LOI and 4.2, 3.4, and 3.4% TOC, respectively.

The sediments were found to have a relatively low level of anthropogenic contamination. According to the consensus based sediment quality criteria of MacDonald et al. (2000), the mean quotient of measured contaminant concentrations to predicted effect concentrations (PEC[2]; above which effects are predicted), mean PEC-Q, was < 0.3 for all sediments (Table 4), which thus were predicted to be not toxic (MacDonald et al., 2000). For most samples even the threshold effect concentration (TEC; below which no effect can be expected) were not or only slightly exceeded (maximal TEC-Q: 0.35–1.6). Only AA-R was considered as moderately polluted, as the concentrations of the majority of heavy metals exceeded the TEC (Hg by a factor of 3.2).

Cluster analysis showed that the investigated sediments could be assigned to three groups (Fig. 2a). Cluster 1 consisted only of AA-R, which is characterized by very fine texture (74% silt, 23% clay; Table 3) but also by degree of pollution higher than that of other samples (Table 4). Cluster 2 consisted of four sediments (PA-R, BA-R, JO-R, DM-L) that also showed high proportions of silt and clay (mean: 81%; Table 3) as well as mildly elevated contaminant concentrations with maximal TEC-Qs of 1.1 to 1.6 (Table 4). Cluster 3 comprised sandier sediments with very low pollution (PO-L, ST-L, LO-L, N1-L, N2-L). With the exception of DM-L, all lake sediments could be assigned to cluster 3 and all river sediments to clusters 1 and 2.

[2] Acronym not to be mistaken for PEC = Predicted Environmental Concentration.

Table 4
Measured contaminant concentrations (based on sediment dry weight) of investigated sediments and classification according to sediment quality guidelines (SQG); LOD = limit of detection, TEC = consensus based threshold effect concentration, PEC = consensus based predicted effect concentration according to MacDonald et al. (2000) except for nonylphenol value,[a] TEC-Q and PEC-Q = quotients of measured concentrations and TEC or PEC, respectively; n.a. = value not available.

Parameter	Unit	LOD	PA-R	PO-L	ST-L	BA-R	JO-R	DM-L	LO-L	N1-L	AA-R	N2-L	TEC	PEC
As	mg kg^{-1}	3.0	14	<3.0	< 3.0	8.0	10	11	4.0	2.0	10	4.0	9.8	33
Pb	mg kg^{-1}	0.50	38	7.0	19	24	18	34	22	18	53	29	36	128
Cd	mg kg^{-1}	0.30	0.40	<0.30	<0.30	<0.30	<0.30	<0.30	0.30	<0.30	0.40	<0.3.0	0.99	5
Cr	mg kg^{-1}	1.0	21	5.0	8.0	35	33	27	6.0	4.0	53	5.0	43	111
Cu	mg kg^{-1}	1.0	33	11	11	36	32	22	15	4.0	58	9.0	32	149
Ni	mg kg^{-1}	1.0	11	2.0	4.0	23	29	18	3.0	< 1.0	35	1.0	23	49
Hg	mg kg^{-1}	0.01	0.29	0.04	0.09	0.23	0.13	0.15	0.07	0.04	0.58	0.07	0.18	1.1
Zn	mg kg^{-1}	1.0	162	19	45	179	171	135	61	24	205	37	121	459
HCH (α-, β-, γ-)[c]	µg kg^{-1}	0.30 (1.0)	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<1.0	<1.0	<1.0	<1.0	2.4	5
HCB[b,c]	µg kg^{-1}	0.30 (1.0)	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<1.0	<1.0	8.7 (2.6)	<1.0	n.a.	n.a.
p,p DDT and homologues[b]	µg kg^{-1}	1.8	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	3.4 (0.55)	<1.8	12 (3.7)	1.5 (0.18)	5.3	572
Nonylphenol[b,c]	µg kg^{-1}	220 (110)	<220	<220	<220	<220	<220	<220	<110	<110	157 (46)	<110	1.400[a]	n.a.
Octa-chlorostyrol[c]	µg kg^{-1}	0.50 (1.0)	<0.50	<0.50	<0.50	<0.5	<0.50	<0.50	<1.0	<1.0	11	<1.0	n.a.	n.a.
ΣPAH (16 US EPA)[b]	mg kg^{-1}	1.0	1.8 (0.13)	<1.0	1.1 (0.13)	<1.0	<1.0	<1.0	5.3 (0.85)	<1.0	2.9 (0.85)	<1.0	1.6	23
ΣPCB (7 congeners)[b]	µg kg^{-1}	5.0	<5.0	<5.0	1.1 (0.13)	<5.0	<5.0	<5.0	<5.0	<5.0	43	1.7	60	676
Mineral oil	mg kg^{-1}	100	640	<100	220	<100	110	380	<100	<100	<100	<100	n.a.	n.a.
TBT	µg Sn kg^{-1}	0.40	13	1.2	39	0.50	<0.40	15	159	<0.40	14	<0.40	n.a.	n.a.
Maximal TEC-Q			1.6	0.35	0.53	1.5	1.4	1.1	0.61	0.50	3.2	0.81		
Mean PEC-Q			0.17	0.03	0.05	0.18	0.17	0.15	0.07	0.03	0.29	0.06		

