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of Ministers

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Preface

This report describes the results of a two-year project called “Evaluation and selection of test methods for the assessment of contaminated sediments in the Baltic Sea” (CONTEST), funded by the Marine Group (HAV) under the Nordic Council of Ministers in 2015–2016. Additional funding for the execution of the project was received from the Finnish Ministry of the Environment.

There is a growing need to establish reliable, science-based methods to assess and classify the degree of contamination in marine sediments affected by human activities. This is necessary for decision-making concerning regulatory actions towards the reduction of pollution from different sources, removal and relocation of contaminated materials, and on-site remediation measures and their follow-up. The aim of the CONTEST project was to test and evaluate suitable biological methods for the quantitative and qualitative assessment of toxicity of anthropogenically contaminated sediments in the Baltic Sea marine environment. Results of toxicity testing of different dilutions of a toxic harbour sediment using a battery of different biotest organisms and biological endpoints are presented here. However, the main outcome of the work carried out within the project was not only the comparison of the sensitivity of the different biotests but also an expert judgement based analysis of various other selection criteria such as ecological relevance, practicality, and costs. This analysis is expected to assist in objective and optimal selection of biotests for each specific case.

Summary

Sediments contaminated by human activities usually contain a mixture of chemicals that produce unforeseen combined toxic effects in organisms. Thus, traditional risk assessments based on the concentrations of chemicals are unlikely to produce realistic data on toxicity. In the CONTEST project, 19 biotests were evaluated using a contaminated model sediment from the Baltic Sea. Most of the biotests applied showed concentration-dependent toxicity related to the degree of chemical pollution measured in the test sediment with some variability in the sensitivity of the test organism and the endpoint. The different biotests were analysed according to specially designed assessment criteria, and the results are foreseen to be useful for end-user groups including environmental authorities, private companies and industries, environmental laboratories, consultants, and the scientific community.

1. Introduction

Many marine coastal areas are heavily affected by various human activities leading to serious chemical pollution. As the use of the coastline and building of diverse offshore constructions keep on accelerating, the pollution status of these areas is likely to deteriorate in a global scale. Contaminated sediments cannot support the proper functioning and diversity of communities of marine organisms, and are also an obvious threat to the health of ecosystems of adjacent areas due to the potential release and transport of toxic substances. Thus, for effective and safe management of contaminated sediments there is a growing need to establish dependable, science-based methods to assess and classify their degree of contamination.

A reliable testing of the toxicity of allegedly contaminated sediments is essential for several reasons, including:

- assessment of the degree of contamination of impacted marine coastal areas (e.g., for marine spatial planning)
- deciding if deposition (of dredged materials) to sea is possible
- monitoring/testing the development of sediment quality in an acutely/accidentally polluted site (including the effectiveness of remediation actions)
- detecting chronic pollution (e.g., diffuse pollution from land, leaking municipal or industrial waste water, harbours)
- periodical checking if routine chemical monitoring of contamination is adequate (i.e., change in toxicity may require a new chemical screening).

Toxicity assessments are often based on the measurement of selected hazardous chemicals in sediments and toxicity threshold values established for single substances using standard laboratory species, with agreed safety factors. Traditional sediment toxicity assessments are based on measurements of concentrations of a very limited number of selected chemicals. This procedure is problematic since (a) contaminated

sediments contain a complex mixture of chemicals that produce unknown combined toxic effects on organisms, (b) only the bioavailable fraction of each chemical poses a risk, which is not correctly assessed by measuring total concentrations, and (c) in the most severe contamination cases there are multiple interacting stressors such as salinity and anoxia/hypoxia. However, due to the enormous variety of chemicals currently present in the environment and their interactions (mixture toxicity), biological testing must be considered an essential component of reliable and realistic toxicity assessment protocols. Various types of sensitive biotests have been developed to detect contaminant effects on test organisms but so far they have seldom been used to their full potential to obtain reliable evaluations of sediment toxicity. Thus, there is an urgent need to develop guidelines to combine chemical and biological measurements in these assessments.

During the past decades, a relatively large number of biotests have been developed to detect effects of single contaminants or mixtures on various test organisms. The common methods in current use include, e.g., tests with bacteria, algae, invertebrates, fish, and different life-stages of these organisms. In testing of sediment toxicity, the test matrix can be the whole sediment, sediment extracts, or elutriates. The traditional endpoints of effects are survival, behaviour, development, and growth, but recently also the use of molecular markers has increased.

Despite the obvious value of biotests in sediment toxicity assessment, their consistent use in different countries and sea areas is still badly lacking. This is due to various reasons, including disputes on their suitability and relevance in different environmental conditions, different sensitivities of species or endpoints and the following disagreements on the interpretation and legitimacy of the data, lack of standardised protocols for ecologically most relevant species in different areas, presumed high cost of biotesting, and others. In many cases the difficulty in choosing between the available biotests has led them not being used at all.

The CONTEST project set out to tackle the problems mentioned above specifically in regard to the application of biotests in the Baltic Sea region. The main target was to generate new data and knowledge on the applicability of a large number of biotests relevant for the Baltic Sea by testing and evaluating their suitability in quantitative and qualitative assessment of toxicity of anthropogenically contaminated sediments. This is in line with the generally accepted goals concerning the development of knowledge of biological and ecological effects of contaminants in water and sediment in the Baltic Sea, and supports the requests of the Baltic Sea Action Plan (BSAP) concerning the

improvement of the ecological status of the Baltic Sea. The project outputs were aimed at supporting development of new national and international legislation concerning the management of contaminated sediments.

In the project, a battery of biotest methods currently in use in toxicity assessments were applied using a contaminated Baltic Sea harbour sediment as a model matrix. Various biological endpoints (survival, reproduction, behaviour, growth, stress responses) were measured in selected species, most of them ecologically relevant for the Baltic Sea. In addition to the data obtained on the sensitivity of each biotest, an objective evaluation of each method was carried out using expert judgment over a set of specifically designed assessment criteria; the outcome of the evaluation is expected to assist in the selection of biotests for different situations and needs.

2. Background

Biotests: the general concept

Ecotoxicological testing methods using selected species or cell lines grown in the laboratory over several generations are called biotests (Mothersill and Austin, 2003). Under carefully standardised conditions, the test systems are exposed for a defined period of time to the test medium, which can be different concentrations or dilutions of single chemicals, mixtures of chemicals, water collected from a field study site, whole sediments, or sediment pore water extracts and elutriates. The most relevant international standardized protocols for biotests include guidelines for testing of chemicals of the Organization for Economic Cooperation and Development (OECD) and those provided by the International Organization for Standardization (ISO).

At its simplest, the measured test parameter, in ecotoxicology often called “endpoint”, is the death of the organism used. The concentration or dilution required to kill 50% of the test organisms during a fixed time, e.g., 24 or 96 h in short-term acute toxicity tests and up to several months in long-term chronic exposure tests, is referred to as “lethal concentration” (LC_{50}). This is similar to the parameter “lethal dose” (LD_{50}) used in the testing of chemicals by oral dosing or injection (e.g., using mice and rats). However, today’s trend in biotesting is to use more sensitive endpoints than mortality, and the so-called sublethal effects are now more often measured and expressed using the term “effective concentration” (EC). Sublethal endpoints include changes in behaviour, growth, reproduction, and also biomarkers representing various biological effect levels from gene expression to population effects. Usually, based on a dose-response curve, an EC_{50} value is calculated expressing the concentration giving a 50% effect in comparison to a control (zero concentration). Any other EC value such as EC_{10} or EC_{20} may also be calculated. Besides EC values, also other parameters in common use in toxicity evaluation can be obtained, e.g., “predicted effect concentration” (PEC), “predicted no-effect concentration” (PNEC), “lowest observed effect concentration” (LOEC), and “no observed effect concentration” (NOEC), as well as their ratios.

Biotests are commonly used to monitor the toxicity of municipal and industrial wastewaters. Water samples taken from the effluent or the receiving water body are subjected to standardized toxicity tests that reveal negative effects at different biological levels such as muta-, geno-, and cytotoxicity, reduced growth, impaired reproduction, disturbances in behaviour, and mortality. The groups of organisms used in common standardised biotests for environmental samples currently include microbes, algae, invertebrates, and small fish. The majority of the test protocols have been standardised for freshwater conditions but also marine biotests are available. Due to the large variability in different test organisms, endpoints and experimental requirements the comparison of toxicity of a sample using different methods is often far from straightforward. Thus, to enable some degree of comparability, specific assessment criteria and thresholds must be developed for each test.

