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Hazard identification of contaminated sites—ranking potential toxicity of organic sediment extracts in crustacean and fish

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Abstract
Background, aim, and scope It is well known that contaminated sediments represent a potential long-term source of pollutants to the aquatic environment. To protect human and ecosystem health, it is becoming common to remediate contaminated sites. However, the great cost associated with, e.g., dredging in combination with the large numbers of contaminated sites makes it crucial to pinpoint those sites that are in greatest need of remediation. In most European countries, this prioritization process has almost exclusively been based on chemical analyses of known substances; only seldom toxicity data has been considered. The main objective of the current study was therefore to develop a tool for hazard identification of sediment by ranking potential toxicity of organic sediment extracts in a crustacean and a fish. A secondary objective was to investigate the difference in potential toxicity between compounds with different polarities.
Materials and methods Early life stages of the crustacean *Nitocra spinipes* and the fish *Oncorhynchus mykiss*, which represent organisms from different trophic levels (primary and secondary consumer) and with different routes of exposure (i.e., ingestion through food, diffusive uptake, and maternal transfer), were exposed to hexane and acetone fractions (semi-polar compounds) of sediment from five locations, ranging from heavily to low contaminated. Preliminary tests showed that the extracts were non-bioavailable to the crustacean when exposed via water, and the extracts were therefore loaded on silica gel. Rainbow trout embryos were exposed using nano-injection technique.
Results and discussion Clear concentration–response relationships of both mortality and larval development were observed in all tests with *N. spinipes*. Also for rainbow trout, the observed effects (e.g., abnormality, hemorrhage, asymmetric yolk sac) followed a dose-related pattern. Interestingly, our results indicate that some of the locations contained toxic semi-polar compounds, which are normally not considered in risk assessment of sediment since they are focused on compounds isolated in the hexane fraction.
Conclusions The ranking of the five sediments followed the expected pattern of potential toxicity in both organisms, i.e., sediments with known pollution history caused major effects while reference sediments caused minor effects in the two test systems. Silica gel turned out to be an excellent carrier for exposure of *N. spinipes* to very hydrophobic and otherwise non-bioavailable sediment extracts.
Recommendations and perspectives Since both test systems demonstrated that a substantial part of the potential toxicity was caused by semi-polar compounds in the acetone fractions, this study enlightens our poor understanding of which compounds are causing adverse effects in environmental samples. Therefore, by investigating potential toxicity (i.e., hazard identification) as a first screening step in prioritizing processes, these implications could be avoided. For proper sediment risk assessment, we however recommend whole sediment toxicity tests to be used for selected sites at following tiers.

Keywords Crustacea · Fish · PAH · PCB · PCDD/Fs · Sediment extract · Sediment toxicity · Semi-polar · Sublethal

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1 Background, aim, and scope

Sediments were for a long time considered as a relative secure sink for pollutants discharged into the water body. However, with increasing knowledge of sediment toxicity,

this concept has changed, and sediments are in a regulatory context currently regarded as a potential risk to both ecosystem and human health (Hollert et al. 2003; SedNet 2004). Although many contaminated sites are in need of remediation, the great numbers as well as limited financial resources make it impossible for sediment managers to perform remedial activities at all places. Hence, reliable prioritizing tools are required to pinpoint locations in most need of remedial actions.

Sediment Quality Assessment (SQA) preferably should rely on several assessment tools, such as sediment toxicity testing, sediment chemistry and bioaccumulation tests, which used in concert make up multiple lines of evidence (LOE; McCauley et al. 2000; Wenning et al. 2005). The European Sediment Research Network, SedNet, recommends the use of Sediment Quality Triad (SQT) when assessing sediment quality (SedNet 2004). The SQT approach compares conaminated and reference sites using information derived from sediment chemistry, sediment toxicity and in situ studies (Long and Chapman 1985; Chapman 1986; SedNet 2004). Still, most European countries predominantly rely on mere chemical analyses when assessing sediment toxicity (Stuer-Lauridsen et al. 2001; Nendza 2002; DelValls et al. 2004; Casado-Martínez et al. 2006; SEPA 2006). Chemical analysis is however not sufficient to provide adequate knowledge necessary to evaluate and assess the potential toxicity of contaminated sites in the sense that it is impossible to identify and measure the concentration of all the potential toxicants present in the sediments (e.g., Baudo et al. 1999). Several studies have also demonstrated a poor relationship between analyzed pollutants and their contribution to potential toxicity (e.g., Sundberg et al. 2005b; Olajire et al. 2005; Sundberg et al. 2006; Bihari et al. 2006). In addition, interactive toxicological effects (synergistic and antagonistic) or the bioavailability of toxicants to organisms, which is a key factor for ecotoxicological effects, cannot be established with mere chemical analysis (Fent 2004). Several European countries, e.g., Netherlands and UK, are therefore making attempts to improve their SQA (Sednet 2004; Allen et al. 2007). One such attempt is to include sediment toxicity testing into the SQA paradigm (McCauley et al. 2000; Ahlf et al. 2002; Fent 2004; Kammann et al. 2005).