[a] Interim SQG taken from CCME (2002).
[b] Concentrations in parentheses, TEC, and PEC are normalized to TOC of 1% according to SQG (MacDonald et al., 2000).
[c] LODs in parentheses refer to LO-L, N1-L, AA-R and N2-L.

Fig. 2. Cluster analysis (a) and principal component analysis (PCA; b) for the 10 native sediments based on geochemical properties and contaminant concentrations; for definitions of abbreviations, see Tables 1, 3 and 4; MO = mineral oil; OCS = Octa-chlorostyrol.

PCA showed that the geochemical sediment properties were highly intercorrelated with contaminant concentrations (Fig. 2b). Along the horizontal axis (PC1; explaining 41% of the variance), the samples were separated in terms of their grain size distribution (clay minerals: Al, Li; particle size fractions) and metal contents, so that cluster 3 sediments appeared on the left side of the plot, and cluster 1 and 2 sediments on the right side. Along the vertical axis (PC2; explaining 18% of the variance), the samples were separated according to the content of organic matter (N, P, S, TOC, LOI), mineral oil, and dry weight. Accordingly, PA-R and DM-L, organically rich samples with low dry weights and slightly elevated contents of mineral oil were positioned in the upper part of the plot N1-L, PO-L and AA-R, samples with low organic contents and high dry weights, in the lower part. PCA was used to reduce the large number of variables to four principal components (PC1 to 4) that, due to their strong intercorrelations, correlated with multiple variables (multivariate correlation: $r > 0.95$ or $r < -0.95$; Table 5).

3.2. Response to sediment samples

The toxicity endpoints of the various sediment-contact tests varied considerably among the different investigated sediments, with several of the sediments differing significantly from the control (Table 6; $p < 0.05$, one-way ANOVA, post-hoc Dunnett). Only plant growth in any native sediment was not significantly different from that in the control (Table 6; $p > 0.05$, one-way ANOVA). Compared to the respective control sediment, the various toxicity endpoints reached relative values of 32–130% (bacterial enzyme activity), 1–106% (yeast fermentation), 70–102% (nematode growth), 43–230% (nematode reproduction), 69–155% (oligochaete reproduction), 83–123% (plant growth rate), and

Table 5
Variables that significantly correlated with factors of PCA (multivariate correlation; $r > 0.95$; $r < -0.95$); PC = principal component; for definitions of abbreviations see Table 3; OCS = Octa-chlorostyrol.

PCs	Eigenvalues	Significant variables	
		$r > 0.95$	$r < -0.95$
PC 1	0.409	• Fine particles (clay, silt, Al, Li, Fe) • Heavy metals (Cu, Cr, Ni, Hg, Zn)	• Coarse particles (sand)
PC 2	0.176	• Organic matter (LOI, TOC, N, P, S) • Mineral oil	• Dry weight
PC 3	0.141	• Chloroorganic chemicals (DDT, HCB, PCB, OCS); Cd • N, Li	• Mineral oil • Silt • S
PC 4	0.100	• TN, DOC (Pore water) • Particles 63–200 μm (sa63)	• TOC

0–105% (fish egg survival) (Fig. 3). For fish egg survival, one extreme value (0% survival) was observed for PA-R.