Sediment biotests

Sediment biotests are useful, e.g., in the evaluation of the degree of contamination in harbours and outside industrial areas, along marine shipping routes, and dredged materials. Sediment toxicity can be assessed by exposing organisms to a whole sediment sample, sediment suspensions, aqueous elutriates or pore water samples, or organic solvent extracts obtained in different ways (Nendza, 2002). The sample preparation method can significantly affect the chemicals ultimately present in the test media used in the biotests. Simple mixing with water without any specific physical or chemical treatment such as addition of organic chemicals will inherently result in different (lower) extraction efficiency of the substances present in the sediment matrix. For example, elements that are tightly bound in the matrix are not directly bioavailable to organisms and thus do not account for any toxicity. Thus, extraction using organic solvents may lead to an overestimation of the real toxicity of a sediment sample. Another aspect to consider is that the toxicity of a sample is seldom due to one chemical compound alone.

In sediment toxicity biotesting, benthic invertebrates such as crustacean amphipods, oligo- and polychaete worms, gastropods, and larvae or embryos of bivalves (e.g., oysters and mussels) and echinoderms (e.g., sea urchins), are widely used. Nendza (2002) compiled marine biotests for sediments and dredged materials, and in addition to the groups above it includes also species of rotifers, insects, fish and

amphibians. As can be seen from the above, the list of species applied for biotesting covers almost the whole range of phyla.

In the Baltic Sea, biotesting of contaminated sediments has not been widely in use, partly due to the lack of methods suitable for local species. However, extensive research on the use of the benthic amphipod *Monoporeia affinis*, a key species in the area, has been carried out. Effects of contaminants on reproduction of *M. affinis* have been studied using the organism for biotesting of sediments and also in field studies in contaminated areas (Sundelin and Eriksson, 1998; Sundelin et al., 2008 a, b; Reutgard et al., 2012; Löf et al., 2014). The method is recommended by International Council for the Exploration of the Seas (ICES) to detect biological effects of contaminated sediments in the field (Sundelin et al., 2008b) and also included in the Swedish marine monitoring programme since 1994.

Since *M. affinis* reproduces only once in its lifetime (in winter), its practicality as a biotest species for reproductive disorders is limited. However, the specific value of these studies is that since all amphipods have a similar reproduction and embryo development, the method described for *M. affinis* can be applied to other Baltic Sea species that have a more continuous reproductive mode such as *Corophium volutator* and *Gmelinoides fasciatus* (Fig. 1). Several species of amphipods including *Pontogammarus robustoides*, *Gammarus tigrinus*, *Bathyporeia pilosa*, and *G. fasciatus* are important components of the zoobenthos in coastal areas of the Baltic Sea (e.g., Gulf of Riga, Gulf of Finland, Curonian and Vistula Lagoons). Accordingly, these species have recently been used in sediment toxicity biotesting in the Baltic Sea (Berezina et al., 2017; Strode et al., 2017). Most of the common amphipod test species (e.g., *Hyalella azteca* and *Ampelisca* sp.) as well as echinoderms do not occur in most parts of the Baltic Sea.

The macroalgae *Ceramium tenuicorne*, indigenous for the Baltic Sea, was used with good results for the testing of harbour sediments in the Stockholm area (Eklund et al., 2010). More recently, the growth inhibition test using *C. tenuicorne* was used for toxicity assessments of sediments collected from small town harbours and natural harbours from the west coast of Sweden (Eklund et al., 2016). The test has also shown an increasing degree of toxicity along a ship lane gradient towards the Stockholm city centre (Eklund et al., 2017).

Using widespread zooplankton species, biotests applying the epibenthic harpacticoid copepod *Nitocra spinipes* have been developed (Perez-Landa and Simpson, 2011), also in the Baltic Sea (Ribbenstedt et al., 2017). This organism has been extensively used to study the effects of a wide range of environmental contaminants

using physiological, developmental and reproductive endpoints as well as responses in population growth and genetics (Ribbenstedt et al., 2017). Among snails, the freshwater gastropod *Potamopyrgus antipodarum*, which is a nonindigenous species in the Baltic Sea, has been shown to be a useful biotest species especially with regard to endocrine disruption (Duft et al., 2003)

In addition to the use of whole organisms, molecular techniques for ecotoxicological biotesting have been developed. In many of these applications the target cells used, usually microorganisms such as bacteria and yeasts, have been genetically tailored to produce light in a dose-responsive way when exposed to chemicals. Among these methods is the detection of dioxins and/or dioxin-like PCBs, (anti)oestrogen compounds and (anti)androgen compounds by using CALUX (Chemical Activated LUciferase gene eXpression) assays (Murk et al., 1996). Other molecular approaches include the application of multigene expression profiles (microarrays, at best containing the whole genome of the test species) and proteomics by using the induction profiles of proteins synthesised (e.g., Kosmehl et al., 2012). However, the application of these methods is so far relatively rare in routine testing but this is expected to increase.

Figure 1: A male specimen of the benthic gammarid amphipod *Gmelinoidea fasciatus*, a non-indigenous species in the Baltic Sea currently inhabiting low-salinity coastal regions of the eastern Gulf of Finland



Remarks on the use of biotests

Exposing standard test organisms under highly-controlled experimental conditions greatly increases reproducibility and reduces internal variability of the measurements but poorly reflects the situation that prevails under natural conditions where various abiotic and biotic factors also contribute to toxicity. Interactions between the chemical compounds present in the sample and their bioavailability under varying physico-chemical conditions (salinity, temperature, light, pH, oxygen content, redox state, etc.), which are carefully controlled in the laboratory, significantly modify the toxicity of water or sediments under the specific field conditions that the resident “wild” biota is facing. Interactions can occur also at the biological level, i.e., the organisms themselves respond to contaminants differently depending on the environmental conditions. To increase ecological relevance it is also important to develop biotests using local species, preferably of key importance to the ecosystem.

Biotests at their best are sensitive to detect different types of toxic effects caused by chemicals and their mixtures and therefore have great value as screening tools for environmental samples. To further develop this, protocols such as Toxicity Identification and Evaluation (TIE) and Effect-Directed Analysis (EDA) have been designed to identify the actual compounds or sample fractions causing the observed toxic effects (U.S. EPA, 2007; Brack et al., 2007). The development of these types of methods to link, e.g., sediment toxicity to its specific causes is of great benefit for practical environmental management.

Even if it would be preferable to carry out the testings directly at the contaminated sites this is not practically feasible. Sediments are usually brought to the laboratory and the testing is performed under controlled conditions, which may be more or less similar to the natural, on-site situation. In biotesting, an important issue is that the test is performed at optimal conditions in regard to the test species in question; this is to ensure that the observed effect is caused by the test sediment and not by any other factor, e.g., poor food conditions or too low or high temperature.

3. Materials and methods

3.1 The study design

The basic concept of the project was to use a single sediment sample from a known contaminated site (Copenhagen harbour, Denmark) to be tested in the partner laboratories using a variety of available biotest methods. A reference sediment was collected from an open sea area in the middle of the Bothnian Sea (northern Baltic Sea). Both sediments were analysed for selected chemical contaminants consisting of organotins, polycyclic aromatic hydrocarbons (PAH), and trace metals.

A preliminary experiment using the amphipod *Corophium volutator* 96-h survival test was carried out to examine the toxicity of the contaminated harbour sediment. As its toxicity turned out to be extremely high, several dilutions using the reference sediment were prepared accordingly for the actual experiments carried out in the different laboratories. The diluted test sediments were distributed for each project partner for biotesting using the specific methods available in each laboratory.

Nineteen biotests/endpoints with different test species, most of them ecologically relevant for the Baltic Sea, were applied (Tables 1).

Figure 2: A simple whole-sediment biotest set-up using benthic amphipods. Constant temperature room and light aeration of the water in the jars containing a 3 cm layer of the tested sediments



Most of the biotests used here were performed according to the protocols and guidelines published in numerous previous publications. Therefore, due to the large number of tests used, detailed information on the tests is not given here but is easily available in the literature. Basic information about the some of the biotests used is found in the Appendix part ("CONTEST Biotest Fact Sheets").

