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Environmental Toxicology and Chemistry, Vol. 32, No. 1, pp. 144–155, 2013

Printed in the USA
DOI: 10.1002/etc.2024

Environmental Toxicology

SEDIMENT CONTACT TESTS AS A TOOL FOR THE ASSESSMENT OF SEDIMENT QUALITY IN GERMAN WATERS

Ute Feiler,*† Sebastian Höss,‡ Wolfgang Ahlf,§ Daniel Gilberg,‖ Monika Hammers-Wirtz,# Henner Hollert,†† Michael Meller,‖ Helga Neumann-Hensel,‡‡ Richard Ottermanns,†† Thomas-Benjamin Seiler,†† Denise Spira,† and Peter Heininger†

†Federal Institute of Hydrology, Koblenz, Germany
‡Institute for Biodiversity-Network, Regensburg, Germany
§Technical University Hamburg-Harburg, Hamburg, Germany
‖ECT Oekotoxikologie, Flörsheim, Germany
#Research Institute for Ecosystem Analysis and Assessment, Aachen, Germany
††Rhenish-Westphalian Technical University, Aachen University, Institute for Environmental Research, Aachen, Germany
‡‡Eurofins Fintelmann & Meyer GMP, Hamburg, Germany

(*Submitted* 23 *May* 2012; *Returned for Revision* 9 *July* 2012; *Accepted* 23 *August* 2012)

Abstract—A sediment contact test (SCT) battery consisting of five ecotoxicological test systems was applied to 21 native freshwater sediments characterized by a broad variety of geochemical properties and anthropogenic contamination. Higher plants (*Myriophyllum aquaticum*), nematodes (*Caenorhabditis elegans*), oligochaetes (*Lumbriculus variegatus*), zebrafish embryos (*Danio rerio*), and bacteria (*Arthrobacter globiformis*), representing various trophic levels and exposure pathways, were used as test organisms. The test battery detected sediment toxicity caused by anthropogenic pollution, whereas the various tests provided site-specific, nonredundant information to the overall toxicity assessment. Based on the toxicity pattern derived from the test battery, the sediments were classified according to a newly proposed classification system for sediment toxicity assessment. The SCT-derived classification generally agreed well with the application of consensus-based sediment quality guidelines (SQGs), especially with regard to sediments with high toxic potential. For sediments with low to medium toxic potential, the SQGs often underestimated the toxicity that was detected by the SCTs, underpinning the need for toxicity tests in sediment quality assessment. Environ. Toxicol. Chem. 2013;32:144–155. © 2012 SETAC

Keywords—Sediment quality assessment Sediment toxicity Contact tests Test battery Classification system

INTRODUCTION

During the last century, the contamination of river and lake sediments increased steadily. Sediments are now recognized both as a major sink and as a potential source of persistent toxic substances in the aquatic environment [1–3]. At the same time, sediments play a key role in the ecological status of aquatic ecosystems, as they are a habitat of diverse communities and a compartment where important biochemical transformations take place. Therefore, sediment studies are very suitable for highlighting the extent, the history, and the trend of water pollution. In Germany, approximately 4 million m^3 of sediments are dredged every year alone in inland waterways. Among other criteria, toxicity criteria are used to decide on the acceptability of dredged material relocation within the waters or the need for other disposal options, which may be considerably higher in cost. Therefore, thorough sediment characterization is essential. At present, weight-of-evidence approaches, such as the sediment quality triad [4,5], are widely accepted to assess the ecological risk of sediment-bound contaminants [6]. In addition to chemical analysis and in situ benthic community assessment, toxicity testing with single species forms the third part of the sediment quality triad. Despite broad consensus in the scientific community that whole-sediment exposure protocols are indispensable for realistic scenarios simulating in situ exposure conditions [5,7], the respective environmental regulations and guidelines in Germany predominantly demand aquatic bioassays for testing aqueous extracts or porewater obtained from the sediments. For example, a successful battery of standardized bioassays using aquatic organisms from the three trophic levels (algae [green algae; ISO 8692], bacteria [luminescent bacteria; ISO 11348], and invertebrates [*Daphnia magna*; ISO 6341]) is part of the guideline for the assessment of dredged material in German Federal Waterways [8,9]. In the United States and Canada, macroinvertebrates have mostly been used for whole sediment toxicity testing [10–12]; standardized sediment contact tests (SCTs) with organisms from other trophic levels did not exist until recently. However, within the last two decades, new SCTs have been developed in Europe using a broader range of organisms, such as bacteria [13–15], yeast [16], nematodes [17,18], fish embryos [19,20], and macrophytes [21], with some of them being standardized (ISO 10872; ISO/DIS 16191; ISO/DIS 10871) and approved for assessing the toxicity of contaminated freshwater sediments [22,23].

The more realistic exposure conditions of SCTs compared to porewater or extract testing, however, also raise uncertainties in the interpretability of test results, as the test organisms are influenced not only by contaminants, but also by geochemical properties of the sediments, such as grain size distribution or organic matter. The response of a test organism to these sediment properties can lead to variability in the toxicity endpoint

All Supplemental Data may be viewed on the online version of this article.

* To whom correspondence may be addressed (feiler@bafg.de).

Published online 1 October 2012 in Wiley Online Library (wileyonlinelibrary.com).

that might be interpreted as a toxic effect. Therefore, Höss et al. [22] set up test-specific toxicity thresholds for a set of SCTs that consider the natural variability of each test system and toxicity endpoint, based on two criteria: (1) the power of a test to detect a significant difference to the control (measured as minimal detectable difference), and (2) the variability of the toxicity endpoint in lightly polluted sediments compared to the control sediment (measured as maximal tolerable inhibition). Thus, an inhibitory effect of a certain sediment sample is regarded as a toxic effect, if it exceeds the endpoint-specific toxicity threshold (expressed as percentage of inhibition).

The aim of the joint research project SeKT (funded by the German Federal Ministry of Education and Research), was to validate a battery of SCTs for assessing the toxicity of native freshwater sediments, including tests using plants (*Myriophyllum aquaticum*), nematodes (*Caenorhabditis elegans*), oligochaetes (*Lumbriculus variegatus*), bacteria (*Arthrobacter globiformis*), yeast (*Saccharomyces cervisiae*), and fish embryos (*Danio rerio*) as test organisms [24,25]. The present study represents the second part of the SeKT project and applies the recommended battery of SCTs to classify the toxicity of polluted freshwater sediments from rivers and lakes, using the toxicity thresholds that were elaborated in the first part of the project [22]. The SCT-based classification was compared with sediment quality guidelines (SQGs) that are derived from chemical data and often used to describe the toxic potential of sediments [26,27].

The following hypotheses were tested: (1) SCTs are suitable to detect anthropogenic pollution in sediment; (2) a test battery, rather than single toxicity tests, is required to reliably identify the risk of contaminated sediments; and (3) the SCT battery-based classification is a useful line of evidence in a weight-of-evidence approach.

MATERIALS AND METHODS

Sediment contact tests

All SCTs were performed according to standard procedures or published test protocols (Table 1). Suitable toxicity thresholds had been defined for all test systems and calculated on the basis of the minimal detectable difference and the maximal tolerable inhibition as described by Höss et al. [22] during the joint research project SeKT [24,25]. In the following, inhibitory effects that exceeded the respective toxicity thresholds were defined as toxic effects. In the plant test, a lightly contaminated native sediment (from the Rhine River) was used as the negative control, instead of the artificial control sediment that was used in former studies [21,22]. Therefore the minimal detectable difference (10%) and maximal tolerable inhibition (21%) had to be recalculated, which had, however, no influence on the toxicity threshold of 20%. All relevant test conditions of the SCTs used are summarized in Table 1.