The response of the various test organisms was compared with the principal components of the PCA, by testing a linear model, to explain the variability of the toxicity endpoints with the sediment properties. The results show that nematode growth and reproduction, as well as plant growth were significantly influenced by variables that were correlated with PC1, whereas nematode growth and reproduction showed a negative and plant growth a positive coefficient (Table 7). Nematodes might have been negatively influenced by the high contents of fine sediments, however also by slightly elevated concentrations of metals. Comparisons of the response of the organisms (Table 6) with the clusters in Fig. 2a clearly showed that both the highest values for nematode growth and reproduction were found in samples of cluster 3 (left side of the PCA plot: PO-L, ST-L, LO-L, N1-L, N2-L; Fig. 2b). The plants, however, preferred the fine sediments, despite their slightly elevated contamination. *M. aquaticum* showed the highest growth rates in sediments belonging to clusters 1 and 2 (AA-R, BA-R, JO-R, DM-L; Fig. 2a; Table 6) positioned on the right side of the PCA plot (Fig. 2b). Additionally, plant growth was significantly related to PC4 (Table 7; positive coefficient), which is positively correlated with dissolved carbon and nitrogen concentrations in the pore water (Table 5). According to the linear model, fish egg survival was significantly influenced by variables that correlated with PC2, with a negative coefficient (Table 8). This was perhaps due to PC2-related factors, i.e. the relatively high contents of organic matter, elevated concentrations of mineral oil, and low dry weights (Table 5). The linear model did not reveal significant correlations of PC3 with the toxicity endpoints. Thus, variables that were correlated with PC3 (e.g. chloroorganic chemicals) did not significantly influence the organisms (Table 7). Neither for yeast fermentation, nor for oligochaete reproduction did the linear model reveal a significant relation to any of the principal components (Table 7).

3.3. Variation coefficients and toxicity thresholds

For calculations of variation coefficients, MDD, and MTI, and in the subsequent definition of the toxicity thresholds only those samples were considered in which pollution effects could be excluded. Therefore, the moderately polluted sample AA-R was omitted. For fish egg survival also PA-R was omitted, because due to the significant correlation with PC2, a toxic effect of mineral oil could not be excluded as a cause for the strong effect in this sample.

Bacterial enzyme activity showed a very low test-inherent variation either in the artificial control sediment or in the native sediments, with a CVi < 5% (Table 8). The yeast contact test showed also a very low CVi in the artificial control ($CVi_C = 1.4\%$), whereas in the native sediments the CVi was quite high with an average value of 61% (Table 8). Nematode growth varied marginally between the replicates of the different treatments: this was the case for the artificial control sediment as well as for the native sediments, since under either condition the CVi did not exceed 10% (Table 8). Nematode reproduction showed a higher test inherent variation: here, the CVi was higher in the artificial control sediment ($CVi_C = 31\%$) than in most native sediments (mean $CVi_S = 22.7\%$) (Table 8). Oligochaete reproduction was characterized by a maximal CVi_C of 17% for the artificial control sediment and an average CVi_S of

Table 6
Response of toxicity endpoints of the various sediment-contact tests to the investigated native sediments (see Table 1); C = artificial control sediment (C1 = test 1; C2 = test 2); * and +indicate significant lower and higher values compared to the control, respectively ($p < 0.05$; one-way ANOVA, posthoc Dunnett, two-sided).

