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Toxicity and neurotoxicity profiling of contaminated sediments from Gulf of Bothnia (Sweden): a multi-endpoint assay with Zebrafish embryos

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Abstract

Background: The toxicological characterization of sediments is an essential task to monitor the quality of aquatic environments. Many hazardous pollutants may accumulate in sediments and pose a risk to the aquatic community. The present study provides an attempt to integrate a diagnostic whole mixture assessment workflow based on a slightly modified *Danio rerio* embryo acute toxicity test with chemical characterization. *Danio rerio* embryos were directly exposed to sieved sediment (≤ 63 µm) for 96 h. Sediment samples were collected from three polluted sites (Kramfors, Sundsvall and Örnsköldsvik) in the Gulf of Bothnia (Sweden) which are characterized by a long history of pulp and paper industry impact. Effect data were supported by chemical analyses of 237 organic pollutants and 30 trace elements.

Results: The results show that malformations and neurotoxic compounds are the main drivers of differentiation in chemical and effects analyses, respectively. Specific spinal cord malformations and delayed hatching were observed only in sediments from Kramfors while light hyperactivity was seen only after exposure to sediments from Sundsvall.

Conclusions: Our experiments demonstrate that specific chemical profiles lead to specific effect patterns in *Danio rerio* embryos. In fact, behavioral endpoints could help detect the exposure to neurotoxins, and the observation of body malformations seems to be a potential tool for the identification of site-specific pollutants as polychlorinated biphenyl (PCBs), brominated flame retardants (BFRs) and several pesticides. Overall, results show the suitability of *Danio rerio* embryos for the fast screening of sediment samples.

Keywords: *Danio rerio* embryos, Sediments, Monitoring, Neurotoxicity, Behavior, Mixtures

Background

Sediments are a well-known sink for a large variety of pollutants that may cause distress to benthic and pelagic species in case of their remobilization to the water-phase [1]. Risk assessment of complex mixtures may involve component-based approaches by applying chemical analysis together with measured or predicted toxicity data of individual components and mixture risk modeling or whole mixture approaches using bioassays. Both are complementary and thus their integration using chemical and bioanalytical tools for the characterization of sediment contamination should provide a more comprehensive picture of ecotoxicological risks to aquatic organisms [2]. In addition to effect concentrations, bioassays may provide information on modes of action (MoA) and may thus improve the diagnosis of risks in sediment samples [3–10]. In the last years, batteries of sensitive, rapid and cost-effective in vitro bioassays have been proposed as promising tools for the toxicological profiling

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of environmental samples and were also successfully applied for the characterization of aquatic sediments [11–17]. However, despite the undoubted advantages of cell-based assays for the analysis of toxicological patterns in sediments, in vitro results cannot directly predict the biological responses in more complex organisms and aquatic communities [18]. Moreover, only a minority of MoA covered by in vitro assays are suited to test complex environmental mixtures [19] including important toxic endpoints such as neurotoxicity [20]. Thus, in vitro tools need to be complemented with diagnostic in vivo monitoring. However, accepted and validated workflows for the in vivo toxicological profiling of complex mixtures including sediment contamination are still lacking.

One of the most promising organisms for diagnostic in vivo testing of sediment extracts is zebrafish (*Danio rerio*) embryos, which are a versatile model suitable for high-throughput analysis while keeping several advantages of in vitro approaches (i.e., low-cost, sensitivity, short duration of the test). The fish embryo toxicity test (FET) with *Danio rerio* has been considered as a good surrogate for the acute toxicity fish test [21] and was successfully used in several studies for the detection of toxicity and neurotoxicity in sediments samples [9, 22–24]. One of the major advantages of the FET with *Danio rerio* is the possibility to monitor several toxic endpoints including the modification of molecular processes and malformations which can be related to the exposure to specific pollutants [25–28]. Further, the FET with *Danio rerio* offers the possibility to monitor changes in behavioral patterns (i.e., swimming activity, early spontaneous movements), which may be relevant also for the population level [29, 30].

The present study provides a first attempt to integrate a diagnostic whole mixture assessment workflow based on in vivo toxicological profiling of *Danio rerio* after exposure to sediments for 4 days with a component-based approach applying chemical analysis of a wide target list of organic and inorganic chemicals. The objectives of the present study were (1) to validate this approach with sediments collected from the coast of Gulf of Bothnia (GoB, Baltic Sea, Sweden) (2) to identify effect patterns with multi-endpoint diagnosis in zebrafish embryos and support them with chemical analyses and (3) to offer a first in vivo ecotoxicological profile of sediment samples.

Methods

Sampling and sample preparation

Sediments were collected in GoB in an extensive sampling campaign in 2013 as part of the Swedish research project REACT. Three sites were selected in the bays of the city of Kramfors, Örnsköldsvik and Sundsvall, Sweden. With a grab sampler, samples from the top layers of the sediments with 5–10 cm thickness were taken. The three sites have a long history of pulp and paper industry, saw mills, as well as other industrial activities with possibly elevated levels of a series of legacy contaminants in the sediments, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCBs), organochlorine insecticides (OCPs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and metals. The three sites are also characterized by the input of waste water treatment plants (WWTPs) and, thus, may receive additional micropollutants. After sampling, the samples were stored in triplicate in plastic containers, transported to the laboratory on ice and stored at − 20 °C until freeze-drying. The freeze-dried sediments were sieved to 63 µm and stored in brown glass bottles at − 20 °C until further treatment. Details about sampling sites, total organic carbon, total inorganic carbon and black carbon are provided in Additional file 1: Table S1.