Sediment sampling

The sediment sampling sites were chosen according to the following criteria, in which the sediments should: (1) bear different loads of anthropogenic contamination; (2) vary in geochemical properties (mainly grain size and organic content); (3) originate from lotic (rivers) and lentic (lakes) systems; and (4) come from different river basins. The samples were taken from 21 selected sites (Fig. 1) during three sampling campaigns. Eleven sediment samples (Altrip, Rhine river, old arm [AA-R]; Bad Abbach river, Danube [BA-R]; Drontermeer lake, The Netherlands [DM-L]; Ehrenbreitstein, Rhine, harbor [EB-R];

Table 1. Experimental designs of the sediment contact tests

Sediment contact test	Plants	Bacteria	Nematodes		Oligochaetes	Fish embryos
Test species	*Myriophyllum aquaticum*	*Arthrobacter Globiformis*	*Caenorhabditis elegans*		*Lumbriculus variegatus*	*Danio rerio*
Standardization	ISO/DIS 16191	ISO/DIS 10871	ISO 10872		OECD 225	Based on DIN 38415-6
Reference	[21]	[13,15]	[18,50]		[51,52]	[19]
Test endpoint	Growth rate (fresh wt)	Enzyme activity (Resorufin formation; fluorescence/min)	Growth (body length, μm)	Reproduction (offspring per organism)	Reproduction (total number of organisms)	Mortality (%)
Control sediment	Natural sediment	Quartz sand	Artificial sediment		OECD-218	Artificial water
Replicates	3 (control 6)	3	4 (control 9)		6	2–3
Validity criteria	Growth rate ≥ 0.09	Fivefold increase in fluorescence	≥80% fertility; ≥30 offspring per test organism		Reproduction ≥ 18 organisms	Survival ≥ 90%
Toxicity threshold (%)[a]	20	60	25	50	25	20

[a] Calculation of the toxicity thresholds is described in Höss et al. [22].

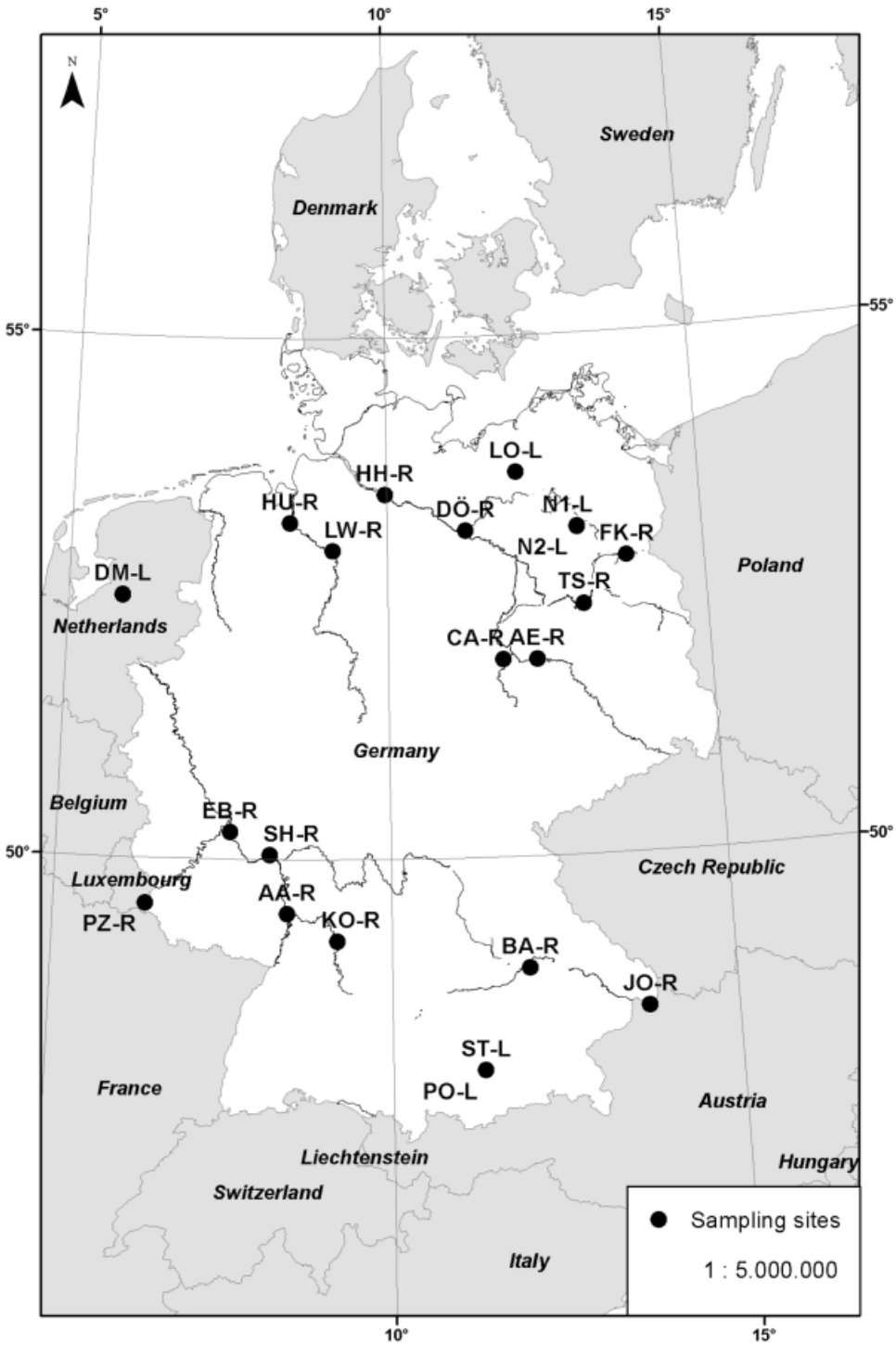

Fig. 1. Selected sampling sites of freshwater sediments. R = river; L = lake; AA-R = Altrip, Rhine, old arm; AE-R = Alte Elbe, Elbe, old arm; BA-R = Bad Abbach, Danube; CA-R = Calbe Saale, littoral zone; DM-L = Drontermeer, The Netherlands; DÖ-R = Dömitz, Müritz-Elde-Waterway (MEW), lock; EB-R = Ehrenbreitstein, Rhine, harbor; FK-R = Finow, Finow-canal; HH-R = Hamburg, Elbe, harbor; HU-R = Hunte, Weser, harbor; JO-R = Jochenstein, Danube, lock; KO-R = Kochendorf, Neckar; LO-L = Lohmen, Lake Lohmer, profundal zone; LW-R = Langwedel, Weser, barrage; N1-L = Neuglobsow1, Lake Stechlin, littoral zone; N2-L = Neuglobsow2, Lake Stechlin, profundal zone; PO-L = Possenhofen, Lake Starnberg, littoral zone; PZ-R = Palzem, Moselle, lock; SH-R = Schierstein, Rhine, harbor; ST-L = Starnberg, Lake Starnberg, profundal zone; TS-L = Tiefer See, Untere Havel-waterway (UHW), profundal zone.

Jochenstein, Danube, lock [JO-R]; Lohmen, Lake Lohmer, profundal zone [LO-L]; Langwedel, Weser, barrage [LW-R]; Neuglobsow1, Lake Stechlin, littoral zone [N1-L]; Neuglobsow2, Lake Stechlin, profundal zone [N2-L]; Possenhofen, Lake Starnberg, littoral zone [PO-L]; Starnberg, Lake Starnberg, profundal zone [ST-L]) were collected and tested in 2005, four samples in 2006 (Alte Elbe, Elbe, old arm [AE-R]; Calbe Saale, littoral zone [CA-R]; Dömitz Müritz-Elde-Waterway, lock [DÖ-R]; Tiefer See, Untere Havel-waterway, profundal zone [TS-L]), and six samples in 2007 (Finow, Finow-canal [FK-R]; Hamburg, Elbe, harbor [HH-R]; Hunte, Weser, harbor [HU-R]; Kochendorf, Neckar [KO-R]; Palzem, Moselle, lock [PZ-R]; Schierstein, Rhine, harbor [SH-R]). All freshwater sediment samples were taken at sites where the sediment is permanently covered by

water. Sediment sampling, dispatch, and testing were carefully scheduled, so that sample storage was kept to a minimum (maximum two weeks from sampling to testing). Some of the sediments were collected in the course of routine monitoring programs in Germany (Federal Institute of Hydrology, Koblenz, Germany). Surface samples (0–10 cm) were taken with a stainless steel Van Veen grab sampler. After homogenization, the samples were stored in plastic jars at $4 \pm 2^{\circ}C$ in the dark until use. The sediments were chemically analyzed and tested with the SCT battery in up to four test series.