Sample	Bacteria		Yeast		Nematodes				Oligochaetes		Plants		Fish eggs
	Enzyme activity (fluorescence min^{-1})		Fermentation (ml CO_2 h^{-1})		Growth (μm)		Reproduction (offspring per test organism)		Reproduction (total number of organisms)		Growth rate		Survival (%)
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
C1	184.3	4.4	51.5	0.7	1313.4	109.8	57.0	17.6	31.5	5.3	0.080	0.009	95.0
PA-R	113.7 *	2.9	7.3 *	3.1	1083.1 *	56.7	50.7	11.3	38.8	9.4	0.081	0.003	0.0 *
PO-L	114.0 *	6.5	17.8 *	21.2	1162.0 *	80.8	53.2	8.0	37.0	3.8	0.086	0.013	90.0
ST-L	79.0 *	2.0	9.3 *	12.7	1192.5	25.6	50.5	3.0	48.8 +	1.9	0.077	0.002	85.7
BA-R	89.3 *	0.6	25.3	2.5	917.1 *	64.1	24.5 *	8.0	38.0	5.5	0.099	0.011	93.3
JO-R	58.8 *	4.6	50.0	6.7	1000.6 *	90.4	32.0	10.6	31.3	3.9	0.098	0.013	100.0
DM-L	112.2 *	8.4	0.7 *	1.2	1067.3 *	57.4	46.9	11.1	40.3 +	2.9	0.095	0.004	81.8
C2	146.7	2.3							35.7	2.2	0.100	0.006	100.0
LO-L	153.0	3.3	63.0	4.6	1255.9	84.9	72.5	15.9	38.7	4.8	0.108	0.009	100.0
N1-L	172.4 +	8.3	54.7	12.2	1273.4	73.8	93.0 +	30.1	24.7 *	3.8	0.088	0.006	93.3
AA-R	93.0 *	12.3	45.7	3.1	956.0 *	81.4	33.4 *	5.0	40.2	5.8	0.118	0.003	100.0
N2-L	190.9 +	3.8	32.0	7.6	1341.6	57.3	130.8 +	23.0	33.0	4.3	0.083	0.003	100.0

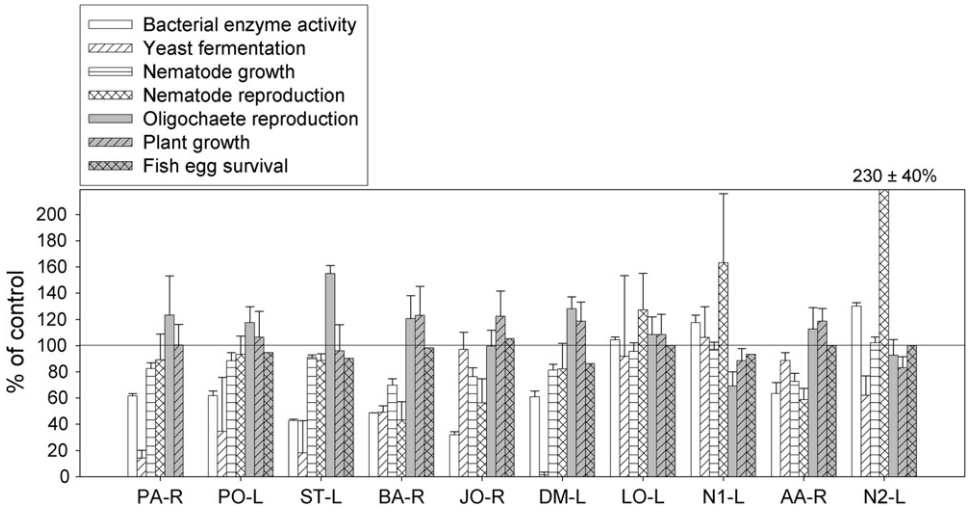

Fig. 3. Response of the various sediment contact tests to the 10 investigated native sediments as percentage of the respective control; for definitions of abbreviations, see Table 1.

13% for the native sediments (Table 8). Plant growth showed a lower test-inherent variation, with a maximal CVi_C of 11% and a mean CVi_S of 8% (Table 8). For the fish egg contact test the CVi was not calculated because only one replicate was set up.

The response of plant growth, nematode growth, oligochaete reproduction and fish egg survival to the native sediments showed the least variability, with a coefficient of variance (CVs) of <25%, followed by bacterial enzyme activity (48%), nematode reproduction (54%) and yeast fermentation (74%) (Table 8).