Evaluating sediment quality is recommended to follow a tiered approach, which already at screening tiers should involve physico-chemical studies and sediment toxicity tests for hazard identification (Nendza 2002; Wenning et al. 2005). Several standardized sediment tests are available, covering a variety of species and endpoints describing both lethal and sublethal effects (e.g., Ingersoll et al. 1998; Ingersoll et al. 2001; Nendza 2002; Feiler et al. 2005; Allen et al. 2007). Chronic tests assessing effects on, e.g., reproduction are on, the other hand, seldom applied for hazard identification at screening tiers since they are time-consuming (exposure 28–42 days or even longer) and consequently more costly (Ingersoll et al. 1997; Nendza 2002; Allen et al. 2007). Hence, early life-stages, which are often considered more sensitive life stages than the adult stage (Barata et al. 2002; Medina et al. 2002; Kiparissis et al. 2003), are rarely considered for hazard identification purposes. To identify the hazard potential of sediments, it is common to use organic sediment extracts (e.g., Gagné et al. 1996; Strmac et al. 2002; Hollert et al. 2003; Viganó et al. 2003; Kammann et al. 2004; Keiter et al. 2006; Kosmehl et al. 2007; Seiler et al. 2008), especially in situations when there is a need to identify those compounds actually causing adverse effects in the environmental samples (Sundberg et al. 2005b, 2006). The use of extracts simplifies the exposure but also increases the bioavailability of especially hydrophobic substances compared to intact sediment (e.g., Eriksson Wiklund et al. 2005). However, as the bioavailability increases, possible long-term effects could be investigated in a much shorter time frame (Seiler et al. 2008), thereby overcoming some of the problems that may occur when testing sediments that contain hydrophobic substances that are strongly bound. Porewater and elutriate extracts are also commonly used for assessing sediment toxicity (Ankley 1991; Lau and Chu 1999; Chapman et al. 2002; Davoren et al. 2005). Although these phases can provide important information on sediment toxicity (e.g., simulate remobilization of sediments), they may under- or overestimate the bioavailability and hence toxicity of contaminants present in intact sediment (e.g. Chapman et al. 2002; Hollert et al. 2003; Davoren et al. 2005). For assessing actual risks of sediment, the most straightforward and relevant scenario is to use whole sediment tests (Ankley 1991; Carr and Nipper 2001; Chapman et al. 2002; Feiler et al. 2005; Chapman and Hollert 2006). However, the complex physico-chemical composition of sediments (e.g., related to pH, salinity, water, organic content) is known to affect the bioavailability of contaminants (van Leeuwen and Hermens 1995; Fent 2004) and consequently places high requirements on the test to be used for actual risk assessment (Feiler et al. 2005). Whole sediment tests used at screening tiers may also be too short to show effect in receptor organisms and thereby underestimate the toxicity of the tested sediment (Solomon and Sibley 2002). Thus, different test phases (solid or liquid) may be needed to obtain the varying information that is needed to assess hazard versus risk in SQA.

The main objective of the present study was to develop a tool for hazard identification of sediment by ranking potential toxicity of organic sediment extracts in early life stages of two organisms, the crustacean *Nitocra spinipes* and the fish *Oncorhynchus mykiss*. Compared to mere chemical analyses, which may be associated with a number of pitfalls (see above), ranking different sediments' toxic potential, offers a more straightforward comparison between different sedi-

ments' abilities to cause actual adverse effects. To investigate the difference in potential toxicity between compounds with different polarity, both hexane and acetone fractions (isolated from a toluene extract) from the selected contaminated locations were studied.

2 Materials and methods

2.1 Sediment sampling

To compare the potential toxicity of sediments with different contaminant characteristics, we collected bottom sediment using a Kajak type gravity corer from five locations (Table 1). Örserumsviken (A—Polluted Bay) on Sweden's Baltic coast served as positive control since the bay has recently been dredged (Sundberg et al. 2007) due to high polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and mercury pollution. In this investigation, we therefore used sediment collected before dredging. Frierfjorden (B—Polluted Fjord) is located in southern Norway and is highly contaminated by PAHs and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) owing to discharges form a magnesium production plant at the inner fjord (Oehme et al. 1989). Riddarfjärden (C—Stockholm City) is the water body located outside the City Hall of Stockholm and receives pollutants from anthropogenic activities including traffic and local industries. Björkskär (D—Stockholm Archipelago) is located in the outer part of the Stockholm archipelago, and Slingsviken (E—Reference Bay) is located in an agricultural area on Sweden's Baltic coast. Both Reference Bay and Stockholm Archipelago served as low contaminated areas.

2.2 Preparation of exposure solutions—sediment extraction and fractionation

Sediment samples were thawed at room temperature, and to get a representative sample, samples from the same location were pooled in a glass beaker and mixed with a stainless steel spoon. Aliquots of wet sediment samples were extracted for 24–50 h in toluene using a Soxhlet apparatus coupled to a Dean–Stark trap for water removal (Lamparski and Nestrick 1980). Dry weights were gravimetrically determined after extraction. After solvent exchange to *n*-hexane, elemental sulfur was removed by the addition of a small amount of elemental copper, ultrasonification-bath (4×15 min), and overnight incubation at room temperature.

A hexane fraction of each sediment extract was isolated by eluting the extracts through a deactivated (10% H_2O) silica gel column (I.D. 1 cm, 10 g of silica gel/g extractable organic matter) with *n*-hexane (10 ml of *n*-hexane/g silica gel). To investigate the toxic potential of those compounds that are insoluble to *n*-hexane (retained in the column), which we designated as semi-polar, an acetone fraction from each sediment extract was isolated by eluting the silica gel column with acetone (10 ml of acetone/g silica gel).