Sediment extraction for chemical analyses

For LC- and GC-HRMS analyses for organic compounds, samples were extracted according to Massei et al. [31] with slight modifications. Extraction and analysis were based on the amount of total organic carbon (TOC) rather than on dry weight assuming TOC as the predominant phase for the accumulation of chemicals and to keep matrix effects in MS on a similar level. Further details of the pressurized liquid extraction (PLE) method and the preparation of the normal phase (NP) for clean-up are given in Additional file 1: S1.

For trace element analysis the sequential extraction procedure followed the three-steps sequential extraction of Rauret et al. [32]. Blanks and certified reference material (CRM) BCR-701 were used at every step of the extraction process to evaluate the instrumental method and for calibration. The element recovery rates from the CRM in each extraction solution were between 85 and 112% and similar to values published by Margui et al. [33] and Tokalıoğlu and Kartal [34]. All samples were finally digested in aqua regia as follows. Aliquots of 250 mg of sample material were funneled into quartz microwave vessel with aqua regia (6 mL HCl; 37%, 2 mL HNO_3; 65%) in a pressure- and temperature-controlled microwave. The elements in the soil extracts and digests were determined by inductively coupled plasma MS (ICP-MS/MS; Agilent 8800, Agilent Technologies, Germany) according to the norm for application of inductively coupled plasma MS (ICP-MS) EN ISO 17294-2:2017-01 [35].

Targeted chemical analysis of organic and inorganic pollutants

A list of 237 organic compounds likely to occur in sediments [i.e., pesticides, industrial chemicals, steroids, persistent organic pollutants (POPs)] and 30 trace elements including the main heavy metals were selected for chemical analysis covering a wide range of physicochemical properties. Details on equipment, target compounds list, LC, GC, and MS conditions, quantification, method detection limits (MDLs), and internal standards are provided in Additional file 1: S2–S4, Tables S2–S6, S9; Additional file 2: Tables S7, S8. Additional methodological details on target chemical analyses are described in Massei et al. [31].

Fish culture, fish embryo production and selection

Adult zebrafish of the strain UFZ-OBI had been originally established from a wild type strain purchased from a local supplier (OBI hardware store, Leipzig) and had been bred at the UFZ for more than 13 generations. Fish were kept in 14-L aquaria with 25–30 fish each with a sex distribution between female to male of 1:2. The light–dark rhythm was 14:10 h and the water temperature was 26 ± 1 °C. Water parameters were measured frequently (pH 7–8; water hardness 2–3 mmol/L, conductivity 540–560 µS/cm, nitrate < 2.5 mg/L, nitrite < 0.025 mg/L, ammonia < 0.6 mg/L, oxygen saturation 87–91%). Within 30 min after spawning, eggs were collected using a grid covered dish and successively cleaned with aerated ISO standard dilution water (ISO-water) as specified in ISO 7346-3. Developmental stages were identified according to Kimmel (1995). The fish embryo acute toxicity test was conducted as described in the OECD TG 236 and in Bittner et al. [36], but additional endpoints were included.

Danio rerio multi-endpoints toxicity assay with sediments contact test

Twenty fertilized eggs were directly exposed to the freeze-dried sediments fine fraction (≤ 63 µm) in 40 mL of ISO-water (1 embryo/2 mL ISO-water) for 105 h at a temperature of 26 ± 1 °C and a photoperiod 12:12 light/dark according to the sediment contact protocol by Hollert et al. [22] with few modifications. Sediments were completely covering the bottom of the crystallization dish (total amount between 0.25 and 0.5 g) and agitated with 55 rpm. Negative control consisted in pure ISO-Water without adding inert particle or blank quartz powder. Table 1 gives an overview on the toxicological endpoints registered during exposure. Endpoints were selected according to their toxicological relevance, and their characterization in previous studies [37].

At 48-h post-fertilization (hpf) the heart is formed in two chambers and it is possible to count a regular heartbeat. The heartbeat rate was assessed by direct observation of the heart of the 5 randomly chosen embryos for 10 s. Heartbeat was counted manually and measurement time did not exceed 2 min to avoid temperature drops. From 72 hpf the hatching rate becomes stable for most of the larvae. The numbers of hatched embryos were counted at 24, 48, 72, and 96 hpf. An embryo was considered hatched when its body completely left the chorion. After 96-h embryos were selected for further locomotor activity (LA) determination [38]. For the evaluation of LA, 16 embryos for each sample and negative control were transferred to a 96 wheel plate with rectangular angles (one embryo per wheel, with 500 µL of clean ISO water). A randomized plate set-up was used to avoid interference in behavioral analyses. LA was only assessed for samples with mortality below 50% and a hatching rate above 50%. LA was registered over 50 min at a light/dark regime of 10-min dark, 20-min light and 20-min dark. Embryonic movement was tracked using the ZebraBox video tracking system (Viewpoint, Lyon, France) at a temperature of 28 ± 1 °C. Prior to the measurements, embryos were acclimated in the device for at least 10 min.

After LA evaluation, embryos were pooled together and used for acetylcholinesterase (AChE) analysis according to Küster [39]. Mortality and sublethal effects (i.e., yolk and pericardial edema, body deformations) were registered every 24 h and dead embryos were removed from the exposure media. The test media was not renewed during exposure.