The appropriate toxicity threshold for each test system was determined based on the maximal MDD and the MTI, whereas always the higher value of the two coefficients was used. The various toxicity endpoints showed maximal MDD and MTI values ranging from 7 to 46%, and 9 to 86%, respectively (Table 8; Fig. 4). Only for oligochaete reproduction and plant growth, the MDD was higher than the MTI, and thus was taken as basis for the toxicity threshold. For bacterial enzyme activity, as well as nematode growth and reproduction, the MTI was the decisive coefficient. The respective MDD or MTI value was then rounded to the next multiple of five, to obtain feasible toxicity thresholds. As a result, the toxicity thresholds were set to 20% inhibition for plant growth, 25% inhibition for nematode growth and oligochaete reproduction, 50% inhibition for nematode reproduction, and 60% inhibition for bacterial enzyme activity. For yeast fermentation, no toxicity threshold was defined due to the high variability of the test system (MTI > 80%). In spite of a MTI of 10 for fish egg survival, the toxicity threshold was set to 20% (following the validity criterion), as no information on the test inherent variability was available. Statistical analyses showed that the power for detecting significant differences at the various toxicity thresholds were sufficiently high (>0.8; SigmaStat, SPSS Inc, Chicago, IL, USA).

4. Discussion

4.1. Influence of sediment properties

The response of the test organisms to the different native sediments with low to moderate contamination varied considerably. The sediment coefficients of variance were markedly lower for nematode growth, oligochaete reproduction, plant growth and fish-egg survival (CV_S of 12, 22, 13, and 6.4%, respectively), than for bacteria, yeast and nematode reproduction (CV_S of 48, 74 and 54, respectively). For nematode growth, the variability was comparable to that determined in a study with freshwater sediments, in which *C. elegans* was exposed to 26 low-level-polluted sediments ($CV_S = 10.1$; Höss et al., 1999). In reference soils, *C. elegans* had a lower variability with a CV_{Soil} of 4% for growth and 31% for reproduction (Höss et al., 2009). The variability of *L. variegatus* reproduction in the native sediments was comparable to the values reported for *Tubifex tubifex*. In that study, Reynoldson et al. (2002) investigated the variability of sublethal toxicity endpoints for *T. tubifex* exposed to 105 reference sediments: CV_S of 12% and 34% were determined for the number of cocoons and offspring per adult, respectively. The variability of *Eisenia andrei*, a soil oligochaete, in reference soils, however, was found to be considerably higher (CV_{Soil} of 44%; Römbke et al., 2006). In the same study, a CV_{Soil} of > 50% was determined for the growth of turnip rape (*Brassica rapa*) in reference soils (Römbke et al., 2006), which is considerably higher than the variability of the growth rate of *Myriophyllum* in the present study (Table 8).

A linear model suggested that grain-size distribution, organic matter and anthropogenic pollution might have influenced the response of the various organisms to the native sediments (Tables 5

Table 7
Linear models for organism response (% of control) against principle components (lm (formula = "organism response" ~ PC1 + PC2 + PC3 + PC4)); AIC = akaike information criterion (criterion for model selection); significant coefficients and overall models, p(F), are printed bold (alpha = 0.05); Bac = bacterial enzyme activity; Yeast = yeast fermentation; Nema-G: nematode growth; Nema-R: nematode reproduction; Oligo: oligochaete reproduction; Plant: plant growth; Fish: fish egg survival.

Endpoint	Intercept		PC1		PC2		PC3		PC4		p(F)	AIC
	Coeff.	p (interc.)	Coeff.	p (coeff.)	Coeff.	p (coeff.)	Coeff.	p (coeff.)	Coeff.	p (coeff.)		
Bac	0.56	0.000	−0.13	0.061	0.04	0.565	0.14	0.058			0.081	−0.48
Yeast	0.49	0.004	−0.02	0.827	−0.14	0.239	0.15	0.205			0.370	11.32
Nema-G	0.84	0.000	**−0.09**	**0.002**	0.02	0.323	0.03	0.148			**0.008**	−26.04
Nema-R	0.45	0.000	**−0.16**	**0.033**	0.04	0.500	0.07	0.251	0.094	0.141	0.104	−1.90
Oligo	0.73	0.000	0.04	0.422							0.422	−5.78
Plant	0.87	0.000	**0.08**	**0.004**	0.03	0.167	0.03	0.126	**0.059**	**0.013**	**0.011**	−26.63
Fish	0.85	0.000	−0.05	0.437	**−0.23**	**0.004**					**0.012**	−2.41

Table 8
Relevant characteristics of all sediment contact tests: CVi_c = coefficient of test inherent variation of control sediment; CVi_{rs} = mean CVi of reference sediments; T1/T2 = Test 1/Test 2; Min = minimal value; Max = maximal value; MDD = mean minimal detectable difference (one way); CVs = coefficient of sediment variation; MTI = maximal tolerable inhibition; n.d. = not determined.