Table 1 Description of collected sediment samples and literature data of some pollutants in each location

Parameter	A—Polluted Bay (Örserumsviken)	B—Polluted Fjord (Frierfjorden)	C—Stockholm City (Riddarfjärden)	D—Stockholm Arch. (Björkskär)	E—Reference Bay (Slingsviken)
Position[a]	57°43.60′ N, 16°40.00′ E	59°07.00′ N, 9°36.50′ E	59°19.50′ N, 18°03.25′ E	59°21.93 N, 19°08.27′ E	57°37.50′ N, 16°30.88′ E
Water depth (m)	1–2	56	15–21	78	3–4
Sediment depth (cm)	0–10	0–15	0–3	0–5	0–10
Pooled samples (n)	10	1	20	4	7
ΣPAH[b] (μg/kg dw)	8,700[c]	4,900[d]	11,000[e]	530[f]	2,100[c]
ΣPCB[g] (μg/kg dw)	7,800[h]	29[i]	100[e]	2.2[f]	n.a.[j]
ΣPCDD/F[k] (μg/kg dw)	n.a.	7,000[i]	n.a.	n.a.	n.a.

[a] Coordinates in World Geodectic System 1984 (WGS-84)
[b] Sum concentration of the following compounds: phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, triphenylene, benzo[b+k+j] fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[ghi]pyrene
[c] From Sundberg et al. 2005a
[d] From Nœs 1999
[e] From Sternbeck et al. 2003
[f] From Hansson. et al. 2005
[g] Sum concentration of the following PCB congeners (IUPAC no): 28, 31, 52, 118, 123, 132, 153, 138, 180, 193
[h] From Sundberg et al. 2005b
[i] From Persson et al. 2005
[j] Not analysed
[k] Sum concentration of 24 PCDD/Fs congeners, from Persson et al. 2005

2.3 Test systems

Early life stages of the primary consumer *N. spinipes* and the secondary consumer rainbow trout (*O. mykiss*) were used to study potential toxicity of the isolated sediment fractions.

2.3.1 N. spinipes—mortality and larval development ratio

The benthic harpacticoid *N. spinipes* is a species with well-documented biology (Lang 1948; Wulff 1972; Abraham and Gopalan 1975), which have been used in ecotoxicological research for many years (e.g., Breitholtz and Bengtsson 2001; Breitholtz and Wollenberger 2003; Breitholtz et al. 2003; Dahl et al. 2006). *N. spinipes* used in the present study were collected from ITM (Department of Applied Environmental Science, Stockholm University, Sweden) stock culture, which origins from one female isolated from a sediment sample about 30 years ago (Bengtsson 1978).

To facilitate homogenous exposure of the organic sediment extracts and to increase the surface area to which the animals were exposed, the bottoms of each exposure beaker (20 ml) were covered with 100 mg silica gel (Merck, silica gel 60, $\varnothing$= 0.063–0.200 mm; Area: ~500 m^2/g; Breitholtz et al. 2007). The spiking of the extracts was prepared the day before the start of the test to avoid residues from the solvents (Ulfsdotter Turesson et al. 2007). Extracts were first added to the solvent, whereupon they were transferred to the test beakers containing the thin layer of silica gel. In all treatments and experiments, including the solvent controls (hereafter referred to as control), the same volume of solvent was added (500–600 μl, except in the acetone treatment for the Reference Bay (1,200 μl)) as in the highest treatments tested. Fractions corresponding to 1.5, 4.5, 13.5, and 40.5 mg sediment were used. In two tests, acetone fractions of Polluted Bay and Stockholm Archipelago, 0.0166 and 121.5 mg sediment were also used as exposure concentrations.

The design of the silica gel based test, which measures Larval Development Ratio (LDR) and mortality, has been presented elsewhere (Breitholtz et al. 2007). Briefly, LDR is calculated as the percentage of copepodites among all offspring at the end of the test (~6 days). About 300 gravid females were isolated 24 h before starting the test. Nauplii released within 24 h were used in the test, and eight of those were randomly transferred to each beaker, containing 5 ml natural brackish seawater (salinity 6.5‰ collected at the Askö laboratory on the coast of the Baltic Sea) and 100 mg of spiked silica gel. Ten replicates per treatment and control were used. The crustaceans were fed with the red micro alga *Rhodomonas salina* (Chrysophycea) three times, i.e., on day 0, 2 or 3 and 5 (final density of 5×10^7 cells/ml). Evaporation was compensated for in connection with feeding by adding distilled water. The test vials were incubated in darkness at 22±1°C.

2.3.2 Rainbow trout—mortality and abnormalities

Rainbow trout are pharmacologically well-characterized and commercially available; their eggs are fairly large, which facilitates handling and injection. To increase the homogeneity of the biological material, only one family pair was used. Eggs and seminal fluid from rainbow trout were collected and shipped to our laboratory from Vilstena fiskodling (Fjärdhundra, Sweden) on the day of fertilization. Artificial fertilization and water swelling were performed at 8.2°C, whereupon eggs were placed in cup-shaped depressions in 1% agarose gel cast in square Petri dishes (Falcon, Becton Dickinson Labware, Franklin Lake, NJ, USA) with maximum 36 eggs/dish (Åkerman and Balk 1995).