One negative (ISO-Water) and one positive control (3,4-dichloroaniline, 3.4 mg/L) were tested in each experiment. The experiments were conducted in three independent replicates ($n = 3$). No fungal or biofilm growth was observed during or at the end of the exposure.

FET quality control

At the end of exposure, mortality of the negative control did not exceed 10%. Mortality of positive controls was in the expected toxicity range (between 30 and 80%). Oxygen concentration and pH were above the limits described by Andrade et al. [40] for developmental retardation and other sublethal effects (dissolved oxygen ≥ 6 mg/L; pH between 6 and 8). Thus, observed effects have to be considered effects of the exposure to sediment-bound chemicals and no other physical stressors.

Table 1 The different endpoints observed along the 105-h exposure in *Danio rerio* embryos, their associated mode of action and major classes of contaminants associated

Endpoints	Observation stage (hpf)	Type of toxicity	References	Active compounds
Modulation of heart rate	48	Cardiotoxicity: Decrease (bradycardia) or increase (tachycardia) of the heartrate. Blocks of the β-adrenergic receptor, mainly on the myocardium	Carlsson et al. [73] Fraysse, Mons and Garric [25]	Mainly β-blockers (i.e., propranolol, metoprolol), PPCPs, several insecticides
Delayed hatching	From 72 to 96	Inhibition of high choriolytic enzymes and physical movement of the embryo	Jin et al. [74] De Gaspar et al. [75]	Several inorganic and organic pollutants
Inhibition of locomotor activity	96	Neurotoxicity: decrease or increase of the swimming distance and dark/light stimulation	Irons et al. [76] Selderslaghs et al. [77]	Several neuroactive pollutants
Inhibition of AChE activity	105	Neurotoxicity: inhibition of the acetylcholisterase and increase of the duration of action of neurotransmitter acetylcholine	Küster [39] Strmac et al. [78] Kais Stengel, Batel and Braunbeck [9]	Organophosphates and carbamates insecticides, organophosphates flame retardants (OPFR), polycyclic aromatic hydrocarbons (PAHs)
Sublethal and mortality effects	From 24 to 105	Embryotoxicity and teratogenicity	Scholz et al. [79]	Several inorganic and organic pollutants

Data treatment, multivariate, and statistical analysis

Multivariate statistical analyses and *k*-clustering were performed to group sites according to their chemical pattern. Data were log transformed, centered and normalized to avoid misclassification due to the differences in data dimensionality [41]. For *k*-means clustering, the Euclidean distance between sites was used to evaluate similarity and the Ward's method was used in the linkage between sites and chemical patterns [41]. Statistical difference between control and treatments for lethality, heartbeat, hatching, and AchE inhibition were detected using a Tuckey multiple pairwise-comparisons test. For behavioral analysis, statistical differences between treatments and controls were detected by a non-parametric Kruskal–Wallis test with post-Bonferroni correction. All statistical analyses were performed using Microsoft Excel 2010®, Sigma Plot® (version 12.0.0.182), the software RStudio® (version 1.0.136) and the R packages drc, MASS, FactoMineR, factoextra, FitAR, chemometrics,ggplot2, dplyr, ggpubr, magrittr, and car.

Results

Occurrence and patterns of organic and inorganic pollutants

Organic pollutants

Out of the 237 selected target compounds analyzed (Additional file 2: Table S8), 71 compounds (27 PAHs, 10 PCBs, 14 pesticides and biocides (PEST), 7 compounds from industrial origin, 7 brominated flame retardants (BFR) and other brominated compounds (BC), and 6 more compounds from various groups were detected.

Among the three sites, the area of Kramfors showed the highest loads of chemicals with 53 identified compounds and a cumulative concentration of 3.6 µg/mg TOC. Native sediments from Örnsköldsvik and Sundsvall showed lower cumulative concentration with 160 and 163 ng/mg TOC, respectively.

The site of Kramfors showed higher cumulative concentrations of PAHs (3.3 µg/mg) with the 3- and 4-ringed phenanthrene and fluoranthene detected at concentrations of 830 and 535 ng/mg TOC. The sites of Sundsvall and Örnsköldsvik showed cumulative concentrations of PAHs of 133 and 146 ng/mg TOC, respectively. Additionally, BFRs were detected only in Kramfors with concentrations ranging from 0.02 to 1.1 ng/mg TOC.

The two fungicides irgarol and fenpropimorph (20 and 6 ng/g TOC), the herbicide chloridazon (0.03 ng/mg TOC) and the insecticide cyhalothrin (1.1 ng/mg TOC) were detected only in the area of Örnsköldsvik and not at other two sites of the GoB. Moreover, we identified in Örnsköldsvik the two herbicide transformations products, 2-hydroxyatrazine and 2-hydroxy-terbuthylazine- (both 0.1 ng/mg TOC).

Concentrations of organic pollutants for each site and compound are provided in Additional file 2: Table S10.

Trace elements

Overall, native sediments from the three sites of GoB showed similar total metal concentrations [from 50 up to 57 g/kg dry weight (d.w.)]. In particular, the pseudo total concentrations (aqua regia digested) of the seven priority elements proposed by the Swedish EPA monitoring list [42] (As, Cd, Cr, Cu, Ni, Pb and Zn) ranged from 0.33 to 165 mg/kg d.w. The metal concentrations (Additional file 2: Table S10) in the three fractions with different degrees of bioavailability F1 (weekly associated to carbonates, considered as readily bioavailable), F2 (reducible fraction and potentially bioavailable after perturbation) and F3 (bound to humic substances and sulfides and not bioavailable) are shown and discussed in Additional file 1: S5.