The samples were taken from rivers and lakes all over Germany, from main river basins the Elbe (northeast), the Rhine (west), and the Danube (southeast), as well as the Warnow-Peene river basin in the north, the Weser basin in the middle, and the Odra basin in the east. The additional sampling site DM-L is located in the Netherlands.

Sediment analysis

Sediments were characterized in terms of priority pollutants, nutrients, and basic geochemical properties by standard procedures (German Institute for Standardization [DIN], European Norm [EN], and International Organization of Standardization [ISO]). The list of parameters includes anthropogenic contaminants that are typically enriched in sediments, such as toxic metals, arsenic, and persistent organic pollutants. Analyses of priority pollutants were performed with freeze-dried, sieved, and milled sediments, with a particle size of <2 mm. The heavy metals lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), mercury (Hg), and zinc (Zn), arsenic (As), and the major mineral elements lithium (Li), calcium (Ca), magnesium (Mg), iron (Fe), and aluminum (Al) were determined after microwave-assisted digestion with aqua regia at 180°C in closed vessels by inductively coupled plasma optical emission spectroscopy, atomic fluorescence spectroscopy (Hg), and hydride atomic absorption spectroscopy (As). The polycyclic aromatic hydrocarbons (U.S. Environmental Protection Agency list of 16 compounds) were determined by high-performance liquid chromatography (gradient elution) with diode array and fluorescence detection (DIN 38414 S21). Polychlorinated biphenyls (28, 52, 101, 118, 138, 153, 180), hexachlorobenzene, hexachlorohexane (HCH), and dichlorodiphenyltrichloroethane and its derivates (*p*,*p*′-DDE, *o*,*p*′-DDE, *p*,*p*′-DDD, *o*,*p*′-DDD, *p*,*p*′-DDT, *o*,*p*′-DDT) were analyzed after Soxhlet extraction by a gas chromatograph equipped with two ^{63}Ni electron-capture detectors and two capillary columns of different polarity (DIN 38414 S20 and DIN 38407-F2). The mineral oil content (petroleum-derived hydrocarbons) was measured by gas chromatography using a flame ionization detector, according to ISO TR 11046. Organotin (mono-, di-, tri-, and tetrabutyltin, mono- and dioctyltin, tricyclohexyltin, triphenyltin) was alkylated, extracted with hexane, and analyzed using gas chromatography atomic emission detection (DIN 19744).

Porewater 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 constant weight was obtained (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 (DIN EN 12879 S3a) and total organic carbon (DIN ISO 10694). Nitrogen (N), phosphorus (P), sulfur (S), and minerals were analyzed according to DIN ISO 11261, DIN 38414 S12, DIN ISO 15178, and DIN ISO 11466, respectively. In porewater, dissolved organic carbon, total phosphorus, and nitrogen compounds (NO_3–N, NO_2–N, NH_4–N, summed up as total nitrogen) were analyzed according to DIN 38409 H3, DIN EN ISO 6878, DIN EN ISO 10304-2, DIN EN 26777, and DIN 38406 E5-1, respectively.

Use of consensus-based sediment quality guidelines

The results of the SCTs were compared to consensus-based SQGs that were calculated from the measured concentrations of selected metals (and arsenic) and persistent organic pollutants according to MacDonald et al. [26]. The concentrations of the analyzed organic contaminants in the sediments were normalized to 1% total organic carbon for reasons of comparability of the bioavailable fraction in the various types of sediment [28]. In cases of chemical concentration below the limit of detection, $0.5 \times$ limit of detection was used for calculations.

Threshold effect concentration (TEC; below which no adverse effects are expected to occur) and probable effect concentration (PEC; above which harmful effects are predicted) for selected chemicals were used as thresholds to calculate quotients (measured concentration divided by the respective SQG for each chemical and each sample [TEC-Q; PEC-Q]). Maximal TEC-Q values (for all contaminants: TEC-Q_{max}total; for metals: TEC-Q_{max}m; for organic chemicals: TEC-Q_{max}o), as well as maximal and mean PEC-Q values were used to describe the toxic potential of the various sediment samples.

Data analysis and statistics

The following statistics were considered to describe the test results of the SCTs: mean, standard deviation, and coefficient of variation for all measured toxicity endpoints, as well as percentage of inhibition (I) with respect to the corresponding controls. To test for statistical differences between the response in natural sediments and the control sediment, one-way analysis of variance tests were performed, and treatments were compared with a post hoc Dunnett test ($\alpha = 0.05$, two-sided). If the tests for normal distribution (Kolmogorow–Smirnow test) and homogeneity of variance (Levene's test) failed, Dunn's test was used ($\alpha = 0.05$).

For the fish embryo test, Fischer's exact binominal test was used to determine statistical differences between the test responses in each sediment, except for the results of samples AA-R, BA-R, EB-R, DM-L, JO-R, LO-L, LW-R, N1-L, N2-L, PO-R, and ST-L, for which no statistical differences could be calculated, as only one replicate per treatment was available. All univariate statistical analyses were performed with the software SigmaStat for Windows Version 3.0 (SPSS).

Principal component analysis (PCA) [29] maps information from a large number of variables onto a smaller number of linear combinations, thereby simplifying the data interpretation. The eigenvalues of the resulting ordination axes are a measure for the information content, the ecological relevance, and the amount of variance explained by the axes. A PCA was used to visualize sediment characteristics and their correlation patterns. The PCA was calculated using CANOCO for Windows Version 4.53 (Microcomputer Power) [30]. Sediment characteristics were standardized by variables standard deviation (PCA based on correlation matrix, centering by species). For the PCA, HCH was excluded from the set of environmental variables because all concentrations were below the limit of detection. Furthermore, loss on ignition, total organic carbon, N, organotin (except tributyltin), and gravel were excluded due to their low information input or redundant information (intercorrelations with other variables, unpublished results). The grain size fractions (sand 630–2,000 μm, sand 200–630 μm, sand 125–200 μm, sand

63–125 μm, silt 20–63 μm, silt 6.3–20 μm, silt 2.0–6.3 μm) were pooled to one sand fraction (sand 63–2,000 μm), and one silt fraction (silt 2.0–63 μm). The data for *p*,*p*′-DDE, *o*,*p*′-DDE, *p*,*p*′-DDD, *o*,*p*′-DDD, *p*,*p*′-DDT, and *o*,*p*′-DDT were summed up as dichlorodiphenyltrichloroethane and derivates. Total nitrogen, total phosphorus, and dissolved organic carbon concentrations in the sediment porewater were only determined to confirm that sufficient nutrients were present in the natural sediments to ensure *Myriophyllum* growth and were therefore not considered for multivariate analyses.

Redundancy analysis (RDA) [31] is an enhancement of PCA. Ordination axes are not only a linear combination of primary variables, but also a linear combination of further external variables. By an additional regression step within the algorithm, only the amount of variance that can be attributed to the external variables is mapped on the ordination axes. The RDA was calculated using CANOCO for Windows. Effect data (target variables) as well as sediment characteristics (descriptors) were standardized by variables standard deviation (PCA based on correlation matrix, centering by species). Significances of environmental variables in RDA were calculated by a Monte Carlo permutation test (499 permutations). Preliminary variable selection of sediment characteristics was done by manual forward selection by means of the significance [30]. Only significant variables ($p \leq 0.05$) were used as environmental variables in a second run. All nonsignificant sediment characteristics were used as supplementary variables. Natural sediment characteristics that are known to have a significant effect on SCT results (Ca, Mg; [22]) were used as covariables to exclude their effects and to constrain the analysis to contamination variables, resulting in partial RDA (RDA based on correlation matrix, centering by species, significance of ordination axes tested by Monte Carlo permutation test, 499 permutations under reduced model). The same grain size fractions as for the PCA were applied. Instead of contaminant concentrations of the single chemicals, maximal TEC-Q_{metal} and TEC-$Q_{organic}$ values were used to describe the ecotoxicologically more relevant toxic potential and to reduce the number of variables in the analysis. As effect data, the percentage of inhibition (percentage of mortality for fish embryos) values were included in the RDA analysis. All data are available either directly in the manuscript (Tables 2, 3, and 4) or in Supplemental Data, Tables S1 and S2.