Graded concentrations of the exposure solutions were prepared so that the desired dose required an injection volume of less than 1‰ of the egg (<100 nl). The hexane fractions and benzo[a]pyrene (positive control) were dissolved in triolein and the acetone fractions in tricaprylin: lecithin (1:1), followed by organic solvent evaporation using a gentle stream of N_2 (g). Triolein (T-7140, 99%) and tricaprylin (T-9126) were purchased from Sigma (St. Louis, MO, USA) and lecithin was bought from Bioforce GmbH (Konstanz, Germany). The carrier substances were investigated by injecting solely triolein (triolein control) or tricaprylin:lecithin (tri:lec control) into eggs. To investigate effects from the organic solvents, hexane and acetone, the two solvents were dissolved in their respective carrier substance followed by evaporation analogously to the exposure solutions. Uninjected controls were used for investigating effects from the injection. Exposure solutions were transferred, using a vacuum suction pump, into sharpened aluminum silicate capillaries (Sutter Instrument CO., Novato, CA, USA). The day after fertilization, rainbow trout eggs were exposed to the solutions using the nano-injection technique as described previously (Sundberg et al. 2005a).

Control and exposure groups were kept in darkness in identical individual flow-through systems (36 individuals/system) with an average temperature of 8.8°C (7.8–9.5°C), containing 2 l of Stockholm municipal drinking water filtered in three consecutive steps (nominal pore size: 50 μm—active carbon—10 μm) and aerated. Mortality was recorded every second day during the experiment. Hemorrhage, asymmetric yolk sac (Sundberg et al. 2005a), and other abnormalities, including scoliosis, edema, and craniofacial deformities were investigated among newly hatched larvae under a stereomicroscope (Leica MZ8, Leica Microscopy and Scientific Instruments Group, Heerburg, Switzerland). The experiment was terminated 28 days post-hatch when surviving larvae were euthanized by cervical dislocation using forceps.

2.4 Ranking

The potential toxicities of the sediment fractions from the five locations were ranked by comparing their abilities to affect the investigated endpoints. The location with the least toxic potential for a specific endpoint was given the score 1. For each specific endpoint, the locations received scores based on their ability to affect that endpoint. In some cases, several locations were equally potent and therefore received equal score. All scores from the investigated endpoints in the two test systems were summed up, and the location with the highest total score was ranked as potentially most toxic for crustacean and fish. For simplicity, all investigated endpoints in the two test systems applied were weighed equal. To receive the total sum of rank of the locations, the ranking of the two systems were summed up. The potentially most toxic sediment received the lowest total sum of rank and was consequently ranked as number one in Total Rank.

In the *N. spinipes* experiments, the following criteria were used when ranking each location. The location/s that received the lowest score (1) for a specific endpoint did not differ statistically from the solvent control. To score a higher ranking (≥2), the location/s did diverge significantly from the control in one or more concentrations. If there was a significant difference at a lower concentration compared to another location, this location scored higher. Therefore, the number of groupings for each endpoint decided the possible number of scores for each endpoint. Theoretically, the highest score for each endpoint was 5, which could be gained if all locations showed different toxicities in comparison with each other.

In the rainbow trout experiments, the following criteria were used when ranking each location. The location/s that received the lowest score (1) for a specific endpoint did not differ statistically from the carrier control, nor was any evident dose–response effect observed. To score a higher ranking (2) the fraction did, at minimum, induce an evident dose–response effect and at least 75% more rainbow trout than carrier controls was affected from this endpoint. If a fraction from another location should score higher (3), the effect needed to be significant. To score higher than that, at least 75% more rainbow trout was affected than the previous ranked location.

2.5 Statistical analysis

All data related to mortality and LDR in *N. spinipes* were analyzed by using the nonparametric Kruskal–Wallis test, and the Mann–Whitney *U* test was used as post hoc test. Bonferroni correction was made for mortality and LDR data, where a significant difference was considered at the 0.05 level if *p*-value was lower than 0.0125.

In the rainbow trout experiment, statistical differences in mortalities and abnormalities between exposure and control groups were determined using Fisher's exact test. As α-level for statistically significant differences between groups, a *p*-value of less than 0.05 was used.

3 Results

3.1 *N. spinipes*

LDR and mortality in *N. spinipes* after 6 days exposure to the hexane fraction of the five sediments are presented in Table 2. The only hexane fraction that caused significantly increased mortality compared with the control was the Polluted Bay. At the highest concentration (corresponding to 40.5 mg sediment) the mortality was 65% ($p<0.0125$) compared to 3.5% mortality in the control. Polluted Bay also caused the most significant decrease in LDR compared to the control. Already 1.5 mg sediment, there was a significant decrease in LDR, since only 31% of the surviving nauplii had reached the copepodite stage compared to over 60% in the control. At the three highest concentrations (4.5, 13.5, and 40 mg), 38, 4.4, and 2.0% of the nauplii reached the copepodite stage, respectively. Polluted Fjord caused a significant decrease in LDR at the two highest concentrations (13.5 and 40.5 mg; see Table 2). The highest concentration of the hexane fraction from the Stockholm City sediment caused significantly decreased LDR compared with the control. Stockholm Archipelago did neither cause any significant mortality nor affected LDR. Although the hexane fraction corresponding to 4.5 mg sediment from the Reference Bay caused a significant increase in LDR, further studies are needed to investigate a possible stimulating effect on larval development. At the highest concentration, there was a significant decrease in LDR.