3.2 Data evaluation

Evaluation of the biotests for selection purposes was based on a set of assessment criteria (AC) applied for each biotest/endpoint. The seven AC formulated for the evaluation were (i) sensitivity (weighting factor [WF]=5), (ii) multi-endpoint organisms/assays (WF=1), (iii) practicality in collection and maintenance of test

organisms (WF=2), (iv) Quality Assurance (WF=3), (v) Baltic Sea ecological relevance (WF=4), (vi) practicality in conducting the assay (WF=5), and (vii), costs (WF=3). Each of the AC consisted of several subcriteria that were ranked scored at 1, 3 or 5, and in some cases only at 1 and 5. The average value of all subcriteria within each AC formed the final AC values.

Table 1: Biotests/endpoints evaluated in the CONTEST project

Test species	Endpoint	Duration	Matrix	Biotest code
<i>Vibrio fischerii</i> (bacterium)	Luminescence (viability)	30 min	SE	VIBRIO_LUMI
Salmonella (UMU genotoxicity)	Induction of SOS response	4 h	SE, EXTR	UMU_GENO
<i>Pseudokirchneriella subcapitata</i> * (green microalga)	Growth rate	72 h	SE	PSEUDO_GR
<i>Ceramium tenuicorne</i> (red macroalga)	Growth rate	7 d	SE	CERAMIUM_GR
<i>Daphnia magna</i> (water flea)	Immobilization	48 h	SE	DAPHNIA_IM
<i>Nitocra spinipes</i> (harpacticoid copepod)	Survival rate	96 h	SE	NITOCRA_SURV
<i>Potamopyrgus antipodarum</i> (snail)	Survival rate	28 d	WS	POTAMO_SURV
	Reproductive output	28 d	SE, WS	POTAMO_REPRO
<i>Nassarius reticulata</i> (snail)	Survival rate	28 d	WS	NASSA_SURV
	Reburial in sediment	24 h	WS	NASSA_REBUR
	Imposex in females	28 d	WS	NASSA_IMPO
<i>Hyalella azteca</i> (amphipod)	Survival rate	10 d	WS	HYALELLA_SURV
<i>Gmelinoides fasciatus</i> (amphipod)	Survival rate	10 d	WS	GMEL_SURV
	Malformation frequency	28 d	WS	GMEL_MALFORM
	Fecundity	28 d	WS	GMEL_FECU
<i>Corophium volutator</i> (amphipod)	Survival rate	10 d	WS	CORO_SURV
	Malformation frequency	28 d	WS	CORO_MALFORM
	Fecundity	28 d	WS	CORO_FECU
	Avoidance	24 h	WS	CORO_AVOID

Note: SE=sediment elutriate; EXTR = extract; WS=whole sediment.

**Pseudokirchneriella subcapitata* is currently regarded as a synonym of *Raphidocelis subcapitata*. *R. subcapitata* is currently the accepted name for the type species (Krienitz et al., 2011) and therefore it is the most appropriate one for this species. However, considering that in ecotoxicology the use of the name *P. subcapitata* is more general than *R. subcapitata*, we chose to use it here.

3.3 Remarks and considerations for the assessment criteria

Sensitivity. One of the most important considerations is the sensitivity of the endpoint in environmentally relevant concentrations. Furthermore, the ecological relevance of the endpoint is crucial, meaning that if the parameter is reversible, i.e., the observed effect returns to the normal level soon after the exposure terminates, or it indicates a permanent damage on the tested organisms, e.g., in terms of failure in reproduction or death.

Multi-endpoint organisms/assays. One useful characteristic of a biotest is the possibility to measure several endpoints from a single test set-up, e.g., a behavioural effect together with fecundity or embryo damage, or molecular biomarkers in connection with higher biological level indicators (see also subchapter 4.4.).

Practicality in collection and maintenance of test organisms. Species that can be cultivated in the laboratory are usually available the year around, which means that the test can be performed whenever needed. These test organisms are usually genetically more uniform and also in more comparable condition compared to field collected organisms since they are cultivated under optimal conditions for the respective species. Species collected from the wild may vary in availability in different times of the year. Also, their reproductive and physiological status may vary, which may set limitations to their use, e.g., in reproduction based tests in case the test species breeds only once a year.

On the practical side, the easiness of handling and maintenance in the laboratory is a valuable asset concerning both laboratory and wild strains. Some species can be used for testing of different kinds of matrices, i.e., water, pore-water elutriates or whole sediments while some are more restricted to one type of exposure type. For the laboratory-reared organisms an important question is if the organism has to be kept as a continuous culture or it can be stored as frozen (e.g., bacteria) or dry eggs (e.g., some crustaceans), thus reducing a considerable amount of work. Regarding the wild-caught organisms a corresponding criterion concerns the easiness of field collection.

Table 2: The biotest Assessment Criteria scoring sheet used in the CONTEST project

Endpoint name		L	M	H	Scoring guide
		1	3	5	
1. Sensitivity	Endpoint sensitivity in environmentally relevant concentrations				5=effects detected in low concentrations (<1/128), 3= in medium (1/64), 1=only in high (1/32) concentrations
	Ecological relevance of the endpoint (reversible vs. damage)				5=mortality and reproduction, 3=behaviour and growth, 1=biomarkers
	SCORE	0,0			
2. Multi-endpoint organisms/ assays	Several endpoints can be obtained from a single test				1=difficult, 3=possible, 5=easy
	SCORE	0,0			
3a. Practicality (cultured test organisms)	Consistency of test organism culture				5=same quality all year, 3=intermediate, 1=quality affected by seasonality
	Easiness of handling and maintenance in the lab				5=easy, 3=intermediate, 1=more demanding
	Selection of test matrix				5=works equally well on sediment and elutriate, 3=sediment only, 1=pore water/elutriate only
	Continuous culture needed				1= yes, culture according to standard protocols assumed, 3=intermediate, 5=no, culture can be frozen for later use (e.g., cells, bacteria)
3b. Practicality (test organisms from natural population)	Availability throughout the year				5=always present and abundant, 3=relatively abundant, 1=highly seasonal availability
	Easiness of sampling				5=sampling sites easy to reach, no special equipment needed; 3=intermediate, 1=difficult to reach/find/identify the species
	Selection of test matrix				5=works equally well on sediment and elutriate, 3=sediment only, 1=test works only on pore water/elutriate
	Easiness of handling and maintenance in the lab				5=easy, 3=intermediate, 1=more demanding
	SCORE	0,0			
4. Quality Assurance	Standard protocols/ operating procedures available				5=international guidelines available, 3=national guidelines or scientific papers available, 1=project guidelines only
	Intercalibration framework				5=within last 5 years, 3=some activities, 1=none
	Availability of baseline information(dose-response, mode of action)				5=abundant, 3=available, 1=limited
	SCORE	0,0			

Endpoint name					
Assessment Criteria	Subcriteria	L	M	H	Scoring guide
5. Baltic Sea ecological relevance	Baltic Sea relevant species				5=important species, 3=present but less important, 1=not present in the area
	Relevant exposure conditions for the test organisms				5=natural sediment dwellers (exposed to Baltic Sea conditions), 3=pelagic species, 1="artificial" test systems
	SCORE	0,0			
6. Practicality (conducting the assay)	Amount of special equipment and facilities needed	5	3	1	5=normal lab (room temperature, basic microscope etc.), 3=climate controlled room, 1=climate control and special equipment needed
	Amount of special skills and experience needed				5=no previous experience, 3=relatively quickly learned, 1=training period needed
	Amount of test material				5=tube, 3=jar, 1=aquarium
	Exposure time				5= <96 h, 3= <2 weeks, 1=4 weeks or more
	Need for test organisms to be analysed immediately				5=samples can be stored for later analysis, 1=samples need to be analyzed immediately after exposure
	Amount of lab space needed				1=entire room, 3=intermediate, 5=1 m of table space
	Dependence of season				5=not dependent, 1=heavily dependent on season
	SCORE	0,0			
7. Costs	Equipment and materials costs				5=low, 3=intermediate, 1=high
	Salaries, personnel costs				5=up to 2 workdays, 3=less than a week, 1=more than a week
	Cost of sampling for test organisms				5=only the salary of the sampler, 3=more technical staff needed, 1=cost for sampling in the open-sea and special equipment
	Cost of culture and maintenance of test organisms				5=less than one workday per week, 3=three days per week, 1=needs constant (every day) monitoring
	SCORE	0,0			
	TOTAL SCORE	0,0			

Note: L=low, M=medium, H=high

Quality Assurance (QA). Standard protocols and/or operation procedures (e.g., ISO and OECD) are available for a number of biotests while for many these are lacking, complicating the comparability of the tests used in different laboratories. Related to the above, some commonly used biotests have established intercalibration frameworks. Finally, for some of the newly-developed biotests there is obviously a lack of sufficient information on the baseline levels and mode of action of the observed toxic response.