RESULTS

Sediment contaminants and geochemical properties

The degree of sediment pollution was described in the present study by a selection of anthropogenic contaminants that are typically enriched in sediments and reflect the situation in densely populated and intensively used Central European river basins [32–34]. The selection includes heavy metals, arsenic, persistent organic pollutants, and phosphorus. For a classification of the sediments in terms of their toxic potential, the consensus-based SQGs described by MacDonald et al. [26] were applied. This classification, together with the individual contaminant data, is shown in Table 2.

According to the SQGs, 12 of the investigated sediments (AA-R, AE-R, BA-R, DÖ-R, DM-L, JO-R, KO-R, LO-L, N1-L, N2-L, PO-L, ST-L) showed a mean PEC-Q below 0.5. Moreover, for nine of these 12 sediments (BA-R, DM-L, JO-R, KO-R, LO-L, N1-L, N2-L, PO-L, ST-L), the even stricter criterion, the TEC, was not or was only slightly exceeded (TEC-$Q_{max} < 1.5$). Therefore, based on these thresholds, these nine sediments were predicted to be nontoxic. Higher toxic potentials were found in CA-R, EB-R, FK-R, HH-R, HU-R, LW-R, PZ-R, SH-R, and TS-L (PEC-$Q_{mean} > 0.5$; TEC-$Q_{max} > 1.5$). Consequently, these sediments were predicted to cause toxic effects.

Regarding their geochemical properties, the sediments investigated in the present study showed considerable variability. For example, the dry weights ranged from 10 (TS-L) to 57% (JO-R), total organic carbon from 23 (DÖ-R) to 140 g kg^{-1} dry weight (FK-R), and contents of sand, silt, and clay from 2 (AA-R) to 90% (DÖ-R), 7 (DÖ-R) to 85% (ST-L), and 4 (DÖ-R) to 39% (HU-R), respectively (detailed data are available in Supplemental Data, Table S1).

Principal component analysis revealed that the samples could be clearly distinguished in terms of their quantity and quality of contamination, as well as their geochemical properties (Fig. 2). Along the horizontal axis, a separation of the sediments due to their contaminant concentrations can be observed, with strongly contaminated sediments (FK-R, HH-R) positioned on the extreme right. The vertical axis separated organic from metal contamination with, for example, FK-R showing a stronger metal contamination than HH-R and vice versa for organic contamination. The contaminants were mainly associated with finer, organic carbon-rich sediments, whereas the coarser sediments showed lower contamination.

Response of sediment contact tests to sediments

The results of the SCTs that were performed with the native sediments are summarized in Table 3. The validity criteria (Table 1) were met for all test controls (Table 3). All test organisms showed a large variation in their response to the various sediments investigated. In the plant test, the growth rate ranged from 0.077 to 0.120, in the nematode test, reproduction varied from 3.6 to 131 and growth from 310 to 1273 μm, reproduction in the oligochaete test ranged from 25 to 70, the fish embryos showed mortalities from 3.4 to 100%, and bacterial enzyme activity ranged from 54 to 251 fluorescence min^{-1}.

The effects of the various sediments revealed in the different test systems (expressed as percentage of inhibition compared to the respective control) are shown in Table 4. Several sediments were identified as toxic, causing effects above or equal to the toxicity thresholds (Table 1 and bold values in Table 4). The effects values ranged from stimulations to almost 100% inhibition, depending on the test system and the sediment. Toxic effects (statistically significant [Table 3] and > toxicity threshold [Table 4]) were induced by five sediments for plant growth (CA-R, DÖ-R, FK-R, N1-L, N2-L), by seven sediments for nematode reproduction (AE-R, BA-R, DÖ-R, HH-R, HU-R, LW-R, PZ-R), by eight sediments for nematode growth (AA-R, AE-R, BA-R, CA-R, HH-R, HU-R, LW-R, PZ-R), by six sediments for fish embryo survival (AE-R, CA-R, DÖ-R, FK-R, HH-R, TS-L), by one sediment for oligochaete reproduction (HH-R), and by two sediments for bacterial enzyme activity (HU-R, JO-R). Thus, the sediment samples could clearly be differentiated in terms of their toxicity with the test battery used.

Synopsis of test data and sediment properties

A multivariate technique (RDA) was used to analyze the relation of the sediment toxicity that was assessed with the various SCTs and the measured sediment properties. Samples that are separated along the horizontal axis of the RDA plot, showed, from left to right, increasing effects on nematodes (inhibition of reproduction and growth) and oligochaetes (inhibition of reproduction), with HH-R showing the strongest effects on these test organisms (Fig. 3). These effects very clearly

Table 2. Measured contaminant concentrations (elements and organic substances) and sediment quality criteria[a]

Code	As mg/kg	Pb mg/kg	Cd mg/kg	Cr mg/kg	Cu mg/kg	Ni mg/kg	Hg mg/kg	Zn mg/kg	PAH Σ16 mg/kg	PAH Σ9 mg/kg	PCB Σ7 µg/kg	MKW mg/kg	HCB µg/kg	DDX µg/kg	TBT µgSn/kg	P g/kg	TEC-Q_{max} total[b]	TEC-Q_{max} metals	TEC-Q_{max} organics	PEC-Q_{mean} total[b]	PEC-Q_{max} total[b]
LOD	3.0	0.5	0.3	1.0	1.0	1.0	0.01	1.0	1.0	0.1	1.0	100	0.3 (0.1)	1.8	1.0	0.001					
AA-R	10	53	0.4	53	58	35	0.58	205	2.9	1.95	42.7	<100	8.7	15.9	14.2	0.7	3.20	3.20	0.88	0.31	0.72
AE-R	36	78	2.2	55	58	37	0.7	446	8.4	7.25	65.9	400	<0.1	182	3.92	2.4	6.17	3.90	6.17	0.50	1.09
BA-R	8	24	<0.3	35	36	23	0.23	179	<1	0.05	<5	<100	<0.3	2.71	0.53	1.0	1.50	1.50	0.12	0.19	0.47
CA-R	41	198	3.4	96	168	42	11	1,095	15.0	12.2	231	1,400	<0.1	44.1	2.29	1.1	61.1	61.1	1.11	1.71	2.39
DM-L	11	34	<0.3	27	22	18	0.15	135	<1	0.1	<5	380	<0.3	<1.8	14.9	0.6	1.10	1.12	0.02	0.17	0.37
DÖ-R	11	30	1.9	156	55	6.8	0.08	164	<1	0.55	10.8	<100	<0.1	19.6	8.25	0.9	3.60	3.60	1.62	0.30	1.41
EB-R	11	93	1	142	57	88	0.48	440	3.8	3.05	77	<100	35	8.48	5.60	1.0	3.90	3.90	0.50	0.56	1.81
FK-R	29	290	33	87	3100	110	96	4,600	24.6	18.3	143	5,700	7.3	155	202	2.3	98.1	98.1	2.09	11.9	87.3
HH-R	73	480	11	130	320	79	8.3	2,000	509	350	286	7,300	40.0	355	132	2.9	46.1	46.1	24.9	2.44	7.55
HU-R	25	140	3.6	80	110	56	0.65	930	5.4	3.8	<1	1,100	<0.1	6.7	319	2.2	7.70	7.70	0.36	0.71	2.03
JO-R	10	18	<0.3	33	32	29	0.13	171	<1	0.33	<5	110	<0.3	0.62	1.00	0.7	1.40	1.40	0.06	0.19	0.60
KO-R	3	17	0.4	11	14	11	0.10	160	6.8	4.7	121	390	2.0	5.1	0.94	0.9	1.30	1.30	0.69	0.11	0.35
LO-L	4	22	0.3	6	15	3	0.07	61	5.3	4.15	<5	<100	<1	27.8	159	2.7	0.85	0.61	0.85	0.07	0.17
LW-R	21	168	4	43	56	36	0.83	701	1.7	1.3	34	<100	1.2	6.3	2.12	2.2	5.80	5.80	0.16	0.60	1.53
N1-L	2	18	<0.3	4	4	<1	0.04	24	<1	0.05	<5	<100	<1	0.7	<1	0.1	0.50	0.50	0.02	0.04	0.14
N2L	4	29	<0.3	5	9	1	0.07	37	<1	0.05	1.7	<100	<1	1.4	<1	0.2	0.81	0.81	0.03	0.06	0.23
PO-L	<3	7	<0.3	5	11	2	0.04	19	<1	0.05	<5	<100	<0.3	<1.8	1.18	0.2	0.35	0.35	0.04	0.03	0.07
PZ-R	32	170	1.2	97	110	55	0.38	960	22.9	15.6	184	1300	0.6	18.4	1.49	1.5	8.00	8.00	1.92	0.72	2.09
SH-R	10	100	2.1	70	100	44	0.70	550	5.5	3.7	91	770	2.1	26.3	28.5	2.0	4.50	4.50	1.13	0.51	1.20
ST-L	<3	19	<0.3	8	11	4	0.09	45	1.1	0.93	1.1	220	<0.3	<1.8	38.6	0.3	0.50	0.50	0.07	0.06	0.15
TS-L	8	143	2.9	52	218	24	1.1	987	11.0	8.1	251.0	960	<0.1	9	40.2	1.3	8.20	8.20	0.39	1.26	2.15
TEC	9.8	35.8	0.99	43.4	31.6	22.7	0.18	121	NA	1.6	59.8	NA	NA	5.3	NA	NA					
PEC	33	128	5	111	149	48.6	1.1	459	NA	22.8	676	NA	NA	572	NA	NA					