LDR and mortality in *N. spinipes* exposed to the acetone fraction of the five sediment extracts are presented in Table 2. Polluted Bay caused significant effects in LDR at as a low concentration as 0.5 mg sediment. The two highest concentrations tested (1.5 and 4.5 mg) caused 49% and 100% mortality, respectively, compared to 17% mortality in the control. Already at a concentration corresponding to 0.5 mg sediment caused significant effects in LDR. In this test, 0.166 mg sediment was also tested, but it did not result in any significant effects in either LDR or mortality, compared to the control. Polluted Fjord caused significant effects in LDR at the two highest concentrations (13.5 and 40.5 mg) and significant effects in mortality at the highest concentrations. Stockholm City did not cause any significant effects on either LDR or mortality. The third most toxic sediment was the Stockholm Archipelago, which showed significant effects in both LDR and mortality at the two highest concentrations (40.5 and 121.5 mg). Like Stockholm City, the Reference Bay did not show any significant effects in either LDR or mortality.

Table 2 Larval development ratio and mortality in *N. spinipes* exposed to hexane and acetone fractions of sediments from five locations

Location	Hexane fraction		Acetone fraction	
	Mortality% (± 95% CI)	LDR% (± 95% CI)	Mortality% (± 95% CI)	LDR% (± 95% CI)
Polluted Bay				
Control	3.5 (±3.5)	63 (±12)	17 (± 13)[b]	80 (± 13)[b]
0.166 (mg sediment)	–	–	9.8 (±5.9)	67 (±8.5)
0.5	–	–	17 (±15)	37 (±10)*
1.5	3.6 (±5.1)	31 (±11)*	49 (±18)*	0 (−)*
4.5	13 (±8.4)	38 (±7.9)*	100 (−)*	0 (−) *
13.5	34 (±20)[a]	4.4 (±6.4)[a,]*	–	–
40.5	65 (±15)*	2.0 (±3.9)*	–	–
Polluted Fjord				
Control	8.1 (±4.7)	70 (±11)	4.2 (±4.2)	47 (±16)
1.5	6.3 (±5.5)	68 (±11)	9.5 (±6.9)	53 (±14)
4.5	4.5 (±5.2)	70 (±12)	5.2 (±5.5)	41 (±7.1)
13.5	13 (±12)	28 (±6.7)*	7.7 (±5.5)	24 (±7.0)*
40.5	13 (8.6)	40 ± (11)*	29 (±10)*	3.7 (±4.8)*
Stockholm City				
Control	3.5 (±3.5)	48 (±12)	9.9 (±6.9)[a]	75 (±13)[a]
1.5	5.0 (±4.0)	32 (±9.9)	9.1 (±6.8)	73 (±13)
4.5	6.0 (±6.3)	46 (±17)	1.3 (±2.4)	72 (±14)
13.5	3.6 (±3.6)	32 (±8.7)	14 (±11.2)	59 (±16)
40.5	2.4 (±3.1)	15 (±5.8)*	3.8 (±3.7)	53 (±11)
Stockholm Arch.				
Control	3.0 (±3.9)[a]	59 (±9.2)[a]	11 (±8.6)	63 (±14)
1.5	3.2 (±4.3)[a]	60 (±12)[a]		
4.5	3.9 (±3.9)	64 (±8.2)	8.3 (±9.1)	44 (±14)
13.5	3.0 (±3.9)[a]	50 (±18)[a]	10 (±3.3)	47 (±11)
40.5	4.1 (±4.1)	42 (±13)	47 (± 15)[a,]*	25 (± 14)[a,]*
121.5	–	–	100 (–)*	0 (–)*
Reference Bay				
Control	2.5 (±3.3)	58 (±11)	7.0 (±4.6)	58 (±11)
1.5	1.1 (±2.2)	50 (±9.5)	9.5 (±8.1)	64 (±18)
4.5	7.9 (±8.6)	90 (±7.1)*	4.1 (±4.1)	71 (±8.4)
13.5	2.5 (±3.3)	44 (±10)	1.4 (±2.8)	64 (±11)
40.5	5.8 (±4.8)	31 (±11)*	2.5 (±3.3)	48 (±14)

*Significant differences from solvent control ($p<0.0125$) are denoted with an asterisk.
[a] Statistics are based on nine replicates instead of 10.
[b] Statistics are based on eight replicates instead of 10.

3.2 Rainbow trout

No significant differences were observed between the solvent, carrier, and uninjected controls, exposure groups were therefore compared with their respective carrier control for statistical analyses.

Mortalities and abnormalities among rainbow trout exposed to the hexane fractions from the five locations and benzo[a]pyrene are shown in Table 3. Polluted Bay caused significant dose-response effects and increased frequencies of larvae suffering from hemorrhages and asymmetric yolk sac or any kind of abnormality. Mortality was slightly enhanced by this exposure (38% higher than the triolein control). Compared to Polluted Bay, Polluted Fjord caused significantly increased mortality, equal frequencies of asymmetric yolk sac, and more than 75% higher amount of rainbow trout were hemorrhagic or suffered from any kind of abnormality. Stockholm City caused nonsignificant dose–response effects in mortality, hemorrhages, and asymmetric yolk; but more than 99% higher amount of rainbow trout in the highest dose were affected compared with the triolein control. No endpoint was affected among rainbow trout exposed to the hexane fractions from Stockholm Archipelago or the Reference Bay. Benzo[a] pyrene—the positive control—caused evident dose–response effects on the four endpoints.