Baltic Sea ecological relevance. Since the main goal should be to protect the local environment and ecosystem it would be optimal to use, as much as possible, species that are natural inhabitants of the region. In case of native species the exposure conditions prevailing in the laboratory should correspond to their physiological ranges, and the species preferably used in their typical niche in the system, e.g., a local sediment-dweller for whole-sediment biotests is favoured over a pelagic species or “artificial” test systems (e.g., bacteria or cell lines).

Practicality in conducting the assay. The requirements of special equipment and facilities (e.g., temperature-controlled rooms), special skills and experience as well as amount of materials needed to carry out the biotests differ greatly between the methods. Some chronic tests require a long exposure time of up to several weeks, often related to the type of damage monitored, while some can be accomplished in hours. Practical benefits include the possibility to store the samples for endpoint analyses at a suitable time, small amount of bench space needed (test tubes vs. aquaria), and also the independence of the time of the year when the testing is performed.

Costs. High costs of performing a specific biotest is sometimes considered a limiting factor for its use. Equipment and material costs, and the cost for personnel carrying out the tests as well as those related to sampling of test organisms from the wild, or, if laboratory strains are used, culturing and maintenance of test organisms can be highly variable depending on the biotest method.

4. Results

4.1 Chemical composition of the test sediment

Chemical analysis of the contaminated sediment confirmed the presence of high concentrations of the measured hazardous substances (Table 3). The reference sediment showed typical low background concentrations observed in the Baltic Sea outside hot-spot areas. As a rough chemical proxy, the highest exposure concentrations (1:32 dilution of the field-collected sediment) were the following: total organotins 2868 $\mu\text{g Sn kg dw}^{-1}$, total 16 PAHs 6064 $\mu\text{g kg dw}^{-1}$, and 352, 115 and 159 mg dw^{-1} of Cu, Pb and Zn, respectively.

4.2 Biotest results

A part of the biotest results are presented in the "CONTEST Biotest Fact Sheet" collection annexed to this report. These contains basic information on some of the methods, their sensitivity in the exposure experiment, and results of the evaluation (AC values).

As expected, the biotests applied showed some variability in their sensitivity to the exposure concentrations. Most of the tests applied showed concentration-dependent effects on the test organisms. However, some common biotests such as *Vibrio fischerii* bioluminescence, UMU genotoxicity, *P. subcapitata* growth, and *D. magna* immobility were not sensitive to the sediment-water extractions. With regard to these biotests the results might have been quite different if some other extraction technique (e.g., solvent extraction) had been used, but in this case the test media would have contained compounds and concentrations that under natural conditions would not readily bioavailable from the sediments to the organisms. The use of different extraction methods in sediment biotests has been debated extensively but it is generally accepted that for ecologically relevant test results one should focus on the bioavailable fraction of the toxicants. However, the issue becomes more complicated in nature where a

number of physico-chemical environmental factors such as redox conditions, hypoxia, salinity and temperature modify the bioavailability of compounds and produce different responses than those recorded under standard laboratory conditions.

Table 3: Concentrations of selected contaminants in different dilutions of the test sediment

Compound	Unit	R	1:128 R	1:64 R	1:32 R
TBT	$\mu\text{g Sn kg dw}^{-1}$	<1	508	1,016	2,030
Sum organotins		<2	717	1,432	2,862
DBT		<1	128	256	512
MBT		<1	54	108	217
Cu	mg kg dw^{-1}	17.1	101	185	352
Pb		25.8	48	70	115
Zn		107.2	120	133	159
TPhT	$\mu\text{g Sn kg dw}^{-1}$	<3	8.5	17	34
DPhT		<3	9.5	19	38
MPhT		<3	8	16	32
Naphthalene	$\mu\text{g kg}^{-1}$	<1	4.8	9.6	19.3
Acenaphthylene		0.2	3.6	7.1	14.3
Acenaphthene		<0.98	43.8	87.65	175.3
Fluorene		<0.64	20.8	41.6	83.2
Phenanthrene		<4.72	116.8	233.6	467.3
Anthracene		0.5	19.6	39.1	78.1
Fluoranthene		7.6	269.1	530.6	1,053.6
Pyrene		4.9	214.6	424.25	843.6
Benzo (a) anthracene		6.0	137.5	269.1	532.2
Chrysene		11.2	106.4	201.7	392.2
Benzo (b) fluoranthene		22.0	186.5	351.1	680.2
Benzo (k) fluoranthene		5.0	64.2	123.5	242.0
Benzo (a) pyrene		4.7	165.2	325.6	646.6
Indeno (1,2,3-cd) pyrene		7.9	109.4	211	414.1
Benzo (ghi) perylene		9.7	97.3	184.8	360.0
Dibenzo (a,h) anthracene		0.9	16.2	31.4	61.9
Cd	mg kg dw^{-1}	0.6	0.47	0.34	0.09
Al		27,420	21,287	15,155	2891
Li		21.9	16.75	11.6	1.3
DW content	%	58.7	64.3	69.95	81.2
LOI		2.4	1.975	1.55	0.7
TOC		0.63	0.535	0.44	0.25

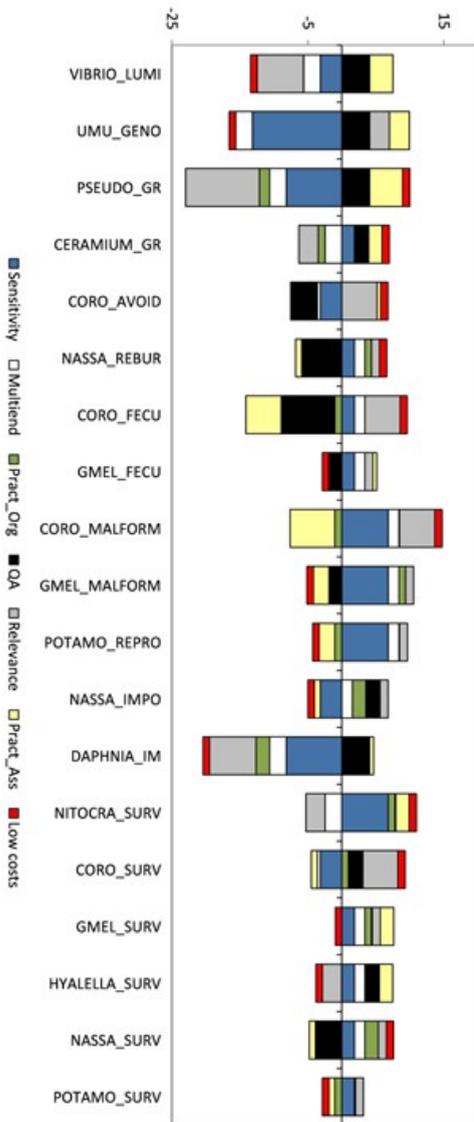
Note: NB! Only the 1:32 dilution and reference sediment (R) concentrations were measured, others (1:64) and 1:128 were obtained by calculation. TBT – tributyltin; DBT – dibutyltin; MBT – monobutyltin, TPhT – triphenyltin, DPhT – diphenyltin, MPhT – monophenyltin, Sum organotins – butyltins+phenyltins; Cu – copper; Pb – lead; Zn – zinc; Cd – cadmium; Al – aluminum; Li – lithium; DW – dry weight; LOI – Loss-on-ignition; TOC – total organic carbon.

In this study, a general observation was that most of the biotests using whole-sediment exposures produced responses at a surprisingly similar concentration range despite the fact that the organisms varied from plants to crustaceans and molluscs. This indicates that the choice of organism might not be that crucial selection criteria for a sediment biotest. Regarding the endpoints used, increased mortality occurred, as expected, at higher concentrations compared to the various sublethal parameters such as behaviour or reproductive disorders, the latter endpoints being therefore considerably more sensitive toxicity indicators.