Test inherent criteria	Bacteria	Yeast	Nematodes		Oligochaetes	Plants	Fish eggs
	Enzyme activity	Fermentation	Growth	Reproduction	Reproduction	Growth rate	Survival
No. of samples used for calculations	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 9$	$n = 8$
Excluded samples	AA-R	AA-R	AA-R	AA-R	AA-R	AA-R	AA-R, PA-R
% CVi_C (T1/T2)	2.4/1.6	1.4	8.4	31.0	16.9/6.1	11.2/10.0	n.d.
% CVi_S (Min; Max)	3.5 (0.6; 7.9)	60.8 (7.3; 173.2)	5.8 (2.1; 9.0)	22.7 (5.8; 33.1)	12.6 (4.0; 24.2)	7.8 (2.8; 15.0)	n.d.
% MDD (Min; Max)	3.8 (1.9; 7.2)	19.0 (3.8; 44.0)	6.5 (5.3; 7.5)	27.8 (19.1; 45.9)	14.7 (9.1; 25.4)	14.4 (9.2; 20.1)	n.d.
Response to sediments	Relative value (% of control)						
Minimum	31.9	1.3	69.8	43.1	69.2	83.2	86.1
Maximum	130.2	106.1	102.1	229.6	155.0	123.0	105.3
Mean	73.3	52.7	87.1	108.0	112.7	105.2	96.0
Standard deviation	35.1	38.9	10.5	57.8	24.3	14.5	6.1
% CVs	47.8	73.8	12.1	53.5	21.6	13.7	6.4
% MTI	61.8	86.2	23.4	49.7	11.6	9.3	10.1
Toxicity threshold (% Inhibition to control)	60	n.d.	25	50	25	20	20

and 7). As the study parameters were strongly intercorrelated, the influence on the organisms could not be unequivocally attributed to individual properties of the sediments. However, due to the relatively low contaminant concentrations in most of the sediments, toxic effects on the organisms were not likely. Indeed, with the exception of AA-R, all sediments showed contaminant concentrations that were below or close to threshold values that are considered as not harmful for benthic invertebrates (threshold effect concentrations, TEC; Table 4; MacDonald et al., 2000). However, in the AA-R sample, by contrast, part of the effect on bacteria (36% inhibition compared to control) and nematodes (growth: 27%, reproduction: 41% inhibition compared to control), might have been caused by an elevated Hg concentration (Table 4). For bacteria, EC50 values for Hg in water of 0.3 mg l^{-1} and 0.9 mg l^{-1} were reported for *Pseudomonas fluorescens* and *Vibrio fischeri*, respectively (Brown et al., 1996; McCloskey et al., 1996). Although, in sediments bioavailability of Hg is assumed to be lower than in water, effects of Hg cannot be excluded at a concentraion of 0.6 mg kg^{-1} (AA-R; Table 4). For *C. elegans*, the LOEC for Hg in water was found to be 2 mg l^{-1} (Donkin and Williams, 1995). Thus, effects caused by Hg in AA-R are not likely. Regarding the strong effect of PA-R on fish egg survival, an effect of mineral oil that was found in comparably high concentrations in this sample (Table 4), cannot be excluded. In water, for mineral oil a LC50 of 100 mg l^{-1} was reported for rainbow trout and blue gill (Office of Pesticide Programs, 2000).

In the low-level-contaminated sediments, it was more likely that organisms were influenced by particle size distribution and the content of organic matter. Similar results were reported in a previous study involving *C. elegans*, in which growth was found to

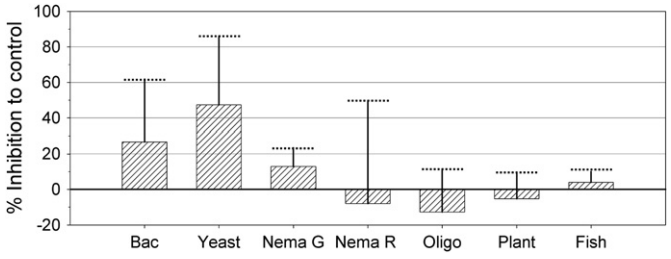

Fig. 4. Means (error bars = standard deviation) of %inhibition (compared to control sediments) of toxicity endpoints in native sediments ($n = 9$; fish: $n = 8$; see Table 8); dotted lines mark the MTI (maximal tolerable inhibition); Bac = bacterial enzyme activity; Yeast = yeast fermentation; Nema-G: nematode growth; Nema-R: nematode reproduction; Oligo: oligochaete reproduction; Plant: plant growth; Fish: fish-egg survival.