Table 3 Effects in early life stages of rainbow trout exposed to hexane and acetone fractions of sediments from five locations

	Hexane fraction				Acetone fraction			
	Hemorrhage	Asymmetric yolk sac	Abnormal larvae	Total mortality	Hemorrhage	Asymmetric yolk sac	Abnormal larvae	Total mortality
Control[a]	3.5	–	14	22	3.6	–	3.6	25
Polluted Bay								
2.4 g	3.1	–	9.4	11	3.2[b]	–[b]	16[b]	18[b]
12 g	4.2[c]	–[c]	8.3[c]	34[c]	–	–	14	22
60 g	24*	28*	48*	31*	n.i.[d]	n.i.	n.i.	n.i.
Polluted Fjord								
2.4 g	–	–	7.7	64*	–	–	20	40
12 g	4	–	20	42	–	–	4	33
60 g	79*	26*	90*	81*	60*	27*	87*	69*
Stockholm City								
2.4 g	–	–	18	22	–	–	5.7	8.3
12 g	–	–	29	22	–	–	6.3	14
60 g	9.5[b]	9.5[b]	29[b]	44[b]	–	–	10	44
Stockholm Archipelago								
2.4 g	–[c]	–[c]	7.1[c]	23[c]	–[b]	–[b]	4[b]	29[b]
12 g	8	–	8	31	–[b]	–[b]	–[b]	32[b]
60 g	3.2	–	19	17	9.1	–	23	42
Reference Bay								
2.4 g	3.1	–	9.4	11	–[c]	–[c]	6.9[c]	20[c]
12 g	6.5[c]	–[c]	9.7[c]	14[c]	–	–	14	25
60 g	3.0	–	9.1	11	7.4[c]	–[c]	19[c]	26[c]
Benzo[*a*]-pyrene[e]								
0.2 mg	3.2	–	3.2	14				
1.0 mg	4[b]	4[b]	20[b]	35[b]				
2.5 mg	3.6	3.6	18	25				
5.0 mg	19	12	39	33				
10 mg	29*	29	–	53*				

Fish were exposed as newly fertilized eggs using the nanoinjection technique; when hatched malformed larvae were recorded. Values are given in percent, 36 eggs were injected for each exposure and doses are given as g dry sediment/kg wet egg or as mg/kg wet egg

*Significantly different ($p<0.05$, Fisher's exact test) compared with the corresponding injection control

[a] Triolein was used as injection control for the hexane fractions and tricaprylin:lecithin for the acetone fractions

[b] 34 eggs were injected

[c] 35 eggs were injected

[d] Not injected since the desired concentration of the Polluted Bay's acetone fraction could not be properly dissolved in the carrier substance (tricaprylin:lecithin) to obtain the highest dose

[e] Positive control dissolved in triolein

The highest dose of the acetone fraction from the Polluted Bay was not injected into rainbow trout eggs since the desired concentration could not be properly dissolved in tricaprylin: lecithin and no evident effect from the two lower doses was observed. The acetone fraction from the Polluted Fjord was slightly less potent than the hexane fraction but still able to cause significant effects on all four endpoints. Mortality and frequencies of abnormal larvae were induced in a dose–response manner in rainbow trout exposed to Stockholm City; the highest dose caused 78% more deaths and 180% more abnormal larvae than tri:lec controls. Even though a dose–response effect on mortality was observed in rainbow trout exposed to Stockholm Archipelago, only 67% more rainbow trout died compared with tri:lec control. Hemorrhages and abnormalities were induced in a dose–response manner, and more than 75% more larvae were affected compared with tri: lec control. These endpoints were also induced (>75% more than tri:lec control) in rainbow trout exposed to the highest dose of Reference Bay; asymmetric yolk sac and mortality were not affected.

3.3 Ranking

The results from both the tests with *N. spinipes* and rainbow trout are compiled in a ranking table (Table 4). If only considering the tests with *N. spinipes*, Polluted Bay was ranked most toxic with the score 13, followed by Polluted Fjord and Stockholm Archipelago with total score of 9 and

6, respectively. The least toxic locations were Stockholm City and Reference Bay with the score 5. The tests with rainbow trout showed a somewhat different pattern. The location with the most toxic potential was Polluted Fjord (score 25) and second most toxic were Polluted Bay and Stockholm City with the score 14. Stockholm Archipelago and Reference Bay were on shared third place (score 10). Note that more endpoints were investigated in the rainbow trout test, resulting in higher values of the scores compared with the *N. spinipes* test. When total sum of rank from both tests was used, the locations with the most toxic potential were Polluted Fjord and Polluted Bay. Stockholm City and Stockholm Archipelago were both ranked second most toxic and Reference Bay least toxic.