It may be summarized that metal concentrations at all three sites of GoBs were similar to background values at the river Elbe [43] as well as with the standard from Turekian and Wedepohl [44]. Total priority element concentrations detected in our study were in average 22 times lower than the one reported for contaminated surface sediments from Oskarshamn harbor (Sweden) [45] and are thus considered as unlikely to drive toxic effects.

K-means clustering of organic chemicals sediment contamination highlighted the presence of ubiquitous and site-specific pollutants (Fig. 1). We identified one cluster with compounds that are ubiquitous in all the sites of GoB (green cluster, UBI).

The green cluster contains 23 PAHs and six additional compounds (2,6-diisopropylnaphthalene, dioctyldiphenylamine, diphenyl sulfone, m-Terphenyl, hexachlorobenzene and alpha-tocopherol acetate). A second cluster (red cluster, KR) is composed by compounds that were detected mostly in the area of Kramfors. The site was characterized by 5 BCs, 3-bromocarbazole, the two PCBs 118 and 52, the PAH indeno (1,2,3cd)fluoranthene and three sulfonated compounds. A third cluster (green cluster, SU&OR) contains chemicals which characterized the sites Sundsvall and Örnsköldsvik. The green cluster was characterized by the insecticide DDT and its metabolites, 8 PCBs congeners, 4 fungicides (carbendazim, fenpropimorph, irgarol and chloridazon), the biocide triclocarban, the transformation products of atrazine (2-hydroxyatrazine) and terbuthylazine (2-hydroxy-terbuthylazine), the PAHs 1-phenylnaphthalene, 9-vinylanthracene, cis-stilbene, *o*-terphenyl, hexachlorobenzene, and the pyrethroid cyhalothrin.

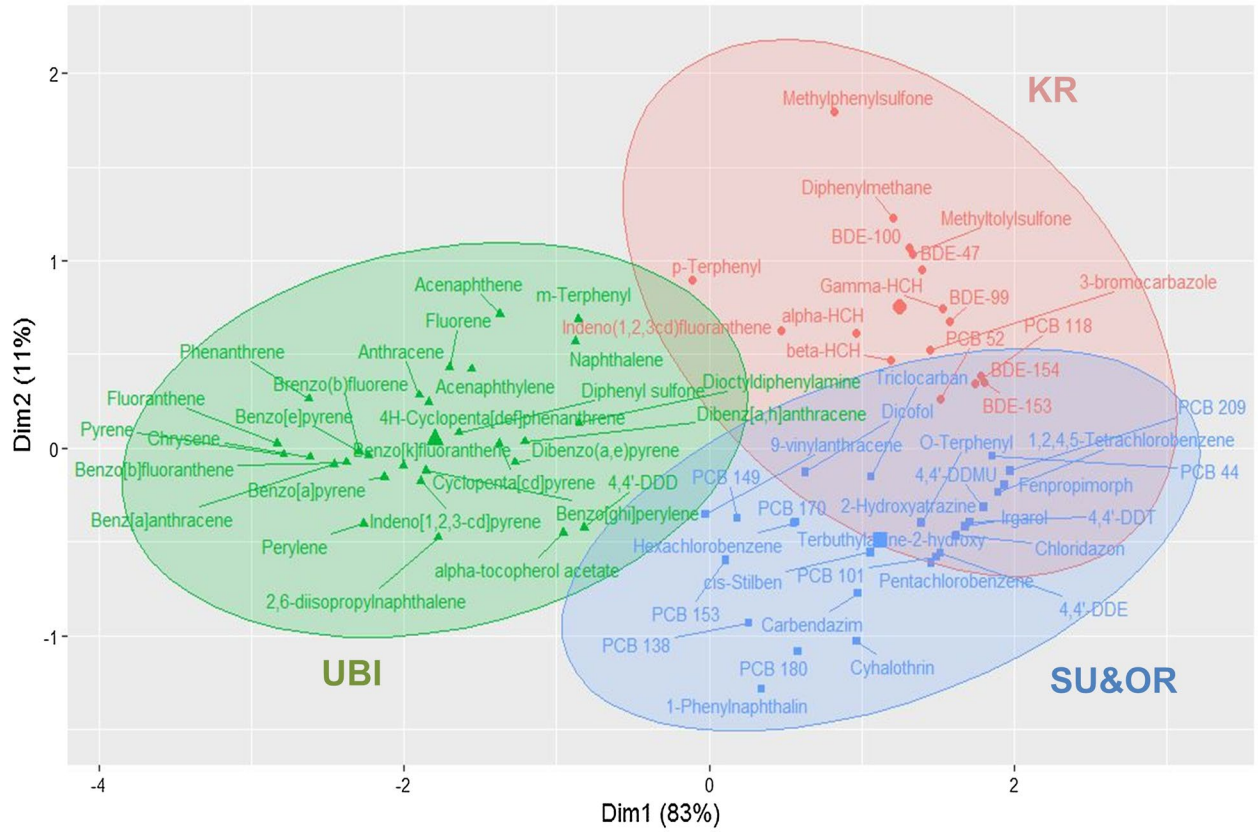

Fig. 1 K-means cluster of 71 detected organic chemicals (UBI: Ubiquitous; KR: Specific in Kramfors; SU&OR: Specific for Sundsvall and Örnsköldsvik). Data were log normalized and centered before analysis (Normal probability ≥ 0.99). No specific contamination patterns were highlighted for inorganic chemicals by k-means cluster analyses (data not shown)

No specific contamination patterns were highlighted for inorganic chemicals by *k*-means cluster analyses due to similar trace element concentrations in the background range among the three sites (data not shown).