4.3 Evaluation of the biotest methods

Evaluation of the biotest methods using the specific AC is presented in Fig. 3. From the figure it is possible to distinguish biotests that have better scores in characteristics required and preferred by each user. In addition, some of the tests fall on average clearly below the average level (zero line) in regard to many of the ACs and can thus be regarded as suboptimal in general. However, if some specific characteristic is preferred over some other, one may choose a test showing a high score in this category. As an example, the *V. fischerii* bioluminescence, *P. subcapitata* growth, and *D. magna* immobilisation tests score poorly with regard to sensitivity and ecological relevance but do better than average for QA and practicality of the assessment procedure. As another example, the amphipod tests (*C. volutator* and *G. fasciatus*) using malformations as endpoints are more sensitive than most others but score below average in regard to practicality of the assessment procedure. Finally, it is interesting to note that, according to the AC and information used this evaluation, the costs of performing the biotests does not seem to vary much.

Figure 1: Evaluation of the biotest methods using the specific assessment criteria



Note: The zero line represents the average value for each biotest, and positive and negative values higher and lower scores for each of the seven components of the total score, respectively. See Table 1 and Chapter 4 for details on the biotests (with abbreviations) and assessment criteria.

4.4 A special study: coupled use of embryo aberrations and biochemical biomarkers in toxicity testing

In addition to the application of single endpoints measured in a selected test organism, a special study using a combination of endpoints representing different biological effect levels was carried out during the project. "Early warning" toxic effects recorded using so-called biomarkers are often applied to detect exposure and effects of hazardous substances. Despite their usefulness in detection of deleterious effects, biomarker responses are in many cases reversible and often difficult to link directly with negative effects at higher level of biological organization such as growth, fitness and reproduction that have true significance for the wellbeing and development of populations and communities. Empirically established linkages between "early warning" effects with damage, e.g., on reproduction, can thus enable a reliable prognostic of higher-level effects using these relatively simple and high-throughput methods in toxicity assessments.

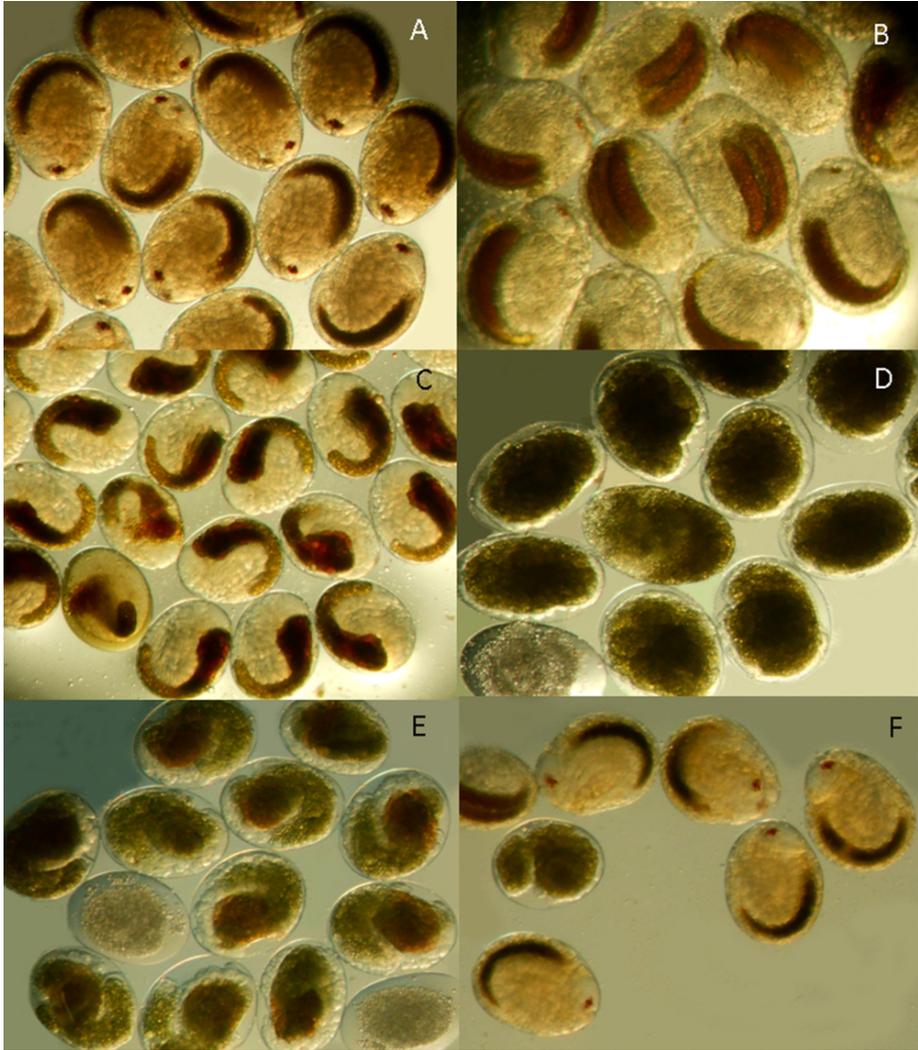
In the current study, the amphipod *G. fasciatus* was exposed to the different dilutions of the test sediment (described in the "Material and methods" chapter) and samples were taken for the analysis of biochemical biomarkers and reproductive disorders. Biomarkers representing effects on the antioxidant defence system (ADS) including glutathione S-transferase activity (GST), catalase activity (CAT) and lipid peroxidation level (LPO) were determined from females after an exposure time of 28 d. From the same batches of exposed females, the following types of aberrations in eggs and embryos were measured: (1) embryos with aberrant cleavages in the early embryogenesis stage; (2) impaired malformed eyespot; (3) enlarged embryos with no other visible damage; (4) malformed midgut; (5) membrane dysfunction; (6) dead eggs; and (7) undifferentiated embryos (Fig. 4). These types of aberrations in embryos have been described in detail in amphipod species from the Baltic Sea by Sundelin et al. (2008 b).

The details of the methodology used and results of the study are found in Berezina et al. (submitted manuscript). Briefly, after a 28 d exposure, aberrations in embryos were observed in >60% of individuals in the 1:64 and 1:128 sediment dilutions, significantly more compared to the group exposed to the reference sediment (<5%). In the 1:256 dilution the total number of aberrations was lower (31%) compared to the two other dilutions while also the frequency of malformed embryos remained at an elevated level (>11%), indicating that effects occur already at this exposure level. In comparison, a low number of malformed embryos per female were observed in the reference (< 5%)

and the 1:1024 dilution (6%). Regarding the ADS biomarkers, a significantly higher level of GST activity was recorded in the 1:1024 sediment compared to the 1:256 treatment, reflecting accelerated function of the cellular detoxification machinery in the former dilution and a possible inhibition of protein synthesis in the latter (the so-called bell-shape response curve). However, in the 1:024 dilution an elevated level of LPO indicated that the increased metabolism of xenobiotics is causing elevated levels of reactive oxygen species (ROS), which are causing oxidative damage to cell membranes that the ADS cannot fully compensate. Apparently, at the toxicity level prevailing in the 1:1024 treatment the protection mechanisms are still working adequately to prevent effects on the developing offspring.

The results obtained in this additional study illustrated the effectiveness of the application of molecular biomarkers in combination with effects measured at higher biological levels in the assessment of sediment toxicity. Established linkages of early warning responses such as biomarkers to biologically relevant effects such as disturbed reproduction of local organisms provide a powerful tool of assessing sediment toxicity in a more realistic way.

Figure 4: Reproductive disorders in the amphipod *Gmelinoides fasciatus*



Note: Normal embryos (A) and examples of malformed embryos (B-F) found in the marsupium of females after exposure to the test sediment dilutions. B and C: embryos with a malformed eyespot and/or midgut; D: undifferentiated embryos; E: embryos with membrane damage and unfertilized eggs (opaque mass); F: a dead embryo and one with a malformed midgut among normal ones.

5. Discussion and conclusions

The current work focused on comparing a set of biomarkers using various AC that were developed during the project. The AC were formulated in an attempt to cover all main aspects of sediment biotesting and they were applied according to previous knowledge of each of the biotest as well as experiences in using them gained during the project. This integrated multicriteria assessment of a set of sediment biotest methods suitable for use specifically for the Baltic Sea is the main result of the project.

Most of the biotests and endpoints applied in the CONTEST project showed concentration-dependent responses to exposure to dilutions of a toxic sediment, with some variability in sensitivity. This result confirms that biotests are valuable tools for sediment toxicity assessment and able to provide reliable data for decision-making concerning the management of contaminated sediments. It also suggests that the chosen organism used in the toxicity assessment can vary without the biotest approach losing its power. More importantly, it may be argued that it is the endpoint chosen that needs more consideration than the test organism itself. Therefore, choosing an optimal sediment biotest method for a specific case or purpose is greatly dependent on other factors in addition to the sensitivity of the organisms to chemicals.