4 Discussion

A simple ranking system based on potential toxicity of organic sediment extracts in a crustacean and a fish was developed with the aim to facilitate hazard identification of sediment for commencing remedial actions. The sediments' total ranking differed to some extent if the tests with the two organisms was ranked separately or combined (see Table 4). The Polluted Fjord was ranked the second most potent in the test with *N. spinipes*, while it was ranked highest in the rainbow trout test (see Table 4). The hexane fraction from Stockholm City caused minor effects in *N. spinipes* but pronounced effects in rainbow trout, including asymmetric yolk sac, which has only been observed in rainbow trout exposed to polycyclic aromatic compounds (Sundberg et al. 2005a). The acetone fraction from Stockholm Archipelago caused strong effects in *N. spinipes* but only slightly enhanced effects in rainbow trout. The observed discrepancies are likely owing to species-specific differences, different routes of exposure, and the different test variables, illustrating the importance of including organisms with different exposure routes to characterize sediment toxicity (e.g., Keiter et al. 2006). As expected, the combined ranking (total sum of rank) showed that sediments from the highly contaminated sites Polluted Bay and Polluted Fjord were the most toxic to both organisms, and were accordingly ranked most toxic. Sediments from Stockholm City and Stockholm Archipelago were both ranked second most toxic. Surprisingly, the Stockholm Archipelago, which is situated in the outer archipelago of Stockholm and therefore considered a pristine location, showed some toxic effects

Table 4 Ranking of the five different sediments based on their potential toxicity to *N. spinipes* and rainbow trout

Species	Fraction	Endpoint	Locations				
			(A)Polluted Bay	(B)Polluted Fjord	(C)Stockholm City	(D)Stockholm Arch	(E)Reference Bay
N. spinipes	Hexane	Mortality	2	1	1	1	1
		LDR	4	3	2	1	2
	Acetone	Mortality	3	2	1	2	1
		LDR	4	3	1	2	1
	Sum		13	9	5	6	5
	Rank		1	2	4	3	4
Rainbow trout	Hexane	Hemorrhage	3	4	2	1	1
		Asymmetric yolk sac	3	3	2	1	1
		Abnormal	3	4	2	1	1
		Mortality	1	3	2	1	1
	Acetone[a,b]	Hemorrhage	1	3	1	2	2
		Asymmetric yolk sac	1	2	1	1	1
		Abnormal	1	3	2	2	2
		Mortality	1	3	2	1	1
	Sum		14	25	14	10	10
	Rank		2	1	2	3	3
N. spinipes and rainbow trout	Total sum of rank [a]		3	3	6	6	7
	Total Rank		1	1	2	2	3

A low score in the ranking line indicates a high potential toxicity. The total sum of rank is received by summation of the ranking of the two tests
[a] The desired concentration of the Polluted Bay's acetone fraction could not be properly dissolved in the carrier substance (tricaprylin:lecithin) to obtain the highest dose. This exposure's score might therefore be underestimated, see "Discussion" section.
[b] The location with the lowest total sum of rank were ranked as potentially most toxic to both *N. spinipes* and rainbow trout.

in the tests with the crustacean. However, Hansson et al. (2006) found out that perch in this area suffered from negative biochemical effects likely induced by pollutants. Reference Bay was ranked least toxic and had sediment causing low toxic effects in both organisms.

Silica gel has earlier been shown to be a suitable carrier of single hydrophobic substances using the same test system as was used for the tests with larval and juvenile *N. spinipes* in the present study (Breitholtz et al. 2007; Ulfsdotter Turesson et al. 2007). Here, we have shown that the silica gel-based test system with *N. spinipes* works equally well for hydrophobic sediment extracts; the exposure was simple and practical in the sense that the animals were easy to retrieve (compared to testing intact sediments with the same species). In preliminary experiments (not presented here) where silica gel was not used as a carrier, the extracts rapidly formed droplets, which were non-bioavailable to the copepods. The clear concentration–response relationship of both mortality and larval development in all tests proved silica gel to be a good way to expose these benthic crustaceans. In fact, toxic effects were observed for all the five locations even though the magnitude and response differed between the locations and fractions. Certainly, the bioavailability of the hydrophobic substances in the extracts was enhanced and likely overestimated the true toxicity, but for the purpose of hazard identification, generating false positives is better than generating false negatives (Hansson and Rudén 2007). For proper SQA, we however recommend that whole sediment toxicity tests be used for selected sites at subsequent tiers.

Toxicants enter fish via gills, skin, and through ingestion of food. In early life stages, maternal transfer is an important route of exposure for hydrophobic xenobiotics (Niimi 1983). The rainbow trout test system is demonstrated being a reproducible and reliable tool for investigating potential toxicity of hydrophobic substances (Ishaq et al. 1999; Sundberg et al. 2005a, b, 2006). In order to expose rainbow trout to the semi-polar acetone fractions, tricaprylin:lecithin was used as carrier substance. This carrier has successfully been used when exposing rainbow trout to polybrominated diphenyl ethers (unpublished). The evident dose–response effects and no observed effects in tri:lec controls in the present investigation advocates the capability of using this carrier when investigating semi-polar compounds.

A major benefit with biological testing in SQA is clearly demonstrated if only literature data of, e.g., PAH concentration at respective location would have been used for hazard identification (see Table 1; it should be noted that in this table, the levels of PCDs, PAHs, and PCDDs were analyzed in the same sediment samples (Stockholm City excluded) as have been used for toxicity testing in the current paper). For instance, Stockholm City would be considered more contaminated than both the Polluted Bay and the Polluted Fjord. If including also other pollutants, such as PCBs and PCDD/Fs, the interpretation would be even more complex.