Sediments toxicological profiling with *Danio rerio* embryos

Lethal and sublethal effects

After 96 h, all tested sediments showed low lethality comparable to negative control (10%), see Fig. 2a.

In all of the tested samples the embryos showed clear developmental retardation or yolk/pericardial edemas. All embryos exposed to sediments from Kramfors exhibited bent spines (Additional file 1: Fig. S3) and slow movements with twitching spasms. Embryos exposed to sediments from Sundsvall did not show clear sublethal effects while embryos exposed to sediments from Örnsköldsvik occasionally exhibited side swimming and slightly slower movements as compared to the control.

Heartbeat

After 48 h, native sediments from all sites caused a decrease of the embryos heart beat (average decrease 39%). The inhibition of the heartbeat was particularly strong in embryos exposed to sediments from Kramfors (47%). All three sites were statically different from the control ($p \leq 0.01$). Results are shown in Fig. 2b.

Hatching rate

Embryos exposed to sediments from Örnsköldsvik and Sundsvall showed a hatching rate similar to the negative control. Embryos exposed to Kramfors had lower hatching rates at 48 (no hatching) and 72 (decrease of 84%) hours compared to those exposed to sediments from the other sites ($p \leq 0.01$). After 96 h of exposure, the hatching rate of all exposed embryos was comparable in all samples. Results are shown in Fig. 2c.

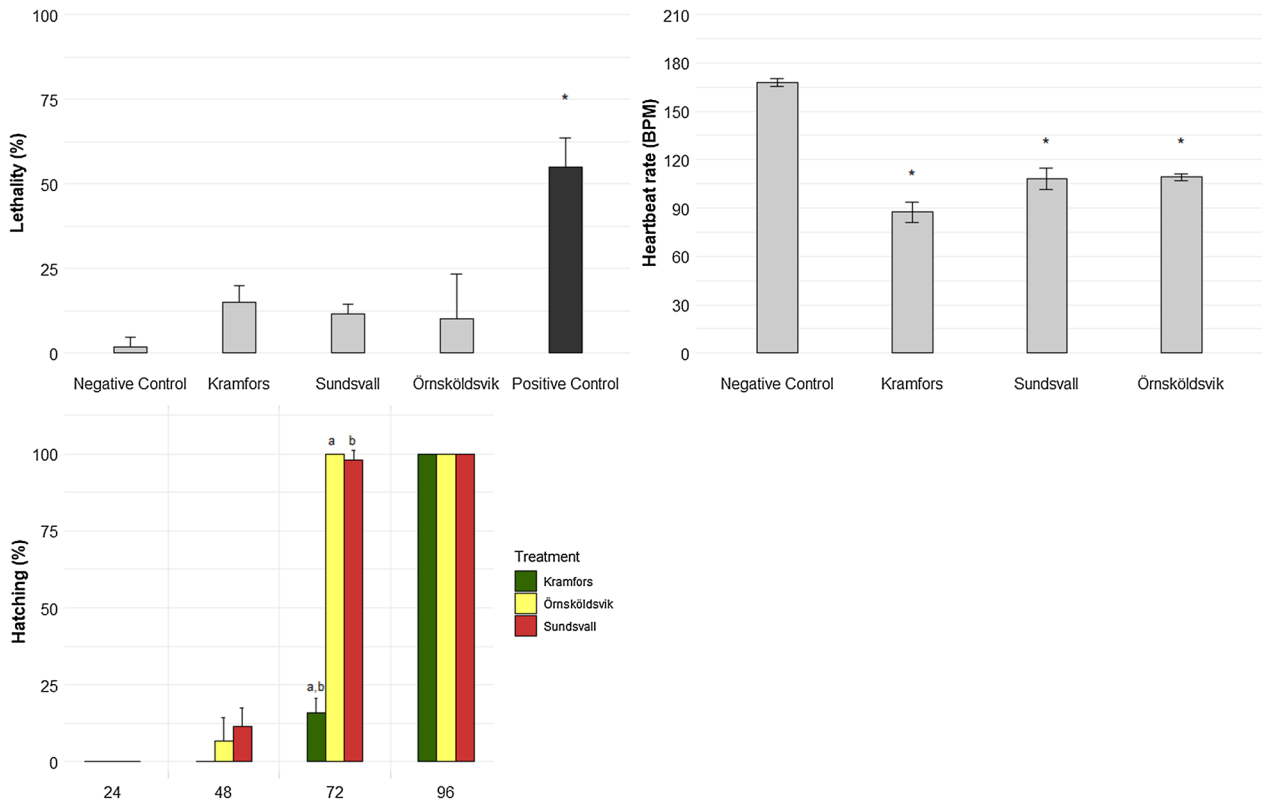

Fig. 2 Embryo toxicity (**a**), heart beat (**b**) and cumulative hatching (**c**) of *Danio rerio* embryos after exposure to native sediments of Gulf of Bothnia. Experiment was performed in 3 independent replicates (20 embryos). ISO-Water was used as negative control and 3,4-dichloroaniline was used as positive control (3.4 mg/L). The symbol * indicates a statistical difference between treatments or between treatments and the control ($p \leq 0.01$). The symbols a and b indicates a statistical difference between treatments ($p \leq 0.01$). Error bars represent standard deviation

LA

Negative controls showed low swimming activity during both light phases. However, the first 10 min of the dark phase were characterized by a peak of activity followed by a steady decrease of total LA in the next 10 min. This trend was already observed by other authors in previous studies [46–48].Considering this trend, we analyzed the movement of fish embryos at four different phases: first light phase (L1), first 10 min of the dark phase (D1), second 10 min of the dark phase (D2) and second light phase (L2).