It should also be noted, however, that only one type of contaminated sediment was tested in the present study; the sensitivity of different organisms to various kinds of contaminants and their relative proportions in the sediment are highly likely to differ from mixture to another. However, the compounds measured from the test sediment in the present study (PAHs, organotins and trace metals) are very frequently found in greatly elevated concentrations especially in harbour sediments. In addition, elevated levels of some other types of contaminants such as polychlorinated biphenyls (PCBs) were also likely to be present in the test sediment. Therefore, the present test matrix probably represents a relatively common and realistic situation with regard to the composition of contaminated sediments, at least in harbours, and the results obtained can be to a certain extent extrapolated to other similar situations.

Following these lines, the selection of the method depends on the purpose of the testing, e.g., assessing the degree of contamination of impacted marine coastal areas

(e.g., for various aspects of marine spatial planning), deciding if deposition (of dredged materials) to the sea is acceptable, monitoring or testing the development of sediment quality in an acutely or accidentally polluted site, including the effectiveness of remediation measures, detection of problems with chronic pollution (e.g., diffuse pollution from land, leaking municipal or industrial waste water, harbours), or periodical checking if routine chemical monitoring of contamination is adequate (i.e., a change in toxicity may require a new chemical screening). Each of these cases may present different kinds of requirements for a biotest. For example, in cases where results are needed for quick decision-making (e.g., in cases of chemical accidents) rapid screening biotest tools could be the preferable choice while in deciding whether a dredged sediment mass could be safely deposited at sea a well-chosen battery of chronic multispecies biotests would be more suitable.

Conclusively, the results of the CONTEST project assist in selecting feasible biotests and endpoints for the testing of contaminated sediments. The sensitivity of most of the applied biotests to different dilutions of the multi-contaminated harbour sediment turned out to be relatively similar in the concentration range tested. Thus, differences in other selection criteria may serve as a basis for the selection of the biotest for each specific need. The use of biotests in sediment toxicity evaluation is considered an absolute necessity when decisions about the management actions on contaminated sediment are made.

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Sammanfattning

Sediment som kontaminerats på grund av mänsklig aktivitet innehåller vanligen en mix av kemikalier som orsakar okända toxiska kombinationseffekter hos organismer. Därför är det osannolikt att traditionella riskbedömningsmetoder som enbart baserar sig på koncentrationsmätningar av specifika kemikalier i ett sediment skulle ge realistisk information om toxiciteten hos dessa och därmed bidra till korrekta vetenskapsbaserade rekommendationer om förfaringsätt gällande hur materialet borde tas om hand. I projektet CONTEST testades toxiciteten hos kontaminerat hamnsediment i olika utspädningar på olika testorganismer och biologiska parametrar för att utvärdera biologiska metoder som används för kvantitativ och kvalitativ uppskattning av toxicitet hos kontaminerat sediment i Östersjön. I denna studie granskades ett urval av biotest som för tillfället används för uppskattning av toxicitet. De flesta av de testade metoderna visade koncentrationsberoende respons beroende på föroreningsgraden av sedimentet med små variationer i sensitivitet beroende på organism och mätningsparameter. Erhållna mätningsresultat och praktiska erfarenheter tillsammans med vedertagen information analyserades för att jämföra de olika biotesterna enligt särskilda evalueringskriterier. Resultaten av projektet förväntas vara till nytta för olika slutanvändare som miljömyndigheter, privata företag och industrier, miljölaboratorier, konsulter samt det vetenskapliga samfundet.

Appendix

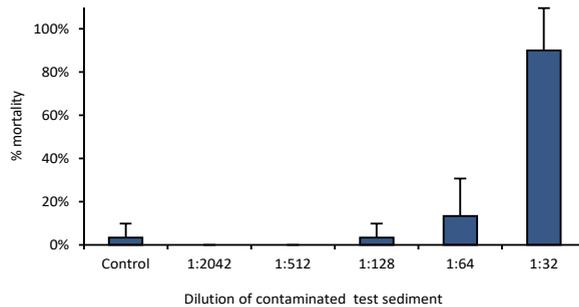
Figure 5: Contest Biotest Fact Sheet No. 1

CONTEST Biotest Fact Sheet No. 1

Survival of the amphipod *Corophium volutator*

Indicator species	<i>Corophium volutator</i> is a sediment-dwelling amphipod inhabiting shallow waters, occurring in the Baltic Sea mainly in the western parts including the Belt Sea.
Source	Field collection.
Endpoint	Mortality is the final endpoint for severe population effects.
Matrix	Whole sediment samples are recommended in protocols, but the test can also be used with elutriates or porewater samples.
Duration	10 d
Key literature	International protocols (e.g. PARCOM 2002, ICES 2010), Schipper et al. (2010).

Results from CONTEST experiments



Description of sediments used in the test with selected contamination proxies

Dilution of original sediment: 1:32. TBT: 2030 $\mu\text{g Sn kg dw}^{-1}$; PAH₁₆: 6064 $\mu\text{g kg dw}^{-1}$, Cu: 352 mg kg dw^{-1} .

Evaluation of methodology

Assessment criteria	Score	Comment
1. Sensitivity	3.0	Response only in more polluted sediments Two endpoints: mortality and avoidance
2. Multi-endpoint organisms/assays	3.0	
3. Practicality (test organisms)	4.5	
4. Quality Assurance	4.3	
5. Baltic Sea ecological relevance	5.0	
6. Practicality (conducting the assay)	3.6	
7. Cost	5.0	

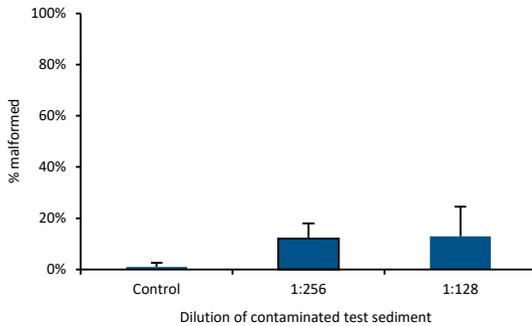
Figure 6: Contest Biotest Fact Sheet No. 2

CONTEST Biotest Fact Sheet No. 2

Embryo malformations in the amphipod *Corophium volutator*

Indicator species	<i>Corophium volutator</i> is a sediment-dwelling amphipod inhabiting shallow waters, occurring in the Baltic Sea mainly in the western parts including the Belt Sea.
Source	Field collection.
Endpoint	Embryo malformation is a very sensitive endpoint for developmental toxicity.
Matrix	Whole sediment samples are recommended in protocols but the test can also be used with elutriates or porewater samples
Duration	28 d
Key literature	Sundelin et al. (2008b); Strode et al. (2017)

Results from CONTEST experiments



Description of sediments used in the test with selected contamination proxies

Dilution of original sediment: 1:32. TBT: 2030 µg Sn kg dw⁻¹; PAH₁₆: 6064 µg kg dw⁻¹, Cu: 352 mg kg dw⁻¹.

Evaluation of methodology

Assessment criteria	Score	Comment
1. Sensitivity	5.0	
2. Multi-endpoint organisms/assays	5.0	Fecundity and embryo malformations were monitored during the same test
3. Practicality (test organisms)	3.5	
4. Quality Assurance	3.7	
5. Baltic Sea ecological relevance	5.0	
6. Practicality (conducting the assay)	2.4	
7. Cost	5.0	

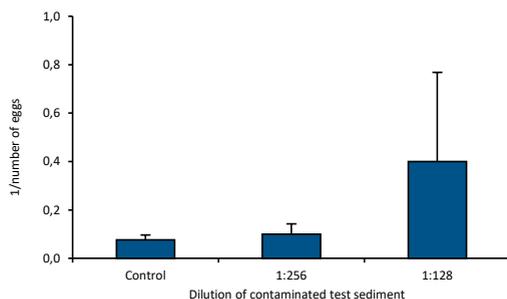
Figure 7: Contest Biotest Fact Sheet No. 3

CONTEST Biotest Fact Sheet No. 3

Fecundity of the amphipod *Corophium volutator*

Indicator species	<i>Corophium volutator</i> is a sediment-dwelling amphipod inhabiting shallow waters, occurring in the Baltic Sea mainly in the western parts including the Belt Sea.
Source	Field collection.
Endpoint	Fecundity is the final endpoint for severe population effects.
Matrix	Whole sediment samples are recommended in protocols, but the test can also be used with elutriates or porewater samples.
Duration	10 d
Key literature	International protocols (e.g. PARCOM 2002; ICES 2010); Schipper et al. (2010)

Results from CONTEST experiments



Description of sediments used in the test with selected contamination proxies

Dilution of original sediment: 1:32. TBT: 2030 µg Sn kg dw⁻¹; PAH₁₆: 6064 µg kg dw⁻¹, Cu: 352 mg kg dw⁻¹.