The hexane fractions from Polluted Bay and Polluted Fjord caused the most severe effects in *N. spinipes*. Also, hexane fractions from Stockholm City and Reference Bay showed some toxicity. One explanation for the observed toxicity could be the high concentrations of PAHs measured in sediments from Stockholm City, Polluted Bay, and Polluted Fjord (see Table 1). Also, Reference Bay contained elevated concentrations of PAHs. Several investigations have demonstrated that PAHs cause toxic effects to crustaceans (e.g. Lotufo 1997; Lotufo 1998; Street et al. 1998). In addition, Polluted Bay contained high concentrations of PCBs, which in previous studies have shown to inhibit molting of both *Daphnia magna* and in *Uca pugilator* (Zou and Fingerman 1997, 1999). Polluted Fjord also contained high concentrations of PCDD/Fs, which could contribute to the observed toxicity. Notably, the hexane fractions from Stockholm City and Reference Bay were equally potent to *N. spinipes* even though the PAH levels were five times higher in Stockholm City, meaning that the toxicity cannot be explained by the analyzed PAHs.

The hexane fractions from Polluted Fjord and Polluted Bay were potentially most toxic to rainbow trout. Although further studies are warranted to investigate the causative agents of the asymmetric yolk sac in sediments from the Polluted Fjord and Stockholm City, three- and four-ringed polycyclic aromatic compounds in the sediment from the Polluted Bay caused this disorder (Sundberg et al. 2005a). The most potent PCDD congener to rainbow trout is 2,3,7,8- tetrachlorinated dienzo-*p*-dioxin (TCDD) with an LD_{50} value as low as 230 pg/kg egg (Walker and Peterson 1991). Persson et al. (2005) found 0.11 μg 2,3,7,8-TCDD/kg sediment in the Polluted Fjord, which corresponds to 6.6 ng/kg egg in the highest dose. Although the sum PCDD/Fs concentration in the Polluted Fjord adds up to 7,000 μg/kg sediment, the major part is represented by less potent PCDD/Fs congeners (Walker and Peterson 1991; Tanguay et al. 2003), suggesting that other compounds than those analyzed induces the toxicopathic responses in rainbow trout.

Acetone fractions (given prior elution using hexane) contain more polar compounds than are routinely analyzed in risk assessment and monitoring, e.g., parent PAHs, PCBs, and PCDD/Fs, which are isolated in the hexane fraction (Alsberg et al. 1985; Zebühr et al. 1993). Such semi-polar polar compounds may be oxidation products of PAHs, e.g., ketones, quinones, and acid anhydrides (Stenberg and Alsberg 1981). The acetone fractions from the majority of the investigated locations in the present study were potentially more toxic to *N. spinipes* than the corresponding hexane fractions. The semi-polar compounds in the acetone fraction are most likely thermodynamically more suitable for passing the water phase before entering the animals than those compounds in the

hexane fraction, which might make the compounds in the acetone fraction more bioavailable to *N. spinipes*. Semi-polar compounds in the sediments, except for the Polluted Bay (the highest dose of the acetone fraction could not be exposed to rainbow trout), did also cause adverse effects in rainbow trout. In the most contaminated locations, Stockholm City and Polluted Fjord, the sediments contained semi-polar compounds, which caused adverse effects on the same magnitude as those compounds isolated in the corresponding hexane fractions. In the more pristine locations, Stockholm Archipelago and Reference Bay, the sediments contained semi-polar compounds, which were more potent than those isolated in the corresponding hexane fractions. Although analyzes of the compounds found in the acetone fraction is warranted, this task was beyond the scope of this study. In addition, to investigate the analyzed pollutants' contribution to the observed toxicity, exposure of artificial mixtures must be performed. This strategy provides, however, poor explanatory background for the observed effects (Sundberg et al. 2006), demonstrating that other compounds than those pollutants commonly analyzed must be given greater attendance. Summing up, since both test systems demonstrated that a substantial part of the potential toxicity was caused by semi-polar compounds in the acetone fractions and that the analyzed pollutants did only explain a minor part of the effects caused by the hexane fraction, this study enlightens our poor understanding of which compounds cause adverse effects in environmental samples. Therefore, by investigating potential toxicity (i.e., hazard identification) as a first screening step in a prioritizing process, these implications could be avoided.

5 Conclusions and recommendations

Two major conclusions can be made from the present study. First, the clear concentration–response relationship of both mortality and larval development in all tests with *N. spinipes* shows that silica gel is an excellent way to expose this benthic crustacean to very hydrophobic and otherwise non-bioavailable sediment extracts. Second, it is crucial to include toxicological information at lower tiers in SQA. The sediments from the Polluted Bay, the Polluted Fjord, and Stockholm Archipelago contain presently unknown toxic semi-polar compounds (isolated in the acetone fraction), which are normally not considered in SQA since the focus mainly is on those compounds that are analyzed by mere chemical analyses of the hexane fraction. This enlightens our poor understanding of which compounds cause adverse effects in environmental samples. Therefore, by investigating potential toxicity (i.e., hazard identification) as a first screening step in a prioritizing process, these implications could be avoided. Since the ranking of the five locations followed the expected pattern of toxicity in both test organisms, we are convinced that the proposed ecotoxicological approach may serve as a useful screening tool in prioritizing processes of contaminated sites. For proper SQA, we however recommend that whole sediment toxicity tests be used for selected sites at following tiers.

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