Embryos exposed to native sediments from GoB caused a general increase of the movement during L2. The increase was particularly strong for native sediments for Kramfors (3.1, $p \leq 0.001$) and Sundsvall (3.7, $p \leq 0.001$). Embryos exposed to native sediments from Sundsvall exhibited a general average increase of movement during D2 (2.6 times, $p \leq 0.001$). Moreover, sediments from Sundsvall caused an increase of movements during L1 (2.4, $p \leq 0.001$). Embryos exposed to sediments from Kramfors showed stronger inhibition of the movement (0.3 times, $p \leq 0.001$) during D1 and increase during L1 (2.7, $p \leq 0.001$). Lastly, native sediments from Örnsköldsvik exhibit values that are comparable with the controls and a slightly higher activity during D1 (1.4, $p \leq 0.001$) and D2 (2.3, $p \leq 0.001$). Results are shown in Fig. 3.

Inhibition of AChE

At 105 h, there was no statistical significance difference in the AChE activity in *Danio rerio* embryos exposed to native sediments from GoB and negative controls (Additional file 1: Figure S1).

An overview on the toxicological patterns in sediments from the three sites is given in Table 2.

Discussion

The major goal of this study was to validate a multi-endpoints in vivo assay to perform a fast identification of toxicological patterns in aquatic sediments. In this context, our results confirmed that different chemical mixtures lead to specific effects patterns in *Danio rerio* embryos. In particular, we found that the chemical mixture at site Kramfors mainly resulted in developmental malformation, while the ones detected in Sundsvall and