Evaluation of methodology

Assessment criteria	Score	Comment
1. Sensitivity	4.0	
2. Multi-endpoint organisms/assays	5.0	
3. Practicality (test organisms)	3.5	
4. Quality Assurance	1.0	
5. Baltic Sea ecological relevance	5.0	
6. Practicality (conducting the assay)	2.7	
7. Cost	5.0	

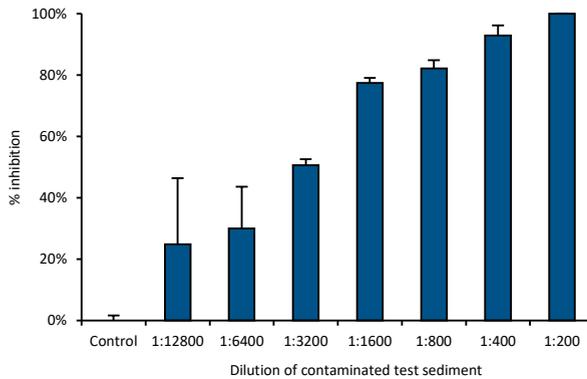
Figure 8: Contest Biotest Fact Sheet No. 4

CONTEST Biotest Fact Sheet No. 4

Growth inhibition in the macroalga *Ceramium tenuicorne*

Indicator species	<i>Ceramium tenuicorne</i> is an abundant macroalga species in the Baltic Sea.
Source	Field collection or laboratory culture.
Endpoint	Growth inhibition is an indicator of moderate toxicity.
Matrix	Elutriate or water exposure is a standard protocol, but also whole sediment testing is possible.
Duration	10 d
Key literature	ISO standard 10710

Results from CONTEST experiments



Description of sediments used in the test with selected contamination proxies

Dilution of original sediment: 1:32. TBT: 2030 µg Sn kg dw⁻¹; PAH₁₆: 6064 µg kg dw⁻¹, Cu: 352 mg kg dw⁻¹.

Evaluation of methodology

Assessment criteria	Score	Comment
1. Sensitivity	4.0	Shows the highest sensitivity of the test organisms used in the CONTEST study.
2. Multi-endpoint organisms/assays	1.0	
3. Practicality (test organisms)	3.5	
4. Quality Assurance	4.3	
5. Baltic Sea ecological relevance	3.0	
6. Practicality (conducting the assay)	4.1	
7. Cost	5.0	

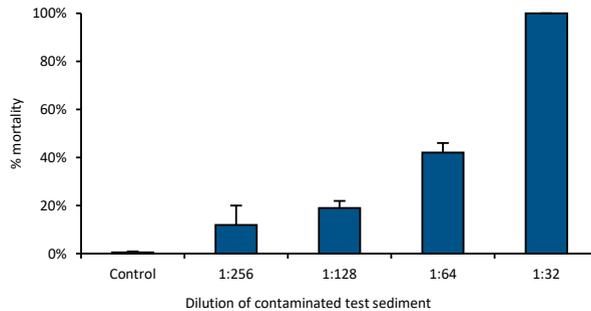
Figure 9: Contest Biotest Fact Sheet No. 5

CONTEST Biotest Fact Sheet No. 5

Survival of the amphipod *Gmelinoides fasciatus*

Indicator species	<i>Gmelinoides fasciatus</i> is a freshwater amphipod found as an invasive species in the eastern part of the Gulf of Finland.
Source	Field collection.
Endpoint	Mortality is the final endpoint for severe population effects.
Matrix	Sediment exposure is possible but it is not the natural environment for the species.
Duration	10 d
Key literature	Berezina et al. (2013)

Results from CONTEST experiments



Description of sediments used in the test with selected contamination proxies

Dilution of original sediment: 1:32. TBT: 2030 µg Sn kg dw⁻¹; PAH₁₆: 6064 µg kg dw⁻¹, Cu: 352 mg kg dw⁻¹.

Evaluation of methodology

Assessment criteria	Score	Comment
1. Sensitivity	4.0	
2. Multi-endpoint organisms/assays	5.0	
3. Practicality (test organisms)	4.5	
4. Quality Assurance	3.7	
5. Baltic Sea ecological relevance	4.0	
6. Practicality (conducting the assay)	4.1	
7. Cost	4.3	

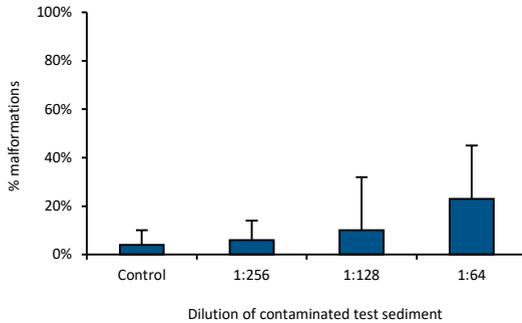
Figure 10: Contest Biotest Fact Sheet No. 6

CONTEST Biotest Fact Sheet No. 6

Embryo malformations in the amphipod *Gmelinoides fasciatus*

Indicator species	<i>Gmelinoides fasciatus</i> is a freshwater amphipod found as an invasive species in the eastern part of the Gulf of Finland.
Source	Field collection.
Endpoint	Mortality is the final endpoint for severe population effects.
Matrix	Sediment exposure is possible but it is not the natural environment for the species.
Duration	28 d
Key literature	Sundelin et al. (2008b); Berezina et al. (submitted manuscript)

Results from CONTEST experiments



Description of sediments used in the test with selected contamination proxies

Dilution of original sediment: 1:32. TBT: 2030 µg Sn kg dw⁻¹; PAH₁₆: 6064 µg kg dw⁻¹; Cu: 352 mg kg dw⁻¹.

Evaluation of methodology

Assessment criteria	Score	Comment
1. Sensitivity	5.0	
2. Multi-endpoint organisms/assays	5.0	
3. Practicality (test organisms)	4.5	
4. Quality Assurance	3.0	
5. Baltic Sea ecological relevance	4.0	
6. Practicality (conducting the assay)	3.3	
7. Cost	4.3	

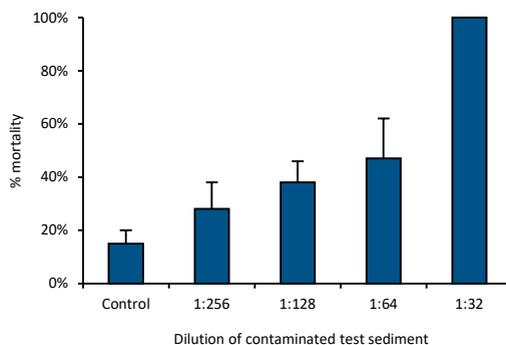
Figure 11: Contest Biotest Fact Sheet No. 7

CONTEST Biotest Fact Sheet No. 7

Survival of the amphipod *Hyalella azteca*

Indicator species	<i>Hyalella azteca</i> is an amphipod inhabiting shallow fresh waters.
Source	Field collection.
Endpoint	Mortality is the final endpoint for severe population effects.
Matrix	Sediment exposure is possible but it is not the natural environment for the species.
Duration	10 d
Key literature	Steevens & Benson (1998)

Results from CONTEST experiments



Description of sediments used in the test with selected contamination proxies

Dilution of original sediment: 1:32. TBT: 2030 $\mu\text{g Sn kg dw}^{-1}$; PAH₁₆: 6064 $\mu\text{g kg dw}^{-1}$, Cu: 352 mg kg dw^{-1} .