[a] Measurement based on dry weight in the 2-mm fraction.

[b] Code abbreviations for sediment sample can be found in Figure 1 legend.

LOD = limit of detection; PAH = polycyclic aromatic hydrocarbons (U.S. Environmental Protection Agency list of 16 compounds and 9 according to [26]); PCB = polychlorinated biphenyl; MKW = petroleum-derived hydrocarbon; HCB = hexachlorobenzene; DDX = dichlorodiphenyltrichloroethane and derivates; TBT = tributyltin; P = phosphorous; TEC = threshold effect concentration; PEC = probable effect concentration; Q = quotient of measured concentration divided by TEC or PEC (TEC-Q and PEC-Q of organic substances calculated on a dry wt normalized basis at 1% organic carbon); total = organic substances and metals); NA = not available.

Table 3. Response of the test parameters of the five sediment contact tests to the investigated sediment samples

	Plants Growth rate			Nematodes Reproduction			Growth (mm)			Oligochaetes Reproduction			Fish embryos Mortality (%)	Bacteria Enzyme activity (fluorescence min^{-1})		
Code[a]	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	M	Mean	SD	CV
C1	0.092	0.002	2.3	57.0	17.6	31.0	1,313.4	109.8	8.4	31.5	5.3	16.9	2,5	184.3	4.4	2.4
PO-L	0.086	0.013	15.0	53.2	8.0	15.0	1,162.0[c]	80.8	7.0	37.0	3.8	10.4	5.0[b]	114.0[c]	6.5	5.7
ST-L	0.077	0.002	2.8	50.5	3.0	5.8	1,192.5	25.6	2.1	48.8[c]	1.9	4.0	7.1[b]	79.0[c]	2.0	2.5
BA-R	0.099	0.011	11.4	24.5[c]	8.0	32.7	917.1[c]	64.1	7.0	38.0	5.5	14.5	10.0[b]	89.3[c]	0.6	0.6
JO-R	0.098	0.013	13.6	32.0[c]	10.6	33.1	1,000.6[c]	90.4	9.0	31.3	3.9	12.4	3.3[b]	58.8[c]	4.6	7.9
DM-L	0.095	0.004	4.5	46.9	11.1	23.6	1,067.3[c]	57.4	5.4	40.3[c]	2.9	7.1	19.1[b]	112.2[c]	8.4	7.4
C2	0.118	0.003	2.3	–	–	–	–	–	–	35.7	2.2	6.1	–	146.7	2.3	1.6
LO-L	0.108	0.009	8.4	72.5	15.9	21.9	1,255.9	84.9	6.8	38.7	4.8	12.5	6.7[b]	153.0	3.3	2.2
N1-L	0.088[c]	0.006	6.9	93.0	30.1	32.4	1,273.4	73.8	5.8	24.7[c]	3.8	15.5	3.3[b]	172.4[c]	8.3	4.8
EB-R	0.118	0.007	5.7	89.0	33.0	37.1	1,137.4[c]	113.4	10.0	45.3[c]	5.6	12.4	3.3[b]	165.5[c]	10.1	6.1
N2-L	0.083[c]	0.003	4.0	130.8	23.0	17.5	1,341.6	57.3	4.3	33.0	4.3	13.0	5.0[b]	190.9[c]	3.8	2.0
LW-R	0.107	0.019	17.3	26.9[c]	5.2	19.3	846.2[c]	69.1	8.2	38.3	4.6	12.0	16.7[b]	113.5[c]	10.1	8.9
AA-R	0.118	0.003	2.3	33.4[c]	5.0	15.0	956.0[c]	81.4	8.2	40.2	5.8	14.6	0.0[b]	92.9	12.3	13.2
C3	0.121	0.012	10.2	68.1	18.6	27.3	1,242.7	123.0	9.9	36.2	3.3	9.0	2.5	158.6	7.7	4.9
TS-L	0.102[c]	0.009	8.9	71.5	23.0	32.2	1,109.3[c]	25.5	2.3	63.7[c]	3.7	5.7	98.3[c]	171.5[c]	9.5	5.5
CA-R	0.086[c]	0.002	2.8	45.8[c]	17.3	37.8	921.5[c]	33.3	3.6	55.3[c]	8.2	14.9	82.6[c]	99.0[c]	6.5	6.5
DÖ-R	0.070[c]	0.002	2.4	27.8[c]	7.7	27.7	1,076.3[c]	109.5	10.2	69.7[c]	2.6	3.7	100.0[c]	250.9[c]	91.9	36.6
AE-R	0.106[c]	0.005	4.5	13.9[c]	3.7	26.2	931.3[c]	121.6	13.1	54.8[c]	11.2	20.4	46.2[c]	92.7[c]	4.7	5.0
C4	0.124	0.002	1.4	107.0	20.0	18.7	1,370.2	37.4	2.7	37.2	4.1	11.1	5.0	143.1	8.7	6.1
SH-R	0.100[c]	0.003	2.7	131.8	57.3	43.5	1,173.9[c]	51.1	4.4	42.7	7.3	17.0	20.0	76.1[c]	7.2	9.5
HU-R	0.112[c]	0.006	5.1	17.6[c]	4.3	24.4	788.5[c]	57.1	7.2	38.8	8.3	21.2	25.0	53.8[c]	6.9	12.8
PZ-R	0.120	0.001	1.1	27.3[c]	10.8	39.5	820.1[c]	48.7	5.9	47.0[c]	6.4	13.7	3.4	74.4[c]	8.2	11.0
HH-R	0.100[c]	0.001	1.2	3.6[c]	4.9	135.3	309.8[c]	105.1	33.9	26.5[c]	3.8	14.5	89.3[c]	103.3[c]	9.1	8.8
FK-R	0.094[c]	0.005	5.4	101.3	10.5	10.4	1,194.7[c]	35.4	3.0	42.7	7.2	16.9	46.2[c]	105.2[c]	10.5	10.0
KO-R	0.106[c]	0.007	6.7	91.5	20.9	22.8	1,028.9[c]	19.3	1.9	50.5[c]	3.8	7.6	13.3	74.8[c]	5.8	7.7

[a] For code abbreviations of sediment samples, see Figure 1 legend.
[b] No test on significance performed due to number of replicates.
[c] Significantly reduced with regard to the respective control ($p < 0,05$, one-way analysis of variance, Fisher's exact binominal test for the fish embryo test).
C = test-specific control; 1–4 = test series 1–4; SD = standard deviation; CV = coefficient of variation; M = mortality.