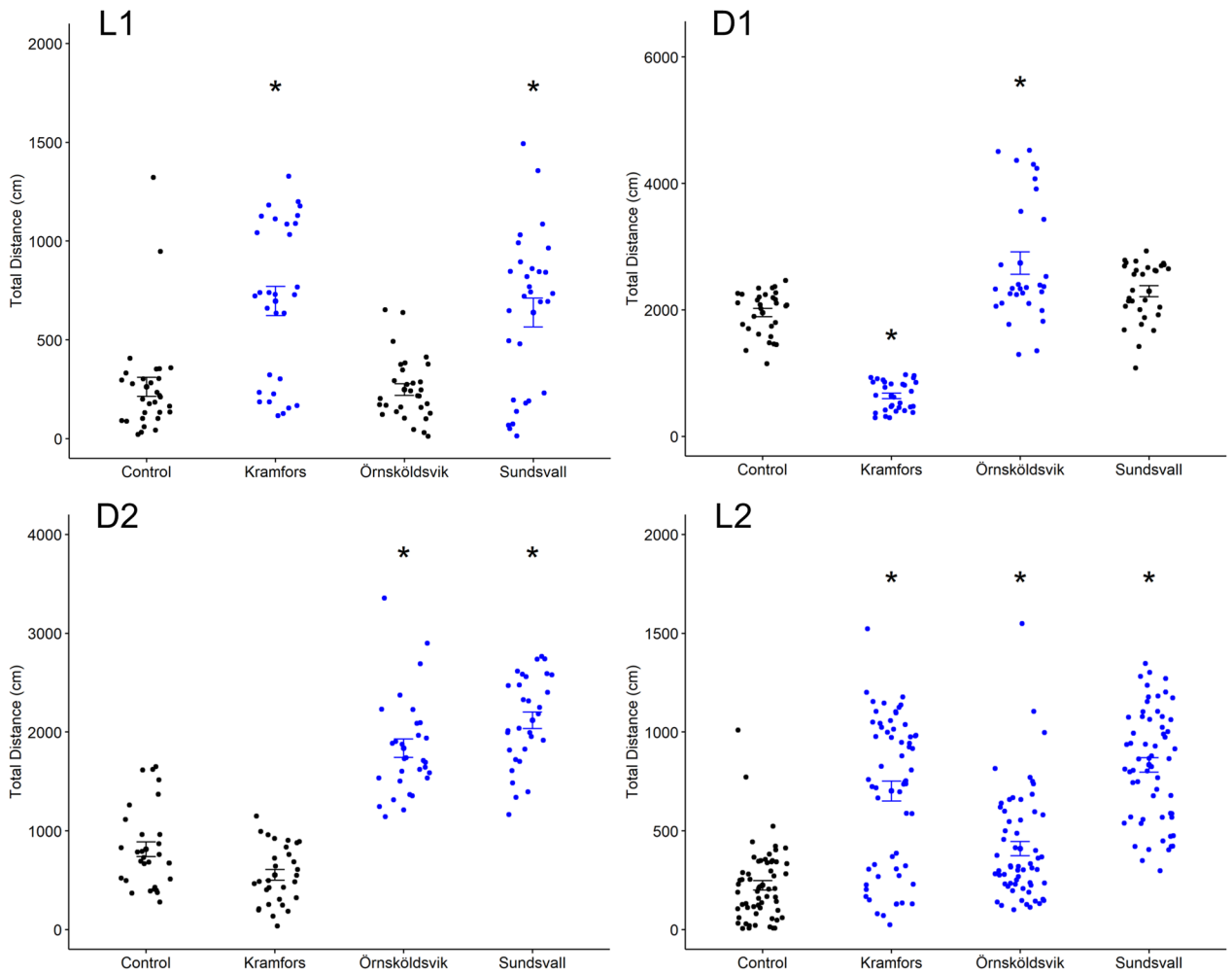

Fig. 3 Total distance (cm) of 96 hpf Danio rerio embryos during first light phase (L1), first 10 min of the dark phase (D1), second 10 min of the dark phase (D2) and second light phase (L2). Single dots represent the summed total distance of 16 embryos during each light/dark phase. The blue data sets market with * highlights statistical differences between control and treatments ($p \leq 0.001$). Centre point show mean and error bars 95% confidence intervals

Table 2 Toxicological pattern observed *Danio rerio* embryos after 96-h exposure to surface sediments from Gulf of Bothnia (fraction ≤ 63 µm)

Endpoints	Kramfors	Örnsköldsvik	Sundsvall
Lethality	Comparable with control	Comparable with control	Comparable with control
Body malformations	Bended spine	Comparable with control	Comparable with control
Heartbeat	Decrease ($p \leq 0.01$)	Decrease ($p \leq 0.01$)	Decrease ($p \leq 0.01$)
Movements increase	L1 (2.7-fold increase) and L2 (3.1-fold increase)	D1, 2 and L2 (1.4-, 2.3-, and 1.8-fold increase)	L1, D2, and L2 (2.4-, 2.6- and 3.7-fold increase)
Movements decrease	D1 (0.4-fold decrease)	Comparable with control	Comparable with control
AChE inhibition	Comparable with control	Comparable with control	Comparable with control
Hatching	Retarded at 72 hpf ($p \leq 0.01$)	Comparable with control	Comparable with control

Örnsköldsvik acted primarily on the swimming activity (Table 2).

Overall, the selected endpoints offered a comprehensive toxicological characterization of complex environmental mixtures. In particular, developmental malformations and delay in hatching were valid endpoints for the characterization of sediment pollution. In fact, abnormal spinal curvatures (i.e., kyphosis) and hatching problems were observed only in embryos exposed to sediments from Kramfors. However, it is important to underline that several chemicals that were detected in the three sites of GoB (i.e., PAHs, BDEs, heavy metals) were reported to cause spinal curvatures, general developmental toxicity, degeneration of myocytes, neural cell death and delayed hatching [45–55]. Thus, it may be hypothesized that the higher concentrations of PAHs in Kramfors (24-fold higher compared to those measured in Sundsvall and Örnsköldsvik) may actually be responsible of the observed effect. In fact, it has been shown that teratogenic effects in early fish life stages are mainly caused by high PAHs concentrations [26, 56].

In this case, effect-directed analysis (EDA) or toxicity identification evaluation (TIE) could help to reduce complexity of the environmental mixture and allow for the identification of specific causative chemicals [57]. Moreover, additional non-destructive morphological endpoints (i.e., craniofacial, eyes malformations, tail and somite malformations) may be included in future studies to increase the screening power and specificity of the present workflow. As an example, innovative vertebrate automated screening technology (VAST) may be used to obtain high-content automatic imaging of *Danio rerio* embryos phenotypes [58].

Our workflow with *Danio rerio* embryos confirmed also the possibility to obtain a comprehensive neurotoxicological profile of environmental samples. As recently discussed by Legradi et al. [59] in a recent review, the identification of neuroactive chemicals in the environmental is an important emerging issue. In fact, more than 30,000 commercially used chemicals may have a neurotoxic potential and they could induce changes in the organism behavior leading to severe effects on the ecosystem [59].

In our study, sediments from Kramfors caused an inhibition of the movement during the dark phase, while sediments from Örnsköldsvik and Sundsvall induced an increase of the movement during the dark phases.