be negatively correlated with clay content (Höss et al., 1999), perhaps due to lower food availability for the nematodes. While the particles <2 μm can be swallowed by the nematodes together with the food bacteria, they are less nutritious. *L. variegatus* is a sediment ingester that can take up larger particles than *C. elegans*. Although choice experiments with freshwater oligochaetes showed that the worms (*Tubifex*, *Limnodrilus*, *Stylodrilus*) avoided coarse sands and headed for fine, muddy sediments (Wachs, 1967), grain size did not influence *L. variegatus* in the present study. For plants water uptake depends on the water capacity of the substrate. In finer substrates, for example silty sediments, more water is capillary bound and thus available for the plant. Furthermore, nutrients (minerals, e.g. Mg) are usually bound to fine-grained sediment particles. Therefore, the observed positive correlation of minerals and grain size reflect the better supply of water and nutrients in fine-grained sediments (Barko and Smart, 1986). This possibility is supported by positive correlations of plant growth to nitrogen and DOC concentrations in the pore water (PC4; Tables 4 and 7). Fish egg survival, by contrast, was not influenced by grain size distribution but was related to the content of organic matter. It was previously shown that *D. rerio* is affected by organic matter, such as humic substances (Cazenave et al., 2006). For the bacteria no significant correlation to the measured geochemical sediment properties were found (Table 7). Instead, the relatively high variability of the enzyme activity observed in the bacteria contact test might have been due to other factors, not measured in this study. It might also be at least partly explained by the varying quenching effects of the different native sediments. A fluorescent dye can lose energy without emitting light during contact with other substances, resulting in a reduced fluorescent signal. Therefore, a calibration method was developed and tested and is described in detail in Heise and Ahlf (2005). In the yeast contact test the reasons behind the strong inhibition excerted by some of the sediments remain unclear, as it could not be explained by the measured sediment properties (Table 7).

4.2. Toxicity thresholds

The need for test-specific thresholds or limits that set the boundary between reference conditions or natural variability and toxic effects has already been stated in other studies (Hunt et al., 2001; Reynoldson et al., 2002; Thursby et al., 1997). Based on the variability of lethal and sublethal toxicity endpoints in reference sediments, Reynoldson et al. (2002) established three categories of responses to toxicity for four benthic invertebrate species (*Chironomus riparius*, *Hyalella azteca*, *Hexagenia* spp., *Tubifex tubifex*): not

toxic, potentially toxic and toxic. Similar to the approach of the present study, the delineations for the three categories were developed from the standard statistical parameters of mean and standard deviation (mean ± SD) of an endpoint measured in reference sediments. In contrast to the present study, however, Reynoldson et al. (2002) inserted a "buffer zone" of potential toxicity between the not toxic and toxic categories, instead of defining sharp threshold. A comparable approach was used by Hunt et al. (2001) who set up individual "sediment toxicity tolerance limits" for the survival of marine amphipods and the development sea urchin embryo/larval, to determine elevated toxicity relative to reference conditions. Thursby et al. (1997) presented a toxicity threshold approach that considers the entire test system by using a historical database of minimal significant differences (MSD), rather than searching only for a statistically significant difference between a sample and the control in a single test run. These toxicity thresholds were applied to a large data set and were shown to be useful for interpreting sediment toxicity data (Phillips et al., 2001). Samples were regarded as toxic, if both statistical significance and detectable significance (below the MSD derived threshold) indicated a toxic effect.