Table 4. Inhibition or mortality values calculated from each sediment contact test endpoint and resulting toxicity classification of the sediments

	Plants		Nematodes			Oligochaetes		Fish embryos		Bacteria		
	Growth rate		Reproduction	Growth (mm)		Reproduction		Mortality (%)		Enzyme activity (fluorescence min^{-1})		
Code[a]	I (%)	Category	I (%)	I (%)	Category	I (%)	Category	M	Category	I (%)	Category	Toxicity class
AA-R	0.0	1	41.4	27.2[b]	2	−12.7	1	0.0	1	36.7	1	II
AE-R	12,4	1	79.5[b]	25.1[b]	2	−51.6	1	46.2[b]	3	41.5	1	IV
BA-R	−6.9	1	56.9[b]	30.2[b]	2	−20.6	1	10.0	1	51.6	1	II
CA-R	28.9[b]	2	32.8	25.9[b]	1	−52.9	1	82.6[b]	3	37.6	1	IV
DM-L	−3.1	1	17.7	18.7	1	−28.0	1	19.1	1	39.1	1	I
DÖ-R	42.2[b]	3	59.2[b]	13.4	2	−92.6	1	100[b]	3	−58	1	V
EB-R	0.0	1	−56.1	13.4	1	−43.9	1	3.3	1	10.2	1	I
FK-R	24.2[b]	2	5.3	12.8	1	−14.8	1	46.2[b]	3	26.5	1	IV
HH-R	19.4	1	96.6[b]	77.4[b]	3	28.7[b]	2	89.3[b]	3	27.8	1	V
HU-R	9.7	1	83.5[b]	42.5[b]	3	−4.5	1	25.0[b]	2	62.4[b]	2	V
JO-R	−6.4	1	43.8	23.8	1	0.5	1	3.3	1	68.1[b]	2	II
KO-R	14.5	1	14.5	24.9	1	−35.9	1	13.3	1	47.7	1	I
LO-L	8.5	1	−27.2	4.4	1	−22.8	1	6.7	1	17.0	1	I
LW-R	9.3	1	52.3[b]	35.6[b]	2	−21.7	1	16.7	1	38.4	1	II
N1-L	25.4[b]	2	−63.1	3.0	1	21.7	1	3.3	1	6.5	1	II
N2-L	29.7[b]	2	−129	−2.5	1	−4.8	1	5.0	1	−3.6	1	II
PO-L	7.4	1	6.8	11.5	1	−17.5	1	5.0	1	38.2	1	I
PZ-R	3.2	1	74.5[b]	40.1[b]	2	−26.5	1	3.4	1	47.9	1	II
SH-R	19.4	1	−23.2	14.3	1	−14.8	1	20.0[b]	2	46.8	1	II
ST-L	16.5	1	11.4	9.2	1	−55.0	1	7.1	1	57.1	1	I
TS-L	15.7	1	−4.9	10.7	1	−76.0	1	98.3[b]	3	−8.1	1	IV

[a] For code abbreviations of sediment samples, see Figure 1 legend.
[b] Inhibitory effect ≥ toxicity threshold. I = inhibition; M = mortality.

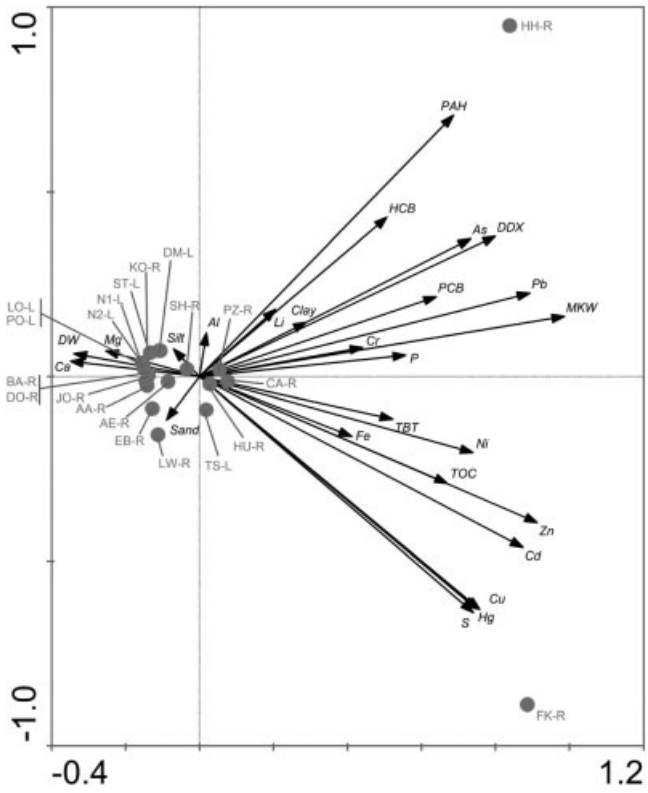

Fig. 2. Principal component analysis of the 21 sediments based on geochemical properties and contaminant concentrations. Eigenvalues 1st axis = 0.906; 2nd axis = 0.086; cumulative percentage variance of data for 1st and 2nd axis = 99.2%. Gray dots = sediment samples. PAH = polycyclic aromatic hydrocarbons; PCB = polychlorinated biphenyls; MKW = petroleum-derived hydrocarbons; DDX = dichlorodiphenyltrichloroethane and derivates; TBT = tributyltin; HCB = hexachlorobenzene; TOC = total organic carbon; P = phosphorus. See Figure 1 legend for code abbreviations of sediment samples.

related to the toxic potential of the organic chemicals, measured in the sediment samples (TEC-Q_{max}o; $p = 0.006$; Monte Carlo permutation test), which strongly correlates with the horizontal axis of the RDA. Samples were separated along the vertical axis with stronger effects on plants (inhibition of growth) and fish embryos (mortality) in the upper part of the plot and stronger effect on bacteria (inhibition of enzyme activity) and partly also on nematodes in the lower part of the plot (Fig. 3). Effects on plants (inhibition of growth) and fish embryo (mortality) tests were related to the toxic potential of the metals, measured in the sediment samples (TEC-Q_{max}m), although this relation was not statistically significant ($p > 0.05$; Monte Carlo permutation test). Inhibition of bacterial enzyme activity was more related to the Li, Al, P, and clay content, rather than to the toxic potential. Also, part of the nematodes' response might have been caused by the clay content (Fig. 3).

Proposal of a sediment classification system

One purpose of the present study was to propose a classification system based on the results of the SCT battery. Through adaptation of an existing classification system that was proposed for a test battery including two aquatic tests and two SCTs [35], the sediments were ranked into five toxicity classes based on the results of the SCT battery used in the present study (cf. Table 4).

First, the effects of the single tests were classified into three effect categories (Table 5). For this purpose, the toxicity thresholds were considered that had been derived from the natural variability of the SCT responses in reference sediments [22]. Using these effect categories from the different SCTs, sediments were then categorized into five toxicity classes (Table 6).

All test results with inhibition values below the toxicity threshold were grouped in category 1 (no toxic effect). Category 2 (medium effect) consists of those values that ranged between the toxicity threshold value and its twofold value. Exceptions were made in defining the threshold values of the second category for bacterial enzyme activity and nematode reproduction, because here the toxicity threshold values were already quite high (60 and 50%, respectively). Accordingly, the second limit was set to 80% for these two tests. Category 3 (strong effect) now comprises all values above the upper threshold of category 2. In case there were two toxicity endpoints for one test system (nematode growth and reproduction), the higher effect class was always used.