The higher swimming activity observed when the embryos were exposed to sediment extracts from Kramfors was probably influenced by spine malformations, occurring as visual impairments in the embryos. In fact, problems with the development of the retinal cells may lead to increased sensitivity to light and impaired behaviors [60]. In this case, the high concentrations of PAHs in Kramfors may also cause developmental defects of the retina, by up-regulating the aryl hydrocarbon receptor (AhR) [61]. Previous findings that environmental concentrations of phenanthrene caused the reduction of cell proliferation in the retina of zebrafish [61] support this hypothesis. It is also possible that the increase of activity during light phase may be related to a specific effect on the nervous system of the embryos. The hypothesis is confirmed by the similar chemical profiles of Örnsköldsvik and Sundsvall (Fig. 1), despite showing a slightly different effect patterns on the swimming behavior during the dark phase. As shown in previous studies, behavioral patterns were suggested to be a sensitive measure of ecotoxicological effects since they respond earlier than other endpoints [62]. Moreover, changes in behavior can be often linked to alteration at higher levels of biological organization [62]. Behavioral patterns were shown to be a robust, sensitive and reliable tool that can be used as early indicator of stress, and thus were used also to understand the effect at population and ecosystem level [29, 30]. Behavioral endpoints were also successfully applied for the identification of toxicity of several compound classes with neurotoxic MoA, including pesticides mixtures, pharmaceuticals and personal care products (PPCPs), metals and classical POPs [63–66]. However, it may be expected that neurotoxic effects are not restricted to these chemicals, but may be exhibited by many more. Thus, it will be hardly possible to identify individual drivers of neurotoxicity using effects on behavioral endpoints. In fact, many lipophilic neurotoxins accumulating in sediments primarily target membrane sodium channels and cause excitability of the nerves and muscles [67, 68]. Moreover, several anti-inflammatory drugs (steroidal glucocorticoids, nonsteroidal anti-inflammatory drugs, phosphodiesterase inhibitors), and dopaminergic antagonists may increase the waking activity during the light period [69]. However, the alteration of behavior may not only be related to the presence of neuroactive compounds. Since the development of the nervous system is influenced by several upstream molecular events (i.e., cellular replication, migration, differentiation, synaptogenesis), xenobiotics may influence processes that are not directly related to functional impairment, but interference with neurodevelopment [70]. Accordingly, potential effects on cardiac and muscle development could affect behavior as well [70]. The detection of these developmental effects would require appropriate detailed microscopical observations and markers that have not been considered in our screening.

Finally, our results highlight the fact that the heart beat rate may be considered a good marker of general stress, but not a good endpoint for the identification of specific

toxicological patterns. It was shown that several PAHs (phenanthrene, naphthalene and benzo(k)fluoranthene) may induce a general bradycardia in zebrafish embryos and defects in the heart structure [71, 72], but also heavy metals for example may exhibit cardiovascular disturbance as heart underdevelopment and, in extreme cases, absence of cardiac muscles [53].

Conclusions

The present study highlights the added value of in vivo toxicological profiling with multi-endpoint FET with a specific focus on sublethal effects and neurotoxicity. This approach may integrate behavioral effects and malformations to discriminate toxic environmental mixtures (here sediment extracts) based on the symptoms they cause. Parallel chemical analysis may provide some insight into possible drivers. However, it is necessary to develop new methods and approaches to better link the observed effect to chemical drivers. The required efforts include testing of a broader range of chemicals and mixtures in multi-endpoints FET together with mechanistic research to better understand the development of the symptoms. An increasing number of mechanistically linked endpoints are needed including specific phenotypic endpoints and modified gene expression to target specific toxicity pathways. EDA and TIE studies may help unravel drivers of toxicity in environmental mixtures.

Additional files

Additional file 1: S1. Sediments extraction and clean-up. **S2**. GC-HRMS and LC-HRMS methods. **S3**. Trace Finder parameters. **S4**. Method detection limits. **S5**. Trace elements analyses. **Table S1**. Sediments characteristics and sampling spot information. **Table S2**. GC oven program. **Table S3**. Injection details for pulsed split less injection for GC-analysis. **Table S4**. Parameters of the GC-QExactive HRMS method. **Table S5**. LC gradient program. **Table S6**. Settings of the Trace Finder software. **Table S9**. Chemicals and Equipment. **Figure S1**. Sampling spot map. **Figure S2**. Acetylcholisterase inhibition in Danio rerio embryos exposed to sediments of Gulf of Bothnia. **Figure S3**. Zebrafish embryos after 96 hpf exposed to sediments of Kramfors and Örnsköldsvik.

Additional file 2: Table S7. Method detection limits (MDLs) for the detected compounds in sediment samples. **Table S8**. Target compounds list, their log D values at pH 7 and the internal standard used for quantification. **Table S10**. Concentrations of detected compounds in sediments of Gulf of Bothnia.

Abbreviations

AChE: acetylcholisterase; BFR: brominated flame retardants; BR: brominated compounds; CRM: certified reference material; EDA: effect-directed analysis; FET: fish embryo test; GC: gas chromatography; GoB: Gulf of Bothnia; Hpf: hours post-fertilization; HR: high resolution; ICP: inductively coupled plasma; LC: liquid chromatography; MDLs: method detection limits; MoA: mode of action; MS: mass spectrometer; NP: normal phase; OCPs: organochlorine insecticides; PAHs: polycyclic aromatic hydrocarbons; PCBs: polychlorinated biphenyl; PCDDs: polychlorinated dibenzo-*p*-dioxins; PCDFs: polychlorinated dibenzofurans; PEST: pesticides and biocides; PLE: pressurized liquid extraction; POPs: persistent organic pollutants; PPCPs: pharmaceuticals and personal care products; TIE: toxicity identification evaluation; TOC: total organic carbon; VAST: vertebrate automated screening technology; WWTPs: waste water treatment plants.

Authors' contributions

RM performed all experiments and been has involved in the whole process of analysis and interpretation of the data. HH has been involved in critically revising the FET section. MK contributed to the analysis and interpretation of the LC-HRMS data. WV contributed to the analysis and interpretation of the trace elements data. CW has been involved in the acquisition and analysis of GC-HRMS data. PH, CG and MT have been critically involved in revising the whole chemical analyses and sampling sections. They also gave an important contribution to the characterization of the sampling area. EK critically contributed to the understanding of the FET data in revising the FET section. WB made substantial contributions to the whole conception, design and interpretation of data. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional information files.

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Not applicable.

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