The present study combines these approaches and considers the statistical power of a test system as well as the influence on the organisms of natural sediment properties that increase the background noise, from which a toxic effect has to be distinguished. For toxicity endpoints showing relatively low variability within treatments (between replicates; low CVi and MDD) and between sediments (low CVs and MTI), such as plant growth, oligochaete reproduction and nematode growth, the toxicity thresholds were set quite low, at 20 and 25% inhibition (Table 5). The high toxicity threshold of the bacterial enzyme activity, despite a very low MDD of <10%, derived from the high variability between the various sediments (CVs: 48%) and the fact that the control enzyme activity in quartz sand higher than in natural sediments. This combination led to the high MTI of 62% and a toxicity threshold of 60%. The use of an alternative, more realistic, artificial control sediment might help to get a lower MTI and thus also a lower toxicity threshold for the bacteria contact test. The yeast contact test showed an exceptionally high variability between the various native sediments that could not be explained by the measured sediment properties or contaminant concentrations. An MTI close to 90% did not allow the definition of a reasonable toxicity threshold that is able to detect a contaminant effect out of the large background noise.

The MTI as the basis, albeit not the only one, for the toxicity threshold, generally accounts for differences in an organism's response to an artificial sediment vs. natural sediments. Formulated, artificial sediments are often designed to yield optimal performance of the test organism but this might also exceed the performance in natural reference sediments (Fig. 3; Kemble et al., 1999). Therefore, in addition to the control sediment, the use of a reference sediment that is similar to the native test sediment, but free of contamination is always recommended (ASTM, 1990; US EPA, 1998). However, as a suitable reference site is not always available, a reliable toxicity threshold, established on the basis of a permanently available control sediment, is crucial for accurate interpretation of the toxicity of native sediments.

4.3. Battery of sediment contact tests

With the exception of the yeast contact test, all studied contact tests appear to be promising tools for sediment toxicity assessments. As the organisms tolerated different types of freshwater sediments from lakes and rivers with a considerable range of geo-chemical properties, it can be broadly applied for assessing sediment toxicity. Moreover, a battery of sediment-contact tests with organisms from different organizational and trophic levels, as proposed in the SeKT-project (Feiler et al., 2005), has several advantages over a test battery using only macro-invertebrates (ASTM, 2005). First, different exposure routes can be considered. *A. globiformis* (bacteria), *M. aquaticum* (plant), and eggs of *D. rerio* (fish) are exposed to sediment-associated contaminants mainly via the dissolved phase in pore water as well as by direct contact with contaminant-loaded particles. Although the adult zebrafish might not come into contact with contaminated sediments, fish embryos in their eggs very likely do, as cyprinids commonly spawn on finely grained sediment. Meiobenthic nematodes, represented by *C. elegans*, are relatively small and live in the interstitial space. Thus, the pore water, containing all dissolved and colloidal substances, and fine particles are relevant for the nematode's uptake of pollutants (Höss et al., 2001). *L. variegatus* takes up the sediment particles with all bound contaminants which become newly available in the gut of these oligochaetes (e.g. Leppänen and Kukkonen, 1998). Second, the use of organisms from different organizational and trophic levels, and thus with a broad variety of receptors for environmental chemicals, allows the assessment of chemicals with different modes of action. Third, in the proposed battery, short-term (few hours to few days: bacteria, nematodes, fish eggs) and longer-term (days to weeks: plants and oligochaetes) tests are included, which allows rapid screening but also the evaluation of long-term effects.

5. Conclusions

This study investigated the response of several sediment-contact tests on freshwater sediments with both low to moderate contamination considerable variability in terms of their geochemical properties. The variability of the test systems that partly could be explained by individual sediment properties was considered as natural variability and used as a basis for defining toxicity thresholds. Only one test system, the yeast contact test, showed an exceptionally high variability in sediments with low-level contamination and thus cannot be recommended for testing native sediments. In all other contact tests, the variability between the lowly contaminated sediments was low enough to define reasonable toxicity thresholds. Thus, the tests fulfilled an important prerequisite for assessing the toxicity of freshwater sediments with a broad range of geochemical properties. However, the presented toxicity threshold should not be regarded as "set in stone". With a growing data base for lowly contaminated sediments, it probably will be necessary to adjust the toxicity thresholds for these contact tests. Overall, an ecologically relevant battery of sediment contact tests can be recommended in which test organisms of different trophic levels and with various exposure routes are used: bacteria, nematodes, oligochaetes, plants and fish eggs. When carried out with reasonable toxicity thresholds, these test systems offer a pragmatic approach to sediment risk assessment.

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