Using the algorithm shown in Tables 5 and 6, the five toxicity effect values (categories) obtained per sample (Table 4) were merged into one toxicity class from I to V, with the highest class representing the highest ecotoxicological potential.

The resulting toxicity classes assigned to the sediments (Table 4) represented the full span of toxicity classes. Only class III (medium toxicity) was not represented within the given selection of sediment samples. The highest class (V) was assigned to the sediments from DÖ-R, HU-R, and HH-R, indicating high toxicity. The sediments from AE-R, CA-R, FK-R, and TS-R were grouped in class IV, indicating toxic effects of the samples. The sediments from AA-R, BA-R, JO-R, LW-R, N1-L, N2-L, PZ-R, and SH-R caused only minor toxic effects and were therefore grouped in class II. The sediments from DM-L, EB-R, KO-R, LO-L, PO-L, and ST-L were assigned to toxicity class I, indicating the SCT battery did not detect any toxic effect.

DISCUSSION

In the present study, the sediment contact test battery used was able to detect toxicity in sediments differing in quality and quantity of chemical contamination. Fifteen of 21 sediments were identified as toxic by at least one test system within the SCT battery, whereas even the most sensitive single tests detected toxicity in eight sediment samples maximum (Table 4). Thus, the application of a heterogeneous test battery seems to be considerably more protective to sediment organisms than using single toxicity tests. This confirms the finding of other studies that used test batteries with a variety of organisms

Table 5. Test-specific effect categories

Category	Description	Plants (%)	Nematode growth (%)	Nematode reproduction (%)	Oligochaetes (%)	Fish embryos (%)	Bacteria (%)
1	No significant effect	<20	<25	<50	<25	<20	<60
2	Medium effect	20–40	25–50	50–80	25–50	20–40	60–80
3	Strong effect	>40	>50	>80	>50	>40	>80

Table 6. Algorithm for classification system

Toxicity class	Criterion	Code	Color code
I	All test results in category 1	No toxicity detectable	Blue
II	1 test result in category 2 and none in category 3	Slightly toxic	Green
III	≥2 test results in category 2 and none in category 3	Medium toxic	Yellow
IV	≥3 test results in category 2 or 1 test result in category 3	Toxic	Orange
V	≥3 test results show toxic effects (categories 2 and 3)	Highly toxic	Red

from different trophic and organizational levels [23,35–37]. Tuikka et al. [23], who investigated seven natural sediments using a test battery similar to that of the present study (SCTs with invertebrates, fish, and bacteria), demonstrated differences in the sensitivities of the species and highlighted the need for data on multiple species, when estimating the effect of sediment pollution on the benthic community. Though it is common to use a battery of assays with different benthic macroinvertebrates to assess the toxicity of sediments [7,10–12,38,39], the variety of organisms to choose from is often limited. The advantage of a test battery with diverse organisms, as in the present study, is the ability to consider a broader range of toxicity pathways, from the uptake route of contaminants into the organism to the final mode of action that triggers the toxic response.

Although several geochemical sediment properties (e.g., clay, Al, Li) influenced the various test organisms (e.g., nematodes, bacteria) to a certain extent, the toxic potential of organic chemicals (TEC-Q_{max}o) was the only variable that was significantly related to the response of organisms (Fig. 3; $p < 0.05$, Monte Carlo permutation test). The RDA revealed that the different test systems responded to sediments characterized by different types of pollution. Although the toxic potential of metals (TEC-Q_{max}m) did not significantly correlate with the effect pattern of the test battery, it was shown that the plants (inhibition of growth) and fish embryos (mortality) responded more to metal contamination (TEC-Q_{max}m; Fig. 3), whereas the nematodes (inhibition of growth and reproduction) and oligochaetes (inhibition of reproduction) seemed to be more affected by organic contamination (TEC-Q_{max}o; Fig. 3). However, statements on cause–effect relationships should be made with caution, as most highly contaminated samples showed metal and organic contamination.

Nevertheless, a previous study on the toxicity of contaminated soils also showed that *C. elegans* was more sensitive to organic (mainly polycyclic aromatic hydrocarbons) than to metal contamination [40]. A higher sensitivity to organic chemicals compared to metals could also partly be confirmed by studies on sediment spiked with single substances for *C. elegans* [41] and nematodes in general [42–44].

In the case of oligochaetes, it should be noted that only one sediment (HH-R) had a toxic effect on reproduction, and was therefore responsible for the correlation with TEC-Q_{max}o. The chemical analysis for HH-R, however, revealed not only a high content of organic substances, but also a high metal contamination. Therefore, metals were possibly also responsible for the toxic effects—metals were shown to be toxic for oligochaetes in other studies (e.g., [45]). From experiments within artificial and natural sediments spiked with mixtures of either organic substances or metals, *L. variegatus* appears to be similarly sensitive to both types of contamination [25].

The inhibition of growth of *M. aquaticum* was positively related with TEC-Q_{max}m. This became particularly clear in the case of the sediment from DÖ-R, which generally showed low contamination but was specifically polluted by chromium, which might have caused a high toxicity to *M. aquaticum*. (Table 2, Fig. 2, Table 4). This finding agrees with the results of previous studies that found a quite high sensitivity of *M. aquaticum* to chromium [46] or heavy metals in general (especially chromium and copper) [47].

Similar to the plant test, results of the fish embryo test correlated with the TEC-Q_{max}m, indicating that the heavy metals caused the toxicity to the fish embryos. Studies with spiked sediments showed no evidence for a specific sensitivity of fish embryos to heavy metals [25]. Perhaps in the native sediments, pore-water concentrations were higher for metals than for strongly particle-bound organic chemicals, and thus metals were more bioavailable for unhatched fish embryos, being only exposed to contaminants passing the chorion.

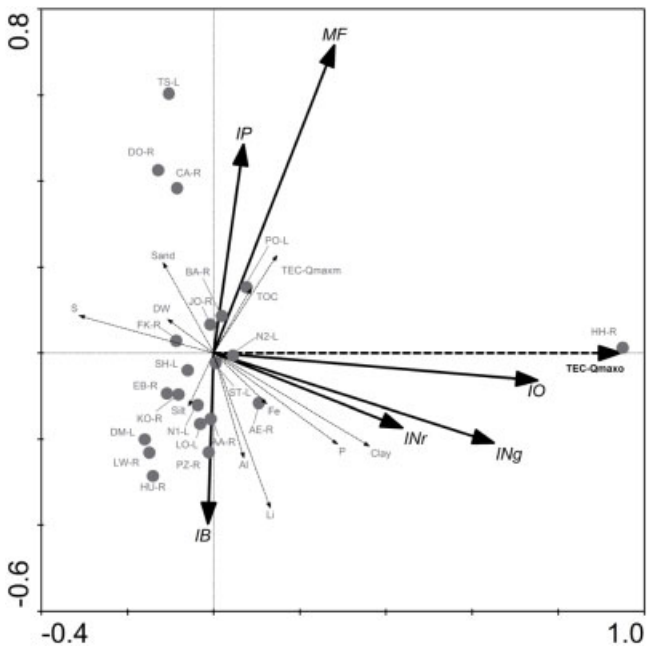

Fig. 3. Partial redundancy analysis of the sediment samples based on the results of the sediment contact tests and the sediment properties (geochemical and contaminant properties). Gray dots = sediment samples. Bold black arrows = sediment contact tests (IP = inhibition plant growth; INr = inhibition nematode reproduction; INg = inhibition nematode growth; IO = inhibition oligochaete reproduction; IB = inhibition bacterial enzyme activity; MF = mortality fish embryos). Dashed arrows = supplementary environmental variables (elements; sand; silt; clay; TOC = total organic carbon; P = phosphorous; S = sulfur; DW = dry weight; TEC-Qmaxm = maximum threshold effect concentration quotient based on metals). Bold dashed arrow = significant environmental variable from preliminary forward selection ($\alpha = 0.05$), with Ca and Mg excluded as covariables; TEC-Qmaxo = maximum threshold effect concentration quotient based on organic substances. Eigenvalues 1st axis = 0.144; 2nd axis = 0.236; sum of all eigenvalues = 0.621; sum of all canonical eigenvalues = 0.144; cumulative percentage of variance of data 1st and 2nd axis = 61.3%). See Figure 1 legend for code abbreviations of sediment samples.