Evaluation of methodology

Assessment criteria	Score	Comment
1. Sensitivity	4.0	
2. Multi-endpoint organisms/assays	5.0	
3. Practicality (test organisms)	4.0	
4. Quality Assurance	4.3	
5. Baltic Sea ecological relevance	3.0	
6. Practicality (conducting the assay)	4.1	
7. Cost	4.3	

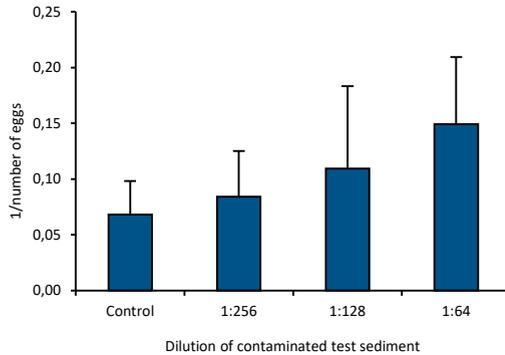
Figure 12: Contest Biotest Fact Sheet No. 8

CONTEST Biotest Fact Sheet No. 8

Fecundity of the amphipod *Gmelinoides fasciatus*

Indicator species	<i>Gmelinoides fasciatus</i> is a freshwater amphipod found as an invasive species in the eastern part of the Gulf of Finland.
Source	Field collection.
Endpoint	Fecundity is a sensitive endpoint even for low toxicity.
Matrix	Sediment exposure is possible but it is not the natural environment for the species.
Duration	28 d
Key literature	Sundelin et al. (2008b); Berezina et al. (submitted manuscript)

Results from CONTEST experiments



Description of sediments used in the test with selected contamination proxies

Dilution of original sediment: 1:32. TBT: 2030 µg Sn kg dw⁻¹; PAH₁₆: 6064 µg kg dw⁻¹, Cu: 352 mg kg dw⁻¹.

Evaluation of methodology

Assessment criteria	Score	Comment
1. Sensitivity	4.0	
2. Multi-endpoint organisms/assays	5.0	
3. Practicality (test organisms)	4.0	
4. Quality Assurance	3.0	
5. Baltic Sea ecological relevance	4.0	
6. Practicality (conducting the assay)	3.9	
7. Cost	4.3	

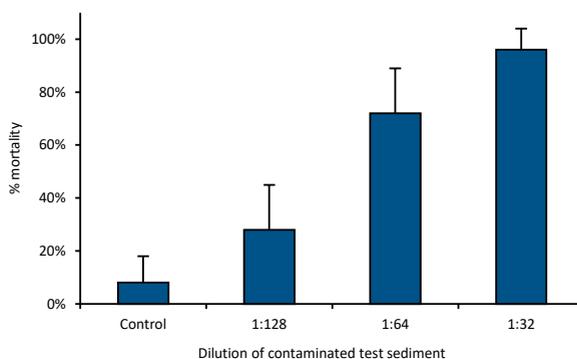
Figure 13: Contest Biotest Fact Sheet No. 9

CONTEST Biotest Fact Sheet No. 9

Survival of the copepod *Nitocra spinipes*

Indicator species	<i>Nitocra spinipes</i> is a small Baltic Sea benthic harpacticoid copepod species.
Source	Field collection.
Endpoint	Mortality is the final endpoint for severe population effects.
Matrix	Whole sediment samples are recommended in protocols but the test can also be used with elutriates or porewater samples.
Duration	96 h
Key literature	Perez-Landa & Simpson (2011).

Results from CONTEST experiments



Description of sediments used in the test with selected contamination proxies

Dilution of original sediment: 1:32. TBT: 2030 µg Sn kg dw⁻¹; PAH₁₆: 6064 µg kg dw⁻¹; Cu: 352 mg kg dw⁻¹.

Evaluation of methodology

Assessment criteria	Score	Comment
1. Sensitivity	5.0	
2. Multi-endpoint organisms/assays	1.0	
3. Practicality (test organisms)	4.5	
4. Quality Assurance	3.7	
5. Baltic Sea ecological relevance	3.0	
6. Practicality (conducting the assay)	4.1	
7. Cost	5.0	

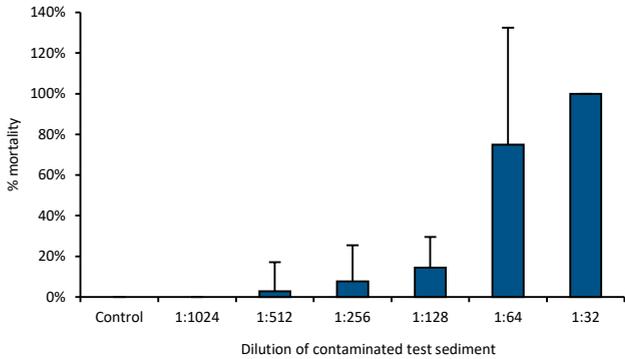
Figure 14: Contest Biotest Fact Sheet No. 10

CONTEST Biotest Fact Sheet No. 10

Survival of the gastropod *Potamopyrgus antipodarum*

Indicator species	<i>Potamopyrgus antipodarum</i> is a sediment-dwelling gastropod originating from New Zealand and inhabits shallow fresh or brackish waters.
Source	Field collection or laboratory culture.
Endpoint	Mortality is the final endpoint for severe population effects.
Matrix	Standard protocols use water exposure but whole sediment testing was found successful in this experiment.
Duration	28 d
Key literature	OECD (2010); Oehlmann et al. (2014)

Results from CONTEST experiments



Description of sediments used in the test with selected contamination proxies

Dilution of original sediment: 1:32. TBT: 2030 µg Sn kg dw⁻¹, PAH₁₆: 6064 µg kg dw⁻¹, Cu: 352 mg kg dw⁻¹.

Evaluation of methodology

Assessment criteria	Score	Comment
1. Sensitivity	4.0	
2. Multi-endpoint organisms/assays	5.0	
3. Practicality (test organisms)	3.5	
4. Quality Assurance	3.7	
5. Baltic Sea ecological relevance	4.0	
6. Practicality (conducting the assay)	3.6	
7. Cost	4.3	

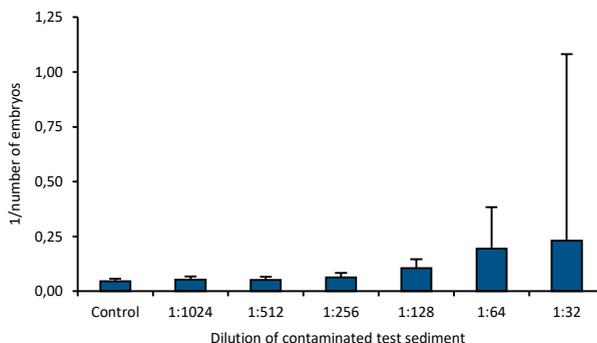
Figure 15: Contest Biotest Fact Sheet No. 11

CONTEST Biotest Fact Sheet No. 11

Fecundity of the gastropod *Potamopyrgus antipodarum*

Indicator species	<i>Potamopyrgus antipodarum</i> is a sediment-dwelling gastropod originating from New Zealand and inhabits shallow fresh or brackish waters.
Source	Field collection or laboratory culture.
Endpoint	Reproduction is a sensitive endpoint and an early warning indicator.
Matrix	Standard protocols use water exposure but whole sediment testing was found successful in this experiment.
Duration	28 d
Key literature	OECD (2010)

Results from CONTEST experiments



Description of sediments used in the test with selected contamination proxies

Dilution of original sediment: 1:32. TBT: 2030 $\mu\text{g Sn kg dw}^{-1}$; PAH₁₆: 6064 $\mu\text{g kg dw}^{-1}$, Cu: 352 mg kg dw^{-1} .

Evaluation of methodology

Assessment criteria	Score	Comment
1. Sensitivity	5.0	
2. Multi-endpoint organisms/assays	5.0	
3. Practicality (test organisms)	3.5	
4. Quality Assurance	3.7	
5. Baltic Sea ecological relevance	4.0	
6. Practicality (conducting the assay)	3.3	
7. Cost	4.3	



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Sediment biotesting in the Baltic Sea

Sediments contaminated by human activities usually contain a mixture of chemicals that produce unforeseen combined toxic effects in organisms. Thus, traditional risk assessments based on the concentrations of chemicals are unlikely to produce realistic data on toxicity. In the CONTEST project, 19 biotests were evaluated using a model contaminated sediment from the Baltic Sea. Most of the biotests applied showed concentration-dependent toxicity related to the degree of chemical pollution measured in the test sediment with some variability in the sensitivity of the test organism and the endpoint. The different biotests were analysed according to specially designed assessment criteria, and the results are foreseen to be useful for end-user groups including environmental authorities, private companies and industries, environmental laboratories, consultants, and the scientific community.



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