The various SCTs could be ranked in terms of their power to detect toxic effects. The SCTs using nematodes and fish embryos were the most sensitive test systems; both detected eight sediment samples as toxic (category > 1), with two and six of them highly toxic (category 3) to nematodes and fish embryos, respectively. For the plant test, five sediments turned out to be toxic, one of them highly toxic. The SCTs with oligochaetes and bacteria turned out to be the least powerful test systems for detecting toxic effects, indicating toxicity only for one and two samples, respectively. However, some methodological issues might have biased the outcomes of these test systems. For the oligochaetes test, the toxicity threshold is quite low (25%), illustrating the robustness of the toxicity endpoint reproduction. However, the high number of stimulating effects observed suggests that reproduction of *L. variegatus* in this artificial sediment (OECD 218) might be limited. More samples might have been detected as toxic, if the performance of oligochaete reproduction had been better in the control sediment. One possible explanation might be the low nutrient content of the artificial sediment. Therefore, there is an obvious need to optimize the artificial control sediment, or the feeding regime, or both to improve the suitability of the SCT with *L. variegatus* for discriminating between contaminated field sediments. For the bacteria contact test with *A. globiformis*, a relatively high toxicity threshold (60%) was set, because of the large variation in enzyme activity in native sediments with low levels of anthropogenic contamination ([22]). Therefore, most of the measured effects were below the toxicity threshold and, thus not distinguishable from the influence of the geochemical sediment properties. These results are in contrast to findings from a study of approximately 250 sediments, in which an effect >60% was classified as "strong effect" and the threshold level was <25% effect level [35]. However, the RDA (Fig. 3), which also considered effects below the toxicity threshold, showed that effects on the bacteria were better related to natural sediment properties rather than to the toxic potential, which justifies the use of the high toxicity threshold. Probably the test system used in this project (modified as a test kit) [15] should be improved to consider the influence of these natural properties.

The multiple toxicity data derived from the test battery allowed a gradual toxicity classification of the sediments investigated (Tables 4 and 6). This integrated information on the various SCTs provides a reliable estimate of the toxicity of the sediment samples assessed. Six sediments were classified as nontoxic (class I), meaning that none of the SCTs used detected a toxic effect. Eight sediments were categorized as slightly toxic (class II), and seven sediments were classified as toxic (class IV) or highly toxic (class V). In a comparison of the toxicity classification with consensus-based SQGs [26], a good concordance was generally found (Fig. 4).

Severe toxicity (classes IV and V) only occurred at maximal PEC quotients (PEC-Q_{max}) > 1, which means that the predicted effect concentration was exceeded at least for one contaminant, and thus an effect was expected. In sediments with maximal TEC quotients (TEC-Q_{max}) < 1, meaning that no measured chemical exceeded the threshold effect concentration, 60% of the samples showed no toxicity (class I), and 40% showed low toxicity (class II), thus confirming the prediction of low toxicity below TEC-Q_{max}. However, in the transition zone between "no effects predicted" and "effects predicted" (TEC-Q_{max} > 1; PEC-Q_{max} < 1), one toxic effect occurred in the test battery in 60% of the samples. Moreover, MacDonald et al. [26] revealed that a mean PEC-Q < 0.5, correctly predicted

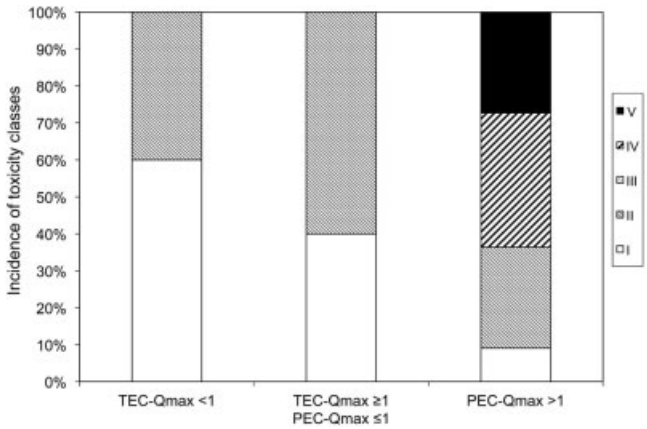

Fig. 4. Comparison of the toxicity classification based on the bioassay battery results (toxicity classes I–V) and the threshold effect concentration (TEC-) and probable effect concentration (PEC-) quotient classification (based on MacDonald et al. [26]). TEC-Qmax = maximum threshold effect concentration quotient; PEC-Qmax = maximum probable effect concentration quotient.

"no toxic effect" in 80% of the cases, when verifying the toxicity with whole sediment toxicity tests using freshwater invertebrates. By applying this threshold (mean PEC-Q < 0.5), a toxic effect was still detected by at least one SCT in 55% of the samples in the present study. Thus, particularly for sediments with low to medium contamination, the toxic potential might be underestimated when one is only using SQGs, which are not able to consider unknown toxicants that were not chemically analyzed, the actual bioavailability of a chemical in a sample, or mixture effects between different toxicants. These uncertainties might not be relevant in the case of sediments with a high contamination of known toxicants. In the case of low to medium contamination levels, effects of unknown chemicals or chemical interactions might provide a considerable proportion to the overall toxicity. Therefore, the use of an SCT battery to assess the toxicity of sediments is most advisable for sediments with low to medium toxic potential. However, well-standardized tests with robust toxicity thresholds, as used in the present study, are a prerequisite to reliably assess the toxicity of sediments.

CONCLUSIONS

The test battery, consisting of five SCTs—plants (*Myriophyllum aquaticum*), nematodes (*Caenorhabditis elegans*), oligochaetes (*Lumbriculus variegatus*), fish embryos (*Danio rerio*), bacteria (*Arthrobacter globiformis*)—representing various trophic levels and exposure routes, appeared to be a suitable tool to assess the toxicity of contaminated sediments, and thus to contribute to an ecotoxicological sediment assessment concept, such as the sediment quality triad. Most of the tested sediments with high toxic potential, according to sediment quality guidelines, were identified as toxic by the test battery, but not by single toxicity tests. This result confirmed the benefit of a test battery as an integrative tool. Within the battery, SCTs using nematodes and fish embryos were the most sensitive, whereas SCTs with oligochaetes and plants appeared to be robust, as reflected by their low toxicity thresholds.

Generally, a good concordance of the bioassay-based classification developed here with SQGs was shown. However, for sediments with low to medium toxic potential, the toxicity was underestimated in >50% of the cases by the SQG approach,

stressing the need for SCTs in sediment toxicity evaluations, as they assess all bioavailable toxic compounds, even unknown chemicals, considering all relevant interactions of toxicants, sediment properties, and organisms. Using standardized SCTs together with robust toxicity thresholds [22], an SCT battery can contribute a reliable line of evidence in weight-of-evidence approaches [4,48,49].

SUPPLEMENTAL DATA

Table S1. Geochemical properties of investigated sediments.

Table S2. Measured concentrations of dichlorodiphenyltrichloroethane and its derivates, organotin compounds, and hexachlorohexan (α-, β-, γ-HCH) (164 KB DOC).

Acknowledgement—The present study was funded by the German Federal Ministry of Education and Research (grants 02WU0598 to 02WU0604). We acknowledge the valuable input of H–J. Pluta (German Federal Environment Agency) and U. Förstner (Technical University Hamburg-Harburg), who were part of the project as members of the scientific advisory board. We also gratefully acknowledge the gifts of *Caenorhabditis elegans* and *Escherichia coli* from the Caenorhabditis Genetic Center (T. Stiernagle), which is funded by the National Center for Research Resources, U.S. National Institutes of Health, of quartz sand from Quarzwerke, and of sphagnum peat from Klassmann-Deilmann.

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